PRIMARY IMMUNODEFICIENCIES WORLDWIDE

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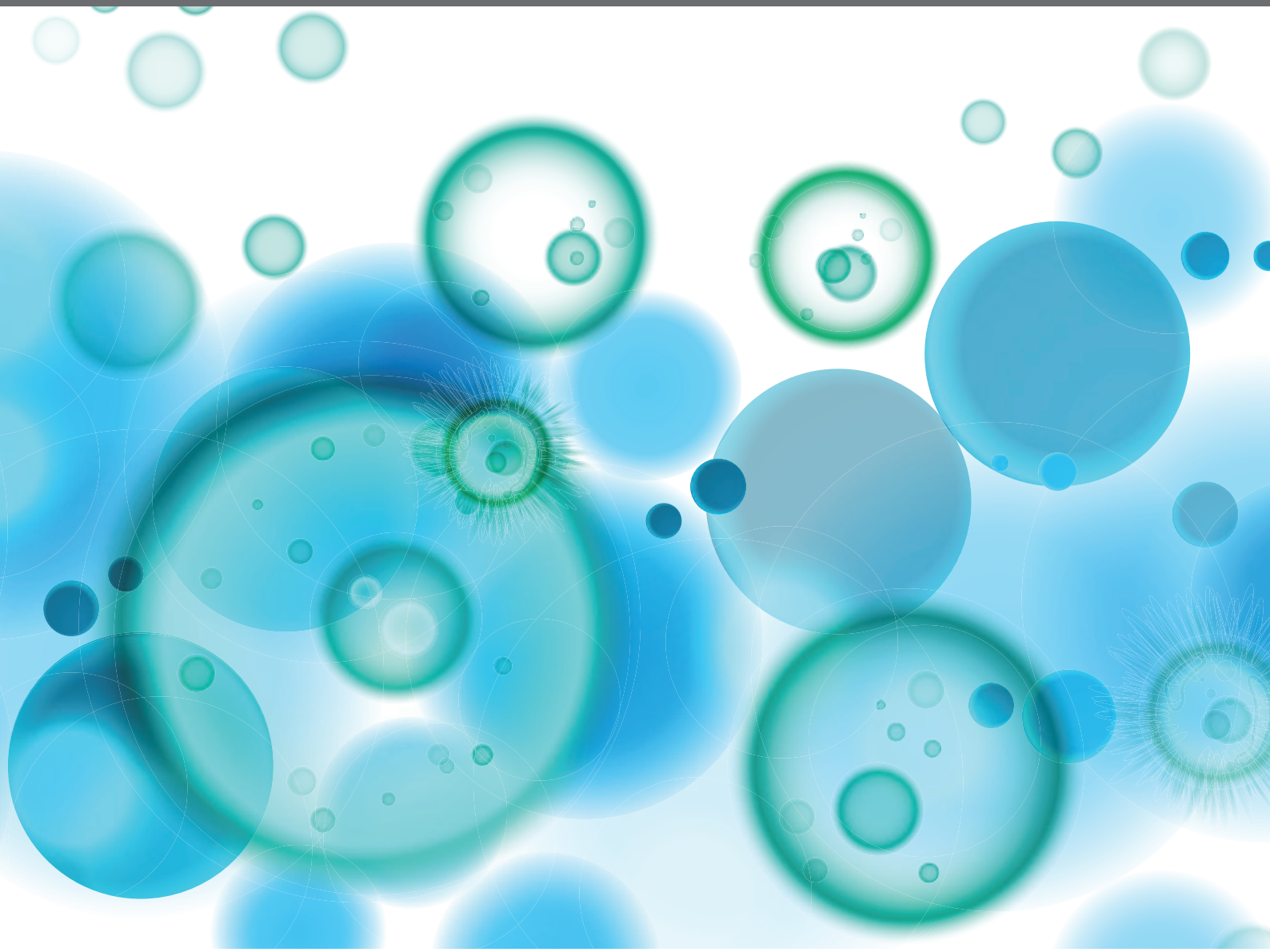

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PRIMARY IMMUNODEFICIENCIES WORLDWIDE

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Editorial: Primary Immunodeficiencies Worldwide

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Keywords: primary immunodeficencies (PID), intravenous immunoglobulin (IVIg), infectious disease, newborn screen (NBS), poliovirus (PV), Bacille Calmette-Guérin vaccine (BCG), developing and transition countries, consanguinity

Editorial on the Research Topic

Primary Immunodeficiencies Worldwide

The field of Primary Immunodeficiencies (PID) is growing fast. Over 400 diseases are defined and characterized by recurrent or chronic infections, autoimmunity, allergy, inflammation, or cancer as a consequence of genetic alterations affecting the immune system (1). The overall incidence of PID is around 1:10,000 and the majority presents early in childhood. Over the past decades, there has been an enormous increase in understanding of disease pathology, as well as clinical expertise and patient awareness worldwide, as a result of educational initiatives and scientific meetings by medical societies, supporting agencies, and patient associations.

Humans are genetically heterogeneous, and the environmental characteristics and social habits differ dramatically in the several geographic regions of the world. Consequently, the prevalence and distribution of the nine groups of primary immunodeficiencies vary worldwide, and the clinical profile of a particular PID will also vary.

Most of the literature and discoveries about Primary Immunodeficiencies come from North America and Europe and refer to these geographical areas. This Frontiers Research Topic was developed to bring together original clinical and basic research from authors representing various geographical areas to highlight challenges and developments in epidemiology, diagnosis, and treatment of Primary Immunodeficiencies worldwide.

Twenty-two articles are included in this Research Topic, and are categorized into the following types: 9 Original Research (Aghamohammadi et al.; de Albuquerque et al.; Goel et al.; Macklin et al.; Pietrucha et al.; Rechavi et al.; Schlechter et al.; Segundo et al.; Slade et al.), 2 Case Reports (Bradshaw et al.; Sogkas et al.), 2 Mini-reviews (Al-Mousa and Al-Saud; Barbouche et al.), 6 Reviews (de Jesus Nunes-Santos and Rosenzweig; Lee and Lau; Marciano and Holland; Ruffner et al.; Seleman et al.; Thakar et al.), 2 Perspectives (Jindal et al.; Quinti and Mitrevski), and 1 Opinion (Sorensen).

Within the context of epidemiology, several papers present and discuss infectious complications in international cohorts. It is discussed how cellular and molecular defects of the immune system predispose to invasive endemic fungal infections worldwide (Lee and Lau). Furthermore, it is shown that PID patients excrete poliovirus for extended periods (Macklin et al.), and that these may form a reservoir that forms a risk to poliovirus eradication strategies (Aghamohammadi et al.). Ruffner et al. provide a comprehensive overview of viral complications of PIDs. On top of infections, vaccination with live strains such as BCG are a major risk for complications in those

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countries where it is provided, including in India (Jindal et al.), and these complications are also associated with newly identified genetic causes of PID (de Jesus Nunes-Santos and Rosenzweig).

In addition to infectious complications, non-infectious complications are more frequently documented worldwide as exemplified in papers presenting the PID field in India (Jindal et al.), and an antibody-deficient cohort in Australia (Slade et al.). These complications most frequently involve autoimmunity, gastrointestinal disease, granulomatous inflammation, and malignancy. Disease complications vary even between individuals with the same genetic defect as illustrated by two siblings with RNF168 deficiency, highlighting roles for additional genetic or epidemiological factors (Pietrucha et al.). Countries in the Middle East and North Africa (MENA) with high rates of consanguinity face additional challenges (Al-Mousa and Al-Saud; Barbouche et al.), as some autosomal recessive disorders can be unexpectedly frequent and disease presentation can be complicated by other genetic changes.

Diagnostic challenges vary worldwide. Access to genetic genetics in developing countries is often limited, and shipment of patient material can be costly. The use of dried bloodspots for sequence analysis of genomic DNA will be a means to overcome the cost limitation (Segundo et al.). In developed countries, the implementation of next-generation sequencing technologies has taken flight (Seleman et al.), and facilitates quick diagnosis such as in the case of X-Linked Moesin-Associated Immunodeficiency (Bradshaw et al.) and MAP3K14 deficiency (Schlechter et al.).

The most optimal timing of PID diagnosis is before disease presentation and that is aimed at in newborn screening programs. A lot of experience has been obtained with the T cell receptor excision circle (TREC) assay in the USA (Thakar et al.), and many countries worldwide, including Israel, have now adopted it (Rechavi et al.).

Still, more insights into PID pathogenesis and genetics are needed. Diagnosis of antibody deficiency remains challenging, especially in the context of normal total IgG serum levels (Sorensen), and this can result in substantial diagnostic delays (Slade et al.). Functional assays for diagnostics require more insights into disease pathogenesis, and this lies at the heart of basic research into PID. Examples included in this Research Topic are examination of STAT phosphorylation upon cytokine receptor engagement (Goel et al.; Sogkas et al.), and *in vitro* models of innate immune responses to Candida (de Albuquerque et al.).

The ultimate focus of the Research Topic concerns treatment of PID. Immunoglobulin replacement therapy is already provided for over 60 years to supplement IgG, but has additional immunomodulatory roles that are still not completely understood (Quinti and Mitrevski), and might warrant the use even beyond antibody deficiency. More recently developed biologicals will hold even more promise over the coming years (Quinti and Mitrevski). Corrective therapy would be ultimate form of treatment, and hematopoietic stem cell transplantation is currently more widely applied in combined immunodeficiencies and in immune dysregulation disorders. Although the rarity of specific PIDs could hamper clinical trials, the genetic definition of disease should provide the best rationale for compassionate access to enable precision medicine.

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REFERENCES

1. Picard C, Bobby Gaspar H, Al-Herz W, Bousfiha A, Casanova JL, Chatila T, et al. International Union of immunological societies: 2017 primary immunodeficiency diseases committee report on inborn errors of immunity. *J Clin Immunol.* (2018) 38:96–128. doi: 10.1007/s10875-017-0464-9

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Patients with Primary Immunodeficiencies Are a Reservoir of Poliovirus and a Risk to Polio Eradication

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Immunodeficiency-associated vaccine-derived polioviruses (iVDPVs) have been isolated from primary immunodeficiency (PID) patients exposed to oral poliovirus vaccine (OPV). Patients may excrete poliovirus strains for months or years; the excreted viruses are frequently highly divergent from the parental OPV and have been shown to be as neurovirulent as wild virus. Thus, these patients represent a potential reservoir for transmission of neurovirulent polioviruses in the post-eradication era. In support of WHO recommendations to better estimate the prevalence of poliovirus excreters among PIDs and characterize genetic evolution of these strains, 635 patients including 570 with primary antibody deficiencies and 65 combined immunodeficiencies were studied from 13 OPV-using countries. Two stool samples were collected over 4 days,

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tested for enterovirus, and the poliovirus positive samples were sequenced. Thirteen patients (2%) excreted polioviruses, most for less than 2 months following identification of infection. Five (0.8%) were classified as iVDPVs (only in combined immunodeficiencies and mostly poliovirus serotype 2). Non-polio enteroviruses were detected in 30 patients (4.7%). Patients with combined immunodeficiencies had increased risk of delayed poliovirus clearance compared to primary antibody deficiencies. Usually, iVDPV was detected in subjects with combined immunodeficiencies in a short period of time after OPV exposure, most for less than 6 months. Surveillance for poliovirus excretion among PID patients should be reinforced until polio eradication is certified and the use of OPV is stopped. Survival rates among PID patients are improving in lower and middle income countries, and iVDPV excreters are identified more frequently. Antivirals or enhanced immunotherapies presently in development represent the only potential means to manage the treatment of prolonged excreters and the risk they present to the polio endgame.

Keywords: poliovirus eradication, immunodeficiency-associated vaccine-derived polioviruses, oral poliovirus vaccine, humoral immunodeficiency, combined immunodeficiency, primary immunodeficiency

INTRODUCTION

Primary immunodeficiencies (PIDs) are a heterogeneous group of inherited disorders due to developmental defects or dysfunction of the immune system components (1). PID patients can potentially be infected by immunizations if they receive live vaccines (2). Attenuated oral poliovirus vaccine (OPV) immunization has been associated with poliovirus infection in patients with primary antibody deficiencies and combined immunodeficiencies, which can lead to paralysis (3–5).

Paralysis is not the only risk of OPV immunizations in PID patients since some vaccinated PID patients may shed vaccine-derived polioviruses (VDPVs) due to a prolonged period of intestinal replication. VDPV variants of OPV serotypes (PV1, PV2, and PV3) show increased nucleotide divergence in the viral protein 1 (VP1) coding region associated with increased neuropathogenicity (6). Although neonatal screening has begun to expedite the early diagnosis of patients with severe combined immunodeficiencies (SCIDs) and agammaglobulinemia to modify their vaccination program and therapeutic management, these screening tests do not diagnose T or B cell dysfunction, nor have they been implemented worldwide. On the other hand, approximately 150 countries still use OPV in the national childhood immunization schedule, which can spread and be transmitted to PID patients incidentally. Approximately 100 VDPV infections have been reported in PID patients worldwide to date (4, 7, 8). As a potential reservoir for neurovirulent VDPV strains, infected PID patients represent a global risk to unimmunized contacts and to the Global Polio Eradication Initiative (4, 9).

The significant impact of OPV on the elimination of poliomyelitis due to wild poliovirus (WPV) and its additional beneficial properties (e.g., economic costs, easy administration, and superior mucosal antibody response) are evident. However, because WPV type 2 has been eradicated and 90% of circulating VDPV, and approximately 40% of VAPP cases are caused by type 2, type 2 was removed from OPV in all countries globally in April 2016. As a result, screening for immunodeficiency-associated VDPV (iVDPV) shedding of known PID patients is more critical to completing elimination of live type 2 poliovirus from the world (10, 11).

Thus, assessing the risk associated with prolonged iVDPV excretion among PID patients by estimating the prevalence in a worldwide study is of critical importance for stakeholders and decision-makers to build an effective strategy for the polio endgame, including development of potential treatments such as antivirals. Based on several reports showing that not only patients with primary antibody deficiencies but also combined immunodeficiencies are very susceptible to persistent polio and non-polio enterovirus (NPEV) infections (4, 12), this multicenter study has been designed to determine the prevalence of iVDPV in patients with both types of immunodeficiencies and characterize the genetic properties of associated virus strains.

MATERIALS AND METHODS

Patients

All patients enrolled in this study were diagnosed with PID from 19 Jeffrey Modell Foundation (JMF) sites in 13 OPV-using countries from January 2014 to November 2015 (**Table 1**). Inclusion criteria encompassed patients 6 weeks of age or greater and having a diagnosis of SCID, combined immunodeficiency, agammaglobulinemia, or common variable immunodeficiency (CVID). Other types of PID patients were not included in this study.

Assessment of each patient at the different JMF sites met the updated criteria introduced by the European Society for Immunodeficiencies[1] (13) and/or the American Academy of

[1]http://esid.org/Working-Parties/Registry/Diagnosis-criteria.

TABLE 1 | 19 Jeffrey Modell Foundation sites from 13 countries enrolled PID patients.

Country[a]	City	Site no.[b]	Enrolled patients	Culture results	National polio vaccination[c]	PID screening
India	Chandigarh	Site 30	23	23	5 OPV doses (birth-6w-10w-14w-16m)	No
Tunisia	Tunis	Site 12	40	40	1 IPV dose (6m) 7 OPV doses (2m-3m-6m-18m-6y-12y-18y)	No
China	Shanghai	Site 23	52	51	4 OPV doses (2m-3m-4m-4y)	No
	Hong Kong	Site 21	11	11	6 OPV doses before 2007 (birth-3m-5m-18m-6y-11y)	No
Colombia	Medellin	Site 24	25	25	5 OPV doses (2m-4m-6m-18m-5y)	No
Iran	Tehran	Site 14	102	102	6 OPV doses (birth-2m-4m-6m-18m-6y)	No
Mexico	Mexico City	Site 25	20	20	2 OPV doses (>6m-<5y)	No
Poland	Warsaw	Site 17	29	29	3 IPV doses (3m-5m-16m) 1 OPV dose (6y)	No
Russia	Moscow	Site 18	35	35	2 IPV doses (3m-4.5m) 4 OPV doses (6m-18m-20m-14y)	No
	Moscow	Site 28	20	20		
	Yekaterinburg	Site 19	28	28		
Turkey	Ankara	Site 27	30	29	2 IPV doses (2m-4m) 2 OPV doses (6m-18m)	No
	Ankara	Site 26	43	43		
	Izmir	Site 11	75	75		
	Istanbul	Site 20	24	24		
Israel	Tel Hashomer	Site 29	24	24	2 OPV doses (6m-18m)	No
Japan	Tokyo	Site 13	9	8	2 OPV doses before 2012 (3m-18m)	No

OPV, oral poliovirus vaccine; IPV, inactivated polio vaccine; PID, primary immunodeficiency; w, weeks; m, months; y, years.

[a]*Countries are organized in order of World Bank income classification (lower middle, upper middle, and upper income).*

[b]*Site numbers are provided to correspond with subject numbers in* ***Tables 3*** *and* ***4****.*

[c]*WHO vaccine-preventable diseases: monitoring system. 2016 Global summary.*

Allergy, Asthma & Immunology for the diagnosis of PID (14). In all studied cases, other defined secondary causes of immunodeficiency were excluded. Other types of PID patients were not included in this study. The standard criteria for diagnosis of patients were summarized in Table S1 in Supplementary Material.

Written informed consent (if 18 years of age or older), assent (if 7–17 years of age) or consent from the parents/guardian (for those less than 7 years) was obtained, in accordance with the principles of the ethics committees or Institutional Review Board of the local institutes using common forms designed by The Task Force for Global Health (TFGH, protocol no: CVE-001, WIRB® Protocol #20130957; Data Sheet S1 in Supplementary Material) translated to the participant JMF sites' local languages.

Study Design

Each selected JMF study site identified patients who met the inclusion criteria at the time of enrollment. After collecting consent or assent forms, the JMF study site collected the relevant information to complete the patient's log (to track enrollment and to maintain the link between the unique identifiers) and case report form (CRF), and assigned a coded identification number (ID) under a standardized procedure. IDs, names, and contact information was recorded in the patient's log, which was stored securely at the site until completion of the study. All specimens and CRFs were labeled with ID only and not any personal identifiers. The CRF included region (country based on JMF site), demographic characteristics (including date of birth and gender), clinical features (type of PID, age of onset and medical records including signs or symptoms of poliovirus infection), polio vaccination history (date of first and last OPV exposure and type of exposure), PID treatment modalities, and specimen collection information.

Each JMF study site chose the optimal procedure for collection of two stool samples (about 8 g using provided kits) over a 4-day period depending on the proximity of the patient to the JMF study site, the practicality of patient travel to the study site and the availability of adequate cold storage of the stool sample (Table S2 in Supplementary Material).

After collection, the stool samples were kept refrigerated at a temperature of 2–8°C and shipped to the local or regional Global Polio Laboratory Network (GPLN) laboratory along with supporting documentation. The JMF study site provided an electronic copy of the CRF by encrypted means (using a secure FTP server at TFGH) to Centers for Disease Control and Prevention (CDC) for secure data management. Stool specimens were sent to the GPLN laboratory for isolation of poliovirus and NPEVs. Isolates were sent to the CDC polio/enterovirus laboratory using standard shipping protocols for further characterization and genomic sequencing. GPLN member laboratories in all six WHO regions followed standardized protocols to identify and

isolate poliovirus and NPEV. They also differentiated the three poliovirus serotypes, Sabin-like poliovirus, and vaccine derived (VDPV). For VDPVs, they conducted genomic sequencing to determine how long the virus had been circulating or had been excreted in the case of iVDPV excreters. This was done by comparing the nucleotide sequence of the VP1 region of the genome from PV isolates according to published protocols (15, 16). The number of mutations compared to the parent Sabin strain is approximately proportional to the duration of excretion. The accepted definition of an iVDPV is >1% nucleotide divergence from Sabin types 1 and 3 or >0.6% for Sabin type 2 in the VP1 coding region (6).

If testing results were positive for poliovirus or NPEV, the JMF site physician assured that family members were appropriately vaccinated against polio and educated about principles of good hygiene to prevent fecal–oral transmission. The physician also followed-up any patient found to be excreting poliovirus periodically (every 1–2 months) until the cessation of excretion and the procedures for testing and reporting above were followed each time. Patients were considered as having cleared an infection if two consecutive stool specimens in 2 months were negative according to the WHO-recommended surveillance standard of poliomyelitis.[2]

The TFGH study manager ensured coordination of specimen shipment along with the JMF scientific program director, and laboratory results were communicated to CDC both by the GPLN laboratory through the GPLN global coordinator and by the TFGH study manager and entered into the secure database at CDC.

Statistical Analysis

Statistical analysis was performed using a commercially available software package (SPSS Statistics 17.0.0, SPSS, Chicago, IL, USA). The one-sample Kolmogorov–Smirnov test was applied to estimate whether data distribution is normal. Parametric and non-parametric analyses were performed based on the finding of this evaluation. A p-value of 0.05 or less was considered statistically significant.

[2] http://www.who.int/immunization/monitoring_surveillance/burden/vpd/surveillance_type/active/poliomyelitis_standards/en/.

RESULTS

Characteristics of Studied PID Cases and OPV Vaccination

During the study period, a total of 635 patients (444 males and 191 females) were included into this study. CVID was the most common diagnosis of studied patients ($n = 320$) followed by agammaglobulinemia ($n = 250$), SCID ($n = 54$) and combined immunodeficiency [$n = 11$; all with a diagnosis of major histocompatibility complex (MHC) class II deficiency]. Age at time of study enrollment was 21.1 ± 15.7 years (mean ± SD) for patients with CVID, 18.0 ± 8.5 years for patients with agammaglobulinemia, 7.9 ± 7.8 years for patients with MHC class II deficiency, and 3.8 ± 4.9 years for SCID patients. None of the patients with SCID or MHC class II deficiency were transplanted prior to the study entry.

Among all enrolled patients from these OPV-using countries, 44 individuals did not receive OPV vaccination based on the prior family history of PID and early diagnosis or modified inactivated polio vaccine-based national childhood immunization schedule (**Table 2**). However, the median age at first OPV dose was approximately 2 months (range from birth to 6 years) for the 584 patients who did receive OPV. Past medical history of paralytic disease was identified in 14 patients (1.25% of CVID and 4% of agammaglobulinemia patients), but none of the SCID or MHC class II deficiency cases had a history of paralytic disease. Agammaglobulinemia patients were younger than SCID and CVID patients when they received their last dose of OPV, consistent with the cessation of further OPV doses after an earlier clinical diagnosis of PID (**Table 2**).

Use of Intravenous and Subcutaneous Immunoglobulin

The majority (97%) of enrolled subjects were receiving either intravenous immune globulin (IVIG) or subcutaneous immune globulin (SCIG) while participating in the study. For 19 subjects, no IVIG or SCIG use was noted.

Frequency of Poliovirus and NPEV Isolation

Thirteen patients (2%) excreted poliovirus with most patients excreting for less than 2 months following identification of infection (**Table 3**). The median age of poliovirus-excreting

TABLE 2 | Demographic data summary for all 635 PID patients.

Parameters	All patients	CVID	Agammaglobulinemia	SCID	MHC class II deficiency
Number of patients	635	320	250	54	11
Gender (M/F)	444/191	179/141	237/13	24/30	4/7
Current age, mean; years (SD)	12.7 (8.2)	21.1 (15.7)	18.0 (8.5)	3.8 (4.9)	7.9 (7.8)
Age at first OPV dose, mean; years (SD)	0.6 (0.8)	0.5 (1.6)	0.4 (1.2)	1.0 (2.6)	0.6 (0.9)
Age at last dose, mean; years (SD)	2.3 (2.0)	4.3 (4.9)	3.0 (3.6)	2.2 (3.4)	NA
Exposure to OPV (Y/N)	584/51	303/17	233/17	37/17	11/0
Paralytic disease	14	4	10	0	0

OPV, oral poliovirus vaccine; CVID, common variable immune deficiency; SCID, severe combined immunodeficiency; PID, primary immunodeficiency; MHC, major histocompatibility complex.

TABLE 3 | Data summary for all 13 PID patients with isolated poliovirus.

Patient ID	Gender	Age at study entry	Diagnosis	Site[b]	Most recent known OPV exposure	Virus	Excretion duration—no. of nucleotide changes
12-017	F	7 years	CVID	Tunisia	6 years	Sabin 1	1 month—3 nucleotide changes
12-007[a]	M	11 years	MHC II deficiency	Tunisia	1 month	Sabin 2	5 months excretion—9 nucleotide changes (0.9%)
12-025	F	7 years	MHC II deficiency	Tunisia	Unknown	Sabin 3	1 month—3 mutations
14-057	M	6 years	Agammaglobulinemia	Iran	1 week	Sabin 2	1 month—no mutations
14-108[a]	F	10 months	MHC II deficiency	Iran	6 months	Sabin 2	10 months excretion on study—12 nucleotide changes (1.2%)
14-116[a]	M	3 months	SCID	Iran	2 months	Sabin 2	6 nucleotide changes (0.6%)
14-117[a]	F	1 year	SCID	Iran	6 months	Sabin 2	10 nucleotide changes (1%)
26-012	M	22 years	CVID	Ankara, Turkey	7 years	Sabin 2	<3 months—no nucleotide changes
27-030	F	2.5 years	MHC II deficiency	Ankara, Turkey	2 years	Sabin 3	Sequence pending
20-003	M	8 months	Agammaglobulinemia	Istanbul, Turkey	6 months	Sabin 2	5 months excretion on study—3–4 nucleotide changes
11-031[a]	F	11 months	SCID	Izmir, Turkey	6 months	Sabin 3	15 nucleotide changes
19-021	M	13 years	CVID	Yekaterinburg, Russia	5 years	Sabin 3	No nucleotide changes
19-023	F	6.5 years	Agammaglobulinemia	Yekaterinburg, Russia	Not known	Sabin 2	No nucleotide changes

OPV, oral poliovirus vaccine; CVID, common variable immune deficiency; SCID, severe combined immunodeficiency; M, male; F, female; PID, primary immunodeficiency; MHC, major histocompatibility complex.

[a]*Patients with immunodeficiency-associated vaccine-derived polioviruses (iVDPV).*

[b]*Countries are organized in order of World Bank income classification (lower middle and upper middle income).*

patients was 6.5 years (range 3 months–22 years). Patients with combined immunodeficiencies (three SCID and four MHC class II deficiency) were at 10-fold increased risk of excreting poliovirus (10.7%) compared to patients with predominantly antibody defects (1.0%; three CVID and three agammaglobulinemia; $p < 0.001$). The average age of patients with combined immunodeficiencies excreting poliovirus was significantly lower than in antibody deficient patients (3.3 ± 3.0 vs. 9.2 ± 7.3 years; $p = 0.044$). None of these 13 patients were coinfected with NPEV. Poliovirus excretion was not observed in any of patients with a history of paralytic disease.

Non-polio enteroviruses were isolated from 4.7% of evaluated patients (17 CVID, 8 agammaglobulinemia, 2 SCID, and 3 MHC class II deficiency), with a higher prevalence of NPEV infection in MHC class II deficient patients compared to patients with other forms of PIDs ($p = 0.012$; **Figure 1**). There was no difference in NPEV prevalence by gender (5.1% of males and 3.6% of females). **Table 4** summarizes the clinical and virologic information for NPEV excreters. Echoviruses were the most commonly isolated virus (41%), followed by coxsackieviruses (24%).

The mean age of poliovirus excreting patients was significantly lower than NPEV excreters (5.9 ± 4.3 vs. 10.4 ± 9.2 years, $p = 0.005$). Samples from nine patients (1.4% of 635 studied patients) were collected at the time of PID diagnosis and prior to administration of immunoglobulin replacement therapy. Of note, two of these nine cases (22.2%) were NPEV positive, both were diagnosed with antibody deficiency and were from the same center (12-006 and 12-019; $p = 0.06$). No poliovirus was isolated from these nine patients.

Predominance of iVDPV among Combined Immunodeficiency

Five (0.8% of all patients) of 13 poliovirus-excreting patients were considered iVDPV excreters based on the number of VP1 nucleotide changes (median of 10 nucleotide changes; range, 6–15). Of note, all iVDPV excreters had combined immunodeficiency (three SCID and two MHC class II deficiency), and four of these five patients excreted Sabin 2 strains (**Table 3**). The median number of mutations did not differ between SCID (1%; range 0.6–1.5%) and MHC II deficiency (1.05%; range, 0.9–1.2%) patients. None of the iVDPV excreters presented with acute flaccid paralysis, but chronic diarrhea was a common clinical manifestation. OPV vaccination had occurred in all of iVDPV excreters, and the median time from the first OPV dose to identification of iVDPV was 10 months (range 3 months–11 years). The sequence divergence suggests that secondary exposure may have been the source of infection in some cases. Further vaccination was avoided in all patients due to the diagnosis of PID.

Clinical Presentation and Genetic Studies of iVDPV Excreters

One MHC class II deficiency patient (12-007) was followed for several years prior to enrollment in the current study showing intermittent VDPV excretion episodes and reinfection with various VDPV strains and with several NPEV from a sibling

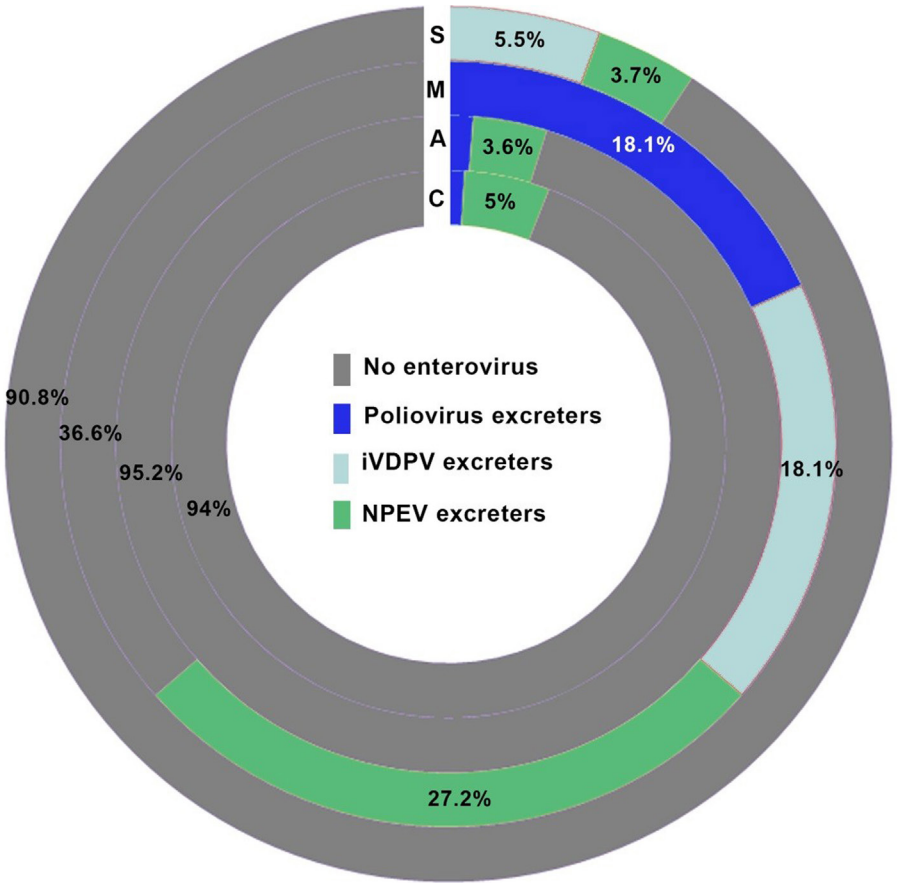

FIGURE 1 | Prevalence of enterovirus excreters among different groups of primary immunodeficiency patients. C, common variable immunodeficiency; M, major histocompatibility complex II deficiency; A, agammaglobulinemia; S, severe combined immunodeficiencies.

and from the community (17). During the current study, he was infected with Sabin 2 strain (**Table 3**). The second MHC class II patient (14-108) was a 10-month-old girl with disseminated granulomatous disease (BCGosis) after BCG vaccination at age 4 months who excreted poliovirus for 5 months. The mutation in *CIITA* gene (homozygous c.3242-3244delACA, p.Asn1082del) was confirmed in this patient using targeted PID genes panel sequencing.

The only Sabin serotype-3 iVDPV excreter was a girl with SCID (11-031; *RAG1* c.1682G>A, p.R561H) enrolled at age 11 months. This poliovirus had the highest number of nucleotide changes (15 nucleotides) observed in the study. Virus elimination was evident 3 months after hematopoietic stem cell transplantation. Two other SCID patients infected by serotype-2 iVDPV had the molecular diagnosis confirmed with a mutation in *ADA* (14-116; homozygous c.415G>T, p.Glu139X) and a mutation in *RAG1* (14-117; homozygous c.1180C>T, p.Arg394Trp) genes, respectively.

DISCUSSION

Primary immunodeficiency patients having defects in humoral or cellular immunity are at risk of chronic/prolonged infection with enteroviruses, including polioviruses. These patients can excrete iVDPV after receiving OPV or after being exposed to a household or community contact excreting poliovirus. Patients who are excreting polioviruses are at risk of developing paralytic poliomyelitis. Such patients must be identified, and their infection resolved, to protect the patients and achieve global eradication of poliovirus. The present study brings together the JMF network, TFGH, WHO, and CDC to estimate the prevalence of poliovirus excretion in selected PID patients who have been exposed to OPV. Two of the participant countries are high income (Israel and Japan); the remainder are lower (two countries) or upper (seven countries) middle income countries according to the World Bank classification. Three poliovirus excreters were from a lower middle income country (Tunisia), and the remainder were from upper middle income countries. NPEVs were isolated from patients from countries from all three economic strata.

Although few cross-sectional studies have been conducted to identify iVDPV among PID patients without paralysis (12, 17–21), this study identified the largest number of PID patients both with antibody and combined immune deficiencies. This study also constituted a large number of countries with worldwide distribution from four continents (Asia, Africa, Europe, and South America). Approximately 2% of the studied PID patients excreted

TABLE 4 | Data summary for all 30 PID patients with isolated non-polio enteroviruses (NPEVs).

Patient ID	Gender	Age at study entry (year)	Diagnosis	Site[b]	Class of NPEV
12-031	M	5	MHC II deficiency	Tunisia	NA
12-037	M	0.4	MHC II deficiency	Tunisia	NA
12-006[a]	M	5	CVID	Tunisia	Negative/E2
15-014	M	10	XLA	Argentina	E25
23-003	M	7	Agammaglobulinemia	China	NA
23-004	M	14	Agammaglobulinemia	China	NA
24-020	M	22	CVID	Columbia	NA
24-023	M	4	Agammaglobulinemia	Columbia	NA
14-079	F	28	CVID	Iran	EV11
14-020	M	7	Agammaglobulinemia	Iran	EV20
14-019	M	41	CVID	Iran	EV6
14-121	M	17	CVID	Iran	NA
25-020	M	8	CVID	Mexico	NA
25-019[a]	M	8	Agammaglobulinemia	Mexico	NA
27-021	F	2	MHC II deficiency	Ankara, Turkey	CA5/no second sample
20-025	M	1	Agammaglobulinemia	Istanbul, Turkey	EV9
20-007	F	18	CVID	Istanbul, Turkey	CA4
20-006	M	20	CVID	Istanbul, Turkey	EV 33
11-018	M	5	SCID	Izmir, Turkey	CA10
11-050	F	6	CVID	Izmir, Turkey	CA2
11-048	M	0.5	CVID	Izmir, Turkey	CB
11-042	M	11	CVID	Izmir, Turkey	CB4
11-016	M	17	CVID	Izmir, Turkey	E6
11-075	F	0.7	CVID	Izmir, Turkey	NA
11-068	M	7	CVID	Izmir, Turkey	E7
29-012	F	3	Agammaglobulinemia	Israel	CB5
29-006	F	12	SCID	Israel	EV01
29-016	M	5	CVID	Israel	EV11
29-024	M	20	Agammaglobulinemia	Israel	EV13
29-008	M	9	Agammaglobulinemia	Israel	NA

OPV, Oral poliovirus vaccine; CVID, common variable immune deficiency; SCID, severe combined immunodeficiency; M, male; F, female; CA, coxsackie A virus; CB, coxsackie B virus; EV, enterovirus; E, echovirus; PID, primary immunodeficiency; MHC; major histocompatibility complex.

[a]*Samples were collected prior to immunoglobulin replacement therapy.*

[b]*Countries are organized in order of World Bank income classification (lower middle, upper middle, and upper income).*

poliovirus. PID survival rates are improving in lower and middle income countries (22). Furthermore, iVDPV excreters are being identified at a markedly increased rate due to improved surveillance that includes polio symptom-free excreters (3). This reinforces the need for enhanced surveillance of PID patients until polio eradication is certified and the use of OPV is stopped. The findings of this study could help estimate the prevalence of iVDPV excreters, which is needed to assess the global risk to eradication posed by these excreters. Undiagnosed PID patients and cases with delayed diagnosis should be considered as potential contributors to underestimation.

In 5% of the studied patients, NPEV was isolated, and none of them were concomitantly excreting poliovirus. Although this prevalence was lower than in a previously reported single-center study (20%) (12), it demonstrates the susceptibility of patients with humoral and cellular immunodeficiency to prolonged excretion of all enteroviruses. Higher prevalence of NPEV compared to OPV strains might be due to the multiplicity of circulating NPEV serotypes. Moreover, immunoglobulin preparations used for treatment of PID patients do not contain high titers of immunoglobulin against all NPEVs. Of note, immunoglobulin replacement might contribute to the relatively short-term excretion of OPV strains observed in some PID patients; however, we could not test this phenomenon as all poliovirus excreters in this study were receiving IVIG or SCIG treatment.

Poliovirus 2 was the most prevalent serotype detected in iVDPV excreting PID patients (80%), followed by serotype 3 (20%); consistent with the findings in PID patients with paralysis (4, 8). Concurrent infection with more than one iVDPV serotype has been documented in other studies; but we did not observe simultaneous shedding of more than one iVDPV serotype in any patient. Possible explanations for the predominance of poliovirus 2 include the high frequency of recombination that can occur with poliovirus 1 and 3 in OPV vaccinated individuals, resulting in a virus with a higher replication efficiency (23–26). Sabin poliovirus 2 also replicates longer and is transmitted more readily due to greater replication fitness or the ability to outcompete the other two serotypes for the binding to the CD155 receptor in the host cells (27, 28). Although these characteristics are potential factors for an increased risk of iVDPV2 in PID, they contributed to the eradication of WPV type 2 (29) and poliovirus 2 has since been removed from the trivalent OPV vaccine (30).

All SCID poliovirus excreters and 50% of MHC class II deficient poliovirus excreters became iVDPV excreters. While the number of iVDPV excreters was small, this result suggests

that cellular immunity may contribute effectively to clearance of enterovirus infections including cessation of poliovirus excretion. Both humoral (antigen-specific plasma cells and memory B cells) and cellular (memory T cells) immunities should be developed following an effective immunization especially for complex vaccines against viruses. In addition to the capability of OPV-infected dendritic cells to engage specific CD8 T cells, the presence of OPV-specific CD4 T cells in vaccinated individuals is crucial. CD4 T cells are involved in serotype-specific antibody production (B cell priming by follicular helper T cells) and interferon gamma production to lyse infected target cells (virus clearance by cytolytic effector CD4 cells as well as cytolytic effector CD8 T cells). Antigen presentation in both functions of CD4 T cells is through MHC class II (31). Therefore, patients with combined immunodeficiencies who lack both humoral and cellular immunities may be more likely to be unable to stop virus excretion. However, the presence of six patients with antibody deficiency with long-term poliovirus shedding (approximately 9 years) highlights the important role of viral specific-antibodies in complete clearance of the virus (32). Considering the several reports of antibody deficient patients with paralysis caused by iVDPV, the identification and treatment of poliovirus symptom-free virus excreting patients is necessary (3–5).

Wild poliovirus or iVDPV infections were not observed in the patients with a history of the paralytic disease, and none of their neurologic symptoms were linked to poliovirus infection. Moreover, paralytic disease in the context of PID has several potential etiologies including neurologic autoimmune disorders including multiple sclerosis, myalgic encephalomyelitis, myasthenia gravis, Guillain–Barré syndrome, chronic inflammatory demyelinating polyneuropathy, and vasculitic neuropathies. Moreover, vitamin deficiency (B12) and paraneoplastic neuropathy should be considered as differential diagnosis for paralysis in PID patients (33).

This study provides an estimate of the global iVDPV prevalence among PID patients without paralytic disease and supports expanded screening for iVDPV excretion in these patients. Although most previous studies focused on the risk of long-term iVDPV infection in antibody deficient patients, the predominance of risk in patients with combined immunodeficiencies included in the current study highlights the importance of considering this group of PID patients in any surveillance program. Reinfection with poliovirus and NPEV excretion in PID patients described elsewhere demonstrates the need for prolonged follow-up (17).

The Global Polio Eradication Initiative plans to cease use of OPV worldwide once WPV has been certified as eradicated, which will end the generation of new iVDPVs. However, there is currently no means for addressing the threat posed by existing immunodeficient persons infected with iVDPVs, either to the infected individual's risk of paralytic disease, or to the community of a continuing source of poliovirus transmission. Antivirals represent a potential means to manage the treatment of iVDPV excreters and the risk they present to the eradication effort (32, 34). Two safe virus-specific antivirals acting by differing mechanisms are now being developed and may be used as a combination (e.g., pocapavir and V-7404). This strategy may resolve the individual's infection, stop iVDPV excretion, and serve to eliminate the risk of poliovirus transmission in the community. Currently, pocapavir is being considered for use in poliovirus excreting PID patients on a compassionate use basis.

The limitations of the existing study include that no low-income level country participated, which may bias the generalization of these findings. Moreover, other forms of less profound combined immunodeficiency should be evaluated in future studies since this study recruited only patients with MHC class II deficiency.

The potential risk posed by iVDPV excreters to the polio eradication effort indicates the immediate need to develop and implement a global iVDPV surveillance strategy. Utilizing this approach, individuals at risk of prolonged poliovirus excretion can be identified and antiviral treatment can be initiated.

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The following JMF centers network investigators and study collaborators contributed to the conduct of this study.

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ETHICS STATEMENT

This study was approved by Western Institutional Review Board.

AUTHOR CONTRIBUTIONS

AA, HA, and MRB performed clinical investigation, analyzed data, and wrote the manuscript. MM, MP, SW, RS, SK, JQ, FM, and VM were responsible for conception and design of the study, interpreted data, and supervised the study. NK, XW, OS, TL, AI, EB, IT, BC-C, JF, RS, EK-A, SS, LB, FE-R, AS, Y-LL, and SN recruited patients and performed clinical investigation. All the authors read and approved the final manuscript. Task Force for Global Health prepared the protocol, developed the study, and coordinated study implementation and reporting. US Centers for Disease Control and Prevention sequenced isolated enterovirus and managed database of patients. World Health Organization Global Polio Laboratory Network developed standard operating procedures, study forms, and training material and analyzed stool samples for presence of enterovirus. Jeffrey Modell Foundation selected clinical sites and facilitated clinical investigator enrollment and communication with patients.

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SUPPLEMENTARY MATERIAL

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REFERENCES

1. Picard C, Al-Herz W, Bousfiha A, Casanova JL, Chatila T, Conley ME, et al. Primary immunodeficiency diseases: an update on the classification from the International Union of Immunological Societies Expert Committee for Primary Immunodeficiency 2015. *J Clin Immunol* (2015) 35(8):696–726. doi:10.1007/s10875-015-0201-1
2. Eibl MM, Wolf HM. Vaccination in patients with primary immune deficiency, secondary immune deficiency and autoimmunity with immune regulatory abnormalities. *Immunotherapy* (2015) 7(12):1273–92. doi:10.2217/IMT.15.74
3. Guo J, Bolivar-Wagers S, Srinivas N, Holubar M, Maldonado Y. Immunodeficiency-related vaccine-derived poliovirus (iVDPV) cases: a systematic review and implications for polio eradication. *Vaccine* (2015) 33(10):1235–42. doi:10.1016/j.vaccine.2015.01.018
4. Shaghaghi M, Shahmahmoodi S, Abolhassani H, Soleyman-Jahi S, Parvaneh L, Mahmoudi S, et al. Vaccine-derived polioviruses and children with primary immunodeficiency, Iran, 1995-2014. *Emerg Infect Dis* (2016) 22(10):1712–9. doi:10.3201/eid2210.151071
5. Abolhassani H, Vitali M, Lougaris V, Giliani S, Parvaneh N, Parvaneh L, et al. Cohort of Iranian patients with congenital agammaglobulinemia: mutation analysis and novel gene defects. *Expert Rev Clin Immunol* (2016) 12(4):479–86. doi:10.1586/1744666X.2016.1139451
6. Centers for Disease Control and Prevention (CDC). Update on vaccine-derived polioviruses – worldwide, April 2011-June 2012. *MMWR Morb Mortal Wkly Rep* (2012) 61:741–6.
7. Diop OM, Burns CC, Sutter RW, Wassilak SG, Kew OM; Centers for Disease Control and Prevention (CDC). Update on vaccine-derived polioviruses – worldwide, January 2014-March 2015. *MMWR Morb Mortal Wkly Rep* (2015) 64(23):640–6.
8. Centers for Disease Control and Prevention (CDC). Laboratory surveillance for wild and vaccine-derived polioviruses – worldwide, January 2008-June 2009. *MMWR Morb Mortal Wkly Rep* (2009) 58(34):950–4.

9. Kew OM, Sutter RW, de Gourville EM, Dowdle WR, Pallansch MA. Vaccine-derived polioviruses and the endgame strategy for global polio eradication. *Annu Rev Microbiol* (2005) 59:587–635. doi:10.1146/annurev.micro.58.030603.123625
10. Trimble R, Atkins J, Quigg TC, Burns CC, Wallace GS, Thomas M, et al. Vaccine-associated paralytic poliomyelitis and BCG-osis in an immigrant child with severe combined immunodeficiency syndrome – Texas, 2013. *MMWR Morb Mortal Wkly Rep* (2014) 63(33):721–4.
11. Morales M, Tangermann RH, Wassilak SG. Progress toward polio eradication – worldwide, 2015-2016. *MMWR Morb Mortal Wkly Rep* (2016) 65(18):470–3. doi:10.15585/mmwr.mm6518a4
12. Driss N, Ben-Mustapha I, Mellouli F, Ben Yahia A, Touzi H, Bejaoui M, et al. High susceptibility for enterovirus infection and virus excretion features in Tunisian patients with primary immunodeficiencies. *Clin Vaccine Immunol* (2012) 19(10):1684–9. doi:10.1128/CVI.00293-12
13. Conley ME, Notarangelo LD, Etzioni A. Diagnostic criteria for primary immunodeficiencies. Representing PAGID (Pan-American Group for Immunodeficiency) and ESID (European Society for Immunodeficiencies). *Clin Immunol* (1999) 93(3):190–7. doi:10.1006/clim.1999.4799
14. Bonilla FA, Bernstein IL, Khan DA, Ballas ZK, Chinen J, Frank MM, et al. Practice parameter for the diagnosis and management of primary immunodeficiency. *Ann Allergy Asthma Immunol* (2005) 94(5 Suppl 1):S1–63. doi:10.1016/S1081-1206(10)61142-8
15. Centers for Disease Control and Prevention (CDC). Laboratory surveillance for wild and vaccine-derived polioviruses – worldwide, January 2006–June 2007. *MMWR Morb Mortal Wkly Rep* (2007) 56(37):965–9.
16. Centers for Disease Control and Prevention (CDC). Evaluating surveillance indicators supporting the Global Polio Eradication Initiative, 2011–2012. *MMWR Morb Mortal Wkly Rep* (2013) 62(14):270–4.
17. Driss N, Mellouli F, Ben Yahia A, Touzi H, Barbouche MR, Triki H, et al. Sequential asymptomatic enterovirus infections in a patient with major histocompatibility complex class II primary immunodeficiency. *J Clin Microbiol* (2014) 52(9):3486–9. doi:10.1128/JCM.01122-14
18. Sazzad HM, Rainey JJ, Kahn AL, Mach O, Liyanage JB, Alam AN, et al. Screening for long-term poliovirus excretion among children with primary immunodeficiency disorders: preparation for the polio posteradication era in Bangladesh. *J Infect Dis* (2014) 210(Suppl 1):S373–9. doi:10.1093/infdis/jiu221
19. de Silva R, Gunasena S, Ratnayake D, Wickremesinghe GD, Kumarasiri CD, Pushpakumara BA, et al. Prevalence of prolonged and chronic poliovirus excretion among persons with primary immune deficiency disorders in Sri Lanka. *Vaccine* (2012) 30(52):7561–5. doi:10.1016/j.vaccine.2012.10.035
20. Halsey NA, Pinto J, Espinosa-Rosales F, Faure-Fontenla MA, da Silva E, Khan AJ, et al. Search for poliovirus carriers among people with primary immune deficiency diseases in the United States, Mexico, Brazil, and the United Kingdom. *Bull World Health Organ* (2004) 82(1):3–8.
21. El-Sayed ZA, Mach O, Hossny EM, Galal NM, El-Sawy I, Elmarsafy A, et al. Poliovirus excretion among persons with primary immune deficiency disorders: summary of data from enhanced poliovirus surveillance in Egypt, 2011–2014. *J Vaccines Vaccin* (2016) 7(4):331–6.
22. Modell V, Quinn J, Orange J, Notarangelo L, Modell F. Primary immunodeficiencies worldwide: an updated overview from the Jeffrey Modell Centers Global Network. *Immunol Res* (2016) 64(3):736–53. doi:10.1007/s12026-016-8784-z
23. Chetverin AB. The puzzle of RNA recombination. *FEBS Lett* (1999) 460(1):1–5. doi:10.1016/S0014-5793(99)01282-X
24. Guillot S, Caro V, Cuervo N, Korotkova E, Combiescu M, Persu A, et al. Natural genetic exchanges between vaccine and wild poliovirus strains in humans. *J Virol* (2000) 74(18):8434–43. doi:10.1128/JVI.74.18.8434-8443.2000
25. Georgopoulou A, Markoulatos P. Sabin type 2 polioviruses with intertypic vaccine/vaccine recombinant genomes. *Eur J Clin Microbiol Infect Dis* (2001) 20(11):792–9. doi:10.1007/s100960100610
26. Karakasiliotis I, Markoulatos P, Katsorchis T. Site analysis of recombinant and mutant poliovirus isolates of Sabin origin from patients and from vaccinees. *Mol Cell Probes* (2004) 18(2):103–9. doi:10.1016/j.mcp.2003.09.009
27. Puligedda RD, Kouiavskaia D, Adekar SP, Sharma R, Devi Kattala C, Rezapkin G, et al. Human monoclonal antibodies that neutralize vaccine and wild-type poliovirus strains. *Antiviral Res* (2014) 108:36–43. doi:10.1016/j.antiviral.2014.05.005
28. He Y, Mueller S, Chipman PR, Bator CM, Peng X, Bowman VD, et al. Complexes of poliovirus serotypes with their common cellular receptor, CD155. *J Virol* (2003) 77(8):4827–35. doi:10.1128/JVI.77.8.4827-4835.2003
29. World Health Organization. Polio vaccines: WHO position paper, March 2016-recommendations. *Vaccine* (2017) 35(9):1197–9. doi:10.1016/j.vaccine.2016.11.017
30. Duintjer Tebbens RJ, Thompson KM. Comprehensive screening for immunodeficiency-associated vaccine-derived poliovirus: an essential oral poliovirus vaccine cessation risk management strategy. *Epidemiol Infect* (2017) 145(2):217–26. doi:10.1017/S0950268816002302
31. Wahid R, Cannon MJ, Chow M. Virus-specific CD4+ and CD8+ cytotoxic T-cell responses and long-term T-cell memory in individuals vaccinated against polio. *J Virol* (2005) 79(10):5988–95. doi:10.1128/JVI.79.10.5988-5995.2005
32. McKinlay MA, Collett MS, Hincks JR, Oberste MS, Pallansch MA, Okayasu H, et al. Progress in the development of poliovirus antiviral agents and their essential role in reducing risks that threaten eradication. *J Infect Dis* (2014) 210(Suppl 1):S447–53. doi:10.1093/infdis/jiu043
33. Dehkordy SF, Aghamohammadi A, Ochs HD, Rezaei N. Primary immunodeficiency diseases associated with neurologic manifestations. *J Clin Immunol* (2012) 32(1):1–24. doi:10.1007/s10875-011-9593-8
34. De Palma AM, Pürstinger G, Wimmer E, Patick AK, Andries K, Rombaut B, et al. Potential use of antiviral agents in polio eradication. *Emerg Infect Dis* (2008) 14(4):545–51. doi:10.3201/eid1404.070439

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Modulatory Effects of Antibody Replacement Therapy to Innate and Adaptive Immune Cells

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Intravenous immunoglobulin administered at replacement dosages modulates innate and adaptive immune cells in primary antibody deficiencies (PAD) in a different manner to what observed when high dosages are used or when their effect is analyzed by *in vitro* experimental conditions. The effects seem to be beneficial on innate cells in that dendritic cells maturate, pro-inflammatory monocytes decrease, and neutrophil function is preserved. The effects are less clear on adaptive immune cells. IVIg induced a transient increase of Treg and a long-term increase of CD4 cells. More complex and less understood is the interplay of IVIg with defective B cells of PAD patients. The paucity of data underlies the need of more studies on patients with PAD before drawing conclusions on the *in vivo* mechanisms of action of IVIg based on *in vitro* investigations.

Keywords: common variable immune disorders, X-linked agammaglobulinemia, IVIg replacement, innate immunity, adaptive immunity, *in vivo*

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INTRODUCTION

Polyvalent intravenous immunoglobulin (IVIg) are used as replacement therapy in patients with primary antibody deficiencies (PAD), a group of defects characterized by a failure to mount protective antibody responses, including X-linked agammaglobulinemia (XLA) and common variable immune disorders (CVID), and as immunomodulatory treatment with high IVIg doses in patients with inflammatory-autoimmune diseases. IVIg contain a broad spectrum of antibody specificities against microorganism antigens able to opsonize and neutralize microbes and toxins. IVIg also contain functionally relevant natural autoantibodies toward a wide range of self-motifs such as Siglec 9, Fas, and BAFF, together with a wide range of specificities including idiotypes of immunoglobulins, T cell receptor, HLA molecules, and other cell surface molecules of immunological importance such as CD4, CD5, BAFF, Fas, cytokines, cytokine receptors, and chemokine receptors that may participate in regulation of the immune response (1).

There are many reports on the various immunological effects of high-dose IVIg treatment in patients with inflammatory diseases. Clearly, the significantly smaller doses of immunoglobulin prescribed to PAD patients for replacement therapy may convey different effects (2). The possible immunomodulatory effect of IVIg administered at replacement dosages on innate and adaptive immune cells in patients with PAD needs to be addressed by detailed studies since the peak plasma IgG level reached in patients on replacement administration is much lower that the peak reached in patients with autoimmune-inflammatory disorders. Moreover, *in vitro* studies might not be a suitable system to replicate the *in vivo* effects of IVIg. In fact, it is possible that the *in vitro* effects of IVIg do not recapitulate the *in vivo* effects since many cellular and mediator interactions are lacking when

IVIg are added *in vitro* to experimental conditions. Moreover, *in vivo* studies might help to analyze the immunomodulatory short- and long-term effects of immunoglobulin on immune cells and the beneficial effects due to the reduction of the infection-associated immune activation that is likely to occur as a result of immunoglobulin replacement.

Several theories have been postulated about the mechanisms through which IVIg preparations exert their immune-regulatory properties at replacement dosages possibly involving different type of cells acting in concert (3). Moreover, the diversity of CVID immunological and clinical phenotype could affect the results of some of the experiments. In addition, the commercial IVIg preparation used to study the *in vivo* or *in vitro* effect should be considered, in that IVIg consist mainly of monomeric IgG, but if a residual amount of dimers is present in the preparation, the biological effects might be different (4).

POLYMORPHONUCLEAR NEUTROPHILS (PMN)

In response to pathogens, PMN rapidly migrate to the site of inflammation, release proteolytic enzymes and antimicrobial peptides as well as reactive oxygen species. IVIg might modulate PMN activity by a saturating and an activating/inhibiting effect on PMN FcγRs (5). Almost 20 years ago, the first demonstration that IVIg administered at low dosages in patients affected by PAD did not alter neutrophils functions was published (6). Phagocytosis, intracellular bactericidal activity, and chemotaxis of PMN in PAD patients treated at very low dosages (IVIg 200 mg/kg/month) and at replacement dosages (IVIg 600 mg/kg/month) were comparable to those of healthy controls (6). We have recently confirmed these data showing that in CVID and XLA patients, PMN were capable *in vivo* to perform efficient migration, degranulation, phagocytosis, and oxidative burst at baseline and shortly after IVIg administration (7, 8). Moreover, IVIg infusion-administered *in vivo* at replacement dosages did not alter the PMN expression of receptors involved in PMN functions, such as CD181, CD66b, CD11b, CD11c, CD16, and Siglec 9 (7, 8).

In contrast with the *in vivo* data obtained from CVID and XLA patients infused with IVIg, experiments performed with IVIg added *in vitro* on isolated PMN or to whole blood (9) showed that IVIg (1–25 mg/ml) might affect the overall activity of PMN by (1) inducing apoptosis; (2) decreasing the pro-inflammatory activity; (3) inhibiting or activating PMN degranulation (10–15) (**Figure 1**).

Thus, *in vitro* experiments provided conflicting results of immunomodulatory effects on PMN activity depending on the concentration of IVIg added *in vitro*, while *in vivo* data showed that PMN remained fully functional in patients treated with replacement IVIg dosages (**Figure 1**).

MONOCYTES

Monocytes are now classified according to their expression of CD14, the receptor of LPS, and CD16, a low affinity Fcγ receptor, into three different subsets: classical monocytes, non-classical monocytes, and intermediate monocytes also called pro-inflammatory monocytes based on gene expression profiling and cytokine production, such as TNF-α, IL-1β, and IL-6 (16, 17). We have recently shown that CVID and XLA patients displayed an increased frequency of $CD14^{bright}CD16^{+}$ pro-inflammatory intermediate monocytes (8, 18). In CVID and XLA patients, shortly after IVIg infusion, we observed a transient reduction of about one-fourth of peripheral monocytes, a decrease involving mainly pro-inflammatory intermediate monocytes. These data are in agreement with other observations showing that IVIg infusion induced a transient decrease in the number of pro-inflammatory monocytes that returned at baseline levels after 20 h and suppressed the production of pro-inflammatory cytokines (19). These phenomena might be an additional mechanism through which IVIg infusion exert an anti-inflammatory effect, even when infused at replacement dosages. It is possible that an apoptotic process is involved. In fact, IVIg preparations contain agonistic and antagonistic anti-CD95 antibodies interacting with $CD16^{+}$ monocytes that constitutively upregulated proapoptotic genes (20). Thus, these naturally occurring autoantibodies might contribute to the anti-inflammatory effects of IVIg *via* cell death regulation. In CVID and XLA shortly after IVIg infusion, we also observed a reduction of expression of functional monocyte receptors such as CD11b and Siglec 9 on classical monocytes associated, as expected, with a slight reduction of phagocytosis and oxidative burst functions that even if decreased remained in the normal range (8, 18). As described for anti-CD95, the reduction of Siglec 9 receptor might also be related to the binding of anti-Siglec 9 antibodies contained in IVIg preparations (20). It is then possible that IVIg bind to the Fc receptors on monocytes' surface and transiently limit the availability to bind opsonized bacteria.

In conclusion as shown in **Figure 1**, taken together our results showed that in XLA and CVID patients IVIg exert *in vivo* a short-term anti-inflammatory effect and do not severely affect the monocytes ability to appropriately respond to pathogens (8).

DENDRITIC CELLS

Dendritic cells are professional antigen-presenting cells essential for priming immune response. When immature, DC capture antigen efficiently, but only mature DC are potent T-cell activators. Maturation process modifies the expression of MHC molecules, co-stimulatory molecules and cytokines. CD1 proteins are MHC I-like markers of differentiation of DC. The groups 1 (CD1a, CD1b, and CD1c) and 2 (CD1d) are involved in lipid antigen presentation to the T cells (21). There are diverse subsets of DC: monocyte-related DC, blood myeloid (mDC), and plasmacytoid DC (pDC), precursors of tissue and lymphoid DC (22). In the presence of GM-CSF and IL-4, it is possible to induce the production *in vitro* of monocyte-derived DC (MdDC) (23, 24). Different subsets of DC express distinct CD1 profiles, suggesting that microenvironment could regulate diverse antigen presentation pathways.

Common variable immune disorder patients have a reduction of both pDC and mDC subsets (25). Their MdDC display disturbed differentiation, maturation, and function (24). *In vitro* and

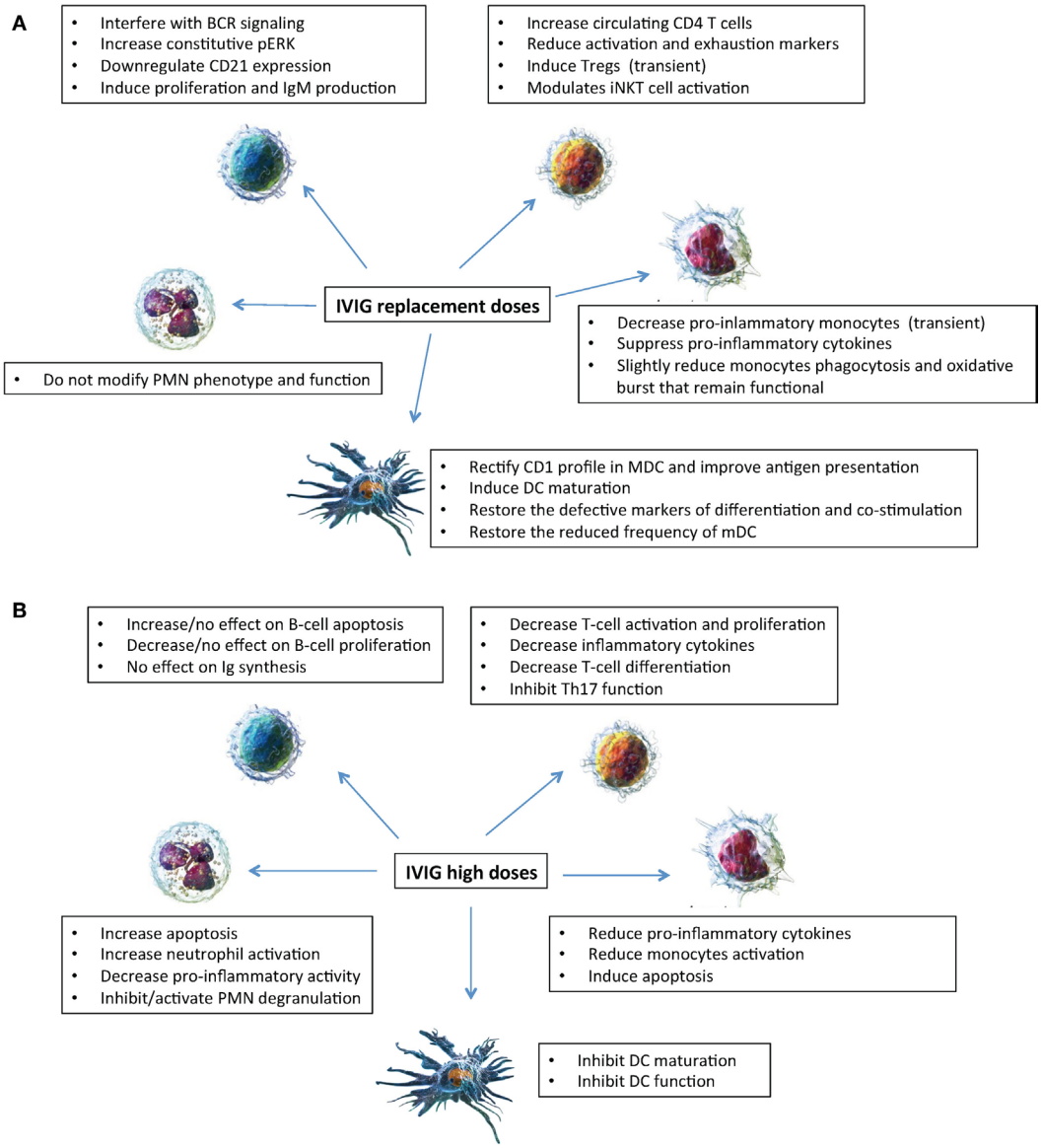

FIGURE 1 | Mechanisms of action of intravenous immunoglobulin (IVIg) on innate (neutrophils, monocytes, and dendritic cells) and adaptive (B and T lymphocytes) immune cells. Effects of IVIg (replacement dosages) **(A)**; effects of IVIg (high dosages) **(B)**. Cell images from Ref. (55).

in vivo experiments showed similar effects of IVIg on DC: the addition *in vitro* of IVIg at a concentration of 10 mg/ml to DC cultures of CVID patients partially restored the defective markers of differentiation (CD1a) and co-stimulation (CD80, CD86, and CD40) (26); a recent *in vivo* study in CVID demonstrated that after 6–12 months from initiation of replacement, IVIg therapy partially restored the reduced frequency of mDC, the altered expression of their co-stimulatory molecules and the CD4 T cell count (27).

Moreover, *in vitro* studies showed that different concentrations of IVIg added to MdDC influenced the expression pattern of CD1 molecules (28); similarly, *in vivo* studies found that IVIg therapy normalized the elevated levels of CD1a and CD1b on mDCs of CVID patients (29). By regulating CD1 expression pattern on DC, IVIg treatment affects the balance between CD1d-restricted antigen presentation to iNKT cells and group 1 CD1-restricted antigen presentation to T cells (28). Thus, by fine tuning of the CD1 antigen presentation pathways, IVIg could provide suitable activation signals to the immune system.

Also XLA patients have reduced DC myeloid subset (25). Moreover, it was suggested that their DC failed to differentiate and to mature because of a hypogammaglobulinemic environment (30). These abnormalities were partially restored by adding IVIg *in vitro* during the differentiation of DC from monocytes. Natural CD40 reactive antibodies are likely to exert this effect through an agonistic action on CD40 (30).

Taken together, as shown in **Figure 1**, IVIg at replacement dosages has a number of beneficial effects on DC differentiation and

function as demonstrated by *in vivo* and *in vitro* studies, which are different from the effects of IVIg (0.15 mM) administered at high dosages that inhibited maturation and function of DC (24).

B CELLS

The B cell compartment is variably disturbed in patients with CVID. The total number of peripheral B cells is reduced in about 40–50% patients (31, 32). Class-switched memory B cells are reduced in 80–90% of CVID patients (31) often in association with defects of B cell receptor (BCR) activation (33) as well as the TLRs (34).

Therapeutic immunoglobulins interplay with B cells both directly, with surface receptors or intracellular molecules, and indirectly, influencing cytokines, survival factors or through other immune cells (35). Nevertheless, there is a shortage of studies that could unveil the effects of IVIg on B lymphocytes, particularly when administered at replacement dosages.

In vitro studies provided conflicting results. Graded concentration of IVIg (from low dose to high dose) demonstrated that IVIg did not affect the proliferative capacity of B cells, did not cause significant apoptosis of B cells, neither affected mRNA synthesis of both IgM and IgG (36). Other *in vitro* studies showed that IVIg at high dose (0.15 mM) can directly inhibit B cell activation and proliferation (37) and can induce apoptosis in B lymphocytes by inducing a functional silencing program similar to anergy (38, 39) (**Figure 1**).

A recent study demonstrated that upon stimulation with physiological IVIg concentration added *in vitro* to experimental conditions, B cells from CVID patients were capable to proliferate and produce IgM (40). The authors suggested that antibodies within IVIg preparations rectified the defective signaling of B cells provided by T cells and delivered T-independent signals. We confirmed a signal dysregulation intrinsic to B cells in a subgroup of CVID patients. CVID patients with expanded CD21low B cells have high constitutive ERK activation [BCR signaling pathway important for B cell anergy (41)], low responsiveness to TLR9 and BCR stimuli, defective calcium signaling (42, 43), and propensity to apoptosis. IVIg infusion administered *in vivo* transiently increased constitutive pERK, reduced the pERK increment induced by BCR cross-linking (44), and drove B cells to downregulate CD21 expression (45).

Thus, data on IVIg and B cells remained unclear and need to be elucidated. Moreover, the long-term consequences of IVIg on B cells of PAD patients have never been addressed due to the complex relationship between IgG and a number of factors that might contribute to the diverse B cell abnormalities observed over time in heterogeneous disorders like CVID.

T CELLS

A relative loss of T-cell function in many CVID patients has been widely demonstrated, including low circulating CD4 T cells, low naive CD4 T cells, low antigen-specific T cells, impaired proliferation, activation, and secretion of cytokines (46). Many CVID patients present a persistent low CD4 T cell count. For the group of CVID patients with persistently low CD4 counts, it has been suggested to change the PID classification from CVID to late-onset combined immune deficiency (47).

An elegant study addressed the modulation of T cells in CVID patients treated with IVIG on a long-term basis. Following IVIg initiation, CD4 counts increase in the majority of CVID patients and can reach normal levels in some cases (48). IVIg reduced the expression of activation and exhaustion markers on CD4 and CD8 T cells, which might remain elevated for up to 1 year from IVIg treatment initiation. This suggests that IgG replacement control with time infection-associated factors implicated in T cell chronic activation (27). The authors concluded that an early initiation of IgG replacement therapy in CVID patients may be beneficial to prevent T cell activation by switching off the inflammatory status. The same effect was demonstrated in *in vitro* studies when IVIg were added to cultures even at physiological concentrations (49).

In CVID patients, Tregs frequency (50) and function have been shown to be reduced, and this defect might have a role in the pathogenesis of CVID-associated autoimmune or inflammatory complications. IVIg administration modulates Tregs as demonstrated by the transient increase in Tregs 30 min after IVIg infusion (51). However, no sustained effect on Treg frequency was observed, and thus additional studies are needed to address the *in vivo* long-term effect on Treg and the clinical impact of this finding.

In addition, CVID patients show a reduction of iNKT cells (52), a subset that control bacterial and viral infections by recognizing endogenous and bacterial-derived glycolipids presented by CD1d molecules. In CVID, iNKT cells showed an elevated expression of markers of activation and exhaustion such as HLA-DR, CD161, and PD-1. IVIg treatment did not improve the frequency of iNKT cells but reduced CD161and PD-1 expression and possibly reduced iNKT cell activation and exhaustion (27).

As shown in **Figure 1**, these effects differ from the data obtained *in vitro* when higher IVIg concentrations (0.15 mmol/L) were added to experimental conditions showing suppression of T cell proliferation (53), of amplification of human Th17 cells (54) and of multiple key effector/inflammatory cytokines (49). Taken together, IVIg at replacement dosages have a beneficial short-lived effect on Tregs and a beneficial long-term effect on T cells and possibly on iNKT cells.

CONCLUSION

IVIg administered at replacement dosages modulate innate and adaptive immune cells in PAD (**Figure 1**) differently to what observed when the effects of high dosages are evaluated. The effects seem to be beneficial on innate cells in that dendritic cells maturate, pro-inflammatory monocytes decrease, and neutrophil function is preserved. The effects are less clear on adaptive immune cells where a transient increase of Treg and a long-term increase of CD4 were demonstrated. More complex and less understood is the interplay of IVIg with defective B cells of PAD patients. The paucity of data underlies the need of more studies before drawing conclusions on the *in vivo* mechanisms of action of IVIg based on *in vitro* investigations. Moreover, should be noted that all studies presented refer to IVIg only and no data

are available on *in vivo* studies of subcutaneous administration. Moreover, studies on SCIg might help to discriminate between short-term effect of IVIg administration due to the IgG peak reached at the time of the infusion and long-term effect due a constant IgG replacement.

AUTHOR CONTRIBUTIONS

IQ and MM contributed to this paper by designing the perspective, by contributing with experimental data, by analyzing the data in the literature, and by writing the manuscript.

REFERENCES

1. Farrugia A, Quinti I. Manufacture of immunoglobulin products for patients with primary antibody deficiencies – the effect of processing conditions on product safety and efficacy. *Front Immunol* (2014) 5:665. doi:10.3389/fimmu.2014.00665
2. Kerr J, Quinti I, Eibl M, Chapel H, Späth PJ, Sewell WA, et al. Is dosing of therapeutic immunoglobulins optimal? A review of a three-decade long debate in Europe. *Front Immunol* (2014) 5:629. doi:10.3389/fimmu.2014.00629
3. Kaveri SV, Maddur MS, Hegde P, Lacroix-Desmazes S, Bayry J. Intravenous immunoglobulins in immunodeficiencies: more than mere replacement therapy. *Clin Exp Immunol* (2011) 164(Suppl 2):2–5. doi:10.1111/j.1365-2249.2011.04387.x
4. Durandy A, Kaveri SV, Kuijpers TW, Basta M, Miescher S, Ravetch JV, et al. Intravenous immunoglobulins – understanding properties and mechanisms. *Clin Exp Immunol* (2009) 158(Suppl 1):2–13. doi:10.1111/j.1365-2249.2009.04022.x
5. Nagelkerke SQ, Kuijpers TW. Immunomodulation by IVIg and the role of Fc gamma receptors: classic mechanisms of action after all? *Front Immunol* (2015) 5:674. doi:10.3389/fimmu.2014.00674
6. Van T, Sussman G, Pruzanski W. Impact of intravenous infusions of low and high doses of gamma globulins (IVIG) on phagocytic functions in adults with primary humoral immunodeficiency. *Inflammation* (1994) 18(4):419–26. doi:10.1007/BF01534439
7. Prezzo A, Cavaliere FM, Bilotta C, Iacobini M, Quinti I. Intravenous immunoglobulin replacement treatment does not alter polymorphonuclear leukocytes function and surface receptors expression in patients with common variable immunodeficiency. *Cell Immunol* (2016) 306-307:25–34. doi:10.1016/j.cellimm.2016.05.006
8. Cavaliere FM, Prezzo A, Bilotta C, Iacobini M, Quinti I. The lack of BTK does not impair monocytes and polymorphonuclear cells functions in X-linked agammaglobulinemia under treatment with intravenous immunoglobulin replacement. *PLoS One* (2017) 12(4):e0175961. doi:10.1371/journal.pone.0175961
9. Casulli S, Topçu S, Fattoum L, von Gunten S, Simon HU, Teillaud JL, et al. A differential concentration-dependent effect of IVIg on neutrophil functions: relevance for anti-microbial and anti-inflammatory mechanisms. *PLoS One* (2011) 6(10):e26469. doi:10.1371/journal.pone.0026469
10. Aschermann S, Lux A, Baerenwaldt A, Biburger M, Nimmerjahn F. The other side of immunoglobulin G: suppressor of inflammation. *Clin Exp Immunol* (2010) 160(2):161–7. doi:10.1111/j.1365-2249.2009.04081.x
11. van Mirre E, Teeling JL, van der Meer JW, Bleeker WK, Hack CE. Monomeric IgG in intravenous Ig preparations is a functional antagonist of FcgammaRII and FcgammaRIIIb. *J Immunol* (2004) 173(1):332–9. doi:10.4049/jimmunol.173.1.332
12. von Gunten S, Schaub A, Vogel M, Stadler BM, Miescher S, Simon HU. Immunologic and functional evidence for anti-Siglec-9 autoantibodies in intravenous immunoglobulin preparations. *Blood* (2006) 108(13):4255–9. doi:10.1182/blood-2006-05-021568
13. Pallister I, Bhatia R, Katpalli G, Allison D, Parker C, Topley N. Alteration of polymorphonuclear neutrophil surface receptor expression and migratory activity after isolation: comparison of whole blood and isolated PMN preparations from normal and post fracture trauma patients. *J Trauma* (2006) 60(4):844–50. doi:10.1097/01.ta.0000215583.08765.ce
14. François S, El Benna J, Dang PM, Pedruzzi E, Gougerot-Pocidalo MA, Elbim C. Inhibition of neutrophil apoptosis by TLR agonists in whole blood: involvement of the phosphoinositide 3-kinase/Akt and NF-kB signaling pathways, leading to increased levels of Mcl-1, A1, and phosphorylated Bad. *J Immunol* (2005) 174(6):3633–42. doi:10.4049/jimmunol.174.6.3633
15. Casulli S, Coignard-Biehler H, Amazzough K, Shoai-Tehrani M, Bayry J, Mahlaoui N, et al. Defective functions of polymorphonuclear neutrophils in patients with common variable immunodeficiency. *Immunol Res* (2014) 60(1):69–76. doi:10.1007/s12026-014-8555-7
16. Ziegler-Heitbrock L. Blood monocytes and their subsets: established features and open questions. *Front Immunol* (2015) 6:423. doi:10.3389/fimmu.2015.00423
17. Wong KL, Tai JJ, Wong WC, Han H, Sem X, Yeap WH, et al. Gene expression profiling reveals the defining features of the classical, intermediate, and nonclassical human monocyte subsets. *Blood* (2011) 118(5):e16–31. doi:10.1182/blood-2010-12-326355
18. Cavaliere FM, Prezzo A, Conti V, Bilotta C, Pulvirenti F, Iacobini M, et al. Intravenous immunoglobulin replacement induces an in vivo reduction of inflammatory monocytes and retains the monocyte ability to respond to bacterial stimulation in patients with common variable immunodeficiencies. *Int Immunopharmacol* (2015) 28(1):596–603. doi:10.1016/j.intimp.2015.07.017
19. Siedlar M, Strach M, Bukowska-Strakova K, Lenart M, Szaflarska A, Węglarczyk K, et al. Preparations of intravenous immunoglobulins diminish the number and proinflammatory response of CD14+CD16++ monocytes in common variable immunodeficiency (CVID) patients. *Clin Immunol* (2011) 139(2):122–32. doi:10.1016/j.clim.2011.01.002
20. von Gunten S, Simon HU. Cell death modulation by intravenous immunoglobulin. *J Clin Immunol* (2010) 30(Suppl 1):S24–30. doi:10.1007/s10875-010-9411-8
21. Barral DC, Brenner MB. CD1 antigen presentation: how it works. *Nat Rev Immunol* (2007) 7:929–41. doi:10.1038/nri2191
22. Collin M, McGovern N, Haniffa M. Human dendritic cell subsets. *Immunology* (2013) 140(1):22–30. doi:10.1111/imm.12117
23. Sallusto F, Lanzavecchia A. Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor alpha. *J Exp Med* (1994) 179(4):1109–18. doi:10.1084/jem.179.4.1109
24. Bayry J, Lacroix-Desmazes S, Carbonneil C, Misra N, Donkova V, Pashov A, et al. Inhibition of maturation and function of dendritic cells by intravenous immunoglobulin. *Blood* (2003) 101(2):758–65. doi:10.1182/blood-2002-05-1447
25. Yong PF, Workman S, Wahid F, Exley A, Webster AD, Ibrahim MA. Selective deficits in blood dendritic cell subsets in common variable immunodeficiency and X-linked agammaglobulinaemia but not specific polysaccharide antibody deficiency. *Clin Immunol* (2008) 127(1):34–42. doi:10.1016/j.clim.2007.12.007
26. Bayry J, Lacroix-Desmazes S, Hermine O, Oksenhendler E, Kazatchkine MD, Kaveri SV. Amelioration of differentiation of dendritic cells from CVID patients by intravenous immunoglobulin. *Am J Med* (2005) 118(12):1439–40. doi:10.1016/j.amjmed.2005.06.028
27. Paquin-Proulx D, Santos BA, Carvalho KI, Toledo-Barros M, Barreto de Oliveira AK, Kokron CM, et al. IVIg immune reconstitution treatment alleviates the state of persistent immune activation and suppressed CD4 T cell counts in CVID. *PLoS One* (2013) 8(10):e75199. doi:10.1371/journal.pone.0075199
28. Smed-Sörensen A, Moll M, Cheng TY, Loré K, Norlin AC, Perbeck L, et al. IgG regulates the CD1 expression profile and lipid antigen-presenting function in human dendritic cells via FcgammaRIIa. *Blood* (2008) 111(10):5037–46. doi:10.1182/blood-2007-07-099549
29. Paquin-Proulx D, Santos BA, Carvalho KI, Toledo-Barros M, Oliveira AK, Kokron CM, et al. Dysregulated CD1 profile in myeloid dendritic cells in CVID is normalized by IVIg treatment. *Blood* (2013) 121(24):4963–4. doi:10.1182/blood-2013-04-499442
30. Bayry J, Lacroix-Desmazes S, Donkova-Petrini V, Carbonneil C, Misra N, Lepelletier Y, et al. Natural antibodies sustain differentiation and maturation

of human dendritic cells. *Proc Natl Acad Sci U S A* (2004) 101(39):14210–5. doi:10.1073/pnas.0402183101
31. Wehr C, Kivioja T, Schmitt C, Ferry B, Witte T, Eren E, et al. The EUROclass trial: defining subgroups in common variable immunodeficiency. *Blood* (2008) 111:77–85. doi:10.1182/blood-2007-06-091744
32. Chapel H, Lucas M, Lee M, Bjorkander J, Webster D, Grimbacher B, et al. Common variable immunodeficiency disorders: division into distinct clinical phenotypes. *Blood* (2008) 112:277–86. doi:10.1182/blood-2007-11-124545
33. Foerster C, Voelxen N, Rakhmanov M, Keller B, Gutenberger S, Goldacker S, et al. B cell receptor-mediated calcium signaling is impaired in B lymphocytes of type Ia patients with common variable immunodeficiency. *J Immunol* (2010) 184:7305–13. doi:10.4049/jimmunol.1000434
34. Yu JE, Knight AK, Radigan L, Marron TU, Zhang L, Sanchez-Ramon S, et al. Toll-like receptor 7 and 9 defects in common variable immunodeficiency. *J Allergy Clin Immunol* (2009) 124:349–56. doi:10.1016/j.jaci.2009.05.019
35. Tha-In T, Bayry J, Metselaar HJ, Kaveri SV, Kwekkeboom J. Modulation of the cellular immune system by intravenous immunoglobulin. *Trends Immunol* (2008) 29(12):608–15. doi:10.1016/j.it.2008.08.004
36. Heidt S, Roelen DL, Eijsink C, Eikmans M, Claas FH, Mulder A. Intravenous immunoglobulin preparations have no direct effect on B cell proliferation and immunoglobulin production. *Clin Exp Immunol* (2009) 158(1):99–105. doi:10.1111/j.1365-2249.2009.03996.x
37. Maddur MS, Hegde P, Sharma M, Kaveri SV, Bayry J. B cells are resistant to immunomodulation by 'IVIg-educated' dendritic cells. *Autoimmun Rev* (2011) 11(2):154–6. doi:10.1016/j.autrev.2011.08.004
38. Séïté JF, Cornec D, Renaudineau Y, Youinou P, Mageed RA, Hillion S. IVIg modulates BCR signaling through CD22 and promotes apoptosis in mature human B lymphocytes. *Blood* (2010) 116:1698–704. doi:10.1182/blood-2009-12-261461
39. Séïté JF, Goutsmedt C, Youinou P, Pers JO, Hillion S. Intravenous immunoglobulin induces a functional silencing program similar to anergy in human B cells. *J Allergy Clin Immunol* (2014) 133(1):181–8.e1–9. doi:10.1016/j.jaci.2013.08.042
40. Bayry J, Fournier EM, Maddur MS, Vani J, Wootla B, Sibéril S, et al. Intravenous immunoglobulin induces proliferation and immunoglobulin synthesis from B cells of patients with common variable immunodeficiency: a mechanism underlying the beneficial effect of IVIg in primary immunodeficiencies. *J Autoimmun* (2011) 36(1):9–15. doi:10.1016/j.jaut.2010.09.006
41. Rui L, Vinuesa CG, Blasioli J, Goodnow CC. Resistance to CpG DNA-induced autoimmunity through tolerogenic B cell antigen receptor ERK signaling. *Nat Immunol* (2003) 4:594–600. doi:10.1038/ni924
42. Rakhmanov M, Keller B, Gutenberger S, Foerster C, Hoenig M, Driessen G, et al. Circulating CD21low B cells in common variable immunodeficiency resemble tissue homing, innate-like B cells. *Proc Natl Acad Sci U S A* (2009) 106(32):13451–6. doi:10.1073/pnas.0901984106
43. Visentini M, Cagliuso M, Conti V, Carbonari M, Mancaniello D, Cibati M, et al. Telomere-dependent replicative senescence of B and T cells from patients with type 1a common variable immunodeficiency. *Eur J Immunol* (2011) 41(3):854–62. doi:10.1002/eji.201040862
44. Visentini M, Marrapodi R, Conti V, Mitrevski M, Camponeschi A, Lazzeri C, et al. Dysregulated extracellular signal-regulated kinase signaling associated with impaired B-cell receptor endocytosis in patients with common variable immunodeficiency. *J Allergy Clin Immunol* (2014) 134(2):401–10. doi:10.1016/j.jaci.2014.03.017
45. Mitrevski M, Marrapodi R, Camponeschi A, Lazzeri C, Todi L, Quinti I, et al. Intravenous immunoglobulin replacement therapy in common variable immunodeficiency induces B cell depletion through differentiation into apoptosis-prone CD21(low) B cells. *Immunol Res* (2014) 60(2–3):330–8. doi:10.1007/s12026-014-8599-8
46. Bonilla FA, Barlan I, Chapel H, Costa-Carvalho BT, Cunningham-Rundles C, de la Morena MT, et al. International Consensus Document (ICON): common variable immunodeficiency disorders. *J Allergy Clin Immunol Pract* (2016) 4(1):38–59. doi:10.1016/j.jaip.2015.07.025
47. Malphettes M, Gerard L, Carmagnat M, Mouillot G, Vince N, Boutboul D, et al. Late-onset combined immune deficiency: a subset of common variable immunodeficiency with severe T cell defect. *Clin Infect Dis* (2009) 49(9):1329–38. doi:10.1086/606059
48. Paquin-Proulx D, Sandberg JK. Persistent immune activation in CVID and the role of IVIg in its suppression. *Front Immunol* (2014) 5:637. doi:10.3389/fimmu.2014.00637
49. Issekutz AC, Rowter D, Miescher S, Käsermann F. Intravenous IgG (IVIG) and subcutaneous IgG (SCIG) preparations have comparable inhibitory effect on T cell activation, which is not dependent on IgG sialylation, monocytes or B cells. *Clin Immunol* (2015) 160(2):123–32. doi:10.1016/j.clim.2015.05.003
50. Melo KM, Carvalho KI, Bruno FR, Ndhlovu LC, Ballan WM, Nixon DF, et al. A decreased frequency of regulatory T cells in patients with common variable immunodeficiency. *PLoS One* (2009) 4(7):e6269. doi:10.1371/journal.pone.0006269
51. Kasztalska K, Ciebiada M, Cebula-Obrzut B, Górski P. Intravenous immunoglobulin replacement therapy in the treatment of patients with common variable immunodeficiency disease: an open-label prospective study. *Clin Drug Investig* (2011) 31(5):299–307. doi:10.2165/11586710-000000000-00000
52. Fulcher DA, Avery DT, Fewings NL, Berglund LJ, Wong S, Riminton DS, et al. Invariant natural killer (iNK) T cell deficiency in patients with common variable immunodeficiency. *Clin Exp Immunol* (2009) 157(3):365–9. doi:10.1111/j.1365-2249.2009.03973.x
53. MacMillan HF, Lee T, Issekutz AC. Intravenous immunoglobulin G-mediated inhibition of T-cell proliferation reflects an endogenous mechanism by which IgG modulates T-cell activation. *Clin Immunol* (2009) 132(2):222–33. doi:10.1016/j.clim.2009.04.002
54. Maddur MS, Vani J, Hegde P, Lacroix-Desmazes S, Kaveri SV, Bayry J. Inhibition of differentiation, amplification, and function of human Th17 cells by intravenous immunoglobulin. *J Allergy Clin Immunol* (2011) 127:823–30. doi:10.1016/j.jaci.2010.12.1102
55. Blausen.com staff. Medical gallery of Blausen Medical 2014. *Wiki J Med* (2014) 1(2):1–79. doi:10.15347/wjm/2014.010

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REVIEW
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Recurrent and Sustained Viral Infections in Primary Immunodeficiencies

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Viral infections are commonplace and often innocuous. Nevertheless, within the population of patients with primary immunodeficiencies (PIDDs), viral infections can be the feature that drives a diagnostic evaluation or can be the most significant morbidity for the patient. This review is focused on the viral complications of PIDDs. It will focus on respiratory viruses, the most common type of viral infection in the general population. Children and adults with an increased frequency or severity of respiratory viral infections are often referred for an immunologic evaluation. The classic teaching is to investigate humoral function in people with recurrent sinopulmonary infections, but this is often interpreted to mean recurrent bacterial infections. Recurrent or very severe viral infections may also be a harbinger of a primary immunodeficiency as well. This review will also cover persistent cutaneous viral infections, systemic infections, central nervous system infections, and gastrointestinal infections. In each case, the specific viral infections may drive a diagnostic evaluation that is specific for that type of virus. This review also discusses the management of these infections, which can become problematic in patients with PIDDs.

Keywords: virus, primary immunodeficiency, morbidity, herpes, papillomavirus, norovirus, enterovirus

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INTRODUCTION

Frequent infections are a common reason for physician visits. Distinguishing a pattern or a type of infection that suggests an immunodeficiency as opposed to part of the normal susceptibility to infection can be a challenge. Common causes of recurrent infections are allergies, anatomical contributions, secondary immune deficiency, and an unusual burden of exposures. Primary immunodeficiencies (PIDDs) are much less common and therefore difficult to appreciate during the wealth of infections that are typically seen in a physician's practice. During the first 5 years of life, children can experience six to eight respiratory tract infections per year. These tend to peak in the winter months and daycare attendance, exposure to smokers, and atopy can increase this frequency significantly (1–4). Respiratory tract infections in adults are somewhat less common; however, three to five respiratory tract infections per year in adults are typical (5). Recurrent sinus infections, pneumonia, and bronchitis are common signs of an immunodeficiency, recognizing that frequent bacterial infections of the respiratory track are often a harbinger of antibody disorders, the most common type of primary immunodeficiency. This review will address recurrent and sustained viral infections for which there are fewer studies to assist the physician in the identification of patients with potential immunodeficiency. This review will address unusual viral respiratory tract infections, systemic viral infections, infections of the brain and meninges, and cutaneous viral infections. Unusual viral infections can be a sign or complication of PIDD. There are several excellent reviews that address the

overall approach to suspected PIDD (6–8). Bacterial infections are generally highlighted, and therefore this review will focus on unusual and severe viral infections.

RESPIRATORY VIRAL INFECTIONS IN PIDD

Respiratory viruses are extremely common in most patients with PIDDs (9, 10). In most cases, they represent nuisance infections that can be a predisposing condition leading to bacterial superinfection. In patients with antibody defects, respiratory infections fall into this category. Although defense against recurrence of respiratory tract viruses is mediated largely by antibody, eradication of an infecting virus is mediated largely by the T cell compartment. Respiratory viral infections are therefore more significant in patients with T cell immune deficiencies. Today, many newborns with severe combined immune deficiency (SCID) are detected by newborn screening; however, this is not true in all parts of the world, nor is it true in all states in the USA. A study by the PIDTC found that 21% of their cohort had a respiratory infection prior to transplant with the most common being parainfluenza followed by RSV, rhinovirus, and influenza (11). Although other types of infections were more common as presenting features in this cohort, respiratory infections were among the least likely to resolve prior to transplant. All viral infections are typically prolonged in patients with T cell defects. However, in SCID, there are no T cells and a simple respiratory virus will progress relentlessly unless a hematopoietic stem cell transplant (HSCT) allows the infant to develop a competent immune system (**Figure 1**) (11). The specific pathogens to which children with T cell defects are susceptible include all of those common in the general population. RSV, influenza, and rhinovirus are typically the most prevalent during the respiratory season (12). Coronavirus and metapneumovirus have been increasingly recognized as causing respiratory infections. Exposures will dictate the pattern of viral infections in patients with T cell defects. The severity of disease is a result of both the degree of T cell compromise and the nature of the infecting virus. Any patient with prolonged viral infections is at risk for bacterial superinfection. It is not, therefore, uncommon to see a mixed picture of viral and bacterial infections. Additionally, severe T cell defects are associated with compromised antibody production, also contributing to a mixed infection picture.

T cell defects are associated with a generally increased predisposition to viral infections. IRF7 deficiency, in contrast, is associated with a selective susceptibility to influenza (13). It is otherwise uncommon to see an isolated susceptibility to a respiratory virus. The single reported patient had a severe primary infection with influenza associated with poor production of type I and type III interferons.

Diagnostic Approach

There are many specific genetic types of immunodeficiencies associated with T cell deficiency, and the diagnostic considerations are different depending on the age of the patient. Infants with a prolonged or severe respiratory viral infection should be evaluated for SCID, and patients of any age with dysmorphic features or other associated features should be evaluated for chromosome 22q11.2 deletion syndrome (14). Most states now have a newborn screening program to detect SCID. The results can be accessed to identify significant T cell lymphopenia in early infancy. Newborn screening has significantly improved survival of infants with SCID. There are, however, significant T cell disorders not identified by this test, and therefore, T cell disorders still represent a concern in the setting of a prolonged viral infection. T cell enumeration is often the quickest way to screen for T cell defects. The vast majority of T cell deficiency conditions will have

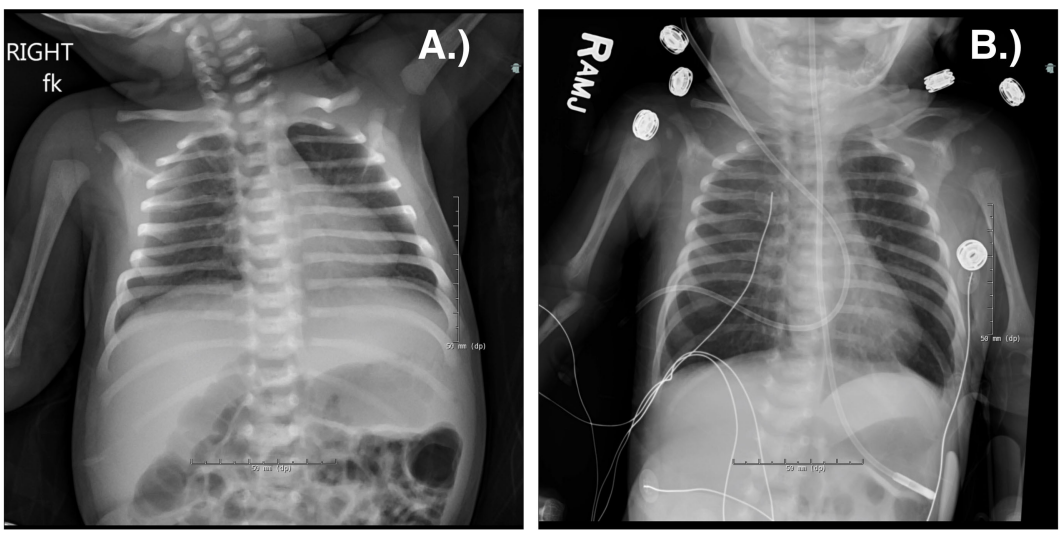

FIGURE 1 | Chest radiograph of a term male infant with X-linked severe combined immune deficiency and RSV pneumonitis, which was rapidly fatal despite adjunctive use of IVIG and inhaled ribavirin. He was treated with an infusion of maternal haplo-identical hematopoietic stem cells at 18 days of life. He was admitted at 10 days old **(A)** and died at 27 days old **(B)** due to worsening respiratory status. Note the absent thymus.

low T cell numbers or at least low CD4/CD45RA (naïve) T cell counts. Additional studies include proliferative studies, exclusion of HIV, and sequencing panels to identify inborn errors of immunity.

Management

Management of respiratory tract infections in non-SCID T cell defects is largely supportive with optimization of bronchodilators, antiviral therapy if available and attention to nutrition (12). Management of respiratory tract infections in SCID is highly problematic. There is a race to replace the immune system before the virus can progress to the point of no return. This race is highly dependent on the type of transplant donor, type of conditioning, and type of transplant, but respiratory infections clearly impact the transplant outcome (11). Any adjunctive measure to improve respiratory status should be sought.

SYSTEMIC VIRAL INFECTIONS IN PIDD

Children with severe T cell defects are also susceptible to systemic viral infections. Patients with SCID are extremely susceptible to progressive infection with cytomegalovirus (CMV) as well as other systemic viral infections. Infants with suspected SCID should be protected from exposures such as breast milk, transfusions, potentially infected siblings, live viral vaccines, and caregivers.

There is another circumstance in which susceptibility to gamma-herpes viruses such as CMV and Epstein–Barr virus (EBV) occurs. **Table 1** lists conditions in which gamma-herpes virus susceptibility dominates the clinical picture. Patients with cytolytic T cell defects (with or without concomitant NK cell defects) exhibit a unique susceptibility to these gamma-herpes viruses (15). In some circumstances, the susceptibility is almost entirely limited to susceptibility to either severe mononucleosis or hemophagocytic lymphohistiocytosis (HLH). HLH is characterized by excessive immune activation and can be diagnosed either by a molecular diagnosis consistent with HLH or clinically when patients meet five out of eight criteria: fever, splenomegaly, cytopenias affecting two or more blood lineages, hypertriglyceridemia and/or hypofibrinogenemia, hemophagocytosis, low/absent natural killer cell activity, hyperferritinemia, and high soluble interleukin-2 receptor levels (16). Any patient presenting with exceptionally severe mononucleosis or HLH should be screened for the HLH defects.

TABLE 1 | EBV susceptibility.

Phenotype	Gene defect	Viral susceptibility	Other features
EBV viremia	*ITK*	EBV	Lymphoma
EBV viremia	*MAGT1*	EBV	Lymphoma
EBV viremia	*CD27*	EBV	Low IgG
EBV viremia	*CORO1A*	Many viruses	Lymphoma
EBV HLH	*SH2D1A*	EBV	Lymphoma, dysgammaglobulinemia, and vasculitis
EBV HLH	*XIAP*	EBV	Hypogammaglobulinemia
EBV lymphoma	*MCM4*	EBV, CMV	Malignancy, short stature, adrenal insufficiency
Primary familial HLH	*PRF1*, *UNC13D*, *STX11*, *STXBP3*	EBV, CMV, others	
Pigmentary dilution with HLH	*LYST*, *RAB27A*, *AP3B1*, *BLOC1S6*	EBV, CMV, others	Pigmentary dilution
EBV susceptibility with broad infectious susceptibility	Leaky SCID, most combined immunodeficiencies, *WASP*, *WIPF1*, *PLCG2*, *PRKCD*, *ORAI1*, *STIM1*, *IKBKG*, *CASP8*, *STAT1* GOF, *DOCK8*, *GATA2*	Many viral susceptibilities	Gene dependent

GOF, gain of function; LOF, loss of function; EBV, Epstein–Barr virus; HLH, hemophagocytic lymphohistiocytosis; CMV, cytomegalovirus; SCID, severe combined immune deficiency; IgG, immunoglobulin G.

A second phenotype with susceptibility to EBV has a smoldering or even asymptomatic presentation. These patients generally have an increased risk of lymphoma due to chronic EBV, but the manifestations of EBV may be subtle or absent. The X-linked disorder due to deficiency of *MAGT1* is associated with a mild susceptibility to other infections but chronic EBV. Similar conditions include deficiencies of *CD27*, *CTPS1*, *RASGRP1*, *CD70*, and *MCM4*. The importance in recognizing this group is due to their unpredictable capacity to control EBV and the risk of lymphoreticular malignancies.

Nearly all of the leaky SCID types and the combined immunodeficiencies are associated with an increased risk of CMV and EBV (17, 18). This set of disorders can have a broad phenotype including Omenn's phenotype, autoimmunity, granulomas, and infections (18–23). In these patients, EBV and CMV can drive progression to malignancy, and they require careful monitoring. Leaky SCID has been defined as T cell lymphopenia (CD3 300–1,500 cells/mm^3); functional impairment as defined by proliferative responses, absence of maternal engraftment, and most often having identified hypomorphic mutations in genes associated with SCID.

Diagnostic Approach

When the consideration is prolonged infection with gamma-herpes viruses without HLH, T cell counts and function can be helpful as supporting information but often genetic testing is the quickest approach. For HLH disorders, enumerating the HLH criteria is a useful exercise. CD163 staining of the bone marrow can be a sensitive way to identify active hemophagocytosis, but this is not required for the diagnosis (15, 16, 24). Additional maneuvers are measurement of IL-2R in the serum and CD107a on the surface as a marker for degranulation. HLH can occur without an underlying PIDD, and thus genetic analysis is often central to the management. Nearly always an underlying PIDD will require HSCT as definitive therapy, whereas HLH due to uncommon infections such as *Leishmania*, certain influenza viruses, and arboviruses will not require HSCT.

Management

Management of systemic viral infections relies on the availability of antiviral compounds. For CMV, therapy is often begun with ganciclovir or valganciclovir (25, 26). Foscarnet may be added if

the virus is resistant or progressive in spite of adequate ganciclovir (27). Bone marrow toxicity from ganciclovir may also require a change to foscarnet. EBV in some cases is treated with rituximab to eliminate one important reservoir of virus (28). When HLH is present, a systemic approach to stabilize the patient and treat the underlying inflammation is essential (29). Risks and benefits of antiviral therapy must be carefully weighed as all approaches can have significant adverse events. Management decisions are often impacted by subsequent transplant strategies.

CHRONIC VIRAL SKIN INFECTIONS IN PRIMARY IMMUNODEFICIENCY

Cutaneous manifestations are common in PIDD. As many as two-thirds of the patients have cutaneous manifestations at some point. Atopy, infection, and inflammatory lesions have all been described, and there may be interplay between the features (30). Awareness of common skin infections is important both to aid in the early diagnosis and also in the treatment of potentially life-threatening infections that can begin in the skin. Bacterial infections are one of the most common findings in PIDD. For example, folliculitis, abscesses, and impetigo are typical in neutrophil defects. Similarly, a significant subset of PIDD diagnoses is associated with fungal infections. These can be seen both in T cell defects as well as defects of the myeloid compartment. Chronic mucocutaneous candidiasis is most often due to defects that affect the Th17 cell production or function. These diseases generally do not overlap those with a susceptibility to cutaneous viral infections. One exception is *STAT1* gain-of-function (GOF) mutations that render patients susceptible to a broad range of cutaneous infections. Viral infections of the skin are not nearly as common but are much more suggestive of PIDD. Severe herpes infections and papillomavirus are particularly characteristic of PIDD and can become the most notable feature in a patient. Chronic herpes virus and papillomavirus, in turn, predispose to cutaneous carcinoma and surveillance becomes important for this evolution. In this section, we will provide a brief synopsis about the individual disorders associated with susceptibility to papillomavirus.

Papillomaviridae

There are more than 200 strains of human papillomavirus (HPV). The diverse strains have variable malignant potential and tissue tropism. HPV causes warts in the general population with an incidence of cutaneous warts (**Figure 2**) that range from 1 to 12% (31–33). School age children have been estimated to have a cutaneous warts prevalence of over 40% (34). While sexually active women 20–24 years of age have a prevalence of genital papillomavirus of nearly 50% (35), clinically significant genital warts occur in only 5% of women (36). Genital HPV infection has been associated with malignant transformation, leading to the development of the first vaccine intended to prevent cancer. Worldwide, 5% of cancer is caused by HPV (37). Nearly all T cell disorders can be associated with increased susceptibility to warts; however, there is a small group of PIDDs that have warts as a cardinal feature (38). In these patients, the warts are recurrent, severe, and resistant to therapy. In most cases, the specific papillomaviruses are identical to those in the general population, and the appearance of the warts is the same as in the general population. In epidermodysplasia verruciformis (EV), however, there is a broader susceptibility to HPV types. In addition, the cutaneous manifestations are often atypical. **Table 2** compares the features of the different conditions described below.

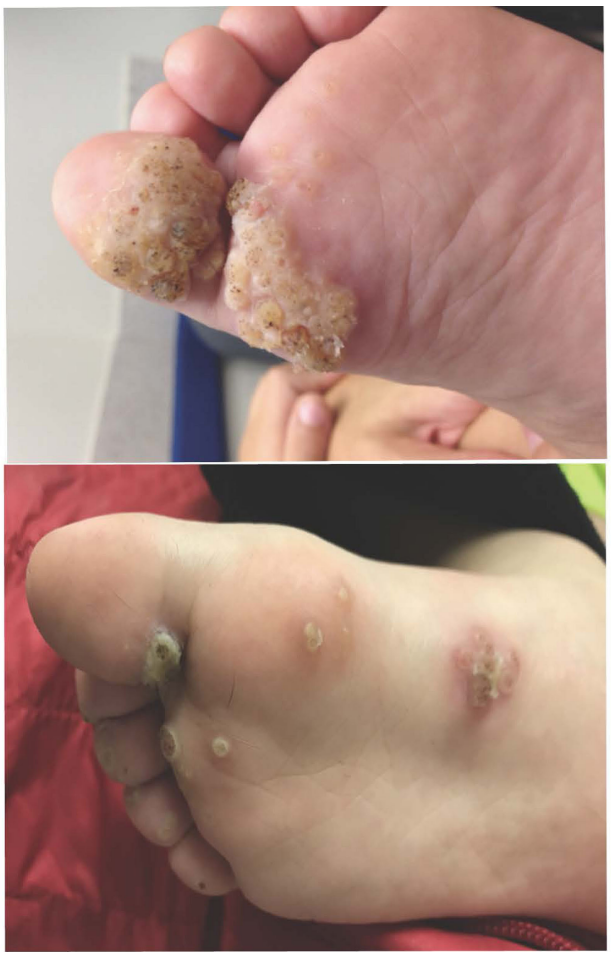

FIGURE 2 | Bilateral plantar warts on a patient who had experienced 5 years of immune suppression for a cardiac transplant. Deep palmoplantar warts such as those in the top panel are referred to as myrmecila and can be painful. The small black markings are characteristic and represent small blood vessels that have grown into the exophytic lesion. Photo credit: Marissa J. Perman, MD.

DOCK8 Deficiency

Patients with *DOCK8* deficiency have a complex combined immunodeficiency secondary to disrupted cytoskeletal rearrangement (39). This includes an inability to properly assemble the immune synapse that fosters the signaling cascades required for lymphocyte memory differentiation (40). Lymphocyte migration through tissues is also compromised, contributing to the susceptibility to cutaneous infections (39). T cell counts are typically low for age, and there is impaired memory differentiation that may be progressive with age (41). Clinical features resemble that seen in Wiskott–Aldrich syndrome (WAS) including low IgM

TABLE 2 | Cutaneous viral infections in primary immunodeficiencies.

Viral family	Virus	Increased susceptibility in which PID	Other features
Papillomaviridae	HPV	Ataxia telangiectasia; *DOCK8*; EV (*EVER1*, *EVER2*, *RHOH*; *LCK*); *GATA2*; Idiopathic T cell lymphopenia; Netherton syndrome; *STK4/MST1*; WHIM (*CXCR4*); WILD, *CARMIL2/RLTPR*, Clouston's syndrome	EV: warts are often flat, appearing as actinic keratosis or seborrhea-like lesions and can have increased susceptibility to unusual HPV strains. No other infectious susceptibility DOCK8, GATA2: also include susceptibility to HSV. Progressive lymphopenia seen
Herpesviridae	HHV8/KSHV	IFNGR1, OX40	Susceptibility to mycobacteria
	HSV	*DOCK8*; *GATA2*; *NEMO*; *STAT1 GOF*; *STK4*; *CXCR4*; Wiskott–Aldrich syndrome (WAS)	*DOCK8*, *NEMO*, *STAT1* GOF: broad infectious susceptibility *CXCR4*: pancytopenia, abnormal neutrophils WAS: thrombocytopenia, eczema
	VZV	*DOCK8*; *GATA2*; *STAT3* GOF; *IFNGR1*; *RHOH*; *STAT*1 GOF; *STK4*	*DOCK8*, *NEMO*, *STAT1* GOF: broad infectious susceptibility *CXCR4*: pancytopenia, abnormal neutrophils WAS: thrombocytopenia, eczema
Poxviridae	MCV	*DOCK8*; *GATA2*; *IKBKG*; *STAT1* GOF; *STK4*; *CXCR4*; *CARMIL2/RLTPR*	*DOCK8*, *IKBKG*, *STAT1* GOF: broad infectious susceptibility *CXCR4*: pancytopenia, abnormal neutrophils WAS: thrombocytopenia, eczema
	Orf virus	*STAT1* GOF	Broad infectious susceptibility

GOF, gain of function; LOF, loss of function; HPV, human papilloma virus; HSV, herpes simplex virus; EV, epidermodysplasia verruciformis; WHIM, warts, hypogammaglobulinemia, infections, and myelokathexis.

and elevated IgE and IgA. Patients have significant atopy, infections, impaired specific antibody responses and poor memory B cell responses, increased rates of malignancy, elevated IgE, and eosinophilia and are highly susceptible to cutaneous viral infections. Early in life, the atopic manifestations may dominate while the infectious susceptibility evolves. Skin infections are most often caused by HPV (including increased risk of malignant transformation of skin lesions), herpes simplex virus (HSV), molluscum contagiosum virus, and varicella zoster virus (42). HSCT has been shown to be curative (43).

Epidermodysplasia Verruciformis

The term EV refers to a group of disorders in which patients are susceptible to beta-HPV with severe diffuse warts, and there is a striking increase in the rate of skin carcinomas (44). *EVER1/TMC6* and *EVER2/TMC8* inactivating mutations cause autosomal recessive (AR) EV. EVER proteins are intracellular zinc transporters, and mutations lead to altered cell activation and a more permissive environment for HPV. Interestingly, the only infectious susceptibility is to HPV. Most patients present in childhood, but the appearance of the warts can lead to misdiagnosis as seborrhea or tinea versicolor. The warts are generally worse on sun-exposed skin for reasons that are not clear. The specific HPV types are not necessarily those seen in the general population as wart-associated. The therapy in EV is usually local control since the susceptibility does not relate to hematopoietic cell dysfunction; there is no role for HSCT.

LCK Deficiency

LCK deficiency causes atypical EV with CD4 T cell deficiency as well as recurrent pneumonia and severe warts complicated by non-melanoma skin cancer (45). To date, only a single patient with *LCK* deficiency has been described, leading to uncertainty in the full spectrum of infectious susceptibility. Therapy is not clear for the same reason, but HSCT would be expected to be curative.

CARMIL2/RLTPR

Three Norwegian families have been identified with increased viral cutaneous infections (warts and molluscum contagiosum) as well as dermatitis and pneumonia. The four affected family members were found to share a single variant in this gene, with some evidence for a role in T cell activation (46).

RHOH Deficiency

Mutations in the *RHOH* gene (an atypical Rho GTPase) cause susceptibility to EV-type HPV strains, due to alterations in T cell activation and homing (47). Naïve T cell counts are low, and there is poor skin homing of T cells, with an increase in effector memory T cells in the setting of altered T cell receptor signaling. *RHOH* deficiency infectious susceptibility was largely limited to HPV in the two siblings identified. In mice, the defect was correctable by transfer of wild-type bone marrow, suggesting that this is a potential treatment.

IKBKG Deficiency (*NEMO*)

Hypomorphic mutations in the central kinase of the canonical NFκB pathway lead to increased susceptibility to warts (48). This PIDD is associated with fine sparse hair, dental defects, and a broad susceptibility to infections including opportunistic infections. The spectrum of phenotypes is among the broadest among PIDDs. Lymphedema, osteopetrosis, susceptibility to pneumocystis and other fungi, mycobacterial susceptibility, and viral susceptibility call all be associated with *IKBKG* deficiency. Inflammatory bowel disease is also common. Cutaneous viral infections are common in this population but are not usually the dominant feature. Among the PIDD in this section, this is a disorder where the benefit of HSCT is unclear because HSCT outcomes have been poor.

GATA2 Deficiency

GATA2 mutations yield a complex set of phenotypes. Patients tend to have monocytopenia and low NK and B cell counts

and have susceptibility to HPV, among other infections (49). The laboratory defects are progressive with age and correlate with increasing infection burden. Clinical features include lymphedema, risk of malignancy, and pulmonary alveolar proteinosis. Infections include fungal infections and cutaneous viral infections. The cutaneous viral infections can be the most prominent feature but there is a wide-ranging phenotypic heterogeneity (50, 51). The cutaneous viral infections and genital HPV are associated with a high rate of malignant transformation.

STAT1 GOF

Patients with *STAT1* GOF mutations can present with dramatic autoimmunity, usually including enteropathy and endocrinopathies, or they can have a picture with a pronounced infectious susceptibility. Susceptibility to candida is common, but cutaneous viral infections can also be problematic (52, 53). The viral infections can become more problematic as immune suppression is used to control the enteropathy.

Netherton Syndrome

Patients with Netherton syndrome (secondary to *SPINK5* mutations) demonstrate congenital ichthyosis and have higher susceptibility to EV-associated HPV strains (53, 54). Although Netherton syndrome can have a mild humoral immune deficiency, the susceptibility to warts seems to be a result of disrupted local response to infection (55). The warts are generally controllable with local measures.

MST1 Deficiency

Serine–threonine kinase 4 (STK4), encoded by a gene called *MST1*, affects the FOXO1 transcription factor and thus impacts T cell lifespan and has been shown to increase susceptibility to HPV infections (56), as well as causing B cell lymphopenia. The phenotype also includes susceptibility to EBV and cutaneous viral infections.

Warts, Hypogammaglobulinemia, Infections, and Myelokathexis (WHIM) Syndrome

Warts, hypogammaglobulinemia, infections, and myelokathexis syndrome is caused by an autosomal dominant (AD) GOF mutation in *CXCR4*, which leads to increased susceptibility to HPV (57, 58). Varying degrees of pancytopenia can be seen, and hypogammaglobulinemia occurs in many (but not all) patients. Warts can be the most prominent susceptibility, but neutropenia and hypogammaglobulinemia can drive diverse infectious susceptibilities. COPD and cutaneous carcinoma have been observed (59). WHIM can be treated with plerixafor or topical control measures for the warts.

WILD Syndrome

WILD (warts, depressed cell-mediated immunity, primary lymphedema, and anogenital dysplasia) is also correlated with severe warts without a known genetic etiology (60). Of note, this diagnosis does not lead to EV-defining HPV strain infections. A recent study demonstrates a case of a patient whose warts improved after quadrivalent HPV vaccination (61).

Clouston Syndrome

An ectodermal dysplasia syndrome with alterations in hair and nails (not generally in teeth) which can present with eccrine syringofibroadenomatosis that is reminiscent of EV and is associated with HPV infection (62).

Other Settings

Idiopathic CD4 T cell lymphopenia is associated with increased risk of cutaneous warts (63), and SCID patients post-HSCT can have an increased risk of warts, especially with specific underlying mutations (64, 65).

Management

Management of cutaneous warts typically progresses from low level removal approaches involving topical therapy (freezing, electrosurgery, curettage, laser, chemical softening, and cantharidin) to immune stimulants (imiquimod and antigens), bleomycin, and antiviral therapy including cidofovir (66). Genital warts are treated with conceptually the same approach. Management of genital warts also includes special considerations for pregnancy, partners, and screening for malignancy (67–69). Interferon a2 and GM-CSF have been used successfully for papillomavirus (70, 71). In severe cases, therapy can be very unsatisfying. HSCT can result in prompt eradication, though *JAK3* mutations and common γ chain SCID can have significant warts post-HSCT, which may be in part due to poor NK cell function (64). Topical cidofovir has recently been shown in a case report to be effective in some of these patients (72). In one case, regression was observed after papillomavirus vaccination (61).

Herpesviridae

The Herpes family of DNA viruses includes nine viruses pathogenic for humans; CMV, EBV, HHV6a/b, HHV7, HSV1, HSV2, and KSHV, not all of which have skin tropism. Almost any type of T cell dysfunction is associated with an increased frequency of cutaneous HSV. The PIDD with a high likelihood of chronic or disfiguring cutaneous herpes are *DOCK8*, *GATA2*, *NEMO*/*IKBKG*, *STK4*, *WAS*, *STAT1* GOF, and WHIM (*CXCR4* mutations) as described above in association with warts. These same syndromes can manifest with severe primary varicella and an increased frequency and severity of zoster (73). Varicella and zoster can also be seen in settings with altered proportions of T cell subsets, as in *STAT3* LOF deficient patients (AD-hyper IgE syndrome) (74) or cytokine signaling defects as seen in *IFNGR1* mutations (75). Thus, susceptibility to herpes viruses occurs in a broad range of T cells defects and is often part of a complex of susceptibility to many cutaneous viral infections. Herpes viruses can be particularly persistent and cause significant morbidity and mortality. Carcinoma is a feared consequence of recurrent or persistent infection and can be difficult to distinguish from persistently infected skin.

Management

Management includes acyclovir, valacyclovir, or famciclovir as initial options. IL-2 therapy has been used successfully in WAS (76). In WAS, it specifically improves NK cell function (77). Type

I and type II interferons have been used successfully in model systems (78, 79). Small studies of non-PIDD populations have supported its use in patients (80–83). Management should focus on prevention of recurrences and healing of cutaneous lesions. Malignant transformation relates in complex ways to persistence of infection.

Poxviridae

Poxviridae are double stranded DNA viruses. Molluscipoxvirus (i.e., molluscum contagiosum virus or MCV), orthopoxvirus (i.e., smallpox), parapoxvirus, and yatapoxvirus are the four genera that can infect humans. Susceptibility to poxviridae tends to be associated with susceptibility to other cutaneous viral infections. Therefore, many of the disorders described above have an increased susceptibility to poxviridae. Molluscum contagiosum in the most common pox virus infection in humans and in the general population is a self-limited infection with minimal residua (84). Severe molluscum contagiosum can be seen in *DOCK8* deficiency, *STK4* deficiency (56), *GATA2*, *NEMO*, *STAT1* GOF, and WHIM syndrome. Molluscum can be a significant issue for patients with WAS, as described in the Herpesviridae section. In addition, Orf virus, found in pasture animals, was found in a single patient with *STAT1* GOF (85). Similar to herpes virus susceptibility described above, molluscum can be seen with nearly any T cell defect and typically arises in that setting as part of a susceptibility to many cutaneous viruses.

Management

Initial management of molluscum in a PIDD patient is control of spread through curettage, topical therapy such as salicylate, cantharidin, or immune stimulation with imiquimod (86, 87). Other topical approaches have also been used successfully. Retreatment 2–4 weeks later is often required. If addressed early, spread may be controlled and the outbreak contained. For diffuse disease or disease that spreads despite all attempts at control, type I interferon (interferon a2) has been suggested (88, 89). Intralesional immunotherapy with live antigen has been promoted but is contraindicated in PIDD patients with T cell defects (90, 91).

Persistent Vaccine-Stain Rubella Infection

Three studies have identified persistent vaccine-strain rubella in patients with moderate T cell defects (91–93). Most of the patients have had ataxia telangiectasia but a wide range of PIDD diagnoses have been seen. Generally, the patients have had sufficient T cell function to be leading relatively normal lives and the immune deficiency might not even be recognized at the time of the MMR vaccine administration. The manifestations have been largely cutaneous granulomas although chronic inflammation at other sites has been observed (91). Persistence of virus due to compromised T cell control and acquisition of mutations that may further impact clearance is the proposed mechanism.

Diagnostic Considerations for Patients with Cutaneous Viral Infections

The above conditions are derived from defects in T cell, NK cell, and local tissue immunity. It is therefore nearly impossible to systematically screen for gene defects related to cutaneous viral infection susceptibility. A reasonable start is to define T cells both quantitatively and functionally. If that is unrevealing and the phenotype suggests a PIDD, then whole exome sequencing may be appropriate.

GASTROINTESTINAL (GI) VIRAL INFECTIONS

Chronic diarrhea (>6 weeks) is a frequent finding in PIDD patients. Given that the etiologies of chronic diarrhea in immunodeficient patients can be diverse, it is important to first distinguish if the diarrhea is infectious, malabsorptive, or inflammatory in nature as there are multiple types of PIDD that can present with autoimmune enteropathy or inflammatory bowel disease (94–96). PIDD patients are susceptible to multiple types of GI pathogens, and this section will focus on GI viruses. The concerted action of both the innate and adaptive immune system is necessary for viral clearance (97, 98). Therefore, there are a number of combined immunodeficiency phenotypes that result in susceptibility to GI viral pathogens, which manifest as prolonged illness as well as prolonged asymptomatic viral shedding.

Norovirus

Persistent infection with norovirus resulting in prolonged viral shedding and symptomatic disease has been noted in patients with SCID and various secondary immunodeficiency states (99). In a series of pediatric PIDD patients, it was the most frequently isolated virus at 20.6%, with patients with SCID, major histocompatibility complex II deficiency, CD40L deficiency, and agammaglobulinemia represented in this series (100). Norovirus shedding can be prolonged in the stool of patients who were immunosuppressed following infection and norovirus can be part of multiple infections in the GI tract (101). In CVID, norovirus infection has been linked to development of severe enteropathy with prolonged viral carriage over the course of years (102). In several patients in this series, clearance of norovirus resulted in normalization of the GI enteropathy.

A concern about norovirus is the great difficulty in public health containment. A patient with PIDD who is shedding for a prolonged period of time is not only themselves at risk but also places those around at risk. Norovirus is a common pathogen in the general population, and exposures are therefore common. Norovirus is spread from the moment of illness to several days after clinical recovery. Both vomit and feces can spread virus. The virus lives on surfaces for up to 20 days, and alcohol-based cleaners are not completely effective. Patients with chronic norovirus should use bleach to clean surfaces. Vigorous hand washing with soap and water is also effective.

Hepatitis C

In the early 1990s, the United States FDA recommended that hepatitis C positive donors be excluded from the plasma donor pool resulting in loss of neutralizing hepatitis C viral antibodies from IVIG (103). Hepatitis C virus (HCV) infection was subsequently reported from several countries, which was due to the

presence of contaminating HCV virus from the small numbers of seronegative HCV positive donors (103–106). The severity of hepatitis seen in immunodeficient patients was variable, but subsets of patients with primary hypogammaglobulinemia were observed to have a more severe course of hepatitis, which in some cases was rapidly fatal (103, 105, 106). Younger age and early treatment with IFN were associated with better overall outcomes (103, 106). Following adoption of PCR screening for HCV and viral inactivation processes with solvent–detergent or pasteurization there have been no subsequent reports of IVIG-associated HCV since 1996 (107). This cautionary tale supports surveillance of PIDD patients who have risk factors for HCV: blood product exposure, including IV drug use, infants born to HCV positive mothers, high-risk sexual behavior, shared personal items with potential blood exposure among HCV positive individuals. Today, therapy for HCV should improve outcomes compared to the cohort in the early 1990s.

Other GI Viruses

Adenovirus, enterovirus, and rotavirus have been isolated from single PIDD patients with chronic diarrhea, and the true incidence is not known (100). Chronic rotavirus infection has been described in patients with SCID and agammaglobulinemia (108). In patients with immunodeficiency, rotavirus can be poorly contained within the GI tract. On investigation at autopsy, active rotavirus replication has been identified in the liver and kidney of patients with SCID, complete DiGeorge syndrome, and acquired-immunodeficiency syndrome, illustrating poor control of viral replication in the setting of profound immunodeficiency (109). An important consideration is that SCID has been associated with susceptibility to the live rotavirus vaccine (110). Indeed, vaccine-strain illness is cleared only after immune reconstitution (110, 111).

CVID and agammaglobulinemia can rarely have prolonged asymptomatic shedding of vaccine-strain polio following immunization with live-attenuated oral polio vaccine, which can pose risk to other immunocompromised members of the community (112–114). Additionally, central nervous system (CNS) infection can occur in agammaglobulinemia (see below). These are the main reasons that live polio vaccination is no longer used in the USA.

Management

Care for chronic GI viral infections in PIDD is primarily supportive: optimizing hydration and nutrition. Orally administered immunoglobulin G (IgG) has been demonstrated to be effective as a therapy for chronic infectious diarrhea in antibody deficient patients (115, 116). Oral IgG survives passage through the stomach and is bioavailable (116). Antiviral therapies are untested; however, they could be considered in severe disease.

VIRAL INFECTIONS OF THE CNS IN PIDD

Viral infections of the CNS confer significant morbidity and mortality in the general population (117–119). Therefore, they are not often considered to be indicators of primary immunodeficiency. There are two circumstances where an infection of the CNS is often associated with a primary immunodeficiency: atypical herpes simplex encephalitis and CNS enteroviral disease.

Herpes Simplex Encephalitis

Herpes simplex encephalitis in the general population is most typically seen in newborns and is typically caused by herpes simplex type 2. Infection occurs at the time of delivery and infants present in the second week of life with agitation, obtundation, or seizures. Adults can develop herpes simplex encephalitis (120). Underlying immune compromise can be a risk factor for adult-onset herpes simplex encephalitis, and today HIV is the most common associated condition in adults. Herpes simplex encephalitis outside of the neonatal period may therefore suggest an immunodeficiency. Among PIDD, defects in the toll-like receptor pathway are most strongly associated with this infection (**Table 3**) (121). Approximately 5% of children with herpes simplex encephalitis have defects in the toll-like receptor pathway (122). Patients with these defects may present in childhood or adulthood, and some patients with just keratitis have been described (123). Recognition is important because therapy can be tailored if the defect is known. Surveillance and prevention of relapses is important. Several of these defects are inherited in an AD fashion, and therefore recognition of these PIDD is critical not only for management of the patient but also surveillance for other family members. A population study suggested that there may be additional defects inherited in an AR fashion yet to be defined (123). A key consideration is that the described toll-like receptor pathway defects are due to loss of local control in the CNS. Antibody and T cell responses are normal, and indeed, local mucosal recurrences are uncommon. As a consequence, testing of the hematopoietic cells is not revealing typically.

Enteroviral Meningoencephalitis

Viral meningoencephalitis due to a prolonged infection with enterovirus is strongly suggestive of a specific class of PIDD. Enteroviruses are the most common cause of viral meningitis in the general population manifesting as acute onset headache with gradual resolution over days to a few weeks. In patients with agammaglobulinemia, manifestations are quite different (124). These children typically present with regression of developmental milestones. Ataxia or clumsiness may be noted by parents or on examination. Features early on are subtle, and the slow progression can lead to efforts at mitigation with physical therapy or behavioral strategies. In a patient with a known humoral immune deficiency, the index of suspicion should be high and a workup should not be delayed if there are clear neurologic signs or symptoms. CNS infection in patients with agammaglobulinemia has a

TABLE 3 | Causes of increased susceptibility to herpes simplex encephalitis.

TLR3 [autosomal dominant (AD)]
TRIF [autosomal recessive (AR)]
UNC93B1 (AR)
TRAF3 (AD)
TBK1 (AD)
IRF3 (AD)
STAT1 (AR)
IKBKG (XL)

very poor prognosis. There can be other phenotypes associated with enteroviral disease in patients with agammaglobulinemia; however, CNS infection is the most common. Dermatomyositis and hepatitis have been described and have progressed in some cases to CNS infection. Treatment for enteroviral disease includes high dose immunoglobulin and when available, drugs directed at enterovirus.

A unique subset of CNS enteroviral infections occurs in either SCID or agammaglobulinemia with live-attenuated polio vaccine. Wild-type polio, occurring in three serotypes, has been nearly eradicated. Even early on, it was recognized that the live-attenuated vaccine could cause disease (125) and that patients with hypogammaglobulinemia could excrete virus for years (126, 127). Currently, circulating wild-type polio is seen only in Afghanistan and Pakistan although virus can be isolated in sewage from other countries supporting ongoing risk for immunodeficient individuals (128). Vaccine-associated poliomyelitis can be due to infection of an immune deficient individual and spread to the CNS or to revertants of vaccine-strain virus (129, 130). In the latter case, even normal hosts can have overt paralytic disease. Vaccine-associated poliomyelitis can appear as acute flaccid paralysis or with a meningoencephalitis in immunodeficient individuals. The prognosis has generally been poor (131).

Diagnostic Approaches

Testing for defects related to herpes simplex encephalitis often involves genetic sequencing although functional analyses are available on a research basis. **Table 3** lists the currently recognized genetic causes of susceptibility to herpes simplex encephalitis.

The diagnosis of enteroviral meningoencephalitis in PIDD patients requires a specific description. In a patient with agammaglobulinemia detection of enterovirus is surprisingly difficult. PCR analysis of cerebrospinal fluid or stool (less specific) should be performed. However, it is not unusual for children with agammaglobulinemia and suggestive clinical features to require a brain biopsy for diagnosis. The biopsy tissue can be tested for enterovirus by PCR. In a patient who presents with CNS enteroviral disease, identification of an immune deficiency is critical because of the prognostic implications. The strong association of CNS enteroviral disease with agammaglobulinemia supports a strategy that begins with enumeration of peripheral blood B cells by flow cytometry. Only if that is negative and there are no other secondary immune deficiencies should alternatives such as CD40L or CVID be sought. A reasonable secondary screen would be to measure immunoglobulin levels and responses to vaccines.

Management

Management of herpes simplex encephalitis requires specific antiviral approaches as well as attention to seizures, increased intracranial pressure, and a comprehensive intensive care approach. Acyclovir delivered intravenously is the cornerstone of management. One should consider a prolonged course of oral therapy after initial management that could include oral acyclovir or valacyclovir because the relapse rate is high in these toll-like receptor pathway defects. The role of steroids is controversial. One small study of immune competent children supported the use of beta-interferon (132), and it could be argued that interferons specifically mitigate the underlying defect in the toll-like receptor pathway disorders.

Management of CNS enteroviral disease in agammaglobulinemia has recently been reviewed (124). In the USA, antiviral drugs are not available, but pocapavir is under study and may become available. High dose IVIG has been proposed as therapeutic, but survival rates remain dismal and functional outcomes are poor.

SUMMARY

Viral infections are a common cause of morbidity in patients with PIDDs. They can be a clue to the diagnosis when persistent or unusually severe and can represent a significant management challenge.

AUTHOR CONTRIBUTIONS

MR, SH, and KS collectively conceived and wrote the manuscript.

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REFERENCES

1. Campbell H. Acute respiratory infection: a global challenge. *Arch Dis Child* (1995) 73(4):281–3. doi:10.1136/adc.73.4.281
2. Gruber C, Keil T, Kulig M, Roll S, Wahn U, Wahn V, et al. History of respiratory infections in the first 12 year among children from a birth cohort. *Pediatr Allergy Immunol* (2008) 19(6):505–12. doi:10.1111/j.1399-3038.2007.00688.x
3. Monto AS. Viral respiratory infections in the community: epidemiology, agents, and interventions. *Am J Med* (1995) 99(6B):24S–7S. doi:10.1016/S0002-9343(99)80307-6
4. Aligne CA, Stoddard JJ. Tobacco and children. An economic evaluation of the medical effects of parental smoking. *Arch Pediatr Adolesc Med* (1997) 151(7):648–53. doi:10.1001/archpedi.1997.02170440010002
5. Kirkpatrick GL. The common cold. *Prim Care* (1996) 23(4):657–75. doi:10.1016/S0095-4543(05)70355-9
6. Aghamohammadi A, Abolhassani H, Mohammadinejad P, Rezaei N. The approach to children with recurrent infections. *Iran J Allergy Asthma Immunol* (2012) 11(2):89–109. doi:011.02/ijaai.89109
7. Costa-Carvalho BT, Grumach AS, Franco JL, Espinosa-Rosales FJ, Leiva LE, King A, et al. Attending to warning signs of primary immunodeficiency diseases across the range of clinical practice. *J Clin Immunol* (2014) 34(1):10–22. doi:10.1007/s10875-013-9954-6
8. Bousfiha A, Jeddane L, Al-Herz W, Ailal F, Casanova JL, Chatila T, et al. The 2015 IUIS phenotypic classification for primary immunodeficiencies. *J Clin Immunol* (2015) 35(8):727–38. doi:10.1007/s10875-015-0198-5
9. Lanari M, Vandini S, Capretti MG, Lazzarotto T, Faldella G. Respiratory syncytial virus infections in infants affected by primary immunodeficiency. *J Immunol Res* (2014) 2014:850831. doi:10.1155/2014/850831
10. Rezaei N, Hedayat M, Aghamohammadi A, Nichols KE. Primary immunodeficiency diseases associated with increased susceptibility to viral infections and malignancies. *J Allergy Clin Immunol* (2011) 127(6):1329–41.e2; quiz 42–3. doi:10.1016/j.jaci.2011.02.047
11. Pai SY, Logan BR, Griffith LM, Buckley RH, Parrott RE, Dvorak CC, et al. Transplantation outcomes for severe combined immunodeficiency, 2000–2009. *N Engl J Med* (2014) 371(5):434–46. doi:10.1056/NEJMoa1401177

12. Tregoning JS, Schwarze J. Respiratory viral infections in infants: causes, clinical symptoms, virology, and immunology. *Clin Microbiol Rev* (2010) 23(1):74–98. doi:10.1128/CMR.00032-09
13. Ciancanelli MJ, Huang SX, Luthra P, Garner H, Itan Y, Volpi S, et al. Infectious disease. Life-threatening influenza and impaired interferon amplification in human IRF7 deficiency. *Science* (2015) 348(6233):448–53. doi:10.1126/science.aaa1578
14. McDonald-McGinn DM, Sullivan KE, Marino B, Philip N, Swillen A, Vorstman JA, et al. 22q11.2 deletion syndrome. *Nat Rev Dis Primers* (2015) 1:15071. doi:10.1038/nrdp.2015.71
15. Chandrakasan S, Filipovich AH. Hemophagocytic lymphohistiocytosis: advances in pathophysiology, diagnosis, and treatment. *J Pediatr* (2013) 163(5):1253–9. doi:10.1016/j.jpeds.2013.06.053
16. Henter JI, Horne A, Arico M, Egeler RM, Filipovich AH, Imashuku S, et al. HLH-2004: diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. *Pediatr Blood Cancer* (2007) 48(2):124–31. doi:10.1002/pbc.21039
17. Speckmann C, Doerken S, Aiuti A, Albert MH, Al-Herz W, Allende LM, et al. A prospective study on the natural history of patients with profound combined immunodeficiency: an interim analysis. *J Allergy Clin Immunol* (2017) 139(4):1302–10.e4. doi:10.1016/j.jaci.2016.07.040
18. Filipovich AH, Mathur A, Kamat D, Kersey JH, Shapiro RS. Lymphoproliferative disorders and other tumors complicating immunodeficiencies. *Immunodeficiency* (1994) 5(2):91–112.
19. Wahn V, Yokota S, Meyer KL, Janssen JW, Hansen-Hagge TE, Knobloch C, et al. Expansion of a maternally derived monoclonal T cell population with CD3+/CD8+/T cell receptor-gamma/delta+ phenotype in a child with severe combined immunodeficiency. *J Immunol* (1991) 147(9):2934–41.
20. de Villartay JP, Lim A, Al-Mousa H, Dupont S, Dechanet-Merville J, Coumau-Gatbois E, et al. A novel immunodeficiency associated with hypomorphic RAG1 mutations and CMV infection. *J Clin Invest* (2005) 115(11):3291–9. doi:10.1172/JCI25178
21. Ehl S, Schwarz K, Enders A, Duffner U, Pannicke U, Kuhr J, et al. A variant of SCID with specific immune responses and predominance of gamma delta T cells. *J Clin Invest* (2005) 115(11):3140–8. doi:10.1172/JCI25221
22. Schuetz C, Huck K, Gudowius S, Megahed M, Feyen O, Hubner B, et al. An immunodeficiency disease with RAG mutations and granulomas. *N Engl J Med* (2008) 358(19):2030–8. doi:10.1056/NEJMoa073966
23. Walter JE, Rosen LB, Csomos K, Rosenberg JM, Mathew D, Keszei M, et al. Broad-spectrum antibodies against self-antigens and cytokines in RAG deficiency. *J Clin Invest* (2015) 125(11):4135–48. doi:10.1172/JCI80477
24. Weitzman S. Approach to hemophagocytic syndromes. *Hematology Am Soc Hematol Educ Program* (2011) 2011:178–83. doi:10.1182/asheducation-2011.1.178
25. Len O, Gavalda J, Aguado JM, Borrell N, Cervera C, Cisneros JM, et al. Valganciclovir as treatment for cytomegalovirus disease in solid organ transplant recipients. *Clin Infect Dis* (2008) 46(1):20–7. doi:10.1086/523590
26. Boeckh M. Complications, diagnosis, management, and prevention of CMV infections: current and future. *Hematology Am Soc Hematol Educ Program* (2011) 2011:305–9. doi:10.1182/asheducation-2011.1.305
27. Razonable RR. Antiviral drugs for viruses other than human immunodeficiency virus. *Mayo Clin Proc* (2011) 86(10):1009–26. doi:10.4065/mcp.2011.0309
28. Chellapandian D, Das R, Zelley K, Wiener SJ, Zhao H, Teachey DT, et al. Treatment of Epstein-Barr virus-induced haemophagocytic lymphohistiocytosis with rituximab-containing chemo-immunotherapeutic regimens. *Br J Haematol* (2013) 162(3):376–82. doi:10.1111/bjh.12386
29. Jordan MB, Allen CE, Weitzman S, Filipovich AH, McClain KL. How I treat hemophagocytic lymphohistiocytosis. *Blood* (2011) 118(15):4041–52. doi:10.1182/blood-2011-03-278127
30. Langan SM, Abuabara K, Henrickson SE, Hoffstad O, Margolis DJ. Increased risk of cutaneous and systemic infections in atopic dermatitis – a cohort study. *J Invest Dermatol* (2017) 137:1375–7. doi:10.1016/j.jid.2017.01.030
31. Beliaeva TL. [The population incidence of warts]. *Vestn Dermatol Venerol* (1990) 2:55–8.
32. Johnson MT, Roberts J. Skin conditions and related need for medical care among persons 1–74 years. United States, 1971–1974. *Vital Health Stat 11* (1978) 212:i–v, 1–72.
33. Loo SK, Tang WY. Warts (non-genital). *BMJ Clin Evid* (2009) 2009:1710.
34. de Koning MNC, Quint KD, Bruggink SC, Gussekloo J, Bouwes Bavinck JN, Feltkamp MCW, et al. High prevalence of cutaneous warts in elementary school children and the ubiquitous presence of wart-associated human papillomavirus on clinically normal skin. *Br J Derm* (2015) 172:196–201. doi:10.1111/bjd.13216
35. Hariri S, Unger ER, Sternberg M, Dunne EF, Swan D, Patel S, et al. Prevalence of genital human papillomavirus among females in the United States, the national health and nutrition examination survey, 2003–2006. *J Infect Dis* (2011) 204(4):566–73. doi:10.1093/infdis/jir341
36. Flagg EW, Schwartz R, Weinstock H. Prevalence of anogenital warts among participants in private health plans in the United States, 2003–2010: potential impact of human papillomavirus vaccination. *Am J Public Health* (2013) 103(8):1428–35. doi:10.2105/AJPH.2012.301182
37. Tota JE, Chevarie-Davis M, Richardson LA, Devries M, Franco EL. Epidemiology and burden of HPV infection and related diseases: implications for prevention strategies. *Prev Med* (2011) 53(Suppl 1):S12–21. doi:10.1016/j.ypmed.2011.08.017
38. Dropulic LK, Cohen JI. Severe viral infections and primary immunodeficiencies. *Clin Infect Dis* (2011) 53(9):897–909. doi:10.1093/cid/cir610
39. Zhang Q, Dove CG, Hor JL, Murdock HM, Strauss-Albee DM, Garcia JA, et al. DOCK8 regulates lymphocyte shape integrity for skin antiviral immunity. *J Exp Med* (2014) 211(13):2549–66. doi:10.1084/jem.20141307
40. Randall KL, Lambe T, Johnson AL, Treanor B, Kucharska E, Domaschenz H, et al. Dock8 mutations cripple B cell immunological synapses, germinal centers and long-lived antibody production. *Nat Immunol* (2009) 10(12):1283–91. doi:10.1038/ni.1820
41. Lambe T, Crawford G, Johnson AL, Crockford TL, Bouriez-Jones T, Smyth AM, et al. DOCK8 is essential for T-cell survival and the maintenance of CD8+ T-cell memory. *Eur J Immunol* (2011) 41(12):3423–35. doi:10.1002/eji.201141759
42. Zhang Q, Davis JC, Lamborn IT, Freeman AF, Jing H, Favreau AJ, et al. Combined immunodeficiency associated with DOCK8 mutations. *N Engl J Med* (2009) 361(21):2046–55. doi:10.1056/NEJMoa0905506
43. Al-Herz W, Chu JI, van der Spek J, Raghupathy R, Massaad MJ, Keles S, et al. Hematopoietic stem cell transplantation outcomes for 11 patients with dedicator of cytokinesis 8 deficiency. *J Allergy Clin Immunol* (2016) 138(3):852–9.e3. doi:10.1016/j.jaci.2016.02.022
44. Orth G. Genetics of epidermodysplasia verruciformis: insights into host defense against papillomaviruses. *Semin Immunol* (2006) 18(6):362–74. doi:10.1016/j.smim.2006.07.008
45. Li SL, Duo LN, Wang HJ, Dai W, Zhou EH, Xu YN, et al. Identification of LCK mutation in a family with atypical epidermodysplasia verruciformis with T-cell defects and virus-induced squamous cell carcinoma. *Br J Dermatol* (2016) 175(6):1204–9. doi:10.1111/bjd.14679
46. Sorte HS, Osnes LT, Fevang B, Aukrust P, Erichsen HC, Backe PH, et al. A potential founder variant in CARMIL2/RLTPR in three Norwegian families with warts, molluscum contagiosum, and T-cell dysfunction. *Mol Genet Genomic Med* (2016) 4(6):604–16. doi:10.1002/mgg3.237
47. Crequer A, Troeger A, Patin E, Ma CS, Picard C, Pedergnana V, et al. Human RHOH deficiency causes T cell defects and susceptibility to EV-HPV infections. *J Clin Invest* (2012) 122(9):3239–47. doi:10.1172/JCI62949
48. Huppmann AR, Leiding JW, Hsu AP, Raffeld M, Uzel G, Pittaluga S, et al. Pathologic findings in NEMO deficiency: a surgical and autopsy survey. *Pediatr Dev Pathol* (2015) 18(5):387–400. doi:10.2350/15-05-1631-OA.1
49. Spinner MA, Sanchez LA, Hsu AP, Shaw PA, Zerbe CS, Calvo KR, et al. GATA2 deficiency: a protean disorder of hematopoiesis, lymphatics, and immunity. *Blood* (2014) 123(6):809–21. doi:10.1182/blood-2013-07-515528
50. Hsu AP, Johnson KD, Falcone EL, Sanalkumar R, Sanchez L, Hickstein DD, et al. GATA2 haploinsufficiency caused by mutations in a conserved intronic element leads to MonoMAC syndrome. *Blood* (2013) 121(19):3830–7, S1–7. doi:10.1182/blood-2012-08-452763
51. Hsu AP, McReynolds LJ, Holland SM. GATA2 deficiency. *Curr Opin Allergy Clin Immunol* (2015) 15(1):104–9. doi:10.1097/ACI.0000000000000126
52. Toubiana J, Okada S, Hiller J, Oleastro M, Lagos Gomez M, Aldave Becerra JC, et al. Heterozygous STAT1 gain-of-function mutations underlie an

unexpectedly broad clinical phenotype. *Blood* (2016) 127(25):3154–64. doi:10.1182/blood-2015-11-679902
53. Furio L, Pampalakis G, Michael IP, Nagy A, Sotiropoulou G, Hovnanian A. KLK5 inactivation reverses cutaneous hallmarks of Netherton syndrome. *PLoS Genet* (2015) 11(9):e1005389. doi:10.1371/journal.pgen.1005389
54. Weber F, Fuchs PG, Pfister HJ, Hintner H, Fritsch P, Hoepfl R. Human papillomavirus infection in Netherton's syndrome. *Br J Dermatol* (2001) 144(5):1044–9. doi:10.1046/j.1365-2133.2001.04196.x
55. Hannula-Jouppi K, Laasanen SL, Ilander M, Furio L, Tuomiranta M, Marttila R, et al. Intrafamily and interfamilial phenotype variation and immature immunity in patients with Netherton syndrome and Finnish SPINK5 Founder Mutation. *JAMA Dermatol* (2016) 152(4):435–42. doi:10.1001/jamadermatol.2015.5827
56. Abdollahpour H, Appaswamy G, Kotlarz D, Diestelhorst J, Beier R, Schaffer AA, et al. The phenotype of human STK4 deficiency. *Blood* (2012) 119(15):3450–7. doi:10.1182/blood-2011-09-378158
57. Balabanian K, Lagane B, Pablos JL, Laurent L, Planchenault T, Verola O, et al. WHIM syndromes with different genetic anomalies are accounted for by impaired CXCR4 desensitization to CXCL12. *Blood* (2005) 105(6):2449–57. doi:10.1182/blood-2004-06-2289
58. Kawai T, Malech HL. WHIM syndrome: congenital immune deficiency disease. *Curr Opin Hematol* (2009) 16(1):20–6. doi:10.1097/MOH.0b013e32831ac557
59. Beaussant Cohen S, Fenneteau O, Plouvier E, Rohrlich PS, Daltroff G, Plantier I, et al. Description and outcome of a cohort of 8 patients with WHIM syndrome from the French Severe Chronic Neutropenia Registry. *Orphanet J Rare Dis* (2012) 7:71. doi:10.1186/1750-1172-7-71
60. Kreuter A, Hochdorfer B, Brockmeyer NH, Altmeyer P, Pfister H, Wieland U, et al. A human papillomavirus-associated disease with disseminated warts, depressed cell-mediated immunity, primary lymphedema, and anogenital dysplasia: WILD syndrome. *Arch Dermatol* (2008) 144(3):366–72. doi:10.1001/archderm.144.3.366
61. Kreuter A, Waterboer T, Wieland U. Regression of cutaneous warts in a patient with WILD syndrome following recombinant quadrivalent human papillomavirus vaccination. *Arch Dermatol* (2010) 146(10):1196–7. doi:10.1001/archdermatol.2010.290
62. Carlson JA, Rohwedder A, Daulat S, Schwartz J, Schaller J. Detection of human papillomavirus type 10 DNA in eccrine syringofibroadenomatosis occurring in Clouston's syndrome. *J Am Acad Dermatol* (1999) 40(2 Pt 1):259–62. doi:10.1016/S0190-9622(99)70201-X
63. Tobin E, Rohwedder A, Holland SM, Philips B, Carlson JA. Recurrent 'sterile' verrucous cyst abscesses and epidermodysplasia verruciformis-like eruption associated with idiopathic CD4 lymphopenia. *Br J Dermatol* (2003) 149(3):627–33. doi:10.1046/j.1365-2133.2003.05543.x
64. Laffort C, Le Deist F, Favre M, Caillat-Zucman S, Radford-Weiss I, Debre M, et al. Severe cutaneous papillomavirus disease after haemopoietic stem-cell transplantation in patients with severe combined immune deficiency caused by common gammac cytokine receptor subunit or JAK-3 deficiency. *Lancet* (2004) 363(9426):2051–4. doi:10.1016/S0140-6736(04)16457-X
65. Kamili QU, Seeborg FO, Saxena K, Nicholas SK, Banerjee PP, Angelo LS, et al. Severe cutaneous human papillomavirus infection associated with natural killer cell deficiency following stem cell transplantation for severe combined immunodeficiency. *J Allergy Clin Immunol* (2014) 134(6):1451–3.e1. doi:10.1016/j.jaci.2014.07.009
66. Sterling JC, Gibbs S, Haque Hussain SS, Mohd Mustapa MF, Handfield-Jones SE. British Association of Dermatologists' guidelines for the management of cutaneous warts 2014. *Br J Dermatol* (2014) 171(4):696–712. doi:10.1111/bjd.13310
67. Karnes JB, Usatine RP. Management of external genital warts. *Am Fam Physician* (2014) 90(5):312–8.
68. Yanofsky VR, Patel RV, Goldenberg G. Genital warts: a comprehensive review. *J Clin Aesthet Dermatol* (2012) 5(6):25–36.
69. Lopaschuk CC. New approach to managing genital warts. *Can Fam Physician* (2013) 59(7):731–6.
70. Ozarmagan G, Didem Yazganoglu K, Agacfidan A. Hyper-IgE syndrome with widespread premalign oral papillomas treated with interferon alpha2b. *Acta Derm Venereol* (2005) 85(5):433–5. doi:10.1080/00015550510030041
71. Gaspari AA, Zalka AD, Payne D, Menegus M, Bunce LA, Abboud CN, et al. Successful treatment of a generalized human papillomavirus infection with granulocyte-macrophage colony-stimulating factor and interferon gamma immunotherapy in a patient with a primary immunodeficiency and cyclic neutropenia. *Arch Dermatol* (1997) 133(4):491–6. doi:10.1001/archderm.1997.03890400091012
72. Henrickson SE, Treat JR. Topical cidofovir for recalcitrant verrucae in individuals with severe combined immunodeficiency after hematopoietic stem cell transplantation. *Pediatr Dermatol* (2017) 34(1):e24–5. doi:10.1111/pde.12992
73. Wade NA, Lepow ML, Veazey J, Meuwissen HJ. Progressive varicella in three patients with Wiskott-Aldrich syndrome: treatment with adenine arabinoside. *Pediatrics* (1985) 75(4):672–5.
74. Siegel AM, Heimall J, Freeman AF, Hsu AP, Brittain E, Brenchley JM, et al. A critical role for STAT3 transcription factor signaling in the development and maintenance of human T cell memory. *Immunity* (2011) 35(5):806–18. doi:10.1016/j.immuni.2011.09.016
75. Dorman SE, Uzel G, Roesler J, Bradley JS, Bastian J, Billman G, et al. Viral infections in interferon-gamma receptor deficiency. *J Pediatr* (1999) 135(5):640–3. doi:10.1016/S0022-3476(99)70064-8
76. Azuma H, Sakata H, Saijyou M, Okuno A. Effect of interleukin 2 on intractable herpes virus infection and chronic eczematoid dermatitis in a patient with Wiskott-Aldrich syndrome. *Eur J Pediatr* (1993) 152(12):998–1000. doi:10.1007/BF01957224
77. Orange JS, Roy-Ghanta S, Mace EM, Maru S, Rak GD, Sanborn KB, et al. IL-2 induces a WAVE2-dependent pathway for actin reorganization that enables WASp-independent human NK cell function. *J Clin Invest* (2011) 121(4):1535–48. doi:10.1172/JCI44862
78. Smith PM, Wolcott RM, Chervenak R, Jennings SR. Control of acute cutaneous herpes simplex virus infection: T cell-mediated viral clearance is dependent upon interferon-gamma (IFN-gamma). *Virology* (1994) 202(1):76–88. doi:10.1006/viro.1994.1324
79. Weiner N, Williams N, Birch G, Ramachandran C, Shipman C Jr, Flynn G. Topical delivery of liposomally encapsulated interferon evaluated in a cutaneous herpes guinea pig model. *Antimicrob Agents Chemother* (1989) 33(8):1217–21. doi:10.1128/AAC.33.8.1217
80. Shalev Y, Berrebi A, Green L, Levin S, Frumkin A, Hurwitz N, et al. Progressive cutaneous herpes simplex infection in acute myeloblastic leukemia. Successful treatment with interferon and cytarabine. *Arch Dermatol* (1984) 120(7):922–6. doi:10.1001/archderm.1984.01650430108020
81. Cardamakis E, Relakis K, Kotoulas IG, Michopoulos J, Metallinos K, Mantouvalos H, et al. Treatment of recurrent genital herpes with interferon alpha-2alpha. *Gynecol Obstet Invest* (1998) 46(1):54–7. doi:10.1159/000009998
82. Shupack J, Stiller M, Davis I, Kenny C, Jondreau L. Topical alpha-interferon ointment with dimethyl sulfoxide in the treatment of recurrent genital herpes simplex. *Dermatology* (1992) 184(1):40–4. doi:10.1159/000247497
83. Shupack J, Stiller M, Knobler E, Ackerman C, Jondreau L, Kenny C. Topical alpha-interferon in recurrent genital herpes simplex infection. A double-blind, placebo-controlled clinical trial. *Dermatologica* (1990) 181(2):134–8. doi:10.1159/000247902
84. Laxmisha C, Thappa DM, Jaisankar TJ. Clinical profile of molluscum contagiosum in children versus adults. *Dermatol Online J* (2003) 9(5):1.
85. Kilic SS, Puel A, Casanova JL. Orf infection in a patient with Stat1 gain-of-function. *J Clin Immunol* (2015) 35(1):80–3. doi:10.1007/s10875-014-0111-7
86. National guideline for the management of molluscum contagiosum. Clinical effectiveness group (Association of Genitourinary Medicine and the Medical Society for the Study of Venereal Diseases). *Sex Transm Infect* (1999) 75(Suppl 1):S80–1.
87. Nguyen HP, Franz E, Stiegel KR, Hsu S, Tyring SK. Treatment of molluscum contagiosum in adult, pediatric, and immunodeficient populations. *J Cutan Med Surg* (2014) 18(5):299–306. doi:10.2310/7750.2013.13133
88. Bohm M, Luger TA, Bonsmann G. Disseminated giant molluscum contagiosum in a patient with idiopathic CD4+ lymphocytopenia. Successful eradication with systemic interferon. *Dermatology* (2008) 217(3):196–8. doi:10.1159/000141649
89. Kilic SS, Kilicbay F. Interferon-alpha treatment of molluscum contagiosum in a patient with hyperimmunoglobulin E syndrome. *Pediatrics* (2006) 117(6):e1253–5. doi:10.1542/peds.2005-2706
90. Na CH, Kim DJ, Kim MS, Kim JK, Shin BS. Successful treatment of molluscum contagiosum with intralesional immunotherapy by measles, mumps,

and rubella vaccine: a report of two cases. *Dermatol Ther* (2014) 27(6):373–6. doi:10.1111/dth.12158
91. Perelygina L, Plotkin S, Russo P, Hautala T, Bonilla F, Ochs HD, et al. Rubella persistence in epidermal keratinocytes and granuloma M2 macrophages in patients with primary immunodeficiencies. *J Allergy Clin Immunol* (2016) 138(5):1436–9.e11. doi:10.1016/j.jaci.2016.06.030
92. Bodemer C, Sauvage V, Mahlaoui N, Cheval J, Couderc T, Leclerc-Mercier S, et al. Live rubella virus vaccine long-term persistence as an antigenic trigger of cutaneous granulomas in patients with primary immunodeficiency. *Clin Microbiol Infect* (2014) 20(10):O656–63. doi:10.1111/1469-0691.12573
93. Neven B, Perot P, Bruneau J, Pasquet M, Ramirez M, Diana JS, et al. Cutaneous and visceral chronic granulomatous disease triggered by a rubella virus vaccine strain in children with primary immunodeficiencies. *Clin Infect Dis* (2017) 64(1):83–6. doi:10.1093/cid/ciw675
94. Kelsen JR, Dawany N, Moran CJ, Petersen BS, Sarmady M, Sasson A, et al. Exome sequencing analysis reveals variants in primary immunodeficiency genes in patients with very early onset inflammatory bowel disease. *Gastroenterology* (2015) 149(6):1415–24. doi:10.1053/j.gastro.2015.07.006
95. Barmettler S, Otani IM, Minhas J, Abraham RS, Chang Y, Dorsey MJ, et al. Gastrointestinal manifestations in X-linked agammaglobulinemia. *J Clin Immunol* (2017) 37:287–94. doi:10.1007/s10875-017-0374-x
96. Uzzan M, Ko HM, Mehandru S, Cunningham-Rundles C. Gastrointestinal disorders associated with common variable immune deficiency (CVID) and chronic granulomatous disease (CGD). *Curr Gastroenterol Rep* (2016) 18(4):17. doi:10.1007/s11894-016-0491-3
97. Donaldson EF, Lindesmith LC, Lobue AD, Baric RS. Norovirus pathogenesis: mechanisms of persistence and immune evasion in human populations. *Immunol Rev* (2008) 225:190–211. doi:10.1111/j.1600-065X.2008.00680.x
98. Desselberger U, Huppertz HI. Immune responses to rotavirus infection and vaccination and associated correlates of protection. *J Infect Dis* (2011) 203(2):188–95. doi:10.1093/infdis/jiq031
99. Green KY. Norovirus infection in immunocompromised hosts. *Clin Microbiol Infect* (2014) 20(8):717–23. doi:10.1111/1469-0691.12761
100. Frange P, Touzot F, Debre M, Heritier S, Leruez-Ville M, Cros G, et al. Prevalence and clinical impact of norovirus fecal shedding in children with inherited immune deficiencies. *J Infect Dis* (2012) 206(8):1269–74. doi:10.1093/infdis/jis498
101. Henke-Gendo C, Harste G, Juergens-Saathoff B, Mattner F, Deppe H, Heim A. New real-time PCR detects prolonged norovirus excretion in highly immunosuppressed patients and children. *J Clin Microbiol* (2009) 47(9):2855–62. doi:10.1128/JCM.00448-09
102. Woodward JM, Gkrania-Klotsas E, Cordero-Ng AY, Aravinthan A, Bandoh BN, Liu H, et al. The role of chronic norovirus infection in the enteropathy associated with common variable immunodeficiency. *Am J Gastroenterol* (2015) 110(2):320–7. doi:10.1038/ajg.2014.432
103. Razvi S, Schneider L, Jonas MM, Cunningham-Rundles C. Outcome of intravenous immunoglobulin-transmitted hepatitis C virus infection in primary immunodeficiency. *Clin Immunol* (2001) 101(3):284–8. doi:10.1006/clim.2001.5132
104. Centers for Disease Control and Prevention. Outbreak of hepatitis C associated with intravenous immunoglobulin administration – United States, October 1993–June 1994. *MMWR Morb Mortal Wkly Rep* (1994) 43(28):505–9.
105. Bjoro K, Froland SS, Yun Z, Samdal HH, Haaland T. Hepatitis C infection in patients with primary hypogammaglobulinemia after treatment with contaminated immune globulin. *N Engl J Med* (1994) 331(24):1607–11. doi:10.1056/NEJM199412153312402
106. Chapel HM, Christie JM, Peach V, Chapman RW. Five-year follow-up of patients with primary antibody deficiencies following an outbreak of acute hepatitis C. *Clin Immunol* (2001) 99(3):320–4. doi:10.1006/clim.2001.5036
107. Stiehm ER. Adverse effects of human immunoglobulin therapy. *Transfus Med Rev* (2013) 27(3):171–8. doi:10.1016/j.tmrv.2013.05.004
108. Saulsbury FT, Winkelstein JA, Yolken RH. Chronic rotavirus infection in immunodeficiency. *J Pediatr* (1980) 97(1):61–5. doi:10.1016/S0022-3476(80)80131-4
109. Gilger MA, Matson DO, Conner ME, Rosenblatt HM, Finegold MJ, Estes MK. Extraintestinal rotavirus infections in children with immunodeficiency. *J Pediatr* (1992) 120(6):912–7. doi:10.1016/S0022-3476(05)81959-6
110. Bakare N, Menschik D, Tiernan R, Hua W, Martin D. Severe combined immunodeficiency (SCID) and rotavirus vaccination: reports to the vaccine adverse events reporting system (VAERS). *Vaccine* (2010) 28(40):6609–12. doi:10.1016/j.vaccine.2010.07.039
111. Patel NC, Hertel PM, Estes MK, de la Morena M, Petru AM, Noroski LM, et al. Vaccine-acquired rotavirus in infants with severe combined immunodeficiency. *N Engl J Med* (2010) 362(4):314–9. doi:10.1056/NEJMoa0904485
112. Fiore L, Plebani A, Buttinelli G, Fiore S, Donati V, Marturano J, et al. Search for poliovirus long-term excretors among patients affected by agammaglobulinemia. *Clin Immunol* (2004) 111(1):98–102. doi:10.1016/j.clim.2003.11.011
113. Galal NM, Bassiouny L, Nasr E, Abdelmeguid N. Isolation of poliovirus shedding following vaccination in children with antibody deficiency disorders. *J Infect Dev Ctries* (2012) 6(12):881–5. doi:10.3855/jidc.2372
114. Halsey NA, Pinto J, Espinosa-Rosales F, Faure-Fontenla MA, da Silva E, Khan AJ, et al. Search for poliovirus carriers among people with primary immune deficiency diseases in the United States, Mexico, Brazil, and the United Kingdom. *Bull World Health Organ* (2004) 82(1):3–8.
115. Melamed I, Griffiths AM, Roifman CM. Benefit of oral immune globulin therapy in patients with immunodeficiency and chronic diarrhea. *J Pediatr* (1991) 119(3):486–9. doi:10.1016/S0022-3476(05)82070-0
116. Losonsky GA, Johnson JP, Winkelstein JA, Yolken RH. Oral administration of human serum immunoglobulin in immunodeficient patients with viral gastroenteritis. A pharmacokinetic and functional analysis. *J Clin Invest* (1985) 76(6):2362–7. doi:10.1172/JCI112248
117. McGavern DB, Kang SS. Illuminating viral infections in the nervous system. *Nat Rev Immunol* (2011) 11(5):318–29. doi:10.1038/nri2971
118. Rantakallio P, Leskinen M, von Wendt L. Incidence and prognosis of central nervous system infections in a birth cohort of 12,000 children. *Scand J Infect Dis* (1986) 18(4):287–94. doi:10.3109/00365548609032339
119. Kelly TA, O'Lorcain P, Moran J, Garvey P, McKeown P, Connell J, et al. Underreporting of viral encephalitis and viral meningitis, Ireland, 2005–2008. *Emerg Infect Dis* (2013) 19(9):1428–36. doi:10.3201/eid1909.130201
120. Raschilas F, Wolff M, Delatour F, Chaffaut C, De Broucker T, Chevret S, et al. Outcome of and prognostic factors for herpes simplex encephalitis in adult patients: results of a multicenter study. *Clin Infect Dis* (2002) 35(3):254–60. doi:10.1086/341405
121. Zhang SY, Abel L, Casanova JL. Mendelian predisposition to herpes simplex encephalitis. *Handb Clin Neurol* (2013) 112:1091–7. doi:10.1016/B978-0-444-52910-7.00027-1
122. Lim HK, Seppanen M, Hautala T, Ciancanelli MJ, Itan Y, Lafaille FG, et al. TLR3 deficiency in herpes simplex encephalitis: high allelic heterogeneity and recurrence risk. *Neurology* (2014) 83(21):1888–97. doi:10.1212/WNL.0000000000000999
123. Abel L, Plancoulaine S, Jouanguy E, Zhang SY, Mahfoufi N, Nicolas N, et al. Age-dependent Mendelian predisposition to herpes simplex virus type 1 encephalitis in childhood. *J Pediatr* (2010) 157(4):623–9, 629.e1. doi:10.1016/j.jpeds.2010.04.020
124. Bearden D, Collett M, Quan PL, Costa-Carvalho BT, Sullivan KE. Enteroviruses in X-linked agammaglobulinemia: update on epidemiology and therapy. *J Allergy Clin Immunol Pract* (2016) 4(6):1059–65. doi:10.1016/j.jaip.2015.12.015
125. Minor PD. Comparative biochemical studies of type 3 poliovirus. *J Virol* (1980) 34(1):73–84.
126. Nkowane BM, Wassilak SG, Orenstein WA, Bart KJ, Schonberger LB, Hinman AR, et al. Vaccine-associated paralytic poliomyelitis. United States: 1973 through 1984. *JAMA* (1987) 257(10):1335–40. doi:10.1001/jama.257.10.1335
127. MacCallum FO. Hypogammaglobulinaemia in the United Kingdom. VII. The role of humoral antibodies in protection against and recovery from bacterial and virus infections in hypogammaglobulinaemia. *Spec Rep Ser Med Res Counc (G B)* (1971) 310:72–85.
128. Etsano A, Damisa E, Shuaib F, Nganda GW, Enemaku O, Usman S, et al. Environmental isolation of circulating vaccine-derived poliovirus after interruption of wild poliovirus transmission – Nigeria, 2016. *MMWR Morb Mortal Wkly Rep* (2016) 65(30):770–3. doi:10.15585/mmwr.mm6530a4
129. Kew O, Morris-Glasgow V, Landaverde M, Burns C, Shaw J, Garib Z, et al. Outbreak of poliomyelitis in Hispaniola associated with circulating type 1 vaccine-derived poliovirus. *Science* (2002) 296(5566):356–9. doi:10.1126/science.1068284

130. Jorba J, Diop OM, Iber J, Sutter RW, Wassilak SG, Burns CC. Update on vaccine-derived polioviruses – worldwide, January 2015–May 2016. *MMWR Morb Mortal Wkly Rep* (2016) 65(30):763–9. doi:10.15585/mmwr.mm6530a3
131. Guo J, Bolivar-Wagers S, Srinivas N, Holubar M, Maldonado Y. Immunodeficiency-related vaccine-derived poliovirus (iVDPV) cases: a systematic review and implications for polio eradication. *Vaccine* (2015) 33(10):1235–42. doi:10.1016/j.vaccine.2015.01.018
132. Wintergerst U, Belohradsky BH. Acyclovir monotherapy versus acyclovir plus beta-interferon in focal viral encephalitis in children. *Infection* (1992) 20(4):207–12. doi:10.1007/BF02033060

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Primary Immunodeficiency Disorders in India—A Situational Review

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Primary immunodeficiency disorders (PIDs) are a group of genetic defects characterized by abnormalities of one or more components of the immune system. While there have been several advances in diagnosis, management, and research in the field of PIDs, they continue to remain underdiagnosed, especially in the less affluent countries. Despite several limitations and challenges, India has advanced significantly in the field of PIDs in the last few years. In this review, we highlight the progress in the field of PIDs in India over the last 25 years, the difficulties faced by clinicians across the country, the current state of PIDs in India and the future prospects.

Keywords: primary immunodeficiencies, intravenous immunoglobulin, hematopoietic stem cell transplantation, India, newborn screening

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INTRODUCTION

Primary immunodeficiency disorders (PIDs) are a group of genetic defects characterized by abnormalities of one or more components of the immune system. More than 300 genetically defined single-gene inborn errors of immunity are now recognized as a cause of PID (1). The International Union of Immunological Societies Expert Committee on classification of PIDs has recently updated the phenotypic classification for PIDs (2). These diseases may affect innate immunity and/or adaptive immunity, and they result in a wide range of manifestations including, but not limited to, susceptibility to infections, autoimmunity, inflammation, allergy, and malignancy. Contrary to common perception PIDs are not uncommon and may have prevalence rates as high as 1:1,200 in the general population (3). The diagnosis of PIDs is often delayed, or even missed altogether, especially in areas where infectious diseases are common as is the case in most developing countries.

Abbreviations: ALPS, autoimmune lymphoproliferative syndrome; APSID, Asia Pacific Society for Immunodeficiencies; CAR, Centre for Advanced Research; CDC, Centers for Disease Control and Prevention; CGD, chronic granulomatous disease; CMC, Christian Medical College; CME, Continued Medical Education; CVID, common variable immunodeficiency; FPID, Foundation for Primary Immunodeficiency Diseases; HLH, hemophagocytic lymphohistiocytosis; HSCT, hematopoietic stem cell transplantation; ICMR, Indian Council of Medical Research; IPOPI, International Patient Organization for Primary Immunodeficiencies; IPSPI, Indian Patients Society for Primary Immunodeficiency; ISPID, Indian Society for Primary Immune Deficiency; IUIS, International Union of Immunological Societies; IVIg, intravenous immunoglobulin; JIPMER, Jawaharlal Institute of Postgraduate Institute of Medical Education and Research; JMF, Jeffrey Modell Foundation; KGMU, King George's Medical University; LAD, leukocyte adhesion defect; MMC, Madras Medical College; NBS, newborn screening; NIIH, National Institute of Immunohaematology; NIMS, Nizam's Institute of Medical Sciences; PGIMER, Postgraduate Institute of Medical Education and Research; PID, Primary Immunodeficiency disorder; PIDPWS, Primary Immunodeficiency Patients Welfare Society; SCID, severe combined immunodeficiency; SGPGIMS, Sanjay Gandhi Postgraduate Institute of Medical Sciences; SGRH, Sir Ganga Ram Hospital; TREC, T-cell receptor excision circle; VAPP, vaccine-associated paralytic poliomyelitis; VDPV, vaccine-derived poliovirus; WAS, Wiskott–Aldrich syndrome; XLA, X-linked agammaglobulinemia.

While there have been several advances in diagnosis, management, and research in the field of PIDs, they continue to remain underdiagnosed, especially in the less affluent countries. This is largely because of lack of awareness about these conditions both among the laity and medical professionals.

Although there are no nationwide data on prevalence of PIDs in India, based on statistical projections it is estimated that the number of patients with PID is likely to be more than one million (4, 5). Hitherto, there was also paucity of published literature about PIDs from India as very few centers in the country had the clinical experience, laboratory facilities, and technical wherewithal for diagnosis and management of these conditions. As a result, an overwhelming majority of these patients continued to remain undiagnosed and untreated.

However, despite these limitations and challenges, India has advanced significantly in the field of PIDs in the last few years. The Indian Society for Primary Immune Deficiency (ISPID) was founded in 2011 in close liaison with the Foundation for Primary Immunodeficiency Diseases (FPID), USA. The Indian Council of Medical Research (ICMR) has also been at the forefront of activities related to PIDs in India. In this review, we highlight the progress in the field of PIDs in India over the last 25 years, the difficulties faced by clinicians across the country, the current state of PIDs in India and the future prospects.

STATUS OF PIDs IN INDIA PRIOR TO 2011

The first two cases of PIDs in India were reported in the year 1964—these were two case reports of boys with Wiskott–Aldrich syndrome (WAS) and agammaglobulinemia, respectively (6, 7). Subsequently, five families with ataxia telangiectasia were reported in the year 1973 (8). Since then several cases of PIDs have been reported from Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh and many other institutions across the country; however, the diagnosis was largely clinical and the immunological workup rather limited. Prior to 1992 (between 1975 and 1991), 59 cases of PIDs had been diagnosed in the country (5). Gupta et al. published their experience of the spectrum of PIDs seen at two large pediatric centers in the country [Advanced Pediatrics Centre, PGIMER, Chandigarh and the National Institute of Immunohaematology (NIIH) and B.J. Wadia Children's Hospital, Mumbai] and highlighted the difficulties in identification, evaluation (including molecular diagnosis) and management of these patients (5). The data were collected from 1992 onward. Between 1992 and 2010, a total of 153 and 122 patients had been diagnosed to have PIDs at Chandigarh and Mumbai respectively. There were important differences in spectrum of PIDs seen at both these centers. Antibody deficiency disorders [including X-linked agammaglobulinemia (XLA), common variable immunodeficiency (CVID), selective IgA deficiency with or without IgG2 subclass deficiency, isolated IgG2 subclass deficiency, and transient hypogammaglobulinemia of infancy] were the most common PIDs diagnosed in Chandigarh; however, the most common PID diagnosed at Mumbai was familial hemophagocytic lymphohistiocytosis (HLH). Cases of disorders of immune regulation (including HLH and autoimmune lymphoproliferative syndrome) were predominantly seen at Mumbai while only a few cases had been diagnosed at Chandigarh. Similarly, phagocyte defects [including neutropenia, leukocyte adhesion defect (LAD), and disorders of IFNγ-IL12 pathway] were predominantly being diagnosed at Mumbai with only a handful of cases at Chandigarh. On the other hand, WAS, hyper-IgE syndrome, ataxia telangiectasia, hereditary angioedema, and chronic mucocutaneous candidiasis were more common at Chandigarh. There could be several reasons for the difference in spectrum of PID seen at these two centers. These could be (1) difference in genetic constitution of the population in North and West India due to differences in racial ethnicities; (2) referral bias (the Mumbai centre is primarily a hemato-oncology unit and tends to attract more cases of HLH and phagocytic defects; the Chandigarh facility on the other hand is a pediatric center and sees the entire spectrum of PIDs); and (3) difference in rate of consanguineous marriages (much higher in Western and Southern India as compared to North India). The diagnosis of PIDs at both centers was being established based on clinical manifestations supplemented by few laboratory investigations including protein expression by flow cytometry and a few functional assays. Facilities for molecular analysis were limited at that time. However, both centers had been able to get some molecular analyses done on individual patients for diagnostic purposes through collaborative efforts with overseas laboratories specializing in PIDs. Major challenges in the diagnosis and management of cases of PID in India prior to 2011 were lack of awareness among general population and physicians, lack of appropriate diagnostic facilities and cost constraints in management of patients diagnosed to have PIDs especially with regard to access to immunoglobulin replacement and hematopoietic stem cell transplantation (HSCT).

The FPID[1]

The FPID was co-founded by Dr. Sudhir Gupta and Dr. Abha Gupta in the USA. This foundation has the avowed aim of supporting education, early diagnosis, genetic counseling, therapy, and research in PID in both India and the USA. The FPID is spearheading efforts at establishing PID centers in India and is currently supporting activities at five institutions in the country, i.e., at PGIMER, Chandigarh; Sir Ganga Ram Hospital (SGRH), New Delhi; Sanjay Gandhi Postgraduate Institute of Medical Sciences (SGPGIMS), Lucknow; Nizam's Institute of Medical Sciences (NIMS), Hyderabad; and NIIH, Mumbai (Figure S1 in Supplementary Material). It is also supporting treatment of some patients with hypogammaglobulinemia in India by provision of intravenous immunoglobulin (IVIg) replacement and partial support toward HSCT.

Indian Society for Primary Immune Deficiency[2]

The ISPID was registered in March 2011. Very few developing countries have a dedicated society for PID—India is one of them. ISPID has been working toward increasing awareness regarding

[1] www.fpid.org.
[2] www.ispid.org.in.

PIDs and establishment of diagnostic support and research centers in the country. ISPID organizes a national and an international conference on PID every alternate year and a Continuing Medical Education program every year with the support of FPID, USA. Till date four such national and international conferences have been organized by the ISPID in India. The faculty at these conferences has consisted of world renowned scientists at the cutting edge of research in PID.

Indian Patients Society for Primary Immunodeficiency (IPSPI)[3]

Indian Patients Society for Primary Immunodeficiency is a national non-profit organization for PIDs, which was established in 2004 and registered in 2005. It was the first patient society for PIDs in our country. It is based at Bhubaneshwar in Eastern India. IPSPI is affiliated with the International Patient Organization for Primary Immunodeficiencies. This organization works to improve the quality of life of individuals with PIDs in India.

Primary Immunodeficiency Patients Welfare Society (PIDPWS)[4]

The second patient care society for PID in India was founded in April 2012 and registered in September 2012. Like the IPSPI, this society has also been established by parents of the children suffering from PIDs. PIDPWS is based at Bengaluru in South India and has been working for the improvement in quality of life of PID patients.

STATUS OF PIDs IN INDIA AFTER 2011

Since the inception of ISPID in 2011, there has been a significant progress in the spread of awareness and setting up of diagnostic facilities for PIDs. The PGIMER, Chandigarh initiated a 3 years postdoctoral (DM) training course in Pediatric Clinical Immunology and Rheumatology in January 2014. This course is the first of its kind in India. The basic aim of this program is to train pediatric fellows and to provide them enough expertise in the field of immunology that can be disseminated across the country. Two fellows have already completed their training and are now working in Guwahati (East India) and Bengaluru (South India), respectively. In addition, six more trainees are currently pursuing this course at the institute. The institute has also started a 3 years postdoctoral (DM) training course for adult physicians in July 2014. Though several other institutes in the country have been running similar postdoctoral (DM) courses in Clinical Immunology (e.g., SGPGIMS, Lucknow and Jawaharlal Institute of Postgraduate Institute of Medical Education and Research, Puducherry) and in Rheumatology (e.g., Madras Medical College, Chennai; NIMS, Hyderabad; Christian Medical College, Vellore; and King George's Medical University, Lucknow). However, the major focus of these institutes has been clinical rheumatology rather than PIDs.

[3] www.ipspiindia.org.

[4] www.pidindia.net.

CENTRE FOR ADVANCED RESEARCH (CAR) FOR PIDs IN INDIA

Clearly, PID care in India is still in its infancy, and we have a long way to go. There are very few centers in the country with clinical expertise and diagnostic facilities for PIDs. As a result, majority of patients with PIDs continue to remain undiagnosed. The ICMR helped set up the CAR in primary immunodeficiency disorders at PGIMER, Chandigarh in January 2015. The second such ICMR CAR in PID has recently been set up at the NIIH, Mumbai. The major objectives for these CARs are training of physicians/scientists, development and standardization of diagnostic facilities, establishment of normograms, and creation of state of the art facilities for diagnosis and management of PIDs.

Asia Pacific Society for Immunodeficiencies (APSID)[5]

Asia Pacific Society for Immunodeficiencies was established in April 2015 by a group of Asian pediatricians and immunologists working in the field of PIDs. The aim of this society is to improve management of PIDs through increasing awareness and promoting collaboration among member countries. The APSID has been organizing Spring Schools for young clinicians who are in training for immunology and infectious diseases. The first conference of the APSID was held in 2016 in Hong Kong from 30th April to 1st May 2016. The APSID would provide a much needed fillip to PID-related activities across countries in the large Asia Pacific region which is home to ~60% of the world population.

PUBLICATIONS ON PID FROM INDIA—A MAJOR THRUST IN LAST 6 YEARS

There has been a significant increase in the number and quality of publications on PID from India in the last 6 years. Publications prior to 2011 were mostly case reports and clinical reviews; however, the more recent ones contain a plethora of molecular and genetic details. This is a welcome change.

Search Strategy

The following search terms were used to find out the number of PubMed publications in PID in the last 50 years in India: India and Primary immune deficiency; Wiskott–Aldrich syndrome and India; Severe combined immunodeficiency and India; X-linked agammaglobulinemia and India; Chronic granulomatous disease and India; Neutropenia and India; Leucocyte adhesion defect and India; Hyper IgE syndrome and India; Hyper-IgM syndrome and India; Chédiak–Higashi syndrome and India; Hemophagocytic lymphohistiocytosis and India; DOCK-8 deficiency and India; Papillon–Lefevre syndrome and India; Ataxia telangiectasia and India; Mendelian susceptibility to mycobacterial diseases and India; Chronic mucocutaneous candidiasis and India; HSCT and Primary Immune deficiency and India; Intravenous immunoglobulin and India and Primary immunodeficiency.

[5] www.paed.hku.hk.

Based on this search strategy, we were able to access 311 publications on PID from India (**Figure 1**).

IMMUNOGLOBULIN REPLACEMENT THERAPY IN INDIA

Immunoglobulin replacement therapy is the cornerstone of treatment for antibody deficiency diseases, arguably the most common clinically significant group of PIDs. Immunoglobulin can be administered by both intravenous and subcutaneous routes; however, subcutaneous immunoglobulin preparations are presently not being marketed in India. Cost of IVIg therapy is a major constraint in the management of children with PIDs in India, as also in other developing countries. However, with recent policy changes initiated by the Federal Government there has been a significant price reduction in IVIg formulations in India. A 5 g vial of IVIg presently costs around 120–140 USD. With the help of philanthropist organizations, FPID and several state governments, IVIg therapy is now being offered to many patients with hypogammaglobulinemia in India. The state government of Punjab (in North India) has taken a major step in this context and is freely providing IVIg to all patients with hypogammaglobulinemia. The state government of Karnataka (in South India) has also taken a major policy initiative and has agreed, in principle, to provide IVIg to patients with PIDs. Because of cost constraints, several of our patients with hypogammaglobulinemia are unable to get the recommended dose of IVIg. Suri et al. recently analyzed the trough levels of IgG in a cohort of 14 boys with XLA at a single center in Chandigarh and correlated the levels with the monthly replacement dose of IVIg and risk of infections (9). This study revealed that the mean dose of IVIg was 414 mg/kg/month and the mean trough IgG level of the entire cohort was 435 mg/dl. It was further observed that a median dose of IVIg of 397 mg/kg and a median trough IgG level of 354 mg/dl was protective for majority of the children. These cutoff levels are much lower that what has hitherto been considered the norm in patients with hypogammaglobulinemia. However, these findings need to be corroborated by clinical results on long-term follow-up.

HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR PIDs

Management of children with PIDs requires a multidisciplinary approach and HSCT plays a major role in improving the outcome of patients with PID. HSCT provides a definitive cure for several primary immune deficiencies including severe combined immunodeficiency (SCID), WAS, chronic granulomatous disease (CGD), HLH, and many other immunodeficiency disorders (10). There are little published data regarding the outcome of HSCT for PID from India (11). The major hurdles so far have been the lack of awareness about diagnosis and availability of expertise for carrying out this procedure in the setting of PID. Even though many centers in India are now routinely performing HSCT for a variety of malignant diseases in both children and adults, there is only a minority

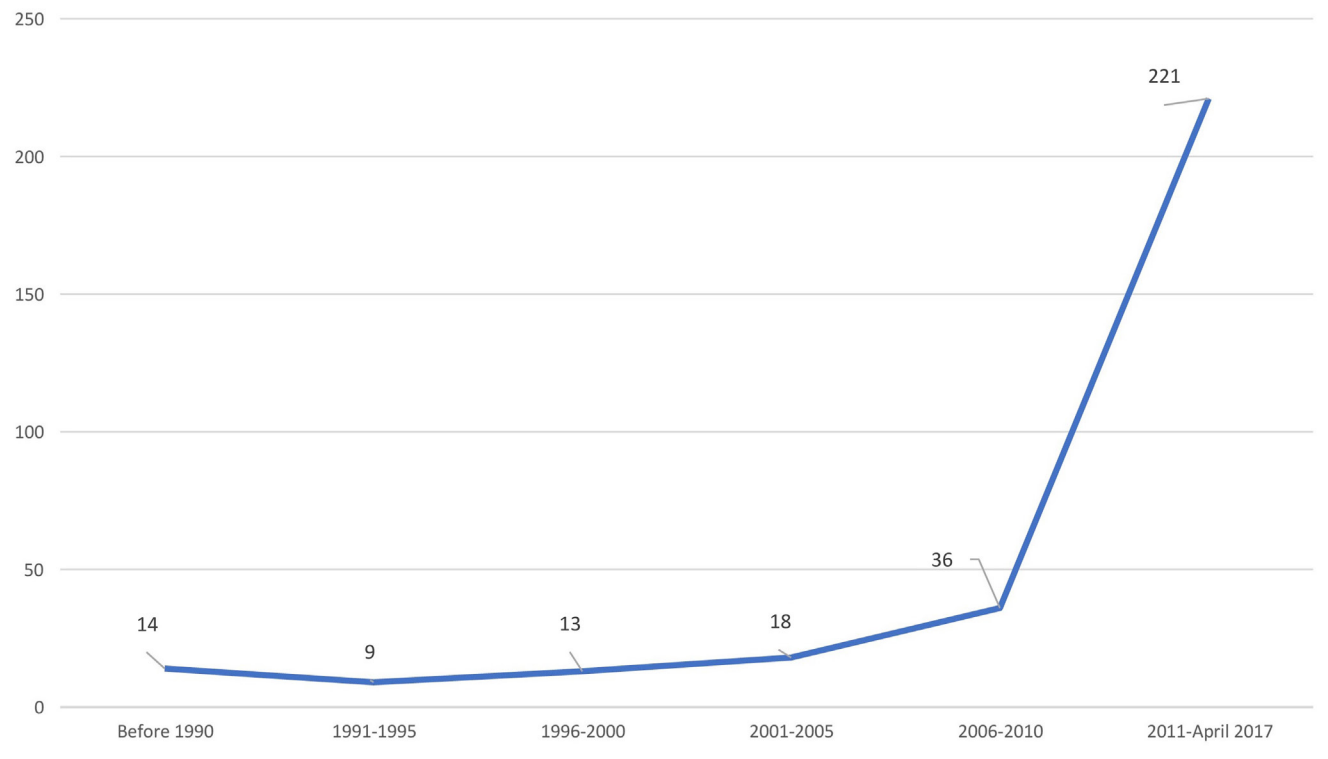

FIGURE 1 | Publications on PIDs from India.

that have the requisite expertise for carrying out transplant for PIDs (Figure S1 in Supplementary Material). Kapoor and Raj have recently published the data on HSCT in PID from India (11). Approximately 100 transplants have so far been carried out in 10 centers across the country. The indications included SCID, HLH and WAS.

VACCINES IN PID

Children with PIDs may not mount an immune response to a given vaccine (e.g., response to pneumococcal vaccine in children with XLA), and these vaccines may not be indicated (12). On the other hand, a live vaccine may be deleterious as once a live bacterium or virus from a vaccine strain is administered to these patients, it is very difficult to eradicate this organism from the body of an immunocompromised child (12). Oral live polio vaccine is still being used in India as a part of Polio Eradication and Endgame Strategic Plan and majority of children receive the first dose at birth. Subsequently, about three to four doses are given in the first year of life either as part of routine immunization or pulse polio immunization program (which is conducted on two designated days per year, and all children below 5 years are given a dose of oral polio on these days). Similarly, all babies born in India receive BCG vaccination at birth. This contrasts with the policy in some of the developed countries where BCG is either not administered at all or, if administered, it is deferred until results of T-cell receptor excision circle (TREC) assay are available. Similarly, OPV has been discontinued in most of the developed countries. Thus, babies born in India with a significant cell mediated immune defect are at risk of developing disseminated BCG infection as well as vaccine-associated paralytic poliomyelitis (VAPP) (4). Recently, a case was reported from India in whom VAPP caused by vaccine-derived poliovirus (VDPV) (strain type 2) was the first presenting manifestation of CVID (13). The recent update on VDPV from India (January 2015–May 2016) revealed three cases of VDPV (one each in XLA, CVID, and SCID) (14). The Jeffrey Modell Foundation in collaboration with Centers for Disease Control and Prevention, WHO—Global Polio Laboratory Network and The Task Force for Global Health recently conducted a surveillance to determine the prevalence of poliovirus excretion in immunodeficient children across 13 different countries (15). Twenty-three children with XLA from Chandigarh, India, were recruited in this multicenter study, and stool samples were cultured for detection of poliovirus excretion. While none of the patients from Chandigarh were excreting poliovirus, 13 patients from Iran, Tunisia, Turkey, and the Russian Federation were found to be doing so. These findings have implications for health planners and vaccinologists all over the world.

NEWBORN SCREENING (NBS) IN PID

Early diagnosis of PIDs is of paramount importance before these children acquire a significant infection (16). HSCT is a curative treatment for several PIDs and the best outcome can be achieved if the transplant is performed before these children acquire an infection (17). SCID is usually not apparent at birth and these children can only be detected by NBS before they get any major illness. NBS for SCID is usually done using TREC assay, which is a measure of thymic output of T cells and is a sensitive assay (18, 19). NBS for SCID is now being routinely carried in all states of USA and many other developed countries. However, the cost of TREC assay is a major constraint for its inclusion in the NBS program in India. In the current scenario, where routine NBS is not available even for more common disorders such as hypothyroidism, NBS for SCID in the near future appears far-fetched. Early screening for SCID will also prevent administration of BCG and other live vaccines to these patients. The FPID is actively considering pilot studies on NBS for SCID in India.

NON-INFECTIOUS COMPLICATIONS IN PID

Patients with PID are also predisposed to develop autoimmune, inflammatory, and allergic complications. While children with CGD have significant inflammatory disorders (20), autoimmune complications are characteristic of CVID and WAS. PIDs can also be associated with several malignancies. We recently reported a case of WAS with Hodgkin lymphoma from Chandigarh (21). A case of diffuse large B-cell lymphoma was reported in another child with WAS from Vellore in 2014 (22) and a case of multifocal extranodal non-Hodgkin lymphoma in a child with combined immunodeficiency and Epstein–Barr virus infection (23). We have also seen leiomyoma of liver in a boy with ataxia telangiectasia (unpublished data).

STATUS AND PROSPECTS OF PID INDIA

The spectrum of PIDs diagnosed at a single center in India (PGIMER, Chandigarh) is shown in **Figure 2**. It is apparent that this spectrum is perhaps no different from that in any other developed country. In addition, PGIMER and many other institutions in India have now developed the facility for genetic analysis for common immunodeficiency diseases. At Chandigarh, facilities for genetic testing for WAS, CGD, XLA, HLH (PRF-1), and X-linked hyper-IgM syndrome are in place. Similarly, genetic testing for HLH, LAD, and several other PIDs is available at NIIH, Mumbai. In addition, a prenatal screening program is being offered to patients with PIDs at PGIMER, Chandigarh as also at NIIH, Mumbai and SGRH, New Delhi (Figure S1 in Supplementary Material). This has been a major advance in the field of PIDs in India. An effort is being made to establish a PID registry for the country; however, it has not been established till date.

ETHNIC CONSTITUTION AND CONSANGUINITY IN INDIA

India has large variation in ethnicity across various geographic regions with six different major religions being followed in the country (24). There is a huge burden of consanguineous and endogamous marriages in India (25, 26). The figure varies from 1% in some states to as high as 38% in others. The rate of

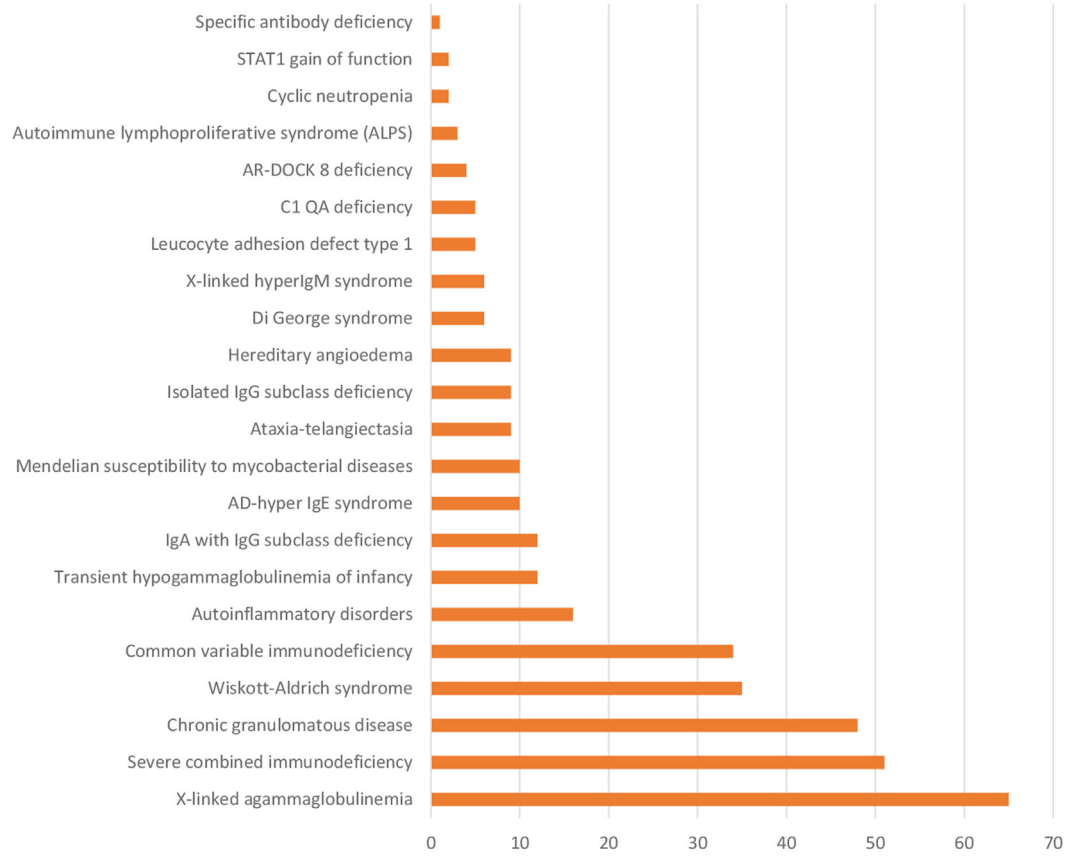

FIGURE 2 | Number of patients with various PIDs at Postgraduate Institute of Medical Education and Research, Chandigarh (January 1990–March 2017).

consanguineous marriages is much higher in southern parts of India (e.g., 38% in Tamil Nadu, 29% in Andhra Pradesh, and 28% in Karnataka) as compared to northern states (27). Several autosomal recessive disorders (as for instance thalassemia) are more commonly reported from regions in India that have increased rate of consanguineous and endogamous marriages, data on PIDs are scare. Our own data from PGIMER, Chandigarh have shown that proportion of autosomal recessive CGD is higher than the X-linked CGD and this is likely due to the high incidence of endogamous and consanguineous marriages in this part of the world (28). However, the increased likelihood of finding an HLA-matched donor in immediate and extended families appears to be the "silver lining" amidst the baneful effects of consanguinity and endogamy (29, 30).

In conclusion, the outcome of patients with PIDs in India appears to be brighter with an increased awareness of these disorders among physicians and specialists. Availability of diagnostic facilities including candidate gene sequencing and prenatal diagnosis for common PIDs in few centers is also a big step forward from the last decade. HSCT is also being performed at more centers now. Although progress has been made during the last few years, we still have a long arduous journey ahead to provide PID patients in India the standard of care that they rightfully deserve.

AUTHOR CONTRIBUTIONS

AJ and RP—initial draft of manuscript, editing of manuscript, revision of manuscript, and data collection. AR and SS—editing and critical revision of manuscript, data collection, and final approval of manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at http://journal.frontiersin.org/article/10.3389/fimmu.2017.00714/full#supplementary-material.

FIGURE S1 | Geographical map of India depicting PID centers across the country with different levels of care and facilities.

REFERENCES

1. Bousfiha A, Jeddane L, Al-Herz W, Ailal F, Casanova J-L, Chatila T, et al. The 2015 IUIS phenotypic classification for primary immunodeficiencies. *J Clin Immunol* (2015) 35(8):727–38. doi:10.1007/s10875-015-0198-5
2. Picard C, Al-Herz W, Bousfiha A, Casanova J-L, Chatila T, Conley ME, et al. Primary immunodeficiency diseases: an update on the classification from the International Union of Immunological Societies Expert Committee for primary immunodeficiency 2015. *J Clin Immunol* (2015) 35(8):696–726. doi:10.1007/s10875-015-0201-1
3. Boyle JM, Buckley RH. Population prevalence of diagnosed primary immunodeficiency diseases in the United States. *J Clin Immunol* (2007) 27(5):497–502. doi:10.1007/s10875-007-9103-1
4. Madkaikar M, Aluri J, Gupta S. Guidelines for screening, early diagnosis and management of severe combined immunodeficiency (SCID) in India. *Indian J Pediatr* (2016) 83(5):455–62. doi:10.1007/s12098-016-2059-5
5. Gupta S, Madkaikar M, Singh S, Sehgal S. Primary immunodeficiencies in India: a perspective. *Ann N Y Acad Sci* (2012) 1250:73–9. doi:10.1111/j.1749-6632.2011.06353.x
6. Gupta MC, Agarwal VK, Mittal AK, Rajvanshi VS. Wiskott-Aldrich syndrome. A case report. *J Assoc Physicians India* (1964) 12:531–3.
7. Mehta SR, Sarin LR, Sanghvi LM. Agammaglobulinaemia. *J Indian Med Assoc* (1964) 1(42):539–41.
8. Malaviya AN, Sachdeva KK, Singh N. Ataxia telangiectasia: immunological abnormalities in probands and first degree relatives in 5 families. *J Assoc Physicians India* (1973) 21(8):701–5.
9. Suri D, Bhattad S, Sharma A, Gupta A, Rawat A, Sehgal S, et al. Serial serum immunoglobulin G (IgG) trough levels in patients with X-linked agammaglobulinemia on replacement therapy with intravenous immunoglobulin: its correlation with infections in Indian children. *J Clin Immunol* (2017) 37(3):311–8. doi:10.1007/s10875-017-0379-5
10. Hagin D, Burroughs L, Torgerson TR. Hematopoietic stem cell transplant for immune deficiency and immune dysregulation disorders. *Immunol Allergy Clin North Am* (2015) 35(4):695–711. doi:10.1016/j.iac.2015.07.010
11. Kapoor N, Raj R. Hematopoietic stem cell transplantation for primary immune deficiency disorders. *Indian J Pediatr* (2016) 83(5):450–4. doi:10.1007/s12098-015-2012-z
12. Sobh A, Bonilla FA. Vaccination in primary immunodeficiency disorders. *J Allergy Clin Immunol Pract* (2016) 4(6):1066–75. doi:10.1016/j.jaip.2016.09.012
13. Gomber S, Arora V, Dewan P. Vaccine associated paralytic poliomyelitis unmasking common variable immunodeficiency. *Indian Pediatr* (2017) 54(3):241–2. doi:10.1007/s13312-017-1038-x
14. Jorba J, Diop OM, Iber J, Sutter RW, Wassilak SG, Burns CC. Update on vaccine-derived polioviruses—worldwide, January 2015–May 2016. *MMWR Morb Mortal Wkly Rep* (2016) 65(30):763–9. doi:10.15585/mmwr.mm6530a3
15. Barbouche MR, Wassilak SG, Pallansch SA, Kluglein S, Quinn J, Sutter RW, et al. Patients with primary immunodeficiencies are reservoir for neurovirulent vaccine derived polio virus strain and represent a risk to polio virus eradication initiative. *Poster Presented at: 17th Biennial Meeting of the European Society for Immunodeficiencies*; Sep 21; Barcelona, Spain (2016).
16. Kwan A, Abraham RS, Currier R, Brower A, Andruszewski K, Abbott JK, et al. Newborn screening for severe combined immunodeficiency in 11 screening programs in the United States. *JAMA* (2014) 312(7):729–38. doi:10.1001/jama.2014.9132
17. Pai S-Y, Logan BR, Griffith LM, Buckley RH, Parrott RE, Dvorak CC, et al. Transplantation outcomes for severe combined immunodeficiency, 2000–2009. *N Engl J Med* (2014) 371(5):434–46. doi:10.1056/NEJMoa1401177
18. de Felipe B, Olbrich P, Lucenas JM, Delgado-Pecellin C, Pavon-Delgado A, Marquez J, et al. Prospective neonatal screening for severe T- and B-lymphocyte deficiencies in Seville. *Pediatr Allergy Immunol* (2016) 27(1):70–7. doi:10.1111/pai.12501
19. Kwan A, Puck JM. History and current status of newborn screening for severe combined immunodeficiency. *Semin Perinatol* (2015) 39(3):194–205. doi:10.1053/j.semperi.2015.03.004
20. Jindal AK, Rawat A, Suri D, Sharma M, Shandilya JK, Goel S, et al. Severe *Aspergillus* pneumonia and pulmonary artery hypertension in a child with autosomal recessive chronic granulomatous disease and selective IgA deficiency. *J Clin Immunol* (2017) 37(4):333–5. doi:10.1007/s10875-017-0386-6
21. Vignesh P, Suri D, Rawat A, Lau YL, Bhatia A, Das A, et al. Sclerosing cholangitis and intracranial lymphoma in a child with classical Wiskott-Aldrich syndrome. *Pediatr Blood Cancer* (2017) 64(1):106–9. doi:10.1002/pbc.26196
22. Senapati J, Devasia AJ, David S, Manipadam MT, Nair S, Jayandharan GR, et al. Diffuse large B cell lymphoma in Wiskott-Aldrich syndrome: a case report and review of literature. *Indian J Hematol Blood Transfus* (2014) 30(Suppl 1):309–13. doi:10.1007/s12288-014-0377-1
23. Bal A, Gupta A, Sodhi KS, Das A, Singh S. Multifocal extranodal non-Hodgkin lymphoma involving both the lungs and brain in a child with primary immunodeficiency. *J Pediatr Hematol Oncol* (2008) 30(4):317–9. doi:10.1097/MPH.0b013e318161aa3b
24. Bittles AH. The impact of consanguinity on the Indian population. *Indian J Hum Genet* (2002) 8(2):45–51.
25. Pemberton TJ, Li F-Y, Hanson EK, Mehta NU, Choi S, Ballantyne J, et al. Impact of restricted marital practices on genetic variation in an endogamous Gujarati group. *Am J Phys Anthropol* (2012) 149(1):92–103. doi:10.1002/ajpa.22101
26. Kumaramanickavel G, Joseph B, Vidhya A, Arokiasamy T, Shridhara Shetty N. Consanguinity and ocular genetic diseases in South India: analysis of a five-year study. *Community Genet* (2002) 5(3):182–5. doi:10.1159/000066334
27. Kuntla S, Goli S, Shekar TV, Doshi R. Consanguineous marriages and their effect on pregnancy outcomes in India. *Presented at: XXVII IUSSP International Population Conference*; Aug 26–31; Busan, South Korea (2013).
28. Rawat A, Vignesh P, Sharma A, Shandilya JK, Sharma M, Suri D, et al. Infection profile in chronic granulomatous disease: a 23-year experience from a tertiary care center in North India. *J Clin Immunol* (2017) 37(3):319–28. doi:10.1007/s10875-017-0382-x
29. Elbjeirami WM, Abdel-Rahman F, Hussein AA. Probability of finding an HLA-matched donor in immediate and extended families: the Jordanian experience. *Biol Blood Marrow Transplant* (2013) 19(2):221–6. doi:10.1016/j.bbmt.2012.09.009
30. Hajeer AH, Algattan M, Anizi A, Alaskar AS, Jarrar MS. Chances of finding a matched parent-child in hematopoietic stem cell transplantation in Saudi Arabia. *Am J Blood Res* (2012) 2(3):201–2.

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Primary Immunodeficiency Diseases in Highly Consanguineous Populations from Middle East and North Africa: Epidemiology, Diagnosis, and Care

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Middle East and North Africa region (MENA)[1] populations are of different ethnic origins. Consanguineous marriages are common practice with an overall incidence ranging between 20 and 50%. Primary immunodeficiency diseases (PIDs) are a group of heterogeneous genetic disorders caused by defects in the immune system that predisposes patients to recurrent infections, autoimmune diseases, and malignancies. PIDs are more common in areas with high rates of consanguineous marriage since most have an autosomal recessive mode of inheritance. Studies of PIDs in the region had contributed into the discovery and the understanding of several novel immunodeficiency disorders. Few MENA countries have established national registries that helped in estimating the prevalence and defining common PID phenotypes. Available reports from those registries suggest a predominance of combined immunodeficiency disorders in comparison to antibody deficiencies seen in other populations. Access to a comprehensive clinical immunology management services is limited in most MENA countries. Few countries had established advanced clinical immunology service, capable to provide extensive genetic testing and stem cell transplantation for various immunodeficiency disorders. Newborn screening for PIDs is an essential need in this population considering the high incidence of illness and can be implemented and incorporated into existing newborn screening programs in some MENA countries. Increased awareness, subspecialty training in clinical immunology, and establishing collaborating research centers are necessary to improve patient care. In this review, we highlight some of the available epidemiological data, challenges in establishing diagnosis, and available therapy for PID patients in the region.

Keywords: immunodeficiency, Middle East, North Africa, consanguinity, primary immunodeficiency, SCID, hematopoietic stem cell transplantation

[1] MENA countries include the following: Saudi Arabia, Kuwait, Bahrain, Qatar, United Arab Emirates, Oman, Yemen, Jordan, Iraq, Israel, Palestine, Syria, Lebanon, Iran, Turkey, Egypt, Sudan, Tunisia, Libya, Algeria, Morocco, and Mauritania.

INTRODUCTION

Primary immunodeficiency diseases (PIDs) are a group of inherited heterogeneous disorders caused by monogenetic immune defects that predispose patients to infections (1). In addition, PID patients have non-infectious manifestation related to disturbed immune regulation that might cause lymphoproliferative and/or autoimmune manifestations (2). In 1952, Bruton described the first case of agammaglobulinemia (3). Since then, over 300 forms of PIDs have been described and characterized. The International Union of Immunological Societies PIDs Classification Committee (4) classified PID in to immunodeficiencies affecting cellular and humoral immunity, combined immunodeficiencies with associated or syndromic features, predominantly antibody deficiencies, disease of immune dysregulation, congenital defects of phagocyte number, function, or both, defects in intrinsic and innate immunity, autoinflammatory disorders, complement deficiencies, or phenocopies of PID. PIDs are considered to be rare disorders. Worldwide databases have shown geographical and racial variation in the epidemiology of PIDs. Published data from highly consanguineous population's like the Middle East/Northern Africa (MENA) region showed that PIDs are not uncommon. A consanguineous marriage is usually defined in clinical genetic as a marriage between two couples who are second cousins or closer (5). Consanguineous marriages are common practice in MENA region with an overall incidence ranging between 20 and 50% (6) [Bittles A. H. and Black M. L. (2015) Global Patterns & Tables of Consanguinity http://consang.net]. This has provided a background where autosomal recessive (AR) diseases are abundant. For example, there are 955 genetic diseases that have been identified in Arabs from the MENA region, of which 586 (60%) are reported to be recessive diseases (7). In addition to high rates of consanguinity, the large family size and the rapid population growth all are factors responsible for the high prevalence of rare genetic diseases in the MENA region (8). Here, we present a review of PIDs status in a highly consanguineous population from the MENA region with particular emphasis on epidemiology, diagnosis, and care.

MENA DEFINITION, POPULATION, AND ETHNICITY

The MENA region covers a surface area of nearly 15 million square kilometers from Morocco in the west to Iran in the east. The MENA region includes 22 countries and territories and accounts for 385 million people representing 6% of the world's population (9). The MENA region has an annual population growth rate of 1.8% compared to a 1.2% average global population growth rate (United Nations, Department of Economic and Social Affairs-Population Division, Population Estimates and Projections Section-World Population Prospects, 2015 Revision). The pediatric age group (0–14 years) represents 31.1% of the total population in the MENA region in comparison to 26.1% globally. The MENA population has a mix of Asian, Caucasian, Arab, and African racial ancestries. MENA region captures pan-ethnic geographically defined groups that include Arab, Persian, Turkish, Kurdish, Berber, Amazigh, Assyrian, Chaldean, Armenian and others.

CONSANGUINITY

20% of world populations live in countries with a preference for consanguineous marriages (6). Among these, are the MENA region countries where consanguineous marriage is a normal practice for multiple sociocultural factors (10–15). The global consanguinity rate is 1–9% while it is 20–56% in the MENA region (4). PID population from the MENA region display a higher rate of consanguinity compared to their general population. In addition, AR inheritance is predominant. The T-B-NK+ SCID represents the most common SCID phenotype in 90, 87, and 50% of Saudi Arabia, Kuwait, and Egypt SCID patients, respectively. Family history suggestive of PID is common among patients in MENA region as captured in several registries at a rate of 30, 44, 48, 61, and 80% in Tunisia, Oman, Iran, Saudi Arabia, and Egypt, respectively (16–24). Moreover, a significant number of these AR PIDs were first described in patients living in the MENA region (25).

EPIDEMIOLOGICAL DATA AND REGISTRIES

Ten countries from the MENA region have published epidemiological data in medical literature. These data result from a national registry or survey as those from Morocco (21), Tunisia (20), Israel (26), Kuwait (17), and Iran (22), or from a major referral centers in; Egypt (19), Turkey (23), Saudi Arabia (16), Qatar (24), and Oman (18). A total number of 4,918 patients were included. The male to female ratio ranged (1.1 to 2). The overall prevalence of PID was ranging from 0.81 to 30.5 in 100,000 populations (**Table 1**). In most registries more than 80% of patients were diagnosed at pediatric age group. The mean age at symptoms onset was within the first 2 years of life, except in Turkey, where it was at 4 years of age. That is probably explained by the fact that 70% of the patients of the Turkish registry are from a subgroup of predominantly antibody deficiencies while combined immunodeficiencies are commoner among other registries. Family history of PID was documented in seven registries and ranged from 17.2 to 61% (**Table 1**). Eight main PID categories based on IUIS classification were captured in various MENA registries as shown in **Figure 1A**. The disease distribution was variable among registries from the different MENA countries, where combined immunodeficiencies were the predominant category among patients registered in Saudi Arabia, Kuwait, Iran, Egypt, Israel, Tunisia, and Morocco. Predominantly antibodies deficiency was noticeably the commonest PID type among patients registered in Turkey. In addition, other PID categories were variably distributed in the registries, as, for example, congenital defect of phagocyte had a higher percentage of registered patients in both Oman and Tunisia while autoinflammatory disorder was higher in Turkey and Egypt in comparison to the other countries (**Figure 1B**).

TABLE 1 | Patient's characteristics from MENA region primary immunodeficiency disease registries.

Country (reference)	Source of cases (no. of sources)	Period (number of years)	Number of patients	Prevalence/ 100,000	Gender ratio male: female	Pediatrics age at diagnosis (%)	Mean age in months at onset of symptoms	Mean age in months at diagnosis	Family history (%)
Morocco (21)	National	1998–2012 (15)	421	0.81	1.17	94.1	16.8	74.5	19.1
Tunisia (20)	National	1988–2012 (25)	710	4.3	1.4	N/A	6 (median)	24 (median)	30
Egypt (19)	Center (1)	2010–2014 (5)	476	N/A	1.2	83	13	39	80
Israel (26)	National	1994–2000 (6)	294	4.9	2	82	N/A	N/A	N/A
Turkey (23)	Center (2)	2004–2010 (6)	1,435	30.5	1.6	94	49	62	N/A
Iran (22)	National	2006–2013 (7)	731	Incidence (9.7/1 million/year)	1.6	89	12 (median)	42 (median)	17.2
Kuwait (17)	National	2004–2006 (2)	76	11.9	1.6	98	15	43	43
Saudi Arabia (16)	Center (1)	2010–2013	504	7.2	1.1	93	17	39	61
Qatar (24)	Center (1)	1998–2012 (15)	131	4.7	1.3	N/A	24	42	N/A
Oman (18)	Center (1)	2005–2015 (10)	140	7	1.6	N/A	23	46	44

DIAGNOSTIC FACILITIES

The extreme clinical, phenotypic, and genetic heterogeneity of many patients with PIDs represents significant diagnostic challenges to physicians in the MENA region. The capability to perform extensive immunologic investigations and mutation analysis is variable from one country to the other. In Saudi Arabia, an advanced clinical diagnostic immunology service at King Faisal Specialist Hospital and Research Centre (KFSHRC) had been established in the last 30 years that allowed access to diagnose most of the known PIDs. Extensive genetic testing that includes Sanger sequencing, Targeted next-generation sequencing PID gene panel, and whole exome sequencing is offered to affected patients (16, 27, 28). Sultan Qaboos University Hospital, the Royal Hospital, and Hamad General Hospital are the main PID centers in Oman and Qatar, respectively. AlSabah, Mubarak Al-Kabeer, and AlRahsid hospitals have PID centers in Kuwait. Genetic testing is performed through international diagnostic laboratories or through collaborative research facilities. The first immunology center in Turkey was established in 1972 at Hacettepe University in Ankara. There are now more than 10 immunology centers in different cities around Turkey with facilities to diagnose common PIDs (29). Departments of Pediatric Immunology at Uludag University Medical Faculty and Ege University Medical Faculty are the major centers reporting PID patients to European Society for Immunodeficiencies database from Turkey (23). Targeted next-generation sequencing PID genes panel and whole exome sequencing are offered to patients in Turkey. In Iran, there is at least one Medical University and immunology center at each of the 30 states of the country that could manage PIDs. National PID network is active in Iran, which allowed referring patients to the Research Center for Immunodeficiencies at Tehran University of Medical Sciences for further immunological and genetic testing (22). Queen Rania Children Hospital is the major pediatric immunology center in Jordan. In Lebanon, PID care is provided primarily by Pediatric infectious disease service at the American University of Beirut Medical center and few other medical centers. Several medical centers provide care and have diagnostic facilities to diagnose various types of PIDs in Israel with access to Sanger and whole exome sequencing (30). The Pasteur Institute of Tunisia is the main diagnostic facility in Tunisia with the ability to perform genetic testing for common PIDs. Ain Shams University center and Cairo University center are the main PID referral centers in Egypt; both are capable to diagnose major PID types while genetic testing is done at research diagnostic facilities in USA. In Algeria, there are five medical centers capable to diagnose and manage major types of PIDs. All are located in northern part of the country. Genetic testing for agammaglobulinemia, LAD, and MHC II deficiency is available and collaborates with research centers in France and USA to diagnose atypical PIDs. In other MENA countries, access is often limited because of either financial or personnel limitations.

PID CARE AND MANAGEMENT

Specialist clinical immunology services and hematopoietic stem cell transplantation (HSCT) facilities emerged in a few countries driven by the health demands of the population. In Saudi Arabia, an HSCT program for PID had been established in 1984 at KFSHRC in Riyadh. More than 500 PID cases had been transplanted with an average of 35–40 transplants per year (16, 31–33). 70% of HSCTs are from HLA matched related donors whereas the remaining donor sources were either unrelated umbilical cord blood or haploidentical bone marrow. Another two centers recently started performing HSCT for PID at National Guard Hospital, Riyadh and KFSHRC, Jeddah. There are five centers performing HSCT for PID in Turkey, in Ankara (Hacettepe and Ankara University.), in Izmir (Ege University), in Antalya and in Istanbul (Medical Park Hospital). In Iran, there are two HSCT centers in Tahran (Hematology, Oncology and Stem Cell Transplantation Research Centre and Children's Medical Center), and more than 50 HSCTs for PIDs had been performed over last 10 years. In Jordan, Queen Rania Children Hospital is the main transplanting center with an average of 8–10/year in addition to King Hussein Cancer Center (34). HSCTs are offered at several medical centers in Israel. Twenty eight HSCTs had been performed for PIDs in the last 8 years at bone marrow transplantation center of Tunis (27 genoidentical and 1 haploidentical). Sultan Qaboos University hospital in Oman performs two to four HSCTs for PID per year.

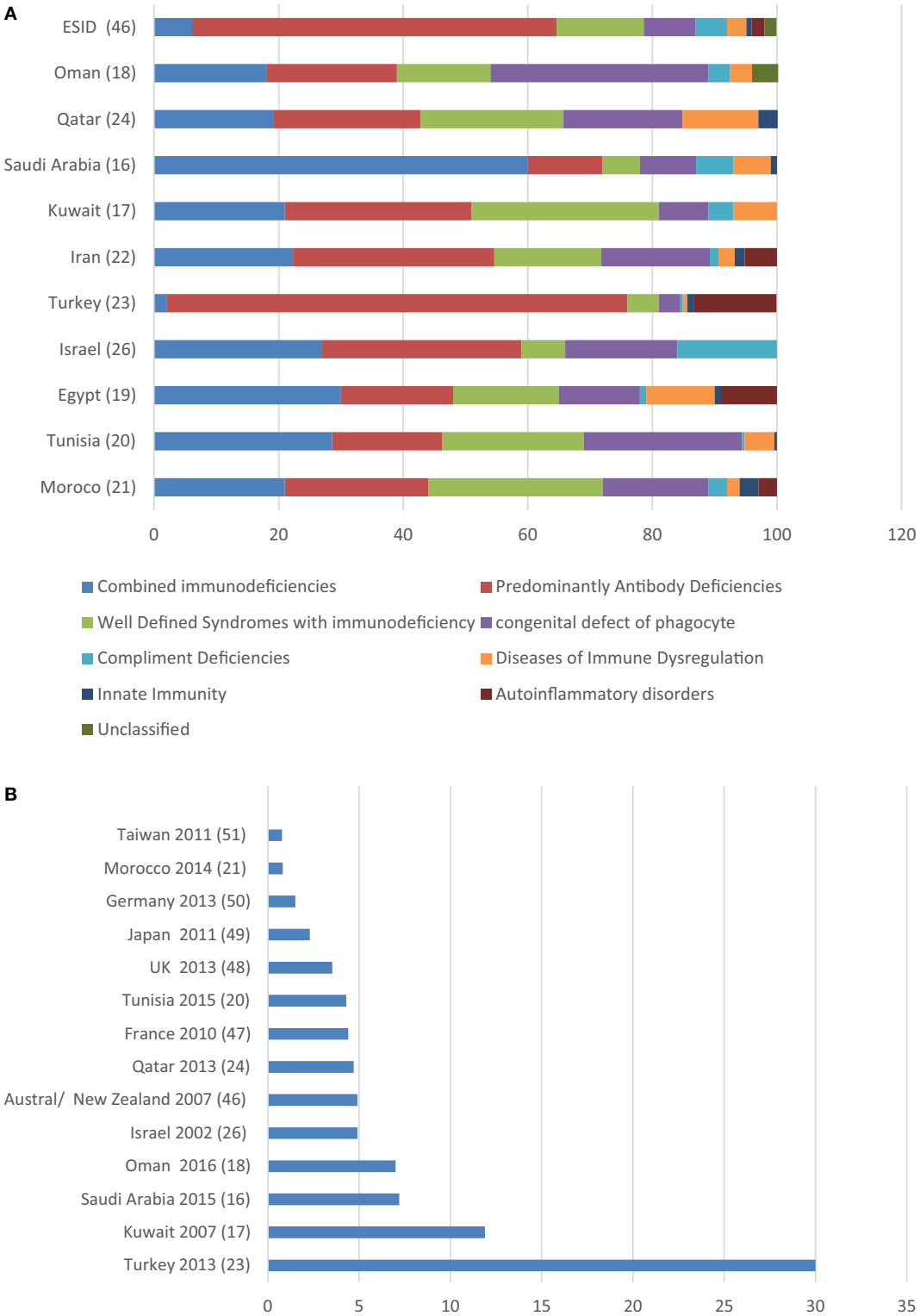

FIGURE 1 | (A) Distribution of primary immunodeficiency disorders in different primary immunodeficiency disease registries from the MENA region compared to the European Society for Immunodeficiencies registry. (B) Primary immunodeficiency disease prevalence in 100,000 inhabitants in the MENA region compared with counties from other world region.

A few HSCTs trials had been performed in Algeria, Egypt, and Morocco. Access to immunoglobulin replacement therapy and HSCT is very limited or unaffordable for many patients in some of the MENA countries.

NOVEL PID DISCOVERY

In the last three decades, more than 300 novel PIDs were discovered, and the list is continuously expanding. The inbred population of the MENA region provides a great opportunity to identify monogenic PIDs through novel next-generation sequencing technology. Studies of PIDs in the region had contributed into the discovery and the understanding of large numbers of these disorders. For example, more than 12 novel PID genes were discovered through studying patients from the MENA region in the last 2 years, which include DOCK2 (35), HOIP (36), IL-17RC (37), RORC (38), RLTPR (39), POLE2 (40), NEIL3 (41), TFRC (42), INO80 (43), LAT (44), MYSM1 (45), and CD70 (46).

GENETIC COUNSELING AND DISEASE PREVENTION

In the MENA countries, the majority of patients has an AR mode of inheritance and come from families known to have the disease. Appropriate genetic counseling for affected families is an essential part of the management. In Saudi Arabia, Turkey, Iran, Israel, and Kuwait genetic counseling, prenatal and preimplantation genetic diagnosis and premarriage screening to identify carriers are offered to affected families. Such services are costly and require sophisticated diagnostic facilities not available in most of the MENA countries.

EDUCATION AND TRAINING

Establishing a structural immunology educational and fellowship programs is essential to improve available immunology services in MENA region. In Saudi Arabia, allergy and immunology fellowship training program had been established since 1989. Structured clinical immunology training had been also established in Iran 30 years ago. A 3-year fellowship training is available in several universities in Turkey. Allergy and immunology fellowship program had been recently launched in Kuwait. Immunological centers at MENA region are actively involved in providing education sessions and training for general practitioners, pediatricians, and internists. Dedicated conferences and workshops on PID had been organized in various MENA countries aiming to increase the health providers' awareness.

NEWBORN SCREENING FOR PID

Most patients with PIDs are asymptomatic at birth. Early diagnosis and initiation of therapy improve the outcome. SCID have been recognized as candidates for population-based newborn screening using the T-cell receptor recombination excision circle assay (TREC) and found to be cost-effective means to improve the quality and duration of life for children with SCID. The high disease incidence as seen in MENA region is a critical driving force that would affect the incremental cost-effectiveness ratio. Implementing such a program in the MENA region is possible in resource-rich countries. However, the health authorities should recognize the seriousness of the health problem and provide all required resources. Israel is the only MENA country performing universal SCID NBS. A NBS pilot study is ongoing in Saudi Arabia to identify the real incidence of SCID in Saudi population.

DISCUSSION

The MENA region has a diverse ethnic background, but consanguineous marriages are common practice among this population. This practice is derived by cultural and socioeconomic interests aiming to strengthen family relations and structure. PIDs are more common in areas with high rates of consanguineous since most PIDs are inherited in an AR pattern and hence the observed increased incidence of combined immunodeficiencies among MENA populations in comparison to populations from the European countries (47) (**Figure 1A**). The higher incidence of combined immunodeficiency seen in Saudi Arabia in comparison to other MENA countries might be related to the availability of diagnostic facilities and to the fact that this was a single center registry. The lower rate of consanguineous marriage in Turkey had contributed to the reduced incidence of such disorders in their population. Moreover registries from the MENA region (16–21, 23, 24, 26) showed a high prevalence of PIDs when compared to the rest of the world (48–53) (**Figure 1B**). The variable low or high PID reported prevalence might be a reflection of the accessibility and the availability for diagnostic facilities in this population among MENA countries.

The majority of patients with PID in MENA region have a family history suggestive of PID. It would be cost effective to establish a family-based preventive program for PID in this region, which allows identifying all family carriers, offer genetic counseling, and premarriage screening. Affected couples can be offered preimplantation or prenatal genetic diagnosis. However, such program should respect social and religious beliefs.

A lot of excitements are observed among clinicians taking care of PID in MENA region, but they are confronted with lack of specialized centers dedicated to the care of PID patients, limited access to a diagnostic facilities and the costly burden for the therapeutic modalities. Molecular genetic testing is essential diagnostic tool for PIDs, it provides a definitive diagnosis, assists in genetic counseling, establishes the diagnosis in atypical cases, provides genotype–phenotype correlation and guide therapy for affected patients. Molecular genetic testing is complex, expensive, and unaffordable in most of MENA countries. It will be important to establish multicenter collaborations in the region to provide and share diagnostic facilities and therapy. This will require strategic support from heath authorities in the region. Governmental support and charity funding are essential to establish clinical services and ensure that patients can receive long term therapy

such as immunoglobulins replacement therapy or stem cell transplantation.

Establishment of a MENA primary immunodeficiency network is essential to promote collaborations and experience sharing. Collaboration with various international research centers through implementing advanced molecular genetic testing had led into several novel genetic discoveries in the field of PID. Multicenter collaborations especially between clinicians and basic scientists can contribute in development of efficient PID research in the region. Clinicians' training is important to promote PID care in MENA region. This can be enforced through including clinical immunology in the undergraduate and postgraduate educational curriculum, implementing formal subspecialty training for pediatric and adult immunologist and through providing continuous medical education courses in clinical immunology.

This report highlights the need to improve awareness about PID in MENA region, enhances structural training in clinical immunology, and establishes national registries and centers of excellence in PID care and stem cell transplantation.

AUTHOR CONTRIBUTIONS

HA-M and BA-S participated in data collection, analysis and interpretation, and drafting and final approval of manuscript.

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REFERENCES

1. Rosen FS, Cooper MD, Wedgwood RJ. The primary immunodeficiencies. *N Engl J Med* (1995) 333(7):431–40. doi:10.1056/NEJM199508173330707
2. Fischer A. Human primary immunodeficiency diseases: a perspective. *Nat Immunol* (2004) 5(1):23–30. doi:10.1038/ni1023
3. Bruton OC, Apt L, Gitlin D, Janeway CA. Absence of serum gamma globulins. *AMA Am J Dis Child* (1952) 84(5):632–6.
4. Picard C, Al-Herz W, Bousfiha A, Casanova JL, Chatila T, Conley ME, et al. Primary immunodeficiency diseases: an update on the classification from the International Union of Immunological Societies Expert Committee for primary immunodeficiency 2015. *J Clin Immunol* (2015) 35(8):696–726. doi:10.1007/s10875-015-0201-1
5. Modell B, Darr A. Science and society: genetic counselling and customary consanguineous marriage. *Nat Rev Genet* (2002) 3(3):225–9. doi:10.1038/nrg754
6. Tadmouri GO, Nair P, Obeid T, Al Ali MT, Al Khaja N, Hamamy HA. Consanguinity and reproductive health among Arabs. *Reprod Health* (2009) 6:17. doi:10.1186/1742-4755-6-17
7. Zayed H. The Arab genome: health and wealth. *Gene* (2016) 592(2):239–43. doi:10.1016/j.gene.2016.07.007
8. Teebi AS, Teebi SA. Genetic diversity among the Arabs. *Community Genet* (2005) 8(1):21–6. doi:10.1159/000083333
9. Nair SC, Ibrahim H, Celentano DD. Clinical trials in the Middle East and North Africa (MENA) region: grandstanding or grandeur? *Contemp Clin Trials* (2013) 36(2):704–10. doi:10.1016/j.cct.2013.05.009
10. El-Mouzan MI, Al-Salloum AA, Al-Herbish AS, Qurachi MM, Al-Omar AA. Regional variations in the prevalence of consanguinity in Saudi Arabia. *Saudi Med J* (2007) 28(12):1881–4.
11. Al-Awadi SA, Moussa MA, Naguib KK, Farag TI, Teebi AS, el-Khalifa M, et al. Consanguinity among the Kuwaiti population. *Clin Genet* (1985) 27(5):483–6. doi:10.1111/j.1399-0004.1985.tb00236.x
12. Hafez M, El-Tahan H, Awadalla M, El-Khayat H, Abdel-Gafar A, Ghoneim M. Consanguineous matings in the Egyptian population. *J Med Genet* (1983) 20(1):58–60. doi:10.1136/jmg.20.1.58
13. Ben Halim N, Ben Alaya Bouafif N, Romdhane L, Kefi Ben Atig R, Chouchane I, Bouyacoub Y, et al. Consanguinity, endogamy, and genetic disorders in Tunisia. *J Community Genet* (2013) 4(2):273–84. doi:10.1007/s12687-012-0128-7
14. Saadat M, Ansari-Lari M, Farhud DD. Consanguineous marriage in Iran. *Ann Hum Biol* (2004) 31(2):263–9. doi:10.1080/03014460310001652211
15. Akbayram S, Sari N, Akgun C, Dogan M, Tuncer O, Caksen H, et al. The frequency of consanguineous marriage in eastern Turkey. *Genet Couns* (2009) 20(3):207–14.
16. Al-Saud B, Al-Mousa H, Al Gazlan S, Al-Ghonaium A, Arnaout R, Al-Seraihy A, et al. Primary immunodeficiency diseases in Saudi Arabia: a tertiary care hospital experience over a period of three years (2010–2013). *J Clin Immunol* (2015) 35(7):651–60. doi:10.1007/s10875-015-0197-6
17. Al-Herz W. Primary immunodeficiency disorders in Kuwait: first report from Kuwait National Primary Immunodeficiency Registry (2004–2006). *J Clin Immunol* (2008) 28(2):186–93. doi:10.1007/s10875-007-9144-5
18. Al-Tamemi S, Naseem SU, Al-Siyabi N, El-Nour I, Al-Rawas A, Dennison D. Primary immunodeficiency diseases in Oman: 10-year experience in a tertiary care hospital. *J Clin Immunol* (2016) 36(8):785–92. doi:10.1007/s10875-016-0337-7
19. Galal N, Meshaal S, Elhawary R, ElAziz DA, Alkady R, Lotfy S, et al. Patterns of primary immunodeficiency disorders among a highly consanguineous population: Cairo University Pediatric Hospital's 5-year experience. *J Clin Immunol* (2016) 36(7):649–55. doi:10.1007/s10875-016-0314-1
20. Mellouli F, Mustapha IB, Khaled MB, Besbes H, Ouederni M, Mekki N, et al. Report of the Tunisian registry of primary immunodeficiencies: 25-years of experience (1988–2012). *J Clin Immunol* (2015) 35(8):745–53. doi:10.1007/s10875-015-0206-9
21. Bousfiha AA, Jeddane L, El Hafidi N, Benajiba N, Rada N, El Bakkouri J, et al. First report on the Moroccan registry of primary immunodeficiencies: 15 years of experience (1998–2012). *J Clin Immunol* (2014) 34(4):459–68. doi:10.1007/s10875-014-0005-8
22. Aghamohammadi A, Mohammadinejad P, Abolhassani H, Mirminachi B, Movahedi M, Gharagozlou M, et al. Primary immunodeficiency disorders in Iran: update and new insights from the third report of the national registry. *J Clin Immunol* (2014) 34(4):478–90. doi:10.1007/s10875-014-0001-z
23. Kilic SS, Ozel M, Hafizoglu D, Karaca NE, Aksu G, Kutukculer N. The prevalences [correction] and patient characteristics of primary immunodeficiency diseases in Turkey – two centers study. *J Clin Immunol* (2013) 33(1):74–83. doi:10.1007/s10875-012-9763-3
24. Ehlayel MS, Bener A, Laban MA. Primary immunodeficiency diseases in children: 15 year experience in a tertiary care medical center in Qatar. *J Clin Immunol* (2013) 33(2):317–24. doi:10.1007/s10875-012-9812-y
25. Barbouche MR, Galal N, Ben-Mustapha I, Jeddane L, Mellouli F, Ailal F, et al. Primary immunodeficiencies in highly consanguineous North African populations. *Ann NY Acad Sci* (2011) 1238:42–52. doi:10.1111/j.1749-6632.2011.06260.x
26. Golan H, Dalal I, Garty BZ, Schlesinger M, Levy J, Handzel Z, et al. The incidence of primary immunodeficiency syndromes in Israel. *Isr Med Assoc J* (2002) 4(11 Suppl):868–71.
27. Al-Mousa H, Abouelhoda M, Monies DM, Al-Tassan N, Al-Ghonaium A, Al-Saud B, et al. Unbiased targeted next-generation sequencing molecular approach for primary immunodeficiency diseases. *J Allergy Clin Immunol* (2016) 137(6):1780–7. doi:10.1016/j.jaci.2015.12.1310

28. Al-Mousa H, Hawwari A, Al-Ghonaium A, Al-Saud B, Al-Dhekri H, Al-Muhsen S, et al. Hematopoietic stem cell transplantation corrects WIP deficiency. *J Allergy Clin Immunol* (2017) 139(3):1039.e–40.e. doi:10.1016/j.jaci.2016.08.036
29. Sanal O, Tezcan I. Thirty years of primary immunodeficiencies in Turkey. *Ann N Y Acad Sci* (2011) 1238:15–23. doi:10.1111/j.1749-6632.2011.06242.x
30. Geller-Bernstein C, Etzioni A. Pediatric allergy and immunology in Israel. *Pediatr Allergy Immunol* (2013) 24(2):187–94. doi:10.1111/pai.12044
31. Al-Saud B, Al-Mousa H, Al-Ahmari A, Al-Ghonaium A, Ayas M, Alhissi S, et al. Hematopoietic stem cell transplant for hyper-IgM syndrome due to CD40L defects: a single-center experience. *Pediatr Transplant* (2015) 19(6):634–9. doi:10.1111/petr.12538
32. Al-Dhekri H, Al-Mousa H, Ayas M, Al-Muhsen S, Al-Ghonaium A, Al-Ghanam G, et al. Allogeneic hematopoietic stem cell transplantation in leukocyte adhesion deficiency type 1: a single center experience. *Biol Blood Marrow Transplant* (2011) 17(8):1245–9. doi:10.1016/j.bbmt.2010.12.714
33. Al-Mousa H, Al-Shammari Z, Al-Ghonaium A, Al-Dhekri H, Al-Muhsen S, Al-Saud B, et al. Allogeneic stem cell transplantation using myeloablative and reduced-intensity conditioning in patients with major histocompatibility complex class II deficiency. *Biol Blood Marrow Transplant* (2010) 16(6):818–23. doi:10.1016/j.bbmt.2010.01.002
34. Amayiri N, Al-Zaben A, Ghatasheh L, Frangoul H, Hussein AA. Hematopoietic stem cell transplantation for children with primary immunodeficiency diseases: single center experience in Jordan. *Pediatr Transplant* (2013) 17(4):394–402. doi:10.1111/petr.12081
35. Dobbs K, Dominguez Conde C, Zhang SY, Parolini S, Audry M, Chou J, et al. Inherited DOCK2 deficiency in patients with early-onset invasive infections. *N Engl J Med* (2015) 372(25):2409–22. doi:10.1056/NEJMoa1413462
36. Boisson B, Laplantine E, Dobbs K, Cobat A, Tarantino N, Hazen M, et al. Human HOIP and LUBAC deficiency underlies autoinflammation, immunodeficiency, amylopectinosis, and lymphangiectasia. *J Exp Med* (2015) 212(6):939–51. doi:10.1084/jem.20141130
37. Ling Y, Cypowyj S, Aytekin C, Galicchio M, Camcioglu Y, Nepesov S, et al. Inherited IL-17RC deficiency in patients with chronic mucocutaneous candidiasis. *J Exp Med* (2015) 212(5):619–31. doi:10.1084/jem.20141065
38. Okada S, Markle JG, Deenick EK, Mele F, Averbuch D, Lagos M, et al. IMMUNODEFICIENCIES. Impairment of immunity to *Candida* and *Mycobacterium* in humans with bi-allelic RORC mutations. *Science* (2015) 349(6248):606–13. doi:10.1126/science.aaa4282
39. Wang Y, Ma CS, Ling Y, Bousfiha A, Camcioglu Y, Jacquot S, et al. Dual T cell- and B cell-intrinsic deficiency in humans with biallelic RLTPR mutations. *J Exp Med* (2016) 213(11):2413–35. doi:10.1084/jem.20160576
40. Frugoni F, Dobbs K, Felgentreff K, Al-Dhekri H, Al-Saud BK, Arnaout R, et al. A novel mutation in the POLE2 gene causing combined immunodeficiency. *J Allergy Clin Immunol* (2016) 137(2):635–8.e1. doi:10.1016/j.jaci.2015.06.049
41. Massaad MJ, Zhou J, Tsuchimoto D, Chou J, Jabara H, Janssen E, et al. Deficiency of base excision repair enzyme NEIL3 drives increased predisposition to autoimmunity. *J Clin Invest* (2016) 126(11):4219–36. doi:10.1172/JCI85647
42. Jabara HH, Boyden SE, Chou J, Ramesh N, Massaad MJ, Benson H, et al. A missense mutation in TFRC, encoding transferrin receptor 1, causes combined immunodeficiency. *Nat Genet* (2016) 48(1):74–8. doi:10.1038/ng.3465
43. Kracker S, Di Virgilio M, Schwartzentruber J, Cuenin C, Forveille M, Deau MC, et al. An inherited immunoglobulin class-switch recombination deficiency associated with a defect in the INO80 chromatin remodeling complex. *J Allergy Clin Immunol* (2015) 135(4):998–1007.e6. doi:10.1016/j.jaci.2014.08.030
44. Keller B, Zaidman I, Yousefi OS, Hershkovitz D, Stein J, Unger S, et al. Early onset combined immunodeficiency and autoimmunity in patients with loss-of-function mutation in LAT. *J Exp Med* (2016) 213(7):1185–99. doi:10.1084/jem.20151110
45. Bahrami E, Witzel M, Racek T, Puchalka J, Hollizeck S, Greif-Kohistani N, et al. Myb-like, SWIRM, and MPN domains 1 (MYSM1) deficiency: genotoxic stress-associated bone marrow failure and developmental aberrations. *J Allergy Clin Immunol* (2017). doi:10.1016/j.jaci.2016.10.053
46. Abolhassani H, Edwards ES, Ikinciogullari A, Jing H, Borte S, Buggert M, et al. Combined immunodeficiency and Epstein-Barr virus-induced B cell malignancy in humans with inherited CD70 deficiency. *J Exp Med* (2017) 214(1):91–106. doi:10.1084/jem.20160849
47. Gathmann B, Binder N, Ehl S, Kindle G; Party ERW. The European internet-based patient and research database for primary immunodeficiencies: update 2011. *Clin Exp Immunol* (2012) 167(3):479–91. doi:10.1111/j.1365-2249.2011.04542.x
48. Kirkpatrick P, Riminton S. Primary immunodeficiency diseases in Australia and New Zealand. *J Clin Immunol* (2007) 27(5):517–24. doi:10.1007/s10875-007-9105-z
49. Group CTFPs. The French national registry of primary immunodeficiency diseases. *Clin Immunol* (2010) 135(2):264–72. doi:10.1016/j.clim.2010.02.021
50. Edgar JD, Buckland M, Guzman D, Conlon NP, Knerr V, Bangs C, et al. The United Kingdom Primary Immune Deficiency (UKPID) registry: report of the first 4 years' activity 2008–2012. *Clin Exp Immunol* (2014) 175(1):68–78. doi:10.1111/cei.12172
51. Ishimura M, Takada H, Doi T, Imai K, Sasahara Y, Kanegane H, et al. Nationwide survey of patients with primary immunodeficiency diseases in Japan. *J Clin Immunol* (2011) 31(6):968–76. doi:10.1007/s10875-011-9594-7
52. Gathmann B, Goldacker S, Klima M, Belohradsky BH, Notheis G, Ehl S, et al. The German national registry for primary immunodeficiencies (PID). *Clin Exp Immunol* (2013) 173(2):372–80. doi:10.1111/cei.12105
53. Lee WI, Huang JL, Jaing TH, Shyur SD, Yang KD, Chien YH, et al. Distribution, clinical features and treatment in Taiwanese patients with symptomatic primary immunodeficiency diseases (PIDs) in a nationwide population-based study during 1985–2010. *Immunobiology* (2011) 216(12):1286–94. doi:10.1016/j.imbio.2011.06.002

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Lessons from Genetic Studies of Primary Immunodeficiencies in a Highly Consanguineous Population

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During the last decades, the study of primary immunodeficiencies (PIDs) has contributed tremendously to unravel novel pathways involved in a variety of immune responses. Many of these PIDs have an autosomal recessive (AR) mode of inheritance. Thus, the investigation of the molecular basis of PIDs is particularly relevant in consanguineous populations from Middle East and North Africa (MENA). Although significant efforts have been made in recent years to develop genetic testing across the MENA region, few comprehensive studies reporting molecular basis of PIDs in these settings are available. Herein, we review genetic characteristics of PIDs identified in 168 patients from an inbred Tunisian population. A spectrum of 25 genes involved was analyzed. We show that AR forms compared to X-linked or autosomal dominant forms are clearly the most frequent. Furthermore, the study of informative consanguineous families did allow the identification of a novel hyper-IgE syndrome linked to phosphoglucomutase 3 mutations. We did also report a novel form of autoimmune lymphoproliferative syndrome caused by homozygous FAS mutations with normal or residual protein expression as well as a novel AR transcription factor 3 deficiency. Finally, we identified several founder effects for specific AR mutations. This did facilitate the implementation of preventive approaches through genetic counseling in affected consanguineous families. All together, these findings highlight the specific nature of highly consanguineous populations and confirm the importance of unraveling the molecular basis of genetic diseases in this context. Besides providing a better fundamental knowledge of novel pathways, their study is improving diagnosis strategies and appropriate care.

Keywords: primary immunodeficiencies, consanguinity, autosomal recessive, founder effect, genetic counseling

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INTRODUCTION

The expression of recessive genes inherited from a common ancestor, in consanguineous populations, underlies higher frequency of otherwise rare genetic diseases (1–3). Indeed, the molecular basis of many monogenic disorders has been first characterized in highly inbred populations. Furthermore, in a significant proportion of cases, a strong founder effect for specific mutations has been reported in these settings; some mutations might be unique to a particular endogamous community (4, 5).

Primary immunodeficiencies (PIDs) are a heterogeneous group of genetic disorders of the immune system that predispose patients to infections, autoimmune diseases, lymphoproliferation,

and malignancy. The exact prevalence of PIDs worldwide is unknown but is expected to be more common in areas with high rates of consanguinity (6). The autosomal recessive (AR) forms of these disorders represent the most frequent mode of inheritance as compared to X-linked (XL) or autosomal dominant (AD) forms. Indeed, according to the 2015 IUIS classification of PIDs, 206 out of 289 known forms of PIDs are AR (7). The occurrence of the disease in the progeny of unaffected consanguineous parents is highly suggestive of an AR mode of inheritance. However, molecular studies are required to confirm the AR determinism of disease in these PIDs affected families.

Although significant efforts have been made in recent years to develop genetic testing across the Middle East and North Africa (MENA) region, very few comprehensive studies reporting molecular basis of PIDs in these settings are available. Herein, we review genetic characteristics of PIDs identified in a large series of inbred Tunisian (North-African) patients. We outline the molecular basis of disease in this population and its potential contribution to a better care through genetic counseling. Furthermore, we discuss the relevance of such studies in the discovery of new PIDs genes and novel modes of inheritance for known PIDs.

PIDs DISTRIBUTION, PARENTAL CONSANGUINITY, AND FAMILIAL HISTORY

This review analyzes data collected from different genetic studies performed in a total of 168 PIDs patients belonging to 122 kindreds. This is at the best of our knowledge, one of the largest molecular studies of PIDs patients from highly consanguineous MENA populations along with the study from Saudi Arabia (8). The most frequently observed PIDs in this series include combined T and B cell immunodeficiencies and congenital defects of phagocyte that account for 52 (30.95%) and 37 (22%) patients, respectively, contrasting with data from European series showing the predominance of antibody deficiencies (9). Indeed, only 15.47% of the patients had predominantly antibody deficiencies. Similar observations have been reported in others series from MENA region (10, 11). This could be due to the less severe clinical phenotype and to the lack of adult physicians' awareness particularly with regard to common variable immunodeficiency. Moreover, the high frequency of particular AR forms of combined immunodeficiencies among North-African PIDs patients could also account for such findings. Indeed, major histocompatibility complex (MHC) class II deficiency is the most frequently reported PID entity in this series with 27 patients, and the majority of patients reported worldwide are of North-African origin (Algeria, Tunisia, and Morocco) (12). Other categories of PIDs included defects in intrinsic and innate immunity (23 cases), combined immunodeficiencies with associated or syndromic features (19 cases), and diseases of immune dysregulation (11 cases). Furthermore, few patients presenting rare PIDs with peculiar clinical and/or immunological phenotype did also undergo genetic characterization. Altogether, the pattern of PIDs distribution in patients with established molecular diagnosis in this series is representative of the actual distribution of PIDs reported by the few national registries available from MENA region (13).

Interestingly, Tunisian and other MENA region countries are unique with regard to high prevalence of consanguinity in the general population varying between 20 and 50% of all marriages (13). Consistently, the patients studied in this series show a high rate of parental consanguinity that reaches 61.9% accounting for the high frequency of family history (55.35%) including early deaths, similar clinical features, and/or previously identified PIDs in relatives. Consanguinity rate was particularly high in patients with Omenn syndrome (88.8%), phosphoglucomutase 3 (PGM3) deficiency (85.7%), leukocyte adhesion deficiency type 1 (LAD I) (76.4%), and MHC class II deficiency (70.3%) (**Table 1**). This is in accordance with the results obtained for one of the largest series of MHC class II North-African patients reporting a consanguinity rate of 81.8% (12), as well as for LAD I and Omenn syndrome in other patients series originating from MENA region (14–16).

MOLECULAR STUDIES AND MODE OF INHERITANCE

In total, mutational studies of 25 candidate genes identified 58 different mutations as detailed in **Table 1**. Among them, 30 were novel (53.57%) with no records in three major databases including HGMD (The Human Gene Mutation Database), LOVD (Leiden Open Variation Database), and IDbases (databases for immunodeficiency-causing variations). Three mutations have been already reported for the first time in other patients from Tunisian origin (17–19) and nine mutations were recurrent. The identified mutations include 21 missense mutations, 10 nonsense mutations, 10 splice-site mutations, 12 deletions, 1 duplication, and 1 insertion. Interesting and rare mutational mechanisms included one complex mutation (2 pb insertion and 5 bp deletion) in *IGHM* gene (20), and one *de novo STAT1* mutation was identified in AD chronic mucocutaneous candidiasis disease (21). Mutations generating a premature stop codon were frequently observed in severe combined immunodeficiencies patients (71%), particularly those with *IL2RG* gene defect. All novel missense mutations were predicted to be possibly or probably damaging by Polyphen2 and/or SIFT algorithms. Furthermore, a deleterious effect was confirmed by appropriate functional testing for several gene mutations including *TCF3* (22), *AICDA* (23), *NCF2* (24), *FAS* (25), *STAT1* (21), *IL12B* (26), and *PGM3* (27).

These molecular studies confirm that the AR mode of inheritance is the most common in Tunisian patients accounting for 73% of all PIDs entities investigated. XL and AD modes were identified in only four and three different disorders, respectively. The deeply rooted tradition of parental consanguinity in the Tunisian general population, which remained relatively constant during the last four decades (28), has resulted in an elevated burden of AR PIDs since consanguinity favors the expression of recessive alleles (29). This is the case for two

TABLE 1 | Mode of inheritance and molecular studies in the 170 Tunisian patients investigated.

PID	Gene	Mode of inheritance	Number of kindred	Number of patients	Confirmed parental consanguinity	Molecular defects		Recurrent mutation	Novel mutation
						cDNA	Protein		
Immunodeficiencies affecting cellular and humoral immunity (*n* = 53)									
SCID	*IL2RG*	XL	1	2	–	c.865C>T	R289X	–	–
			1	3	–	c.710G>A	W237X	–	+
			1	2	–	c.222G>A	W74X	–	+
	RAG 2	AR	1	1	1	c.1219G>T	E407X	–	–
			2	2	2	c.1338C>G	C446W	–	–
	IL7RA	AR	1	1	1	c.616 C>T	R206X	–	–
	PNP	AR	1	1	1	c.181 + 1G>A	–	–	+
Omenn syndrome	*RAG1*	AR	7	9	8	631delT	T173TfsX28	+	–
MHC-II deficiency	*RFXANK*	AR	23	27	19	c.338-25_338del26	I5E6-25_I5E6 + 1	+	–
HIGE—DOCK8 deficiency	*DOCK8*	AR	1	1	1	Ex1-43 del	–	–	+
HIGM—CD40 ligand deficiency	*CD40LG*	XL	1	1	–	c.348_351dup	Q118Vfs*5	–	+
			1	1	–	c.782_*2del	L261Qfs*50	–	+
			1	1	–	c.[356G>A; 299_356del]	p.([116G>S, D97_K115del])	–	–
HIGM—CD40 deficiency	*CD40*	AR	1	1	1	c.109T>G	C37G	–	+
Combined immunodeficiencies with associated or syndromic features (*n* = 19)									
HIES	*STAT 3*	AD	1	4	–	c.1298A>G	M329V	–	+
			1	1	–	c.1858A>G	T620A	–	–
	PGM3	AR	3	12	10	c.1018_1020del	E340del	+	+
			1	2	2	c.248T>C	L83S	–	+
Predominantly antibody deficiencies (*n* = 26)									
XLA	*BTK*	XL	2	2	–	c.1762T>G	W588G	–	+
			1	1	1	c.863G>A	R288Q	–	–
			2	2	1	c.435C>A	C145X	–	–
			1	1	–	c.1117C>T	L373V	–	+
			1	1	–	c.1567 – 1G>A	–	–	+
			1	1	1	c.1181C>G	S394X	–	+
			1	2	2	c.1631 + 1G>A	–	–	–
			1	1	1	c.653delA	K218fsX228	–	–
			1	1	–	c.1845_1846insGT	L616fsX649	–	+
	IGHM	AR	1	1	1	c.1789insCC1792-1796del CCAGC	V378AfsX1	–	+
	TCF3	AR	1	2	2	c.808C>T	Q270X	–	+
HIGM—AID deficiency	*AICDA*	AR	1	1	–	c.91T>C	Y31H	–	+
			4	5	4	c.389A>C	H130P	+	+
			3	5	3	c.156 + 1G>T	([N53Lfs*15, N53Lfs*19])	+	–[a]
Diseases of immune dysregulation (*n* = 10)									
ALPS	*FAS*	AD	1	1	–	c.266G>A	A16T	–	–
			1	1	–	c.926G>A	E194K	–	–
			1	1	–	c.365C>T	T122I	–	–
			1	1	–	c.1009A>G	E256G	–	–
		AR	1	4	3	c.1017A>G	N266S	–	+
			1	2	1	c.581C>T	R121W	–	+

(*Continued*)

TABLE 1 | Continued

PID	Gene	Mode of inheritance	Number of kindred	Number of patients	Confirmed parental consanguinity	Molecular defects		Recurrent mutation	Novel mutation
						cDNA	Protein		
Congenital defects of phagocyte number, function, or both (n = 37)									
CGD	*NCF2*	AR	6	11	9	c.257 + 2T>C	A59IfsX2	+	–[a]
			1	1	1	c.78A>T	N419I	–	+
	NCF1	AR	5	5	2	c.75_76delGT	–	–	–
	CYBA	AR	1	1	–	295-301delGTGCCCG	–	–	+
			1	1	–	c.70G>A	G24R	–	–
	CYBB	XL	1	1	–	c.1359G>A	W453X	–	+
LAD I	*CD18*	AR	10	15	11	c.119_128delGGCCCGGCTG	G40A fsX7	+	–
			2	2	2	c.1777C>T	R593C	–	–
Defects in intrinsic and innate immunity (n = 23)									
MSMD	*IL12B*	AR	7	9	4	c.298_305del	–	+	+
	IL12RB1	AR	1	1	–	622C>A	C185X	–	+
			1	1	1	64 + 5G → A	–	–	+
			2	2	2	64 + 2T>G	–	–	–
			1	2	2	1386-1387delGT	–	–	+
			1	1	1	550-2A>G	–	–	+
			2	2	–	1791 + 2T>G	–	–	–
	IFNGR1	AR	2	3	2	c.131delC	–	+	–[a]
CMC	*STAT1* (GOF)	AD	1	1	1	c.876T4G	L163R	–	+
			1	1	–	c.820C>T	R274W	–	–

[a]*These mutations have been already reported for the first time in other patients from Tunisian origin.*

XL, X-linked inheritance; AR, autosomal recessive inheritance; AD, autosomal dominant inheritance; SCID, severe combined immunodeficiencies; ALPS, autoimmune lymphoproliferative syndrome; AID, activation-induced cytidine deaminase; CGD, chronic granulomatous disease; MSMD, Mendelian susceptibility to mycobacterial disease; LAD, leukocyte adhesion deficiency; CMC, chronic mucocutaneous candidiasis; PID, primary immunodeficiency; MHC, major histocompatibility complex; LAD I, leukocyte adhesion deficiency type 1; HIGM, hyper-IgM syndrome; HIES, hyper-IgE syndrome.

otherwise rare AR PIDs, namely, MHC class II deficiency and LAD I, diagnosed in fewer than 200 and 300 patients worldwide (12, 30) but accounting for 56 and 30 patients in Tunisia, respectively (31, 32). One practical implication related to this high frequency is to recommend, at least for the MHC class II deficiency, routine investigation of DR expression for North-African patients presenting symptoms suggestive of combined deficiency. Furthermore, for PIDs with more than one known mode of transmission, the AR trait was the most frequent or proportionately more represented in our settings than in other series and registries from non-consanguineous populations. Indeed, this mode of transmission accounted for most kindreds with chronic granulomatous disease (CGD) (93%), hyper-IgM syndrome (HIGM) (75%), and hyper-IgE syndrome (HIES) (71.42%). Consistently, among CGD patients, only one had a mutation in *CYBB* gene whereas the remaining patients (19/20) bear mutations in AR CGD genes. This is in accordance with previous reports showing a higher frequency of AR CGD in consanguineous populations (13, 33). In contrast, the XL form caused by *CYBB* gene mutation is the most common CGD form accounting for 70% of all patients according to the European registry data (34). Similar findings have also been observed in Tunisian patients with HIGM. Indeed, we demonstrate that 11 out of 15 patients are assigned to the AR form of the disease due to mutations in *AICDA* gene (20, 23). In contrast, the XL CD40L deficiency defines the most frequent type of HIGM in other series as it has been reported in 75% in North American and Asian patients (35), 42% in European patients (9), and 94% in Latin American patients (36). Thus, AR and XL forms for these diseases should be equally suspected in males originating from consanguineous regions. For HIES, PGM3 homozygous mutations account for 70% of Tunisian patients whereas the AD form due to mutations in signal transducer and activator of transcription 3 is the most common form reported worldwide (37).

IDENTIFICATION OF NOVEL GENE AND OF NOVEL MODE OF INHERITANCE FOR KNOWN GENES

Because a majority of PIDs are inherited as AR traits, the identification of patients with singular clinical and immunological phenotype within informative consanguineous families has tremendously contributed to the discovery of novel disease-causing genes. Accordingly, we did recently identify *PGM3* gene defect due to homozygous hypomorphic mutations in patients from two Tunisian consanguineous families with hyper-IgE like syndrome (27). This gene has not been previously associated with human disease. The patients' clinical phenotype included classical features of HIES; however, they showed neurologic impairment with a developmental delay and psychomotor retardation (27). PGM3 enzyme plays an important role in the glycosylation pathway by catalyzing a key step in the synthesis of UDP-GlcNAc required for the biosynthesis of N-glycans (38). Thus, leukocytes from PGM3 deficient patients showed aberrant pattern of glycosylation due to altered PGM3 enzymatic activity (**Table 2**). This new congenital disorder of glycosylation accounts probably for the patients' clinical and immunological phenotype, although the underlying mechanisms remain to be fully understood. Concurrently, additional patients with heterogeneous clinical phenotypes carrying distinct *PGM3* mutations have also been described (39, 40). Given that some congenital disorders of glycosylation are treatable with supplements of enzyme substrates (41), such an approach in these patients with hypomorphic mutations and residual enzymatic activity could be proposed to improve their condition while waiting the generally difficult access to bone marrow transplantation in our settings. Many other gene discoveries have been previously made following the study of large consanguineous families from the MENA region. Indeed, as already reported by Barbouche and Eley (42), during the period 1994–2013, at least 21 novel underlying genes were first described in PIDs families living or originating from this region.

Families from areas with a high rate of consanguinity are important not only for the discovery of novel disease-causing genes but also for the identification of novel forms of known PIDs. Indeed, in autoimmune lymphoproliferative syndrome (ALPS), heterozygous germline mutations in the *FAS* gene inherited in an AD mode and associated with preserved protein expression are the most common cause of ALPS (25). These heterozygous mutations reported mainly in outbred human populations alter protein functioning by dominant-negative effect or by haploinsufficiency mechanisms (43–45). Very rare cases of total absence of Fas protein expression caused by homozygous *FAS*

TABLE 2 | Identification of novel gene and novel mode of inheritance for known genes in Tunisian consanguineous families.

Identification strategy		Mutation	Protein expression	Functional consequences	Reference
Novel gene					
PGM3	Homozygosity mapping/linkage analysis and selector-based sequencing	c.1018_1020del (Hypomorphic) c.248T>C (Hypomorphic)	Reduced	Reduced PGM3 enzymatic activity	(27)
Novel mode of inheritance					
FAS	Sanger sequencing	c.1017A>G (Loss of function) c.581C>T (Loss of function)	Present	Resistance to Fas-mediated cell death	(25)
TCF3	Whole exome sequencing	c.808C>T (Loss of function)	Absent	Hypogammaglobulinemia	(22)

mutations have been reported (46). Interestingly, we identified in two unrelated consanguineous Tunisian kindreds the first example of a human AR ALPS characterized by homozygous *FAS* gene mutations in either intracellular or extracellular domains associated with normal or residual Fas expression, respectively. Both mutations are associated with resistance to Fas-mediated cell-death (25) (**Table 2**).

In addition, we identified another mode of inheritance for the previously reported AD agammaglobulinemia due to heterozygous dominant-negative *de novo* mutation in transcription factor 3 (*TCF3*) gene (47). Indeed, we did recently report a patient with a homozygous nonsense mutation in *TCF3* gene, who presented with severe hypogammaglobulinemia, very low number of B cells and developed B-cell acute lymphoblastic leukemia (22) (**Table 2**).

Both novel modes of inheritance herein reported are supported by parents' consanguinity, familial history, and segregation of clinical and immunological features with the homozygous status. Such findings expand the spectrum of ALPS and TCF3 deficiency types and should prompt clinicians to search for such patients in highly endogamous populations for appropriate clinical and immunological follow-up.

INVESTIGATIONS OF HOMOZYGOSITY MAPPING AND AGE ESTIMATION OF FOUNDER MUTATIONS

Another striking feature pinpointed in the study population is the high frequency of founder effects accounting for the recurrence of mutations, which were unique for several PIDs. The patterns of founder mutation distribution disclosed two types: those that are shared with other populations particularly from the North-African countries and those that are specific to Tunisia. One of the most illustrative PIDs examples for a regional founder effect is the AR MHC class II combined immunodeficiency that has been considered to be a "North-African disease." Indeed, we have identified a founder effect for the highly frequent c.338-25_338del26 mutation (also known as 752delG-25) in the RFXANK gene resulting in a 26-bp deletion (31) that has been reported in other North-African studies (4, 5, 48). The founder event responsible for this mutation has been estimated to have arise approximately 2,250 years ago (12), a period concurrent to the Berber civilization. This is consistent with previous reports showing that the population of the North Africa particularly in Tunisia, Algeria, and Morocco has a common substantial genetic background and that founder mutations could be shared in some of these countries (49). Additional MHC class II mutations have been since reported in the Middle East and other world regions (50–52). Another recurrent mutation in *RAG1* gene (631delT) has been identified in nine Tunisian patients with Omenn syndrome; its description was limited to patients originating from North Africa as well (53–56) suggesting a possible founder effect for this variant. Furthermore, a recent study reported the recurrence of the same homozygous mutation (Q289X) in *CARD9* gene in eight Algerian and four Tunisian patients from seven unrelated families with deep dermatophytosis (57). Such finding was due to a founder effect with the common ancestor living approximately 975 years ago (57).

Interestingly, other identified founder effects seem to be limited to Tunisian regions (49). Indeed, a founder effect for c.298_305del mutation in the *IL12B* gene has been reported in patients originating from the same Tunisian village. This mutation resulting in Mendelian susceptibility to mycobacterial disease is inherited as a common founder mutation arousing 1,100 years ago (26). In addition, we have recently reported a recurrent homozygous mutation in *NCF2* (c.257 + 2T>C) gene in 11 Tunisian patients CGD (24). The founder mutational event responsible of the recurrence of this mutation seems to be more recent since it was estimated to have occurred approximately 175 years ago (24).

Deleterious founder mutations have been reported in consanguineous populations to be the underlying cause of a large spectrum of monogenic AR diseases (28). In Tunisia, more than 300 genetic disorders were reported. Among them, 42% were associated to the presence of potential founder effect including PIDs (49, 58). Several clinical implications of the existence of the founder effect are the implementation of preventive approaches through genetic counseling and prenatal diagnosis in affected families. Furthermore, early genetic diagnosis in patients originating from the same geographical area will help propose HSCT, which is often the unique curative treatment, prior to the development of a severe clinical phenotype.

CONCLUSION

This review shows evidence that AR forms of PIDs are frequent in our settings characterized by a high rate of consanguineous marriages. The presence of recurrent mutations and strong founder effects are shown to be common characteristics of PIDs patients from this population. Furthermore, these findings clearly demonstrate that the presence of an extended genetic homozygosity has the potential to reveal unusual patterns of inheritance. Thus, classical dominant disorders may assume a recessive pattern of inheritance in such population. Finally, it becomes obvious that PIDs in this region of the world is a relatively important health priority. The role of accurate molecular diagnosis and appropriate genetic counseling will help moderate the burden of PIDs and its associated costs for the community.

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M-RB, NM, MB-A, and IB-M did contribute to the collection, analysis, and interpretation of the data as well as editing of the manuscript and approval of the submitted version.

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REFERENCES

1. Campbell H, Rudan I, Bittles AH, Wright AF. Human population structure, genome autozygosity and human health. *Genome Med* (2009) 1(9):91. doi:10.1186/gm91
2. Curtis D, Vine AE, Knight J. Study of regions of extended homozygosity provides a powerful method to explore haplotype structure of human populations. *Ann Hum Genet* (2008) 72(Pt 2):261–78. doi:10.1111/j.1469-1809.2007.00411.x
3. Wang S, Haynes C, Barany F, Ott J. Genome-wide autozygosity mapping in human populations. *Genet Epidemiol* (2009) 33(2):172–80. doi:10.1002/gepi.20344
4. Wiszniewski W, Fondaneche MC, Lambert N, Masternak K, Picard C, Notarangelo L, et al. Founder effect for a 26-bp deletion in the RFXANK gene in North African major histocompatibility complex class II-deficient patients belonging to complementation group B. *Immunogenetics* (2000) 51(4–5):261–7. doi:10.1007/s002510050619
5. Naamane H, El Maataoui O, Ailal F, Barakat A, Bennani S, Najib J, et al. The 752delG26 mutation in the RFXANK gene associated with major histocompatibility complex class II deficiency: evidence for a founder effect in the Moroccan population. *Eur J Pediatr* (2010) 169(9):1069–74. doi:10.1007/s00431-010-1179-6
6. Al-Herz W, Aldhekri H, Barbouche MR, Rezaei N. Consanguinity and primary immunodeficiencies. *Hum Hered* (2014) 77(1–4):138–43. doi:10.1159/000357710
7. Picard C, Al-Herz W, Bousfiha A, Casanova JL, Chatila T, Conley ME, et al. Primary immunodeficiency diseases: an update on the classification from the International Union of Immunological Societies Expert Committee for primary immunodeficiency 2015. *J Clin Immunol* (2015) 35(8):696–726. doi:10.1007/s10875-015-0201-1
8. Al-Mousa H, Abouelhoda M, Monies DM, Al-Tassan N, Al-Ghonaium A, Al-Saud B, et al. Unbiased targeted next-generation sequencing molecular approach for primary immunodeficiency diseases. *J Allergy Clin Immunol* (2016) 137(6):1780–7. doi:10.1016/j.jaci.2015.12.1310
9. Gathmann B, Binder N, Ehl S, Kindle G; ESID Registry Working Party. The European internet-based patient and research database for primary immunodeficiencies: update 2011. *Clin Exp Immunol* (2012) 167(3):479–91. doi:10.1111/j.1365-2249.2011.04542.x
10. Al-Saud B, Al-Mousa H, Al Gazlan S, Al-Ghonaium A, Arnaout R, Al-Seraihy A, et al. Primary immunodeficiency diseases in Saudi Arabia: a tertiary care hospital experience over a period of three years (2010–2013). *J Clin Immunol* (2015) 35(7):651–60. doi:10.1007/s10875-015-0197-6
11. Galal N, Meshaal S, Elhawary R, ElAziz DA, Alkady R, Lotfy S, et al. Patterns of primary immunodeficiency disorders among a highly consanguineous population: Cairo University Pediatric Hospital's 5-year experience. *J Clin Immunol* (2016) 36(7):649–55. doi:10.1007/s10875-016-0314-1
12. Ouederni M, Vincent QB, Frange P, Touzot F, Scerra S, Bejaoui M, et al. Major histocompatibility complex class II expression deficiency caused by a RFXANK founder mutation: a survey of 35 patients. *Blood* (2011) 118(19):5108–18. doi:10.1182/blood-2011-05-352716
13. Barbouche MR, Galal N, Ben-Mustapha I, Jeddane L, Mellouli F, Ailal F, et al. Primary immunodeficiencies in highly consanguineous North African populations. *Ann N Y Acad Sci* (2011) 1238:42–52. doi:10.1111/j.1749-6632.2011.06260.x
14. Rezaei N, Aghamohammadi A, Moin M, Pourpak Z, Movahedi M, Gharagozlou M, et al. Frequency and clinical manifestations of patients with primary immunodeficiency disorders in Iran: update from the Iranian Primary Immunodeficiency Registry. *J Clin Immunol* (2006) 26(6):519–32. doi:10.1007/s10875-006-9047-x
15. Al-Herz W. Primary immunodeficiency disorders in Kuwait: first report from Kuwait National Primary Immunodeficiency Registry (2004–2006). *J Clin Immunol* (2008) 28(2):186–93. doi:10.1007/s10875-007-9144-5
16. Reda SM, Afifi HM, Amine MM. Primary immunodeficiency diseases in Egyptian children: a single-center study. *J Clin Immunol* (2009) 29(3):343–51. doi:10.1007/s10875-008-9260-x
17. Tanugi-Cholley LC, Issartel JP, Lunardi J, Freycon F, Morel F, Vignais PV. A mutation located at the 5' splice junction sequence of intron 3 in the p67phox gene causes the lack of p67phox mRNA in a patient with chronic granulomatous disease. *Blood* (1995) 85(1):242–9.
18. Jouanguy E, Altare F, Lamhamedi S, Revy P, Emile JF, Newport M, et al. Interferon-gamma-receptor deficiency in an infant with fatal bacille Calmette-Guerin infection. *N Engl J Med* (1996) 335(26):1956–61. doi:10.1056/NEJM199612263352604
19. Fiorini C, Jilani S, Losi CG, Silini A, Giliani S, Ferrari S, et al. A novel activation-induced cytidine deaminase gene mutation in a Tunisian family with hyper IgM syndrome. *Eur J Pediatr* (2004) 163(12):704–8. doi:10.1007/s00431-004-1540-8
20. Ouadani H, Ben-Mustapha I, Ben-ali M, Ben-khemis L, Largueche B, Boussoffara R, et al. Novel and recurrent AID mutations underlie prevalent autosomal recessive form of HIGM in consanguineous patients. *Immunogenetics* (2016) 68(1):19–28. doi:10.1007/s00251-015-0878-6
21. Mekki N, Ben-Mustapha I, Liu L, Boussofara L, Okada S, Cypowyj S, et al. IL-17 T cells' defective differentiation in vitro despite normal range ex vivo in chronic mucocutaneous candidiasis due to STAT1 mutation. *J Invest Dermatol* (2014) 134(4):1155–7. doi:10.1038/jid.2013.480
22. Ben-Ali M, Yang J, Chan KW, Ben-Mustapha I, Mekki N, Benabdesselem C, et al. Homozygous TCF3 mutation is associated with severe hypogammaglobulinemia and B-cell acute lymphoblastic leukemia. *J Allergy Clin Immunol* (2017). doi:10.1016/j.jaci.2017.04.037
23. Ouadani H, Ben-Mustapha I, Ben-Ali M, Largueche B, Jovanic T, Garcia S, et al. Activation induced cytidine deaminase mutant (AID-His130Pro) from hyper IgM 2 patient retained mutagenic activity on SHM artificial substrate. *Mol Immunol* (2016) 79:77–82. doi:10.1016/j.molimm.2016.09.025
24. Ben-Farhat K, Ben-Mustapha I, Ben-Ali M, Rouault K, Hamami S, Mekki N, et al. A founder effect of c.257 + 2T > C mutation in NCF2 gene underlies severe chronic granulomatous disease in eleven patients. *J Clin Immunol* (2016) 36(6):547–54. doi:10.1007/s10875-016-0299-9
25. Agrebi N, Sfaihi Ben-Mansour L, Medhaffar M, Hadiji S, Fedhila F, Ben-Ali M, et al. Autoimmune lymphoproliferative syndrome caused by homozygous FAS mutations with normal or residual protein expression. *J Allergy Clin Immunol* (2017). doi:10.1016/j.jaci.2016.11.033
26. Ben-Mustapha I, Ben-Ali M, Mekki N, Patin E, Harmant C, Bouguila J, et al. A 1,100-year-old founder effect mutation in IL12B gene is responsible for Mendelian susceptibility to mycobacterial disease in Tunisian patients. *Immunogenetics* (2014) 66(1):67–71. doi:10.1007/s00251-013-0739-0
27. Sassi A, Lazaroski S, Wu G, Haslam SM, Fliegauf M, Mellouli F, et al. Hypomorphic homozygous mutations in phosphoglucomutase 3 (PGM3) impair immunity and increase serum IgE levels. *J Allergy Clin Immunol* (2014) 133(5):1410–9, 1419.e1–13. doi:10.1016/j.jaci.2014.02.025
28. Ben Halim N, Ben Alaya Bouafif N, Romdhane L, Kefi Ben Atig R, Chouchane I, Bouyacoub Y, et al. Consanguinity, endogamy, and genetic disorders in Tunisia. *J Community Genet* (2013) 4(2):273–84. doi:10.1007/s12687-012-0128-7

29. Anwar WA, Khyatti M, Hemminki K. Consanguinity and genetic diseases in North Africa and immigrants to Europe. *Eur J Public Health* (2014) 24 (Suppl 1):57–63. doi:10.1093/eurpub/cku104
30. Harris ES, Weyrich AS, Zimmerman GA. Lessons from rare maladies: leukocyte adhesion deficiency syndromes. *Curr Opin Hematol* (2013) 20(1):16–25. doi:10.1097/MOH.0b013e32835a0091
31. Ben-Mustapha I, Ben-Farhat K, Guirat-Dhouib N, Dhemaied E, Largueche B, Ben-Ali M, et al. Clinical, immunological and genetic findings of a large Tunisian series of major histocompatibility complex class II deficiency patients. *J Clin Immunol* (2013) 33(4):865–70. doi:10.1007/s10875-013-9863-8
32. Mellouli F, Mustapha IB, Khaled MB, Besbes H, Ouederni M, Mekki N, et al. Report of the Tunisian registry of primary immunodeficiencies: 25-years of experience (1988–2012). *J Clin Immunol* (2015) 35(8):745–53. doi:10.1007/s10875-015-0206-9
33. Koker MY, Camcioglu Y, van Leeuwen K, Kilic SS, Barlan I, Yilmaz M, et al. Clinical, functional, and genetic characterization of chronic granulomatous disease in 89 Turkish patients. *J Allergy Clin Immunol* (2013) 132(5):1156–63. e5. doi:10.1016/j.jaci.2013.05.039
34. van den Berg JM, van Koppen E, Ahlin A, Belohradsky BH, Bernatowska E, Corbeel L, et al. Chronic granulomatous disease: the European experience. *PLoS One* (2009) 4(4):e5234. doi:10.1371/journal.pone.0005234
35. Lee WI, Torgerson TR, Schumacher MJ, Yel L, Zhu Q, Ochs HD. Molecular analysis of a large cohort of patients with the hyper immunoglobulin M (IgM) syndrome. *Blood* (2005) 105(5):1881–90. doi:10.1182/blood-2003-12-4420
36. Cabral-Marques O, Klaver S, Schimke LF, Ascendino EH, Khan TA, Pereira PV, et al. First report of the hyper-IgM syndrome Registry of the Latin American Society for immunodeficiencies: novel mutations, unique infections, and outcomes. *J Clin Immunol* (2014) 34(2):146–56. doi:10.1007/s10875-013-9980-4
37. Freeman AF, Holland SM. Clinical manifestations of hyper IgE syndromes. *Dis Markers* (2010) 29(3–4):123–30. doi:10.1155/2010/580197
38. Li C, Rodriguez M, Banerjee D. Cloning and characterization of complementary DNA encoding human N-acetylglucosamine-phosphate mutase protein. *Gene* (2000) 242(1–2):97–103. doi:10.1016/S0378-1119(99)00543-0
39. Zhang Y, Yu X, Ichikawa M, Lyons JJ, Datta S, Lamborn IT, et al. Autosomal recessive phosphoglucomutase 3 (PGM3) mutations link glycosylation defects to atopy, immune deficiency, autoimmunity, and neurocognitive impairment. *J Allergy Clin Immunol* (2014) 133(5):1400–9, 1409.e1–5. doi:10.1016/j.jaci.2014.02.013
40. Stray-Pedersen A, Backe PH, Sorte HS, Morkrid L, Chokshi NY, Erichsen HC, et al. PGM3 mutations cause a congenital disorder of glycosylation with severe immunodeficiency and skeletal dysplasia. *Am J Hum Genet* (2014) 95(1):96–107. doi:10.1016/j.ajhg.2014.05.007
41. Harms HK, Zimmer KP, Kurnik K, Bertele-Harms RM, Weidinger S, Reiter K. Oral mannose therapy persistently corrects the severe clinical symptoms and biochemical abnormalities of phosphomannose isomerase deficiency. *Acta Paediatr* (2002) 91(10):1065–72. doi:10.1111/j.1651-2227.2002.tb00101.x
42. Barbouche MR, Eley B. Considerations for primary immune deficiency disorders in Africa and the Middle East. In: Sullivan KE, Stiehm ER, editors. *Stiehm's Immune Deficiencies*. Elsevier (2014). p. 957–64.
43. Fisher GH, Rosenberg FJ, Straus SE, Dale JK, Middleton LA, Lin AY, et al. Dominant interfering Fas gene mutations impair apoptosis in a human autoimmune lymphoproliferative syndrome. *Cell* (1995) 81(6):935–46. doi:10.1016/0092-8674(95)90013-6
44. Jackson CE, Fischer RE, Hsu AP, Anderson SM, Choi Y, Wang J, et al. Autoimmune lymphoproliferative syndrome with defective Fas: genotype influences penetrance. *Am J Hum Genet* (1999) 64(4):1002–14. doi:10.1086/302333
45. Kuehn HS, Caminha I, Niemela JE, Rao VK, Davis J, Fleisher TA, et al. FAS haploinsufficiency is a common disease mechanism in the human autoimmune lymphoproliferative syndrome. *J Immunol* (2011) 186(10):6035–43. doi:10.4049/jimmunol.1100021
46. Worth A, Thrasher AJ, Gaspar HB. Autoimmune lymphoproliferative syndrome: molecular basis of disease and clinical phenotype. *Br J Haematol* (2006) 133(2):124–40. doi:10.1111/j.1365-2141.2006.05993.x
47. Boisson B, Wang YD, Bosompem A, Ma CS, Lim A, Kochetkov T, et al. A recurrent dominant negative E47 mutation causes agammaglobulinemia and BCR(-) B cells. *J Clin Invest* (2013) 123(11):4781–5. doi:10.1172/JCI71927
48. Djidjik R, Messaoudani N, Tahiat A, Meddour Y, Chaib S, Atek A, et al. Clinical, immunological and genetic features in eleven Algerian patients with major histocompatibility complex class II expression deficiency. *Allergy Asthma Clin Immunol* (2012) 8(1):14. doi:10.1186/1710-1492-8-14
49. Romdhane L, Kefi R, Azaiez H, Ben Halim N, Dellagi K, Abdelhak S. Founder mutations in Tunisia: implications for diagnosis in North Africa and Middle East. *Orphanet J Rare Dis* (2012) 7:52. doi:10.1186/1750-1172-7-52
50. Al-Herz W, Alsmadi O, Melhem M, Recher M, Frugoni F, Notarangelo LD. Major histocompatibility complex class II deficiency in Kuwait: clinical manifestations, immunological findings and molecular profile. *J Clin Immunol* (2013) 33(3):513–9. doi:10.1007/s10875-012-9831-8
51. Clarridge K, Leitenberg D, Loechelt B, Picard C, Keller M. Major histocompatibility complex class II deficiency due to a novel mutation in RFXANK in a child of Mexican descent. *J Clin Immunol* (2016) 36(1):4–5. doi:10.1007/s10875-015-0219-4
52. Prod'homme T, Dekel B, Barbieri G, Lisowska-Grospierre B, Katz R, Charron D, et al. Splicing defect in RFXANK results in a moderate combined immunodeficiency and long-duration clinical course. *Immunogenetics* (2003) 55(8):530–9. doi:10.1007/s00251-003-0609-2
53. Corneo B, Moshous D, Gungor T, Wulffraat N, Philippet P, Le Deist FL, et al. Identical mutations in RAG1 or RAG2 genes leading to defective V(D)J recombinase activity can cause either T-B-severe combined immune deficiency or Omenn syndrome. *Blood* (2001) 97(9):2772–6. doi:10.1182/blood.V97.9.2772
54. de Villartay JP, Lim A, Al-Mousa H, Dupont S, Dechanet-Merville J, Coumau-Gatbois E, et al. A novel immunodeficiency associated with hypomorphic RAG1 mutations and CMV infection. *J Clin Invest* (2005) 115 (11):3291–9. doi:10.1172/JCI25178
55. IJspeert H, Driessen GJ, Moorhouse MJ, Hartwig NG, Wolska-Kusnierz B, Kalwak K, et al. Similar recombination-activating gene (RAG) mutations result in similar immunobiological effects but in different clinical phenotypes. *J Allergy Clin Immunol* (2014) 133(4):1124–33. doi:10.1016/j.jaci.2013.11.028
56. Martinez-Martinez L, Vazquez-Ortiz M, Gonzalez-Santesteban C, Martin-Nalda A, Vicente A, Plaza AM, et al. From severe combined immunodeficiency to Omenn syndrome after hematopoietic stem cell transplantation in a RAG1 deficient family. *Pediatr Allergy Immunol* (2012) 23(7):660–6. doi:10.1111/j.1399-3038.2012.01339.x
57. Lanternier F, Pathan S, Vincent QB, Liu L, Cypowyj S, Prando C, et al. Deep dermatophytosis and inherited CARD9 deficiency. *N Engl J Med* (2013) 369(18):1704–14. doi:10.1056/NEJMoa1208487
58. Romdhane L, Abdelhak S; Research Unit on Molecular Investigation of Genetic Orphan Diseases, Collaborators. Genetic diseases in the Tunisian population. *Am J Med Genet A* (2011) 155A(1):238–67. doi:10.1002/ajmg.a.33771

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REVIEW
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Cellular and Molecular Defects Underlying Invasive Fungal Infections—Revelations from Endemic Mycoses

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The global burden of fungal diseases has been increasing, as a result of the expanding number of susceptible individuals including people living with human immunodeficiency virus (HIV), hematopoietic stem cell or organ transplant recipients, patients with malignancies or immunological conditions receiving immunosuppressive treatment, premature neonates, and the elderly. Opportunistic fungal pathogens such as *Aspergillus, Candida, Cryptococcus, Rhizopus,* and *Pneumocystis jiroveci* are distributed worldwide and constitute the majority of invasive fungal infections (IFIs). Dimorphic fungi such as *Histoplasma capsulatum*, *Coccidioides* spp., *Paracoccidioides* spp., *Blastomyces dermatiditis*, *Sporothrix schenckii*, *Talaromyces (Penicillium) marneffei*, *and Emmonsia* spp. are geographically restricted to their respective habitats and cause endemic mycoses. Disseminated histoplasmosis, coccidioidomycosis, and *T. marneffei* infection are recognized as acquired immunodeficiency syndrome (AIDS)-defining conditions, while the rest also cause high rate of morbidities and mortalities in patients with HIV infection and other immunocompromised conditions. In the past decade, a growing number of monogenic immunodeficiency disorders causing increased susceptibility to fungal infections have been discovered. In particular, defects of the IL-12/IFN-γ pathway and T-helper 17-mediated response are associated with increased susceptibility to endemic mycoses. In this review, we put together the various forms of endemic mycoses on the map and take a journey around the world to examine how cellular and molecular defects of the immune system predispose to invasive endemic fungal infections, including primary immunodeficiencies, individuals with autoantibodies against interferon-γ, and those receiving biologic response modifiers. Though rare, these conditions provide importance insights to host defense mechanisms against endemic fungi, which can only be appreciated in unique climatic and geographical regions.

Keywords: endemic mycoses, primary immunodeficiencies, human immunodeficiency virus, histoplasmosis, coccidioidomycosis, paracoccidioidomycosis, blastomycosis, *Taloromyces marneffei*

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INTRODUCTION

Endemic mycoses are infections caused by a diverse group of fungi that occupy specific ecologic niche in the environment (1). The major pathogenic fungi in this group, including *Blastomyces dermatiditis, Coccidioides immitis* and *C. posadasii*, *Paracoccidioides brasiliensis* and *P. lutzii, Histoplasma capsulatum, Sporothrix schenckii, Talaromyces marneffei* (formerly known as

Penicillium marneffei) and *Emmonsia* spp., belong to the phylum *Ascomycota* and are evolutionary related (2) (**Table 1**). They share the common characteristic of thermal dimorphism—they grow as saprophytic molds in the environment at temperatures ranging from 25 to 30°C, and undergo morphological switch to the yeast form, or spherules in *Coccidioides*, at body temperatures of mammalian hosts. The yeast form serves to accommodate intracellular growth within host phagocytes (3). Majority of these organisms are primary pathogens that are able to cause disease in healthy human individuals. However, they may cause severe, disseminated infections in immunocompromised hosts, such as patients with human immunodeficiency virus (HIV) infection, organ transplant, or hematopoietic stem cell transplant (HSCT) recipients, and those with autoimmune disorders receiving immunosuppressants (4–7). In particular, *T. marneffei* and *Emmonsia* spp. more typically cause disease in HIV-infected individuals (5–8). The HIV pandemic and the increasing use of immunosuppressive medications, such as calcineurin and tumor necrosis factor (TNF) inhibitors, have resulted in a rising trend of histoplasmosis and coccidioidomycosis in endemic regions (9, 10). Exposure to the specific environmental niche, either residential, occupational, or travel precedes the development of disease.

GEOGRAPHICAL DISTRIBUTION OF ENDEMIC MYCOSES

Endemic mycoses occur predominantly in specific climate zones. Coccidioidomycosis is present in semidesert areas, histoplasmosis and paracoccidioidomycosis (PCM) are prevalent in tropical regions while *T. marneffei* is endemic in subtropical regions, and blastomycosis belongs to temperate climates (6–8). Coccidioioidomycosis (11–13) and histoplasmosis (14–18) are widely distributed in the American continent and some tropical regions, while PCM is limited to Central and South America (19–21). *T. marneffei* is unique to Southeast Asia (22–24), and blastomycosis is found in North America, and Central and East Africa (25–27). *S. schenckii* is distributed around the world, mainly reported in those tropical and temperate zones with high humidity and mild temperatures (22–27°C) (28). Recently, an emerging thermally dimorphic fungus within the genus *Emmonsia* that is most closely related to *E. pasteuriana* has been recognized to be uniquely associated with in HIV infection in South Africa (29–31). The geographical distribution of endemic mycoses is shown in **Figure 1** (1, 12–14, 18–20, 27, 28, 32–39).

The biological niche is specific for each endemic fungus and knowledge of their natural habitat provides understanding about the risk factors for exposure to these pathogens (**Table 2**). *C. immitis* and *C. posadasii* are saprophytic fungi, which exist in their mycelial form in dry, alkaline soil in deserts with very low precipitation and extreme temperature variations (11–13). Coccidioidomycosis is most prevalent in Arizona and California in the United States (US) (40, 41). In contrast, *H. capsulatum* thrive in the tropical zones with high relative humidity, and its growth is favored by soil contaminated by bird and chicken excreta or bat guano, which creates an environment with high nitrogen content (14, 15). *P. brasiliensis* is also found in the tropical and very humid regions, especially in acidic soil where coffee and sugar canes are cultivated (20). PCM is prevalent in South America (Brazil, Columbia, Venezuela, Paraguay) and some regions of Central America and Mexico (19–21). *B. dermatitidis* exists in wet soils, and the most significant endemic epicenter is in Eastern US between the Ohio and Mississippi River valleys (25–27). *T. marneffei* is highly endemic in Thailand, Vietnam, Southern China, and other subtropical areas in Southeast Asia (22–24). Bamboo rats (*Rhizomys* spp. and *Cannomys* spp.) and soil from their burrows are important enzootic and environmental reservoirs of *T. marneffei*, respectively (39). *S. schenckii* is found in the soil containing decaying vegetation such as dead wood,

TABLE 1 | Endemic regions, natural habitats and risk factors of exposure to endemic mycoses.

	Phylum	Order	Endemic regions	Animal hosts	Disease in human
Coccidioides immitis, C. posadasii	Ascomycota	Onygenales	Southwestern USA, northern Mexico, Central and South America	Non-human primates, domesticated or wide mammals, dogs, cats, horses, llamas, snakes	Coccidioidomycosis
Paracoccidioides brasiliensis, P. lutzii	Ascomycota	Onygenales	South America	Domesticated and wild animals (monkeys and armadillos), dogs	Paracoccidioidomycosis
Histoplasma capsulatum	Ascomycota	Onygenales	Worldwide; hyperendemic in Mississippi and Ohio river valleys in USA	Cattle, sheep, horses	Histoplasmosis
Blastomyces dermatitidis	Ascomycota	Onygenales	Worldwide (endemic in North America, autochthonous in Africa, South America, and Asia)	Dogs, cats, horses, marine mammals	Blastomycosis
Emmonsia spp.	Ascomycota	Onygenales	South Africa	Wild rodents	Emmonsiosis
Sporothrix schenckii, S. brasiliensis	Ascomycota	Ophiostomales	Worldwide	Cats, occasionally dogs, horses, cows, goats, mules, pigs, rats, armadillos, camels, dolphins, birds	Sporotrichosis
Talaromyces (Penicillium) marneffei	Ascomycota	Eurotiales	Southwest and southern China; Southeast Asia	Bamboo rats, domestic animals such as dogs and cats	Penicilliosis

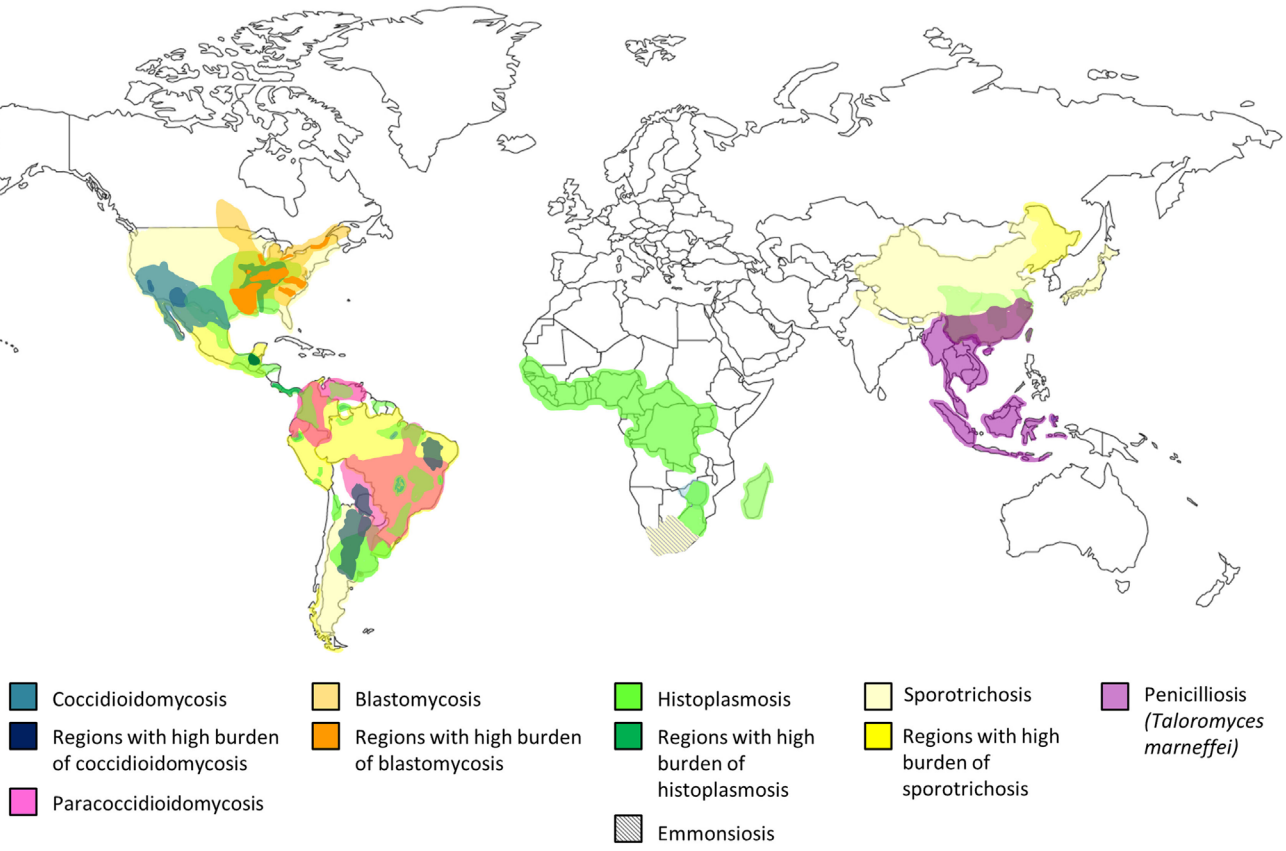

FIGURE 1 | Global distribution of endemic mycosis (1, 13, 14, 18–20, 27, 28, 32–39).

mosses, hay, and cornstalks. Sporotrichosis is also widely prevalent in warm-blooded animals including cats, dogs, armadillos, birds, and parrots, which constitute a source of zoonotic transmission (28). To date, disseminated emmonsiosis associated with HIV infection caused by the new *Emmonsia* spp. has only been described in South Africa (29–31).

PATHOGENESIS AND CLINICAL MANIFESTATIONS

The acquisition of endemic fungi relates to human activities and climatic conditions that increase the risk of exposure to these organisms in susceptible individuals. Coccidioidomycosis, PCM, histoplasmosis, blastomycosis, and *T. marneffei* infection are acquired by the respiratory route. Occupational or recreational activities causing disturbance of the soil environment lead to aerosolization of the conidia, which could then be inhaled by exposed individuals to cause infection (1, 6–8, 14, 20, 22, 23, 27). In contrast, inhalation is not the major route by which *S. schenkii* is acquired. Instead, it typically occurs after traumatic inoculation or through microscopic breaks in the skin caused by pricks with plants, although the mode of transmission was not obvious in 60% of patients with sporotrichosis. Infection of the skin and subcutaneous tissues develops at the site of penetrating trauma and may spread to the muscles, fascia, cartilage, and bones (42, 43). The clinical features of these endemic mycoses are summarized in **Table 3**.

After entry to the body, inhaled conidia converts to the yeast form, which are taken up by tissue-resident macrophages. In most individuals, the pathogenic yeasts can be eliminated by the macrophages and the infection is usually asymptomatic or mild, and self-limiting in most cases. In patients whose immunity is compromised, the yeasts continue to proliferate in the macrophages, which if uncontained, systemic dissemination may occur *via* the reticuloendothelial system (12, 23, 44–46). HIV infection is the most important risk factor for disseminated endemic mycoses (5). Other risk factors include malignancy and immunosuppression, as listed in **Table 3** (4–7, 9, 10).

ENDEMIC MYCOSES: INSIGHTS FROM THE HIV EPIDEMIC

Human immunodeficiency virus infection is the most common cause for disseminated or extrapulmonary forms of histoplasmosis, coccidioidomycosis, and *T. marneffei* infection, particularly in patients with profound T-cell lymphopenia (CD4+ lymphocytes <200/μL) (12, 47–50). They are considered as acquired immunodeficiency syndrome (AIDS)-defining conditions in the World Health Organization (WHO) clinical staging of HIV/AIDS for adults and adolescents (51). The association between PCM and

TABLE 2 | Endemic regions, natural habitats, and risk factors of exposure to endemic mycoses.

	Main endemic regions	Other areas	Natural habitat	Human activities/ conditions associated with increased risk of exposure	Occupations associated with increased risk of exposure
Coccidioidomycosis	Arizona and California in the US	Other parts of Southwestern US: New Mexico, Nevada, Utah and Texas Central America: Mexico, Guatemala, Honduras South America: Venezuela, Brazil, Argentina, Paraguay	Alkaline soils in dry desert climates	Soil excavations Dust storms, earthquakes	Construction site workers, farmers, military personnel, excavators, archeologists, inmates, and officers in correctional facilities
Histoplasmosis	*Histoplasma capsulatum var. capsulatum*: Ohio and Mississippi River Valleys in the Upper Midwest and Southeastern US *H. capsulatum var. duboisii* (African histoplasmosis): between 20° North and 20° South of the equator, and Madagascar	Southern Mexico Central and South America, e.g., Brazil, Uruguay, Paraguay, Argentina, Venezuela Mainland China: provinces along the Yangtze River (Yunnan, Sichuan, Hubei, Hunan, Jiangsu, Zhejiang) Southeast Asia, e.g., Thailand India, especially West Bengal and Uttar Pradesh along the Gangetic plains Europe: Italy (Po River Valley), Spain, Germany	Soil contaminated by bird and chicken excreta, or bat guano; bat caves	Walking on contaminated grounds, setting up tents Excavation, clearing foliage in a bird-roosting site	Miners, cave explorers, guano workers, farmers, beekeepers, archeologists
Paracoccidioidomycosis	*Paracoccidioides brasiliensis*: Brazil, Columbia, Venezuela, Paraguay *P. lutzii*: Center-West of Brazil	Central America and Mexico	Acid soils in area of coffee and sugar cane plantations	Soil exposure	Farmers, outdoor workers Women are less likely to develop clinical disease as estrogens inhibit conidial transformation to yeast cells
Blastomycosis	US: Mississippi and Ohio River valleys, Midwestern states Canada: provinces that border the Great Lakes and the Saint Lawrence Riverway, including Manitoba and northwestern Ontario	Middle and East Africa India	Warm, moist soil with high organic content, e.g., animal droppings	Occupational, residential, or recreational exposures to wildlife, soil, or bodies of freshwater	Occupational, residential, or recreational exposures that occur in close proximity to bodies of freshwater
Talaromyces marneffei infection	Thailand, Vietnam, Southern China	Laos, Malaysia, Myanmar, Cambodia, Hong Kong, Taiwan, Northeastern India	Soil, particularly burrows of bamboo rats	Soil exposure during rainy season	Agricultural workers
Sporotrichosis	Peru, Brazil, Mexico (Jalisco and Puebla mountain ranges)	Worldwide distribution in temperate and tropical regions—US, Asia (China, India, Japan), Australia	Soil and decaying vegetation, e.g., dead wood, sphagnum moss, cornstalks, hay	Cutaneous trauma with wound contamination by plants or soil; contact with reeds after flooding, bites from mice, armadillos, squirrels, cats, and dogs	Farming, gardening, flower vending, handling hay, animal husbandry, armadillo hunting (in Uruguay), mining

AIDS is relatively rare in contrast to the higher incidence of other systemic mycosis. HIV coinfection has been detected in about 5% of patients with PCM (52, 53). Although PCM and sporotrichosis are not included as AIDS-defining conditions, they are increasingly recognized as an emerging neglected opportunistic infections in HIV patients in Latin America (7, 53, 54). Systemic sporotrichosis with organ involvement or widespread cutaneous lesions may occur in these patients, causing a mortality rate of up to 30% (54, 55). Blastomycosis infrequently develops in HIV patients, but disease tends to be more severe with increased risk of central nervous system (CNS) involvement with high mortality (46, 56). The new species of *Emmonsia* spp. discovered in South

TABLE 3 | Clinical manifestations of endemic mycoses and risk factors for disseminated disease.

	Asymptomatic infections	Sites of initial infection	Distant spread/disseminated disease	Conditions predisposing to disseminated disease
Coccidioidomycosis	Asymptomatic infections in majority of immunocompetent individuals	Pneumonia, often as mild respiratory illness Rarely primary cutaneous lesions at the site of inoculation due to injury	Fungemia, lymphadenopathy, skin lesions (in the vicinity of infected lymph nodes manifesting as abscesses, ulcers, gummata, retracting scars), osteoarticular involvement, meningitis	HIV infection PID Patients on anti-TNF-alpha monoclonal antibodies Chemotherapy, organ transplant and HSCT, immunosuppressants Diabetes mellitus, cardiopulmonary disease, pregnancy Higher risk of dissemination in African-American and Filipino
Histoplasmosis	Mostly acquired during childhood as asymptomatic infection	Most are self-limiting Acute pulmonary histoplasmosis: fever, cough, dyspnea, enlarged mediastinal, or hilar lymph nodes Chronic pulmonary histoplasmosis: cavitating lung lesions Rarely primary cutaneous lesions by injury—chancre, lymphangitis, nodular gummata	Fungemia, hepatomegaly, splenomegaly, bone marrow involvement, pancytopenia, reactive hemophagocytosis, oropharyngeal ulcers, gastrointestinal bleeding, endocarditis, skin lesions (molluscum-like papules, nodular/gummatous lesions), meningeal involvement, adrenal (Addison's disease)	HIV infection PID Patients on anti-TNF-alpha monoclonal antibodies Chemotherapy, organ transplant, and HSCT, immunosuppressants
Paracoccidioidomycosis	Asymptomatic infections in majority of immunocompetent individuals	Juvenile form: generalized lymphadenopathy, hepatosplenomegaly, lesions in the skin, oral and intestinal mucosa, bone involvement Chronic ("adult") form: pneumonia, mucosal lesions in the oropharyngeal or nasal region, palatal ulceration extending to the gums and tongue	Involvement of the digestive tract, pancreas and adrenal glands; hepatomegaly, splenomegaly	HIV infection PID
Blastomycosis	Asymptomatic infections in majority of immunocompetent individuals	Pneumonia Rarely primary skin involvement at the site of inoculation due to injury, manifesting as lymphangitis, ulcers, nodules, verruca	Skin involvement (nodules, gummata, abscesses, ulcers)	Uncommon association with acquired immunodeficiencies; no case of PID identified in individuals with blastomycosis
Talaromyces marneffei infection	Asymptomatic infections in majority of immunocompetent individuals	Localized skin disease due to direct inoculation Lymphadenitis Pneumonia	Fungemia, pneumonia, hepatomegaly, splenomegaly, lymphadenopathy, bone marrow involvement, osteoarticular involvement, cutaneous lesions, neurological manifestations	HIV infection PID Individuals with autoantibodies against IFN-gamma Splenectomy, diabetes mellitus, autoimmune disease Chemotherapy, organ transplant and HSCT, immunosuppressants Novel anti-cancer target therapies, e.g., anti-CD20 monoclonal antibodies, kinase inhibitors
Sporotrichosis	Most cases are acquired through traumatic implantations, often with spontaneous resolution	Skin infections may progress into chronic cutaneous, subcutaneous, or deeper infections involving the lymphatics, fascia, muscles, cartilage and bones	Occasional cases of pulmonary or disseminated disease: multiple skin lesions at non-contiguous sites, mucosal (nasal, oral, conjunctival), osteoarticular, pulmonary and meningeal involvement	HIV infection PID Chemotherapy, organ transplant and HSCT, immunosuppressants Diabetes mellitus, alcoholism, cirrhosis, malnutrition

HIV, human immunodeficiency virus; HSCT, hematopoietic stem cell transplant; IFN, interferon; TNF, tumor necrosis factor; PID, primary immunodeficiencies.

Africa was found to cause disseminated infection almost exclusively in patients with AIDS (29–31).

The first case series of histoplasmosis in HIV-infected patients was described in 1982 in the US (57). Extrapulmonary or disseminated histoplasmosis became an AIDS-defining disease in 1987 (58). The increase in morbidity and mortality from histoplasmosis has been largely contributed by the HIV pandemic. In the US, the availability of highly active anti-retroviral therapy (HAART) and lipid formulations of amphotericin B, the increased awareness of the disease, and the development of rapid, non-invasive diagnostic methods led to decrease in the incidence and mortality associated with histoplasmosis in patients with

AIDS (16). On the other hand, extrapulmonary or disseminated histoplasmosis is becoming an important health issue in the increasing number of patients receiving chemotherapy, solid organ or HSCT, immunosuppressive treatment especially TNF-α blockade (59), as well as a rare group of patients with primary immunodeficiencies (PIDs). In contrast, in endemic areas of Latin America histoplasmosis occurs in up to 25% of HIV-infected patients and represents the first manifestation of AIDS in up to 50–75% of patients (60, 61). Due to the lack of diagnostic facilities and algorithm, histoplasmosis is undiagnosed in many HIV-infected patients and is considered as an "invisible burden" of AIDS in less resourced countries (62, 63). These phenomena illustrate how demographics of histoplasmosis could be shaped by HIV burden and socioeconomic forces in different endemic regions.

Shortly after the description of increased susceptibility to histoplasmosis in AIDS, *Coccidioides* infection emerged as an important form of mycosis in patients with HIV infection (64–66). A prospective study at an Arizona HIV clinic in 1988 showed a cumulative incidence of active coccidioidomycosis of 25% during 41 months of follow-up, corresponding to an average annual incidence of 7.3% (67). In contrast, in a retrospective review at the same clinic during 2003–2008, only 11.3% of HIV-infected patients ($n = 257$) had coccidioidomycosis, and the annual incidence was only 0.9% when compared to the previous study (47). Both studies showed that CD4 count was the only predictor for developing active coccidioidomycosis; factors such as a history of coccidioidomycosis and duration of residence in an endemic area or age, sex, race, ethnicity, plasma HIV RNA level, or receipt of HAART were not associated with increased risk for coccidioidomycosis (11, 47, 67).

While histoplasmosis and coccidioidomycosis are well recognized as human pathogens long before the HIV epidemic, the importance of *T. marneffei* as a human disease was not recognized until the outbreak of HIV in Asia (22, 68, 69). In 1973, the first naturally occurring human case of *T. marneffei* infection was reported in an American minister with Hodgkin's disease who had been residing in Southeast Asia (70). No more than 50 cases were reported in the literature during the early 1980s (68–76). From 1988, *T. marneffei* infection was increasingly observed in patients with advanced HIV infection, initially in foreign visitors who have been to endemic regions, and later in local residents who were native to endemic parts of Thailand and Vietnam (22, 23, 77–79). In Northern Thailand, *T. marneffei* infection is the third most common opportunistic infection, accounting for 15–20% of all AIDS-related illness, after tuberculosis (TB) and cryptococcosis. *T. marneffei* infection is estimated to occur in 2.3% of new AIDS cases, compared with 0.39% for histoplasmosis (80). The trend of *T. marneffei* infection closely paralleled that of HIV, and in areas where reduction of HIV transmission and availability of HAART have improved, a decrease in the prevalence of *T. marneffei* infection has been observed (23, 81).

Together with *Emmonsia* spp., which causes disseminated infection almost exclusively in advanced HIV infection in South Africa (29–31), it is apparent that the epidemiology of coccidioidomycosis, histoplasmosis, and *P. marneffei* evolves with HIV epidemic. The close relationship between disease manifestation and severity with CD4+ cell count confirms the central importance of cell-mediated immunity against endemic fungi.

PIDs IN ENDEMIC MYCOSES: NEEDLES IN A HAYSTACK?

Primary immunodeficiencies are rare inborn errors of immunity. Defects of T-cell development and differentiation, phagocytic functions, and pathways involved in the innate recognition of pathogens and downstream signaling are associated with increased risk of fungal infections, the most common being candidiasis and aspergillosis. Endemic mycoses are rarely described in patients with PID, and little is known about the spectrum of PID associated with increased susceptibility to endemic fungi. On the other hand, as individuals who are apparently healthy can also develop disease caused by endemic fungi, recognition of those who may have an underlying PID could be a challenge.

Talaromyces marneffei infection is mostly seen in advanced HIV infection with CD4+ cell count <100/μL, and in fact, up to 80% or more of the cases have CD4+ count <50/μL (22, 81, 82). Only small proportion of disseminated *T. marneffei* infection occurs in patients with secondary immunodeficiencies (22, 23, 83, 84). It is otherwise rare in healthy persons, especially in children. The close epidemiological relationship between HIV and *T. marneffei*, and the fact that *T. marneffei* is an AIDS-defining illness (51) suggests that individuals who are HIV negative and without secondary immunodeficiencies may have underlying immune defects that are unrecognized. A systematic review by Lee et al (85) on more than 500 articles published in English and Chinese from 1970 to 2011 on penicilliosis revealed 32 patients aged 3 months to 16 years with *T. marneffei* infection but without known HIV infection. Twenty-four patients (75%) had disseminated disease, and 55% died of *T. marneffei* infection. Eight patients had PID or blood disorders, while four others had abnormal immune functions. Immune evaluations of the remaining patients were unstated. This observation highlights the knowledge gap in the immunological susceptibility to *T. marneffei*.

Two systematic reviews on PID in histoplasmosis and coccidioidomycoses were recently published. Lovell et al. (86) summarized all published cases of histoplasmosis in patients with PID up to August 2015 and revealed 47 patients with underlying PID, defined either molecularly or clinically. Together with the four patients described in their report, more than 50 PID patients have been documented to have *Histoplasma* infection. Disseminated histoplasmosis occurred in 68% of cases, and two deaths occurred because of progressive disease. Another systematic literature search on disseminated coccidioidomycosis yielded 370 case reports, and 8 cases of PID were identified (87). The frequency of PID underlying endemic mycoses is unknown. Given the rarity of PID, the proportion of PID accounting for disseminated endemic mycoses is likely to be small. However, the cellular and molecular defects of these PID can provide important mechanistic insights into host defense mechanisms against endemic fungi. More importantly, disseminated or extrapulmonary forms of endemic mycoses can be utilized as unique indicators for PIDs, which is of particular relevance to clinicians working in respective endemic areas (88).

PIDs UNDERLYING FUNGAL INFECTIONS

Host immune response toward fungal pathogens is initiated by the recognition of invading fungi *via* pattern recognition receptors (PRRs) expressed on neutrophils, monocytes, macrophages, and dendritic cells (DCs). Receptor-mediated signaling induces downstream events such as cytokine and chemokine release, phagocytosis, and respiratory burst ultimately leading to fungal killing (89–93). In addition, the cytokine responses shape the induction of Th-1 and Th-17 adaptive immune response. IL-12 drives IFN-γ production by T-helper 1 (Th1) cells, which is crucial for phagocyte activation. On the other hand, IL-1β, IL-6, and IL-23 promotes Th17 differentiation (89–91). The various PRRs that recognize fungal pathogen-associated molecular patterns and the downstream signaling pathways leading to induction of Th1 and Th17 response are shown in **Figure 2A**.

Defects of the dectin-1/CARD9-MALT1-BCL10 signaling pathway are associated with chronic mucocutaneous candidiasis (CMC) (94). Patients with CARD9 deficiency have defects in Th17 differentiation and impaired neutrophil killing (95), and they are susceptible to CMC (96), deep dermatophytoses (97), and invasive fungal infection, particularly *Candida* meningitis. Other monogenic disorders causing CMC include autosomal recessive (AR) IL-17 receptor A (IL17RA), AR IL-17RC, AR ACT1, and autosomal-dominant (AD) IL-17F deficiencies (98). These patients display deficiency of IL-17F and IL-17A/F (*IL-17F* mutations) or dysfunctional responses to IL-17A, IL-17A/F, and IL-17F (*IL17RA*, *IL-17RC*, and *ACT1* mutations). In patients with autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy, high plasma titers of neutralizing autoantibodies against IL-17A, IL-17F, and IL-22 can be detected, as a result of the lack of AIRE expression in the thymus causing impaired T-cell tolerance (99). However, endemic mycoses have not been reported in patients with these genetic defects.

Opportunistic fungal infections are common in patients with severe combined immunodeficiencies (SCIDs) and phagocytic disorders. Infants with classical SCID often have recurrent or persistent mucosal and/or cutaneous candidiasis involving the orodigestive tract, genital area, nails, and skin. *Pneumocystis jiroveci* pneumonia (PCP) and invasive fungal infections (IFIs) including systemic candidiasis and aspergillosis are often life-threatening (100, 101). Interestingly, endemic mycoses have not been described in SCID babies, at least in the English literature. It may be due to the fact that it is uncommon for infants to be exposed to the natural habitats containing those endemic fungi, but further epidemiological data would be required to address this. Numerical and functional defects of phagocytes such as severe congenital neutropenia, chronic granulomatous disease (CGD), and leukocyte adhesion deficiency (LAD) are major groups of PIDs predisposing to systemic candidiasis and invasive aspergillosis (102–104). Filamentous fungi other than *Aspergillus* causing pulmonary infections in CGD include *Geosmithia argillacea* and *Trichosporon inkin*. Osteomyelitis can also be caused by rare non-*Aspergillus* filamentous fungi, including *Cladophialophora arxii*, *Inonotus tropicalis*, *Scedospoium apiospermum*, *Penicillium piceum*, and *P. variotii*. Cerebral abscesses caused by dematiaceous molds such as *Exophiala* spp., *Phaeoacremonium* spp., and *Alternaria* spp. have been reported (105–107). Interestingly, endemic mycoses have not been reported in CGD and LAD.

In the following sections, we highlight the spectrum of PIDs, which are associated with increased susceptibility to disseminated endemic mycoses. The genetic defects are summarized in **Figure 2B**.

Combined Immunodeficiencies: CD40 Ligand, Nuclear Factor Kappa B (NF-κB) Essential Modulator (NEMO), and DOCK8 Deficiencies

Mucosal candidiasis is common in combined immunodeficiencies e.g. CD40 ligand (CD40L) deficiency, NEMO (IKBG) deficiency, IKBA gain-of-function (GOF) mutation and DOCK8 deficiency, in addition to a broad range of viral, bacterial, and IFI (102, 103). CD40L is expressed on activated T-cells and signals through NEMO/NF-κB to induce IL-12 production. CD40L deficiency, also known as hyper-IgM syndrome, is inherited in an X-linked recessive manner. Patients are susceptible to opportunistic infections including PCP, cryptosporidiosis, and mycobacterial infections due to impaired interaction between T-cells and antigen-presenting cells (APCs). In addition, the failure of B-cell immunoglobulin (Ig) isotype switching results in markedly low serum IgG and IgA, while IgM is elevated (108, 109). Disseminated and cutaneous forms of histoplasmosis have been reported in eight patients in patients with X-linked hyper-IgM disorder (**Table 4**). Five cases had disseminated histoplasmosis, while two had lymphadenitis and one had cutaneous involvement only (86, 110–115). All of them responded well to antifungal therapy, and only two patients had recurrent histoplasmosis (86, 111). One case of PCM was reported in Brazil (116). It was shown that mature DCs from patients with CD40L deficiency exhibited markedly reduced IL-12 and increased IL-10 production in response to *P. brasiliensis* and *C. albicans* compared with normal controls, and T cells had significantly reduced IFN-γ production when cocultured with their DCs, whereas IL-4 and IL-5 production was increased. In contrast, T-cell proliferation and generation of TGF-β and IL-17 were comparable with normal controls. These findings suggested that the absence of CD40L during monocyte/DC differentiation leads to functional DC abnormalities, which may contribute to the susceptibility to fungal infections in patients with CD40L deficiency (117). Four cases of *T. marneffei* infection were reported in CD40L deficiency (118–120). One patient who had disseminated *T. marneffei* infection had rapid deterioration due to late diagnosis and died, and CD40L deficiency was diagnosed after he passed away (120).

One patient with NEMO deficiency was reported to have persistent nodal histoplamosis at the age of 52 years. Symptoms including worsening dyspnea and intermittent night sweats that lasted for 1 year, and imaging studies revealed perihilar mass and mediastinal lymphadenopathy. Positive culture of *H. capsulatum* was obtained from paratracheal lymph node biopsy. He responded well to posaconazole (86). A patient with DOCK8 deficiency had miliary pneumonia caused by *H. capsulatum*. She also had numerous infectious caused by viruses (molluscum contagiosum, recurrent herpes zoster, cutaneous human papilloma

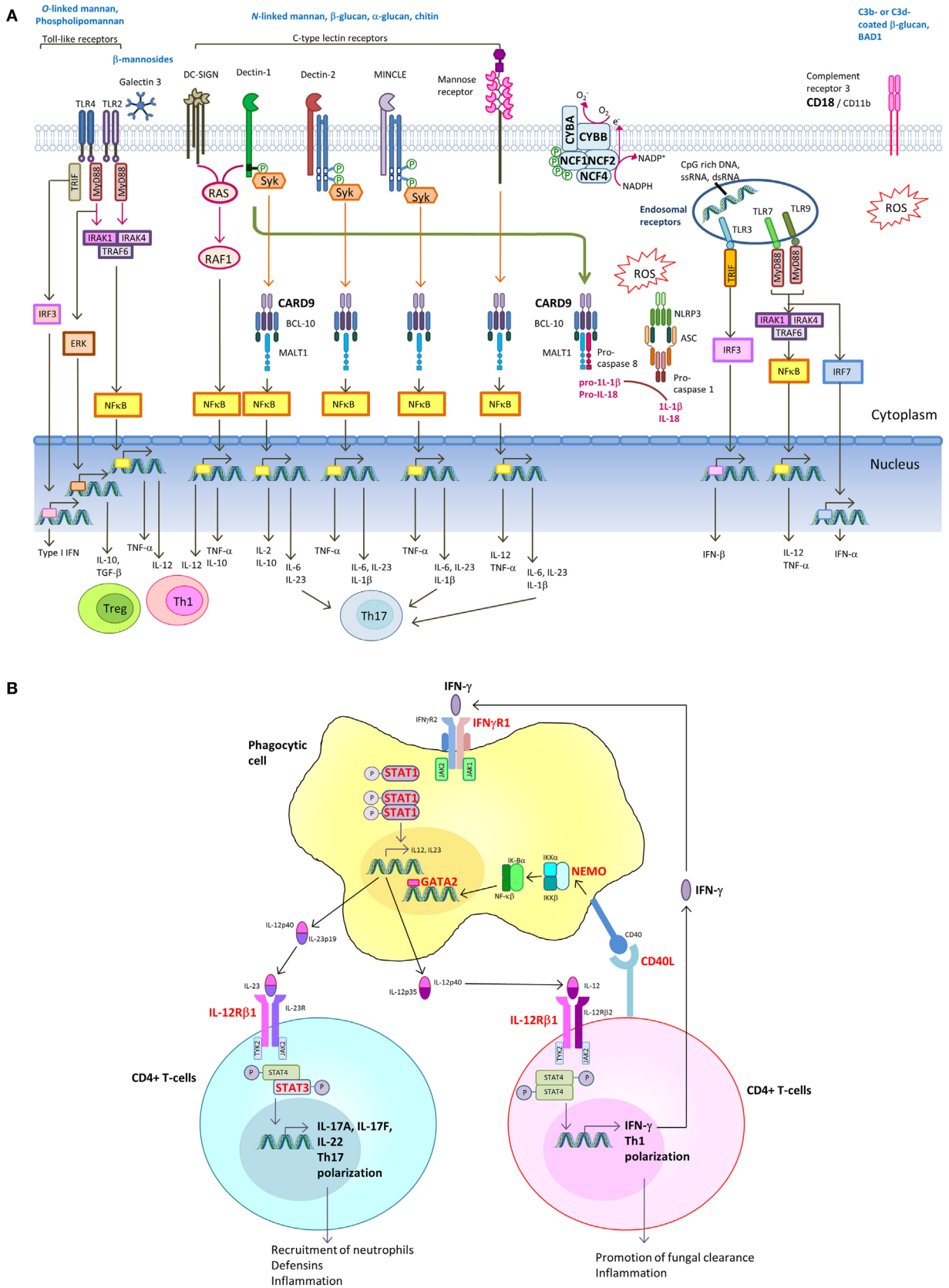

FIGURE 2 | Continued

FIGURE 2 | Continued
Signaling pathways in innate recognition of fungal pathogens and differentiation of CD4+ T helper cells. **(A)** Pathogen-associated molecular patterns (PAMPs) expressed by fungi are recognized by host pattern recognition receptors (PRRs), including toll-like receptors (TLRs), C-type lectin receptors (CLRs) [e.g., dendritic cell (DC)-specific ICAM3-grabbing non-integrin (DC-SIGN), Dectin-1, Dectin-2, MINCLE, and mannose receptor] and complement receptor 3 (CR3). TLRs and CLRs activate multiple intracellular signaling pathways upon binding to specific fungal PAMPs, including β-glucans, chitin, *O*-linked mannan and *N*-linked mannan, and nucleic acids. These signals activate canonical or non-canonical nuclear factor-κB and the NOD-, LRR- and pyrin domain-containing 3 (NLRP3) inflammasome. The integration of simultaneously activated PRRs occurs at the level of intracellular adaptors and transcription factors shared between overlapping pathways. The resulting cytokine responses shape the activation of adaptive immune response. Induction of IL-12 drives IFN-γ production by T-helper 1 (Th1) cells, which is crucial for phagocyte activation. Induction of IL-1β, IL-6, and IL-23 promotes Th17 differentiation. Regulatory T-cells (Treg) act as host-driven homeostatic response to keep inflammation under control. **(B)** Th1 and Th17 differentiation. Polarization of naive T cells into Th1 leads to IFNγ production, and its signaling is mediated through the Janus kinase (JAK)–signal transducer and activator of transcription 1 (STAT1) pathway, leading to transcription of IFNγ-inducible genes. IL-6 and IL-21 upregulate the expression of the retinoic acid-related orphan receptor RORγt and RORα, leading to expression of the inducible component of the IL-23 receptor (IL-23R) and further Th17 development. IL-17A and IL-17F produced by Th17 cells augments neutrophil production in the bone marrow and their recruitment to the site of infection. IL-17A, IL-17F, and IL-22 promote production of antimicrobial peptides in epithelial cells. Molecules in which genetic defects have been identified to be associated with increased susceptibility to endemic mycoses are marked in bold red.

virus infection), and dermatitis with *Staphylococcus aureus* superinfection (86, 121).

GATA2 Deficiency

A syndrome of monocytopenia with susceptibility to non-tuberculous mycobacterial (NTM) infections, often termed "MonoMAC," is caused by haploinsufficiency of the hematopoietic transcription factor GATA2 (122). Majority of the patients have monocytopenia, natural killer (NK), and B lymphocytopenia, while CD4 lymphocytopenia and neutropenia are also common but less marked. Affected individuals are susceptible to a broad range of viral (human herpes virus and human papillomavirus), disseminated NTM, bacterial, and fungal infections (123, 124). In a cohort of 57 patients with *GATA2* mutations evaluated at the National Institutes of Health in the US (124), severe fungal infections were observed in 16%, including invasive aspergillosis (9%), disseminated histoplasmosis (5%), and recalcitrant mucosal candidiasis (5%). Another patient with GATA2 deficiency and disseminated histoplasmosis was reported by Lovell et al. (86). All the patients diagnosed to have GATA deficiency with disseminated histoplamosis were adults (86, 124). Apart from infections, other clinical features of GATA2 deficiency include congenital lymphedema, pulmonary alveolar proteinosis, and predisposition to myelodysplastic syndrome or acute myeloid leukemia, but considerable clinical heterogeneity exists.

Inborn Errors of IFN-γ-Dependent Immunity

Host defense against intracellular bacterial and fungal pathogens depends on effective cell-mediated immunity, which is coordinated by APC and T-lymphocytes (89, 90, 125). Following phagocytosis, macrophages, monocytes, and DCs secrete IL-12p70, a heterodimer of IL-12p40 (IL12B) and IL-12p35 (IL-12A) that stimulates T and NK cells through its receptor IL-12R, a heterodimer of IL-12Rβ1 and IL-12Rβ2. IL-12Rβ1 is bound to tyrosine kinase 2 (TYK2), and IL-12Rβ2 is bound to Janus kinase-2 (JAK2). IL-12 receptor signaling induces phosphorylation, dimerization, and nuclear translocation of signal transducer and activator of transcription-4 (STAT4) to induce IFN-γ production in T-cells and NK cells, and drives Th1 polarization of CD4+ T-cells. Binding of IFN-γ to its heterodimeric receptor consisting of IFN-γR1 and IFN-γR2 leads to signal transducer and activator of transcription 1 (STAT1) phosphorylation by Janus kinase 1 (JAK1) and JAK2. The phospho-STAT1 (p-STAT1) homodimer translocates to the nucleus and modifies gene expression regulated by the γ-regulated sequencing, resulting in phagocyte activation including production of bactericidal ROS by NADPH oxidase, further IL-12 production, and killing of intracellular pathogens (126, 127). IL-12 production is augmented by a T-cell dependent pathway through interaction of CD40 on the surface of APC with CD40L expressed on activated T-cells (128). The signaling pathway is shown in **Figure 2B**.

IL-23 shares the p40 component with IL-12p70, and IL-12Rβ1 combines with IL-23 receptor (IL-23R) to form the IL-23R complex (129). IL-23R signaling leads to STAT3/STAT4 heterodimer phosphorylation by TYK2 and JAK2, supporting the proliferation of Th17 cells, which are critical mediators of immunity at the mucosal surface (130). Activated Th17 cells produce IL-17 and IL-22, which induce antimicrobial peptide production in epithelial cells, and the recruitment and activation of inflammatory cells, especially neutrophils (131). These effector functions are critical in the control of mycobacteria, fungi, and bacterial pathogens such as *salmonella* (131–133).

Genetic defects of the IFN-γ-dependent immunity are collectively known as the Mendelian susceptibility to mycobacterial disease (MSMD) (126, 127). These disorders encompass defects of IFN-γ production or response to IFN-γ, caused by mutations in *IL12B*, *IL12RB1*, *ISG15, NEMO*, *IFNGR1*, *IFNGR2*, *STAT1*, *NEMO*, *IRF8*, and *CYBB.* Altogether, they constitute 18 genetic etiologies of MSMD based on the mode of inheritance, complete or partial defect, expression of the mutant allele, and the functional aberrations. Mycobacterial infection is the sole infectious phenotype in some of these disorders (AD IRF8 deficiency, AR ISG15 deficiency), while others have increased susceptibility to a broader range of pathogens (126, 127). Endemic fungal infections have been reported in AR IL12Rβ1, AR IFN-γR1, and AD GOF STAT1 defects.

AR IL12Rβ1 Deficiency

Autosomal recessive IL12Rβ1 deficiency is the most common form of MSMD, accounting for approximately half of the cases in which a genetic cause has been identified (126, 127, 134). Patients with IL12Rβ1 deficiency are recognized by their susceptibility to

TABLE 4 | Endemic mycoses in CD40 ligand deficiency.

	Genetic defect	Endemic fungal pathogen	Gender/age, residence	Clinical manifestations	Other infections and comorbidities	Treatment and outcome
Tu et al. (110)	Not stated	*Histoplasma* spp.	M/3 years, US	Disseminated histoplasmosis with esophageal ulcers and bone marrow involvement	Cyclical neutropenia and anemia	Not stated
Hostoffer et al. (111)	Not stated	*Histoplasma capsulatum*	M/19 years, US	Disseminated histoplasmosis with pulmonary infiltrates, pancytopenia and splenomegaly	Tongue and per-rectal ulcers	Treated with amphotericin B, recurrence due to poor compliance to itraconazole prophylaxis
Yilmaz et al. (112)	Not stated	*Histoplasma* spp.	M/5 years, Turkey	Facial lesions, cervical lymphadenopathy, bilateral pulmonary infiltration and bronchiectasis	Recurrent pulmonary infections	Treated with ketoconazole
Danielian et al. (113)	p.R11X	*H. capsulatum*	M/6 months, Argentina	Histoplasma lymphadenitis	PCP and parvovirus B19 infection, recurrent pneumonia, adenitis, anemia	Not stated
Dahl and Eggebeen (114)	Not stated	*Histoplasma* spp.	M/14 years, US	Disseminated histoplasmosis complicated by fungemia and macrophage activation syndrome	Recurrent sinopulmonary infections and neutropenia	Liposomal amphotericin B for 14 days followed by oral itraconazole; macrophage activation syndrome treated with steroid and anakinra with prompt improvement
Lovell et al. (86)	c.289-15T > A	*Histoplasma* spp.	M/6 years (patient 2)	Disseminated histoplasmosis with fever, hepatomegaly; Histoplasma identified from bone marrow biopsy	Recurrent otitis media, streptococcal pharyngitis	Amphotericin B, itraconazole; recurrence 2 years later with abdominal histoplasmosis
	c.289-15T > A	*Histoplasma* spp.	M/4 years (patient 3)	Lymphadenitis	Recurrent otitis media, streptococcal pharyngitis, bronchitis	Amphotericin B, itraconazole
Pedroza et al. (115)	c.233_234 delinsAA, p.S78*	*H. capsulatum*	M/2.5 years, Ecuador	Cutaneous histoplasmosis	*Cryptosporidium parvum* enteritis, oral candidiasis, pneumonia caused by *Pseudomonas aeruginosa* and *Candida albicans*	Amphotericin B for 4 weeks followed by itraconazole prophylaxis
Cabral-Marques et al. (116)	c.345_402del	*Paracoccidioides brasiliensis*	M/11 years, Sao Paulo, Brazil	Prolonged fever and cough, mediastinal lymphadenopathy, bone marrow hypoplasia and tuberculoid granuloma	PCP, recurrent otitis media, and sinopulmonary infections	Treated with 8 months of itraconazole and recovered
Kamchaisatian et al. (118)	Complex mutation in exon 5	*Talaromyces marneffei*	M/14 months, Northeastern Thailand	Prolonged fever, cough, neck pain and bloody sputum; neck imaging showed prevertebral soft tissue swelling. Throat swab, sputum, blood and bone marrow cultures yielded *T. marneffei*	Recurrent pneumonia, oral ulcers, cyclical neutropenia	Treated with amphotericin B for 21 days, followed by itraconazole for 10–12 weeks
	Not stated	*T. marneffei*	M/1 year, Northern Thailand	Fever, cough, dyspnea, lymphadenopathy and pleural effusion; lymph node biposy yielded *T. marneffei*	PCP	Treated with amphotericin B for 21 days, followed by itraconazole for 10–12 weeks
Sripa et al. (119)	Not stated	*T. marneffei*	M/3 years, Thailand	Pneumonia, positive *T. marneffei* culture from tracheal aspirate	PCP, cyclical neutropenia	Treated with itraconazole with good response
Liu et al. (120)	g.IVS1-3T > G	*T. marneffei*	M/2 years, China	Disseminated *T. marneffei* infection with airway granuloma, hepatosplenomegaly and fungemia	BCG-itis, pneumonia	Died of multi-organ failure

Location of residence is indicated wherever information is available.
PCP, Pneumocystis jiroveci pneumonia.

mycobacterial infections (*M. bovis BCG*, NTM, and *M. tuberculosis*) and non-typhoidal salmonellosis of unusual severity or frequency, but some patients are also susceptible to *Candida*, *Klebsiella*, *Nocardia*, *Leishmania*, *Histoplasma*, *Coccicioides*, *and Paracoccidioides* (134). Peripheral blood mononuclear cells of these patients do not respond to IL-12 and IL-23, resulting in

impaired IFN-γ production by T and NK cells. The development of IL-17-producing T-cells is also impaired, due to defect of the IL-23R complex, which is composed of IL-12Rβ1 (135–137). This accounts for the susceptibility to develop CMC observed in 23% of patients (134–137).

The seven reported cases of coccidioidomycosis, PCM, and histoplasmosis in patients with IL12Rβ1 deficiency are summarized in **Table 5** (134, 138–141). The age at which disseminated mycoses developed varied from childhood to adulthood, some with past history of mycobacterial infection and salmonellosis. Two cases had recurrence disease, but could be controlled by antifungal treatment.

AR IFNγ Receptor Deficiency

IFN-γR1 and IFN-γ2 are the ligand-binding and transducing receptor chains of the INF-γ receptor, respectively. Biallelic null mutations in the *IFNGR1* gene result in AR complete IFNγR1 deficiency, which is characterized by high plasma concentration of IFN-γ and a lack of response to IFNγ *in vitro* (142, 143). These patients have early onset, life-threatening disseminated mycobacterial infections and the overall prognosis is poor with high rate of fatality. Other infections caused by viruses (cytomegalovirus, respiratory syncytial virus, varicellar zoster virus, human herpes virus 8) and bacteria (*Listeria monocytogenes*) have been described. In AR partial IFN-γR1 deficiency, the clinical phenotype is less severe (144). Apart from mycobacterial infections, bacterial, viral, and parasitic organisms have been reported (126, 127, 142, 144).

Autosomal dominant partial IFN-γR1 deficiency is caused by mono-allelic mutations affecting exon 6 and exon 7. A hotspot mutation, 818del4, accounts for over 80% of patients with AD IFN-γR1 deficiency (126, 127). In contrast with AR IFN-γR1 deficiency, these is an increase in IFN-γR1 protein expression on the cell surface, due to the accumulation of truncated IFN-γR1 receptors lacking the recycling domain. Despite the presence of receptors encoded by the wild-type *IFNGR1* allele, the non-functioning IFN-γR1 protein impedes the normal function of IFN-γR1 dimers by negative dominance and impairs the response to IFN-γ *in vitro* (145). The clinical features are less severe than those seen in patients in AR complete IFN-γR1 deficiency. Most

TABLE 5 | Endemic mycoses in IL12RB1 deficiency and INFGR1 deficiency.

	Genetic defect	Endemic fungal pathogen	Gender/age, residence	Clinical manifestations	Other infections and comorbidities	Treatment and outcome
IL12RB1 deficiency						
Moraes-Vasconcelos et al. (138)	Homozygous p.L77F	*Paracoccidioides brasiliensis*	M/24 years, Brazil	20 years: fever, hepatosplenomegaly, generalized lymphadenpathy	BCG cervical adenopathy at 7 m, relapse at 2 years 6 years; disseminated non-typhoidal salmonellosis which lasted for 7 years	Treated with trimethoprim-sulfamethoxazole for 5 years with clinical resolution
de Beaucoudrey et al. (137)	Homozygous p.R521X	*Histoplasma* spp.	F/5 years	Disseminated histoplasmosis	Tuberculosis	Not mentioned
Vinh et al. (139)	Homozygous p.C186Y	*Coccidioides* spp.	Patient 1: F/22 years, US	Diffuse lymphadenopathy (cervical, supraclavicular, hilar, mediastinal, retroperitoneal)	Non-typhoidal salmonellosis (bacteremia and lymphadenopathy)	Fluconazole for 1.5 years without recurrence
			Patient 2 (brother of Patient 1): M/6 years, Arizona, US	6 years: coccidioidal pneumonia 14 years: right supraclavicular lymphadenopathy and a nasal lesion 16 years: osteomyelitis of the right proximal tibia	Nil	Received fluconazole for 2 years, developed osteomyelitis 2 months after stopping fluconazole, treated with itraconazole with improvement
Hwangpo et al. (140)	IL12-receptor defect (by functional studies)	*Histoplasma* spp.	M/8 years	Disseminated histoplasmosis with miliary infiltration of the lungs, mediastinal lymphadenopathy, splenomegaly	Not mentioned	Itraconazole
Falcão et al. (141)	Homozygous p.R283X	*Histoplasma capsulatum*	M/4 years, Brazil	4 years: fever, hepatosplenomegaly, generalized lymphadenopathy, bone marrow involvement 6 years: CNS histoplasmosis complicated by hydrocephalus	Tuberculous adenitis	Antifungal treatment and itraconazole prophylaxis
			Brother of the proband	Disseminated histoplasmosis	Tuberculous adenitis, disseminated salmonellosis	Not mentioned

(*Continued*)

TABLE 5 | Continued

	Genetic defect	Endemic fungal pathogen	Gender/age, residence	Clinical manifestations	Other infections and comorbidities	Treatment and outcome
IFNGR1 defect						
Zerbe and Holland (146)	Heterozygous c.818del4	*H. capsulatum*	M/3 years, Tennessee, US	3 years: fever, pneumonia, hepatosplenomegaly, cervical and paratracheal lymphadenopathy 4.5 years: fever, pneumonia, generalized lymphadenopathy, sinusitis; lymph node biopsy yielded *H. capsulaum* 4.8 years: right paranasal mass and osteomyelitis of the facial bones requiring debridement	Coexisting MAC infection: 3 years: MAC found in gastric aspirate 7 years: cervical lymphadenopathy and osteomyelitis of rib, biopsy yielded MAC	Repeated courses of intensive antifungal and antimycobacterial therapy, subcutaneous IFN-γ injection led to clearing of all bone lesions. Remained well on prophylactic itraconazole, azithromycin, and IFN-γ
Vinh et al. (147)	Heterozygous c.818del4	*Coccidioides* spp.	M/11 years, Arizona, US	Lobar pneumonia, mediastinal and hilar lymphadenopathy; later developed osteomyelitis involving the vertebral spine and pelvic bone	*Mycobacterium chelonae* pulmonary infection at 11 months; *M. kansasii* abscess involving the cervical spine and retropharyngeal space	Refractory coccidioidomycosis with progressive skeletal lesions despite prolonged use of antifungal therapy (amphotericin B and azoles). Surgical debridement with implantation of amphotericin B-impregnated beads. Adjunctive IFN-γ injection for *M. kansasii* infection with good response
Lee and Lau (manuscript in preparation)	Homozygous c.182dupT, p.V61fs	*Talaromyces marneffei*	F/5 months, Chiang Mai, Thailand	Generalized papular skin lesions, hepatosplenomegaly, osteolytic lesions in the skull, fungemia	11 months: fulminant salmonella septicemia	*T. marneffei* infection resolved with amphotericin B followed by oral itraconazole; died of salmonellosis and massive lower gastrointestinal bleeding

Location of residence is indicated wherever information is available.
IFN, interferon; MAC, Mycobacterium avium complex.

patients have BCG or NTM infections, and salmonella infection was reported in only 5% of cases (126, 127, 142). Disseminated histoplasmosis (146) and coccidioidomycosis (147) were reported in two patients with IFN-γR1 deficiency and both had a refractory or relapsing course (**Table 5**). Our group diagnosed AR IFN-γ receptor 1 deficiency in a Burmese infant suffering from disseminated *T. marneffei* infection, and she eventually died of salmonellosis (manuscript in preparation).

IFN-γR2 deficiency is less common than IFN-γR1 deficiency. Similarly, AR complete or partial IFN-γR2 deficiency cause increased susceptibility to mycobacterial infections (148–150); other infections are rare and include salmonellosis in one patient and cytomegalovirus disease in three patients (126), but mycosis has not been reported. AD form of partial IFN-γR2 deficiency was diagnosed in a patient with mild BCG disease, and clinical penetrance is very low (151).

AD STAT1 Defect

Signal transducer and activator of transcription 1 (STAT1) is a transcription factor involved in cellular responses mediated by type I (IFNα/β), type II (IFN-γ), and type III (IFN-λ) IFNs (152). AR complete STAT1 deficiency is characterized by the absence of STAT1 expression and abolished cellular response to IFN-γ as well as IFN-α/β and IFN-λ, resulting in severely impaired antimycobacterial and antiviral immunity (153, 154). Patients with complete STAT1 deficiency caused by null mutations have increased susceptibility to mycobacterial, viral, and bacterial infections, whereas biallelic hypomorphic mutations in AR partial STAT1 deficiency are associated with milder clinical severity (153–155). AD partial STAT1 deficiency with mono-allelic loss-of-function (LOF) *STAT1* mutation predispose to mycobacterial infection (156); in contrast, AD GOF *STAT1* mutation is recognized as the most common cause of CMC disease, accounting for half of the cases (157–159). Majority of the mutations affect the coiled-coil domain or DNA-binding domain of STAT1 (159). They increase STAT1 phosphorylation by impairing nuclear dephosphorylation. They are GOF for the STAT1-dependent cytokines including IFN-α, IFN-β, IFN-γ, and IL-27, which repress Th17 development, accounting for the low numbers of IL-17-producing T-cells in these patients (160).

In addition to CMC, invasive mycoses caused by a variety of yeasts (e.g., *Cryptococcus*), molds (*Aspergillus*, *Fusarium*), and endemic fungi (*Histoplasma, Coccidioides, T. marneffei*) have been reported in patients with GOF *STAT1* mutations, as summarized in **Table 6** (161, 162). The two patients who developed disseminated coccidioidomycosis during childhood had progressive disease, which persisted into teenage despite intensive treatment; one had spinal cord compression and one died of overwhelming coccidioidomycosis at 17 years. Of note, the latter patient did not have CMC or other unusual infections, implying that coccidioidomycosis could be the sole infection in patients with GOF STAT1 defect. Three patients had disseminated histoplasmosis and two of them had recurrent disease. All of them responded well to antifungal treatment (161).

TABLE 6 | Endemic mycoses in AD signal transducer and activator of transcription 1 defect.

	Genetic defect	Endemic fungal pathogen	Gender/ age, residence	Clinical manifestations	Other infections and comorbidities	Treatment and outcome
Sampaio et al. (161)	Heterozygous p.E353K	*Coccidioides* spp.	Patient 1: F/17 years, AZ, USA	Coccidioidal pneumonia, mediastinal lymphadenopathy, and sternocleidomastoid abscess; progressive disease with osteomyelitis of the vertebral spine and lesions in the skin, liver, and spleen at 20 years	Extensive persistent tinea capitis and kerion caused by *T. tonsurans*	Progressive disease despite prolonged antifungal therapy including amphotericin B, azoles and caspofungin. Developed spinal cord compression at 20 years due to intramedullary lesion
	Heterozygous p.A267V	*Coccidioides immitis*	Patient 2: F/9 years, AZ, USA	Coccidioidal pneumonia and intrathoracic lymphadenopathy, osteomyelitis of the vertebral spine; progressive disease with CNS involvement, lymphadenopathy, retinal mass, and multifocal osteomyelitis	Nil	Progressive disease despite prolonged antifungal therapy including amphotericin B, azoles, and caspofungin, suboptimal response to adjunctive IFN-γ therapy. Died of overwhelming coccidioidomycosis at 17 years
	Heterozygous p.T385M	*Histoplasma capsulatum*	Patient 3: M/21 years	Disseminated histoplasmosis at 12 years	CMC, *M. fortuitum* cervical lymphadenopathy, recurrent pneumonia and herpes zoster, bronchiecatasis Recurrent fractures, progressive bilateral upper limb muscle atrophy	Histoplasmosis treated with itraconazole with good response
	Heterozygous p.R274G	*H. capsulatum*	Patient 4: M/31 years	17 years: disseminated histoplasmosis presenting with fever, weight loss, lymphadenopathy with liver, and bone marrow involvement 30 years: CNS histoplasmosis	CMC, warts, recurrent *Salmonella* septicemia Type 1 DM at 24 years 31 years: PML caused by JC virus	Histoplasmosis treated with amphotericin B for 6 months followed by fluconazole with multiple relapses that responded to intensified treatment
	Heterozygous p.F172L	*H. capsulatum*	Patient 5: F/25 years	7 years: disseminated histoplasmosis presenting with fever, hepatosplenomegaly, lymphadenopathy, and dyspnea; recurrence at 8 years	CMC Subclinical hypothyroidism at 14 years, ovarian failure at 24 years	Histoplasmosis treated with itraconazole with good response
Lee et al. (162)	Heterozygous p.A267V	*Talaromyces marneffei*	Patient 1: M/14 years, Hong Kong	Disseminated *T. marneffei* infection at 15 years with generalized lymphadenopathy, positive culture of *T. marneffei* from lymph node biopsy	CMC	Amphotericin B, itraconazole prophylaxis
	Heterozygous p.L358F	*T. marneffei*	Patient 2: F/8 years, Hong Kong	Cavitating pneumonia with cystic cavities; mediastinal and hilar lymphadenopathy, positive culture of *T. marneffei* from BAL	CMC Recurrent sinopulmonary infections Influenza A (H1N1)	Liposomal amphotericin, itraconazole prophylaxis
	Heterozygous p.T288I	*T. marneffei*	Patient 3: F/16 years, Hong Kong	Cervical lymphadenopathy, positive culture of *T. marneffei* and *M. tuberculosis* from lymph node biopsy; concomitant axillary, mesenteric, and retroperitoneal lymphadenopathy	CMC Recurrent sinopulmonary infections and herpes zoster EBV-associated HLH Disseminated aspergillosis	*T. marneffei* infection responded well to itraconazole Disseminated aspergillosis and EBV-associated HLH at 16 years, died of massive gastrointestinal hemorrhage

(*Continued*)

TABLE 6 | Continued

	Genetic defect	Endemic fungal pathogen	Gender/ age, residence	Clinical manifestations	Other infections and comorbidities	Treatment and outcome
Lee and Lau, unpublished	Heterozygous p.M390I	*T. marneffei*	M/40 years, Hong Kong	10 years: cervical lymphadenopathy complicated by ulcerations; perforation of the hard palate, mediastinal lymphadenopathy causing SVC obstruction and sternal erosion, tissue culture yielded *T. marneffei* 17 years: osteomyelitis of the thumb, forearm, and tibia	CMC	Relapsing and remitting disease course on prolonged treatment of amphotericin B and fluconazole till 20 years; infection cleared with residual scarring of the skin and dilated veins on the chest

Location of residence is indicated wherever information is available.

BAL, bronchoalveolar lavage; CMC, chronic mucocutaneous candidiasis; CNS, central nervous system; DM, diabetes mellitus; EBV, Epstein–Barr virus; IFN, interferon; PML, progressive multifocal leukoencephalopathy; SVC, superior vena cava.

In Hong Kong, five pediatric patients were diagnosed to have *T. marneffei* infection from 1983 to 2009 in a single center (74, 85, 162, 163). One patient was lost to follow-up after complete recovery from *T. marneffei* infection (163), while the remaining four patients underwent thorough immunological investigations and genetic studies, and all were found to have GOF STAT1 defect (162). They all had CMC, and two had recurrent sinopulmonary infections, herpes virus infections (cytomegalovirus, Epstein–Barr virus and varicella zoster), TB, and disseminated aspergillosis (85, 162). The patient with chronic relapsing *T. marneffei* infection first reported by Yuen et al. in 1986 was subsequently investigated when his son was referred for CMC, and both were confirmed to have AD GOF STAT1 defect in 2015.

An increased incidence of herpes virus infections as well as TB and NTM infections (e.g., *M. bovis BCG*) has been observed in patients with GOF STAT1 defect, and it was thought that the enhancement of signaling downstream to IFN-α/IFN-β and IFN-γ caused by GOF *STAT1* mutations could lead to exhaustion of virus-specific T-cells and refractory response to IFN-γ. Other clinical manifestations of GOF *STAT1* mutations include autoimmunity (e.g., type 1 diabetes mellitus, thyroid disease, autoimmune cytopenia, and hepatitis), vascular aneurysms, and malignancies, particularly squamous cell carcinoma of the esophagus (159, 160, 164).

Inborn Errors of Immunity Associated with Impaired Th-17-Mediated Immunity

Autosomal-dominant hyper-IgE syndrome caused by LOF *STAT3* mutation, also known as Job's syndrome, is characterized by recurrent staphylococcal cold abscesses, pneumonia, and eczema. In addition, patients often display joint hyperextensibility, skeletal abnormalities and pathological fractures, delayed dental deciduation, coronary artery aneurysms, brain lesions, and Chiari's malformation (165, 166). Pneumonia is typically caused by *S. aureus, Haemophilus influenzae*, or *Streptococcus pneumoniae*, and is often complicated by pneumoatocele formation. Approximately 20% of patients with AD hyper-IgE syndrome develop invasive infection caused by Aspergillus, which has angioinvasive properties with tendency to cause hematogenous dissemination (167–170). STAT3 promotes the expression of the gene encoding the retinoic acid receptor-related orphan receptor-γt (RORγt), and important transcription factor that drives differentiation of naïve CD4+ T-cells to Th17 cells. Dominant negative *STAT3* mutation leads to impaired RORγt induction in response to IL-1β, IL-6, and IL-23, causing severe deficiency of IL-17-producing effector cells (171, 172).

Three cases of coccidioidomycosis, 10 cases of histoplasmosis and two cases of *T. marneffei* infection were reported in patients with hyper-IgE syndrome, as summarized in **Table 7** (85, 87, 173–183). The three cases of hyper-IgE syndrome with coccidioidomycosis all had CNS involvement (87, 173, 174), while those with IL-12Rβ1 deficiency (139), IFN-γR1 deficiency (147), and GOF STAT1 defect (161) mainly had lymphadenopathy and osteomyelitis, so it appears that there is a predilection for *Coccidioides* to disseminate to the CNS in hyper-IgE syndrome. In contrast, disseminated histoplasmosis occurred in IL-12Rβ1 deficiency (141), IFN-γR1 deficiency (146), and GOF STAT1 defect (161), but 7 out of 10 cases of histoplasmosis in hyper-IgE syndrome involved the aerodigestive tract only (175–182). Two cases of *T. marneffei* infection were reported in hyper-IgE syndrome (85, 183).

To summarize, the susceptibility to endemic mycoses in CD40L deficiency, IL12Rβ1 deficiency, and IFN-γR1 deficiency highlights the critical role of the IL-12/IFN-γ crosstalk in macrophage activation and killing of these endemic fungi, while the deficiency of Th17 cells in patients with GOF STAT1 defect and AD hyper-IgE syndrome puts them at risk for both CMC and IFIs, and they frequently have CMC due to impaired mucosal immunity against *C. albicans*. Defective oxidative burst alone, as in CGD, is not sufficient to cause increased risk to endemic mycoses, suggestive that other mechanisms of phagosomal killing may compensate for the lack of NADPH oxidase activity to control these endemic fungi, distinguishing them from many other invasive fungi to which CGD patients are susceptible.

PROTECTIVE IMMUNITY AGAINST ENDEMIC MYCOSES CONFERRED BY CYTOKINES: INSIGHTS FROM BIOLOGICS AND ANTI-IFN-γ AUTOANTIBODIES

The development of biologic response modifiers (BRMs), such as monoclonal antibodies and receptor antagonists that target

TABLE 7 | Endemic mycoses in AD hyper-IgE syndrome (Job syndrome).

	Genetic defect	Endemic fungal pathogen	Gender/age, residence	Clinical manifestations	Other infections and comorbidities	Treatment and outcome
Stanga and Dajud (173)	Not stated	*Coccidioides immitis*	F/4 years	Coccidioidal meningitis with cerebral infarct at multiple sites, gross left hemiplegia	Recurrent sinus and skin infections, eczema	Treated with amphotericin B and fluconazole, minimal residual deficits
Powers et al. (174)	Heterozygous p.T412S	*C. immitis*	F/17 years, UT, USA	Coccidioidal meningitis and cerebral abscess, altered mental status requiring temporary intubation	*Staphylococcus aureus* skin and soft tissue infections, recurrent sinopulmonary infections	Improved with liposomal amphotericin, followed by fluconazole prophylaxis
Odio et al. (87)	Heterozygous p.V713M	*C. immitis*	F/4 years, AZ, USA	Coccidioidal meningitis and pulmonary infection presenting with fever, headache, and seizure	Recurrent pneumonia and otitis, skin infections, eczema, thrush	Complicated by cerebral vascular accident and hydrocephalus, treated with liposomal amphotericin and fluconazole Residual left hemiparesis
Alberti-Flor et al. (175)	Not stated	*Histoplasma capsulatum*	M/16 years	Histoplasmosis with ileocecal involvement	Not stated	Resection of terminal ileum and right colon, treated with ketoconazole with good response
Cappell et al. (176)	Not stated	*H. capsulatum*	F/27 years	Disseminated histoplasmosis with cecum, colon, and bone marrow involvement	Not stated	Treated with amphotericin B and ketoconazole
Desai et al. (177)	Not stated	*H. capsulatum*	M/33 years	Histoplasmosis with pulmonary and tongue involvement	Eczema, recurrent sinopulmonary infections, thrush and onychomycosis, *Staphylococcal* septic arthritis, *Cryptococcus* meningitis at 37 years	Histoplasmosis treated with amphotericin B and ketoconazole; lobectomy for bronchiectasis
Steiner et al. (178)	Not stated	*H. capsulatum*	F/14 years	Histoplasmosis with ileocecal involvement	Staphylococcal pneumonitis complicated by cystic changes and bronchopleural fistula	Treated with 12 months of itraconzole with good response
Robinson et al. (179)	Heterozygous p.K591M	*H. capsulatum*	M/33 months	Disseminated histoplasmosis with pneumonia and hepatosplenomegaly	Pneumonia, otitis, thrush, eczema, folliculitis, gastroenteritis, multiple fractures, pneumatocele, multiple allergy, developmental delay	Not stated
Rana et al. (180)	Not stated	*H. capsulatum*	F/4 years, India	Histoplasmosis with rectal involvement	Recurrent subcutaneous abscess, giardiasis, *Entamoeba* infection, molluscum contagiosum, milk allergy	Good response to treatment
Jiao et al. (181)	Not stated	*H. capsulatum*	M/21 years	Terminal ileal perforation, histopathology showed *H. capsulatum* within histiocytes	Not stated	Partial small bowel resection Liposomal amphotericin, itraconazole
Odio et al. (182)	Heterozygous p.V432M	*H. capsulatum*	M/10 years, US	Disseminated histoplasmosis with pulmonary, liver, and spleen involvement	Not stated	Treated with liposomal amphotericin, itraconzaole, and posaconazole
	Heterozygous p.F621V	*H. capsulatum*	F/15 years, US	Histoplasmosis with gastrointestinal involvement, complicated by duodenal stricture	Not stated	Treated with liposomal amphotericin and itraconazole
	Heterozygous p.W479C	*H. capsulatum*	F/22 years, US	Histoplasmosis with laryngeal involvement requiring reconstructive largyngoplasty	Not stated	Treated with ketoconazole
Ma et al. (183)	Not stated	*Talaromyces marneffei*	M/30 years, Hong Kong	Lung abscess, massive hemoptysis	*Stenotrophomonas maltophilia* lung abscess, recurrent pneumonia, skin infections	Treated with amphotericin B, died of respiratory failure due to rapid disease progression
Lee et al. (85)	Heterozygous p.D374G	*T. marneffei*	F/12 months, Guangzhou, China	Disseminated *T. marneffei* infection with pancytopenia and hepatosplenomegaly, positive culture of *T. marneffei* from bone marrow	Pulmonary aspergillosis, *Staphylococcus* septicemia, pneumatocele, and pneumothorax	*T. marneffei* infection treated with itraconazole with good response

Location of residence and mutation of STAT3 gene are provided wherever information is available.

pro-inflammatory cytokines and their receptors has led to major advances in the treatment of autoimmune and malignant disorders. However, they have the potential to suppress host immune response and increase the risk of infections. The use of biologics is associated with a small but important risk of IFI. Histoplasmosis is the most common IFI associated with TNF-α inhibitors (10, 59, 184, 185). In a survey of infectious disease specialists, histoplasmosis was second only to *S. aureus* as the cause of serious infection complicating anti-TNF and other BRM (184). In most cases, patients reside in areas where the fungus is endemic and have received other immunosuppressants concurrently. Up to 2% of patients receiving BRMs will develop coccidioidomycosis if they reside in an endemic region (186). The American Academy of Pediatrics (AAP) Committee on Infectious Diseases recommends that patients on BRM should be enquired about epidemiologic risk factors and possible exposures to histoplasmosis and coccidioidomycosis, which have symptoms and signs that significantly overlap with TB. If there is suspicion of signs or symptoms compatible with acute histoplasmosis or coccidioidomycosis, BRM should be discontinued immediately and patients will require evaluation with a combination of chest radiography and serologic, antigen detection, and culture tests, which are best conducted in consultation with an infectious diseases expert (186, 187).

Other targeted therapies have also been implicated as risk factors for endemic mycoses. In Hong Kong, four cases of disseminated *T. marneffei* infection were diagnosed in adult hematology patients receiving anti-CD20 monoclonal antibodies (rituximab and obinutuzumab) and kinase inhibitors (84, 188, 189). The observation is revealing, as the importance of B-lymphocytes and humoral immune response against fungus is not well defined, and *T. marneffei* infection has so far not been reported in patients with congenital agammaglobulinemia. Depletion of B-lymphocytes may lead to profound deficiency in the production of neutralizing antibodies against key virulence factors of *T. marneffei*. Kinase inhibitors such as ruxolitinib and sorafenib are increasingly used in treating hematological malignancies, solid tumors, psoriasis, and alopecia areata. Ruxolitinib is a selective JAK1 and JAK2 inhibitor that interferes with the IFN-γ and its downstream JAK-STAT signaling. Sorafenib is a multi-kinase inhibitor that exhibits immunomodulatory effect by impairing T-lymphocyte proliferation, production of IFN-γ and other pro-inflammatory cytokines, NK cell, and DC functions. The suppression of IFN-γ signaling pathway poses risk to develop *T. marneffei* infection (84). It would be important for clinicians to have a high index of suspicion on *T. marneffei* infection in patients receiving these targeted therapies to avoid delay in diagnosis and treatment.

Autoantibody against IFN-γ has been reported to be associated with adult-onset immunodeficiency in patients from Asian countries (190–195). Disseminated NTM is the most common clinical presentation. In a cross-sectional, case-control study conducted in Chiang Mai, Thailand showed that patients with opportunistic infections including disseminated NTM, disseminated *T. marneffei* infection, melioidosis and non-typhoidal Salmonellosis had anti-IFN-γ autoantibody level above 99th percentile of cut-off for healthy individuals, and the level of autoantibody in patients who had active opportunistic infection was relatively higher than those without active infection (193). Similar observations were also reported in Hong Kong and Taiwan (190, 192, 194, 195). HLA class II molecules HLA-DRB1*15:02–HLA-DQB1*05:01 and HLA-DRB1*16:02–HLA-DQB1*05:02 are specifically associated with anti-IFN-γ autoantibodies and NTM (196, 197). The high frequency of such alleles in Southeast Asia might account for the relatively high prevalence this condition in the Asian population. The study by Lin et al. showed that anti-IFN-γ autoantibody from patients recognizes an epitope at the C terminus of IFN-γ, and binding of the autoantibody neutralizes IFNγ-induced signaling. This epitope displays a high degree of sequence homology to the Aspergillus Noc2 protein. It was postulated that in the warm and humid environment of Southeast Asia where exposure to Aspergillus species is common in everyday life, some individuals might develop anti-IFN-γ autoantibodies due to molecular mimicry (198). The co-evolution of anti-IFN-γ autoantibody production as the susceptibility trait amongst Southeast Asians, and the high prevalence of *T. marneffei*, NTM, and mellioidosis in this region is a unique combination not observed in the rest of the world, and it is so interesting that exposure to a common environmental fungal agent could indirectly induce susceptibility to other pathogens.

FUTURE PERSPECTIVES

The understanding about inborn errors of immunity predisposing to endemic mycoses is limited. First, exposure to these environmental fungal pathogens is often a chance event that depends on the natural habitat and climate, as well as the circumstances and activities in which the individual is engaged. Thus, the number of cases of endemic mycoses associating with PID is likely to be low. The proportion of patients with PID amongst those with disseminated endemic mycoses is unknown, due to the lack of information about the population incidence (i.e., the "denominator"). Second, some forms of endemic mycoses, particularly *T. marneffei* are prevalent in less resourced countries where well-developed clinical service for PID is lacking, and diagnosis is often delayed or missed. Third, the global health impact of these geographically restricted endemic fungi is probably less than those opportunistic fungal pathogens of worldwide distribution causing high disease burden in immunocompromised patients (e.g., *Candida*, *Aspergillus*, *P. jiroveci*, *Cryptococcus*, and *Rhizopus*), and they probably generate less attractions and interests in the global public and scientific community (7, 199, 200). Regional and global effort in establishing registries on disseminated endemic mycoses is crucial, in order to collect patient demographic data and determine their true population incidence.

While endemic mycoses are geographically restricted to certain regions, clinicians looking after patients with PID or acquired immunodeficiencies should gain knowledge about these rare fungal infections so that appropriate advice can be given to their patients when planning for travels, and to have heightened awareness of such diagnostic possibility when they return from endemic areas. Climate, environment, and exposure (behavior) are the "triad" that determine the risk of endemic fungal infection in susceptible hosts. High-risk activities that increase the chance

of exposure to fungal conidia should be avoided, otherwise, precautionary measures should be taken.

The discovery of PID predisposing to endemic mycoses is a fascinating journey, as it illuminates the key molecules and signaling pathways that are crucial in host defense against this group of dimorphic fungi, which are closely related in phylogeny. As disseminated coccidioidomycosis, histoplasmosis, PCM, and *T. marneffei* infection are recognized as AIDS-defining illness, they should also be regarded as indicator diseases for PID in individuals who are HIV-negative, and without known risk factors and secondary immunosuppression, particularly in children. There is a need to design an algorithm to evaluate such patients, with stepwise immunological investigations. A detailed history on previous infections, CMC, autoimmune manifestations, and family history should be taken. We recommend a basic panel of immunological evaluations including Ig pattern (IgG, IgA, IgM, and IgE), lymphocyte subset, and nitroblue tetrazolium, or dihydrorhodamine tests to assess oxidative burst activity. These patients should be assessed by immunologists. Abnormal results obtained during acute illness should be repeated upon full recovery. A systematic approach will facilitate clinicians to identify patients who warrant candidate gene studies or functional delineation of the pathways involved in immune recognition, T-cell activation and differentiation, cytokine signaling, and phagocytic killing. The presence of anti-IFN-γ autoantibody should be excluded. Functional evaluation of the IL-12/IFN-γ axis, STAT1 phosphorylation studies, and Th17 enumeration will be particularly relevant in this context. The utilization of next-generation sequencing techniques may lead to discoveries of novel monogenic disorders causing unique susceptibility to endemic mycoses.

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REFERENCES

1. Hsu LY, Ng ES, Koh LP. Common and emerging fungal pulmonary infections. *Infect Dis Clin North Am* (2010) 24:557–77. doi:10.1016/j.idc.2010.04.003
2. Sil A, Andrianopoulos A. Thermally dimorphic human fungal pathogens – polyphyletic pathogens with a convergent pathogenicity trait. *Cold Spring Harb Perspect Med* (2014) 5:a019794. doi:10.1101/cshperspect.a019794
3. Boyce KJ, Andrianopoulos A. Fungal dimorphism: the switch from hyphae to yeast is a specialized morphogenetic adaptation allowing colonization of a host. *FEMS Microbiol Rev* (2015) 39:797–811. doi:10.1093/femsre/fuv035
4. Kauffman CA, Freifeld AG, Andes DR, Baddley JW, Herwaldt L, Walker RC, et al. Endemic fungal infections in solid organ and hematopoietic cell transplant recipients enrolled in the transplant-associated infection surveillance network (TRANSNET). *Transpl Infect Dis* (2014) 16:213–24. doi:10.1111/tid.12186
5. Ramos-e-Silva M, Lima CM, Schechtman RC, Trope BM, Carneiro S. Systemic mycoses in immunodepressed patients (AIDS). *Clin Dermatol* (2012) 30:616–27. doi:10.1016/j.clindermatol.2012.01.008
6. Bonifaz A, Vázquez-González D, Perusquía-Ortiz AM. Endemic systemic mycoses: coccidioidomycosis, histoplasmosis, paracoccidioidomycosis and blastomycosis. *J Dtsch Dermatol Ges* (2011) 9:705–14. doi:10.1111/j.1610-0387.2011.07731.x
7. Seyedmousavi S, Guillot J, Tolooe A, Verweij PE, de Hoog GS. Neglected fungal zoonoses: hidden threats to man and animals. *Clin Microbiol Infect* (2015) 21:416–25. doi:10.1016/j.cmi.2015.02.031
8. Chakrabarti A, Slavin MA. Endemic fungal infections in the Asia-Pacific region. *Med Mycol* (2011) 49:337–44. doi:10.3109/13693786.2010.551426
9. Bryant PA, Baddley JW. Opportunistic infections in biological therapy, risk and prevention. *Rheum Dis Clin North Am* (2017) 43:27–41. doi:10.1016/j.rdc.2016.09.005
10. Vallabhaneni S, Chiller TM. Fungal infections and new biologic therapies. *Curr Rheumatol Rep* (2016) 18:29. doi:10.1007/s11926-016-0572-1
11. Brown J, Benedict K, Park BJ, Thompson GR III. Coccidioidomycosis: epidemiology. *Clin Epidemiol* (2013) 5:185–97. doi:10.2147/CLEP.S34434
12. Nguyen C, Barker BM, Hoover S, Nix DE, Ampel NM, Frelinger JA, et al. Recent advances in our understanding of the environmental, epidemiological, immunological, and clinical dimensions of coccidioidomycosis. *Clin Microbiol Rev* (2013) 26:505–25. doi:10.1128/CMR.00005-13
13. Malo J, Luraschi-Monjagatta C, Wolk DM, Thompson R, Hage CA, Knox KS. Update on the diagnosis of pulmonary coccidioidomycosis. *Ann Am Thorac Soc* (2014) 11:243–53. doi:10.1513/AnnalsATS.201308-286FR
14. Wheat LJ, Azar MM, Bahr NC, Spec A, Relich RF, Hage C. Histoplasmosis. *Infect Dis Clin North Am* (2016) 30:207–27. doi:10.1016/j.idc.2015.10.009
15. Teixeira Mde M, Patané JS, Taylor ML, Gómez BL, Theodoro RC, de Hoog S, et al. Worldwide phylogenetic distributions and population dynamics of the genus *Histoplasma*. *PLoS Negl Trop Dis* (2016) 10:e0004732. doi:10.1371/journal.pntd.0004732
16. Bahr NC, Antinori S, Wheat LJ, Sarosi GA. Histoplasmosis infections worldwide: thinking outside of the Ohio River valley. *Curr Trop Med Rep* (2015) 2:70–80. doi:10.1007/s40475-015-0044-0
17. Benedict K, Mody RK. Epidemiology of histoplasmosis outbreaks, United States, 1938–2013. *Emerg Infect Dis* (2016) 22:370–8. doi:10.3201/eid2203.151117
18. Pan B, Chen M, Pan W, Liao W. Histoplasmosis: a new endemic fungal infection in China? Review and analysis of cases. *Mycoses* (2013) 56:212–21. doi:10.1111/myc.12029
19. Theodoro RC, Teixeira Mde M, Felipe MS, Paduan Kdos S, Ribolla PM, San-Blas G, et al. Genus *Paracoccidioides*: species recognition and biogeographic aspects. *PLoS One* (2012) 7:e37694. doi:10.1371/journal.pone.0037694
20. Martinez R. New trends in paracoccidioidomycosis epidemiology. *J Fungi* (2017) 3:1. doi:10.3390/jof3010001
21. Arantes TD, Theodoro RC, Teixeira Mde M, Bosco Sde M, Bagagli E. Environmental mapping of *Paracoccidioides* spp. in Brazil reveals new clues into genetic diversity, biogeography and wild host association. *PLoS Negl Trop Dis* (2016) 10:e0004606. doi:10.1371/journal.pntd.0004606
22. Supparatpinyo K, Khamwan C, Baosoung V, Nelson KE, Sirisanthana T. Disseminated *Penicillium marneffei* infection in southeast Asia. *Lancet* (1994) 344:110–3. doi:10.1016/S0140-6736(94)91287-4
23. Vanittanakom N, Cooper CR Jr, Fisher MC, Sirisanthana T. *Penicillium marneffei* infection and recent advances in the epidemiology and molecular biology aspects. *Clin Microbiol Rev* (2006) 19:95–110. doi:10.1128/CMR.19.1.95-110.2006
24. Hu Y, Zhang J, Li X, Yang Y, Zhang Y, Ma J, et al. *Penicillium marneffei* infection: an emerging disease in mainland China. *Mycopathologia* (2013) 175:57–67. doi:10.1007/s11046-012-9577-0
25. Seitz AE, Adjemian J, Steiner CA, Prevots DR. Spatial epidemiology of blastomycosis hospitalizations: detecting clusters and identifying environmental risk factors. *Med Mycol* (2015) 53:447–54. doi:10.1093/mmy/myv014

26. Bradsher RW Jr. The endemic mimic: blastomycosis an illness often misdiagnosed. *Trans Am Clin Climatol Assoc* (2014) 125:188–202.
27. Castillo CG, Kauffman CA, Miceli MH. Blastomycosis. *Infect Dis Clin North Am* (2016) 30:247–64. doi:10.1016/j.idc.2015.10.002
28. Chakrabarti A, Bonifaz A, Gutierrez-Galhardo MC, Mochizuki T, Li S. Global epidemiology of sporotrichosis. *Med Mycol* (2015) 53:3–14. doi:10.1093/mmy/myu062
29. Kenyon C, Bonorchis K, Corcoran C, Meintjes G, Locketz M, Lehloenya R, et al. A dimorphic fungus causing disseminated infection in South Africa. *N Engl J Med* (2013) 369:1416–24. doi:10.1056/NEJMoa1215460
30. van Hougenhouck-Tulleken WG, Papavarnavas NS, Nel JS, Blackburn LY, Govender NP, Spencer DC, et al. HIV-associated disseminated emmonsiosis, Johannesburg, South Africa. *Emerg Infect Dis* (2014) 20:2164–6. doi:10.3201/eid2012.140902
31. Schwartz IS, Govender NP, Corcoran C, Dlamini S, Prozesky H, Burton R, et al. Clinical characteristics, diagnosis, management, and outcomes of disseminated emmonsiosis: a retrospective case series. *Clin Infect Dis* (2015) 61:1004–12. doi:10.1093/cid/civ439
32. Centers for Disease Control and Prevention. *Environmental Fungal Diseases in the United States*. (2016). Available from: https://www.cdc.gov/features/fungalinfections/
33. Centers for Disease Control and Prevention. *Areas Endemic for Coccidioidomycosis*. (2016). Available from: https://www.cdc.gov/fungal/diseases/coccidioidomycosis/causes.html
34. Centers for Disease Control and Prevention. *Areas Endemic for Histoplasmosis*. (2016). Available from: https://www.cdc.gov/fungal/diseases/histoplasmosis/causes.html
35. Leading International Fungal Education (LIFE). *Paracoccidioidomycosis*. (2016). Available from: http://www.life-worldwide.org/fungal-diseases/paracoccidioidomycosis
36. Leading International Fungal Education (LIFE). *Blastomycosis*. (2016). Available from: http://www.life-worldwide.org/fungal-diseases/blastomyces
37. Leading International Fungal Education (LIFE). *Histoplasmosis*. (2016). Available from: http://www.life-worldwide.org/fungal-diseases/histoplasmosis-chronic-cavitary-pulmonary
38. Leading International Fungal Education (LIFE). *Talaromyces marneffei Infectiton*. (2016). Available from: http://www.life-worldwide.org/fungal-diseases/talaromyces-marneffei-infection
39. Cao C, Liang L, Wang W, Luo H, Huang S, Liu D, et al. Common reservoirs for *Penicillium marneffei* infection in humans and rodents, China. *Emerg Infect Dis* (2011) 17:209–14. doi:10.3201/eid1702.100718
40. Centers for Disease Control and Prevention (CDC). Increase in reported coccidioidomycosis – United States, 1998–2011. *MMWR Morb Mortal Wkly Rep* (2013) 62:217–21.
41. Hector RF, Rutherford GW, Tsang CA, Erhart LM, McCotter O, Anderson SM, et al. The public health impact of coccidioidomycosis in Arizona and California. *Int J Environ Res Public Health* (2011) 8:1150–73. doi:10.3390/ijerph8041150
42. Téllez MD, Batista-Duharte A, Portuondo D, Quinello C, Bonne-Hernández R, Carlos IZ. *Sporothrix schenckii* complex biology: environment and fungal pathogenicity. *Microbiology* (2014) 160:2352–65. doi:10.1099/mic.0.081794-0
43. Mahajan VK. Sporotrichosis: an overview and therapeutic options. *Dermatol Res Pract* (2014) 2014:272376. doi:10.1155/2014/272376
44. Kauffman CA. Histoplasmosis: a clinical and laboratory update. *Clin Microbiol Rev* (2007) 20:115–32. doi:10.1128/CMR.00027-06
45. de Oliveira HC, Assato PA, Marcos CM, Scorzoni L, de Paula E Silva AC, Da Silva Jde F, et al. *Paracoccidioides*-host interaction: an overview on recent advances in the paracoccidioidomycosis. *Front Microbiol* (2015) 6:1319. doi:10.3389/fmicb.2015.01319
46. Saccente M, Woods GL. Clinical and laboratory update on blastomycosis. *Clin Microbiol Rev* (2010) 23:367–81. doi:10.1128/CMR.00056-09
47. Masannat FY, Ampel NM. Coccidioidomycosis in patients with HIV-1 infection in the era of potent antiretroviral therapy. *Clin Infect Dis* (2010) 50:1–7. doi:10.1086/648719
48. Adenis A, Nacher M, Hanf M, Vantilcke V, Boukhari R, Blachet D, et al. HIV-associated histoplasmosis early mortality and incidence trends: from neglect to priority. *PLoS Negl Trop Dis* (2014) 8:e3100. doi:10.1371/journal.pntd.0003100
49. Zheng J, Gui X, Cao Q, Yang R, Yan Y, Deng L, et al. A clinical study of acquired immunodeficiency syndrome associated *Penicillium marneffei* infection from a non-endemic area in China. *PLoS One* (2015) 10:e0130376. doi:10.1371/journal.pone.0130376
50. Kawila R, Chaiwarith R, Supparatpinyo K. Clinical and laboratory characteristics of penicilliosis marneffei among patients with and without HIV infection in Northern Thailand: a retrospective study. *BMC Infect Dis* (2013) 13:464. doi:10.1186/1471-2334-13-464
51. World Health Organization (WHO). *WHO Case Definitions of HIV for Surveillance and Revised Clinical Staging and Immunological Classification of HIV-Related Disease in Adults and Children*. Geneva: WHO (2007).
52. Morejón KM, Machado AA, Martinez R. Paracoccidioidomycosis in patients infected with and not infected with human immunodeficiency virus: a case-control study. *Am J Trop Med Hyg* (2009) 80:359–66.
53. Sarti EC, de Oliveira SM, dos Santos LF, de Camargo ZP, Paniago AM. Paracoccidioidal infection in HIV patients at an endemic area of paracoccidioidomycosis in Brazil. *Mycopathologia* (2012) 173:145–9. doi:10.1007/s11046-011-9495-6
54. Freitas DF, Valle AC, da Silva MB, Campos DP, Lyra MR, de Souza RV, et al. Sporotrichosis: an emerging neglected opportunistic infection in HIV-infected patients in Rio de Janeiro, Brazil. *PLoS Negl Trop Dis* (2014) 8:e3110. doi:10.1371/journal.pntd.0003110
55. Moreira JA, Freitas DF, Lamas CC. The impact of sporotrichosis in HIV-infected patients: a systematic review. *Infection* (2015) 43:267–76. doi:10.1007/s15010-015-0746-1
56. Witzig RS, Hoadley DJ, Greer DL, Abriola KP, Hernandez RL. Blastomycosis and human immunodeficiency virus: three new cases and review. *South Med J* (1994) 87:715–9. doi:10.1097/00007611-199407000-00008
57. Jones PG, Cohen RL, Batts DH, Silva J Jr. Disseminated histoplasmosis, invasive pulmonary aspergillosis, and other opportunistic infections in a homosexual patient with acquired immune deficiency syndrome. *Sex Transm Dis* (1983) 10:202–4. doi:10.1097/00007435-198311000-00010
58. Centers for Disease Control (CDC). Revision of the CDC surveillance case definition for acquired immunodeficiency syndrome. Council of State and Territorial Epidemiologists; AIDS program, Center for Infectious diseases. *MMWR Suppl* (1987) 36(Suppl 1):1S–15S.
59. Luckett K, Dummer JS, Miller G, Hester S, Thomas L. Histoplasmosis in patients with cell-mediated immunodeficiency: human immunodeficiency virus infection, organ transplantation, and tumor necrosis factor-α inhibition. *Open Forum Infect Dis* (2015) 2:ofu116. doi:10.1093/ofid/ofu116
60. Wheat J. Endemic mycoses in AIDS: a clinical review. *Clin Microbiol Rev* (1995) 8:146–59.
61. Adenis AA, Aznar C, Couppié P. Histoplasmosis in HIV-infected patients: a review of new developments and remaining gaps. *Curr Trop Med Rep* (2014) 1:119–28. doi:10.1007/s40475-014-0017-8
62. Nacher M, Adenis A, Mc Donald S, Do Socorro Mendonca Gomes M, Singh S, Lopes Lima I, et al. Disseminated histoplasmosis in HIV-infected patients in South America: a neglected killer continues on its rampage. *PLoS Negl Trop Dis* (2013) 7:e2319. doi:10.1371/journal.pntd.0002319
63. The Neglected Histoplasmosis in Latin America Group. Disseminated histoplasmosis in Central and South America, the invisible elephant: the lethal blind spot of international health organizations. *AIDS* (2016) 30:167–70. doi:10.1097/QAD.0000000000000961
64. Abrams DI, Robia M, Blumenfeld W, Simonson J, Cohen MB, Hadley WK. Disseminated coccidioidomycosis in AIDS. *N Engl J Med* (1984) 310:986–7. doi:10.1056/NEJM198404123101511
65. Roberts CJ. Coccidioidomycosis in acquired immune deficiency syndrome: depressed humoral as well as cellular immunity. *Am J Med* (1984) 76:734–6. doi:10.1016/0002-9343(84)90304-8
66. Bronnimann DA, Adam RD, Galgiani JN, Habib MP, Petersen EA, Porter B, et al. Coccidioidomycosis in the acquired immunodeficiency syndrome. *Ann Intern Med* (1987) 106:372–9. doi:10.7326/0003-4819-106-3-372
67. Ampel NM, Dols CL, Galgiani JN. Coccidioidomycosis during human immunodeficiency virus infection: results of a prospective study in a coccidioidal endemic area. *Am J Med* (1993) 94:235–40. doi:10.1016/0002-9343(93)90054-S

68. Li PC, Yeoh EK. Current epidemiological trends of HIV infection in Asia. *AIDS Clin Rev* (1992):1–23.
69. Kaldor JM, Sittitrai W, John TJ, Kitamura T. The emerging epidemic of HIV infection and AIDS in Asia and the Pacific. *AIDS* (1994) 8:S1–2.
70. DiSalvo AF, Fickling AM, Ajello L. Infection caused by *Penicillium marneffei*: description of first natural infection in man. *Am J Clin Pathol* (1973) 60:259–63. doi:10.1093/ajcp/60.2.259
71. Pautler KB, Padhye AA, Ajello L. Imported penicilliosis marneffei in the United States: report of a second human infection. *Sabouraudia* (1984) 22:433–8. doi:10.1080/00362178485380691
72. Jayanetra P, Nitiyanant P, Ajello L, Padhye AA, Lolekha S, Atichartakarn V, et al. Penicilliosis marneffei in Thailand: report of five human cases. *Am J Trop Med Hyg* (1984) 33:637–44. doi:10.4269/ajtmh.1984.33.637
73. Deng ZL, Connor DH. Progressive disseminated penicilliosis caused by *Penicillium marneffei*. Report of eight cases and differentiation of the causative organism from *Histoplasma capsulatum*. *Am J Clin Pathol* (1985) 84:323–7. doi:10.1093/ajcp/84.3.323
74. Yuen WC, Chan YF, Loke SL, Seto WH, Poon GP, Wong KK. Chronic lymphadenopathy caused by *Penicillium marneffei*: a condition mimicking tuberculous lymphadenopathy. *Br J Surg* (1986) 73:1007–8. doi:10.1002/bjs.1800731224
75. Deng ZL. [Penicilliosis marneffei]. *Zhonghua Bing Li Xue Za Zhi* (1987) 16(306–8):46.
76. Deng Z, Ribas JL, Gibson DW, Connor DH. Infections caused by *Penicillium marneffei* in China and Southeast Asia: review of eighteen published cases and report of four more Chinese cases. *Rev Infect Dis* (1988) 10:640–52. doi:10.1093/clinids/10.3.640
77. Peto TE, Bull R, Millard PR, Mackenzie DW, Campbell CK, Haines ME, et al. Systemic mycosis due to *Penicillium marneffei* in a patient with antibody to human immunodeficiency virus. *J Infect* (1988) 16:285–90. doi:10.1016/S0163-4453(88)97700-6
78. Chierakul W, Rajanuwong A, Wuthiekanun V, Teerawattanasook N, Gasiprong M, Simpson A, et al. The changing pattern of bloodstream infections associated with the rise in HIV prevalence in northeastern Thailand. *Trans R Soc Trop Med Hyg* (2004) 98:678–86. doi:10.1016/j.trstmh.2004.01.011
79. Nga TV, Parry CM, Le T, Lan NP, Diep TS, Campbell JI, et al. The decline of typhoid and the rise of non-typhoid salmonellae and fungal infections in a changing HIV landscape: bloodstream infection trends over 15 years in southern Vietnam. *Trans R Soc Trop Med Hyg* (2012) 106:26–34. doi:10.1016/j.trstmh.2011.10.004
80. Chayakulkeeree M, Denning DW. Serious fungal infections in Thailand. *Eur J Clin Microbiol Infect Dis* (2017) 36:931–35. doi:10.1007/s10096-017-2927-6
81. Le T, Wolbers M, Chi NH, Quang VM, Chinh NT, Lan NP, et al. Epidemiology, seasonality, and predictors of outcome of AIDS-associated *Penicillium marneffei* infection in Ho Chi Minh City, Viet Nam. *Clin Infect Dis* (2011) 52:945–52. doi:10.1093/cid/cir028
82. Wu TC, Chan JW, Ng CK, Tsang DN, Lee MP, Li PC. Clinical presentations and outcomes of *Penicillium marneffei* infections: a series from 1994 to 2004. *Hong Kong Med J* (2008) 14:103–9.
83. Wong SS, Wong KH, Hui WT, Lee SS, Lo JY, Cao L, et al. Differences in clinical and laboratory diagnostic characteristics of penicilliosis marneffei in human immunodeficiency virus (HIV)- and non-HIV-infected patients. *J Clin Microbiol* (2001) 39:4535–40. doi:10.1128/JCM.39.12.4535-4540.2001
84. Chan JF, Lau SK, Yuen KY, Woo PC. *Talaromyces (Penicillium) marneffei* infection in non-HIV-infected patients. *Emerg Microbes Infect* (2016) 5:e19. doi:10.1038/emi.2016.18
85. Lee PP, Chan KW, Lee TL, Ho MH, Chen XY, Li CH, et al. Penicilliosis in children without HIV infection – are they immunodeficient? *Clin Infect Dis* (2012) 54:e8–19. doi:10.1093/cid/cir754
86. Lovell JP, Foruraghi L, Freeman AF, Uzel G, Zerbe CS, Su H, et al. Persistent nodal histoplasmosis in nuclear factor kappa B essential modulator deficiency: report of a case and review of infection in primary immunodeficiencies. *J Allergy Clin Immunol* (2016) 138:903–5. doi:10.1016/j.jaci.2016.02.040
87. Odio CD, Marciano BE, Galgiani JN, Holland SM. Risk factors for disseminated coccidioidomycosis, United States. *Emerg Infect Dis* (2017) 23:308–11. doi:10.3201/eid2302.160505
88. Lee PP, Lau YL. Endemic infections in Southeast Asia provide new insights to the phenotypic spectrum of primary immunodeficiency disorders. *Asian Pac J Allergy Immunol* (2013) 31:217–26.
89. Romani L. Immunity to fungal infections. *Nat Rev Immunol* (2011) 11:275–88. doi:10.1038/nri2939
90. Drummond RA, Gaffen SL, Hise AG, Brown GD. Innate defense against fungal pathogens. *Cold Spring Harb Perspect Med* (2014) 5:a019620. doi:10.1101/cshperspect.a019620
91. Plato A, Hardison SE, Brown GD. Pattern recognition receptors in antifungal immunity. *Semin Immunopathol* (2015) 37:97–106. doi:10.1007/s00281-014-0462-4
92. Sukhithasri V, Nisha N, Biswas L, Anil Kumar V, Biswas R. Innate immune recognition of microbial cell wall components and microbial strategies to evade such recognitions. *Microbiol Res* (2013) 168:396–406. doi:10.1016/j.micres.2013.02.005
93. Barreto-Bergter E, Figueiredo RT. Fungal glycans and the innate immune recognition. *Front Cell Infect Microbiol* (2014) 4:145. doi:10.3389/fcimb.2014.00145
94. Turvey SE, Durandy A, Fischer A, Fung SY, Geha RS, Gewies A, et al. The CARD11-BCL10-MALT1 (CBM) signalosome complex: stepping into the limelight of human primary immunodeficiency. *J Allergy Clin Immunol* (2014) 134:276–84. doi:10.1016/j.jaci.2014.06.015
95. Drewniak A, Gazendam RP, Tool AT, van Houdt M, Jansen MH, van Hamme JL, et al. Invasive fungal infection and impaired neutrophil killing in human CARD9 deficiency. *Blood* (2013) 121:2385–92. doi:10.1182/blood-2012-08-450551
96. Glocker EO, Hennigs A, Nabavi M, Schäffer AA, Woellner C, Salzer U, et al. A homozygous CARD9 mutation in a family with susceptibility to fungal infections. *N Engl J Med* (2009) 361:1727–35. doi:10.1056/NEJMoa0810719
97. Lanternier F, Pathan S, Vincent QB, Liu L, Cypowyj S, Prando C, et al. Deep dermatophytosis and inherited CARD9 deficiency. *N Engl J Med* (2013) 369:1704–14. doi:10.1056/NEJMoa1208487
98. Okada S, Puel A, Casanova JL, Kobayashi M. Chronic mucocutaneous candidiasis disease associated with inborn errors of IL-17 immunity. *Clin Transl Immunol* (2016) 5:e114. doi:10.1038/cti.2016.71
99. Puel A, Döffinger R, Natividad A, Chrabieh M, Barcenas-Morales G, Picard C, et al. Autoantibodies against IL-17A, IL-17F, and IL-22 in patients with chronic mucocutaneous candidiasis and autoimmune polyendocrine syndrome type I. *J Exp Med* (2010) 207:291–7. doi:10.1084/jem.20091983
100. Chinn IK, Shearer WT. Severe combined immunodeficiency disorders. *Immunol Allergy Clin North Am* (2015) 35:671–94. doi:10.1016/j.iac.2015.07.002
101. Cirillo E, Giardino G, Gallo V, D'Assante R, Grasso F, Romano R, et al. Severe combined immunodeficiency – an update. *Ann N Y Acad Sci* (2015) 1356:90–106. doi:10.1111/nyas.12849
102. Vinh DC. Insights into human antifungal immunity from primary immunodeficiencies. *Lancet Infect Dis* (2011) 11:780–92. doi:10.1016/S1473-3099(11)70217-1
103. Lanternier F, Cypowyj S, Picard C, Bustamante J, Lortholary O, Casanova JL, et al. Primary immunodeficiencies underlying fungal infections. *Curr Opin Pediatr* (2013) 25:736–47. doi:10.1097/MOP.0000000000000031
104. Dinauer MC. Primary immune deficiencies with defects in neutrophil function. *Hematology Am Soc Hematol Educ Program* (2016) 2016:43–50. doi:10.1182/asheducation-2016.1.43
105. Falcone EL, Holland SM. Invasive fungal infection in chronic granulomatous disease: insights into pathogenesis and management. *Curr Opin Infect Dis* (2012) 25:658–69. doi:10.1097/QCO.0b013e328358b0a4
106. Henriet S, Verweij PE, Holland SM, Warris A. Invasive fungal infections in patients with chronic granulomatous disease. *Adv Exp Med Biol* (2013) 764:27–55. doi:10.1007/978-1-4614-4726-9_3
107. Haidar G, Zerbe CS, Cheng M, Zelazny AM, Holland SM, Sheridan KR. *Phellinus* species: an emerging cause of refractory fungal infections in patients with X-linked chronic granulomatous disease. *Mycoses* (2017) 60:155–60. doi:10.1111/myc.12573
108. Davies EG, Thrasher AJ. Update on the hyper immunoglobulin M syndromes. *Br J Haematol* (2010) 149:167–80. doi:10.1111/j.1365-2141.2010.08077.x
109. de la Morena MT. Clinical phenotypes of hyper-IgM syndromes. *J Allergy Clin Immunol Pract* (2016) 4:1023–36. doi:10.1016/j.jaip.2016.09.013
110. Tu RK, Peters ME, Gourley GR, Hong R. Esophageal histoplasmosis in a child with immunodeficiency with hyper-IgM. *AJR Am J Roentgenol* (1991) 157:381–2. doi:10.2214/ajr.157.2.1853826

111. Hostoffer RW, Berger M, Clark HT, Schreiber JR. Disseminated *Histoplasma capsulatum* in a patient with hyper IgM immunodeficiency. *Pediatrics* (1994) 94:234–6.
112. Yilmaz GG, Yilmaz E, Coşkun M, Karpuzoğlu G, Gelen T, Yeğin O. Cutaneous histoplasmosis in a child with hyper-IgM. *Pediatr Dermatol* (1995) 12:235–8. doi:10.1111/j.1525-1470.1995.tb00166.x
113. Danielian S, Oleastro M, Eva Rivas M, Cantisano C, Zelazko M. Clinical follow-up of 11 Argentinian CD40L-deficient patients with 7 unique mutations including the so-called "milder" mutants. *J Clin Immunol* (2007) 27:455–9. doi:10.1007/s10875-007-9089-8
114. Dahl K, Eggebeen A. Hyper IgM, histoplasmosis and MAS. *J Clin Immunol* (2012) 32:355.
115. Pedroza LA, Guerrero N, Stray-Pedersen A, Tafur C, Macias R, Muñoz G, et al. First case of CD40LG deficiency in Ecuador, diagnosed after whole exome sequencing in a patient with severe cutaneous histoplasmosis. *Front Pediatr* (2017) 5:17. doi:10.3389/fped.2017.00017
116. Cabral-Marques O, Schimke LF, Pereira PV, Falcai A, de Oliveira JB, Hackett MJ, et al. Expanding the clinical and genetic spectrum of human CD40L deficiency: the occurrence of paracoccidioidomycosis and other unusual infections in Brazilian patients. *J Clin Immunol* (2012) 32:212–20. doi:10.1007/s10875-011-9623-6
117. Cabral-Marques O, Arslanian C, Ramos RN, Morato M, Schimke L, Soeiro Pereira PV, et al. Dendritic cells from X-linked hyper-IgM patients present impaired responses to *Candida albicans* and *Paracoccidioides brasiliensis*. *J Allergy Clin Immunol* (2012) 129:778–86. doi:10.1016/j.jaci.2011.10.026
118. Kamchaisatian W, Kosalaraksa P, Benjaponpitak S, Hongeng S, Direkwattanachai C, Lumbiganon P, et al. Penicilliois in patients with X-linked hyperimmunoglobulin M syndrome (XHIGM), case reports from Thailand. *J Allergy Clin Immunol* (2006) 117:S282. doi:10.1016/j.jaci.2005.12.1166
119. Sripa C, Mitchai J, Thongsri W, Sripa B. Diagnostic cytology and morphometry of *Penicillium marneffei* in the sputum of a hypogammaglobulinemia with hyper-IgM patient. *J Med Assoc Thai* (2010) 93:S69–72.
120. Liu D, Zhong LL, Li Y, Chen M. Recurrent fever, hepatosplenomegaly and eosinophilia in a boy [article in Chinese]. *Zhongguo Dang Dai Er Ke Za Zhi* (2016) 18:1145–9.
121. Chu EY, Freeman AF, Jing H, Cowen EW, Davis J, Su HC, et al. Cutaneous manifestations of DOCK8 deficiency syndrome. *Arch Dermatol* (2012) 148:79–84. doi:10.1001/archdermatol.2011.262
122. Hsu AP, McReynolds LJ, Holland SM. GATA2 deficiency. *Curr Opin Allergy Clin Immunol* (2015) 15:104–9. doi:10.1097/ACI.0000000000000126
123. Collin M, Dickinson R, Bigley V. Haematopoietic and immune defects associated with GATA2 mutation. *Br J Haematol* (2015) 169:173–87. doi:10.1111/bjh.13317
124. Spinner MA, Sanchez LA, Hsu AP, Shaw PA, Zerbe CS, Calvo KR, et al. GATA2 deficiency: a protean disorder of hematopoiesis, lymphatics, and immunity. *Blood* (2014) 123:809–21. doi:10.1182/blood-2013-07-515528
125. Erwig LP, Gow NA. Interactions of fungal pathogens with phagocytes. *Nat Rev Microbiol* (2016) 14:163–76. doi:10.1038/nrmicro.2015.21
126. Bustamante J, Boisson-Dupuis S, Abel L, Casanova JL. Mendelian susceptibility to mycobacterial disease: genetic, immunological, and clinical features of inborn errors of IFN-γ immunity. *Semin Immunol* (2014) 26:454–70. doi:10.1016/j.smim.2014.09.008
127. Boisson-Dupuis S, Bustamante J, El-Baghdadi J, Camcioglu Y, Parvaneh N, El Azbaoui S, et al. Inherited and acquired immunodeficiencies underlying tuberculosis in childhood. *Immunol Rev* (2015) 264:103–20. doi:10.1111/imr.12272
128. Trinchieri G. Interleukin-12 and the regulation of innate resistance and adaptive immunity. *Nat Rev Immunol* (2003) 3:133–46. doi:10.1038/nri1001
129. Floss DM, Schröder J, Franke M, Scheller J. Insights into IL-23 biology: from structure to function. *Cytokine Growth Factor Rev* (2015) 26:569–78. doi:10.1016/j.cytogfr.2015.07.005
130. Boniface K, Blom B, Liu YJ, de Waal Malefyt R. From interleukin-23 to T-helper 17 cells: human T-helper cell differentiation revisited. *Immunol Rev* (2008) 226:132–46. doi:10.1111/j.1600-065X.2008.00714.x
131. Gaffen SL, Jain R, Garg AV, Cua DJ. The IL-23-IL-17 immune axis: from mechanisms to therapeutic testing. *Nat Rev Immunol* (2014) 14:585–600. doi:10.1038/nri3707
132. Espinosa V, Rivera A. Cytokines and the regulation of fungus-specific CD4 T cell differentiation. *Cytokine* (2012) 58:100–6. doi:10.1016/j.cyto.2011.11.005
133. Jin W, Chen D. IL-17 cytokines in immunity and inflammation. *Emerg Microbes Infect* (2013) 2:e60. doi:10.1038/emi.2013.58
134. de Beaucoudrey L, Samarina A, Bustamante J, Cobat A, Boisson-Dupuis S, Feinberg J, et al. Revisiting human IL-12Rβ1 deficiency: a survey of 141 patients from 30 countries. *Medicine (Baltimore)* (2010) 89:381–402. doi:10.1097/MD.0b013e3181fdd832
135. Ouederni M, Sanal O, Ikinciogullari A, Tezcan I, Dogu F, Sologuren I, et al. Clinical features of candidiasis in patients with inherited interleukin 12 receptor β1 deficiency. *Clin Infect Dis* (2014) 58:204–13. doi:10.1093/cid/cit722
136. Fieschi C, Bosticardo M, de Beaucoudrey L, Boisson-Dupuis S, Feinberg J, Santos OF, et al. A novel form of complete IL-12/IL-23 receptor {beta}1 deficiency with cell surface-expressed nonfunctional receptors. *Blood* (2004) 104:2095–101. doi:10.1182/blood-2004-02-0584
137. de Beaucoudrey L, Puel A, Filipe-Santos O, Cobat A, Ghandil P, Chrabieh M, et al. Mutations in STAT3 and IL12RB1 impair the development of human IL-17-producing T cells. *J Exp Med* (2008) 205:1543–50. doi:10.1084/jem.20080321
138. Moraes-Vasconcelos DD, Grumach AS, Yamaguti A, Andrade ME, Fieschi C, de Beaucoudrey L, et al. *Paracoccidioides brasiliensis* disseminated disease in a patient with inherited deficiency in the beta1 subunit of the interleukin (IL)-12/IL-23 receptor. *Clin Infect Dis* (2005) 41:e31–7. doi:10.1086/432119
139. Vinh DC. Coccidioidal meningitis: disseminated disease in patients without HIV/AIDS. *Medicine (Baltimore)* (2011) 90:87. doi:10.1097/MD.0b013e3182073ae3
140. Hwangpo TA, Harriw WT, Atkinson P, Cassady K, Kankirawatana S. IL-12 receptor defect predisposes to histoplasmosis. *Ann Allergy Asthma Immunol* (2012) 109:A80.
141. Falcão ACAM, Marques PTL, Santos AR, Oliveira JB. Disseminated histoplasmosis caused by IL12RB1 gene mutations in two Brazilian siblings. *J Clin Immunol* (2012) 32:405.
142. Dorman SE, Picard C, Lammas D, Heyne K, van Dissel JT, Baretto R, et al. Clinical features of dominant and recessive interferon gamma receptor 1 deficiencies. *Lancet* (2004) 364:2113–21. doi:10.1016/S0140-6736(04)17552-1
143. Fieschi C, Dupuis S, Picard C, Smith CI, Holland SM, Casanova JL. High levels of interferon gamma in the plasma of children with complete interferon gamma receptor deficiency. *Pediatrics* (2001) 107:E48. doi:10.1542/peds.107.4.e48
144. Sologuren I, Boisson-Dupuis S, Pestano J, Vincent QB, Fernández-Pérez L, Chapgier A, et al. Partial recessive IFN-γR1 deficiency: genetic, immunological and clinical features of 14 patients from 11 kindreds. *Hum Mol Genet* (2011) 20:1509–23. doi:10.1093/hmg/ddr029
145. Jouanguy E, Lamhamedi-Cherradi S, Lammas D, Dorman SE, Fondanèche MC, Dupuis S, et al. A human IFNGR1 small deletion hotspot associated with dominant susceptibility to mycobacterial infection. *Nat Genet* (1999) 21:370–8. doi:10.1038/7701
146. Zerbe CS, Holland SM. Disseminated histoplasmosis in persons with interferon-gamma receptor 1 deficiency. *Clin Infect Dis* (2005) 41:e38–41. doi:10.1086/432120
147. Vinh DC, Masannat F, Dzioba RB, Galgiani JN, Holland SM. Refractory disseminated coccidioidomycosis and mycobacteriosis in interferon-gamma receptor 1 deficiency. *Clin Infect Dis* (2009) 49:e62–5. doi:10.1086/605532
148. Dorman SE, Holland SM. Mutation in the signal-transducing chain of the interferon-gamma receptor and susceptibility to mycobacterial infection. *J Clin Invest* (1998) 101:2364–9. doi:10.1172/JCI2901
149. Döffinger R, Jouanguy E, Dupuis S, Fondanèche MC, Stephan JL, Emile JF, et al. Partial interferon-gamma receptor signaling chain deficiency in a patient with bacille Calmette-Guérin and *Mycobacterium abscessus* infection. *J Infect Dis* (2000) 181:379–84. doi:10.1086/315197
150. Moncada-Vélez M, Martinez-Barricarte R, Bogunovic D, Kong XF, Blancas-Galicia L, Tirpan C, et al. Partial IFN-γR2 deficiency is due to protein misfolding and can be rescued by inhibitors of glycosylation. *Blood* (2013) 122:2390–401. doi:10.1182/blood-2013-01-480814

151. Kong XF, Vogt G, Itan Y, Macura-Biegun A, Szaflarska A, Kowalczyk D, et al. Haploinsufficiency at the human IFNGR2 locus contributes to mycobacterial disease. *Hum Mol Genet* (2013) 22:769–81. doi:10.1093/hmg/dds484
152. Schneider WM, Chevillotte MD, Rice CM. Interferon-stimulated genes: a complex web of host defenses. *Annu Rev Immunol* (2014) 32:513–45. doi:10.1146/annurev-immunol-032713-120231
153. Dupuis S, Jouanguy E, Al-Hajjar S, Fieschi C, Al-Mohsen IZ, Al-Jumaah S, et al. Impaired response to interferon-alpha/beta and lethal viral disease in human STAT1 deficiency. *Nat Genet* (2003) 33:388–91. doi:10.1038/ng1097
154. Chapgier A, Wynn RF, Jouanguy E, Filipe-Santos O, Zhang S, Feinberg J, et al. Human complete Stat-1 deficiency is associated with defective type I and II IFN responses in vitro but immunity to some low virulence viruses in vivo. *J Immunol* (2006) 176:5078–83. doi:10.4049/jimmunol.176.8.5078
155. Chapgier A, Kong XF, Boisson-Dupuis S, Jouanguy E, Averbuch D, Feinberg J, et al. A partial form of recessive STAT1 deficiency in humans. *J Clin Invest* (2009) 119:1502–14. doi:10.1172/JCI37083
156. Dupuis S, Dargemont C, Fieschi C, Thomassin N, Rosenzweig S, Harris J, et al. Impairment of mycobacterial but not viral immunity by a germline human STAT1 mutation. *Science* (2001) 293:300–3. doi:10.1126/science.1061154
157. Smeekens SP, Plantinga TS, van de Veerdonk FL, Heinhuis B, Hoischen A, Joosten LA, et al. T1 hyperphosphorylation and defective IL12R/IL23R signaling underlie defective immunity in autosomal dominant chronic mucocutaneous candidiasis. *PLoS One* (2011) 6:e29248. doi:10.1371/journal.pone.0029248
158. van de Veerdonk FL, Plantinga TS, Hoischen A, Smeekens SP, Joosten LA, Gilissen C, et al. STAT1 mutations in autosomal dominant chronic mucocutaneous candidiasis. *N Engl J Med* (2011) 365:54–61. doi:10.1056/NEJMoa1100102
159. Toubiana J, Okada S, Hiller J, Oleastro M, Lagos Gomez M, Aldave Becerra JC, et al. Heterozygous STAT1 gain-of-function mutations underlie an unexpectedly broad clinical phenotype. *Blood* (2016) 127:3154–64. doi:10.1182/blood-2015-11-679902
160. Liu L, Okada S, Kong XF, Kreins AY, Cypowyj S, Abhyankar A, et al. Gain-of-function human STAT1 mutations impair IL-17 immunity and underlie chronic mucocutaneous candidiasis. *J Exp Med* (2011) 208:1635–48. doi:10.1084/jem.20110958
161. Sampaio EP, Hsu AP, Pechacek J, Bax HI, Dias DL, Paulson ML, et al. Signal transducer and activator of transcription 1 (STAT1) gain-of-function mutations and disseminated coccidioidomycosis and histoplasmosis. *J Allergy Clin Immunol* (2013) 131:1624–34. doi:10.1016/j.jaci.2013.01.052
162. Lee PP, Mao H, Yang W, Chan KW, Ho MH, Lee TL, et al. *Penicillium marneffei* infection and impaired IFN-γ immunity in humans with autosomal-dominant gain-of-phosphorylation STAT1 mutations. *J Allergy Clin Immunol* (2014) 133:894–6.e5. doi:10.1016/j.jaci.2013.08.051
163. Kwan EY, Lau YL, Yuen KY, Jones BM, Low LC. *Penicillium marneffei* infection in a non-HIV infected child. *J Paediatr Child Health* (1997) 33:267–71. doi:10.1111/j.1440-1754.1997.tb01596.x
164. Uzel G, Sampaio EP, Lawrence MG, Hsu AP, Hackett M, Dorsey MJ, et al. Dominant gain-of-function STAT1 mutations in FOXP3 wild-type immune dysregulation-polyendocrinopathy-enteropathy-X-linked-like syndrome. *J Allergy Clin Immunol* (2013) 131:1611–23. doi:10.1016/j.jaci.2012.11.054
165. Farmand S, Sundin M. Hyper-IgE syndromes: recent advances in pathogenesis, diagnostics and clinical care. *Curr Opin Hematol* (2015) 22:12–22. doi:10.1097/MOH.0000000000000104
166. Woellner C, Gertz EM, Schäffer AA, Lagos M, Perro M, Glocker EO, et al. Mutations in STAT3 and diagnostic guidelines for hyper-IgE syndrome. *J Allergy Clin Immunol* (2010) 125:424–32.e8. doi:10.1016/j.jaci.2009.10.059
167. Freeman AF, Holland SM. The hyper-IgE syndromes. *Immunol Allergy Clin North Am* (2008) 28:277–91, viii. doi:10.1016/j.iac.2008.01.005
168. Vinh DC, Sugui JA, Hsu AP, Freeman AF, Holland SM. Invasive fungal disease in autosomal-dominant hyper-IgE syndrome. *J Allergy Clin Immunol* (2010) 125:1389–90. doi:10.1016/j.jaci.2010.01.047
169. Chandesris MO, Melki I, Natividad A, Puel A, Fieschi C, Yun L, et al. Autosomal dominant STAT3 deficiency and hyper-IgE syndrome: molecular, cellular, and clinical features from a French national survey. *Medicine (Baltimore)* (2012) 91:e1–19. doi:10.1097/MD.0b013e31825f95b9
170. Roxo P, Menezes UP, Tucci S Jr, Andrade MF, Silva GE, Melo JM. Renal abscess in hyper-IgE syndrome. *Urology* (2013) 81:414–6. doi:10.1016/j.urology.2012.10.035
171. Milner JD, Brenchley JM, Laurence A, Freeman AF, Hill BJ, Elias KM, et al. Impaired T(H)17 cell differentiation in subjects with autosomal dominant hyper-IgE syndrome. *Nature* (2008) 452:773–6. doi:10.1038/nature06764
172. Al Khatib S, Keles S, Garcia-Lloret M, Karakoc-Aydiner E, Reisli I, Artac H, et al. Defects along the T(H)17 differentiation pathway underlie genetically distinct forms of the hyper IgE syndrome. *J Allergy Clin Immunol* (2009) 124:342–8, 348.e1–5. doi:10.1016/j.jaci.2009.05.004
173. Stanga SD, Dajud MV. Visual changes in a 4-year-old. *Clin Pediatr (Phila)* (2008) 47:959–61. doi:10.1177/0009922808319788
174. Powers AE, Bender JM, Kumánovics A, Ampofo K, Augustine N, Pavia AT, et al. *Coccidioides immitis* meningitis in a patient with hyperimmunoglobulin E syndrome due to a novel mutation in signal transducer and activator of transcription. *Pediatr Infect Dis J* (2009) 28:664–6. doi:10.1097/INF.0b013e31819866ec
175. Alberti-Flor JJ, Granda A. Ileocecal histoplasmosis mimicking Crohn's disease in a patient with Job's syndrome. *Digestion* (1986) 33:176–80. doi:10.1159/000199290
176. Cappell MS, Manzione NC. Recurrent colonic histoplasmosis after standard therapy with amphotericin B in a patient with Job's syndrome. *Am J Gastroenterol* (1991) 86:119–20.
177. Desai K, Huston DP, Harriman GR. Previously undiagnosed hyper-IgE syndrome in an adult with multiple systemic fungal infections. *J Allergy Clin Immunol* (1996) 98:1123–4. doi:10.1016/S0091-6749(96)80202-8
178. Steiner SJ, Kleiman MB, Corkins MR, Christenson JC, Wheat LJ. Ileocecal histoplasmosis simulating Crohn disease in a patient with hyperimmunoglobulin E syndrome. *Pediatr Infect Dis J* (2009) 28:744–6. doi:10.1097/INF.0b013e31819b65e0
179. Robinson WS, Arnold SR, Michael CF, Vickery JD, Schoumacher RA, Pivnick EK, et al. Case report of a young child with disseminated histoplasmosis and review of hyper immunoglobulin e syndrome (HIES). *Clin Mol Allergy* (2011) 9:14. doi:10.1186/1476-7961-9-14
180. Rana C, Krishnani N, Kumari N, Shastri C, Poddar U. Rectal histoplasmosis in Job's syndrome. *Indian J Gastroenterol* (2013) 32:64–5. doi:10.1007/s12664-012-0275-0
181. Jiao J, Horner CC, Kau AL. Terminal ileum perforation in a patient with hyper-IgE syndrome. *Ann Allergy Asthma Immunol* (2014) 113:S61.
182. Odio CD, Milligan KL, McGowan K, Rudman Spergel AK, Bishop R, Boris L, et al. Endemic mycoses in patients with STAT3-mutated hyper-IgE (Job) syndrome. *J Allergy Clin Immunol* (2015) 136:1411–3.e1–2. doi:10.1016/j.jaci.2015.07.003
183. Ma BH, Ng CS, Lam R, Wan S, Wan IY, Lee TW, et al. Recurrent hemoptysis with *Penicillium marneffei* and *Stenotrophomonas maltophilia* in Job's syndrome. *Can Respir J* (2009) 16:e50–2. doi:10.1155/2009/586919
184. Salvana EM, Salata RA. Infectious complications associated with monoclonal antibodies and related small molecules. *Clin Microbiol Rev* (2009) 22:274–90. doi:10.1128/CMR.00040-08
185. Ordonez ME, Farraye FA, Di Palma JA. Endemic fungal infections in inflammatory bowel disease associated with anti-TNF antibody therapy. *Inflamm Bowel Dis* (2013) 19:2490–500. doi:10.1097/MIB.0b013e31828f1fba
186. Davies HD; Committee on Infectious Diseases. Infectious complications with the use of biologic response modifiers in infants and children. *Pediatrics* (2016) 138:e20161209. doi:10.1542/peds.2016-1209
187. Galgiani JN, Ampel NM, Blair JE, Catanzaro A, Geertsma F, Hoover SE, et al. Executive summary: 2016 Infectious Diseases Society of America (IDSA) clinical practice guideline for the treatment of coccidioidomycosis. *Clin Infect Dis* (2016) 63:717–22. doi:10.1093/cid/ciw538
188. Chan JF, Chan TS, Gill H, Lam FY, Trendell-Smith NJ, Sridhar S, et al. Disseminated infections with *Talaromyces marneffei* in non-AIDS patients given monoclonal antibodies against CD20 and kinase inhibitors. *Emerg Infect Dis* (2015) 21:1101–6. doi:10.3201/eid2107.150138
189. Tse E, Leung RY, Kwong YL. Invasive fungal infections after obinutuzumab monotherapy for refractory chronic lymphocytic leukemia. *Ann Hematol* (2015) 94:165–7. doi:10.1007/s00277-014-2120-2

190. Tang BS, Chan JF, Chen M, Tsang OT, Mok MY, Lai RW, et al. Disseminated penicilliosis, recurrent bacteremic nontyphoidal salmonellosis, and burkholderiosis associated with acquired immunodeficiency due to autoantibody against gamma interferon. *Clin Vaccine Immunol* (2010) 17:1132–8. doi:10.1128/CVI.00053-10
191. Kampitak T, Suwanpimolkul G, Browne S, Suankratay C. Anti-interferon-γ autoantibody and opportunistic infections: case series and review of the literature. *Infection* (2011) 39:65–71. doi:10.1007/s15010-010-0067-3
192. Browne SK, Burbelo PD, Chetchotisakd P, Suputtamongkol Y, Kiertiburanakul S, Shaw PA, et al. Adult-onset immunodeficiency in Thailand and Taiwan. *N Engl J Med* (2012) 367:725–34. doi:10.1056/NEJMoa1111160
193. Wongkulab P, Wipasa J, Chaiwarith R, Supparatpinyo K. Autoantibody to interferon-gamma associated with adult-onset immunodeficiency in non-HIV individuals in Northern Thailand. *PLoS One* (2013) 8:e76371. doi:10.1371/journal.pone.0076371
194. Chan JF, Trendell-Smith NJ, Chan JC, Hung IF, Tang BS, Cheng VC, et al. Reactive and infective dermatoses associated with adult-onset immunodeficiency due to anti-interferon-gamma autoantibody: Sweet's syndrome and beyond. *Dermatology* (2013) 226:157–66. doi:10.1159/000347112
195. Chi CY, Lin CH, Ho MW, Ding JY, Huang WC, Shih HP, et al. Clinical manifestations, course, and outcome of patients with neutralizing anti-interferon-γ autoantibodies and disseminated nontuberculous mycobacterial infections. *Medicine (Baltimore)* (2016) 95:e3927. doi:10.1097/MD.0000000000003927
196. Chi CY, Chu CC, Liu JP, Lin CH, Ho MW, Lo WJ, et al. Anti-IFN-γ autoantibodies in adults with disseminated nontuberculous mycobacterial infections are associated with HLA-DRB1*16:02 and HLA-DQB1*05:02 and the reactivation of latent varicella-zoster virus infection. *Blood* (2013) 121:1357–66. doi:10.1182/blood-2012-08-452482
197. Ku CL, Lin CH, Chang SW, Chu CC, Chan JF, Kong XF, et al. Anti-IFN-γ autoantibodies are strongly associated with HLA-DR*15:02/16:02 and HLA-DQ*05:01/05:02 across Southeast Asia. *J Allergy Clin Immunol* (2016) 137:945–8.e8. doi:10.1016/j.jaci.2015.09.018
198. Lin CH, Chi CY, Shih HP, Ding JY, Lo CC, Wang SY, et al. Identification of a major epitope by anti-interferon-γ autoantibodies in patients with mycobacterial disease. *Nat Med* (2016) 22:994–1001. doi:10.1038/nm.4158
199. Dujardin JC, Herrera S, do Rosario V, Arevalo J, Boelaert M, Carrasco HJ, et al. Research priorities for neglected infectious diseases in Latin America and the Caribbean region. *PLoS Negl Trop Dis* (2010) 4:e780. doi:10.1371/journal.pntd.0000780
200. Brown GD, Denning DW, Gow NA, Levitz SM, Netea MG, White TC. Hidden killers: human fungal infections. *Sci Transl Med* (2012) 4:165rv13. doi:10.1126/scitranslmed.3004404

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REVIEW
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Uses of Next-Generation Sequencing Technologies for the Diagnosis of Primary Immunodeficiencies

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Primary immunodeficiencies (PIDs) are genetic disorders impairing host immunity, leading to life-threatening infections, autoimmunity, and/or malignancies. Genomic technologies have been critical for expediting the discovery of novel genetic defects underlying PIDs, expanding our knowledge of the complex clinical phenotypes associated with PIDs, and in shifting paradigms of PID pathogenesis. Once considered Mendelian, monogenic, and completely penetrant disorders, genomic studies have redefined PIDs as a heterogeneous group of diseases found in the global population that may arise through multigenic defects, non-germline transmission, and with variable penetrance. This review examines the uses of next-generation DNA sequencing (NGS) in the diagnosis of PIDs. While whole genome sequencing identifies variants throughout the genome, whole exome sequencing sequences only the protein-coding regions within a genome, and targeted gene panels sequence only a specific cohort of genes. The advantages and limitations of each sequencing approach are compared. The complexities of variant interpretation and variant validation remain the major challenge in wide-spread implementation of these technologies. Lastly, the roles of NGS in newborn screening and precision therapeutics for individuals with PID are also addressed.

Keywords: primary immunodeficiency, next-generation sequencing, whole exome sequencing, gene panels, genomics

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INTRODUCTION

Primary immunodeficiencies (PIDs) are genetic diseases that affect the development and/or function of the immune system, thereby increasing the susceptibility to infectious pathogens, autoimmunity (1), and malignancies (2). Classically, PIDs have been considered monogenic disorders that follow the principles of Mendelian inheritance. However, advances in DNA sequencing technologies have facilitated the identification of multigenic and somatic causes of PIDs and have revealed the wide phenotypic variability of these diseases (3, 4).

The global incidence of PIDs has been estimated to be 1:10,000 live births (5) although this is considered an underestimation due to limited patient access to diagnostic technologies and the challenges of diagnosing patients with atypical clinical presentations (6). Although PIDs are rare diseases from a global perspective, PIDs are more prevalent in areas with highly consanguineous populations due to the predominance of autosomal recessive PIDs. The incidence of consanguineous unions is 65% in the Middle East, significantly higher than what has been found in Europe, the Western Pacific region, and Latin America (5.6, 2.3, and 0.96%, respectively) (7, 8). Correspondingly, the incidence of PID is 20 times greater in Middle Eastern countries compared with North America and Europe (9). Attempts in delineating the epidemiology of PIDs have utilized different data collection methodologies, including clinician registries, hospital/health insurance databases, and population

surveys (10–14). These epidemiologic studies have revealed a great need for more clinicians trained in the diagnosis of PIDs as well as access to inexpensive diagnostic technologies, particularly in resource-limited areas of the world (15). To compensate for the uneven distribution of clinical and technical expertise focused on PID, collaborative networks of PID specialists have been established throughout the globe. These networks have facilitated collaborative efforts for identifying novel genetic defects underlying PIDs and improving the diagnosis of PIDs (16).

Traditionally, the initial evaluation of patients with suspected PIDs has consisted of both quantitative and qualitative analysis of the immune system. Laboratories with expertise in the diagnosis of PIDs perform enumeration of lymphocyte subpopulations, lymphocyte proliferation studies, quantification of immunoglobulin levels and vaccine-specific antibody titers, evaluation of complement levels and function, as well as tests for specific pathways (e.g., assays for investigating Toll-like receptor function, neutrophil oxidative burst, or T cell receptor signaling pathways). This approach enables clinicians categorize the phenotype of a patient's PID, with the aim of prioritizing the most likely causative genetic defects and guiding therapeutic decision (17, 18). However, this approach is time-consuming, costly, and requires viable cells from patients as well as personnel trained in a diversity of laboratory techniques. The shipping of patient blood to tertiary referral centers results in impaired cellular responses and viability that can compromise the accuracy of diagnostic tests (19, 20). Furthermore, the field of PIDs advances rapidly, since mutations in over 200 genes are known to cause PIDs and over 10 novel PIDs are discovered annually (17, 21). Sanger sequencing, which is the conventional approach for gene sequencing, is much slower and more costly than next-generation DNA sequencing (NGS) (22). Therefore, there is a great need for improving patient access to leading-edge diagnostic technologies.

IMPACT OF NGS ON THE GENOTYPE–PHENOTYPE CORRELATION IN PIDs

Next-generation DNA sequencing has significantly changed our understanding of PIDs, which are no longer considered purely monogenic diseases following Mendelian patterns of inheritance. By enabling sequencing of the entire exome or genome, NGS has demonstrated the breadth of unusual phenotypes caused by mutations in genes known to cause PID. This is exemplified by patients who have a common variable immunodeficiency (CVID)-like phenotype due to hypomorphic mutations in genes classically associated with severe combined immunodeficiency (SCID). These include *RAG1* (23), *DCLRE1C* (24, 25), and *JAK3* (26). The reported patient with the hypomorphic mutation in *DCLRE1C* did not receive a molecular diagnosis until his second decade of life. There are also reports of hypomorphic mutations in *RAG1* and *RAG2* resulting in a combined immunodeficiency less severe than classical SCID, thus permitting survival into adulthood (27, 28).

The unbiased nature of whole exome sequencing (WES) and whole genome sequencing (WGS) has facilitated the discovery of multigenic PIDs (4). In 28 patients with abnormal degranulation assays, Zhang et al. found heterozygous mutations in two genes associated with familial hemophagocytic lymphohistiocytosis (29). Additionally, the broad spectrum of clinical phenotypes in patients with PIDs may reflect the effects of modifier mutations. As an example, WGS of a patient with LRBA deficiency identified a homozygous mutation in the base excision repair enzyme *NEIL3* (30). Studies of Neil3-deficent mice demonstrated its critical role in maintaining peripheral B cell tolerance and in providing protection against autoimmunity. Synergy of NEIL3 deficiency and LRBA deficiency was proposed as the cause of the patient's markedly severe clinical phenotype (30).

The high-throughput approach of NGS enables deep sequencing coverage, which refers to the average number of times any given nucleotide is sequenced. This is essential for identifying somatic variants, which occur in only a small subpopulation of cells. Although PIDs are classically considered to be germline defects, somatic mutations have now been recognized as a cause of PIDs (31). For example, autoimmune lymphoproliferative syndrome can arise from somatic mutations in *TNFRSF6*, *KRAS*, or *NRAS*. Certain mutations in *TNFRSF6*, which encodes the Fas receptor, in the lymphoid lineage have a dominant negative effect that allows increased survival and lymphoproliferation mimicking the phenotype of germline *TNFRSF6* mutations (32, 33). Somatic gain-of-function (GOF) mutations affecting *KRAS* and *NRAS* have been reported to cause RAS-associated leukoproliferative disease, a syndrome that presents with autoimmunity, splenomegaly, and lymphadenopathies, with or without expansion of alpha beta double negative T cells (34, 35). Importantly, some of these somatic mutations have been found only after enriching for specific lymphocyte populations prior to DNA isolation, thus highlighting the importance of cell-specific NGS in some disorders (32).

NGS TECHNOLOGIES

Next-generation DNA sequencing has revolutionized the diagnosis of genetic diseases by providing high-throughput and increasingly cost-efficient diagnostic technologies (**Figure 1**). The most comprehensive NGS technique is WGS, which sequences a patient's entire genome and enables the identification of variants in exonic and non-coding regions (36). WES is a more focused technology that sequences only the protein-coding regions within a genome, which contain approximately 85% of disease-causing mutations (37). The most focused NGS approach is the targeted gene panel (TGP), which sequences a specific cohort of genes. These three approaches differ primarily in the comprehensiveness of genetic sequencing, which translates into differences in the complexity of data analysis and cost (**Table 1**). Since the exome contains approximately 30,000 genes, or <2% of the human genome, the exome can be sequenced at a greater depth than the genome at a lower price. The cost of an average exome has been estimated at $800 (38), although the fee for a clinical-grade exome typically exceeds several thousand dollars due to the certifications and regulations applied to clinical testing. A TGP focused on genes specific to a clinical phenotype is even more cost efficient, with studies reporting a range of $250–500 per sample for targeted PID gene panels (39). Barcoding and batching samples in sequencing runs is the key to cost efficiency: increasing the number of samples per sequencing run minimizes

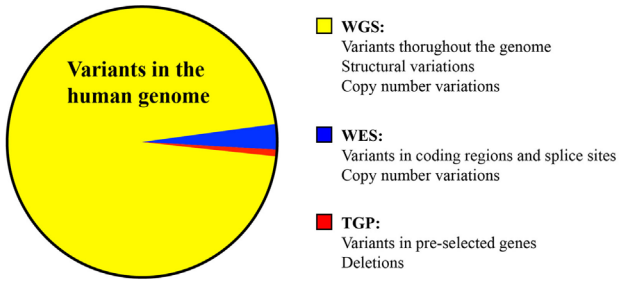

FIGURE 1 | Comparison of the variants identified by whole genome sequencing, whole exome sequencing, and targeted panel sequencing.

TABLE 1 | Comparison of targeted panels, whole exome sequencing (WES), and whole genome sequencing (WGS).

	Targeted panel	WES	WGS
Target	300 genes	2% of genome	Entire genome
Cost per sample (USD)	$250–500	$800	$1,400–1,600
Variants detected	Variable: depends on the panel size	~20,000	~4,000,000
Advantages	1. Customizable 2. Lowest cost	1. Identifies novel genetic causes of primary immunodeficiencies (PIDs) in coding regions 2. Low cost	1. Identifies novel genetic causes of PIDs in coding and non-coding regions 2. Detects structural variants 3. Most uniform depth of sequencing
Limitations	1. Variants limited to the pre-selected gene panel 2. Requires updates as new diseases are discovered 3. Cannot detect structural variants	1. Sequencing depth affected by poor/incomplete exome capture 2. Cannot detect non-coding or structural variants	1. Highest cost 2. Largest volume of data and the most complex analysis

the use of NGS reagents, which account for a significant amount of the overall operating costs (22). The decreasing costs of NGS, coupled with the stability of DNA, render NGS a potentially powerful tool even for resource-limited areas.

Diagnostic Yield

The diagnostic yield of NGS technologies is determined by the limitations specific to each approach. WES and TGP rely on the preparation of libraries containing fragments of patient DNA complementary to the exome or panel of selected genes, respectively (40). Most NGS technologies require PCR amplification of libraries to generate sufficient quantities of DNA for high-throughput sequencing (41). The preparation of incomplete libraries will lead to gaps in sequencing coverage that can potentially miss pathogenic variants. Furthermore, the detection of structural variations, such as large insertions or deletions, translocations, inversions, and copy-number variations, is much more difficult by WES or TGP because the target regions are not contiguous, as they are in WGS (42). The identification of structural variations is important for the diagnosis of PIDs, since pathogenic variations have been commonly identified in large, repetitive genes, such as *DOCK8* and *LRBA* (43).

Studies utilizing patients with PIDs have reported wide variability in the diagnostic yield of NGS approaches. The only study directly comparing WES with WGS in patients with immunodeficiency demonstrated that WGS identified 656 more coding variants than WES in six patient studies; furthermore, WES in this small cohort was not reliable for the detection of copy-number variants, all of which involved non-coding regions (44). WES of patients from 278 families with PIDs achieved a diagnostic yield of 40%, resulting in a modified clinical diagnosis for 50% of patients, and alterations in the therapeutic management for 25% of patients (45). Another study applying WES to 50 patients with CVID found a similar diagnostic yield of 30% (46). The reported diagnostic yields using TGP in patients with PIDs are lower. A TGP of 170 genes associated with PIDs identified a diagnosis in 15% of 26 patients sequenced (47); another study using a TGP of 162 PID genes achieved a diagnostic yield of 25% in 139 patients with PIDs (22). Since TGPs are composed of pre-selected genes, these panels will not identify an unexpected or novel genetic cause of PID, thus leading to a lower diagnostic yield.

Pitfalls of NGS

Previously published studies utilizing WES or TGPs as diagnostic tools for patients with PIDs show that at least 60% of patients remain undiagnosed (22, 45–47). By comparison, conventional genetic testing, such as Sanger sequencing for single genes, karyotyping, and chromosomal microarrays identifies a diagnosis in only ~15% of patients, thus leaving 85% without a diagnosis (48). Despite the increased diagnostic yield of NGS compared with conventional genetic testing, the fact that the majority of patients lack a diagnosis indicates that deficits in the technologies, data analysis, or our understanding of PIDs remain.

Depending on the depth of sequencing, NGS detects 20,000–50,000 variants per patient sample (49). Sequencing a familial trio or quartet, consisting of the proband and his parents and/or siblings, is a common approach used to narrow the list of candidate mutations (49). However, this approach assumes complete penetrance of the disease. Misidentification of an ostensibly healthy family member as "unaffected" will eliminate all variants in this individual from the candidate variant list, including those pathogenic mutation in the individual that are incompletely penetrant. While incomplete penetrance is well-known to occur in autosomal dominant PIDs, such as CTLA4 haploinsufficiency (50), studies have begun to show variability in the genotype–phenotype association for autosomal recessive diseases as well. For example, a homozygous mutation in *ICOS* resulted in a combined immunodeficiency in the proband, but only mild hypogammaglobulinemia and

decreased antibody titers to some but not all vaccines in his sister (51). Both the proband and his sister had absent ICOS expression and severely decreased T follicular helper cells, demonstrating that a deleterious homozygous mutation can translate to clinical variability (51).

Potentially pathogenic variants are prioritized using computational pipelines, comparisons with public databases of polymorphisms, software for predicting the effect of a given variant *in silico* (Polyphen, SIFT, MutationTaster, among others), and knowledge of genetically modified cell lines and animal models (52, 53). Synonymous mutations, which do not alter the amino acid sequence of the protein in production, are typically considered benign variants and eliminated from the candidate mutation list. However, recent studies have shown that synonymous mutations can result in PIDs. A patient with $T^-B^+NK^{low}$ SCID was found to have a synonymous mutation in exon 19 of JAK3, which served as a cryptic donor splice site that generated an unstable JAK3 mutant protein (54). In another report, a synonymous mutation in exon 3 of *IL7R* was found to cause aberrant splicing, leading to $T^{low}B^+NK^+$ SCID (55). Both of these studies demonstrate that NGS cannot serve as the only diagnostic tool for patients with PIDs, since functional studies are needed to determine the biologic effect of novel mutations.

Interpretation of Variants

Clinical criteria have been established to standardize the approach for interpreting genetic variants (56). The classification of variants as pathogenic, likely pathogenic, benign, likely benign, or a variant of uncertain significance integrates genetic and biologic criteria. Strong criteria supporting pathogenicity include: a null variant in a gene in which loss of function has been previously shown to cause human disease, studies demonstrating loss of altered protein expression and/or function, previously published evidence of a genotype-disease correlation. Additional criteria include the identification of the variant only in individuals demonstrating the disease phenotype, location of the variant in a highly conserved genomic locus, and *in silico* predictions of pathogenicity. In contrast, benign variants include those with a high (>5%) minor allelic frequency in established databases, those that fail to segregate with the disease phenotype, those with no demonstrated biologic effect through *in vitro* testing. Additionally, mutations in genes expressed strictly in organs without immune function (e.g., genes encoding olfactory receptors) are unlikely to be the cause of PIDs. Variants of uncertain significance are those that do not meet sufficient criteria for classification as pathogenic, likely pathogenic, benign, or likely benign. These variants of uncertain significance include mutations in genes whose relevance to human disease is not yet known. This is particularly relevant to patients with PIDs, since the pace of discovery in the field is rapid: 34 novel genetic causes of PIDs were discovered between 2013 and 2015 (18). GOF variants constitute another challenging category. These mutations are typically predicted to be benign by *in silico* algorithms because these mutations enhance, rather than impair gene function. However, GOF variants in *PIK3CD*, *STAT1*, *STAT3*, and *CARD11* result in immune dysfunction or dysregulation, and thus, biologic assays delineating the mechanisms linking a GOF mutation to a disease phenotype are essential (18).

The primary limitation of genomic diagnostics is the lack of functional evidence provided by sequencing alone: functional assays are required to demonstrate the biologic effect of a variant. This is particularly important for non-coding variants whose effects are challenging to predict *in silico*. The importance of functional validation has been underscored in a study of 33 missense mutations in 23 genes essential for immune function. Only 15–20% of those predicted to be deleterious *in silico* were shown to have a pathogenic effect *in vivo* using mouse models (57). Due to the rarity of PIDs, novel defects often occur in single patients and thus lack the burden of proof provided by multiple unrelated patients sharing the same genotype–phenotype correlation. Therefore, criteria have been proposed for establishing the causal relationship between the patient's genotype and phenotype: firstly, the candidate genotype must not be found in healthy individuals; secondly, the variant must be proven to destroy or significantly impair the expression or function of the gene product; thirdly, the causal relationship between the patient's genotype and phenotype must be replicated in a relevant cellular or animal model (3). The increasing breadth of public databases, such as the dbSNP, 1,000 genomes, and ExAC databases, enables researchers and clinicians to determine the prevalence of a given mutation in the general population. A diverse array of assays may be required to confirm the biologic effect of mutations identified by NGS. For example, defects impairing gene expression can be evaluated by Western blotting or flow cytometry (58, 59). Receptor activation can be assessed by the phosphorylation of downstream proteins or upregulation of target gene expression (60). Mutations in genes important for lymphocyte activation can be evaluated by assessing upregulation of activation markers, quantifying proliferation in response to mitogens and antigen stimulation, and measure the secretion of cytokines and immunoglobulins after T or B cell activation, respectively (61). Cellular modeling requires the use of patient-derived cells that may not be readily available. The use of induced pluripotent stem cells (iPSCs) is one way to circumvent this obstacle. iPSCs are pluripotent cells derived from terminally differentiated patient cells that can be subsequently re-differentiated into relevant cell types (62–66). As an example, the reprogramming of iPSCs from dermal fibroblasts of TLR3- or UNC-93-deficient patients into neural stem cells, astrocytes, oligodendrocytes, and neurons provided *in vitro* evidence that deficiencies in TLR3 and UNC-93B result in neuronal cell susceptibility to herpes simplex virus 1 (67).

Animal models of PIDs complement cellular studies by enabling investigations of a molecular defect within the context of an *in vivo* immune system on a uniform genetic background. In the field of PIDs, the mouse and zebrafish are the two most commonly used animal models for demonstrating the biologic effect of mutations. Mice are the most frequently used animal model system due to the high level of homology between the mouse and human immune systems, their rapid reproductive rate, and their small size. Mouse models are particularly useful for delineating the contribution of genes with poorly understood contributions to human immunity. This was the

case for transferrin receptor 1 (TfR1), a ubiquitously expressed cell surface receptor known to be essential for erythropoiesis (68). A missense mutation impairing TfR1 internalization was shown to result in combined immunodeficiency due to its role in lymphocyte proliferation and class-switching, but permitted normal erythroid development due to the presence of an erythroid cell-specific accessory pathway for TfR1 endocytosis (68). Zebrafish are another *in vivo* system for studying the biologic effect of novel mutations because the majority of human genes have orthologs in the zebrafish genome and their rapid development, high reproductive rate, and transparent bodies are conducive to gene editing and live-imaging studies (69). Zebrafish have been used to model multiple types of SCID using mutants lacking RAG1, ZAP-70, TBX1, JAK3, IL7R, AK2, BCL11B, or EXTL3 (64, 70–72), as well as warts–hypogammaglobulinemia–immunodeficiency–myelokathexis (WHIM) syndrome (73). While these animal models provide invaluable opportunities for delineating the mechanisms driving PIDs *in vivo*, the genetic differences among species constitutes the major limitation of these models. To circumvent this, recent studies have begun to used humanized mice to model PIDs. Humanized mice are generated by transplanting human hematopoietic stem cells (HSCs) into immunodeficient mice, such as the *Il2rg* knockout mice, which then generate human immune cells *de novo.* By combining this approach with iPSC technology, dermal fibroblasts or PBMCs from patients with PIDs can be de-differentiated into iPSCs, which are then re-differentiated into HSCs to generate a humanized mouse model of the patient's PID. This approach has been used to generate a humanized mouse model of JAK3 deficiency; gene editing using clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR-associated protein (Cas) successfully corrected the JAK3 mutation and restored T cell development in this model (74). This humanized mouse model therefore provided *in vivo* proof of concept for CRISPR/Cas9 gene editing as a therapeutic strategy for JAK3 deficiency.

Collectively, these studies demonstrate that NGS is only one of many steps in the diagnosis of a PID (**Figure 2**). DNA, due to its stability, can be easily shipped to diagnostic centers with genomics expertise for NGS and *in silico* interpretation of variants. Therefore, a TGP can serve as a screening test. However, the validation of previously unreported mutations even in well-described genes requires multidisciplinary expertise in molecular and cellular biology, biochemistry, and immunology. Biologic outcomes from a mutation in a poorly characterized or novel gene require cellular and animal modeling of the mutation. This remains a major limitation in the diagnosis of the PIDs in resource-limited areas of the world.

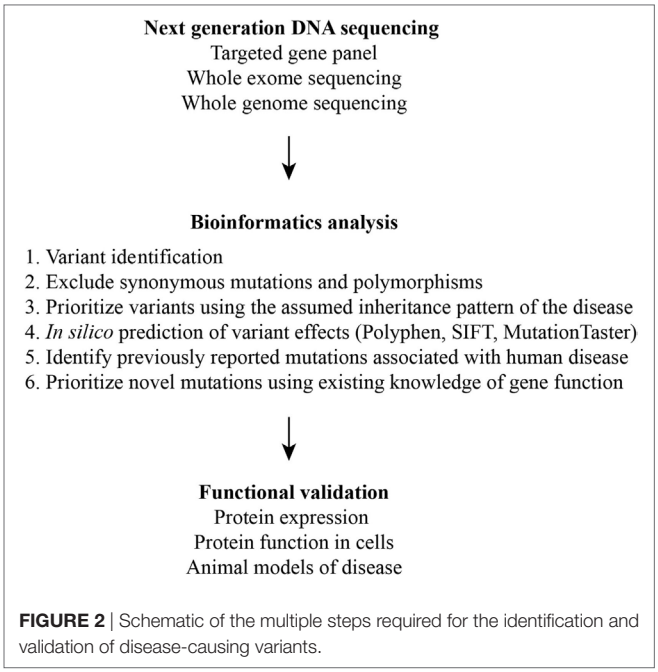

FIGURE 2 | Schematic of the multiple steps required for the identification and validation of disease-causing variants.

NGS AS A TOOL FOR PRECISION MEDICINE IN THE FIELD OF PIDs

Newborn Screening (NBS)

The global prevalence and the severity of PIDs indicate a need for improved diagnosis of these disorders, particularly in resource-limited areas of the world. Hematopoietic stem cell transplant (HSCT), the standard cure for patients with SCID, has better outcomes in younger patients. The largest multicenter study of transplantation outcomes in patients with SCID found that the 5-year survival rate and reconstitution were significantly better in patients who received matched sibling donor HSCT, but that the survival rate among infants transplanted prior to 3.5 months of age was high, independent of donor type (75). This has increased the momentum for SCID NBS. The current method of NBS for SCID quantifies T cell receptor excision circles (TRECs), which reflect generation of naïve T cells. Likewise, kappa light chain-deleting recombination excision circles (KRECs) assays are used to measure the generation of naïve B cells. Although relatively inexpensive, these tests do not provide a molecular diagnosis and are limited to defects affecting naïve T and B cell generation. While the current cost of NGS greatly exceeds that of TREC/KREC assays, the falling cost of sequencing may enable targeted panels to serve as an early diagnostic tool, as demonstrated by several feasibility studies. A 2015 study performed NGS on DNA extracted from the dried blood spot to screen for 48 different *CFTR* mutations in 67 newborns with known pathogenic mutations in *CFTR* (76). NGS was in complete concordance with the previously confirmed mutations in this cohort. Another study, in which NGS was used for the screening of inherited metabolic diseases, used a custom gene panel to sequence 97 genes. This panel identified 244 variants in 269 infants, 94% of which were validated by Sanger sequencing (77). Previously undetected pathogenic mutations were also identified in 10 newborns in the same study, suggesting that NGS may increase the sensitivity of NBS.

Precision Therapeutics

The rapid identification of pathogenic mutation facilitates the use of gene therapy as a potential treatment option, an approach

that has been used for ADA-SCID, X-linked SCID, chronic granulomatous disease, and Wiskott–Aldrich Syndrome, with varying degrees of success (78). Additionally, a molecular diagnosis also enables the selection of biologics that target the affected signaling pathway. For example, abatacept, a soluble CTLA4 fusion protein, has been shown to improve autoimmune complications seen in patients with CTLA4 haploinsufficiency or LRBA deficiency, two disorders characterized by immune dysregulation due to impaired expression of the inhibitory molecule CTLA4 (79). Diseases caused by GOF mutations have been shown to be amenable to treatment by targeted inhibitors. Ruxolitinib, an inhibitor of JAK1 and JAK2, improved mucocutaneous candidiasis and autoimmune disease in a patient with a GOF mutations in STAT1 (80). Tocilizumab, an IL-6 inhibitor, improved arthritis and contractures in a patient with an STAT3 GOF mutation, a finding notable because the two patients who were treated with HSCT in this study died of transplant-related complications (81). Patients with GOF mutations in *CXCR4*, resulting in WHIM syndrome, have been treated successfully with the CXCR4 antagonist plerixafor (82). Ongoing clinical trials are underway to assess the efficacy of selective PI3K delta inhibitors in patients with GOF mutations in *PIK3CD*, resulting in activated PI3K delta syndrome (83). Precision therapeutics do not provide germline correction of the molecular defect, but provide an important alternative for patients who have no matched HSC donor, who have a PID with a high rate of HSCT-related mortality, or who have no access to a medical center with expertise in the PID-focused HSCT. The possibility of developing and utilizing targeted biologics depends on the efficient identification of pathogenic mutations in patients with PIDs, thus opening a therapeutic niche for NGS beyond HSCT.

CONCLUSION

While NGS has been used extensively in PID-related research, it has a relatively nascent role in clinical immunology. This is due, at least partly, to the time needed for sequencing costs to decrease and to validate the technologies in patients with PIDs. Although the cost of whole genome/exome sequencing remains high for most clinical labs, the field of oncology has shown the effectiveness of targeted NGS panels (84). Targeted NGS allows for a high-throughput, low-cost pipeline with a very short turnaround time due to the limited number of sequenced genes. DNA is inexpensive to isolate and can be easily shipped from remote areas of the world to centers with NGS capabilities, thus circumventing the difficulty inherent in shipping viable cells. The genetic basis of PIDs and the field's focus on molecular mechanisms, along with the available corrective therapies, render patients with these diseases ideal candidates for NGS. The integration of TGPs into NBS protocols will enable the early diagnosis and treatment of PIDs in a comprehensive manner that is yet achieved by current standard of care.

AUTHOR CONTRIBUTIONS

MS, RH-B, RG, and JC wrote and edited the manuscript.

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REFERENCES

1. Allenspach E, Torgerson TR. Autoimmunity and primary immunodeficiency disorders. *J Clin Immunol* (2016) 36:57–67. doi:10.1007/s10875-016-0294-1
2. Mortaz E, Tabarsi P, Mansouri D, Khosravi A, Garssen J, Velayati A, et al. Cancers related to immunodeficiencies: update and perspectives. *Front Immunol* (2016) 7:365. doi:10.3389/fimmu.2016.00365
3. Casanova J-L, Conley ME, Seligman SJ, Abel L, Notarangelo LD. Guidelines for genetic studies in single patients: lessons from primary immunodeficiencies. *J Exp Med* (2014) 211:2137–49. doi:10.1084/jem.20140520
4. Germeshausen M, Zeidler C, Stuhrmann M, Lanciotti M, Ballmaier M, Welte K. Digenic mutations in severe congenital neutropenia. *Haematologica* (2010) 95:1207–10. doi:10.3324/haematol.2009.017665
5. Notarangelo LD. Primary immunodeficiencies. *J Allergy Clin Immunol* (2010) 125:S182–94. doi:10.1016/j.jaci.2009.07.053
6. Bousfiha AA, Jeddane L, Ailal F, Benhsaien I, Mahlaoui N, Casanova J-L, et al. Primary immunodeficiency diseases worldwide: more common than generally thought. *J Clin Immunol* (2013) 33:1–7. doi:10.1007/s10875-012-9751-7
7. Hadizadeh H, Salehi M, Khoramnejad S, Vosoughi K, Rezaei N. The association between parental consanguinity and primary immunodeficiency diseases: a systematic review and meta-analysis. *Pediatr Allergy Immunol* (2017) 28:280–7. doi:10.1111/pai.12685
8. Liascovich R, Rittler M, Castilla EE. Consanguinity in South America: demographic aspects. *Hum Hered* (2000) 51:27–34. doi:10.1159/000022956
9. Al-Muhsen S, Alsum Z. Primary immunodeficiency diseases in the Middle East. *Ann N Y Acad Sci* (2012) 1250:56–61. doi:10.1111/j.1749-6632.2011.06379.x
10. Edgar JDM, Buckland M, Guzman D, Conlon NP, Knerr V, Bangs C, et al. The United Kingdom primary immune deficiency (UKPID) registry: report of the first 4 years' activity 2008–2012. *Clin Exp Immunol* (2014) 175:68. doi:10.1111/cei.12172
11. Errante PR, Franco JL, Espinosa-Rosales FJ, Sorensen R, Condino-Neto A. Advances in primary immunodeficiency diseases in Latin America: epidemiology, research, and perspectives. *Ann N Y Acad Sci* (2012) 1250:62–72. doi:10.1111/j.1749-6632.2011.06289.x
12. Resnick ES, Bhatt P, Sidi P, Cunningham-Rundles C. Examining the use of ICD-9 diagnosis codes for primary immune deficiency diseases in New York State. *J Clin Immunol* (2013) 33:40–8. doi:10.1007/s10875-012-9773-1
13. Boyle JM, Buckley RH. Population prevalence of diagnosed primary immunodeficiency diseases in the United States. *J Clin Immunol* (2007) 27:497–502. doi:10.1007/s10875-007-9103-1
14. Joshi AY, Iyer VN, Hagan JB, St. Sauver JL, Boyce TG. Incidence and temporal trends of primary immunodeficiency: a population-based cohort study. *Mayo Clin Proc* (2009) 84:16–22. doi:10.4065/84.1.16
15. Latif AH, Tabassomi F, Abolhassani H, Hammarström L. Molecular diagnosis of primary immunodeficiency diseases in a developing country: Iran as an example. *Expert Rev Clin Immunol* (2014) 10:385–96. doi:10.1586/1744666X.2014.880654
16. Al-Herz W, Notarangelo LD, Sadek A, Buckley R. Combined immunodeficiency in the United States and Kuwait: comparison of patients' characteristics and molecular diagnosis. *Clin Immunol* (2015) 161:170–3. doi:10.1016/j.clim.2015.07.013
17. Bousfiha A, Jeddane L, Al-Herz W, Ailal F, Casanova J, Chatila T, et al. The 2015 IUIS phenotypic classification for primary immunodeficiencies. *J Clin Immunol* (2015) 35:727–38. doi:10.1007/s10875-015-0198-5
18. Picard C, Al-Herz W, Bousfiha A, Casanova J-L, Chatila T, Conley ME, et al. Primary immunodeficiency diseases: an update on the classification from

the International Union of Immunological Societies Expert Committee for primary immunodeficiency 2015. *J Clin Immunol* (2015) 35:696–726. doi:10.1007/s10875-015-0201-1

19. Posevitz-Fejfár A, Posevitz V, Gross CC, Bhatia U, Kurth F, Schütte V, et al. Effects of blood transportation on human peripheral mononuclear cell yield, phenotype and function: implications for immune cell biobanking. *PLoS One* (2014) 9:e115920. doi:10.1371/journal.pone.0115920
20. Olson WC, Smolkin ME, Farris EM, Fink RJ, Czarkowski AR, Fink JH, et al. Shipping blood to a central laboratory in multicenter clinical trials: effect of ambient temperature on specimen temperature, and effects of temperature on mononuclear cell yield, viability and immunologic function. *J Transl Med* (2011) 9:26. doi:10.1186/1479-5876-9-26
21. Bousfiha AA, Jeddane L, Ailal F, Herz WA, Conley ME, Cunningham-Rundles C, et al. A phenotypic approach for IUIS PID classification and diagnosis: guidelines for clinicians at the bedside. *J Clin Immunol* (2013) 33:1078. doi:10.1007/s10875-013-9901-6
22. Al-Mousa H, Abouelhoda M, Monies DM, Al-Tassan N, Al-Ghonaium A, Al-Saud B, et al. Unbiased targeted next-generation sequencing molecular approach for primary immunodeficiency diseases. *J Allergy Clin Immunol* (2016) 137:1780–7. doi:10.1016/j.jaci.2015.12.1310
23. Abolhassani H, Wang N, Aghamohammadi A, Rezaei N, Lee YN, Frugoni F, et al. A hypomorphic RAG1 mutation resulting in a phenotype resembling common variable immunodeficiency. *J Allergy Clin Immunol* (2014) 134:1375. doi:10.1016/j.jaci.2014.04.042
24. Volk T, Pannicke U, Reisli I, Bulashevska A, Ritter J, Björkman A, et al. DCLRE1C (ARTEMIS) mutations causing phenotypes ranging from atypical severe combined immunodeficiency to mere antibody deficiency. *Hum Mol Genet* (2015) 24:7361. doi:10.1093/hmg/ddv437
25. Tahiat A, Badran YR, Chou J, Cangemi B, Lefranc G, Labgaa Z-M, et al. Epidermodysplasia verruciformis as a manifestation of ARTEMIS deficiency in a young adult. *J Allergy Clin Immunol* (2017) 139:372–5.e4. doi:10.1016/j.jaci.2016.07.024
26. Abolhassani H, Cheraghi T, Rezaei N, Aghamohammadi A, Hammarström L. Common variable immunodeficiency or late-onset combined immunodeficiency: a new hypomorphic JAK3 patient and review of the literature. *J Investig Allergol Clin Immunol* (2015) 25:218–20.
27. Geier CB, Piller A, Linder A, Sauerwein KMT, Eibl MM, Wolf HM. Leaky RAG deficiency in adult patients with impaired antibody production against bacterial polysaccharide antigens. *PLoS One* (2015) 10:e0133220. doi:10.1371/journal.pone.0133220
28. Schröder C, Baerlecken N, Pannicke U, Dörk T, Witte T, Jacobs R, et al. Evaluation of RAG1 mutations in an adult with combined immunodeficiency and progressive multifocal leukoencephalopathy. *Clin Immunol* (2017) 179:1–7. doi:10.1016/j.clim.2016.12.013
29. Zhang K, Chandrakasan S, Chapman H, Valencia CA, Husami A, Kissell D, et al. Synergistic defects of different molecules in the cytotoxic pathway lead to clinical familial hemophagocytic lymphohistiocytosis. *Blood* (2014) 124:1331–4. doi:10.1182/blood-2014-05-573105
30. Massaad MJ, Zhou J, Tsuchimoto D, Chou J, Jabara H, Janssen E, et al. Deficiency of base excision repair enzyme NEIL3 drives increased predisposition to autoimmunity. *J Clin Invest* (2016) 126:4219–36. doi:10.1172/JCI85647
31. Al-Herz W, Bousfiha A, Casanova J-L, Chatila T, Conley ME, Cunningham-Rundles C, et al. Primary immunodeficiency diseases: an update on the classification from the international union of immunological societies expert committee for primary immunodeficiency. *Front Immunol* (2014) 5:162. doi:10.3389/fimmu.2014.00162
32. Holzelova E, Vonarbourg C, Stolzenberg M-C, Arkwright PD, Selz F, Prieur A-M, et al. Autoimmune lymphoproliferative syndrome with somatic Fas mutations. *N Engl J Med* (2004) 351:1409–18. doi:10.1056/NEJMoa040036
33. Dowdell KC, Niemela JE, Price S, Davis J, Hornung RL, Oliveira JB, et al. Somatic FAS mutations are common in patients with genetically undefined autoimmune lymphoproliferative syndrome. *Blood* (2010) 115:5164–9. doi:10.1182/blood-2010-01-263145
34. Takagi M, Shinoda K, Piao J, Mitsuiki N, Takagi M, Matsuda K, et al. Autoimmune lymphoproliferative syndrome-like disease with somatic KRAS mutation. *Blood* (2011) 117:2887–90. doi:10.1182/blood-2010-08-301515
35. Shiota M, Yang X, Kubokawa M, Morishima T, Tanaka K, Mikami M, et al. Somatic mosaicism for a NRAS mutation associates with disparate clinical features in RAS-associated leukoproliferative disease: a report of two cases. *J Clin Immunol* (2015) 35:454–8. doi:10.1007/s10875-015-0163-3
36. Royer-Bertrand B, Rivolta C. Whole genome sequencing as a means to assess pathogenic mutations in medical genetics and cancer. *Cell Mol Life Sci* (2015) 72:1463–71. doi:10.1007/s00018-014-1807-9
37. Rabbani B, Mahdieh N, Hosomichi K, Nakaoka H, Inoue I. Next-generation sequencing: impact of exome sequencing in characterizing Mendelian disorders. *J Hum Genet* (2012) 57:621–32. doi:10.1038/jhg.2012.91
38. van Nimwegen KJM, van Soest RA, Veltman JA, Nelen MR, van der Wilt GJ, Vissers LELM, et al. Is the $1000 genome as near as we think? A cost analysis of next-generation sequencing. *Clin Chem* (2016) 62:1458–64. doi:10.1373/clinchem.2016.258632
39. Stoddard JL, Niemela JE, Fleisher TA, Rosenzweig SD. Targeted NGS: a cost-effective approach to molecular diagnosis of PIDs. *Front Immunol* (2014) 5:531. doi:10.3389/fimmu.2014.00531
40. Robinson PN, Krawitz P, Mundlos S. Strategies for exome and genome sequence data analysis in disease-gene discovery projects. *Clin Genet* (2011) 80:127–32. doi:10.1111/j.1399-0004.2011.01713.x
41. Luthra R, Patel KP, Routbort MJ, Broaddus RR, Yau J, Simien C, et al. A targeted high-throughput next-generation sequencing panel for clinical screening of mutations, gene amplifications, and fusions in solid tumors. *J Mol Diagn* (2017) 19:255–64. doi:10.1016/j.jmoldx.2016.09.011
42. Meienberg J, Bruggmann R, Oexle K, Matyas G. Clinical sequencing: is WGS the better WES? *Hum Genet* (2016) 135:359–62. doi:10.1007/s00439-015-1631-9
43. Alkhairy OK, Abolhassani H, Rezaei N, Fang M, Andersen KK, Chavoshzadeh Z, et al. Spectrum of phenotypes associated with mutations in LRBA. *J Clin Immunol* (2016) 36:33–45. doi:10.1007/s10875-015-0224-7
44. Belkadi A, Bolze A, Itan Y, Cobat A, Vincent QB, Antipenko A, et al. Whole-genome sequencing is more powerful than whole-exome sequencing for detecting exome variants. *Proc Natl Acad Sci USA* (2015) 112:5473–8. doi:10.1073/pnas.1418631112
45. Stray-Pedersen A, Sorte HS, Samarakoon P, Gambin T, Chinn IK, Coban Akdemir ZH, et al. Primary immunodeficiency diseases: genomic approaches delineate heterogeneous Mendelian disorders. *J Allergy Clin Immunol* (2017) 139:232–45. doi:10.1016/j.jaci.2016.05.042
46. Maffucci P, Filion CA, Boisson B, Itan Y, Shang L, Casanova J-L, et al. Genetic diagnosis using whole exome sequencing in common variable immunodeficiency. *Front Immunol* (2016) 7:220. doi:10.3389/fimmu.2016.00220
47. Nijman IJ, van Montfrans JM, Hoogstraat M, Boes ML, van de Corput L, Renner ED, et al. Targeted next-generation sequencing: a novel diagnostic tool for primary immunodeficiencies. *J Allergy Clin Immunol* (2014) 133:529–34. doi:10.1016/j.jaci.2013.08.032
48. Yang Y, Muzny DM, Reid JG, Bainbridge MN, Willis A, Ward PA, et al. Clinical whole-exome sequencing for the diagnosis of Mendelian disorders. *N Engl J Med* (2013) 369:1502–11. doi:10.1056/NEJMoa1306555
49. Meyts I, Bosch B, Bolze A, Boisson B, Itan Y, Belkadi A, et al. Exome and genome sequencing for inborn errors of immunity. *J Allergy Clin Immunol* (2016) 138:957–69. doi:10.1016/j.jaci.2016.08.003
50. Kuehn HS, Ouyang W, Lo B, Deenick EK, Niemela JE, Avery DT, et al. Immune dysregulation in human subjects with heterozygous germline mutations in CTLA4. *Science* (2014) 345:1623–7. doi:10.1126/science.1255904
51. Chou J, Massaad MJ, Cangemi B, Bainter W, Platt C, Badran YR, et al. A novel mutation in ICOS presenting as hypogammaglobulinemia with susceptibility to opportunistic pathogens. *J Allergy Clin Immunol* (2015) 136:794–7.e1. doi:10.1016/j.jaci.2014.12.1940
52. Platt C, Geha RS, Chou J. Gene hunting in the genomic era: approaches to diagnostic dilemmas in patients with primary immunodeficiencies. *J Allergy Clin Immunol* (2014) 134:262–8. doi:10.1016/j.jaci.2013.08.021
53. Chou J, Ohsumi TK, Geha RS. Use of whole exome and genome sequencing in the identification of genetic causes of primary immunodeficiencies. *Curr Opin Allergy Clin Immunol* (2012) 12:623–8. doi:10.1097/ACI.0b013e3283588ca6
54. Platt CD, Massaad MJ, Cangemi B, Schmidt B, Aldhekri H, Geha RS. Janus kinase 3 deficiency caused by a homozygous synonymous exonic mutation that creates a dominant splice site. *J Allergy Clin Immunol* (2016) 140:268–71.e6. doi:10.1016/j.jaci.2016.09.057
55. Gallego-Bustos F, Gotea V, Ramos-Amador JT, Rodríguez-Pena R, Gil-Herrera J, Sastre A, et al. A case of IL-7R deficiency caused by a novel synonymous mutation and implications for mutation screening in SCID diagnosis. *Front Immunol* (2016) 7:443. doi:10.3389/fimmu.2016.00443

56. Rehm HL, Bale SJ, Bayrak-Toydemir P, Berg JS, Brown KK, Deignan JL, et al. ACMG clinical laboratory standards for next-generation sequencing. *Genet Med* (2013) 15:733–47. doi:10.1038/gim.2013.92
57. Miosge LA, Field MA, Sontani Y, Cho V, Johnson S, Palkova A, et al. Comparison of predicted and actual consequences of missense mutations. *Proc Natl Acad Sci USA* (2015) 112:E5189–98. doi:10.1073/pnas.1511585112
58. Lopez-Herrera G, Tampella G, Pan-Hammarström Q, Herholz P, Trujillo-Vargas CM, Phadwal K, et al. Deleterious mutations in LRBA are associated with a syndrome of immune deficiency and autoimmunity. *Am J Hum Genet* (2012) 90:986–1001. doi:10.1016/j.ajhg.2012.04.015
59. Sobh A, Chou J, Schneider L, Geha RS, Massaad MJ. Chronic mucocutaneous candidiasis associated with an SH2 domain gain-of-function mutation that enhances STAT1 phosphorylation. *J Allergy Clin Immunol* (2016) 138:297–9. doi:10.1016/j.jaci.2015.12.1320
60. Chang JJ, Lacas A, Lindsay RJ, Doyle EH, Axten KL, Pereyra F, et al. Differential regulation of toll-like receptor pathways in acute and chronic HIV-1 infection. *AIDS* (2012) 26:533–41. doi:10.1097/QAD.0b013e32834f3167
61. Tan W, Yu S, Lei J, Wu B, Wu C. A novel common gamma chain mutation in a Chinese family with X-linked severe combined immunodeficiency (X-SCID; T(-)NK(-)B(+)). *Immunogenetics* (2015) 67:629–39. doi:10.1007/s00251-015-0871-0
62. Takahashi K, Yamanaka S. A decade of transcription factor-mediated reprogramming to pluripotency. *Nat Rev Mol Cell Biol* (2016) 17:183–93. doi:10.1038/nrm.2016.8
63. Halevy T, Akov S, Bohndorf M, Mlody B, Adjaye J, Benvenisty N, et al. Chromosomal instability and molecular defects in induced pluripotent stem cells from Nijmegen breakage syndrome patients. *Cell Rep* (2016) 16:2499–511. doi:10.1016/j.celrep.2016.07.071
64. Rissone A, Weinacht KG, la Marca G, Bishop K, Giocaliere E, Jagadeesh J, et al. Reticular dysgenesis-associated AK2 protects hematopoietic stem and progenitor cell development from oxidative stress. *J Exp Med* (2015) 212:1185–202. doi:10.1084/jem.20141286
65. Jang J, Yoo J-E, Lee J-A, Lee DR, Kim JY, Huh YJ, et al. Disease-specific induced pluripotent stem cells: a platform for human disease modeling and drug discovery. *Exp Mol Med* (2012) 44:202–13. doi:10.3858/emm.2012.44.3.015
66. Park I-H, Arora N, Huo H, Maherali N, Ahfeldt T, Shimamura A, et al. Disease-specific induced pluripotent stem cells. *Cell* (2008) 134:877–86. doi:10.1016/j.cell.2008.07.041
67. Lafaille FG, Pessach IM, Zhang S-Y, Ciancanelli MJ, Herman M, Abhyankar A, et al. Impaired intrinsic immunity to HSV-1 in human iPSC-derived TLR3-deficient CNS cells. *Nature* (2012) 491:769–73. doi:10.1038/nature11583
68. Jabara HH, Boyden SE, Chou J, Ramesh N, Massaad MJ, Benson H, et al. A missense mutation in TFRC, encoding transferrin receptor 1, causes combined immunodeficiency. *Nat Genet* (2016) 48:74–8. doi:10.1038/ng.3465
69. Iwanami N. Zebrafish as a model for understanding the evolution of the vertebrate immune system and human primary immunodeficiency. *Exp Hematol* (2014) 42:697–706. doi:10.1016/j.exphem.2014.05.001
70. Moore JC, Mulligan TS, Torres Yordán N, Castranova D, Pham VN, Tang Q, et al. T cell immune deficiency in zap70 mutant zebrafish. *Mol Cell Biol* (2016) 36:2868–76. doi:10.1128/MCB.00281-16
71. Iwanami N, Mateos F, Hess I, Riffel N, Soza-Ried C, Schorpp M, et al. Genetic evidence for an evolutionarily conserved role of IL-7 signaling in T cell development of zebrafish. *J Immunol* (2011) 186:7060–6. doi:10.4049/jimmunol.1003907
72. Punwani D, Zhang Y, Yu J, Cowan MJ, Rana S, Kwan A, et al. Multisystem anomalies in severe combined immunodeficiency with mutant BCL11B. *N Engl J Med* (2016) 375:2165–76. doi:10.1056/NEJMoa1509164
73. Walters KB, Green JM, Surfus JC, Yoo SK, Huttenlocher A. Live imaging of neutrophil motility in a zebrafish model of WHIM syndrome. *Blood* (2010) 116:2803–11. doi:10.1182/blood-2010-03-276972
74. Chang C-W, Lai Y-S, Westin E, Khodadadi-Jamayran A, Pawlik KM, Lamb LS, et al. Modeling human severe combined immunodeficiency and correction by CRISPR/Cas9-enhanced gene targeting. *Cell Rep* (2015) 12:1668–77. doi:10.1016/j.celrep.2015.08.013
75. Pai S-Y, Logan BR, Griffith LM, Buckley RH, Parrott RE, Dvorak CC, et al. Transplantation outcomes for severe combined immunodeficiency, 2000–2009. *N Engl J Med* (2014) 371:434–46. doi:10.1056/NEJMoa1401177
76. Baker MW, Atkins AE, Cordovado SK, Hendrix M, Earley MC, Farrell PM. Improving newborn screening for cystic fibrosis using next-generation sequencing technology: a technical feasibility study. *Genet Med* (2016) 18:231–8. doi:10.1038/gim.2014.209
77. Park K-J, Park S, Lee E, Park J-H, Park J-H, Park H-D, et al. A population-based genomic study of inherited metabolic diseases detected through newborn screening. *Ann Lab Med* (2016) 36:561–72. doi:10.3343/alm.2016.36.6.561
78. Kuo CY, Kohn DB. Gene therapy for the treatment of primary immune deficiencies. *Curr Allergy Asthma Rep* (2016) 16:39. doi:10.1007/s11882-016-0615-8
79. Vignesh P, Rawat A, Singh S. An update on the use of immunomodulators in primary immunodeficiencies. *Clin Rev Allergy Immunol* (2016) 52:287–303. doi:10.1007/s12016-016-8591-2
80. Higgins E, Al Shehri T, McAleer MA, Conlon N, Feighery C, Lilic D, et al. Use of ruxolitinib to successfully treat chronic mucocutaneous candidiasis caused by gain-of-function signal transducer and activator of transcription 1 (STAT1) mutation. *J Allergy Clin Immunol* (2015) 135:551–3. doi:10.1016/j.jaci.2014.12.1867
81. Milner JD, Vogel TP, Forbes L, Ma CA, Stray-Pedersen A, Niemela JE, et al. Early-onset lymphoproliferation and autoimmunity caused by germline STAT3 gain-of-function mutations. *Blood* (2015) 125:591–9. doi:10.1182/blood-2014-09-602763
82. McDermott DH, Liu Q, Velez D, Lopez L, Anaya-O'Brien S, Ulrick J, et al. A phase 1 clinical trial of long-term, low-dose treatment of WHIM syndrome with the CXCR4 antagonist plerixafor. *Blood* (2014) 123:2308–16. doi:10.1182/blood-2013-09-527226
83. Coulter TI, Chandra A, Bacon CM, Babar J, Curtis J, Screaton N, et al. Clinical spectrum and features of activated phosphoinositide 3-kinase δ syndrome: a large patient cohort study. *J Allergy Clin Immunol* (2017) 139:597–606.e4. doi:10.1016/j.jaci.2016.06.021
84. Hyman DM, Taylor BS, Baselga J. Implementing genome-driven oncology. *Cell* (2017) 168:584–99. doi:10.1016/j.cell.2016.12.015

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frontiers in Immunology

CASE REPORT
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First Association of Interleukin 12 Receptor Beta 1 Deficiency with Sjögren's Syndrome

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Introduction: Interleukin 12 receptor beta 1 (IL12Rβ1) deficiency is a primary immunodeficiency resulting mainly in susceptibility to opportunistic infection by non-tuberculous, environmental mycobacteria and severe infection caused by *Salmonella* spp. Till now, less than 300 patients with IL12Rβ1 deficiency have been reported. Among them, only three have been described to develop autoimmunity.

Case presentation: We present the case of a 50-year-old male with IL12Rβ1 deficiency due to compound *heterozygosity [c. 1623_1624delGCinsTT (pGln542Stop) and c.1791 + 2T > C (donor splice site)]*, who—18 months after diagnosis of disseminated BCGitis—presented with recurrent fever and sicca syndrome. No indication of an infectious origin of these symptoms could be found at that point. The diagnosis of a Sjögren's syndrome (SS) was made on the basis of fulfilled American-European consensus classification criteria, including a positive minor salivary gland biopsy.

Conclusion: Apart from persistent antigenic stimulation, which may drive autoimmune inflammation in primary immunodeficiency, evidence on the involvement of interleukin 12 in pathogenesis of SS suggests that the same immunological mechanism may underlie both defense against infection and the maintenance of tolerance. To our knowledge, this is the first report of a case of autoimmunity in the form of SS in a patient with a primary immunodeficiency and one of the rare cases of IL12Rβ1 deficiency with manifested autoimmunity.

Keywords: interleukin-12, interleukin-12 receptor beta 1 subunit, Sjögren's syndrome, primary immunodeficiency, autoimmunity, Mendelian susceptibility to mycobacterial disease, interleukin-12 receptor beta 1 subunit deficiency

INTRODUCTION

The capability to produce or respond to interferon γ (IFNγ) is important in controlling infection by intracellular bacteria such as mycobacteria and *Salmonella* spp. (1, 2). Mutations in genes encoding for type I cytokines and molecules involved in their signaling have been identified in patients with infection due to environmental mycobacteria. In particular, mutations of the *IL12RB1*-gene resulting in Interleukin 12 receptor beta 1 (IL12Rβ1) deficiency are the most common cause of Mendelian susceptibility to mycobacterial disease (MSMD) (2). IL12Rβ1 deficiency follows an

autosomal recessive pattern of inheritance, although the clinical outcome of IL12Rβ1-deficient individuals, carrying two mutant alleles, is variable, ranging from early death to an asymptomatic outcome. This incomplete penetrance may reflect differences in the genetic background of IL12Rβ1-deficient individuals and/or the environmental exposure to relevant pathogens. According to the study by de Beaucoudrey et al., including over 140 IL12Rβ1-deficient individuals, in more than 90% of cases, IL12Rβ1-deficiency was symptomatic with mycobacterial infections representing the most common phenotype, found in 77% of IL12Rβ1-deficient individuals. The second most common infection was Salmonellosis, affecting 40% of IL12Rβ1-deficient individuals. Disease onset occurred in early and middle childhood in most cases and a lethal outcome was observed in up to a third of patients (3).

Interleukin 12 receptor beta 1 physically associates with p40, which is a common subunit of interleukin 12 (IL-12) and interleukin 23 (IL-23) (4). IL-12 and IL-23 are proinflammatory cytokines, whose biological activities depend on IL12Rβ1. IL12Rβ1 is bound to the non-receptor protein tyrosine kinase 2, whereas IL12Rβ2—its IL-12 receptor (IL-12R) counterpart chain—is bound to the Janus kinase 2. Activation of IL-12R results in the phosphorylation, homodimerization, and nuclear translocation of the signal transducer and activator of transcription 4 (STAT4), which induces the expression of IFNγ. IL12Rβ1 deficiency and the consequent reduced IL-12 responsivity result in impaired production of IFNγ by NK cells and T cells, which impairs control of infection by mycobacteria and other intracellular bacteria (1).

Primary immunodeficiency diseases are often associated with autoimmunity, which may reflect the fact that common mechanisms account for both defense against possible pathogens and the maintenance of tolerance (5, 6). Apart from that, it has been suggested that persistent or recurrent antigenic stimulation as a consequence of infectious diseases in patients with primary immunodeficiency may cause or contribute to breaking of tolerance.

In contrast to other primary immunodeficiency diseases, there is only scarce evidence for association of defects of the IL-12/IFN-γ axis, including IL12Rβ1 deficiency with autoimmunity. Here, we present the case of a 50-year-old male with IL12Rβ1-deficiency, who presented with fever episodes and sicca syndrome 18 months after starting of an antimycobacterial therapy due to the diagnosis of a disseminated infection with Bacillus Calmette–Guérin (BCG). In the absence of evidence of infectious origin of these symptoms and on the basis of fulfilled American-European consensus classification criteria (7), we diagnosed a Sjögren's syndrome (SS).

CASE REPORT

A male infant, only child born to non-consanguineous Caucasian parents of German descent in 1966, developed after birth an inguinal lymphadenopathy, which was treated with a single antibiotic for approximately 2 weeks. At the age of 20 years, he developed a left-sided axillary lymphadenopathy, which led to the dissection of an axillary lymph node. Histological examination yielded no relevant pathological findings. A 2-week-treatment with antibiotics resulted in regression of the lymphadenopathy.

At the age of 47, the patient developed a disseminated lymphadenopathy; a computer tomography (CT) scan revealed a pathological enlargement of abdominal, mediastinal, supraclavicular, and axillary lymph nodes (**Figure 1**, left column). An infection by BCG was diagnosed after a biopsy of a right axillary lymph node in a peripheral hospital of our region. Detection of BCG was achieved by culture of the biopsy sample and analysis of the genomic "region of difference 1" (RD1) of the cultured mycobacteria. In line with this diagnosis, is the fact that the patient as an infant had received vaccination with BCG. An antimycobacterial therapy with rifampicin, isoniazid, ethambutol, and azithromycin was started. A macrolide antibiotic, i.e., azithromycin was added to achieve coverage of other environmental mycobacteria. At that time, the patient was referred to our department for Clinical Immunology due to a suspected immunodeficiency. On admission, the C-reactive protein (CRP) was elevated (82 mg/l, normal <5 mg/l). The rest of routine laboratory tests were normal, except for a slight increase of gammaglutamyltransferase (gGT, 100 U/l, normal <55 U/l). A serum protein electrophoresis revealed a polyclonal hypergammaglobulinemia. Immunoglobulin classes revealed increased levels for serum immunoglobulin G (27.7 g/l, normal range: 7–16 g/l). Serum and urine immunofixation were negative. Immunophenotyping of blood lymphocyte subsets revealed a slight absolute reduction in B cell count with normal absolute and relative counts for the rest of lymphocyte subsets

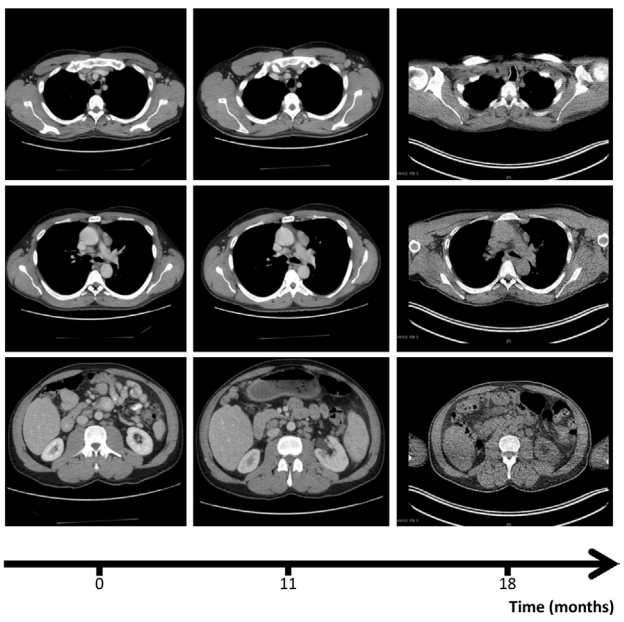

FIGURE 1 | Computer tomography (CT) scans, revealing axillary (upper row), mediastinal (mid row), and abdominal lympadenopathy (lower row) at the three different time points: diagnosis of disseminated infection with Bacillus Calmette–Guérin (0, left column), escalation of antimycobacterial therapy (11, mid column), and diagnosis of Sjögren's syndrome (18, right column). CT scan of right row was performed in the context of a positron emission tomography/CT (see **Figure 4**).

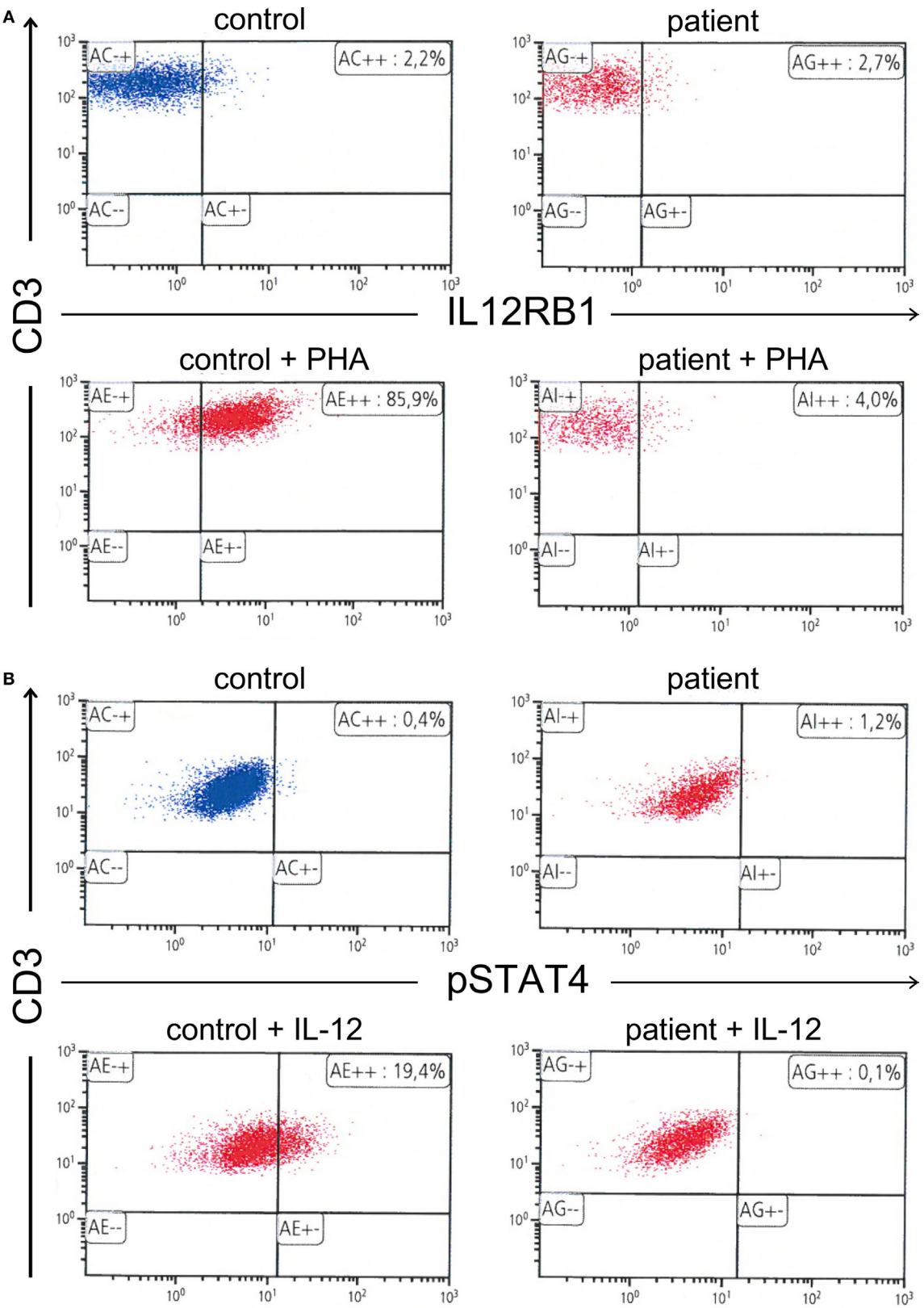

FIGURE 2 | **(A)** Absence of interleukin 12 receptor beta 1 expression on the surface of phytohemagglutinin-stimulated T cells. **(B)** Further, absence of signal transducer and activator of transcription 4 phosphorylation in interleukin 12 (IL-12)-stimulated T cells.

and an increased expression of human leukocyte antigen-antigen D related (HLA-DR), indicating lymphocyte activation. T cell activation with distinct stimuli (including interleukin 2, anti-CD3-antibody, phytohemagglutinin (PHA), concanavalin A, and pokeweed mitogen) resulted in an adequate T cell proliferation. Testing of granulocytes for oxidative burst and phagocytic potential yielded adequate responses. Searching for the underlying cause of a BCGitis, we also tested for an abnormality of the IL-12/IFNγ-pathway, which showed the absence of IL12Rβ1 on the surface of PHA-stimulated T cells. Further, there was no STAT4 phosphorylation after T cell stimulation with IL-12. Results of flow cytometric analysis of IL12Rβ1 expression/STAT4 phosphorylation and the rest of immunological investigations are presented in **Figure 2** and **Table 1**, respectively.

TABLE 1 | Immunological findings on admission, directly after diagnosis of disseminated infection with Bacillus Calmette–Guérin in a 47-year-old male with interleukin 12 receptor beta 1 deficiency.

Immunological test	Value	Normal range (or control value)
Serum Ig concentration		
IgG (g/l)	27.7	7.0–16.0
IgA (g/l)	4.4	0.7–4.0
IgM (g/l)	1.8	0.4–2.3
IgE (IE/ml)	680	1–100
Complement		
Total complement activity CH50 (%)	>165	90–150
C3c (g/l)	1.91	0.9–1.8
C4 (g/l)	0.14	0.1–0.4
Lymphocyte subset analysis		
Lymphocyte count (cells/μl)	4,030	1,000–2,800/μl
CD3+ (relative %/cells/μl)	89%/3,909	700–2,100
CD3+/CD4+ (relative %/cells/μl)	34%/1,370	500–1,400
CD3+/CD8+ (relative %/cells/μl)	44%/1,773	200–900
CD3+/human leukocyte antigen-antigen D related+ (relative %/cells/μl)	13%/524	30–200
CD3−/CD16+/CD56+ (relative %/cells/μl)	8%/322	90–600
CD19+ (relative %/cells/μl)	2%/81	100–500
Lymphocyte proliferation assay		
Medium control (cells/μl)	2,741	2,379
Phytohemagglutinin (cells/μl)	42,100	34,295
Concanavalin A (cells/μl)	26,008	7,928
Pokeweed mitogen (cells/μl)	13,487	10,151
Tuberculin purified protein derivative (cells/μl)	2,580	1,484
Interleukin 2 (cells/μl)	4,887	2,505
Isotype control (cells/μl)	601	218
Anti-CD3-antibody (cells/μl)	26,326	24,235
Granulocyte phenotyping		
CD16 (%)	98	99
CD32 (%)	99	99
CD64 (%)	3	1
CD18 (%)	100	100
CD11b (%)	100	100
CD15s (%)	100	99
Granulocyte function tests		
Phagocytosis of *E. coli* (%)	94	96
Oxidative burst induction (oxidation of 2′,7′-dichlorofluorescin diacetate)		
Medium control (%)	2	1
N-formylmethionyl-leucyl-phenylalanine (%)	4	3
E. coli (%)	100	98
Phorbol 12-myristate 13-acetate (%)	100	100

We then performed genetic analysis focused on the *IL12RB1*-gene (NM_005535), which revealed a compound heterozygosity [*c. 1623_1624delGCinsTT* (*pGln542Stop*) and *c.1791 + 2T > C* (donor splice site)], explaining the absence of IL12Rβ1 expression (2).

Two months later, ethambutol was stopped and 8 months later, B symptoms (fever, weight loss, and night sweats), which were present at the first presentation of the patient in our clinic, reappeared. CRP, which had been steadily decreasing after beginning of the antimycobacterial therapy, was again elevated (78 mg/l). A CT-scan revealed persistence of the lymphadenopathy (**Figure 1**, mid column), so that we assumed a relapse of the BCGitis. We, therefore, escalated the antimycobacterial therapy by adding ethambutol, terizidone, and moxifloxacin to the at that time point ongoing therapy with rifampicin, isoniazid, and azithromycin.

Despite initial regression of B symptoms under the six-drug antimycobacterial therapy, 6 months later, the patient developed once more fever with night sweating. Serologic inflammation activity was again increased as exemplified by elevated CRP (117 mg/l). In a follow-up, we performed a CT scan combined with positron emission tomography. We detected a regression of the lymphadenopathy (**Figure 1**, right column), with diffuse increased uptake of F-18 fluoro-2-deoxyglucose (18FDG) in bone marrow (**Figure 3**). Increased uptake of 18FDG was also localized in the area of the right lacrimal gland. With these findings, we performed a bone marrow biopsy, which revealed a minor hyperplastic granulopoiesis and megakaryopoiesis with a non-specific inflammatory reaction. Further, a biopsy of the right lacrimal gland revealed focal lymphocytic infiltrates with periductal distribution (**Figure 4**). Microbiological testing of bone marrow and lacrimal gland samples, including polymerase chain reactions to detect mycobacteria, remained negative.

Taken the absence of evidence for an infectious cause of the aforementioned symptoms and findings, we suspected an autoimmune inflammation. Serum immunological tests showed an increased titer of antinuclear antibodies (ANA 1:320, normal <1:160) with no specification in the extractable nuclear antigen screening test. The test for rheumatoid factor was highly positive (2,750 IE/ml, normal <15.9 IE/ml) with a negative test for anti-cyclic citrullinated peptide antibody. Further, anti-alpha-fodrin antibodies of IgG and IgA class could be detected (IgA anti-alpha-fodrin 16 U/ml, normal <15 U/ml, and IgG anti-alpha-fodrin 38, normal <15 U/ml). The rest of performed autoantibody tests [anti-double-stranded DNA, antineutrophil cytoplasmic antibodies (pANCA and cANCA), IgG and IgM anti-cardiolipin, anti-Smith antigen (Sm), anti-ribonucleoprotein, anti-Ro (SSA), anti-La (SSB), anti-Jo1, and anti-topoisomerase I (Scl-70)] were negative. The patient complained of xerophthalmia, which could be confirmed by a positive Schirmer's test. Suspecting a SS, we also performed a labial salivary gland biopsy, which revealed a focal lymphocytic sialadenitis of grade 3 according to the Chisholm–Mason classification (**Figure 4**). On the basis of fulfilled American-European consensus classification criteria (chronic xerophthalmia, positive Schirmer's test, positive rheumatoid factor and ANA titer of 1:320, positive labial salivary gland biopsy), we made the diagnosis of a SS and started a treatment with methotrexate (p.o. 15 mg 1×/week)

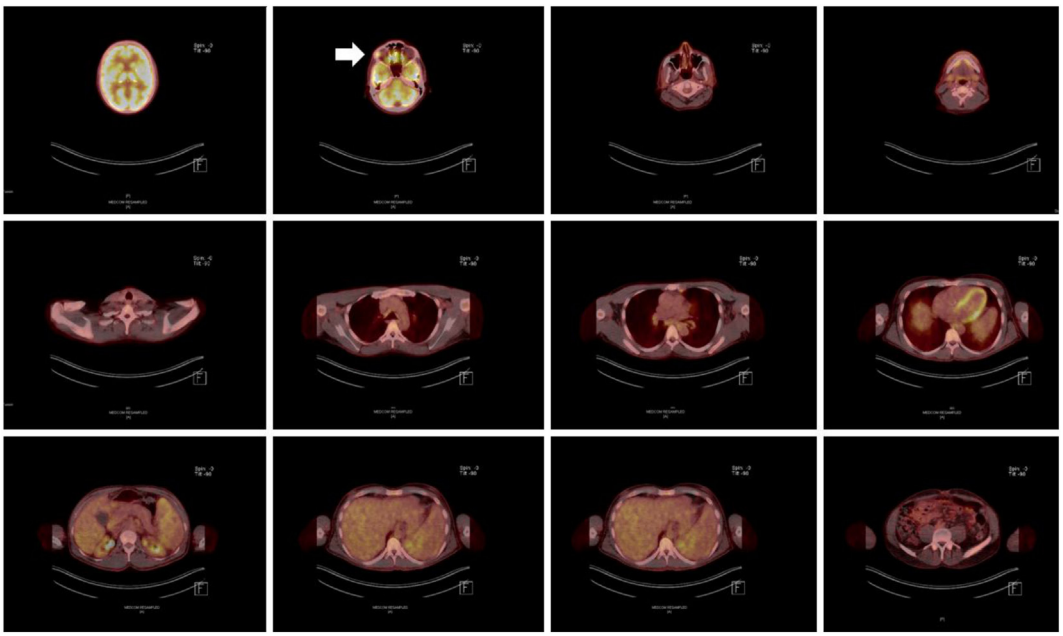

FIGURE 3 | Positron emission tomography/computer tomography scan after completion of an 18-month antimycobacterial therapy, including a 6-month 6-drug regimen, revealing significant homogenous hypermetabolism in the area of the right lacrimal gland (marked with an arrow).

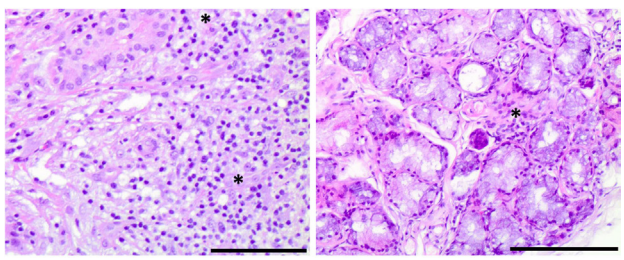

FIGURE 4 | Histological findings of Sjögren's syndrome in an interleukin 12 receptor beta 1-deficient male. Right panel: lymphocytic infiltrates with periductal distribution in a lacrimal gland biopsy section, stained with hematoxylin and eosin (10× magnification, bar size: 0.5 μg) Left panel: focal lymphocytic infiltration in a minor salivary gland biopsy section, stained with hematoxylin and eosin (10× magnification, bar size: 0.5 μg).

and prednisolone (initially 20 mg and after a week gradual dose reduction till a maintenance dose of 5 mg daily). The intensified 6-drug antimycobacterial regimen was discontinued. Starting an immunosuppressive therapy in the presence of a primary immunodeficiency was done with caution, under close monitoring of the patient in the hospital setting. Considering the absence of evidence of a residual infection with BCG after completion of an 18-month antimycobacterial regimen, the diagnosis of SS and the sustained response to the immusuppressive therapy with maintenance of normal body temperature and CRP reduction as well as the rarity of recurrence or of multiple mycobacterial infections in case of IL12Rβ1-deficiency, especially after an infection with BCG (3), we did not start a prophylactic antimycobacterial therapy. However, we suggested close monitoring though family physician, including testing for inflammatory markers. In the follow-up in our outpatient clinic, 2 and 8 months after starting the treatment with methotrexate the patient remained afebrile and the CRP was reduced (46 mg/l).

DISCUSSION

We describe a patient with an inherited IL12Rβ1 deficiency, who developed SS approximately 18 months after the diagnosis of a disseminated infection with BCG. A few hundred patients with IL12Rβ1 deficiency have been reported in the literature (3). To our knowledge, till now only three patients with IL12Rβ1-deficiency and clinically significant autoimmune manifestations, including SLE-like disease and autoimmune hemolytic anemia, have been presented (8, 9). This is the first association of a primary immunodeficiency with autoimmunity in the form of SS and a very rare case of manifested autoimmunity in case of IL12Rβ1-deficiency.

Human and mouse-derived evidence implicates IL-12-signaling in the development of SS-like/SS-autoimmunity. *Il-12rb2*-knockout mice, which produce but cannot respond to IL-12, develop SS-like disease with lymphoid infiltration involving the salivary glands and ANA-positivity in serum (10). Interestingly, autoimmunity develops late in these mice, starting at the age of 4 months and worsens as the mice get older. Although not a direct model of IL12Rβ1-deficiency, as IL12Rβ2 is an exclusive constituent chain of IL-12 receptor and not of IL-23 receptor, *il-12rb2*-knockout mice apart from developing late–onset autoimmunity, exhibit similarly to human IL12Rβ1-deficiency a propensity to infection with intracellular pathogens (11). The largest available genome-wide association study on SS recognized variants in

genomic regions encoding for constituents of the type I cytokine pathway, such as *IL12A* (encoding the p35 subunits of IL12), *IRF5* (encoding a transcription factor that activates among others the gene for the p40 subunit of IL-12), and *STAT4* (encoding the homonymous transcription factor that activates IFNγ expression) as risk factors in SS (12). Further, autoantibodies against IL-12, which exhibited a neutralizing effect, were found in a subset of patients with primary SS (13). In contrast to the aforementioned findings, suggesting a regulatory role for IL-12 signaling in SS, mice overexpressing IL-12, have been shown to develop SS-like disease, including lymphocytic infiltration of lacrimal glands and anti-SSB/La antibody positivity (14). This may reflect the fact that SS is a heterogeneous disease, with different—even diverging—pathways involved in its pathogenesis.

Further, the present case exemplifies the already described association between mycobacterial infections and autoimmune phenomena, including the emergence of diverse autoantibodies, such as ANA, antineutrophil cytoplasmic antibodies, and anti-SSA/Ro antibodies (15). Recently, Chao et al. have described a significant association between non-tuberculous mycobacterial infection and SS (16), which can be reflecting the pathogenic involvement of mycobacterial infections in SS. However, the fact that non-tuberculous mycobacterial infections, but not infection with mycobacterium tuberculosis associated with increased risk for SS makes it is tempting to speculate that some of these patients may have had an abnormality in the IL-12/IFNγ-pathway, and especially in IL-12 signaling.

Taken the existing literature, the association between IL12Rβ1 deficiency and SS can be reflecting the regulatory role of IL-12. However, before we can be sure, more cases of adult patients with IL12Rβ1-deficiency need to be investigated. Apart from evidence on the pathogenic involvement of IL-12 in SS, persistent antigenic stimulation as a consequence of recurrent or persistent infection—especially of a mycobacterial infection—in IL12Rβ1-deficiency, may contribute or lead to autoimmunity (6).

CONCLUDING REMARKS

This is the first report of a case of autoimmunity in form of SS in a patient with a primary immunodeficiency and a rare case of manifested autoimmunity in a patient with IL12Rβ1 deficiency, suggesting that the same immunological mechanisms may underlie both defense against infection and the maintenance of tolerance.

ETHICS STATEMENT

This is a report on a single patient, which complies with the Declaration of Helsinki. This study was carried out in accordance with the recommendations of the Ethic committee of the Hannover Medical University. The patient gave a written informed consent to publish the report.

AUTHOR CONTRIBUTIONS

GS wrote the paper under the guidance of TW and RS. RS, TW, MS, GS, VS, and AJ were involved in the diagnostic and/or therapeutic course. RJ and AF were performed the laboratory studies for the diagnosis of IL12RB12 deficiency. All coauthors revised the paper and approved its final version for publication.

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REFERENCES

1. Rosenzweig SD, Holland SM. Defects in the interferon-gamma and interleukin-12 pathways. *Immunol Rev* (2005) 203:38–47. doi:10.1111/j.0105-2896.2005.00227.x
2. van de Vosse E, Haverkamp MH, Ramirez-Alejo N, Martinez-Gallo M, Blancas-Galicia L, Metin A, et al. IL-12Rβ1 deficiency: mutation update and description of the IL12RB1 variation database. *Hum Mutat* (2013) 34(10):1329–39. doi:10.1002/humu.22380
3. de Beaucoudrey L, Samarina A, Bustamante J, Cobat A, Boisson-Dupuis S, Feinberg J, et al. Revisiting human IL-12Rβ1 deficiency: a survey of 141 patients from 30 countries. *Medicine (Baltimore)* (2010) 89(6):381–402. doi:10.1097/MD.0b013e3181fdd832
4. Robinson RT. IL12Rβ1: the cytokine receptor that we used to know. *Cytokine* (2015) 71(2):348–59. doi:10.1016/j.cyto.2014.11.018
5. Fischer A, Provot J, Jais JP, Alcais A, Mahlaoui N; Members of the CEREDIH French PID Study Group. Autoimmune and inflammatory manifestations occur frequently in patients with primary immunodeficiencies. *J Allergy Clin Immunol* (in press). doi:10.1016/j.jaci.2016.12.978
6. Grimbacher B, Warnatz K, Yong PF, Korganow AS, Peter HH. The crossroads of autoimmunity and immunodeficiency: lessons from polygenic traits and monogenic defects. *J Allergy Clin Immunol* (2016) 137(1):3–17. doi:10.1016/j.jaci.2015.11.004
7. Shiboski CH, Shiboski SC, Seror R, Criswell LA, Labetoulle M, Lietman TM, et al. 2016 American College of Rheumatology/European League against rheumatism classification criteria for primary Sjögren's syndrome: a consensus and data-driven methodology involving three international patient cohorts. *Ann Rheum Dis* (2017) 76(1):9–16. doi:10.1136/annrheumdis-2016-210571
8. Ling G, Ling E, Broides A, Poran Feldman H, Levy J, Garty BZ, et al. IL-12 receptor 1β deficiency with features of autoimmunity and photosensitivity. *Autoimmunity* (2016) 49(3):143–6. doi:10.3109/08916934.2015.1134513
9. Göktürk B, Reisli İ, Çalışkan Ü, Oleaga-Quintas C, Deswarte C, Turul-Özgür T, et al. Infectious diseases, autoimmunity and midline defect in a patient with a novel bi-allelic mutation in IL12RB1 gene. *Turk J Pediatr* (2016) 58(3):331–6. doi:10.24953/turkjped.2016.03.019
10. Airoldi I, Di Carlo E, Cocco C, Sorrentino C, Fais F, Cilli M, et al. Lack of Il12rb2 signaling predisposes to spontaneous autoimmunity and malignancy. *Blood* (2005) 106(12):3846–53. doi:10.1182/blood-2005-05-2034
11. Wu C, Wang X, Gadina M, O'Shea JJ, Presky DH, Magram J. IL-12 receptor beta 2 (IL-12R beta 2)-deficient mice are defective in IL-12-mediated signaling despite the presence of high affinity IL-12 binding sites. *J Immunol* (2000) 165(11):6221–8. doi:10.4049/jimmunol.165.11.6221
12. Lessard CJ, Li H, Adrianto I, Ice JA, Rasmussen A, Grundahl KM, et al. Variants at multiple loci implicated in both innate and adaptive immune responses are associated with Sjögren's syndrome. *Nat Genet* (2013) 45(11):1284–92. doi:10.1038/ng.2792
13. Gupta S, Tatouli IP, Rosen LB, Hasni S, Alevizos I, Manna ZG, et al. Distinct functions of autoantibodies against interferon in systemic lupus erythematosus: a comprehensive analysis of anticytokine autoantibodies in common rheumatic diseases. *Arthritis Rheumatol* (2016) 68(7):1677–87. doi:10.1002/art.39607

14. Vosters JL, Landek-Salgado MA, Yin H, Swaim WD, Kimura H, Tak PP, et al. Interleukin-12 induces salivary gland dysfunction in transgenic mice, providing a new model of Sjögren's syndrome. *Arthritis Rheum* (2009) 60(12):3633–41. doi:10.1002/art.24980
15. Machado Ribeiro F, Goldenberg T. Mycobacteria and autoimmunity. *Lupus* (2015) 24(4–5):374–81. doi:10.1177/0961203314559634
16. Chao WC, Lin CH, Liao TL, Chen YM, Chen DY, Chen HH. Association between a history of mycobacterial infection and the risk of newly diagnosed Sjögren's syndrome: a nationwide, population-based case-control study. *PLoS One* (2017) 12(5):e0176549. doi:10.1371/journal.pone.0176549

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frontiers in Immunology

REVIEW
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Primary Immunodeficiency Diseases: Current and Emerging Therapeutics

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Primary immunodeficiency diseases (PID) result from defects in genes affecting the immune and other systems in many and varied ways (1, 2). Until the last few years, treatments have been largely supportive, with the exception of bone marrow transplantation. However, recent advances in immunobiology, genetics, and the explosion of discovery and commercialization of biologic modifiers have drastically altered the landscape and opportunities in clinical immunology. Therapeutic options and life expectancy of PID patients have also improved dramatically, in large part as a result of better prevention and treatment of infections as well as better understanding and treatment of autoimmune complications (3). As early-life infection-related mortality declines we should anticipate the emergence of other conditions that were previously not appreciated, including malignancies and degenerative disorders unmasked by increasing longevity (4). The genomic revolution has identified literally hundreds of new genetic etiologies of immune dysfunction, many of which are or will soon be eligible for targeted therapies. These emerging immunomodulatory agents represent new therapeutic options in PIDs (5).

Keywords: immunodeficiency, immune modulation, chronic granulomatous disease, leukocyte adhesion deficiency, interferon gamma

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THERAPY BASED ON CLINICAL PRACTICE

Prophylaxis

Invasive bacterial, fungal, viral, and mycobacterial infections carry a high morbidity and mortality in the immunocompromised, and therefore, enormous effort has been directed at prevention. The advent of antibiotics in the last century was critical for the survival of patients with primary immunodeficiency disease (PID) (6). Even before the advent of antibiotic prophylaxis in advanced HIV infection, which transformed AIDS from a rapidly fatal to a more manageable disease, long-term prophylactic antibiotics were widely implemented in chronic granulomatous disease (CGD), with excellent effects (7). CGD also served as the first PID for which cytokine therapy with interferon gamma (IFNγ) (8) and antifungal prophylaxis was indicated and approved (9). The reasons that CGD played such a prominent role in the development and study of prophylaxis were that it was relatively survivable with medical management and bone marrow transplantation was relatively infrequent until recently. Therefore, a relatively large population of patients was able to participate in clinical trials, which markedly assisted the exploration and development of therapeutics. This paradigm has been missing from several other diseases, either because they are not as survivable or because bone marrow transplantation has been more aggressively practiced [e.g., severe combined immune deficiency, Wiskott–Aldrich syndrome (WAS), hyper-IgM syndrome]. For these reasons, many of the approaches discussed below will not have significant prospective clinical validation, making it necessary to rely upon mechanistic explanations and anecdotal reports. While it is likely that in the relatively near future treatments to replace (transplantation) or repair (gene therapy) the underlying

B cell, T cell, and NK cell defects will be available, it is most likely that immunopathology will continue to be a prominent and recurring cause for PID patients to seek care.

The dual and somewhat conflicting demands of immune deficiency, with its recurrent and severe infections, and immune dysregulation, with its associated autoimmunity or autoinflammation, are now well appreciated as a hallmark of PID (10). This means that balancing close attention to infection susceptibility against needs for immunosuppression poses a major therapeutic challenge (11).

Steroids

Corticosteroids have been the backbone of immune modulation since their discovery in the 1930s and their use in rheumatoid arthritis (RA) in 1948 (12, 13). Despite their long use, their long-term toxicity remains significant and their specific mechanisms poorly understood. However, they continue to be a mainstay and first-line approach to immune modulation, especially when needed urgently. Their broad, rapid onset of effects on all the major actors of immune response (T, B, NK, neutrophils) as well as nominally non-immune pathways (e.g., wound healing, glucose metabolism, adrenal suppression) make them both highly effective and very difficult to clearly understand. Their significant complications such as polyphagia, diabetes, cataracts, osteopenia, and infections limit their utility and keep provider and patient anxiety levels high.

Despite these concerns, corticosteroids effectively manage the hollow viscera obstructive and inflammatory disorders in CGD and are surprisingly well tolerated with minimal additional infectious complications at low doses (14). In CGD, steroids have also been used in the treatment of "mulch pneumonitis," the acute inflammatory and necrotizing granulomatous lung disease that follows inhalation of organic matter, such as mulch, compost, dirt, or hay (15). In the CGD mouse model, fatal pulmonary granulomatous inflammation can be caused by either live *Aspergillus* hyphae, which cause invasive fungal infection, or, more surprisingly, by dead *Aspergillus* hyphae, which cause a severe fatal granulomatous response (16). Further, in CGD, steroids have been used in the setting of staphylococcal liver abscess, where they can largely obviate the need for surgery and better preserve long-term liver function (17). They have also helped with the management of necrotizing *Nocardia* pneumonias (18). Therefore, in CGD both invasive infection and hyper-inflammation vie as causes of morbidity and mortality and can be successfully managed with judicious coadministration of both steroids and antibiotics.

Cytokine Therapy

Interferon Alpha (IFNα)

Defects in the TLR3 pathway have been clearly defined as causes of recurrent herpes simplex encephalitis (HSE) in children due to mutations in TLR3, UNC-93B, TRIF, TBK-1, TRAF-3, and IRF3 (19). All of these genes converge on the pathways for neuronal IFNα production, mutations in which put neurons at risk for uncontrolled herpesviral replication. Importantly, in some of these defects (*TBK1, TLR3, TRAF3*) peripheral blood mononuclear cell IFNα production is normal, even though fibroblast and neuronal production are impaired. Therefore, the search for defects involved in HSE should be done genetically and not based entirely on the *in vitro* responses found in peripheral blood samples. Exogenous IFNα or IFNβ therapy clearly rescue the viral phenotype *in vitro,* suggesting that IFNα or IFNβ therapy might be useful in cases of HSE associated with defects in the TLR3 pathway (20, 21). Whether IFNα therapy might have activity in cases of HSE without defects in the TLR3–IRF3 pathway is unclear at this point.

Interferon Gamma (IFNγ)

After its early cloning and production, expectations for the clinical applications of IFNγ were high. Unfortunately, very few of those expectations were realized. However, the observation that IFNγ increased superoxide production from monocyte-derived macrophages *in vitro* led to interest in using it in therapy for chronic granulomatous disease (CGD). An international, prospective, randomized double-blind trial in CGD patients showed clear reduction in severe infections in the IFNγ (50 μg/m^2 subcutaneously three times weekly) group without exacerbation of granulomatous or inflammatory complications (22). IFNγ is essential for the killing of many intracellular microbes and has been used to enhance anti-mycobacterial immunity in patients with partial dominant IFNγ receptor 1 deficiency and chemotherapy-resistant mycobacterial infection (23). Higher doses of IFNγ (200 μg/m^2) have been used in those with mycobacterial infections in the dominant partial forms of IFNγ receptor deficiency and in recessive IL12Rβ1 deficiency (24).

THERAPY BASED ON MECHANISM

Interleukin-2

Interleukin-2 is secreted from T cells and supports T cell proliferation, NK cell activation, and can promote activation-induced cell death (25, 26). However, at low does, recombinant IL-2 has also been shown to selectively increase T regulatory (Treg) cells. In the WAS, IL-2 therapy was recognized to enhance killing activity *in vitro* (27). In a prospective study WAS patients responded to low-dose IL-2 (0.5 MU/m^2 for 5 days every 3 weeks) with modest increases in lymphocyte counts. However, consistent with the narrow dose range for many cytokines, at the 1 MU dose several patients had worsened thrombocytopenia (28). In the setting of bone marrow transplantation low-dose and very low-dose IL-2 have been shown to increase Treg cells, but their long-term value in preventing graft-versus-host disease (GVHD) is still being determined (29).

Cell Depletion

CD 20

Rituximab (anti-CD20) is active in the treatment of lymphoma and in many autoimmune diseases, presumably through B cell depletion and also through disruption of autoantibody production. However, depletion of CD20+ B cells also removes potent antigen presenting cells, so it may have more than one mechanism of action. Improved quality of life has been observed in those with B-cell class-switch defects (hyper-IgM syndrome), who received

rituximab for the treatment of autoimmune manifestations and generalized lymphadenopathy (30). Rituximab has been used as immunomodulatory therapy, especially as part of the treatment of granulomatous lymphocytic interstitial lung disease (31) in CVID as well as in CVID-associated autoimmune cytopenias (32–34). In patients with anti-IFNγ autoantibodies who have severe disseminated infections with intracellular pathogens, especially non-tuberculous mycobacteria, rituximab has helped to reduce antibody levels allowing clearance of infection (35).

Cytokine Antagonists

Anti-IL-17 and Anti-p40 (Anti-IL-12/23)

IL-17 is an important mediator of inflammation, especially at epithelial surfaces. It is itself induced in CD4 + T cells by IL-23 and in turn induces the generation of G-CSF and IL-22. IL-17 neutralization has profoundly beneficial effects on the clinical courses of psoriasis and colitis (36). IL-23 is formed by the heterodimerization of IL-23p19 and IL-12p40, while IL-12 is formed by the heterodimerization of IL-12p35 and IL-12p40. In a mouse model of colitis, neutralization of IL-23 using an IL-23-specific anti-p19 antibody significantly alleviated both emerging and established colitis, through the downstream inhibition of IL-17 expression, leading to diminished neutrophil infiltration (37). One case of severe CGD colitis treated with ustekinumab (anti-p40, the common chain of IL-12 and IL-23) showed clinical improvement but developed a severe infection (38).

Leukocyte adhesion defect (LAD) 1 is characterized by a severe periodontal disease and premature loss of teeth. Moutsopoulos et al. showed that excessive IL-17 expressing T cells in periodontal tissue is responsible for the inflammation and bone loss in human and mouse models of LAD1. These observations support targeting IL17 production through inhibition of IL-12/23 p40 (39).

IL-1

IL-1 is induced in response to numerous inflammatory stimuli and is the critical mediator of fever; it was previously known as "endogenous pyrogen." Its natural antagonist, IL1RA, inhibits IL-1's activation of its receptor and, therefore, blocks IL1-mediated inflammation. IL-1 is generated from proIL-1 by the action of the IL-1 inflammasome, mediated by NLRP3, previously known as CIAS. Gain-of-function (GOF) mutations in NLRP3 lead to autoinflammatory diseases, so-called because they do not depend on antigen-specific triggers or T or B responses. Anakinra has been used extensively in disorders of inflammation. de Luca et al. showed that inhibition of IL-1 receptor activation using the receptor blocker anakinra resulted in inflammasome inactivation, restoration of autophagy, improvement in colitis, and protection from invasive aspergillosis in p47*phox*$^{-/-}$ mice. Studies in human subjects are essential to derive data on safety, dosage, and duration and complete efficacy of anakinra therapy in patients with CGD.

IL-6

IL-6 is a cytokine that induces signal transducer and activator of transcription (STAT) 3 phosphorylation and leads to fever and the acute phase response. Interestingly, it is also a myokine that is produced by muscle during activity. IL6 is elevated in RA and has been successfully targeted in that disease by tocilizumab, which inhibits downstream STAT3-mediated effects. In the disease caused by GOF STAT3 mutations, signaling is pathologically augmented, leading to fever, arthritis, rashes and lung disease. Milner et al. showed that tocilizumab led to marked improvement of peripheral arthritis and scleroderma-like skin lesions in a patient who had failed multiple other immunosuppressive therapies (40).

Signal Blockade

CTLA4

CTLA4 is expressed on Treg cells and activated T cells, providing an inhibitory signal to effector T lymphocytes through its binding to CD80/CD86. Therefore, reduced CTLA4 expression leads to reduced Treg activity, which in turn leads to autoimmunity. CTLA4 deficiency is characterized by cytopenias and the triad of granulomatous brain lesions, granulomatous lung lesions and gut involvement; these manifestations can be separate or together, and either immunodeficient or autoimmune phenomena may predominate. Abatacept is a protein formed by the fusion of the Fc domain of IgG1 to the extracellular domain of CTLA4, thereby mimicking the binding of CTLA4 to CD80/CD86 and helping to tamp down autoimmunity. Lee et al. reported the effectiveness of abatacept in an adolescent girl with a mutation in CTLA4 and severe gut and other disease (41).

T Cell-Directed Therapies

In ALPS-associated autoimmune cytopenias steroids are the first line of treatment, followed by mycophenolate mofetil (a prodrug of mycophenolic acid that inhibits inosine monophosphate dehydrogenase and suppresses T and B cells) and sirolimus [an mechanistic target of rapamycin (mTOR) inhibitor] that more effectively targets double-negative T cells (32, 42).

In other PIDs, such as CGD, methotrexate and cyclosporine have been used to control T-cell-mediated complications. Hydroxychloroquine, a moderately effective but less immunosuppressive drug, can be used as a single drug therapy or in combination with low-dose steroids. Hydroxychloroquine may enhance CLTLA4 expression through the inhibition of lysosomal CTLA4 degradation (43).

Janus-Associated Kinase/STAT Inhibitors

Gain-of-function mutations in STAT1 lead to sustained levels of phosphorylation of STAT1 upon stimulation, which result in increased expression of interferon-simulated genes. Autoimmunity and infection caused by STAT1 GOF mutations are thought to be the result of dysregulated T cell responses. Janus kinase inhibitors may be effective targeted treatments for long-term disease control of severely affected patients for whom hematopoietic stem cell transplantation is not available (44–46). The experience with transplantation for STAT1 GOF has so far been disappointing, suggesting that there are complex issues that will need novel approaches in terms of preparative regimen, in particular (47).

mTOR Inhibitors

Mechanistic Target of Rapamycin is a serine/threonine protein kinase that regulates a dizzying array of cellular and metabolic

activities, especially including T cell proliferation. Conditions that constitutively or aberrantly activate the mTOR pathway lead to excess signaling, and are associated with abnormal cell proliferation and autoimmunity. Rapamycin is a small molecule that blocks mTOR activity and has found extensive clinical application in the maintenance of transplant tolerance. It has also achieved good clinical responses in patients with immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome, probably related to the decrease in proliferation of T effector cells with relative preservation of Treg cells. It leads to reduction in hepatosplenomegaly and lymphadenopathy, improvement in naive T cell counts, and restitution of lymphocyte IL-2 secretion (48, 49).

In activating mutations in PIK3CD, Lucas et al. demonstrated that rapamycin restored the abundance of naive T cells, largely "rescued" the *in vitro* T cell defects and improved the clinical course (50). In *LRBA* deficiency, which is associated with impaired CTLA4 display due to LRBA's role in CTLA4 recycling, the CTLA4 mimetic abatacept caused dramatic and sustained improvement (43).

Apoptosis

Programmed cell death protein 1, PD-1, binds to PD-1 ligand, PDL-1, and downregulates the activity of inflammatory T cells. This interaction forms the basis of one of the immune checkpoints, a node at which immune reactivity can either be encouraged (PD-1 inhibition) or diminished (PD-1 expression). This fundamental recognition has served as the basis for the newer immune therapies in cancer, which use PD-1 inhibition to disinhibit high PD-1 expression leading to antitumor immune response. High PD-1 expression is generally associated with exhaustion of T cells, such as during chronic viral infections, and is associated with poor responses to antigen activation. Importantly, blockade of this PD-1/PD-1-ligand interaction restores antigen-specific responses *in vitro* (51). PD-1 receptor blockade increased JC virus-specific T-cell immune responses in a subgroup of progressive multifocal leukoencephalopathy (PML) patients *in vitro*, suggesting that this pathway might be important in the control of JC virus-associated PML (52).

Miscellaneous Agents

Magnesium

LOF mutations in the gene encoding the X-linked magnesium transporter 1 (*MAGT1*) result in an immunodeficiency characterized by EBV infection and lymphoma (XMEN), due to impaired magnesium-dependent intracellular signaling, which is especially important in NK cell function (53). Treatment with high-dose magnesium, supplied by the oral preparation magnesium threanate has been able to restore NK activity and reduce EBV viral loads in a small number of cases.

Pioglitazone

Peroxisome proliferator-activated receptor γ (PPARγ) is a central mediator of metabolic responses, which has made it a target for endocrine therapies. PPARγ is involved in numerous cellular pathways, including reactive oxygen species (ROS) generation in mitochondria, fatty acid metabolism, and gluconeogenesis. Activation of intracellular PPARγ by agonists such as pioglitazone improved neutrophil ROS production and enhanced microbicidal action against *Staphylococcus aureus* in a murine CGD model (54). Apparently, pioglitazone leads to increased mitochondrial ROS, which then supplements the intracellular killing of certain microbes. Pioglitazone may also regulate other pathways, including IL-17, which is aberrantly high in CGD.

STATE-OF-THE-ART TREATMENTS

Chronic and refractory viral infections remain a cause of significant mortality both before and after HSCT in patients with PID. Reconstitution of T cell immunity is critical for control of viral infections. Adoptive immunotherapy with virus-specific T lymphocytes (VSTs) is an attractive option for addressing the underlying impaired T cell immunity (55). Infusion reactions are uncommon, mild and likely related to the cryopreservation additive rather than the VST themselves. To date, with limited phase 1 and 2 studies, cytokine release syndrome has not been described, though it remains a theoretical risk, particularly in patients with disseminated viral disease. And also it is unclear if the rate of GVHD in those receiving VST therapy is different from the background rate of GVHD in patients undergoing HSCT.

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REFERENCES

1. Stray-Pedersen A, Sorte HS, Samarakoon P, Gambin T, Chinn IK, Coban Akdemir ZH, et al. Primary immunodeficiency diseases: genomic approaches delineate heterogeneous Mendelian disorders. *J Allergy Clin Immunol* (2017) 139:232–45. doi:10.1016/j.jaci.2016.05.042
2. Bousfiha AA, Jeddane L, Ailal F, Benhsaien I, Mahlaoui N, Casanova J-L, et al. Primary immunodeficiency diseases worldwide: more common than generally thought. *J Clin Immunol* (2013) 33(1):1–7. doi:10.1007/s10875-012-9751-7
3. Notarangelo LD. Primary immunodeficiencies. *J Allergy Clin Immunol* (2010) 125(2 Suppl 2):S182–94. doi:10.1016/j.jaci.2009.07.053
4. Rezaei N, Hedayat M, Aghamohammadi A, Nichols KE. Primary immunodeficiency diseases associated with increased susceptibility to viral infections and malignancies. *J Allergy Clin Immunol* (2011) 127:1329–41. doi:10.1016/j.jaci.2011.02.047
5. Vignesh P, Rawat A, Singh S. An update on the use of immunomodulators in primary immunodeficiencies. *Clin Rev Allergy Immunol* (2017) 52(2):287–303. doi:10.1007/s12016-016-8591-2
6. Ochs HD, Hitzig WH. History of primary immunodeficiency diseases. *Curr Opin Allergy Clin Immunol* (2012) 12(6):577–87. doi:10.1097/ACI.0b013e32835923a6
7. Kuruvilla M, de la Morena MT. Antibiotic prophylaxis in primary immune deficiency disorders. *J Allergy Clin Immunol Pract* (2013) 1:573–82. doi:10.1016/j.jaip.2013.09.013
8. Gallin JI, Malech HL, Curnutte J, Quie P, Jaffe H, Ezekowitz R. A controlled trial of interferon gamma to prevent infection in chronic granulomatous

disease. *N Engl J Med* (1991) 324:509–16. doi:10.1056/NEJM199102213240801
9. Gallin JI, Alling DW, Malech HL, Wesley R, Koziol D, Marciano B, et al. Itraconazole to prevent fungal infections in chronic granulomatous disease. *N Engl J Med* (2003) 348:2416–22. doi:10.1056/NEJMoa021931
10. Walter JE, Farmer JR, Foldvari Z, Torgerson TR, Cooper MA. Mechanism-based strategies for the management of autoimmunity and immune dysregulation in primary immunodeficiencies. *J Allergy Clin Immunol Pract* (2016) 4:1089–100. doi:10.1016/j.jaip.2016.08.004
11. Kuijpers T, Lutter R. Inflammation and repeated infections in CGD: two sides of a coin. *Cell Mol Life Sci* (2012) 69:7–15. doi:10.1007/s00018-011-0834-z
12. Benedek TG. History of the development of corticosteroid therapy. *Clin Exp Rheumatol* (2011) 29(5 Suppl 68):S5–12.
13. Hench PS, Kendall EC, Slocumb CH, Polley HF. The effect of a hormone of the adrenal cortex (17-hydroxy-11-dehydrocorticosterone: compound E) and of pituitary adrenocortical hormone in arthritis: preliminary report. *Ann Rheum Dis* (1949) 8(2):97–104.
14. Chin TW, Stiehm ER, Falloon J, Gallin JI. Corticosteroids in treatment of obstructive lesions of chronic granulomatous disease. *J Pediatr* (1987) 111(3):349–52. doi:10.1016/S0022-3476(87)80452-3
15. Siddiqui S, Anderson VL, Hilligoss DM, Abinun M, Kuijpers TW, Masur H, et al. Fulminant mulch pneumonitis: an emergency presentation of chronic granulomatous disease. *Clin Infect Dis* (2007) 45:673–81. doi:10.1086/520985
16. Morgenstern DE, Gifford MA, Li LL, Doerschuk CM, Dinauer MC. Absence of respiratory burst in X-linked chronic granulomatous disease mice leads to abnormalities in both host defense and inflammatory response to *Aspergillus fumigatus*. *J Exp Med* (1997) 185:207–18. doi:10.1084/jem.185.2.207
17. Leiding JW, Freeman AF, Marciano BE, Anderson VL, Uzel G, Malech HL, et al. Corticosteroid therapy for liver abscess in chronic granulomatous disease. *Clin Infect Dis* (2012) 54(5):694–700. doi:10.1093/cid/cir896
18. Freeman AF, Marciano BE, Anderson VL, Uzel G, Costas C, Holland SM. Corticosteroids in the treatment of severe *Nocardia* pneumonia in chronic granulomatous disease. *Pediatr Infect Dis J* (2011) 30(9):806–8. doi:10.1097/INF.0b013e318218181d
19. Maglione PJ, Simchoni N, Cunningham-Rundles C. Toll-like receptor signaling in primary immune deficiencies. *Ann N Y Acad Sci* (2015) 1356:1–21. doi:10.1111/nyas.12763
20. Lafaille FG, Pessach IM, Zhang SY, Ciancanelli MJ, Herman M, Abhyankar A, et al. Impaired intrinsic immunity to HSV-1 in human iPSC-derived TLR3-deficient CNS cells. *Nature* (2012) 491:769–73. doi:10.1038/nature11583
21. McGlasson S, Jury A, Jackson A, Hunt D. Type I interferon dysregulation and neurological disease. *Nat Rev Neurol* (2015) 11:515–23. doi:10.1038/nrneurol.2015.143
22. Weening RS, Leitz GJ, Seger RA. Recombinant human interferon-gamma in patients with chronic granulomatous disease – European follow up study. *Eur J Pediatr* (1995) 154:295–8. doi:10.1007/s004310050292
23. Newport MJ, Huxley CM, Huston S, Hawrylowicz CM, Oostra BA, Williamson R, et al. A mutation in the interferon-gamma-receptor gene and susceptibility to mycobacterial infection. *N Engl J Med* (1996) 335(26):1941–9. doi:10.1056/NEJM199612263352602
24. Alangari AA, Al-Zamil F, Al-Mazrou A, Al-Muhsen S, Boisson-Dupuis S, Awadallah S, et al. Treatment of disseminated mycobacterial infection with high-dose IFN-gamma in a patient with IL-12Rbeta1 deficiency. *Clin Dev Immunol* (2011) 2011:691956. doi:10.1155/2011/691956
25. Cook KD, Waggoner SN, Whitmire JK. NK cells and their ability to modulate T cells during virus infections. *Crit Rev Immunol* (2014) 34:359–88. doi:10.1615/CritRevImmunol.2014010604
26. Bachmann MF, Oxenius A. Interleukin 2: from immunostimulation to immunoregulation and back again. *EMBO Rep* (2007) 8:1142–8. doi:10.1038/sj.embor.7401099
27. Azuma H, Sakata H, Saijyou M, Okuno A. Effect of interleukin 2 on intractable herpes virus infection and chronic eczematoid dermatitis in a patient with Wiskott-Aldrich syndrome. *Eur J Pediatr* (1993) 152:998–1000. doi:10.1007/BF01957224
28. Jyonouchi S, Gwafila B, Gwalani LA, Ahmad M, Moertel C, Holbert C, et al. Phase I trial of low-dose interleukin 2 therapy in patients with Wiskott-Aldrich syndrome. *Clin Immunol* (2017) 179:47–53. doi:10.1016/j.clim.2017.02.001
29. Kennedy-Nasser AA, Ku S, Castillo-Caro P, Hazrat Y, Wu MF, Liu H, et al. Ultra low-dose IL-2 for GVHD prophylaxis after allogeneic hematopoietic stem cell transplantation mediates expansion of regulatory T cells without diminishing antiviral and antileukemic activity. *Clin Cancer Res* (2014) 20:2215–25. doi:10.1158/1078-0432.CCR-13-3205
30. Hennig C, Baumann U, Ilginus C, Horneff G, Foell J, Hansen G. Successful treatment of autoimmune and lymphoproliferative complications of patients with intrinsic B-cell immunodeficiencies with rituximab. *Br J Haematol* (2010) 148:445–8. doi:10.1111/j.1365-2141.2009.07987.x
31. Chase NM, Verbsky JW, Hintermeyer MK, Waukau JK, Tomita-Mitchell A, Casper JT, et al. Use of combination chemotherapy for treatment of granulomatous and lymphocytic interstitial lung disease (GLILD) in patients with common variable immunodeficiency (CVID). *J Clin Immunol* (2013) 33:30–9. doi:10.1007/s10875-012-9755-3
32. Rao VK, Price S, Perkins K, Aldridge P, Tretler J, Davis J, et al. Use of rituximab for refractory cytopenias associated with autoimmune lymphoproliferative syndrome (ALPS). *Pediatr Blood Cancer* (2009) 52(7):847–52. doi:10.1002/pbc.21965
33. Cunningham-Rundles C. Key aspects for successful immunoglobulin therapy of primary immunodeficiencies. *Clin Exp Immunol* (2011) 164(Suppl 2):16–9. doi:10.1111/j.1365-2249.2011.04390.x
34. Gobert D, Bussel JB, Cunningham-Rundles C, Galicier L, Dechartres A, Berezne A, et al. Efficacy and safety of rituximab in common variable immunodeficiency-associated immune cytopenias: a retrospective multicentre study on 33 patients. *Br J Haematol* (2011) 155(4):498–508. doi:10.1111/j.1365-2141.2011.08880.x
35. Browne SK, Zaman R, Sampaio EP, Jutivorakool K, Rosen LB, Ding L, et al. Anti-CD20 (rituximab) therapy for anti-IFN-gamma autoantibody-associated nontuberculous mycobacterial infection. *Blood* (2012) 119:3933–9. doi:10.1182/blood-2011-12-395707
36. Yamagata T, Skepner J, Yang J. Targeting Th17 effector cytokines for the treatment of autoimmune diseases. *Arch Immunol Ther Exp (Warsz)* (2015) 63:405–14. doi:10.1007/s00005-015-0362-x
37. Wang R, Hasnain SZ, Tong H, Das I, Che-Hao Chen A, Oancea I, et al. Neutralizing IL-23 is superior to blocking IL-17 in suppressing intestinal inflammation in a spontaneous murine colitis model. *Inflamm Bowel Dis* (2015) 21:973–84. doi:10.1097/MIB.0000000000000353
38. Butte MJ, Park KT, Lewis DB. Treatment of CGD-associated colitis with the IL-23 blocker ustekinumab. *J Clin Immunol* (2016) 36:619–20. doi:10.1007/s10875-016-0318-x
39. Moutsopoulos NM, Konkel J, Sarmadi M, Eskan MA, Wild T, Dutzan N, et al. Defective neutrophil recruitment in leukocyte adhesion deficiency type I disease causes local IL-17-driven inflammatory bone loss. *Sci Transl Med* (2014) 6:229ra40. doi:10.1126/scitranslmed.3007696
40. Milner JD, Vogel TP, Forbes L, Ma CA, Stray-Pedersen A, Niemela JE, et al. Early-onset lymphoproliferation and autoimmunity caused by germline STAT3 gain-of-function mutations. *Blood* (2015) 125:591–9. doi:10.1182/blood-2014-09-602763
41. Lee S, Moon JS, Lee CR, Kim HE, Baek SM, Hwang S, et al. Abatacept alleviates severe autoimmune symptoms in a patient carrying a de novo variant in CTLA-4. *J Allergy Clin Immunol* (2016) 137:327–30. doi:10.1016/j.jaci.2015.08.036
42. Teachey DT, Greiner R, Seif A, Attiyeh E, Bleesing J, Choi J, et al. Treatment with sirolimus results in complete responses in patients with autoimmune lymphoproliferative syndrome. *Br J Haematol* (2009) 145(1):101–6. doi:10.1111/j.1365-2141.2009.07595.x
43. Lo B, Zhang K, Lu W, Zheng L, Zhang Q, Kanellopoulou C, et al. Patients with LRBA deficiency show CTLA4 loss and immune dysregulation responsive to abatacept therapy. *Science* (2015) 349(6246):436–40. doi:10.1126/science.aaa1663
44. Weinacht KG, Charbonnier LM, Alroqi F, Plant A, Qiao Q, Wu H, et al. Ruxolitinib reverses dysregulated T helper cell responses and controls autoimmunity caused by a novel signal transducer and activator of transcription 1 (STAT1) gain-of-function mutation. *J Allergy Clin Immunol* (2017) 139(5):1629–40.e2. doi:10.1016/j.jaci.2016.11.022
45. Harel S, Higgins CA, Cerise JE, Dai Z, Chen JC, Clynes R, et al. Pharmacologic inhibition of JAK-STAT signaling promotes hair growth. *Sci Adv* (2015) 1:e1500973. doi:10.1126/sciadv.1500973

46. Xing L, Dai Z, Jabbari A, Cerise JE, Higgins CA, Gong W, et al. Alopecia areata is driven by cytotoxic T lymphocytes and is reversed by JAK inhibition. *Nat Med* (2014) 20:1043–9. doi:10.1038/nm.3645
47. Leiding JW, Okada S, Hagin D, Abinun M, Shcherbina A, Balashov DN, et al. Hematopoietic stem cell transplantation in patients with gain of function STAT1 mutation. *J Allergy Clin Immunol* (2017). doi:10.1016/j.jaci.2017.03.049
48. Bacchetta R, Barzaghi F, Roncarolo MG. From IPEX syndrome to FOXP3 mutation: a lesson on immune dysregulation. *Ann N Y Acad Sci* (2016). doi:10.1111/nyas.13011
49. Yong PL, Russo P, Sullivan KE. Use of sirolimus in IPEX and IPEX-like children. *J Clin Immunol* (2008) 28:581–7. doi:10.1007/s10875-008-9196-1
50. Lucas CL, Kuehn HS, Zhao F, Niemela JE, Deenick EK, Palendira U, et al. Dominant-activating germline mutations in the gene encoding the PI(3)K catalytic subunit p110delta result in T cell senescence and human immunodeficiency. *Nat Immunol* (2014) 15:88–97. doi:10.1038/ni.2771
51. Hong JJ, Amancha PK, Rogers K, Ansari AA, Villinger F. Re-evaluation of PD-1 expression by T cells as a marker for immune exhaustion during SIV infection. *PLoS One* (2013) 8:e60186. doi:10.1371/journal.pone.0060186
52. Tan CS, Bord E, Broge TA Jr, Glotzbecker B, Mills H, Gheuens S, et al. Increased program cell death-1 expression on T lymphocytes of patients with progressive multifocal leukoencephalopathy. *J Acquir Immune Defic Syndr* (2012) 60:244–8. doi:10.1097/QAI.0b013e31825a313c
53. Ravell J, Chaigne-Delalande B, Lenardo M. X-linked immunodeficiency with magnesium defect, Epstein-Barr virus infection, and neoplasia disease: a combined immune deficiency with magnesium defect. *Curr Opin Pediatr* (2014) 26:713–9. doi:10.1097/MOP.0000000000000156
54. Fernandez-Boyanapalli RF, Frasch SC, Thomas SM, Malcolm KC, Nicks M, Harbeck RJ, et al. Pioglitazone restores phagocyte mitochondrial oxidants and bactericidal capacity in chronic granulomatous disease. *J Allergy Clin Immunol* (2015) 135:517–27.e12. doi:10.1016/j.jaci.2014.10.034
55. McLaughlin LP, Bollard CM, Keller M. Adoptive T cell immunotherapy for patients with primary immunodeficiency disorders. *Curr Allergy Asthma Rep* (2017) 17:3. doi:10.1007/s11882-017-0669-2

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Prolonged Excretion of Poliovirus among Individuals with Primary Immunodeficiency Disorder: An Analysis of the World Health Organization Registry

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Individuals with primary immunodeficiency disorder may excrete poliovirus for extended periods and will constitute the only remaining reservoir of virus after eradication and withdrawal of oral poliovirus vaccine. Here, we analyzed the epidemiology of prolonged and chronic immunodeficiency-related vaccine-derived poliovirus cases in a registry maintained by the World Health Organization, to identify risk factors and determine the length of excretion. Between 1962 and 2016, there were 101 cases, with 94/101 (93%) prolonged excretors and 7/101 (7%) chronic excretors. We documented an increase in incidence in recent decades, with a shift toward middle-income countries, and a predominance of poliovirus type 2 in 73/101 (72%) cases. The median length of excretion was 1.3 years (95% confidence interval: 1.0, 1.4) and 90% of individuals stopped excreting after 3.7 years. Common variable immunodeficiency syndrome and residence in high-income countries were risk factors for long-term excretion. The changing epidemiology of cases, manifested by the greater incidence in recent decades and a shift to from high- to middle-income countries, highlights the expanding risk of poliovirus transmission after oral poliovirus vaccine cessation. To better quantify and reduce this risk, more sensitive surveillance and effective antiviral therapies are needed.

Keywords: polio eradication, primary immunodeficiency, vaccine-derived poliovirus, oral poliovirus vaccine, immunodeficiency-related vaccine-derived poliovirus

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INTRODUCTION

Since the launch of the Global Polio Eradication Initiative in 1988, there has been substantial progress toward eradication, documented by a decline in the incidence of cases from >350,000 in 1988 to 37 in 2016, and a decrease in the number of endemic countries from 125 to 3 (1). Although wild poliovirus type 1 continues to circulate in Afghanistan, Nigeria, and Pakistan, no wild poliovirus type 2 or wild poliovirus type 3 have been detected globally since October 1999 (2) and November 2012 (3), periods of 17 and 4 years, respectively.

Abbreviations: AFP, acute flaccid paralysis; CVID, common variable immunodeficiency; iVDPV, immunodeficiency-related vaccine-derived poliovirus; OPV, oral poliovirus vaccines; PID, primary immunodeficiency disorder; VDPV, vaccine-derived poliovirus; WHO, World Health Organization.

These achievements have been accomplished through the extensive use of oral poliovirus vaccines (OPV), which are easy to administer, suitable for mass campaigns, and able to induce both humoral and mucosal immunity (4). However, in rare instances, live Sabin strains contained in OPV can cause vaccine-associated paralytic poliomyelitis, with paralytic manifestations indistinguishable from those caused by wild poliovirus (4). Furthermore, these viruses have the potential to revert to neurovirulence and re-acquire the transmissibility characteristics of wild poliovirus, resulting in outbreaks of circulating vaccine-derived poliovirus (VDPV) (5). In the presence of high population immunity, VDPVs rarely emerge and cause outbreaks. However, in areas with low population immunity, these viruses could potentially re-establish endemic transmission (6).

Immunodeficiency-related vaccine-derived poliovirus (iVDPV) is a type of VDPV in which individuals with a primary immunodeficiency disorder (PID) excrete Sabin polioviruses; in some cases, for substantially longer periods than immunocompetent individuals (4). After exposure to OPV, immunocompetent individuals usually excrete the vaccine virus for 4–8 weeks (7). However, in immunodeficient individuals, an inability to mount an adequate immune response can result in persistence of the intestinal infection with poliovirus and prolonged viral shedding (8). In the process, the virus can mutate to re-acquire the neurovirulence and transmissibility characteristics of wild poliovirus (9–12).

To address the risks associated with OPV use, the Strategic Plan of Action 2013–2018 of the Global Polio Eradication Initiative calls for a sequential global withdrawal of OPV, starting with the Sabin type 2 component that was removed in April 2016 and followed by Sabin type 1 and Sabin type 3 after certification of wild type 1 and 3 eradication, respectively (13). Although this strategy may minimize the long-term risk of circulating VDPV outbreaks, iVDPV cases will remain a potential source of live poliovirus in communities after the withdrawal of OPV and in the post eradication era, and could pose a substantial risk of poliovirus reintroduction in the population. The post-OPV cessation risks of long-term excretors have been modeled previously (14–16).

A number of case reports and case series of iVDPV excretors have been published with a systematic review of published cases undertaken (17), and more recently, studies have assessed iVDPV excretion among persons with PIDs (18–20). However, many of these works focus on only a subset of iVDPV cases, with case reports often limited to upper-middle income and high-income countries and patients with paralysis.

We present analysis of iVDPV cases known to the World Health Organization (WHO), from the inception of widespread OPV use (1962–2016). The main objectives of this study are to conduct demographic, risk factor, and survival analysis of reported iVDPV cases to determine the populations most at risk and to better understand the threat posed by iVDPV cases to global poliovirus eradication.

MATERIALS AND METHODS

Study Population

This study analyzed cases in the iVDPV case registry maintained by WHO, containing reported cases from 1962 to 2016, irrespective of reporting source or paralysis status (see Table S2 in Supplementary Table). The main sources contributing cases to the registry include: (1) the acute flaccid paralysis (AFP) surveillance system, which is the routine way countries report cases; (2) the regional polio laboratory network; (3) specific studies and pilot poliovirus surveillance projects targeting PID patients; and (4) regularly conducted systematic literature reviews of scientific journals reporting on iVDPV cases, where key search terms include VDPV, iVDPV, immunodeficiency, immunocomprised, excretion, polio, and "vaccine-associated paralytic poliomyelitis." In order to obtain the most recent information on ongoing cases, corresponding authors of case reports and/or relevant scientific and medical personnel, including regional laboratory coordinators, were contacted.

Inclusion criteria comprised: excretion of poliovirus for greater than 6 months; confirmed PID; and laboratory-confirmed VDPV. A *prolonged excretor* was defined as a person excreting virus for ≥6 months and ≤5 years, and a *chronic excretor* was defined as excreting for >5 years (14).

Primary immunodeficiency disorder in the registry includes individuals with congenital onset of B-cell deficiency, T-cell deficiency, major histocompatibility complex deficiency, or a combination of the above. For the purposes of these analyses, immunodeficiency disorders were divided into the following broad categories:

- Antibody disorder, including hypogammaglobulinemia, agammaglobulinemia, X-linked agammaglobulinemia, and other antibody deficiencies.
- Severe combined immunodeficiency disorder and other combined humoral/T-cell deficiencies.
- Common variable immunodeficiency disorder (CVID).
- Other, including major histocompatibility complex deficiencies, the immunodeficiency, centromere instability, and facial anomalies syndrome, as well as other unknown or undiagnosed causes of immunodeficiency.

Laboratory Processing of Poliovirus Isolates

Until the establishment of sensitive surveillance for polio eradication, viruses were mainly isolated in a few specialized laboratories. With implementation of surveillance for polio eradication, poliovirus was increasingly isolated from patient stool samples by laboratories participating in the WHO Global Polio Laboratory Network. Isolates were identified by several steps: first, specimens were grown in tissue culture; second, molecular methods were used to identify the serotype; and third, intratypic differentiation to determine vaccine or wild strains was done either by enzyme-linked immunosorbent assay using highly specific cross-absorbed antisera and/or by diagnostic reverse transcriptase-polymerase chain reaction (21). If the enzyme-linked immunosorbent assay and molecular intratypic differentiation methods yielded discordant results, the ~900-nucleotide interval encoding the major capsid protein, the viral protein 1 (VP1), was sequenced. Poliovirus genomes appear to evolve at a rate of ~1.1% mutations in VP1 region per year (22) and VDPVs are defined as having a VP1 nucleotide divergence >1% for type 1 and 3, and >0.6%

for type 2, from the corresponding parenteral OPV strain (23), consistent with prolonged replication or transmission.

Demographic and Clinical Variables

Cases were recorded with the following demographic and clinical parameters: year of detection; country of residence and its income classification [based on the 2016 World Bank classifications (24)]; date of birth; date of onset; age at onset; gender; PID; presence or absence of paralysis; OPV vaccination history; clinical outcome (alive, alive and stopped excreting, dead or unknown); and first and most recent positive specimen dates, serotype of virus, percentage VP1 divergence from the parenteral Sabin strain, and recombination with other non-polio enteroviruses.

Statistical Analysis

Descriptive analysis was conducted to identify demographic and clinical characteristics of iVDPV cases. According to available information, duration of excretion was estimated following the algorithm provided in Table S1 in Supplementary Material. The algorithm bases the length of excretion on either the time period between OPV administration (or exposure) and the last iVDPV isolate, the time period calculated by VP1 divergence from parental Sabin strain based on an evolution rate of ~1.1% divergence per year (22), or where this information was not available, the excretion time under observation. To model the length of excretion, Kaplan–Meier survival analysis was employed and log-rank tests used to determine difference in survival times between groups. To allow for multiple comparisons, the *P*-value for significance was set at $P < 0.01$. All analyses were conducted using the statistical software R 3.3.1 (25) and Stata MP 13 (26).

RESULTS

Demographics

As of July 2016, the WHO registry included 110 suspected iVDPV cases with onset of excretion or paralytic symptoms during 1962–2016. Of these, 101 (91.8%) met the definition of prolonged or chronic iVDPV excretion and were included in the analysis. **Figure 1** shows the number of reported iVDPV cases over time since 1962. There has been an increase in the number of reported cases since the year 2000, with the highest number the period since 2010 (56/101). Within this, there are two divergent trends: an increase in cases reported from low- and middle-income countries and a decrease in the number of cases reported from high-income countries (**Figure 1**).

Figure 2 provides the geographic location of these cases by associated serotype, illustrating a large concentration of cases around the Middle East. The characteristics of the 101 prolonged and chronic iVDPV cases are presented in **Table 1**. The residence of cases by worldwide region shows the highest proportion of cases 43/101 (43%) occurred in the Eastern Mediterranean Region. There were 24/101 (24%) cases in lower-middle-income countries, 47/101 (47%) in upper-middle income countries, and 29/101 (29%) in high-income countries, with all seven chronic excretor cases resident in high-income countries. Only one case was reported from a low-income country, which was in Afghanistan in 2013.

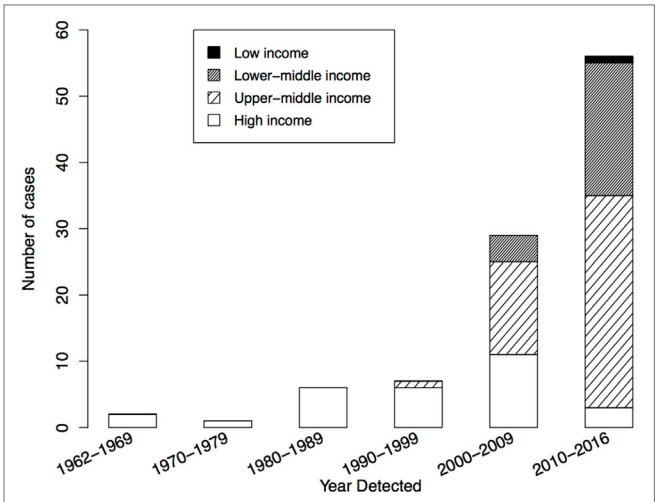

FIGURE 1 | Year of detection of 101 reported chronic and prolonged immunodeficiency-related vaccine-derived poliovirus cases from 1962 to 2016, by income classification of country of residence: low income ($n = 1$), lower-middle income ($n = 24$), upper-middle income ($n = 47$), and high-income ($n = 29$). Income classification based on 2016 World Bank Classification.

Out of the 101 cases, 63/98 (64%) were males, 71/100 (71%) presented with paralytic manifestations, as defined by AFP, and the most common age of onset was <1 year in 46/100 (46%) cases, followed by 1 to <5 years old in 37/100 (37%) (**Table 1**).

Underlying PID

The underlying immunodeficiency disorders of cases are shown in **Table 1**. Antibody disorders were the most frequent, accounting for 31/91 (34%) of the cases, primarily composed of hypogammaglobulinemia, agammaglobulinemia, and X-linked agammaglobulinemia. Severe combined immunodeficiency disorder and CVID were also common, present in 25/92 (27%) and 21/92 (23%) of cases, respectively. All the seven chronic excretors had CVID.

Virological Factors

The serotype of excreted virus was predominantly type 2 poliovirus, in 68/101 cases (67%), followed by type 1 in 15/101 cases (15%) and type 3 in 13/101 cases (13%) (**Table 1**). Among the six cases with multi-serotype infections, two were type 1 and 2 co-infections and three were type 2 and 3 co-infections, resulting in 73/101 (72%) cases in the database associated with type 2 poliovirus.

Survival Analysis

The length of excretion was calculated for all 101 iVDPV cases, of which 94 (93%) were prolonged excretors and 7 (7%) were chronic excretors. At the time of analysis, 48/101 (48%) individuals had died and 36/101 (36%) had stopped excreting. There were 8/101 (8%) individuals who were still alive and excreting at last specimen and 9/101 (9%) were lost to follow-up (**Table 1**).

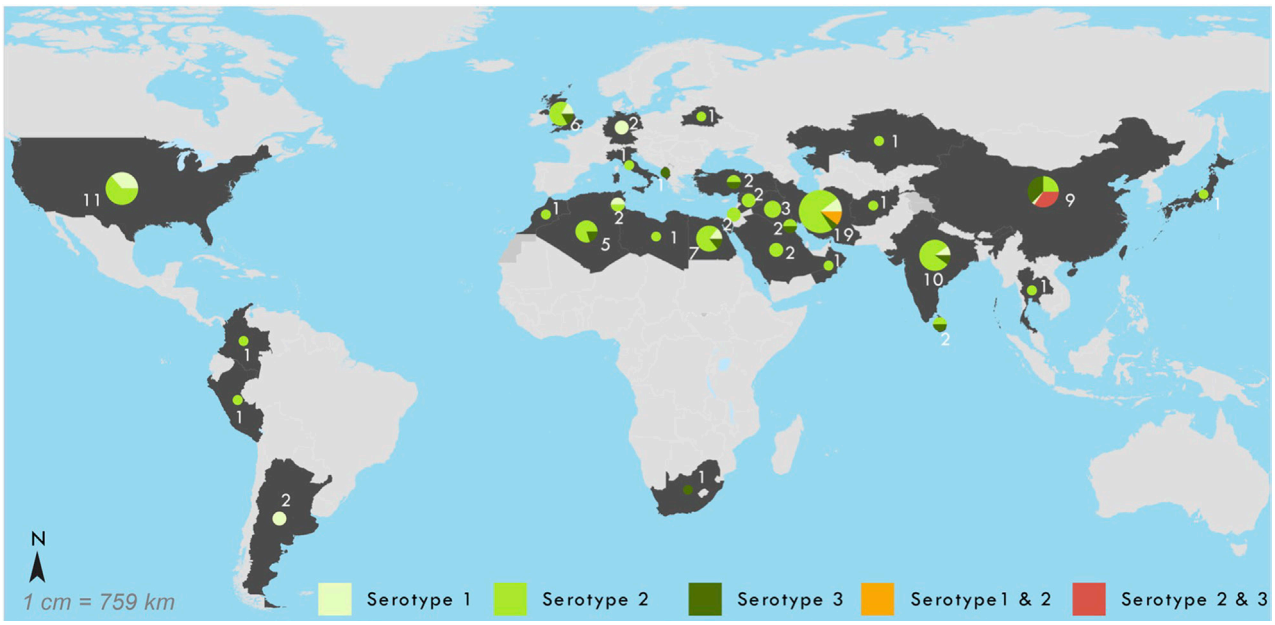

FIGURE 2 | Geographic location of 101 reported chronic and prolonged immunodeficiency-related vaccine-derived poliovirus cases, 1962–2016. Shown by serotype of virus in most recent specimen available: 1 ($n = 15$), 2 ($n = 68$), 3 ($n = 13$), 1 + 2 ($n = 2$), and 2 + 3 ($n = 3$).

The median length of excretion for all 101 cases was 1.3 years [95% confidence interval (CI): 1.0–1.4], with a range of 0.5–28.6 years. **Figure 3** shows the Kaplan–Meier survival curves for time to cessation of poliovirus excretion, stratified by PID and country income classification. After 2.4 and 3.7 years, 80 and 90% of individuals had stopped excreting, respectively (**Figure 3A**). There was a significant difference in the Kaplan–Meier curves for length of excretion between different PIDs ($P < 0.001$), with individuals with CVID having the longest length of excretion, a median of 3.0 years (95% CI: 1.6–6.7) (**Figure 3B**). There was also a highly significant difference between different income classification of countries ($P < 0.001$), with a longer median length of excretion for high-income countries (2.5 years, 95% CI: 1.3–3.7) than upper-middle income countries (1.0 year, 95% CI: 0.9–1.3), lower-middle income countries (1.0 years, 95% CI: 0.8–1.5), and low-income countries (0.8 years) (**Figure 3C**). No statistical significance was found in the Kaplan–Meier curves for sex ($P = 0.68$) or poliovirus serotype ($P = 0.21$) (results not shown).

Evolution of Length of Excretion

To understand the risk of iVDPV2 to the communities, we stratified the length of excretion by the date of onset (**Table 2**). Only those cases that were known to have stopped excreting (outcome alive and stopped excreting or dead) were included in this analysis to ensure comparability of the length of excretion between the two time periods 1962–2010 and 2011–2016. There was a decrease in the median length of excretion from cases with onset in 1962–2010 to 2011–2016: 1.75 years (95% CI: 1.3–2.5) and 1.0 years (95% CI: 0.8–1.4), respectively. The difference in length of excretion for the two time periods was found to be highly statistically significant when all serotypes ($P < 0.001$) were considered and specifically for type 2 ($P < 0.001$).

DISCUSSION

Our analyses document the changing epidemiology of iVDPV cases since the turn of the millenium, when the incidence of reported cases dramatically increased. The cases reside primarily from middle-income countries and are concentrated in the Middle East and North Africa. We confirm that the preponderance of cases are excreting poliovirus type 2, and demonstrate that the length of virus excretion has decreased in recent years. We also estimated that the last reported chronic excretor (greater than 5 years of excretion) had onset in 1998 and, finally, identified CVID as the major risk factor for chronic excretion.

The documented increase in number of reported iVDPV cases since the year 2000 was consistent with previous reports (17) and could be attributed to better case ascertainment, improved health care allowing immunodeficient patients to survive longer, or a combination of these factors. The surveillance sensitivity for AFP has risen in parallel with case incidence since 2000; however, this system is designed to capture cases with paralytic manifestations only (28), including those with PID. During the same period, capabilities of PID diagnostics have been enhanced and may now be available in many low- and middle-income countries. Therefore, it is possible that the increase in iVDPV cases is a surveillance artifact.

The shift in iVDPV cases from high-income to middle-income countries likely reflects two different trends: (1) high-income countries have changed their immunization schedules and switched to exclusive inactivated poliovirus vaccine use (thus

TABLE 1 | Baseline characteristics of 101 reported chronic and prolonged immunodeficiency-related vaccine-derived poliovirus cases at time of detection, 1962–2016.

Characteristic	Number of cases, *n/N* (%)	Prolonged excretor cases[a], *n/N* (%)	Chronic excretor cases[b], *n/N* (%)
Total	101	94	7
Gender			
Male	63/98 (64)	59/91 (65)	4/7 (57)
Female	35/98 (36)	32/91 (35)	3/7 (43)
Age at onset or first positive specimen			
<1 year	46/100 (46)	46/93 (49)	0/7 (0)
1 to <5 years	37/100 (37)	37/93 (40)	0/7 (0)
5 to <10 years	7/100 (7)	6/93 (6)	1/7 (14)
10 to <20 years	5/100 (5)	2/93 (2)	3/7 (43)
20 to <30 years	3/100 (3)	1/93 (1)	2/7 (29)
≥30 years	2/100 (2)	1/93 (1)	1/7 (14)
Paralysis			
Yes	71/100 (71)	67/93 (72)	4/7 (57)
No	29/100 (29)	26/93 (28)	3/7 (43)
Country of residence income classification[c]			
High income	29/101 (29)	22/94 (23)	7/7 (100)
Upper-middle income	47/101 (47)	47/94 (50)	0/7 (0)
Lower-middle income	24/101 (24)	24/94 (26)	0/7 (0)
Low income	1/101 (1)	1/94 (1)	0/7 (0)
WHO region of residence			
Eastern Mediterranean Region	43/101 (43)	43/94 (46)	0/7 (0)
African Region	6/101 (6)	6/94(6)	0/7 (0)
Western Pacific Region	10/101 (10)	7/94 (7)	3/7 (43)
European Region	14/101 (14)	10/94 (11)	4/7 (57)
South-East Asian Region	13/101 (13)	13/94 (14)	0/7 (0)
Region of the Americas	15/101 (15)	15/94 (16)	0/7 (0)
Immunodeficiency disorder			
SCID and other combined humoral/T-cell deficiencies	27/92 (29)	27/85 (32)	0/7 (0)
SCID	25/92 (27)	25/85 (29)	0/7 (0)
CVID	21/92 (23)	14/85 (16)	7/7 (100)
Antibody disorders	31/92 (33.7)	31/85 (36)	0/7 (0)
HGG	10/92 (11)	10/85 (12)	0/7 (0)
XLA	12/92 (13)	12/85 (14)	0/7 (0)
AGG	5/92 (5)	5/85 (6)	0/7 (0)
Other disorders	13/92 (14)	13/85 (15)	0/7 (0)
MHC II	6/92 (7)	6/85 (7)	0/7 (0)
HLA-DR	4/92 (4)	4/85 (5)	0/7 (0)
Serotype[d]			
1	15/101 (15)	12/94 (13)	3/7 (43)
2	68/101 (67)	64/94 (68)	4/7 (57)
3	13/101 (13)	13/94 (14)	0/7 (0)
1 + 2	2/101 (2)	2/94 (2)	0/7 (0)
2 + 3	3/101 (3)	3/94 (3)	0/7 (0)
All type 2 associated	73/101 (72)	69/94 (73)	4/7 (57)
Outcome status			
Dead	48/101 (48)	45/94 (48)	3/7 (43)
Stopped excreting	36/101 (36)	34/94 (36)	2/7 (29)
Alive (and excreting at last specimen)	8/101 (8)	7/94 (7)	1/7 (14)[e]
Unknown	9/101 (9)	8/94 (9)	1/7 (14)

AGG, agammaglobulinemia; CVID, common variable immunodeficiency disorder; HGG, hypogammaglobulinemia; HLA-DR, human leukocyte antigen-antigen D related; MHC II; major histocompatibility complex type II; SCID, severe combined immunodeficiency disorder; WHO, World Health Organization; XLA, X-linked agammaglobulinemia.

[a]*Defined as excreting virus for ≥6 months and ≤5 years.*

[b]*Defined as excreting for >5 years.*

[c]*Based on 2016 World Bank Classification.*

[d]*Serotype of virus in most recent specimen available.*

[e]*This case has been published (27).*

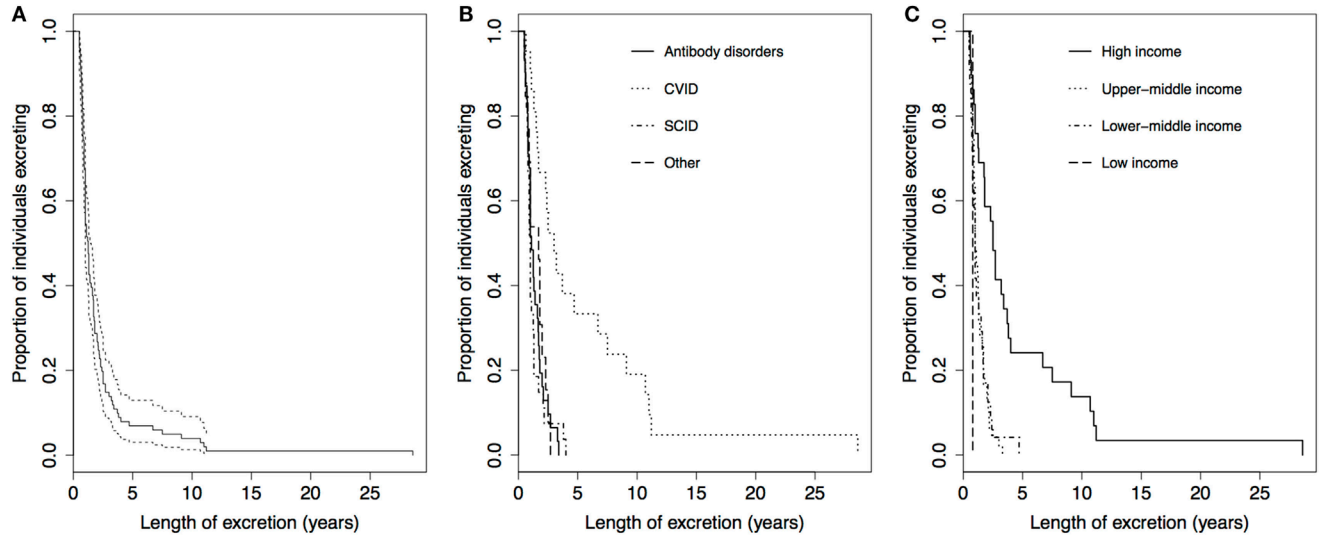

FIGURE 3 | Kaplan–Meier curves for the length of poliovirus excretion in reported chronic and prolonged immunodeficiency-related vaccine-derived poliovirus cases, 1962–2016. **(A)** For all reported cases ($n = 101$, dotted lines: 95% Confidence Limits). **(B)** Comparison by income classification of country of residence: low income ($n = 1$), lower-middle income ($n = 24$), upper-middle income ($n = 47$), and high-income ($n = 29$). Two-tailed $P < 0.001$ for log-rank test for equality of Kaplan–Meier curves. **(C)** Comparison by primary immunodeficiency disorder: antibody disorders ($n = 31$), CVID ($n = 21$), SCID and other combined humoral T-cell deficiencies ($n = 27$), and other disorders ($n = 13$). Two-tailed $P < 0.001$ for log-rank test for equality of Kaplan–Meier curves. Abbreviation: CVID, common variable immunodeficiency disorder; SCID, severe combined immunodeficiency disorder.

TABLE 2 | Kaplan–Meier Estimates of the length of excretion for 84 prolonged and chronic immunodeficiency-related vaccine-derived poliovirus cases with outcome dead or stopped excreting, stratified by date of onset, 1962–2016.

Serotype[a]	Total number of cases	Date of onset 1962–2010			Date of onset 2011–2016			*P*-value[c]
		Number of cases	Median length of excretion, years	95% CI	Number of cases	Median length of excretion, years	95% CI	
1	13	9	3.20[b]		4	0.96[b]		0.002
2	54	27	1.50	1.1, 2.3	27	0.82	0.8, 1.0	<0.001
3	12	3	1.25[b]		9	1.30[b]		0.24
All	84	41	1.75	1.3, 2.5	43	1.00	0.8, 1.4	<0.001

CI, confidence interval.

[a]*Serotype of most recent specimen available.*

[b]*No upper CI.*

[c]*P-value for log-rank test for difference in Kaplan–Meier survival curves; tests of significance were two-sided.*

preventing new iVDPVs from being generated); (2) middle-income countries have established increasingly sensitive surveillance for AFP, which better captures PIDs with paralysis; and (3) health systems in middle-income countries provide better medical care for PIDs, allowing these cases to be diagnosed and treated. However, this shift in case distribution causes new challenges to polio eradication.

The striking concentration of iVDPV cases in the Middle East and North Africa may be due to: (1) an increased risk of PID due to co-sanguinity (29) and (2) an interest of immunologists in these Regions to strengthen surveillance for PID and create immunodeficiency registries. In particular, iVDPV surveillance projects have taken place in Egypt (30), Iran (31), and Tunisia (20).

Our analysis is consistent with previous studies documenting that iVDPV type 2 constitutes more than 70% of all iVDPV cases (17), including a case that has excreted poliovirus type 2 for almost 30 years (27). Since the removal of Sabin type 2 from OPV was implemented in April 2016, no further Sabin type 2 are being introduced into populations, except for outbreak control (13, 32), which should prevent the generation of almost all new iVDPV type 2 cases.

On average, the duration of excretion of poliovirus among iVDPV cases is relatively short (approximately 1 year) and has further declined in recent years. This declining length of poliovirus excretion for all serotypes, but especially type 2, is likely to be associated with the shift in cases from high to middle income countries. This may be caused by shorter survival of iVDPV cases in middle-income countries or a higher likelihood of spontaneous cessation of excretion. However, we have little evidence to support the latter assumption.

We also document that chronic excretors are all associated with CVID in our case series. This is not surprising since CVID consists of a variety of underlying pathologies associated with

later diagnosis and longer survival than some of the other PIDs diagnoses (15, 33). What is more surprising is that all chronic excretors originated from high-income countries, and that no new chronic cases have been detected since 2009. Whether this is a reflection of the quality of the health systems in developing countries to both detect and care for PID patients remains an open question.

In our case series, only eight iVDPV cases were documented to be alive and excreting. The rest either had a fatal outcome or spontaneously stopped excretion. An additional nine cases were lost to follow-up or had no information on excretion. However, if we apply the observed mortality to these cases, very few would be expected to be alive and excreting. Therefore, the known prevalence of actively excreting iVDPV cases (i.e., those captured by current surveillance systems) is small.

In terms of limitations, our analyses could only focus on those iVDPV cases reflected in the WHO registry. Our study does not address the unknown number of PIDs who excrete poliovirus but have not been captured by the AFP surveillance system because they are not paralyzed and did not come to the attention of immunologists because of either a lack of suspicion or specialist immunological services are not available. This reflects the broader challenge of tracking iVDPV cases and limitations of registries in the absence of routine screening of PID patients for poliovirus infection and excretion. Furthermore, the information available in the registry is limited by the quality of reported data. In the calculation for length of excretion, cases that were still excreting or lost to follow-up were censored at the time of last positive sample and may continue to excrete for longer time periods. Furthermore, the analysis assumed a standard evolution rate of 1.1% mutations in VP1 region of the poliovirus genome per year.

Our results have important implications for the polio endgame. The withdrawal of Sabin type 2 poliovirus in April 2016 went well (32), and future immunity to type 2 poliovirus will be induced solely by inactivated poliovirus vaccine. Our duration of excretion analysis suggests that the highest risk period for iVDPV cases re-seeding communities is in the next 2–3 years.

However, there are ways in which the Global Polio Eradication Initiative can actively decrease the risk of iVDPV cases. Generating effective therapeutic options for clearing poliovirus infections is critical. Progress has been made in this field over the last decade or more, and antiviral drugs are nearing practical applicability (34). The first drug, a capsid inhibitor, is now ready for deployment under an Investigational New Drug protocol (35). Because of high levels of drug resistance, a second drug is needed for a combination treatment (35) and related studies with a protease inhibitor are in progress (36). These drugs, as well as specific high-titer monoclonal antibodies, should provide effective treatment for iVDPV excretors. In this context, identifying PIDs with poliovirus to enable treatment with these new drugs becomes an urgent priority, not only for public health but also because these patients are at risk of developing paralytic poliomyelitis, which has a high mortality burden.

In parallel, we need to establish sensitive surveillance systems to identify and report iVDPV cases with or without paralytic symptoms, which constitute the last remaining sources of poliovirus type 2. Recent modeling by Tebbens et al. has shown that treating iVDPVs with antiviral drugs alone will have limited impact and requires integration with expanded surveillance (15). This surveillance system would be complementary to the AFP surveillance system that captures children with paralytic manifestations. The new system would also target children with PIDs (without paralytic manifestations) and would use the 10 Warning Signs of the Jeffrey Modell Foundation as a screening case definition (37). Children meeting at least two signs would then be included in the AFP surveillance system and followed up accordingly (with two stool samples collected and related laboratory investigations). The Strategic Advisory Group of Experts on Immunization Polio Working Group has endorsed this approach, and pilot country studies are being established (38). Furthermore, analysis suggests that extended surveillance could save between US$0.7 to 1.5 billion and identify 25–90% of asymptomatic iVDPV excretors (16).

This dual approach of developing therapeutic options to clear poliovirus infection and establishing sensitive surveillance for PIDs should further quantify the risks and would allow the Global Polio Eradication Initiative to actively mitigate these risks, so that global polio eradication cannot be undone by few iVDPV cases that may inadvertently re-seed communities with poliovirus, potentially leading to re-establishment of endemic or epidemic transmission. With these enhancements to the surveillance and treatment system for PIDs, we can make the final steps to a world free of polio.

AUTHOR CONTRIBUTIONS

Participated in research design: GM, YL, MT, OM, OK, and RS. Collected data: GM, YL, MT, KD, OM, and RS. Performed data analysis: GM, MT, YL, and SG. Wrote or contributed to drafting of early manuscripts: GM, YL, MT, KD, and RS. Critically revised manuscript: GM, YL, MT, KD, SG, OM, OK, and RS.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at http://journal.frontiersin.org/article/10.3389/fimmu.2017.01103/full#supplementary-material.

REFERENCES

1. World Health Organisation. *Acute Flaccid Paralysis (AFP) Surveillance Data and Polio Case Count*. Geneva: World Health Organisation (2017). Available from: https://extranet.who.int/polis/public/CaseCount.aspx
2. Centers for Disease Control and Prevention. Apparent global interruption of wild poliovirus type 2 transmission. *MMWR Morb Mortal Wkly Rep* (2001) 50(12):222–4.
3. Kew OM, Cochi SL, Jafari HS, Wassilak SG, Mast EE, Diop OM, et al. Possible eradication of wild poliovirus type 3 – worldwide, 2012. *MMWR Morb Mortal Wkly Rep* (2014) 63(45):1031–3.
4. Kew OM, Sutter RW, de Gourville EM, Dowdle WR, Pallansch MA. Vaccine-derived polioviruses and the endgame strategy for global polio eradication. *Annu Rev Microbiol* (2005) 59:587–635. doi:10.1146/annurev.micro.58.030603.123625
5. Sutter RW, Kew OM, Cochi SL, Aylward RB. Poliovirus vaccine – live. 6th ed. In: Plotkin SA, Orenstein WA, Offit PA, editors. *Vaccines*. Philadelphia, PA: Saunders Elsevier (2013). p. 599–645.
6. Kew OM, Wright PF, Agol VI, Delpeyroux F, Shimizu H, Nathanson N, et al. Circulating vaccine-derived polioviruses: current state of knowledge. *Bull World Health Organ* (2004) 82(1):16–23.
7. Alexander JP Jr, Gary HE Jr, Pallansch MA. Duration of poliovirus excretion and its implications for acute flaccid paralysis surveillance: a review of the literature. *J Infect Dis* (1997) 175(Suppl 1):S176–82. doi:10.1093/infdis/175.Supplement_1.S176
8. Martin J. Vaccine-derived poliovirus from long term excretors and the end game of polio eradication. *Biologicals* (2006) 34(2):117–22. doi:10.1016/j.biologicals.2006.02.005
9. Kew OM, Sutter RW, Nottay BK, McDonough MJ, Prevots DR, Quick L, et al. Prolonged replication of a type 1 vaccine-derived poliovirus in an immunodeficient patient. *J Clin Microbiol* (1998) 36(10):2893–9.
10. Martín J, Dunn G, Hull R, Patel V, Minor PD. Evolution of the Sabin strain of type 3 poliovirus in an immunodeficient patient during the entire 637-day period of virus excretion. *J Virol* (2000) 74(7):3001–10. doi:10.1128/JVI.74.7.3001-3010.2000
11. Bellmunt A, May G, Zell R, Pring-Akerblom P, Verhagen W, Heim A. Evolution of poliovirus type I during 5.5 years of prolonged enteral replication in an immunodeficient patient. *Virology* (1999) 265(2):178–84. doi:10.1006/viro.1999.0003
12. Buttinelli G, Donati V, Fiore S, Marturano J, Plebani A, Balestri P, et al. Nucleotide variation in Sabin type 2 poliovirus from an immunodeficient patient with poliomyelitis. *J Gen Virol* (2003) 84(Pt 5):1215–21. doi:10.1099/vir.0.18974-0
13. Global Polio Eradication Initiative. *Polio Eradication & Endgame Strategic Plan 2013-2018*. Geneva: World Health Organisation (2013). (WHO/POLIO/13.02).
14. Tebbens RJ, Pallansch MA, Kew OM, Cáceres VM, Jafari H, Cochi SL, et al. Risks of paralytic disease due to wild or vaccine-derived poliovirus after eradication. *Risk Anal* (2006) 26(6):1471–505. doi:10.1111/j.1539-6924.2006.00827.x
15. Duintjer Tebbens RJ, Pallansch MA, Thompson KM. Modeling the prevalence of immunodeficiency-associated long-term vaccine-derived poliovirus excretors and the potential benefits of antiviral drugs. *BMC Infect Dis* (2015) 15:379. doi:10.1186/s12879-015-1115-5
16. Duintjer Tebbens RJ, Thompson KM. Comprehensive screening for immunodeficiency-associated vaccine-derived poliovirus: an essential oral poliovirus vaccine cessation risk management strategy. *Epidemiol Infect* (2017) 145(2):217–26. doi:10.1017/S0950268816002302
17. Guo J, Bolivar-Wagers S, Srinivas N, Holubar M, Maldonado Y. Immunodeficiency-related vaccine-derived poliovirus (iVDPV) cases: a systematic review and implications for polio eradication. *Vaccine* (2015) 33(10):1235–42. doi:10.1016/j.vaccine.2015.01.018
18. Li L, Ivanova O, Driss N, Tiongco-Recto M, da Silva R, Shahmahmoodi S, et al. Poliovirus excretion among persons with primary immune deficiency disorders: summary of a seven-country study series. *J Infect Dis* (2014) 210(Suppl 1):S368–72. doi:10.1093/infdis/jiu065
19. de Silva R, Gunasena S, Ratnayake D, Wickremesinghe GD, Kumarasiri CD, Pushpakumara BA, et al. Prevalence of prolonged and chronic poliovirus excretion among persons with primary immune deficiency disorders in Sri Lanka. *Vaccine* (2012) 30(52):7561–5. doi:10.1016/j.vaccine.2012.10.035
20. Driss N, Ben-Mustapha I, Mellouli F, Ben Yahia A, Touzi H, Bejaoui M, et al. High susceptibility for enterovirus infection and virus excretion features in Tunisian patients with primary immunodeficiencies. *Clin Vaccine Immunol* (2012) 19(10):1684–9. doi:10.1128/CVI.00293-12
21. World Health Organisation. *Polio Laboratory Manual*. 4th ed. Geneva: World Health Organisation (2004). (WHO/IVB/04.10).
22. Jorba J, Campagnoli R, De L, Kew O. Calibration of multiple poliovirus molecular clocks covering an extended evolutionary range. *J Virol* (2008) 82(9):4429–40. doi:10.1128/JVI.02354-07
23. World Health Organization. Update on vaccine-derived polioviruses detected worldwide, April 2011–June 2012. *Wkly Epidemiol Rec* (2012) 87(38):358–68.
24. The World Bank. *Country and Lending Groups*. (2016). Available from: http://data.worldbank.org/about/country-and-lending-groups
25. Core Team R. *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing (2016).
26. Stata Corporation. *Stata Statistical Software: Release 13*. College Station, TX: Stata Corporation (2013).
27. Dunn G, Klapsa D, Wilton T, Stone L, Minor PD, Martin J. Twenty-eight years of poliovirus replication in an immunodeficient individual: impact on the global polio eradication initiative. *PLoS Pathog* (2015) 11(8):e1005114. doi:10.1371/journal.ppat.1005114
28. World Health Organisation. *WHO–Recommended Standards for Surveillance of Selected Vaccine-Preventable Diseases*. Geneva: World Health Organisation (2003). (WHO/V&B/03.01).
29. Rezaei N, Pourpak Z, Aghamohammadi A, Farhoudi A, Movahedi M, Gharagozlou M, et al. Consanguinity in primary immunodeficiency disorders; the report from Iranian Primary Immunodeficiency Registry. *Am J Reprod Immunol* (2006) 56(2):145–51. doi:10.1111/j.1600-0897.2006.00409.x
30. El-Sayed ZA, Mach O, Hossny EM, Galal NM, El-Sawy I, Elmarsafy A, et al. Poliovirus excretion among persons with primary immune deficiency disorders: summary of data from enhanced poliovirus surveillance in Egypt, 2011–2014. *J Vaccines Vaccin* (2016) 7(4):1000331. doi:10.4172/2157-7560.1000331
31. Shaghaghi M, Shahmahmoodi S, Abolhassani H, Soleyman-Jahi S, Parvaneh L, Mahmoudi S, et al. Vaccine-derived polioviruses and children with primary immunodeficiency, Iran, 1995-2014. *Emerg Infect Dis* (2016) 22(10):1712–9. doi:10.3201/eid2210.151071
32. Hampton LM, Farrell M, Ramirez-Gonzalez A, Menning L, Shendale S, Lewis I, et al. Cessation of trivalent oral poliovirus vaccine and introduction of inactivated poliovirus vaccine – worldwide, 2016. *MMWR Morb Mortal Wkly Rep* (2016) 65(35):934–8. doi:10.15585/mmwr.mm6535a3
33. Quinti I, Soresina A, Spadaro G, Martino S, Donnanno S, Agostini C, et al. Long-term follow-up and outcome of a large cohort of patients with common variable immunodeficiency. *J Clin Immunol* (2007) 27(3):308–16. doi:10.1007/s10875-007-9075-1
34. Couzin J. Infectious diseases. Report concludes polio drugs are needed – after disease is eradicated. *Science* (2006) 311(5767):1539. doi:10.1126/science.311.5767.1539a
35. Collett MS, Hincks JR, Benschop K, Duizer E, van der Avoort H, Rhoden E, et al. Antiviral activity of pocapavir in a randomized, blinded, placebo-controlled human oral poliovirus vaccine challenge model. *J Infect Dis* (2017) 215(3):335–43.

36. McKinlay MA, Collett MS, Hincks JR, Oberste MS, Pallansch MA, Okayasu H, et al. Progress in the development of poliovirus antiviral agents and their essential role in reducing risks that threaten eradication. *J Infect Dis* (2014) 210(Suppl 1):S447–53. doi:10.1093/infdis/jiu043
37. Jeffrey Modell Foundation. *10 Warning Signs of Primary Immunodeficiency*. (2017). Available from: http://www.info4pi.org/library/educational-materials/10-warning-signs
38. World Health Organisation. *12th Meeting of the SAGE Polio Working Group*. Geneva: World Health Organisation (2016). Available from: http://www.who.int/immunization/sage/meetings/2016/october/1_12th_meeting_of_the_SAGE_polio_WG_Note_for_the_Record_final_August_2016.pdf?ua=1

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First Year of Israeli Newborn Screening for Severe Combined Immunodeficiency—Clinical Achievements and Insights

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Severe combined immunodeficiency (SCID), the most severe form of T cell immunodeficiency, is detectable through quantification of T cell receptor excision circles (TRECs) in dried blood spots obtained at birth. Herein, we describe the results of the first year of the Israeli SCID newborn screening (NBS) program. This important, life-saving screening test is available at no cost for every newborn in Israel. Eight SCID patients were diagnosed through the NBS program in its first year, revealing an incidence of 1:22,500 births in the Israeli population. Consanguine marriages and Muslim ethnic origin were found to be a risk factor in affected newborns, and a founder effect was detected for both *IL7Rα* and *DCLRE1C* deficiency SCID. Lymphocyte subset analysis and TREC quantification in the peripheral blood appear to be sufficient for confirmation of typical and leaky SCID and ruling out false positive (FP) results. Detection of secondary targets (infants with non-SCID lymphopenia) did not significantly affect the management or outcomes of these infants in our cohort. In the general, non-immunodeficient population, TREC rises along with gestational age and birth weight, and is significantly higher in females and the firstborn of twin pairs. Low TREC correlates with both gestational age and birth weight in extremely premature newborns. Additionally, the rate of TREC increase per week consistently accelerates with gestational age. Together, these findings mandate a lower cutoff or a more lenient screening algorithm for extremely premature infants, in order to reduce the high rate of FPs within this group. A significant surge in TREC values was observed between 28 and 30 weeks of gestation, where median TREC copy numbers rise by 50%

over 2 weeks. These findings suggest a maturational step in T cell development around week 29 gestation, and imply moderate to late preterms should be screened with the same cutoff as term infants. The SCID NBS program is still in its infancy, but is already bearing fruit in the early detection and improved outcomes of children with SCID in Israel and other countries.

Keywords: severe combined immunodeficiency, newborn screening, T cell development, preterm, immunodeficiency

INTRODUCTION

The purpose of newborn screening (NBS) is to identify newborns that are at risk for a panel of metabolic, endocrine, or hematologic diseases in which early diagnosis and prompt treatment will dramatically change disease outcome of affected patients (1). One such disease that has been recently added to NBS panels worldwide, is severe combined immunodeficiency (SCID), the most profound inherited immunodeficiency (2). SCID encompasses a heterogeneous group of genetic disorders manifest by increased susceptibility to life-threatening and opportunistic infections at early life and is characterized by arrest of T lymphocyte maturation and variable alterations in B and natural killer (NK) cells differentiation (3). It was shown that SCID infants diagnosed and treated with hematopoietic stem cell transplantation in the first 3.5 months of life had improved survival and reduced morbidity (4). This observation has made SCID a very attractive target for NBS (5). Screening for SCID is of value particularly in communities, such as in Israel, where a relatively high frequency of consanguineous marriages is known to exist (6). While the accepted frequency of SCID in most western countries is 1:58,000 births, it is estimated that in such communities the frequency of SCID will be higher (7–10). SCID can be detected early in life with the use of T cell receptor excision circle (TREC) quantification on dry blood spots obtained from a Guthrie card (11). TREC, a DNA marker that is formed as a byproduct during the normal process of T cell receptor (TCR) development, is a highly sensitive and specific tool to estimate T cell immunity in any medical conditions where T cells are known to be affected (12). For SCID detection, it can obviously be used in patients with no T cells but can also identify patients with clonally expended T cells or with a limited TCR repertoire (13). Normal production of TREC begins in early embryonic development, around 13 weeks of gestation, and rises progressively throughout pregnancy, reaching decent and easy to detect levels at birth (14). TREC quantification has been established as the most useful and inexpensive high-throughput assay to screen newborns for SCID (15, 16). This screening test is currently being used successfully across the US and in several additional countries (8, 17–19). The primary target of the screening is to identify SCID patients. As a secondary target, the screening identifies other causes of T cell immunodeficiencies including specific syndromes, severe prematurity, secondary T cell lymphopenia or undefined causes of T cell lymphopenia (20). A recent successful pilot study that was conducted by us was able to retrospectively identify seven Israeli patients with SCID (13). This paved the way toward adding NBS for SCID to the national NBS program by the Israeli Ministry of Health (21). As of October 1, 2015, this test is available at no cost for all newborns in Israel and the confirmatory process is performed in one center. Here, we summarize our experience with 1 year of NBS for SCID and report several perspectives regarding T cell development in non-immunodeficient newborns that emerged from the accumulated data.

MATERIALS AND METHODS

Population

Data comprised of TREC results and accompanying epidemiologic parameters (sex, gestational age, birth weight, singleton/twins/triplets) for newborns born in Israel between October 1, 2015 and September 30, 2016, drawn from the computer archives of the Israeli National Center for NBS. Data entries with missing information or containing apparent typing errors were removed from analysis, as were entries for samples with poor DNA amplification (beta-actin <16 copies). Additionally, gestational age/birth weight norms were calculated and newborns for which birth weight was above 5 SD or below −5 SD for their respective gestational age were removed from analysis ($n = 93$). Finally, analysis was performed for 177,277 newborns.

The primary target of the screening program for newborns was to identify those with SCID (defined by us as less than 300/μl $CD3^+$ T cells in peripheral blood) or leaky SCID (T lymphopenia but >300/μl $CD3^+$ T cells). The secondary target was to identify newborns with non-SCID lymphopenia (classified into groups as syndromic patients, lymphopenia due to secondary causes, prematurity, or unknown etiology). False positives (FPs) were defined as newborns with consecutive positive screening results, whose clinical presentation was unremarkable and immunological workup was negative for lymphopenia of any etiology.

The study was approved by both the Sheba Medical Center institutional review board as well as the Israeli national research committee. Informed consent was obtained from all individual participants included in the study where genetic evaluation was needed.

Specimen Testing

The Israeli SCID NBS program uses the commercial EnLiteTM Neonatal TREC kit (Wallac Oy, Mustionkatu 6, FI-20750 Turku, Finland). Briefly, Dried Blood Spot (DBS) punches of 1.5 mm diameter are inserted into a black, 96-well PCR plate. DNA is eluted without extraction. Next, PCR amplification of TREC and beta-actin, an internal control for each specimen, is performed.

Four PCR plates are processed in parallel, each plate containing a standard TREC curve in triplicates, as well as positive and negative controls for both targets.

Mutation Analyses

Severe combined immunodeficiency mutations were identified using either Whole Exome Sequencing (WES) or direct Sanger sequencing (where family history allowed targeted sequencing of suspected genes). Results were than validated through Sanger sequencing of patients' parents, healthy siblings, or both.

Lymphocyte Subset Determination

Cell surface markers of peripheral blood mononuclear cells (PBMCs) were determined by immunofluorescent staining and flow cytometry (Navios, Beckman Coulter, Brea, CA, USA) using anti-CD3, anti-CD19, anti-CD16, and anti-CD56 from BD Biosciences; and anti-CD4 and anti-CD8 from Beckman Coulter.

Proliferation Assay

T cell proliferation was tested by standard H^3-thymidine uptake assays (1 μCi/well) by culturing 10^5 PBMCs with phytohemagglutinin (1 μg/ml; Sigma-Aldrich) or plastic bound anti-CD3 (10 μg/ml UCHT-1 from BD) for 72 h.

TCR Repertoire Analysis

Surface expression of individual TCRVβ families was analyzed using flow cytometry and a set of Vβ-specific fluorochrome-labeled monoclonal antibodies as previously described (22). Normal control values were obtained from the IOTest Beta Mark-Quick Reference Card (Beckman Coulter).

Quantification of TREC in Peripheral Blood

T cell receptor excision circle copy numbers were determined using quantitative real-time PCR (qRQ-PCR). PCRs were performed as previously described (22) using as template 0.5-μg genomic DNA (gDNA) extracted from PBMCs. RQ-PCR was carried out using an ABI PRISM 7900 Sequence Detector System (Applied Biosystems). A standard curve was constructed by using serial dilutions containing 10^3–10^6 copies of a plasmid with known TREC copy numbers. Patient and control samples were tested in triplicate, and the number of TRECs in a given sample was calculated by comparing the obtained cycle threshold value of the sample to the standard curve using an absolute quantification algorithm.

Statistical Analyses

Statistical analyses were performed using SPSS software (IBM Corp. Released 2016. IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY, USA: IBM Corp.). Given the skewed distribution of the data, the Mann–Whitney *U* test and the Kruskal–Wallis test were used to compare continuous variables between groups. Correlation between continuous variables was assessed using the Spearman's rank correlation coefficient. All statistical tests were two-tailed. Differences were considered statistically significant when the p value was less than 0.05. Whenever applicable, results were presented as median, with 25th to 75th percentiles in brackets.

RESULTS

Screening Overview

A total of 188,162 newborns were screened between October 1, 2015 and September 30, 2016. After subtracting entries with missing data, errors, and poor DNA amplification, data of a total of 177,277 newborns were analyzed. Of these, 51.5% were male. Median TREC copy number/blood spot for all DBS samples was 107 (69–169, 25th to 75th percentiles), median gestational age 39 weeks, median birth weight 3,240 g (2,925–3,545). 12,880 (7.26%) infants were born prematurely (<37 weeks gestation). 11,316 (87.8% of all preterm newborn) were moderate to late preterm (per WHO definition, 32 to <37 weeks), 1,126 (8.7%) very preterm (28 to <32 weeks), and 438 (3.4%) extremely preterm (<28 weeks). 1,614 (0.9%) were born Very Low Birth Weight (VLBW; per WHO definition, weighing ≤1,500 g), 12,293 (6.9%) were born Low Birth Weight (LBW; >1,500 to ≤2,500 g).

T cell receptor excision circle cutoff for retesting was initially set at 36 copies per blood spot and was gradually lowered to 23 copies per blood spot by year's end. The Israeli SCID screening algorithm and the rate of positive results with different cutoffs are reviewed elsewhere (23). In 561 instances (0.3%), a second Guthrie card was requested following an initial positive result. Forty-six infants (0.02%) were referred to the national center for SCID screening confirmation following consecutive positive results on two separate Guthrie cards.

Primary Target

The primary target of the screening program was to identify infants with SCID or leaky SCID and to distinguish them from infants with FP screening results. During the first year of the screening program, 8 infants were diagnosed as SCID or leaky SCID (**Table 1**) and 11 infants received a diagnosis of FP. Consanguine marriage and Arab-Muslim origin were more frequent in the SCID patients (7 of 8) compared to the FP group (1 and 3, respectively). Of note, while consanguineous marriages are relatively high in Israel compared to other developed countries as a whole, the rate of consanguinity is particularly high among Arab-Muslims. Three patients belonged to the same extended family, though not immediately related. Two patients had positive family histories for SCID. The Israeli confirmation protocol consists of TREC measurement in peripheral blood, proliferation in response to mitogen stimuli, and flow cytometry analysis for total lymphocyte profile and the expression of TCRVβ repertoire. Per definition, all confirmatory tests and outcome measurements (growth and development, infections, hospitalization, and overall general appearance) were completely normal in newborns with FP results. All SCID patients had lymphopenia (**Table 2**). They could be classified as true SCID (5 patients) and leaky SCID (3 patients) based on the number of autologous $CD3^+$ T cells (more or less than 300 cells/μl). Four patients had SCID variants with normal B cell counts (B^+ SCID) and four had SCID variants with decreased B cell counts (B^- SCID). All had normal numbers of NK cells. T cell proliferation was relatively normal in three patients and reduced or absent in the rest (3 and 2, respectively). Similarly, assaying TCRVβ

TABLE 1 | Severe combined immunodeficiency patients clinical and genetic data.

Patient	Major infection	Diagnosis	Mutation	Outcome
P1	None	*DCLRE1C*[a]	c.1299_1306dup-AGGATGCT (homozygous)	A/W, Post-BMT
P2	None	*DCLRE1C*[a]	c.1299_1306dup-AGGATGCT (homozygous)	A/W, Post-BMT
P3	None	*DCLRE1C*[a]	c.1299_1306dup-AGGATGCT (homozygous)	A/W, Post-BMT
P4	None	*IL7Rα*[b]	c.120C > G; p. F40L (homozygous)	A/W, No-BMT
P5	None	*DCLRE1C*[c]	del. ex1-3 (homozygous)	A/W, Post-BMT
P6	Yes	*IL7Rα*[b]	c.120C > G; p. F40L (homozygous)	A/W, Post-BMT
P7	None	Complete DGS	Unknown	Deceased
P8	None	RMRP[d]	ins.17bp TIS-4 TCTGTGAAGCTGAGGAC TIS + 239 C > T	A/W, Post-BMT

DGS, DiGeorge syndrome; A/W, alive and well; BMT, bone marrow transplant; RMRP, RNA component of mitochondrial RNA processing endoribonuclease, causative gene for Cartilage-Hair Hypoplasia; TIS, transcription initiation site.

[a]*Accession no. NM_001033855.*

[b]*Accession no. Chromosome 10, NC_000010.*

[c]*Accession no. NM_002185.*

[d]*Accession no. NG_017041.*

TABLE 2 | Severe combined immunodeficiency patients confirmatory tests results.

Patient	DBS TREC[a]	PB TREC[a]	Total Lymphocytes[b]	$CD3^{+}$[b]	$CD4^{+}$[b]	Proliferation[c] (%)	TCR repertoire
P1	0	74	1,695	1,135	728	100	Normal
P2	0	0	393	35	35	0	Skewed
P3	0	105	1,392	807	626	50	N/D
P4	0	57	2,568	360	257	70	Normal
P5	0	0	1,162	0	70	0	N/D
P6	0	9	924	101	64	30	Normal
P7	0	0	2,000	4	0	0	N/D
P8	0	50	886	88	35	25	Skewed

[a]*Copy numbers per 3 mm blood spot for DBS, per 0.5 µg DNA for peripheral blood.*

[b]*Cells/µl.*

[c]*Proliferation assay results are displayed as percent of healthy age matched control's result.*

DBS, dried blood spot; TREC, T cell receptor excision circles; PB, peripheral blood; TCR, T cell receptor; N/D, not done.

repertoire did not prove sufficient for diagnosis as three of the SCID patients had only mildly abnormal results. The remaining patients had either a skewed repertoire (two patients) or the test could not be performed due to absence of T cells (three patients). TREC in DBS, obtained during the NBS, was undetectable in all SCID patients and in 7/11 infants in the FP group. The remaining four FP had an average of 10.4 ± 8.6 copies per blood spot. TREC quantification in peripheral blood was significantly reduced in all SCID patients compared to the FP group (36.8 ± 40.4 vs $1{,}984.6 \pm 1{,}944.5$, normal >400 copies per 0.5 µl DNA). Test sensitivity and specificity were calculated for each confirmation test with regards to its ability to distinguish between SCID patients and all other newborns referred to confirmation testing (FP and secondary targets). TREC in peripheral blood, total lymphocytes, and $CD3^{+}$ are more sensitive and less specific than $CD4^{+}$, TCRVβ, or proliferation assay (**Table 3**).

For seven of the eight SCID patients, a genetic etiology was revealed (**Table 1**) suggesting a founder genetic effect for both *IL7rα* and *DCLRE1C* (encoding ARTEMIS) SCID. Six of the seven identified mutations were homozygous, whereas P8 was found to harbor compound heterozygote mutations (**Table 1**). For the eighth patient, though phenotypically consistent with DiGeorge syndrome (typical facies, cardiac defect, hypocalcemia, and severe lymphopenia), a genetic etiology was not identified even with the use of chromosomal microarray (CMA) and WES. Seven of the eight SCID patients detected through the screening program are currently alive, six have already undergone successful bone marrow transplantation (BMT). One patient succumbed to cardiac complications associated with her syndrome. One patient despite having genetically verified SCID, has displayed a spontaneous recovery of her immune system due to yet unknown reasons. No typical SCID patients "missed" by the screening have been reported in Israel since the initiation of the screening program. All infants defined as FPs are currently alive and well, not requiring special medical attention over a follow-up period of at least 1 year.

TABLE 3 | Statistical measures of confirmatory tests.

Test	Sensitivity (confidence interval)	Specificity (confidence interval)
Total lymphocytes	87.5% (52.9–97.8)	75% (56.6–87.3)
$CD3^{+}$	87.5% (52.9–97.8)	82.1% (64.4–92.1)
$CD3^{+}CD4^{+}$	75% (40.9–92.9)	89.3% (72.8–96.3)
Proliferation	62.5% (30.6–86.3)	95.2% (77.3–99.2)
TCR repertoire	62.5% (30.6–86.3)	93.1% (78–98.1)
PB TREC	100% (67.6–100)	72.4% (54.3–85.3)

PB, peripheral blood; TREC, T cell receptor excision circles; TCR, T cell receptor.

Secondary Targets

Clinical assessment and results of confirmation tests identified 27 newborns that were considered to be part of the secondary target of the program. These could be categorized into four groups. Nine newborns were diagnosed with congenital syndromes with variable degrees of T-cell impairment (four with Down syndrome, two with partial DiGeorge syndrome, one with multiple congenital anomalies, two with unknown syndromes). Complete confirmation tests were performed in only five of these syndromic patients. In nine newborns, the abnormal results were attributed to extreme prematurity with slow recovery of the immune system. Complete confirmation tests were available in only five of them. Four patients were diagnosed with secondary T cell immunodeficiency (three cases of chylothorax and one case of an infant born to a mother who was treated with immunosuppressive agents during pregnancy). Finally, five newborns, for which some of confirmation tests were abnormal (thus excluding them as FP) could not be classified. By 1 year, all of these children had normal repeat workup. They required no medical intervention.

Correlation between Gestational Age and TREC

T cell receptor excision circle copy numbers rose consistently and significantly along with gestational age in our healthy newborn cohort (Figure S1 in Supplementary Material). There was great variability in TREC results within each birth week, resulting in a mild positive correlation (0.256, $p < 0.001$) between gestational age and TREC only in extreme preterms, and no correlation between age and TREC for all other age groups. When looking at median TREC for age groups, due to the large sample size, there was a significant difference in median TREC values between each preterm group and term infants (**Figure 1A**). However, while the difference between term and moderate to late preterm TREC was significant but not meaningful, 108 (70–170) vs 101 (64–164) median TREC ($p = 0.0017$), the differences between term and extremely preterm, 49 (29–92), or very preterm, 88 (52–149), were both highly significant ($p = 1E^{-16}$, $p = 4E^{-6}$, respectively) and meaningful. When looking at each birth week separately, one can observe a steady increase in median TREC from week to week between 23 and 28 weeks gestation, followed by a surge in TREC values between 28 and 30 weeks gestation, when median TREC rises by 32 (a 150% increase), another period of incremental increases from week 30 on until reaching a plateau around gestational week 38 (**Figure 1B**).

Correlation between Birth Weight and TREC

T cell receptor excision circle copy numbers rose consistently and significantly along with birth weight (**Figure 2**). Newborns with VLBW had a median TREC of 73.6 (41.8–131), compared to 98.6 (62.9–160) in newborns with LBW and 108.6 (70.2–170) in newborns with NBW. All differences were statistically significant (VLBW vs LBW $p = 1.7E^{-8}$, VLBW vs NBW $p = 5.2E^{-19}$, LBW vs NBW $p = 9.8E^{-13}$). The correlation between birth weight and TREC was more pronounced for extremely premature newborns (0.309) than for newborns of other age groups (0.147 for very premature newborns and no correlation for late preterm and term infants). All correlations were statistically significant, $p < 0.001$.

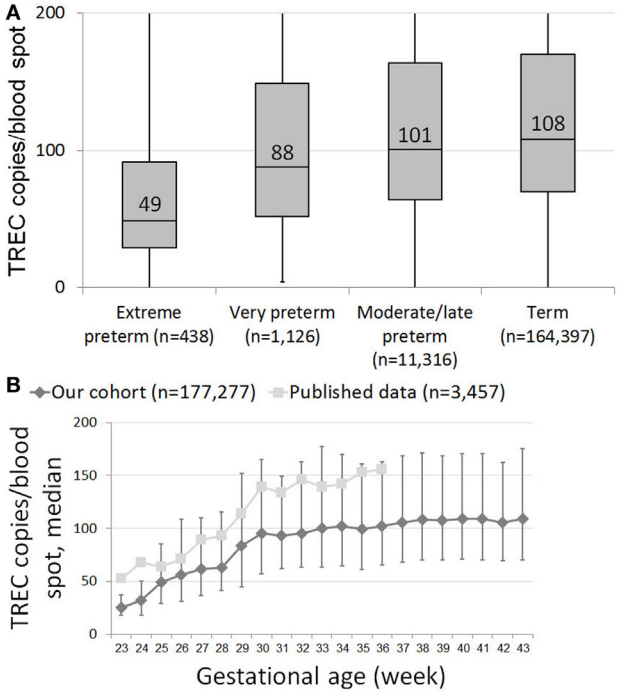

FIGURE 1 | **(A)** Box and whiskers plot showing T cell receptor excision circle (TREC) levels for each gestational age group. Extreme preterms = below 28 GAW (gestational age weeks, $n = 438$); very preterm = 29–32 GAW ($n = 1,126$); moderate/late preterm = 33–36 GAW (11,316); term = 37–45 GAW ($n = 164,397$). **(B)** Median TREC copy numbers/blood spot for each gestational week. Results from our cohort are marked as diamonds, results from Barbaro et al. are marked as squares (18). Error bars indicate 25th and 75th percentile TREC copy numbers for each gestational week.

Correlation between Sex and TREC

Median TREC for female neonates was 115.2 (74.6–179.7), compared to 100.8 (65.4–158.6) for male neonates. The difference was extremely significant ($p = 7E^{-202}$). The groups were similar in terms of gestational age (39.0, 38.9, respectively) and birth weight (3,147 g, 3,268 g, respectively). Correlations between gestational age and TREC, as reported above, were maintained when stratified into female (Figure S2 in Supplementary Material) and male (Figure S3 in Supplementary Material) infants. Correlations between birth weight and TREC, similarly, were maintained when sectioning the data into female (Figure S4 in Supplementary Material) and male (Figure S5 in Supplementary Material) infants.

TREC Recovery Rate in Preterm Infants

Per the Israeli SCID screening algorithm, repeat DBS were collected and tested for TREC for preterm infants who remain hospitalized for an extended time period, regardless of their initial TREC result. We examined the rate of TREC increase per week for 4,212 preterm infants, for whom TREC results from

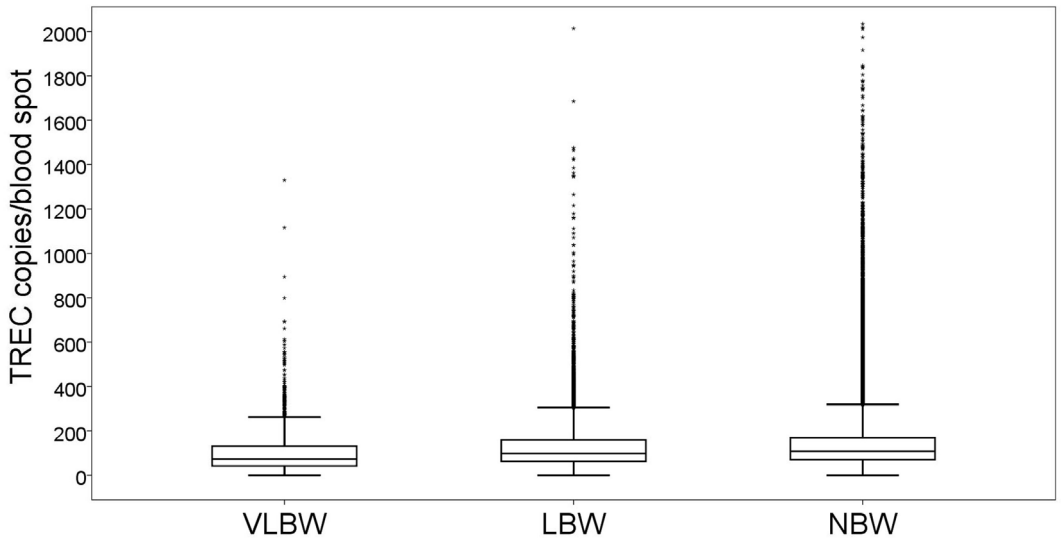

FIGURE 2 | Box and whiskers plot showing T cell receptor excision circle (TREC) levels for each birth weight group (total n = 177,277). VLBW = very low birth weight, below 1,500 g; LBW = low birth weight, 1,500–2,500 g; NBW = normal birth weight, above 2,500 g. Box = 25th and 75th percentiles, whiskers = 1.5 times height of box or, if no case has a value in that range, the min/max value, asterisks = outliers.

multiple time points were available. Individual TREC dynamics were extremely variable. For all preterm infants, the median rate of TREC increase per week was 24 (−7.6 to +79). However, TREC increase rate changes dramatically with birth week (**Figure 3**). For infants born at 23 weeks gestation, median TREC decreased by 0.07 (−4.2 to +9.6) per week, whereas for infants born at 35 weeks gestation, median TREC increased by 48 (−2.6 to +113) per week. TREC increase rate was very well correlated with gestational age ($R^2 = 0.9229$). These data are limited by the fact that it pertains to a hospitalized population and may not represent the general population.

Twins

Our cohort included 4,786 sets of twins. The average gestational age of all twin pairs was 35.77 weeks; the average birth weight was 2,338 g. The median TREC for all twins was 103 (64–172), significantly lower than the median TREC for singletons, as expected due to lower gestational ages and birth weight. Despite similar average birth weights between twins (2,359 and 2,318, first and second twin accordingly), the first twin in each set had a median TREC of 107 (67–177), compared to only 100 (62–166) for the second twin ($p = 0.003$). The correlation between TREC values of twin sets was low (**Figure 4A**), highlighting the great inter-individual variability in TREC. The different TREC levels according to birth order appear to be unrelated to differences in birth weight in our cohort, as no correlation was found between birth weight and TREC copies/blood spot in the twin cohort (**Figure 4B**).

DISCUSSION

The NBS program for SCID achieves its primary goal, of detecting infants with SCID mere days after birth so that they may receive prompt, disease course altering treatment, and it does so with resounding success (8, 24). However, as any major program in its infancy, there is great potential benefit in fine-tuning the screening algorithm, in order to minimize FP results, better define secondary targets, and glean information about normal and abnormal immune development from an ever growing database of patients and healthy infants.

T cell receptor excision circle analysis has been the method of choice for screening of newborns for severe forms of primary T cell lymphopenia. Our one year experience has reiterated the feasibility of NBS followed by confirmatory tests as a means to successfully identify SCID. Eight SCID patients were identified during the first year of the Israeli NBS program. In the US, NBS for SCID revealed an incidence of 1.72:100,000 births in the general population (8). A similar incidence (~1.69:100,000) was found in a recent meta-analysis, where 13 relevant studies were included (24). Due to high rates of consanguine marriages in Israel, it was assumed the incidence of SCID would be higher, and indeed, according to these data the incidence of SCID in Israel is 4.25:100,000 births (95% CI 1.835–8.377), consistent with the high prevalence recently observed by Broides et al. (7). As this was the first year where such extended and accurate data were available, the true incidence should be re-evaluated in the coming years. There was a predominance of patients of Arab-Muslim decent among our SCID patients (7/8). Genetic founder effects were observed for mutations in *DCLRE1C* and *IL7rα*, which may allow incorporating these mutations into the Israeli Carrier Genetic Screening program for couples of Arab-Muslim decent (25). The high incidence of autosomal recessive SCID in Israel is in contrast with the known high incidence of X-linked SCID (the common γ chain deficiency) in most of the world, and is clearly attributable to the high rate of consanguine marriages and founder genetic effects in our area.

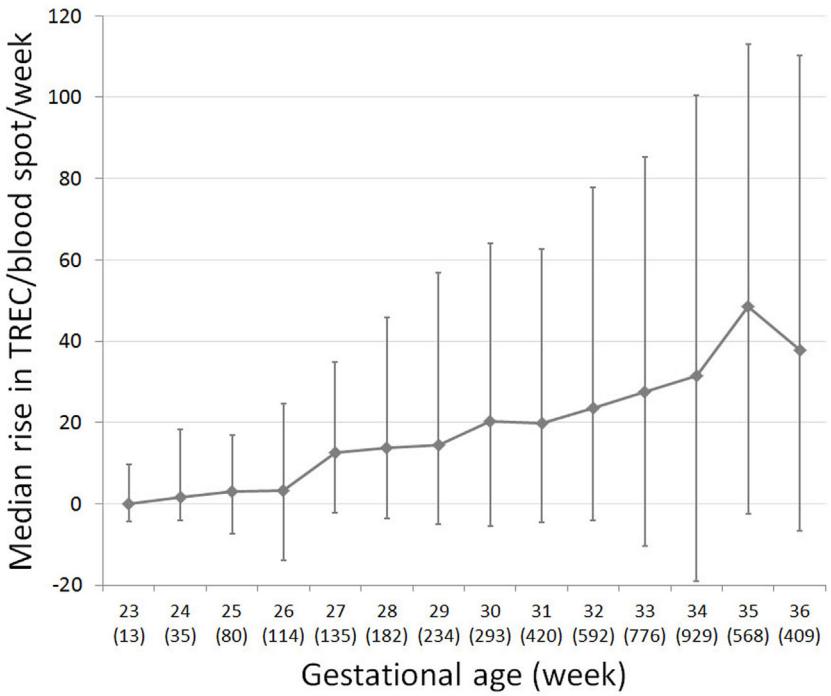

FIGURE 3 | T cell receptor excision circle (TREC) recovery rate in preterm infants, for which multiple TREC results were collected at separate time points (total $n = 4,780$). Median change in TREC copies/blood spot per week for each gestational week. Number of subjects per week listed in brackets below gestational week. Error bars indicate 25th and 75th percentile TREC change for each gestational week.

Confirmation Testing

Currently, the Israeli confirmation algorithm includes a broad range of assays, namely TREC measurement in peripheral blood, proliferation in response to mitogen stimuli and flow cytometry analysis for total lymphocytes, $CD3^+$, $CD4^+$, and TCR repertoire. In California and Wisconsin, confirmatory tests include CBC and lymphocyte profile only (11, 20), and in New York, mitogen stimulation is performed for select cases (26). In some cases, identification of maternal T cells by various means is needed to diagnose patients with SCID and relatively normal T cell counts including maternally engrafted cells (27). To better evaluate the optimal confirmation algorithm, we have used the extended panel of tests listed above for the duration of the first year of the NBS program. Given the overlapping confidence intervals for the various assays, conclusions on performance data should be treated with caution. Unsurprisingly, TREC in peripheral blood is the most sensitive, but least specific confirmation assay. Mitogen stimulation test and TCR repertoire were the most specific assays in our cohort but not sensitive, unable to identify (by themselves) several SCID patients. Overall, lymphocyte subset analysis (total lymphocytes, $CD3^+$, $CD4^+$, and $CD8^+$) were of satisfactory specificity to be used as standalone confirmation tests. In our cohort, lack of $CD20^+$cells was also very informative to identify an underlying genetic defect that is associated with such immune phenotype (such as in *DCLRE1C*). It is important to remember, also, that TCR repertoire analysis could be of great value when lymphocyte profiling is inconclusive and clinical entities such as Omenn's syndrome or SCID with maternal engraftment are suspected (typical rash, lymphadenopathy, alopecia, etc.). Since the TCR repertoire test is not routinely available in many labs and the TREC assay by itself is known to be very sensitive also in diagnosing patients with these clinical phenotypes (28), we suggest an initial confirmatory panel that will include complete blood count, full lymphocyte profile and TREC quantification in the peripheral blood.

Secondary Targets

The secondary target of the screening, detection of newborns with T lymphopenia due to non-SCID etiologies, poses several issues that warrant consideration. First, the management of children with genetic syndromes accompanied by lymphopenia, extreme preterms or patients with idiopathic T cell lymphopenia is very often unaffected by a positive NBS result. Many of them recover spontaneously (11). A recent study by Albin-Leeds et al. (29) has reported that while additional infants with T cell lymphopenia are identified in NBS panels, they seem to do well clinically. Children with severe genetic syndromes often present other, more urgent symptoms at birth. Several of these children detected as secondary targets in our cohort were designated by their families as DNR (do not resuscitate), prohibiting any interventions even if medically warranted. Extreme preterms are hospitalized and closely monitored regardless of their screening results, and are often treated as immunocompromised even in the absence of lymphopenia. The same applies for newborns with secondary immunodeficiency as a result of chylothorax, maternal immune suppression, or other causes. Second, while early detection of syndromes with primary

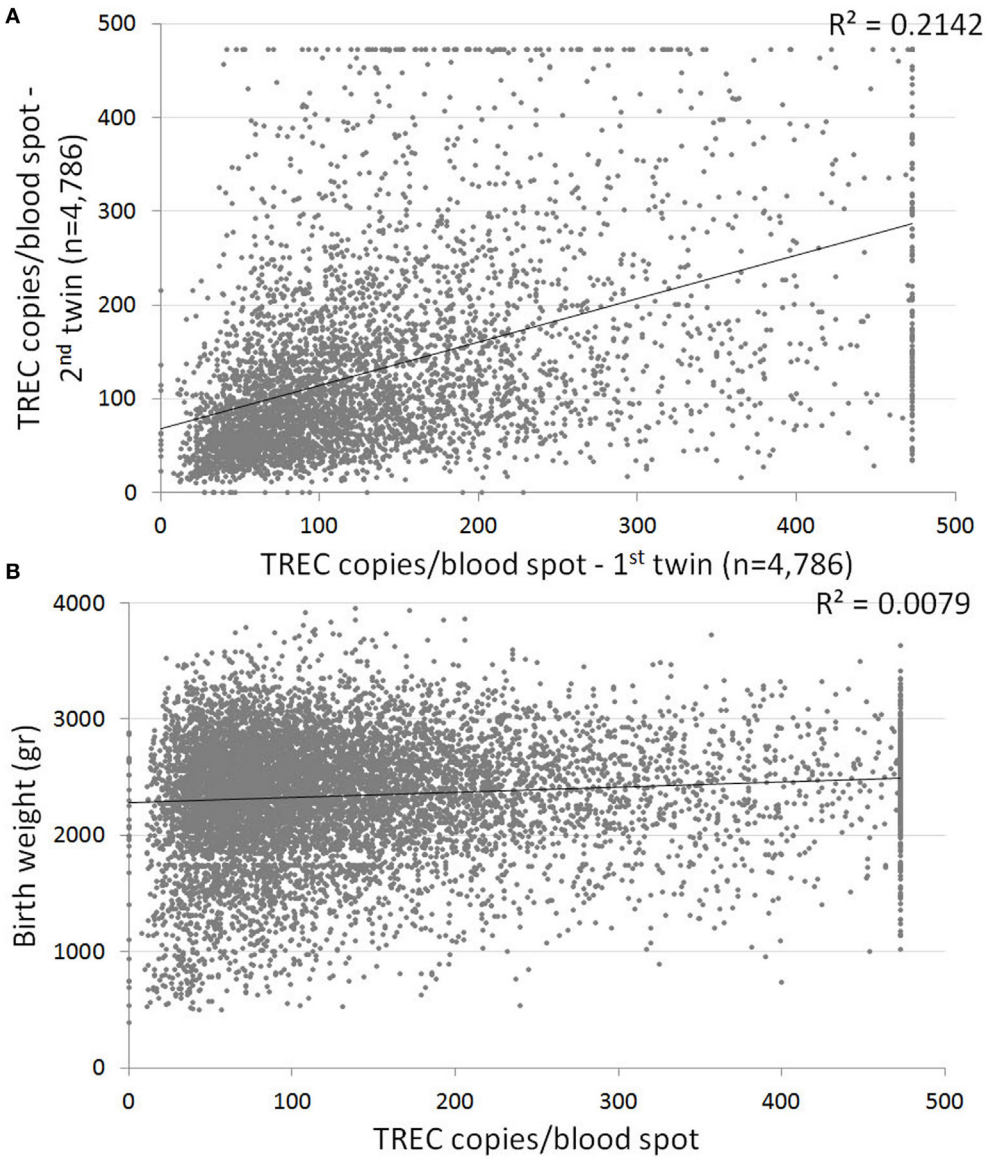

FIGURE 4 | **(A)** Correlation between T cell receptor excision circle (TREC) values of twins. Each dot represents a twin set. Linear trend line indicates a weak correlation between twin TREC results, with a 0.2 coefficient of determination. *n* = 4,786 sets of twins. **(B)** Correlation between birth weight and TREC values for the twin cohort. Linear trend line indicates no correlation (0.0079 coefficient of determination).

immunodeficiencies, such as DiGeorge syndrome, is possible through the NBS program, most newborns with these syndromes have TREC birth levels above the threshold. Barry et al. retrospectively examined NBS results of 1,350 DiGeorge syndrome and found positive screening results in only 11. Five out of these 11 would have been diagnosed with DiGeorge syndrome without NBS (30). Thus, reliance on the screening for secondary targets would be problematic. Nevertheless, because of its well-known relevancy to immunodeficiency, DiGeorge syndrome should be considered in every non-SCID newborn with a positive screening result, even if the typically associated features are absent.

Further studies are required to assess the efficacy and cost–benefit of screening for Non-SCID T cell lymphopenia. Active screening for secondary targets should be pursued if one has sufficient resources to properly workup these children, while keeping in mind that the majority of children with mild T cell abnormalities will return a negative NBS result.

Considerations Based on Gestational Age/Birth Weight

As expected, TREC correlates to both gestational age and birth weight. However, there is great variability in TREC results between newborns of similar birth age, newborns of similar birth weight and even twins, resulting in only a weak correlation between these parameters and TREC. Nevertheless, several important observations arise from the data.

Both gestational age and birth weight correlate with low TREC in extreme preterms (<28 weeks). This is compounded further by a slower recovery rate in preterms, with infants born 26 weeks gestation or younger in our cohort exhibiting a recovery rate of below four TREC copies/blood spot per week. Thus, for extreme preterms, a lower cutoff or a lenient confirmation approach (whereby extreme preterms are referred to confirmation only if still positive at 37 weeks corrected age), is acceptable (26).

In both our cohort and previously published results by Barbaro et al., TREC copy numbers/blood spot rise significantly over a 2-week period between 28 and 30 weeks gestation (18). Though week-specific data are unavailable, de Felipe et al. describe a similar surge between newborns of 29–31 and 32–36 weeks of gestation (19). This "leap" in TREC increase, preceded and followed by milder, incremental increases, could signal an important maturational period in T cell development. Pragmatically, these data suggest that while a lenient approach toward extreme preterms with positive screening results is acceptable, moderate to late preterms should be screened under the same scrutiny as term infants.

Further studies and analysis of relation between gestational age, birth weight, and TREC are required. As data from similar studies are aggregated, it may be possible to comprise a normal distribution chart for TREC based on gestational age and birth weight, in order to minimize FP results while allowing for prompt assessment of true positives.

Concluding Remarks

The purpose of NBS is to enable early diagnosis and treatment of life-threatening conditions. As reported above, seven of the eight SCID patients diagnosed through the Israeli screening program in its first year are currently alive and well, and are either post- or awaiting BMT. Before the initiation of the NBS program, such positive clinical outcomes were impossible for children with SCID.

AUTHOR CONTRIBUTIONS

ER analyzed the data and wrote the paper; AL and AS performed all confirmatory and genetic tests and analyzed the results; SD, TS-L, and SA performed the screening for TREC and beta-actin and collected the results; TS, AB, AN, NM, OT, SH, ID, PS, and RS followed the patients; AE and RS analyzed the immunological results and supervised the writing of the paper. All authors approved the final version for submission and are accountable for all aspects of the work.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at http://www.frontiersin.org/article/10.3389/fimmu.2017.01448/full#supplementary-material.

REFERENCES

1. Therrell BL, Padilla CD, Loeber JG, Kneisser I, Saadallah A, Borrajo GJC, et al. Current status of newborn screening worldwide : 2015. *Semin Perinatol* (2015) 39:171–87. doi:10.1053/j.semperi.2015.03.002
2. Kwan A, Puck JM. History and current status of newborn screening for severe combined immunodeficiency. *Semin Perinatol* (2015) 39:194–205. doi:10.1038/nbt.3121.ChIP-nexus
3. Dvorak CC, Cowan MJ, Logan BR, Notarangelo LD, Griffith LM, Puck JM, et al. The natural history of children with severe combined immunodeficiency: baseline features of the first fifty patients of the primary immune deficiency treatment consortium prospective study 6901. *J Clin Immunol* (2014) 33:1156–64. doi:10.1007/s10875-013-9917-y.The
4. Railey MD, Lokhnygina Y, Buckley RH. Long term clinical outcome of patients with severe combined immunodeficiency who received related donor bone marrow transplants without pre-transplant chemotherapy or post-transplant GVHD prophylaxis. *J Pediatr* (2009) 155:834–40. doi:10.1016/j.jpeds.2009.07.049.Long
5. Chan K, Davis J, Pai S-Y, Bonilla FA, Puck JM, Apkon M. A Markov model to analyze cost-effectiveness of screening for severe combined immunodeficiency (SCID). *Mol Genet Metab* (2011) 104:383–9. doi:10.1016/j.ymgme.2011.07.007
6. Zlotogora J, Leventhal A, Amitai Y. The impact of congenital malformations and Mendelian diseases on infant mortality in Israel. *Isr Med Assoc J* (2003) 5:416–8.
7. Broides A, Nahum A, Mandola AB, Rozner L, Pinsk V, Ling G, et al. Incidence of typically severe primary immunodeficiency diseases in consanguineous and non-consanguineous populations. *J Clin Immunol* (2017) 37:295–300. doi:10.1007/s10875-017-0378-6
8. Kwan A, Abraham RS, Currier R, Brower A, Andruszewski K, Abbott JK, et al. Newborn screening for severe combined immunodeficiency in 11 screening programs in the United States. *JAMA* (2014) 312:729–38. doi:10.1001/jama.2014.9132
9. Kwan A, Hu D, Song M, Gomes H, Brown DR, Bourque T, et al. Successful newborn screening for SCID in the Navajo Nation. *Clin Immunol* (2015) 158:29–34. doi:10.1016/j.clim.2015.02.015
10. Rozmus J, Junker A, Thibodeau ML, Grenier D, Turvey SE, Yacoub W, et al. Severe combined immunodeficiency (SCID) in Canadian children: a national surveillance study. *J Clin Immunol* (2013) 33:1310–6. doi:10.1007/s10875-013-9952-8
11. Verbsky J, Thakar M, Routes J. The Wisconsin approach to newborn screening for severe combined immunodeficiency. *J Allergy Clin Immunol* (2012) 129:622–7. doi:10.1016/j.jaci.2011.12.004
12. Douek DC, McFarland RD, Keiser PH, Gage EA, Massey JM, Haynes BF, et al. Changes in thymic function with age and during the treatment of HIV infection. *Nature* (1998) 396:690–5. doi:10.1038/25374
13. Somech R, Lev A, Simon AJ, Korn D, Garty BZ, Amariglio N, et al. Newborn screening for severe T and B cell immunodeficiency in Israel : a pilot study. *Isr Med Assoc J* (2013) 15:404–9.
14. Rechavi E, Lev A, Lee YN, Simon AJ, Yinon Y, Lipitz S, et al. Timely and spatially regulated maturation of B and T cell repertoire during human fetal development. *Sci Transl Med* (2015) 7:1–12. doi:10.1126/scitranslmed.aaa0072
15. Audrain M, Thomas C, Mirallie S, Bourgeois N, Sebille V, Rabetrano H, et al. Evaluation of the T-cell receptor excision circle assay performances for

severe combined immunodeficiency neonatal screening on Guthrie cards in a French single centre study. *Clin Immunol* (2014) 150:137–9. doi:10.1016/j.clim.2013.11.012
16. Puck JM. Neonatal screening for severe combined immune deficiency. *Curr Opin Pediatr* (2011) 23:667–73. doi:10.1097/MOP.0b013e32834cb9b0
17. Adams SP, Rashid S, Premachandra T, Harvey K, Ifederu A, Wilson MC, et al. Screening of neonatal UK dried blood spots using a duplex TREC screening assay. *J Clin Immunol* (2014) 34:323–30. doi:10.1007/s10875-014-0007-6
18. Barbaro M, Ohlsson A, Borte S, Jonsson S, Zetterstrom RH, King J, et al. Newborn screening for severe primary immunodeficiency diseases in Sweden – a 2-year pilot TREC and KREC screening study. *J Clin Immunol* (2017) 37:51–60. doi:10.1007/s10875-016-0347-5
19. de Felipe B, Olbrich P, Lucenas JM, Delgado-Pecellin C, Pavon-Delgado A, Marquez J, et al. Prospective neonatal screening for severe T- and B-lymphocyte deficiencies in Seville. *Pediatr Allergy Immunol* (2016) 27:70–7. doi:10.1111/pai.12501
20. Kwan A, Church JA, Cowan MJ, Agarwal R, Kapoor N, Kohn DB, et al. Newborn screening for SCID and T cell lymphopenia in California: results of the first two years. *J Allergy Clin Immunol* (2013) 132:140–50. doi:10.1016/j.immuni.2010.12.017.Two-stage
21. Somech R, Etzioni A. A call to include severe combined immunodeficiency in newborn screening program. *Rambam Maimonides Med J* (2014) 5:e0001. doi:10.5041/RMMJ.10135
22. Lev A, Simon AJ, Bareket M, Bielorai B, Hutt D, Amariglio N, et al. The kinetics of early T and B cell immune recovery after bone marrow transplantation in RAG-2-deficient SCID patients. *PLoS One* (2012) 7:e30494. doi:10.1371/journal.pone.0030494
23. Rechavi E, Lev A, Saraf-Levy T, Etzioni A, Almashanu S, Somech R. Newborn screening for severe combined immunodeficiency in Israel. *Int J Neonatal Screen* (2017) 3:13. doi:10.3390/ijns3020013
24. van der Spek J, Groenwold RHH, van der Burg M, van Montfrans JM. TREC based newborn screening for severe combined immunodeficiency disease: a systematic review. *J Clin Immunol* (2015) 35:416–30. doi:10.1007/s10875-015-0152-6
25. Na'amnih W, Romano-Zelekha O, Kabaha A, Rubin LP, Bilenko N, Jaber L, et al. Continuous decrease of consanguineous marriages among Arabs in Israel. *Am J Hum Biol* (2015) 27:94–8. doi:10.1002/ajhb.22610
26. Vogel BH, Bonagura V, Weinberg GA, Ballow M, Isabelle J, Diantonio L, et al. Newborn screening for SCID in New York state: experience from the first two years. *J Clin Immunol* (2014) 34:289–303. doi:10.1007/s10875-014-0006-7
27. Chase NM, Verbsky JW, Routes JM. Newborn screening for SCID: three years of experience. *Ann N Y Acad Sci* (2011) 1238:99–105. doi:10.1111/j.1749-6632.2011.06241.x
28. Lev A, Simon AJ, Trakhtenbrot L, Goldstein I, Nagar M, Stepensky P, et al. Characterizing T cells in SCID patients presenting with reactive or residual T lymphocytes. *Clin Dev Immunol* (2012) 2012:1–9. doi:10.1155/2012/261470
29. Albin-Leeds S, Ochoa J, Mehta H, Vogel BH, Caggana M, Bonagura V, et al. Idiopathic T cell lymphopenia identified in New York state newborn screening. *Clin Immunol* (2017) 183:36–40. doi:10.1016/j.clim.2017.07.002
30. Barry JC, Crowley TB, Jyonouchi S, Heimall J, Zackai EH, Sullivan KE, et al. Identification of 22q11.2 deletion syndrome via newborn screening for severe combined immunodeficiency. *J Clin Immunol* (2017) 37(5):1–10. doi:10.1007/s10875-017-0403-9

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frontiers in Immunology

REVIEW
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A Practical Approach to Newborn Screening for Severe Combined Immunodeficiency Using the T Cell Receptor Excision Circle Assay

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Severe combined immunodeficiency (SCID) is a life-threatening condition of newborns and infants caused by defects in genes involved in T cell development. Newborn screening (NBS) for SCID using the T cell receptor excision circle (TREC) assay began in Wisconsin in 2008 and has been adopted or is being implemented by all states in 2017. It has been established that NBS using the TREC assay is extremely sensitive to detect SCID in the newborn period. Some controversies remain regarding how screening positives are handled by individual states, including when to perform confirmatory flow cytometry, what is the necessary diagnostic workup of patients, what infection prophylaxis measures should be taken, and when hematopoietic stem cell transplantation should occur. In addition, the TREC can also assay detect infants with T cell lymphopenia who are not severe enough to be considered SCID; management of these infants is also evolving.

Keywords: severe combined immunodeficiency, T cell receptor excision circles, newborn screening, bone marrow transplantation, antibiotic prophylaxis

NEWBORN SCREENING (NBS) FOR SEVERE COMBINED IMMUNODEFICIENCY (SCID): AN EFFECTIVE SCREENING PROGRAM

Numerous studies from individual states, as well as a multi-state summary of NBS results, have been published summarizing the results of SCID screening with the T cell receptor excision circle (TREC) assay (1–10). Several conclusions can be made from these studies. First, the TREC analysis is capable of detecting known genetic causes of SCID, including *IL2RG*, *RAG1*, *ADA*, *IL7R*, *JAK3*, *DCLRE1C*, and *CD3D* (4). The percentage of cases of X-linked SCID due to mutations in *IL2RG* (19%) is lower than expected based on prior studies (~50%). In addition to known causes of SCID, a number of infants are detected without genetic causes but with clinically defined SCID (1, 2, 4). Thus, it is important to remember that the lack of a genetic diagnosis does not preclude a diagnosis of SCID. Curative treatment is recommended if the infant has a persistently low T cell count (<300/μl autologous T cells) and poor proliferative response to phytohemagglutinin (PHA) (<10% of normal), which are the diagnostic criteria of SCID. Preliminary data from these studies have been

used to generate an incidence of SCID in the US population of approximately 1:60,000 (2, 4, 9). Importantly, there have been no cases of documented SCID that have been missed by NBS with the TREC assay, confirming its high sensitivity for detection. It is important to note that some significant immune deficiencies can be missed by the TREC assay, such as late onset ADA deficiency, bare lymphocyte syndrome due to lack of MHC class II, ZAP70 deficiency, CD40 ligand deficiency, and NF-kappa-B essential modulator deficiency. In these cases, the T cells do develop in the thymus, but the defect occurs after VDJ recombination.

In addition to SCID, the NBS using the TREC assay detects a variety of non-SCID disorders that also result in T cell lymphopenia (TCL) (2, 4, 9). Some of these disorders are known to affect normal T cell development, including 22q11.2 deletion syndrome, ataxia telangiectasia, Nijmegen breakage syndrome, and CHARGE syndrome. Interestingly, a variety of chromosomal disorders not classically thought to affect T cell development have also been detected, including Trisomy 21, Jacobsen syndrome, and other cytogenetic anomalies. Congenital anomalies, including cardiac defects, gastrointestinal malformation, and infants with multiple congenital malformations, can be associated with secondary lymphopenia. Most of these disorders, except for complete DiGeorge syndrome and CHARGE syndrome, typically do not result in T cell counts low enough to be considered SCID.

PRACTICAL CARE OF INFANTS WITH A POSITIVE NBS FOR SCID

When NBS for SCID is implemented, an algorithm regarding how to care for screening positives is essential to the success of the screening program. Each state that performs screening has its own algorithm, but from our experience and published reports, they all are fairly similar (1, 9, 11–13). In Wisconsin, when an infant fails the NBS test for SCID, the primary care doctor and one of the Clinical Immunologists is contacted, and arrangements are made for confirmatory flow cytometry. The TREC level can be informative as to the urgency of this step, as can the current health of the baby. Our approach differs slightly based on whether an infant presents with other concurrent medical issues (e.g., prematurity, medical disorders) or is an otherwise well full-term baby (**Table 1**). Also, an absent or very low TREC level (e.g., <20% of the cutoff value for TRECs) in an infant is typically treated with more urgency than an infant with a higher TREC level, as these infants are less likely to have SCID.

The evaluation of an infant with a failed TREC assay includes a thorough medical history to determine if there were any prenatal exposures or perinatal illnesses that could have contributed to false-positive screening. In some states, premature infants with positive screens are followed clinically, and the TREC assay repeated every 2 weeks until they reach 37 weeks adjusted gestational age, at which time lymphocyte enumeration *via* flow cytometry is performed (2, 9). Other states may perform flow cytometry at an earlier gestational age, as SCID can be diagnosed at this time (5). Similarly, infants with significant infections or congenital anomalies such as cardiac defects are followed and assessed when clinically stable. Chylous effusions or lymphatic malformations can also lead to T cell loss, and flow cytometry should be delayed until the underlying cause is treated. Since secondary lymphopenia is common in these instances, this limits unnecessary costs of repeated lymphocyte enumerations.

For well-appearing full-term infants, the family history is reviewed specifically for young childhood deaths and significant infectious history in siblings and close family members. A social history with focus on caregivers is elicited. A thorough physical exam is performed including evaluation for dysmorphic features, skin rashes, presence of absence of lymphoid tissue, and growth. Laboratory evaluations include lymphocyte enumeration to confirm the diagnosis. A limited flow cytometry panel is performed to

TABLE 1 | Recommended diagnostic evaluation for suspected severe combined immunodeficiency (SCID)/T cell lymphopenia (TCL).

Test	Frequency	Details/rationale
All patients		
T cell receptor excision circles (TRECs)	At initial visit and every 2 weeks if premature or other illness	To confirm results of initial screening test To determine if TRECs will normalize once illness or prematurity resolves
Lymphocyte enumeration	At initial visit (if full term and healthy), monthly thereafter	To confirm initial result of screening test. Minimum flow panel includes numbers of CD4 and CD8 T cells, CD56 NK cells, and CD19 B cells. CD45RA and CD45RO analysis to determine the numbers of naïve T cells.
Chimerism testing	OnceTCL is confirmed	To test for material engraftment, infant's buccal swabs are compared to whole blood using variable number tandem repeats. If male, XX/XY FISH from blood can be performed
T cell mitogens, (e.g., PHA)	Once TCL is confirmed	To test for function of T cells
IgG, IgA, IgM	Once prior to first dose of IVIG/SCIG. IgA and IgM are repeated over time if on IVIG/SCIG	To test for function of B cells
Genetic sequencing of SCID-causing genes	Once severe TCL is confirmed	To establish a genetic diagnosis of SCID, we utilize clinically available SCID panels that cover many SCID-causing genes
DNA duplication/deletion array	Once TCL is confirmed	To rule out 22q11 deletion syndrome, CHARGE caused by deletions, and other chromosomal anomalies

enumerate absolute CD4 and CD8 positive T cells, CD56-positive NK cells, and CD19-positive B cells. Naïve and memory T cells are enumerated by CD45RA and CD45RO analyses, respectively. Since maternal engraftment and hypomorphic mutations in SCID-causing genes can result in a higher T cell count than would be expected with typical SCID, it is important to verify whether the T cell repertoire is skewed toward a memory phenotype. To assess for maternal engraftment, chimerism testing using variable number tandem repeats is sent. This can be done by comparing the DNA obtained from the infant's buccal swabs to whole blood. Typically, there are insufficient numbers of lymphocytes present to perform a sorted CD3 chimerism test. If the infant is a male, a XX/XY chimerism assay by FISH can be performed. If the lymphocyte numbers are consistent with SCID and there is no cause for secondary lymphopenia, these infants are labeled as "presumed SCID" (**Table 1**).

All infants with presumed SCID then undergo a diagnostic and confirmatory workup during these initial visits. T cell mitogen studies are performed to evaluate T cell function. Genetic testing is pursued to determine if there is a genetic cause for their TCL. Gene panels are cost-effective and preferred for evaluating the many causes of SCID in order to prevent delays associated with single gene testing. DNA deletion/duplication array is performed to exclude 22q11.2 deletion syndrome, microdeletions of CHD7, or other chromosomal anomalies. Due to blood volume issues in an infant, this testing is performed over several visits. It is important to evaluate for the presence of thymic tissue in cases of T-B + SCID, and the genetic tests listed above may not detect all cases of CHARGE or 22q11. Therefore, other imaging can be performed to look for thymic tissue, including a chest ultrasound or a CT scan of the chest. Some centers consider thymic biopsy to confirm the diagnosis of SCID versus athymia (14).

As the diagnostic workup is taking place, evaluation and prophylaxis of infectious diseases are undertaken (**Table 2**) (5, 11, 12). Baseline infectious disease testing is imperative in all infants suspected of SCID whether symptomatic or not. The frequency of surveillance increases based on clinical suspicion of new infection (e.g., fever, irritability) or if any of the initial results are positive. Infants who test positive for viral infection are hospitalized and anti-viral therapy implemented. Serological assays, with the exception of HIV-1 infection, are not obtained, as specific antibody production is absent in infants with SCID and variably present in infants with TCL. Infants are screened for antibodies to HIV-1, which would indicate maternal HIV infection, and require immediate treatment. We have found that the burden of blood testing is high during this period, with limitations imposed based on patient size. As mentioned above, we typically prioritize infectious disease testing (based on clinical suspicion) over multiple visits.

Infants identified as possible SCID undergo a prophylaxis regimen designed to prevent bacterial, fungal, and viral infections (**Table 3**) (11, 12). Subcutaneous or intravenous immunoglobulin is started to aid in preventing the acquisition of infections. Infants with SCID do not receive any live vaccinations, and we withhold other routine immunizations as well since these children are on antibody replacement. We instruct family members and close contacts to be up-to-date on immunizations against influenza and pertussis. Although transmission of varicella, measles, and rotavirus through vaccination is also highly unlikely (15), we do not recommend that close contacts or family members be vaccinated with live attenuated vaccines unless there is a community outbreak, or if there are other special circumstances. Our supportive care and infectious disease prophylaxis guidelines are largely based on national consensus guidelines, and this supportive care is continued through the immunologic diagnostic workup until

TABLE 2 | Recommended screening tests at initial newborn screening (NBS) referral and pre-hematopoietic stem cell transplantation (HSCT).

Test	At referral for NBS	Monitoring during pre-HSCT period	During pre-transplant evaluation (<3 weeks prior to HSCT)
Blood PCR tests			
CMV, EBV, adenovirus, HHV6	X	Every 4 weeks	X
Hepatitis B, hepatitis C, HSV1/2, HIV-1	X		X (omit if performed <30 days prior to HSCT and negative)
NMDP, WNV			X
Blood antibody testing			
HIV-1[a]	X		X
NMDP, HIV-2, HTLV 1/2, HepBsAg, Hep B core, Hep C, CMV, *T. cruzi*, STS			X
Blood serum or plasma			
Frozen for potential future diagnostic purposes	X		
Nasopharyngeal swabs for viral PCR and viral culture (regardless of symptoms)			
Influenza A and B, parainfluenza 1, 2, and 3, RSV, adenovirus, and HMPV	X		X
Imaging evaluation			
Chest X-ray; CT scans of head, neck, chest, abdomen, pelvis; urinalysis	Only if clinically indicated to evaluate for infection; if diagnosis is under question, chest ultrasound is informative to evaluate for presence of thymus	Only if clinically indicated	X

CMV, cytomegalovirus; EBV, Epstein–Barr virus; HHV6, human herpes virus 6; HSV1/2, herpes simplex virus 1/2; HIV-1/2, human immunodeficiency virus 1/2; WNV, West Nile virus; NMDP, National Marrow Donor Program; HTLV 1/2, human T-lymphotrophic virus 1/2; HepBsAg, hepatitis B surface antigen; HepB core, hepatitis B core antibody; T. cruzi, Trypanosoma cruzi antibody; STS, serological test for syphilis; RSV, respiratory syncytial virus; HMPV, human metapneumovirus.

[a]*HIV-2 testing is not routinely performed as part of the baseline testing due to its low prevalence in the United States; should there be concern for HIV infection, both HIV-1 and HIV-2 should be tested at baseline.*

TABLE 3 | Recommended infectious disease prophylaxis for newborns with suspected of severe combined immunodeficiency.

Prophylaxis	Drug (dose)	Time of initiation	Alternatives	Comments
In newborn				
PCP	TMP-SMX orally (5 mg TMP/kg once a day for 2 consecutive days weekly)	1 month old	Atovaquone orally (30 mg/kg once a day)	Verify that bilirubin is <2X's upper limit of normal before starting. Monitor ALT, AST, and bilirubin every 2–4 weeks
HSV and VSV	Acyclovir orally (20 mg/kg/dose 3 times a day)	At first visit		Follow BUN and creatinine every 2–4 weeks
Respiratory syncytial virus (RSV)	Palivizumab (15 mg/kg I.M.)	1 month old		Given during peak RSV season, typically November–March in the northern hemisphere
General (bacterial/viral)	IVIG (0.4–0.5 g/kg every month) or SCIG	1 month old		Monitor troughs monthly and maintain Ig > 600 mg/dl; Based on subcutaneous fat and body surface area to volume of medication administered, could consider SCIG in select patients
Fungal	Fluconazole (6 mg/kg once daily)	1 month old	Micafungin or discontinue fungal prophylaxis	Follow AST, ALT, and bilirubin every 2–4 weeks
In family members or close contacts				
Influenza	Inactivated influenza vaccine	Seasonally		
Pertussis	Tdap vaccine	Per routine childhood vaccinations		One booster for adolescents (11–12 years age); adults 19–64 years age and adults >65 years age

transplant occurs (16). Additionally, complete blood counts and kidney and liver function are followed while on antimicrobials at least every 2–4 weeks. Lymphocyte counts continue to be followed monthly. This is particularly important for those infants whose genetic diagnosis has not yet been confirmed, in order to monitor for any potential for T cell recovery.

It is during these initial visits that education is provided regarding hematopoietic stem cell transplantation (HSCT). We also request that families maintain continuity with their primary care provider during this time, who can continue monitoring the infant for normal developmental milestones and nutrition. We advise the primary care provider to schedule office visits as the first appointment of the morning, in order to limit waiting room exposures. If not previously done, human leukocyte antigen (HLA) typing is sent on the patient and immediate family members. If blood volume limits are being met due to diagnostic and infectious disease testing, buccal swabs can be used for HLA typing. The transplant service initiates an unrelated donor search when a suitable donor with an HLA match is not found within the family. At our center frequent meetings between the Clinical Immunology and the Bone Marrow Transplant teams occur to discuss any diagnostic dilemmas and to update each other on the patient's clinical status. Once a diagnosis of SCID is confirmed by genetic testing, or for patients without a genetic diagnosis but with persistent T cell counts below 300 cell/μl and abnormal mitogenic responses, care is formally transferred to Bone Marrow Transplant Service.

If a newborn has any concern for infection, including fever (a one-time fever of 100.4°F/38°C, or low-grade fevers that do not resolve), admission to the inpatient unit is mandatory. At our center, patients are admitted to the Bone Marrow Transplant Unit in positive pressure rooms. Mandatory hand washing and limited visitation including no sick contacts is strictly implemented. Workup for fevers is broad to include cultures and PCR of the blood, cerebrospinal fluid, and nasopharyngeal swabs with imaging as clinically warranted (**Table 2**). If the infant is hospitalized, the Infectious Disease service is consulted, as is a Neonatologist or Pediatric Hospitalist for a general pediatrics evaluation.

DECISION OF TRANSPLANT TIMING

Once the diagnosis of SCID has been confirmed, we proceed to curative HSCT using the best available donor. The timing of transplant depends on many factors such as the type of SCID, ability to clear/treat any underlying infections, and the need for and type of conditioning. In some types of SCID caused by DNA repair defects, such as DCLRE1C (artemis) deficiency, ionizing radiation, and certain conditioning regimens can be dangerous, and treatment should aim to minimize exposure to alkylating agents and ionizing radiation (17). In other cases of SCID, such as IL2RG deficiency, HSCT can occur without conditioning and can be performed within the first several months of life (18, 19). Additionally, if there is a concurrent infection, there may be a decision made to proceed to transplant without prior conditioning in order to reduce the risk of transplant-related mortality. However, if conditioning is used, it is our practice to wait until 3–6 months of age to allow for further growth, development, and organ maturity. In patients where the diagnosis has not been confirmed by genetic testing, the severity of TCL is verified through serial testing and the decision to transplant may be delayed until at least 6–9 months of age, if not later, based on evaluation of T cell recovery. In our experience, if an infant remains on prophylactic measures with excellent compliance and close follow-up, short delays in referring to transplant are well tolerated.

FAMILY GUIDANCE FOR INFANTS DETECTED WITH SCID/TCL

While the NBS has allowed prompt identification of newborns with SCID/TCL, families confronting this disease are typically

overwhelmed with emotion, confusion, and uncertainty when first informed of the result. It is challenging for most families, who see a well-child who is thriving, to consider the underlying concerns of an immune deficiency. Anticipatory guidance and education on preventing infections is essential, although there are controversies regarding this. Some recommendations are routine and should be implemented, including strict hand hygiene, limited exposure to visitors, and no exposure to anyone who is ill. We typically keep these infants at home until transplant as long as the social situation is stable. Other issues are more controversial, such as recommendations regarding breastfeeding and contact precautions, particularly in regards to the chance of acquiring cytomegalovirus (CMV). Our current policy is to immediately halt breastfeeding in any child with absent TRECs until the immune status to CMV of the mother is obtained. CMV can be transferred through saliva or breast milk, so at our center, we test the parents for prior CMV exposure by serology. If the mother is CMV seropositive (IgG or IgM), breastfeeding is discouraged, and formula feeding using boiled water is recommended. Furthermore, if either parent is CMV seropositive, he or she is educated regarding the transmission of this virus through saliva, although we do not insist that families use masks or other contact precautions at home. This is a difficult issue, as we strive to balance the need of families to bond with their newborns, while concurrently preventing any infection exposures that may affect the success of the HSCT.

One of the first jobs of the medical team is to focus on a family's understanding of the diagnosis and their ability to conform to protective measures and prophylactic antimicrobials, and this can affect disposition of management of the infant (i.e., inpatient versus outpatient). If there are any concerns from a social standpoint in regards to poor care or lack of understanding of prophylactic measures, the patient is admitted for continued management and education. If there are no infectious or social concerns, as mentioned earlier, infants suspected of SCID are cared for at home. We employ several guidelines in order to reduce the risk infection (**Table 4**). We also require that the infant live within 1-h drive of our hospital, or another appropriate hospital well-versed in taking care of sick children, from where transfer to our hospital would occur should there be a fever or sign of infection. We also send the family home with a handout explaining the diagnosis, current medications, and our contact information should the infant be seen at an outside hospital. A family is educated at the first in-person visit that a high degree of suspicion for infections is critical, because infections can be clinically asymptomatic. With the above precautions, we have had no infants acquire serious or opportunistic infections while awaiting transplant or while immunologic investigations were ongoing.

TABLE 4 | Recommended supportive care anticipatory guidelines for infants with suspected severe combined immunodeficiency.

Guideline	Reason
Avoid public places, daycare Limited contact with young children Strict hand washing	Prevent transmission of community-acquired diseases
No breastfeeding[a]	Prevent transmission of cytomegalovirus (CMV)
Boil ingestible water, including bottled water	Prevent cryptosporidium infection
Avoid all live and live attenuated vaccines (MMR, Varicella, Rotavirus, Flu-Mist)	Prevent infection with vaccine-related viruses
Blood products—leukodepletion and irradiation essential; CMV negative when available	Prevent transmission of CMV and graft versus host disease

[a]*Breastfeeding could be reinitiated if maternal CMV demonstrates no prior exposure (IgG and IgM are both negative, and CMV PCR is negative). If CMV IgG is positive, breastfeeding is strongly discouraged.*

IDIOPATHIC TCL—A UNIQUE MANAGEMENT PROBLEM FOR NBS

A number of infants who failed the NBS for SCID are diagnosed with idiopathic T cell lymphopenia (ITL) (1, 9, 20). These patients have low T cell numbers, but not low enough to be considered SCID (>300 autologous T cells/mm^3), and lack a genetic diagnosis that results in SCID or a known cause of TCL. Evaluation and treatment of these patients is evolving as more is known about this condition. First, it is important to investigate for maternal factors that can influence T cell development. Mothers on immunosuppressants, such as azathioprine, can give birth to infants with lymphopenia that may resolve over time (6, 21). Since hypomorphic mutations in SCID-causing genes can result in severe TCL (also known as leaky SCID), genetic sequencing typically with a "SCID panel" that includes all genes known to cause SCID should be performed (22, 23). With new sequencing technologies, it is possible to screen hundreds of known immune defects that could potentially affect TREC levels, and these studies have led to the description of a variety of genetic defects leading to idiopathic TCL, including *ATM* and *ITK* mutations (6, 24, 25).

T cell counts are followed to determine if the TCL is improving. A controversial issue is what constitutes a "protective" T cell count, or a T cell count that is sufficient to prevent infections. However, the absolute number of T cells is not the only factor, as T cell function also needs to be considered. We perform T cell mitogen studies to determine if the T cells are capable of expanding and obtaining effector function. The acquisition of normal antibody levels in infants with ITL can be informative as a surrogate of T cell function, and we follow IgA, IgM, and IgG. IgG levels will not be accurate once replacement antibody is started, and can be complicated by maternal IgG during the first 4–6 months of life. Protective serum vaccination titers can eventually be helpful, but in the early stages of ITL evaluation, we do not recommend routine immunizations. There are several possible outcomes of patients with ITL. First, the T cell counts may normalize over time, but in our and others experience, the timing of this can vary greatly and may take up to 9 months (9, 20). In other cases, the T cell counts may be persistently low and decrease over time, which would necessitate consideration of proceeding to HSCT (9, 20).

SUMMARY

Newborn screening for SCID/TCL using the TREC assay has been proven to be extremely effective to detect SCID and other severe forms of TCL, with a sensitivity approaching 100% for SCID. Initial evaluation of infants with a positive TREC assay should include flow cytometry to assess the numbers of naïve T cells, as well as NK cells and B cells, and T cell response to mitogens to evaluate function. Genetic testing is important, and any child with two known variants that cause SCID, undergoes HSCT. Infants with T cell counts not consistent with SCID are followed clinically to see if the TCL will resolve over time. Infectious prophylaxis with antimicrobial medications and immune globulin is important as these children are evaluated. Family counseling is essential, not only to prevent the acquisition of new infections but also to educate the family on the diagnosis and the complexities of HSCT. With these issues in mind, outcomes of children detected with abnormal TREC assays are excellent, as morbidity and mortality has been greatly reduced.

AUTHOR CONTRIBUTIONS

MT, JR, JV, and nurse practitioners MH and MG were all involved in the writing and editing of the manuscript.

REFERENCES

1. Routes JM, Grossman WJ, Verbsky J, Laessig RH, Hoffman GL, Brokopp CD, et al. Statewide newborn screening for severe T-cell lymphopenia. *JAMA* (2009) 302(22):2465–70. doi:10.1001/jama.2009.1806
2. Verbsky JW, Baker MW, Grossman WJ, Hintermeyer M, Dasu T, Bonacci B, et al. Newborn screening for severe combined immunodeficiency; the Wisconsin experience (2008–2011). *J Clin Immunol* (2012) 32(1):82–8. doi:10.1007/s10875-011-9609-4
3. Hale JE, Bonilla FA, Pai SY, Gerstel-Thompson JL, Notarangelo LD, Eaton RB, et al. Identification of an infant with severe combined immunodeficiency by newborn screening. *J Allergy Clin Immunol* (2010) 126(5):1073–4. doi:10.16/j.jaci.2010.08.043
4. Kwan A, Abraham RS, Currier R, Brower A, Andruszewski K, Abbott JK, et al. Newborn screening for severe combined immunodeficiency in 11 screening programs in the United States. *JAMA* (2014) 312(7):729–38. doi:10.1001/jama.2014.9132
5. Kwan A, Church JA, Cowan MJ, Agarwal R, Kapoor N, Kohn DB, et al. Newborn screening for severe combined immunodeficiency and T-cell lymphopenia in California: results of the first 2 years. *J Allergy Clin Immunol* (2013) 132(1):140–50. doi:10.1016/j.jaci.2013.04.024
6. Barbaro M, Ohlsson A, Borte S, Jonsson S, Zetterstrom RH, King J, et al. Newborn screening for severe primary immunodeficiency diseases in Sweden-a 2-year pilot TREC and KREC screening study. *J Clin Immunol* (2017) 37(1):51–60. doi:10.1007/s10875-016-0347-5
7. Kanegae MP, Barreiros LA, Mazzucchelli JT, Hadachi SM, de Figueiredo Ferreira Guilhoto LM, Acquesta AL, et al. Neonatal screening for severe combined immunodeficiency in Brazil. *J Pediatr (Rio J)* (2016) 92(4):374–80. doi:10.1016/j.jped.2015.10.006
8. Chien YH, Chiang SC, Chang KL, Yu HH, Lee WI, Tsai LP, et al. Incidence of severe combined immunodeficiency through newborn screening in a Chinese population. *J Formos Med Assoc* (2015) 114(1):12–6. doi:10.1016/j.jfma.2012.10.020
9. Vogel BH, Bonagura V, Weinberg GA, Ballow M, Isabelle J, DiAntonio L, et al. Newborn screening for SCID in New York State: experience from the first two years. *J Clin Immunol* (2014) 34(3):289–303. doi:10.1007/s10875-014-0006-7
10. Somech R, Lev A, Simon AJ, Korn D, Garty BZ, Amariglio N, et al. Newborn screening for severe T and B cell immunodeficiency in Israel: a pilot study. *Isr Med Assoc J* (2013) 15(8):404–9.
11. Verbsky J, Thakar M, Routes J. The Wisconsin approach to newborn screening for severe combined immunodeficiency. *J Allergy Clin Immunol* (2012) 129(3):622–7. doi:10.1016/j.jaci.2011.12.004
12. Dorsey MJ, Dvorak CC, Cowan MJ, Puck JM. Treatment of infants identified as having severe combined immunodeficiency by means of newborn screening. *J Allergy Clin Immunol* (2017) 139(3):733–42. doi:10.1016/j.jaci.2017.01.005
13. van der Spek J, Groenwold RH, van der Burg M, van Montfrans JM. TREC based newborn screening for severe combined immunodeficiency disease: a systematic review. *J Clin Immunol* (2015) 35(4):416–30. doi:10.1007/s10875-015-0152-6
14. Roifman CM. Studies of patients' thymi aid in the discovery and characterization of immunodeficiency in humans. *Immunol Rev* (2005) 203:143–55. doi:10.1111/j.0105-2896.2005.00236.x
15. Pickering LK, Baker CJ, Kimberlin DW, Long SS, editors. Immunocompromised children. *The Red Book: 2009 Report to the Committee on Infections Diseases*. 28th ed. Elk Grove Village, IL: American Academy of Pediatrics (2009). p. 72–86.
16. Griffith LM, Cowan MJ, Notarangelo LD, Puck JM, Buckley RH, Candotti F, et al. Improving cellular therapy for primary immune deficiency diseases: recognition, diagnosis, and management. *J Allergy Clin Immunol* (2009) 124:1152–60.e12. doi:10.1016/j.jaci.2009.10.022
17. Cowan MJ, Gennery AR. Radiation-sensitive severe combined immunodeficiency: the arguments for and against conditioning before hematopoietic cell transplantation – what to do? *J Allergy Clin Immunol* (2015) 136(5):1178–85. doi:10.016/j.jaci.2015.04.027
18. Dvorak CC, Hassan A, Slatter MA, Honig M, Lankester AC, Buckley RH, et al. Comparison of outcomes of hematopoietic stem cell transplantation without chemotherapy conditioning by using matched sibling and unrelated donors for treatment of severe combined immunodeficiency. *J Allergy Clin Immunol* (2014) 134(4):935–43.e15. doi:10.1016/j.jaci.2014.06.021
19. Haddad E, Leroy S, Buckley RH. B-cell reconstitution for SCID: should a conditioning regimen be used in SCID treatment? *J Allergy Clin Immunol* (2013) 131(4):994–1000. doi:10.16/j.jaci.2013.01.047
20. Albin-Leeds S, Ochoa J, Mehta H, Vogel BH, Caggana M, Bonagura V, et al. Idiopathic T cell lymphopenia identified in New York state newborn screening. *Clin Immunol* (2017) 183:36–40. doi:10.1016/j.clim.2017.07.002
21. de Felipe B, Olbrich P, Lucenas JM, Delgado-Pecellin C, Pavon-Delgado A, Marquez J, et al. Prospective neonatal screening for severe T- and B-lymphocyte deficiencies in Seville. *Pediatr Allergy Immunol* (2016) 27(1):70–7. doi:10.1111/pai.12501
22. Abraham RS, Recher M, Giliani S, Walter JE, Lee YN, Frugoni F, et al. Adult-onset manifestation of idiopathic T-cell lymphopenia due to a heterozygous RAG1 mutation. *J Allergy Clin Immunol* (2013) 131(5):1421–3. doi:10.016/j.jaci.2012.09.016
23. Kuijpers TW, Ijspeert H, van Leeuwen EM, Jansen MH, Hazenberg MD, Weijer KC, et al. Idiopathic CD4+ T lymphopenia without autoimmunity or granulomatous disease in the slipstream of rag mutations. *Blood* (2011) 117(22):5892–6. doi:10.1182/blood-2011-01-329052
24. Serwas NK, Cagdas D, Ban SA, Bienemann K, Salzer E, Tezcan I, et al. Identification of ITK deficiency as a novel genetic cause of idiopathic CD4+ T-cell lymphopenia. *Blood* (2014) 124(4):655–7. doi:10.1182/blood-2014-03-564930
25. Rios X, Chinn IK, Orange JS, Hanson CI, Rider NL. T-cell lymphopenia detected by newborn screening in two siblings with an Xq13.1 duplication. *Front Pediatr* (2017) 5:156. doi:10.3389/fped.2017.00156

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Exome Sequencing Identifies a Novel *MAP3K14* Mutation in Recessive Atypical Combined Immunodeficiency

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Primary immunodeficiency disorders (PIDs) render patients vulnerable to infection with a wide range of microorganisms and thus provide good *in vivo* models for the assessment of immune responses during infectious challenges. Priming of the immune system, especially in infancy, depends on different environmental exposures and medical practices. This may determine the timing and phenotype of clinical appearance of immune deficits as exemplified with early exposure to Bacillus Calmette-Guérin (BCG) vaccination and dissemination in combined immunodeficiencies. Varied phenotype expression poses a challenge to identification of the putative immune deficit. Without the availability of genomic diagnosis and data analysis resources and with limited capacity for functional definition of immune pathways, it is difficult to establish a definitive diagnosis and to decide on appropriate treatment. This study describes the use of exome sequencing to identify a homozygous recessive variant in *MAP3K14*, $NIK^{Val345Met}$, in a patient with combined immunodeficiency, disseminated BCG-osis, and paradoxically elevated lymphocytes. Laboratory testing confirmed hypogammaglobulinemia with normal CD19, but failed to confirm a definitive diagnosis for targeted treatment decisions. $NIK^{Val345Met}$ is predicted to be deleterious and pathogenic by two *in silico* prediction tools and is situated in a gene crucial for effective functioning of the non-canonical nuclear factor-kappa B signaling pathway. Functional analysis of $NIK^{Val345Met}$- versus NIK^{WT}-transfected human embryonic kidney-293T cells showed that this mutation significantly affects the kinase activity of NIK leading to decreased levels of phosphorylated IkappaB kinase-alpha (IKKα), the target of NIK. BCG-stimulated RAW264.7 cells transfected with $NIK^{Val345Met}$ also presented with reduced levels of phosphorylated IKKα, significantly increased p100 levels and significantly decreased p52 levels compared to cells transfected with NIK^{WT}. Ideally, these experiments would have been conducted in patient-derived immune cells, but we were unable to source these cells from the patient. The functional analysis described in this paper supports

previous illustrations of the importance of NIK in human immune responses and demonstrates the involvement of function-altering mutations in *MAP3K14* in PIDs. The genomic approach used for this patient demonstrates its value in the diagnosis of an unusual PID and as a tool for detecting rarer mutations to help guide treatment approaches.

Keywords: nuclear factor-kappa B-inducing kinase, primary immunodeficiency, tuberculosis, whole exome sequencing, BCG dissemination

INTRODUCTION

Primary immunodeficiency disorders (PIDs) are heritable genetic errors of the immune system that, if left undiagnosed and untreated, may lead to serious, chronic, and in some cases fatal infections and manifestations of autoimmunity (1, 2). PIDs provide *in vivo* models for identifying factors crucial for human host defense and immune regulation. The nuclear factor-kappa B (NF-κB) family of transcription factors found in mammals is crucial for the expression of developmental, inflammatory, as well as survival genes (3). This pathway consists of a canonical as well as a non-canonical arm, with the former involved in expression of pro-inflammatory genes, and the latter responsible for persistent, slower responses generally not associated with innate immune responses (4, 5).

The non-canonical NF-κB signaling pathway uses NF-κB-inducing kinase (NIK), encoded by mitogen-activated protein kinase (*MAP3K14*), to integrate signals from various membrane receptors, such as tumor necrosis factor alpha receptor family members (6). Several ligands can activate this pathway, including B cell-activating factor, CD40 ligand (CD40L), lymphotoxin beta, receptor activator of NF-κB ligand (RANKL), and TNF-like weak inducer of apoptosis (TWEAK) (7). Binding of these ligands to their appropriate receptors cause NIK to phosphorylate IkappaB kinase-alpha (IKKα), which activates and targets IKKα to p100, its substrate. p100 is in turn phosphorylated by IKKα, which prompts the ubiquitination and partial degradation of p100 to first produce p52 and second permit the formation of RelB-p52 complexes. These heterodimeric complexes move to the nucleus to activate target genes (3). This non-canonical NF-κB pathway controls lymphoid organogenesis, activation of dendritic cells and B cell maturation and survival, and errors in this pathway are associated with lymphoid disorders (5).

Although mortality rates due to *Mycobacterium tuberculosis* infections are increased in mice with genetically disrupted NF-κB, the role NF-κB plays in human immune responses to *M. tuberculosis* is not well understood (8). It has been speculated that some bacterial pathogens misuse specific NF-κB-mediated pathways to promote their survival (9). Activation of autophagy and apoptosis are two processes through which inhibition of NF-κB decreases the amount of intracellular bacilli after *M. tuberculosis* infection (10, 11). Autophagy is associated with innate and adaptive immune responses, as well as inflammation regulation (12). Ineffective autophagy has been implicated in several human diseases, including infectious diseases and inflammatory disorders (13–16). IkappaB kinase (IKK), the regulator of the NF-κB pathway, is required for autophagy activation in mammals, and inhibition of NF-κB increases autophagosome formation (17). Classic NF-κB is not involved in this response, and the mechanism by which IKK promotes stimulus-induced autophagy is largely unknown (18). NIK as well as IKKα are degraded by autophagy when the function of heat shock protein 90, required for the folding and maturation of certain signaling proteins, is inhibited (19). The processing of p100 and NF-κB activity is thus inhibited (20). However, when heat shock stress activates NF-κB, the autophagy pathway is in turn activated, indicating a close interaction and tight regulation between these two pathways (21).

This study identifies a novel potentially disease-causing variant in *NIK* in a South African PID patient using whole exome sequencing (WES). *In silico* analysis predicts the homozygous variant $NIK^{Val345Met}$ to be deleterious and pathogenic. Functional studies with human embryonic kidney (HEK)-293T cells and RAW264.7 cells transfected with either NIK^{WT} or $NIK^{Val345Met}$ showed that this mutation significantly affects the kinase activity of NIK, as well as p100 and p52 levels. The relationship between the non-canonical NF-κB signaling pathway and autophagy was also investigated in an attempt to shed some light on their poorly understood interaction. However, $NIK^{Val345Met}$ did not affect the autophagy pathway.

MATERIALS AND METHODS

Case Report

The proband is a white South African female, from a non-consanguineous marriage, initially diagnosed with humoral immunodeficiency after presenting with a Bacillus Calmette-Guérin (BCG) abscess on the upper leg at the age of 2 years. She received intravenous immunoglobulin (IVIG) replacement therapy. A year later she developed BCG meningitis and received standard treatment in the acute phase with isoniazid (INH), rifampicin (RIF), ethionamide (ETA), and dexamethasone, and thereafter only INH and RIF. At the age of 4 years, diffuse granulomas were identified in her brain. INH, RIF, ETA, and levofloxacin were prescribed for a year, after which the levofloxacin was discontinued. In 2014, at the age of 6 years, she presented with acute loss of consciousness and raised intracranial pressure. *Mycobacterium bovis* BCG genotypically sensitive to INH and RIF was subsequently cultured from the patient. She was started on a very aggressive 18-month treatment regimen of levofloxacin, terizidone, amikacin IV, linezolid, RIF, INH, para-aminosalisylic acid, as well as continued IVIG replacement therapy. With

continued dissemination of BCG in spite of the above treatment and in the absence of a confirmed PID diagnostic category, the patient was not selected for bone marrow transplantation. She proceeded to develop severe neurological, motor, as well as cognitive impairment and is now in total dependency care 6 years after initial presentation.

WES Analysis

The study was approved by the Health Research Ethics Committee of Stellenbosch University (approval no. N13/05/075). Written informed consent was granted by the parents of the patient, and this included genetic evaluation of the patient. The parents also consented to the publication of any molecular findings. The study adhered to the ethical guidelines as set out in the "Declaration of Helsinki, 2013" (22). Venous blood for DNA extraction and WES was drawn from the patient and both of her parents. DNA was purified from blood using the Nucleon BACC3 Kit (Amersham Biosciences, Buckinghamshire, UK).

Enrichment and sequencing of the exomes of the proband and both of her healthy parents were performed with Illumina's TruSeq Exome Enrichment Kit. It targets >20,000 genes with >200,000 exons as well as 9 Mb of predicted microRNA targets with a total size of 62 Mb. Paired-end WES of the three samples was carried out on the Illumina HiSeq 2000, yielding an average of 120 million reads per sample and resulting in an average coverage of the target regions of 60–80× after duplicate removal. The resulting FASTQ file containing the sequencing data underwent quality control in FastQC and was mapped against the human reference genome hg38 using Burrows-Wheeler Aligner (BWA). PCR duplicates were removed using Picard, while SamTools and Genome Analysis Toolkit (GATK) were used in parallel for the detection of single-nucleotide variants (SNVs). The variant calls from both callers were pooled into a single set, after which ANNOVAR was used for SNV annotation and filtering, and to interrogate a number of programs and databases for each called position to generate more evidence of deleterious mutations. The basic filtering options in ANNOVAR used are (1) filtering out common SNVs unlikely to be disease-causing based on a frequency threshold of >1% in the 1000 Genomes Project (1000GP) data (23) and the Exome Sequencing Project 6500 (ESP6500) data (24); (2) restricting the SNVs to those causing amino acid changes in the protein; (3) assessing the impact on protein structure through prediction tools; and (4) the presence of a gene or SNV in Online Mendelian Inheritance in Man (OMIM) or Human Gene Mutation Database (HGMD), which shows known disease associations. All variants with negative Genomic Evolutionary Rate Profiling (GERP)+++ scores as well as all variants with Functional Analysis through Hidden Markov Models (FATHMM) scores greater than 0.1 were also removed. GERP estimates evolutionary constraints at specific positions in an exome and identifies "constrained elements," where several positions combine to produce a signal indicative of a putative functional element (25). FATHMM is a high-throughput webserver that can predict the functional consequences of coding and non-coding variants (26). Finally, variants were also removed if they were homozygous in either of the healthy parents of the proband.

Frequency Investigation

The 1000GP data, ESP6500, and ExAC Browser were investigated for the presence of all potentially disease-causing variants. Only those present in less than 1% of the population in these databases were considered as candidate variants.

In Silico Prediction

Project HOPE [Have (y)Our Protein Explained] (27), PolyPhen-2 (28), Sorting Tolerant From Intolerant (SIFT) (29), and MutationTaster2 (30) were used to predict the functional and structural causes of the amino acid changes on proteins.

Variant Verification

Sanger sequencing was used to validate the WES results and verify whether the potentially disease-causing variants identified were true variants or sequencing artifacts. Only one potentially disease-causing variant was identified, for which the forward and reverse primers F: 5′AGCCCTGGAAACCTCACC and R: 5′TGAGATTGGCGGAATAAGAGA were used to produce a fragment of 455 bp. This was bi-directionally sequenced at the Central Analytical Facility of Stellenbosch University using the BigDye® Terminator v3.1 Cycle Sequencing kit (Perkin-Elmer, Applied Biosystems Inc., Foster City, CA, USA), followed by electrophoresis on an ABI 3130XL Genetic Analyzer (Perkin-Elmer, Applied Biosystems Inc., Foster City, CA, USA).

In Vitro Functional Analysis

Two plasmids, pWZL-Neo-Myr-Flag-MAP3K14 and pCR-Flag-IKKalpha, were obtained from the non-profit plasmid repository Addgene (https://www.addgene.org/). pWZL-Neo-Myr-Flag-MAP3K14 was a gift from William Hahn & Jean Zhao (Addgene plasmid # 20640) (31) and pCR-Flag-IKKalpha was a gift from Hiroyasu Nakano (Addgene plasmid # 15467) (32). To generate the pWZL-Neo-Myr-Flag-MAP3K14^{Val345Met} mutant construct, the mutation-specific oligo nucleotide primers F: 5′AAGGCAGCGTGAGCTC and R: 5′CAGAGCATGCACTAGGTAT were used together with the Q5® Site Directed Mutagenesis Kit (New England Biolabs Inc., UK) as per the manufacturer's instructions. Sanger sequencing was used to confirm successful mutagenesis.

Cell Culture and Transfection

Human embryonic kidney-293T cells and RAW264.7 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) containing 4.5 g/L glucose with L-glutamine and supplemented with 10% FBS and 1% penicillin/streptomycin. One day before transfection, the cells were trypsinized and seeded into six-well culture plates so that the cells were 50–80% confluent the following day. Plasmid DNA was purified from the IKKα as well as the wild-type and mutant MAP3K14 plasmids using the PureYield™ Plasmid Miniprep System (Promega Corp., USA) according to the manufacturer's guidelines. The IKKα plasmid DNA combined with either the wild-type or mutant MAP3K14 plasmid DNA were then transfected into the seeded HEK293T, while only the wild-type or mutant MAP3K14 plasmids were transfected into RAW264.7 cells using Lipofectamine™ LTX

Reagent and PLUS™ Reagent (Invitrogen, USA) according to the manufacturer's instructions.

BCG Treatment of Cells

The RAW264.7 cells were divided into stimulated and unstimulated groups, each group consisting of untransfected, $NIK^{Val345Met}$-transfected, and NIK^{WT}-transfected sub-groups. After transfection of the appropriate plasmids into each sub-group, BCG was added to each well in the six-well plates making up the stimulated group, at a multiplicity of infection of 5. A stock solution BCG with a concentration of 1.46×10^6 CFUs/mL was prepared using BCG Vaccine SSI (Statens Serum Institut, Denmark) and Diluted Sauton SSI (Statens Serum Institut, Denmark). Per well containing 1×10^6 cells growing in 6 mL growing media, 34 µL of this BCG stock solution was added. Cells were stimulated for 16 h at 37°C.

Bafilomycin Treatment of Cells

The transfected HEK293T cells used for investigation of autophagy were divided into two groups. One received Bafilomycin A1 (Baf) treatment while the other remained untreated. Baf is a known inhibitor of the late phase of autophagy and prevents maturation of autophagic vacuoles by inhibiting fusion between autophagosomes and lysosomes (33). A Baf stock solution with a concentration of 1 mM was made using DMSO and Bafilomycin A1, which was further diluted to 1 µM by adding PBS. Each well in one six-well plate seeded with HEK293T cells received 150 µL of the 1 µM Baf stock and 1,350 µL growth media, and constituted the Baf treatment group. Another plate received only 1,500 µL growth media and constituted the control group. The plates were incubated for 16 h at 37°C.

Cell Lysis and Western Blotting

All cells were lysed with lysis buffer [0.05 M Hepes, 0.1 M NaCl, 0.01 M EDTA, 0.17 mM Triton X-100, 4 mM Nappi, 2 mM Na_3VO_4, and protease inhibitors (Roche)] at 95°C for 5 min and all lysates were stored at −80°C. The lysates were subjected to Sodium dodecyl sulfate polyacrylamide gel electrophoresis SDS/PAGE on Mini-PROTEAN® TGX™ precast polyacrylamide gels [Bio-Rad Laboratories (Pty) Ltd., RSA] containing 1% SDS, after which the proteins were transferred to 0.2-µm-pore polyvinylidine difluoride membranes using the iBlot® Dry Blotting system (Invitrogen, RSA). Membranes were blocked in 5% BSA (w/v) or 5% low fat milk and subsequently probed with the rabbit antibodies phospho-IKKα/β (Ser176/180) (16A6), IKKα, NF-κB p100/p52, NIK (Cell Signaling Technology, Inc., USA; Abcam Inc., UK) and LC3B (Abcam Inc., UK), as well as mouse GAPDH antibody (Santa Cruz Biotechnology Inc., USA). Membranes were then exposed to horseradish peroxidase-conjugated goat anti-rabbit and donkey anti-mouse secondary antibodies (Santa Cruz Biotechnology, Inc., USA) and the proteins were subsequently visualized by enhanced chemiluminescence using Clarity™ Western ECL Substrate (Bio-Rad Laboratories, Inc., USA).

Statistics

The open platform image analysis tool ImageJ was used for analysis of all western blots. All experiments were done in true biological triplicates and under the same conditions. One-tailed unpaired *t*-tests were used to determine significant differences between samples. All western blots were normalized using GAPDH.

RESULTS

The patient tested negative for the human immunodeficiency virus as well as Herpes simplex virus and presented with severe hypogammaglobulinemia with decreased immunoglobulin (Ig) levels (IgA < 0.06 G/L, IgM = 0.14 G/L, IgG = 0.42 G/L), increased lymphocyte subset numbers for her age and normal lymphocyte proliferation to mitogens and recall antigens. She was investigated for Mendelian susceptibility to mycobacterial disease (MSMD) by screening *STAT1*, *LRBA*, *IL12RB1*, *IL12B*, and *IFNGR1* for possible disease-causing mutations. No mutations were found in any of these genes. Longitudinal immunological investigation of this patient with persistent, disseminated, treatment-resistant *M. bovis* BCG infection indicated a dramatic decrease of natural killer (NK) cells, B cells, and CD8 cells over time (**Table 1**). Her $CD27^+IgD^+$ cell population was decreased (2.96%) and class-switched memory B-cells ($CD27^+IgD^-$) were also low (0.12%), while CD40 ligand (CD40L) was detected as present. Memory T cells were low in relation to naïve T cells and reduced levels of γ/δ T cells were observed—cells known to be involved in the innate immune reaction against mycobacteria. The patient's phytohemagglutinin control lymphocyte proliferation was normal on the T Spot TB test with negative proliferation to specific TB antigens. This is what would be expected, since the test is specific for TB antigens, not BCG—the cause of disease dissemination observed in this patient. Upregulation of CD69 on NK cells after interleukin-2 stimulation

TABLE 1 | Normal cell counts versus patient total cell counts at different ages.

Cell counts of the patient at different ages						**Reference**
Subsets	2 years, 1 month	2 years, 3 months	4 years, 8 months	5 years, 1 months	6 years	2–6 years
Lymphocytes	15,774 (H)	9,522 (H)	6,720 (H)	3,999	2,366	2,340–5,028
T cells	10,719 (H)	6,785 (H)	4,682 (H)	2,655	1,886	1,578–3,707
CD4+	8,743 (H)	5,523 (H)	3,954 (H)	2,258 (H)	1,516	870–2,144
CD8+	1,584 (H)	959	813	533	369 (L)	472–1,107
NK cells	2,549 (H)	1,263 (H)	214	778 (H)	70 (L)	155–565
B cells	2,156 (H)	1,339 (H)	1,783 (H)	398 (L)	362 (L)	434–1,274

Values are given in cells per microliter.

CD, cluster of differentiation; H, high counts; L, low counts; NK, natural killer.

was slightly decreased. T cell receptor excision circles (TRECs) and kappa-deleting recombination excision circles were clearly visible (AMPATH Laboratories, South Africa). Investigation by WES was thus pursued to assist with establishing a diagnosis for this patient.

Identification of a Homozygous Mutation in NIK

Whole exome sequencing of the proband revealed a total of 23,939 variants, which were reduced to 708 candidate variants after filtering and annotation using ANNOVAR (**Table 2**). Upon exclusion of all variants present in a homozygous state in either of the healthy parents, 9 homozygous and 23 heterozygous variants remained (**Table 3**). After interrogating OMIM and HGMD, only one variant was identified as potentially disease-causing according to the function of the gene it is situated in. This homozygous c.G1033A p.Val345Met nucleotide variant is situated in exon 5 of *NIK* (**Table 4**). SIFT and PolyPhen-2 predicted this change to be pathogenic and damaging, while MutationTaster2 was not able to generate any results, indicating the uncharacterized state of *NIK*. The variant is situated at a position that is highly conserved across several different species (**Figure 1**).

This variant was covered 65× during WES, improving its likelihood of being a true variant rather than a sequencing artifact. The average coverage for all the variants identified in the proband was 105×. Sanger sequencing confirmed this to be a true variant present in a homozygous state in the patient and heterozygous in both unaffected parents (**Figure 2**). $NIK^{Val345Met}$ was absent from all interrogated databases and therefore previously unidentified in all investigated populations. No missense or loss-of-function mutations in *NIK* are listed in these databases.

In Silico Variant Prediction

A schematic representation of the amino acid exchange caused by this variant, with wild-type valine (Val) on the left and mutated methionine (Met) on the right, is shown in **Figure 3A**. Met and Val are both non-polar, hydrophobic, and aliphatic amino acids. **Figure 3B** shows the difference in size between Val and Met. The main difference is the presence of a C-beta branch in Val: two non-hydrogen substituents attached to its C-beta carbon, instead of only one as for Met. Met also has an additional sulfur atom, which forms very strong amide N–H·S hydrogen bonds crucial for controlling the conformational setting of this amino acid (35). Val is bulkier near the protein backbone and more restricted in the conformations the main-chain can adopt. The Val side chain is extremely non-reactive and is rarely directly involved in protein function, although it can play a role in substrate recognition. The site of variation is situated near a conserved site.

The HOPE analysis indicated that Val345Met is located 45 amino acid positions before the interpro domain known as mitogen-activated protein kinase kinase kinase 14 (IPR017425), associated with protein kinase and transferase activity. This kinase domain stretches from positions 390 to 660 (**Figure 4**). Because of its close proximity to the kinase domain, it is tempting to speculate that Val345Met may have an effect on the ability of NIK to phosphorylate IKKα. This domain is in contact with residues from other domains, implying the possibility for the variant to influence correct protein function by inhibiting/altering these interactions.

TABLE 2 | Variants identified by WES in the proband and both parents.

	Father	Mother	Patient
Total variants	23,440	23,474	23,939
All synonymous and non-frameshifts removed	11,707	11,693	11,925
Remove all variants with a frequency >1% in 1KGP	2,357	2,377	2,495
Remove all variants with a frequency >1% in ESP6500	2,077	2,107	2,160
Remove all variants with negative GERP+++ scores	1,514	1,481	1,535
Remove all variants with positive FATHMM scores	703	688	708
Novel variants	108	113	114
Variants with rs numbers	595	575	594

ESP6500, Exome Sequencing Project 6500; GERP, Genomic Evolutionary Rate Profiling; FATHMM, Functional Analysis through Hidden Markov Models; WES, whole exome sequencing; 1KGP, 1000 Genome Project.

Plasmid Transfection into Human Cells

To experimentally assess the effect of the mutation of interest, we analyzed the kinase activity of $NIK^{Val345Met}$ compared to NIK^{WT} by testing NIK-dependent phosphorylation of IKKα. Sanger sequencing first confirmed that the mutagenesis occurred correctly (Figure S1 in Supplementary Material). IKKα in combination with either wild-type or mutant NIK were transfected into HEK293T cells, while only NIK, in either its wild-type or mutant form, was transfected into RAW264.7 cells. The NIK and IKKα antibodies were then used to show that transfection of the plasmids expressing IKKα and mutant and wild-type NIK into HEK293T and RAW264.7 cells was successful. Untransfected HEK293T cells do not contain endogenous NIK, although they do contain IKKα, while RAW264.7 cells contain both NIK and IKKα endogenously. As seen in Figure S2 in Supplementary Material, there was an increase of both the expression of IKKα in both wild-type and mutant NIK cotransfected HEK 293T cells (although this increase did not reach statistical significance; $p = 0.0638$). Similarly, there was an increase in NIK levels in RAW264.7 cells following transfection with wild-type and mutant NIK constructs (Figure S3 in Supplementary Material). No significant differences are observed between the transfection efficiencies of wild-type- and mutant-transfected cells in either of these cell types.

Phosphorylation Assay

IkappaB kinase-alpha and phospho-IKKα/β (Ser176/180) antibodies were used to investigate the difference between IKKα and phospho-IKKα levels in NIK^{WT} compared to $NIK^{Val345Met}$. $NIK^{Val345Met}$ was not thought to affect the production of IKKα and as expected, the level of IKKα was not substantially altered by this mutation (**Figure 5A**: $p = 0.3879$; **Figures 6A,C,E**; unstimulated: $p = 0.4757$; stimulated: $p = 0.1844$). However,

TABLE 3 | Final list of variants identified in the proband after filtering.

Gene	Variant	ExAC	dbSNP	CADD_phred scores
Homozygous variants				
CELA1	c.6_7insC; p.V3fs	0.383	–	–
FOXD4	c.748_749del:p.G250fs	0.189	–	–
FOXD4	c.753_754insCG:p.G252fs	0.189	–	–
GJD3	c.C523T:p.H175Y	0.00401	rs202055764	13.57
GJD3	c.G758C:p.R253P	0.00286	rs532965992	–
LRRC46	c.10_11insGT:p.G4fs	0.000602	rs536101939	–
MAP3K14	c.G1033A:p.V345M	–	–	18.77
NBPF1	Unknown	0.52	rs2990550	–
SYN2	Unknown	0.000729	–	–
Heterozygous variants				
C2CD4C	c.C205A:p.L69M	0.00134	rs200204713	10.36
CASP5	c.67delA:p.R23fs	–	rs372526393	–
CCDC150	c.839delA:p.Q280fs	–	rs376590781	–
CD36	c.G1016T:p.G339V	0.000602	rs146027667	21.3
CDC27	c.C1697T:p.A566V	–	–	36
CEP164	c.337delA:p.K113fs	–	–	–
CES1	c.A145G:p.I49V	0.303	rs3826193	10.91
CES1	c.G53T:p.G18V	0.285	rs3826190	18.35
CXorf40B	c.T159G:p.C53W	0.00747	rs140921811	1,305
FBXW10	c.T2552C:p.V851A	0.000155	rs199779085	9,638
FOXD4L1	c.A463G:p.I155V	0.000705	rs199845792	18.09
KRT18	c.C300G:p.S100R	–	–	16.24
KRT18	c.C308A:p.T103N	–	–	14.54
KRT18	c.C316T:p.R106W	–	rs11551638	14.74
MTCH2	c.G196A:p.G66R	–	–	27.9
OPALIN	c.G52T:p.A18S	0.00449	rs35821065	25.2
PAK2	c.A383G:p.K128R	0.028	rs78043821	21.6
PLEC	c.G8992A:p.E2998K	0.000982	rs200898220	7,527
RASA4, RASA4B	c.A1054G:p.M352V	0.124	rs144395384	7.05
SERPINA4	c.C403T:p.R135C	0.0000244	–	15.31
SLC11A2	c.C1291A:p.L431I	0.00251	rs144863268	19.37
SPDYE6	c.C890T:p.P297L	0.00348	rs202078839	–
UBXN11	c.1104_1181del:p.368_394del	0.301	–	–

TABLE 4 | Details of the putative disease-causing variant identified in the proband.

Chromosome	17
Position	45286550
Gene name	*MAP3K14/NIK*
Refseq	NM_003954.4
Reference sequence	G
PROBAND: number of reads with reference	0
FATHER: number of reads with reference	22
MOTHER: number of reads with reference	17
Alternative sequence	A
PROBAND: number of reads with alternative	65
FATHER: number of reads with alternative	26
MOTHER: number of reads with alternative	20
Mutation type	Nonsynonymous SNV
Mutation: DNA (HGVS nomenclature_c.)	c.G1033A
Mutation: protein (HGVS nomenclature_p.)	p.VAL345MET (NP_003945.2)
Prediction < SIFT	Damaging
Prediction < PolyPhen-2	Probably damaging
Prediction < MutationTaster2	N/A
Sanger verification	Yes

NIK, NF-κB-inducing kinase; SNV, single-nucleotide variant; SIFT, Sorting Tolerant From Intolerant; HGVS, Human Genome Variation Society; N/A, not applicable.

phosphorylated IKKα levels were significantly reduced by $NIK^{Val345Met}$ in both HEK293T (**Figure 5A**; $p = 0.0353$) and RAW264.7 (**Figures 6B,D,F**; unstimulated: $p = 0.0010$; stimulated: $p < 0.0001$) cells, indicating that this mutation alters the kinase activity of NIK. The ratio of phosphorylated IKKα to un-phosphorylated IKKα is shown in **Figure 6G**, with a significant difference observed between the wild-type-transfected and the mutant-transfected cell groups that have been stimulated with BCG ($p = 0.0090$).

Downstream Functional Effects of $NIK^{Val345Met}$

Phosphorylated IKKα results in the ubiquitination and proteosomal degradation of p100 to produce p52. The difference in p100 and p52 levels between cells expressing wild-type and mutant NIK was thus also investigated to determine whether $NIK^{Val345Met}$ has any downstream effects. The ideal would have been to use patient-derived immune cells for these experiments to more accurately measure the downstream effects of the identified mutation. Unfortunately, the patient deteriorated to such an extent that we could not obtain more blood samples from her. HEK293T cells, that are incapable of eliciting an immune response upon infection, were first used. However, no quantifiable levels of p52 were detected (**Figure 5B**), while no significant differences in the levels of p100 were observed between wild-type- and mutant-transfected groups (**Figure 5B**; $p = 0.7409$). p100 and p52 levels were also measured in

```
Human              LEPSCLSRGAHEKFSVEEYLVHALQGSVSSGQAHSLTSLAKTWAARGSRSREPSPKTEDN
King cobra         SKCHQSAKNTSDTFSMDEFLVDALKGNVILGAPKNLACLAKTWKDGSSSKV-CLQEINEN
Philippine tarsier LEPSYVCRGPYKQFSVEEYLVHALQGSVSSGPAHSLASLAKTWAAGGSRPWEPSPETEDS
House mouse        LESSCPSRGALEKVPVEEYLVHALQGSVSSGQAHSLASLAKTWSSGSAKLQRLGPETEDN
Mongolian gerbil   LESSYPAQGAQEKVPVEEYLIHALQGSVSSGQANSLASLAKTWSSGSAKLQRLSPETEDN
Red deer           LKPSCPSRGSSDKLSVEEYLVHALQGSVSSGQAHSLASLAKTWSVGGSRPQEPNPETEDS
Monk seal          LEPGCPSRGPREKFSVEEYLVHALQGSVSSGQAHSLASLAKTWSAGGSKPRKPSPETEDS
Ground squirrel    LEPSFPTQSSHEKF-VEEYLVHALQGSVSSGQAHSLASLAKTWSAGGSRAQRPSPETEDN
Night monkey       LEPSCPSRSAHEKFSVEEYLVHALQGSVSSGQAHSLTSLAKTWAAGGSRPREPSPKTEDN
Olive baboon       LEPSCPSRGAHEKFSVEEYLVHALQGSVSSGQAHSLTSLAKTWAAGGSRPREPSPKTEDN
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FIGURE 1 | ClustalW multiple sequence alignment. The c.G1033A (p.Val345Met) homozygous mutation identified in this patient is situated at a highly conserved position in *MAP3K14* (34).

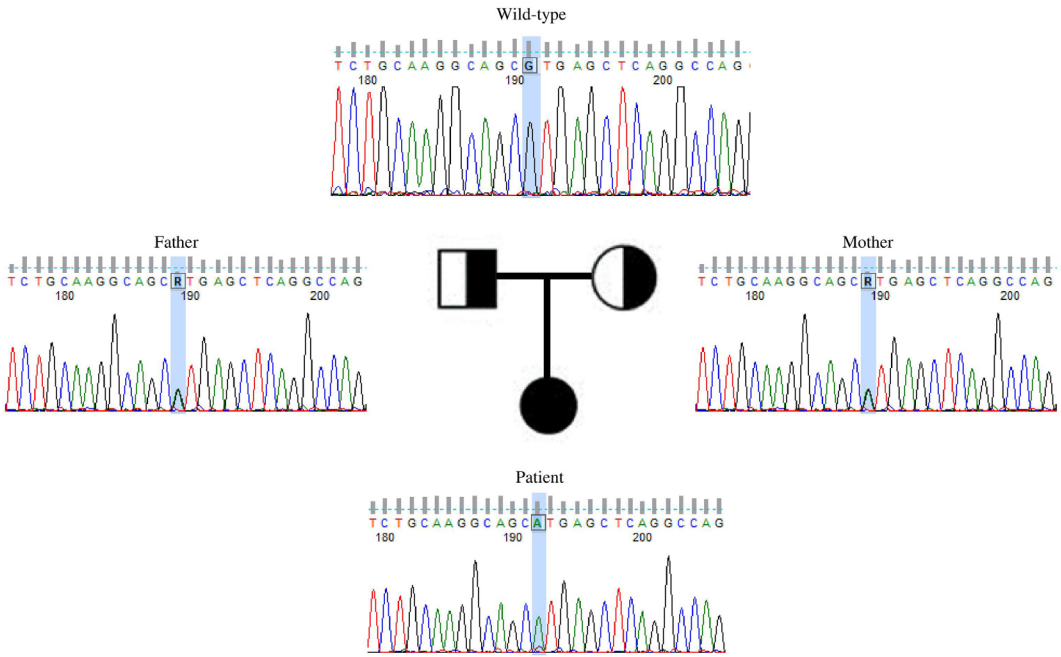

FIGURE 2 | Validation by Sanger sequencing of *NIK*^{Val345Met} found by whole exome sequencing. Patient's c.G1033A homozygous mutation, with one mutated allele inherited from each of the healthy, heterozygous parents.

BCG-stimulated and unstimulated RAW264.7 cells transfected with NIK^{Val345Met} and NIK^{WT}. In BCG-stimulated cells, p100 levels were significantly increased ($p = 0.0458$) and p52 levels decreased ($p = 0.0208$) in the mutant compared to the wild-type groups (**Figures 6C,H,I**). The difference in p100 levels between the mutant and wild-type groups was not significant in BCG-unstimulated RAW264.7 cells (**Figures 6B,I**; $p = 0.1745$), although the decrease in p52 levels remained significant in the unstimulated cells (**Figures 6B,H**; $p = 0.0330$). The NIK^{Val345Met} variant thus significantly affects the downstream functioning of the non-canonical NF-κB signaling pathway. The ratio of p52 to p100 is shown in **Figure 6J**, with significant differences observed between the wild-type-transfected and the mutant-transfected cell groups in both BCG-stimulated and unstimulated cell populations (unstimulated: $p = 0.0077$; stimulated: $p = 0.0053$).

Effect of NIK^{Val345Met} on Autophagy

Autophagy is a dynamic catabolic process during which double-membrane vesicles (autophagosomes) form by engulfing parts of the cytoplasm and subsequently fuse with lysosomes to degrade and recycle their contents (36). LC3 immunoblotting is widely used to measure autophagic activity (37–39). SDS-PAGE and immunoblotting detects two bands of endogenous LC3, namely LC3-I and LC3-II. LC3-I is present in the cytosol, while LC3-II is bound to phosphatidylethanolamine and localized to autophagosomal membranes (40, 41), thus directly correlating with the number of autophagosomes (42). The effect of NIK^{Val345Met} on autophagy was also investigated in this study by measuring LC3-II levels. The western blots and corresponding bar graphs are shown in **Figure 5C**. Significant differences in LC3-II were observed when comparing cells transfected with NIK^{WT} before and after Baf treatment ($p = 0.0418$), as well as those infected

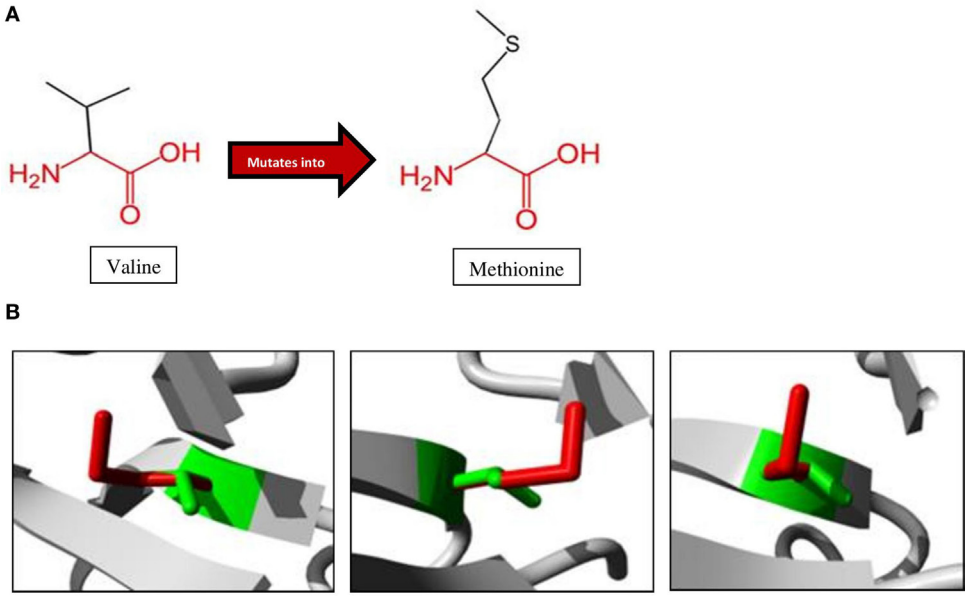

FIGURE 3 | Amino acid changes caused by the mutation identified in the proband. **(A)** Mutation of Val to Met, as observed at position 345 in *NIK* of the patient. The conserved backbone is indicated in red, with the unique side-chain in black. **(B)** Mutant Met (red) is larger than wild-type Val (green), potentially influencing interactions within the protein as well as with other proteins (27).

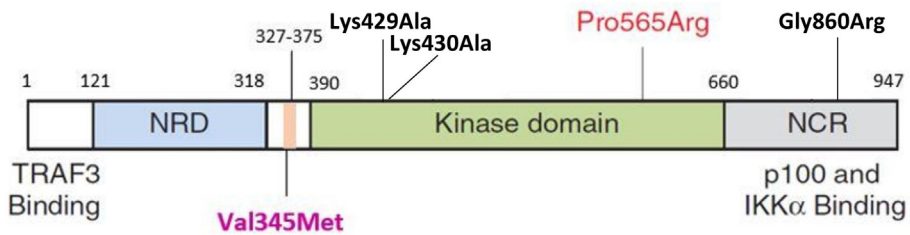

FIGURE 4 | Schematic representation of NIK protein domain structures. The NRD (light blue) contains the BR and the P-RR domains (darker blue). The conserved domain containing V345M (purple label) is indicated in pink and situated before the kinase domain (green). This is followed by the NCR (gray). The P565R amino acid change observed in P1 and P2 by Willmann et al. is situated in the kinase domain. Black labels indicate the mutations previously observed in the murine studies (Lys429Ala; Lys430Ala; Gly860Arg). TRAF3, tumor necrosis factor receptor-associated factor 3; NRD, negative regulatory domain; BR, basic region; P-RR, proline-rich repeat; NCR, non-catalytic region; Lys, lysine; Ala, alanine; Pro, proline; Arg, arginine; Gly, glycine; Val, valine; Met, methionine; IKKα, IkappaB kinase-alpha; NIK, NF-κB inducing kinase (44).

with $NIK^{Val345Met}$ before and after treatment ($p = 0.0366$), thus indicating effective functioning of the autophagic pathway. When comparing the difference in LC3-II levels between mutant and wild-type groups (**Figure 5C**), however, no significant difference was observed ($p = 0.9506$), indicating that $NIK^{Val345Met}$ has no effect on autophagy.

DISCUSSION

Phenotype heterogeneity is often observed in patients with mutations in the same gene, and this may in part depend on the patient's environment (43). We identified a patient with a mutation in *NIK* where the phenotype was modified by early BCG exposure as part of routine vaccination in the first days of life.

Only one previous study to date has described mutations in *NIK* in humans and identified a bi-allelic mutation as the cause of a primary immunodeficiency characterized by multifaceted aberrant lymphoid immunity in two patients (44). Patient 1 (P1) was born to consanguineous healthy parents and had a younger brother who died at the age of 2 years from suspected combined immunodeficiency. Decreased IgG and IgA and elevated IgM levels were identified on a single occasion before any treatment was prescribed. Despite treatment (such as regular intravenous Ig substitution and ganciclovir), the patient continued to suffer from multiple episodes of bacterial and viral infections. There was also one documented episode of granulomatous hepatitis and tuberculosis osteomyelitis due to dissemination after BCG vaccination. An allogeneic hematopoietic stem cell transplantation (HSCT) after reduced toxicity conditioning was performed at the

age of 9 years, and upon last report in 2014 she remained clinically well. Patient 2 (P2) is a first-degree cousin of P1 also born to consanguineous healthy parents. She presented with severe chronic diarrhea, recurrent lower respiratory tract infections, as well as oral and esophageal candidiasis. She tested positive for *Cryptosporidium* on one occasion. Her human leukocyte antigen-identical mother was used as a donor for an allogeneic HSCT performed without conditioning at the age of 3 years. As no engraftment was observed after 50 days, the same donor was used and a second transplant was performed. However, the patient died on day 6 following the second HSCT due to rapidly accelerated septic shock and multi-organ failure.

We describe a patient with similar symptoms: initial diagnosis of humoral immunodeficiency with severe hypogammaglobulinemia, decreased memory B cells, B cell lymphopenia, normal T cell proliferation to mitogens and recall antigens, as well as normal IFN-γ production by T cells (**Table 1**). However, while the patients from the previous study presented with recurrent

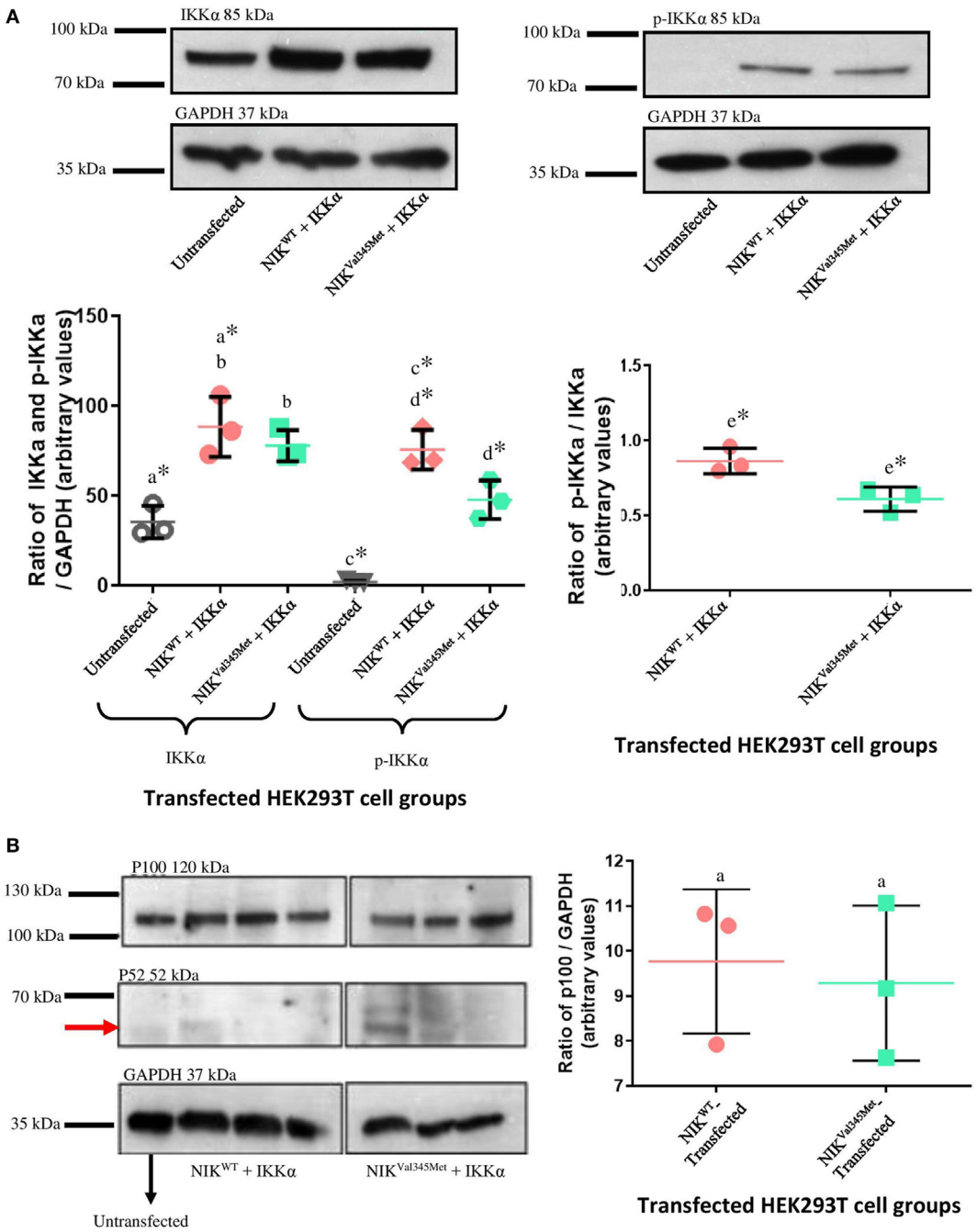

FIGURE 5 | Continued

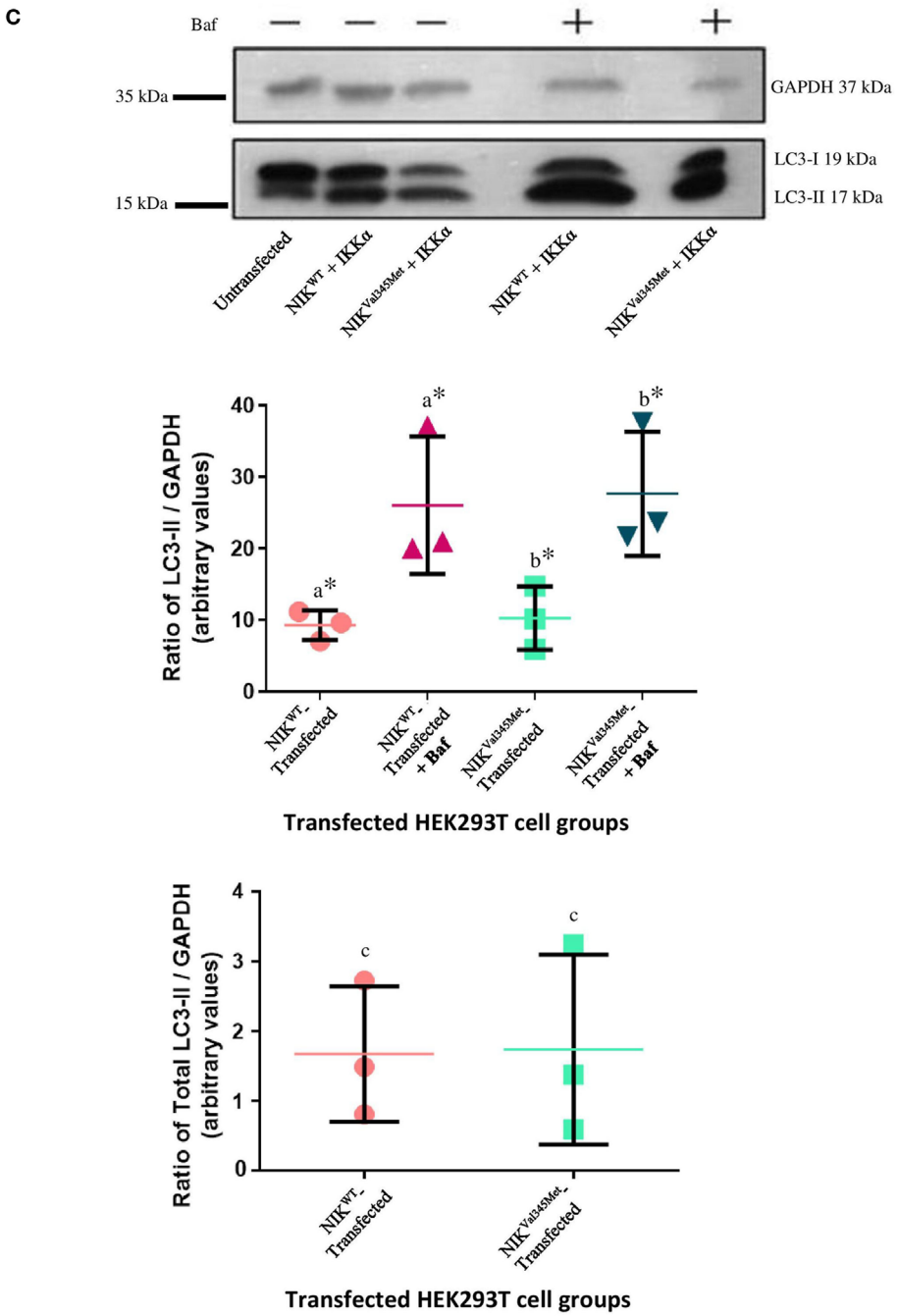

FIGURE 5 | Comparison between NIK^{Val345Met}- and NIK^{WT}-transfected HEK293T cells. **(A)** No phospho-IKKα was detected in untransfected HEK293T cells, with significant amounts present in transfected cells, proving efficient transfection. A significant decrease in phospho-IKKα is observed in cells transfected with *NIK*^{Val345Met} compared to *NIK*^{WT}. The ratio of phospho-IKKα over IKKα is also significantly affected by *NIK*^{Val345Met}. a: $p = 0.0084$*; b: $p = 0.3879$; c: $p = 0.0003$*; d: $p = 0.0353$*; e: $p = 0.0199$*. **(B)** No quantifiable p52 levels were observed in untransfected, wild-type-transfected, or mutant-transfected samples. p100 was detectable at significant amounts, but no differences between the wild-type and mutant groups were observed. a: $p = 0.7409$. **(C)** Significant differences were seen when comparing cells transfected with wild-type *NIK* and *IKKα* before and after Baf treatment, as well as mutant *NIK* and *IKKα* before and after treatment. However, the difference in autophagy between wild-type-transfected and mutant-transfected groups was not significant. a: $p = 0.0418$*; b: $p = 0.0366$*; c: $p = 0.9506$. Each image is representative of three experiments conducted independently. * indicates significance.

viral, bacterial, and *Cryptosporidium* infections, the phenotype of our patient seems to be confined to BCG-osis. Our patient did not present with any other infections. WES and subsequent bioinformatics analysis identified a novel putative disease-causing homozygous variant, c.G1033A, situated in *NIK*. *NIK* encodes the 947 residue protein mitogen-activated protein kinase 14, which

is a serine/threonine protein kinase involved in NF-κB activity (NM_003954.4). The *NIK*^{Val345Met} variant identified in this patient has never been described and is predicted to be disease-causing and deleterious by two *in silico* prediction tools. It is difficult to predict the exact result of this variant on protein function, since *NIK* is poorly characterized. The variant is situated in a

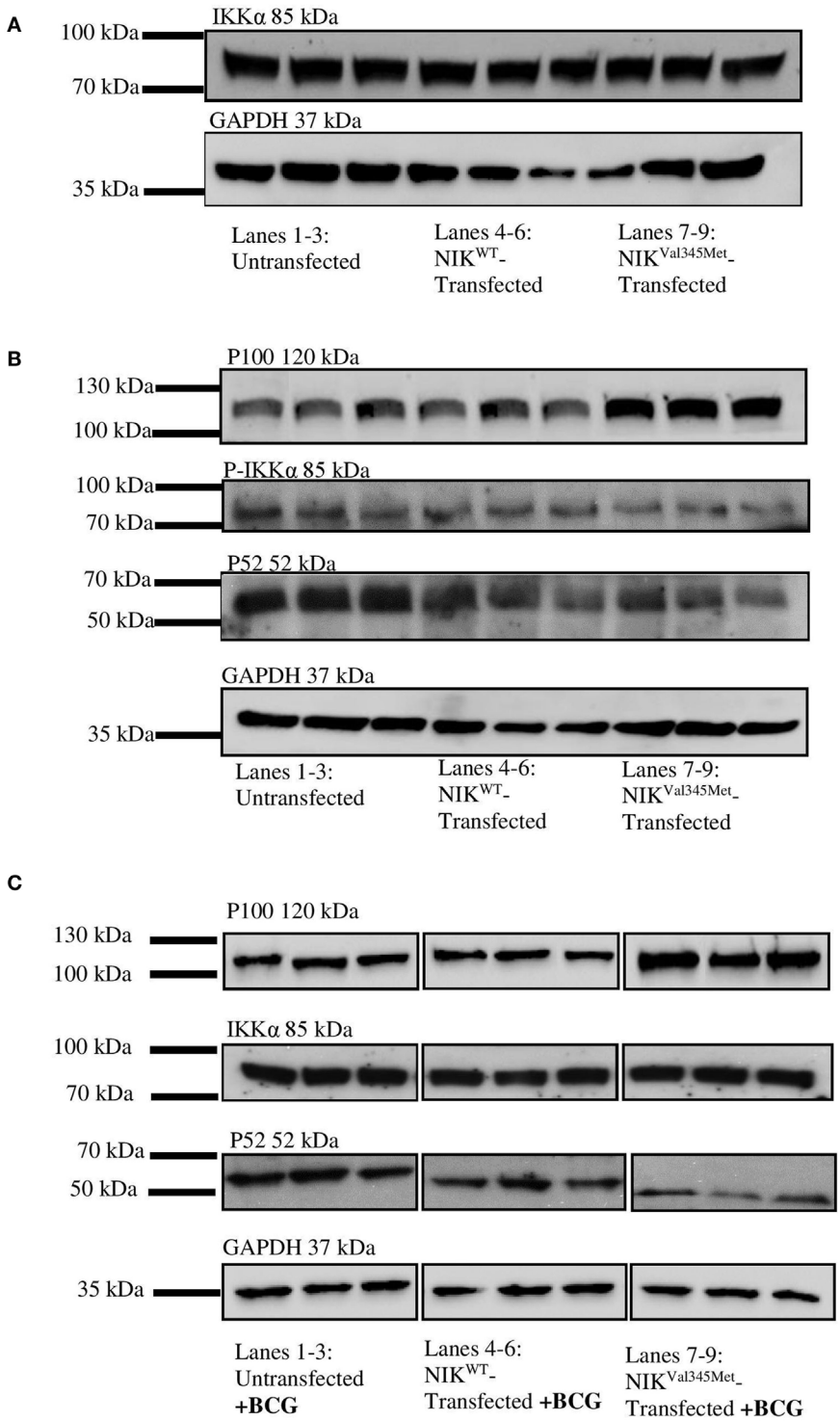

FIGURE 6 | Continued

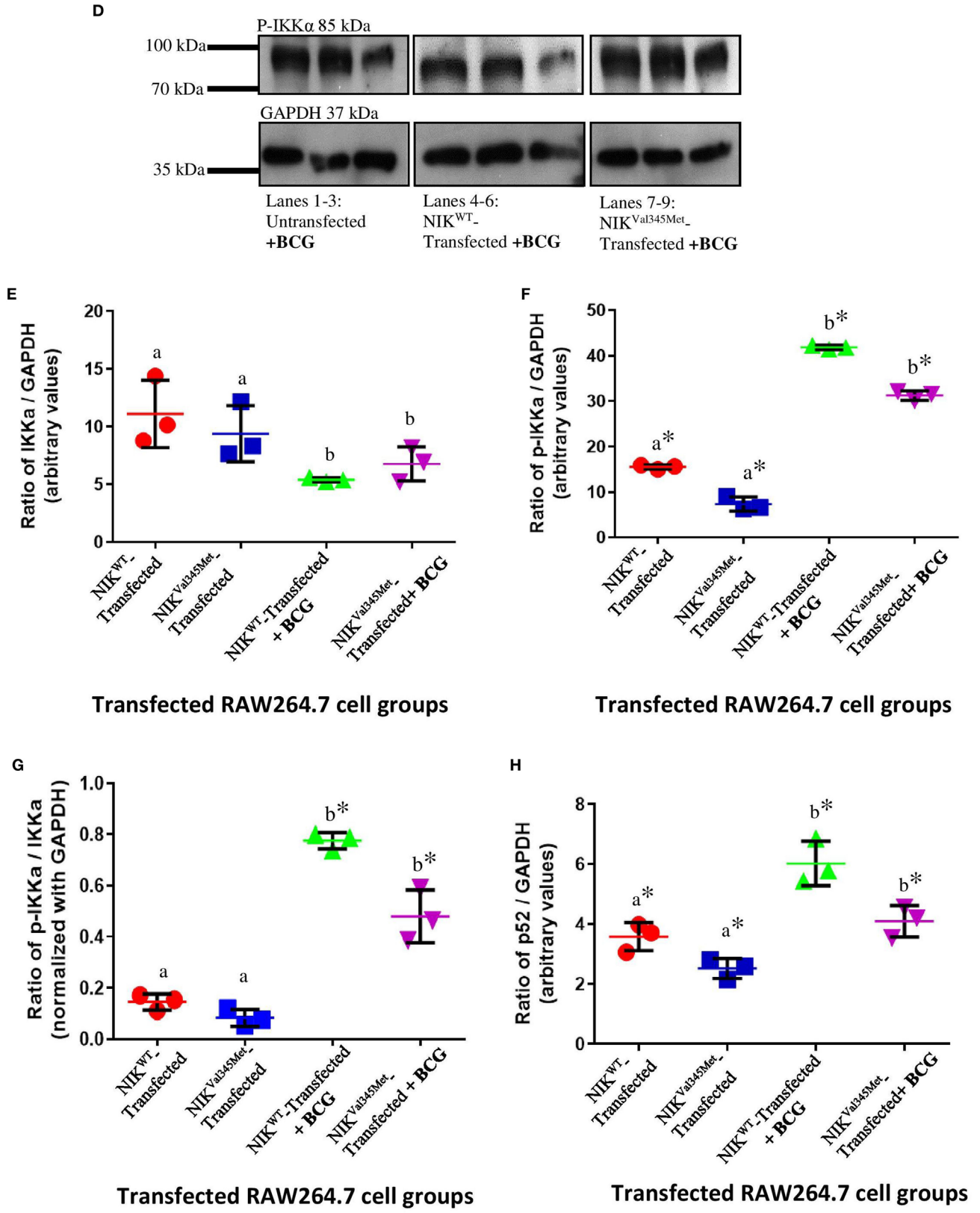

FIGURE 6 | Continued

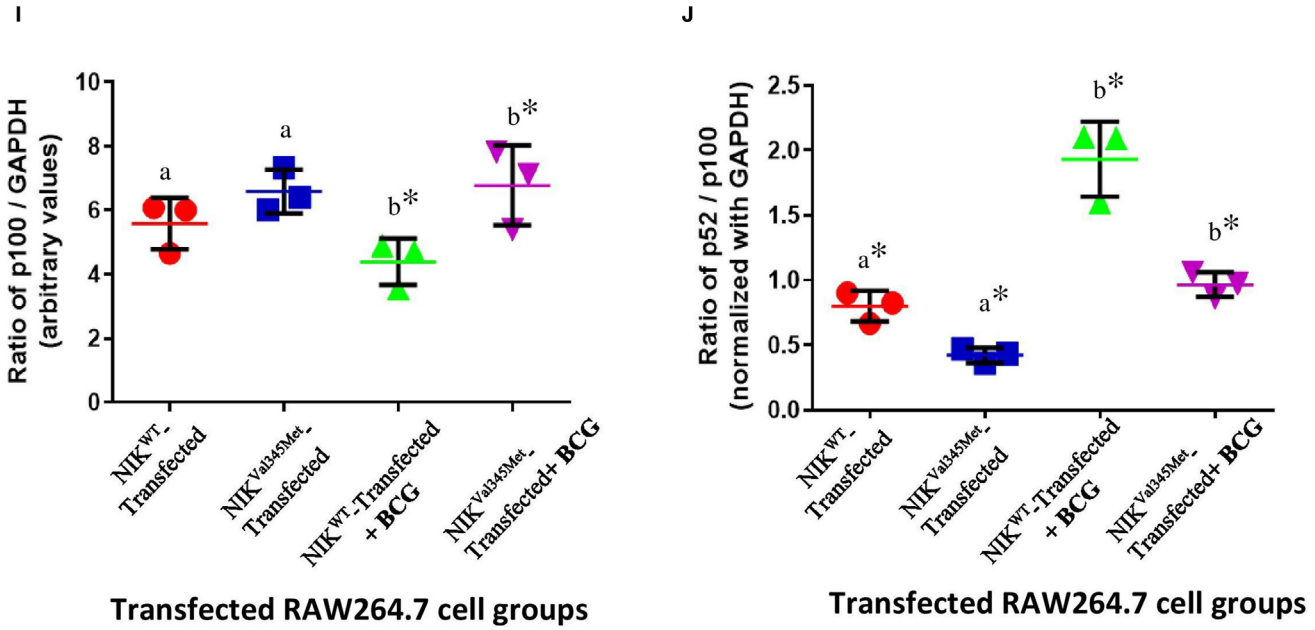

FIGURE 6 | Comparison between NIK^{Val345Met}- and NIK^{WT}-transfected RAW264.7 cells. **(A,B)** Similar amounts of IKKα was detected in the wild-type and mutant cell groups, indicating that NIK^{Val345Met} had no effect on IKKα production. p100 levels were similar in the wild-type and mutant RAW264.7 cell line groups, with reduced levels of phospho-IKKα and p52 observed in cells transfected with *NIK^{Val345Met}* compared to *NIK^{WT}*, without BCG stimulation. **(C,D)** p100, p52, and phospho-IKKα levels were affected by the Val345Met mutation, with increased p100, and decreased p52 and phospho-IKKα levels observed in BCG-stimulated cells transfected with *NIK^{Val345Met}* compared to *NIK^{WT}*. IKKα levels were not affected. **(E)** Quantification of western blots in **(A,C)** showed similar amounts of IKKα in wild-type and mutant cell groups, both without and with BCG stimulation. a: $p = 0.4757$; b: $p = 0.1844$; **(F)** quantification of western blots in **(B,D)** show significant reductions in phospho-IKKα in the mutant-transfected cell groups compared to the wild-type-transfected groups, both without and with BCG stimulation. a: $p = 0.0010$*; b: $p < 0.0001$*. **(G)** The ratio of phosphorylated IKKα over un-phosphorylated IKKα was similar between the wild-type- and mutant-transfected cell groups in the absence of BCG stimulation, but significantly reduced in the mutant-transfected groups compared to the wild-type-transfected groups when stimulated with BCG. a: $p = 0.0760$; b: $p = 0.0090$*. **(H)** Significantly reduced levels of p52 was observed in cells transfected with *NIK^{Val345Met}* compared to *NIK^{WT}*, both in the absence and in the presence of BCG stimulation. a: $p = 0.0330$*; b: $p = 0.0208$*. **(I)** p100 levels were similar in cells transfected with *NIK^{Val345Met}* compared to *NIK^{WT}* without BCG stimulation. However, p100 levels significantly increased in the *NIK^{Val345Met}*-transfected groups upon BCG stimulation. a: $p = 0.1745$; b: $p = 0.0458$*. **(J)** The ratio of p52 over p100 differed significantly between cells transfected with *NIK^{Val345Met}* compared to *NIK^{WT}*, both without and with BCG stimulation. a: $p = 0.0077$*; b: $p = 0.0053$*. The data for each image in this figure is representative of three experiments conducted independently. * indicates significance.

highly conserved region (**Figure 4**). The domain it is located in is important for NIK's activity and interacts with residues from other domains, making it possible for this mutation to inhibit the correct functioning of NIK. The involvement of *NIK* in B cell development and maturation makes it a perfect candidate gene to investigate further for association with primary immunodeficiency and increased susceptibility to TB.

Phenotypic heterogeneity may be due to different effects of a mutation on protein function (43). However, clinical phenotypic variation cannot always be attributed to different functional consequences of a mutation. As an example, the first patient in whom a mutation in *TYK2* (member of the JAK family of tyrosine kinases) was identified was diagnosed with hyper-IgE syndrome accompanied by lesions of the skin, BCG disease, as well as fungal and viral infections (45). A second patient, also with small deletions in *TYK2* and the absence of protein on western blots like the first patient, presented with BCG disease and brucellosis, but had normal IgE levels and no skin lesions (46). In a further example, three Chinese siblings with intracranial calcifications and epileptic seizures, without severe infectious diseases, were exome sequenced and a mutation in *ISG15* was identified (47). Mutations in this gene were previously found to cause MSMD in three unrelated children from two families in Iran and Turkey (48). Subsequently, intracranial calcifications were also identified in other ISG15-deficient patients. The Chinese individuals never received BCG vaccinations, which could explain why they did not have the MSMD phenotype. Even within a single family, mutations in the same gene have been shown to cause very different clinical phenotypes (35). This is due to the range of infectious or environmental exposures, age of exposure and various modifying epigenetic factors that can affect disease presentation. Therefore, clinical outcomes of patients with immunodeficiencies, even with previously described phenotype/genotype associations, cannot always be accurately predicted.

Phenotypic differences observed between the proband in this study and P1 and P2 described by Willmann and colleagues can also be attributed to the location, and thus the effect, of the identified mutations. The previously identified mutation is situate in the kinase domain of NIK, and completely abolishes the

functioning of this protein. The mutation described in this study, however, is situated just before the kinase domain (**Figure 4**) and does not affect the kinase activity of NIK as critically as the mutation previously described (44). This was proven by the decreased, and not abolished, kinase activity of NIK observed in this study.

The genomic approach in unusual presentations like in the presented patient illustrates its value for successful identification of a novel mutation situated in a PID-causing gene. A confirmed molecular diagnosis directs potential treatment approaches such as the indication for stem cell therapy. Moreover, genetic predisposition combined with protein dysfunction studies are already used to tailor-make patient-specific approaches in mycobacterial disease (49). In the era of personalized medical treatment, based on unique genetic features, it is realistic to anticipate that this will also be extended to the specific treatment of variants of genetically susceptible TB.

WEB RESOURCES

1000GP, http://www.1000genomes.org
ADMIXTURE, http://www.genetics.ucla.edu/software
WANNOVAR, http://wannovar.wglab.org (accessed 3.10.16)
BWA, https://github.com/lh3/bwa
ClustalW, https://www.ebi.ac.uk/Tools/msa/clustalw2/
ESP6500, http://evs.gs.washington.edu/EVS/
ExAC Browser http://exac.broadinstitute.org/
FastQC, http://www.bioinformatics.babraham.ac.uk/projects/fastqc/
GATK suite, www.broadinstitute.org/gatk
HGMD, http://www.hgmd.cf.ac.uk/ac/index.php
MutationTaster-2, http://www.mutationtaster.org/
OMIM, http://www.omim.org/
Picard, http://picard.sourceforge.net
PLINK, http://zzz.bwh.harvard.edu/plink/
PolyPhen-2, http://genetics.bwh.harvard.edu/pph2/
Project HOPE, http://www.cmbi.ru.nl/hope/
SamTools, http://www.htslib.org/
SIFT, http://sift.jcvi.org/

Accession numbers: NM_003954.4 and NP_003945.2.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of the Health Research Ethics Committee of Stellenbosch University (approval no. N13/05/075) with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the Health Research Ethics Committee of Stellenbosch University.

AUTHOR CONTRIBUTIONS

CK, EH, PH, and MM conceived the project. Y-LL carried out all testing on MSMD genes. NS carried out all laboratory work and wrote the first draft of the manuscript. B-SP and AF performed the whole exome sequencing and assisted with the bioinformatics analysis. BG and NS performed the bioinformatics analysis and interpreted the data with CK and MM. MS and MU assisted with the genetic counseling. ME was involved in patient recruitment. All authors read and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at http://www.frontiersin.org/article/10.3389/fimmu.2017.01624/full#supplementary-material.

REFERENCES

1. Modell F. Immunology today and new discoveries: building upon legacies of Dr. Robert A. Good. *Immunol Res* (2007) 38(1–3):48–50. doi:10.1007/s12026-007-0049-4
2. Cunningham-Rundles C, Ponda PP. Molecular defects in T- and B-cell primary immunodeficiency diseases. *Nat Rev Immunol* (2005) 5(11):880–92. doi:10.1038/nri1713
3. Cildir G, Low KC, Tergaonkar V. Noncanonical NF-κB signaling in health and disease. *Trends Mol Med* (2016) 22(5):414–29. doi:10.1016/j.molmed.2016.03.002
4. Razani B, Reichardt AD, Cheng G. Non-canonical NF-κB signaling activation and regulation: principles and perspectives: non-canonical NF-κB signaling activation and regulation. *Immunol Rev* (2011) 244(1):44–54. doi:10.1111/j.1600-065X.2011.01059.x
5. Sun S-C. Non-canonical NF-κB signaling pathway. *Cell Res* (2011) 21(1):71–85. doi:10.1038/cr.2010.177
6. Pieper K, Grimbacher B, Eibel H. B-cell biology and development. *J Allergy Clin Immunol* (2013) 131(4):959–71. doi:10.1016/j.jaci.2013.01.046
7. Sun S-C. The noncanonical NF-κB pathway. *Immunol Rev* (2012) 246(1):125–40. doi:10.1111/j.1600-065X.2011.01088.x
8. Yamada H, Mizuno S, Reza-Gholizadeh M, Sugawara I. Relative importance of NF-kappaB p50 in mycobacterial infection. *Infect Immun* (2001) 69(11):7100–5. doi:10.1128/IAI.69.11.7100-7105.2001
9. Tato CM, Hunter CA. Host-pathogen interactions: subversion and utilization of the NF-kappa B pathway during infection. *Infect Immun* (2002) 70(7):3311–7. doi:10.1128/IAI.70.7.3311-3317.2002
10. Gutierrez MG, Master SS, Singh SB, Taylor GA, Colombo MI, Deretic V. Autophagy is a defense mechanism inhibiting BCG and *Mycobacterium tuberculosis* survival in infected macrophages. *Cell* (2004) 119(6):753–66. doi:10.1016/j.cell.2004.11.038
11. Yuk JM, Yoshimori T, Jo EK. Autophagy and bacterial infectious diseases. *Exp Mol Med* (2012) 44(2):99–108. doi:10.3858/emm.2012.44.2.032

12. Levine B, Mizushima N, Virgin HW. Autophagy in immunity and inflammation. *Nature* (2011) 469(7330):323–35. doi:10.1038/nature09782
13. Kroemer G, White E. Autophagy for the avoidance of degenerative, inflammatory, infectious, and neoplastic disease. *Curr Opin Cell Biol* (2010) 22(2): 121–3. doi:10.1016/j.ceb.2010.02.003
14. Vece TJ, Watkin LB, Nicholas SK, Canter D, Braun MC, Guillerman RP, et al. Copa syndrome: a novel autosomal dominant immune dysregulatory disease. *J Clin Immunol* (2016) 36(4):377–87. doi:10.1007/s10875-016-0271-8
15. Hooper KM, Barlow PG, Stevens C, Henderson P. Inflammatory bowel disease drugs: a focus on autophagy. *J Crohns Colitis* (2016) 11(1):118–27. doi:10.1093/ecco-jcc/jjw127
16. Negroni A, Colantoni E, Vitali R, Palone F, Pierdomenico M, Costanzo M, et al. NOD2 induces autophagy to control AIEC bacteria infectiveness in intestinal epithelial cells. *Inflamm Res* (2016) 65(10):803–13. doi:10.1007/s00011-016-0964-8
17. Deretic V, Singh S, Master S, Harris J, Roberts E, Kyei G, et al. *Mycobacterium tuberculosis* inhibition of phagolysosome biogenesis and autophagy as a host defence mechanism. *Cell Microbiol* (2006) 8(5):719–27. doi:10.1111/j.1462-5822.2006.00705.x
18. Comb WC, Cogswell P, Sitcheran R, Baldwin AS. IKK-dependent, NF-κB-independent control of autophagic gene expression. *Oncogene* (2011) 30(14):1727–32. doi:10.1038/onc.2010.553
19. Yan P, Qing G, Qu Z, Wu C-C, Rabson A, Xiao G. Targeting autophagic regulation of NFkappaB in HTLV-I transformed cells by geldanamycin: implications for therapeutic interventions. *Autophagy* (2007) 3(6):600–3. doi:10.4161/auto.4761
20. Qing G, Yan P, Qu Z, Liu H, Xiao G. Hsp90 regulates processing of NF-kappa B2 p100 involving protection of NF-kappa B-inducing kinase (NIK) from autophagy-mediated degradation. *Cell Res* (2007) 17(6):520–30. doi:10.1038/cr.2007.47
21. Nivon M, Richet E, Codogno P, Arrigo A-P, Kretz-Remy C. Autophagy activation by NFkappaB is essential for cell survival after heat shock. *Autophagy* (2009) 5(6):766–83. doi:10.4161/auto.8788
22. World Medical Association. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. *JAMA* (2013) 310(20):2191–4. doi:10.1001/jama.2013.281053
23. The 1000 Genomes Project Consortium, Auton A, Brooks LD, Durbin RM, Garrison EP, Kang HM, et al. A global reference for human genetic variation. *Nature* (2015) 526(7571):68–74. doi:10.1038/nature15393
24. *Exome Variant Server*. Seattle, WA: NHLBI GO Exome Sequencing Project (ESP) (2014). Available from: http://evs.gs.washington.edu/EVS/
25. Davydov EV, Goode DL, Sirota M, Cooper GM, Sidow A, Batzoglou S. Identifying a high fraction of the human genome to be under selective constraint using GERP++. *PLoS Comput Biol* (2010) 6(12):e1001025. doi:10.1371/journal.pcbi.1001025
26. Shihab HA, Gough J, Cooper DN, Stenson PD, Barker GLA, Edwards KJ, et al. Predicting the functional, molecular, and phenotypic consequences of amino acid substitutions using hidden Markov models. *Hum Mutat* (2013) 34(1):57–65. doi:10.1002/humu.22225
27. Venselaar H, Te Beek TAH, Kuipers RKP, Hekkelman ML, Vriend G. Protein structure analysis of mutations causing inheritable diseases. An e-Science approach with life scientist friendly interfaces. *BMC Bioinformatics* (2010) 11:548. doi:10.1186/1471-2105-11-548
28. Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, et al. A method and server for predicting damaging missense mutations. *Nat Methods* (2010) 7(4):248–9. doi:10.1038/nmeth0410-248
29. Ng PC, Henikoff S. Accounting for human polymorphisms predicted to affect protein function. *Genome Res* (2002) 12(3):436–46. doi:10.1101/gr.212802
30. Schwarz JM, Rödelsperger C, Schuelke M, Seelow D. MutationTaster evaluates disease-causing potential of sequence alterations. *Nat Methods* (2010) 7(8):575–6. doi:10.1038/nmeth0810-575
31. Boehm JS, Zhao JJ, Yao J, Kim SY, Firestein R, Dunn IF, et al. Integrative genomic approaches identify IKBKE as a breast cancer oncogene. *Cell* (2007) 129(6):1065–79. doi:10.1016/j.cell.2007.03.052
32. Nakano H, Shindo M, Sakon S, Nishinaka S, Mihara M, Yagita H, et al. Differential regulation of IkappaB kinase alpha and beta by two upstream kinases, NF-kappaB-inducing kinase and mitogen-activated protein kinase/ERK kinase kinase-1. *Proc Natl Acad Sci U S A* (1998) 95(7):3537–42. doi:10.1073/pnas.95.7.3537
33. Sarkar S, Davies JE, Huang Z, Tunnacliffe A, Rubinsztein DC. Trehalose, a novel mTOR-independent autophagy enhancer, accelerates the clearance of mutant huntingtin and alpha-synuclein. *J Biol Chem* (2007) 282(8):5641–52. doi:10.1074/jbc.M609532200
34. Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, et al. Clustal W and Clustal X version 2.0. *Bioinformatics* (2007) 23(21):2947–8. doi:10.1093/bioinformatics/btm404
35. Mundlapati VR, Ghosh S, Bhattacherjee A, Tiwari P, Biswal HS. Critical assessment of the strength of hydrogen bonds between the sulfur atom of methionine/cysteine and backbone amides in proteins. *J Phys Chem Lett* (2015) 6(8):1385–9. doi:10.1021/acs.jpclett.5b00491
36. Loos B, Hofmeyr J-HS, Müller-Nedebock K, Boonzaaier L, Kinnear C. Autophagic flux, fusion dynamics, and cell death. *Autophagy: Cancer, Other Pathologies, Inflammation, Immunity, Infection, and Aging*. Elsevier (2014). p. 39–56. Available from: http://linkinghub.elsevier.com/retrieve/pii/B9780124055292000020
37. Kirkegaard K, Taylor MP, Jackson WT. Cellular autophagy: surrender, avoidance and subversion by microorganisms. *Nat Rev Microbiol* (2004) 2(4):301–14. doi:10.1038/nrmicro865
38. Klionsky DJ, Cuervo AM, Seglen PO. Methods for monitoring autophagy from yeast to human. *Autophagy* (2007) 3(3):181–206. doi:10.4161/auto.3678
39. Mizushima N. Methods for monitoring autophagy. *Int J Biochem Cell Biol* (2004) 36(12):2491–502. doi:10.1016/j.biocel.2004.02.005
40. Kabeya Y, Mizushima N, Ueno T, Yamamoto A, Kirisako T, Noda T, et al. LC3, a mammalian homologue of yeast Apg8p, is localized in autophagosome membranes after processing. *EMBO J* (2000) 19(21):5720–8. doi:10.1093/emboj/19.21.5720
41. Kabeya Y, Mizushima N, Yamamoto A, Oshitani-Okamoto S, Ohsumi Y, Yoshimori T. LC3, GABARAP and GATE16 localize to autophagosomal membrane depending on form-II formation. *J Cell Sci* (2004) 117(Pt 13):2805–12. doi:10.1242/jcs.01131
42. Loos B, du Toit A, Hofmeyr J-HS. Defining and measuring autophagosome flux – concept and reality. *Autophagy* (2014) 10(11):2087–96. doi:10.4161/15548627.2014.973338
43. Conley ME, Casanova J-L. Discovery of single-gene inborn errors of immunity by next generation sequencing. *Curr Opin Immunol* (2014) 30:17–23. doi:10.1016/j.coi.2014.05.004
44. Willmann KL, Klaver S, Doğu F, Santos-Valente E, Garncarz W, Bilic I, et al. Biallelic loss-of-function mutation in NIK causes a primary immunodeficiency with multifaceted aberrant lymphoid immunity. *Nat Commun* (2014) 5:5360. doi:10.1038/ncomms6360
45. Minegishi Y, Saito M, Morio T, Watanabe K, Agematsu K, Tsuchiya S, et al. Human tyrosine kinase 2 deficiency reveals its requisite roles in multiple cytokine signals involved in innate and acquired immunity. *Immunity* (2006) 25(5):745–55. doi:10.1016/j.immuni.2006.09.009
46. Kilic SS, Hacimustafaoglu M, Boisson-Dupuis S, Kreins AY, Grant AV, Abel L, et al. A patient with tyrosine kinase 2 deficiency without hyper-IgE syndrome. *J Pediatr* (2012) 160(6):1055–7. doi:10.1016/j.jpeds.2012.01.056
47. Zhang X, Bogunovic D, Payelle-Brogard B, Francois-Newton V, Speer SD, Yuan C, et al. Human intracellular ISG15 prevents interferon-α/β over-amplification and auto-inflammation. *Nature* (2015) 517(7532):89–93. doi:10.1038/nature13801
48. Bogunovic D, Byun M, Durfee LA, Abhyankar A, Sanal O, Mansouri D, et al. Mycobacterial disease and impaired IFN-γ immunity in humans with inherited ISG15 deficiency. *Science* (2012) 337(6102):1684–8. doi:10.1126/science.1224026
49. Mirsaeidi M. Personalized medicine approach in mycobacterial disease. *Int J Mycobacteriol* (2012) 1(2):59–64. doi:10.1016/j.ijmyco.2012.03.001

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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ORIGINAL RESEARCH
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Clinical and Biological Manifestation of RNF168 Deficiency in Two Polish Siblings

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Germline mutations in the RING finger protein gene *RNF168* have been identified in a combined immunodeficiency disorder called RIDDLE syndrome. Since only two patients have been described with somewhat different phenotypes, there is need to identify further patients. Here, we report on two Polish siblings with RNF168 deficiency due to homozygosity for a novel frameshift mutation, c.295delG, that was identified through exome sequencing. Both patients presented with immunoglobulin deficiency, telangiectasia, cellular radiosensitivity, and increased alpha-fetoprotein (AFP) levels. The younger sibling had a more pronounced neurological and morphological phenotype, and she also carried an *ATM* gene mutation in the heterozygous state. Immunoblot analyses showed absence of RNF168 protein, whereas ATM levels and function were proficient in lymphoblastoid cells from both patients. Consistent with the absence of RNF168 protein, 53BP1 recruitment to DNA double-strand breaks (DSBs) after irradiation was undetectable in lymphoblasts or primary fibroblasts from either of the two patients. γH2AX foci accumulated normally but they disappeared with significant delay, indicating a severe defect in DSB repair. A comparison with the two previously identified patients indicates immunoglobulin deficiency, cellular radiosensitivity, and increased AFP levels as hallmarks of RNF168 deficiency. The variability in its clinical expression despite similar cellular phenotypes suggests that some manifestations of RNF168 deficiency may be modified by additional genetic or epidemiological factors.

Keywords: DNA repair, chromosome instability, radiosensitivity, immunodeficiency syndrome, double-strand break repair

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INTRODUCTION

Primary immunodeficiency syndromes commonly associate with functional impairments in DNA double-strand break (DSB) repair, highlighting the critical nature of this pathway for the development and maturation of the immune system (1, 2). A genetic disorder including "radiosensitivity, immunodeficiency, dysmorphic features, and learning difficulties," coined RIDDLE syndrome, has initially been described in a single patient whose cells lacked the ability to recruit 53BP1 to DSB sites (3). Subsequent work revealed that the *RNF168* gene was mutated in this patient (4).

RNF168 encodes an E3 ubiquitin ligase that orchestrates the accumulation of 53BP1 or BRCA1 to DNA lesions (4, 5). RNF168 binds to ubiquitinated linker histone H1 at sites of DNA damage (6)

where it then ubiquitinates histones H2A and H2AX, a prerequisite for accurate DSB repair (5, 7–10). RNF168-dependent ubiquitination of histones generates docking sites for RAP80 that mediates binding of BRCA1 to sites flanking the break (11) and also generates one of the histone marks required for 53BP1 recruitment (12, 13). RNF168 has been reported to further promote the initial recruitment of 53BP1 through its direct polyubiquitination (14). 53BP1 in turn suppresses end resection and promotes non-homologous end-joining in antagonism with BRCA1 (15). Consistent with its role in 53BP1 activation, RNF168 is required for class switch recombination and for V(D)J recombination (16, 17).

The second patient with RNF168 deficiency has been described in the literature with a somewhat different clinical phenotype including mild gait ataxia, ocular telangiectasia, elevated AFP, immunodeficiency, microcephaly, growth retardation, and terminal respiratory failure (18). Again, patient cells lacked RNF168 expression and the ability to recruit 53BP1 to sites of DNA DSBs. By contrast with the originally described patient, the clinical description of this patient indicated normal intelligence but a phenotype resembling ataxia–telangiectasia (18).

Given the rarity and non-overlapping features of the syndrome, it will be important to identify additional patients, describe consistent clinical phenotypes or differences, and better define the clinical spectrum of this disorder. In this work, we report on two Polish siblings with RNF168 deficiency.

PATIENTS AND METHODS

Case Reports

The older boy, now aged 21 years, was born at term with asphyxia, hypotrophy (2,100 g), and second-degree intraventricular hemorrhage. He was rehabilitated until 2 years of age. He started to walk alone at the age of 1.5 years, then slightly abnormal gait and some "motor clumsiness" were observed. Since the neonatal period he was suffering from recurrent respiratory infection including pneumonia, a few events of bronchitis and chronic sinusitis. At the age of 7 years, he was hospitalized due to suspicion of seizure disorders, to be differentiated with tics. He was found to have reduced level of eye–hand coordination and elevated levels of emotional tension. The severity of health problems increased by the age of 13, when he was again hospitalized several times because of recurrent respiratory infections, sinusitis, and nocturnal enuresis. By age 14, he was hospitalized because of recurrent fever, generalized chronic lymphadenopathy, gait disturbances, mild arthritis, and erythematous, scaly skin lesions on lower limbs. Based on biopsies of lymph nodes and bone marrow and imaging tests, a proliferative process was excluded. Although the skin lesions clinically resembled those of vascular origin, a pathological examination of skin biopsy did not reveal any signs of vasculitis. Due to an increased level of serum AFP [59.1 IU/mL (normal range < 5 IU/mL)], cancer of testis was excluded. MRI of brain showed lesions localized in white matter to be differentiated with inflammation, vasculitis, or demyelination. EMG revealed slight chronic neurogenic, axonal damage of muscles of the lower limbs, suggesting demyelinating component or sensomotor neuropathy. Finally, the only diagnosis the boy was given was mononucleosis but since then he has been suffering from repeated headaches. By the age of 15, he was admitted to the Department of Immunology due to hypogammaglobulinemia. His total IgG was 4.29 g/L (range for age 7.06–14.40 g/L) and his IgA was below 0.06 g/L (range for age 0.85–1.94 g/L). He presented with conjunctival telangiectasia, persistent skin lesion on his lower limbs, chronic sinusitis, recurrent headaches, and stomach pains. His karyotype was 46,XY, but a few translocations were found involving chromosomes 7q and 14. Based on laboratory tests, we suspected ataxia–telangiectasia and regular substitution of intravenous immunoglobulin was commenced. By the age of 16, the boy was again hospitalized due to abdominal pain, prolonged diarrhea, and significant weight loss. Gastroscopy and colonoscopy were performed and *Helicobacter pylori* infection was confirmed but inflammatory bowel disease was excluded.

His younger sister, now aged 12 years, was born at term after an uneventful pregnancy and suffered from her first infection of upper respiratory tract by the age of 6 months. Since early childhood she presented with psycho-motor developmental delay. By the age of 6 years, she was consulted in the Immunology Outpatient Clinic Children's Memorial Health Institute (CMHI) due to recurrent mucosal herpes simplex infections. Up to now she has had a few upper respiratory tract infections including bronchitis and otitis, and for 9 years an increasing number of herpes infections. She had a decent value for total IgG of 8.85 g/L (normal range for age 8.53–14.40 g/L) but reduced IgA < 0.06 g/L (normal range for age 0.38–2.35 g/L). IgG subclass analysis revealed reduced IgG2 at 0.39 g/L (normal range for age 0.71–3.41 g/L). Like her brother, she had markedly increased serum AFP at 41.9 IU/mL (normal range < 5 IU/mL).

At the time of this manuscript, there was no evidence of growth failure in both siblings. The brother's weight was 105.5 kg (3.69 SD, >97c), height was 182.5 cm, head circumference 59.5 cm (1.7 SD), and BMI 31.68 (3.73 SD). The sister's weight was 66 kg (3.04 SD, >97c), height 160.5 (1.56 SD, 90c), head circumference 52 cm (−1.37 SD, 10c), and BMI 25.54 (2.57 SD, >97c). Written informed consent was obtained from the patients for the publication of these case reports and their photographs (Figure S1 in Supplementary Material).

Cell Culture

Lymphoblastoid cell lines (LCLs) were established using routine EBV immortalization of B-lymphocytes from peripheral EDTA blood samples for each of both patients and were grown in RPMI 1640 supplemented with 10% fetal calf serum, 500 U/mL penicillin, 0.5 mg/mL streptomycin, and 2 mM L-glutamine. The LCLs were designated HA591 for the sister and HA592 for her older brother. Previously established LCLs from a healthy donor (HA325) and from a patient with classical ataxia–telangiectasia (HA56) were maintained under the same cell culture conditions. Primary fibroblast cultures were obtained from skin biopsies for each of both patients and were grown in DMEM with the above-mentioned supplements. The fibroblast lines were designated F591 for the sister and F592 for her older brother. The previously described ADP fibroblast line from a healthy male donor was cultured under the same conditions (19). All cell cultures were maintained at 37°C in a humidified atmosphere with 5% CO_2.

For treatment with ionizing radiation, cells were γ-irradiated at the respective doses (1.5 and 6 Gy) using a Mevatron MD-2 accelerator (Siemens, Munich, Germany).

Genetic Analysis

Genomic DNA was extracted from peripheral blood lymphocytes or cultured lymphoblastoid cell lines using proteinase K digestion and phenol–chloroform extraction. Exome sequencing was performed on genomic DNA samples (1 µg) from each of both affected siblings. For this purpose, exonic sequences were enriched using the SureSelect XT Human All Exon V6 library (Agilent Technologies, Santa Clara, CA, USA) and were analysed on an Illumina HiSeq2500 platform using TruSeq SBS Kit v3-HS (200 cycles, paired end run) with an average of 25×10^6 reads per single exome and 100× coverage (Illumina Inc., San Diego, CA, USA). Raw exome sequencing data were called, de-multiplexed, and aligned according to the GATK pipeline and variants were annotated using the SnpEff tool (http://snpeff.sourceforge.net/SnpEff.html). Mutations were filtered according to their minor allele frequencies in the NCBI SNP and/or 1000Genomes databases and according to their predicted effects. The truncating mutation in *RNF168* was then confirmed by Sanger sequencing using BigDye chemistry and a Genetic Analyzer 3100 Avant (Applied Biosystems, Foster City, CA, USA). Primers for validation sequencing of the *RNF168**c.295delG mutation were 5′-GGACAAAATCTTGCCCTTGAC-3′ and 5′-ACCCGAAGAAATTCTCTCGTC-3′. Primers for the sequencing of the *ATM**1402_1403delAA mutation were 5′-CTATGGAAATGATGGTGATTCTC-3′ and 5′-GCATCTGAAATAGAATTTGACATC-3′ (20). Purified PCR products were subjected to direct sequencing using BigDye v1.1 terminator chemistry and a 3100 Avant capillary sequencer (Life Technologies). Sequencing data were analyzed with the Sequencing Analysis 5.1.1 software. Patient and parental genomic DNAs were further genotyped by means of RFLP analysis. For this assay, 10 µL genomic PCR products were generated with the *RNF168* primer pair listed above and were incubated overnight with 1.5 U *Mnl*I (New England Biolabs). Cleavage fragments were separated through electrophoresis on 2% agarose gels supplemented with GelRed and were visualized over an UV transilluminator.

Immunoblotting

Cells were lysed in cell extraction buffer (50 mM Tris pH 7.4, 150 mM NaCl, 2 mM EGTA, 2 mM EDTA, 25 mM NaF, 0.1 mM Na_3VO_4, 0.1 mM PMSF, 2 mg/mL Leupeptin, 2 mg/mL Aprotinin, 0.2% Triton X-100, 0.3% NonidetP-40) for 30 min on ice and centrifuged at 16100 rcf for 15 min. Protein extracts were separated through SDS-PAGE followed by immunoblotting. Primary antibodies against the following proteins were used: ATM (rabbit monoclonal, Epitomics, 1:1,000), DNA-PKcs (mouse monoclonal,

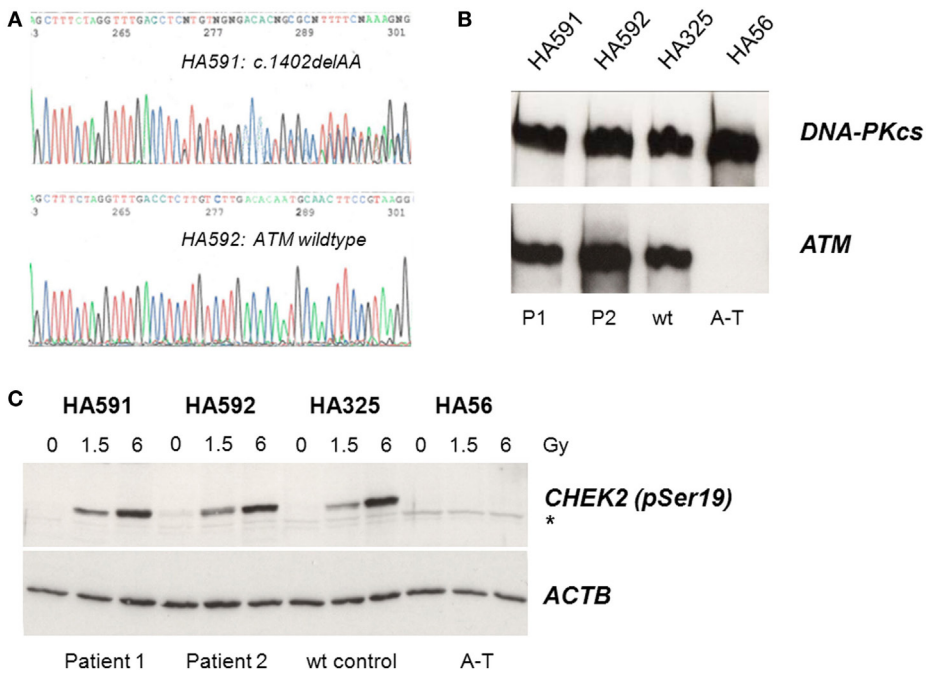

FIGURE 1 | Assessment of ATM mutation **(A)**, ATM protein level **(B)**, and ATM kinase activity **(C)**. **(A)** Direct sequencing of *ATM* exon 12 reveals heterozygosity for the novel frameshift mutation c.1402_1403delAA in genomic DNA from the sister (HA591) but not the brother (HA592). **(B)** Immunoblotting detects ATM protein in lymphoblastoid cells from both patients (HA591, HA592). Lymphoblastoid cell lines (LCLs) from a healthy individual were used as an ATM-proficient control (HA325), and LCLs from a patient with classical ataxia–telangiectasia were used as an ATM-deficient control (HA56). DNA-dependent protein kinase, catalytic subunit, served as the loading control (DNA-PKcs). **(C)** Immunoblotting of cells after irradiation reveals radiation-induced phosphorylation of the ATM substrate KAP1 at Ser824. Cells were irradiated with 0, 1.5, or 6 Gy, respectively, and proteins were extracted at 30 min after irradiation. LCLs from a healthy individual were used as an ATM-proficient control (HA325), and LCLs from a patient with classical ataxia–telangiectasia were used as an ATM-deficient control (HA56). β-actin served as the loading control (ACTB).

Calbiochem, 1:500), pSer824-KAP1 (rabbit monoclonal, Bethyl Laboratories, 1:5,000), RNF168 (rabbit polyclonal, GeneTex, 1:1,000), and β-Actin (mouse monoclonal, Sigma, 1:3,000). Anti-mouse and anti-rabbit horseradish peroxidases labeled secondary antibodies were purchased from GE Healthcare. Enhanced chemiluminescence (Dura ECL, Thermo Scientific/Pierce) was used for visualization of immunoreactive bands.

Immunocytochemistry

Subconfluent fibroblast cells grown on cover glasses in six-well plates or lymphoblastoid cells centrifuged onto slides through cytospin were fixed with 3% (w/v) PFA, 2% (w/v) sucrose for 10 min. Cells were permeabilized with 0.2% (v/v) Triton X-100 in 1× PBS for 3 min and rinsed three times with 1× PBS. Cells were incubated for 1–1.5 h at room temperature with the primary antibodies against Histone H2A.x Phospho(S139) (Millipore, 1:200) and 53BP1 (Bethyl Laboratories, 1:200) in 2% (w/v) normal goat serum (Dianova). After 1× PBS washing, cells were incubated with Alexa Fluor anti-mouse IgG 488 and Alexa Fluor anti-rabbit IgG 546 (Invitrogen) for 45 min to 1 h at room temperature in the dark. Cells were washed with 1× PBS, and DNA was counterstained with 4′,6-diamidino-2-phenylindole (DAPI) (Invitrogen) (1:50,000 in PBS) for 5 min and mounted using Prolong Gold® (Invitrogen). Foci were counted under a Leica DMI 6000B fluorescence microscope, and cells with $n > 4$ foci were considered "positive" cells. Results from $n = 100–150$ cells with two technical replicates of each line were analyzed. Mean values of RNF168-deficient cells and RNF168-proficient reference lines were compared with two-sample *t*-tests. Accounting for multiple testing, *p*-values were considered significant at $\alpha < 0.01$.

RESULTS

Two Polish siblings presented with a primary immunodeficiency of unknown cause at the Department of Immunology, The CMHI, Warsaw (21). They had been born to healthy consanguineous parents (third degree cousins). Pictures at their present age are provided as Figure S1 in Supplementary Material, and extended case reports are provided in the Section "Patents and Methods." Both siblings showed normal growth and no signs of microcephaly or ataxia, no café-au-lait spots or gray hair, although the younger sister has some mild facial dysmorphism and showed clumsy gait at earlier childhood.

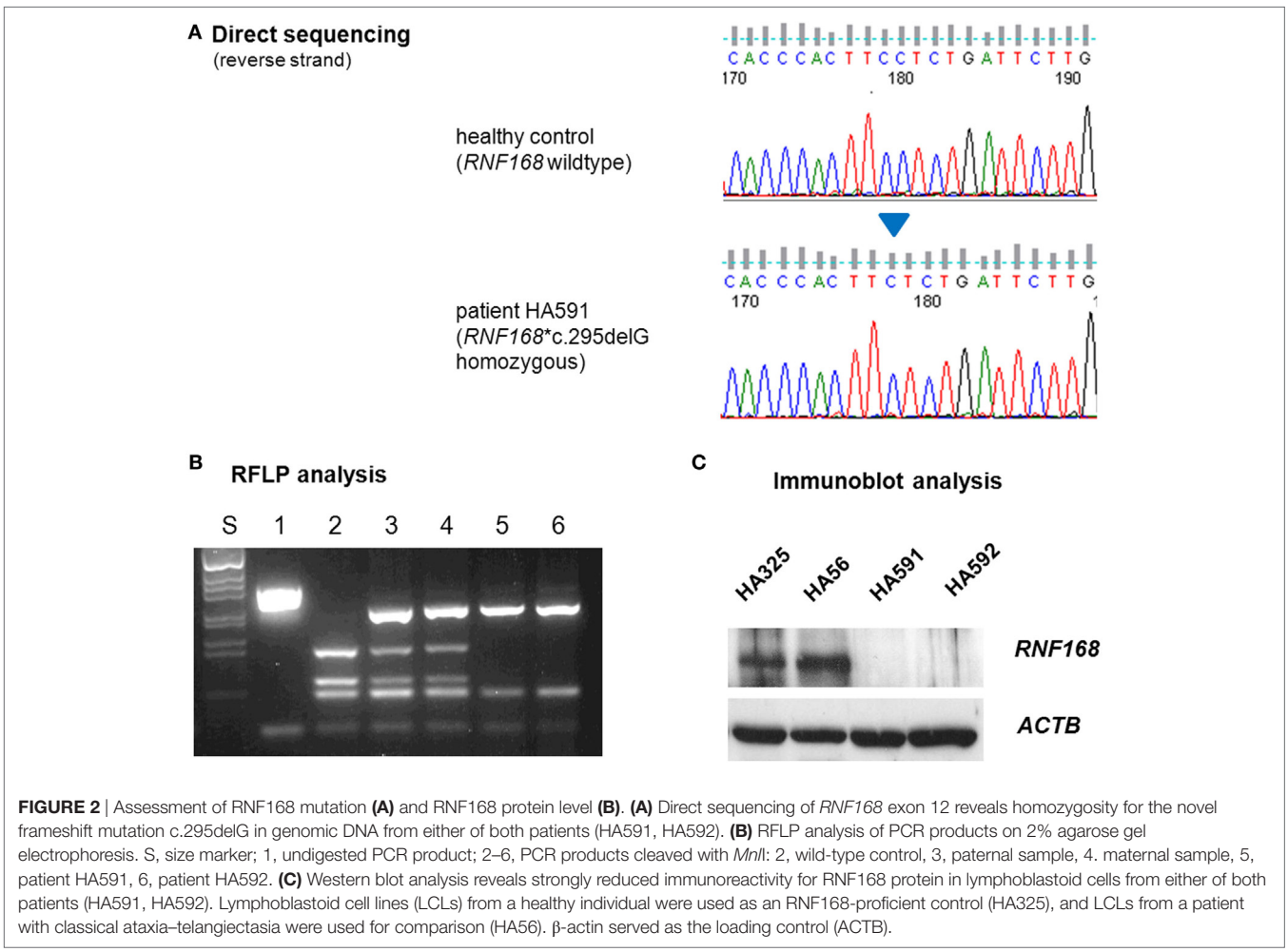

FIGURE 2 | Assessment of RNF168 mutation **(A)** and RNF168 protein level **(B)**. **(A)** Direct sequencing of *RNF168* exon 12 reveals homozygosity for the novel frameshift mutation c.295delG in genomic DNA from either of both patients (HA591, HA592). **(B)** RFLP analysis of PCR products on 2% agarose gel electrophoresis. S, size marker; 1, undigested PCR product; 2–6, PCR products cleaved with *MnlI*: 2, wild-type control, 3, paternal sample, 4. maternal sample, 5, patient HA591, 6, patient HA592. **(C)** Western blot analysis reveals strongly reduced immunoreactivity for RNF168 protein in lymphoblastoid cells from either of both patients (HA591, HA592). Lymphoblastoid cell lines (LCLs) from a healthy individual were used as an RNF168-proficient control (HA325), and LCLs from a patient with classical ataxia–telangiectasia were used for comparison (HA56). β-actin served as the loading control (ACTB).

Conjunctival telangiectasia was noted in both siblings. Mild mental retardation with learning difficulties was apparent for the sister but not for her older brother, both siblings still suffer from nocturnal enuresis. Laboratory measurements revealed IgA and IgG2 deficiency in the sister, while the brother had markedly reduced total IgG which required starting regular IVIG, later SCIG substitution in 2010. While lymphocyte subpopulations were within the normal range for their age, reduced percentage, and number of memory B cells and "switched" B cells were found in both siblings. Both siblings also had strongly elevated serum levels of AFP. Cellular radiosensitivity, as measured by means of colony survival assays in lymphoblastoid cells, was markedly increased for both siblings.

Because of the IgG and IgA deficiency, cellular radiosensitivity, telangiectasia, and elevated AFP, we explored the possibility of an ATM-related dysfunction. Although the siblings showed no ataxia, it is known that very mild form of ataxia–telangiectasia exist where the cerebellar phenotype is attenuated and occurs late in life (22–24). Indeed, direct sequencing of germline DNA demonstrated a novel *ATM* gene mutation, c.1402_1403delAA, in one of the two siblings (**Figure 1A**). However, this mutation was carried by the sister only in the heterozygous state and was not shared with her brother. Subsequent immunoblot analyses of protein extracts obtained from lymphoblastoid cells revealed wild-type levels of ATM protein in the cell lines from both siblings (**Figure 1B**). To further investigate the functional proficiency of the ATM pathway, we irradiated lymphoblastoid cells from both siblings at 1.5 or 6 Gy and monitored the phosphorylation of KAP1 (Ser824), a known target of the ATM kinase (25). We did not observe any significant difference between the patient cells and a wild-type control, demonstrating largely normal ATM kinase activity (**Figure 1C**). Thus, the heterozygosity for an *ATM* mutation in one of the two patients was insufficient to explain their clinical phenotype.

To elucidate the molecular basis for the immunodeficiency, we performed exome sequencing on genomic DNA samples from both patient LCLs. We identified one novel truncating mutation in *RNF168*, c.295delG, that appeared in the homozygous state (**Figure 2A**) and was shared by both siblings. Conceptual translation with this mutation results in a frameshift that starts at codon 99 and generates a premature termination signal 17 codons downstream (p.Glu99Lysfs*17). Because *RNF168* mutations had previously been associated with an immunodeficiency (RIDDLE) syndrome in two unrelated patients (4, 18), this mutation was considered for further analyses. Genomic DNA samples from both parents were genotyped using RFLP analysis and direct sequencing and were

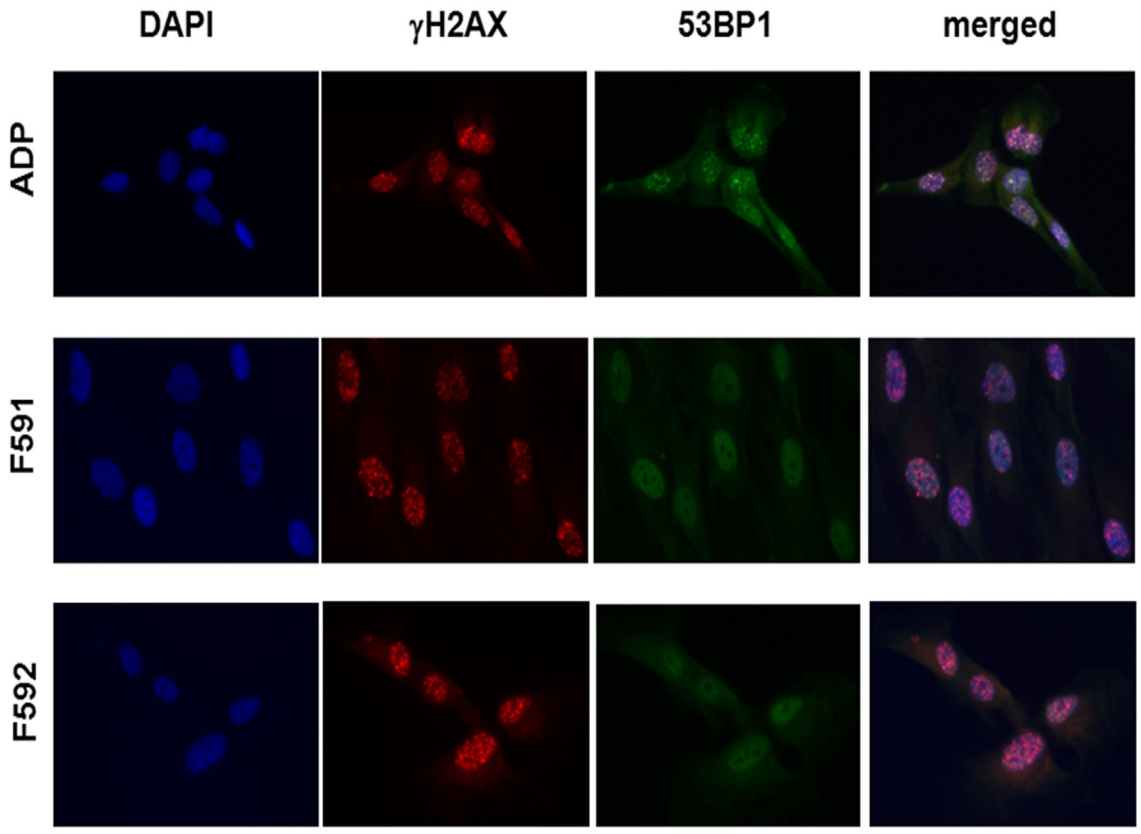

FIGURE 3 | Immunocytochemical analysis of irradiation-induced repair foci in RNF168-deficient fibroblasts and lymphoblastoid cell lines. Detection of γH2AX foci (second column) and 53BP1 foci (third column) in reference ADP fibroblasts (upper panel) compared to patient fibroblasts F591 and F592 (middle and bottom panel) at 1 h after 6 Gy irradiation. DAPI staining and merged pictures are shown in the outer columns as controls for intracellular localization.

confirmed to be heterozygous, thereby proving true homozygosity of the affected patients (**Figure 2B**). We then assessed the amount of RNF168 protein using immunoblot analyses of lymphoblastoid cells from both patients. By contrast with wild-type control and with ataxia–telangiectasia cells, we could not detect RNF168 protein in the LCLs from both patients (**Figure 2C**).

Because the patient cells had shown cellular radiosensitivity in the initial assay and RIDDLE cells had been reported to be defective in 53BP1 recruitment (3), we comparatively monitored the formation and disappearance of γH2AX and 53BP1 repair foci over time in both, lymphoblastoid cells and fibroblasts from both patients. Whereas the initial formation of γH2AX occurred near normally at 1 h after irradiation with 6 Gy, the fibroblasts from both patients failed to form any detectable 53BP1 foci (**Figure 3**; **Table 1**). Similarly, we could not detect any 53BP1 foci after irradiation in lymphoblastoid cells from both patients (data not shown). At later time-points of 48 or 72 h post-irradiation, we observed significantly more residual γH2AX foci in the patient cells than in wild-type control lymphoblasts or fibroblasts ($p \leq 0.002$), consistent with a pronounced defect in the repair of DNA DSB damage. At 72 h, some 38–44% of RNF168-deficient fibroblasts were still γH2AX-positive compared with 1–3% of reference fibroblasts (**Table 1**).

DISCUSSION

Patients suspected of immunodeficiency frequently have underlying DNA DSB repair defects with considerable impact on V(D)J recombination, class switching and lymphocyte maturation, leading to increased infections and cancer risk (1, 2). In addition, the phenotype of cellular radiosensitivity may identify immunologically compromised patients with increased toxicity to radiation and chemotherapeutic agents. Because such disorders are often partial phenocopies of one another, they have been tentatively classified as a "XCIND" group of disorders characterized by X-ray sensitivity, cancer susceptibility, immunodeficiency, neurological abnormalities, and DNA DSB repair dysfunction (1). Modulation and sensing of damage-induced histone ubiquitylation strongly impact on DSB repair pathway choice and genome integrity, as well as cell and organismal fitness, and RNF168 plays a unique role in being a writer as well as a reader of the histone ubiquitylation code (15). In this work, we have identified two siblings with RNF168 deficiency, also termed RIDDLE syndrome, for which only two single cases have been published previously (3, 18). Our genetic testing results for two homozygous siblings and their unaffected heterozygous parents corroborate the autosomal recessive inheritance of this disorder. As a clinical phenotype may be influenced by many genetic and epidemiological factors, it is important to collect clinical data from several patients of a rare syndrome to identify a common theme emerging from a defined molecular deficiency.

TABLE 1 | Monitoring DNA repair protein foci in fibroblasts with RNF168 deficiency.

Fibroblast line	Mean foci per cell			Percentage of foci-positive cells		
	53BP1	γH2AX	$p_{(\gamma H2AX)}$	53BP1	γH2AX	$p_{(\gamma H2AX)}$[a]
ADP reference						
1 h after 6 Gy	11 ± 3	43 ± 5	Ref	28 ± 1	67 ± 1	Ref
24 h after 6 Gy	3 ± 0	6 ± 2	Ref	18 ± 1	39 ± 8	Ref
48 h after 6 Gy	2 ± 0	3 ± 4	Ref	9 ± 1	13 ± 1	Ref
72 h after 6 Gy	1 ± 0	2 ± 2	Ref	7 ± 1	2 ± 1	Ref
F591 RNF168 deficient						
1 h after 6 Gy	0	44 ± 1	0.80	0	71 ± 0	0.01
24 h after 6 Gy	0	13 ± 2	0.08	0	58 ± 1	0.07
48 h after 6 Gy	0	11 ± 3	0.12	0	45 ± 2	**0.002**
72 h after 6 Gy	0	9 ± 1	0.05	0	39 ± 1	**0.0009**
F592 RNF168 deficient						
1 h after 6 Gy	0	52 ± 1	0.13	0	71 ± 2	0.12
24 h after 6 Gy	0	12 ± 1	0.06	0	61 ± 1	0.06
48 h after 6 Gy	0	11 ± 3	0.12	0	59 ± 2	**0.001**
72 h after 6 Gy	0	9 ± 0	0.04	0	43 ± 1	**0.0007**

The number of 53BP1 or γH2AX foci per cell and the percentage of foci-positive cells were assessed in primary fibroblasts from the two patients with RIDDLE syndrome (F591, F592) and wild-type fibroblasts from a healthy donor (ADP) at five time-points (0, 1, 24, 48, 72 h). Shown are the mean values from two technical replicates after normalization to the untreated condition (t = 0). Because no 53BP1 foci were detected in F591 and F592, the monitoring over time was only performed for γH2AX foci and p-values were determined by two-sample t-tests using ADP as the reference.
[a]p < 0.01 marked in bold.

The first patient reported had marked IgG deficiency, mild motor control and learning difficulty, mild facial dysmorphism, and short stature (3). However, the second reported patient featured ataxia, telangiectasia, elevated AFP, IgA deficiency, microcephaly, and pulmonary failure (18). In this study, even though one of the two siblings also was heterozygous for an A–T mutation, the RNF168 deficiency resulted in a much milder neurological phenotype, if any, with the major clinical feature being the immunological defect. The lack of classic ataxia was in line with the ATM proficiency measured *in vitro* in both patients' lymphoblastoid cell lines. **Table 2** compares the features reported for all four hitherto described patients with RNF168 deficiency.

From this comparison, it becomes evident that RNF168 deficiency is consistently associated with immunoglobulin deficiency and cellular radiosensitivity, whereas ataxia, microcephaly, short stature, or learning difficulties were more variable phenotypes and not found in each of the four patients (**Table 2**). It is unlikely that the type of mutation in *RNF168* can explain the variability as all four patients harbor a truncation upstream of the WD40 domain that is crucial for homology-directed repair (26) and showed absence of RNF168 protein *in vitro*. It is possible that mutation in additional genes, including the *ATM* heterozygosity identified in the younger sister, may contribute as modifiers to phenotypic expressions. Interestingly, elevated serum AFP appears to be a common feature and may be of diagnostic use in future screening. We have further confirmed, in patient fibroblasts and in patient lymphoblast cells, a failure to form radiation-induced 53BP1 foci while γH2AX foci remain unaffected (3). Since there is no other human disorder known to show this phenotype, this may provide a rapid and specific diagnostic tool to distinguish RNF168 deficiency from phenocopies.

RNF168 deficiency is presently classified as combined immunodeficiency with syndromic features (27). Our study of two new patients refines reduced immunoglobulin levels and radiosensitivity as the hallmarks of this syndrome, confirms its

TABLE 2 | Comparison of diagnostic features of reported patients with RNF168 deficiency.

	Patient 1 (15-9BI) (3, 4)	Patient 2 (RS66) (18)	Patient 3 (HA591) (this study)	Patient 4 (HA592) (this study)
Country of origin	UK	Turkey	Poland	Poland
Sex	Male	Male	Female	Male
Age	22	16	13	21
RNF168 mutation (cDNA level)	c.397dupG/c.1323_1326delACAA	c.391C>T homozygous	c.295delG homozygous	c.295delG homozygous
RNF168 mutation (protein level)	p.A133Gfs*10/p. N441Rfs*16	p.R131X homozygous	p.E99Kfs*17 homozygous	p.E99Kfs*17 homozygous
ATM mutation	No	No	c.1402_1403delAA heterozygous	No
Cellular radiosensitivity	Yes	Yes	Yes	Yes
Reduced IgG	Yes	No	Yes (IgG2)	Yes
Reduced IgA	(No)[a]	Yes	Yes	Yes
Elevated AFP	n.d.	Yes	Yes	Yes
Ataxia	(No)[b]	Yes, mild	(No)[b]	(No)[b]
Telangiectasia	No	Yes	Yes	Yes
Short stature	Yes	Yes	No	No
Microcephaly	(No)[c]	Yes	(No)[c]	No
Learning difficulties	Yes	No	Yes	No
Respiratory failure	No	Yes	No	No

Clinical and cellular features were summarized for the first (15-9BI) and second (RS66) reported patients with RNF168 deficiency according to the descriptions by Stewart et al. (3) and Devgan et al. (18), and for the two new patients in the present report.

[a]*Borderline in early childhood.*

[b]*Mild motor control difficulties.*

[c]*Mild facial dysmorphism.*

Ig, immunoglobulin; AFP, alpha-fetoprotein.

unique cellular phenotype in the DNA damage response, and suggests elevated serum AFP as a useful laboratory marker. *RNF168* has occasionally been included on sequencing panels for primary immunodeficiencies (28, 29), and in one study two unspecified *RNF168* missense variants were reported in 1/33 patients (28). Another missense variant in *RNF168* has been observed in an adult patient with ZAP70 deficiency and was suggested to modify his phenotype (30). It is anticipated that, with the increasing use of next-generation sequencing in the diagnosis of immunodeficiency disorders (31), additional patients with *RNF168* mutations might be identified and can help to define and perhaps extend the clinical spectrum of RNF168 deficiency.

ETHICS STATEMENT

This study was carried out in accordance with the Declaration of Helsinki. All subjects gave written informed consent, and the protocol was approved by the Bioethical Commission, The Children's Memorial Health Institute, Warsaw.

AUTHOR CONTRIBUTIONS

BP and EH-P: clinical diagnosis and documentation; sample acquisition. RG, JE, BW, and NB: laboratory analyses. TD: data analyses and manuscript drafting. All authors: manuscript writing and approval.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at http://www.frontiersin.org/article/10.3389/fimmu.2017.01683/full#supplementary-material.

FIGURE S1 | Photographs of the Polish siblings with RNF168 deficiency at their age of 12 years (sister) and 21 years (brother).

REFERENCES

1. Nahas SA, Gatti RA. DNA double strand break repair defects, primary immunodeficiency disorders, and 'radiosensitivity'. *Curr Opin Allergy Clin Immunol* (2009) 9(6):510–6. doi:10.1097/ACI.0b013e328332be17
2. Blundred RM, Stewart GS. DNA double-strand break repair, immunodeficiency and the RIDDLE syndrome. *Expert Rev Clin Immunol* (2011) 7(2):169–85. doi:10.1586/eci.10.93
3. Stewart GS, Stankovic T, Byrd PJ, Wechsler T, Miller ES, Huissoon A, et al. RIDDLE immunodeficiency syndrome is linked to defects in 53BP1-mediated DNA damage signaling. *Proc Natl Acad Sci U S A* (2007) 104(43):16910–5. doi:10.1073/pnas.0708408104
4. Stewart GS, Panier S, Townsend K, Al-Hakim AK, Kolas NK, Miller ES, et al. The RIDDLE syndrome protein mediates a ubiquitin-dependent signaling cascade at sites of DNA damage. *Cell* (2009) 136(3):420–34. doi:10.1016/j.cell.2008.12.042
5. Doil C, Mailand N, Bekker-Jensen S, Menard P, Larsen DH, Pepperkok R, et al. RNF168 binds and amplifies ubiquitin conjugates on damaged chromosomes to allow accumulation of repair proteins. *Cell* (2009) 136(3):435–46. doi:10.1016/j.cell.2008.12.041
6. Thorslund T, Ripplinger A, Hoffmann S, Wild T, Uckelmann M, Villumsen B, et al. Histone H1 couples initiation and amplification of ubiquitin signalling after DNA damage. *Nature* (2015) 527(7578):389–93. doi:10.1038/nature15401

7. Pinato S, Scandiuzzi C, Arnaudo N, Citterio E, Gaudino G, Penengo L. RNF168, a new RING finger, MIU-containing protein that modifies chromatin by ubiquitination of histones H2A and H2AX. *BMC Mol Biol* (2009) 10:55. doi:10.1186/1471-2199-10-55
8. Mattiroli F, Vissers JH, van Dijk WJ, Ikpa P, Citterio E, Vermeulen W, et al. RNF168 ubiquitinates K13-15 on H2A/H2AX to drive DNA damage signaling. *Cell* (2012) 150(6):1182–95. doi:10.1016/j.cell.2012.08.005
9. Gatti M, Pinato S, Maspero E, Soffientini P, Polo S, Penengo L. A novel ubiquitin mark at the N-terminal tail of histone H2As targeted by RNF168 ubiquitin ligase. *Cell Cycle* (2012) 11(13):2538–44. doi:10.4161/cc.20919
10. Gatti M, Pinato S, Maiolica A, Rocchio F, Prato MG, Aebersold R, et al. RNF168 promotes noncanonical K27 ubiquitination to signal DNA damage. *Cell Rep* (2015) 10(2):226–38. doi:10.1016/j.celrep.2014.12.021
11. Goldstein M, Kastan MB. Repair versus checkpoint functions of BRCA1 are differentially regulated by site of chromatin binding. *Cancer Res* (2015) 75(13):2699–707. doi:10.1158/0008-5472.CAN-15-0400
12. Fradet-Turcotte A, Canny MD, Escribano-Díaz C, Orthwein A, Leung CC, Huang H, et al. 53BP1 is a reader of the DNA-damage-induced H2A Lys 15 ubiquitin mark. *Nature* (2013) 499(7456):50–4. doi:10.1038/nature12318
13. Wilson MD, Benlekbir S, Fradet-Turcotte A, Sherker A, Julien JP, McEwan A, et al. The structural basis of modified nucleosome recognition by 53BP1. *Nature* (2016) 536(7614):100–3. doi:10.1038/nature18951
14. Bohgaki M, Bohgaki T, El Ghamrasni S, Srikumar T, Maire G, Panier S, et al. RNF168 ubiquitylates 53BP1 and controls its response to DNA double-strand breaks. *Proc Natl Acad Sci U S A* (2013) 110(52):20982–7. doi:10.1073/pnas.1320302111
15. Smeenk G, Mailand N. Writers, readers, and erasers of histone ubiquitylation in DNA double-strand break repair. *Front Genet* (2016) 7:122. doi:10.3389/fgene.2016.00122
16. Ramachandran S, Chahwan R, Nepal RM, Frieder D, Panier S, Roa S, et al. The RNF8/RNF168 ubiquitin ligase cascade facilitates class switch recombination. *Proc Natl Acad Sci U S A* (2010) 107(2):809–14. doi:10.1073/pnas.0913790107
17. Bohgaki T, Bohgaki M, Cardoso R, Panier S, Zeegers D, Li L, et al. Genomic instability, defective spermatogenesis, immunodeficiency, and cancer in a mouse model of the RIDDLE syndrome. *PLoS Genet* (2011) 7(4):e1001381. doi:10.1371/journal.pgen.1001381
18. Devgan SS, Sanal O, Doil C, Nakamura K, Nahas SA, Pettijohn K, et al. Homozygous deficiency of ubiquitin-ligase ring-finger protein RNF168 mimics the radiosensitivity syndrome of ataxia-telangiectasia. *Cell Death Differ* (2011) 18(9):1500–6. doi:10.1038/cdd.2011.18
19. Schröder-Heurich B, Wieland B, Lavin MF, Schindler D, Dörk T. Protective role of RAD50 on chromatin bridges during abnormal cytokinesis. *FASEB J* (2014) 28(3):1331–41. doi:10.1096/fj.13-236984
20. Sandoval N, Platzer M, Rosenthal A, Dörk T, Bendix R, Skawran B, et al. Characterization of ATM gene mutations in 66 ataxia telangiectasia families. *Hum Mol Genet* (1999) 8(1):69–79. doi:10.1093/hmg/8.1.69
21. Wolska-Kuśnierz B, Pac M, Pietrucha B, Heropolitańska-Pliszka E, Klaudel-Dreszler M, Kurenko-Deptuch M, et al. Twenty five years of investigations into primary immunodeficiency diseases in the Department of Immunology, The Children's Memorial Health Institute, Warsaw. *Cent Eur J Immunol* (2005) 30(3–4):104–14.
22. Dörk T, Bendix-Waltes R, Wegner RD, Stumm M. Slow progression of ataxia-telangiectasia with double missense and in frame splice mutations. *Am J Med Genet A* (2004) 126A(3):272–7. doi:10.1002/ajmg.a.20601
23. Claes K, Depuydt J, Taylor AM, Last JI, Baert A, Schietecatte P, et al. Variant ataxia telangiectasia: clinical and molecular findings and evaluation of radiosensitive phenotypes in a patient and relatives. *Neuromolecular Med* (2013) 15(3):447–57. doi:10.1007/s12017-013-8231-4
24. Worth PF, Srinivasan V, Smith A, Last JI, Wootton LL, Biggs PM, et al. Very mild presentation in adult with classical cellular phenotype of ataxia telangiectasia. *Mov Disord* (2013) 28(4):524–8. doi:10.1002/mds.25236
25. Li X, Lee YK, Jeng JC, Yen Y, Schultz DC, Shih HM, et al. Role for KAP1 serine 824 phosphorylation and sumoylation/desumoylation switch in regulating KAP1-mediated transcriptional repression. *J Biol Chem* (2007) 282(50):36177–89. doi:10.1074/jbc.M706912200
26. Luijsterburg MS, Typas D, Caron MC, Wiegant WW, Heuvel DV, Boonen RA, et al. A PALB2-interacting domain in RNF168 couples homologous recombination to DNA break-induced chromatin ubiquitylation. *Elife* (2017) 6:e20922. doi:10.7554/eLife.20922
27. Al-Herz W, Bousfiha A, Casanova JL, Chatila T, Conley ME, Cunningham-Rundles C, et al. Primary immunodeficiency diseases: an update on the classification from the international union of immunological societies expert committee for primary immunodeficiency. *Front Immunol* (2014) 5:162. doi:10.3389/fimmu.2014.00162
28. Moens LN, Falk-Sörqvist E, Asplund AC, Bernatowska E, Smith CI, Nilsson M. Diagnostics of primary immunodeficiency diseases: a sequencing capture approach. *PLoS One* (2014) 9(12):e114901. doi:10.1371/journal.pone.0114901
29. Stoddard JL, Niemela JE, Fleisher TA, Rosenzweig SD. Targeted NGS: a cost-effective approach to molecular diagnosis of PIDs. *Front Immunol* (2014) 5:531. doi:10.3389/fimmu.2014.00531
30. Chinn IK, Sanders RP, Stray-Pedersen A, Coban-Akdemir ZH, Kim VH, Dadi H, et al. Novel combined immune deficiency and radiation sensitivity blended phenotype in an adult with biallelic variations in ZAP70 and RNF168. *Front Immunol* (2017) 8:576. doi:10.3389/fimmu.2017.00576
31. Seleman M, Hoyos-Bachiloglu R, Geha RS, Chou J. Uses of next-generation sequencing technologies for the diagnosis of primary immunodeficiencies. *Front Immunol* (2017) 8:847. doi:10.3389/fimmu.2017.00847

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Dried Blood Spots, an Affordable Tool to Collect, Ship, and Sequence gDNA from Patients with an X-Linked Agammaglobulinemia Phenotype Residing in a Developing Country

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Background: New sequencing techniques have revolutionized the identification of the molecular basis of primary immunodeficiency disorders (PID) not only by establishing a gene-based diagnosis but also by facilitating defect-specific treatment strategies, improving quality of life and survival, and allowing factual genetic counseling. Because these techniques are generally not available for physicians and their patients residing in developing countries, collaboration with overseas laboratories has been explored as a possible, albeit cumbersome, strategy. To reduce the cost of time and temperature-sensitive shipping, we selected Guthrie cards, developed for newborn screening, to collect dried blood spots (DBS), as a source of DNA that can be shipped by regular mail at minimal cost.

Method: Blood was collected and blotted onto the filter paper of Guthrie cards by completely filling three circles. We enrolled 20 male patients with presumptive X-linked agammaglobulinemia (XLA) cared for at the Vietnam National Children's Hospital, their mothers, and several sisters for carrier analysis. DBS were stored at room temperature until ready to be shipped together, using an appropriately sized envelope, to a CLIA-certified laboratory in the US for sequencing. The protocol for Sanger sequencing was modified to account for the reduced quantity of gDNA extracted from DBS.

Result: High-quality gDNA could be extracted from every specimen. Bruton tyrosine kinase (BTK) mutations were identified in 17 of 20 patients studied, confirming the diagnosis of XLA in 85% of the study cohort. Type and location of the mutations were similar to those reported in previous reviews. The mean age when XLA was suspected clinically was 4.6 years, similar to that reported by Western countries. Two of 15 mothers, each with an affected boy, had a normal BTK sequence, suggesting gonadal mosaicism.

Conclusion: DBS collected on Guthrie cards can be shipped inexpensively by airmail across continents, providing sufficient high-quality gDNA for Sanger sequencing overseas. By using this method of collecting gDNA, we were able to confirm the diagnosis of XLA in 17 of 20 Vietnamese patients with the clinical diagnosis of agammaglobulinemia.

Keywords: X-linked agammaglobulinemia, genetic sequencing, primary immunodeficiencies, Btk, dried blood spots

INTRODUCTION

Recent technical advances in gene analysis have facilitated diagnosis and treatment of primary immunodeficiency diseases (PID), with more than 320 genes identified that cause PID when mutated (1). While those technologies are readily available in developed countries, most developing countries do not have the medical facilities to perform gene sequencing and protein analysis (2, 3). Many physicians caring for PID patients in these parts of the world enter into partnerships with researchers in the United States, Europe, and selected Asian countries to initiate genetic evaluation of their patients (2, 3). However, shipping blood or cryopreserved peripheral blood mononuclear cells between continents is hampered by bureaucratic road blocks, is expensive, and is sometimes unsuccessful.

X-linked agammaglobulinemia (XLA), a genetic disorder first described by Bruton in 1952 (4), is caused by mutations in the Bruton tyrosine kinase (*BTK*) gene (5). Patients with XLA have low or absence of circulating B cells and markedly reduced serum immunoglobulin and specific antibody levels, resulting in increased susceptibility to bacterial and selective viral infections. Affected boys present typically with respiratory tract infections, but may also develop colitis, arthritis, and neurologic findings (6–8). The *BTK* gene consists of 18 coding exons and directs the production of a 659 amino acid protein with 5 distinct structural domains (**Figure 1**) that plays a crucial role in B cell development and function (6, 9).

The clinical criteria sufficient for a probable diagnosis of XLA include a male with less than 2% $CD19^+$ B cells in whom other causes of hypogammaglobulinemia have been excluded, who has at least one of the following: onset of recurrent bacterial infections in the first 5 years of life, serum IgG, IgM and IgA that are more than 2 SD below normal for age, and poor responses to vaccines. The "definitive" diagnosis of XLA requires gene sequencing confirming a mutation in BTK and/or absence of BTK protein expression in monocytes or platelets (10). Because analysis of protein expression and sequencing of the *BTK* gene are not commonly available in developing countries, a definitive diagnosis of XLA cannot be established.

To provide an inexpensive and effective method of performing genetic analyses in patients from developing countries with clinical and laboratorial findings compatible with a diagnosis of XLA, we tested the procedure established more than 50 years ago to collect and ship blood samples from hospitals to State laboratories for newborn screening. Originally designed for early detection of metabolic disorders such as phenylketonuria (11), dried blood spots (DBS) collected on filter paper (Guthrie cards) have been used in recent years as a source of gDNA to screen for severe combined immune deficiency, based on the presence or absence of T cell receptor excision circles, representing recent thymic emigrants (12, 13). We used Standard Guthrie cards to collect DBS, which can be shipped in a regular envelop by air mail (20 cards/envelop) from Vietnam to a CLIA-approved laboratory in Seattle, WA, USA, where the DNA was extracted and sequenced for mutations in the *BTK* gene. The approach has been validated by the demonstration that gDNA, stored for years in DBS, is stable and suitable for sequence analysis (14).

MATERIALS AND METHODS

Ethics Statement

The DBS analysis for BTK mutations was approved by the institutional review boards at Seattle Children's Hospital and Vietnam National Children's Hospital. Written informed consent for genetic testing was obtained from the parents of Vietnamese patients by local physicians.

Patients

Twenty patients with a clinical diagnosis of XLA, cared for by physicians at the Vietnam National Children's Hospital, Hanoi, were enrolled in the study from May to July 2017, and DBS were collected on Guthrie cards. A possible clinical diagnosis of XLA was based on the increased susceptibility to bacterial infections at an early age, low serum immunoglobulin levels, and marked decrease or absence of peripheral blood B lymphocytes (10). Mothers and sisters of these patients were invited to provide DBS for investigation of carrier status.

Samples

Vietnamese physicians caring for the patients collected venous blood or blood from a finger stick and completely filled three circles on the paper filter cards (ID Biological, Greenville, SC, USA) used by the Washington State Department of Health for newborn screening. The samples were stored at room temperature until DBS from all 20 patients were collected and were shipped in a single standard envelope by regular air mail to the Immunology Diagnostic Laboratory at Seattle Children's Research Institute. Upon arrival, gDNA was extracted from one of the three blood spots collected from each patient using the Quiagen DNA blood Mini Kit (Quiagen, Germantown, USA). The average yield was 3–20 ng/μl (total 150 μl). The unused DBS were stored at room temperature for future use.

BTK Gene Analysis

The targeted Sanger sequencing protocol for regular DNA analysis was modified, as the final concentration of gDNA extracted from DBS (5–25 ng/μl) was lower than that obtained from peripheral blood.

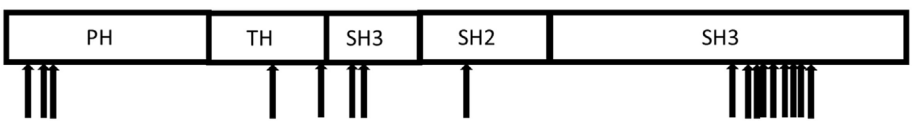

FIGURE 1 | Distribution of the mutations in the Bruton tyrosine kinase gene identified in the 17 Vietnamese X-linked agammaglobulinemia patients.

We optimized PCR amplification using only 2 µl of gDNA per exon or 5 µl gDNA for the longest exons or multiple exons based on the (modified) primers reported previously (15). Briefly, samples were amplified in 10 µl reactions containing 1 µl each of forward and reverse primers (5 mM each), 1 µl of dNTP mix, 1 µl of 10× buffer, 0.5 µl of $MgSO_4$ (50 mM), and 0.1 µl of undiluted Taq DNA Polymerase. All exons were amplified under the same PCR conditions: denaturation at 94°C during 2 min and then 38 cycles at 94°C for 30 s, 55°C for 30 s, and 68°C for 1.5 min, with final extension at 68°C for 5 min. Amplicons were sequenced according to the standard Big Dye protocol, analyzed, and aligned using the Mutation Surveyor and BioEdit software to detect mutations in the coding sequence and exon/intron junctions of *BTK*. The nomenclature for mutations in BTK is according to guidelines by the Human Genome Variation Society as determined by Mutalyzer 2.0.26-Name checker (NM_000061.2).

RESULTS

Table 1 presents clinical findings and laboratorial data of the 20 patients and lists *BTK* mutations identified in 17 participants. The mean age at the onset of symptoms was 9.52 months (range, 1–27 months), and the mean age at diagnosis was 54.70 months (range, 6–153 months). There was no difference between those patients with BTK mutations and those patients without. The mean age when the diagnosis was confirmed by BTK genetic analysis was 84.50 months (range, 21–197 months). The diagnosis of XLA based on the clinical characteristics was suspected at the age of 6 months in an asymptomatic boy (p06) who had an older diseased brother with an XLA phenotype. All patients started to receive IVIG as soon as the diagnosis of XLA was suspected clinically.

The most prevalent infections observed in this cohort of 20 patients were otitis media (121 episodes) and pneumonia (61 episodes), followed by conjunctivitis, arthritis, sepsis, sinusitis, and meningitis (**Figure 2**). The mean IgG level before IVIG therapy was 0.58 g/l (range, 0.01–3.17 g/l).

Pathogenic mutations in the *BTK* gene were identified in 17 of our cohort of 20 unrelated symptomatic patients tested by sequencing the 18 coding exons and exon–intron junctions. The mutations included missense and nonsense mutations, insertions, deletions, and splicing defects and were located throughout the *BTK* gene (**Table 1**; **Figure 1**). Additional samples were provided from 15 mothers of the 17 unrelated patients with *BTK* mutations (**Table 2**). Thirteen of the mothers had the same mutation as their sons and are considered XLA carriers. Of three girls, each having a full brother with a *BTK* mutation, one was found to be a carrier. The daughter of one of the two mothers with normal BTK sequence was found to have the same *BTK* mutation as her brother (p01), suggesting gonadal mosaicism.

DISCUSSION

The diagnosis of agammaglobulinemia can be suspected based on the clinical and laboratory characteristics. While the majority of patients with agammaglobulinemia are male, suggesting X-linked inheritance and mutations in *BTK*, there are a handful of genes that, if mutated, result in autosomal recessive agammaglobulinemia,

TABLE 1 | Clinical findings, laboratory results, and *BTK* mutations in 20 Vietnamese patients with agammaglobulinemia.

Patient number	Birth date	Onset of symptoms, age (months)	Age at diagnosis (months)	Age when genetic analysis was done (months)	IgA (g/l)	IgM (g/l)	IgG (g/l)	CD19/ mm³	CD19 (%)	*BTK* mutation	Mutation reported previously
p01	20/09/2012	4	14	58	0.03	0.07	3.17	5	0	c.1735G>C; p.D579H	No
p02	11/10/2011	6	22	69	0.01	0.09	0.01	1	0	c.1908+2delTAAGTGCTT (splicing defect)	No
p03	21/04/2010	4	75	87	0.01	0.27	0.16	7	0	c.752G>A; p.W251*	YES
p04	12/04/2011	9	42	75	0.05	0.08	0.1	78	1	c.117_119del; p.Y40del	No
P05	25/06/2011	5	23	73	0.01	0.14	0.07	8	0	c.521-1G>A (splicing defect)	No
P06	07/01/2013	[a]	6	54	0.13	0.13	0.22	48	1	c.1744del; p.A582Lfs*5	Yes
P07	08/10/2015	6	11	21	0	0.05	0.01	0	0	c.763C>T; p.R255*	Yes
P08	04/07/2011	27	58	72	0.01	0.17	0.06	0	0	c.1713_1714 del; p.Y571*	No
P09	29/09/2011	9	59	70	0.01	0.01	0.16	0	0	c.1768A>T; p.I590F	Yes
P10	10/02/2001	1	153	197	0.01	0.01	0.06	0	0	c.1782del; p.K595Rfs*54	No
p11	14/01/2003	24	113	174	0.35	0.36	1.58	2	0.1	c.37C>T; p.R13*	Yes
p12	23/10/2008	14	61	105	0.02	0.12	0.02	1	0	c.1610del; p.V537Dfs*19	No
p13	06/06/2008	9	63	109	0.03	0.16	1.2	1	0	c.953C>T; p.S318F	Yes
p14	27/09/2010	6	62	82	0.01	0.01	1.7	15	0.4	c.1657del; p.S553Afs*3	No
p15	17/11/2010	4	72	80	0.03	0.19	0.12	52	1	c.124T>G; p.Y42D	No
p16	06/01/2013	20	51	54	0.01	0.15	0.88	52	1	c.627_628insA; p.P210Tfs*6	No
p17	04/03/2012	8	59	64	0.02	0.21	0.06	20	1	c.1651T>A; p.Y551N	No
p18	07/07/2014	4	25	36	0.02	0.39	0.2	0	0	No mutation[b]	–
p19	22/06/2012	12	50	61	0.18	0.23	1.28	1	0	No mutation[b]	–
p20	10/02/2005	9	79	149	0.01	0.82	0.62	0	0	No mutation[b]	–

[a]*Patient diagnosed before he developed symptoms.*

[b]*In exons and exon/intron junctions.*

TABLE 2 | Carriers found among mothers and sisters of patients with XLA diagnosis.

Patient	Relative tested
p01[a]	Mother not carrier[b]
	Sister carrier
p02	Mother carrier
p03	Mother not carrier[b]
p04	Mother carrier
p05	Mother carrier
p06	Mother carrier
p07	Mother carrier
p08	Mother carrier
p11	Mother carrier
	Sister not carrier[b]
p12	Mother carrier
	Sister not carrier[b]
p13	Mother carrier
p14	Mother carrier
p15	Mother carrier
p16	Mother carrier
p17	Mother carrier

[a]*The presence of a BTK mutation in the brother (p01) and sister while absent in the mother suggests gonadal mosaicism of the mother.*
[b]*Target sequencing directed to the affected exon of the BTK gene did not reveal the mutation of the proband.*
BTK, Bruton tyrosine kinase.

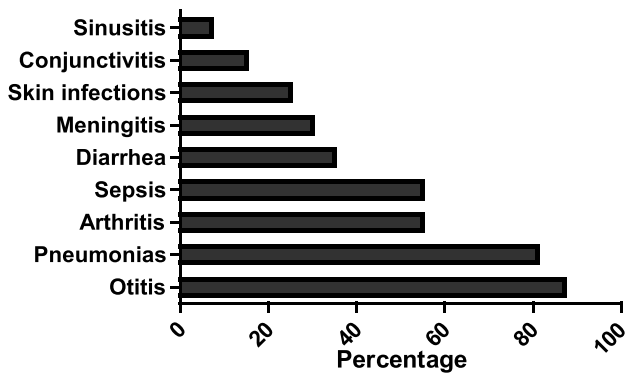

FIGURE 2 | Frequency of infections in a cohort of 20 Vietnamese patients with agammaglobulinemia.

including *IGHM* (μ heavy chain deficiency), *IGLL1* (λ5 deficiency), *CD79A* (Igα deficiency), *CD79B* (Igβ deficiency), *BLNK*, *PIK3R1* (1), *IKAROS* (16), and one gene (*TCF3*, E47 deficiency) causing an autosomal-dominant agammaglobulinemia (17). Because of this heterogeneity, molecular analysis is imperative for optimal therapy, prognostic considerations, and genetic counseling of patients with agammaglobulinemia. While sequence analysis, either by Sanger sequencing of individual genes or by next-generation sequencing is broadly available in the developed world, most developing countries do not have access to these advanced techniques.

This pilot study demonstrates the feasibility of collecting blood on Guthrie cards, shipping the cards at minimal cost from one continent to another, and identifying causative mutations in *BTK* by Sanger sequencing in 17 of 20 (85%) unrelated male patients with clinical and laboratory findings compatible with XLA. We have used this technique to collect and ship DBS from a number of South American countries to identify disease-causing mutations in >25 PID-associated genes including *WAS*, *CD40L*, *PIK3CD, FOXP3, CTLA4, STAT1, STAT3, RAG1*, and *RAG2*. Because of the limited quantity of patient material, we had to slightly modify the PCR amplification technique that we routinely use for gDNA extraction from peripheral blood or saliva.

The type and distribution of the *BTK* mutations were similar to those reported previously. Since only the coding region and the intron/exon junctions of *BTK* were sequenced, mutations in the promoter or Poly A regions, or deep intronic variants, would have been missed. Identifying those mutations will require analysis of mRNA or protein expression, we hope to develop in the future. The clinical characteristics and laboratory findings of the 17 Vietnamese XLA patients with BTK mutations were similar to those observed in Western countries, with episodes of pneumonia reported by 80% of the Vietnamese patients, 62% of US patients (7), 100% of patients from the Netherlands (18), and 77% of patients from China (8).

The mean age at diagnosis, based on clinical and immunological criteria, was similar to Western countries: 4.6 years in Vietnamese patients, compared with 5.37 in the United States (7) and 6.5 years in patients from the Netherlands (18), but slightly lower than in those from China (7.09 years) (8).

In conclusion, DBS are easy to collect, can be stored and shipped at room temperature, and transported inexpensively across continents. DBS are an excellent source of high-quality gDNA in sufficient quantity for Sanger sequencing, making this collection technique an affordable option for developing countries to interact and collaborate with sophisticated laboratories anywhere in the world.

ETHICS STATEMENT

Institutional review boards (IRBs) at Seattle Children's Hospital and Vietnam National Children's Hospital.

AUTHOR CONTRIBUTIONS

HO and TT conceived and designed the study; GS developed the technique and sequenced BTK from DBS; AN, HTML, HT, LN, and HTL identified the patients and collected DBS; RK established contact with the Hanoi group and facilitated the shipment of samples.

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REFERENCES

1. Picard C, Bobby Gaspar H, Al-Herz W, Bousfiha A, Casanova JL, Chatila T, et al. International Union of Immunological Societies: 2017 Primary Immunodeficiency Diseases Committee Report on Inborn Errors of Immunity. *J Clin Immunol* (2017) 38:96–128. doi:10.1007/s10875-017-0464-9
2. Costa-Carvalho B, González-Serrano M, Espinosa-Padilla S, Segundo G. Latin American challenges with the diagnosis and treatment of primary immunodeficiency diseases. *Expert Rev Clin Immunol* (2017) 13:483–9. doi:10.1080/1744666X.2017.1255143
3. Espinosa-Rosales FJ, Condino-Neto A, Franco JL, Sorensen RU. Into action: improving access to optimum care for all primary immunodeficiency patients. *J Clin Immunol* (2016) 36:415–7. doi:10.1007/s10875-016-0277-2
4. Bruton OC. Agammaglobulinemia. *Pediatrics* (1952) 9:722–8.
5. Tsukada S, Saffran DC, Rawlings DJ, Parolini O, Allen RC, Klisak I, et al. Deficient expression of a B cell cytoplasmic tyrosine kinase in human X-linked agammaglobulinemia. *Cell* (1993) 72:279–90. doi:10.1016/0092-8674(93)90667-F
6. Conley ME, Rohrer J, Minegishi Y. X-linked agammaglobulinemia. *Clin Rev Allergy Immunol* (2000) 19:183–204. doi:10.1385/CRIAI:19:2:183
7. Winkelstein JA, Marino MC, Lederman HM, Jones SM, Sullivan K, Burks AW, et al. X-linked agammaglobulinemia: report on a United States registry of 201 patients. *Medicine (Baltimore)* (2006) 85:193–202. doi:10.1097/01.md.0000229482.27398.ad
8. Chen XF, Wang WF, Zhang YD, Zhao W, Wu J, Chen TX. Clinical characteristics and genetic profiles of 174 patients with X-linked agammaglobulinemia: report from Shanghai, China (2000–2015). *Medicine (Baltimore)* (2016) 95:e4544. doi:10.1097/MD.0000000000004544
9. Desiderio S. Role of Btk in B cell development and signaling. *Curr Opin Immunol* (1997) 9:534–40. doi:10.1016/S0952-7915(97)80107-0
10. Conley ME, Notarangelo LD, Etzioni A. Diagnostic criteria for primary immunodeficiencies. Representing PAGID (Pan-American Group for Immunodeficiency) and ESID (European Society for Immunodeficiencies). *Clin Immunol* (1999) 93:190–7. doi:10.1006/clim.1999.4799
11. Guthrie R, Susi A. A simple phenylalanine method for detecting phenylketonuria in large populations of newborn infants. *Pediatrics* (1963) 32:338–43.
12. Hazenberg MD, Verschuren MC, Hamann D, Miedema F, van Dongen JJ. T cell receptor excision circles as markers for recent thymic emigrants: basic aspects, technical approach, and guidelines for interpretation. *J Mol Med (Berl)* (2001) 79:631–40. doi:10.1007/s001090100271
13. Chan K, Puck JM. Development of population-based newborn screening for severe combined immunodeficiency. *J Allergy Clin Immunol* (2005) 115:391–8. doi:10.1016/j.jaci.2004.10.012
14. Hollegaard MV, Grove J, Grauholm J, Kreiner-Møller E, Bønnelykke K, Nørgaard M, et al. Robustness of genome-wide scanning using archived dried blood spot samples as a DNA source. *BMC Genet* (2011) 12:58. doi:10.1186/1471-2156-12-58
15. Chan K-W, Chen T, Jiang L, Fok F-S, Lee T-L, Lee B-W, et al. Identification of Bruton tyrosine kinase mutations in 12 Chinese patients with X-linked agammaglobulinaemia by long PCR-direct sequencing. *Intl J Immunogenet* (2006) 33:205–9. doi:10.1111/j.1744-313X.2006.00598.x
16. Kuehn HS, Boisson B, Cunningham-Rundles C, Reichenbach J, Stray-Pedersen A, Gelfand EW, et al. Loss of B cells in patients with heterozygous mutations in IKAROS. *N Engl J Med* (2016) 374:1032–43. doi:10.1056/NEJMoa1512234
17. Boisson B, Wang YD, Bosompem A, Ma CS, Lim A, Kochetkov T, et al. A recurrent dominant negative E47 mutation causes agammaglobulinemia and BCR(-) B cells. *J Clin Invest* (2013) 123:4781–5. doi:10.1172/JCI71927
18. Van Der Hilst JCH, Smits BW, Van Der Meer JWM. Hypogammaglobulinemia: cumulative experience in 49 patients in a tertiary care institution. *Neth J Med* (2002) 60:140–7.

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CASE REPORT
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Exome Sequencing Diagnoses X-Linked Moesin-Associated Immunodeficiency in a Primary Immunodeficiency Case

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Background: We investigated the molecular etiology of a young male proband with confirmed immunodeficiency of unknown cause, presenting with recurrent bacterial and Varicella zoster viral infections in childhood and persistent lymphopenia into early adulthood.

Aim: To identify causative functional genetic variants related to an undiagnosed primary immunodeficiency.

Method: Whole genome microarray copy number variant (CNV) analysis was performed on the proband followed by whole exome sequencing (WES) and trio analysis of the proband and family members. A >4 kbp deletion identified by repeated CNV analysis of exome sequencing data along with three damaging missense single nucleotide variants were validated by Sanger sequencing in all family members. Confirmation of the causative role of the candidate gene was performed by qPCR and Western Blot analyses on the proband, family members and a healthy control.

Results: CNV identified our previously reported interleukin 25 amplification in the proband; however, the variant was not validated to be a candidate gene for immunodeficiency. WES trio analysis, data filtering and *in silico* prediction identified a novel, damaging (SIFT: 0; Polyphen 1; Grantham score: 101) and disease-causing (MutationTaster) single base mutation in the X chromosome (c.511C > T p.Arg171Trp) *MSN* gene not identified in the UCSC Genome Browser database. The mutation was validated by Sanger sequencing, confirming the proband was hemizygous X-linked recessive (–/T) at this locus and inherited the affected T allele from his non-symptomatic carrier mother (C/T), with other family members (father, sister) confirmed to be wild type (C/C). Western Blot analysis demonstrated an absence of moesin protein in lymphocytes derived from the proband, compared with normal expression in lymphocytes derived from the healthy control, father and mother. qPCR identified significantly lower *MSN* mRNA transcript expression in the proband compared to an age- and sex-matched healthy control subject in whole blood ($p = 0.02$), and lymphocytes ($p = 0.01$). These results confirmed moesin deficiency in the proband, directly causative of his immunodeficient phenotype.

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Conclusion: These findings confirm X-linked moesin-associated immunodeficiency in a proband previously undiagnosed up to 24 years of age. This study also highlights the utility of WES for the diagnosis of rare or novel forms of primary immunodeficiency disease.

Keywords: lymphopenia, whole exome sequencing, moesin, *MSN*, X-linked moesin-associated immunodeficiency

INTRODUCTION

Primary immunodeficiency disorders (PIDs) encompass a diverse group of mostly inherited genetic disorders that compromise immune system function and predispose individuals to recurrent infections and other immune disorders such as autoimmunity, hematological disorders and lymphoid malignancies (1–3). As defined by the International Union of Immunological Societies, there are almost 300 single gene defects described which are causative for the wide range of phenotypes (4), making PIDs a rapidly expanding field of medicine. Loss of function mutations in multiple genes coding for proteins that regulate the actin cytoskeleton are known to cause PID, the most well characterized form being Wiskott-Aldrich (WAS) syndrome (5). This is a rare X chromosome-linked PID where expression and function of the WAS protein results in failure of Arp2/3-mediated actin polymerization causing a combined defect of innate and adaptive immunity associated with microthrombocytopenia, eczema, blood cancers, and increased risk of autoimmunity (5).

Next-generation DNA sequencing technologies such as whole genome sequencing (WGS) and whole exome sequencing (WES) have revolutionized the field of genomics by enabling a high-throughput and increasingly cost-effective method for complex and rare disease diagnosis (6). While WGS is the most comprehensive technique able to identify around 4 million variants within the entire genome, including coding and non-coding regions, WES provides more focus on only protein-coding exons, which harbor around 85% of pathogenic mutations (7, 8). Currently, WES permits lower operating costs (US$800 per sample compared to US$1,500 per sample for WGS) with faster data generation and less complex analysis, however, it is unable to identify structural variants (6, 9). Here, we used WES to elucidate possible disease-causing variants in a case diagnosed previously as immunodeficient of unknown cause.

BACKGROUND

The proband is a 24-year–old male. At age 7 weeks, he had boils in his groin and axillae. At 18 months of age, he had a prolonged episode of bronchitis for which he received antibiotics but was not hospitalized. He suffered repeated upper respiratory tract infections and ear infections for the next few years. He had two episodes of chicken pox (Varicella) at around 18 months and 3 years. The first episode was severe, with widespread skin lesions, but no secondary infection or hemorrhagic lesions. At about 3 years of age, he had a persistent eye infection, which progressed to periorbital cellulitis, secondary to *Pseudomonas aeruginosa* infection. Neutropenia was first noted during that episode. Bone marrow aspirate showed normal myeloid maturation, consistent with an immune etiology. No treatment was started at that stage. At 4 years of age, he had an episode of thrombocytopenia, with platelets falling to <5. A further bone marrow aspirate was consistent with an immune mediated thrombocytopenia, and he responded rapidly to intravenous immunoglobulin. He had some further skin infections and paronychiae at this time, and continued to suffer repeated respiratory infections, although not requiring hospitalization. At 6 years 6 months of age, he had his first episode of pneumonia and had several hospitalizations.

At this stage, a diagnosis of combined immunodeficiency was made: he had persistent absolute lymphopenia, normal T-cell receptor Vβ distribution, low numbers of T-cell receptor excision circles (TRECs), no mutation in the common γ chain gene, low IgG, IgA, and IgM. He was negative for HIV by PCR. He started immunoglobulin replacement (IVIg) and G-CSF treatment in early 2000 and has very much improved symptomatically since then, however, lymphocyte counts have remained severely decreased (**Tables 1**and **2**).

We previously investigated a functional molecular cause for this undiagnosed immunodeficiency through whole genome arrays. Briefly, analysis of Affymetrix 250K SNP microarray data of the case and a matched healthy control subject *via* a copy number analysis tool identified hyperploidy of a region centromeric to chromosome 14q11.2, mapped over the interleukin 25 (*IL25*) gene (10). IL-25 (a member of the IL-17 family of cytokines) induction in mice has been previously associated with a T-helper (Th) 2-like pathological immune response (11) and shown to regulate the development of autoimmune inflammation mediated by IL-17-producing cells (12, 13) and was deemed a plausible candidate for further analyses.

Green et al. (10) paired genetic analysis with transcriptional profiling and found a large number of genes associated with Th1/Th2 profiles to be differentially expressed in peripheral blood lymphocytes of the proband, indicative of a Th2 bias confirmed by flow cytometric analysis. T-cells from the proband and control subject were cultured and activated *in vitro* with qPCR analysis demonstrating higher *IL25* expression in the proband when compared to the control. The skewed Th2 immunity hypothesized in the proband was in line with his susceptibility to infections normally cleared by Th1 responses, such as Varicella and *Pseudomonas*. Further *in vitro* studies were performed in B-cell line models to measure the effect of IL-25 treatment on cellular proliferation and viability, however when proband and control lymphocytes were treated with exogenous IL-25, no differences were observed (data not shown).

TABLE 1 | Hematology laboratory workup for the proband.

Hematology	June 28, 2001	June 17, 2004	June 14, 2005	Units	Ref interval
Age at evaluation	8 years	11 years	12 years		6–12 years
Hemoglobin	118	127	127	g/L	110–15
Hematocrit	0.34	0.36	0.37		0.36–0.46
Red cell count	–	4.3	4.5	$\times10^{12}$/L	4.0–5.6
Mean cell volume	87	84	84	fL	77–95
White cell count	2.4 **L**	2.5 **L**	3.4 **L**	$\times10^{9}$/L	5.0–12.0
Neutrophils	2.04	2.00	2.67	$\times10^{9}$/L	1.5–7.5
Lymphocytes	0.32 **L**	0.40 **L**	0.52 **L**	$\times10^{9}$/L	1.0–6.5
Monocytes	0.04	0.10	0.11	$\times10^{9}$/L	0–1.5
Eosinophils	–	0.00	0.05	$\times10^{9}$/L	0–0.6
Basophils	–	0.00	0.01	$\times10^{9}$/L	0–0.20
Platelets	363	329	401	$\times10^{9}$/L	150–600
Lymphocyte subsets					
Lymphocytes	370 **L**	ND	ND	Cells/μL	1,900–3,700
T-cells CD3+	74 **L**	ND	ND	Cells/μL	600–2,600
T-helper CD4+	33 **L**	ND	ND	Cells/μL	650–1,500
T suppressor CD8+	33 **L**	ND	ND	Cells/μL	370–1,100
B-cells CD19+	15 **L**	ND	ND	Cells/μL	270–860
NK cells CD16+/56+	3 **L**	ND	ND	Cells/μL	4–20
TRECs	<100,000 **L**	ND	ND	Per million PBMC	100,000
Protein studies					
Immunoglobulin G (IgG) on IVIg	805	ND	ND	mg/dL	520–1,098
Immunoglobulin A (IgA)	16 **L**	ND	ND	mg/dL	36–230
Immunoglobulin M (IgM)	30 **L**	ND	ND	mg/dL	42–172

Historical hematology workup showing persistent leucopenia with lymphopenia and hypogammaglobulinemia at 8, 11, and 12 years of age. Neutropenia improved with G-CSF treatment.
PBMC, peripheral blood mononuclear cells; IVIg, intravenous immunoglobulin; ND, not determined.
Red font indicates values lower than the reference interval.

TABLE 2 | Hematology laboratory workup for the proband.

Hematology	December 16, 2016	July 04, 2017	Units	Ref interval
Age at evaluation	23 years	24 years		>12 years
Hemoglobin	138	142	g/L	135–175
Hematocrit	0.42	0.41		0.40–0.54
Red cell count	4.8	4.9	$\times10^{12}$/L	4.5–6.5
Mean cell volume	87	84	fL	80–100
White cell count	1.5 **L**	1.3 **L**	$\times10^{9}$/L	3.5–10.0
Neutrophils	0.96 **L**	0.74 **L**	$\times10^{9}$/L	1.5–6.5
Lymphocytes	0.46 **L**	0.47 **L**	$\times10^{9}$/L	1.0–4.0
Monocytes	0.06	0.08	$\times10^{9}$/L	0–0.9
Eosinophils	0.00	0.00	$\times10^{9}$/L	0–0.6
Basophils	0.01	0.02	$\times10^{9}$/L	0–0.15
Platelets	316	255	$\times10^{9}$/L	150–400
Lymphocyte subsets	0.46 **L**	ND	$\times10^{9}$/L	1.0–4.0
T-cells CD3+	0.43 **L**	ND	$\times10^{9}$/L	0.75–2.50
T-helper CD4+	0.15 **L**	ND	$\times10^{9}$/L	0.50–1.90
T suppressor CD8+	0.24	ND	$\times10^{9}$/L	0.21–1.2
B-cells CD19+	0.01 **L**	ND	$\times10^{9}$/L	0.05–0.60
NK cells CD16+/56+	0.02 **L**	ND	$\times10^{9}$/L	0.05–0.60
Protein studies				
Immunoglobulin G (IgG) on IVIg	9.15	6.98	g/L	5.76–15.36
Immunoglobulin A (IgA)	0.49 **L**	0.45 **L**	g/L	1.24–4.16
Immunoglobulin M (IgM)	0.13 **L**	0.21 **L**	g/L	0.48–3.1

Current hematology results showing persistent leucopenia with neutropenia, lymphopenia, and hypogammaglobulinemia at 23 and 24 years of age. Peripheral blood lymphocyte surface markers shows all lymphocyte subsets are below the normal range excluding T suppressor CD8+ cells which are on the lower level of normal.
IVIg, intravenous immunoglobulin; ND, not determined.
Red font indicates values lower than the reference interval.

MATERIALS AND METHODS

Whole Exome Sequencing

Peripheral blood samples were obtained from all participants and genomic DNA (gDNA) extracted using the QIAamp DNA Blood Maxi Kit (Qiagen) according to the manufacturer's instructions. Exome sequencing of the family trio (case, mother, and father) was performed on the Ion Chef and Ion Proton Next Generation Sequencing platform using the Ion Ampliseq Exome RDY Kit 4 × 2 (Life Technologies). DNA was quantitated using the Agilent 2100 Bioanalyser using the Agilent High Sensitivity DNA kit (Agilent Technologies) with 50 ng gDNA used for library preparation of each family member. Barcode adapters were ligated to each exome library with the Ion Express Barcode Adapters 1-96 Kit (Life Technologies). Using a Qubit 2.0 Fluorometer (Life Technologies), libraries were diluted to a concentration of 100 pM before being clonally amplified on the Ion Chef System using the Ion PI Chip v3 (Life Technologies) prior to loading on an Ion PI Chip v3 for sequencing *via* semiconduction.

WES Analysis

Raw sequences from each library were aligned to the GRCh37/Hg19 reference genome *via* the Ion Torrent Server TMAP alignment algorithm. Trio analysis was performed on the Ion Reporter Suite V.5.0 (Life Technologies) where variant annotation identified included single-nucleotide polymorphisms (SNPs) and indels (insertions and deletions) for each exome library.

At a total read-depth of 20×, the target base coverage was 94.63%. A combined total of 51,997 variants were identified with 482 of these unique to the proband. Variant filtering then focused on deleterious variants (frameshift insertion and deletion, stoploss, missense, and nonsense) followed by screening using the OMIM public database as a filter for genes related to immunological disorders. 13 identified variants were then further filtered using *in silico* missense variant effect prediction tools with the following parameters designated as damaging: SIFT < 0.05, PolyPhen > 0.8, and Grantham Score > 100. SIFT and PolyPhen predict both the structural and functional impact of a variant based on properties such as accessible surface area, ligand contacts, solvent accessible area, and change in residue side change volume (14). The Grantham score focuses on the difference between amino acid atomic composition, polarity, and volume where a radical amino acid substitution gives a score above 100 (15). MutationTaster predicts whether a variant is tolerable, polymorphic, or damaging and disease-causing to identify potential disease-causing variants (16). Three final candidate variants were then verified using the Integrative Genome Viewer (IGV) software (Broad Institute). Copy number variant (CNV) analysis was performed on WES data for the case and family members using the same filtering steps as described. WES was used to indicate the general presence of the CNV, which was validated by touchdown PCR and Sanger sequencing. A more detailed description of the methods used can be found in the BMC Bioinformatics paper by Demidov et al. (17). With the ongoing advances in sequencing technology and improvement in alignment techniques for large-scale data analysis, we were able to confirm that *IL25* does not lie within the previously identified amplified region on chromosome 14, but rather 4 Mb downstream.

Sanger Sequencing

PCR was performed using forward and reverse primers for the *MSN* (F: 5′-TTCTCTCCTGCACAGGGACTTT-3′; R: 5′-ATTCACCCTGTAAGGGAAGTGGG-3′), *TET2* (F:5′-GCCTGATGGAACAGGATAGAAC-3′;R:5′-TTCCCTTCATACAGGGTATTC-3′), and *NLRP8* (F:5′-ATATCCAGCGCCTGATAGCG-3′; R:5′-TCAGGGTGACGGTCAGTT-3′) single nucleotide variants (SNVs). Following amplification, PCR products were treated with Exo-SAP for use in Big Dye Terminator v3.1 sequencing reactions, analyzed on the 3500 Genetic Analyzer (ThermoFisher Scientific, Life Technologies). Sequencing data for each sample chromatogram was assessed using Chromas Lite 2.1.1 software.

Lymphocyte Isolation, Activation, and Expansion *In Vitro*

Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized whole blood from the family trios and a healthy control subject, using the Ficoll-Histopaque centrifugation method (Sigma-Aldrich). Isolated PBMCs were washed in 1X PBS and plated into T-75 culture flasks in RPMI-1640 culture medium (Invitrogen, ThermoFisher) with phytohemagglutinin (PHA) mitogen for up to 24 h to allow monocytes to adhere to the bottom of the flask. After 3 h lymphocytes in suspension were removed and plated into a new T-75 flask with PHA and IL-2 (Sigma-Aldrich). Lymphocytes were allowed to proliferate in RPMI-1640 culture medium at 37°C in 5% CO_2 for up to 5 days, and if necessary further expanded after 2 days. Live lymphocyte counts were performed on a hemocytometer and viability assessed by Trypan blue dye. Cell lysates were prepared for qPCR and Western Blot analyses with 4×10^5 to 3×10^6 cells.

Total RNA Extraction, cDNA Synthesis, and qPCR

Whole blood from a healthy control subject and family members was collected in PAXgene™ vacutainer tubes for immediate stabilization of intracellular RNA at collection. Total RNA was extracted using the PreAnalytiX PAXgene™ Blood RNA Kit according to the manufacturer's instructions. Lymphocytes were harvested in TRIzol and total RNA extracted using the Direct-zol™ RNA MiniPrep kit (Zymo Research). Total RNA from whole blood and lymphocytes was converted to cDNA using the iScript™ cDNA Synthesis Kit (Bio-Rad) according to the manufacturer's instructions. Samples for qPCR were assayed in triplicate using the Promega SYBR Green with 18S expression used as an endogenous control for normalization of the data. The $^{\Delta\Delta}$Ct comparison method was used to measure relative gene expression on the QuantStudio™ 7 instrument (ThermoFisher) and analyzed using Student's *T*-test to calculate statistical significance ($p < 0.05$).

Western Blotting

Total protein was extracted from cell lysates using Runx protein-lysis buffer (containing protease and phosphatase inhibitors) as previously described (18). Protein concentration was measured using the Qubit™ Protein Assay Kit (Invitrogen). A Western Blot using 30 µg protein per sample was performed using an anti-*MSN* primary antibody (ab52490, Abcam) and an HRP-conjugated secondary antibody (anti-Rabbit IgG #7074, Cell Signaling). Sample loading was normalized using HRP-conjugated anti-Beta-actin (#5125S, Cell Signaling). Detection of target protein was carried out with ECL (Clarity™ ECL, Bio-Rad) using the Fusion Spectra chemiluminescent system (Vilber Lourmat, Fisher Biotec) and optical density quantitation assessed using Bio-1D software.

Ethics and Cell Line Validation

This study was performed in accordance with the recommendations of the Queensland University of Technology Human Research Ethics Committee (Approval number 1400000125). All subjects gave written informed consent in accordance with the Declaration of Helsinki. Written informed consent was also obtained from the proband and family members for the publication of this case report. Cell line validations were performed for commercial Toledo non-Hodgkin lymphoma (NHL) B-cell and MCF-7 breast cancer cell lines (data not shown).

RESULTS

Identification and Validation of a SIRPβ1 Deletion

Copy number variant analysis of the WES data identified a large >4 kbp deletion in the *SIRPβ1* gene in the proband (chr20:1, 576,140–1,580,375 Hg38) not present in other family members.

Further inspection suggested the deletion in the proband encompassed exon 2 of the *SIRPβ1* gene. Validation of this deletion by touchdown PCR and Sanger sequencing confirmed a deletion of exon 2, which has high sequence homology with another exon in the same gene (data not shown). Further Sanger validation to determine the size of the deletion was discontinued due to high sequence homology in that region. Furthermore, this exon 2 deletion has also been found in a number of healthy individuals (19); and therefore, this variant was considered to be an unlikely pathogenic candidate for the immunodeficiency.

Identification and Validation of a Hemizygous X-Linked MSN Mutation

Filtering of the WES data on the Ion Reporter Suite yielded three variants in three immune function genes *TET2*, *NLRP8* and *MSN* with damaging SIFT and Polyphen scores predicted to be disease-causing/polymorphic by MutationTaster (**Table 3**). The *TET2* SNP (rs111948941, C > T) has a frequency of 0.15. The homozygous case (T/T) inherited an affected allele from each parent, both of whom are heterozygous (C/T) at this locus. The *NLRP8* variant (rs754128390, A > C) is very rare, occurring in <1/10,000 individuals. The proband was identified to be *de novo* heterozygous (A/C) with both parents homozygous wild type (A/A) at this locus. The *MSN* variant (c.511C > T, p.Arg171Trp at position chrX:64951012, Hg19) was a novel mutation, not reported in the genomic databases or the literature at the time of query. The proband is hemizygous X-linked for the mutation (-/T), inheriting the affected allele from his heterozygous (C/T) mother. The *TET2* SNP and *NLRP8* rare variant occur in healthy individuals with no known disease associations, therefore, they were considered unlikely candidates and not analyzed further. WES coverage for the *MSN* mutation was 145× for the proband, 119× for the father, 150× for the mother, with an average total coverage of 138×. The *MSN* mutation was validated by Sanger sequencing (**Figure 1A**) and confirmed using IGV for whole genome and exome data in all four family members (**Figure 1B**) and as such, considered to be the most likely candidate.

Protein Detection and Quantification

MSN encodes the moesin protein (MSN) which is a member of the ezrin-radixin-moesin (ERM) family of cell structure-related proteins. Western Blot analysis confirmed the presence of the 68 kDa MSN protein in the Toledo NHL B-cell line (positive control) and absence of MSN in the MCF-7 breast cancer cell line (negative control). MSN was shown to be present in the healthy control, father, mother, and is absent in the proband (**Figure 2A**). Relative MSN concentrations were normalized against beta-actin concentrations where the MCF-7 cells and the proband show very low MSN concentrations (<0.5 μg; **Figure 2B**). These data confirm the proband has a MSN protein deficiency, not present in the healthy control or parents, and is the most likely sole genetic cause for the persistent lymphopenia.

MSN mRNA Transcript Expression

MSN gene expression was measured in all samples (healthy control, father, mother and proband). In whole blood the proband

TABLE 3 | Whole exome trio analysis data filtering and *in silico* prediction tools (SIFT, PolyPhen, Grantham Score, and MutationTaster) identified three damaging and disease-causing SNVs in the proband: *MSN* C > T, *TET2* C > T, and *NLRP8* A > C.

Gene	Locus (Hg19)	dbSNP/ transcripts	Nucleotide change	MAF	Proband genotype	Variant effect	Amino acid change	SIFT	PolyPhen	Grantham score	Mutation taster
MSN	ChrX:64951012	Novel mutation NM_002444	c.511C > T	n/a	T/– Hemizygous X-linked	Missense AA change	p.Arg171Trp Basic to non-polar residue change	0 Damaging	1 Damaging	101 Radical amino acid change	Disease-causing
TET2	Chr4:106155199	rs111948941 NM_001127208 NM_017628	c.100C > T	T = 0.015 (dbSNP)	T/T Homozygous Recessive	Missense AA change	p.Leu34Phe Both residues are non-polar	0 Damaging	0.5 Possibly damaging	22 Conservative AA change	Disease-causing
NLRP8	Chr19:56467265	*De novo* variant NM_176811	c.1841A > C	C = < 1/10,000 (gnomAD)	A/C Heterozygous	Missense AA change	p.His614Pro Basic to non-polar residue change	0 Damaging	> 0.8 Damaging	77 Non-conservative AA change	Polymorphic, minor allele frequency; Hg19, GRCh37 reference genome. ly-morphic

MAF, minor allele frequency; Hg19, GRCh37 reference genome.
Red font highlights significant damaging and disease-causing prediction scores.

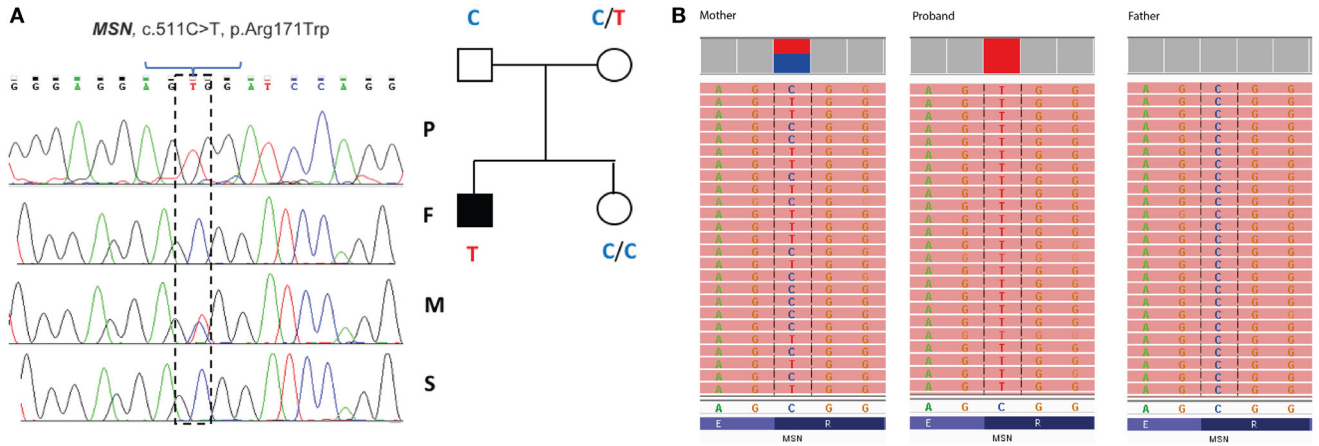

FIGURE 1 | Validation of the ^{R171W}MSN mutation in family members. **(A)** Sanger sequence and pedigree tree diagram for the missense *MSN* C > T mutation. P, proband; F, father; M, mother; S, sister. Mother is heterozygous C/T marked by a double blue and red peak and proband is hemizygous –/T marked by a single red peak. **(B)** IGV software showing *MSN* genotypes for the mother (C/T), proband (–T), and father (C/C), respectively. IGV, integrative genomics viewer.

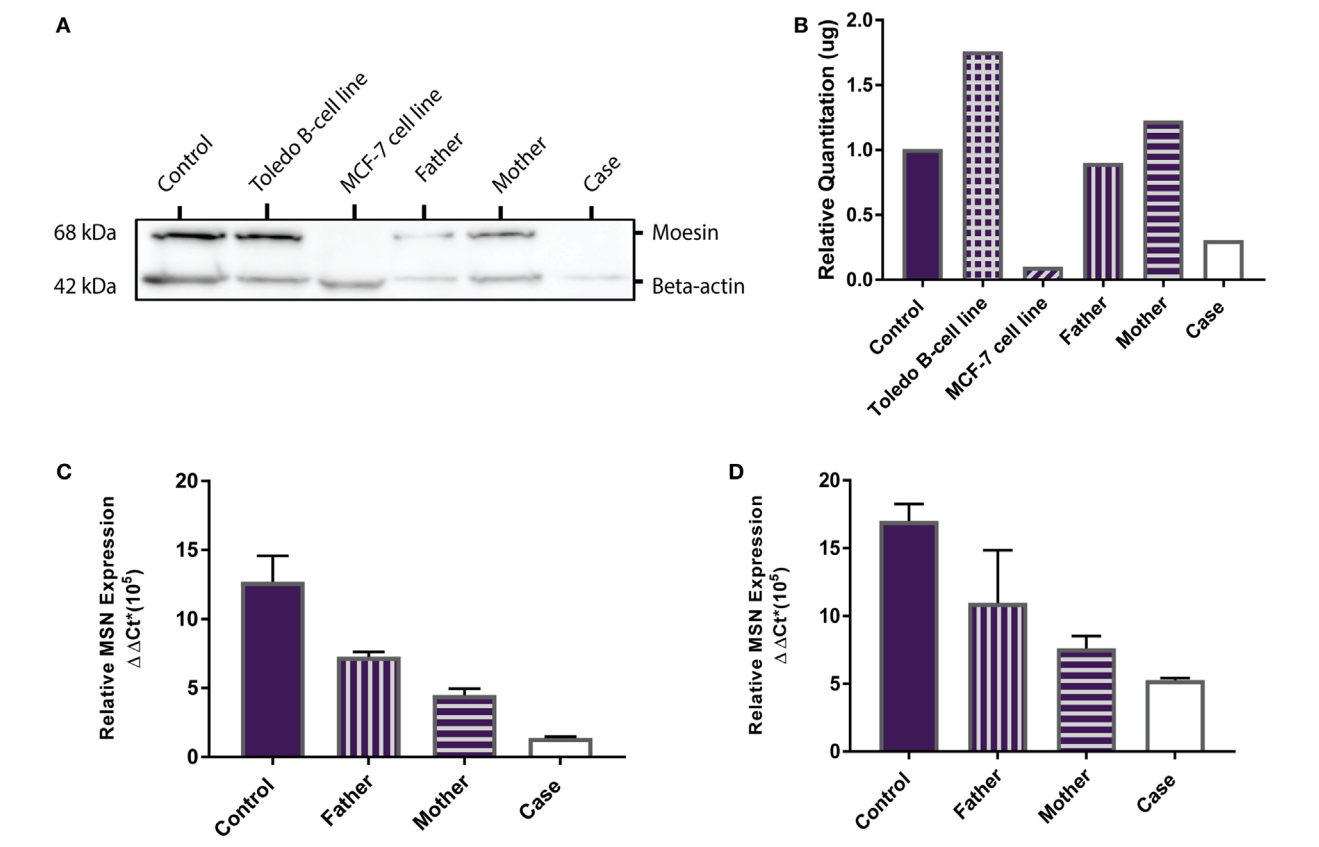

FIGURE 2 | MSN protein detection and *MSN* gene expression. **(A)** Western Blot shows absence of the 68 kDa moesin protein in the MCF-7 cell line (negative control, lane 3) and in the proband (lane 6), and presence of moesin in the healthy control subject (lane 1), Toledo B-cell line (positive control, lane 2), father (lane 4), and mother (lane 5). Beta-actin (42 kDa) was used as the loading control. **(B)** Optical density protein quantitation software showed very low levels of moesin (<0.5 μg) in the MCF-7 cells and the proband, confirming a moesin deficiency. **(C)** qPCR analysis of *MSN* mRNA transcript levels in whole blood (PAXgene) from the control subject, father, mother and proband. Error bars depict standard error of the mean (SEM) between triplicates. **(D)** qPCR analysis of *MSN* mRNA transcript levels in lymphocytes from the control subject, father, mother, and case. Error bars depict SEM between triplicates.

had significantly lower moesin mRNA expression than the control ($p = 0.02$), father ($p = 0.002$) and mother ($p = 0.01$) and the mother had significantly lower expression than the father ($p = 0.01$) and control ($p = 0.04$) (**Figure 2C**). In isolated lymphocytes, the proband ($p = 0.01$) and mother ($p = 0.004$) had significantly lower expression than the control (**Figure 2D**). Overall expression levels in all participants were higher in lymphocytes than in whole blood, with *MSN* mainly expressed in lymphocytes, however, it is also expressed in monocytes, neutrophils and platelets. The lower levels observed in the mother compared to the father and control and higher levels than the proband are likely due to the heterozygous C > T mutation in the *MSN* gene. High levels were observed in the healthy control who was age- and sex-matched to the proband.

DISCUSSION

The variant identified here in the *MSN* gene is a missense, hemizygous, single-base mutation in the proband. This mutation was not identified in current online databases (UCSC Genome Browser, gnomAD) with no reference number and no disease association reported in OMIM at the time of query (March 2016). A genome-wide association study (GWAS) search in the GWAS Catalog (20) produced no results for disease-trait-associated variants in the *MSN* gene, or other polymorphisms within the *MSN* gene.

More recently an OMIM entry for Immunodeficiency-50 was identified describing a novel X-linked moesin-associated immunodeficiency (X-MAID) (21). This was the first study to document a moesin-associated disease in humans and describes six male cases from four unrelated families with the same ^{R171W}MSN missense mutation as identified in the proband. A seventh case had a different mutation, p.R553X, which caused a frameshift and truncation of MSN in the F-actin binding domain. The authors show an impact on lymphocyte function *in vitro* where moesin deficiency impaired proliferation following activation with mitogen, increased adhesion, and reduced migration (21).

Moesin is a member of the ERM family of cell structure-related proteins that regulate the cell's actin cytoskeleton (5, 22). The mutation identified results in a radical amino acid substitution from basic arginine to non-polar tryptophan within the functional four-point-one, ezrin, radixin, moesin domain. However, interestingly both our study and that of Lagresle-Peyrou et al. suggest that rather than interfering with protein function, the ^{R171W}MSN mutation triggers degradation of moesin mRNA in lymphocytes with a loss of expression at the transcript and protein level (21). Moesin has been shown to have a crucial and non-redundant role in lymphocyte homeostasis in mice (23). As such, a lack of moesin protein could prevent efficient lymphocyte migration and egress from lymphoid organs causing persistent absolute lymphopenia in the peripheral blood, as observed in the case. The low TRECs exhibited by the proband at a young age, are an indicator of recent thymic emigrants (24). We compared the Lagresle-Peyrou et al. cases' (P1-P7) and proband phenotypes in more detail and we surmise the P3 case would be the most similar to the proband in terms of clinical outcomes due to both individuals presenting with no eczema, no *Molluscum contagiosum*, and presentation of an autoimmune reaction in the form of TTP

TABLE 4 | Comparison of clinical outcomes for cases P1–P7 (Lagresle-Peyrou et al.) and the proband.

Case	*MSN* mutation	Bacterial infections	Varicella zoster	Eczema	*Molluscum contagiosum*	Auto-immunity	Persistent lymphopenia	Fluctuating neutropenia	IgG therapy	Improvement with G-CSF	Alive and well
P1	R171W	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	No G-CSF	Yes
P2	R171W	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes
P3	R171W	Yes	Yes	No	No	TTP	Yes	Yes	Yes	No GCS-F	Yes
P4	R171W	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	Yes
P5	R171W	Yes	No	Yes	No	No	Yes	Yes	Yes	No G-CSF	Yes
P6	R171W	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	No G-CSF	Yes
P7	R553*	Yes	No	No	No	No	Yes	Yes	Yes	No G-CSF	Yes
Proband	R171W	Yes	Yes	No	No	ITP	Yes	Yes	Yes	Yes	Yes

Red font highlights similarities between case P3 and proband.

(P3) and ITP (proband). Considering we cannot confirm whether the P3 case has an *IL25* or *NLRP8* variant which could play a role in the absence of eczema or *M. contagiosum*, it would be difficult to determine whether these variants are playing a role in the proband. In addition, as there are no outstanding differences between the proband and all of the other cases, we can be more confident that the ^{R171}MSN mutation is the sole molecular cause for this disease in the proband (**Table 4**).

Infants with genetic defects of the immune system that cause severe combined immunodeficiency, or SCID, are effectively identified by population-wide screening practices in the United States (25). Newborns are routinely screened for SCID by using qPCR to quantitate TRECs from DNA extracted from dried blood spots (24); however, this is a laborious assay with a number of limitations. Recently, a newborn case with an ^{R171W}MSN mutation was identified by WES (26) when undergoing screening for SCID. Together with our report of an Australian case with X-MAID due to the ^{R171W}MSN mutation, this data suggests that although rare, it is a site of recurrent mutation.

CONCLUDING REMARKS

Together with our case report, the findings by Lagresle-Peyrou et al. and Delmonte et al. confirm the effectiveness and validity of WES as a diagnostic method for PIDs and SCID, as well as an investigative method for the identification of novel or rare variants causing novel forms of PID not yet been documented. Identification of these rare and novel variants early in the patient's life will not only aid with swift intervention prior to life-threatening infections, but may also provide possibility for application of gene therapies and personalized treatment options for these patients.

ETHICS STATEMENT

This study was performed in accordance with the recommendations of the Queensland University of Technology Human Research Ethics Committee (approval number 1400000125). All subjects gave written informed consent in accordance with the Declaration of Helsinki. Cell line validations were performed for commercial Pfeiffer non-Hodgkin lymphoma (NHL) B-cell and MCF-7 breast cancer cell lines (data not shown).

AUTHOR CONTRIBUTIONS

GB performed candidate gene validation, designed qPCR primers, performed cell culture, functional experiments and analysis, and wrote the first draft of the manuscript; RRL, NM performed exome sequencing trio analysis; RRL performed candidate gene validations and drafted the exome sequencing method and analysis sections of the manuscript; CA, NM, RAL, MB, DE performed sequencing data analysis and interpretation. DR-A, CA, NM, and RS designed PCR primers for candidate gene validations. HS, LH, and LG conceptualized the study, contributed to data interpretation and edited the final version of the manuscript. LG provided funding support and finalized the manuscript for submission.

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REFERENCES

1. Rezaei N, Moazzami K, Aghamohammadi A, Klein C. Neutropenia and primary immunodeficiency diseases. *Int Rev Immunol* (2009) 28(5):335–66. doi:10.1080/08830180902995645
2. Tran H, Nourse J, Hall S, Green M, Griffiths L, Gandhi MK. Immunodeficiency-associated lymphomas. *Blood Rev* (2008) 22(5):261–81. doi:10.1016/j.blre.2008.03.009
3. Su H. Studying human immunodeficiencies in humans: advances in fundamental concepts and therapeutic interventions. *F1000Res* (2017) 6:318. doi:10.12688/f1000research.10594.1
4. Bousfiha A, Jeddane L, Al-Herz W, Ailal F, Casanova JL, Chatila T, et al. The 2015 IUIS phenotypic classification for primary immunodeficiencies. *J Clin Immunol* (2015) 35(8):727–38. doi:10.1007/s10875-015-0198-5
5. Burns SO, Zarafov A, Thrasher AJ. Primary immunodeficiencies due to abnormalities of the actin cytoskeleton. *Curr Opin Hematol* (2017) 24(1):16–22. doi:10.1097/MOH.0000000000000296
6. Seleman M, Hoyos-Bachiloglu R, Geha RS, Chou J. Uses of next-generation sequencing technologies for the diagnosis of primary immunodeficiencies. *Front Immunol* (2017) 8(847). doi:10.3389/fimmu.2017.00847
7. Rabbani B, Mahdieh N, Hosomichi K, Nakaoka H, Inoue I. Next-generation sequencing: impact of exome sequencing in characterizing Mendelian disorders. *J Hum Genet* (2012) 57(10):621–32. doi:10.1038/jhg.2012.91
8. Platt C, Geha RS, Chou J. Gene hunting in the genomic era: approaches to diagnostic dilemmas in patients with primary immunodeficiencies. *J Allergy Clin Immunol* (2014) 134(2):262–8. doi:10.1016/j.jaci.2013.08.021
9. Biesecker LG, Shianna KV, Mullikin JC. Exome sequencing: the expert view. *Genome Biol* (2011) 12(9):128. doi:10.1186/gb-2011-12-9-128
10. Green MR, Camilleri E, Gandhi MK, Peake J, Griffiths LR. A novel immunodeficiency disorder characterized by genetic amplification of interleukin 25. *Genes Immun* (2011) 12(8):663–6. doi:10.1038/gene.2011.50
11. Fort MM, Cheung J, Yen D, Li J, Zurawski SM, Lo S, et al. IL-25 Induces IL-4, IL-5, and IL-13 and Th2-associated pathologies in vivo. *Immunity* (2001) 15(6):985–95. doi:10.1016/S1074-7613(01)00243-6
12. Kleinschek MA, Owyang AM, Joyce-Shaikh B, Langrish CL, Chen Y, Gorman DM, et al. IL-25 regulates Th17 function in autoimmune inflammation. *J Exp Med* (2007) 204(1):161–70. doi:10.1084/jem.20061738
13. Wang Y-H, Angkasekwinai P, Lu N, Voo KS, Arima K, Hanabuchi S, et al. IL-25 augments type 2 immune responses by enhancing the expansion and functions of TSLP-DC–activated Th2 memory cells. *J Exp Med* (2007) 204(8):1837–47. doi:10.1084/jem.20070406
14. Adzhubei I, Jordan DM, Sunyaev SR. Predicting functional effect of human missense mutations using PolyPhen-2. *Curr Protoc Hum Genet* (2013) 76:7.20.1–41. doi:10.1002/0471142905.hg0720s76
15. Tavtigian SV, Greenblatt MS, Lesueur F, Byrnes GB; IARC Unclassified Genetic Variants Working Group. In silico analysis of missense substitutions using

sequence-alignment based methods. *Hum Mutat* (2008) 29(11):1327–36. doi:10.1002/humu.20892
16. Schwarz JM, Rödelsperger C, Schuelke M, Seelow D. MutationTaster evaluates disease-causing potential of sequence alterations. *Nat Methods* (2010) 7(8):575–6. doi:10.1038/nmeth0810-575
17. Demidov G, Simakova T, Vnuchkova J, Bragin A. A statistical approach to detection of copy number variations in PCR-enriched targeted sequencing data. *BMC Bioinformatics* (2016) 17:429. doi:10.1186/s12859-016-1272-6
18. Oikari LE, Okolicsanyi RK, Qin A, Yu C, Griffiths LR, Haupt LM. Cell surface heparan sulfate proteoglycans as novel markers of human neural stem cell fate determination. *Stem Cell Res* (2016) 16(1):92–104. doi:10.1016/j.scr.2015.12.011
19. Kidd JM, Cooper GM, Donahue WF, Hayden HS, Sampas N, Graves T, et al. Mapping and sequencing of structural variation from eight human genomes. *Nature* (2008) 453(7191):56–64. doi:10.1038/nature06862
20. MacArthur J, Bowler E, Cerezo M, Gil L, Hall P, Hastings E, et al. The new NHGRI-EBI Catalog of published genome-wide association studies (GWAS Catalog). *Nucleic Acids Res* (2017) 45(D1):D896–D901. doi:10.1093/nar/gkw1133
21. Lagresle-Peyrou C, Luce S, Ouchani F, Soheili TS, Sadek H, Chouteau M, et al. X-linked primary immunodeficiency associated with hemizygous mutations in the moesin (MSN) gene. *J Allergy ClinImmunol* (2016) 138(6):1681.e–9.e. doi:10.1016/j.jaci.2016.04.032
22. Fernando H, Martin TA, Douglas-Jones A, Kynaston HG, Mansel RE, Jiang WG. Expression of the ERM family members (ezrin, radixin and moesin) in breast cancer. *Exp Ther Med* (2010) 1(1):153–60. doi:10.3892/etm_00000025
23. Hirata T, Nomachi A, Tohya K, Miyasaka M, Tsukita S, Watanabe T, et al. Moesin-deficient mice reveal a non-redundant role for moesin in lymphocyte homeostasis. *Int Immunol* (2012) 24(11):705–17. doi:10.1093/intimm/dxs077
24. Baker MW, Grossman WJ, Laessig RH, Hoffman GL, Brokopp CD, Kurtycz DF, et al. Development of a routine newborn screening protocol for severe combined immunodeficiency. *J Allergy ClinImmunol* (2009) 124(3):522–7. doi:10.1016/j.jaci.2009.04.007
25. Kwan A, Abraham RS, Currier R, Brower A, Andruszewski K, Abbott JK, et al. Newborn screening for severe combined immunodeficiency in 11 screening programs in the United States. *JAMA* (2014) 312(7):729–38. doi:10.1001/jama.2014.9132
26. Delmonte OM, Biggs CM, Hayward A, Comeau AM, Kuehn HS, Rosenzweig SD, et al. First case of X-linked moesin deficiency identified after newborn screening for SCID. *J Clin Immunol* (2017) 37(4):336–8. doi:10.1007/s10875-017-0391-9

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The Role of AIRE in the Immunity Against *Candida Albicans* in a Model of Human Macrophages

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Autoimmune-polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) is a primary immunodeficiency caused by mutations in the autoimmune regulator gene (*AIRE*). Patients with AIRE mutations are susceptible to *Candida albicans* infection and present with autoimmune disorders. We previously demonstrated that cytoplasmic AIRE regulates the Syk-dependent Dectin-1 pathway. In this study, we further evaluated direct contact with fungal elements, synapse formation, and the response of macrophage-like THP-1 cells to *C. albicans* hyphae to determine the role of AIRE upon Dectin receptors function and signaling. We examined the fungal synapse (FS) formation in wild-type and AIRE-knockdown THP-1 cells differentiated to macrophages, as well as monocyte-derived macrophages from APECED patients. We evaluated Dectin-2 receptor signaling, phagocytosis, and cytokine secretion upon hyphal stimulation. AIRE co-localized with Dectin-2 and Syk at the FS upon hyphal stimulation of macrophage-like THP-1 cells. AIRE-knockdown macrophage-like THP-1 cells exhibited less Dectin-1 and Dectin-2 receptors accumulation, decreased signaling pathway activity at the FS, lower *C. albicans* phagocytosis, and less lysosome formation. Furthermore, IL-1β, IL-6, or TNF-α secretion by AIRE-knockdown macrophage-like THP-1 cells and AIRE-deficient patient macrophages was decreased compared to control cells. Our results suggest that AIRE modulates the FS formation and hyphal recognition and help to orchestrate an effective immune response against *C. albicans*.

Keywords: APECED, AIRE, *C. albicans*, hyphae, macrophages, receptor recruitment, Dectin receptor

INTRODUCTION

Immunocompromised patients affected by fungal infections experience high morbidity and mortality. Most of these infections are caused by opportunistic yeasts, such as *Candida spp.*, present in the skin and mucous membranes (1). It is well established that the recognition and destruction of *Candida* by phagocytic cells are pivotal (2). The interactions between different receptors on phagocytic

cells and components of the fungal cell wall are known to trigger phagocytosis and subsequent immune responses (3, 4). However, the receptors directly mediating the initial stage of recognition have not been clearly defined.

Although many immunodeficient patients are susceptible to fungal infections, some deficiencies have a signature disease associated with yeast (5–8). Patients with CARD9 deficiency are susceptible to invasive fungal diseases and chronic mucocutaneous candidiasis (CMC) as a result of primary immune dysfunction (9, 10). Patients with inborn errors of IL-17F, IL-17RA, or at the adaptor protein from IL-17 receptor, ACT1, which abolish the TH17 response, present clinically with CMC (11, 12). Gain-of-function (GOF) mutations in signal transducer and activator of transcription 1 (STAT1) impair dephosphorylation of STAT1 in response to IFN-γ, IFN-α/β, and IL-27 stimulation. This leads to defects of IL-17-producing T cells, and many of these patients develop severe CMC (5, 13). STAT3 plays a central role in signal transduction downstream of IL-6, IL-10, IL-17, IL-22, IL-23, and IL-27 cytokines. STAT3-deficient patients have severely decreased frequency of circulating IL-17A- and IL-22-producing T cells, and as a result also develop CMC (5, 14). The Y238X polymorphism in Dectin-1 do not result in immune deficiencies but it increases predisposition to fungal infections due to low production of cytokines by innate immune cells and a functional defect in T helper (TH) 17 responses (15, 16). Finally, patients with autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) may present with high titers of neutralizing autoantibodies against IL-17A, IL-17F, and IL-22 and subsequent CMC (17, 18).

Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy is a rare syndrome with a worldwide incidence of 1:100,000, albeit higher incidence in certain ethnic groups such as Italians, Iranian Jews, and Finns (1:9,000, 1:14,000, and 1:25,000, respectively) (17, 19). APECED is caused by mutations in the autoimmune regulator (*AIRE*) gene, which encodes a transcription factor involved in thymic antigen expression and T cell education (19–21). However, many studies have shown that AIRE is expressed in extra-thymic cells. These include peripheral lymphoid tissues and monocytes and dendritic cells, and there is evidence that AIRE can modulate the inflammatory response (22–24).

Interestingly, CMC, typically affecting the oral, vaginal, and esophageal mucosa, is the sole infectious disease seen in AIRE-deficient patients (17, 18, 21, 25). This susceptibility can be potentially attributed to the production of autoantibodies against IL-17 and IL-22 cytokines in APECED patients (17, 21). However, some AIRE-deficient patients do not produce autoantibodies against IL-17A, IL17F, and IL-22 but still have CMC, similarly the presence of cytokine autoantibodies does not correlate with the presence of CMC (18, 26). This suggests that other factors may be involved in the profound susceptibility to develop CMC in APECED patients.

Dectin-1 and Dectin-2 receptors recognize yeast and hyphal forms of *C. albicans*, respectively (27). The balance in the activation of these receptors stimulates the phagocytic activity, lysosomal activation, and production of cytokines, which in turn contribute to the anti-fungal response including the generation of TH1 and TH17 cells (28). Although some studies have shown that susceptibility of APECED patients to candidiasis is related to defects in innate immune cells, the molecular mechanisms potentially impairing mononuclear phagocytes remain unclear.

We have previously demonstrated that cytoplasmic AIRE regulates the Syk-dependent Dectin-1 pathway and TNF-α secretion by monocytes in APECED patients (23). The Syk-dependent pathway, however, is shared by other receptors involved in defense against fungal infections, such as Dectin-2 (28). *C. albicans* hyphae possess low concentrations of β-glucan and high concentrations of α-mannan exposed in the cell wall that are recognized, respectively, by Dectin-1 and Dectin-2 (29, 30). Thus, the recruitment and activation of Dectin-1 and Dectin-2 to the interface formed with fungal elements, which we term as fungal synapse (FS), appears to be crucial for an effective response against *C. albicans*.

We hypothesized that AIRE is important for an effective response against *C. albicans* and that this response involves Dectin-1 and Dectin-2 receptors at the FS, influencing further steps of the immunological response.

MATERIALS AND METHODS

Patients and Healthy Donors

We selected adult APECED patients presenting with CMC and autoimmunity, as confirmed by genetic analysis of the *AIRE* gene; two of these patients have a Pro326Leu substitution, one has two deleted residues (exon 5 Ser187 and exon 9 Gln358) and another stop codon in exon 5. Blood samples were collected from patients and healthy donors and were then processed and shipped according to the protocols approved by the Institutional Ethics Committee, the Ministry of Health of Brazil, and the Helsinki Convention.

Reagents

The following reagents were used: puromycin, piceatannol, and polybrene (Sigma-Aldrich); RPMI 1640 culture medium and Dulbecco's PBS (Invitrogen); anti-Dectin-1, anti-Dectin-2 (H-46), anti-AIRE (H-300 and C-2), anti-Syk (4D10), anti-CARD9 (C-17) (Santa Cruz Biotechnology); anti-Dectin-1 (AF1756), anti-Dectin-2 (AF3114) (R&D Systems); and human IgG (Abcam 90285). Lentiviral particles specific for AIRE knockdown and control shRNA Lentiviral particles (shRNA; sc-37669-V and sc-108080) (Santa Cruz Biotechnology) were used in the transduction of THP-1 cell.

Culture Conditions

The myelomonocytic THP-1 cell line was cultured in RPMI 1640 supplemented with 10% inactivated fetal bovine serum (iFBS), 2 mM L-glutamine, 10 U/mL penicillin, 100 μg/mL streptomycin, 10 mM HEPES, 2 mM sodium pyruvate, and 100 μM MEM non-essential amino acids, in a humid 5% CO_2 atmosphere at 37°C. 2.5×10^6 THP-1 cells were differentiated to macrophage-like THP-1 cells using 100 U/mL IFN-γ and 1000 U/mL TNF-α for 24–48 h in 6-well plates, following overnight resting in RPMI media. This time point was selected based upon detection of AIRE

protein by western blotting (data not shown). Only adherent cells were used in the experiments. Peripheral blood mononuclear cells (PBMCs) from healthy donors and AIRE patients were purified using a Ficoll Paque gradient (GE Healthcare Life Sciences). Human monocytes were isolated by adherence and differentiated into macrophages by incubation with 5 ng/mL GM-CSF (R&D Systems) for 6 days. The *C. albicans* strain used was ATCC SC5314 (kindly provided by Dr. Joachim Morschhauser from Institut Fur Molekulare Infektionsbiologie, Wurzburg, Germany) (31), and it was cultured in Sabouraud medium with 10% iFBS for 3 h to induce hyphal differentiation.

Transduction of THP-1 Cells With Lentiviral Particles and AIRE Overexpression in HEK293T Cells

2×10^4 THP-1 cells were cultured in supplemented RPMI medium in 96-well plates for 24 h. Then, 8 mg/mL polybrene was added prior to cell transduction with lentiviral particles at a multiplicity of infection (MOI) of 1:10. Cells were incubated for 5 h, and then the culture medium was replaced with fresh medium. After 48 h culture stabilization, fresh medium containing 1.5 mg/mL puromycin was added for 72 h to select transduced cells. The puromycin concentration was then increased to 3 mg/mL, and the cells were incubated for an additional 72 h. AIRE expression in the transduced clones was monitored by western blotting, flow cytometry, and RT-PCR, using anti-AIRE (H-300 and C-2), donkey anti-rabbit Alexa Fluor 647 (a31573) (Molecular Probes), and human AIRE primers (sc-37669-PR). For overexpression of AIRE, 3×10^6 HEK293T cells were transfected with pLenti-AIRE (NM_000383) Human Tagged ORF, mGFP tagged (RC213497L2, OriGene) using FuGENE 6 Transfection Reagent (Promega) for overexpression of AIRE.

Complementary DNA Preparation and Real-Time PCR

3×10^6 macrophages-like THP-1 cells stimulated with hyphae for 10, 20, or 30 min were submitted to RNA extraction using Trizol reagent (Life Technologies). Preparation of complementary DNA (cDNA) was performed using 2 µg of RNA, 0.8 µL of reverse transcriptase M-MLV, 4 µL of 5× Reaction Buffer M-MLV, 2 µL of 10 mM dNTPs, 32 U/µL of RiboLock™ Ribonuclease Inhibitor (Fermentas), and 320 ng of oligo-dT primer (Integrated DNA Technologies). This mixture was incubated at 42°C for 60 min and then at 70°C for 10 min. cDNA was stored at −20°C. After preparation of cDNA, quantification of gene expression by real-time PCR was performed. Amplification conditions were standardized for each transcript. A comparative relationship between reaction cycles (CT) was used to determine gene expression relative to *PPIA* control (housekeeping gene). The detection of the gene of interest was performed using Quantstudio apparatus (Applied Biosystems). Commercially available probes (Life Technologies) were used for *PPIA* (Hs_99999904). The following sequences of primers, detected using SybrGreen® (Life Technologies) were also used for this study: KiCqStart Primers Human H_*Clec7a* (*Dectin*-1) (NM_022570), H_*Syk* (NM_001135052), H-*Card9* (NM_052813), H_*Clec6* (*Dectin*-2) (NM_001007033), and H_*AIRE* (NM_000383) (Sigma Aldrich). The quantification method was $2 - \Delta\Delta Ct$ (32) using unstimulated THP-1 samples as a normalizer.

Western Blotting and Immunoprecipitation

Wild-type and AIRE-knockdown macrophage-like THP-1 cells (3×10^6 cells) were stimulated with Candida hyphae. Lysates were obtained using Pierce RIPA buffer. The protein concentrations were determined using a Pierce BCA Protein Assay Kit (Life Technologies), and equivalent amounts of protein were subjected to SDS-PAGE. The membrane was incubated with primary antibody for 1 h, followed by incubation with an IRDye 800CW, IRDye 680RD (LI-COR), or horseradish peroxidase (Sigma Aldrich) secondary antibody. Bands were visualized with an Odyssey® CLx Infrared Imaging System (LI-COR) or ImageQuant LAS 500 (GE Healthcare Life Sciences). 18×10^6 macrophage-like THP-1 cells, control, or stimulated with hyphae was lysed with immunoprecipitation buffer (500 mM Tris pH7.4; 500 mM NaCl; 500 mM EDTA pH8.0; 200 mM EGTA; 1% TRITON X-100). Total protein was quantified using Bradford reagent (BioRad) before immunoprecipitation with anti-AIRE (C-2), anti-Dectin-2 (H-46) (Santa Cruz Biotechnology), and control IgG. Macrophage-like THP-1 cells (2.5×10^6 cells) were stimulated with Candida hyphae for 10, 20, or 30 min. The cytoplasmic fraction was separated using Buffer 1 (10 mM HEPES pH7.9, 50 mM NaCl, 0.5 M sucrose, 0.1 mM EDTA, and 0.5% TRITON X-100). The nuclear pellet was washed using 1 mL Wash Buffer (10 mM HEPES, 60 mM KCl, 1 mM EDTA, 1 mM DTT, 1 mM PMSF), and nuclear fraction extraction was performed using Buffer 2 (20 mM Tris-HCl, 1.5 mM $MgCl_2$, 400 mM NaCl, 0,2 mM EDTA, 1 mM PMSF, and 15% Glycerol). Western blotting was performed using anti-AIRE (H-300), anti-AIRE (C-2), anti-CARD9, anti-Syk (4D10), anti-Dectin-1 (Santa Cruz Biotechnology), and anti-Dectin-2 (AF3114) (R&D Systems). Anti-β-Actin (I-19), anti-GAPDH (FL-335) (Santa Cruz Biotechnology), and anti-Lamin B1 (Abcam16048) were used as loading controls.

Confocal Microscopy

After macrophage differentiation, cells were trypsinized and adjusted to 2×10^5 cells/mL. Cells were then incubated for 45 min at 37°C on poly-L-lysine-treated slides to promote cellular adhesion. Macrophage-like THP-1 cells were subsequently stimulated with *C. albicans* hyphae for different times. Fc receptors were blocked using human IgG to prevent non-specific binding, followed by staining for anti-Dectin-1 (AF1756), anti-Dectin-2 (AF3114) (R&D Systems), or anti-Dectin-2 (H-46) (Santa Cruz Biotechnology). Cells were then fixed with 100 µL CytoFix/CytoPerm (BD Bioscience), according to the manufacturer's recommendations, and stained for anti-AIRE (H-300), anti-Syk (4D10), or anti-CARD9 (C-17) (Santa Cruz Biotechnology) on ice to reduce non-specific binding. The cells were washed with wash buffer (BD Bioscience) and stained with fluorescence secondary antibody (Molecular Probes). Slides were mounted with Gel Mount (Vectashield) and sealed with nail polish prior to image acquisition using a Zeiss confocal microscopy equipped with Yokogawa CSU-10 spinning disk and Hamamatsu ORCA-ER camera. Confocal micrographs

were analyzed by Volocity software (PerkinElmer) to obtain area and mean fluorescence intensity (MFI). 30 cells from 3 independent experiments were measured. All antibodies were validated with a species-specific IgG negative control at the same concentration (Figure S1A in Supplementary Material). To validate the specificity of the AIRE antibody used for confocal microscopy, AIRE-mGFP was overexpressed in HEK293T cells as described earlier. Cells were fixed using CytoFix/CytoPerm (BD Biosciences) or FoxP3/Transcription Factor Fix/Perm solution (Thermo Fisher Scientific) at room temperature and gently washed with Perm wash (BD Biosciences) or Perm buffer (Thermo Fisher Scientific). Staining for GFP was performed with anti-GFP (BioLegend) directly conjugated to AlexaFluor 488 (BioLegend). Staining for AIRE was performed with anti-AIRE (Santa Cruz Biotechnology) followed by goat anti-rabbit IgG conjugated to AlexaFluor 568. Coverslips were mounted using using VectaShield with DAPI (Vector Laboratories). Images were acquired in Leica TCS SP8 laser scanning confocal microscope (Leica Microsystems) using LASAFx software (Figure S1B in Supplementary Material).

C. albicans Phagocytosis and Lysosome Production

Wild-type and AIRE-knockdown macrophage-like THP-1 cells were treated with 10 μg/mL piceatannol (selective Syk inhibitor) or vehicle control and stimulated with hyphae or yeast for 20 or 30 min. To evaluate lysosome production, cells were incubated with LysoTracker Red for 30 min and washed and stimulated with *C. albicans*. Next, the cells were fixed with 4% paraformaldehyde in PBS for 10 min and washed and resuspended in 80 μL PBS. Samples were analyzed by Imagestream X Mark II Imaging Flow Cytometer (Amnis). Flow cytometry image data analysis was performed on LysoTracker/Candida-GFP double positive cells, following by the analysis of the internalization function using IDEAS software (Amnis). The internalization gate was generated using THP-1 macrophages stimulated at 4°C. Human macrophages were stimulated with yeast (MOI 1:5) and hyphae (MOI 1:2) for 30 min on coverslips for phagocytosis evaluations using cells from healthy donors and patients. After being washed, the cells were stained with panoptic dye (Laborclin), and *C. albicans* were counted under a light microscope. As control, human macrophages or macrophage-like THP-1 cells were stimulated with *E. coli* (MOI 1:5) for 30 min, incubated with gentamicin for 45 min, washed and cultured in Luria Bertani agar. The number of colony-forming units (CFU) was counted after overnight incubation at 37°C.

Cytokine Evaluation

Macrophage-like THP-1 cells were stimulated with hyphae for 6 h. Supernatants were collected and assessed for IL-1β and TNF-α using BioLegend's LEGENDplex™ kits. Human macrophages were stimulated with hyphae or 100 ng/mL lipopolysaccharide (LPS; Sigma Aldrich), and cytokine secretion was evaluated using IL-6 (eBioscience) and TNF-α (BD Biosciences) ELISA kits. A standard curve was generated using purified protein to obtain a R^2 value of 0.99.

RESULTS

FS Formation in Macrophages After Hyphae Stimulation

To evaluate the FS formation, macrophage-like THP-1 cells were stimulated with *C. albicans* hyphae for 10, 20, or 30 min (**Figure 1A**). Dectin-1 and Dectin-2 expression increased at the membrane following contact with hyphae compared to unstimulated macrophage-like THP-1 cells (**Figures 1B,C**). Analysis of macrophage in contact with hyphae demonstrated higher percentages of Dectin-1 and Dectin-2 at the FS after 20 min stimulation compared to levels in the cells as a whole (**Figures 1D,E**). In addition, expression of Dectin-2 was higher than Dectin-1 at the FS. This was expected since the cell wall of hyphae binds more strongly to Dectin-2 than to Dectin-1. Moreover, the amount of Dectin-1 did increase after 30 min (**Figures 1F,G**) as did the co-localization of Dectin-1 and Dectin-2 at the FS (**Figures 1H,I**).

To determine any potential role of Dectin-2 in the recruitment of Dectin-1 to the nascent FS, we first blocked Dectin receptors in macrophage-like THP-1 cells using either an anti-Dectin-1 or anti-Dectin-2 antibody or both for 30 min prior to hyphal stimulation (Figure S2 in Supplementary Material). Blocking Dectin-1 increased the accumulation of Dectin-2 at the FS compared with control IgG-treated macrophages. Since these receptors likely share a Syk-dependent signaling pathway, blocking Dectin-1 may increase signaling promoting Dectin-2 activation. After blocking Dectin-2, however, there was no significant change in Dectin-1 accumulation at the FS (Figures S2A,C in Supplementary Material). To verify that Dectin-1 recruitment was initiated by its own ligation and signaling, we blocked both Dectin receptors before hyphal stimulation. This blockage resulted in decreased accumulation of both receptors at the FS (Figures S2B,D in Supplementary Material). These findings suggest that Dectin-1 recruitment is mainly attributed to its own ligation and activation.

AIRE Interaction With Dectin-2 and Syk

First, we investigated whether FS activation is modulating the transcription of *Dectin*-1, *Dectin*-2, *Syk*, *Card9*, and *AIRE*. We observed that only *Dectin*-2 and *AIRE* mRNA significantly increased the transcription after hyphae stimulation (Figure S3B in Supplementary Material). Since Dectin-2 localizes to the FS and we observed increased mRNA expression levels of *Dectin*-2 and *AIRE*, we next evaluated whether AIRE might be involved in the Dectin-2 signaling pathway. Macrophage-like THP-1 cells were stimulated with hyphae to evaluate AIRE and Syk recruitment to the FS. This stimulation led to considerable co-localization of Dectin-2, Syk, and AIRE (**Figure 2A**) as well as increased AIRE and Syk expression at the FS (**Figures 2B,C**). Interestingly, the cell surface expression of AIRE, as well as the accumulation of Dectin-1 and Dectin-2 receptors at the FS, reached peaks at 20 min (**Figures 1D,E** and **2B**). However, the percentage of AIRE accumulated at the FS remained relatively constant across all time points (**Figures 2B–D**). Thus, AIRE accumulation did not change because its expression was increased throughout the cell and not only at the FS. Although Syk is important for Dectin receptor activation, Syk expression remained consistent at all

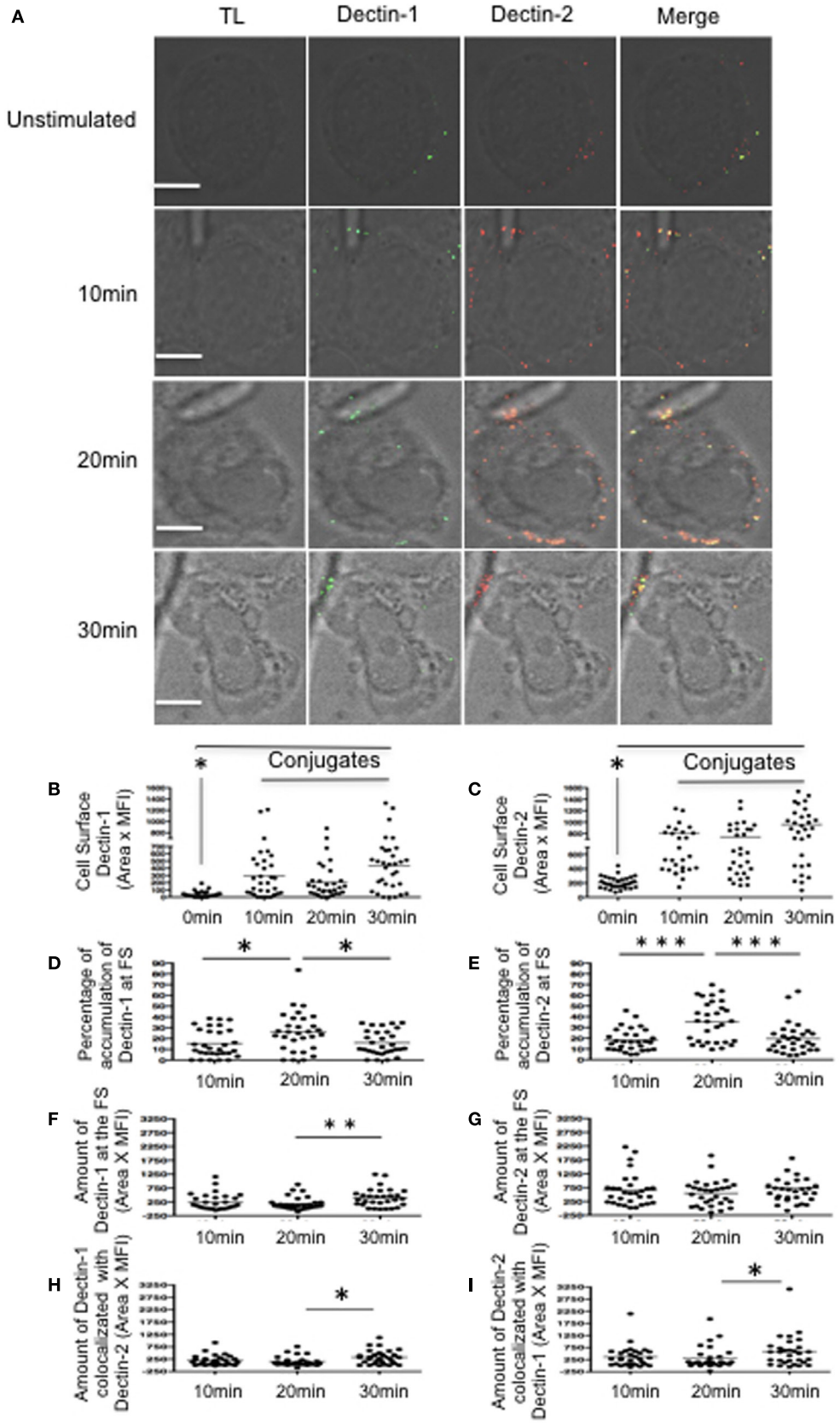

FIGURE 1 | Continued

FIGURE 1 | Dynamics of Dectin-1 and Dectin-2 recruitment to the fungal synapse (FS). **(A)** Representative confocal micrographs of macrophage-like THP-1 cells stimulated with *Candida albicans* hyphae for 10, 20, or 30 min to observe the recruitment of Dectin-1 (green) and Dectin-2 (red) receptors to the FS. **(B–I)** Each dot represents a cell used for the measurement indicated. The horizontal bars denote the mean. * $p < 0.05$ as determined by *t*-test. **(B,C)** Amounts of Dectin-1 **(B)** and Dectin-2 **(C)** on the cell surface. **(D,E)** Relative percentages of total Dectin-1 **(D)** and Dectin-2 **(E)** at the FS compared with that in the whole cell. **(F,G)** Amounts of Dectin-1 **(F)** and Dectin-2 **(G)** at the FS. **(H,I)** Synaptic co-localization of Dectin-1 with Dectin 2 **(H)** or of Dectin-2 with Dectin-1 **(I)**. Representative confocal micrographs of 30 cells counted in 3 independent experiments. Scale bar = 5 μm.

time points (**Figures 2C–E**). An increase in AIRE and Syk co-localization, however, was detected after 20 min of hyphal stimulation (**Figures 2F,G**). Moreover, we separated cytoplasmic and nuclear fractions to confirm AIRE expression in macrophage-like THP-1 cells stimulated with hyphae. AIRE was identified in two isoforms, with higher expression at 20 min in the cytoplasmic fraction while it was increased after hyphae stimulation at the nuclear fraction (**Figure 2H**). To determine whether Dectin-2, Syk, and AIRE actually physically interact, AIRE or Dectin-2 was immunoprecipitated from hyphae-stimulated macrophage-like THP-1 cells. AIRE was identified in molecular complexes with Dectin-2 and Syk, in macrophage-like THP-1 cells that had been activated with *C. albicans* hyphae (**Figure 2I**). Moreover, AIRE was increased compared to unstimulated cells. Thus, Dectin-2 localizes to the FS as part of a molecular complex with both Syk and AIRE.

AIRE Is Required for FS Formation

To evaluate the role of AIRE in macrophages with regard to the FS, AIRE was knocked down in THP-1 cells, and the FS formation compared to that in control cells. There was no overall change in the total expression levels of Dectin-1, Dectin-2, Syk, and CARD9 in the AIRE-knockdown cells (Figures S4A–E in Supplementary Material). Given that these were maintained at physiological levels, we next examined whether AIRE is involved in FS formation. Wild-type and AIRE-knockdown macrophage-like THP-1 cells were stimulated with hyphae for 20 or 30 min, and then Dectin-1 and Dectin-2 recruitment to the FS was analyzed (**Figure 3**). We observed decreased accumulation of both receptors at the FS in 20 and 30 min in AIRE knockdown macrophages (**Figures 3A–G**). To evaluate if this has any functional significance, we asked whether protein signaling was affected in AIRE knockdown cells. Importantly, decreased Syk amount was observed at the FS at both time points in AIRE-knockdown macrophages and decreased CARD9 recruitment was identified at 30 min (**Figures 4A–G**). These results suggest that the delayed FS formation is attributed to decreases in Dectin-1 and Dectin-2 recruitment and protein signaling.

The Role of AIRE in Macrophage Anti-Fungal Activity

Based on the observed delay in FS formation in AIRE-knockdown macrophage-like THP-1 cells, we determined if the phagocytosis of *C. albicans* might be affected. Notably, decreased yeast and hyphal conjugation as well as internalization was observed in AIRE-knockdown macrophage-like THP-1 cells when compared to controls in 20 and 30 min (**Figures 5A–C**). However, *E. coli* phagocytosis was not affected in AIRE-knockdown macrophage-like THP-1 cells (Figure S5 in Supplementary Material). To determine whether this effect of AIRE was upstream of Syk as previously proposed, yeast and hyphal phagocytosis were measured in AIRE-knockdown macrophage-like THP-1 cells treated with piceatannol, a Syk inhibitor. In this setting, the reduced levels were not further altered by Syk inhibition in AIRE-knockdown macrophage-like THP-1 cells, only in control cells (Figures S6A,B in Supplementary Material), showing that AIRE is important for yeast and hyphal phagocytosis and that it requires a Syk-dependent pathway in macrophage-like THP-1 cells.

Since Dectin-1 is also recruited to the FS after 30 min of hyphal stimulation (**Figure 1F**) and it is important for lysosomal maturation (33), we determined if lysosomal production was affected by absence of AIRE. Wild-type and AIRE-knockdown macrophage-like THP-1 cells were stimulated with yeast or hyphae for 20 or 30 min. No difference was observed between the wild-type and AIRE-knockdown macrophage-like THP-1 cells stimulated with hyphae. However, simulation of macrophages with yeast resulted in decreased lysosome production in the AIRE-knockdown macrophage-like THP-1 cells at 30 min compared with that in the wild-type macrophage-like THP-1 cells (**Figures 5D,E**). This suggests that AIRE can be involved in lysosomal production by Dectin-1 in macrophages stimulated with yeast.

Cytokines are pivotal to both innate and adaptive immunity, and their production increases following Dectin ligation. We evaluated IL-1β and TNF-α secretion in wild-type and AIRE-knockdown macrophage-like THP-1 cells stimulated with hyphae. As might be predicted from the interrupted Dectin signaling complex in the absence of normal AIRE function, AIRE-knockdown macrophage-like THP-1 cells secreted lower levels of IL-1β and TNF-α after stimulation with hyphae for 6 h (**Figure 5F**). This demonstrated that the physiologic inflammatory response after hyphal stimulation requires AIRE in macrophage-like THP-1 cells.

APECED Patients Macrophages Responses to Fungal Stimulation

To determine if those mechanisms are relevant to APECED patients, we evaluated human monocyte-derived macrophages from APECED patients with AIRE mutations as well as from healthy donors. Decreased Dectin-2 expression throughout the entire cell surface of APECED patient macrophages was detected after 30 min of stimulation with *C. albicans* hyphae compared to those from healthy donors (**Figure 6**). In addition, the APECED macrophages exhibited low phagocytic activity following stimulation with yeast or hyphae for 30 min compared to that in the healthy donor cells (**Figure 7A**). Macrophages from APECED patients also showed reduced IL-6 and TNF-α secretion after 24 h

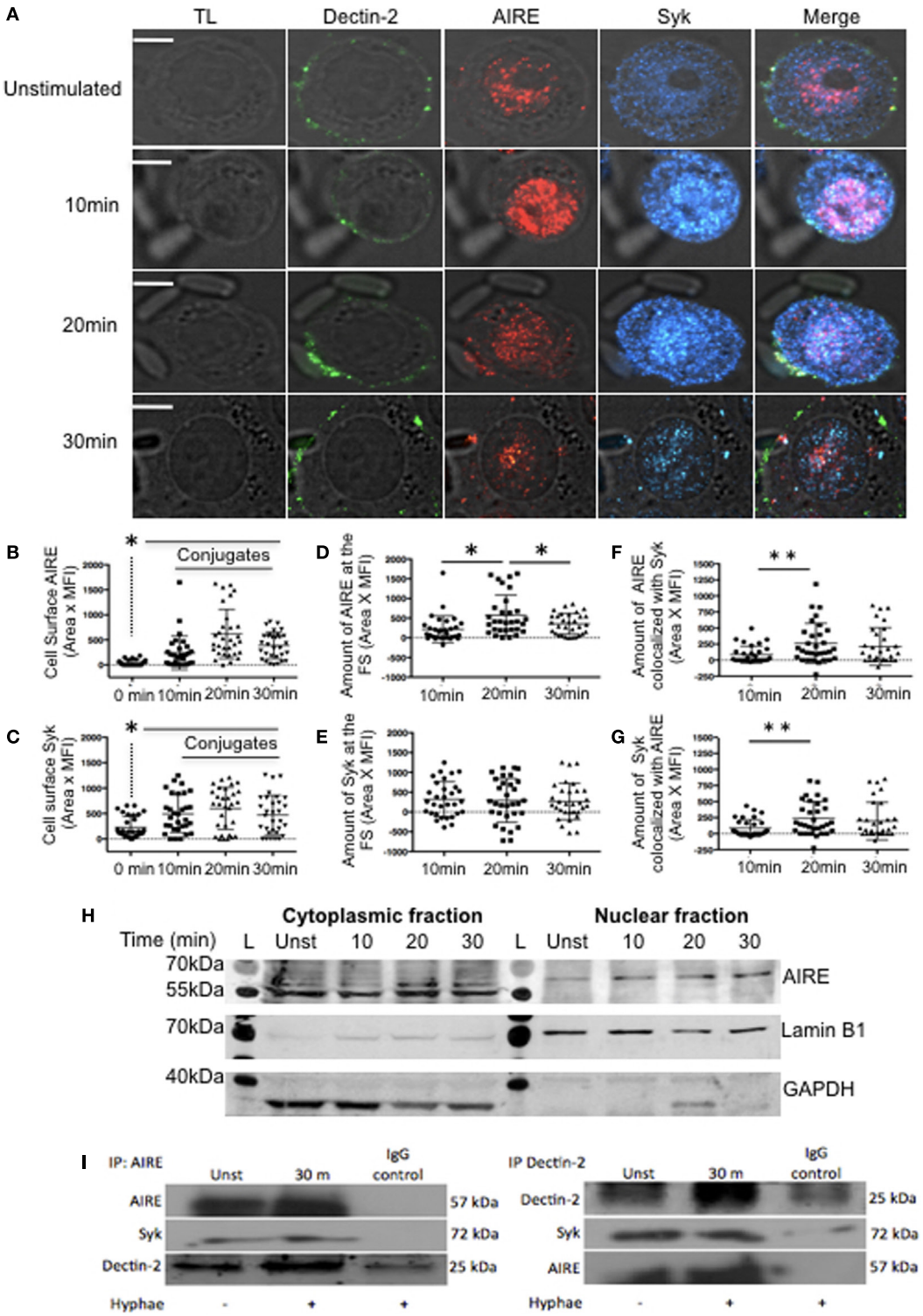

FIGURE 2 | Dectin signaling at fungal synapse (FS). **(A)** Representative confocal micrographs of macrophage-like THP-1 cells, unstimulated (0 min) or stimulated with *C. albicans* hyphae for 10, 20, or 30 min to observe the recruitment of Dectin-2 (green), Syk (blue), and autoimmune regulator (AIRE) (red) to the FS. **(B–G)** Each dot represents a cell used for the measurement indicated. The horizontal bar in each column denotes the mean. *$p < 0.05$ as determined by the *t*-test. **(B,C)** Amounts of AIRE **(B)** and Syk **(C)** on the cell surface. **(D,E)** Amounts of AIRE **(D)** and Syk **(E)** at the FS (conjugated–unconjugated). **(F,G)** Synaptic co-localization of AIRE with Syk **(F)** or of Syk with AIRE **(G)**. **(H)** Western blotting analysis of AIRE in cytoplasmic and nuclear fractions from macrophage-like THP-1 cells, unstimulated (unst) or stimulated with hyphae for 10, 20, or 30 min. (L) Ladder, GAPDH and Lamin B1 was used as loading control. **(I)** Lysates from macrophage-like THP-1 cells, unst or stimulated with hyphae for 30 min, were immunoprecipitated with AIRE (left panel) or Dectin-2 antibodies (right panel) and with a control antibody (IgG). The immunoprecipitated products were probed for AIRE, Dectin-2, and Syk as indicated. Representative confocal micrographs of 30 cells counted in 3 independent experiments. Scale bar = 5 μm.

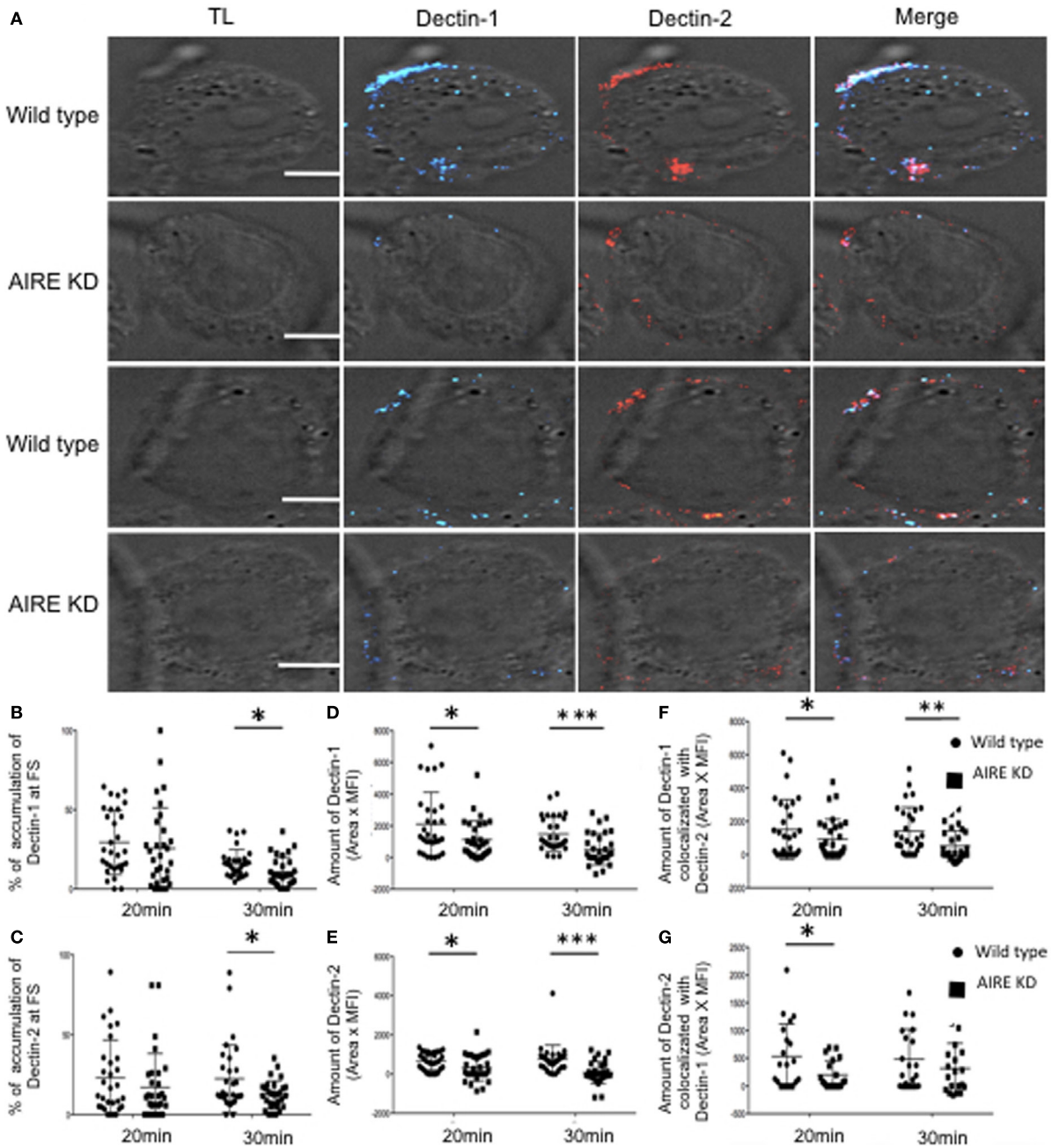

FIGURE 3 | AIRE-mediated recruitment of Dectin receptors to fungal synapse (FS). **(A)** Representative confocal micrographs of wild-type and AIRE-knockdown macrophage-like THP-1 cells stimulated with *C. albicans* hyphae for 20 or 30 min to observe the recruitment of Dectin-1 (blue) and Dectin-2 (red) receptors to the FS. **(B–E)** Each dot represents a cell used for the measurement indicated. The horizontal bars denote the mean. *$p < 0.05$ as determined by the *t*-test. **(B,C)** Percentages of total Dectin-1 **(B)** and Dectin-2 **(C)** at the FS compared with that in the whole cell. **(D,E)** Amounts of Dectin-1 **(D)** and Dectin-2 **(E)** at the FS (conjugated-unconjugated cells). **(F,G)** Synaptic co-localization of Dectin-1 with Dectin-2 **(F)** or Dectin-2 with Dectin-1 **(G)**. Representative confocal micrographs of 30 cells counted in 3 independent experiments. Scale bar = 5 μm.

of hyphal stimulation compared with those from healthy donors (**Figure 7B**). Importantly, they were not globally impaired as there was no detectable difference in cytokine secretion from those macrophages after LPS stimulation (Figure S7 in Supplementary Material). Thus, the CMC observed in AIRE-deficient patients may be associated with the delay in FS formation and associated defective fungal-specific macrophage signal generation, phagocytosis, and cytokine production.

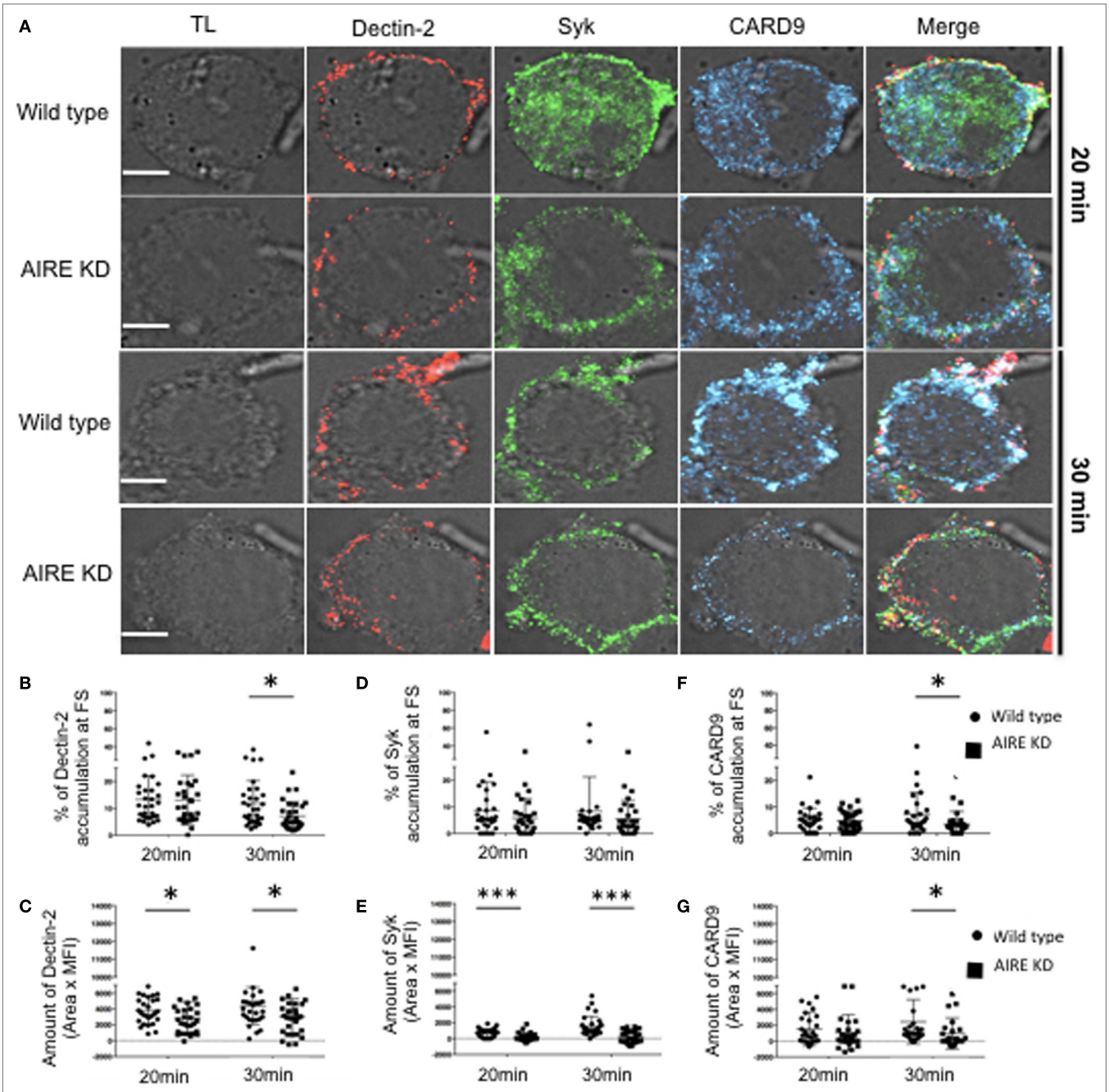

FIGURE 4 | Influence of autoimmune regulator (AIRE) on fungal synapse (FS) formation. **(A)** Representative confocal micrographs of wild-type and AIRE-knockdown macrophage-like THP-1 cells stimulated with *C. albicans* hyphae for 20 or 30 min to observe the recruitment of Dectin-2 (red), Syk (green), and CARD9 (blue) to FS. **(B–E)** Each dot represents a cell used for the measurement indicated. The horizontal bars denote the mean. *$p < 0.05$ as determined by the *t*-test. **(B,D,F)** Percentages of total Dectin-2 **(B)**, Syk **(D)** and **(F)** CARD9 at the FS compared with those in the whole cell. **(C,E,G)** Amount of Dectin-2 **(C)**, Syk **(E),** and CARD9 **(G)** at the FS (conjugated–unconjugated cells). Representative confocal micrographs of 30 cells counted in 3 independent experiments. Scale bar = 5 μm.

DISCUSSION

In this study, we explored the influence of AIRE on Dectin-1 and Dectin-2 dynamic in macrophages stimulated with *C. albicans* hyphae. Although Dectin-1 and Dectin-2 are known to be the key receptors that control the anti-fungal responses against yeast and hyphal forms, respectively (27), the molecular mechanisms underlying the FS formation and function remain unclear. The most important *C. albicans* virulence trait is the reversible capacity to transform into yeast or hyphae. Hyphae promotes invasion, whereas yeast promotes dissemination. They are predominantly recognized by Dectin-2 and Dectin-1, respectively (27, 30). Thus, the process of pathogen recognition may be central to the FS formation, and any ability that it has to modulate the intensity

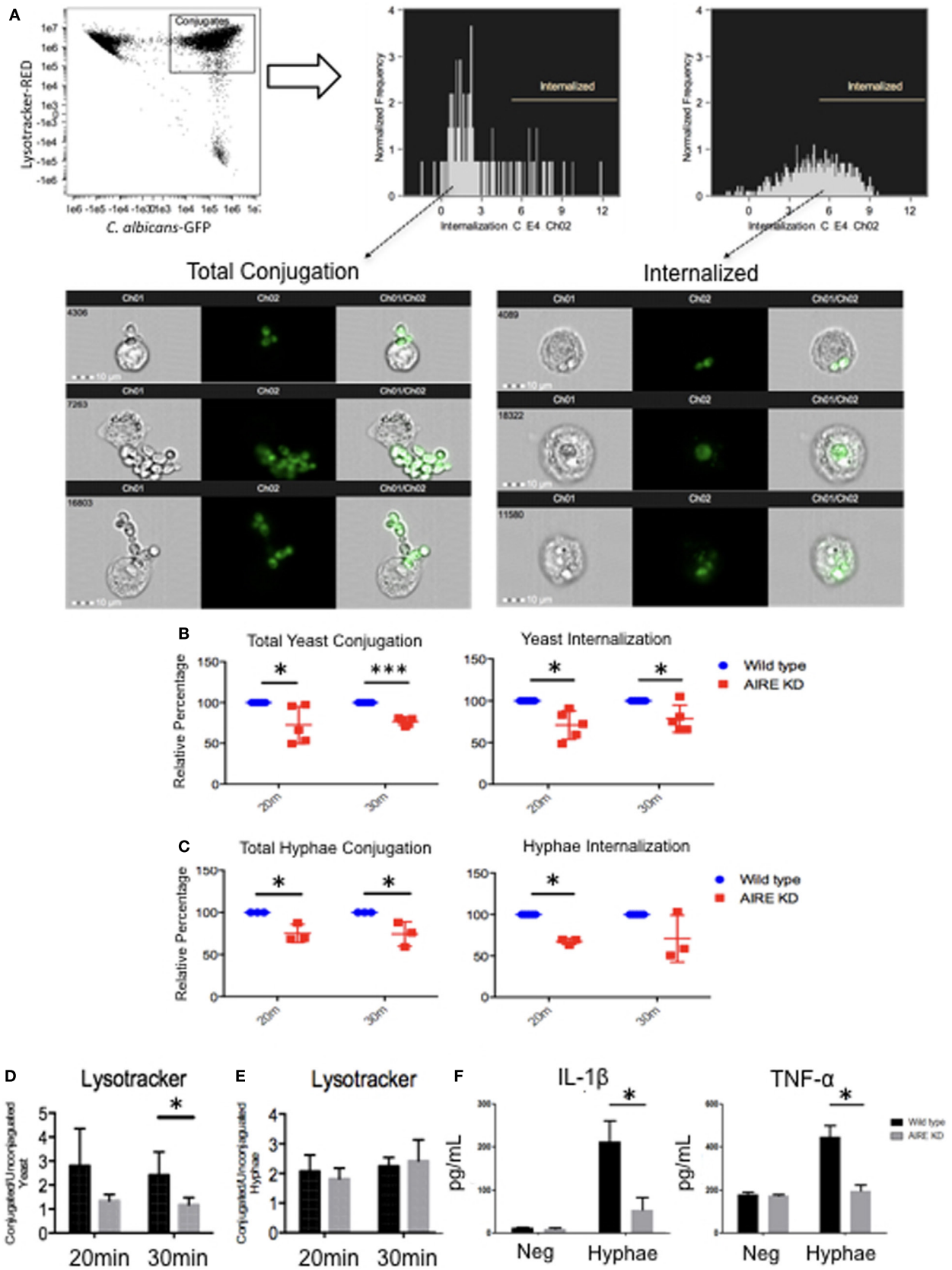

FIGURE 5 | Continued

FIGURE 5 | Candidacidal activity in wild-type and AIRE-knockdown macrophage-like THP-1 cells. Wild-type and AIRE-knockdown macrophage-like THP-1 cells were stimulated with yeast [multiplicity of infection (MOI 1:2)] **(B)** or hyphae (MOI 2:1) GFP *C. albicans* **(C)** for 20 or 30 min. **(A)** Image Stream analysis of fungal phagocytosis by macrophages. GFP fluorescence intensities were gated for further analysis. The cells were divided into subpopulations according to the GFP maximum pixels. Internalization gate was performed for cells incubated with yeast or hyphae at 4°C. Each dot represents one independent experiment used for the measurement indicated. Wild-type (blue circle) and AIRE-knockdown macrophage-like THP-1 cells (red square). **(D,E)** Measurement of cytoplasm acidification in wild-type and AIRE-knockdown macrophage-like THP-1 cells stimulated with yeast (MOI 1:2) **(D)** or hyphae (MOI 2:1) **(E)** for 20 or 30 min by Image Stream analysis. Representative images of five independent experiments are shown. **(F)** IL-1β and TNF-α secretion in supernatants from resting and hyphae-stimulated cells. Wild-type (black bar) and AIRE-knockdown (gray bar) macrophage-like THP-1 cells were stimulated with hyphae (MOI 2:1) for 6 h. The horizontal bars denote the mean. Representative graph of three independent experiments. $^*p < 0.05$ as determined by the *t*-test.

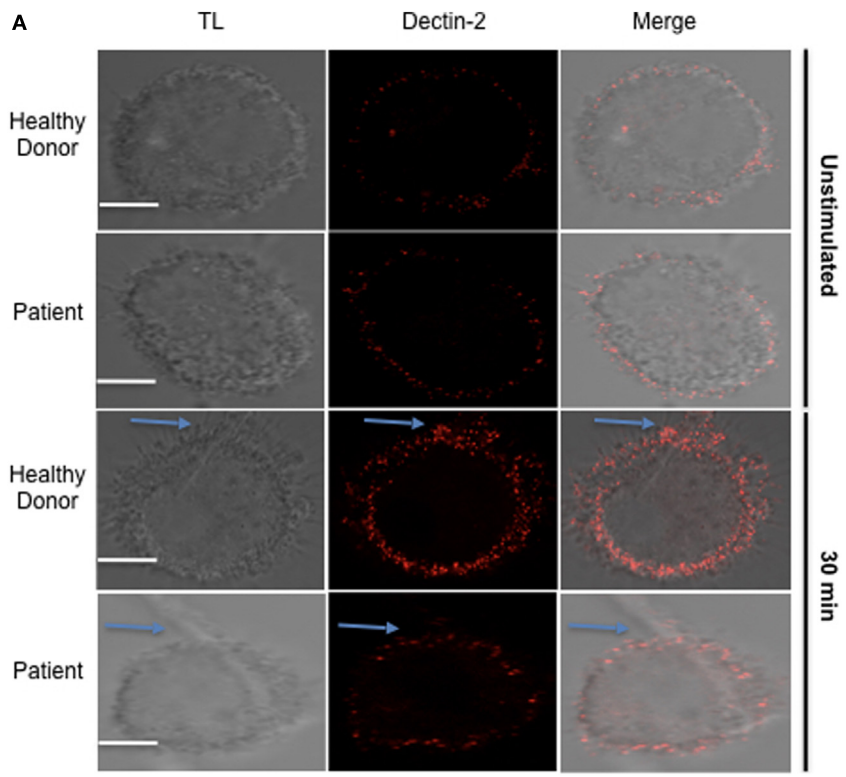

FIGURE 6 | Dectin-2 recruitment in macrophages from healthy donors and autoimmune-polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) patients after hyphal stimulation. **(A)** Representative confocal micrographs of macrophages from healthy donors and AIRE-deficient patients stimulated with *Candidaalbicans* hyphae for 30 min to observe the recruitment of Dectin-2 (red) receptors to the fungal synapse (FS). The blue arrow indicates the FS. Representative image of 4 independent experiments for both healthy donors and APECED patients, 10 pictures acquired per sample. Scale bar = 5 μm.

and appropriateness of the response necessary for *C. albicans* destruction. Concomitantly, patients with defects in this recognition process or in Dectin activity are more susceptible to fungal infection (34–36).

Our study shows that FS formation in hyphae-stimulated THP-1 cells is promoted by Dectin-1 and Dectin-2, followed by AIRE, Syk, and CARD9 recruitment when stimulated by *C. albicans* hyphae. These results suggest that a relationship exists between Dectin-1 and Dectin-2 activation and AIRE in macrophages that are activated by hyphae. These receptors are responsible for activation of cytokine secretion, lysosome production, and phagocytic activity in macrophages (3, 27, 33). Given these findings, the absence of AIRE likely results in altered FS formation due to disrupted interactions among AIRE, Dectin-1, Dectin-2, Syk, and CARD9. The FS is important for the recognition of hyphae by macrophages and for the subsequent generation of an effective immune response (3). Because of abnormal FS formation, AIRE-knockdown macrophage-like THP-1 cells exhibited suppressed phagocytosis of yeast and hyphae. This was due to a reduction in activation of the Syk-dependent pathway following Dectin-1 and Dectin-2 ligation, as illustrated in piceatannol-treated THP-1 cells.

The Dectin-1 Syk-dependent pathway is involved in additional immunological functions necessary for the elimination of Candida, such as lysosomal activation. Efficient phagosome maturation is central for the control of candidiasis and is pivotal to both innate and adaptive immunity (33). As we observed, lysosome production in the AIRE-knockdown macrophage-like THP-1 cells stimulated with yeast was reduced; however, this decrease was not observed in the macrophage-like THP-1 cells stimulated with hyphae. These results may be due to the binding

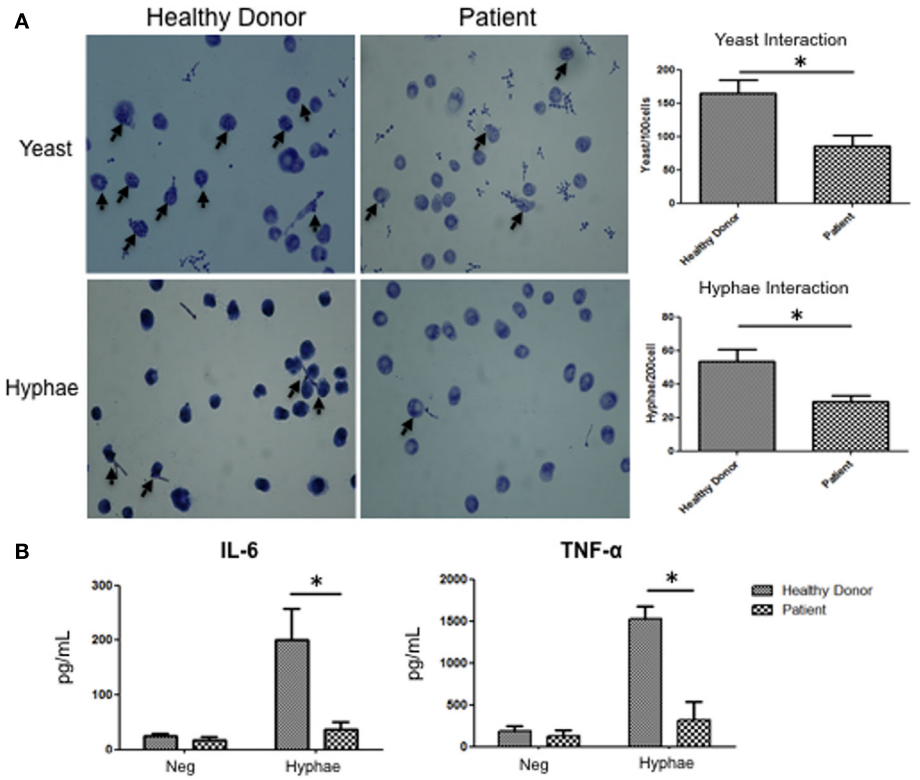

FIGURE 7 | Fungal response in macrophages from autoimmune-polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) patients. **(A)** Representative images of *C andida albicans* phagocytosis in macrophages from healthy donors and AIRE-deficient patients after 30 min of stimulation with yeast or hyphae. The arrow indicates fungal phagocytosis by macrophages. The images on the right show the numbers of yeast and hyphae inside of the macrophages. **(B)** IL-6 and TNF-α secretion by macrophages from a healthy donor or AIRE-deficient patient in cells unstimulated or stimulated with hyphae for 24 h. **(A,B)** The horizontal bars denote the mean. *$p < 0.05$ as determined by the *t*-test. Representative image of four independent experiments for both healthy donors and APECED patients.

of β-glucan to Dectin-1 and the fact that the β-glucan concentration is high in the yeast cell wall and low in the hyphal cell wall. This carbohydrate activates Dectin-1, thereby stimulating lysosome production by the Syk-dependent pathway (3). Moreover, the hyphal form inhibits lysosomal maturation *via* O-mannan, which is present at a high concentration in the cell wall (29). Therefore, reduced lysosome production was only observed in the AIRE-knockdown macrophage-like THP-1 cells stimulated with yeast in this study.

Chronic mucocutaneous candidiasis is frequent in acquired or inherited disorders involving profound T cell defects, especially in those affecting TH17 responses (26, 37). Patients with deficiency in IL-17RA, IL-17F, or ACT1 have defective TH17 function and CMC (11, 14, 18, 26, 37). However, patients with CARD9 deficiency or Dectin-1 Y238X polymorphism are also susceptible to CMC and other fungal infections (8, 15, 35, 38, 39). Defective Dectin-1 expression caused by the Tyr238X polymorphism does not result in immune deficiencies but it is associated with high risk of fungal infections and CMC. This polymorphism generates a truncated Dectin-1 that results in low secretion of IL-1β, IL-6, and TNF-α by peripheral blood mononuclear cell (PBMC), monocytes, and macrophages with impaired IL-17 production in response to *C. albicans* or β-glucan, but normal killing of *C. albicans* by neutrophils (15, 16). On the other hand, CARD9-deficient patients are predisposed to recurrent mucocutaneous and invasive fungal infections with *C. albicans*. These patients show a strong impairment of TNF-α or IL-6 production by neutrophil and monocyte-derived dendritic cells in response to *C. albicans*, whereas IL-17 T-cell production is normal (9, 10, 35).

Autoimmune regulator is essential for proper T cell development and selection in the thymus. While Dectin-1 and CARD9 are expressed in many cell types, studies have shown that AIRE is expressed in peripheral lymphoid tissues, monocytes, and dendritic cells and that it participates in extrathymic functions (23, 24, 40). APECED patients produce variable titers of autoantibodies against IL-17A, IL-17F, or IL-22 associated with CMC (17, 18, 26). However, some patients are susceptible to CMC without these autoantibodies (18), suggesting that other factors may involved in CMC in these patients. AIRE deficiency has been associated with failure in building a proper immune response by monocytes (23, 24, 41). Recently, it has been shown that monocytes from APECED patients have a decrease in IFN-γR2 and STAT1 protein levels that are associated with lower levels of phosphorylated STAT1 molecules after IFN-γ stimulation (24). Another study reported failure of the immune response to *C. albicans*, including a dysregulation of IL-23p19 production in monocytes from APECED patients stimulated with *C. albicans* (41). In 2012, Pedroza et al. established that cytoplasmic AIRE

could regulate a Syk-dependent Dectin-1 pathway and secretion of TNF-α in monocytes from APECED patients stimulated with curdlan, a Dectin-1 agonist (23). These results suggest that other Syk-dependent receptors may be affected by AIRE deficiency. Corroborating these findings, here we demonstrate that AIRE-deficient macrophages exhibited less signaling pathway activation at the FS, lower *C. albicans* phagocytosis, and less lysosome formation. On the other hand, CARD9 signaling induces IL-1β, IL-6, and TNF-α secretion by macrophages upon Dectin-1 receptor activation (42). In our study, AIRE-deficient macrophages show CARD9 and Dectin-1, as well as Dectin-2, recruited to the FS followed by decreased secretion of IL-1β, IL-6, and TNF-α. These cytokines are responsible for increasing the intensity of the innate immune response and are involved in generating the TH17 response (7, 43). IL-1β and IL-6 are pivotal to an efficient TH17 response against *C. albicans* (44). TNFα in combination with IL-22 induce innate immune mechanisms in human keratinocytes and maintain the epidermal barrier integrity during *C. albicans* infection (45). Thus, AIRE downstream of Dectin-1 and Dectin-2 engages a critical pathway contributing to antifungal immunity.

In summary, our results show that AIRE is required for the interaction among Dectin-1 and Dectin-2 receptors and Syk-dependent pathway components in human macrophages upon *C. albicans* stimulation. The susceptibility to CMC observed in APECED patients likely includes direct roles of AIRE in peripheral immunity *via* the FS formation and function, which becomes a part of the overall host defense defect. Although APECED is associated with defects in IL-17 immunity caused by autoantibodies, the additional mechanisms we have identified provide additional insight into CMC so frequently observed in these patients.

ETHICS STATEMENT

Blood samples were collected from the patients and healthy donors and were then processed and shipped according to the protocols approved by the Institutional Ethics Committee, the Ministry of Health of Brazil, and the Helsinki Convention.

AUTHOR CONTRIBUTIONS

JA and PB designed and conducted the experiments and wrote the manuscript. RM, AC, NZ, LY, CA, and MB-C conducted experiments. JC-D, FW, MD-d-S, and ML-C provided human samples. LP provided technical support. NC and EM designed the experiments. JO and AC-N designed the experiments and wrote and reviewed the manuscript.

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SUPPLEMENTARY MATERIAL

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FIGURE S1 | **(A)** Isotype control staining of macrophages THP-1 cells. Representative macrophage-like THP-1 cells stained with AIRE (red), Dectin-1 (purple), Dectin-2 (green), Syk (blue), and its respective IgG control as a negative control for nonspecific staining. **(B)** AIRE overexpression in HEK293T cells. Representative HEK293T cells stained with AIRE (red), nuclei (DAPI, Purple) anti-GFP (green), and respective IgG controls.

FIGURE S2 | Localization of Dectin-1 and Dectin-2 at the FS is independent of the other Dectin receptor activation. **(A)** Representative confocal micrographs of macrophage-like THP-1 cells blocked with anti-Dectin-1, anti-Dectin-2, or IgG control before stimulation with *C. albicans* hyphae for 30 min to observe the recruitment of Dectin-1 (green) or Dectin-2 (red) receptors to the FS. **(B)** Representative confocal micrographs of macrophage-like THP-1 cells blocked with anti-Dectin-1 and anti-Dectin-2 or IgG control before stimulation with *C. albicans* hyphae for 30 min to observe the recruitment of Dectin-1 (green) or Dectin-2 (red) receptors to the FS. **(C,D)** Each dot represents a cell used for the measurement indicated. The horizontal bars denote the mean. $*p < 0.05$ as determined by the *t*-test. (C) Relative percentage of total Dectin-1 and Dectin-2 at the FS in macrophage-like THP-1 cells blocked with anti-Dectin-1, anti-Dectin-2 or IgG control. **(D)** Relative percentage of total Dectin-1 and Dectin-2 at the FS in macrophage-like THP-1 cells blocked with anti-Dectin-1 and anti-Dectin-2 or IgG control. Representative confocal micrographs of 30 cells counted in 3 independent experiments.

FIGURE S3 | **(A)** Real-time PCR measurement of *Dectin*-1, *Dectin*-2, *AIRE*, *Syk,* and *Card9* expression in macrophage-like THP-1 cells stimulated with hyphae for 10, 20, or 30 min. Representative graph of three independent experiments. The horizontal bars denote the mean. $*p < 0.05$ as determined by the ANOVA.

FIGURE S4 | Expression of Dectin receptors and proteins from Syk-dependent pathway in wild-type and AIRE-knockdown macrophage-like THP-1 cells. **(A)** Evaluation of AIRE expression in AIRE-knockdown macrophage-like THP-1 cells: wild-type (black line), AIRE-knockdown (dashed line) and IgG control cells (gray line). **(B)** Relative expression of AIRE mRNA (**Left**) and protein expression (**Right**) in AIRE-knockdown and wild-type macrophage-like THP-1 cells. **(C,D)** Expression of Dectin receptors in wild-type (black line) and AIRE-knockdown (dashed line) macrophage-like THP-1 cells and IgG control cells (gray line). **(E)** Syk-dependent pathway activation in macrophage-like THP-1 cells for Dectin-1, Syk, CARD9, and β-actin. Representative image of three independent experiments.

FIGURE S5 | Phagocytic activity in wild-type and AIRE-knockdown macrophage-like THP-1 cells. Wild-type and AIRE-knockdown macrophage-like THP-1 cells were stimulated with *E. coli* (MOI 1:5) for 20 or 30 min. Cells were incubated for 45 min with gentamicin, washed, lysed, and cultured in LB agar. CFU was counted after overnight incubation at 37°C. Representative graph of three independent experiments.

FIGURE S6 | Decreased phagocytic activity resulting from chemical Syk inhibition in THP-1 cells. Wild-type and AIRE-knockdown macrophage-like THP-1 cells were treated with piceatannol for 45 min, followed by stimulation for 30 min with yeast (MOI 1:2) **(A)** or hyphae (MOI 2:1) **(B)** from GFP-positive *C. albicans*. Phagocytosis was assessed with Image Stream. Each dot represents one independent experiment used for the measurement indicated. The horizontal bars denote the mean. $*p < 0.05$ as determined by the *t*-test.

FIGURE S7 | Cytokine secretion and *E. coli* phagocytosis in healthy donor or APECED patient macrophages stimulated with LPS. **(A)** Healthy donor or AIRE-deficient patient macrophages stimulated with *E. coli* for 30 min, lysed and cultured in LB agar. CFU was counted after overnight incubation at 37°C. **(B)** IL-6 and TNF-α secretion by healthy donor or AIRE-deficient patient macrophages unstimulated or stimulated with LPS for 24 h. The horizontal bars denote the mean of four independent experiments for both healthy donors or APECED patients.

REFERENCES

1. Hanna S, Etzioni A. New host defense mechanisms against *Candida* species clarify the basis of clinical phenotypes. *J Allergy Clin Immunol* (2011) 127(6):1433–7. doi:10.1016/j.jaci.2011.03.026
2. Keppler-Ross S, Douglas L, Konopka JB, Dean N. Recognition of yeast by murine macrophages requires mannan but not glucan. *Eukaryot Cell* (2010) 9(11):1776–87. doi:10.1128/EC.00156-10
3. Goodridge HS, Reyes CN, Becker CA, Katsumoto TR, Ma J, Wolf AJ, et al. Activation of the innate immune receptor Dectin-1 upon formation of a 'phagocytic synapse'. *Nature* (2011) 472(7344):471–5. doi:10.1038/nature10071
4. Niedergang F, Di Bartolo V, Alcover A. Comparative anatomy of phagocytic and immunological synapses. *Front Immunol* (2016) 7:18. doi:10.3389/fimmu.2016.00018
5. Okada S, Puel A, Casanova JL, Kobayashi M. Chronic mucocutaneous candidiasis disease associated with inborn errors of IL-17 immunity. *Clin Transl Immunology* (2016) 5(12):e114. doi:10.1038/cti.2016.71
6. Li J, Vinh DC, Casanova JL, Puel A. Inborn errors of immunity underlying fungal diseases in otherwise healthy individuals. *Curr Opin Microbiol* (2017) 40:46–57. doi:10.1016/j.mib.2017.10.016
7. Maródi L, Cypowyj S, Tóth B, Chernyshova L, Puel A, Casanova JL. Molecular mechanisms of mucocutaneous immunity against *Candida* and *Staphylococcus* species. *J Allergy Clin Immunol* (2012) 130(5):1019–27. doi:10.1016/j.jaci.2012.09.011
8. Lanternier F, Cypowyj S, Picard C, Bustamante J, Lortholary O, Casanova JL, et al. Primary immunodeficiencies underlying fungal infections. *Curr Opin Pediatr* (2013) 25(6):736–47. doi:10.1097/MOP.0000000000000031
9. Drewniak A, Gazendam RP, Tool AT, van Houdt M, Jansen MH, van Hamme JL, et al. Invasive fungal infection and impaired neutrophil killing in human CARD9 deficiency. *Blood* (2013) 121(13):2385–92. doi:10.1182/blood-2012-08-450551
10. Alves de Medeiros AK, Lodewick E, Bogaert DJ, Haerynck F, Van Daele S, Lambrecht B, et al. Chronic and invasive fungal infections in a family with CARD9 deficiency. *J Clin Immunol* (2016) 36(3):204–9. doi:10.1007/s10875-016-0255-8
11. Puel A, Cypowyj S, Bustamante J, Wright JF, Liu L, Lim HK, et al. Chronic mucocutaneous candidiasis in humans with inborn errors of interleukin-17 immunity. *Science* (2011) 332(6025):65–8. doi:10.1126/science.1200439
12. Boisson B, Wang C, Pedergnana V, Wu L, Cypowyj S, Rybojad M, et al. An ACT1 mutation selectively abolishes interleukin-17 responses in humans with chronic mucocutaneous candidiasis. *Immunity* (2013) 39(4):676–86. doi:10.1016/j.immuni.2013.09.002
13. Zheng J, van de Veerdonk FL, Crossland KL, Smeekens SP, Chan CM, Al Shehri T, et al. Gain-of-function STAT1 mutations impair STAT3 activity in patients with chronic mucocutaneous candidiasis (CMC). *Eur J Immunol* (2015) 45(10):2834–46. doi:10.1002/eji.201445344
14. Hiller J, Hagl B, Effner R, Puel A, Schaller M, Mascher B, et al. STAT1 gain of function and STAT3 loss of function mutations impair IL-17 and IL-22 immunity associated with CMC. *J Invest Dermatol* (2017) 138(3):711–4. doi:10.1016/j.jid.2017.09.035
15. Ferwerda B, Ferwerda G, Plantinga TS, Willment JA, van Spriel AB, Venselaar H, et al. Human dectin-1 deficiency and mucocutaneous fungal infections. *N Engl J Med* (2009) 361(18):1760–7. doi:10.1056/NEJMoa0901053
16. Cunha C, Di Ianni M, Bozza S, Giovannini G, Zagarella S, Zelante T, et al. Dectin-1 Y238X polymorphism associates with susceptibility to invasive aspergillosis in hematopoietic transplantation through impairment of both recipient- and donor-dependent mechanisms of antifungal immunity. *Blood* (2010) 116(24):5394–402. doi:10.1182/blood-2010-04-279307
17. Kisand K, Peterson P. Autoimmune polyendocrinopathy candidiasis ectodermal dystrophy. *J Clin Immunol* (2015) 35(5):463–78. doi:10.1007/s10875-015-0176-y
18. Ferre EM, Rose SR, Rosenzweig SD, Burbelo PD, Romito KR, Niemela JE, et al. Redefined clinical features and diagnostic criteria in autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy. *JCI Insight* (2016) 1(13):e88782. doi:10.1172/jci.insight.88782
19. Abramson J, Giraud M, Benoist C, Mathis D. Aire's partners in the molecular control of immunological tolerance. *Cell* (2010) 140(1):123–35. doi:10.1016/j.cell.2009.12.030
20. Weiler FG, Dias-da-Silva MR, Lazaretti-Castro M. Autoimmune polyendocrine syndrome type 1: case report and review of literature. *Arq Bras Endocrinol Metabol* (2012) 56(1):54–66. doi:10.1590/S0004-27302012000100009
21. Kluger N, Jokinen M, Krohn K, Ranki A. Gastrointestinal manifestations in APECED syndrome. *J Clin Gastroenterol* (2013) 47(2):112–20. doi:10.1097/MCG.0b013e31827356e1
22. Poliani PL, Kisand K, Marrella V, Ravanini M, Notarangelo LD, Villa A, et al. Human peripheral lymphoid tissues contain autoimmune regulator-expressing dendritic cells. *Am J Pathol* (2010) 176(3):1104–12. doi:10.2353/ajpath.2010.090956
23. Pedroza LA, Kumar V, Sanborn KB, Mace EM, Niinikoski H, Nadeau K, et al. Autoimmune regulator (AIRE) contributes to Dectin-1-induced TNF-alpha production and complexes with caspase recruitment domain-containing protein 9 (CARD9), spleen tyrosine kinase (Syk), and Dectin-1. *J Allergy Clin Immunol* (2012) 129(2):e1–3. doi:10.1016/j.jaci.2011.08.027
24. Zimmerman O, Rosen LB, Swamydas M, Ferre EMN, Natarajan M, van de Veerdonk F, et al. Autoimmune regulator deficiency results in a decrease in STAT1 levels in human monocytes. *Front Immunol* (2017) 8:820. doi:10.3389/fimmu.2017.00820
25. Kisand K, Bøe Wolff AS, Podkrajsek KT, Tserel L, Link M, Kisand KV, et al. Chronic mucocutaneous candidiasis in APECED or thymoma patients correlates with autoimmunity to Th17-associated cytokines. *J Exp Med* (2010) 207(2):299–308. doi:10.1084/jem.20091669
26. Puel A, Döffinger R, Natividad A, Chrabieh M, Barcenas-Morales G, Picard C, et al. Autoantibodies against IL-17A, IL-17F, and IL-22 in patients with chronic mucocutaneous candidiasis and autoimmune polyendocrine syndrome type I. *J Exp Med* (2010) 207(2):291–7. doi:10.1084/jem.20091983
27. Bi L, Gojestani S, Wu W, Hsu YM, Zhu J, Ariizumi K, et al. CARD9 mediates dectin-2-induced IkappaBalpha kinase ubiquitination leading to activation of NF-kappaB in response to stimulation by the hyphal form of *Candida albicans*. *J Biol Chem* (2010) 285(34):25969–77. doi:10.1074/jbc.M110.131300
28. Dambuza IM, Brown GD. C-type lectins in immunity: recent developments. *Curr Opin Immunol* (2015) 32:21–7. doi:10.1016/j.coi.2014.12.002
29. Bain JM, Louw J, Lewis LE, Okai B, Walls CA, Ballou ER, et al. *Candida albicans* hypha formation and mannan masking of beta-glucan inhibit macrophage phagosome maturation. *MBio* (2014) 5(6):e01874. doi:10.1128/mBio.01874-14
30. Gow NA, van de Veerdonk FL, Brown AJ, Netea MG. *Candida albicans* morphogenesis and host defence: discriminating invasion from colonization. *Nat Rev Microbiol* (2012) 10(2):112–22. doi:10.1038/nrmicro2711
31. Strauss A, Michel S, Morschhäuser J. Analysis of phase-specific gene expression at the single-cell level in the white-opaque switching system of *Candida albicans*. *J Bacteriol* (2001) 183(12):3761–9.
32. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. *Methods* (2001) 25(4):402–8. doi:10.1006/meth.2001.1262
33. Mansour MK, Tam JM, Khan NS, Seward M, Davids PJ, Puranam S, et al. Dectin-1 activation controls maturation of beta-1,3-glucan-containing phagosomes. *J Biol Chem* (2013) 288(22):16043–54. doi:10.1074/jbc.M113.473223
34. Rosentul DC, Plantinga TS, Oosting M, Scott WK, Velez Edwards DR, Smith PB, et al. Genetic variation in the dectin-1/CARD9 recognition pathway and susceptibility to candidemia. *J Infect Dis* (2011) 204(7):1138–45. doi:10.1093/infdis/jir458
35. Lanternier F, Mahdaviani SA, Barbati E, Chaussade H, Koumar Y, Levy R, et al. Inherited CARD9 deficiency in otherwise healthy children and adults with

Candida species-induced meningoencephalitis, colitis, or both. *J Allergy Clin Immunol* (2015) 135(6):1558–68e2. doi:10.1016/j.jaci.2014.12.1930
36. Gavino C, Hamel N, Zeng JB, Legault C, Guiot MC, Chankowsky J, et al. Impaired RASGRF1/ERK-mediated GM-CSF response characterizes CARD9 deficiency in French-Canadians. *J Allergy Clin Immunol* (2016) 137(4):1178–1188e7. doi:10.1016/j.jaci.2015.09.016
37. Puel A, Cypowyj S, Maródi L, Abel L, Picard C, Casanova JL. Inborn errors of human IL-17 immunity underlie chronic mucocutaneous candidiasis. *Curr Opin Allergy Clin Immunol* (2012) 12(6):616–22. doi:10.1097/ACI.0b013e328358cc0b
38. Glocker EO, Hennigs A, Nabavi M, Schäffer AA, Woellner C, Salzer U, et al. A homozygous CARD9 mutation in a family with susceptibility to fungal infections. *N Engl J Med* (2009) 361(18):1727–35. doi:10.1056/NEJMoa0810719
39. Lanternier F, Pathan S, Vincent QB, Liu L, Cypowyj S, Prando C, et al. Deep dermatophytosis and inherited CARD9 deficiency. *N Engl J Med* (2013) 369(18):1704–14. doi:10.1056/NEJMoa1208487
40. Suzuki E, Kobayashi Y, Kawano O, Endo K, Haneda H, Yukiue H, et al. Expression of AIRE in thymocytes and peripheral lymphocytes. *Autoimmunity* (2008) 41(2):133–9. doi:10.1080/08916930701773941
41. Bruserud Ø, Bratland E, Hellesen A, Delaleu N, Reikvam H, Oftedal BE, et al. Altered immune activation and IL-23 signaling in response to *Candida albicans* in autoimmune polyendocrine syndrome type 1. *Front Immunol* (2017) 8:1074. doi:10.3389/fimmu.2017.01074
42. Plato A, Willment JA, Brown GD. C-type lectin-like receptors of the dectin-1 cluster: ligands and signaling pathways. *Int Rev Immunol* (2013) 32(2):134–56. doi:10.3109/08830185.2013.777065
43. McDonald DR. TH17 deficiency in human disease. *J Allergy Clin Immunol* (2012) 129(6):1429–35. doi:10.1016/j.jaci.2012.03.034
44. Zielinski CE, Mele F, Aschenbrenner D, Jarrossay D, Ronchi F, Gattorno M, et al. Pathogen-induced human TH17 cells produce IFN-gamma or IL-10 and are regulated by IL-1beta. *Nature* (2012) 484(7395):514–8. doi:10.1038/nature10957
45. Eyerich S, Wagener J, Wenzel V, Scarponi C, Pennino D, Albanesi C, et al. IL-22 and TNF-alpha represent a key cytokine combination for epidermal integrity during infection with *Candida albicans*. *Eur J Immunol* (2011) 41(7):1894–901. doi:10.1002/eji.201041197

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Delayed Diagnosis and Complications of Predominantly Antibody Deficiencies in a Cohort of Australian Adults

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Background: Predominantly antibody deficiencies (PADs) are the most common type of primary immunodeficiency in adults. PADs frequently pass undetected leading to delayed diagnosis, delayed treatment, and the potential for end-organ damage including bronchiectasis. In addition, PADs are frequently accompanied by comorbid autoimmune disease, and an increased risk of malignancy.

Objectives: To characterize the diagnostic and clinical features of adult PAD patients in Victoria, Australia.

Methods: We identified adult patients receiving, or having previously received immunoglobulin replacement therapy for a PAD at four hospitals in metropolitan Melbourne, and retrospectively characterized their clinical and diagnostic features.

Results: 179 patients from The Royal Melbourne, Alfred and Austin Hospitals, and Monash Medical Centre were included in the study with a median age of 49.7 years (range: 16–87 years), of whom 98 (54.7%) were female. The majority of patients (116; 64.8%) met diagnostic criteria for common variable immunodeficiency (CVID), and 21 (11.7%) were diagnosed with X-linked agammaglobulinemia (XLA). Unclassified hypogammaglobulinemia (HGG) was described in 22 patients (12.3%), IgG subclass deficiency (IGSCD) in 12 (6.7%), and specific antibody deficiency (SpAD) in 4 individuals (2.2%). The remaining four patients had a diagnosis of Good syndrome (thymoma with immunodeficiency). There was no significant difference between the age at diagnosis of the disorders, with the exception of XLA, with a median age at diagnosis of less than 1 year. The median

age of reported symptom onset was 20 years for those with a diagnosis of CVID, with a median age at diagnosis of 35 years. CVID patients experienced significantly more non-infectious complications, such as autoimmune cytopenias and lymphoproliferative disease, than the other antibody deficiency disorders. The presence of non-infectious complications was associated with significantly reduced survival in the cohort.

Conclusion: Our data are largely consistent with the experience of other centers internationally, with clear areas for improvement, including reducing diagnostic delay for patients with PADs. It is likely that these challenges will be in part overcome by continued advances in implementation of genomic sequencing for diagnosis of PADs, and with that opportunities for targeted treatment of non-infectious complications.

Keywords: predominantly antibody deficiency, primary immunodeficiency, diagnostic delay, common variable immunodeficiency, X-linked agammaglobulinemia, immunoglobulin subclass deficiency, specific antibody deficiency

INTRODUCTION

Primary immunodeficiencies (PIDs) are a heterogeneous group of diseases, characterized by an impaired immune response to pathogens, predisposing to more frequent and severe infection, in some instances to a single pathogen, and dysregulated immune function, which may result in autoimmune disease or inflammatory conditions (1). Knowledge of the key molecular processes underpinning these varied disorders continues to evolve with advances in genomic technology. The archetypal classification of immunologic disorders has recognized predominantly antibody deficiency (PAD) as the most prevalent PID, and these patients require lifelong antibody replacement therapy (2, 3). In comparison with other countries, the prevalence of PADs in Australia has not been clearly established. There are only two reports over the last two decades with varied results that are likely to represent significant under reporting, due to ascertainment bias (4, 5).

Diagnosis is challenging because PADs have varied clinical presentations and may present from infancy to late adulthood. The hallmark clinical feature is a history of recurrent sinopulmonary bacterial infections, resulting from an ineffective antibody response, however, in many cases, non-infectious complications, such as autoimmune disease, or malignancy may complicate the clinical presentation.

The most profound PAD is agammaglobulinemia with a complete or near-complete absence of serum Ig and absence of mature B cells in blood. Most of these individuals suffer from X-linked agammaglobulinemia (XLA) which occurs due to mutations in the gene encoding Bruton's tyrosine kinase (*BTK*), an enzyme essential for B-cell development (6). Defects in other genes crucial for B-cell development and survival have been identified in a smaller proportion of patients (7–10), currently leaving only 5–10% without a genetic diagnosis.

Common variable immunodeficiency (CVID) constitutes the majority of PAD cases which require ongoing treatment, with a global incidence of approximately 1:25,000 (11) although this varies according to the population studied. A recent Finnish study found the prevalence to be as high as 6.9 per 100,000 (12). CVID has complex clinical features and pathophysiology, underpinned by identifiable monogenic defects in a minority of patients (13–15). Patients may present with a spectrum of manifestations, including recurrent infections and autoimmune cytopenias. Several diagnostic criteria, based upon a combination of clinical and laboratory features, may be used to aid in diagnosis and classification of patients with these complex presentations (16–18). A diagnosis may be made from 2 years of age (19).

The spectrum of PAD also includes milder syndromes, albeit with variable clinical features that include selective IgA deficiency; unclassified hypogammaglobulinemia (HGG), characterized by reduced levels of IgG in the presence of normal IgA and IgM; IgG subclass deficiency (IGSCD), characterized by a reduction in one or more IgG subclasses with normal total IgG; and specific antibody deficiency (SpAD), characterized by normal serum immunoglobulins with an apparent failure to produce antibody in response to vaccines. Thymoma with immunodeficiency, otherwise eponymously known as Good syndrome, is a rare PID associated with the presence of a thymoma, although the mechanism is not well understood (1, 20–22).

We sought to identify the diagnostic approaches that had been used for PADs and potential obstacles to diagnosis. In addition we aimed to characterize the infectious and non-infectious manifestations in our patients, such as organ-specific or systemic autoimmune disease as well as malignancies such as lymphomas and gastrointestinal (GI) tumors.

MATERIALS AND METHODS

Patients

We performed a comprehensive cross-sectional clinical analysis of adult patients with PADs managed under clinical immunology services in Victoria from January 2001 to February 2017. Patients who were currently receiving, or had previously received replacement immunoglobulin for PADs were identified by physicians specializing in Clinical Immunology at four major teaching hospitals in metropolitan Melbourne, Victoria: The Royal Melbourne Hospital, The Alfred Hospital, Monash Medical Centre and Austin Health. Patients with secondary hypogammaglobulinemia were

excluded, as were individuals with a defined combined immunodeficiency, or a history of hematopoietic stem-cell transplantation. To ensure that the cohort included patients with clinically significant disease, and receiving regular follow-up, we did not include patients who were not deemed to require replacement immunoglobulin therapy. The study was carried out according to the principles of the Declaration of Helsinki and was approved by local human research ethics committees (Melbourne Health projects 2009.162, 2013.245; Walter and Eliza Hall Research Institute projects 10/02, 14/01; Alfred Health 109/15, 277/17). All living patients, or their next of kin, consented to the collection of their medical information. For those individuals who were deceased at the time of the data collection, ethical approval was obtained to review the medical records without the consent of the next of kin.

Data Collection

Data were retrospectively collected from the clinical case notes using a pre-specified template to capture age of reported onset of symptoms, age at diagnosis and organ-specific manifestations that included respiratory, GI, neurological, musculoskeletal, organomegaly, and malignancy. We defined immune dysregulation on clinical notes reporting any of the following diagnoses: autoimmune disease, lymphoproliferative disease, organomegaly, granulomatous inflammation, and/or enteropathy.

Statistical Analysis

We examined differences between groups using one-way ANOVA for comparison of continuous variables; Chi square analysis for comparison of categorical variables with a high frequency, and Fisher's exact test for categorical variables with a frequency of less than 5; and Mantel–Cox log rank for comparing differences in survival between disease groups. As the data for age of onset, diagnosis and diagnostic delay were non-parametrically distributed, we have reported median values for these measures. A p value of less than 0.05 was considered statistically significant. Statistical analyses were performed using GraphPad Prism 7 software.

RESULTS

Patient Demographics

179 patients with PAD were identified from the four centers. 98 patients were females, and 81 were males, with median age of 49 years (**Table 1**). CVID was the most frequent antibody deficiency disorder requiring clinical follow-up with 116 patients. XLA was diagnosed in 21 male patients. 22 patients had HGG, 4 had SpAD, and 12 were diagnosed with IGSCD. We also included four patients with thymoma and immunodeficiency, with a diagnosis of Good syndrome (**Table 1**).

Diagnostic Features

Age at Diagnosis and Diagnostic Delay

The median age at diagnosis for the entire cohort was 36 years, which ranged from diagnoses at birth to 80 years old. Patients with XLA were diagnosed at significantly younger ages than all other antibody deficiency disorders (**Figure 1A**; **Table 1**) and experienced significantly shorter delay from symptom onset to diagnosis (**Table 1**; Table S1 in Supplementary Material). With regards to CVID specifically, the median age of reported onset of symptoms was 20 years, and the median age of diagnosis was 35 years (**Table 1**; **Figures 1A,B**). The median diagnostic delay was available for only 83/116 CVID patients and was 9 years. The four patients with Good syndrome were diagnosed at a median age of 60.75 years (interquartile range 44.3–74), which was significantly older than XLA ($p < 0.0001$) and CVID ($p < 0.05$) diagnoses, but not the other antibody deficiency syndromes (**Table 1**; **Figures 1A,B**).

Serum Immunoglobulin Levels

The serum IgG levels at time of diagnosis of CVID were significantly lower than those recorded for patients with HGG (3.10 ± 1.55 versus 6.32 ± 4.67 g/L, respectively, $p < 0.0001$; **Figure 1D**). However, serum IgG levels for CVID and HGG were both significantly lower than in patients with a diagnosis of IgGSCD and SpAD ($p < 0.0001$; **Figure 1D**). Serum IgA levels were also significantly reduced in CVID patients, compared with other PAD, consistent with the diagnostic criteria (0.23 ± 0.28 versus 1.16 ± 1.05 g/L, $p = < 0.0001$; **Figure 1D**). Serum IgM levels in CVID (0.3 ± 0.36 g/L) were significantly lower compared with HGG (0.97 ± 0.6 g/L; $p < 0.0001$), and IGSCD (1.15 ± 0.66 g/L; $p < 0.0001$) (**Figure 1D**). Serum Ig levels at time of diagnosis were not available for most of the XLA patients, due to the historic nature of their diagnoses.

TABLE 1 | Diagnostic and demographic data of the Victorian adult predominantly antibody deficiency cohort.

Immunologic diagnosis	Number of patients (%)	Male:female ratio	Current age (years; range)	Median age at diagnosis (years)	Median age at symptom onset[a] (years; range)	Median diagnostic delay[a] (years; range)
All	179	0.82	49 (16–87)	36 (0–87)	20 (0–68)	7.5 (0–63)
Common variable immunodeficiency (CVID)	116 (64.8)	0.78	48.5 (16–80)	35 (2–80)	20 (1–65)	9 (1–63)
X-linked agammaglobulinemia (XLA)	21 (11.7)	N/A	31.5 (23–65)	0 (0–15)	0 (0–2)	1 (0–15)
Hypogammaglobulinemia (unclassified) (HGG)	22 (12.29)	0.16	54 (18–87)	43 (5–80)	19.5 (1–50)	4 (1–59)
Specific antibody deficiency (SpAD)	4 (2.23)	1.00	65.5 (43–81)	68 (33–72)	N/A	N/A
IgG subclass deficiency (IGSCD)	12 (6.7)	0.20	56 (30–81)	36 (7–74)	20 (2–68)	9 (6–16)
Good syndrome	4 (2.23)	3.0	69 (46–77)	60 (40–71)	58 (38–68)	2 (0–3)

[a]*Data for age at symptom onset and diagnostic delay available for the following numbers of patients in each group. CVID, 83/116; XLA, 16/21; HGG, 7/22; SpAD, 1/4; IGSCD, 7/12; Good syndrome 2/4.*

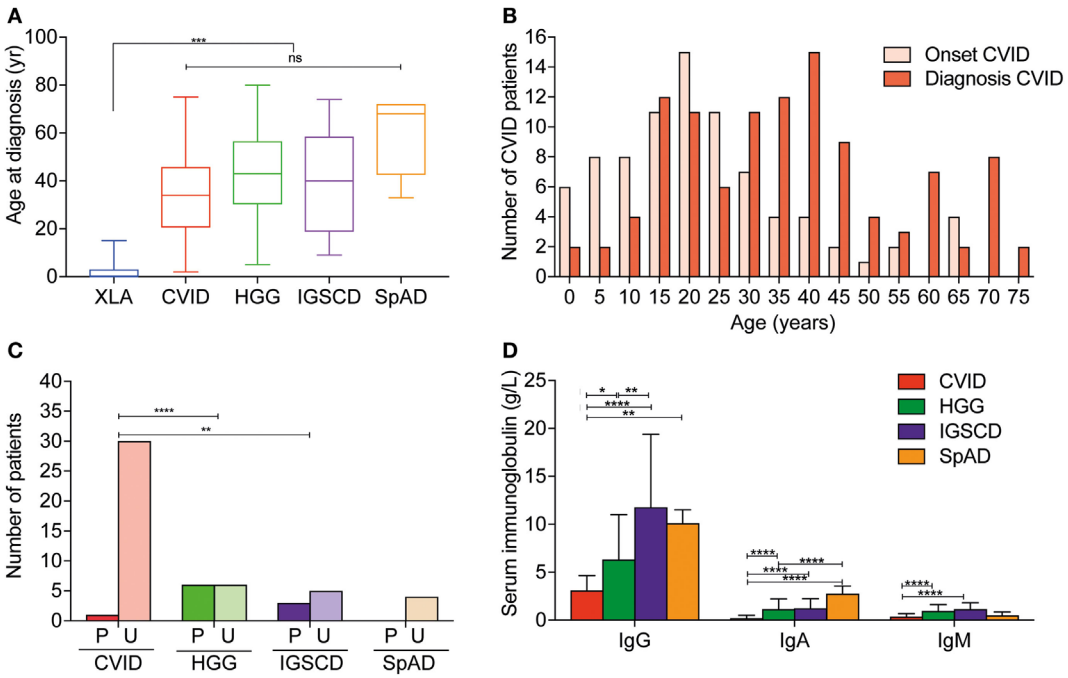

FIGURE 1 | Age at diagnosis and diagnostic features differ between disease groups in Victorian adult patients with predominantly antibody deficiencies. **(A)** Box–whisker plot of age at diagnosis according to disease classification with median and 5–95% percentiles indicated. **(B)** Histogram demonstrating age at reported symptom onset, light red, and age at diagnosis (dark red) in common variable immunodeficiency (CVID) patients. **(C)** Qualitative vaccine responses to pneumococcal polysaccharide vaccine responses measured IgG binding against PPS23 polysaccharides in CVID patients (red), unclassified hypogammaglobulinemia (HGG) patients (green), IgG subclass deficiency (IGSCD) (purple), and specific antibody deficiency (SpAD) (orange). Darker colored columns indicate protective responses, P, and lighter colored columns indicate unprotective responses, U (χ^2 analysis: $^{**}p < 0.01$ and $^{****}p < 0.0001$). **(D)** Serum IgG and IgA (g/L, mean ± SD) at diagnosis according to disease classification ($^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$, using one-way ANOVA).

Specific Antigen Responses

The results of pneumococcal polysaccharide vaccine responses were available for 55 patients with PAD reported here. For CVID, specific pneumococcal polysaccharide antibody titers were available for 30 of 116 patients. From these 30 records, 29 CVID patients were reported to have unprotective vaccine responses (96.7%). Reflecting the heterogeneity of these conditions, the single patient with protective vaccine responses, in retrospect had an atypical presentation for CVID with early-onset chronic mucocutaneous candidiasis, and subsequent adult-onset hypogammaglobulinemia, was identified to have a gain-of-function (GOF) *STAT1* mutation, which is not typically associated with PAD. Furthermore, consistent with the diagnostic criteria for CVID, a higher proportion of HGG and IGSCD patients demonstrated protective vaccine responses compared with the CVID and SpAD groups, who consistently demonstrated suboptimal vaccine responses (χ^2; $p < 0.001$ and $p < 0.01$ respectively; **Figure 1C**).

Genetic Diagnoses

Although genetic testing was not uniformly used in the diagnosis of these patients, 40/178 had an identified genetic cause for PAD (Table S1 in Supplementary Material). This included 21 XLA patients with hemizygous *BTK* mutations. Of the remaining 19 patients with PAD and an identified genetic contribution, 17 had CVID, and 2 had IGSCD. Of the CVID patients, 4 had NFκB1 deficiency, and 3 were heterozygous for the (C104R) variant in *TNFSRF13B*, encoding TACI, a known risk-factor for the development of CVID (23). One of these patients also harbored a pathogenic mutation in *TCF3* (E555K), which causes an autosomal dominant form of agammaglobulinemia (24). In addition, we identified *NFKB2* mutations in three CVID and two IgGSCD patients, who had marked autoimmune clinical manifestations. Two patients initially diagnosed with CVID were found to have *CXCR4* mutations and three patients with CVID were rediagnosed with CTLA4 haploinsufficiency.

Clinical Features and Disease Complications

All patients presented with infections; however, most patients developed complications in addition; only 28% demonstrated an infection-only clinical phenotype (**Table 2**; **Figure 2A**). Respiratory tract involvement was the most common clinical manifestation followed by disease of the GI tract, skin and musculoskeletal system (**Table 2**; **Figure 2A**). Pneumonia and sinusitis were the most common infectious manifestations of the respiratory tract, present in 78% of all PAD patients, and GI infections were present in 19% of patients (data not shown). CVID patients were disproportionately affected and appeared susceptible to a broad range of infections that included *Giardia* in eight patients, *Campylobacter* and *Salmonella* in five, candidiasis in three, *Helicobacter pylori* in three and *Cryptosporidia* spp. in two patients. Bronchiectatic structural lung disease was most

TABLE 2 | Complications in Victorian adults with predominantly antibody deficiency.

	CVID, *n*(%)	XLA	HGG	IGSCD	SpAD	Good syndrome	Total, *n*(%)
Total number of patients	116	21	22	12	4	4	179
Infections only[a]	33 (28)	7 (33)	12 (55)	4 (33)	1 (25)[c]	0	57 (32)
Bronchiectasis	31 (27)	14 (67)	4 (18)	4 (33)	2 (50)	1 (25)	28 (16)
GLILD	5 (4)	0	0	0	0	0	5 (3)
Autoimmunity (total)	44 (38)	0	3 (14)	4 (33)	1 (25)	2 (50)	54 (30)
Musculoskeletal	11 (9)	0	0	2 (17)	0	1 (25)	14 (8)
Cytopenia[b]	24 (21)	0	1 (5)	0	0	1 (25)[b]	26 (15)
Endocrine	9 (8)	0	0	2 (17)	1	0	12 (7)
Gastrointestinal disease (total)	27 (23)	0	2 (9)	1 (8)	0	0	30 (17)
Enteropathy	16 (14)	0	0	1 (8)	0	0	17 (9)
Colitis	11 (9)	0	2 (9)	0	0	1 (25)[c]	14 (8)
Granulomatous disease	8 (7)	0	0	0	0	1 (25)[c]	9 (5)
Autoimmune liver disease	3 (3)	0	1 (5)	0	0	0	4 (2)
Neuropathy	4 (3)	0	0	1 (8)	0	0	5 (3)
Malignancy[c]	20 (17)	0	2 (9)	3 (25)	0	2 (50)	27 (15)
Solid organ	14 (12)	0	2 (9)	2 (17)	0	1 (25)[c]	19 (11)
Hematological	6 (5)	0	0	1 (8)	0	1 (25)[c]	8 (4)

CVID, common variable immunodeficiency; XLA, X-linked agammaglobulinemia; HGG, unclassified hypogammaglobulinemia; IGSCD, immunoglobulin subclass deficiency; GLILD, granulomatous lymphocytic interstitial lung disease; SpAD, specific antibody deficiency.

[a]*Excludes autoimmunity, gastrointestinal disease, granulomatous disease, liver disease, malignancy, and GLILD.*

[b]*Excluding lymphopenia.*

[c]*Excluding thymoma.*

common in XLA with 66.7% patients affected compared with 27% of CVID patients (**Table 2**; **Figure 2A**). Four of these patients with chronic lung disease underwent lung transplantation; one female with CVID, and three males with XLA. One of four patients with Good syndrome also developed bronchiectasis.

Neurologic manifestations were also evident in this cohort. There were five cases of meningitis, which were enterovirus-related in two patients, one of whom succumbed to a fatal progressive meningoencephalitis (**Table 3**). Neuropathy was reported in four CVID patients and a single patient with IGSCD (**Table 2**). The features of the neuropathy were not specified as to severity, pattern or progression. Seizures, optic neuritis, autoimmune hypophysitis were also recognized in individual patients from this cohort. Endocrine manifestations that were recorded included autoimmune thyroid disease in eight CVID patients, one IGSCD and one SpAD, while autoimmune adrenal insufficiency was reported in one CVID and one IGSCD patient.

Overall, manifestations of immune dysregulation were most prevalent in CVID and IGSCD patients (72 and 67%, respectively), compared with only 5/22 hypogammaglobulinemic patients, and 1/4 SpAD (**Table 2**). The proportion of patients with all non-infectious manifestations was significantly higher in the CVID group, compared with the aggregated non-CVID cohort of patients (59/116 versus 12/38, respectively; χ^2, $p < 0.05$).

We reviewed the incidence of specific clinical manifestations in the cohort according to diagnostic group (**Figure 2A**). Compared with the non-CVID antibody deficient patients, the 116 CVID patients more frequently developed cytopenia ($p < 0.001$), enteropathy ($p < 0.01$), splenomegaly ($p < 0.01$), lymphadenopathy ($p < 0.05$), and granulomatous infiltration ($p < 0.05$), but there were no significant differences between the proportions of patients with bronchiectasis, colitis, solid organ or hematological neoplasia and autoimmune liver disease.

To determine if organ complications were cumulative, we assessed the presence or absence of bronchiectasis, autoimmune liver disease, enteropathy, colitis, organomegaly, granulomas and malignancy across the key antibody deficient disease groups. Most patients suffered from <2 complications of disease; however, CVID patients were more likely to have >2 complications (**Figure 2B**).

Management of PAD

Patients were identified on the basis of current or previous immunoglobulin replacement therapy. At the time of data analysis all patients with a diagnosis of XLA, CVID and Good syndrome were receiving replacement immunoglobulin and maintaining IgG mean trough levels of 8.8 g/L (data not shown). Antibiotics were used prophylactically in 10 of 20 CVID patients with available data while systemic immunosuppression was used in 17 CVID patients. These included nine individual patients treated with prednisolone, four patients with azathioprine or methotrexate in combination with prednisolone, whereas rituximab (anti-CD20) was administered in six CVID patients for cytopenias. One patient with CVID and granulomatous lymphocytic interstitial lung disease was treated with rituximab and mycophenolate. Splenectomy was performed on two patients for refractory cytopenia. One patient had undergone liver transplantation due to cirrhosis secondary to nodular regenerative hyperplasia and has tolerated tacrolimus well. One other patient had undergone renal transplantation. Three XLA patients and one CVID patient received lung transplants and were treated with combination immunosuppression including mycophenolate, tacrolimus and prednisolone as prophylaxis against rejection.

Mortality

From this retrospective analysis, we identified 19 PAD patients who had died, including 4 from infections (cytomegalovirus,

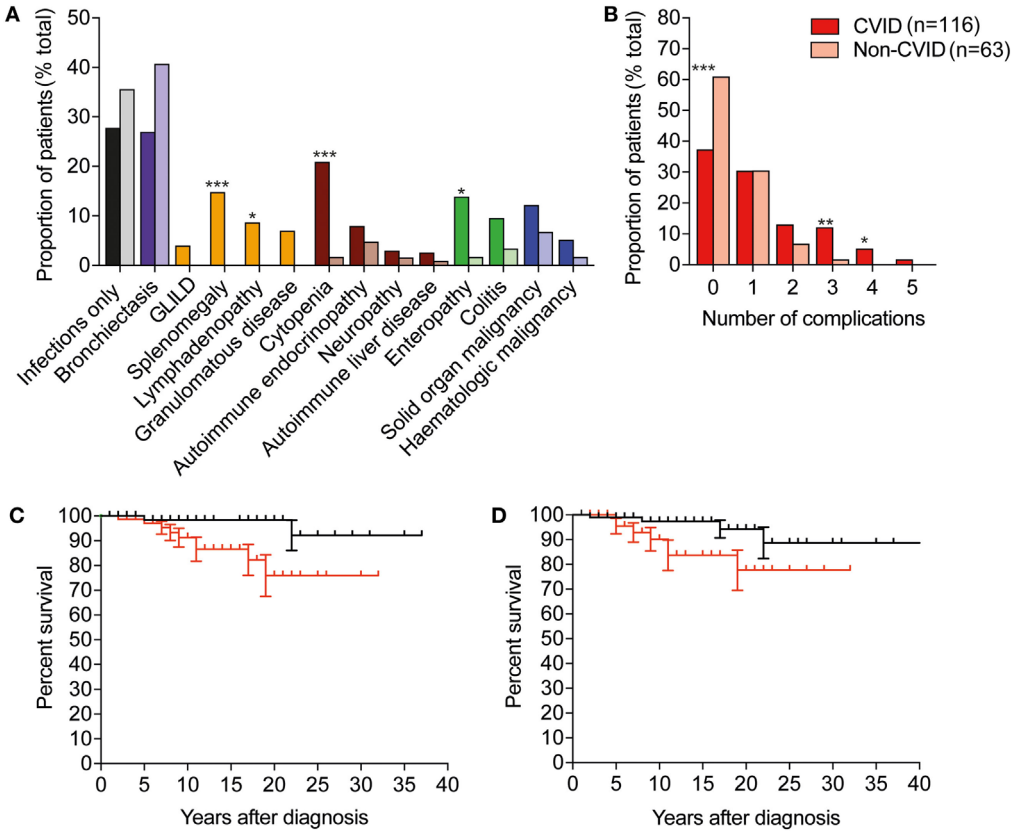

FIGURE 2 | CVID is associated with more frequent non-infectious complications than other predominantly antibody deficiencies, and these complications are associated with reduced survival. **(A)** Histogram depicting frequency of complications in CVID, darker-colored columns, compared with non-CVID cases in lighter-colored columns. Abbreviations: GLILD, granulomatous lymphocytic interstitial lung disease; CVID, common variable immunodeficiency. Purple represents bronchiectasis; yellow represents non-malignant lymphoproliferative complications; brown, autoimmune complications; green, gastrointestinal disease, and blue, malignancies. (Fisher's exact test: ***$p < 0.001$; **$p < 0.01$; and *$p < 0.05$). **(B)** Histogram depicting percentage of CVID and non-CVID patients with 0, 1, or more non-infectious complications of disease. **(C)** Survival after diagnosis in individuals with immune dysregulation is significantly reduced compared with patients with infections only ($p < 0.05$, Mantel–Cox log rank). Black line indicates survival of individuals without features of immune dysregulation, red line indicates survival of individuals with immune dysregulation. **(D)** Survival after diagnosis in individuals with bronchiectasis is significantly reduced compared with patients with infections only ($p < 0.05$, Mantel–Cox log rank). Black line indicates survival of individuals without bronchiectasis, and red indicates survival of individuals with bronchiectasis.

enteroviral encephalitis, sepsis-unspecified, and respiratory infection) and 6 patients died as a result of malignant disease (breast cancer, malignant thymoma, serous ovarian cancer, squamous esophageal cancer, lung adenocarcinoma, and diffuse large B cell lymphoma; **Table 3**). Of those, five had CVID and one had a diagnosis of Good syndrome, with a malignant thymoma. One patient died from an intracerebral hemorrhage, which was thought to be unrelated to PAD. Survival following diagnosis was significantly reduced in patients with immune dysregulation, with a 75% survival at 20 years after diagnosis compared with 98% for those with an infection-only phenotype ($p < 0.05$; **Figure 2C**). Significantly reduced survival was also noted in PAD patients with bronchiectasis as early as 10 years post-diagnosis (**Figure 2D**).

DISCUSSION

Primary immunodeficiencies are a heterogeneous mix of disorders, with >300 defined conditions. The most prevalent of these are PADs, which may present with a broad range of clinical features, age of onset, and population-dependent frequency. As a result, the underlying immunodeficiency diagnosis is typically significantly delayed or missed.

We aimed to characterize how PAD patients have been diagnosed, managed and manifested disease and sought to identify areas for improvement in clinical practice in Victoria, the second most populous state in Australia, with a population aged over 15 years of approximately 4.9 million at the end of 2016 (Australian Bureau of Statistics). Australia is a geographically expansive country, with a relatively small population; posing unique challenges for diagnosis and treatment of rare diseases. The most recent Australian study demonstrated incomplete capture of all PIDs, with the finding of 5.6 cases per 100,000 (5). We found a prevalence of PAD in Victorian adults of approximately 1 in 25,000, which is similar to other population frequencies internationally (11). The prevalence of PAD varies geographically, with a very high prevalence in Finland, a non-diverse population, in contrast to that of Australia, which is more heterogeneous (25).

As with any retrospective study, there are some limitations to this report. Data collection may be incomplete, and inconsistency may arise due to differences in practice between individual

TABLE 3 | Causes of death among antibody deficient patients in Victoria (2000–2017).

Patient no.	Diagnosis	Sex	Clinical phenotype	Age at diagnosis	Age deceased	Cause of death
46	IGSCD[a]	F	Immune dysregulation	9	28	Enteroviral encephalitis
90	CVID	F	Infections only	62	70	Breast cancer
91	Good syndrome	M	Infections only	75	77	Thymoma
92	CVID	F	Immune dysregulation	69	74	Serous ovarian cancer
93	CVID	F	Infections only	47	74	Squamous esophageal cancer
113	CVID[b]	F	Immune dysregulation	67	81	Lung adenocarcinoma
123	CVID	F	Immune dysregulation	40	65	Intracerebral hemorrhage
156	CVID	M	Immune dysregulation	42	62	Diffuse large B cell lymphoma
157	CVID	F	Immune dysregulation	?	48	Sepsis
158	CVID	M	Immune dysregulation	71	78	Respiratory failure—ILD
159	XLA	M	Infections/bronchiectasis	?	58	Infection post lung transplant
160	CVID	F	Infections only	68	73	Not available
161	XLA	M	Infections/bronchiectasis	?	35	Lung transplant complication
162	CVID	M	Immune dysregulation	27	32	Enteropathy/cachexia/sepsis
163	XLA	M	Infections/bronchiectasis	13	59	Respiratory failure
164	XLA	M	Infections/bronchiectasis	?	39	Infection post lung transplant (CMV)
165	XLA	M	Infections/bronchiectasis/cirrhosis	?	36	HCV-related cirrhosis
166	CVID	M	Immune dysregulation	36	53	Respiratory failure and refractory enteropathy
167	CVID	F	Infections/bronchiectasis	71	80	Respiratory failure

IGSCD, immunoglobulin subclass deficiency; CVID, common variable immunodeficiency; XLA, X-linked agammaglobulinemia; ILD, interstitial lung disease; CMV, cytomegalovirus; HCV, hepatitis C virus.

? indicates unknown.

[a]*NFKB2 mutation.*

[b]*NFKB1 mutation.*

physicians and centers. Under-diagnosis is likely to relate to the relative infrequency of these conditions, and therefore lack of suspicion among primary care providers, particularly as the clinical features are varied (25). There is also a significant delay in diagnosis observed in the majority of these disorders, which is associated with an increased risk of mortality, and significant morbidity (26). Thus, increased awareness among medical practitioners and improvements in diagnostic tools are of great clinical importance. Implementation of increased awareness programs is likely to raise the number of patients to be diagnosed and potentially this will lead to identification of a higher prevalence of PAD than 1 in 25,000.

While this cohort represents a small group of patients, our median diagnostic delay of 9 years in the CVID group is longer than other recently reported cohorts. European data from more than 2,000 patients with CVID identified a median diagnostic delay overall of 5 years when the diagnosis was made prior to the year 2000, and 4.2 years when the diagnosis was made during or after the year 2000 (27). However, those patients comprised a large proportion of children, whereas our cohort only included adults, which may introduce a bias to a shorter diagnostic delay in that study. Indeed, in other smaller European adult cohorts a diagnostic delay of 7 years has been reported (28, 29).

Predominantly antibody deficiency remains a diagnostic challenge unless specifically considered in light of the varied non-infectious manifestations, and involvement with physicians with expertise in diagnosis and treatment of primary immunodeficiency. Our data demonstrate that distinguishing primary hypogammaglobulinemia from CVID cannot be readily made using laboratory parameters in isolation, as there is overlap in these parameters between the two conditions. Furthermore, vaccination studies, which are used for diagnosis of PAD, are complicated by the need for paired serum analyses, and are confounded by increasing community use of pneumococcal vaccines and variability in the measurement, and interpretation, of polysaccharide responses. Taken together, there is a need to improve PAD diagnosis, which may entail the use of molecular tests and dialog between internal medicine specialists and clinical immunologists.

Molecular tests that identify pathogenic mutations will improve diagnostic precision in PAD. In this cohort, patients who were later identified to have a genetic contribution to their disease, had a shorter time to a clinical diagnosis (**Table 2**). This may be influenced by the severity of presenting illnesses and/or a positive family history pre-empting screening for antibody deficiency. In addition, molecular tests may help identify patients-at-risk for certain disease complications, as in the case of *NFKB2* mutations which are associated with a high risk of central adrenal insufficiency. Further highlighting the complexities of clinical presentation in PID, we have described a patient who was initially misdiagnosed with CVID, but later found to have a *STAT1* GOF mutation after his diagnosis was reconsidered in light of early-onset mucocutaneous candidiasis.

We also identified one individual with digenic disease due to a pathogenic E555K variant in *TCF3* and C104R variant in *TNFRSF13B*. We have previously reported another kindred affected by PAD and autoimmune disease; the proband harbored a novel non-sense *TCF3* mutation and the C104R variant in *TNFRSF13B*, with a resultant CVID-like disorder and systemic lupus erythematosus (30). We are not aware of other reports of digenic PAD due to the more common pathogenic *TCF3* E555K variant in combination with an additional genetic risk-factor.

Our findings support improved access to diagnostic genomic studies in these patients, although currently the majority of CVID cases are unlikely to be readily diagnosed due to their complex pathogenesis (31, 32). Nevertheless diagnostic rates could

increase to 30% for CVID patients selected for sequencing on the basis of certain clinical or laboratory features (14). Other studies have identified XLA in adult patients previously misdiagnosed as CVID, which has significant implications regarding missed opportunities for genetic counseling (33). The genetic diagnoses we have reported here may have been biased toward patients with more extreme phenotypes, and further work is ongoing to determine the clinical utility of genomic sequencing to aid diagnosis in the cohort more broadly.

Earlier diagnosis of PAD will facilitate earlier IgG replacement therapy and/or antibiotic therapy to mitigate the susceptibility to infections, and delay or prevent end-organ changes from infections such as bronchiectasis. However, optimal management of infections alone may not prevent complications that arise from immune dysregulation such as autoimmune disease, granulomatous inflammation and enteropathy, which affected 68% of our cohort. This is a higher proportion than reported in other cohorts and may reflect a referral bias toward patients with more severe disease, and a failure to include stable PID patients with infection-only phenotypes controlled on immunoglobulin replacement managed by non-Immunology services, or indeed those not receiving immunoglobulin at all.

Prediction of risk for the non-infectious complications of PAD is a major challenge. In a recent US study, unbiased network clustering using two large CVID patient datasets has identified novel biochemical markers of patients with lymphoproliferative, autoimmune and allergic disease (34). Whether these associations apply to the other PADs is unclear. In our cohort CVID patients suffered the most non-infectious complications, but we also identified non-infectious complications in other PADs. Non-infectious complications in IGSCD have also been recognized in other cohorts, although a recent Dutch study did not identify any non-infectious complications in a cohort of patients with HGG (35, 36). Importantly, two IGSCD patients in our cohort harbored *NFKB2* mutations, which may have predisposed these patients to the more severe clinical phenotype.

CONCLUSION

Predominantly antibody deficiencies are the most common of the primary immune deficiency diseases, yet are still likely to be underdiagnosed, or diagnosed after the development of life-threatening complications. Individuals with PADs suffer from diverse and severe complications including infection, bronchiectasis and disorders of immune dysregulation, which are associated with reduced survival. These complications may be averted by earlier and more precise diagnosis, and targeted therapeutic interventions. Early diagnosis relies upon improved knowledge and appropriate clinical suspicion among internal medical physicians and primary care providers, as well as equitable access to genetic diagnosis and the benefits of personalized medicine.

ETHICS STATEMENT

The study was carried out according to the principles of the Declaration of Helsinki and was approved by local human research ethics committees (Melbourne Health projects 2009.162, 2013.245; Walter and Eliza Hall Research Institute projects 10/02, 14/01; Alfred Health 109/15, 277/17). All living patients, or their next of kin, consented to the collection of their medical information. For those individuals who were deceased at the time of the data collection, ethical approval was obtained to review the medical records without the consent of the next of kin.

AUTHOR CONTRIBUTIONS

CS and JB designed the study, collected data, interpreted data, and wrote the manuscript. MZ and VB designed the study, interpreted the data, and wrote the manuscript; TG and EK collected and interpreted data. RS, PC, FH-L, MS, SH, SO, SB, GU, JL, MP, JM, KS, YT, PA, KN, RO, JD, and PH contributed data and assisted writing the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at https://www.frontiersin.org/articles/10.3389/fimmu.2018.00694/full#supplementary-material.

REFERENCES

1. Bousfiha A, Jeddane L, Picard C, Ailal F, Bobby Gaspar H, Al-Herz W, et al. The 2017 IUIS phenotypic classification for primary immunodeficiencies. *J Clin Immunol* (2018) 38(1):129–43. doi:10.1007/s10875-017-0465-8
2. Lucas M, Lee M, Lortan J, Lopez-Granados E, Misbah S, Chapel H. Infection outcomes in patients with common variable immunodeficiency disorders: relationship to immunoglobulin therapy over 22 years. *J Allergy Clin Immunol* (2010) 125(6):1354–1360e4. doi:10.1016/j.jaci.2010.02.040
3. Durandy A, Kracker S, Fischer A. Primary antibody deficiencies. *Nat Rev Immunol* (2013) 13(7):519–33. doi:10.1038/nri3466
4. Baumgart KW, Britton WJ, Kemp A, French M, Roberton D. The spectrum of primary immunodeficiency disorders in Australia. *J Allergy Clin Immunol* (1997) 100(3):415–23. doi:10.1016/S0091-6749(97)70257-4
5. Kirkpatrick P, Riminton S. Primary immunodeficiency diseases in Australia and New Zealand. *J Clin Immunol* (2007) 27(5):517–24. doi:10.1007/s10875-007-9105-z
6. Bruton OC. Agammaglobulinaemia. *Pediatrics* (1952) 9(6):722–8.
7. Minegishi Y, Rohrer J, Coustan-Smith E, Lederman HM, Pappu R, Campana D, et al. An essential role for BLNK in human B cell development. *Science* (1999) 286(5446):1954–7. doi:10.1126/science.286.5446.1954

8. Minegishi Y, Coustan-Smith E, Rapalus L, Ersoy F, Campana D, Conley ME. Mutations in Ig-alpha (CD79a) result in a complete block in B-cell development. *J Clin Invest* (1999) 104(8):1115–21. doi:10.1172/JCI7696
9. Yel L, Minegishi Y, Coustan-Smith E, Buckley RH, Trubel H, Pachman LM, et al. Mutations in the mu heavy-chain gene in patients with agammaglobulinemia. *N Engl J Med* (1996) 335(20):1486–93. doi:10.1056/NEJM199611143352003
10. Minegishi Y, Coustan-Smith E, Wang YH, Cooper MD, Campana D, Conley ME. Mutations in the human lambda5/14.1 gene result in B cell deficiency and agammaglobulinemia. *J Exp Med* (1998) 187(1):71–7.
11. Gathmann B, Grimbacher B, Beauté J, Dudoit Y, Mahlaoui N, Fischer A, et al. The European internet-based patient and research database for primary immunodeficiencies: results 2006-2008. *Clin Exp Immunol* (2009) 157(Suppl 1):3–11. doi:10.1111/j.1365-2249.2009.03954.x
12. Selenius JS, Martelius T, Pikkarainen S, Siitonen S, Mattila E, Pietikäinen R, et al. Unexpectedly high prevalence of common variable immunodeficiency in Finland. *Front Immunol* (2017) 8:1190. doi:10.3389/fimmu.2017.01190
13. Conley ME, Casanova JL. Discovery of single-gene inborn errors of immunity by next generation sequencing. *Curr Opin Immunol* (2014) 30:17–23. doi:10.1016/j.coi.2014.05.004
14. Maffucci P, Filion CA, Boisson B, Itan Y, Shang L, Casanova JL, et al. Genetic diagnosis using whole exome sequencing in common variable immunodeficiency. *Front Immunol* (2016) 7:220. doi:10.3389/fimmu.2016.00220
15. Bogaert DJ, Dullaers M, Lambrecht BN, Vermaelen KY, De Baere E, Haerynck F. Genes associated with common variable immunodeficiency: one diagnosis to rule them all? *J Med Genet* (2016) 53(9):575–90. doi:10.1136/jmedgenet-2015-103690
16. European Society for Immunodeficiencies. *New Clinical Diagnosis Criteria for the ESID Registry*. Geneva (2017). Available from: https://esid.org/Working-Parties/Registry/Diagnosis-criteria
17. Bonilla FA, Barlan I, Chapel H, Costa-Carvalho BT, Cunningham-Rundles C, de la Morena MT, et al. International Consensus Document (ICON): common variable immunodeficiency disorders. *J Allergy Clin Immunol Pract* (2016) 4(1):38–59. doi:10.1016/j.jaip.2015.07.025
18. Ameratunga R, Brewerton M, Slade C, Jordan A, Gillis D, Steele R, et al. Comparison of diagnostic criteria for common variable immunodeficiency disorder. *Front Immunol* (2014) 5:415. doi:10.3389/fimmu.2014.00415
19. Conley ME, Notarangelo LD, Etzioni A. Diagnostic criteria for primary immunodeficiencies. representing PAGID (Pan-American Group for Immunodeficiency) and ESID (European Society for Immunodeficiencies). *Clin Immunol* (1999) 93(3):190–7. doi:10.1006/clim.1999.4799
20. Gabrielsen AE, Good RA. Thymoma. *Ann Intern Med* (1966) 65(3):607–11. doi:10.7326/0003-4819-65-3-607
21. Malphettes M, Gérard L, Galicier L, Boutboul D, Asli B, Szalat R, et al. Good syndrome: an adult-onset immunodeficiency remarkable for its high incidence of invasive infections and autoimmune complications. *Clin Infect Dis* (2015) 61(2):e13–9. doi:10.1093/cid/civ269
22. Picard C, Bobby Gaspar H, Al-Herz W, Bousfiha A, Casanova JL, Chatila T, et al. International union of immunological societies: 2017 primary immunodeficiency diseases committee report on inborn errors of immunity. *J Clin Immunol* (2018) 38(1):96–128. doi:10.1007/s10875-017-0464-9
23. Salzer U, Bacchelli C, Buckridge S, Pan-Hammarström Q, Jennings S, Lougaris V, et al. Relevance of biallelic versus monoallelic TNFRSF13B mutations in distinguishing disease-causing from risk-increasing TNFRSF13B variants in antibody deficiency syndromes. *Blood* (2009) 113(9):1967–76. doi:10.1182/blood-2008-02-141937
24. Boisson B, Wang YD, Bosompem A, Ma CS, Lim A, Kochetkov T, et al. A recurrent dominant negative E47 mutation causes agammaglobulinemia and BCR(-) B cells. *J Clin Invest* (2013) 123(11):4781–5. doi:10.1172/JCI71927
25. Chapel H, Lucas M, Lee M, Bjorkander J, Webster D, Grimbacher B, et al. Common variable immunodeficiency disorders: division into distinct clinical phenotypes. *Blood* (2008) 112(2):277–86. doi:10.1182/blood-2007-11-124545
26. Resnick ES, Moshier EL, Godbold JH, Cunningham-Rundles C. Morbidity and mortality in common variable immune deficiency over 4 decades. *Blood* (2012) 119(7):1650–7. doi:10.1182/blood-2011-09-377945
27. Gathmann B, Mahlaoui N; CEREDIH, Gérard L, Oksenhendler E, Warnatz K, et al. Clinical picture and treatment of 2212 patients with common variable immunodeficiency. *J Allergy Clin Immunol* (2014) 134(1):116–26. doi:10.1016/j.jaci.2013.12.1077
28. Graziano V, Pecoraro A, Mormile I, Quaremba G, Genovese A, Buccelli C, et al. Delay in diagnosis affects the clinical outcome in a cohort of CVID patients with marked reduction of IgA serum levels. *Clin Immunol* (2017) 180:1–4. doi:10.1016/j.clim.2017.03.011
29. Westh L, Mogensen TH, Dalgaard LS, Bernth Jensen JM, Katzenstein T, Hansen AE, et al. Identification and characterization of a nationwide Danish adult common variable immunodeficiency cohort. *Scand J Immunol* (2017) 85(6):450–61. doi:10.1111/sji.12551
30. Ameratunga R, Koopmans W, Woon ST, Leung E, Lehnert K, Slade CA, et al. Epistatic interactions between mutations of TACI (TNFRSF13B) and TCF3 result in a severe primary immunodeficiency disorder and systemic lupus erythematosus. *Clin Transl Immunology* (2017) 6(10):e159. doi:10.1038/cti.2017.41
31. van Schouwenburg PA, Davenport EE, Kienzler AK, Marwah I, Wright B, Lucas M, et al. Application of whole genome and RNA sequencing to investigate the genomic landscape of common variable immunodeficiency disorders. *Clin Immunol* (2015) 160(2):301–14. doi:10.1016/j.clim.2015.05.020
32. Kienzler AK, Hargreaves CE, Patel SY. The role of genomics in common variable immunodeficiency disorders. *Clin Exp Immunol* (2017) 188(3):326–32. doi:10.1111/cei.12947
33. Kanegane H, Tsukada S, Iwata T, Futatani T, Nomura K, Yamamoto J, et al. Detection of bruton's tyrosine kinase mutations in hypogammaglobulinaemic males registered as common variable immunodeficiency (CVID) in the Japanese immunodeficiency registry. *Clin Exp Immunol* (2000) 120(3):512–7. doi:10.1046/j.1365-2249.2000.01244.x
34. Farmer JR, Ong MS, Barmettler S, Yonker LM, Fuleihan R, Sullivan KE, et al. Common variable immunodeficiency non-infectious disease endotypes redefined using unbiased network clustering in large electronic datasets. *Front Immunol* (2017) 8:1740. doi:10.3389/fimmu.2017.01740
35. Barton JC, Bertoli LF, Barton JC. Comparisons of CVID and IgGSD: referring physicians, autoimmune conditions, pneumovax reactivity, immunoglobulin levels, blood lymphocyte subsets, and HLA-A and -B typing in 432 adult index patients. *J Immunol Res* (2014) 2014:542706. doi:10.1155/2014/542706
36. Driessen GJ, Dalm VA, van Hagen PM, Grashoff HA, Hartwig NG, van Rossum AM, et al. Common variable immunodeficiency and idiopathic primary hypogammaglobulinemia: two different conditions within the same disease spectrum. *Haematologica* (2013) 98(10):1617–23. doi:10.3324/haematol.2013.085076

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STAT3-Mediated Transcriptional Regulation of Osteopontin in STAT3 Loss-of-Function Related Hyper IgE Syndrome

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Background: Hyper-IgE syndrome (HIES) caused by loss-of-function (LOF) mutations in STAT3 gene (STAT3 LOF HIES) is associated with dental and facial abnormalities in addition to immunological defects. The role of STAT3 in the pathogenesis of the dental/facial features is, however, poorly elucidated.

Objectives: Since mechanism of cellular resorption of mineralized tissues such as bone and teeth are similar, we attempted to study the expression of genes involved in bone homeostasis in STAT3 LOF HIES.

Methods: Peripheral blood mononuclear cells from healthy controls (HCs), STAT3 LOF HIES patients, $STAT3^{-/-}$ PC-3 cells and $STAT3^{+/+}$ LNCaP cells were stimulated with IL-6 and quantitative PCR array was performed to study the relative mRNA expression of 43 pre-selected genes. PCR array finding were further evaluated after *stattic* induced STAT3 inhibition.

Results: Osteopontin (OPN) gene was seen to be significantly upregulated after IL-6 stimulation in HC (mean fold change 18.6, $p = 0.01$) compared with HIES subjects. Inhibition of STAT3 signaling by *stattic* followed by IL-6 stimulation abrogated the OPN response in HCs suggesting that IL-6-induced STAT3 signaling regulates *OPN* expression. Bioinformatics analysis predicted the presence of STAT3 response element TTCCAAGAA at position -2005 of the OPN gene.

Conclusion: Regulation of OPN gene through IL-6-mediated STAT3 activation and its significant dysregulation in STAT3 LOF HIES subjects could make OPN a plausible candidate involved in the pathogenesis of dental/facial manifestations in HIES.

Keywords: craniosynostosis, dental anomalies, hyper-IgE syndrome, osteopontin, STAT3 signaling, skeletal anomalies

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INTRODUCTION

Hyper-IgE syndrome (HIES) associated with loss-of-function (LOF) mutations in the STAT3 gene (STAT3 LOF HIES) (1, 2) with resultant deficiency of TH17 cells and its secreted cytokine IL-17 (3–5), are now known to underlie majority of the sporadic and autosomal dominant forms of HIES. The syndrome is characterized by varying degrees of immunodeficiency along with

dental, connective tissue, and skeletal defects. Features include high-serum levels of IgE (>2,000 IU/ml), staphylococcal skin abscesses, recurrent bacterial infections, pneumonia with *pneumatocele* formation, mucocutaneous candidiasis, and chronic eczematoid dermatitis manifesting early in life. Coarse facies and mild eosinophilia are variable features (6–10). Though most of the features of autosomal dominant STAT3 LOF HIES reflect a deficient immune system, the non-immunological features *viz*. dental abnormalities (retained primary teeth, non-eruption of permanent teeth, double rows of teeth), anomalies in midline facial development (prominent forehead, wide nasal bridge, broad nose, prominent mandible), and skeletal abnormalities (bone fractures, hyperextensible joints, scoliosis, and craniosynostosis) reflects the multisystem nature of the disease (1, 6, 11–14). The exact mechanism by which the STAT3 defect leads to the dental/craniofacial manifestations is, however, not clearly defined. Recent description of a HIES phenotype with craniosynostosis, and scoliosis in mutations of the IL6ST gene encoding the gp130 receptor, and of isolated craniosynostosis, maxillary hypoplasia, delayed tooth eruption, supernumerary teeth, and minor digital anomalies in IL-11RA mutations (15, 16), strongly suggests involvement of the IL-11/gp130/STAT3 axis in craniofacial and dental features.

It is known that mechanisms in cellular resorption of bone and teeth are similar (17) and delayed shedding of primary teeth is essentially a manifestation of ineffective apical root resorption. On similar lines, the steady state in mature cranial and facial bone sutures represents a balance between osteogenesis and resorption (18). Osteoclasts (OC) and odontoclasts (OdCs) are crucial for resorption of mineralized tissues of the craniofacial complex and the rate of resorption as well as the timing of OC/OdC function during pre-emergent tooth eruption is crucial to support the erupting tooth to move along the appropriate path. Though the precise mechanism of apical root resorption in shedding of primary teeth have been elusive, decreased OdC activity is observed in over-retention of primary teeth (19). Though the maintenance of cranial sutures is genetically much more complex, similar mechanisms of OC function and resorption are thought to be involved (18). Minegishi et al. in an attempt to correlate STAT3 with OC differentiation and function observed that OCs from individuals with STAT3 LOF HIES tend to show higher resorptive activity compared with controls without any effect on OC differentiation (2). While this might explain the skeletal features of osteoporosis and fractures (increased OC activity), it fails to explain retention of teeth and craniosynostosis (decreased OC activity) in HIES. A similar dissociation is observed in the IL-11RA defect and craniosynostosis, as IL-11 is known to have a stimulatory effect in osteoblasts predicting osteopenia in IL-11RA deficiency, but the opposite turns out to be true (18). No detailed study has, however, been performed on OC function, osteoporosis, and fractures till date in HIES. IL-17 has also been found to be involved in bone remodeling by inducing the expression of receptor activator of nuclear factor κB ligand (RANKL) which in turn increase osteoclastogenesis causing osteoporosis (20) implying that TH17 deficiency would lead to decreased osteoclastogenesis. Defects in the IL17 axis, however, are known to cause chronic mucocutaneous candidiasis (21, 22), which do not show any involvement of the dental/skeletal system whatsoever and hence IL17 deficiency as the cause for the dental/skeletal defects in HIES seems unlikely.

The explanation behind the dental/facial involvement in STAT3 LOF HIES would probably lie on a molecule that has both immune and osteogenic/odontogenic functions and is regulated through STAT3. In the light of the above facts, the study was aimed to evaluate plausible molecules involved in bone homeostasis that could be dysregulated in STAT3 LOF HIES.

MATERIALS AND METHODS

Study Design and Subjects

This prospective study was carried out in the department of Immunopathology, Postgraduate Institute of Medical Education and Research, Chandigarh, India. Study protocol was approved by the Institute Ethics Committee (IEC No. 8827-PG 10-1TRG/8450). All subjects were recruited after obtaining a written informed consent prior to enrollment as per IEC guidelines.

Three STAT3 LOF HIES subjects with mutation in STAT3 DNA binding domain (P1; 35 years male; Exon 10, A>C; g.54792; c.1018; p.K340Q), transactivation domain (P4; 3 years male; Exon 22, C>T; g.71311; c.2141; p.T714I), and SH2 domain (P5; 16 years male; Exon 21, T>C; g.66092; c.1979; p.M660T), respectively, were enrolled in the study (**Table 1**). The clinical details and laboratory investigations of the subjects P1, P4, and P5 have been previously reported (23, 24). All three STAT3 LOF HIES subjects had reduced TH17 cell numbers, high serum IgE levels, and NIH score more than 40 (**Table 1**). Healthy volunteers without any history of chronic illness and normal serum IgE levels were recruited as controls.

TABLE 1 | Clinical and genetic profile of hyper IgE syndrome patients.

Patient ID	Age (years)[c]	Sex	NIH score	STAT3 mutation	IgE levels (IU/ml)	Eosinophilia (cells/μl)	Eczema	Skin abscesses	Pneumonia	Skeletal abnormalities[b]
P1	40	M	52	1018A>C, K340Q (DBD)[a]	103,200	295	+	+	+	1, 2
P4	3	M	42	2141C>T, T714I (TA)	5,000	948	+	–	+	1, 2
P5	16	M	49	1979T>C, M660T (SH2)[a]	9,180	968	+	+	+	1, 3, 4

[a]*Novel mutation reported by Saikia et al. (23, 24).*

DBD, DNA binding domain, SH2, Src homology domain; TA, transactivation domain.

[b]*1: coarse facies; 2: increased nasal width; 3: pathological fractures; 4: retained primary teeth.*

[c]*Age of the patients at the time of analysis.*

Sample Collection and Peripheral Blood Mononuclear Cell (PBMC) Isolation and Cell Culture

8 ml of peripheral venous blood was collected in heparin from the patients and controls for PBMC isolation using density gradient centrifugation method. Cell viability was checked by trypan blue dye (Sigma Aldrich, USA) exclusion test and more than 98% viability was obtained. PBMCs (1×10^6) were suspended in RPMI 1640 without fetal bovine serum (FBS) and incubated with or without IL-6 (Peprotech, Rocky Hill, NJ, USA) at a dose of 30 ng/ml for 2 h. For STAT3 inhibition, PBMCs were incubated in the presence of 10 μM of the *stattic* (Sigma Aldrich, USA), a specific STAT3 inhibitor, for 1 h followed by treatment with 30 ng/ml of IL-6 for 2 h in 5% CO_2. Cells were subsequently harvested for RNA extraction. Two prostate cancer cell lines viz. $STAT3^{-/-}$ PC-3 (25, 26) and $STAT3^{+/+}$ LNCaP (25, 27) were procured from National Center for Cell Science, Pune, India. The cells were maintained in RPMI 1640 and Ham's F-12 (Sigma Aldrich, USA) media, respectively, supplemented with 10% FBS and antibiotics. Cells were grown as monolayers at 37°C in 5% CO_2. Confluent cultures were trypsinized (0.25% trypsin + 0.02% EDTA), re-suspended in complete medium, and seeded at a concentration of 1–2 × 10^5 cells/well/ml into 12-well tissue culture plates and incubated for 48 h at 37°C to obtain 70–80% confluency. Once confluent, cells were serum starved for 4 h before stimulation with IL-6 for 2 h, harvested, and RNA was extracted.

RNA Extraction and cDNA Synthesis

Total RNA was isolated from cells using Trizol according to the manufacturer's protocol (Ambion, Life technologies, CA, USA) and stored at −80°C. DNase treatment was carried out using RNase-free DNase kit (Sigma Aldrich, USA). RNA concentration was determined by Spectrophotometer (Bibby Scientific, Jenway, USA) by taking absorbance at 260 nm and quantity was adjusted to >40 μg/ml. Purity was checked by taking absorbance ratio of 260/280 and values of 1.9–2.0 was taken to indicate good quality RNA. cDNA synthesis was carried out using the Revert Aid™ First strand cDNA Synthesis Kit (Fermentas Life Sciences, USA) according to the manufacturer's instructions and stored at −80°C till further use.

Relative Gene Expression Study by Customized PCR Arrays

Forty-three genes, including transcription factors, cytokines, growth factors, negative regulators, receptors, extracellular membrane proteins, and proteases, were selected based on their involvement in bone metabolism and immune function after a careful literature search (**Table 2**). Functional aspects of the selected genes are illustrated in Table S1 in Supplementary Material. Arrays were customized by Molecular Diagnosis & Research Laboratories, India. Each 96-well plate composed of 43 genes (in duplicate) along with 4 housekeeping genes and negative RT-control. Relative expression of these genes was studied in PBMCs from healthy controls (HCs) ($n = 5$) and HIES patients ($n = 3$) together with LNCaP and PC-3 cells after stimulation with IL-6 for 2 h.

TABLE 2 | List of genes studied by PCR array.

	Bone homeostasis related genes	Immune system related genes
Growth factors		Epigen, Epireg
Cytokines	LIF, Onco M	LIF
Negative regulators	OPG, Noggin	Blmp1, SOCS3, PIAS3
Transcription factors	RUNX2, Sp7 (Osterix), TWIST1	RORyt
Proteases	MMP2, MMP8, MMP9, Cath K	Cyst C, Cath E
Protease inhibitors	TIMP2	b3 int
Receptors	V.D3, LIFR	V.D3, LIFR, gp130, T3 zeta chain
Adhesion molecules	VCAM	b3 int, SELL, VCAM
Kinases		MAPK1, ROCK1
Phosphatases		Calcen, SHP2
Others	BSP, DKK1, BGP, DKK2, OPN (osteopontin), SPARC, LRP5	SIRT1, Cam1, FLt3L, MTTF, PLCy2
Housekeeping genes	RPL37A, RPLP1, GAPDH, ACTB	

Briefly, 5 ng template cDNA was mixed with 2× Real Time SYBR Green PCR master mix (Roche Diagnostic, Germany). Equal volumes of this mixture was added to each well of the 96-well PCR array plate containing the pre-dispensed gene-specific primer sets in duplicate, and quantitative real time PCR (qRT-PCR) carried out using Roche LC 480 real time PCR machine (Roche Diagnostic, Germany). The amplification conditions used were initial denaturation at 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 15 s, annealing at 60°C for 10 s, and extension at 72°C for 20 s. The threshold cycle (C_t) values were derived for all genes on all PCR arrays. Finally, the fold-changes in gene expression were calculated using RT^2 PCR Array analysis software, applying the $2^{-}(\Delta C_t)$ formula:

$$\text{Fold Change} = \frac{2^{-}(\Delta C_t)\text{Stim}}{2^{-}(\Delta C_t)\text{Unstim}}$$

where ΔC_t was calculated using the formula: $\Delta C_t = C_t(\text{GOI}) - \text{Ave}\ C_t\ (\text{HKG})$ (GOI: Gene of interest, HKG: Housekeeping gene, Ave: Average).

Relative Gene Expression Analysis by qRT-PCR

Relative expression of *SOCS3, STAT3, and OPN* genes were determined by qRT-PCR using SYBR Green chemistry. Reactions were performed in triplicates with 50 ng of cDNA in 20 μl final reaction volume containing 1× SYBR Green dye (Roche Diagnostics, Switzerland) and 10 pmol of each primer (Sigma, USA). Each sample along with calibrator was normalized with β-actin gene and the ratio of gene expression was obtained by applying $2^{-}\Delta\Delta C_t$ formula: $\Delta\Delta C_t = \Delta C_t$ (un-stimulated) $- \Delta C_t$ (stimulated), where ΔC_t was calculated using the formula: $\Delta C_t = C_t^{\text{GOI}} - C_t^{\text{HKG}}$ (GOI: Gene of interest; HKG: Housekeeping gene). The primer sequences and PCR cycle conditions are mentioned in **Table 3**.

TABLE 3 | Primer sequences and PCR conditions for *STAT3, SOCS3, OPN, and β-actin* genes.

Genes	Primer sequence (5′–3′)	Product size (bp)	Annealing Temp (30 s)	Extension time (72°C)
STAT3	CATATGCGGCCAGCAAAGAA ATACCTGCTCTGAAGAAACT	152	58°C	30 s
OPN	CAGTGATTTGCTTTTGCCTCC ATTCTGCTTCTGAGATGGGTC	149	60°C	30 s
SOCS3	CACCTGGACTCCTATGAGAAAGTCA GGGGCATCGTACTGGTCCAGGAA	74	60°C	20 s
β-*Actin*	AGCACAGAGCCTCGCCTTTGC GGGGCATCGTACTGGTCCAGGAA	280	60°C	40 s

Evaluation of Phosphorylated STAT3 (p.Y705)

Phosphorylation of *STAT3* was analyzed using flow cytometry. Fresh whole blood (100 µl) or PBMCs (5×10^5) from HCs and HIES subjects and cell lines (1×10^6 cells) were incubated with IL-6 with or without *stattic*. Cells were simultaneously fixed and RBCs were lysed (in case of whole blood) using BD fix and lysing solution (BD Biosciences, USA). Cells were then permeabilized for 20 min using Perm III solution (BD Biosciences, USA) and subsequently incubated with Alexa Fluor 647 phospho-STAT3 antibody against phospho-Y705 (BD Biosciences, USA) for 30 min at room temperature. Washing was done twice with stain buffer (PBS + 2% FBS + 0.09% sodium azide) and cells were suspended in 1% paraformaldehyde. The cells were then acquired and analyzed in a BD FACS Aria III, flow cytometer (BD Biosciences, USA) using BD FACS Diva software (version 8).

Serum OPN Quantification by ELISA

Estimation of serum *OPN* was done using pre-coated commercially available ELISA kit (eBiosciences, San Diego, CA, USA) as per the manufacturer's protocol. Plate was read at 570 and 450 nm using an ELISA reader (Tecan, Germany). Standard curve range of the kit was 0.47–30 ng/ml with sensitivity of 0.26 ng/ml.

Statistical Analysis

Statistical analysis was performed using Graph Pad Prism software version 5.0 (GraphPad Software, La Jolla, CA, USA). Skewed and normally distributed data were deduced as median and mean, respectively. Comparisons between groups of related samples were assessed by the paired or unpaired *t*-test (for normally distributed data). Wilcoxon or Mann–Whitney test were performed for non-parametric data. ANOVA test was performed for more than two group comparisons. A p value of ≤ 0.05 was considered to be significant.

RESULTS

STAT3 Phosphorylation and IL-6/STAT3 Mediated SOCS3 Gene Expression in HCs, HIES Subjects, and Cell Lines (LNCaP and PC3)

Presence or absence of *STAT3* was checked in LNCaP and PC-3 cells at protein, mRNA, and genomic level by flow cytometry, qRT-PCR, and sequencing, respectively. No *STAT3* protein or mRNA expression was observed in PC-3 cells while LNCaP showed *STAT3* mRNA as well as protein expression (**Figures 1A,B**). PCR and sequencing of STAT3 gene (Exon 9 to Exon 23) in PC-3 cells showed either absence or faint and/or non-specific amplicons indicating a large deletion or absence of STAT3 gene (**Figure 1C**). PC3 cells have been shown to lack an entire chromosomal region spanning 500 kb, which includes the entire 30 kb STAT3 gene (26). Exons 1–8 were not further examined. *STAT3* phosphorylation was absent in PC-3 cells whereas LNCaP cells showed normal (36%) phosphorylation (**Figure 1D**). These experiments validated the use of the LNCaP and PC3 cell lines as +ve and −ve controls for STAT3. The range of *STAT3* phosphorylation in HCs ($n = 15$) was 35–67% (mean 44.62 ± 8.5, median 42.8). P1 with mutation in DNA binding domain showed normal phosphorylation (46%) while P4 and P5 with mutations in transactivation domain and SH2 domain, respectively, had reduced STAT3 phosphorylation: 6.5 and 25% (**Figure 1D**).

To evaluate the IL-6/STAT3 pathway activation, mRNA expression of *suppressor of cytokine signaling 3* (*SOCS3*), a known downstream target of STAT3 (28) was checked to confirm signaling through the IL-6/STAT3 pathway. For optimizing time and dose of IL-6, experiments were performed with HC PBMCs at different time intervals (0, 2, 12, and 24 h) and various IL-6 dosage (30, 50, and 100 ng/ml) (**Figures 1E,F**). IL-6 at a dose of 30 ng/ml for 2 h showed maximum up-regulation of *SOSC3* mRNA using qRT-PCR and the same was used for the subsequent experiments. *SOCS3* was found to be significantly upregulated in HCs ($n = 15$, mean fold change 3.1 ± 1.6, $p = 0.001$) while all three HIES subjects showed downregulated *SOSC3* mRNA expression. LNCaP cells showed mean fold change of 6 ± 3.3 ($p = 0.03$), while PC-3 cells showed no change in mRNA expression (mean fold change 0.9 ± 0.34, $p = 0.68$) (**Figure 1G**) which indicated an intact IL-6/STAT3 signaling in HC and LNCaP cells but a disrupted signaling in HIES subjects and PC-3 cells.

PCR Array Showed Differential Gene Expression Patterns in HCs, HIES Subjects, and Cell Lines

Of the 43 genes, PCR array in 12 genes showed C_t values beyond 35 and/or non-specific melting peaks and were hence excluded from analysis. Fold change difference in the remaining 31 gene sets in HCs ($n = 5$), HIES subjects, PC-3, and LNCaP is shown

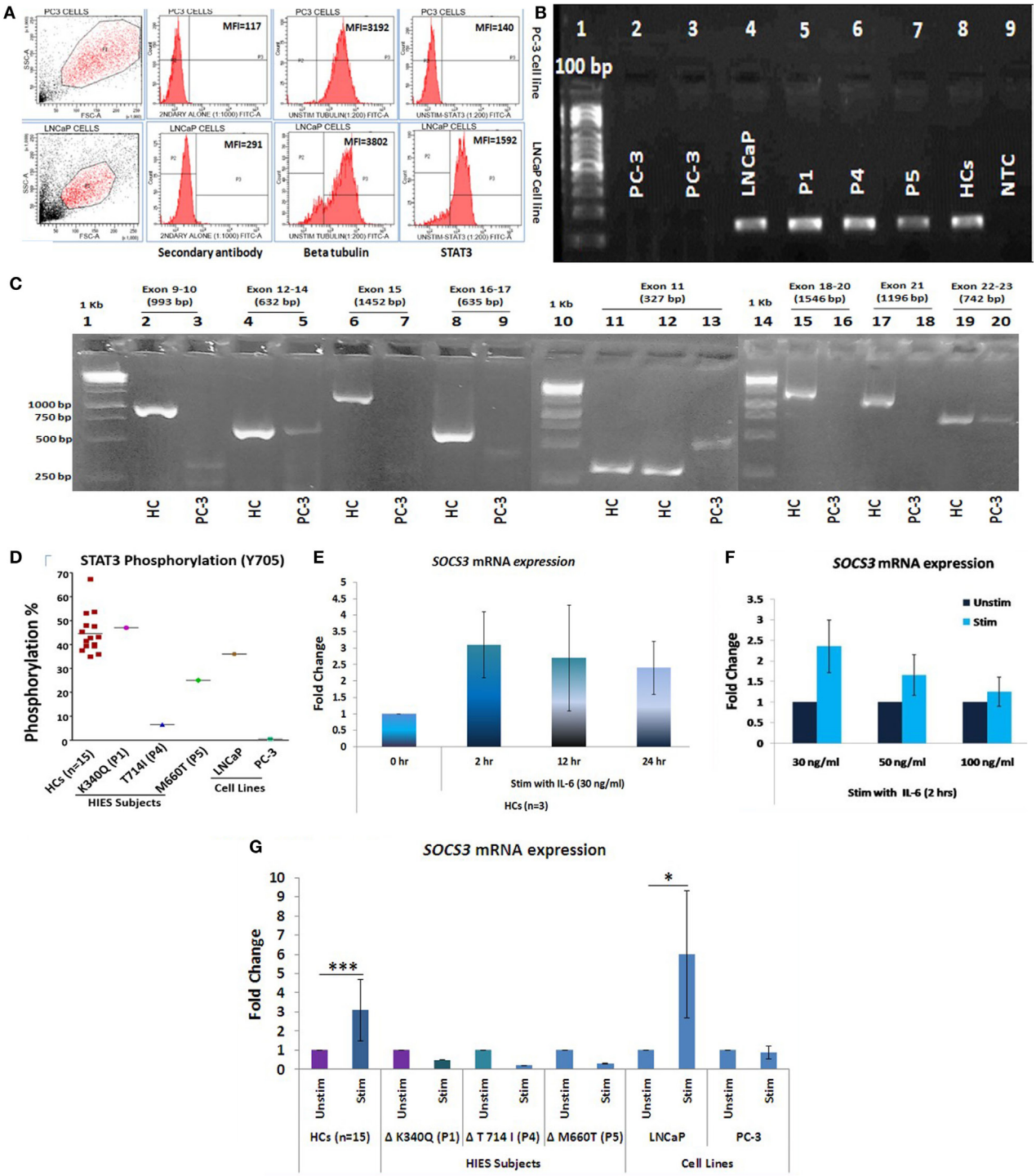

FIGURE 1 | **(A)** Representative flow cytometry histogram of *STAT3* protein expression in PC-3 and LNCaP cells. PC-3 cells had no expression of *STAT3* protein. **(B)** STAT3 mRNA expression in PC3, LNCaP, hyper-IgE syndrome (HIES) subjects and healthy controls (HCs) by conventional PCR. PC-3 cells showed no expression of STAT3 mRNA. **(C)** Agarose gel pictures showing either absence or non-specific/extremely faint bands of STAT3 amplification (exons 9–22) in PC-3 cells. Gel 1 (Exons 9–10/12–24/15 and 16–17), gel 2 (exon 11), and gel 3 (exons 18-20/21/22-23) have been grouped together for representation purpose. **(D)** Scatter plot showing per cent STAT3 phosphorylation in HCs, HIES subjects, LNCaP, and PC3 cells; mean values are shown as horizontal bars. Bar graphs showing quantitative real time PCR (qRT-PCR) of *SOCS3* mRNA expression in HC peripheral blood mononuclear cells stimulated with IL-6 in a **(E)** time-dependent manner at 30 ng/ml and **(F)** dose-dependent manner for 2 h. Data are shown as mean ± SD for three independent experiments. **(G)** Bar graphs showing qRT-PCR results of *SOCS3* expression from HCs, HIES subjects, LNCaP, and PC-3 cells stimulated with IL-6. The primary transcript for each gene were assayed at least in duplicate and normalized to *β-actin* expression. (Data are represented as mean ± SD, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, paired *t*-test.)

in the form of a heat map (**Figure 2A**). Differential fold change expression was observed in *OPN, RORyt, VitD3 receptor, SHP2, SOCS3*, and *gp130* genes between HCs and HIES subjects with respect to their respective unstimulated samples. *RORyt, VitD3 receptor, and SHP2* were found to be ≥2-fold upregulated in HCs, whereas *SOCS3* and *gp130* showed slight upregulation (mean fold change 1.5 and 1.7, respectively). However, PBMCs from HIES subjects showed either an unaltered expression or

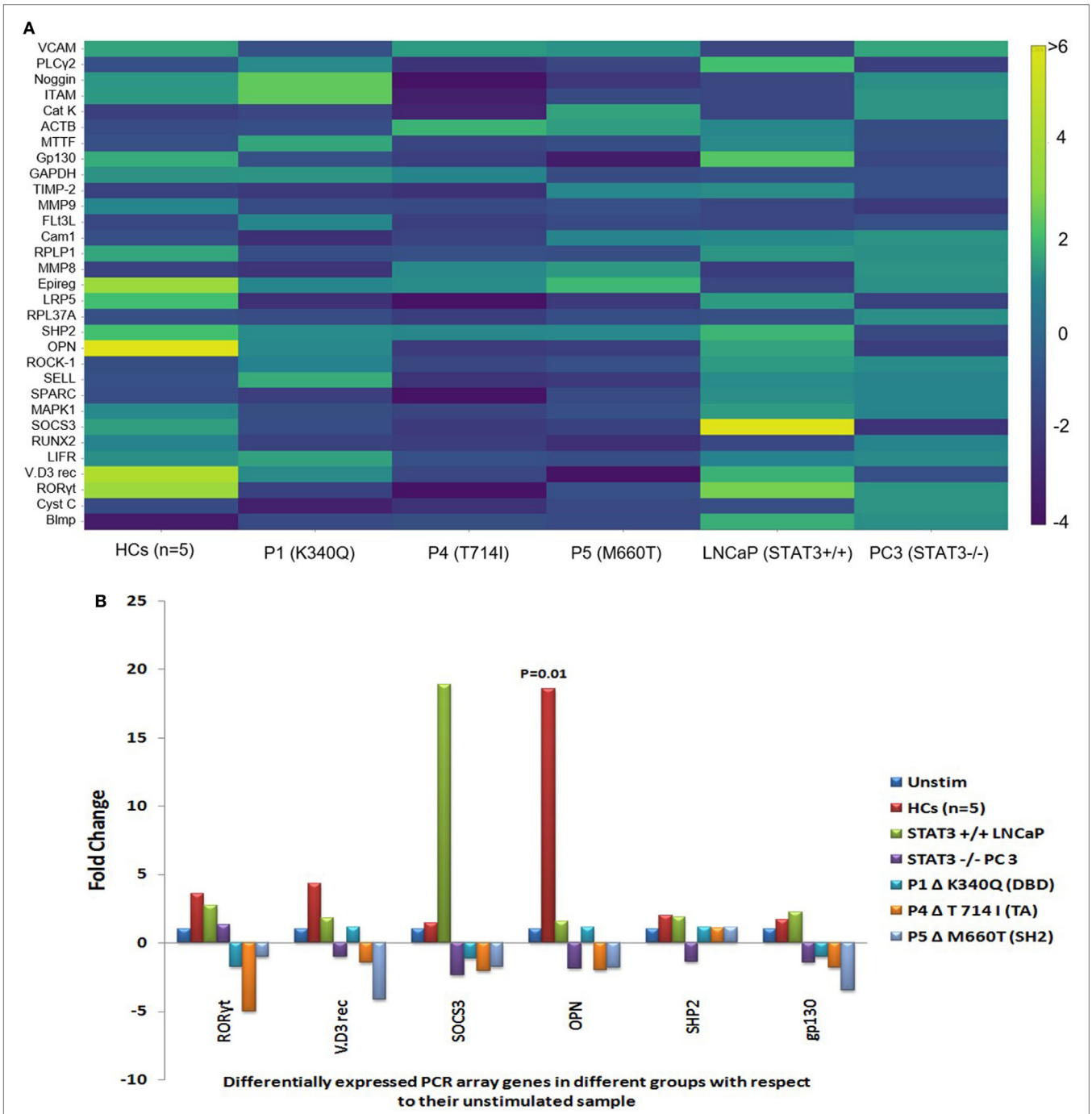

FIGURE 2 | **(A)** Heat map of gene expression profile of HCs, hyper-IgE syndrome (HIES) subjects, LNCaP, and PC-3 cells generated using Plotly software (https://plot.ly). Scale bar −4 to +6 and >6. Fold change values were calculated using RT2 PCR array analysis software (Qiagen), an online tool. **(B)** Bar graphs showing differentially expressed genes in the PCR Array post IL-6 stimulation of HCs, LNCaP cells, PC3 cells, and HIES subjects with respect to their unstimulated samples. Data were normalized to mean C_t value of *β-actin*, *GAPDH*, and *RPL37A* genes. Statistical significance was determined by Student's *t*-test (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$) using RT^2 PCR Array Analysis software, Qiagen.

downregulation of these genes. *OPN* was found to be the most significantly upregulated gene in HCs (mean fold change 18.6, $p = 0.01$) and was not seen in HIES subjects: P1—unchanged, P4—2-fold downregulated, and P5—1.8-fold downregulated (**Figure 2B**). *OPN* was slightly upregulated in LNCaP cells (1.6-fold) and downregulated in PC-3 cells (−1.9-fold). *SOCS3* gene expression was significantly upregulated in LNCaP cells but not in PC-3 cells. Expression of *RORyt, VitD3 receptor, SHP2*, and *gp130* was found to be upregulated (≥2-fold) in LNCaP cells while it was either downregulated or unaltered in PC-3 cells (**Figure 2B**). Taken together, the PCR array results showed a set of 6 genes to be differentially upregulated of which OPN was the most prominent.

Stattic-Induced Inhibition of STAT3 Suppresses OPN Gene Transcription

To verify the PCR array findings, *OPN* gene expression was further validated by qRT-PCR in HCs, HIES subjects, LNCaP, and PC-3 stimulated with IL-6 for 2 h and the effect of *stattic* on *OPN* expression was studied in HC PBMCs. MTT assay was performed to assess toxicity of *stattic* on PBMC viability (**Figures 3A,B**). Dose and time optimization of *stattic* were done to assess its optimal effect on STAT3 phosphorylation by flow cytometry (**Figures 3C,D**). Maximum reduction in *STAT3* phosphorylation was observed at 10 μM concentration and hence 10 μM *stattic* for 1 h was chosen for the subsequent experiments.

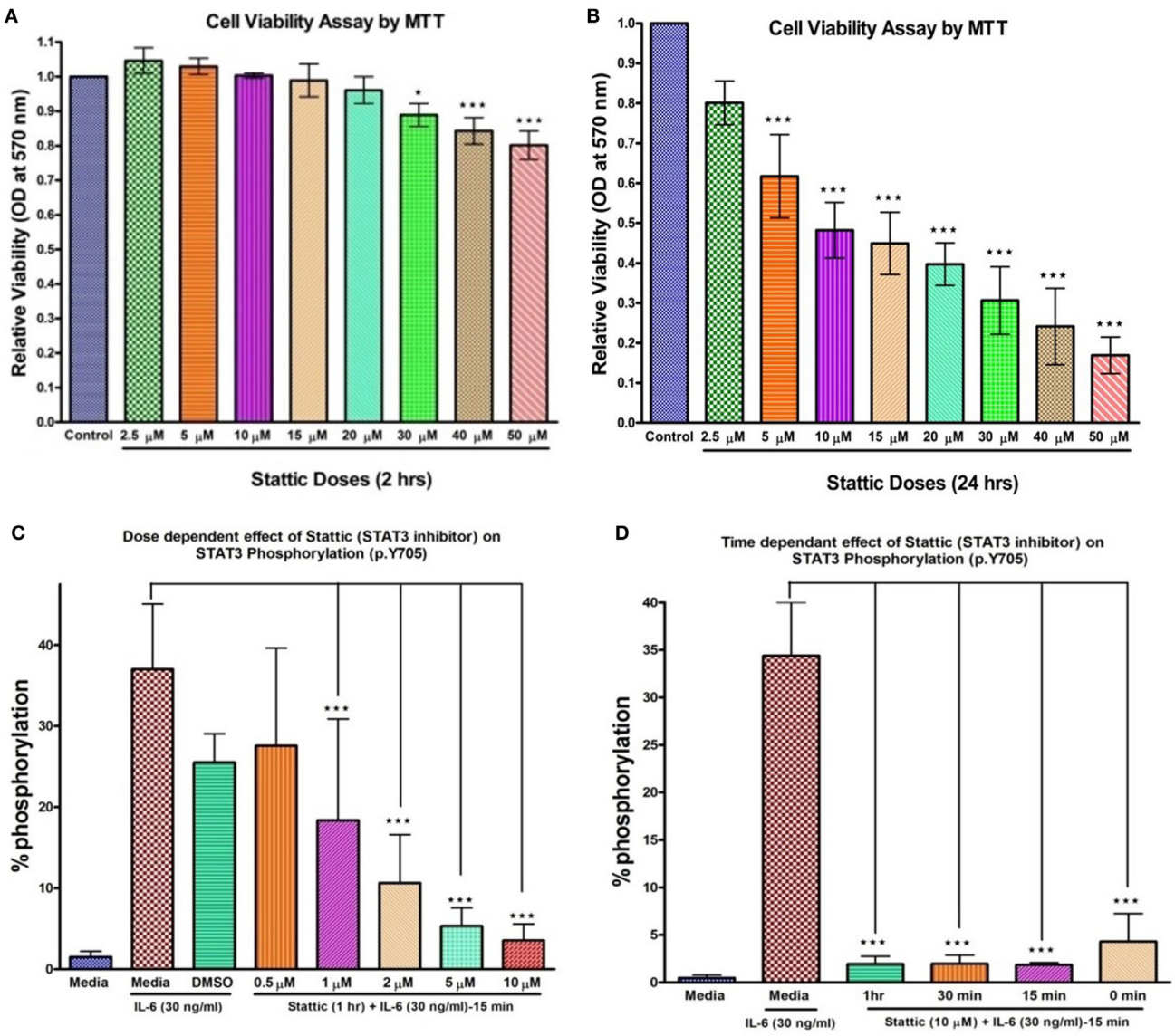

FIGURE 3 | Bar graphs showing effect of *stattic* at different doses on cell viability and STAT3 phosphorylation on healthy controls peripheral blood mononuclear cells. Cell viability: **(A)** at 2 h, no effect on cell viability was observed except at high doses where growth inhibition was evident **(B)** at 24 h, growth was inhibited in a dose-dependent manner. *Stattic* abrogated the STAT3 activity in a dose **(C)** and time **(D)** dependent manner. Data are represented as mean ± SD for three independent experiments. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, one way ANOVA post Tukey's multiple comparison test.

Relative mRNA expression of *SOCS3* and *STAT3* was found to be significantly upregulated in HCs after IL-6 treatment: mean fold change 3.0 ± 1.6 ($p = 0.002$) and 4.7 ± 2.3 ($p = 0.0004$), respectively, while HIES subjects showed either downregulated or unaltered mRNA expression. LNCaP cells showed 46- and 2-fold increase in *SOCS3* and *STAT3* expression while PC-3 cells showed 0.6-fold and no mRNA expression for these genes, respectively (**Figures 4A–F**). *Stattic*, however, failed to downregulate the *SOCS3* expression unlike *STAT3* and *OPN*. Further experiments to clarify this apparent discrepancy showed that this was because

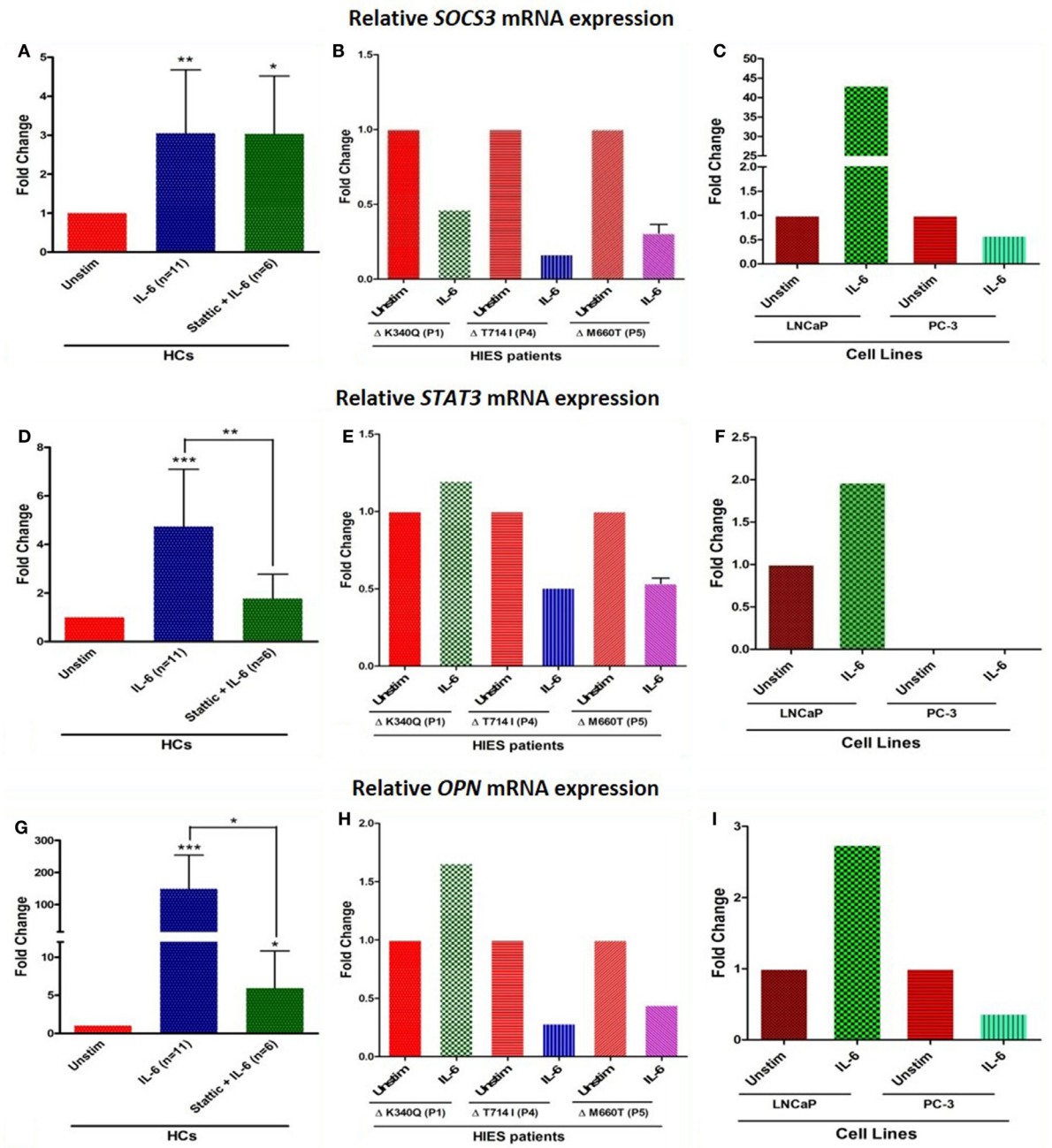

FIGURE 4 | Bar graphs showing relative mRNA expression of *SOCS3, STAT3*, and *OPN* in HC peripheral blood mononuclear cells **(A,D,G)** treated with either IL-6 alone or with *stattic*; in hyper-IgE syndrome (HIES) subjects **(B,E,H)** treated with IL-6 alone and in LNCaP and PC-3 cells **(C,F,I)** treated with IL-6 alone. The primary transcript for each gene were assayed at least in duplicate and normalized to *β-actin* expression. Data represented as mean ± SD, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, paired *t*-test and Wilcoxon test.

of a non-specific action of DMSO in which *stattic* was dissolved (Figure S1 in Supplementary Material). This effect was, however, not observed with *STAT3* or *OPN* mRNA expression. Careful scrutiny of studies looking at SOCS3 mRNA expression with DMSO as vehicle control, showed a significant upregulation of SOCS3 even with DMSO alone, and was more than empty vector/negative controls (29, 30). Diverse effects of DMSO on gene expression profiles have been documented in literature (31).

Osteopontin mRNA expression in HC showed up-regulation (mean fold change 149 ± 105, median 149, $p = 0.001$), whereas cells treated with *stattic* showed markedly diminished expression (mean fold change 6 ± 4.8, median 4, $p = 0.02$). HIES subjects showed 1.6-, 0.3-, and 0.4-fold change in *OPN* gene expression which was markedly reduced compared with HC. *OPN* expression in LNCaP and PC3 cells were 2.7- and 0.4-fold, respectively. LNCaP cells seemed to be somewhat deficient in OPN upregulation compared with HC PBMCs despite an intact STAT3 signaling (**Figures 4G–I**). Androgen-dependent LNCaP cells are known to express OPN to a much lesser extent compared with the androgen-independent counterparts like C4-2, and DU145 prostate cancer cell lines (32).

Serum OPN Levels Do Not Correlate With STAT3 Induced OPN mRNA Expression

Mean serum *OPN* in HCs were 25 ± 9 ng/ml, whereas HIES subjects showed variable levels—3 (P1), 46 (P4), and 118 (P5) ng/ml. This observation suggests that serum *OPN in vivo* is regulated by pathways other than STAT3 and a defect in the IL-6/STAT3 pathway does not cause any apparent deficiency in serum *OPN* levels.

In Silico Demonstration of STAT3 Response Element at Distal Enhancer Region of OPN Gene

Bioinformatic analysis to find a STAT3 transcription factor binding site (TFBS) in the OPN gene was done by YMO using the STAT finder Bioinformatic tool (33, 34), which predicted four response elements in the OPN gene with different binding scores (**Figure 5**). Consensus sequence TTCCAAGAA showed a maximum score of 0.99 and hence was considered to be the most likely TFBS for STAT3 in the human OPN gene. Similar response element with the consensus sequence TTCTGGGAA was observed in mice OPN gene with a score of 0.99.

DISCUSSION

Though both IL-6 and IL-11 signal through the same gp130 receptor β-subunit, and both activate STAT3, specificity is achieved through cell- and tissue-specific expression of the non-signaling α-receptor subunits. While IL6R is expressed in T-cells, monocytes, neutrophils, and pancreatic alpha cells, IL-11R is expressed in cardiac myocytes and endothelial and epithelial

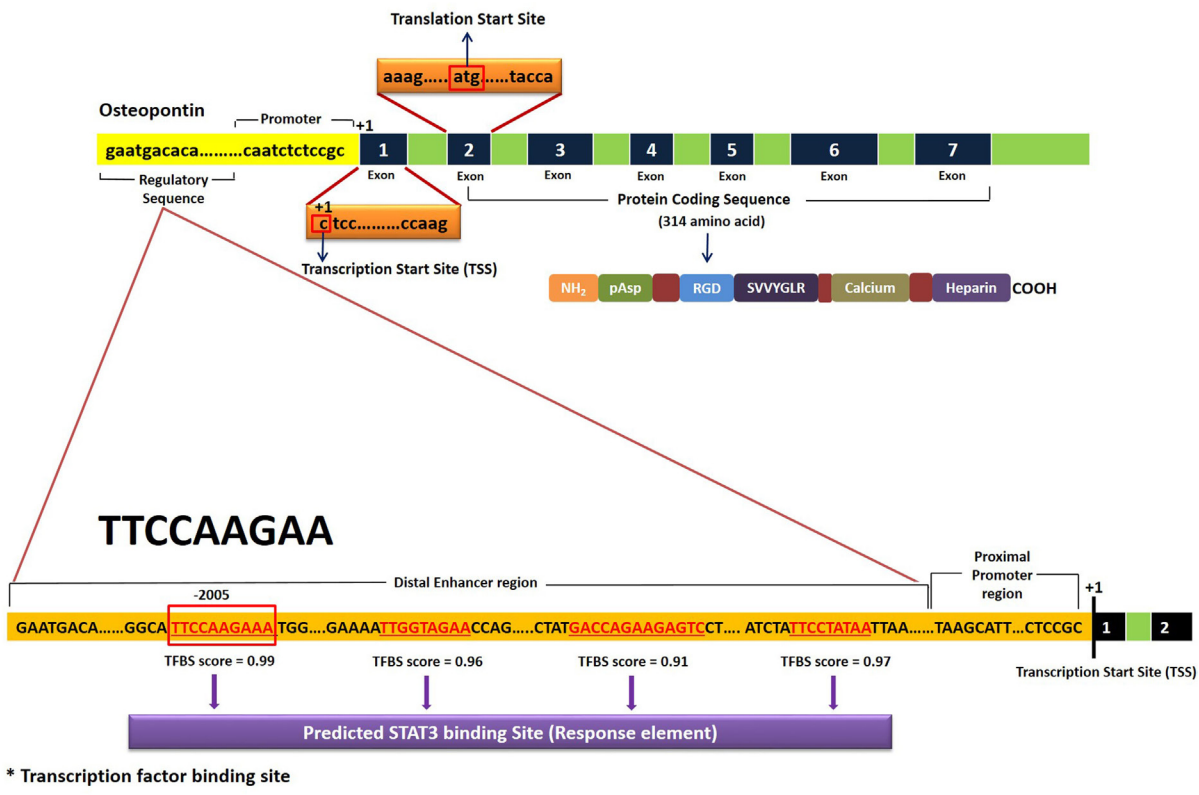

FIGURE 5 | Schematic representation of STAT3 transcription factor binding site (TFBS) in the human OPN gene. Results were obtained using STAT Finder Bioinformatics Tool (33, 34).

cells of the colon (35). In addition, IL-6/IL-6R is known to be the key signaling pathway in prostate cancer (36). Since we employed PBMCs and prostate cancer cells in the study, IL-6 was used as the stimulant. Our observations indicate that OPN gene expression in STAT3 LOF HIES in response to STAT3 activation is markedly diminished and the observation was supported by the PC3 and LNCap cell line data. Since cell lines are prone to genetic instability, they are not considered as good models for studying signaling pathways (37–39). Both LNCaP and PC3 cells used in the study were hence extensively characterized and validated with respect to STAT3 signaling before including them in the experiments. PCR array data pointed to a significant differential upregulation of *OPN* mRNA in HCs after IL-6 stimulation compared with HIES. Intriguingly enough, in the pre-STAT3 era, a marked under expression of *OPN* was observed in six of nine HIES subjects in the study by Chehimi et al. (40), 6 years before STAT3 was implicated in HIES. The finding, however, was ignored since the focus of the study was on traditional cytokine and chemokine dysregulation and TH1/TH2 imbalance in HIES. Sequencing of OPN gene from genomic DNA in that particular study did not reveal any mutations.

Stattic is known to potently and "selectively" inhibit STAT3 activation and nuclear translocation and selectively induce apoptosis of STAT3 dependent cancer cells (41–43). In our inhibition experiments using *stattic* in HC PBMCs, a downregulation of *OPN* gene expression was consistently observed indicating that *OPN* mRNA expression is induced upon IL-6 mediated STAT3 signaling. Though there is abundant literature on *OPN* regulation by different mechanisms (44–48), there was no emphasis in the literature on its regulation through STAT3 till a recent paper by Choi et al. which showed that OPN secreted by TM4SF4/GSK3β/β-catenin signaling activated the JAK2/STAT3 or FAK/STAT3 pathway which also upregulated OPN expression in an autocrine manner (49). Though *OPN* itself is known to induce STAT3 signaling (50, 51), its regulation through STAT3 pathway was a novel finding in our study, and is supported by Choi et al.'s study (49). This is further corroborated by a study that showed low mRNA expression of *OPN* in CD4+ve T cells conditionally knocked out for STAT3 gene (52). Our bioinformatic analysis predicted the presence of STAT3 response element at the distal enhancer region of *OPN* gene with consensus sequence TTCCAAGAA located at position -2005. This, however, needs to be further validated using reporter assay or chromatin immunoprecipitation assay.

OPN deficiency has been shown to suppress appearance of OdCs and resorption of tooth root induced by experimental force application (53). While wild-type mice showed appearance of OdCs around the mesial surface of the tooth root resulting in tooth root resorption, OPN knockout mice showed significantly suppressed force-induced increase in the number of OdCs and suppressed root resorption. Though this application of force also induced increase in the number of OCs in the alveolar bone on the pressure side, the number of OCs in such alveolar bone was similar between the *OPN*-deficient and wild-type mice which indicate that *OPN* deficiency specifically suppresses tooth root resorption by inhibiting the OdCs without affecting the adjacent bone OCs (53). The same study also showed reduced TNFα induced bone resorption using anti-OPN neutralizing antibody. Similar effects could be achieved using αvβ3 integrin receptor blockade using peptide inhibitor echistatin, an RGD containing peptide in a rat model (54, 55). The OdC numbers were, however, not affected implying that the blockade led to a functional defect in the OdCs. *OPN* is also known to be required for OC recruitment and RANKL expression during tooth drift-associated bone remodeling (56). Notably, OPN deficient mice show lower volume and length of OC ruffled borders, indicating lower resorptive capacity (57) despite normal numbers and also are hypomotile with reduced CD44 expression (58).

Though HIES subjects in our study showed low *OPN* mRNA expression, serum OPN levels were not reduced. This implied that the serum OPN was rescued in these patients apparently through other signaling pathways despite a defective STAT3-mediated OPN regulation. Although *OPN* does not seem to be required for skeletal development *per se*, and OPN knockout mice do not show any apparent skeletal malformation (59), its requirement in tooth resorption and cranial suture homeostasis might be a local site specific and time and age-dependent event which do not reflect on the general skeletal development. Moreover, not all patients of STAT3 LOF HIES show dental defects. The mediators of osteoclastogenesis (and osteoporosis) include many other factors like hormones (*viz.* estrogen, parathormone) and inflammatory cytokines (e.g., TNF-α, PGE2, VEGF, etc.). In contrast to alveolar bone of the skeleton which is rich in cells and blood vessels with abundance of these mediators, tooth cementum and dentin as well as cranial sutures are largely matrix dominant tissues and avascular. While the OPN deficiency in the trabecular bone of the skeleton can be easily overcome by the other mediators, the tooth cementum and cranial sutures may be largely dependent on the locally produced OPN by OdCs and OPN deficiency in this milieu may cause a state of deficiency. The osteoporosis observed in STAT3 LOF HIES on the other hand could be a result of the existence of a pro-inflammatory state rather than OPN deficiency.

CONCLUSION

Together with *in vitro* and *in silico* experiments in the study, we concluded that STAT3 has a direct role in the transcriptional regulation of *OPN* gene and thus, STAT3 through *OPN*, might be involved in the development of dental/craniofacial manifestations observed in STAT3 LOF HIES. More detailed multi-centric collaborative studies need to be performed in larger STAT3 LOF HIES cohorts with and without dental/facial manifestations using other appropriate biological systems like primary OCs/OdCs and macrophages to completely understand its exact role.

ETHICS STATEMENT

Institute Ethics Committee (IEC No. 8827-PG 10-1TRG/8450). All subjects were recruited after obtaining an informed consent prior to enrolment as per IEC guidelines.

AUTHOR CONTRIBUTIONS

SG participated in performance of research, data analysis, and writing of paper; SS and DS treated the patients; SmS did the

flow cytometry experiments; RM, ShS, and AR participated in writing of the manuscript; YO did the bioinformatics analysis; BS participated in research design, result interpretation, and writing of manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at https://www.frontiersin.org/articles/10.3389/fimmu.2018.01080/full#supplementary-material.

REFERENCES

1. Holland SM, DeLeo FR, Elloumi HZ, Hsu AP, Uzel G, Brodsky N, et al. STAT 3 mutation in the hyper IgE syndrome. *N Engl J Med* (2007) 357:1608–19. doi:10.1056/NEJMoa073687
2. Minegishi Y, Saito M, Tsuchiya S, Tsuge I, Takada H, Hara T, et al. Dominant-negative mutations in the DNA-binding domain of STAT 3 cause hyper-IgE syndrome. *Nature* (2007) 448:1058–62. doi:10.1038/nature06096
3. Chandesris MO, Melki I, Natividad A, Puel A, Fieschi C, Yun L, et al. Autosomal dominant STAT3 deficiency and hyper-IgE syndrome: molecular, cellular, and clinical features from a French national survey. *Medicine* (2012) 91:e1–19. doi:10.1097/MD.0b013e31825f95b9
4. Milner JD, Brenchley JM, Laurence A, Freeman AF, Hill BJ, Elias KM, et al. Impaired TH17 cell differentiation in subjects with autosomal dominant hyper-IgE syndrome. *Nature* (2008) 452:773–7. doi:10.1038/nature06764
5. Minegishi Y, Saito M, Nagasawa M, Takada H, Hara T, Tsuchiya S, et al. Molecular explanation for the contradiction between systemic TH17 defect & localized bacterial infection in hyper-IgE syndrome. *J Exp Med* (2009) 206:1291–301. doi:10.1084/jem.20082767
6. Grimbacher B, Holland SM, Gallin JI, Greenberg F, Hill SC, Malech HL, et al. Hyper-IgE syndrome with recurrent infections – an autosomal dominant multisystem disorder. *N Engl J Med* (1999) 340:692–702. doi:10.1056/NEJM199903043400904
7. Desai K, Houston DP, Harriman GR. Previously undiagnosed hyper-IgE syndrome in an adult with multiple systemic fungal infections. *J Allergy Clin Immunol* (1996) 98:1123–4. doi:10.1016/S0091-6749(96)80202-8
8. Hochreutener H, Wuthrich B, Huwyler T, Schopfer K, Seger R, Baerlocher K, et al. Variant of hyper IgE syndrome: the differentiation from atopic dermatitis is important because of treatment and prognosis. *Dermatologica* (1991) 182:7–11. doi:10.1159/000247728
9. Hill HR, Ochs HD, Quie PG, Clark RA, Pabst HF, Klebanoff SJ, et al. Defect in neutrophil granulocyte chemotaxis in Job's syndrome of recurrent 'cold' staphylococcal abscesses. *Lancet* (1974) 2:617–9. doi:10.1016/S0140-6736(74)91942-4
10. Thompson RA, Kumararante DS. Hyper IgE syndrome and H2-receptor blockade. *Lancet* (1989) 2:630. doi:10.1016/S0140-6736(89)90759-9
11. Grimbacher B, Holland SM, Puck JM. Hyper-IgE syndromes. *Immunol Rev* (2005) 203:244–50. doi:10.1111/j.0105-2896.2005.00228.x
12. Woellner C, Gertz EM, Schäffer AA, Lagos M, Perro M, Glocker EO, et al. Mutations in STAT3 and diagnostic guidelines for hyper-IgE syndrome. *J Allergy Clin Immunol* (2010) 125:424–32. doi:10.1016/j.jaci.2009.10.059
13. Sowerwine KJ, Holland SM, Freeman AF. Hyper-IgE syndrome update. *Ann N Y AcadSci* (2012) 1250:25–32. doi:10.1111/j.1749-6632.2011.06387.x
14. Höger PH, Boltshauser E, Hitzig WH. Craniosynostosis in hyper-IgE syndrome. *Eur J Pediatr* (1985) 144:414–7. doi:10.1007/BF00441793
15. Schwerd T, Twigg SRF, Aschenbrenner D, Manrique S, Miller KA, Taylor IB, et al. A biallelic mutation in IL6ST encoding the GP130 co-receptor causes immunodeficiency and craniosynostosis. *J Exp Med* (2017) 214(9):2547–62. doi:10.1084/jem.20161810
16. Nieminen P, Morgan NV, Fenwick AL, Parmanen S, Veistinen L, Mikkola ML, et al. Inactivation of IL11 signaling causes craniosynostosis, delayed tooth eruption, and supernumerary teeth. *Am J Hum Genet* (2011) 89(1):67–81. doi:10.1016/j.ajhg.2011.05.024
17. Sasaki T. Differentiation and functions of osteoclasts and odontoclasts in mineralized tissue resorption. *Microsc Res Tech* (2003) 61:483–95. doi:10.1002/jemt.10370
18. Stephen RE, Wilkie AO. A genetic-pathophysiological framework of craniosynostosis. *Am J Hum Genet* (2015) 97:359–77. doi:10.1016/j.ajhg.2015.07.006
19. Bimbstein E, Soskolne WA, Lustmann J. Histomorphologic changes in the gingival and pulp of overretained primary teeth. *ASDC J Dent Child* (2000) 67:403–7.
20. Takayanagi H. New developments in osteoimmunology. *Nat Rev Rheumatol* (2012) 8:684–9. doi:10.1038/nrrheum.2012.167
21. Eyerich K, Foerster S, Rombold S, Seidl HP, Behrendt H, Hofmann H, et al. Patients with chronic mucocutaneous candidiasis exhibit reduced production of Th17-associated cytokines IL-17 and IL-22. *J Invest Dermatol* (2008) 128:2640–5. doi:10.1038/jid.2008.139
22. Puel A, Cypowyj S, Bustamante J, Wright JF, Liu L, Lim HK, et al. Chronic mucocutaneous candidiasis in humans with inborn errors of interleukin-17 immunity. *Science* (2011) 332:65–8. doi:10.1126/science.1200439
23. Saikia B, Suri D, Goel S, Rawat A, Minz RW, Gupta A, et al. Hyper-IgE syndrome with a novel STAT3 mutation – a single center study from India. *Asian Pac J Allergy Immunol* (2014) 32:321–7. doi:10.12932/AP0456.32.4.2014
24. Saikia B, Goel S, Suri D, Minz RW, Rawat A, Singh S, et al. Novel mutation in SH2 domain of STAT3 (p.M660T) in hyper-IgE syndrome with sterno-clavicular and paravertebral abscesses. *Indian J Pediatr* (2017) 84:494–5. doi:10.1007/s12098-017-2336-y
25. Spiotto MT, Chung TD. STAT3 mediates IL-6 induced growth inhibition in the human prostate cancer cell line LNCap. *Prostate* (2000) 41(2):88–9. doi:10.1002/(SICI)1097-0045(20000201)42:2<88::AID-PROS2>3.0.CO;2-P
26. Clark J, Edwards S, Feber A, Flohr P, John M, Giddings I, et al. Genome-wide screening for complete genetic loss in prostate cancer by comparative hybridization onto cDNA microarrays. *Oncogene* (2003) 22(8):1247–52. doi:10.1038/sj.onc.1206247
27. Lou W, Ni Z, Dyer K, Tweardy DJ, Gao AC. Interleukin-6 induces prostate cancer cell growth accompanied by activation of STAT3 signaling pathway. *Prostate* (2000) 42(3):239–42. doi:10.1002/(SICI)1097-0045(20000215)42:3<239::AID-PROS10>3.0.CO;2-G
28. He J, Shi J, Xu X, Zhang W, Wang Y, Chen X, et al. STAT3 mutations correlated with hyper-IgE syndrome lead to blockage of IL-6/STAT3 signalling pathway. *J Biosci* (2012) 37:243–57. doi:10.1007/s12038-012-9202-x
29. Narayana Y, Balaji KN. NOTCH1 up-regulation and signaling involved in *Mycobacterium bovis* BCG-induced SOCS3 expression in macrophages. *J Biol Chem* (2008) 283(18):12501–11. doi:10.1074/jbc.M709960200
30. Huang N, Singh N, Yoon K, Loiacono CM, Kohut ML, Birt DF, et al. The immune-regulatory impact of orally-administered *Hypericum perforatum* on Balb/c mice inoculated with H1N1 influenza virus. *PLoS One* (2013) 8(9):76491. doi:10.1371/journal.pone.0076491
31. Pal R, Mamidi MK, Das AK, Bhonde R. Diverse effects of dimethyl sulfoxide (DMSO) on the differentiation potential of human embryonic stem cells. *Arch Toxicol* (2012) 86(4):651–61. doi:10.1007/s00204-011-0782-2
32. Thalmann GN, Sikes RA, Devoll RE, Kiefer JA, Markwalder R, Klima I, et al. Osteopontin: possible role in prostate cancer progression. *Clin Cancer Res* (1999) 5:2271–7.

33. Oh YM, Kim JK, Choi Y, Choi S, Yoo JY. Prediction and experimental validation of novel STAT3 target genes in human cancer cells. *PLoS One* (2009) 4:e6911. doi:10.1371/journal.pone.0006911
34. Oh YM, Kim JK, Choi S, Yoo JY. Identification of co-occurring transcription factor binding sites from DNA sequence using clustered position weight matrices. *Nucleic Acids Res* (2012) 40:e38. doi:10.1093/nar/gkr1252
35. Garbers C, Scheller J. Interleukin-6 and interleukin-11: same same but different. *Biol Chem* (2013) 349:1145–61. doi:10.1515/hsz-2013-0166
36. Azevedo A, Cunha V, Teixeira AL, Medeiros R. IL-6/IL-6R as a potential key signaling pathway in prostate cancer development. *World J Clin Oncol* (2011) 2:384–96. doi:10.5306/wjco.v2.i12.384
37. Ferreira D, Adega F, Chaves R. The importance of cancer cell lines as in vitro models in cancer methylome analysis and anticancer drugs testing. In: López-Camarillo C, Aréchaga-Ocampo E, editors. *Oncogenomics and cancer proteomics - novel approaches in biomarkers discovery and therapeutic targets in cancer*. In-Tech (2013). p. 140–66.
38. Pilli T, Prasad KV, Jayarama S, Pacini F, Prabhakar BS. Potential utility and limitations of thyroid cancer cell lines as models for studying thyroid cancer. *Thyroid* (2009) 19:1333–42. doi:10.1089/thy.2009.0195
39. Domcke S, Sinha R, Levine DA, Sander C, Schultz N. Evaluating cell lines as tumour models by comparison of genomic profiles. *Nat Commun* (2013) 4:2126. doi:10.1038/ncomms3126
40. Chehimi J, Elder M, Greene J, Noroski L, Stiehm ER, Winkelstein JA, et al. Cytokine and chemokine dysregulation in hyper-IgE syndrome. *Clin Immunol* (2001) 100:49–56. doi:10.1006/clim.2001.5039
41. McMurray J. A new small-molecule Stat3 inhibitor. *Chem Biol* (2006) 13:1123–4. doi:10.1016/j.chembiol.2006.11.001
42. Schust J, Sperl B, Hollis A, Mayer TU, Berg T. Stattic: a small-molecule inhibitor of STAT3 activation and dimerization. *Chem Biol* (2006) 13:1235–42. doi:10.1016/j.chembiol.2006.09.018
43. Pan Y, Zhou F, Zhang R, Claret FX. Stat3 inhibitor stattic exhibits potent antitumor activity and induces chemo- and radio-sensitivity in nasopharyngeal carcinoma. *PLoS One* (2013) 8:e54565. doi:10.1371/journal.pone.0054565
44. Gómez-Ambrosi J, Catalán V, Ramírez B, Rodríguez A, Colina I, Silva C, et al. Plasma osteopontin levels and expression in adipose tissue are increased in obesity. *J Clin Endocrinol Metab* (2007) 92:3719–27. doi:10.1210/jc.2007-0349
45. Sodek J, Batista Da Silva AP, Zohar R. Osteopontin and mucosal protection. *J Dent Res* (2006) 85:404–15. doi:10.1177/154405910608500503
46. Zhang G, Huang Z, Shi R, Lin Y, Hao B. Osteopontin regulation by protein kinase B (AKT) in HepG2 cells. *Exp Oncol* (2006) 28:36–9.
47. Salvi V, Scutera S, Rossi S, Zucca M, Alessandria M, Greco D, et al. Dual regulation of osteopontin production by TLR stimulation in dendritic cells. *J Leukoc Biol* (2013) 94:147–58. doi:10.1189/jlb.0412194
48. Chen M, Chen G, Nie H, Zhang X, Niu X, Zang YC, et al. Regulatory effects of IFN-β on production of osteopontin and IL-17 by CD4+ T cells in MS. *Eur J Immunol* (2009) 39:2525–36. doi:10.1002/eji.200838879
49. Choi SI, Kim SY, Lee JH, Kim JY, Cho EW, Kim IG. Osteopontin production by TM4SF4 signaling drives a positive feedback autocrine loop with the STAT3 pathway to maintain cancer stem cell-like properties in lung cancer cells. *Oncotarget* (2017) 8(60):101284–97. doi:10.18632/oncotarget.21021
50. Rangaswami H, Bulbule A, Kundu GC. Osteopontin: role in cell signaling and cancer progression. *Trends Cell Biol* (2006) 16:79–87. doi:10.1016/j.tcb.2005.12.005
51. Behera R, Kumar V, Lohite K, Karnik S, Kundu GC. Activation of JAK2/STAT3 signaling by osteopontin promotes tumor growth in human breast cancer cells. *Carcinogenesis* (2010) 31:192–200. doi:10.1093/carcin/bgp289
52. Oh HM, Yu CR, Lee Y, Chan CC, Maminishkis A, Egwuagu CE. Autoreactive memory CD4+ T lymphocytes that mediate chronic uveitis reside in the bone marrow through STAT3-dependent mechanisms. *J Immunol* (2011) 187:3338–46. doi:10.4049/jimmunol.1004019
53. Chung CJ, Soma K, Rittling SR, Denhardt DT, Hayata T, Nakashima K, et al. OPN deficiency suppresses appearance of odontoclastic cells and resorption of the tooth root induced by experimental force application. *J Cell Physiol* (2008) 14:614–20. doi:10.1002/jcp.21250
54. Talic NF, Evans C, Zaki AM. Inhibition of orthodontically induced root resorption with echistatin, an RGD-containing peptide. *Am J Orthod Dentofacial Orthop* (2006) 129:252–60. doi:10.1016/j.ajodo.2004.11.030
55. Dolce C, Vakani A, Archer L, Morris-Wiman JA, Holliday LS. Effects of echistatin and an RGD peptide on orthodontic tooth movement. *J Dent Res* (2003) 82:682–6. doi:10.1177/154405910308200905
56. Walker CG, Dangaria S, Ito Y, Luan X, Diekwisch TG. Osteopontin is required for unloading-induced osteoclast recruitment and modulation of RANKL expression during tooth drift-associated bone remodeling, but not for super-eruption. *Bone* (2010) 47:1020–9. doi:10.1016/j.bone.2010.08.025
57. Franzén A, Hultenby K, Reinholt FP, Onnerfjord P, Heinegård D. Altered osteoclast development and function in osteopontin deficient mice. *J Orthop Res* (2008) 26:721–8. doi:10.1002/jor.20544
58. Chellaiah MA, Kizer N, Biswas R, Alvarez U, Strauss-Schoenberger J, Rifas L, et al. Osteopontin deficiency produces osteoclast dysfunction due to reduced CD44 surface expression. *Mol Biol Cell* (2003) 14:173–89. doi:10.1091/mbc.E02-06-0354
59. Rittling SR, Matsumoto HN, McKee MD, Nanci A, An XR, Novick KE, et al. Mice lacking osteopontin show normal development and bone structure but display altered osteoclast formation in vitro. *J Bone Miner Res* (1998) 13:1101–11. doi:10.1359/jbmr.1998.13.7.1101

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REVIEW
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Bacille Calmette–Guerin Complications in Newly Described Primary Immunodeficiency Diseases: 2010–2017

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Bacille Calmette–Guerin (BCG) vaccine is widely used as a prevention strategy against tuberculosis. BCG is a live vaccine, usually given early in life in most countries. While safe to most recipients, it poses a risk to immunocompromised patients. Several primary immunodeficiency diseases (PIDD) have been classically associated with complications related to BCG vaccine. However, a number of new inborn errors of immunity have been described lately in which little is known about adverse reactions following BCG vaccination. The aim of this review is to summarize the existing data on BCG-related complications in patients diagnosed with PIDD described since 2010. When BCG vaccination status or complications were not specifically addressed in those manuscripts, we directly contacted the corresponding authors for further clarification. We also analyzed data on other mycobacterial infections in these patients. Based on our analysis, around 8% of patients with gain-of-function mutations in *STAT1* had mycobacterial infections, including localized complications in 3 and disseminated disease in 4 out of 19 BCG-vaccinated patients. Localized BCG reactions were also frequent in activated PI3Kδ syndrome type 1 (3/10) and type 2 (2/18) vaccinated children. Also, of note, no BCG-related complications have been described in either CTLA4 or LRBA protein-deficient patients; and not enough information on BCG-vaccinated NFKB1 or NFKB2-deficient patients was available to drive any conclusions about these diseases. Despite the high prevalence of environmental mycobacterial infections in GATA2-deficient patients, only one case of BCG reaction has been reported in a patient who developed disseminated disease. In conclusion, BCG complications could be expected in some particular, recently described PIDD and it remains a preventable risk factor for pediatric PIDD patients.

Keywords: mycobacteria, bacille Calmette–Guerin, primary immunodeficiency, live vaccines, complications, adverse reactions

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INTRODUCTION

Based on the World Health Organization (WHO) Global Tuberculosis Report, tuberculosis remains a public health global problem: it is the ninth leading cause of death worldwide, and the leading cause of death from a single pathogen (1). Bacille Calmette–Guerin (BCG) vaccine is widely used as a prevention strategy against tuberculosis. The BCG vaccine was developed between 1908 and 1921

by Albert Calmette and Camille Guerín in France by culturing and attenuating a live strain of *Mycobacterium bovis*. BCG was first administered to humans in 1921 and has been used for more than 95 years until now (2).

Vaccination policies vary around the world, linked mostly to tuberculosis disease prevalence. While tuberculosis endemic areas (mainly in developing countries) adopt universal vaccination, tuberculosis low-prevalence countries either restrict BCG vaccine to high-risk groups or choose not to administer it at all (3). Controversies surrounding the vaccine's efficacy account for variations in vaccination policies. While BCG vaccine is believed to provide a somehow consistent protection against severe forms of tuberculosis (i.e., miliary, meningeal) in childhood, most adult individuals remain susceptible to pulmonary tuberculosis despite vaccination (4). As previous exposure to nontuberculous mycobacteria (NTM) seems to influence vaccine efficacy, and to assure full coverage, BCG is usually given right after birth in the first months of life (5).

Even in the context of its questionable efficacy, BCG vaccine is considered safe in immunocompetent subjects (6). However, being a live vaccine, it can result in serious illness or even fatal disease in immunocompromised hosts (7). For instance, WHO guidelines recommend holding BCG vaccination in high-risk infants until assessment of HIV status (8). Patients with primary immunodeficiency diseases (PIDD) are at equal or even greater risk of complications and represent a challenging group regarding live vaccines in general and BCG vaccination in particular (7, 9).

Primary immunodeficiency diseases are inborn errors of immunity which commonly lead to increased susceptibility to infection (10). Defects impairing cellular immunity, phagocytic function, and interferon-γ-mediated immunity have been classically associated with BCG vaccine complications (11). The advent of next-generation sequencing technology boosted discoveries in the field of PIDD. To date, 354 different disorders affecting 344 genes have been described, nearly one-third of them after 2010 (12). Meanwhile, the potential impact of recently described PIDD on BCG immune response remains blurry, as our understanding of natural history and detailed molecular mechanisms of these defects are still limited and continuously expanding.

The aim of this review is to update and summarize the published data on BCG-related complications in patients diagnosed with PIDD described after 2010. We arbitrarily selected defects affecting either innate (i.e., monocytes, macrophages, or dendritic cells) or adaptive (i.e., T cells) arms of antimycobacterial immunity that had been described in at least 20 patients or 10 unrelated kindreds. Diseases unequivocally classified as having Mendelian susceptibility to mycobacterial diseases (MSMD), were not reanalyzed in this review. Finally, 18 genetic defects or allelic variants associated with particular PIDD were analyzed. Considering the timing of vaccine administration, most patients' PIDD were undiagnosed when vaccinated. As BCG vaccination history is not described in all publications and many reports come from countries where BCG vaccine is not universally applied, we also collected data on any mycobacterial infection, highlighting the occurrence of weakly virulent strains. For those reports where BCG vaccination status was not specifically described, we directly contacted the corresponding author/s for further clarification in terms of patients' BCG vaccination status and complications associated with it. The overall retrieve rate for extra information requests was 17%, range 0.8–100%, depending on the specific PIDD analyzed (**Table 1**).

GAIN-OF-FUNCTION (GOF) MUTATIONS IN SIGNAL TRANSDUCER AND ACTIVATOR OF TRANSCRIPTION 1 (*STAT1*)

STAT1 is a transcription factor involved in several cytokine-dependent signaling pathways, notably IFN-α/β, IFN-γ, and IL-27 (13, 14). Both biallelic and heterozygous loss-of-function (LOF) mutations of *STAT1* have been previously described and associated with either susceptibility to intracellular bacterial/viral infections, or Mendelian susceptibility to mycobacterial disease,

TABLE 1 | Retrieval rate of BCG vaccination status among patients with recently described PIDD.

PIDD	Published cases	BCG vaccination status retrieval
GOF mutations in *STAT1*		
STAT1	348	43 (12%)
GOF mutations in *STAT3*		
STAT3	31	7 (23%)
Activated PI3Kδ syndrome type 1 and 2		
PIK3CD	158	40 (25%)
PIK3R1	64	49 (77%)
Defects affecting the NF-κB pathway		
NFKB1	56	15 (27%)
NFKB2 (DN and GOF)	29	11 (38%)
CBM complex[a]		
CARD11 AR LOF	5	2 (40%)
CARD11 GOF	9	9 (100%)
CARD11 DN/LOF	12	4 (33%)
BCL10	1	–
MALT1	6	4 (67%)
CTLA-4 and LRBA deficiencies		
CTLA4	74	14 (19%)
LRBA	108	21 (19%)
GATA2 deficiency		
GATA2	357	3 (0.8%)
CARMIL2 deficiency		
CARMIL2	21	4 (19%)
PGM3 deficiency		
PGM3	37	4 (11%)
TTC7A deficiency		
TTC7A	53	–
	1,369	230 (17%)

[a]*For the purpose of the inclusion criteria, all members of the CBM complex were considered as a group.*

AR, autosomal recessive; BCG, Bacille Calmette–Guerin; DN, dominant negative; GOF, gain-of-function; LOF, loss-of-function; PIDD, primary immunodeficiency diseases; TB, tuberculosis; STAT, signal transducer and activator of transcription; CARD, caspase activation and recruitment domain; BCL10, B-cell leukemia/lymphoma 10; MALT1, mucosa-associated lymphoid tissue lymphoma translocation gene 1; CTLA-4, cytotoxic T lymphocyte antigen-4; LRBA, lipopolysaccharide-responsive Beige-like anchor protein; PGM3, phosphoglucomutase 3; PIK3CD, phosphoinositide kinase 3 catalytic unit delta; PIK3R1, phosphoinositide kinase 3 regulatory subunit 1; NFKB, nuclear factor kappa-light-enhancer of activated B cells; CBM, CARD11, BCL10, MALT1 complex; CARMIL2, capping protein regulator and myosin 1 linker 2; TTC7A, tetratricopeptide repeat domain 7A.

respectively (15–17). In 2011, Liu et al. and van de Veerdonk et al. demonstrated that an enhanced STAT1 activity, as a result of heterozygous GOF mutations of STAT1, was also deleterious, presenting with a chronic mucocutaneous candidiasis (CMC) phenotype due to impaired IL-17 immunity (18, 19). Afterward, several studies confirmed that these mutations caused a delay in nuclear dephosphorylation of activated STAT1 resulting in reduced production of Th17 cells (20–22), which are pivotal for candida-specific immune responses (23).

While CMC is still the hallmark of this disease—it was present in 98% of patients from an international cohort of 274 STAT1 GOF patients (24)—as more patients were described, the phenotype was proven to be much more diverse than initially assumed (22, 24). These patients are at higher risk of other infections (bacterial/viral/fungal) (25–27), autoimmunity (28, 29), aneurisms (30, 31), and malignancies (32, 33). Many of these manifestations cannot be solely explained by a reduction of Th17 cells, implying that excessive activation of STAT1 potentially impairs immunity through other mechanisms not yet fully understood (29, 34, 35).

Twenty-seven reports of mycobacterial infections could be retrieved from more than 350 published STAT1 GOF patients (20, 24, 25, 27, 29, 31, 33, 34, 36–40). Vaccination status was not addressed in most descriptions (3, 18–22, 24–34, 36–39, 41–72). We were able to retrieve BCG vaccination history from 43 patients, of whom 19 received the vaccine (**Tables 1** and **2**). BCG complications were seen in seven patients. Three patients had local reactions [(29, 37, 38), and Acknowledgments] and four experienced disseminated disease (24, 25, 31). In addition to BCG, other mycobacteria also seem to threaten a subset of these patients (**Table 2**). Disseminated disease was observed in eight more patients (six cases of *Mycobacterium tuberculosis* and two of NTM) (24, 31, 34, 38, 39), four patients had pulmonary infections caused by environmental mycobacteria (24, 29, 33), and two patients had lymphadenitis caused by *Mycobacterium fortuitum* (27, 37). *M. tuberculosis* caused pulmonary disease in five patients (24, 36, 37) and extrapulmonary localized disease in one patient (20).

Overall, our own literature review shows an estimated 8% prevalence of mycobacterial infections among patients carrying *STAT1* GOF mutations. Mycobacterial susceptibility is a classical presentation for LOF *STAT1* mutations (15–17) as decreased STAT1-mediated IFN-γ responses impair immunity against mycobacteria (73). Published data demonstrate that excessive activation of STAT1 also impairs IFN-γ responses and, therefore, increases the risk of infection (27, 38). IFN-γ tachyphylaxis has been proposed as one possible explanation to this effect (27). Tight regulation of STAT1 phosphorylation, and in turn its activity, is likely required to mount a protective response against mycobacteria (73).

GOF MUTATIONS IN SIGNAL TRANSDUCER AND ACTIVATOR OF TRANSCRIPTION 3 (*STAT3*)

Signal transducer and activator of transcription 3 is another member of the STATs protein family of transcription factors. STAT3 is activated by various cytokines and growth factors and plays critical roles in several cell processes, such as cell growth, differentiation, apoptosis, as well as inflammation and oncogenesis (74). LOF-dominant negative (DN) mutations in *STAT3*, cause autosomal-dominant hyper-IgE syndrome, characterized by CMC, bacterial infections, eczema, and connective tissue abnormalities (75, 76). GOF somatic mutations in STAT3 have been associated with some particular types of lymphoproliferative diseases, being found in 40% of large granular lymphocytic leukemia patients (77).

Heterozygous activating germline mutations in *STAT3* were first described in 2014, in a cohort of five patients presenting early-onset autoimmunity (78). One-year later, two groups simultaneously described 14 additional cases, providing informative data on this PIDD phenotype (79, 80). Early-onset autoimmunity was prominent, alongside short stature and lymphoproliferative disease. Recurrent infections were frequent and two patients had malignancies, one adult patient had Hodgkin lymphoma, and one pediatric patient had a T-cell large granular lymphocytic leukemia (79, 80). Immunologically, most patients showed hypogammaglobulinemia, and also decreased numbers of T regulatory cells (79–81).

A NTM infection was reported in one patient with a germline *STAT3* GOF mutation. The patient had received BCG vaccine as a child without any reported complication. However, at the age of 19 years she developed *Mycobacterium avium* pneumonia, followed by dissemination (*M. avium* was isolated from lymph node, feces, and bone marrow samples). The IL-12/IFN-γ pathway was evaluated and found to be normal. The authors hypothesized that the mycobacterial infection could be due to the lack of plasmacytoid dendritic cells detected in the patient (80). A total of four patients received BCG vaccination without complications [(78–80, 82), and Acknowledgments] (**Table 2**).

The underlying molecular mechanisms of increased transcriptional activity of STAT3 are not yet fully elucidated (83, 84). Although BCG complications have not been reported in these patients [(78–82, 84–88), and Acknowledgments], the occurrence of disseminated environmental mycobacterial infection in one patient raises awareness (80).

ACTIVATED PI3Kδ SYNDROME TYPE 1 AND 2

Activated phosphoinositide 3-kinase δ syndrome (APDS) in an immunodeficiency and immune dysregulation syndrome, first described in 2013 (89, 90). It results from pathological hyper activation of PI3Kδ, a lipid kinase responsible for the conversion of phosphatidylinositol 4,5-bisphosphate (PIP_2) to the second messenger phosphatidylinositol 3,4,5-trisphosphate (PIP_3). PI3Kδ is a class IA PI3K, composed of a catalytic (p110δ) and a regulatory subunit (p85). It is predominantly expressed in leukocytes and can be induced by several transmembrane receptors, such as antigen receptors, cytokine receptors, toll-like receptors, and costimulatory molecules. It is important for cell growth, proliferation, motility, and survival (91–93).

Germline heterozygous GOF mutations in *PIK3CD*, that encode the catalytic subunit p110δ, were initially described as the genetic cause of APDS (89, 90). Less than a year later,

TABLE 2 | BCG and other mycobacterial complications in recently described PIDD.

PIDD	BCG vaccinated[a]	BCG reaction		NTM	Pulmonary TB	Extrapulmonary TB		Total[b]	References
		Localized	Disseminated			Localized	Disseminated		
STAT1 GOF	19/43	3	4	8 (2 pulmonary *M. avium;* 1 disseminated *M. avium;* 2 *M. fortuitum* lymphadenitis; 1 disseminated *M. genavense;* and 2 lung NTM infection)	5	1	6	348	(3, 18–22, 24–34, 36–39, 41–72)
STAT3 GOF	4/7	–	–	1 (disseminated *M. avium*)	–	–	–	31	(78–82, 84–88)
PIK3CD	10/40	3	–	–	–	–	–	158	(40, 89, 90, 96, 97, 101, 103–120)
PIK3R1	18/49	2	–	–	–	–	–	64	(94, 95, 98–100, 102, 113, 117, 121–124)
NFKB1	1/15	–	–	2 (*M. avium* complex)	–	–	–	56	(40, 82, 131–137)
NFKB2 DN and GOF	0/11	–	–	1 (*M. kansasii*)	–	–	–	29	(40, 138–148)
CBM complex									
CARD11 AR LOF	0/2	–	–	–	–	–	–	5	(150, 153, 154, 157)
CARD11 GOF	3/9	–	–	–	–	–	–	9	(152, 155, 156, 158)
CARD11 DN/LOF	0/4	–	–	–	1	–	–	12	(159, 160)
MALT1	0/4	–	–	–	–	–	–	6	(162–165)
CTLA4	4/14	–	–	–	2	–	–	74	(82, 111, 166, 167, 169–181)
LRBA	16/21	–	–	–	–	–	–	108	(82, 174, 179, 180, 182–184, 186, 187, 190–210)
GATA2	3/357	–	1	54 (24 *M. avium complex; 12 M. kansasii; 3 M. abscessus; 1 M. fortuitum; 1 M. scrofulaceum; 1 M. massiliense; 1 M. chelonae; 1 M. szulgai; 1 M. malmoense; 9 NTM*)	–	–	1	357	(40, 211–214, 216, 217, 220–284)
CARMIL2	4/21	–	–	–	–	–	2	21	(286–289)
PGM3	4/37	–	–	–	–	–	–	37	(291, 292, 295–301)

[a]*Numerator/denominator based on paper's review and personal communications with corresponding authors.*

[b]*Total number of cases published.*

AR, autosomal recessive; BCG, Bacille Calmette–Guerin; DN, dominant negative; GOF, gain-of-function; LOF, loss-of-function; NTM, nontuberculous mycobacteria; PIDD, primary immunodeficiency diseases; TB, tuberculosis; STAT1, signal transducer and activator of transcription 1; STAT3, signal transducer and activator of transcription 3; CARD, caspase activation and recruitment domain; MALT1, mucosa-associated lymphoid tissue lymphoma translocation gene 1; LRBA, lipopolysaccharide-responsive Beige-like anchor protein; PGM3, phosphoglucomutase 3; BCL10, B-cell leukemia/lymphoma 10; PIK3CD, phosphoinositide kinase 3 catalytic unit delta; PIK3R1, phosphoinositide kinase 3 regulatory subunit 1; NFKB, nuclear factor kappa-light-enhancer of activated B cells; CBM, CARD11, BCL10, MALT1 complex; CARMIL2, capping protein regulator and myosin 1 linker 2; TTC7A, tetratricopeptide repeat domain 7A. BCL10 and TTC7A-deficient patients are not shown in this table as information on their BCG vaccination status could not be retrieved.

heterozygous GOF mutations in *PIK3R1*, which encodes the regulatory subunit p85, were identified as a phenocopy of this PIDD, being designated APDS type 2 (94, 95). The vast majority of patients present with recurrent sinopulmonary infections commonly leading to bronchiectasis. Other clinical manifestations are highly variable and comprise herpesvirus infections, autoimmune disease (mainly cytopenias), benign lymphoproliferation, and an increased risk of lymphoma (96–99). Immunologically, CD4+ T cell and B cell lymphopenia, progressive loss of naïve CD4+ and CD8+ T cells, expansion of senescent CD8+ T cells, reduced class-switched memory B cells, and poor antibody responses are common findings. A subset of patients presents with hyper IgM (96–101).

In a cohort study of 36 APDS2 patients, Elkaim et al. reported two cases of persistent local skin lesions at BCG vaccination injection site, out of 17 patients who had been BCG vaccinated (99). We gathered information regarding BCG vaccination history from 32 more APDS2 patients and only 1 received the vaccine [(94, 95, 100, 102), and Acknowledgments]. In total, 2/18 BCG-vaccinated APDS2 patients developed local reactions to the vaccine. Likewise, Coulter et al. reported two additional cases of persistent granulomatous local skin reactions to BCG vaccine in a large cohort study of 53 APDS1 patients (97). Through our literature search we identified another local reaction to BCG in an APDS1 patient (103). Collectively, among 10 APDS1 BCG vaccinated patients, three of them developed local reactions to BCG (**Table 2**) [(40, 90, 97, 101, 103–109), and Acknowledgments].

The ability to control BCG infection was assessed in one APDS1 patient by Chiriaco et al. Monocyte-derived macrophages from the patient failed to restrict intracellular mycobacterial growth *in vitro*, compared to a healthy control. Treatment with a PI3Kδ inhibitor restored patient's cells ability to kill BCG, suggesting that normal PI3Kδ activity is important to control BCG infection. Despite the failure *in vitro*, this patient received BCG vaccine without complications (104).

There were no reports of disseminated BCG reactions or other mycobacterial infections in the APDS patients reviewed (40, 89, 90, 94–124).

DEFECTS AFFECTING THE NF-κB PATHWAY

Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) represents a protein complex that plays pivotal roles in immune and inflammatory responses, cell development, and survival. It is composed of five transcription factors, NFκB1 (p50/p105), NFκB2 (p52/p100), RelA, RelB, and c-Rel that bind to form homodimers or heterodimers. In resting state these dimers are sequestrated in the cytoplasm, tightly controlled by inhibitory proteins. Once activated, they are released after phosphorylation, ubiquitination, and proteasome degradation of their inhibitors and translocated to the nucleus where they control the transcription of a large set of genes (125, 126).

Mutations that affect the NF-kappa-B inhibitor alpha (NFκBIA or IKBA) or the kinases responsible for its inactivation (IKK-β or IKK-γ/NF-κB essential modifier) are known causes of combined immunodeficiency with increased susceptibility to mycobacteria (11, 127, 128). Several patients carrying these mutations present with disseminated disease after receiving BCG vaccine (129, 130).

More recently, germline mutations in two genes encoding transcription factors of the NF-κB family have been described as new causes of PIDD.

NFKB1 encodes the transcription factor p50 and its precursor p105, which are activated *via* canonical NF-κB signaling pathway (125, 126). Germline heterozygous mutations in *NFKB1* were first described in 2015 (131). They led to haploinsufficiency of NFκB1 and penetrance was shown to be incomplete. The first symptomatic patients reported presented a common variable immunodeficiency (CVID)-like phenotype, suggesting a primarily B-cell disorder. Hypogammaglobulinemia, recurrent infections, autoimmunity, and lymphoproliferation were common findings (82, 131, 132). Finally, new reports expanded the phenotype of this PIDD, highlighting the occurrence of recurrent EBV infection and EBV-driven lymphoproliferative disease, pointing that this defect likely underlies a combined immunodeficiency (133). More than 50 patients carrying *NFKB1* mutations have been published (40, 82, 131–137). No BCG-related complications have been reported but BCG vaccination history from these patients was not described in the literature (40, 82, 131–137). We had access to vaccination status of 15 patients, of whom only one received BCG vaccine, uneventfully (**Table 2**) [(40, 82, 135, 137), and Acknowledgments]. Interestingly, one group reported *M. avium intracellulare* infections in two patients who were not BCG vaccinated (82).

The second gene, *NFKB2*, encodes p52 and its precursor p100, the transcription factor that mediates the noncanonical NF-κB signaling pathway (125, 126). Initial reports described an autosomal-dominant inheritance and DN effect (138–140). Patients presented with a phenotype of early-onset CVID (hypogammaglobulinemia, impaired antigen response, recurrent infections) associated with unique autoimmune manifestations, ectodermal dysplasia (alopecia areata or totalis and trachyonychia) and endocrine defects, most notably central adrenal insufficiency (138–140). While humoral immunity was always affected, impairment of T lymphocytes and NK cells varied among published cases (138–142). Neither BCG vaccination status nor vaccine adverse reactions were mentioned in the reports (40, 138–148), however, one patient had *Mycobacterium kansasii* infection (146). In 2017, Kuehn et al., described three additional patients with novel mutations in *NFKB2* that resulted in constitutive NFκB2 activation because of a GOF effect (144). Clinically, these patients presented manifestations consistent with a combined immunodeficiency, such as *Pneumocystis jirovecii* pneumonia and severe viral infections. Of notice, no endocrine, ectodermal, or autoimmune manifestations were found. None of these patients were BCG vaccinated, but one patient received anti-tuberculous treatment due to pulmonary nodules and caseating granulomas in a lung biopsy, although a mycobacterial infection was not documented [(144), and Acknowledgments].

In the canonical NF-κB signaling pathway, defects in a protein complex upstream NF-κB have also been described as new causes of PIDD. This complex is composed of three proteins: B-cell leukemia/lymphoma 10 (BCL10), mucosa-associated lymphoid

tissue lymphoma translocation gene 1 (MALT1), both ubiquitously expressed, and a member of the caspase activation and recruitment domain (CARD) protein family, which is expressed in a cell-type specific manner (149, 150). In lymphocytes, CARD11, BCL10, and MALT1 bind to form the CBM signalosome complex, which is responsible for triggering NF-κB activation following antigen binding to either T- or B-cell receptor (151).

Germline mutations in *CARD11* have been reported in 26 patients (150, 152–160) leading to three distinct phenotypes. Patients with biallelic LOF mutations presented with a combined immunodeficiency, similarly to what is seen in other mutations affecting the CBM complex (150, 153, 154, 157). Patients with heterozygous GOF mutations showed a lymphoproliferative condition known as "B cell expansion with NF-KB and T-cell anergy" (BENTA) disease (152). This disease is characterized by childhood-onset polyclonal B-cell lymphocytosis, splenomegaly, recurrent bacterial and viral infections, along with impaired vaccine responses (152, 155, 156, 158). In 2017, two groups identified both LOF and dominant-negative heterozygous defects in *CARD11* causing severe atopy and recurrent infections (159, 160). One of these patients had pulmonary tuberculosis (159). BCG-related complications were not mentioned in the reports and BCG vaccination history was not addressed either (150, 152–160). Three BENTA patients were BCG vaccinated without complications (**Table 2**) [(152, 155, 156, 158), and Acknowledgments].

Concerning the other members of the CBM complex, BCL10 deficiency has been identified in one patient (161) and MALT1 deficiency in six (162–165). These two defects, together with *CARD11* LOF mutations, share a similar phenotype of combined immunodeficiency without T cell lymphopenia, poor growth, severe infections, and gastrointestinal disease. Despite the limited number of BCL10 and MALT1 deficiency cases reported, we chose to include them in this review as an exception to our inclusion criteria as they all are a constitutive part of the CBM complex, and help us to expand our understanding on BCG reactions related to new immunodeficiencies in general and CBM complex defects in particular. Neither BCG vaccination status nor BCG-related complications were described in any of the reports (161–165). From our direct contact with the corresponding authors, we got information on the vaccination status of four MALT1 deficient patients, none of them were BCG vaccinated (**Table 2**) [(163, 164), and Acknowledgments].

CYTOTOXIC T LYMPHOCYTE ANTIGEN-4 (CTLA-4) AND LIPOPOLYSACCHARIDE-RESPONSIVE BEIGE-LIKE ANCHOR PROTEIN (LRBA) DEFICIENCIES

CTLA4 haploinsufficiency is an immune dysregulation syndrome characterized by hypogammaglobulinemia, progressive B-cell lymphopenia, autoimmunity, and lymphocytic organ infiltration (166, 167). CTLA-4 is as an inhibitory receptor (168) and, once defective, immune tolerance is disrupted due to impairment of regulatory T cell suppressor function along with an increase of autoreactive B cells, among other immunological abnormalities. It is inherited in an autosomal-dominant manner with incomplete penetrance (166, 167). Seventy-six patients carrying pathogenic mutations in CTLA4 have been published to date (82, 111, 166, 167, 169–181), of whom 62 are symptomatic and 14 asymptomatic mutation carriers due to incomplete disease penetrance. No adverse reactions to BCG vaccine have been reported in their past medical history, although patient's BCG vaccination status information was not available in the publications. We retrieved information on BCG vaccination status of 14 CTLA4-deficient patients, 4 of whom received BCG vaccine without complications (**Table 2**) [(82, 166, 170, 174, 177, 179), and Acknowledgments]. Moreover, there were no reports of infections caused by weakly virulent mycobacteria in this cohort (82, 111, 166, 167, 169–181). Two patients did have pulmonary tuberculosis in their early twenties (167). They both progressed to significant lung morbidity, but recurrent bacterial pneumonias also accounted for their unfavorable pulmonary outcomes.

LRBA protein deficiency is closely related to CLTA-4 haploinsufficiency in terms of its pathophysiology (182). It is an autosomal recessive disease with almost complete penetrance, also characterized by recurrent infections, hypogammaglobulinemia, autoimmunity, and lymphocytic organ infiltration (183, 184). Autoimmune disease is particularly severe in the gut, presenting with inflammatory bowel disease-like manifestations (179, 185, 186). LRBA protein protects CTLA-4 from lysosomal degradation, maintaining its intracellular stores (182). Although symptoms are similar to CTLA-4 haploinsufficiency, LRBA deficiency is usually more severe and presents at an earlier age (184, 187). One possible explanation to these differences is that when biallelic mutations of LRBA occur, CTLA-4 surface levels can be even lower than those seen in CTLA-4 haploinsufficiency (188). Also, LRBA protein is present in more cell types than CTLA-4 and not all of its functions are completely known (189). Despite the broader phenotype, over 100 LRBA-deficient patients have been described so far and no adverse reactions to BCG vaccine or any mycobacterial disease have been highlighted in their reports (82, 174, 179, 180, 182–184, 186, 187, 190–210). Out of 21 LRBA-deficient patients that we had access to BCG vaccination history, 16 received BCG vaccine without adverse reactions (**Table 2**) [(82, 174, 179, 185, 191, 193, 202, 205, 210), and Acknowledgments].

GATA2 DEFICIENCY

GATA2 deficiency was described as a new PIDD in 2011 (211–214). Heterozygous germline mutations in *GATA2*, a highly pleiotropic gene that encodes the hematopoietic transcription factor GATA2 (215), revealed to be the unifying genetic cause of four apparently distinct syndromes: monocytopenia with *M. avium* complex (MonoMAC) (216), dendritic cell, monocyte, B and NK lymphoid deficiency (DCML) (217), primary lymphedema with myelodysplasia (Emberger syndrome) (218), and familial myelodysplastic syndrome/acute myeloid leukemia (219).

As more patients were described, the phenotype was broadened and significant overlap among previously known syndromes was

noticed (220, 221). Childhood-onset cases have been reported (222), but most patients start manifesting symptoms later in life (220, 221). Patients present increased susceptibility to infections, mainly viral (particularly human papillomavirus), mycobacterial, and fungal (220, 221). Lung involvement is common, and a subset of patients present with pulmonary alveolar proteinosis (223, 224). Lymphedema and hearing loss are also common (221, 222). Most patients progress to myelodysplasia and are at increased risk of acute myeloid leukemia (221, 225).

Immunologically, progressive monocytopenia, B and NK lymphocytopenia, and absence of dendritic cells are hallmarks of the disease. CD4+ lymphopenia and neutropenia can also be seen but are less pronounced (220, 221). Bone marrow usually shows multilineage dysplasia and atypical megakaryocytes (221, 226).

Susceptibility to NTM is a remarkable finding in GATA2 deficiency. Fifty-four cases of NTM (212, 213, 216, 217, 220, 221, 224, 227–243) and only one case of disseminated *M. tuberculosis* (244) have been described in more than 350 GATA2-deficient patients published (**Table 2**) (40, 211–214, 216, 217, 220–284).

Information on BCG vaccination was available only for three patients in the reports (217, 241, 245). While two of them did not experience vaccine-related complications, one developed disseminated BCG infection at the age of 12 years, as the initial presentation of his disease. Four years later, he underwent a successful bone marrow transplantation from a matched unrelated donor (285). In the first month post-transplant, the patient had immune reconstitution syndrome (fever and rash) treated with a short course of systemic corticosteroids. Antimycobacterial treatment was stopped within a year.

CARMIL2 DEFICIENCY

Recently, biallelic LOF mutations in *RLTPR*, also known as *CARMIL2*, were described as a new PIDD (286–289). Capping protein regulator and myosin 1 linker 2 (CARMIL2) is a cytosolic protein found to be important for CD28-co-stimulation pathway and migration in T cells, as well as BCR-mediated activation of B cells (289, 290).

CARMIL2-deficient patients presented with recurrent bacterial respiratory and cutaneous infections, widespread warts alongside other viral infections (varicella zoster virus, molluscum contagiosum, and EBV), persistent dermatitis (eczema or psoriasiform hyperkeratotic lesions), and CMC. Inflammatory bowel disease and chronic esophagitis were seen in some patients. Four patients had disseminated EBV+ smooth muscle tumors (286–289).

Immunologically, patients shared a significant reduction in regulatory T cells, CD4+ memory and follicular helper cells. Th1 and Th17 cytokine production were impaired. Switched memory B cells counts were low and antibody responses to vaccines were poor (286–289).

Mycobacterial infections were seen in 2 out of 21 published cases. They both had disseminated tuberculosis (described as multifocal disease in one patient and miliary tuberculosis in the other). BCG vaccine had been given to both patients, uneventfully (289). Two other CARMIL2-deficient patients received BCG vaccine, without complications (287) (**Table 2**). Information on BCG vaccination status of the other CARMIL2-deficient patients was not available (286–289).

PHOSPHOGLUCOMUTASE 3 (PGM3) DEFICIENCY

Recently, biallelic hypomorphic mutations in *PGM3* have been described as a new congenital disorder of glycosylation resulting in PIDD (291, 292). PGM3 is an enzyme that catalyzes the conversion of *N*-acetylglucosamine-6-phosphate (GlcNAc-6-P) to 1-phosphate (GlcNAc-1-P) which is necessary for the generation of uridine diphosphate *N*-acetylglucosamine, an important precursor to multiple glycosylation pathways. Glycosylation is a complex posttranslational enzymatic process responsible for the attachment and trimming of glycans to proteins and lipids, critically affecting their structure and function (293, 294).

Congenital disorders of glycosylation usually manifest with broad, multisystemic symptoms (294). Two distinct phenotypes have been described for *PGM3* mutations, possibly correlated with levels of residual enzymatic activity (291, 292, 295). The majority of patients presented with an AR hyper-IgE syndrome phenotype of eczema and multiple manifestations of atopy, recurrent sinopulmonary and skin infections, failure to thrive, and varying degrees of neurological impairment. Dysmorphic features were present in some patients. Serum IgE levels and eosinophils counts were elevated and T cell lymphopenia was frequent (291, 292, 296). A subset of patients manifested a severe combined immunodeficiency phenotype with profound T- and B-cell lymphopenia but normal IgE levels. Neutropenia was also present. Skeletal dysplasia and multiple dysmorphisms were common findings among these patients (295, 297, 298).

There were no reports of BCG-related complications in the PGM3-deficient patients (291, 292, 295–301). Information on vaccination status was not provided in most reports, including all patients with severe combined immunodeficiency (SCID) presentation. Four patients were BCG vaccinated uneventfully (**Table 2**) (301).

TTC7A DEFICIENCY

The hereditary association of multiple intestinal atresia (MIA) and immunodeficiency has been long reported in the literature (302). However, the discovery of mutations in *TTC7A* as its underlying cause was only possible in 2013, after the advent of whole exome sequencing (303, 304).

Tetratricopeptide repeat domain 7A (TTC7A) protein function was not clear until the description of TTC7A-deficient patients (305). To date, more than 50 cases of biallelic mutations in *TTC7A* have been reported (303, 304, 306–317), and this protein was found critical to gut and immune system development and homeostasis (305).

The initial phenotype recognized is shared by the majority of patients reported. MIA requiring surgical interventions is associated with profound lymphopenia, hypogammaglobulinemia,

severe and recurrent infections, with high incidence of sepsis caused by intestinal microbes (303, 304, 306–309, 313). Some patients present MIA alone (304, 308), and others manifest very early onset inflammatory bowel disease (204, 307). The prognosis is poor and most patients die at a young age. More recently, a milder phenotype of enteropathy and predominantly humoral immunodeficiency has been reported (315).

None of the reports of TTC7A-deficient patients had information regarding BCG vaccination status. Neither BCG vaccine-related complications nor mycobacterial infections were reported (**Table 2**) (303, 304, 306–317).

CONCLUSION

The prevalence of BCG-associated complications in the general population can vary widely depending on the reporting country, the vaccine strain used, and the age at vaccination. Reports of 1 in 2,500 vaccines presenting with localized BCG-associated complications, and 1 in 100,000 presenting with disseminated complications represent a fair estimate of the general prevalence of such side effects. However, when focused on patients with SCID, the most severe forms of PIDD, ~1 in 2 (51%) develop BCG-associated complications after vaccination, 2/3 presenting with disseminated disease, and the remaining 1/3 as localized disease (9). In a recent cohort of 71 chronic granulomatous disease (CGD) patients, 75% presented with BCG-related complications (318). Moreover, among patients with MSMD due to mutations in IL12Rβ1, ~3 in 4 (77%) present with BCG-associated disease, 4/5 as disseminated and 1/5 as localized complications (319). While these diseases are well known for their increased susceptibility to BCG-related side effects, less specific information is available regarding other or more recently described PIDD.

In this review, we focused on PIDD first reported since 2010. In order to assure a fair patient representation, we limited our analysis to those diseases affecting 20 or more unrelated patients belonging to 10 or more families, with defects impairing areas of the immune system known to be involved in the control of mycobacterial infections.

Not surprisingly and as previously shown by Toubiana et al. (24), we found that patients with GOF STAT1 mutations showed an increased susceptibility to mycobacterial diseases, including BCG. Of note, our analysis of the published data also showed that patients with APDS 1 and 2 appear to have an increased risk for BCG-related localized complications. These findings *per se* can involve actionable recommendations as to ban BCG vaccination in STAT1 GOF, APDS1, and APDS2 patients and in their genetically untested newborn relatives until their status is clarified.

Among other newly described PIDD, no BCG-related complications were reported in the relatively frequent LRBA and CTLA4-deficient patients, or in NFKB1 and NFKB2-mutated individuals. As a limitation of this review, and despite our efforts to try to retrieve as much information as possible regarding BCG vaccination status and complications in these diseases, this negative data have to be taken with a grain of salt as the *n* of vaccinated patients might not have been sufficient to capture low but still relevant BCG complication rates in these diseases.

In summary, despite its debatable efficacy against tuberculosis, BCG remains one of the most popular and with higher coverage rate vaccines in many parts of the world. Interestingly, certain vaccines—BCG included—have been recently suggested to provide a significantly positive impact on overall health through heterologous immunity, sometimes even surpassing the specific protection originally intended (320). In any case, as clinicians taking care of a vulnerable population of individuals with inborn errors of their immune system, we should remain vigilant of any preventable medical interventions that can detrimentally impact our patients. As already well described, BCG vaccination should be avoided not only in SCID, MSMD, and CGD patients (321) but also in other particular newly described PIDD (e.g., STAT1 GOF, APDS1, and APDS2) in which its complication rates have shown to markedly exceed when compared to the general population.

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CJNS contributed to the research design and wrote the first draft. SDR contributed to the research design and supervised the whole project.

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REFERENCES

1. World Health Organization. *Global Tuberculosis Report 2017*. Geneva: World Health Organization (2017).
2. Ritz N, Hanekom WA, Robins-Browne R, Britton WJ, Curtis N. Influence of BCG vaccine strain on the immune response and protection against tuberculosis. *FEMS Microbiol Rev* (2008) 32(5):821–41. doi:10.1111/j.1574-6976.2008.00118.x
3. Zwerling A, Behr MA, Verma A, Brewer TF, Menzies D, Pai M. The BCG World Atlas: a database of global BCG vaccination policies and practices. *PLoS Med* (2011) 8(3):e1001012. doi:10.1371/journal.pmed.1001012
4. Dockrell HM, Smith SG. What have we learnt about BCG Vaccination in the last 20 years? *Front Immunol* (2017) 8:1134. doi:10.3389/fimmu.2017.01134
5. Mangtani P, Abubakar I, Ariti C, Beynon R, Pimpin L, Fine PE, et al. Protection by BCG vaccine against tuberculosis: a systematic review of randomized controlled trials. *Clin Infect Dis* (2014) 58(4):470–80. doi:10.1093/cid/cit790
6. World Health Organization. Meeting of the Global Advisory Committee on Vaccine Safety, June 2017. (2017) July, 2017. Report No.: Contract No.: 28.
7. Bonilla FA. Update: vaccines in primary immunodeficiency. *J Allergy Clin Immunol* (2018) 141(2):474–81. doi:10.1016/j.jaci.2017.12.980
8. World Health Organization. Revised BCG vaccination guidelines for infants at risk for HIV infection. *Wkly Epidemiol Rec* (2007) 82(21):193–6.
9. Marciano BE, Huang CY, Joshi G, Rezaei N, Carvalho BC, Allwood Z, et al. BCG vaccination in patients with severe combined immunodeficiency: complications, risks, and vaccination policies. *J Allergy Clin Immunol* (2014) 133(4):1134–41. doi:10.1016/j.jaci.2014.02.028
10. Casanova JL. Severe infectious diseases of childhood as monogenic inborn errors of immunity. *Proc Natl Acad Sci U S A* (2015) 112(51):E7128–37. doi:10.1073/pnas.1521651112
11. Boisson-Dupuis S, Bustamante J, El-Baghdadi J, Camcioglu Y, Parvaneh N, El Azbaoui S, et al. Inherited and acquired immunodeficiencies underlying tuberculosis in childhood. *Immunol Rev* (2015) 264(1):103–20. doi:10.1111/imr.12272
12. Picard C, Bobby Gaspar H, Al-Herz W, Bousfiha A, Casanova JL, Chatila T, et al. International union of immunological societies: 2017 primary immunodeficiency diseases committee report on inborn errors of immunity. *J Clin Immunol* (2018) 38(1):96–128. doi:10.1007/s10875-017-0464-9
13. Ramana CV, Chatterjee-Kishore M, Nguyen H, Stark GR. Complex roles of Stat1 in regulating gene expression. *Oncogene* (2000) 19(21):2619–27. doi:10.1038/sj.onc.1203525
14. Iwasaki Y, Fujio K, Okamura T, Yamamoto K. Interleukin-27 in T cell immunity. *Int J Mol Sci* (2015) 16(2):2851–63. doi:10.3390/ijms16022851
15. Dupuis S, Jouanguy E, Al-Hajjar S, Fieschi C, Al-Mohsen IZ, Al-Jumaah S, et al. Impaired response to interferon-alpha/beta and lethal viral disease in human STAT1 deficiency. *Nat Genet* (2003) 33(3):388–91. doi:10.1038/ng1097
16. Chapgier A, Kong XF, Boisson-Dupuis S, Jouanguy E, Averbuch D, Feinberg J, et al. A partial form of recessive STAT1 deficiency in humans. *J Clin Invest* (2009) 119(6):1502–14. doi:10.1172/JCI37083
17. Chapgier A, Boisson-Dupuis S, Jouanguy E, Vogt G, Feinberg J, Prochnicka-Chalufour A, et al. Novel STAT1 alleles in otherwise healthy patients with mycobacterial disease. *PLoS Genet* (2006) 2(8):e131. doi:10.1371/journal.pgen.0020131
18. Liu L, Okada S, Kong XF, Kreins AY, Cypowyj S, Abhyankar A, et al. Gain-of-function human STAT1 mutations impair IL-17 immunity and underlie chronic mucocutaneous candidiasis. *J Exp Med* (2011) 208(8):1635–48. doi:10.1084/jem.20110958
19. van de Veerdonk FL, Plantinga TS, Hoischen A, Smeekens SP, Joosten LA, Gilissen C, et al. STAT1 mutations in autosomal dominant chronic mucocutaneous candidiasis. *N Engl J Med* (2011) 365(1):54–61. doi:10.1056/NEJMoa1100102
20. Lee PP, Mao H, Yang W, Chan KW, Ho MH, Lee TL, et al. Penicillium marneffei infection and impaired IFN-gamma immunity in humans with autosomal-dominant gain-of-phosphorylation STAT1 mutations. *J Allergy Clin Immunol* (2014) 133(3):894–6.e5. doi:10.1016/j.jaci.2013.08.051
21. Zheng J, van de Veerdonk FL, Crossland KL, Smeekens SP, Chan CM, Al Shehri T, et al. Gain-of-function STAT1 mutations impair STAT3 activity in patients with chronic mucocutaneous candidiasis (CMC). *Eur J Immunol* (2015) 45(10):2834–46. doi:10.1002/eji.201445344
22. Depner M, Fuchs S, Raabe J, Frede N, Glocker C, Doffinger R, et al. The extended clinical phenotype of 26 patients with chronic mucocutaneous candidiasis due to gain-of-function mutations in STAT1. *J Clin Immunol* (2016) 36(1):73–84. doi:10.1007/s10875-015-0214-9
23. Mengesha BG, Conti HR. The role of IL-17 in protection against mucosal candida infections. *J Fungi (Basel)* (2017) 3(4):E52. doi:10.3390/jof3040052
24. Toubiana J, Okada S, Hiller J, Oleastro M, Lagos Gomez M, Aldave Becerra JC, et al. Heterozygous STAT1 gain-of-function mutations underlie an unexpectedly broad clinical phenotype. *Blood* (2016) 127(25):3154–64. doi:10.1182/blood-2015-11-679902
25. Sharfe N, Nahum A, Newell A, Dadi H, Ngan B, Pereira SL, et al. Fatal combined immunodeficiency associated with heterozygous mutation in STAT1. *J Allergy Clin Immunol* (2014) 133(3):807–17. doi:10.1016/j.jaci.2013.09.032
26. Toth B, Mehes L, Tasko S, Szalai Z, Tulassay Z, Cypowyj S, et al. Herpes in STAT1 gain-of-function mutation [corrected]. *Lancet* (2012) 379(9835):2500. doi:10.1016/S0140-6736(12)60365-1
27. Sampaio EP, Hsu AP, Pechacek J, Bax HI, Dias DL, Paulson ML, et al. Signal transducer and activator of transcription 1 (STAT1) gain-of-function mutations and disseminated coccidioidomycosis and histoplasmosis. *J Allergy Clin Immunol* (2013) 131(6):1624–34. doi:10.1016/j.jaci.2013.01.052
28. Hori T, Ohnishi H, Teramoto T, Tsubouchi K, Naiki T, Hirose Y, et al. Autosomal-dominant chronic mucocutaneous candidiasis with STAT1-mutation can be complicated with chronic active hepatitis and hypothyroidism. *J Clin Immunol* (2012) 32(6):1213–20. doi:10.1007/s10875-012-9744-6
29. Uzel G, Sampaio EP, Lawrence MG, Hsu AP, Hackett M, Dorsey MJ, et al. Dominant gain-of-function STAT1 mutations in FOXP3 wild-type immune dysregulation-polyendocrinopathy-enteropathy-X-linked-like syndrome. *J Allergy Clin Immunol* (2013) 131(6):1611–23. doi:10.1016/j.jaci.2012.11.054
30. Tanimura M, Dohi K, Hirayama M, Sato Y, Sugiura E, Nakajima H, et al. Recurrent inflammatory aortic aneurysms in chronic mucocutaneous candidiasis with a gain-of-function STAT1 mutation. *Int J Cardiol* (2015) 196:88–90. doi:10.1016/j.ijcard.2015.05.183
31. Dadak M, Jacobs R, Skuljec J, Jirmo AC, Yildiz O, Donnerstag F, et al. Gain-of-function STAT1 mutations are associated with intracranial aneurysms. *Clin Immunol* (2017) 178:79–85. doi:10.1016/j.clim.2017.01.012
32. Koo S, Kejariwal D, Al-Shehri T, Dhar A, Lilic D. Oesophageal candidiasis and squamous cell cancer in patients with gain-of-function STAT1 gene mutation. *United European Gastroenterol J* (2017) 5(5):625–31. doi:10.1177/2050640616684404
33. Romberg N, Morbach H, Lawrence MG, Kim S, Kang I, Holland SM, et al. Gain-of-function STAT1 mutations are associated with PD-L1 overexpression and a defect in B-cell survival. *J Allergy Clin Immunol* (2013) 131(6):1691–3. doi:10.1016/j.jaci.2013.01.004
34. Kataoka S, Muramatsu H, Okuno Y, Hayashi Y, Mizoguchi Y, Tsumura M, et al. Extrapulmonary tuberculosis mimicking Mendelian susceptibility to mycobacterial disease in a patient with signal transducer and activator of transcription 1 (STAT1) gain-of-function mutation. *J Allergy Clin Immunol* (2016) 137(2):619–22.e1. doi:10.1016/j.jaci.2015.06.028
35. Zimmerman O, Rosler B, Zerbe CS, Rosen LB, Hsu AP, Uzel G, et al. Risks of ruxolitinib in STAT1 gain-of-function-associated severe fungal disease. *Open Forum Infect Dis* (2017) 4(4):ofx202. doi:10.1093/ofid/ofx202
36. Baris S, Alroqi F, Kiykim A, Karakoc-Aydiner E, Ogulur I, Ozen A, et al. Severe early-onset combined immunodeficiency due to heterozygous gain-of-function mutations in STAT1. *J Clin Immunol* (2016) 36(7):641–8. doi:10.1007/s10875-016-0312-3
37. Leiding JW, Okada S, Hagin D, Abinun M, Shcherbina A, Balashov DN, et al. Hematopoietic stem cell transplantation in patients with gain-of-function signal transducer and activator of transcription 1 mutations. *J Allergy Clin Immunol* (2018) 141(2):704–17.e5. doi:10.1016/j.jaci.2017.03.049
38. Pedraza-Sanchez S, Lezana-Fernandez JL, Gonzalez Y, Martinez-Robles L, Ventura-Ayala ML, Sadowinski-Pine S, et al. Disseminated tuberculosis and chronic mucocutaneous candidiasis in a patient with a gain-of-function mutation in signal transduction and activator of transcription 1. *Front Immunol* (2017) 8:1651. doi:10.3389/fimmu.2017.01651

39. Zerbe CS, Marciano BE, Katial RK, Santos CB, Adamo N, Hsu AP, et al. Progressive multifocal leukoencephalopathy in primary immune deficiencies: Stat1 gain of function and review of the literature. *Clin Infect Dis* (2016) 62(8):986–94. doi:10.1093/cid/civ1220
40. Rae W, Ward D, Mattocks CJ, Gao Y, Pengelly RJ, Patel SV, et al. Autoimmunity/inflammation in a monogenic primary immunodeficiency cohort. *Clin Transl Immunology* (2017) 6(9):e155. doi:10.1038/cti.2017.38
41. Al Rushood M, McCusker C, Mazer B, Alizadehfar R, Grimbacher B, Depner M, et al. Autosomal dominant cases of chronic mucocutaneous candidiasis segregates with mutations of signal transducer and activator of transcription 1, but not of toll-like receptor 3. *J Pediatr* (2013) 163(1):277–9. doi:10.1016/j.jpeds.2013.02.040
42. Aldave Becerra JC, Cachay Rojas E. A 3-year-old girl with recurrent infections and autoimmunity due to a STAT1 gain-of-function mutation: the expanding clinical presentation of primary immunodeficiencies. *Front Pediatr* (2017) 5:55. doi:10.3389/fped.2017.00055
43. Aldave JC, Cachay E, Nunez L, Chunga A, Murillo S, Cypowyj S, et al. A 1-year-old girl with a gain-of-function STAT1 mutation treated with hematopoietic stem cell transplantation. *J Clin Immunol* (2013) 33(8):1273–5. doi:10.1007/s10875-013-9947-5
44. Breuer O, Daum H, Cohen-Cymberknoh M, Unger S, Shoseyov D, Stepensky P, et al. Autosomal dominant gain of function STAT1 mutation and severe bronchiectasis. *Respir Med* (2017) 126:39–45. doi:10.1016/j.rmed.2017.03.018
45. Dhalla F, Fox H, Davenport EE, Sadler R, Anzilotti C, van Schouwenburg PA, et al. Chronic mucocutaneous candidiasis: characterization of a family with STAT-1 gain-of-function and development of an ex-vivo assay for Th17 deficiency of diagnostic utility. *Clin Exp Immunol* (2016) 184(2):216–27. doi:10.1111/cei.12746
46. Dotta L, Scomodon O, Padoan R, Timpano S, Plebani A, Soresina A, et al. Clinical heterogeneity of dominant chronic mucocutaneous candidiasis disease: presenting as treatment-resistant candidiasis and chronic lung disease. *Clin Immunol* (2016) 164:1–9. doi:10.1016/j.clim.2015.12.010
47. Eren Akarcan S, Ulusoy Severcan E, Edeer Karaca N, Isik E, Aksu G, Migaud M, et al. Gain-of-function mutations in STAT1: a recently defined cause for chronic mucocutaneous candidiasis disease mimicking combined immunodeficiencies. *Case Reports Immunol* (2017) 2017:2846928. doi:10.1155/2017/2846928
48. Eslami N, Tavakol M, Mesdaghi M, Gharegozlou M, Casanova JL, Puel A, et al. A gain-of-function mutation of STAT1: a novel genetic factor contributing to chronic mucocutaneous candidiasis. *Acta Microbiol Immunol Hung* (2017) 64(2):191–201. doi:10.1556/030.64.2017.014
49. Frans G, Moens L, Schaballie H, Van Eyck L, Borgers H, Wuyts M, et al. Gain-of-function mutations in signal transducer and activator of transcription 1 (STAT1): chronic mucocutaneous candidiasis accompanied by enamel defects and delayed dental shedding. *J Allergy Clin Immunol* (2014) 134(5):1209–13.e6. doi:10.1016/j.jaci.2014.05.044
50. Giardino G, Somma D, Cirillo E, Ruggiero G, Terrazzano G, Rubino V, et al. Novel STAT1 gain-of-function mutation and suppurative infections. *Pediatr Allergy Immunol* (2016) 27(2):220–3. doi:10.1111/pai.12496
51. Higgins E, Al Shehri T, McAleer MA, Conlon N, Feighery C, Lilic D, et al. Use of ruxolitinib to successfully treat chronic mucocutaneous candidiasis caused by gain-of-function signal transducer and activator of transcription 1 (STAT1) mutation. *J Allergy Clin Immunol* (2015) 135(2):551–3. doi:10.1016/j.jaci.2014.12.1867
52. Kilic SS, Puel A, Casanova JL. Orf infection in a patient with Stat1 gain-of-function. *J Clin Immunol* (2015) 35(1):80–3. doi:10.1007/s10875-014-0111-7
53. Kobbe R, Kolster M, Fuchs S, Schulze-Sturm U, Jenderny J, Kochhan L, et al. Common variable immunodeficiency, impaired neurological development and reduced numbers of T regulatory cells in a 10-year-old boy with a STAT1 gain-of-function mutation. *Gene* (2016) 586(2):234–8. doi:10.1016/j.gene.2016.04.006
54. Kumar N, Hanks ME, Chandrasekaran P, Davis BC, Hsu AP, Van Wagoner NJ, et al. Gain-of-function signal transducer and activator of transcription 1 (STAT1) mutation-related primary immunodeficiency is associated with disseminated mucormycosis. *J Allergy Clin Immunol* (2014) 134(1):236–9. doi:10.1016/j.jaci.2014.02.037
55. Martinez-Martinez L, Martinez-Saavedra MT, Fuentes-Prior P, Barnadas M, Rubiales MV, Noda J, et al. A novel gain-of-function STAT1 mutation resulting in basal phosphorylation of STAT1 and increased distal IFN-gamma-mediated responses in chronic mucocutaneous candidiasis. *Mol Immunol* (2015) 68(2 Pt C):597–605. doi:10.1016/j.molimm.2015.09.014
56. Meesilpavikkai K, Dik WA, Schrijver B, Nagtzaam NM, van Rijswijk A, Driessen GJ, et al. A novel heterozygous mutation in the STAT1 SH2 domain causes chronic mucocutaneous candidiasis, atypically diverse infections, autoimmunity, and impaired cytokine regulation. *Front Immunol* (2017) 8:274. doi:10.3389/fimmu.2017.00274
57. Mekki N, Ben-Mustapha I, Liu L, Boussofara L, Okada S, Cypowyj S, et al. IL-17 T cells' defective differentiation in vitro despite normal range ex vivo in chronic mucocutaneous candidiasis due to STAT1 mutation. *J Invest Dermatol* (2014) 134(4):1155–7. doi:10.1038/jid.2013.480
58. Mizoguchi Y, Tsumura M, Okada S, Hirata O, Minegishi S, Imai K, et al. Simple diagnosis of STAT1 gain-of-function alleles in patients with chronic mucocutaneous candidiasis. *J Leukoc Biol* (2014) 95(4):667–76. doi:10.1189/jlb.0513250
59. Mossner R, Diering N, Bader O, Forkel S, Overbeck T, Gross U, et al. Ruxolitinib induces interleukin 17 and ameliorates chronic mucocutaneous candidiasis caused by STAT1 gain-of-function mutation. *Clin Infect Dis* (2016) 62(7):951–3. doi:10.1093/cid/ciw020
60. Nielsen J, Kofod-Olsen E, Spaun E, Larsen CS, Christiansen M, Mogensen TH. A STAT1-gain-of-function mutation causing Th17 deficiency with chronic mucocutaneous candidiasis, psoriasiform hyperkeratosis and dermatophytosis. *BMJ Case Rep* (2015) 2015:1–5. doi:10.1136/bcr-2015-211372
61. Sampaio EP, Ding L, Rose SR, Cruz P, Hsu AP, Kashyap A, et al. Novel signal transducer and activator of transcription 1 mutation disrupts small ubiquitin-related modifier conjugation causing gain of function. *J Allergy Clin Immunol* (2017) 141(5):1844–53.e2. doi:10.1016/j.jaci.2017.07.027
62. Second J, Korganow AS, Jannier S, Puel A, Lipsker D. Rosacea and demodicidosis associated with gain-of-function mutation in STAT1. *J Eur Acad Dermatol Venereol* (2017) 31(12):e542–4. doi:10.1111/jdv.14413
63. Smeekens SP, Plantinga TS, van de Veerdonk FL, Heinhuis B, Hoischen A, Joosten LA, et al. STAT1 hyperphosphorylation and defective IL12R/IL23R signaling underlie defective immunity in autosomal dominant chronic mucocutaneous candidiasis. *PLoS One* (2011) 6(12):e29248. doi:10.1371/journal.pone.0029248
64. Sobh A, Chou J, Schneider L, Geha RS, Massaad MJ. Chronic mucocutaneous candidiasis associated with an SH2 domain gain-of-function mutation that enhances STAT1 phosphorylation. *J Allergy Clin Immunol* (2016) 138(1):297–9. doi:10.1016/j.jaci.2015.12.1320
65. Soltesz B, Toth B, Shabashova N, Bondarenko A, Okada S, Cypowyj S, et al. New and recurrent gain-of-function STAT1 mutations in patients with chronic mucocutaneous candidiasis from Eastern and Central Europe. *J Med Genet* (2013) 50(9):567–78. doi:10.1136/jmedgenet-2013-101570
66. Tabellini G, Vairo D, Scomodon O, Tamassia N, Ferraro RM, Patrizi O, et al. Impaired natural killer cell functions in patients with signal transducer and activator of transcription 1 (STAT1) gain-of-function mutations. *J Allergy Clin Immunol* (2017) 140(2):553–64.e4. doi:10.1016/j.jaci.2016.10.051
67. Takezaki S, Yamada M, Kato M, Park MJ, Maruyama K, Yamazaki Y, et al. Chronic mucocutaneous candidiasis caused by a gain-of-function mutation in the STAT1 DNA-binding domain. *J Immunol* (2012) 189(3):1521–6. doi:10.4049/jimmunol.1200926
68. Wang X, Lin Z, Gao L, Wang A, Wan Z, Chen W, et al. Exome sequencing reveals a signal transducer and activator of transcription 1 (STAT1) mutation in a child with recalcitrant cutaneous fusariosis. *J Allergy Clin Immunol* (2013) 131(4):1242–3. doi:10.1016/j.jaci.2012.11.005
69. Weinacht KG, Charbonnier LM, Alroqi F, Plant A, Qiao Q, Wu H, et al. Ruxolitinib reverses dysregulated T helper cell responses and controls autoimmunity caused by a novel signal transducer and activator of transcription 1 (STAT1) gain-of-function mutation. *J Allergy Clin Immunol* (2017) 139(5):1629–40.e2. doi:10.1016/j.jaci.2016.11.022
70. Wildbaum G, Shahar E, Katz R, Karin N, Etzioni A, Pollack S. Continuous G-CSF therapy for isolated chronic mucocutaneous candidiasis: complete clinical remission with restoration of IL-17 secretion. *J Allergy Clin Immunol* (2013) 132(3):761–4. doi:10.1016/j.jaci.2013.04.018

71. Yamazaki Y, Yamada M, Kawai T, Morio T, Onodera M, Ueki M, et al. Two novel gain-of-function mutations of STAT1 responsible for chronic mucocutaneous candidiasis disease: impaired production of IL-17A and IL-22, and the presence of anti-IL-17F autoantibody. *J Immunol* (2014) 193(10):4880–7. doi:10.4049/jimmunol.1401467
72. Faitelson Y, Bates A, Shroff M, Grunebaum E, Roifman CM, Naqvi A. A mutation in the STAT1 DNA-binding domain associated with hemophagocytic lymphohistiocytosis. *LymphoSign J* (2014) 1(2):87–95. doi:10.14785/lpsn-2014-0004
73. Boisson-Dupuis S, Kong XF, Okada S, Cypowyj S, Puel A, Abel L, et al. Inborn errors of human STAT1: allelic heterogeneity governs the diversity of immunological and infectious phenotypes. *Curr Opin Immunol* (2012) 24(4):364–78. doi:10.1016/j.coi.2012.04.011
74. Qi QR, Yang ZM. Regulation and function of signal transducer and activator of transcription 3. *World J Biol Chem* (2014) 5(2):231–9. doi:10.4331/wjbc.v5.i2.231
75. Minegishi Y, Saito M, Tsuchiya S, Tsuge I, Takada H, Hara T, et al. Dominant-negative mutations in the DNA-binding domain of STAT3 cause hyper-IgE syndrome. *Nature* (2007) 448(7157):1058–62. doi:10.1038/nature06096
76. Holland SM, DeLeo FR, Elloumi HZ, Hsu AP, Uzel G, Brodsky N, et al. STAT3 mutations in the hyper-IgE syndrome. *N Engl J Med* (2007) 357(16):1608–19. doi:10.1056/NEJMoa073687
77. Koskela HL, Eldfors S, Ellonen P, van Adrichem AJ, Kuusanmaki H, Andersson EI, et al. Somatic STAT3 mutations in large granular lymphocytic leukemia. *N Engl J Med* (2012) 366(20):1905–13. doi:10.1056/NEJMoa1114885
78. Flanagan SE, Haapaniemi E, Russell MA, Caswell R, Allen HL, De Franco E, et al. Activating germline mutations in STAT3 cause early-onset multi-organ autoimmune disease. *Nat Genet* (2014) 46(8):812–4. doi:10.1038/ng.3040
79. Milner JD, Vogel TP, Forbes L, Ma CA, Stray-Pedersen A, Niemela JE, et al. Early-onset lymphoproliferation and autoimmunity caused by germline STAT3 gain-of-function mutations. *Blood* (2015) 125(4):591–9. doi:10.1182/blood-2014-09-602763
80. Haapaniemi EM, Kaustio M, Rajala HL, van Adrichem AJ, Kainulainen L, Glumoff V, et al. Autoimmunity, hypogammaglobulinemia, lymphoproliferation, and mycobacterial disease in patients with activating mutations in STAT3. *Blood* (2015) 125(4):639–48. doi:10.1182/blood-2014-04-570101
81. Weinreich MA, Vogel TP, Rao VK, Milner JD. Up, down, and all around: diagnosis and treatment of novel STAT3 variant. *Front Pediatr* (2017) 5:49. doi:10.3389/fped.2017.00049
82. Maffucci P, Filion CA, Boisson B, Itan Y, Shang L, Casanova JL, et al. Genetic diagnosis using whole exome sequencing in common variable immunodeficiency. *Front Immunol* (2016) 7:220. doi:10.3389/fimmu.2016.00220
83. Consonni F, Dotta L, Todaro F, Vairo D, Badolato R. Signal transducer and activator of transcription gain-of-function primary immunodeficiency/immunodysregulation disorders. *Curr Opin Pediatr* (2017) 29(6):711–7. doi:10.1097/MOP.0000000000000551
84. Gutierrez M, Scaglia P, Keselman A, Martucci L, Karabatas L, Domene S, et al. Partial growth hormone insensitivity and dysregulatory immune disease associated with de novo germline activating STAT3 mutations. *Mol Cell Endocrinol* (2018). doi:10.1016/j.mce.2018.01.016
85. Khoury T, Molho-Pessach V, Ramot Y, Ayman AR, Elpeleg O, Berkman N, et al. Tocilizumab promotes regulatory T-cell alleviation in STAT3 gain-of-function-associated multi-organ autoimmune syndrome. *Clin Ther* (2017) 39(2):444–9. doi:10.1016/j.clinthera.2017.01.004
86. Nabhani S, Schipp C, Miskin H, Levin C, Postovsky S, Dujovny T, et al. STAT3 gain-of-function mutations associated with autoimmune lymphoproliferative syndrome like disease deregulate lymphocyte apoptosis and can be targeted by BH3 mimetic compounds. *Clin Immunol* (2017) 181:32–42. doi:10.1016/j.clim.2017.05.021
87. Sediva H, Dusatkova P, Kanderova V, Obermannova B, Kayserova J, Sramkova L, et al. Short stature in a boy with multiple early-onset autoimmune conditions due to a STAT3 activating mutation: could intracellular growth hormone signalling be compromised? *Horm Res Paediatr* (2017) 88(2):160–6. doi:10.1159/000456544
88. Velayos T, Martinez R, Alonso M, Garcia-Etxebarria K, Aguayo A, Camarero C, et al. An activating mutation in STAT3 results in neonatal diabetes through reduced insulin synthesis. *Diabetes* (2017) 66(4):1022–9. doi:10.2337/db16-0867
89. Angulo I, Vadas O, Garçon F, Banham-Hall E, Plagnol V, Leahy TR, et al. Phosphoinositide 3-kinase δ gene mutation predisposes to respiratory infection and airway damage. *Science* (2013) 342(6160):866–71. doi:10.1126/science.1243292
90. Lucas CL, Kuehn HS, Zhao F, Niemela JE, Deenick EK, Palendira U, et al. Dominant-activating germline mutations in the gene encoding the PI(3)K catalytic subunit p110δ result in T cell senescence and human immunodeficiency. *Nat Immunol* (2014) 15(1):88–97. doi:10.1038/ni.2771
91. Vanhaesebroeck B, Welham MJ, Kotani K, Stein R, Warne PH, Zvelebil MJ, et al. P110delta, a novel phosphoinositide 3-kinase in leukocytes. *Proc Natl Acad Sci U S A* (1997) 94(9):4330–5. doi:10.1073/pnas.94.9.4330
92. Fung-Leung WP. Phosphoinositide 3-kinase delta (PI3Kdelta) in leukocyte signaling and function. *Cell Signal* (2011) 23(4):603–8. doi:10.1016/j.cellsig.2010.10.002
93. Fruman DA, Bismuth G. Fine tuning the immune response with PI3K. *Immunol Rev* (2009) 228(1):253–72. doi:10.1111/j.1600-065X.2008.00750.x
94. Deau MC, Heurtier L, Frange P, Suarez F, Bole-Feysot C, Nitschke P, et al. A human immunodeficiency caused by mutations in the PIK3R1 gene. *J Clin Invest* (2014) 124(9):3923–8. doi:10.1172/JCI75746
95. Lucas CL, Zhang Y, Venida A, Wang Y, Hughes J, McElwee J, et al. Heterozygous splice mutation in PIK3R1 causes human immunodeficiency with lymphoproliferation due to dominant activation of PI3K. *J Exp Med* (2014) 211(13):2537–47. doi:10.1084/jem.20141759
96. Elgizouli M, Lowe DM, Speckmann C, Schubert D, Hulsdunker J, Eskandarian Z, et al. Activating PI3Kdelta mutations in a cohort of 669 patients with primary immunodeficiency. *Clin Exp Immunol* (2016) 183(2):221–9. doi:10.1111/cei.12706
97. Coulter TI, Chandra A, Bacon CM, Babar J, Curtis J, Screaton N, et al. Clinical spectrum and features of activated phosphoinositide 3-kinase δ syndrome: a large patient cohort study. *J Allergy Clin Immunol* (2017) 139(2):597–606.e4. doi:10.1016/j.jaci.2016.06.021
98. Olbrich P, Lorenz M, Cura Daball P, Lucena JM, Rensing-Ehl A, Sanchez B, et al. Activated PI3Kdelta syndrome type 2: two patients, a novel mutation, and review of the literature. *Pediatr Allergy Immunol* (2016) 27(6):640–4. doi:10.1111/pai.12585
99. Elkaim E, Neven B, Bruneau J, Mitsui-Sekinaka K, Stanislas A, Heurtier L, et al. Clinical and immunologic phenotype associated with activated phosphoinositide 3-kinase delta syndrome 2: a cohort study. *J Allergy Clin Immunol* (2016) 138(1):210–8.e9. doi:10.1016/j.jaci.2016.03.022
100. Lougaris V, Faletra F, Lanzi G, Vozzi D, Marcuzzi A, Valencic E, et al. Altered germinal center reaction and abnormal B cell peripheral maturation in PI3KR1-mutated patients presenting with HIGM-like phenotype. *Clin Immunol* (2015) 159(1):33–6. doi:10.1016/j.clim.2015.04.014
101. Crank MC, Grossman JK, Moir S, Pittaluga S, Buckner CM, Kardava L, et al. Mutations in PIK3CD can cause hyper IgM syndrome (HIGM) associated with increased cancer susceptibility. *J Clin Immunol* (2014) 34(3):272–6. doi:10.1007/s10875-014-0012-9
102. Petrovski S, Parrott RE, Roberts JL, Huang H, Yang J, Gorentla B, et al. Dominant splice site mutations in PIK3R1 cause hyper IgM syndrome, lymphadenopathy and short stature. *J Clin Immunol* (2016) 36(5):462–71. doi:10.1007/s10875-016-0281-6
103. Tsujita Y, Mitsui-Sekinaka K, Imai K, Yeh TW, Mitsuiki N, Asano T, et al. Phosphatase and tensin homolog (PTEN) mutation can cause activated phosphatidylinositol 3-kinase delta syndrome-like immunodeficiency. *J Allergy Clin Immunol* (2016) 138(6):1672–80.e10. doi:10.1016/j.jaci.2016.03.055
104. Chiriaco M, Brigida I, Ariganello P, Di Cesare S, Di Matteo G, Taus F, et al. The case of an APDS patient: defects in maturation and function and decreased in vitro anti-mycobacterial activity in the myeloid compartment. *Clin Immunol* (2017) 178:20–8. doi:10.1016/j.clim.2015.12.008
105. Hartman HN, Niemela J, Hintermeyer MK, Garofalo M, Stoddard J, Verbsky JW, et al. Gain of function mutations of PIK3CD as a cause of primary sclerosing cholangitis. *J Clin Immunol* (2015) 35(1):11–4. doi:10.1007/s10875-014-0109-1
106. Rae W, Gao Y, Ward D, Mattocks CJ, Eren E, Williams AP. A novel germline gain-of-function variant in PIK3CD. *Clin Immunol* (2017) 181:29–31. doi:10.1016/j.clim.2017.05.020
107. Heurtier L, Lamrini H, Chentout L, Deau MC, Bouafia A, Rosain J, et al. Mutations in the adaptor-binding domain and associated linker region of

p110delta cause activated PI3K-delta syndrome 1 (APDS1). *Haematologica* (2017) 102(7):e278–81. doi:10.3324/haematol.2017.167601
108. Rae W, Ramakrishnan KA, Gao Y, Ashton-Key M, Pengelly RJ, Patel SV, et al. Precision treatment with sirolimus in a case of activated phosphoinositide 3-kinase delta syndrome. *Clin Immunol* (2016) 171:38–40. doi:10.1016/j.clim.2016.07.017
109. Rao VK, Webster S, Dalm VASH, Šedivá A, van Hagen PM, Holland S, et al. Effective "activated PI3Kδ syndrome"-targeted therapy with the PI3Kδ inhibitor leniolisib. *Blood* (2017) 130(21):2307–16. doi:10.1182/blood-2017-08-801191
110. Kracker S, Curtis J, Ibrahim MA, Sediva A, Salisbury J, Campr V, et al. Occurrence of B-cell lymphomas in patients with activated phosphoinositide 3-kinase δ syndrome. *J Allergy Clin Immunol* (2014) 134(1):233–6. doi:10.1016/j.jaci.2014.02.020
111. Buchbinder D, Seppanen M, Rao VK, Uzel G, Nugent D. Clinical challenges: identification of patients with novel primary immunodeficiency syndromes. *J Pediatr Hematol Oncol* (2017). doi:10.1097/MPH.0000000000001003
112. Dulau Florea AE, Braylan RC, Schafernak KT, Williams KW, Daub J, Goyal RK, et al. Abnormal B-cell maturation in the bone marrow of patients with germline mutations in PIK3CD. *J Allergy Clin Immunol* (2017) 139(3):1032–5.e6. doi:10.1016/j.jaci.2016.08.028
113. Nademi Z, Slatter MA, Dvorak CC, Neven B, Fischer A, Suarez F, et al. Hematopoietic stem cell transplant in patients with activated PI3K delta syndrome. *J Allergy Clin Immunol* (2017) 139(3):1046–9. doi:10.1016/j.jaci.2016.09.040
114. Mettman D, Thiffault I, Dinakar C, Saunders C. Immunodeficiency-associated lymphoid hyperplasia as a cause of intussusception in a case of activated PI3K-δ syndrome. *Front Pediatr* (2017) 5:71. doi:10.3389/fped.2017.00071
115. Saettini F, Pelagatti MA, Sala D, Moratto D, Giliani S, Badolato R, et al. Early diagnosis of PI3Kδ syndrome in a 2 years old girl with recurrent otitis and enlarged spleen. *Immunol Lett* (2017) 190:279–81. doi:10.1016/j.imlet.2017.08.021
116. Takeda AJ, Zhang Y, Dornan GL, Siempelkamp BD, Jenkins ML, Matthews HF, et al. Novel PIK3CD mutations affecting N-terminal residues of p110δ cause activated PI3Kδ syndrome (APDS) in humans. *J Allergy Clin Immunol* (2017) 140(4):1152–6.e10. doi:10.1016/j.jaci.2017.03.026
117. Wentink M, Dalm V, Lankester AC, van Schouwenburg PA, Schölvinck L, Kalina T, et al. Genetic defects in PI3Kδ affect B-cell differentiation and maturation leading to hypogammaglobulineamia and recurrent infections. *Clin Immunol* (2017) 176:77–86. doi:10.1016/j.clim.2017.01.004
118. Jou ST, Chien YH, Yang YH, Wang TC, Shyur SD, Chou CC, et al. Identification of variations in the human phosphoinositide 3-kinase p110delta gene in children with primary B-cell immunodeficiency of unknown aetiology. *Int J Immunogenet* (2006) 33(5):361–9. doi:10.1111/j.1744-313X.2006.00627.x
119. Kannan JA, Davila-Saldana BJ, Zhang K, Filipovich AH, Kucuk ZY. Activated phosphoinositide 3-kinase delta syndrome in a patient with a former diagnosis of common variable immune deficiency, bronchiectasis, and lymphoproliferative disease. *Ann Allergy Asthma Immunol* (2015) 115(5):452–4. doi:10.1016/j.anai.2015.08.009
120. Goto F, Uchiyama T, Nakazawa Y, Imai K, Kawai T, Onodera M. Persistent impairment of T-cell regeneration in a patient with activated PI3K delta syndrome. *J Clin Immunol* (2017) 37(4):347–50. doi:10.1007/s10875-017-0393-7
121. Kuhlen M, Honscheid A, Loizou L, Nabhani S, Fischer U, Stepensky P, et al. De novo PIK3R1 gain-of-function with recurrent sinopulmonary infections, long-lasting chronic CMV-lymphadenitis and microcephaly. *Clin Immunol* (2016) 162:27–30. doi:10.1016/j.clim.2015.10.008
122. Martinez-Saavedra MT, Garcia-Gomez S, Dominguez Acosta A, Mendoza Quintana JJ, Paez JP, Garcia-Reino EJ, et al. Gain-of-function mutation in PIK3R1 in a patient with a narrow clinical phenotype of respiratory infections. *Clin Immunol* (2016) 173:117–20. doi:10.1016/j.clim.2016.09.011
123. Bravo Garcia-Morato M, Garcia-Minaur S, Molina Garicano J, Santos Simarro F, Del Pino Molina L, Lopez-Granados E, et al. Mutations in PIK3R1 can lead to APDS2, SHORT syndrome or a combination of the two. *Clin Immunol* (2017) 179:77–80. doi:10.1016/j.clim.2017.03.004
124. Hauck F, Magg T, Krolo A, Bilic I, Hirschmugl T, Laass M, et al. Variant PIK3R1 hypermorphic mutation and clinical phenotypes in a family with short statures, mild immunodeficiency and lymphoma. *Klin Padiatr* (2017) 229(3):113–7. doi:10.1055/s-0043-104218
125. Hayden MS, Ghosh S. NF-κB, the first quarter-century: remarkable progress and outstanding questions. *Genes Dev* (2012) 26(3):203–34. doi:10.1101/gad.183434.111
126. Mitchell S, Vargas J, Hoffmann A. Signaling via the NFκB system. *Wiley Interdiscip Rev Syst Biol Med* (2016) 8(3):227–41. doi:10.1002/wsbm.1331
127. Puel A, Picard C, Ku CL, Smahi A, Casanova JL. Inherited disorders of NF-kappaB-mediated immunity in man. *Curr Opin Immunol* (2004) 16(1):34–41. doi:10.1016/j.coi.2003.11.013
128. Paciolla M, Pescatore A, Conte MI, Esposito E, Incoronato M, Lioi MB, et al. Rare mendelian primary immunodeficiency diseases associated with impaired NF-kappaB signaling. *Genes Immun* (2015) 16(4):239–46. doi:10.1038/gene.2015.3
129. Karaca NE, Aksu G, Ulusoy E, Cavusoglu C, Oleaga-Quintas C, Nieto-Patlan A, et al. Disseminated BCG infectious disease and hyperferritinemia in a patient with a novel NEMO mutation. *J Investig Allergol Clin Immunol* (2016) 26(4):268–71. doi:10.18176/jiaci.0068
130. Imamura M, Kawai T, Okada S, Izawa K, Takachi T, Iwabuchi H, et al. Disseminated BCG infection mimicking metastatic nasopharyngeal carcinoma in an immunodeficient child with a novel hypomorphic NEMO mutation. *J Clin Immunol* (2011) 31(5):802–10. doi:10.1007/s10875-011-9568-9
131. Fliegauf M, Bryant VL, Frede N, Slade C, Woon ST, Lehnert K, et al. Haploinsufficiency of the NF-kappaB1 subunit p50 in common variable immunodeficiency. *Am J Hum Genet* (2015) 97(3):389–403. doi:10.1016/j.ajhg.2015.07.008
132. Keller B, Cseresnyes Z, Stumpf I, Wehr C, Fliegauf M, Bulashevska A, et al. Disturbed canonical nuclear factor of kappa light chain signaling in B cells of patients with common variable immunodeficiency. *J Allergy Clin Immunol* (2017) 139(1):220–31.e8. doi:10.1016/j.jaci.2016.04.043
133. Boztug H, Hirschmugl T, Holter W, Lakatos K, Kager L, Trapin D, et al. NF-kappaB1 haploinsufficiency causing immunodeficiency and EBV-driven lymphoproliferation. *J Clin Immunol* (2016) 36(6):533–40. doi:10.1007/s10875-016-0306-1
134. Kaustio M, Haapaniemi E, Goos H, Hautala T, Park G, Syrjanen J, et al. Damaging heterozygous mutations in NFKB1 lead to diverse immunologic phenotypes. *J Allergy Clin Immunol* (2017) 140(3):782–96. doi:10.1016/j.jaci.2016.10.054
135. Lougaris V, Patrizi O, Baronio M, Tabellini G, Tampella G, Damiati E, et al. NFKB1 regulates human NK cell maturation and effector functions. *Clin Immunol* (2017) 175:99–108. doi:10.1016/j.clim.2016.11.012
136. Schipp C, Nabhani S, Bienemann K, Simanovsky N, Kfir-Erenfeld S, Assayag-Asherie N, et al. Specific antibody deficiency and autoinflammatory disease extend the clinical and immunological spectrum of heterozygous NFKB1 loss-of-function mutations in humans. *Haematologica* (2016) 101(10):e392–6. doi:10.3324/haematol.2016.145136
137. Lougaris V, Moratto D, Baronio M, Tampella G, van der Meer JWM, Badolato R, et al. Early and late B-cell developmental impairment in nuclear factor kappa B, subunit 1-mutated common variable immunodeficiency disease. *J Allergy Clin Immunol* (2017) 139(1):349–52.e1. doi:10.1016/j.jaci.2016.05.045
138. Chen K, Coonrod EM, Kumánovics A, Franks ZF, Durtschi JD, Margraf RL, et al. Germline mutations in NFKB2 implicate the noncanonical NF-κB pathway in the pathogenesis of common variable immunodeficiency. *Am J Hum Genet* (2013) 93(5):812–24. doi:10.1016/j.ajhg.2013.09.009
139. Lee CE, Fulcher DA, Whittle B, Chand R, Fewings N, Field M, et al. Autosomal-dominant B-cell deficiency with alopecia due to a mutation in NFKB2 that results in nonprocessable p100. *Blood* (2014) 124(19):2964–72. doi:10.1182/blood-2014-06-578542
140. Lindsley AW, Qian Y, Valencia CA, Shah K, Zhang K, Assa'ad A. Combined immune deficiency in a patient with a novel NFKB2 mutation. *J Clin Immunol* (2014) 34(8):910–5. doi:10.1007/s10875-014-0095-3
141. Lougaris V, Tabellini G, Vitali M, Baronio M, Patrizi O, Tampella G, et al. Defective natural killer-cell cytotoxic activity in NFKB2-mutated CVID-like disease. *J Allergy Clin Immunol* (2015) 135(6):1641–3. doi:10.1016/j.jaci.2014.11.038
142. Montin D, Licciardi F, Giorgio E, Ciolfi A, Pizzi S, Mussa A, et al. Functional evaluation of natural killer cell cytotoxic activity in NFKB2-mutated patients. *Immunol Lett* (2018) 194:40–3. doi:10.1016/j.imlet.2017.12.006
143. Brue T, Quentien MH, Khetchoumian K, Bensa M, Capo-Chichi JM, Delemer B, et al. Mutations in NFKB2 and potential genetic heterogeneity

in patients with DAVID syndrome, having variable endocrine and immune deficiencies. *BMC Med Genet* (2014) 15:139. doi:10.1186/s12881-014-0139-9

144. Kuehn HS, Niemela JE, Sreedhara K, Stoddard JL, Grossman J, Wysocki CA, et al. Novel nonsense gain-of-function *NFKB2* mutations associated with a combined immunodeficiency phenotype. *Blood* (2017) 130(13):1553–64. doi:10.1182/blood-2017-05-782177
145. Lal RA, Bachrach LK, Hoffman AR, Inlora J, Rego S, Snyder MP, et al. A case report of hypoglycemia and hypogammaglobulinemia: DAVID syndrome in a patient with a novel NFKB2 mutation. *J Clin Endocrinol Metab* (2017) 102(7):2127–30. doi:10.1210/jc.2017-00341
146. Liu Y, Hanson S, Gurugama P, Jones A, Clark B, Ibrahim MA. Novel NFKB2 mutation in early-onset CVID. *J Clin Immunol* (2014) 34(6):686–90. doi:10.1007/s10875-014-0064-x
147. Ramakrishnan KA, Rae W, Barcenas-Morales G, Gao Y, Pengelly RJ, Patel SV, et al. Anticytokine autoantibodies in a patient with a heterozygous NFKB2 mutation. *J Allergy Clin Immunol* (2017) 141(4):1479–82.e6. doi:10.1016/j.jaci.2017.11.014
148. Shi C, Wang F, Tong A, Zhang XQ, Song HM, Liu ZY, et al. NFKB2 mutation in common variable immunodeficiency and isolated adrenocorticotropic hormone deficiency: a case report and review of literature. *Medicine (Baltimore)* (2016) 95(40):e5081. doi:10.1097/MD.0000000000005081
149. Perez de Diego R, Sanchez-Ramon S, Lopez-Collazo E, Martinez-Barricarte R, Cubillos-Zapata C, Ferreira Cerdan A, et al. Genetic errors of the human caspase recruitment domain-B-cell lymphoma 10-mucosa-associated lymphoid tissue lymphoma-translocation gene 1 (CBM) complex: molecular, immunologic, and clinical heterogeneity. *J Allergy Clin Immunol* (2015) 136(5):1139–49. doi:10.1016/j.jaci.2015.06.031
150. Turvey SE, Durandy A, Fischer A, Fung SY, Geha RS, Gewies A, et al. The CARD11-BCL10-MALT1 (CBM) signalosome complex: stepping into the limelight of human primary immunodeficiency. *J Allergy Clin Immunol* (2014) 134(2):276–84. doi:10.1016/j.jaci.2014.06.015
151. Meininger I, Krappmann D. Lymphocyte signaling and activation by the CARMA1-BCL10-MALT1 signalosome. *Biol Chem* (2016) 397(12):1315–33. doi:10.1515/hsz-2016-0216
152. Snow AL, Xiao W, Stinson JR, Lu W, Chaigne-Delalande B, Zheng L, et al. Congenital B cell lymphocytosis explained by novel germline CARD11 mutations. *J Exp Med* (2012) 209(12):2247–61. doi:10.1084/jem.20120831
153. Greil J, Rausch T, Giese T, Bandapalli OR, Daniel V, Bekeredjian-Ding I, et al. Whole-exome sequencing links caspase recruitment domain 11 (CARD11) inactivation to severe combined immunodeficiency. *J Allergy Clin Immunol* (2013) 131(5):1376–83.e3. doi:10.1016/j.jaci.2013.02.012
154. Stepensky P, Keller B, Buchta M, Kienzler AK, Elpeleg O, Somech R, et al. Deficiency of caspase recruitment domain family, member 11 (CARD11), causes profound combined immunodeficiency in human subjects. *J Allergy Clin Immunol* (2013) 131(2):477–85.e1. doi:10.1016/j.jaci.2012.11.050
155. Buchbinder D, Stinson JR, Nugent DJ, Heurtier L, Suarez F, Sukumar G, et al. Mild B-cell lymphocytosis in patients with a CARD11 C49Y mutation. *J Allergy Clin Immunol* (2015) 136(3):819–21.e1. doi:10.1016/j.jaci.2015.03.008
156. Brohl AS, Stinson JR, Su HC, Badgett T, Jennings CD, Sukumar G, et al. Germline CARD11 mutation in a patient with severe congenital B cell lymphocytosis. *J Clin Immunol* (2015) 35(1):32–46. doi:10.1007/s10875-014-0106-4
157. Fuchs S, Rensing-Ehl A, Pannicke U, Lorenz MR, Fisch P, Jeelall Y, et al. Omenn syndrome associated with a functional reversion due to a somatic second-site mutation in CARD11 deficiency. *Blood* (2015) 126(14):1658–69. doi:10.1182/blood-2015-03-631374
158. Outinen T, Syrjanen J, Rounioja S, Saarela J, Kaustio M, Helminen M. Constant B cell lymphocytosis since early age in a patient with CARD11 mutation: a 20-year follow-up. *Clin Immunol* (2016) 165:19–20. doi:10.1016/j.clim.2016.02.002
159. Ma CA, Stinson JR, Zhang Y, Abbott JK, Weinreich MA, Hauk PJ, et al. Germline hypomorphic CARD11 mutations in severe atopic disease. *Nat Genet* (2017) 49(8):1192–201. doi:10.1038/ng.3898
160. Dadi H, Jones TA, Merico D, Sharfe N, Ovadia A, Schejter Y, et al. Combined immunodeficiency and atopy caused by a dominant negative mutation in caspase activation and recruitment domain family member 11 (CARD11). *J Allergy Clin Immunol* (2017) 141(5):1818–30.e2. doi:10.1016/j.jaci.2017.06.047
161. Torres JM, Martinez-Barricarte R, Garcia-Gomez S, Mazariegos MS, Itan Y, Boisson B, et al. Inherited BCL10 deficiency impairs hematopoietic and non-hematopoietic immunity. *J Clin Invest* (2014) 124(12):5239–48. doi:10.1172/JCI77493
162. Jabara HH, Ohsumi T, Chou J, Massaad MJ, Benson H, Megarbane A, et al. A homozygous mucosa-associated lymphoid tissue 1 (MALT1) mutation in a family with combined immunodeficiency. *J Allergy Clin Immunol* (2013) 132(1):151–8. doi:10.1016/j.jaci.2013.04.047
163. McKinnon ML, Rozmus J, Fung SY, Hirschfeld AF, Del Bel KL, Thomas L, et al. Combined immunodeficiency associated with homozygous MALT1 mutations. *J Allergy Clin Immunol* (2014) 133(5):1458–62, 62.e1–7. doi:10.1016/j.jaci.2013.10.045
164. Punwani D, Wang H, Chan AY, Cowan MJ, Mallott J, Sunderam U, et al. Combined immunodeficiency due to MALT1 mutations, treated by hematopoietic cell transplantation. *J Clin Immunol* (2015) 35(2):135–46. doi:10.1007/s10875-014-0125-1
165. Charbit-Henrion F, Jeverica AK, Begue B, Markelj G, Parlato M, Avcin SL, et al. Deficiency in mucosa-associated lymphoid tissue lymphoma translocation 1: a novel cause of IPEX-like syndrome. *J Pediatr Gastroenterol Nutr* (2017) 64(3):378–84. doi:10.1097/MPG.0000000000001262
166. Kuehn HS, Ouyang W, Lo B, Deenick EK, Niemela JE, Avery DT, et al. Immune dysregulation in human subjects with heterozygous germline mutations in CTLA4. *Science* (2014) 345(6204):1623–7. doi:10.1126/science.1255904
167. Schubert D, Bode C, Kenefeck R, Hou TZ, Wing JB, Kennedy A, et al. Autosomal dominant immune dysregulation syndrome in humans with CTLA4 mutations. *Nat Med* (2014) 20(12):1410–6. doi:10.1038/nm.3746
168. Tivol EA, Borriello F, Schweitzer AN, Lynch WP, Bluestone JA, Sharpe AH. Loss of CTLA-4 leads to massive lymphoproliferation and fatal multiorgan tissue destruction, revealing a critical negative regulatory role of CTLA-4. *Immunity* (1995) 3(5):541–7. doi:10.1016/1074-7613(95)90125-6
169. Zeissig S, Petersen BS, Tomczak M, Melum E, Huc-Claustre E, Dougan SK, et al. Early-onset Crohn's disease and autoimmunity associated with a variant in CTLA-4. *Gut* (2015) 64(12):1889–97. doi:10.1136/gutjnl-2014-308541
170. Hayakawa S, Okada S, Tsumura M, Sakata S, Ueno Y, Imai K, et al. A patient with CTLA-4 haploinsufficiency presenting gastric cancer. *J Clin Immunol* (2016) 36(1):28–32. doi:10.1007/s10875-015-0221-x
171. Lee S, Moon JS, Lee CR, Kim HE, Baek SM, Hwang S, et al. Abatacept alleviates severe autoimmune symptoms in a patient carrying a de novo variant in CTLA-4. *J Allergy Clin Immunol* (2016) 137(1):327–30. doi:10.1016/j.jaci.2015.08.036
172. Shields CL, Say EA, Mashayekhi A, Garg SJ, Dunn JP, Shields JA. Assessment of CTLA-4 deficiency-related autoimmune choroidopathy response to abatacept. *JAMA Ophthalmol* (2016) 134(7):844–6. doi:10.1001/jamaophthalmol.2016.1013
173. Slatter MA, Engelhardt KR, Burroughs LM, Arkwright PD, Nademi Z, Skoda-Smith S, et al. Hematopoietic stem cell transplantation for CTLA4 deficiency. *J Allergy Clin Immunol* (2016) 138(2):615–9.e1. doi:10.1016/j.jaci.2016.01.045
174. Alroqi FJ, Charbonnier LM, Baris S, Kiykim A, Chou J, Platt CD, et al. Exaggerated follicular helper T-cell responses in patients with LRBA deficiency caused by failure of CTLA4-mediated regulation. *J Allergy Clin Immunol* (2017) 141(3):1050–9.e10. doi:10.1016/j.jaci.2017.05.022
175. Greil C, Roether F, La Rosee P, Grimbacher B, Duerschmied D, Warnatz K. Rescue of cytokine storm due to HLH by hemoadsorption in a CTLA4-deficient patient. *J Clin Immunol* (2017) 37(3):273–6. doi:10.1007/s10875-017-0377-7
176. Kucuk ZY, Charbonnier LM, McMasters RL, Chatila T, Bleesing JJ. CTLA-4 haploinsufficiency in a patient with an autoimmune lymphoproliferative disorder. *J Allergy Clin Immunol* (2017) 140(3):862–4.e4. doi:10.1016/j.jaci.2017.02.032
177. Moraes-Fontes MF, Hsu AP, Caramalho I, Martins C, Araujo AC, Lourenco F, et al. Fatal CTLA-4 heterozygosity with autoimmunity and recurrent infections: a de novo mutation. *Clin Case Rep* (2017) 5(12):2066–70. doi:10.1002/ccr3.1257
178. Navarini AA, Hruz P, Berger CT, Hou TZ, Schwab C, Gabrysch A, et al. Vedolizumab as a successful treatment of CTLA-4-associated autoimmune

enterocolitis. *J Allergy Clin Immunol* (2017) 139(3):1043–6.e5. doi:10.1016/j.jaci.2016.08.042
179. Tegtmeyer D, Seidl M, Gerner P, Baumann U, Klemann C. Inflammatory bowel disease caused by primary immunodeficiencies-clinical presentations, review of literature, and proposal of a rational diagnostic algorithm. *Pediatr Allergy Immunol* (2017) 28(5):412–29. doi:10.1111/pai.12734
180. Besnard C, Levy E, Aladjidi N, Stolzenberg MC, Magerus-Chatinet A, Alibeu O, et al. Pediatric-onset Evans syndrome: heterogeneous presentation and high frequency of monogenic disorders including LRBA and CTLA4 mutations. *Clin Immunol* (2018) 188:52–7. doi:10.1016/j.clim.2017.12.009
181. Hou TZ, Olbrich P, Soto JML, Sanchez B, Moreno PS, Borte S, et al. Study of an extended family with CTLA-4 deficiency suggests a CD28/CTLA-4 independent mechanism responsible for differences in disease manifestations and severity. *Clin Immunol* (2018) 188:94–102. doi:10.1016/j.clim.2018.01.001
182. Lo B, Zhang K, Lu W, Zheng L, Zhang Q, Kanellopoulou C, et al. AUTOIMMUNE DISEASE. Patients with LRBA deficiency show CTLA4 loss and immune dysregulation responsive to abatacept therapy. *Science* (2015) 349(6246):436–40. doi:10.1126/science.aaa1663
183. Lopez-Herrera G, Tampella G, Pan-Hammarstrom Q, Herholz P, Trujillo-Vargas CM, Phadwal K, et al. Deleterious mutations in LRBA are associated with a syndrome of immune deficiency and autoimmunity. *Am J Hum Genet* (2012) 90(6):986–1001. doi:10.1016/j.ajhg.2012.04.015
184. Gamez-Diaz L, August D, Stepensky P, Revel-Vilk S, Seidel MG, Noriko M, et al. The extended phenotype of LPS-responsive beige-like anchor protein (LRBA) deficiency. *J Allergy Clin Immunol* (2016) 137(1):223–30. doi:10.1016/j.jaci.2015.09.025
185. Alangari A, Alsultan A, Adly N, Massaad MJ, Kiani IS, Aljebreen A, et al. LPS-responsive beige-like anchor (LRBA) gene mutation in a family with inflammatory bowel disease and combined immunodeficiency. *J Allergy Clin Immunol* (2012) 130(2):481–8.e2. doi:10.1016/j.jaci.2012.05.043
186. Serwas NK, Kansu A, Santos-Valente E, Kuloglu Z, Demir A, Yaman A, et al. Atypical manifestation of LRBA deficiency with predominant IBD-like phenotype. *Inflamm Bowel Dis* (2015) 21(1):40–7. doi:10.1097/MIB.0000000000000266
187. Alkhairy OK, Abolhassani H, Rezaei N, Fang M, Andersen KK, Chavoshzadeh Z, et al. Spectrum of phenotypes associated with mutations in LRBA. *J Clin Immunol* (2016) 36(1):33–45. doi:10.1007/s10875-015-0224-7
188. Lo B, Fritz JM, Su HC, Uzel G, Jordan MB, Lenardo MJ. CHAI and LATAIE: new genetic diseases of CTLA-4 checkpoint insufficiency. *Blood* (2016) 128(8):1037–42. doi:10.1182/blood-2016-04-712612
189. Park MY, Sudan R, Srivastava N, Neelam S, Youngs C, Wang JW, et al. LRBA is essential for allogeneic responses in bone marrow transplantation. *Sci Rep* (2016) 6:36568. doi:10.1038/srep36568
190. Burns SO, Zenner HL, Plagnol V, Curtis J, Mok K, Eisenhut M, et al. LRBA gene deletion in a patient presenting with autoimmunity without hypogammaglobulinemia. *J Allergy Clin Immunol* (2012) 130(6):1428–32. doi:10.1016/j.jaci.2012.07.035
191. Charbonnier LM, Janssen E, Chou J, Ohsumi TK, Keles S, Hsu JT, et al. Regulatory T-cell deficiency and immune dysregulation, polyendocrinopathy, enteropathy, X-linked-like disorder caused by loss-of-function mutations in LRBA. *J Allergy Clin Immunol* (2015) 135(1):217–27. doi:10.1016/j.jaci.2014.10.019
192. Revel-Vilk S, Fischer U, Keller B, Nabhani S, Gamez-Diaz L, Rensing-Ehl A, et al. Autoimmune lymphoproliferative syndrome-like disease in patients with LRBA mutation. *Clin Immunol* (2015) 159(1):84–92. doi:10.1016/j.clim.2015.04.007
193. Seidel MG, Hirschmugl T, Gamez-Diaz L, Schwinger W, Serwas N, Deutschmann A, et al. Long-term remission after allogeneic hematopoietic stem cell transplantation in LPS-responsive beige-like anchor (LRBA) deficiency. *J Allergy Clin Immunol* (2015) 135(5):1384–90.e1–8. doi:10.1016/j.jaci.2014.10.048
194. Bakhtiar S, Ruemmele F, Charbit-Henrion F, Lévy E, Rieux-Laucat F, Cerf-Bensussan N, et al. Atypical manifestation of LPS-responsive beige-like anchor deficiency syndrome as an autoimmune endocrine disorder without enteropathy and immunodeficiency. *Front Pediatr* (2016) 4:98. doi:10.3389/fped.2016.00098
195. Lévy E, Stolzenberg MC, Bruneau J, Breton S, Neven B, Sauvion S, et al. LRBA deficiency with autoimmunity and early onset chronic erosive polyarthritis. *Clin Immunol* (2016) 168:88–93. doi:10.1016/j.clim.2016.03.006
196. Schreiner F, Plamper M, Dueker G, Schoenberger S, Gámez-Díaz L, Grimbacher B, et al. Infancy-onset T1DM, short stature, and severe immunodysregulation in two siblings with a homozygous LRBA mutation. *J Clin Endocrinol Metab* (2016) 101(3):898–904. doi:10.1210/jc.2015-3382
197. Shokri S, Nabavi M, Hirschmugl T, Aghamohammadi A, Arshi S, Bemanian MH, et al. LPS-responsive beige-like anchor gene mutation associated with possible bronchiolitis obliterans organizing pneumonia associated with hypogammaglobulinemia and normal IgM phenotype and low number of B cells. *Acta Med Iran* (2016) 54(10):620–3.
198. Tesi B, Priftakis P, Lindgren F, Chiang SC, Kartalis N, Löfstedt A, et al. Successful hematopoietic stem cell transplantation in a patient with LPS-responsive beige-like anchor (LRBA) gene mutation. *J Clin Immunol* (2016) 36(5):480–9. doi:10.1007/s10875-016-0289-y
199. Azizi G, Abolhassani H, Yazdani R, Mohammadikhajehdehi S, Parvaneh N, Negahdari B, et al. New therapeutic approach by sirolimus for enteropathy treatment in patients with LRBA deficiency. *Eur Ann Allergy Clin Immunol* (2017) 49(5):235–9. doi:10.23822/EurAnnACI.1764-1489.22
200. Bakhtiar S, Gámez-Díaz L, Jarisch A, Soerensen J, Grimbacher B, Belohradsky B, et al. Treatment of infantile inflammatory bowel disease and autoimmunity by allogeneic stem cell transplantation in LPS-responsive beige-like anchor deficiency. *Front Immunol* (2017) 8:52. doi:10.3389/fimmu.2017.00052
201. Kostel Bal S, Haskologlu S, Serwas NK, Islamoglu C, Aytekin C, Kendirli T, et al. Multiple presentations of LRBA deficiency: a single-center experience. *J Clin Immunol* (2017) 37(8):790–800. doi:10.1007/s10875-017-0446-y
202. Bratanič N, Kovač J, Pohar K, Trebušak Podkrajšek K, Ihan A, Battelino T, et al. Multifocal gastric adenocarcinoma in a patient with LRBA deficiency. *Orphanet J Rare Dis* (2017) 12(1):131. doi:10.1186/s13023-017-0682-5
203. Johnson MB, De Franco E, Lango Allen H, Al Senani A, Elbarbary N, Siklar Z, et al. Recessively inherited *LRBA* mutations cause autoimmunity presenting as neonatal diabetes. *Diabetes* (2017) 66(8):2316–22. doi:10.2337/db17-0040
204. Kammermeier J, Dziubak R, Pescarin M, Drury S, Godwin H, Reeve K, et al. Phenotypic and genotypic characterisation of inflammatory bowel disease presenting before the age of 2 years. *J Crohns Colitis* (2017) 11(1):60–9. doi:10.1093/ecco-jcc/jjw118
205. Seidel MG, Bohm K, Dogu F, Worth A, Thrasher A, Florkin B, et al. Treatment of severe forms of LPS-responsive beige-like anchor protein deficiency with allogeneic hematopoietic stem cell transplantation. *J Allergy Clin Immunol* (2018) 141(2):770–5.e1. doi:10.1016/j.jaci.2017.04.023
206. Şıklar Z, de Franco E, Johnson MB, Flanagan SE, Ellard S, Ceylaner S, et al. Monogenic diabetes not caused by mutations in mody genes: a very heterogenous group of diabetes. *Exp Clin Endocrinol Diabetes* (2017). doi:10.1055/s-0043-120571
207. Al Sukaiti N, AbdelRahman K, AlShekaili J, Al Oraimi S, Al Sinani A, Al Rahbi N, et al. Agammaglobulinaemia despite terminal B-cell differentiation in a patient with a novel *LRBA* mutation. *Clin Transl Immunology* (2017) 6(5):e144. doi:10.1038/cti.2017.20
208. Vairo FP, Boczek NJ, Cousin MA, Kaiwar C, Blackburn PR, Conboy E, et al. The prevalence of diseases caused by lysosome-related genes in a cohort of undiagnosed patients. *Mol Genet Metab Rep* (2017) 13:46–51. doi:10.1016/j.ymgmr.2017.08.001
209. Ye Z, Zhou Y, Huang Y, Wang Y, Lu J, Tang Z, et al. Phenotype and management of infantile-onset inflammatory bowel disease: experience from a tertiary care center in China. *Inflamm Bowel Dis* (2017) 23(12):2154–64. doi:10.1097/MIB.0000000000001269
210. Sari S, Dogu F, Hwa V, Haskologlu S, Dauber A, Rosenfeld R, et al. A successful HSCT in a girl with novel LRBA mutation with refractory celiac disease. *J Clin Immunol* (2016) 36(1):8–11. doi:10.1007/s10875-015-0220-y
211. Dickinson RE, Griffin H, Bigley V, Reynard LN, Hussain R, Haniffa M, et al. Exome sequencing identifies GATA-2 mutation as the cause of dendritic cell, monocyte, B and NK lymphoid deficiency. *Blood* (2011) 118(10):2656–8. doi:10.1182/blood-2011-06-360313
212. Hahn CN, Chong CE, Carmichael CL, Wilkins EJ, Brautigan PJ, Li XC, et al. Heritable GATA2 mutations associated with familial myelodysplastic syndrome and acute myeloid leukemia. *Nat Genet* (2011) 43(10):1012–7. doi:10.1038/ng.913

213. Hsu AP, Sampaio EP, Khan J, Calvo KR, Lemieux JE, Patel SY, et al. Mutations in GATA2 are associated with the autosomal dominant and sporadic monocytopenia and mycobacterial infection (MonoMAC) syndrome. *Blood* (2011) 118(10):2653–5. doi:10.1182/blood-2011-05-356352
214. Ostergaard P, Simpson MA, Connell FC, Steward CG, Brice G, Woollard WJ, et al. Mutations in GATA2 cause primary lymphedema associated with a predisposition to acute myeloid leukemia (Emberger syndrome). *Nat Genet* (2011) 43(10):929–31. doi:10.1038/ng.923
215. Bresnick EH, Katsumura KR, Lee HY, Johnson KD, Perkins AS. Master regulatory GATA transcription factors: mechanistic principles and emerging links to hematologic malignancies. *Nucleic Acids Res* (2012) 40(13):5819–31. doi:10.1093/nar/gks281
216. Vinh DC, Patel SY, Uzel G, Anderson VL, Freeman AF, Olivier KN, et al. Autosomal dominant and sporadic monocytopenia with susceptibility to mycobacteria, fungi, papillomaviruses, and myelodysplasia. *Blood* (2010) 115(8):1519–29. doi:10.1182/blood-2009-03-208629
217. Bigley V, Haniffa M, Doulatov S, Wang XN, Dickinson R, McGovern N, et al. The human syndrome of dendritic cell, monocyte, B and NK lymphoid deficiency. *J Exp Med* (2011) 208(2):227–34. doi:10.1084/jem.20101459
218. Mansour S, Connell F, Steward C, Ostergaard P, Brice G, Smithson S, et al. Emberger syndrome-primary lymphedema with myelodysplasia: report of seven new cases. *Am J Med Genet A* (2010) 152A(9):2287–96. doi:10.1002/ajmg.a.33445
219. Owen C, Barnett M, Fitzgibbon J. Familial myelodysplasia and acute myeloid leukaemia – a review. *Br J Haematol* (2008) 140(2):123–32. doi:10.1111/j.1365-2141.2007.06909.x
220. Dickinson RE, Milne P, Jardine L, Zandi S, Swierczek SI, McGovern N, et al. The evolution of cellular deficiency in GATA2 mutation. *Blood* (2014) 123(6):863–74. doi:10.1182/blood-2013-07-517151
221. Spinner MA, Sanchez LA, Hsu AP, Shaw PA, Zerbe CS, Calvo KR, et al. GATA2 deficiency: a protean disorder of hematopoiesis, lymphatics, and immunity. *Blood* (2014) 123(6):809–21. doi:10.1182/blood-2013-07-515528
222. Kazenwadel J, Secker GA, Liu YJ, Rosenfeld JA, Wildin RS, Cuellar-Rodriguez J, et al. Loss-of-function germline GATA2 mutations in patients with MDS/AML or MonoMAC syndrome and primary lymphedema reveal a key role for GATA2 in the lymphatic vasculature. *Blood* (2012) 119(5):1283–91. doi:10.1182/blood-2011-08-374363
223. Ballerie A, Nimubona S, Meunier C, Gutierrez FL, Desrues B, Delaval P, et al. Association of pulmonary alveolar proteinosis and fibrosis: patient with GATA2 deficiency. *Eur Respir J* (2016) 48(5):1510–4. doi:10.1183/13993003.00252-2016
224. Sanges S, Prévotat A, Fertin M, Terriou L, Lefèvre G, Quesnel B, et al. Haemodynamically proven pulmonary hypertension in a patient with GATA2 deficiency-associated pulmonary alveolar proteinosis and fibrosis. *Eur Respir J* (2017) 49(5):1700407. doi:10.1183/13993003.00178-2017
225. Wlodarski MW, Hirabayashi S, Pastor V, Starý J, Hasle H, Masetti R, et al. Prevalence, clinical characteristics, and prognosis of GATA2-related myelodysplastic syndromes in children and adolescents. *Blood* (2016) 127(11):1387–97; quiz 1518. doi:10.1182/blood-2015-09-669937
226. Ganapathi KA, Townsley DM, Hsu AP, Arthur DC, Zerbe CS, Cuellar-Rodriguez J, et al. GATA2 deficiency-associated bone marrow disorder differs from idiopathic aplastic anemia. *Blood* (2015) 125(1):56–70. doi:10.1182/blood-2014-06-580340
227. Cuellar-Rodriguez J, Gea-Banacloche J, Freeman AF, Hsu AP, Zerbe CS, Calvo KR, et al. Successful allogeneic hematopoietic stem cell transplantation for GATA2 deficiency. *Blood* (2011) 118(13):3715–20. doi:10.1182/blood-2011-06-365049
228. Plagnol V, Curtis J, Epstein M, Mok KY, Stebbings E, Grigoriadou S, et al. A robust model for read count data in exome sequencing experiments and implications for copy number variant calling. *Bioinformatics* (2012) 28(21):2747–54. doi:10.1093/bioinformatics/bts526
229. Pasquet M, Bellanné-Chantelot C, Tavitian S, Prade N, Beaupain B, Larochelle O, et al. High frequency of GATA2 mutations in patients with mild chronic neutropenia evolving to MonoMac syndrome, myelodysplasia, and acute myeloid leukemia. *Blood* (2013) 121(5):822–9. doi:10.1182/blood-2012-08-447367
230. Camargo JF, Lobo SA, Hsu AP, Zerbe CS, Wormser GP, Holland SM. MonoMAC syndrome in a patient with a GATA2 mutation: case report and review of the literature. *Clin Infect Dis* (2013) 57(5):697–9. doi:10.1093/cid/cit368
231. Hsu AP, Johnson KD, Falcone EL, Sanalkumar R, Sanchez L, Hickstein DD, et al. GATA2 haploinsufficiency caused by mutations in a conserved intronic element leads to MonoMAC syndrome. *Blood* (2013) 121(19):3830–7, S1–7. doi:10.1182/blood-2012-08-452763
232. Chou J, Lutskiy M, Tsitsikov E, Notarangelo LD, Geha RS, Dioun A. Presence of hypogammaglobulinemia and abnormal antibody responses in GATA2 deficiency. *J Allergy Clin Immunol* (2014) 134(1):223–6. doi:10.1016/j.jaci.2014.01.041
233. Fujiwara T, Fukuhara N, Funayama R, Nariai N, Kamata M, Nagashima T, et al. Identification of acquired mutations by whole-genome sequencing in GATA-2 deficiency evolving into myelodysplasia and acute leukemia. *Ann Hematol* (2014) 93(9):1515–22. doi:10.1007/s00277-014-2090-4
234. West ES, Kingsbery MY, Mintz EM, Hsu AP, Holland SM, Rady PL, et al. Generalized verrucosis in a patient with GATA2 deficiency. *Br J Dermatol* (2014) 170(5):1182–6. doi:10.1111/bjd.12794
235. Griese M, Zarbock R, Costabel U, Hildebrandt J, Theegarten D, Albert M, et al. GATA2 deficiency in children and adults with severe pulmonary alveolar proteinosis and hematologic disorders. *BMC Pulm Med* (2015) 15:87. doi:10.1186/s12890-015-0083-2
236. Brondfield S, Reid M, Patel K, Ten R, Dhaliwal G. Disseminated mycobacterial infection and scabies infestation. *Am J Med* (2015) 128(10):e41–2. doi:10.1016/j.amjmed.2015.06.008
237. Maciejewski-Duval A, Meuris F, Bignon A, Aknin ML, Balabanian K, Faivre L, et al. Altered chemotactic response to CXCL12 in patients carrying GATA2 mutations. *J Leukoc Biol* (2016) 99(6):1065–76. doi:10.1189/jlb.5MA0815-388R
238. Crall C, Morley KW, Rabinowits G, Schmidt B, Dioun Broyles A, Huang JT. Merkel cell carcinoma in a patient with GATA2 deficiency: a novel association with primary immunodeficiency. *Br J Dermatol* (2016) 174(1):169–71. doi:10.1111/bjd.14062
239. Koegel AK, Hofmann I, Moffitt K, Degar B, Duncan C, Tubman VN. Acute lymphoblastic leukemia in a patient with MonoMAC syndrome/GATA2 haploinsufficiency. *Pediatr Blood Cancer* (2016) 63(10):1844–7. doi:10.1002/pbc.26084
240. Cohen JI, Dropulic L, Hsu AP, Zerbe CS, Krogmann T, Dowdell K, et al. Association of GATA2 deficiency with severe primary Epstein-Barr virus (EBV) infection and EBV-associated cancers. *Clin Infect Dis* (2016) 63(1):41–7. doi:10.1093/cid/ciw160
241. Vila A, Dapás JI, Rivero CV, Bocanegra F, Furnari RF, Hsu AP, et al. Multiple opportunistic infections in a woman with GATA2 mutation. *Int J Infect Dis* (2017) 54:89–91. doi:10.1016/j.ijid.2016.11.408
242. Shah NN, Parta M, Baird K, Rafei H, Cole K, Holland SM, et al. Monozygotic twins with GATA2 deficiency: same haploidentical-related donor, different severity of GvHD. *Bone Marrow Transplant* (2017) 52(11):1580–2. doi:10.1038/bmt.2017.180
243. Schlums H, Jung M, Han H, Theorell J, Bigley V, Chiang SC, et al. Adaptive NK cells can persist in patients with. *Blood* (2017) 129(14):1927–39. doi:10.1182/blood-2016-08-734236
244. Johnson KD, Hsu AP, Ryu MJ, Wang J, Gao X, Boyer ME, et al. Cis-element mutated in GATA2-dependent immunodeficiency governs hematopoiesis and vascular integrity. *J Clin Invest* (2012) 122(10):3692–704. doi:10.1172/JCI61623
245. Ishida H, Imai K, Honma K, Tamura S, Imamura T, Ito M, et al. GATA-2 anomaly and clinical phenotype of a sporadic case of lymphedema, dendritic cell, monocyte, B- and NK-cell (DCML) deficiency, and myelodysplasia. *Eur J Pediatr* (2012) 171(8):1273–6. doi:10.1007/s00431-012-1715-7
246. Bödör C, Renneville A, Smith M, Charazac A, Iqbal S, Etancelin P, et al. Germline GATA2 p.THR354MET mutation in familial myelodysplastic syndrome with acquired monosomy 7 and ASXL1 mutation demonstrating rapid onset and poor survival. *Haematologica* (2012) 97(6):890–4. doi:10.3324/haematol.2011.054361
247. Holme H, Hossain U, Kirwan M, Walne A, Vulliamy T, Dokal I. Marked genetic heterogeneity in familial myelodysplasia/acute myeloid leukaemia. *Br J Haematol* (2012) 158(2):242–8. doi:10.1111/j.1365-2141.2012.09136.x

248. Mutsaers PG, van de Loosdrecht AA, Tawana K, Bödör C, Fitzgibbon J, Menko FH. Highly variable clinical manifestations in a large family with a novel GATA2 mutation. *Leukemia* (2013) 27(11):2247–8. doi:10.1038/leu.2013.105
249. Mace EM, Hsu AP, Monaco-Shawver L, Makedonas G, Rosen JB, Dropulic L, et al. Mutations in GATA2 cause human NK cell deficiency with specific loss of the CD56(bright) subset. *Blood* (2013) 121(14):2669–77. doi:10.1182/blood-2012-09-453969
250. Maeurer M, Magalhaes I, Andersson J, Ljungman P, Sandholm E, Uhlin M, et al. Allogeneic hematopoietic cell transplantation for GATA2 deficiency in a patient with disseminated human papillomavirus disease. *Transplantation* (2014) 98(12):e95–6. doi:10.1097/TP.0000000000000520
251. Gao J, Gentzler RD, Timms AE, Horwitz MS, Frankfurt O, Altman JK, et al. Heritable GATA2 mutations associated with familial AML-MDS: a case report and review of literature. *J Hematol Oncol* (2014) 7:36. doi:10.1186/1756-8722-7-36
252. Lübking A, Vosberg S, Konstandin NP, Dufour A, Graf A, Krebs S, et al. Young woman with mild bone marrow dysplasia, GATA2 and ASXL1 mutation treated with allogeneic hematopoietic stem cell transplantation. *Leuk Res Rep* (2015) 4(2):72–5. doi:10.1016/j.lrr.2015.10.001
253. Wang X, Muramatsu H, Okuno Y, Sakaguchi H, Yoshida K, Kawashima N, et al. GATA2 and secondary mutations in familial myelodysplastic syndromes and pediatric myeloid malignancies. *Haematologica* (2015) 100(10):e398–401. doi:10.3324/haematol.2015.127092
254. Cortés-Lavaud X, Landecho MF, Maicas M, Urquiza L, Merino J, Moreno-Miralles I, et al. GATA2 germline mutations impair GATA2 transcription, causing haploinsufficiency: functional analysis of the p.Arg396Gln mutation. *J Immunol* (2015) 194(5):2190–8. doi:10.4049/jimmunol.1401868
255. Mir MA, Kochuparambil ST, Abraham RS, Rodriguez V, Howard M, Hsu AP, et al. Spectrum of myeloid neoplasms and immune deficiency associated with germline GATA2 mutations. *Cancer Med* (2015) 4(4):490–9. doi:10.1002/cam4.384
256. Churpek JE, Pyrtel K, Kanchi KL, Shao J, Koboldt D, Miller CA, et al. Genomic analysis of germ line and somatic variants in familial myelodysplasia/acute myeloid leukemia. *Blood* (2015) 126(22):2484–90. doi:10.1182/blood-2015-04-641100
257. Svobodova T, Mejstrikova E, Salzer U, Sukova M, Hubacek P, Matej R, et al. Diffuse parenchymal lung disease as first clinical manifestation of GATA-2 deficiency in childhood. *BMC Pulm Med* (2015) 15:8. doi:10.1186/s12890-015-0006-2
258. Zhang MY, Keel SB, Walsh T, Lee MK, Gulsuner S, Watts AC, et al. Genomic analysis of bone marrow failure and myelodysplastic syndromes reveals phenotypic and diagnostic complexity. *Haematologica* (2015) 100(1):42–8. doi:10.3324/haematol.2014.113456
259. Spinner MA, Ker JP, Stoudenmire CJ, Fadare O, Mace EM, Orange JS, et al. GATA2 deficiency underlying severe blastomycosis and fatal herpes simplex virus-associated hemophagocytic lymphohistiocytosis. *J Allergy Clin Immunol* (2016) 137(2):638–40. doi:10.1016/j.jaci.2015.07.043
260. Delgado-Márquez AM, Zarco C, Ruiz R, Simarro A, Vanaclocha F. Severe disseminated primary herpes simplex infection as skin manifestation of GATA2 deficiency. *J Eur Acad Dermatol Venereol* (2016) 30(7):1248–50. doi:10.1111/jdv.13183
261. Mallhi K, Dix DB, Niederhoffer KY, Armstrong L, Rozmus J. Successful umbilical cord blood hematopoietic stem cell transplantation in pediatric patients with MDS/AML associated with underlying GATA2 mutations: two case reports and review of literature. *Pediatr Transplant* (2016) 20(7):1004–7. doi:10.1111/petr.12764
262. Seo SK, Kim KY, Han SA, Yoon JS, Shin SY, Sohn SK, et al. First Korean case of Emberger syndrome (primary lymphedema with myelodysplasia) with a novel GATA2 gene mutation. *Korean J Intern Med* (2016) 31(1):188–90. doi:10.3904/kjim.2016.31.1.188
263. Saida S, Umeda K, Yasumi T, Matsumoto A, Kato I, Hiramatsu H, et al. Successful reduced-intensity stem cell transplantation for GATA2 deficiency before progression of advanced MDS. *Pediatr Transplant* (2016) 20(2):333–6. doi:10.1111/petr.12667
264. Buchbinder D, Sassoon A. You GATA look at the marrow! *Blood* (2016) 128(4):603. doi:10.1182/blood-2016-04-712547
265. Keel SB, Scott A, Sanchez-Bonilla M, Ho PA, Gulsuner S, Pritchard CC, et al. Genetic features of myelodysplastic syndrome and aplastic anemia in pediatric and young adult patients. *Haematologica* (2016) 101(11):1343–50. doi:10.3324/haematol.2016.149476
266. Ciullini Mannurita S, Vignoli M, Colarusso G, Tucci F, Veltroni M, Frenos S, et al. Timely follow-up of a GATA2 deficiency patient allows successful treatment. *J Allergy Clin Immunol* (2016) 138(5):1480–3.e4. doi:10.1016/j.jaci.2016.06.004
267. Nováková M, Žaliová M, Suková M, Wlodarski M, Janda A, Froňková E, et al. Loss of B cells and their precursors is the most constant feature of GATA-2 deficiency in childhood myelodysplastic syndrome. *Haematologica* (2016) 101(6):707–16. doi:10.3324/haematol.2015.137711
268. Ding LW, Ikezoe T, Tan KT, Mori M, Mayakonda A, Chien W, et al. Mutational profiling of a MonoMAC syndrome family with GATA2 deficiency. *Leukemia* (2017) 31(1):244–5. doi:10.1038/leu.2016.256
269. Kurata T, Shigemura T, Muramatsu H, Okuno Y, Nakazawa Y. A case of GATA2-related myelodysplastic syndrome with unbalanced translocation der(1;7)(q10;p10). *Pediatr Blood Cancer* (2017) 64(8):e26419. doi:10.1002/pbc.26419
270. Rastogi N, Abraham RS, Chadha R, Thakkar D, Kohli S, Nivargi S, et al. Successful nonmyeloablative allogeneic stem cell transplant in a child with emberger syndrome and GATA2 mutation. *J Pediatr Hematol Oncol* (2017). doi:10.1097/MPH.0000000000000995
271. Kuriyama Y, Hattori M, Mitsui T, Nakano H, Oikawa D, Tokunaga F, et al. Generalized verrucosis caused by various human papillomaviruses in a patient with GATA2 deficiency. *J Dermatol* (2017) 45(5):e108–9. doi:10.1111/1346-8138.14149
272. Álvarez-Chinchilla P, Poveda I, Marco FM, López-Fernández JA, Peiro G, Illán F, et al. Vulvar lymphedema and refractory VIN-III heralding GATA2 deficiency syndrome. *Eur J Obstet Gynecol Reprod Biol* (2017) 218:138–40. doi:10.1016/j.ejogrb.2017.09.016
273. Hamadou WS, Mani R, Besbes S, Bourdon V, Youssef YB, Eisinger F, et al. GATA2 gene analysis in several forms of hematological malignancies including familial aggregations. *Ann Hematol* (2017) 96(10):1635–9. doi:10.1007/s00277-017-3076-9
274. Polat A, Dinulescu M, Fraitag S, Nimubona S, Toutain F, Jouneau S, et al. Skin manifestations among GATA2-deficient patients. *Br J Dermatol* (2017) 178(3):781–5. doi:10.1111/bjd.15548
275. Dorn JM, Patnaik MS, Van Hee M, Smith MJ, Lagerstedt SA, Newman CC, et al. WILD syndrome is GATA2 deficiency: a novel deletion in the GATA2 gene. *J Allergy Clin Immunol Pract* (2017) 5(4):1149–52.e1. doi:10.1016/j.jaip.2017.02.010
276. Brambila-Tapia AJL, García-Ortiz JE, Brouillard P, Nguyen HL, Vikkula M, Ríos-González BE, et al. GATA2 null mutation associated with incomplete penetrance in a family with Emberger syndrome. *Hematology* (2017) 22(8):467–71. doi:10.1080/10245332.2017.1294551
277. Perez Botero J, Rodriguez V. Primary lymphedema and viral warts in GATA2 haploinsufficiency. *Mayo Clin Proc* (2017) 92(3):482. doi:10.1016/j.mayocp.2016.12.003
278. Berry D, Fekrat S. Central retinal vein occlusion in GATA2 deficiency. *Retin Cases Brief Rep* (2017). doi:10.1097/ICB.0000000000000558
279. Ramzan M, Lowry J, Courtney S, Krueger J, Schechter Finkelstein T, Ali M. Successful myeloablative matched unrelated donor hematopoietic stem cell transplantation in a young girl with GATA2 deficiency and Emberger syndrome. *J Pediatr Hematol Oncol* (2017) 39(3):230–2. doi:10.1097/MPH.0000000000000737
280. González-Lara MF, Wisniowski-Yáñez A, Pérez-Patrigeon S, Hsu AP, Holland SM, Cuellar-Rodríguez JM. Pneumocystis jiroveci pneumonia and GATA2 deficiency: expanding the spectrum of the disease. *J Infect* (2017) 74(4):425–7. doi:10.1016/j.jinf.2017.01.005
281. Burroughs LM, Shimamura A, Talano JA, Domm JA, Baker KK, Delaney C, et al. Allogeneic hematopoietic cell transplantation using treosulfan-based conditioning for treatment of marrow failure disorders. *Biol Blood Marrow Transplant* (2017) 23(10):1669–77. doi:10.1016/j.bbmt.2017.06.002
282. Ruiz-García R, Rodríguez-Vigil C, Marco FM, Gallego-Bustos F, Castro-Panete MJ, Diez-Alonso L, et al. Acquired senescent T-cell phenotype correlates with clinical severity in GATA binding protein 2-deficient patients. *Front Immunol* (2017) 8:802. doi:10.3389/fimmu.2017.00802
283. Kazamel M, Klein CJ, Benarroch EE, Patnaik MM, Tracy JA. Subacute demyelinating polyradiculoneuropathy complicating Epstein-Barr virus infection

in GATA2 haploinsufficiency. *Muscle Nerve* (2018) 57(1):150–6. doi:10.1002/mus.25581
284. Azevedo L, Jay A, Lorenzana A, Keel S, Abraham RS, Horwitz M, et al. Case report of an adolescent male with unexplained pancytopenia: GATA-2 associated bone marrow failure and genetic testing. *Glob Pediatr Health* (2017) 4:1–4. doi:10.1177/2333794X17744947
285. Tholouli E, Sturgess K, Dickinson RE, Gennery A, Cant AJ, Jackson G, et al. In vivo T-depleted reduced-intensity transplantation for. *Blood* (2018) 131(12):1383–7. doi:10.1182/blood-2017-10-811489
286. Alazami AM, Al-Helale M, Alhissi S, Al-Saud B, Alajlan H, Monies D, et al. Novel CARMIL2 mutations in patients with variable clinical dermatitis, infections, and combined immunodeficiency. *Front Immunol* (2018) 9:203. doi:10.3389/fimmu.2018.00203
287. Schober T, Magg T, Laschinger M, Rohlfs M, Linhares ND, Puchalka J, et al. A human immunodeficiency syndrome caused by mutations in CARMIL2. *Nat Commun* (2017) 8:14209. doi:10.1038/ncomms14209
288. Sorte HS, Osnes LT, Fevang B, Aukrust P, Erichsen HC, Backe PH, et al. A potential founder variant in CARMIL2/RLTPR in three Norwegian families with warts, molluscum contagiosum, and T-cell dysfunction. *Mol Genet Genomic Med* (2016) 4(6):604–16. doi:10.1002/mgg3.237
289. Wang Y, Ma CS, Ling Y, Bousfiha A, Camcioglu Y, Jacquot S, et al. Dual T cell- and B cell-intrinsic deficiency in humans with biallelic RLTPR mutations. *J Exp Med* (2016) 213(11):2413–35. doi:10.1084/jem.20160576
290. Roncagalli R, Cucchetti M, Jarmuzynski N, Gregoire C, Bergot E, Audebert S, et al. The scaffolding function of the RLTPR protein explains its essential role for CD28 co-stimulation in mouse and human T cells. *J Exp Med* (2016) 213(11):2437–57. doi:10.1084/jem.20160579
291. Sassi A, Lazaroski S, Wu G, Haslam SM, Fliegauf M, Mellouli F, et al. Hypomorphic homozygous mutations in phosphoglucomutase 3 (PGM3) impair immunity and increase serum IgE levels. *J Allergy Clin Immunol* (2014) 133(5):e1–13. doi:10.1016/j.jaci.2014.02.025
292. Zhang Y, Yu X, Ichikawa M, Lyons JJ, Datta S, Lamborn IT, et al. Autosomal recessive phosphoglucomutase 3 (PGM3) mutations link glycosylation defects to atopy, immune deficiency, autoimmunity, and neurocognitive impairment. *J Allergy Clin Immunol* (2014) 133(5):1400–9, 9.e1–5. doi:10.1016/j.jaci.2014.02.013
293. Pang H, Koda Y, Soejima M, Kimura H. Identification of human phosphoglucomutase 3 (PGM3) as N-acetylglucosamine-phosphate mutase (AGM1). *Ann Hum Genet* (2002) 66(Pt 2):139–44. doi:10.1046/j.1469-1809.2002.00103.x
294. Monticelli M, Ferro T, Jaeken J, Dos Reis Ferreira V, Videira PA. Immunological aspects of congenital disorders of glycosylation (CDG): a review. *J Inherit Metab Dis* (2016) 39(6):765–80. doi:10.1007/s10545-016-9954-9
295. Stray-Pedersen A, Backe PH, Sorte HS, Morkrid L, Chokshi NY, Erichsen HC, et al. PGM3 mutations cause a congenital disorder of glycosylation with severe immunodeficiency and skeletal dysplasia. *Am J Hum Genet* (2014) 95(1):96–107. doi:10.1016/j.ajhg.2014.05.007
296. Ben-Khemis L, Mekki N, Ben-Mustapha I, Rouault K, Mellouli F, Khemiri M, et al. A founder mutation underlies a severe form of phosphoglutamase 3 (PGM3) deficiency in Tunisian patients. *Mol Immunol* (2017) 90:57–63. doi:10.1016/j.molimm.2017.06.248
297. Pacheco-Cuellar G, Gauthier J, Desilets V, Lachance C, Lemire-Girard M, Rypens F, et al. A novel PGM3 mutation is associated with a severe phenotype of bone marrow failure, severe combined immunodeficiency, skeletal dysplasia, and congenital malformations. *J Bone Miner Res* (2017) 32(9):1853–9. doi:10.1002/jbmr.3173
298. Bernth-Jensen JM, Holm M, Christiansen M. Neonatal-onset T(-)B(-)NK(+) severe combined immunodeficiency and neutropenia caused by mutated phosphoglucomutase 3. *J Allergy Clin Immunol* (2016) 137(1):321–4. doi:10.1016/j.jaci.2015.07.047
299. Lundin KE, Hamasy A, Backe PH, Moens LN, Falk-Sorqvist E, Elgstoen KB, et al. Susceptibility to infections, without concomitant hyper-IgE, reported in 1976, is caused by hypomorphic mutation in the phosphoglucomutase 3 (PGM3) gene. *Clin Immunol* (2015) 161(2):366–72. doi:10.1016/j.clim.2015.10.002
300. Moens LN, Falk-Sorqvist E, Asplund AC, Bernatowska E, Smith CI, Nilsson M. Diagnostics of primary immunodeficiency diseases: a sequencing capture approach. *PLoS One* (2014) 9(12):e114901. doi:10.1371/journal.pone.0114901
301. Bjorksten B, Lundmark KM. Recurrent bacterial infections in four siblings with neutropenia, eosinophilia, hyperimmunoglobulinemia A, and defective neutrophil chemotaxis. *J Infect Dis* (1976) 133(1):63–71. doi:10.1093/infdis/133.1.63
302. Ali YA, Rahman S, Bhat V, Al Thani S, Ismail A, Bassiouny I. Hereditary multiple intestinal atresia (HMIA) with severe combined immunodeficiency (SCID): a case report of two siblings and review of the literature on MIA, HMIA and HMIA with immunodeficiency over the last 50 years. *BMJ Case Rep* (2011) 2011:1–7. doi:10.1136/bcr.05.2010.3031
303. Chen R, Giliani S, Lanzi G, Mias GI, Lonardi S, Dobbs K, et al. Whole-exome sequencing identifies tetratricopeptide repeat domain 7A (TTC7A) mutations for combined immunodeficiency with intestinal atresias. *J Allergy Clin Immunol* (2013) 132(3):656–64.e17. doi:10.1016/j.jaci.2013.06.013
304. Samuels ME, Majewski J, Alirezaie N, Fernandez I, Casals F, Patey N, et al. Exome sequencing identifies mutations in the gene TTC7A in French-Canadian cases with hereditary multiple intestinal atresia. *J Med Genet* (2013) 50(5):324–9. doi:10.1136/jmedgenet-2012-101483
305. Notarangelo LD. Multiple intestinal atresia with combined immune deficiency. *Curr Opin Pediatr* (2014) 26(6):690–6. doi:10.1097/MOP.0000000000000159
306. Agarwal NS, Northrop L, Anyane-Yeboa K, Aggarwal VS, Nagy PL, Demirdag YY. Tetratricopeptide repeat domain 7A (TTC7A) mutation in a newborn with multiple intestinal atresia and combined immunodeficiency. *J Clin Immunol* (2014) 34(6):607–10. doi:10.1007/s10875-014-0067-7
307. Avitzur Y, Guo C, Mastropaolo LA, Bahrami E, Chen H, Zhao Z, et al. Mutations in tetratricopeptide repeat domain 7A result in a severe form of very early onset inflammatory bowel disease. *Gastroenterology* (2014) 146(4):1028–39. doi:10.1053/j.gastro.2014.01.015
308. Bigorgne AE, Farin HF, Lemoine R, Mahlaoui N, Lambert N, Gil M, et al. TTC7A mutations disrupt intestinal epithelial apicobasal polarity. *J Clin Invest* (2014) 124(1):328–37. doi:10.1172/JCI71471
309. Fernandez I, Patey N, Marchand V, Birlea M, Maranda B, Haddad E, et al. Multiple intestinal atresia with combined immune deficiency related to TTC7A defect is a multiorgan pathology: study of a French-Canadian-based cohort. *Medicine (Baltimore)* (2014) 93(29):e327. doi:10.1097/MD.0000000000000327
310. Guanà R, Garofano S, Teruzzi E, Vinardi S, Carbonaro G, Cerrina A, et al. The complex surgical management of the first case of severe combined immunodeficiency and multiple intestinal atresias surviving after the fourth year of life. *Pediatr Gastroenterol Hepatol Nutr* (2014) 17(4):257–62. doi:10.5223/pghn.2014.17.4.257
311. Lemoine R, Bigorgne A, Farin H, Saint Basile G. [TTC7A, a critical effector for the intestinal and immune system homeostasis]. *Med Sci (Paris)* (2014) 30(6–7):616–8. doi:10.1051/medsci/20143006006
312. Woutsas S, Aytekin C, Salzer E, Conde CD, Apaydin S, Pichler H, et al. Hypomorphic mutation in TTC7A causes combined immunodeficiency with mild structural intestinal defects. *Blood* (2015) 125(10):1674–6. doi:10.1182/blood-2014-08-595397
313. Yang W, Lee PP, Thong MK, Ramanujam TM, Shanmugam A, Koh MT, et al. Compound heterozygous mutations in TTC7A cause familial multiple intestinal atresias and severe combined immunodeficiency. *Clin Genet* (2015) 88(6):542–9. doi:10.1111/cge.12553
314. Kammermeier J, Lucchini G, Pai SY, Worth A, Rampling D, Amrolia P, et al. Stem cell transplantation for tetratricopeptide repeat domain 7A deficiency: long-term follow-up. *Blood* (2016) 128(9):1306–8. doi:10.1182/blood-2016-01-696385
315. Lawless D, Mistry A, Wood PM, Stahlschmidt J, Arumugakani G, Hull M, et al. Bialellic mutations in tetratricopeptide repeat domain 7A (TTC7A) cause common variable immunodeficiency-like phenotype with enteropathy. *J Clin Immunol* (2017) 37(7):617–22. doi:10.1007/s10875-017-0427-1
316. Lien R, Lin YF, Lai MW, Weng HY, Wu RC, Jaing TH, et al. Novel mutations of the tetratricopeptide repeat domain 7A gene and phenotype/genotype comparison. *Front Immunol* (2017) 8:1066. doi:10.3389/fimmu.2017.01066
317. Neves JF, Afonso I, Borrego L, Martins C, Cordeiro AI, Neves C, et al. Missense mutation of TTC7A mimicking tricho-hepato-enteric (SD/THE) syndrome in a patient with very-early onset inflammatory bowel disease. *Eur J Med Genet* (2018) 61(4):185–8. doi:10.1016/j.ejmg.2017.11.014

318. Conti F, Lugo-Reyes SO, Blancas Galicia L, He J, Aksu G, Borges de Oliveira E Jr, et al. Mycobacterial disease in patients with chronic granulomatous disease: a retrospective analysis of 71 cases. *J Allergy Clin Immunol* (2016) 138(1):241–8.e3. doi:10.1016/j.jaci.2015.11.041
319. de Beaucoudrey L, Samarina A, Bustamante J, Cobat A, Boisson-Dupuis S, Feinberg J, et al. Revisiting human IL-12Rbeta1 deficiency: a survey of 141 patients from 30 countries. *Medicine (Baltimore)* (2010) 89(6):381–402. doi:10.1097/MD.0b013e3181fdd832
320. Aaby P, Roth A, Ravn H, Napirna BM, Rodrigues A, Lisse IM, et al. Randomized trial of BCG vaccination at birth to low-birth-weight children: beneficial nonspecific effects in the neonatal period? *J Infect Dis* (2011) 204(2):245–52. doi:10.1093/infdis/jir240
321. Medical Advisory Committee of the Immune Deficiency Foundation, Shearer WT, Fleisher TA, Buckley RH, Ballas Z, Ballow M, et al. Recommendations for live viral and bacterial vaccines in immunodeficient patients and their close contacts. *J Allergy Clin Immunol* (2014) 133(4):961–6. doi:10.1016/j.jaci.2013.11.043

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A Critical View of Specific Antibody Deficiencies

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In thisopinion manuscript the author postulates that the present definition of Specific Antibody Deficiency (SAD) needs to be revised and expanded. It is presently defined as a syndrome of low IgG antibody responses to purified *Streptococcus pneumoniae* capsular polysaccharides vaccines with normal concentrations of IgG, IgA, IgM, and IgG subclasses. Responses to protein antigens and to pneumococcal polysaccharides conjugated to proteins are normal (1, 2). Severe, moderate and memory SAD forms have been arbitrarily defined by expert committees. These definitions miss the imperfections of a diagnosis based on mainly poorly standardized laboratory tests and curtail the search for many other clinically relevant specific deficiencies of antibodies to bacterial, viral and fungal pathogens.

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THE LABORATORY TESTS ON WHICH THE DIAGNOSES OF SAD ARE BASED ARE VARIABLE AND IMPERFECT

The assessment of IgG antibodies against *S. pneumonia* serotype-specific capsular vaccine antigens are widely used to diagnose a patient's ability to develop IgG antibody responses. Available vaccines include a 23-valent purified capsular polysaccharide vaccine that includes the 23 most prevalent serotypes (PPV-23) and several polysaccharide-protein conjugated vaccines that include antibiotic resistant serotypes. These are a 7 serotypes vaccine (PCV-7) that was replaced by a 13 serotypes vaccine (PCV-13) and a different 10-valent vaccine (PCV-10). The use of both purified polysaccharide and combined protein-polysaccharide vaccines places evaluation of anti-pneumococcal immunity among the most valuable tools used to assess an important component of antibody-mediated immunity.

Different laboratory methods are used to define normal and abnormal antibody responses. The diagnosis of SAD is given to patients older than 2 years with recurrent infections who fail to mount an arbitrarily defined normal response to purified pneumococcal serotypes (3, 4). If the patient was immunized with PCV-7, PCV-13, or PCV-10, the diagnosis of SAD is only possible based on the response to serotypes not present in conjugate vaccines. A response to conjugated serotypes does not preclude unresponsiveness to pure polysaccharide antigens. Correlation of the extent of antibody deficiencies and clinical presentation is poor, the diagnostic methods used at the present time are unreliable, and having low responses as defined for SAD does not necessarily lead to recurrent infections (5).

The methods used to measure anti-pneumococcal, serotype-specific IgG antibodies that confer long-term protection are summarized in **Table 1** (6, 7).

The World Health Organization ELISA (WHO, ELISA) is well-standardized and reproducible. It defines a specific way of performing this test. It was developed under the auspices of the World Health Organization, to evaluate conjugate vaccine effectiveness. Results are expressed as μg/ml

TABLE 1 | Methods to measure IgG anti-S. *pneumonia* surface polysaccharide antibodies.

Test features	WHO ELISA	Luminex®	Global test
Performance	Cumbersome, in few, mostly research laboratories	Easier and faster. Widely used in Commercial laboratories	Easier and cheaper to perform, used in many parts of world
Serotype-specific antibodies measured	Limited, variable 12–16 serotypes	Usually to each of 23 serotypes in PPV- 23	All 23 serotypes used as one antigen
Reproducibility	High worldwide	Low among different laboratories and with WHO-ELISA	Questionable relationship to serotype-specific tests

WHO ELISA and Luminex® are pneumococcal serotype-specific antibody assays (PSSA) with results expressed as weight /volume.

after calibration against an FDA standard now replaced by 007sp and by laboratory standards (7).

An easier to perform method that simultaneously measures antibodies to each of the 23 serotypes in the PPV-23 is based on a multiplex fluorescent bead assay, Luminex®. It is used by most reference laboratories in the United States. Luminex results are consistent within laboratories but there are significant differences among laboratories and its correlation with ELISA test results is variable (7, 8).

A "global test" measures antibodies against all 23 serotypes in the 23-valent PPV as one antigen (9). The information obtained with the global test does not correlate well with results obtained when measuring antibodies against each serotype individually.

The only functional assay of anti-pneumococcal antibodies is opsonophagocytosis (OPA). It measures antibodies of all immunoglobulin classes against polysaccharide and protein antigens on the surface of intact bacteria. This test is not generally available for clinical use although vaccine manufacturers use it extensively when testing vaccine antigenicity.

There is not a strict relationship between the weight by volume antibody concentration results obtained by ELISA and OPA results (10). In clinical practice, there are patients who have normal ELISA titers but who improve clinically when given therapeutic IgG, suggesting that the lower function observed in the elderly (11) may also be present in some individuals at an earlier age.

When vaccines are used to evaluate a response to *S. pneumoniae* polysaccharides, interpretation of results is based on a combination of the following: (1) increase in specific antibody concentration over pre-immunization levels, (2) the final concentration of antibodies after immunization, and (3) the percentage of serotypes to which the patient developed an arbitrarily defined antibody concentration.

There are shortcomings with each of these criteria:

1. The requirement for a minimum two- and four-fold increase disregards the fact that high pre-immunization concentrations may not increase with immunization. The desired concentration to prove effective antibody production is subject to variable interpretation. If the value is set at 1.0 instead of 1.3 μg/ml, (12) the number of responses considered normal can differ, without proof that these differences are clinically significant.
2. The relevance of the percentages of serotypes inducing a given antibody concentration is also subject to interpretation. Different combinations of high, medium, and low antibodies can be seen in the same patient sample. The serotypes that may elicit these different antibody concentrations vary from patient to patient. It is therefore not surprising that attempts to identify a response to one or several selected serotypes as representative of the response to all or most serotypes included in the PPV-23 have failed. The possibility of using serotype-specific threshold values could be applicable for some defined, uniform populations (13). However, in a diverse clinical practice this kind of definition of antibody responses is unrealistic.

The history of prior immunization with *S. pneumonia* vaccines is essential in the interpretation of antibody measurement results. Responses to conjugate polysaccharides in PCV are considered T cell-dependent while responses to purified polysaccharides in the PPV do not involve T cell activation (14–18). The response to PCV matures earlier and is developed in the first months of life while the response to PPV is considered fully developed only by 2 years of age. Prior PCV immunization induces antibodies indistinguishable from those induced by purified polysaccharides**.** When using PPV to assess specific antibodies to *S. pneumoniae* polysaccharides in PCV immunized individuals, antibodies to PCV serotypes do not document a normal response to PPV. The additional response to PPV may be influenced by conditioning produced be the exposure to conjugate polysaccharides (19). Conversely, a response to PPV may occur even in patients who fail to respond to PCV, documenting that conjugate and purified polysaccharides induce antibodies through different activation pathways.

The injectable polysaccharide S. typhi vaccine (Typhi Vi) has been studied as an alternative to the PPV-23 to assess the polysaccharide response. Typhi Vi antibodies are usually absent in the population. Therefore, they reflect a new immune response but their results are not concordant with results of anti *S. Pneumonia* polysaccharides (20–22). Like other vaccines, the typhi does not test the same molecular pathways activated by *S. pneumonia* capsular polysaccharides.

THERE ARE DIFFERENT FORMS OF SPECIFIC ANTIBODY DEFICIENCIES

An experience-based list of abnormalities in the response to various forms of exposure to pneumococcal polysaccharides is presented below. Correctly identifying these patterns of anti-*S. pneumonia* polysaccharide responses in patients with increased susceptibility to infections allows the clinician to define patient-based management options.

The response patterns to conjugate and purified polysaccharide vaccines that suggest an immune dysfunction are as follows:

1. Deficient response to pure polysaccharides (SAD or PPV-SAD)
2. Deficient response to conjugate polysaccharides (PCV-SAD), frequent in children
3. Deficient response to PPV and PCV.

Options 2 and 3 are not accepted as an antibody deficiency syndrome in present disease classification

For each of the two kinds of vaccine polysaccharide antigens, the abnormality may be:

- Absent response to any serotype tested Low antibody concentration (below 1.3 μg/ml in the USA. Other cut-off values are used in different regions of the world).
- Incomplete antibody repertoire. Protective titers to <50–80% of serotypes. This could be a strong response to only 1 serotype.
- Poor memory after adequate response to immunization.
- Deficient opsonophagocytosis. Serological response may appear normal, but antibodies are not protective.

*All these situations may be the only detected immune abnormality or part of other immune abnormalities. They may also be present in children and adults who do not experience any form of severe or recurrent infections.

The multiplicity of immunological phenotypes and conditions in which a specific antibody deficiency can be observed suggests that different pathogenic mechanisms cause this defect. A lack of response is different from a deficient immunological memory capable of maintaining protective IgG concentrations. Antibodies against purified or conjugate polysaccharides do develop through different cellular pathways likely to be affected by different abnormalities (23, 24). Additionally, the biologic diversity of serotype-specific polysaccharides leads to very specific lack of responses to some polysaccharides in some individuals. The lack of responses to *S. pneumonia* polysaccharides may be linked to unresponsiveness to unrelated antigens as well. In some young patients the selective antibody deficiency may just represent an extension of an immunological status that is normal in infants (25).

Further variability in the pathogenesis of anti-polysaccharide antibody deficiencies is suggested by the large number of more general immunodeficiencies, such as IgG subclass deficiencies (26–30), and by the wide array of known immunodeficiency syndromes that may have normal IgG concentrations, and poor or absent polysaccharide responses. Examples are patients with asplenia, hyper-IgE syndrome, or selective IgA deficiency (31–36). Additionally, congenital molecular abnormalities like Bruton's tyrosine kinase deficiency, commonly associated with agammaglobulinemia, may have SAD (37). Similar unique associations between molecular abnormalities and deficient specific antibody responses are increasingly identified as evaluation of anti-*S. pneumoniae* antibodies has become part of the evaluation of patients with different forms of immunodeficiencies (38, 39).

VACCINES ARE IMPORTANT TREATMENT ELEMENTS

The management of abnormalities of the response to pneumococcal polysaccharides includes, foremost, an adequate identification of infections, infection complications and their impact on cost and quality of life. Based on the type of abnormality detected it is possible to use additional immunization, preventive antibiotics and, in some patients, the use of IgG replacement.

S. pneumoniae vaccines are a cost-effective part of treatment. Below are the recommended additional immunizations of patients with an identified abnormal antibody response to pneumococcal polysaccharides:

PPV non-responders (classic SAD) PCV 13 (29)

PCV-non-responders PPV (40)

Normally, unimmunized older children and adults have develop antibodies to most *S. pneumonia* serotypes in response to clinical and subclinical infections. Such unimmunized patients usually respond serologically and clinical to immunization with PCV-13 followed by PPV-23.

The use of PPV-23 in patients unresponsive to a full complement of conjugate vaccines is one of the most useful and cost effective form of treatment of this frequently observed condition. Estrada el al. observed that the PPV-23 vaccine serologically and clinically improved children who had failed to develop strong antibody responses and had recurrent infections despite a full complement of PCV vaccines (40). A general stimulating effect of immunity was also reported (41). Notably, it did not matter if the infections were caused by pneumococci, other bacterial pathogens, or respiratory viruses. Although the PPV-23 vaccine is recommended only after the second year of life, Tang et al. observed strong responses in unimmunized 12 month-olds (42). Personal observation confirms strong antibody responses to the PPV-23 in patients between 1 and 2 years of age. While not recommending this course of action, considering the large difference in cost of one dose of PPV-23 as opposed to several doses of the PCV-10 and PCV-13, in economically strapped situations or areas in which PCV is not available, the earlier use of PPV-23 may be advisable.

TREATMENT TRIALS WITH HUMAN GAMMAGLOBULIN SHOULD BE CONSIDERED IN SPECIAL CLINICAL CIRCUMSTANCES

In patients with recurrent infections and laboratory diagnosed forms of Specific Antibody Deficiencies, IgG replacement is an accepted treatment option. This could be used for a period of time in young children and probably for life in SAD forms detected in adolescents and adults.

It is also of note that patients without a clear immunoglobulin-deficiency syndrome or immunologically mild forms of SAD are very likely to experience a very significant reduction of infections and improvement in quality of life if they are treated with IgG replacement. IgG replacement should be based on a

complete assessment of the patient's condition and not only on the presence or absence of anti-pneumococcal antibodies (43). A therapeutic trial of 6–12 months of treatment with subcutaneous or intravenous IgG with a rigorous assessment of infections and quality of life is an option strongly recommended by this author. It is important, however, to use all available treatment approaches before resorting to IgG replacement.

In summary, our present diagnosis and treatment of specific antibody deficiencies need reevaluation and improvement. The most reliable evidence of a failure of antibody-mediated immunity is the continued proven presence of infections that improve with intensified treatment. Treatment can be with antibiotics in the case of bacterial infections or with human gammaglobulin when antibiotics fail or the infections are caused by viruses. Given the complexity of assessing true antibody function, rather than further attempts to define numeric criteria for the normality or deficiency of antibodies, it is important to develop a better definition of infections that signal a deficient antibody function. Acceptable criteria for therapeutic trials of IgG replacement of sufficient length and duration, and objective criteria to assess the patient's clinical response and the cost effectiveness of treatment need to be developed with the aim of helping patients and preventing the abuse of treatment with IgG.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

REFERENCES

1. Orange J, Ballow M, Stiehm E, Ballas ZK, Chinen J, De La Morena M, et al. Use and interpretation of diagnostic vaccination in primary immunodeficiency: a working group report of the Basic and Clinical Immunology Interest Section of the American Academy of Allergy, Asthma & Immunology. *J Allergy Clin Immunol.* (2012) 130:S1–24. doi: 10.1016/j.jaci.2012.07.002
2. Perez E, Orange J, Bonilla F, Chinen J, Chinn IK, Dorsey M, et al. Update on the use of immunoglobulin in human disease: a review of evidence. *J Allergy Clin Immunol.* (2017) 139:S1–46. doi: 10.1016/j.jaci.2016.09.023
3. Bonilla F, Barlan I, Chapel H, Costa-Carvalho B, Cunningham-Rundles C, de la Morena M, et al. International consensus document (ICON): common variable immunodeficiency disorders. *J Allergy Clin Immunol Pract.* (2016) 4:38–59. doi: 10.1016/j.jaip.2015.07.025
4. Bonilla F, Khan D, Ballas Z, Chinen J, Frank M, Hsu J, et al. Practice parameter for the diagnosis and management of primary immunodeficiency. *J Allergy Clin Immunol.* (2015) 136:1186–205.e1–78. doi: 10.1016/j.jaci.2015.04.049
5. van de Vosse E, van Ostaijen-Ten Dam MM, Vermaire R, Verhard EM, Waaijer JL, Bakker JA, et al. Recurrent respiratory tract infections (RRTI) in the elderly: a late onset mild immunodeficiency? *Clin Immunol.* (2017) 180:111–9. doi: 10.1016/j.clim.2017.05.008
6. Sorensen RU, Leiva LE. Measurement of pneumococcal polysaccharide antibodies. *J Clin Immunol.* (2014) 34:127–8. doi: 10.1007/s10875-013-9977-z
7. Sorensen RU, Edgar D. Specific antibody deficiencies in clinical practice. *J Allergy Clin Immunol Pract.* (2019) 7:801–8. doi: 10.1016/j.jaip.2019.01.024
8. Balloch A, Licciardi PV, Tang ML. Serotype-specific anti-pneumococcal IgG and immune competence: critical differences in interpretation criteria when different methods are used. *J Clin Immunol.* (2013) 33:335–41. doi: 10.1007/s10875-012-9806-9
9. Lopez B, Bahuaud M, Fieschi C, Mehlal S, Jeljeli M, Rogeau S, et al. Value of the overall pneumococcal polysaccharide response in the diagnosis of primary humoral immunodeficiencies. *Front Immunol.* (2017) 8:1862. doi: 10.3389/fimmu.2017.01862
10. Concepcion NF, Frasch CE. Pneumococcal type 22f polysaccharide absorption improves the specificity of a pneumococcal-polysaccharide enzyme-linked immunosorbent assay. *Clin Diagnos Lab Immunol.* (2001) 8:266–72. doi: 10.1128/CDLI.8.2.266-272.2001
11. Romero-Steiner S, Musher D, Cetron M, Pais L, Groover J, Fiore A, et al. Reduction in functional antibody activity against *Streptococcus pneumoniae* in vaccinated elderly individuals highly correlates with decreased IgG antibody avidity. *Clin Infect Dis.* (1999) 29:281–8. doi: 10.1086/520200
12. Hoffman T, van Kessel D, Rijkers G. Impact of using different response criteria for pneumococcal polysaccharide vaccination for assessment of humoral immune status. *J Clin Immunol.* (2018) 38:149–52. doi: 10.1007/s10875-017-0470-y
13. Schaballie H, Bosch B, Schrijvers R, Proesmans M, de Boeck K, Boon M, et al. Fifth percentile cutoff values for antipneumococcal polysaccharide and anti-salmonella typhi vi IgG describe a normal polysaccharide response. *Front Immunol.* (2017) 8:546. doi: 10.3389/fimmu.2017.00546
14. Uddin S, Borrow R, Haeney MR, Moran A, Warrington R, Balmer P, et al. Total and serotype-specific pneumococcal antibody titres in children with normal and abnormal humoral immunity. *Vaccine.* (2006) 24:5637–44. doi: 10.1016/j.vaccine.2006.03.088
15. Mond JJ, Lees A, Snapper CM. T cell-independent antigens type 2. *Annu Rev Immunol.* (1995) 13:655–92. doi: 10.1146/annurev.iy.13.040195.003255
16. Choo S, Seymour L, Morris R, Quataert S, Lockhart S, Cartwright K, et al. Immunogenicity and reactogenicity of a pneumococcal conjugate vaccine administered combined with a haemophilus influenzae type B conjugate vaccine in United Kingdom infants. *Pediatr Infect Dis J.* (2000) 19:854–62. doi: 10.1097/00006454-200009000-00009
17. Rose MA, Schubert R, Strnad N, Zielen S. Priming of immunological memory by pneumococcal conjugate vaccine in children unresponsive to 23-valent polysaccharide pneumococcal vaccine. *Clin Diagnos Lab Immunol.* (2005) 12:1216–22. doi: 10.1128/CDLI.12.10.1216-1222.2005
18. Bernth-Jensen JM, Sogaard OS. Polysaccharide responsiveness is not biased by prior pneumococcal-conjugate vaccination. *PLoS ONE.* (2013) 8:e75944. doi: 10.1371/journal.pone.0075944
19. Schaballie H, Wuyts G, Dillaerts D, Frans G, Moens L, Proesmans M, et al. Effect of previous vaccination with pneumococcal conjugate vaccine on pneumococcal polysaccharide vaccine antibody responses. *Clin Exp Immunol.* (2016) 185:180–9. doi: 10.1111/cei.12784
20. Sanchez-Ramon S, de Gracia J, Garcia-Alonso AM, Rodriguez Molina JJ, Melero J, de Andres A, et al. Multicenter study for the evaluation of the antibody response against salmonella typhi Vi vaccination (EMPATHY) for the diagnosis of Anti-polysaccharide antibody production deficiency in patients with primary immunodeficiency. *Clin Immunol.* (2016) 169:80–4. doi: 10.1016/j.clim.2016.05.006
21. Bausch-Jurken MT, Verbsky JW, Gonzaga KA, Elms NP, Hintermeyer MK, Gauld SB, et al. The use of salmonella typhim vaccine to diagnose antibody deficiency. *J Clin Immunol.* (2017) 37:427–33. doi: 10.1007/s10875-017-0406-6
22. Evans C, Bateman E, Steven R, Ponsford M, Cullinane A, Shenton C, et al. Measurement of Typhi Vi antibodies can be used to assess adaptive immunity in patients with immunodeficiency. *Clin Exp Immunol.* (2018) 192:292–301. doi: 10.1111/cei.13105
23. Siber GR. Pneumococcal disease: prospects for a new generation of vaccines. *Science.* (1994) 265:1385–7. doi: 10.1126/science.8073278
24. Pletz MW, Maus U, Krug N, Welte T, Lode H. Pneumococcal vaccines: mechanism of action, impact on epidemiology and adaption of the species. *Int J Antimicrob Agents.* (2008) 32:199–206. doi: 10.1016/j.ijantimicag.2008.01.021
25. Rijkers GT, Sanders LA, Zegers BJ. Anti-capsular polysaccharide antibody deficiency states. *Immunodeficiency.* (1993) 5:1–21.
26. Siber G, Schur P, Aisenberg A, Weitzman S, Schiffman G. Correlation between serum IgG-2 concentrations and the antibody

response to bacterial polysaccharide antigens. *N Engl J Med*. (1980) 303:178–82. doi: 10.1056/NEJM198007243030402
27. Lim PL, Lau YL. Occurrence of IgG subclass antibodies to ovalbumin, avidin, and pneumococcal polysaccharide in children. *Int Arch Allergy Immunol*. (1994) 104:137–43. doi: 10.1159/000236721
28. Barrett DJ, Ayoub EM. IgG2 subclass restriction of antibody to pneumococcal polysaccharides. *Clin Exp Immunol*. (1986) 63:127–34.
29. Sorensen RU, Leiva LE, Giangrosso PA, Butler B, Javier FC III, Sacerdote DM, et al. Response to a heptavalent conjugate *Streptococcus pneumoniae* vaccine in children with recurrent infections who are unresponsive to the polysaccharide vaccine. *Pediatr Infect Dis J*. (1998) 17:685–91. doi: 10.1097/00006454-199808000-00005
30. Sanders LA, Rijkers GT, Tenbergen-Meekes AM, Voorhorst-Ogink MM, Zegers BJ. Immunoglobulin isotype-specific antibody responses to pneumococcal polysaccharide vaccine in patients with recurrent bacterial respiratory tract infections. *Pediatr Res*. (1995) 37:812–9. doi: 10.1203/00006450-199506000-00023
31. Gross S, Blaiss MS, Herrod HG. Role of immunoglobulin subclasses and specific antibody determinations in the evaluation of recurrent infection in children. *J Pediatrics*. (1992) 121:516–22. doi: 10.1016/S0022-3476(05)81137-0
32. Sanders LA, Rijkers GT, Kuis W, Tenbergen-Meekes AJ, de Graeff-Meeder BR, Hiemstra I, et al. Defective antipneumococcal polysaccharide antibody response in children with recurrent respiratory tract infections. *J Allergy Clin Immunol*. (1993) 91(1 Pt 1):110–9. doi: 10.1016/0091-6749(93)90303-W
33. Zora J, Silk H, Tinkelman D. Evaluation of postimmunization pneumococcal titers in children with recurrent infections and normal levels of immunoglobulin. *Ann Allergy*. (1993) 70:283–8.
34. Knutsen A. Patients with IgG subclass and/or selective antibody deficiency to polysaccharide antigens: initiation of a controlled clinical trial of intravenous immune globulin. *J Allergy Clin Immunol*. (1989) 84:640–7. doi: 10.1016/0091-6749(89)90203-0
35. Herer B, Labrousse F, Mordelet-Dambrine M, Durandy A, Offredo-Hemmer C, Ekindjian O, et al. Selective IgG subclass deficiencies and antibody responses to pneumococcal capsular polysaccharide antigen in adult community-acquired pneumonia. *Am Rev Respir Dis*. (1990) 142:854–7. doi: 10.1164/ajrccm/142.4.854
36. Bernatowska-Matuszkiewicz E, Pac M, Pum M, Liszka K, Leibl H, Eibl M. IgG subclasses and antibody response topneumococcal capsular polysaccharides in children with severe sinopulmonary infections and asthma. *Immunol Invest*. (1991) 20:173–85. doi: 10.3109/08820139109050785
37. Wood P, Mayne A, Joyce H, Smith C, Granoff D, Kumararatne D. A mutation in Bruton's tyrosine kinase as a cause of selective anti-polysaccharide antibody deficiency. *J Pediatrics*. (2001) 139:148–51. doi: 10.1067/mpd.2001.115970
38. Zhao Y P-HmQ, Zhao Z, Wen S, Hammarström L. Selective IgG2 deficiency due to a point mutation causing abnormal splicing of the Cgamma2 gene. *Int Immunol*. (2005) 17:95–101. doi: 10.1093/intimm/dxh192
39. Wall LA, Dimitriades VR, Sorensen RU. Specific antibody deficiencies. *Immunol Allergy Clin North Am*. (2015) 35:659–70. doi: 10.1016/j.iac.2015.07.003
40. Estrada J, Najera M, Pounds N, Catano G, Infante A. Clinical and serologic response to the 23-valent polysaccharide pneumococcal vaccine in children and teens with recurrent upper respiratory tract infections and selective antibody deficiency. *Pediatr Infect Dis J*. (2016) 35:205–8. doi: 10.1097/INF.0000000000000964
41. Leiva L, Butler B, Hempe J, Ortigas A, Sorensen R. Up-regulation of CD40L and induction of a Th2 response in children immunized with pneumococcal polysaccharide vaccines. *Clin Diag Lab Immunol*. (2001) 8:233–40. doi: 10.1128/CDLI.8.2.233-240.2001
42. Balloch A, Licciardi P, Russell F, Mulholland EK, Tang ML. Infants aged 12 months can mount adequate serotype-specific IgG responses to pneumococcal polysaccharide vaccine. *J Allergy Clin Immunol*. (2010) 126:395–7. doi: 10.1016/j.jaci.2010.05.008
43. Edgar J, Richter A, Huissoon A, Kumararatne D, Baxendale H, Bethune C, et al. Prescribing immunoglobulin replacement therapy for patients with non-classical and secondary antibody deficiency: an analysis of the practice of clinical immunologists in the UK and Republic of Ireland. *J Clin Immunol*. (2018) 38:204–13. doi: 10.1007/s10875-017-0469-4

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