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Reviews in pediatric primary immunodeficiencies

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Reviews in pediatric primary immunodeficiencies

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Editorial: Reviews in pediatric primary immunodeficiencies

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Editorial on the Research Topic
Reviews in pediatric primary immunodeficiencies

Inborn errors of immunity (IEI) increase susceptibility to infections, severe allergic disorders, and malignancies due to deficiencies in specific immune components. The International Union of Immunological Societies Expert (IUIS) Committee updated the IEI classification, encompassing 555 IEIs and 17 phenocopies due to mutations in 504 genes (1). Autoinflammation is one of the ten main groups of immune system disorders in the IUIS classification and remains at the forefront of new discoveries, with the boundaries between immunodeficiencies and rheumatology becoming increasingly blurred. Autoinflammatory disorders (AIDs) are primarily driven by innate immune system abnormalities rather than autoimmune mechanisms. They are characterized by recurrent inflammatory episodes due to immune system dysfunction, with diverse underlying mechanisms. The most well-known pathways leading to autoinflammation include inflammasomopathies, relopathies, and interferonopathies (2). This issue provides an overview of childhood AIDs, where fever and skin manifestations are often prominent symptoms, detailing the clinical manifestations, pathophysiology, diagnosis, and management of each AID syndrome.

Patients with IEI often exhibit increased susceptibility to infections; however, not all are prone to *Staphylococcus aureus* infections (3). In this special issue, Kurz et al. examine the complex relationship between *Staphylococcus aureus* virulence and host immune susceptibility in *S. aureus*-susceptible patients. While the significance of neutrophil numbers and function is well established, the role of specific cytokines, such as functional interleukin (IL)-6 signaling, is less widely recognized. This review explores host-pathogen interactions in *S. aureus* infections in susceptible individuals, potentially paving the way for more effective management and preventive treatment strategies.

In patients with severe and recurrent infections, a minimal diagnostic workup for IEI includes a complete blood count and serum immunoglobulin levels. While antibody responses to protein antigens are commonly assessed through vaccine antibodies, anti-polysaccharide IgG antibodies are not routinely measured. This limitation can lead to significant delays in diagnosing monogenic IEI, which may initially present with an impaired IgG response to polysaccharide antigens, with or without IgG subclass deficiency (4). This issue highlights the importance of evaluating IgG responses to polysaccharide antigens to prevent delays in diagnosing immunodeficiency.

Chronic granulomatous disease (CGD) primarily results from inherited defects in components of the nicotinamide adenine dinucleotide phosphate oxidase enzyme

complex (5). The authors report the first case of CGD with a homozygous loss-of-function variant in the Cytochrome B-245 chaperone 1 gene (CYBC1) reported from Nepal, and draw attention to the bacterial and fungal infections that may be caused by this genetic disorder, as well as inflammatory manifestations (such as inflammatory bowel disease, acute pancreatitis, hemophagocytic lymphohistiocytosis, systemic granulomatosis).

The Ikaros family of transcription factors has important functions in immune regulation, lymphomagenesis, and the hypothalamic-pituitary axis. Ikaros family zinc finger 1 (IKZF1) is a family of hemopoietic-specific zinc finger proteins that play an essential regulatory role in multiple stages of B lymphocyte development (6). IKZF1 mutations lead to CVID, a severe B cell deficiency (7). In this issue, increasing evidence of pathophysiological genotype-phenotype correlations caused by this disorder due to 6 cases with IKZF1 defects helps us understand IKAROS-associated diseases.

Immunoglobulin (IG) replacement therapy is the standard of care for immunodeficiencies that result in impaired antibody production. Intravenous (IVIG) and subcutaneous (SCIG) administration routes are commonly used, with comparable efficacy (8). Highly purified human Ig preparations are administered to restore serum IgG levels to physiological concentrations, providing broad-spectrum polyclonal antibodies for immune support. Facilitated subcutaneous immunoglobulin (fSCIG) infusion, enhanced with recombinant human hyaluronidase (rHuPH20), is an innovative treatment approach that delivers large volumes with minimal needle insertions (9). Conventional SCIG (cSCIG) therapy typically involves weekly subcutaneous injections to prevent fluctuations in IgG threshold levels (10). Furthermore, highly concentrated IgG formulations (20%) enable the administration of the required dosage in smaller volumes than less concentrated products. These formulations can be infused quickly and in larger quantities at a single site, offering a more efficient and convenient alternative to traditional subcutaneous preparations (10). While many studies have compared the safety and efficacy of various Ig administration routes, there remains a need for more research on the incidence of infections in patients receiving IG replacement therapy via intravenous, subcutaneous, and facilitated subcutaneous routes.

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The author declares 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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Granulomatous inflammation in inborn errors of immunity

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Granulomas have been defined as inflammatory infiltrates formed by recruitment of macrophages and T cells. The three-dimensional spherical structure typically consists of a central core of tissue resident macrophages which may merge into multinucleated giant cells surrounded by T cells at the periphery. Granulomas may be triggered by infectious and non-infectious antigens. Cutaneous and visceral granulomas are common in inborn errors of immunity (IEI), particularly among patients with chronic granulomatous disease (CGD), combined immunodeficiency (CID), and common variable immunodeficiency (CVID). The estimated prevalence of granulomas in IEI ranges from 1%–4%. Infectious agents causing granulomas such Mycobacteria and Coccidioides presenting atypically may be 'sentinel' presentations for possible underlying immunodeficiency. Deep sequencing of granulomas in IEI has revealed non-classical antigens such as wild-type and RA27/3 vaccine-strain Rubella virus. Granulomas in IEI are associated with significant morbidity and mortality. The heterogeneity of granuloma presentation in IEI presents challenges for mechanistic approaches to treatment. In this review, we discuss the main infectious triggers for granulomas in IEI and the major forms of IEI presenting with 'idiopathic' non-infectious granulomas. We also discuss models to study granulomatous inflammation and the impact of deep-sequencing technology while searching for infectious triggers of granulomatous inflammation. We summarize the overarching goals of management and highlight the therapeutic options reported for specific granuloma presentations in IEI.

KEYWORDS

granuloma - etiology, combined immune deficiency, GLILD, rubella (MMR) vaccine, coccidiodomycosis, autoinflammatory and autoimmunological diseases

Introduction

Granulomas represent the result of an immune response induced by an encounter between antigen presenting cells (predominantly monocytes and macrophages) and T cells (both CD4 + and CD8+ T cells) (1, 2). In this regard, granulomas offer an opportunity to explore an interface between the innate and adaptive immune system. However, unlike lymph nodes there is no prerequisite anatomic scaffold for granulomas to develop. Classically, granulomas in mammals have been described as having a spherical structure consisting of a central core of tissue resident macrophages which may merge into multinucleated giant cells surrounded by T cells at the periphery (3). In some cases, the central core of the granulomas shows evidence of necrosis (caseating granulomas), while other forms of granulomas do not show this feature

Abbreviations

AT, ataxia-telangiectasia; BCG, Bacille Calmette-Guérin; CGD, chronic granulomatous disease; CID, combined immunodeficiency; CVID, common variable immunodeficiency; GLILD, granulomatous lung interstitial lung disease; HCST, hematopoietic stem cell transplantation; IEI, inborn errors of immunity; iVDRVs, immunodeficiency-related vaccine-derived rubella viruses; NTM, non-tuberculous mycobacteria; PLAID, phospholipase C gamma 2–associated antibody deficiency and immune dysregulation; RuV, Rubella virus.

(non-caseating granulomas). These two histological types are exemplified by granulomas occurring in the course of Mycobacterial infection and sarcoidosis, respectively (2, 4). Granulomas may be triggered by infectious and non-infectious antigens (5). Despite significant advances in imaging, histopathology, antimicrobials, and immunomodulation, granulomatous inflammation is a cause for morbidity and mortality in both adults and pediatric patients, particularly as described in common variable immunodeficiency (CVID) (6). Inborn errors of immunity (IEIs) provide a unique insight into the pathophysiology of granulomas and immune susceptibility to specific pathogens that trigger granulomatous inflammation. Tuberculous and non-tuberculous Mycobacterial infection (7) and fungal infections (8) presenting with granulomas, for example, may uncover new forms of IEI. Recently patients with disseminated Coccidioidomycosis, a dimorphic fungal infection endemic to the Southwestern United States, have helped to identify novel variants in genes affecting the immune function and inform on fungal pathogenesis (8). Rubella virus (including the attenuated live vaccine strain) is usually well controlled by the immune system in healthy individuals; however, it has been frequently identified in granulomas of patients with various forms of combined immunodeficiency (CID) (9) and has been associated with significant morbidity and mortality in IEIs (10). In a section of this review, we will highlight the current understanding of the role of infectious agents mentioned and how they interface in the development of granulomas in patients with IEIs.

Furthermore, various IEIs present with granulomas of presumed non-infectious origin involving skin and other organs. The overall prevalence of presumed non-infections granulomas in patients with IEI is estimated to be 1 to 4% with the highest prevalence being among CVID, CID, and CGD (11). In a retrospective single center pediatric study from Turkey, among 82 patients with granulomas who underwent immunological evaluation, 62 carried a diagnosis of IEI. CID was the most common diagnosis and hypogammaglobulinemia was present in 50% of the subjects (12). Granulomas have also been described in autoinflammatory diseases and primary atopic disorders (13).

In this review we will discuss updates in understanding IEIs and variants affecting host defense and predisposing to presumably non-infectious granulomas. The focus of the review will center on non-infectious granulomatous inflammation in CVID, CID, CGD and autoinflammatory disorders. We will highlight the role of infectious agents such mycobacteria and Coccidioides as 'sentinel' granulomatous infectious to assist with further workup of possible underlying immunodeficiency. Further, we explore the recent data pertaining to the identification of Rubella virus vaccine-strain in IEI granulomas and discuss how this discovery could shed further mechanistic insights into granuloma pathogenesis.

Granulomatous inflammation in CVID

CVID is a clinical diagnosis characterized by severe and/or recurrent oto-sino-pulmonary infections, low serum immunoglobulins and impaired vaccine responses (14, 15). Non-infectious complications of CVID, more than infectious history are associated with decreased CVID survival (6). Granulomas in lungs, liver, spleen, lymph nodes and skin were identified in 46 CVID patients within a cohort of 473 subjects (9.7%) (6). A systematic review by van Stigt et al. (16) showed that 50% of CVID patients with granulomatous disease displays extrapulmonary granulomatous manifestation. Granulomatous and lymphocytic interstitial lung disease (GLILD) instead occurs in around 20%–30% of CVID patients (17). GLILD is a clinical, radiologic and pathologic entity characterized by lymphocytic infiltration and/or granuloma of the lung whenever other infectious causes have been excluded. Risk factors for GLILD include history of cytopenias, female gender, age between 20 and 50 years, concomitant hypersplenism and polyarthritis (17, 18). It is associated with increased risk for non-Hodgkin's lymphoma (19). Dysregulated B cells, with increased expression of B cell activating factor (BAFF), which upregulates IFN-γ signaling, have been reported in CVID with interstitial lung diseases (20). A recent study identified increased numbers of CD14 + CD16- monocytes and memory T cells, and prominent inflammation in peripheral blood of CVID patients with non-infectious complications. Further, the same patients had higher serum levels of IFN-γ, IL-6, IL-18, TNF and T-cell activation markers in peripheral blood (21). These abnormalities improved with T-cell-targeted therapy (21). While the study included various CVID patients with non-infectious complications and not only granulomas, it highlighted the role of T cell dysregulation in the pathogenesis of inflammatory manifestations associated with CVID. Screening high-resolution computerized tomogram (HRCT) is recommended for all CVID patients and should be repeated after 4–5 years if initial screening is unremarkable (22). Further, annual spirometry and 6 min-walk test are considered a cost-effective approach to identify patients who may rapidly progress to GLILD. Immunoglobulin replacement should be targeted to reach and maintain a trough IgG level >1000 mg/dl (22, 23). Increasingly, symptomatic patients may be treated with oral glucocorticoids even though the clinical response to this treatment has shown to be poor (24). Glucocorticoid-sparing agents such as weekly Rituximab 375 mg/m^2 for 4 weeks repeated every 4 months for 3–4 courses (25), azathioprine 1–2 mg/kg/day or mycophenolate mofetil 250–1000 mg twice daily can be also considered (25). With such treatment, remission of extrapulmonary granulomas affecting the skin, liver and lymph nodes has been reported in 86% of patients (16). Anti-TNF-α therapy has been successful to treat extrapulmonary granulomas in CVID (16, 26).

Combined immunodeficiencies and granulomatous inflammation

The study of combined immunodeficiencies provides mechanistic insights into the pathophysiologic role of adaptive immune cells in granuloma formation (27, 28). Hypomorphic *RAG* mutations, for example, offer an interesting model to understand granulomatous inflammation. The RAG 1/2 heterotetramer is crucial for the *VDJ* recombination process and for the generation of a diverse repertoire of antigen-specific T-and B-cell receptors (28). The severity of the clinical phenotype correlates with residual

RAG1/2 catalytic activity and may range from severe combined immunodeficiency (SCID) in null *RAG 1/2* variants with absent T or B lymphocyte to a less severe phenotype with milder infections, autoimmunity, and granulomas (CID-G/AI) typically with a later onset in life (27). The latter group of patients display preservation of circulating T cells (albeit in reduced numbers and with a predominance of memory cells), and often have normal levels of immunoglobulins and variable response to immunization. High throughput sequencing analysis revealed significant abnormalities of TCR beta repertoire, especially in T regulatory cells, and of BCR repertoire, supporting the notion that relatively higher levels of RAG protein function may allow for partial preservation of the diversity of TCR and BCR repertoires, which however are enriched in self-reactive specificities that may be possibly implicated in granuloma pathogenesis (29). Granulomas in RAG deficiency infiltrate the skin, bones and/or internal organs (30, 31) and can lead to significant morbidity and physical disfiguration (32). In a cohort of 85 patients with RAG deficiency, 30 patients had CID-G/AI phenotype with 15/30 displaying granulomas. Of note, most of these patients had concomitant autoimmunity, with autoimmune cytopenias being the most prevalent autoimmune manifestation (33). In another series of 68 patients with CID-G/AI, granulomatous lesions were identified in 35% of patients with the most common location being lungs and skin but also multiple other tissues (liver, spleen, bone marrow, oropharynx, gut, testis, pancreas); usually more than one site was involved in the same individual (34). In contrast with what reported in CVID, patients with RAG deficiency showed to be refractory to glucocorticoids and biologics (i.e., anti-TNF) requiring allogeneic hematopoietic stem cell transplantation (HSCT) which appears to be the only definitive management for granulomatous inflammation in this disease (33, 35).

Skin and visceral granulomas have been described in several other IEIs with predominance of DNA repair defects including ataxia telangiectasia [AT], Artemis deficiency, Nijmegen-breakage syndrome [NBS], PRKCD deficiency (36, 37) and ligase IV deficiency (38–41) (**Table 1**). Cutaneous non-infectious granulomas have been extensively described in AT (**Table 1**) (42). Rarely, patients with AT have had granulomas detected in bones and joints (43). Granulomas are typically observed in AT patients with elevated IgM (44). In a cohort of 44 AT patients, those with granulomas had significantly decreased naïve CD8 T cells in peripheral blood (43). A skewed T cell repertoire has also been reported in AT patients with granulomas (45). IVIG, topical and systemic glucocorticoids, tacrolimus, TNF-α inhibitors have been used with variable success however a patient who underwent allogeneic hematopoietic stem cell transplantation (HCT) had complete remission from granulomas (43).

The recent identification of RA27/3 Rubella virus (RuV) vaccine strain in some patients has led to the inquiry as to whether RuV antigens may play a causative role for granuloma formation in CID (10, 38). It is unclear as to whether the virus triggers granulomatous inflammation or the impaired host defense allows viral persistence in M2 skewed macrophages and neutrophils (38, 46, 47). Infiltrative granulomas have been described also in TAP1 and TAP2 deficiency (48–52). Recently, 2 cases from Iran of TAP 2 deficiency presenting with granulomas for more than 2 decades before the genetic diagnosis were described. Three relatives of the probands that carried the same homozygous mutation had no clinical manifestation of disease supporting variable expressivity and multifactorial pathogenesis of granulomatous formations (50). Out of 17 cases of TAP2 deficiency reported in the literature, 30% manifested with skin granulomas. Immunomodulatory or immunosuppressant medications are not recommended in this disease because they may cause granuloma exacerbation (50). Data regarding HCT in MHC-I deficiency is limited; one subject displayed persistent regression of skin granulomas 15 years after HCT (53). Cartilage hair hypoplasia patients have granulomas among their clinical manifestations as well (41, 54–56). Rubella virus-associated granulomas have also been described in 21 IEI patients having cytotoxicity defects with significant frequency in MUNC13–4 and RAB27A (Griscelli syndrome type 2) deficiency (57). Of note, GLILD has also been described in Griscelli Syndrome type 2 (58). These data suggest that impaired T cell function allows persistence of macrophages to perpetuate granulomatous inflammation.

Inflammatory granulomas in phagocytic disorders

CGD is the archetypal IEI for granulomatous inflammation (59, 60). The disorder is characterized by defects in NADPH oxidase due to mutations in 6 known genes – *CYBB, CYBA, NCF1, NCF2, NCF4, and CYBC1* (59)**.** Granulomatous lesions are observed in both autosomal recessive forms (61) and in X-linked forms of CGD (62). Most granulomas in CGD are secondary to infections by organisms predisposing to granuloma formation. The infectious susceptibility and natural history of CGD is described extensively in other reviews (59, 60). However, granulomas of presumed non-infectious origin and post-infectious hyperinflammatory granulomatous inflammation are also prevalent in CGD (63). In a cohort of 71 patients with CGD around 10% suffered from post-infectious granulomas in multiple organs (64). Moreover, in a single center study on inflammatory complications of CGD in 98 patients from France, histological analysis showed presence of granulomatous formation (liver, skin, testes, and ocular) in 22 of 44 patients analyzed. This hyperinflammatory state is often associated with chronic colitis (65), granulomatous cystitis (66) and infections including Staphylococcal liver abscesses or Nocardia infection (67, 68). In these cases, systemic glucocorticoids are co-administered with empiric antimicrobials (68). It appears that hyperinflammation in CGD is triggered by an infectious antigen, however it is often perpetuated due to dysregulation in immune function – particularly defective neutrophil apoptosis (69), skewed NF-κB signaling (70), impaired leukotriene B4 and C5a degradation (71), and upregulation of pro-inflammatory cytokines TNF-α, IL-1β, IL-8, IL-17, IL-6 and G-CSF (72–75). Hyperinflammatory foci, including abscesses, lymphadenitis or granulomas often require surgical excision (76).

Granulomas caused by *Mycobacterium tuberculosis* and non-tuberculous mycobacteria (NTM) are significantly prevalent in IEIs due to phagocytic disorders, and T cell signaling disorders including defects in IFN-γ/Il-12 signaling (7, 77). In endemic areas

TABLE 1 Well-described inborn errors of immunity (IEIs) presenting with non-infectious granuloma – presentation, immunophenotype and reported therapies.

	Type	Skin granulomas	Non cutaneous granulomas	Age of presentation (approximative)	Immunologic Findings	Treatment (effect)	References
CVID	Non-caseating, tuberculoid, necrobiotic granuloma with perineural invasion	Face, lip, buccal mucosa, nose, cheek, limbs, shoulders, trunk, buttocks, hands, feet	Lungs, lymph nodes, liver, spleen and conjunctiva	Childhood - adulthood	Hypogammaglobinemia	IVIG, Anti- TNF-α, systemic glucocorticoids	(Aghamohammadi et al., s.d.; Harp et al., 2015; Nanda et al., 2014; Stigt, A.C., et al. 2020)
CID-G/AI (RAG1/2 deficiency)	Sarcoidal, necrotizing vasculitis, pyoderma gangrenosum, palisades granulomatous dermatitis	Diffuse	Lung, soft tissue, liver, spleen, tongue, gut, testis, bone marrow, adenoids, pancreas, lymph nodes, oropharynx, granulomatous-lymphocytic interstitial lung disease.	Infancy - adulthood (2 −40y).	Mainly T cell lymphopenia. Hypogammaglobulinemia.	Systemic Corticosteroid, cyclosporine, infliximab (partial remission), surgery, HSCT (remission)	(Schuetz et al., 2008), (Delmonte et al., 2018), (De Ravin et al., 2010), (Henderson et al., 2013), (Avila et al., 2010), (Walter et al., 2015), (Sharapova et al., 2013), (Patiroglu et al., 2014), (Buchbinder et al., 2015), (Min et al., 2021), (Farmer et al., 2019), (Geier et al., 2020), (Van Horn et al., 2018)
Artemis deficiency	Necrotizing granuloma	Extremities, nose.	–	Infancy (5y – 7 y)	Hypogammaglobulinemia, T cell lymphopenia	HSCT	(Baumann et al., 2022; de Jager et al., 2008; IJspeert et al., 2011)
PRKCD deficiency	Non-Langerhans cell histiocytosis. epithelioid granulomas	Face and extremities, limb, elbow	Splenic granuloma	Infancy - childhood (6 mo, 9y)	T and B cell lymphopenia, hypogammaglobulinemia.	IVIG, HSCT	(Esenboga et al., 2018; Mathieu et al., 2015)
Ataxia-telangiectasia	Palisading, epithelioid, tuberculoid, necrotizing and non-necrotizing granuloma; Necrobiotic granulomatous inflammation (granuloma annulare); granulomatous acne rosacea.	Limbs, face, trunk, buttocks; trauma-prone areas	Synovial (knee, elbow, wrist), lungs, spleen, liver, larynx, bone marrow, bone (tibia)	Newborn - adulthood (0–31 y)	Decreased IgG, IgA and IgE. Normal/increased IgM, marked decreased B cells and naïve T cells. High AFP	IVIG (progression/ partial remission/ remission); Topical corticosteroid, systemic corticosteroid, tacrolimus, intralesional triamcinolone injections, antibiotics, prednisolone, adalimumab, infliximab, (partial/ transient); HSCT (remission), Isotretinoin [for granulomatous acne rosacea] (remission)	(Amirifar et al., 2020; Cantarutti et al., 2015; Chiam et al., 2011; de Jager et al., 2008; Fleck et al., 1986; Folgori et al., 2010; Joshi et al., 1993; Mitra et al., 2005, 2011; Paller et al., 1991; Privette et al., 2014; ŞentÜrk et al., 1998; Shoimer et al., 2016; Szczawińka-Popłonyk et al., 2020; Woelke et al., 2018)
TAP1/TAP2 deficiency	Epithelioid granuloma, necrotizing granulomatous skin lesion	Extremities, midface, legs, other parts of the body	Septal perforation and cartilage destruction	Infancy - adulthood (3–26y)	Complete absence of HLA I on CD8 + cells, low CD8+, normal/increased CD19 + and CD56 + .	Corticosteroids, methotrexate, clarithromycin (null/ partial improvement), HSCT	(Darazam et al., 2022; Gadola et al., 2008; Konstantinou et al., 2013; Law-Ping-Man et al., 2018; Moins-Teisserenc et al., 1999; Tsilifis et al., 2021)
Cartilage hair hypoplasia	Sarcoidal, tuberculoid, epithelioid, histiocytic palisading	Limb (monomelic), nose, lips, chin, cheeks, scalp, buttocks.	Bone, nasal septum, larynx, lymph node, spleen, Diffuse (fetal) in skeletal	Fetal; infancy - adolescence (1y – 13 y)	T cell lymphopenia, Low IgG and IgA, Intermittent neutropenia	Anti- TNF-α, (partial remission), HSCT (remission)	(Crahes et al., 2013; Leclerc-Mercier et al., 2019; McCann et al., 2014; Moshous et al.,

(*continued*)

TABLE 1 Continued

	Type	Skin granulomas	Non cutaneous granulomas	Age of presentation (approximative)	Immunologic Findings	Treatment (effect)	References
	(necrobiotic) granuloma.		muscle, myocardium, pancreas, spleen, bladder, liver, uterus, thyroid, lungs.				2011; Sathishkumar et al., 2018)
CGD	Non-caseating granuloma	Diffuse; granulomatous acne	Gastrointestinal tract, lung, eye, testis, bladder	Infancy - adulthood	–	Corticosteroid, isotretinoin, surgery, HSCT	(Dunogué et al., 2017; Magnani et al., 2014)
Blau Syndrome	Non-caseating granuloma	Trunk, extremities	Granulomatous uveitis, hepatic and renal granulomatosis, granulomatous arteritis, granulomatous lymphadenitis, Synovia	Childhood - adolescence (3-12 years)	–	Corticosteroid, immunosuppressive agents	(Jabs et al., 1985; Sfriso et al., 2012; Ting et al., s.d.)
PLAID	Non-caseating granuloma	Finger, nose, ears, feet.	Soft palate and larynx.	Birth - childhood.	Low switched memory B-cells. low or low-normal NK cells. Low serum IgM, IgA. poor antibody responses to pneumococcal vaccines. Positive ANA,	Spontaneous disappearance is described; cold avoidance, antihistamines, antibiotic prophylaxis and/or immunoglobulin replacement	(Milner, 2015; Ombrello et al., 2012; Shea et al., 2020)

CVID, common variable immunodeficiency; CID-G/AI, combined immunodeficiency with granulomas and/or autoimmunity; CGD, Chronic Granulomatous Disease; HSCT, hematopoietic stem cell transplant; IVIG, Intravenous immunoglobulin; PLAID, PLCG2 associated antibody deficiency and immune dysregulation; TNF-α, tumor necrosis factor alpha; y, years; mo, months.

where the Bacille Calmette-Guérin (BCG) vaccine, containing live-attenuated *Mycobacterium bovis* bacilli is administered, IEI patients may present with localized granulomatous inflammation termed BCG-itis or disseminated "BCG-osis" (77–79). This may be the first presentation of CGD or SCID and may present challenges in patient management if HSCT is considered since pre-transplant infection and/or inflammation is associated with poor outcomes. Further, granulomatous lesions may only manifest after engraftment and lead to significant morbidity (80, 81).

Very early onset IBD (VEO-IBD) includes a heterogenous group of monogenic IEIs presenting with inflammatory (non-infectious) bowel disease, occasionally with granulomas, prior to age 6 (82). Commercial targeted gene panels for VEO-IBD typically test over 65 genes (83). Readers are referred to comprehensive reviews on monogenic causes of VEO-IBD for further information (83, 84).

Disorders of autoinflammation and primary atopic disorders

Autoinflammatory diseases encompass disorders of pathogenic inflammation secondary to intrinsic immune pathway hyperactivation (85). Autoinflammatory syndromes due to hyperactivation of the NF-κB signaling pathway may be associated with granuloma formation. These disorders are also typically associated with exaggerated TNF activity. The major disorder associated with granulomas in this category is Blau syndrome due autosomal dominant *NOD2* pathogenic variants (86). *NOD2* variants are also associated with susceptibility to Crohn's disease, characterized by non-caseating granulomas within the gastrointestinal tract (87). Patients classically present within the first decade of life with a combination of granulomatous dermatitis, erythema nodosum, uveitis and polyarticular arthritis (86). Granulomas infiltrating the liver and kidney have been described in Blau Syndrome (88). Recently, a Japanese patient with a pathogenic *NOD2* variant was diagnosed with Blau syndrome following BCG vaccination suggesting that infectious triggers may play a role in granuloma formation of this disease (89). Granulomatous inflammation has also been observed in autosomal dominant RelA haploinsufficiency leading to NF-κB hyperactivation (90). Systemic glucocorticoids are used as an initial treatment however TNF-α inhibitors have shown significant therapeutic benefit in both RelA haploinsufficiency and Blau syndrome (91, 92).

Phospholipase C gamma 2–associated antibody deficiency and immune dysregulation (PLAID) is a disorder of autoinflammation, autoimmunity, immunodeficiency, and a primary atopic disorder (93). Phospholipase C gamma 2 (PLCG2) hydrolyzes phosphatidylinositol-4,5-bisphosphate into diacylglycerol and inositol trisphosphate, triggering calcium release from the endoplasmic reticulum to mediate cell activation (94). Heterozygous pathogenic deletions in the autoinhibitory domain of PLCG2 cause a

PLAID phenotype since it leads to constitutive activation of the PLCG2 enzyme (95). Patients present with recurrent sinopulmonary infections, urticaria triggered by evaporative cooling, granulomatous dermatitis, hypogammaglobulinemia and various autoimmune manifestations (93, 96). Cutaneous granulomatous lesions are present in 25% of patients and among these subjects many developed skin lesions on the nose, ears and fingers within the first few days of life. These lesions may spontaneously resolve in the vast majority of patients however sometimes they may persist and lead to tissue damage and destruction of nasal and auricular cartilage (97). Furthermore, in some cases granulomatous dermatitis may have a later onset affect especially cold exposed areas (97). Histological characteristics of granulomas in PLAID are similar to CVID with a core of tissue resident macrophages including multinucleated giant cells surrounded by a lymphocytic infiltrate. In this disease the most likely pathogenetic trigger is the spontaneous activation of neutrophil and monocytes by cold exposure (98). Treatment includes cold avoidance, daily high dose nonsedating antihistamines, antibiotic prophylaxis and/or immunoglobulin replacement (96). The use of anti-inflammatory drugs or immunomodulators has not shown significant clinical efficacy in PLAID (96).

Rubella-associated granulomatous inflammation: a potential trigger for significant IEI morbidity

As described previously, live-attenuated RuV RA27/3 vaccine strain has been identified in cutaneous and visceral granulomas in IEI patients (99) (**Table 2**). This finding highlights the importance for thorough antigen screening in tissue biopsy particularly since treatment of granulomas in IEI has classically focused on use of immunosuppression. The RuV antigen and/or RNA has been identified in at least 66 IEI cases -predominantly cases of AT and CID, and defects of cytotoxicity, but much rarer in primary antibody deficiencies (10, 57). Of note, varicella zoster vaccine, mumps and RuV vaccine strain were all identified in granulomas of a patient with late onset hypomorphic RAG2 deficiency (100). The causal role for RuV in granulomas remains to be defined. Recently, both wild-type and vaccine strain RuV has been isolated from cutaneous granulomas of 4 presumed immunocompetent adults (9, 101). **However, laboratory evaluation in these patients did reveal immunologic abnormalities including lower CD8+ T cells, lower T-cell mitogen responses, reversed ratio of CD8 + to CD4+ T cells, and/or low serum immunoglobulins (101).**

Viral genome sequencing has revealed multiple nucleotide and amino acid substitutions in the RA27/3 vaccine strain identified in IEI granulomas. These vaccine strains have been termed immunodeficiency-related vaccine-derived rubella viruses (iVDRVs) (47). In IEI patients iVDRVs persist within M2 macrophages, neutrophils, and epidermal keratinocytes (34). It is thought that long-term iVDRV reservoir stems from neutrophils and macrophages residing in the bone marrow (46, 47). Further natural history and mechanistic studies are required to further characterize whether iVDRV is causing granulomas in IEIs. Impaired CD8 T cell repertoire could be a plausible mechanistic insight since CD8 T cell memory is critical for Rubella virus control, and CD8 T cell repertoire decreases with age (102, 103). To date no significant clinical improvement for RuV-associated granulomas has been derived by pharmacotherapy and HSCT remains the only definitive management if indicated by the clinical severity of the underlying IEI (10).

TABLE 2 Well-described pathogens identified in IEI granulomas.

Etiology	Common site of presentation	Types of IEI	Common therapy used	References
Mycobacteria	Skin, BCG vaccine site, lymph nodes, bone, lung, bowel, liver, adrenal, aorta, kidney, nerve, muscle, testis, pericardium	MSMD (IL-12/IFN-γ axis), anti IFN-γ autoantibodies, NEMO deficiency, SCID	Depending on the species, a combination of first and second line antitubercular drugs, antibiotics and surgery. (Wi, 2019)	(Abramowsky et al., 1993; Dolezalova, Karolina et al., 2022; O'Connell et al., 2012; Süleyman et al., 2022; Xu et al., 2019)
Rubella RA27/3 vaccine strain	Skin (face limbs, diffuse), lung, spleen, kidney, lymph nodes, bone marrow, and liver.	AT, ADA-SCID and CID (predominant). Also observed in CVID, X-SCID, RAG1/2 deficiency, NBS, XLA, DiGeorge Syndrome, CHH, Artemis deficiency, MHC II deficiency, Marden-Walker syndrome, McKusic syndrome, TAP1 deficiency, WHIM syndrome, Coronin 1A deficiency.	Nitazoxanide, local corticosteroid (no improvement); IVIG (moderate improvement), rapamycin, rituximab, infliximab, interleukin-2 (moderate effect); HSCT (remission).	(Browne et al., 2022; Murguia-Favela et al., 2019; Perelygina et al., 2020; Shoimer et al., 2016)
***Coccidioides* spp.**	Disseminated infection	IL-12/IFN-γ and STAT3 signaling pathways disregulation; CID due to *CTPS1* biallelic variants. Impaired TNF-α signaling due to *CLECL7A*, *PLCG2* variants; Impaired Hydrogen peroxyde production *due to monoallelic* DUOX1/*DUOXA1* variant	Antymicotic drugs (fluconazole), IVIG.	(Hsu et al., 2022; Krase et al., 2022; Odio et al., 2017)

AT, ataxia-telangiectasia; CID, Combined immunodeficiency; CHH, Cartilage hair hypoplasia; IFN-γ, interferon gamma; IVIG, intravenous immunoglobulin; MHC, Major histocompatibility complex; MSMD, Mendelian Susceptibility to mycobacterial disease; NBS, Nijmegen-Breakage Syndrome; HSCT, hematopoietic stem cell transplant; SCID, Severe combined immunodeficiency; XLA, X-Linked agammaglobulinemia.

Coccidioides – an endemic fungus causing granulomatous inflammation increasingly identified in IEI

Coccidioidomycosis, known as Valley Fever, is caused by the pathogenic fungus *Coccidioides*, endemic to the Southwestern United States (104, 105). Symptomatic illness occurs in around 30% of those infected with disseminated disease in <1% (104, 106). Known risk factors for disseminated disease include secondary immunodeficiency due AIDS, chemotherapy, solid organ-and hematopoietic stem cell transplantation, and immunomodulatory biologics (107). Only 14 patients with disseminated coccidioidomycosis (DCM) had been reported in the literature having mutations impairing immune function – 12 within the IL-12/IFN-γ and STAT3 signaling pathways (107–109) and a 5-year-old male with CTPS1 deficiency - a disorder of impaired lymphocyte proliferation (110). A recent publication of a largely adult DCM cohort described mutations in *CLECL7A* and *PLCG2* which impaired TNF-α signaling, and heterozygous variants in *DUOX1* and *DUOXA1* which impaired hydrogen peroxide production (8). A query of the USIDNET database containing information on 5,485 IEI patients in the United States identified 10 patients with a history of Coccidioidomycosis (111). Patients with persistent presumed non-infections granulomatous inflammation can go undiagnosed for coccidioidomycosis (112). In IEI and immunocompromised patients, serology and immunofixation has poor sensitivity thus the diagnosis must be ruled out by tissue biopsy (113, 114). Identification of *Coccidiodes* by tissue biopsy is thus of increasing importance in IEI patients within the Southwestern United states particularly in those where the use of immunomodulators as therapeutics is being considered.

Diagnostic workup and management of granulomas in IEI

A detailed understanding of the etiology, or at least the inflammatory process of an underlying granuloma is key to direct treatment. For this, obtaining a tissue biopsy for histology and culture is imperative. Apart from acid-fast staining for Mycobacteria, and Giemsa staining for fungal etiology, cultures should be prolonged enough to detect possible fastidious organisms. In some cases where biopsy may not be obtained or cultures remain negative, cell-free DNA testing can help identify less common organisms (115) to enable targeted antimicrobial therapy. Antimicrobial target genes have been identified in granulomas of sarcoidosis patients (116). Cytokine gene expression in granulomas can be quantified using techniques such as RNAScope® (117). Complete surgical excision should be considered whenever granulomas may cause anatomic obstruction or if there is insufficient response to pharmacotherapy. Identification of an underlying pathogen warrants guideline-directed antimicrobial therapy. In the case of antimicrobial therapeutics, this is best done in liaison with infectious disease specialists and tailored to the presumed pathogen based on the underlying IEI. Recombinant interferon-gamma (IFNγ) has been used to aid pathogen clearance in IEIs mycobacterial disease, CGD and Coccidioidomycosis. In the case of immunomodulation, there is a longstanding knowledge of using systemic glucocorticoids for inflammatory granulomas in CGD (59, 118). Nitazoxanide, an antiparasitic drug with antiviral properties has been used in IEIs with RuV-associated granulomas (119). Use of oral nitazoxanide was associated with decreased Rubella virus antigen or elimination from granulomas; however, this treatment did not translate into clinically meaningful outcomes (38, 119). Several patients with RuV-associated granulomas underwent allogeneic HCT (120) Patients with lower co-morbid disorders had improved outcomes which suggests that early detection and characterization of RuV-associated granulomas has a bearing on patient outcomes (120).

Perspectives and futures directions

The advancement of minimally invasive surgical procedures has facilitated obtaining tissue biopsy to characterize granulomas in IEI. Further, histopathologic staining and genetic sequencing can help characterize the inflammatory milieux and possible pathogenic triggers for granulomatous inflammation. This has both prognostic and therapeutic implications, particularly since empiric use of immune suppressive agents can worsen underlying latent infections. The granuloma structure has been described in the mammalian superorder *Archonta* (including primates) and *Laurasiatheria* (including carnivores and ungulates) (121). Granulomatous inflammation in preceding animals such as fish does not reflect the complex architecture seen in primates (122). Non-human primates remain a well characterized model of Tuberculosis-induced granulomas however studies are hindered by length of time needed for granuloma formation and requirement of biosafety level 3 laboratories (123). Mouse models of tuberculous granulomas further do not mirror the granuloma architecture seen in humans (124). Inoculation of iVDRV in hypomorphic models of *Rag1*-mutant mice (125) was unsuccessful in eliciting granulomatous inflammation (126). A heterozygous *Stat4*-mutant mouse model shows predisposition to disseminated Coccidioidomycosis however lung histology is characterized by lymphocyte infiltration rather than granulomatous inflammation (109, 127). *In vitro* human granuloma models have been developed to circumvent the difficulties of studying granulomatous inflammation in animal models (128). An *in vitro* human granuloma model may help characterize mechanisms in granuloma formation as has been observed in various IEI from a clinically phenotypic standpoint. IEIs provide a fascinating template to characterize the heterogeneity and kinetics of granulomatous inflammation. Further characterization of granulomas in IEI can facilitate development of diagnostics and targeted therapeutics for more common granulomatous disorders such as sarcoidosis.

Author contributions

KS and OD analyzed evidence from the literature and wrote the manuscript. AG elaborated the tables. LN and OD

supervised the project and critically reviewed the final draft. All the authors approved the submitted manuscript and agreed to be accountable for the content of the work. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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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Long-term follow-up in common variable immunodeficiency: the pediatric-onset and adult-onset landscape

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Introduction: The primary aim of this study is to investigate the evolution of the clinical and laboratory characteristics during the time in a longitudinal cohort of pediatric-onset and adult-onset Common Variable Immunodeficiency (CVID) patients in order to identify early predictive features of the disease and immune dysregulation complications.
Methods: This is a retrospective-prospective monocentric longitudinal study spanning from 1984 to the end of 2021. The data of pediatric-onset vs. adult-onset patients have been compared for immunological features and for infectious and non-infectious complications assessed at diagnosis and follow-up.
Results: Seventy-three CVID patients have been enrolled, with a mean of 10.0 years (SD ± 8.17) of prospective follow-up. At diagnosis, infections were observed in 89.0% of patients and immune dysregulation in 42.5% of patients. At diagnosis, 38.6% of pediatric-onset and 20.7% of adult-onset patients presented with only infections. Polyclonal lymphoid proliferation (62.1%) and autoimmunity (51.7%) were more prevalent in the adult-onset than in the pediatric-onset group (polyclonal lymphoid proliferation 52.3% and autoimmunity 31.8%, respectively). Enteropathy was present in 9.1% of pediatric-onset and 17.2% of adult-onset patients. The prevalence of polyclonal lymphoid proliferation increased during follow-up more in pediatric-onset patients (diagnosis 52.3%—follow-up 72.7%) than in adult-onset patients (diagnosis 62.1%—follow-up 72.7%). The cumulative risk to develop immune dysregulation increases according to the time of disease and the time of diagnostic delay. At the same age, pediatric-onset patients have roughly double the risk of having a complication due to immune dysregulation than adult-onset patients, and it increases with diagnostic delay. The analysis of lymphocyte subsets in the pediatric-onset group showed that CD21 low B cells at diagnosis may be a reliable prognostic marker for the development of immune dysregulation during follow-up, as the ROC curve analysis showed (AUC = 0.796). In the adult-onset group, the percentage of transitional B cells measured at diagnosis showed a significant accuracy (ROC AUC = 0.625) in identifying patients at risk of developing immune dysregulation.

Discussion: The longitudinal evaluation of lymphocyte subsets combined with clinical phenotype can improve the prediction of lymphoid proliferation and allow experts to achieve early detection and better management of such complex disorder.

KEYWORDS

common variable immunodeficiency (CVID), immune dysregulation, autoimmunity, lymphoid proliferation, cytopenia, CD21low B cells, inborn errors of immunity

1. Introduction

Among the inborn errors of immunity, Common Variable Immunodeficiency (CVID) is considered the most common symptomatic primary antibody deficiency with an estimated prevalence between 1:25,000 and 1:50,000 inhabitants (1). CVID is caused by a primary defect in the development and/or functioning of the B cells, resulting in reduced serum levels of immunoglobulins (Ig) with reduced or absent specific antibody production (2–5). CVID clinical spectrum may be wide, and it is characterized predominantly by increased susceptibility to infections and/or by dysregulation of the immune system (1, 5). This background causes an extensive set of different clinical and immunological features with a broad range of comorbidities (6). The non-infectious complications may be evident at presentation or may appear afterward. They include autoimmunity, gastrointestinal inflammatory disease, liver disease, granulomatous lung disease, lymphoid hyperplasia and infiltrative disease, and the development of cancer, especially lymphoma (7, 8). Patients with CVID can be "categorized" into different phenotypes, according to their manifestations (7). This phenotypic distinction has important implications because the risk of death is estimated at 11 times higher for patients with non-infectious complications compared to those without (7, 8). The introduction of Ig replacement therapy has reduced the number of infections and improved their outcomes (9), but it does not seem to prevent or improve manifestations associated with immune dysregulation. CVID seems to have two different peaks of onset in the second or fourth decades of life, mainly between 20 and 45 years of age. The onset during childhood is usually recognized early by pediatricians if recurrent or severe infections are present. In adulthood, a diagnostic delay of 6–7 years (or later) after the onset of symptoms is often seen (10), perhaps due to the very heterogeneous nature of the disease and the importance of excluding secondary causes of hypogammaglobulinemia or lymphoproliferative disorders. This fact also causes a delay in accessing adequate therapy (11, 12), and, if associated with the higher prevalence of non-infectious complications, it contributes to worsening the prognosis of patients. Recently, non-infectious complications are emerging as the major challenge, not only in adult patients but also in the children, requiring a better understanding of pathogenesis and therapies (8). With this perspective, a number of monogenic defects have been recently identified in ∼10%–30% of CVID patients providing potential insights into both pathogenesis and additional therapeutics (8, 13).

The evolution of CVID disorder over time in patients diagnosed near to the onset of the disease, especially if diagnosed in childhood, has been rarely reported (14–16). A large cohort of patients and registry data have provided many insights into non-infectious complications of CVID, but the issue of early identification of patients with a high risk of developing complications or poor prognosis is still an open question (1, 7, 8, 10, 12, 14–17). The aim of this study is to investigate the evolution of the clinical and laboratory characteristics over time in a longitudinal cohort of pediatric-onset and adult-onset patients in order to identify early predictive features of disease complications. This study analyzes the organ-specific pathologies and the immunologic parameters identified in a monocentric cohort of patients with CVID since 1984 in a specialized referral center for the care of pediatric and adult patients with primary immunodeficiencies.

2. Materials and methods

2.1. Patients' selection

In this retrospective-prospective monocentric longitudinal study we have enrolled all patients with a diagnosis of Common Variable Immunodeficiency (CVID) who have been followed up since 1984 until December 2021 at the Primary Immunodeficiency referral Centre in Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, a tertiary and university care hospital in Milan, Italy. All the patients are enrolled in the European Society for Immunodeficiencies (ESID) Registry. The local Ethics Committee has approved this registry (Milan Area 2, Italy; ID # 509-2017). Each patient signed informed consent for data collection and scientific publications. Diagnosis of CVID was made based on current ESID diagnostic criteria (4, 18) or on the criteria of the original ESID/Pan American Immunodeficiency Group (PAGID) of 1999 (19). Diagnosis has also been reviewed and confirmed using the International Consensus Document (ICON) criteria (2). Among those patients originally diagnosed based on the ESID/PAGID criteria, this study included only the ones who had low levels of immunoglobulin G (IgG) and immunoglobulin A (IgA) and/or immunoglobulin M (IgM) plus poor antibody response to vaccines, or absent isohemagglutinin, and/or reduced values of switched memory B cells with CD4+ T cells above 200/μl. Clinical and immunological data were obtained from patients' medical

records. For each patient, we excluded all secondary causes of hypogammaglobulinemia (20) and the presence of a marked cellular T deficiency.

2.2. Clinical and laboratory data

At the time of the study, eleven of the enrolled patients were still cared for at the pediatric center, and ten had been transferred to the adult center after reaching the age of 18 according to the transition path program at our hospital.

Documented clinical data include the clinical history of infections, bronchiectasis (confirmed with computed tomography), autoimmune cytopenias such as autoimmune hemolytic anemia (AIHA) and idiopathic thrombocytopenic purpura (ITP), organ-specific autoimmunity (including vitiligo, psoriasis, thyroid disease, atrophic gastritis), granulomatous disease, enteropathy, and malignancies. CVID-associated granulomatous interstitial lung disease (GLILD) was diagnosed based on typical CT scan results in the absence of evidence of an infectious or alternative cause and in a few cases confirmed by biopsy. Splenomegaly was defined as an increase in spleen size greater than or equal to 12 cm for adults, or above the age reference values for the pediatric population on ultrasound or CT or magnetic resonance (MRI), including the previous splenectomy of an enlarged spleen. Lymphadenopathy was detected on palpation, ultrasound, and CT or MRI. Granulomatous disease was defined by the finding of at least one granuloma not explained by other causes and demonstrated on biopsy, excluding granulomas associated with Crohn's disease. Enteropathy includes all cases of non-inflammatory bowel disease biopsy-proven infection (excluding ulcerative colitis and Crohn's disease) and hyperlymphocytosis (lymphocytic infiltration of the mucosa, lamina propria, and/or submucosa), defined as lymphocytic colitis. Clinical phenotypes were defined according to Chapel's et al. classification (7). Data on human polyclonal immunoglobulin (Ig) replacement therapy (RT) were recorded.

All patients underwent blood tests both at diagnosis and at the periodic follow-up because this investigation "at follow up" has been used as the latest data available. Malignant tumors include hematological malignancies and all other forms of cancer. The tumor diagnosis and staging were performed according to national guidelines currently in force. Gastric cancer was diagnosed with gastrointestinal endoscopy associated with biopsies. Breast cancer was diagnosed with standard imaging tests followed by histological confirmation. Lymphomas were diagnosed with imaging, including CT, MRI, and PET, as well as the histological examination of tissue sections, lymph nodes, and/or bone marrow biopsies, according to the current WHO classification. Diagnosis of non-melanocytic skin cancer (NMSC) was clinically performed and histologically confirmed after tumor excision. Patients were all treated and followed up according to current clinical practice at the time of their management.

2.3. Genetic analysis

Genetic analysis of all 73 patients was performed through sequencing of genes associated with CVID. The analyses were carried out at the Institute of Molecular Medicine Angelo Nocivelli of the ASST Spedali Civili of Brescia. The genetic study of 71 patients was performed using Next-Generation Sequencing (NGS) techniques on the Ion Torrent platform. Patients were screened through a target panel of 52 genes related to CVID and hypogammaglobulinemia. The identified mutations were then confirmed with Sanger sequencing and validated through those already described in the literature. One more patient was diagnosed with NGS performed by another laboratory (Center for Autoinflammatory Diseases and Immunodeficiencies, IRCCS Istituto Giannina Gaslini, Genova), and the last patient was diagnosed using Whole Exome Sequencing (WES) performed by the Laboratory of Medical Genetics of Papa Giovanni XXIII Hospital in Bergamo.

2.4. Definitions

The diagnostic delay was calculated from the age of the onset of CVID to the date of diagnosis. Follow-up time was considered to be the time from the date of CVID diagnosis to the last visit in December 2021. The disease time was defined as the time that elapsed between the age of the onset of the disease and the date of the last visit performed.

2.5. Statistical analysis

All the variables of interest were analyzed with descriptive statistics. Variables with continuous data were presented as mean and standard deviation (SD) and median values and their intervals. Categorical data were presented as frequencies and percentage values. Comparison of the means of continuous variables was performed with the appropriate tests for paired or independent samples, according to Levene's test for variance. Categorical variables were compared with Fisher's exact test. Sensitivity and specificity analysis of the predictive value of continuous variables of interest was performed using Receiver Operating Characteristic (ROC) curves. The correlation coefficient of Spearman was used to analyze the relationships between continuous and/or dichotomous variables. All analyses were performed with IBM SPSS Statistics (SPSS Inc. Chicago, IL, version 27.0). Graphs were made with Windows Excel and SPSS, and the graphs of lymphocyte subpopulations were made with GraphPad Prism version 9.0 for Windows (GraphPad Software, La Jolla, California).

3. Results

A total of 78 patients were initially diagnosed with CVID from 1984 to 2021. The diagnosis was revised and five patients were

excluded: one was diagnosed with X-linked Agammaglobulinemia (XLA) after NGS analysis (pathogenic mutation on BTK gene); one could not be followed up; three did not fulfill the diagnostic criteria for CVID. Finally, this study enrolled 73 patients, 29 men and 44 women (1:1.5) (**Figure 1**).

All patients were of Italian origin except four patients who were from Egypt, Colombia, Albania, and Ukraine. There was a case of consanguinity of parents, first cousins, in one pediatric patient; two patients were familial cases of CVID. In 2017 two patients were lost; one died of post-traumatic cerebral hemorrhage at the age of 71, and 34-year-old patient was transferred to another center after 17 years of follow-up.

At the time of this study, patients were aged between 4 and 71 years (mean age 38.4 years ± 16.59). The mean age of CVID onset was 17.3 years ± 13.84 (range <1 to 63 years), but the mean age at diagnosis was 28.3 years ± 15.94 (range 3 to 67 years) with a mean diagnostic delay of 11.5 years ± 11.71 (median 6.0 years and range <1 to 47 years). The mean of the estimated time of disease was 21.5 ± 13.86 years (range 1 to 59 years) with a mean time of prospective follow-up of 10.0 years ± 8.17 (See **Table 1**). All the patients, except one who refused, were under replacement therapy with intravenous (52.8%) or subcutaneous (47.2%) immunoglobulins.

Table 1 shows that the most frequent clinical manifestation of CVID at onset was recurrent infection (89.0%), especially recurrent respiratory infections, followed by immune dysregulation (42.5%). Five patients had other symptoms (chronic diarrhea, eczema, liver failure, short stature, and asthenia), two patients were asymptomatic, and three patients had associated syndromic features. After the diagnosis, when NGS target panel was available, all the patients underwent genetic analysis. Nineteen patients (26.0%) were found to be carriers of a monogenic pathogenic defect: nine on TNFRSF13B (TACI), two on CTLA-4, one on LRBA, ADA2, TWE-PRIL, CXCR4, CD40, TTC37, NFKB1, and ATP6AP1 gene (**Supplementary Table S1**). The small number of patients does not allow for correlating the genetic result with the phenotype in immune dysregulation in particular. We found that among the 19 patients with the identified gene, the "only infections" phenotype was present in four (21%) and the other 15 had CVID complications (autoimmunity, splenomegaly, polyclonal lymphoid proliferation, and noninfectious enteropathy). Among the 54 patients with no gene identified, 13 (24.1%) had the "only infections" phenotype, while all the other 41 presented complications.

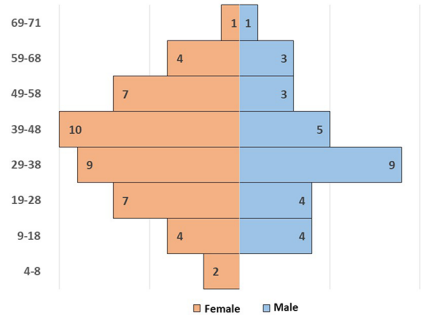

FIGURE 1
Demographic distribution of age and sex of the whole CVID cohort.

We evaluated the overall cohort's disease complications at diagnosis and follow-up (**Figure 2**). Over time, there is an increase in the prevalence of bronchiectasis ($p < 0.001$), splenomegaly ($p < 0.001$), hepatomegaly ($p < 0.001$), and enteropathy ($p < 0.001$). There was an overall increase in immune dysregulation features, such as lymphocytic colitis ($p < 0.001$), nodular lymphoid hyperplasia ($p < 0.001$), granulomas ($p < 0.001$), granulomatous lymphocytic interstitial lung disease (GLILD) ($p < 0.001$), and granulomatous liver disease ($p < 0.001$). Lymphomas arose during follow-up in five patients in adult age.

3.1. Analysis according to age at CVID onset

Patients were divided into a "pediatric-onset cohort", as defined by age of onset of CVID from 2 to 18 ($n = 44$), and an "adult-onset cohort", as defined by age of onset of disease >18 years old ($n = 29$). Early onset (age <11) is prevalent in men, while a later onset was observed in women.

Table 1 details the age at diagnosis and follow up and time of disease and follow up between the two groups. Comparison between the two groups shows that the diagnostic delay is higher in the pediatric-onset rather than in the adult-onset group ($p = 0.031$), and both groups have been followed up at our centers for a similar follow-up time. We analyzed the distribution of patients by year of birth and the time of diagnostic delay considering that before the 1990s CVID disorders were poorly known in Italy. Indeed, the difference between the year of birth and diagnostic delay is less evident for pediatric-onset patients with a diagnosis in the pediatric age (R2 linear = 0.019) and for adult-onset patients with a diagnosis in adulthood (R2 linear = 0.142) (**Figure 3**). For patients with pediatric-onset and diagnosis in adulthood, we can observe the inversely proportional correlation between the two parameters (R2 linear = 0.568) since several patients were born before 1980. Analyzing other differences in diagnostic delay, the subgroup of patients with pediatric-onset and pediatric diagnosis ($n = 21$) had the CVID onset very early, before the age of 5. On the other hand, patients with pediatric-onset but diagnosed in adulthood ($n = 23$) had the CVID onset at the beginning of adolescence (age >11). Failure to thrive was one of the factors that probably led to an earlier diagnosis in the former group than in the latter ($p = 0.019$). At follow-up, patients with pediatric-onset and adult-diagnosis manifested gastrointestinal complications more frequently than the patients with pediatric diagnosis (gastrointestinal infections: $p = 0.011$; colitis lymphocytic: $p = 0.023$), and the endoscopy was found to be pathological in a higher rate of patients with pediatric-onset and adult-diagnosis than in the ones with pediatric-diagnosis ($p = 0.038$).

3.2. Clinical and laboratory characteristics at diagnosis and follow-up

Initial clinical manifestations observed at onset in the two groups of pediatric-onset and adult-onset patients are detailed

TABLE 1 Demographic characteristics, features at CVID onset and diagnosis are detailed.

	Overall CVID cohort (*N* = 73)		Pediatric-onset (*N* = 44)		Adult-onset (*N* = 29)		
Demographic Characteristics	Mean	SD	Mean	SD	Mean	SD	*p*
Age at the latest follow-up	38.4	16.59	42.9	13.45	47.4	12.89	0.213
Age at onset	17.3	13.84	10.3	6.68	30.8	11.29	<0.001
Age at diagnosis	28.4	16.00	31.6	12.17	38.9	12.59	0.041
Diagnostic delay	11.5	11.71	21.7	13.76	8.2	7.76	<0.001
Follow up time	10.0	8.17	11.2	9.45	8.6	7.67	0.279
Time of disease	21.5	13.86	33.0	15.38	16.8	10.23	<0.001
Characteristics at CVID onset	*N*	%	*N*	%	*N*	%	*p*
Immune Dysregulation	31.0	42.47	16.0	36.36	15.0	51.72	0.231
Infections	65.0	89.04	38.0	86.36	27.0	93.10	0.465
Malignancy	0.0	0.00	0.0	0.00	0.0	0.00	NA
Syndromic Features	3.0	4.11	3.0	6.82	0.0	0.00	0.272
Other Symptoms	5.0	6.85	3.0	6.82	2.0	6.90	1
No Symptoms	2.0	2.74	2.0	4.55	0.0	0.00	0.514
Hypogammaglobulinemia	2.0	2.74	2.0	4.55	0.0	0.00	0.514
Characteristics at CVID diagnosis	*N*	%	*N*	%	*N*	%	*p*
Recurrent respiratory infections	65	89.0	42	95.5	24	82.8	0.252
Failure to thrive (infancy)	5	6.8	5	11.4	0	0.0	0.51
Recurrent pyogenic infections	6	8.2	2	4.5	4	13.8	0.207
Unusual infections	7	9.6	4	9.1	3	10.3	ns
Same pathogen recurrent infections	7	9.6	5	11.4	2	6.9	0.696
Autoimmunity, autoinflammation, and lymphoid proliferation	30	41.1	16	36.4	14	48.3	0.34
Pneumonia	44	60.3	26	59.1	18	62.1	ns
Meningitis	6	8.2	3	6.8	3	10.3	0.676
Upper respiratory airways recurrent infections	64	87.7	41	93.2	23	79.3	0.142
Lower respiratory airways recurrent infections	40	54.8	22	50.0	18	62.1	0.345
Gastrointestinal recurrent infections	25	34.2	16	36.4	9	31.0	0.802
Urinary tract infections	14	19.2	6	13.6	8	27.6	0.223
Skin recurrent infections	6	8.2	3	6.8	3	10.3	0.676
Other recurrent infections	4	5.5	2	4.5	2	6.9	ns
Unusual viral infections	11	15.1	7	15.9	4	13.8	ns
Unusual opportunistic infections	4	5.5	2	4.5	2	6.9	ns
Unusual parasitic infections	1	1.4	0	0.0	1	3.4	0.394
Bronchiectasis	31	43.1	18	41.9	13	44.8	0.813
Enteropathy	9	12.3	4	9.1	5	17.2	0.469
Lymphocytic colitis	9	12.3	4	9.1	5	17.2	0.469
Hepatomegaly	16	21.9	8	18.2	8	27.6	0.394
Splenomegaly	38	52.1	21	47.7	17	58.6	0.474
Liver disease	5	6.8	3	6.8	2	6.9	ns
Pulmonary disease	29	39.7	18	40.9	11	37.9	ns
GLILD	1	1.4	0	0.0	1	3.4	0.397
Other organ diseases	10	13.7	7	15.9	3	10.3	0.73
Hypothyroidism	3	4.1	2	4.5	1	3.4	ns
Chronic diarrhea	5	6.8	4	9.1	1	3.4	0.642
Autoimmune cytopenia	17	23.3	10	22.7	7	24.1	ns
Autoimmune hemolytic anemia	8	11.0	6	13.6	2	6.9	0.465
Autoimmune neutropenia	3	4.1	1	2.3	2	6.9	0.559
Immune Thrombocytopenia	17	23.3	10	22.7	7	24.1	ns
Evans syndrome	9	12.3	6	13.6	3	10.3	ns
Other organ autoimmunity	13	17.8	6	13.6	7	24.1	0.35
Thyroiditis	7	9.6	3	6.8	4	13.8	0.425
Atrophic gastritis	1	1.4	1	2.3	0	0.0	ns
Vitiligo	2	2.7	2	4.5	0	0.0	0.514
Psoriasis	3	4.1	1	2.3	2	6.9	0.559
History of malignancy	1	1.4	0	0.0	1	3.4	0.397
Lymphoid Nodular Hyperplasia	7	9.6	4	9.1	3	10.3	ns
Granulomas	3	4.1	1	2.3	2	6.9	0.559

(*continued*)

TABLE 1 Continued

Demographic Characteristics	Overall CVID cohort (*N* = 73)		Pediatric-onset (*N* = 44)		Adult-onset (*N* = 29)		
	Mean	SD	Mean	SD	Mean	SD	*p*
Celiac disease	5	6.8	2	4.5	3	10.3	0.38
Allergy	16	22.2	12	27.9	4	13.8	0.248
Persistent HCV viremia	0	0.0	0	0.0	0	0.0	na
Persistent HIV viremia	0	0.0	0	0.0	0	0.0	na
Persistent EBV viremia	3	4.8	1	2.6	2	8.0	0.557
Persistent CMV viremia	1	1.6	1	2.8	0	0.0	ns
Pathologic HRCT	51	79.7	32	82.1	19	76.0	0.751
Pathologic endoscopy	23	63.9	14	77.8	9	50.0	0.164

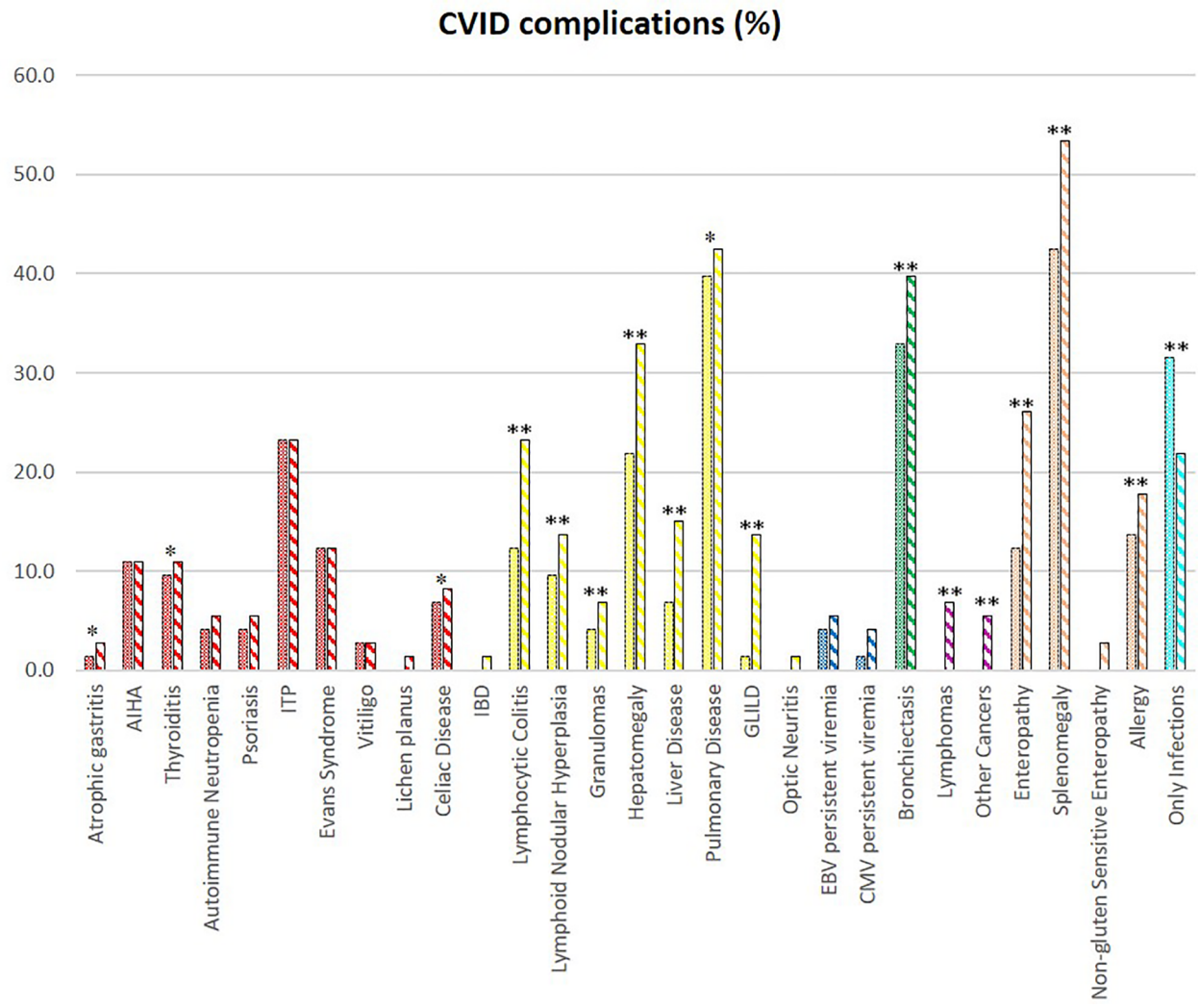

FIGURE 2
CVID complications of the whole cohort. The comparison between manifestations at diagnosis (full colored) and follow-up (colorful stripped) is shown if significate: $^{**}p < 0.001$; $^{*}p < 0.05$.

in Table 1. Infections and immune dysregulation were observed to play the predominant role, but no statistically significant differences were found between the two groups. The analysis of infectious complications at diagnosis shows that recurrent upper and lower respiratory tract infections are predominant in the two groups, but there are no significant differences between them. The longitudinal analysis showed that the two groups have an equal incidence of infectious manifestations

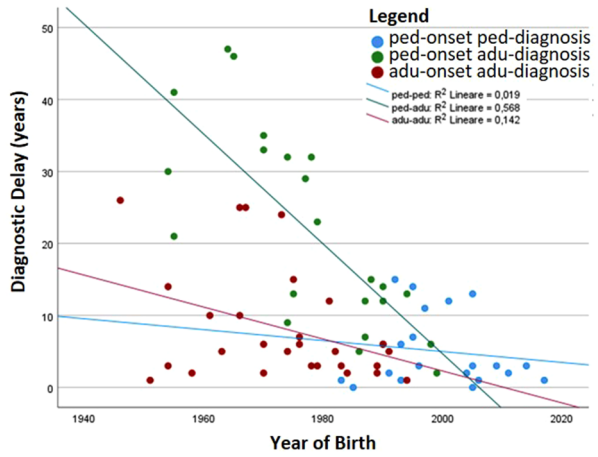

FIGURE 3
The patients' year of birth has been plotted with the time of diagnostic delay. The correlation index (R^2) is also shown.

during follow-up, except for urinary tract infections which are more frequent in adult-onset patients ($p = 0.021$) (**Supplementary Table S2**). At diagnosis, bronchiectasis was already present in 40.9% of pediatric-onset patients and 44.8% of adult-onset patients with no differences between the two groups. Allergic manifestations were found in 12 pediatric-onset patients and 4 adult-onset patients with a similar prevalence.

Table 2 details the serum level of IgA, IgG, and IgM and cell subsets CD19+, and switched memory B cells at diagnosis in the two groups. The lymphocyte subpopulations were further paired and compared between diagnosis and follow-up for each age group onset, evaluating CD4+ and CD8+ lymphocytes, NK cells, CD19+ lymphocytes, switched memory B cells, CD21 low B cells, and transitional B cells (**Figures. 4, 5**). In the pediatric-onset group, the transitional B cells significantly decreased at follow-up ($p = 0.021$), while the other cell subsets did not change over time, although the CD21 low B cells appeared to have increased at follow-up (**Figure 4**). In the adult-onset group, the paired analysis between diagnosis and follow-up revealed no differences, also in this group, CD21 low B cells tended to increase over time (**Figure 5**).

3.3. Clinical phenotypes

Patients were assigned one of the five clinical phenotypes according to the ones by Chapel et al. (7) at diagnosis and follow-up. **Figure 6** shows findings, while **Table 1** and **Supplementary Table S2** detail data. According to the phenotypes' categorization at follow-up, the pediatric-onset group has the main changes in complications, reducing the number of patients who presented with only infections. Complications were due to immune dysregulation as polyclonal lymphoid proliferation and enteropathy increased. In the adult-onset group, the phenotype seemed not to change between onset and follow-up, but lymphoma with a rate of 10.3% at follow-up appeared as the main complication of immune dysregulation. Manifestations of immune dysregulation at diagnosis and follow-up are represented in **Figure 7**. Polyclonal lymphoid proliferation represents the group of complications most developed over time. The prevalence of splenomegaly is high at diagnosis in both groups (40.9% pediatric-onset and 44.8% adult-onset) and increased considerably even at the last follow-up (52.3% pediatric-onset and 55.2% adult-onset). Nodular lymphoid hyperplasia is found at diagnosis in 9.1% pediatric-onset and 10.3% adult-onset, and at follow-up in 13.6% pediatric-onset and 13.8% adult-onset. Granulomas are found at diagnosis in 2.3% pediatric-onset and 6.9% adult-onset and increased at follow up in the pediatric-onset group at 6.9%; one more pediatric-onset patient had granuloma with optic neuritis (21). Pulmonary lung disease, including granulomatous lymphocytic infiltration (GLILD) and thoracic lymphadenopathy, is the most common organ-specific complication both in patients with pediatric-onset (45.5%) and with adult-onset (37.9%). GLILD was already present at the diagnosis of CVID only in one adult-onset patient (1.4%), but its prevalence greatly increases at the last follow-up to 20.7% of adult-onset and 9.1% of pediatric-onset.

3.4. Analysis of the risk for immune dysregulation

Autoimmune complications, including autoimmune cytopenias and/or organ-specific autoimmunity, were seen at diagnosis in 41.1% of the overall cohort. All the patients with autoimmune

TABLE 2 Laboratory findings at CVID diagnosis.

	Overall CVID cohort ($N = 73$)		Pediatric-onset ($N = 44$)				Adult-onset ($N = 29$)	
			Pediatric-onset/ pediatric-diagnosis ($N = 21$)		Pediatric-onset/ adult-diagnosis ($N = 23$)			
Laboratory Characteristics	Mean	SD	Mean	SD	Mean	SD	Mean	SD
IgG (mg/dl)	203.6	148.68	240.3	139.34	313.0	148.43	174.8	151.85
IgM (mg/dl)	11.4	18.19	22.5	18.12	28.5	24.50	18.8	19.37
IgA (mg/dl)	20.5	18.74	6.3	6.11	54.8	173.03	15.4	23.11
CD19+ B cells (cell/µl)	215.2	111.46	238.6	103.30	373.3	284.16	210.7	119.24
Switched memory B cells (%)	3.1	2.07	2.5	1.72	5.8	6.31	3.5	2.17

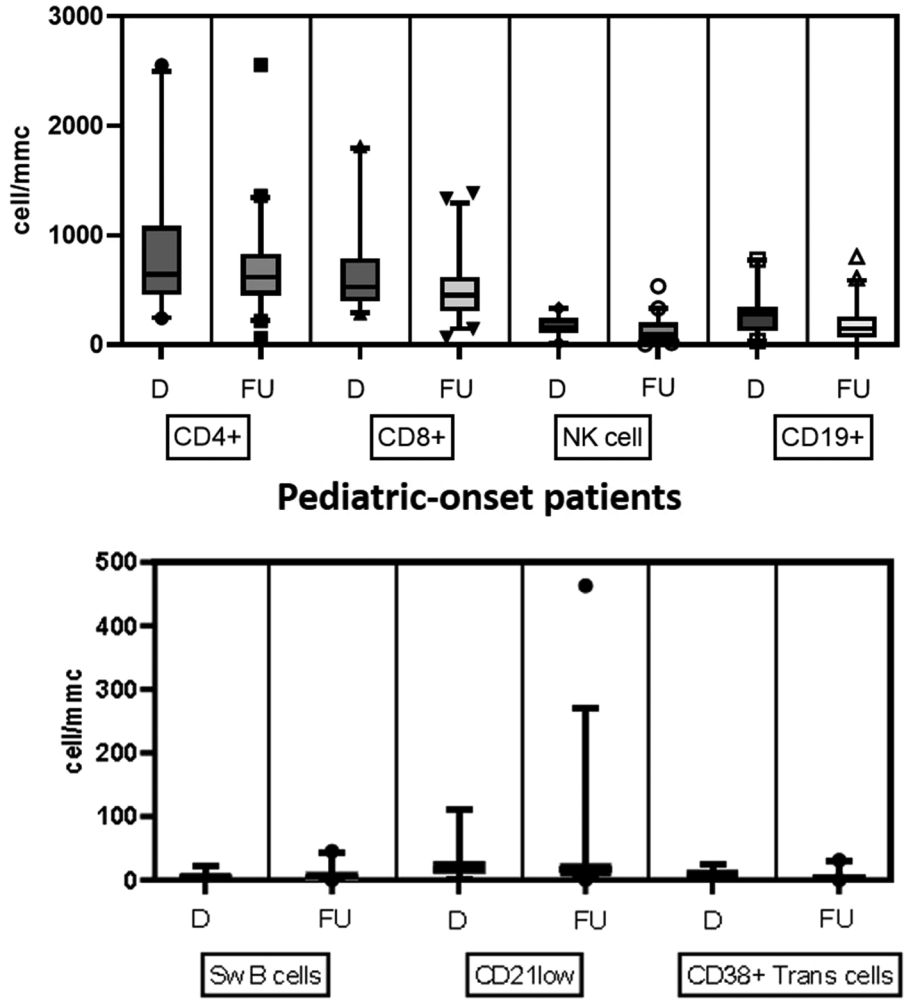

FIGURE 4
Distribution of B, T and NK cell subsets, switched memory B cells, CD21 low B cells, and transitional B cells at diagnosis and follow up in the pediatric-onset group.

cytopenias (23.3%) presented immune thrombocytopenic purpura (ITP), 22.7% of pediatric-onset and 24.1% of adult-onset. Associated with autoimmune hemolytic anemia (AIHA) in 11% of overall subjects and with autoimmune neutropenia in 5.5% of overall patients in the context of Evans syndrome is 12.3% of the whole cohort. There was no change from diagnosis to follow-up on the prevalence of autoimmune cytopenias in both pediatric-onset and adult-onset groups (**Figure 8**). **Table 1** and **Supplementary Table S2** detail findings for organ-specific autoimmunity. Enteropathy at diagnosis was documented in 9.1% of pediatric-onset patients and 17.2% of adult-onset patients. Its prevalence increases considerably at the last follow-up both in patients with pediatric-onset (22.7%) and in patients with adult-onset (31.0%). Lymphocytic colitis affects 20.5% of pediatric-onset and 27.6% of adult-onset patients, furthermore, gluten non-sensitive enteropathy has been diagnosed in 2.3% of pediatric-onset and 3.4% of adult-onset patients. Hepatomegaly was found at diagnosis in 40.9% of pediatric-onset and 44.8% of adult-onset patients, and its prevalence increased during follow-up reaching 52.3% of pediatric-onset and 55.2% of adult-onset patients. Liver disease has been documented at the last follow-up in 21.7% of pediatric-onset and 37.5% of adult-onset patients who have hepatomegaly. As for malignancy, no patients had a history of cancer at diagnosis. Neoplasms occurred during follow-up in 12.3% of the whole cohort, including gastric adenocarcinoma (2 patients), non-melanocytic skin cancer (1 patient), breast cancer (1 patient), and lymphomas (5 patients, 2 in the pediatric-onset and 3 in the adult-onset group). Two were non-Hodgkin's lymphomas, and three were EBV-related lymphomas: one Burkitt's lymphoma and two Hodgkin's lymphomas. These both occurred in patients with pediatric-onset CVID which was complicated by relapsing and refractory autoimmune cytopenia. NGS identified a pathogenic mutation on CTLA-4 gene for both patients. The estimated risk of malignancies in patients affected with CTLA-4 deficiency is approximately 12.9%. The reported cases are mostly EBV-positive lymphomas (HL, DLBCL, and BL) (22). The patient with Burkitt's lymphoma had acute EBV infection documented 5 years before the lymphoma. Splenomegaly, lymphadenopathy, and chronic EBV viremia (very low copies circulating) were

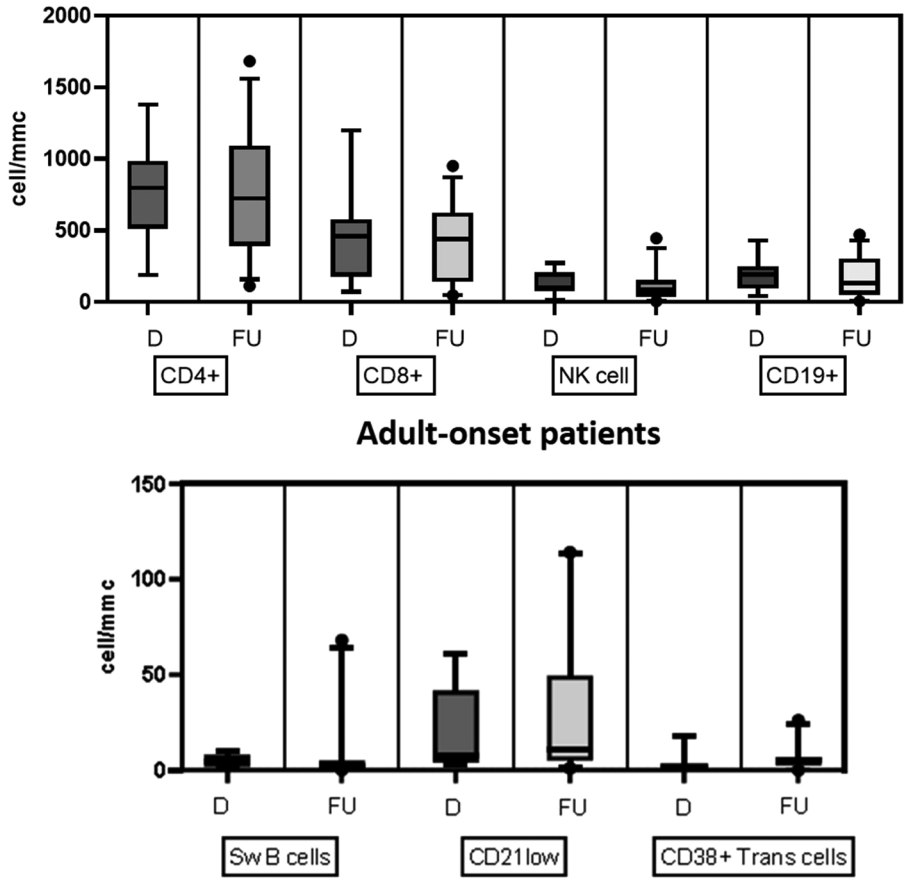

FIGURE 5
Distribution of B, T and NK cell subsets, switched memory B cells, CD21low B cells, and transitional B cells at diagnosis and follow up in the adult-onset group.

persistent for two years before lymphoma. NGS identified two mutations on the TACI gene associated with CVID (23).

3.5. Analysis of the risk for immune dysregulation

The risk of developing at least one complication during follow-up due to immune dysregulation was assessed considering the age at the onset of CVID (**Figure 9A**). At the same age, pediatric-onset patients have about twice the risk of having complications due to immune dysregulation than adult-onset patients. The risk is enhanced in pediatric-onset adult-diagnosis patients, and it increases with diagnostic delay. Considering the time of follow-up since the diagnosis, the early years' patients with pediatric-onset and adult-onset seem to have an overlapping risk of having immune dysregulation features (**Figure 9B**), as they may have those just present at diagnosis. After the first ten years of follow-up, adult-onset patients seem to have double the risk of developing a complication due to immune dysregulation compared to pediatric-onset patients.

In order to identify biomarkers predictive of immune dysregulation development during follow-up, some indices were evaluated based on the data reported in the literature (24–26). In particular, the predictive power of the CD21low B cells assessed at diagnosis to identify subjects who would develop immune dysregulation at follow-up was analyzed by ROC curve. If we analyze the entire cohort of 73 patients with CVID, the area under the curve of CD21low cells is not significant. Considering the pediatric-onset group, the percentage of CD21 low B cells at diagnosis may be a reliable prognostic marker for the development of immune dysregulation during follow-up as the ROC curve analysis showed (AUC = 0.796), illustrated in **Figure 10**. The sample was numerically small ($n = 14$), therefore the identified cut-off value (>3%) is lower than the known data in the literature (>10%) (24). In the group of patients with adult-onset, the very small sample size ($n = 8$) of CD21 low B cell data at diagnosis greatly limits the analysis of the ROC curve (data not shown). Another index evaluated was the percentage of transitional B cells measured at diagnosis, which showed significant accuracy (ROC AUC = 0.625) in identifying adult-onset patients at risk of developing immune dysregulation, despite the limitation of the small sample (data not shown).

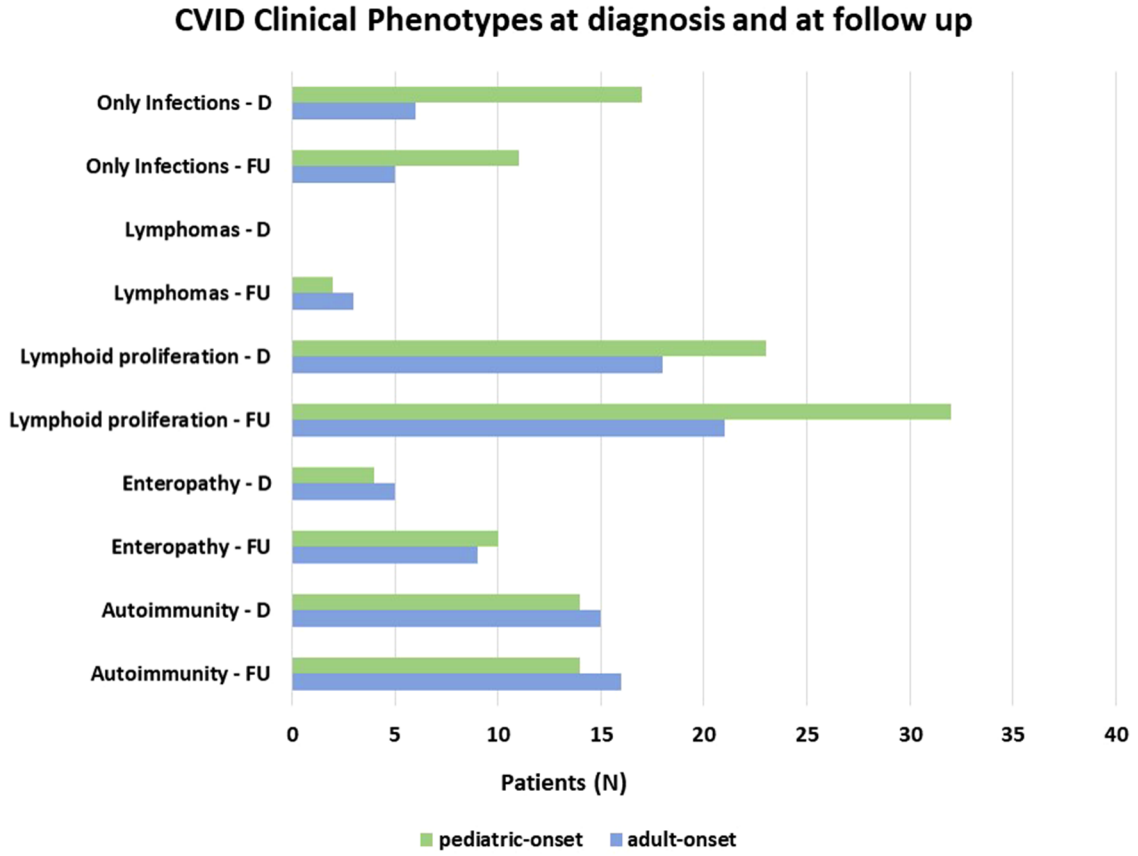

FIGURE 6
Distribution of patients according to Chapel et al clinical phenotypes (7) at diagnosis and follow-up in the pediatric-onset and adult-onset groups.

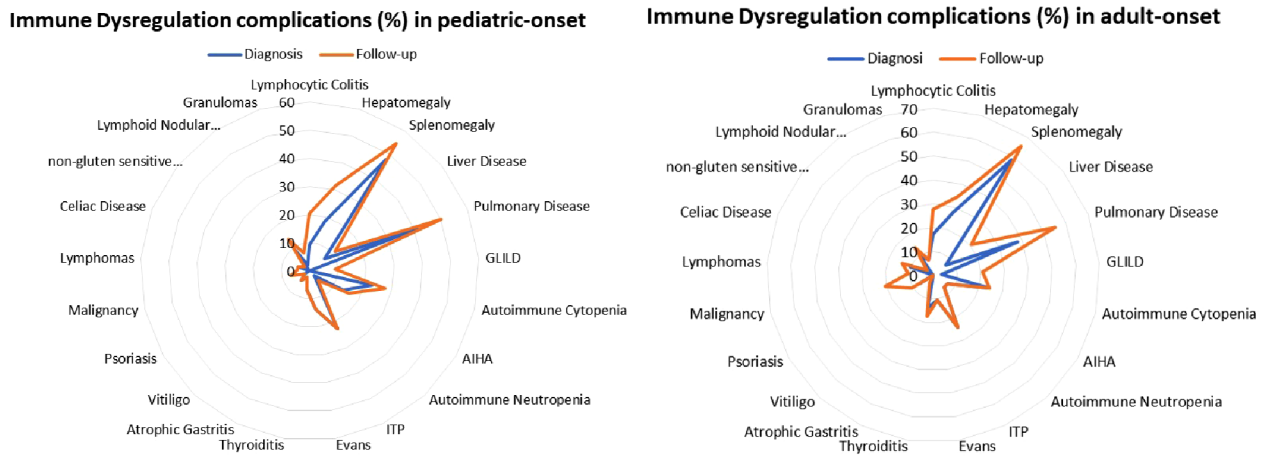

FIGURE 7
Prevalence of complications of immune dysregulation at diagnosis and at follow-up in the pediatric-onset (A) and adult-onset patients (B).

The ROC analysis of the same cell subsets at follow-up shows that in both the pediatric-onset group and the adult-onset group, the percentage of transitional B cells measured at follow up seems to be associated with immune dysregulation (AUC = 0.651 and AUC = 0.722 respectively), while CD21 low B cells do not appear to play a role (**Figure 11**). The sample size of the patient groups limits the interpretation of these data (pediatric-onset group $n = 30$; adult-onset group $n = 21$).

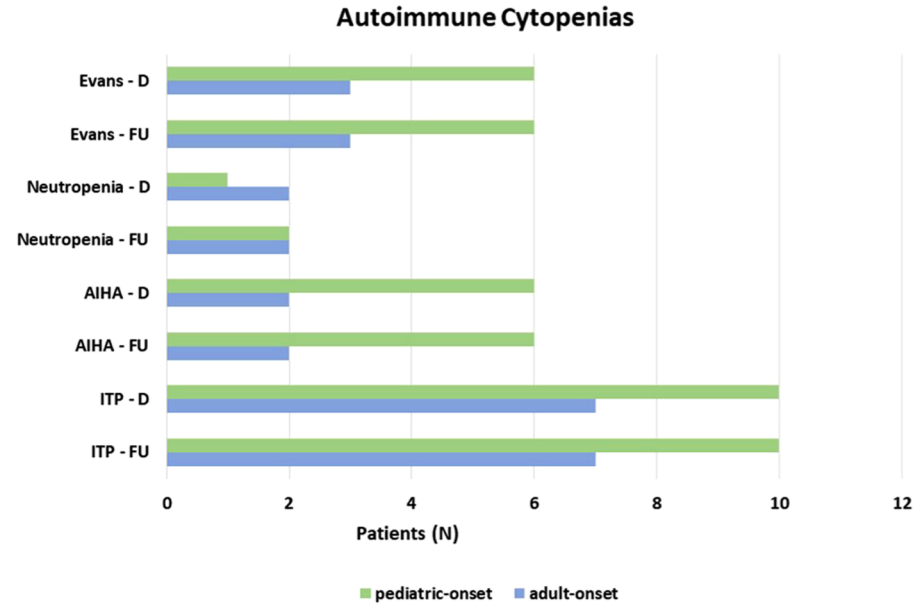

FIGURE 8
Prevalence of autoimmune cytopenias at diagnosis and at follow up in the pediatric-onset and adult-onset groups.

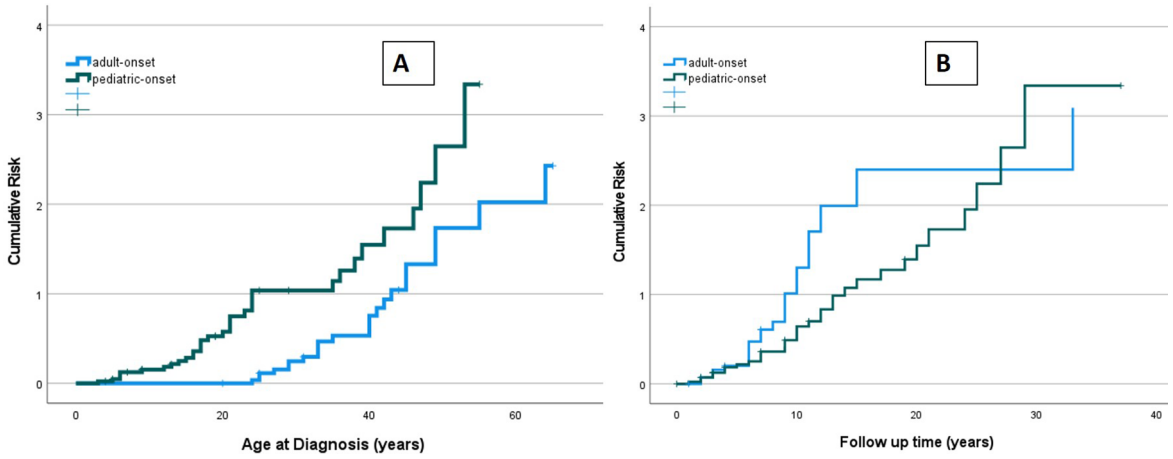

FIGURE 9
Cumulative risk curves of developing immune dysregulation observed in the pediatric-onset group (green line) and the adult-onset group (blue line) according to age at diagnosis of CVID (A) and time of CVID follow-up (B).

3.6. Associated factors to immune dysregulation in the overall cohort of CVID patients

A correlation analysis was performed between clinical features of immune dysregulation and laboratory data, particularly subsets of lymphocytes highlighted by recent studies in the literature (24–26) circulating CD8+ T cells <200 cells/μl and circulating CD21low B cells >10%. We also considered the presence of chronic EBV or CMV viremia during follow-up and the serum IgM levels at diagnosis. We found that circulating CD8+ level <200 cells/μl at follow-up correlated positively with the presence of autoimmunity ($R = 0.313$) and granulomatosis ($R = 0.463$) at follow-up, the persistence of chronic EBV viremia ($R = 0.379$) at follow-up, malignancy ($R = 0.463$) and, in particular, with lymphomas ($R = 0.327$) at follow-up, which in this series are EBV-related in 3 out of 5 subjects. Levels of circulating CD21 low B cells >10% at follow-up correlate positively with circulating CD8+ T cells <200 cells/μl ($R = 0.327$) and circulating CD4+ lymphocytes <300 cells/μl at follow-up. The presence of chronic EBV viremia is associated with the presence of autoimmunity both at diagnosis ($R = 0.297$) and at follow-up ($R = 0.288$), and it

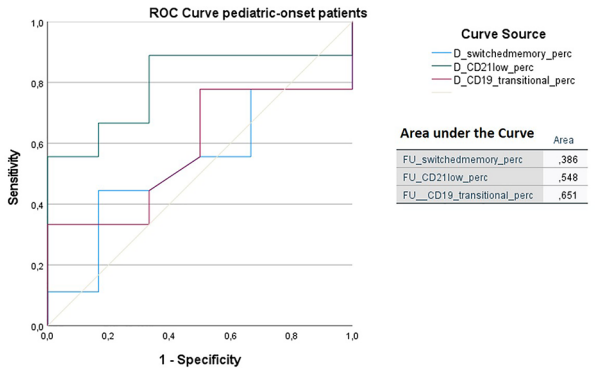

FIGURE 10
ROC curve analysis to predict immune dysregulation at follow-up of switched memory B cells (%), CD21low cells (%), and transitional B cells (%) measured at diagnosis in the pediatric-onset group. Details of the area under the curve (AUC) on the graph.

correlates with lymphoma development ($R = 0.411$). Nodular lymphoid hyperplasia showed a positive correlation with chronic EBV and chronic CMV viremia during follow-up ($R = 0.254$ and $R = 0.550$, respectively). Considering the circulating serum immunoglobulin levels measured at diagnosis, IgM was found to be inversely related to the presence of autoimmunity at diagnosis ($R = -0.202$) and follow-up ($R = -0.218$), as well as with granulomas ($R = -0.196$), GLILD ($R = -0.287$) or splenomegaly ($R = -0.304$).

4. Discussion

4.1. Age at CVID onset

Previous cross-sectional studies investigated the differences in CVID manifestations and complications according to age at onset or at diagnosis (14, 27–30). They reported that pediatric-onset CVID patients were more affected by infections and autoimmunity than adult-onset patients.

The present analysis found no significant differences in diagnosis in the prevalence of infectious disease, autoimmunity, or immune dysregulation, nor in immunoglobulin or lymphocyte profiles between patients with pediatric-onset and adult-onset CVID. Comparison at follow-up showed that patients divided by age of onset did not show significant differences in terms of initial clinical manifestations and clinical features at follow-up (except for urinary tract infections), showing that age of onset does not affect the clinical phenotype. Patients could therefore present more or less severe clinical manifestations, even immune dysregulation, regardless of the age of CVID onset. These findings are consistent with the ones recently reported by the data on the 457 patients of the USIDNET CVID cohort (15) and differ from previous reports. The USIDNET cross-sectional study on the features of patients across the age groups identified few differences between pediatric-onset and adult-onset of the disease. Some of the differences, such as bronchiectasis or granulomatous infiltration, may reflect the long-term consequences of subacute, recurrent, or chronic infections and delay in Ig replacement therapy (15). The largest cross-sectional study on 2,212 patients with CVID enrolled in ESID Registry showed also that males with CVID onset before the age of 10 years were more susceptible to pneumonia and bronchiectasis (14). Both studies did not assess longitudinal follow-up and the effects of the years of disease in the two age groups.

4.2. Clinical phenotypes

The present longitudinal analysis showed that during follow-up the incidence of the "only-infections" phenotype decreases and all the patients skew to the "immune dysregulation phenotype". The increase in immune dysregulation complications is higher in the pediatric-onset group than in the adult-onset group. At

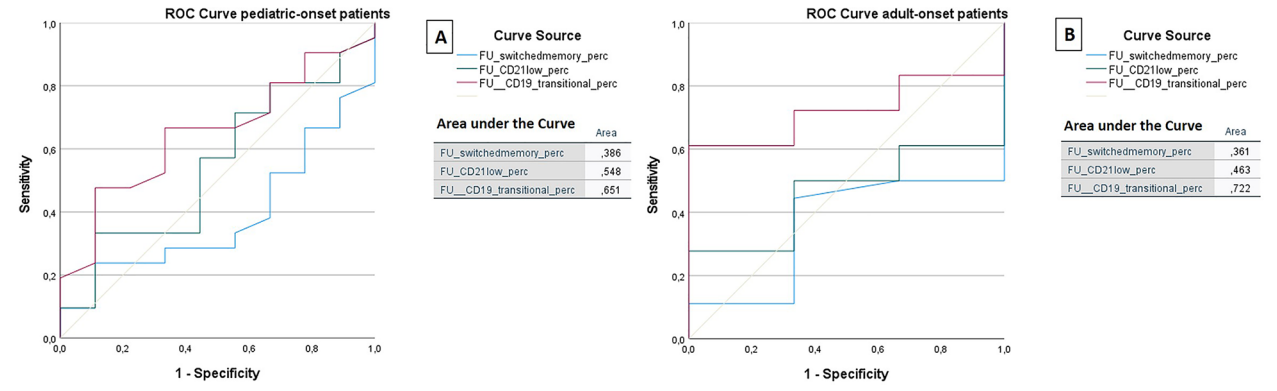

FIGURE 11
ROC curve analysis to predict the presence of immune dysregulation of switched memory B cells (%), CD21low cells (%), and the transitional B cells (%) measured at follow-up in the pediatric-onset group (A) and adult-onset (B) group.

diagnosis, 38.6% of pediatric-onset and 20.7% of adult-onset patients presented with only infections. Polyclonal lymphoid proliferation (62.1%) and autoimmunity (51.7%) were more prevalent at diagnosis in the adult-onset than in the pediatric-onset group (polyclonal lymphoid proliferation 52.3% and autoimmunity 31.8%, respectively). Enteropathy was present at diagnosis in 9.1% of pediatric-onset and 17.2% of adult-onset patients. These findings agree with those of other studies (8, 15, 16). Immune dysregulation seems to be an intrinsic feature of CVID that manifests over time. At follow-up, the sub-analysis of age at diagnosis showed that patients with pediatric-onset and adult-diagnosis manifested gastrointestinal complications more frequently than the patients with pediatric-diagnosis. A recent study comparing 9 pediatric-onset to 13 adult-onset patients with CVID found that the pediatric-onset patients with chronic diarrhea had decreased serum IgA levels and naïve CD4 T-cell and RTE cell percentages compared to the age-matched CVID patients (31).

There is increasing evidence that altered microbiome and gut barrier dysfunction contribute to systemic inflammation in patients with CVID, especially in those with lower levels of IgA and IgM antibodies binding to lipopolysaccharide and low isotype-switched memory B cells. A multi-omics approach has been used to characterize biomarkers of noninfectious complications in CVID patients (32). The data obtained are consistent with a model in which defects in humoral immunity, especially IgA, lead to barrier dysfunction, translocation of bacteria, activation of both the innate and the adaptive arms of the immune system, and tissue infiltration and/or autoimmunity.

A recent meta-analysis on neoplastic complications in patients with CVID showed an overall prevalence of malignancies equal to 8.6% (33). In our population, malignancy occurred in 12.3% of the overall cohort, divided equally between lymphomas (6.8%) and other cancers (5.5%). These data are comparable to those found in other series that report values between 1.8% and 6.7% for lymphomas and between 3% and 6.4% for other neoplasms (7, 8, 17). Lymphomas occurred similarly in both pediatric-onset and adult-onset groups.

4.3. Risk for immune dysregulation

In the present analysis, the risk of having a complication due to immune dysregulation in the pediatric-onset group is about double in the adult-onset patients at the same age. It may be that the longer delay in diagnosis in the pediatric-onset cohort is one of the keys to explaining their higher incidence of enteropathy due to immune dysregulation, as the prevalence of lymphoid proliferation appears to increase with time.

In the last fifteen years, many studies have focused on the description, characterization, diagnosis, and therapy of immune dysregulation in CVID, which is increasingly considered a characteristic of the disease from childhood to adulthood (7, 8, 15, 16). A meta-analysis (34) conducted on the most recent studies of the main centers that have large series of CVID patients showed the prevalence of the various conditions: autoimmune cytopenias 18.9%, gastrointestinal autoimmune diseases 11.5%, skin autoimmune diseases 5.9%, and endocrinopathies 2.5%. The prevalence of the single autoimmune complications observed in our cohort is comparable (autoimmune cytopenias 23.3%, gastrointestinal autoimmune diseases 12.3%, and skin diseases 8.2%), except for the endocrinopathies, which in our series are present in a higher percentage of patients, equal to 11.0%. We found that autoimmunity seems to increase near CVID onset in both groups by age of onset, while lymphoid proliferation and enteropathy increase over time.

A recent study conducted on 16,486 patients suffering from different inborn errors of immunity and enrolled in the ESID Registry evaluated the initial manifestations of the disease. Of the 4,244 patients affected by CVID (including the 73 patients of this cohort), the analysis described a prevalence of immune dysregulation equal to 18%, apart from neoplasms which has a total prevalence of 1% was highlighted (26). Immune dysregulation as the main manifestation at the onset of symptoms in the present overall CVID cohort is higher and reaches 42.5%. The immune dysregulation prevalence highlighted in our cohort, equal to 78%, is comparable with that of other international CVID cohorts (7, 8).

4.4. Biomarkers

Despite the clinical subsets of complications showing important prognostic implications, the effort to identify biomarkers of severe CVID disease for early identification is still ongoing (24). Circulating lymphocyte markers were the first extensively studied biomarkers in CVID in order to identify predisposing conditions or parameters able to predict the development of immune dysregulation. Reduced isotype-switched memory B cells and reduced T cells (CD4) can be utilized to identify those with increased complication risks. The EUROclass trial associated low switched B memory and expanded CD21 low B cell counts with splenomegaly and granulomatous disease and the expansion of transitional B cells to lymphadenopathy (25). The DEFI group found that infection-only patients had low switched memory B cell counts, while decreased naïve CD4+ T-cell counts and increased CD4 + CD95+ cells were associated with lymphoid proliferation, autoimmune cytopenias, or chronic enteropathy (26). These biomarker studies have had limited validation in pediatric cohorts (35).

We found that in the pediatric-onset group, the percentage of CD21 low B cells at diagnosis may be a reliable prognostic marker for the development of immune dysregulation during follow-up (ROC AUC = 0.796). The sample size was small, therefore the identified cut-off value (>3%) is lower than the known data in the literature (>10%) (24). In adult-onset patients, the percentage of transitional B cells measured at diagnosis showed significant accuracy (ROC AUC = 0.625) in identifying individuals at risk of developing immune dysregulation, despite the limitation of the small sample size. Similar to other previous studies (7), the association between a low number of CD8+ T lymphocytes

(<200 cells/μl) and the presence of immune dysregulation and persistence of EBV viremia was also confirmed in this cohort as well as the association between persistent EBV viremia and the development of lymphoid proliferation, in particular lymphomas. EBV may promote autoimmunity not only through cross-reactivity with self-antigens, as it has been observed in SLE or multiple sclerosis, but also by expanding autoreactive age-associated B cells (ABCs) that could continuously stimulate autoreactive T cells and promote chronic inflammation in both secondary lymphoid tissue and target tissue (36).

Unlike other studies (7), no association was found between the increase in serum IgM and the development of lymphomas. In this cohort, serum IgM measured at diagnosis has a negative correlation with the development of immune dysregulation. This aspect requires further analysis and confirmatory studies.

In the latest years, genetic sequencing in CVID has been largely applied, hoping that the identification of specific genes responsible for the CVID phenotype would drive our understanding of the disease. In 10%–30% of CVID patients, pathologic monogenic defects can be identified (9–11). These defects are broadly categorized into (1) genes implicated in various stages of B cell activation, survival, or maturation to the plasma cell stage, and (2) immune-regulatory genes, with autoimmunity and inflammation (CVID) being more characteristic of the latter group. However, genetic causes of at least ~70% of CVID patients remain unidentified to date, including those with complications (37).

Also in our cohort, targeted NGS identified a mutation in genes considered causative of CVID in 26% of patients. Among the 56 patients with complications, the majority (73.2%) did not have a causative genetic mutation. Nine of the patients with the identified gene had mutations on the TACI gene. Among these patients, seven had CVID complications. Moreover, as TACI gene variants commonly found in CVID subjects can be observed in first-degree relatives and healthy individuals with normal immunoglobulin levels, these may be more disease-modifying than disease-causing, but still, are often associated with lymphoproliferation and autoimmunity (24). The small number of patients does not allow us any further consideration.

The results of this study are consistent with other recent reports on a larger cohort of patients with CVID. They show that pediatric-onset and adult-onset do not differ in manifestation at diagnosis or follow up. Autoimmunity, cytopenia in particular, is present in close proximity to the onset of CVID, while other complications of immune dysregulation arose over time in both age-at-onset groups. Therefore, periodic monitoring with tools associated with immune dysregulation, such as CD21 low and transitional B cells, or with lymphoproliferation, such as persistent EBV viremia in the peripheral blood, appear to have a role in predicting immune dysregulation. The sample size and the data relating to the extended phenotype of lymphocyte subsets represent the main limitations of this study, therefore, our data need further investigation and validation.

In conclusion, longitudinal assessment of lymphocyte subsets combined with clinical phenotype can improve the prediction of lymphoid proliferation and allow CVID specialists to achieve earlier detection and better management of such a complex disorder. Additionally, condition-specific markers have also been suggested for lymphoma (normal or elevated IgM) and progressive interstitial lung disease (increased BAFF, normal, or elevated IgM) (24).

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Milan Area 2, Italy. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

MC, MS, RMD, and GF designed the study, performed data analysis, and prepared the manuscript. MC, LB, MS, MZ, SS, MCP, GF, and RMD helped with patient enrollment, data collection, registry records of clinical information, and manuscript preparation. Lymphocyte subsets analysis was performed by ET. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2023.1125994/full#supplementary-material.

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Immunoglobulin replacement therapies in inborn errors of immunity: a review

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Immunoglobulins (Ig) were used as a therapeutic modality for the first time in a patient with X-linked agammaglobulinemia in 1952 by Colonel Ogden Bruton, decades before the molecular mechanisms causing the disease were unraveled. In many autoimmune and inflammatory illnesses, human immunoglobulin has been employed as a significant immunomodulatory and immunosuppressive drug. In patients with inborn errors of immunity (IEI), immunoglobulin remains a cornerstone of management. IEIs are notable causes of recurrent infections and autoimmunity due to inheritable single-gene defects in genes encoding for different components of the immune system. As there is decreased immunoglobulin production in IEIs with antibody defects, immunoglobulin replacement is the mainstay of therapy in these disorders. Although serum immunoglobulin levels may not be low in combined immune defects, immunoglobulin replacement is still necessary in these disorders due to a deficiency of functional antibodies and qualitative defects of immunoglobulins. Commercial immunoglobulin preparations are generated from plasma donated by thousands of donors. Immunoglobulin preparations are usually available in two forms: intravenous and subcutaneous immunoglobulins. In the developed world, both intravenous immunoglobulin (IVIg) and subcutaneous immunoglobulin (SCIg) are available, and SCIg is preferred over IVIg for replacement therapy in patients with IEIs. In developing countries, IVIg remains the mainstay of replacement therapy. The rate of adverse events has significantly reduced over the last few years due to advancements in the production process. In this review article, we discuss different aspects of the use of Ig (indications, dosing, mechanism of action, route, adverse effects) in patients with IEIs.

KEYWORDS

immunoglobulin, inborn errors of immunity (IEI), intravenous immunoglobulin (IVIg), subcutaneous immunoglobulin (SCIg), antibody defects

Background

In many autoimmune and inflammatory illnesses, human immunoglobulins have been proven to be significant immunomodulatory agents. A diverse collection of diseases known as inborn errors of immunity (IEI) can cause autoimmunity, recurring infections, and can even increase the risk of developing malignancy. IEIs are caused by heritable single gene abnormalities in the genes that code for several immune system components. To date, descriptions of more than 485 IEIs have been made (1). The first FDA-approved use of immunoglobulin replacement therapy was for IEIs (2). While immunoglobulin replacement therapy (IGRT) remains the cornerstone of management in antibody deficiency related IEI,

it plays a supporting role in several other IEIs as well (3). Immunoglobulins (Ig) can be administered through intravenous and subcutaneous modes. In this review we have discussed the available preparations of immunoglobulins and their use in IEIs.

History

It was Emil Adolf Von Behring and Shibasaburo Kitasato who first documented transfer of protection against diphtheria and tetanus in animal models in 1890 (4). Paul Ehrlich first used the word "antibody" in 1891 (5). Ig was used as a therapeutic modality for the first time in X-linked agammaglobulinemia (XLA) patient by Colonel Ogden Bruton in 1952 (6). He administered subcutaneous immunoglobulin to an 8-year-old boy who had undetectable gamma globulin fraction in his blood and suffered from repeated pneumococcal infections. Circulating gamma globulin levels improved significantly after subcutaneous immunoglobulin infusions of 3.2 g/month and complete elimination of pneumococcal infections was achieved (6). However, at that time very little was known about the molecular mechanisms of XLA.

Principles of therapy with immunoglobulins

Ig is used in diverse clinical situations. It is often used as an immunomodulatory agent in autoimmune and inflammatory conditions. It is also used as replacement therapy (IGRT) in several IEIs. Main aim of replacement is to achieve functional levels of passive antibodies (IgG) sufficient for opsonization and neutralisation of infectious pathogens, like parasites, viruses and bacteria (5). Commonly used replacement doses are 400–600 mg/kg given every 3 weeks when used intravenously. For subcutaneous (SC) route, the dose is 100–200 mg/kg/week. For immunomodulatory effects, higher dose of Ig (1–2 g/kg) need to be given (7). Immunomodulatory action of Ig is executed by several mutually non-exclusive mechanisms. These include altering Fc receptor expression and function, controlling complement activation, affecting the cytokine network, forming antibodies against pathogens, neutralising *T*-cell superantigens, and controlling the development, activation, differentiation, and effector functions of *T* cells and B cells (8). Immunoglobulin therapy affects the growth, development, and functions of several immune system cells, including monocytes/macrophages, dendritic cells, granulocytes, NK cells, and *T* and B cells (9) (Figure 1).

Putative mechanisms of action of Ig when used for IEIs:

1. Ig stimulates maturation and differentiation of dendritic cells (DC) in low dose. It has been observed that in patients with XLA differentiation of DC is defective (10). Ig at low dose can correct dendritic cell maturation defect and exerts a boosting effect on immune response of the host. Same

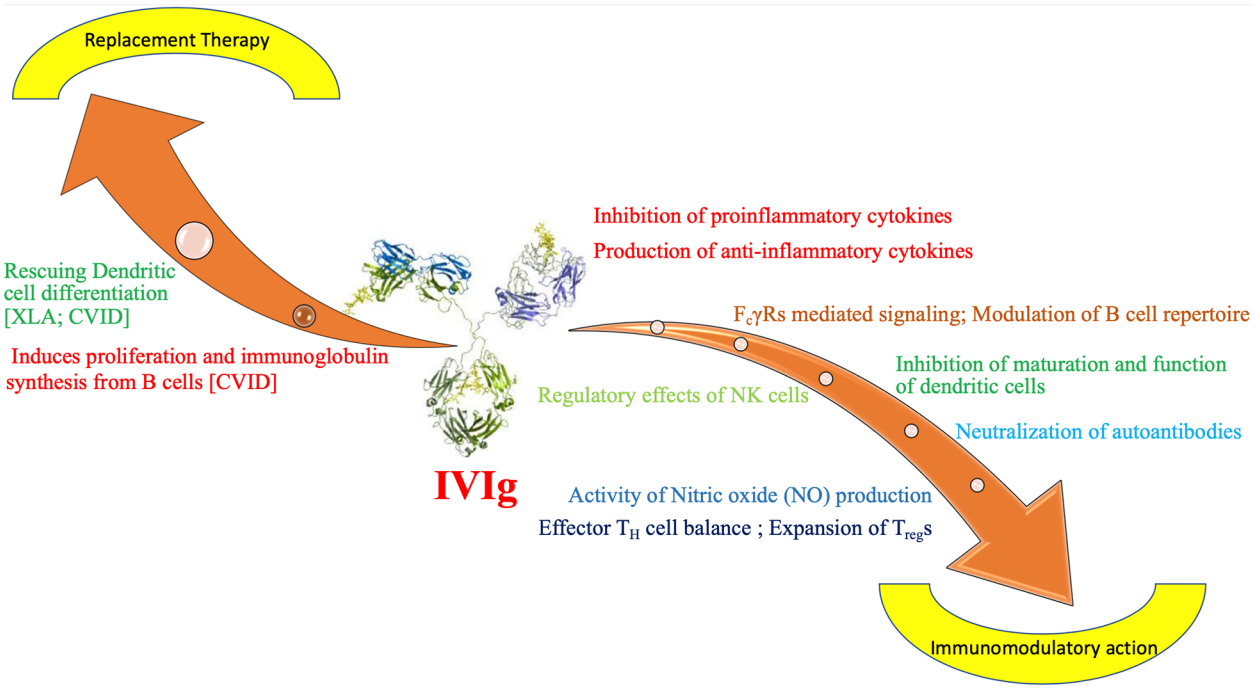

FIGURE 1
Mechanism of action of immunoglobulin as replacement and immunomodulatory therapy in inborn errors of immunity. CVID- Common Variable Immunodeficiency, $F_c\gamma R$, F_c gamma receprtors; IVIg, intravenous immunoglobulin; NK, natural killer cells; T_H, T helper cell; T_{reg}, T regulatory cell; XLA, X linked agammaglobulinemia.

enhancing effect can be observed on differentiation of DC in CVID patients (11).
2. Another important aspect of action of Ig is stimulation of B-cell proliferation and induction of immunoglobulin synthesis. It has been documented in patients with CVID (12).
3. It has important role in modulating the inflammatory manifestations of IEI by reducing secretion of inflammatory cytokines like IL-1β, IL-6 by B cells (12).

❖ Source of Immunoglobulin preparation:

Ig can be obtained from the plasma of donors undergoing plasmapheresis- this is known as "source plasma". Ig can also be recovered from donated blood in blood banks- this is known as "recovered plasma". **Source plasma** is directly processed from plasma donated voluntarily (usually remunerated) donors.

Recovered plasma is a by product of whole blood separated during preparation of blood components. It is collected at blood bank from blood donated by donors meeting whole blood donor requirements (Figure 2).

❖ Donors (13):

- Selection- Selection of donor is based on the World Health Organisation (WHO) criteria:

1. Donor should be healthy (free from transfusion-transmitted infections)
2. Absence of transfusion transmitted infections [Donors should be screened for hepatitis B, C and human immunodeficiency virus (HIV) infections]
3. Appropriate interval since last donation [Donors can be divided based on the frequency of donation: (A) Frequent-Donors can donate maximum twice within a week (B) Infrequent- Donors can donate once every month or less]
4. Physical assessment of the donor: Parameters like body weight, pulse, blood pressure, temperature should be checked and haemoglobin level should also be measured.

- Testing details: Testing should be carried out for syphilis, malaria, cytomegalovirus, HIV, hepatitis B surface antigen (HBsAg), and hepatitis C virus (HCV) serology. It is recommended to utilise molecular (polymerase chain reaction) and serological approaches. Additionally advised testing for retrieved plasma includes human T-lymphotropic virus (HTLV) I and II, West Nile virus, and Chagas disease.

❖ Storage of plasma

Source plasma should be stored at a temperature lesser than −20°C. Regulation for minimum freezing temperature has been set by Food and Drug Administration (FDA). Protein activity can be preserved by rapid freezing following plasmapheresis.

In source plasma, donors are screened for infections and if normal, only then their plasma is considered. Units of plasma are then tested for known blood-borne diseases and if found positive, is discarded. As per FDA 60 days "quarantine" or "inventory hold" procedure is done where donor plasma is stored separately until the donor returns to provide another unit of plasma. Only if the second and subsequent donations test negative then the previous units are released. Tracking of all products can be done with the help of computerized databases. Ig preparations are derived from pooled plasma of 1,000–100,000 healthy donors. Use of such a high number of plasma donors ensure that there is broad spectrum of protective antibodies. IgG fraction is the main component with very low IgA and IgM. The concentration of IgA fraction varies from 0.6–40 mg per 100 ml depending on the preparation (14). Half-life of IgG is 3–4 weeks. Both IVIg and SCIg preparation have IgG1, IgG2, IgG3 and IgG4 levels comparable to human plasma. IgG molecules tend to aggregate in concentrated solution bringing the Fc fragment into proximity. These Fc portions cause activation of complement and cross-linking of Fcγ-receptors. As a result there is production of inflammatory mediators which lead to adverse events during IgG infusions (15). To prevent IgG aggregation, sugar stabilizers are used like sorbitol, glucose, or maltose. Recently, amino acids like proline or glycine are preferred as stabilizers because osmotic tubular injury has been reported with Ig stabilising sugar components (14, 16). Purification process can adversely affect the quality and biological activity of immunoglobulins in terms of efficacy and safety. There is variation in content of salt and IgG concentration, and not all products are licensed for use by different routes. So route of Ig administration should be individualized. Unpredictable idiosyncratic reactions can occur due to use of large donor pools, leading to difference in specific antibody and plasma protein concentration.

Immunoglobulins play a pivotal role in humoral adaptive immunity; IVIg reflects a collective exposure of the donor

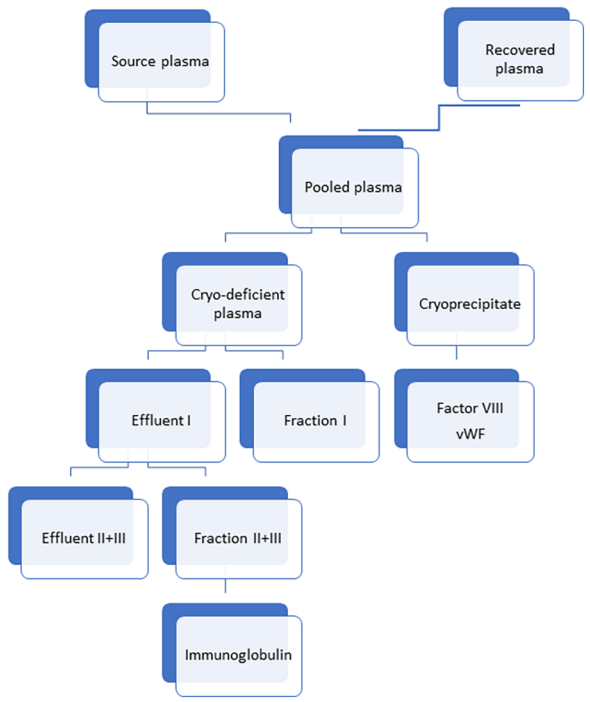

FIGURE 2
Method of preparation of immunoglobulin from different plasma sources. Cryo-deficient plasma: Cryo-deficient plasma is the plasma supernatant remaining following removal of the cryoprecipitate from frozen-thawed plasma (39).

population to their environment and can be expected to contain an antibody repertoire of multiple specificities against a broad spectrum of infectious agents (bacterial, viral, and others), self-antigens and anti-idiotype antibodies.

Types of immunoglobulin preparation

Immunoglobulin preparations are usually available in 3 forms –

1. Intravenous immunoglobulin (IVIg)
2. Subcutaneous immunoglobulins (SCIg).
3. SCIg with hyaluronidase facilitated

There are reports of use of intramuscular IgG (IGIM) in the 1950s, 60s and 70s. However, it was associated with adverse events like chronic pain at the injection site, hypotension, unconsciousness, tightness in the chest, dyspnea, and episodes of facial swelling. Children with low muscle mass used to have difficulty receiving IGIM, necessitating more frequent injections to attain adequate IgG levels. Immunoglobulin preparations are to be used at frequent intervals unlike specific immunoglobulin like tetanus anti-toxin which is mostly used on single occasion. Therefore, its use became obsolete due to pain at the injection site, frequent dosing, and poor compliance (17).

While IVIg preparations are available in 5% and 10% concentrations, SCIg as 10%, 16.5%, and 20% solutions (Table 1). In developed world both IVIg and SCIg are available. Most European countries and the USA SCIg is preferred over IVIg for IGRT in patients with IEIs. In developing world IVIg still remains mainstay of IGRT. Different commercially available FDA approved preparations of immunoglobulin have been mentioned in Table 1.

Subcutaneous immunoglobulin vs. intravenous immunoglobulin

First SCIg in treatment of IEI was used in 1952 (6). Bruton treated his patient with SCIG replacement at "20 cc. containing 3.2 g of gamma globulin" and continued with monthly doses leading to resolution of recurrent infection in the patient (18).

But after that intramuscular immunoglobulin initially, and IVIg later was the standard of care until 1995 and 2006 when SCIg was approved in Europe and United States respectively (18). An international crossover trial that was conducted in 2000 showed that SCIG was not inferior to IVIG, and further research showed that SCIG is as effective in IEIs (18). Enzyme facilitated subcutaneous immunoglobulin (fSCIG) was approved in Europe in 2015 and in the US in 2016 (18).

Advantages of SCIg over other forms

Due to several advantages SCIg has become a standard of care in IEIs. Ease of administration, home-based therapy, no loss of working days, no need of vascular access, low adverse events and maintenance of steady state of IgG level are the advantages of SCIg over IVIg. It is also helpful in patients having unacceptable side-effects with IVIg or whom venous access is difficult. Small and more frequent doses of SCIg help to achieve steady-state level of IgG which provides better protection against infections (19) (Table 2).

Situation where IVIg is preferred over SCIg

If patient or caretaker is physically incapable of administering the infusion or the patient is having severe thrombocytopenia, bruising, bleeding at the sites of infusion or having severe skin diseases then IVIg is preferred to SCIg. However, it is important to note that in a given patient with IEI, choice of Ig should be individualized, and decided after detailed discussion with parents and caregivers (18).

Dosage of IVIg

Dose should be individualised for IGRT and should be titrated to achieve best clinical effect. Dose is best determined by the level required to remain "infection free" (20). Average half-life of total IgG is 25.8 days and the half-life for IgG1, IgG2 and IgG3 was found to be 29.7, 26.9 and 15.7 days respectively (21). The values are consistent with those reported for endogenous IgG, but there are major differences in IgG metabolism. Higher IVIg dosages

TABLE 1 Commercially available FDA approved preparations of immunoglobulin.

Name of brands	Available concentrations	Reconstitution	Routes of administration	IgA content	Sugar content
Gammagard	5% and 10%	Required- available in powder form	Intravenous	≤2.2 μg/ml	Glucose added
Gammagard liquid	10%	Not required- Available in liquid form	Intravenous/ subcutaneous	37 μg/ml	Nil
Carimune	3%, 6%, 12%	Required- available in powder form	Intravenous	720 μg/ml	Sucrose added
Bivigam	10%	Not required- Available in liquid form	Intravenous	≤200 μg/ml	Nil
Flebogamma	5% and 10%	Not required- Available in liquid form	Intravenous	<3 μg/ml	Nil
Octagam	5% and 10%	Not required- Available in liquid form	Intravenous	<100 μg/ml	Maltose added
Gammaplex	5% and 10%	Not required- Available in liquid form	Intravenous	<4 μg/ml for 5% and <20 μg/ml for 10%	D-Sorbitol present in 5% solution
Privigen	10%	Not required- Available in liquid form	Intravenous	≤25 μg/ml	Nil; Stabilized with Proline
Gammaked	10%	Not required- Available in liquid form	Intravenous/Subcutaneous	46 μg/ml	Nil
Gamunex-C	10%	Not required- Available in liquid form	Intravenous/Subcutaneous	46 μg/ml	Nil

TABLE 2 Comparison of intravenous and subcutaneous immunoglobulin.

Characteristics	Intravenous	Subcutaneous
Frequency of infusion	Every 3–4 weeks	Usually given once in 7–10 days
Duration of infusion	Longer (at least over 6–8 h)	Shorter (2–4 h)
Administration of large volume	Is not difficult	Only a limited volume can be given in one sitting
Available concentrations	5%, 10%	10%, 16.5%, and 20%
Pharmacokinetics	Rapid increase initially followed by wearing off effect by 3^{rd}–4^{th} week	Steady serum IgG level. No wearing of effects
Dosing schedule	400–600 mg/kg every 3–4 weeks	100–200 mg/kg/week
Advantages	a. Proven efficacy b. Better monitoring	a. Home administration b. Venous access not required c. Systemic side effects are minimal d. Less needle discomfort and pain e. Less requirement of premedications
Disadvantages	a. Venous access is essential b. It requires hospital admission c. Chances of acute febrile reactions, headache and aseptic meningitis are higher d. Trough levels	a. Local site reactions like erythema, induration or swelling b. More frequent infusions are required

(400–600 mg/kg vs. 100–200 mg/kg q 3–4 weeks) have been shown to be superior in studies on patients with primary hypogammaglobulinemia for lowering infection rates, reducing hospitalizations, reducing antibiotic use, and improving pulmonary outcomes. The recommended dosage of IVIg replacement is therefore 400–600 mg/kg of body weight every three weeks. IVIg dose needs to be titrated to maintain the trough level >500 mg/dl just before the next infusion (22).

Dosage of SCIg

Seven to ten days following the last dose of IVIg, the first dose of SCIg is administered. The dose is at least the same dose of maintenance IVIg therapy and infusion rate is 15 ml/hour/site. Rate of subcutaneous infusion can be increased to 25 ml/hour/site (maximum −50 ml/hour/site) and a maximum of 4–8 sites can be infused at one time. In SCIG, Ig levels remain stable and trough level is also higher than IVIg. There are 2 methods of SCIg application −1] conventional (using infusion pump—rapid-push SCIG) 2], facilitated (fSCIG)(initial administration of human recombinant hyaluronidase in the same needle as IgG) (23). Home SCIG is affordable, secure, therapeutically beneficial, and frequently favoured by patients and medical professionals. Since just a little amount of IgG can be pumped into the subcutaneous tissue, conventional SCIG needs weekly infusion. However, like IVIg, fSCIG can be taken every 4 weeks and delivers a higher dose of IgG (24). It reduces burden of SCIg treatment, improve patients' quality of life and compliance to therapy (25) (Table 2).

Monitoring during transfusion

IVIg should be started at minimal rate and under close observation during first transfusion. Monitoring of vital signs is important. Infusion rate can be doubled every 15–30 min if the patient is stable to a peak of @ 0.08 ml/kg/minute (4 mg/kg/min of 5% or 8 mg/kg/min of 10% solution) (Table 3) (26).

Adverse effects of immunoglobulin

The use of IMIg is restricted by the discomfort and hazards involved with intramuscular injections. Infusion-related adverse effects are more common in treatment naïve cases and in those who are on irregular therapy or are chronically infected. This is caused by the rapid release of lipopolysaccharides or other components of pathogens that are already present in the recipient, as well as the creation of antigen-antibody complexes while immunoglobulin is administered (15, 26).

Common adverse reactions

Common adverse events are chills and rigors, arthralgias, myalgias, headache, anaphylaxis and anaphylactoid reactions. These occur in less than 10% of the cases.

TABLE 3 Checklist to follow during administration of IVIg.

Checklist	1. Consent and counseling regarding adverse events during and after transfusion. 2. Calculate the dose of IVIg to be given; check batch number and expiry date. 3. Intravenous line to be started with running normal saline drip. 4. Prefilled syringes with adrenaline, hydrocortisone with proper labels of dose should be kept ready at bed side. 5. Starting dose is 0.1–0.2 ml/kg/hr for 15 min, then increase every 15 min. Rate of infusion should not exceed 2 ml/kg/hr in the first hour of infusion. 6. Monitor vital parameters (heart rate, respiratory rate, blood pressure and $SpO_{2)}$ every 15 min for first hour and then half hourly for the rest of the period. 7. If the child is clinically stable, final rate of infusion can be increased to 20 ml (smaller child) to 60 ml (older child) per hour. 8. Duration of infusion: in day care regular infusion of 400–800 mg/kg over 3–4 h. The rate should be slower if the child develops any mild reaction to it (like chills, fever). In high dose infusions (2 mg/kg): over 8–12 h; in some situations like small children, cardiovascular instability and mild infusion related reactions as stated above duration may exceed to 18- 20 h. 9. If anaphylaxis develops: stop the drip immediately, give oxygen and inject adrenaline (0.1 mg/kg of 1:1,000) and hydrocortisone (10 mg/kg) immediately. Do not restart again. For minor reactions like fever, chills, headache: infusion should be withheld and then restarted at a slower rate.

Management of headache

Headaches can occur up to 48–72 h of infusion. Headache may be similar to that of migraine in character or in some rare case associated with meningismus, and aseptic meningitis. Adequate fluid intake should be ensured and paracetamol can be used (15).

Methods to reduce infusion related adverse events:

1. This reaction risk can be reduced by transfusing the patient in an afebrile and infection-free state.
2. Incidence of reactions increase if patient receive different brands of IVIg (27). Hence, it is preferrable to continue with the same brand.
3. Rate-related side-effects can be minimised by temporary stoppage of infusion or slowing the rate of infusion [@ 0.01 ml/kg/minute (=0.5 mg/kg/minute of 5% solution; 1 mg/kg/minute of 10% solution)].
4. If these symptoms persist antipyretics, antihistaminics may help.

Anaphylactoid reactions

Anaphylactoid reactions are immediate systemic reactions that have similarities with anaphylaxis but are not mediated by IgE. These can occur even without prior exposure. Anaphylactic reactions are IgE mediated and occur only with prior exposure. It is seen in some patients, who complain of chest tightness, anxiety and a feeling of impending doom and is associated with flushing and tachycardia.

Precautions that can be taken to reduce risk of anaphylaxis

Any medical professional or setting that delivers IVIg needs to be adequately prepared to handle anaphylaxis.

Rare complications

Transfusion-related acute lung injury, thromboses and acute kidney injury are some other rare complications (26).

Thrombotic events, major side-effects of immunoglobulin treatment have an estimated incidence of 1%–16.9% (28, 29). In a survey of thrombotic adverse events made between 2008 and 2010, Daniel et al. discovered that 1.2 percent (122/11,785) of the patients experienced immunoglobulin-induced thrombotic events. The majority (80%) of thrombotic events occurred within 24 h of the end of immunoglobulin delivery and were stroke and myocardial infarction (28). An increase in plasma viscosity, the activation of procoagulant factors, vasospasm, autoimmune vasculitis, and an elevated platelet count are some of the mechanisms that could cause thrombotic events. Contact activation of clotting factors [coagulation factor F(XI)] with Ig during product manufacturing processes may lead to an elevated thrombotic potential.

Renal dysfunctions are rare with the commonest being proximal tubular damage. Majority of renal side effects were due to osmotic effects secondary to sugar (sucrose) used in older preparations of therapeutic immunoglobulin.

It is often seen that a patient may have adverse effects with one particular brand and tolerate another brand relatively well. In these cases switching of brand is indicated but infusions should be initially started at a slow infusion rate.

Side effects encountered during SCIg infusion

SCIg infusion rarely causes side-effects or significant vital sign changes. Subcutaneous injections may carry a risk of cellulitis or local site infections at infusion sites. Most patients will have swelling and redness (30).

Management of the side effects

Before starting infusion, blood return is to be checked to prevent inadvertent intravascular administration. Injected fluid may cause fluctuance and differentiating from abscess can be difficult. The infused medicine is dispersed via increased local circulation brought on by a warm compress or light massage. Within hours after the infusion's completion, the majority of local responses at the infusion site disappear. A healthcare professional's opinion is required if the infusion site exhibits redness, warmth, or swelling that gets worse over time, suggesting an infection (27). These patients ought to be able to get in touch with an on-call doctor or nurse. The patient can use a pen to indicate the size of their local reaction and objectively monitor any prospective growth (15).

Advantages of subcutaneous form in patients with cardiovascular, thrombotic and renal complications

IgG is administered slowly with most subcutaneous regimens, and adsorption from the subcutaneous site is slower than with IV infusions. The total monthly dose is divided into four distinct doses. SCIg is therefore preferable in patients with renal, thrombotic, or cardiovascular problems (30).

Indications of immunoglobulin in IEI

The indications of using immunoglobulin in IEI has widened (31). Antibody deficiencies are the commonest type of IEI described worldwide. According to literature, they account for almost half of the cases of IEI reported. Some of the studies have reported the proportion of antibody deficiencies as high as 78% (32). As there is decreased immunoglobulin production in IEIs with antibody defects,

TABLE 4 Indications of immunoglobulin in inborn errors of immunity.

A. Quantitative Ig deficiency	1. Agammaglobulinemia [eg., X-linked Agammaglobulinamia (XLA)] 2. Hypogammaglobulinemia with poor antibody function [eg. Common Variable Immunodeficiency (CVID)] 3. Hypogammaglobulinemia with normal antibody function (eg. Transient hypogammaglobulinemia of infancy) 4. Combined Immunodeficiency [eg., Severe Combined Immunodeficiency (SCID)]
B. Qualitative Ig deficiency	1. Poor antibody function (eg. Hyper Ig E Syndrome)
C. IEI with autoimmunity	1. Wiskott Aldrich Syndrome 2. Common Variable Immunodeficiency (CVID)

the primary modality of treatment in these disorders is IGRT. Regular IGRT is the mainstay of therapy in humoral immunodeficiencies like XLA, CVID, hyper IgM syndrome and IgG subclass deficiencies. IGRT is also essential in severe combined immunodeficiency (SCID). Although serum immunoglobulin levels may not be low in combined immune defects like autosomal dominant STAT-3 loss of function, autosomal recessive DOCK-8 deficiency, or Wiskott Aldrich Syndrome (WAS), immunoglobulin replacement is still necessary in these disorders due to deficiency of functional antibodies and qualitative defects of immunoglobulins. IGRT is usually required in patients with combined immune defect while awaiting hematopoietic stem cell transplantation (HSCT). However, in patients with milder impairment of *T* cell function, IGRT may remain an important therapeutic armamentarium. IEIs can be divided into different phenotypes for that IGRT is indicated (2). These are:

(1) **Agammaglobulinemia**- This is the definitive indication of Ig replacement. The prototype of this category is XLA. Regular IVIg supplementation showed significant reduction in both acute and chronic infections in patients with B-cell deficiency (33). It has been observed that severe bacterial infections and enteroviral meningoencephalitis can be avoided if IgG trough levels are kept above 800 mg/dl (33). It has been seen that children with primary antibody deficiency like XLA (on regular IGRT) have good prognosis in developing countries in spite of economic and other challenges. Mean trough IgG levels are reported to be much lower in developing nations than Western population (20).

(2) **Hypogammaglobulinemia with impaired antibody function**- This group of disorders are characterised by either decreased production and/or defective response with IgG antibody on antigen challenge. The prototype of this category is CVID. IVIg transfusion in these patients showed decreased prevalence of infections in comparison to the infection rates prior to initiation of IVIg (34) and in long run chronic lung changes and autoimmune complications are also found to be less in these patients (35). B cell proliferation and antibody production are induced by IVIg in low doses. IVIg replacement therapy in CVID induces B cell activation and proliferation in T cell independent manner. Anti-inflammatory action of IVIg is mediated by suppression of production of inflammatory cytokines from B cells. Therefore, IVIg, as a treatment modality, not only aids in replacement of antibody, but also plays an important role in immunomodulation (12).

(3) **Hypogammaglobulinemia with normal antibody function**- Although IgG levels normalize with increasing age in patients with transient hypogammaglobulinemia of infancy, initially antibody function may be partially impaired, requiring treatment with immunoglobulin replacement (36).

(4) **Combined Immunodeficiency**- Studies have demonstrated that IGRT decrease rates of infections and antibiotic use, and improve quality of life even in patients with combined immunodeficiency (37).

(5) **Normal immunoglobulins with impaired antibody function**- Immunoglobulin therapy should be initiated in patients who have normal total IgG levels, but poor response to polysaccharide antigens following vaccination due to defective specific antibody production (38).

(6) **Autoimmunity in IEI**- In primary immunodeficiencies with autoimmunity, IVIg therapy may be beneficial (1). For example, autoimmune cytopenias in Wiskott Aldrich Syndrome shows good response to immunoglobulin treatment. The mechanism and dose of IVIg in autoimmune diseases are not based on good quality evidences. Some anecdotal reports are the principal sources.

Continuously changing mode of immunoglobulin therapy is a life-saving option for patients with IEI, especially those with antibody deficiency and immune dysregulation. Considering the ever-increasing demand of Ig preparations for IEIs, more clinical trials and research projects at the basic level are required for better understanding of mechanisms of action and use of Ig. SCIg is a relatively newer modality of IGRT in developing countries, which is now being increasingly used in clinical practice (Table 4).

Author contributions

AS: Conceptualization, Data curation, Formal Analysis, Writing – original draft, Writing – review & editing. SB: Data curation, Writing – original draft, Writing – review & editing. VJ: Investigation, Writing – review & editing. RP: Conceptualization, Supervision, Writing – review & editing. SaS: Writing – review & editing. DS: Supervision, Writing – review & editing. AR: Supervision, Writing – review & editing. SuS: Supervision, Writing – review & editing.

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Conflict of interest

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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Current genetic diagnostics in inborn errors of immunity

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New technologies in genetic diagnostics have revolutionized the understanding and management of rare diseases. This review highlights the significant advances and latest developments in genetic diagnostics in inborn errors of immunity (IEI), which encompass a diverse group of disorders characterized by defects in the immune system, leading to increased susceptibility to infections, autoimmunity, autoinflammatory diseases, allergies, and malignancies. Various diagnostic approaches, including targeted gene sequencing panels, whole exome sequencing, whole genome sequencing, RNA sequencing, or proteomics, have enabled the identification of causative genetic variants of rare diseases. These technologies not only facilitated the accurate diagnosis of IEI but also provided valuable insights into the underlying molecular mechanisms. Emerging technologies, currently mainly used in research, such as optical genome mapping, single cell sequencing or the application of artificial intelligence will allow even more insights in the aetiology of hereditary immune defects in the near future. The integration of genetic diagnostics into clinical practice significantly impacts patient care. Genetic testing enables early diagnosis, facilitating timely interventions and personalized treatment strategies. Additionally, establishing a genetic diagnosis is necessary for genetic counselling and prognostic assessments. Identifying specific genetic variants associated with inborn errors of immunity also paved the way for the development of targeted therapies and novel therapeutic approaches. This review emphasizes the challenges related with genetic diagnosis of rare diseases and provides future directions, specifically focusing on IEI. Despite the tremendous progress achieved over the last years, several obstacles remain or have become even more important due to the increasing amount of genetic data produced for each patient. This includes, first and foremost, the interpretation of variants of unknown significance (VUS) in known IEI genes and of variants in genes of unknown significance (GUS). Although genetic diagnostics have significantly contributed to the understanding and management of IEI and other rare diseases, further research, exchange between experts from different clinical disciplines, data integration and the establishment of comprehensive guidelines are crucial to tackle the remaining challenges and maximize the potential of genetic diagnostics in the field of rare diseases, such as IEI.

KEYWORDS

inborn errors of immunity, next-generation sequencing, technologies in genetic diagnostics, variants of unknown significance, genes of unknown significance

1 Introduction

Today's standard of care for patients with clinically diagnosed or suspected inborn errors of immunity (IEI) involves genetic testing. The clinical characterization and the selection of the testing method influences the probability of obtaining a molecular diagnosis. In general, several genetic testing methods are available, including Sanger sequencing of single genes, targeted gene sequencing panels (targeted Next Generation Sequencing, tNGS), whole exome sequencing (WES), and whole genome sequencing (WGS), which can all be expanded to trio- or whole-family analyses. The choice of method largely depends on the clinical presentation, the suspected type of IEI, and the access to resources (1). When the patient's symptoms closely match a specific type of IEI, targeted gene panels that test a set of selected genes known to be associated with IEI, can be a quick and cost-effective first-line method (1). On the other hand, when the clinical presentation is less specific, or when initial targeted gene testing was inconclusive, more comprehensive methods such as WES or WGS may be employed. The goal of genetic testing in IEI is not only to confirm the clinical diagnosis but also to improve patient management. A genetic diagnosis can inform about prognosis, guide treatment decisions, enable genetic counselling, and provide the opportunity for predictive family testing for relatives at-risk. Patient characteristics, including their phenotype, family history, and ethnicity, can also influence the selection of the testing method and thus the likelihood of obtaining a diagnosis. This review aims at providing a summary for the clinician of the current genetic diagnostic tools available in the clinics (tNGS, WES, WGS). In addition, we provide an outlook on the more elaborative tools such as RNAseq, epigenetics and proteomics and used widely on research basis today to facilitate the diagnosis of IEI.

1.1 Milestones in developments

The history of genetic diagnostics began in the 20th century with the advent of technologies that allowed scientists to isolate and understand the structure of DNA. The first breakthrough was the discovery of the double helix structure of the DNA in 1953 that paved the way for the field of molecular genetics (2). Cytogenetic techniques visualized chromosomes and found abnormalities, starting clinical genetic diagnostics. Fluorescence *in situ* hybridization (FISH) improved detection of chromosome rearrangements. The chain-termination method developed by Frederick Sanger in 1977 revolutionized DNA sequencing for genetic diagnostics (3). It is primarily used to analyze known disease-associated genes or genomic regions linked to patient symptoms. Sanger sequencing was the method of choice for decades used mainly for identifying single nucleotide variants (SNVs) and is still considered for the validation of variants identified by other sequencing methods. However, the method´s main limitation is that it can only sequence a comparatively small number of bases at a time, making it less suitable for conditions that can be caused by variants in different genes, as is the case for many IEIs. It detects SNVs or small insertions/deletions but struggles with larger structural genomic variations, like extensive deletions or duplications of whole exons. Multiplex ligation-dependent probe amplification (MLPA) is used alongside Sanger sequencing to address these genomic variants. Apart from SNVs, Array-based comparative genomic hybridization (array-CGH) (4), introduced in the 1990s, expedited identifying deletions, duplications, and unbalanced translocations.

The collaborative international Human Genome Project, launched in October 1990 marked a significant breakthrough in the field of genetics (5, 6). By the end of this ambitious project, approximately 92% of the human genome was sequenced in April 2003, mainly using Sanger sequencing. The resulting comprehensive genomic map included most of the estimated 20,000–25,000 human protein-coding genes and their organizational structure. However, repetitive and homologous genomic regions were unresolved. In 2022, the telomere to telomere (T2T) consortium published near-complete sequences of all 24 human chromosomes using advanced sequencing methods, identifying 19.969 protein coding genes. (Box 1).

1.1.1 Next-generation sequencing (NGS)

BOX 1 Take home for clinicians

Sanger sequencing was the method of choice for genetic routine diagnostics for many years; however, it is relatively labor-intensive and cost-expensive. With emerging next-generation sequencing techniques, it is used less frequently nowadays, mostly only for predictive diagnostics. It still plays a role in confirming unclear findings from Next-Generation Sequencing.

The advent of next-generation sequencing (NGS) technologies in the mid to late 2000s dramatically increased the speed and reduced the cost of DNA sequencing by introducing parallel data generation from usually small DNA fragments ("massive parallel high-throughput sequencing"). This greatly facilitated the sequencing of vast amounts of DNA, enabling the comprehensive analysis of human genomes in diagnostic settings and the identification of genetic variants at an unprecedented scale previously not possible. Short-read (or "second generation") NGS, emerging in genetic diagnostics since 2005, sequences patient DNA fragments up to 160 base pairs, producing sequencing "reads". Newer NGS technologies generate up to 20 billion reads within a day, allowing to sequence more than 20 human genomes in a single run. These reads are then compared to a reference genome to detect differences.

Long-read NGS ("third-generation technique"), sequences DNA stretches up to >100,000 base pairs, facilitating complex structural variation analysis. Though currently more common in research due to complexity and cost, decreasing expenses may integrate long-read NGS into genetic diagnostics. However, managing vast data generated, sometimes reaching a terabyte for a single genome, poses challenges in storage, transfer, and analysis, demanding substantial computational resources.

NGS methods (tNGS, WES,) enable cost-effective and swift analysis of multiple genes. tNGS encompasses a certain primer set amplifying a selected group of genes (e.g., 120 primary immunodeficiency genes). This approach offers high-accuracy variant detection but generally does not provide new insights into the role of yet unknown genes. Nevertheless, it can be particularly suitable for the identification of mosaicism due to a high sequencing depth (7). tNGS panels were widely used in the last decade, but many genetic laboratories have switched to whole exome sequencing (amplifying all exons) and then applying virtual "*in silico*" gene panels as filter. This sometimes leads to confusion as the term "gene panel investigation" does not clarify whether a limited set of genes were amplified or whether a WES was performed, but only a limited number thereof analyzed. WES, in contrast to tNGS, enables analysis of almost all genomic protein coding regions, which only represent about 1% of the entire genome but account for about 85% of disease-causing variants (8). WES achieves extensive coverage of coding variants and is useful for the identification of genetic variants in numerous diseases.

Finally, WGS covers almost all genomic regions, including non-coding (intronic) regions and mitochondrial DNA (mtDNA). The non-protein coding portions of the genome correspond to about 99% of the genome. The biological and therefore also clinical evaluation of most variants in non-coding regions is difficult, and often requires extensive functional testing to provide a more definitive assessment of the effects of a variant in these regions. Larger copy number variations (deletions or duplications) that were previously only be detectable by complementary methods such as array-CGH analysis are now reliably detected by WGS.

Different studies focus on evaluating the yield of NGS-based approaches in patients with IEI (9–13), which have been summarized by Vorstefeld et al. (14). The average diagnostic yield of NGS in IEI was found to be 29%, with a range of 10%–70%. For WES, the average yield was 38% (ranging from 15% to 70%), which suggests that in a significant number of cases, NGS-based sequencing approaches such as WES do not effectively diagnose the majority of patients with IEI. Of course, reported percentages expressing a diagnostic yield highly depend on the inclusion criteria, the severity of the phenotype and the depth of the immunological analysis performed prior to genetic testing. However, it is important to highlight that in a considerable number of IEI patients the genetic cause cannot be identified, and a negative genetic test does not rule out the diagnosis of an IEI (Box 2).

BOX 2 Take home for clinicians

For suspected Inborn errors of immunity (IEI), selecting the most suitable genetic diagnostic method is crucial. Today, routine diagnostic of IEI encompasses WES (sometimes amended by complementary array-CGH in order to address structural aberrations and CNVs) or WGS. This is amended by phenotypical and functional investigations. However, despite the advanced in genetic diagnostics, in many IEI patients, no causative genetic variant can be identified. A negative finding does not rule out the diagnosis of IEI.

The challenge is to choose the right diagnostic tool depending on presentation, suspected (group of) diseases, and available resources: Ideally, genetic analysis identifies a broad spectrum of genetic abnormalities, encompassing not only single nucleotide variations, but structural variants such as duplications, deletions, inversions and translocations in a streamlined process and timely manner. It is also increasingly recognized, that most advanced genetic technologies require interdisciplinary collaborations to achieve the best possible results regarding diagnoses and patient management. Geneticists, immunologists, and clinicians can jointly develop personalized treatment plans that refer to both, the distinct genetic variants and the patient's clinical manifestations.

1.2 Emerging technologies in genetic diagnostics

1.2.1 Optical genome mapping

As in most patients with suspected IEI causative variants cannot be identified by routine NGS methods, further efforts are undertaken with the following research methods:

Optical Genome Mapping (OGM) is a genomic technique detecting various chromosomal rearrangements (like balanced translocations, inversions, and insertions) without constraints of traditional methods such as sequencing or probe hybridization. Unlike short read sequencing (100–160 bp), which struggles in complex regions, OGM achieves full genome assembly using long DNA fragments (150 kbp–1 Mbp). It visualizes DNA fragments tagged with a specific sequence motif (CTTAAG) that acts as a "barcode" for comparison to a reference genome. OGM boasts a whole genome analysis with up to 500 bp resolution, surpassing array-CGH's 20 kb–100,000 kb resolution. While promising for diagnostics, it is primarily used in research due to challenges like DNA quality requirements and complex data interpretation. OGM excels in detecting structural variants (SVs) and copy number variations (CNVs) but cannot identify single nucleotide variants or small indels common in genetic disorders. Combining OGM with other methods, like NGS, may offer a comprehensive view of a patient's genome. OGM holds potential for revealing complete genetic variations in critical immune system-related genes, being especially valuable in these highly polymorphic regions. Some publications concerning other diseases have indicated the benefit of its use for IEI. In Sahajpal et al., OGM has been performed on 57 severely ill COVID-19 patients, and seven SVs have been identified as affecting genes that are involved in innate immunity, inflammatory response, and viral replication and spread (15). These examples underline the potential relevance of OGM in immunodeficient phenotypes, especially because of its superiority in the detection and description of complex variants.

1.2.2 RNA sequencing

Coding variants account for over 85% of pathogenic or likely pathogenic variants in clinical databases (16). Nonetheless, it is widely accepted that non-coding variants also play a significant role in human diseases (17). RNA sequencing (RNA-seq) has

emerged as a powerful technique to study gene expression and transcriptomic changes due to non-coding and splicing variants. To date, molecular diagnostic RNA-seq is primarily being used as a research tool. It has been demonstrated to augment the diagnostic yield by approximately 15% compared to WES alone (18, 19). However, selecting an appropriate source (e.g., whole-blood, leukocyte subsets, tissue) for RNA-seq plays an important role in obtaining the optimal diagnostic yield. For example, specific pathogenic splicing variants identified in fibroblast samples have been undetectable in blood samples, indicating the limitations of blood as the sole tissue for certain analyses (20). Furthermore, the analysis of RNA-seq for diagnostic purposes requires normalized samples and a comprehensive control dataset for statistical comparison (21, 22).

In IEI, in which the expression of disease-causing genes is often suppressed, targeted RNA sequencing (T-RNA-seq) is particularly valuable (23, 24). T-RNA-seq focuses on genes of interest, providing exquisite sensitivity for transcript detection and quantification. Numerous studies on IEI have provided compelling evidence for the effectiveness of RNA-seq or T-RNA-seq as powerful tools in the field. For example, intronic variants in the genes *STAT1, DOCK8* and *IL6ST* or in the non-coding gene *RNU4ATAC* have been shown to be pathogenic using RNA-seq (25–28).

1.2.3 Proteomics

Over the past two decades, mass spectronomy-based (MS-based) proteomics has provided significant advances in the field of immunology (29). High-resolution mass spectrometry is a powerful method for profiling and quantifying proteins in tissues, organs, and cells, enabling comprehensive exploration of cellular processes, signaling pathways, post-translational modifications, and protein interaction networks (30). This approach has enhanced our understanding of the dynamic and complex nature of the immune system, shedding light on its functioning and underlying mechanisms. A recent review of the literature has highlighted the significant contributions of MS-based proteomics to our understanding of innate immunity (31).

Proteomics has been employed in a limited number of studies for the genetic diagnosis of IEI (32, 33). Despite the impressive biological insights provided by MS-based proteomics its integration into mainstream diagnostic laboratories is limited by cost and lack of expertise in data analysis and interpretation.

2 Further emerging diagnostic genetic approaches

Besides OGM, RNA-seq and proteomics, other genetic approaches, such as single cell sequencing, epigenomics, metabolomics or multiomics are increasingly relevant in both research and diagnostics. These aspects are briefly discussed here but are beyond the scope of this review.

Single cell sequencing allows the analysis of individual cells to identify their genetic profile, which is particularly useful in heterogeneous cell populations such as immune cells, and could play a crucial role for understanding and diagnosing IEIs (34, 35). It can be divided into single cell DNA (scDNA-seq) and single cell RNA sequencing (RNA-seq), belonging to single-cell genomics and single-cell transcriptomics (36), respectively. One of the advantages of scDNA-seq over bulk DNA sequencing is the higher sensitivity of mosaicism detection. Increasing the depth of bulk DNA sequencing does not eliminate the risk of missing mosaic features, as mosaicism with less than 0.5% cannot be distinguished from sequencing error (36). In addition, single cell analysis allows to further define low-level mosaic variants detected by bulk sequencing and determine their origin (i.e., the same cell or to different cells). Besides, it allows the association of a genetic feature with the phenotypic character of a specific cell type. The review by Evrony et al. (36) gives an overview on the major applications of scDNA-seq.

In the context of IEIs, the importance of understanding the interplay between genetic and epigenetic factors such as DNA methylation, chromatin remodeling, and histone acetylation are crucial. The differentiation of immune system cells relies on the presence of a DNA methylation pattern. Any dysfunction or impairment in the DNA methylation machinery may lead to immune dysfunction and the onset of various diseases. This is excellently summarized in a review by Romano et al. (37). Hypermethylation of genes like *PIK3CD*, *BCL2L1*, *RPS6KB2*, *TCF3* and *KCNN4* and the decreased ability to demethylate them led to an impaired transition from naive to memory cells shown in a study on CVID-discordant monozygotic twins (38). Moreover, in cohorts of CVID-patient and healthy controls, different methylation patterns of relevant genes of B-cell development and function could be observed (39). The immunodeficiency, centromeric instability, facial anomalies syndrome, type 1 (ICF1) can be caused by mutations in *DNMT3B*. The impaired function of this gene leads to changed methylation of regulatory regions of lineage-specific immune-related genes during development which cause the phenotype of ICF1. Correction of *DNMT3B* variants using CRISPR-Cas9 could partially restore the healthy epigenome (40).

Most likely, to provide a holistic view of the molecular basis of diseases, a multiomics approach is preferred. This would include the above-mentioned genomics, transcriptomic, proteomics, metabolomics, epigenetics and other "omics" data. Chu et al. and others provide overviews of the various methodological approaches available for the different omics data layers that are relevant in immunological research (41, 42). In Figure 1 an overview of the most commonly used genetic technologies is given.

2.1 Analytic strategy of genomic data of patients with IEI

The ACMG/AMP (American College of Medical Genetics and Genomics/Association for Molecular Pathology) classification system was established for the evaluation and classification of sequence variants for Mendelian diseases based on single gene defects (43). This system recommends the use of a specific

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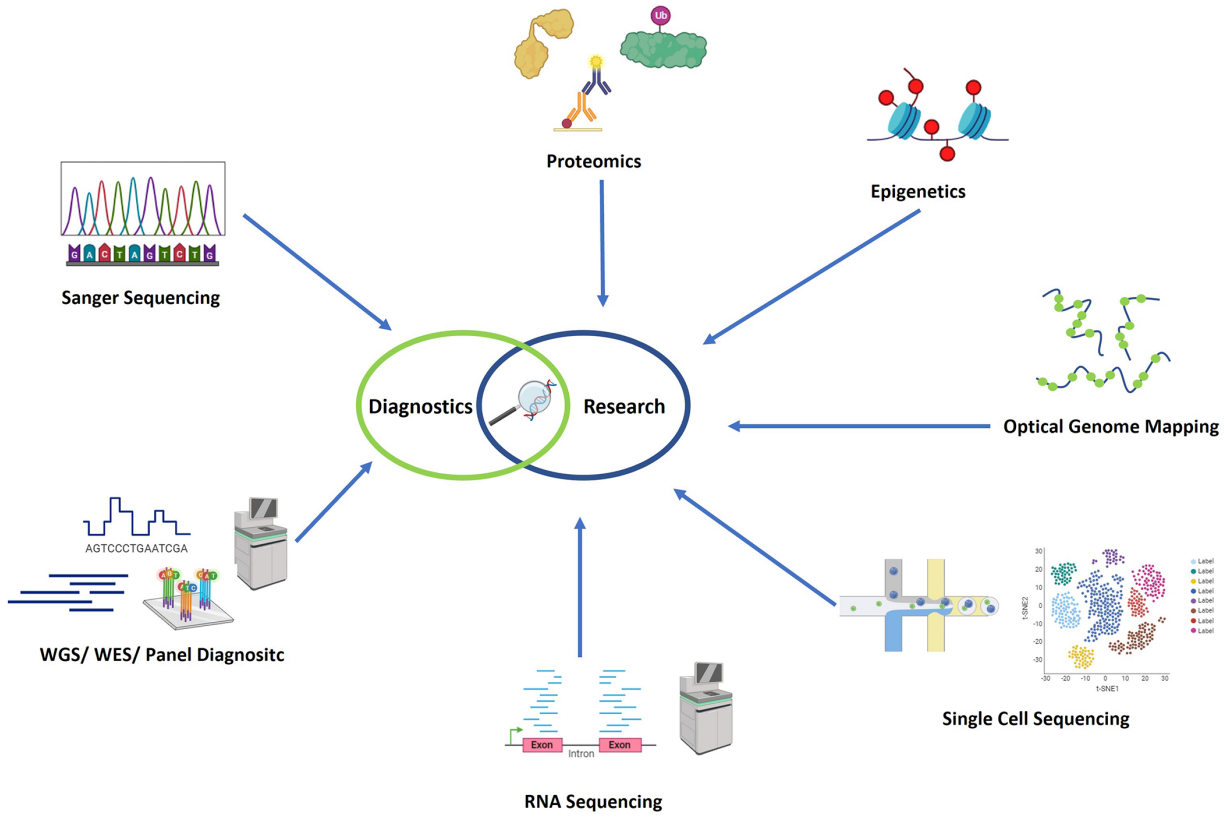

FIGURE 1
Overview of the most commonly used genetic technologies. In the field of genetic diagnostics, Sanger sequencing, panel diagnostics, whole exome sequencing, and whole genome sequencing have recently been employed as technologies. Additionally, in research settings, optical genome mapping (OGM), RNA sequencing (RNA-seq), proteomics, single cell sequencing, and epigenomics are additionally used in research settings. Figure created with Biorender.com.

standard terminology: pathogenic (class 5), likely pathogenic (class 4), unknown significance (class 3), likely benign (class 2), and benign (class 1) (Table 1). To classify a sequence variant, several criteria are used, including the type and location of the variant, frequency in the general population, listing in gene-specific databases, evaluation by bioinformatic prediction programs, and segregation within the family. The use of the ACMG/AMP classification system has become increasingly important in clinical practice to guide patient management and counselling, and to improve the accuracy and consistency of variant interpretation. Clinical consequences are currently recommended only for class 4 and class 5 variants (Table 1).

TABLE 1 Standard terminology of the ACMG/AMP classification system.

ACMG classification	Class	Probability of pathogenicity clinical	Consequences
Pathogenic	5	>99%	e.g., diagnosis, prognosis, therapy as well as testing of family members
Likely pathogenic	4	>90%	
Variant of unknown significance (VUS)	3	10 bis 90%	Currently none, also no testing of family members
Likely benign	2	<10%	No communication, no consequences
Benign	1	<0.1%	

2.2 Gene panels based on the IUIS classification of IEI

In 1973, the International Union of Immunological Societies (IUIS) Committee was established by the World Health Organization with the primary objective of characterizing and categorizing IEIs in humans. Since then, a curated list of immunologic disorders has been authored by the committee, ensuring a standard nomenclature and consistent approach. Following the discovery of genetic defects associated with IEI, the committee has begun to include a list of genes linked to IEI in peer-reviewed publications. This list is updated every two to three years. In October 2022, the most recent update has been released, which includes 485 genes linked to IEI, including 55 additional genes since the 2019 IUIS update (44). These genes are divided into ten categories (45) (Table 2). The implementation of these categories into routine diagnostic as defined *in silico* gene panels would allow the efficient and accurate analysis of genes associated with specific IEI groups. However, there is no unified procedure for this. In the Netherlands, an identical *in silico* gene panel with 389 genes for IEI is used nationwide (46) whereas diagnostic *in silico* panels used in other countries and centers differ across laboratories. As

TABLE 2 Gene panel based on the IUIS classification of inborn errors of immunity.

Table	Genpanel based on the IUIS classification of genetically inherited immunodeficiencies
1	combined immunodeficiencies
2	combined immunodeficiencies with syndromic features
3	predominantly antibody deficiencies
4	diseases of immune dysregulation
5	congenital defects of phagocytes
6	defects in intrinsic and innate immunity
7	autoinflammatory diseases
8	complement deficiencies
9	bone marrow failure
10	phenocopies of inborn errors of immunity

there is also a worldwide standardized classification system of detected variants in genes associated with IEI, the use of a standardized *in silico* gene panel should be recommended. This would also prevent the large variability in diagnostic yield, which has been reported with an average of 38% (with a range of 15%–70%) in context of IEIs (14, 47, 48).

2.3 Gene panels based on the clinical genome resource (ClinGen)

The Clinical Genome Resource (ClinGen) is a collaboration between US-American National Institutes of Health (NIH), academic institutions, and industry partners with over 2,200 contributors from more than 62 countries. It was funded in 2013 to promote the knowledge on clinical relevance of genes and variants for use in precision medicine and research. ClinGen has established several working groups focused on specific rare disease areas, such as neurodevelopmental, cardiovascular, neurological or immunological disorders. These Clinical Domain Working Groups bring together experts from different fields to evaluate the strength of evidence of gene-disease relationships and create a gene curation expert panel. The Clinical Domain Working Group "Immunology" curates clinically relevant and actionable genes causative for diseases of the immune system. To date, the gene curation expert panels for antibody deficiencies, primary immune regulatory disorders and SCID-CID (severe combined immunodeficiency-common variable immunodeficiency) are completed and publicly available (https://www.clinicalgenome.org/working-groups/).

2.4 Human phenotype ontology (HPO) based analysis

The Human Phenotype Ontology (HPO) is a standardized description of human phenotypes, emphasizing those seen in genetic disorders (49, 50). Each HPO term details a specific abnormality in human traits linked to genes causing diseases defined by OMIM (51). With over 13,000 terms, HPO is crucial for analyzing clinical WES and WGS data. Bioinformatics tools integrate an individual's HPO-coded phenotype with sequencing data to prioritize causal genes.

Despite its utility, applying HPOs in clinical practice presents challenges. Patients exhibit not just disease-specific symptoms but also secondary signs shared by various conditions. Moreover, unrelated medical issues may confound diagnosis. Limited availability of comprehensive IEI-related HPO terms hinders its widespread use (52, 53). In genetic testing for IEIs, tools using HPO terms failed to identify disease-causing genes in 37% of patients with monogenic disorders (12).

Efforts from the ESID genetics working party and ERN-RITA aim to refine IEI-related HPO terminology. Haimel et al. have enhanced the HPO vocabulary by generating more comprehensive sets of terms specifically related to IEIs. They have thoroughly examined four distinct branches of the HPO tree, contributing a total of 57 newly developed and extended terms to the HPO. The majority of these terms has been successfully incorporated into the official HPO data set (53).

2.5 ESID classification

ESID, established in 1994, aims to advance knowledge on IEIs through education, research, and best practice guidelines. Its continuously updated registry, launched online in 2004, gathers clinical and research data of IEI patients globally. ESID's "working definitions for clinical diagnosis of primary immunodeficiencies" help diagnose and register IEIs based on standardized criteria, facilitating global communication among scientists and physicians. These criteria categorize immune system disorders (e.g., T-cell deficiencies, B-cell deficiencies), enabling comprehensive understanding and data organization. The criteria consider both clinical and laboratory characteristics, aiding in pattern recognition within disorders for improved IEI management, genetic testing recommendations, and further studies. However, they must be applied considering individual patient characteristics and clinical context. As research evolves, these criteria may require updates to reflect new classifications or insights.

3 Comprehensive approaches for analysis of genomic data

3.1 Family based sequencing

Simultaneous genetic analysis of the patient and their parents [NGS-based Trio (Trio WES or Trio-WGS)] is a useful approach to speed up the process of making a precise genetic diagnosis (54). This is because the parental data and segregation information for each variant are immediately available, facilitating clinical interpretation of the variants. This can be of particular importance in case of severely ill infants who are admitted to neonatal or pediatric intensive care units or for patients who benefit from precision treatments (e.g., patients with SCID and life-threatening infections in infancy). Furthermore, NGS-based Trio analysis allows the reliable detection of *de novo* variants without the addition of further analysis, which leads to a faster turnaround time and a higher detection rate. Farwell et al. have estimated Trio analyses to have a

diagnostic yield of 37%, compared to 21% for single gene analyses (45). Identification of potential new disease-causing genes is also more likely with Trio analysis. There are also a few possible contraindications or disadvantages associated with NGS-based Trio analysis. (1) Cost: NGS-based Trio analysis involves sequencing of three individuals, which is more expensive compared to individual WES or WGS. The increased cost may be a limiting factor, especially in situations where financial resources are limited. (2) Ethical concerns: NGS-based Trio analysis raises ethical questions, particularly when it comes to obtaining informed consent from all individuals involved. It is important to ensure that all patients understand the implications of NGS-based Trio analysis, including the potential identification of genetic conditions or predispositions that might have consequences for the whole family. (3) Privacy concerns: NGS-based Trio analysis involves the analysis of genomic data from multiple individuals within a family, raising privacy concerns. Obtaining comprehensive clinical information and medical history of all individuals undergoing sequencing is essential for meaningful data analysis. (4) Data interpretation: In the process of analysis strategy, it is crucial to consider the presence of variants with variable expressivity and incomplete penetrance within the family. Even if a genetic variant is identified in the individual, it may not necessarily present with the associated condition or disease. Alternatively, even if a genetic variant is inherited from a parent, it may not necessarily be excluded to be causative. This can lead to challenges in determining the clinical significance of the variant. In any case, it must be ensured that the results are interpreted in the context of the individual's clinical and family history.

Certainly, this issue also has to be considered if prenatal testing is an option for affected families. Especially, in the context of genetic alterations with variable clinical expressivity and incomplete penetrance, uncertainty may arise regarding the actual impact of the alteration on the health and development of the unborn child. Overall, navigating the ethical complexities of prenatal diagnosis involves a careful balance between providing parents with the information they need to make informed decisions and respecting their autonomy, all while acknowledging the uncertainties inherent in genetic medicine. Therefore, genetic counselling is an integral part of prenatal testing in families with inborn errors of immunity. However, particularly in families with IEI, early diagnosis can be instrumental in saving lives. Therefore, it is valuable to ascertain, even in unborn children, whether they are highly likely to be affected by an IEI.

3.2 Genematcher approach

NGS-based Trio analysis has played a significant role in expanding our understanding of rare diseases by identifying new disease-causing genes. Web-based tools enable scientists from around the world with an interest in the same genes, variants or phenotypes to collaborate (e.g., GeneMatcher—https://genematcher.org/, Variant Matcher—https://variantmatcher.org/, phenodb—https://phenodb.org/). These collaborative approaches allow researchers to pool their resources, expertise, and patient data, leading to more robust and comprehensive analyses. This enhances the statistical power and accuracy of genetic studies, ultimately increasing the chances of finding disease-causing variants and improving patient outcomes. Through these collaborations, ideas are exchanged, and valuable methodologies are shared (Figure 2). Researchers and physicians worldwide

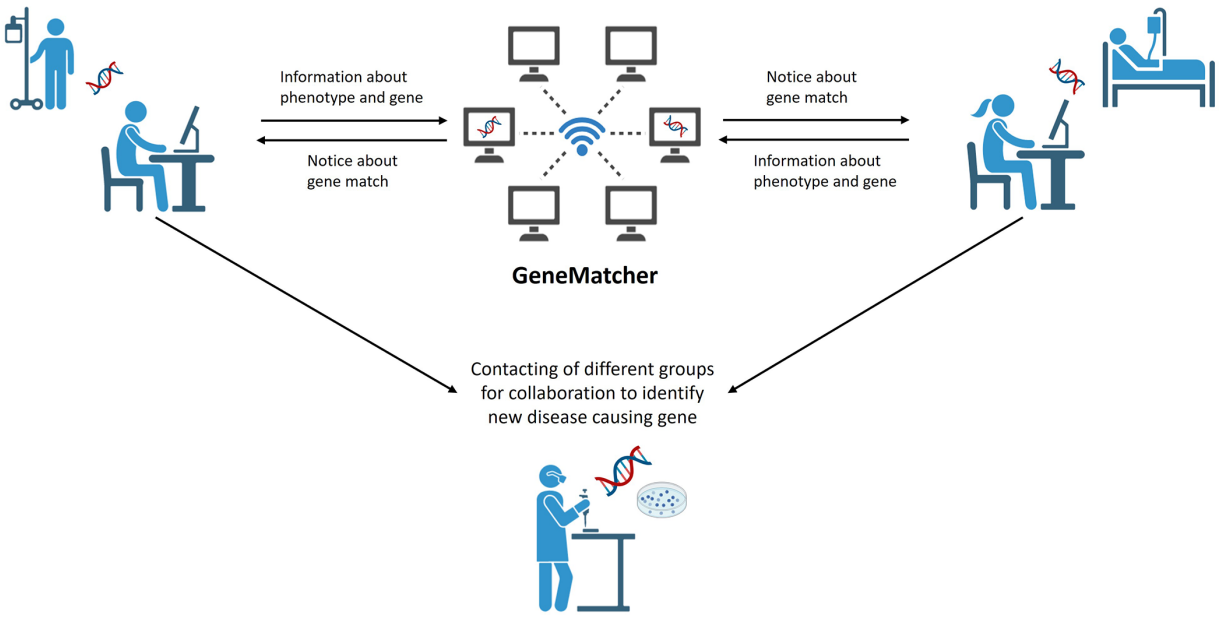

FIGURE 2
Genematcher approach. Web based tools like GeneMatcher are used to share information about phenotypes of patients with IEI and the results of WGS analysis. GeneMatcher informs researchers about a "match"—same gene was uploaded by others—that allows them to get in touch, to exchange about phenotypes and results and to collaborate for further analysis of potential disease-causing genes. Figure created with Biorender.com.

benefit from each other's discoveries, ultimately hastening the pace of research. To assess the impact of these collaborative approaches is challenging; however, the significance is evident through the number of matches and publications they facilitate. For instance, since 2015 GeneMatcher has been cited in 753 publications (https://genematcher.org/statistics/).

Different studies have also demonstrated the advantage of using GeneMatcher in IEI (55–61). An international team, for instance, was able to identify five families with ten patients exhibiting a similar constellation of symptoms, including medically refractory infantile-onset inflammatory bowel disease (IBD), bilateral sensorineural hearing loss and, in most cases, recurrent infections. All patients carry biallelic or monoallelic damaging variants in *STXBP3*. Through GeneMatcher three families with immune-associated defects, poor growth, pancytopenia and skin pigmentation abnormalities have been ascertained. All affected patients carry biallelic *DPP9* rare variants. Another international team, connected through GeneMatcher, has identified a total of 15 patients from eight families to have an autosomal recessive immunodeficiency syndrome characterized by severe infections caused by both RNA and DNA viruses, along with virally triggered inflammatory episodes associated with hemophagocytic lymphohistiocytosis-like disease. These patients also presented with early-onset seizures, as well as renal and lung disease. All of them carry biallelic damaging variants in *ZNFX1*.

3.3 Artificial intelligence and machine learning in genetic diagnostics

Artificial Intelligence (AI) and Machine Learning (ML) tools have demonstrated considerable potential in genomics research. Notable examples include facial analysis for genetic disorder identification and machine learning for variant classification or risk-assessment algorithms. AI programs, such as Face2Gene (https://www.face2gene.com/) have emerged as a valuable aid by analyzing facial features to assist in the diagnosis of specific genetic conditions, potentially improving early detection and patient management. For pediatrician's clinical workflow, the Face2Gene platform has introduced a specialized feature known as the "Pediatrician View." This functionality facilitates the analysis of patients by incorporating facial images. Upon uploading a portrait photo, the system computes a normalized score termed the facial D-Score. The facial D-Score serves as an indicator of the likelihood of dysmorphic features being present in the patient's photo. A higher D-Score corresponds to a higher probability of the existence of such features. This scoring mechanism can assist clinicians in making informed decisions about whether a patient should be referred for further genetic diagnostic evaluation (https://www.face2gene.com/pediatrician-view/). Furthermore AI-based phenotypic scores of facial image data, generated by Face2Gene, GestaltMatcher, Phenoscore etc., could be implemented into variant classification (62). ML algorithms are also being developed to distinguish pathogenic genomic variants from benign ones (63). These deep learning networks predict the pathogenicity of genetic variants from curated datasets and various genomic features, including experimental, population and clinical data, thereby assisting in the interpretation of genetic testing results. Mostly, an automated, streamlined process identifies a concise list of candidate genes for comprehensive evaluation, and reporting (64, 65). The automation of genetic disease diagnosis potentially simplifies and expedites the interpretation of the vast numbers of genetic variants, leading to an increased diagnostic yield while reducing turnaround time and cost. Different studies have already shown a benefit in using these tools (64). A recent publication has presented a prospective cohort study that has successfully validated an ML approach for risk stratification of IEI. This approach exploits ICD codes extracted from electronic health records to discriminate between datasets linked to children diagnosed with IEI and those without. The approach has demonstrated an accuracy rate of 89% in diagnosing patients with IEI (66). Despite all the benefits using AI and ML in genetic approaches, it is important to be cautious about biases as the effectiveness of algorithms depends on the quality of the training data. Recognizing that algorithms are developed by humans with biases and individual perspectives underscores the need for caution.

4 Current challenges

With the vast volume of data generated by NGS, the importance of effective filter techniques cannot be overstated. These are necessary to reduce the multitude of identified variants to a manageable subset of potentially clinically relevant ones. Appropriate filtering strategies consider factors such as variant frequency in population databases, predicted functional impact, inheritance pattern, and consistency with the patient's phenotype. VUS pose a particular challenge, as their impact on protein function and contribution to disease phenotype is uncertain. Novel bioinformatics approaches are continually being developed to predict the potential pathogenicity of these variants, employing machine learning and integrating diverse data types. In particular, when using WES or WGS as a diagnostic method, the possibility exists of identifying pathogenic variants in genes that are not associated with the actual diagnostic request, so called "incidental" or "secondary" findings. Before conducting extensive genetic diagnostics, it should always be clarified between the patient, the requesting physician, and the performing laboratory how to handle incidental or secondary findings. The implementation of accurate filters can minimize the likelyhood of incidental or secondary findings, thus mitigating potential ethical implications. It is worth noting that no filtering strategy is perfect, and rare pathogenic variants can be incorrectly filtered out, stressing the need for continual refinement of these methods based on the latest research findings. Therefore, establishing robust and accurate filter techniques is fundamental to harness the power of NGS in the diagnostics of IEI, striking a balance between sensitivity and specificity to ensure that relevant pathogenic variants are detected while limiting the identification of irrelevant ones.

While technological advances in sequencing and bioinformatics play a significant role in the process of establishing genetic diagnosis, the human factor remains a critical component in the interpretation and application of these results. Genetic diagnostics should ideally be performed in specialized centers with experienced human geneticists who have a deep understanding of IEI genetics. These professionals bring the necessary capacity to integrate complex genetic data with clinical information, including the patient's symptoms, family history, and laboratory findings, to provide a meaningful interpretation of sequencing results and enabling or arrange functional diagnostics in unclear cases. A nuanced understanding of IEIs can enable geneticists to anticipate and recognize atypical presentations and variable expressivity of diseases, to consider the impact of genetic modifiers, and to factor in potential non-genetic causes. Furthermore, they can give guidance on the follow-up functional studies needed to validate the impact of novel variants and to correlate genotype with phenotype. Of note, the geneticists depend on the clinicians who ideally provide clinical and phenotypical information as detailed as possible. Geneticists in these specialized settings can also play a pivotal role in communicating complex genetic information to patients and their families, helping them understand the implications of genetic diagnoses for disease prognosis, management, and family planning. Thus, while we continue to automate and refine our technical capabilities, expertise and judgment of human geneticists remain invaluable in the field of IEI genetic diagnostics.

4.1 Dealing with variants of unknown significance (VUS)

As diagnostic genetic sequencing becomes more comprehensive, the frequency of detecting variants that cannot be classified as either benign or pathogenic, referred to as VUS, is also increasing. A VUS is defined as a variant with an unclear or unknown association with disease risk. In many cases, these variants are very rare in the population so that there is limited information available about them. Additional data (e.g., further phenotypical or functional analyses) are usually required to evaluate its pathogenicity. However, these analyses are not typically performed as part of genetic diagnostics. The detection of VUS can create uncertainty for treating physicians and patients alike, raising questions such as whether the disease's underlying cause has been identified and whether additional analyses are necessary. In order to preemptively alleviate uncertainty for patients and their parents, it is of paramount importance to inform patients during the genetic diagnostic request that there may be findings involving VUS, and that every individual, including healthy individuals, may harbor a number of VUS. It is generally advised not to base clinical recommendations on the presence of a VUS andthe 2015 ACMG/AMP variant classification guidelines state explicitly that a VUS should not be used in clinical decision-making. When a patient is found to have a VUS, any clinical decisions should rely on their individual and family history rather than the presence of the VUS. Genetic analysis of parents or other family members may generate additional evidence for a potential VUS reclassification. The classification of a VUS may evolve over time. Therefore, it is equally important to request a reassessment of a dataset after a specified period, such as two years, to allow for the possibility of a more certain diagnosis through changes in interpretation.

Furthermore, it also may be of importance to functionally validate a variant classified as pathogenic if the variant does not explain the reported phenotype. These variants normally should not be reported by genetic laboratories but such variants, however, could be discussed with clinicians when there are doubts about the specific phenotype of the patient. Nevertheless, it is predicted that a significant number of VUSs in coding regions will be elucidated by 2030. This assumption is driven by the progress in standards for variant classification, the enhancements in the performance of computational variant effect predictors, the scalability of multiplexed assays capable of thoroughly examining variant effects across the genome, and collaborative data-sharing initiatives poised to extract maximum information from each newly sequenced individual and interpreted variant (67). In the majority of cases, immunological functional testing in patients does not result in in a change of classification of the genetic variant. For instance, neutrophil granulocyte dysfunction cannot be employed to reclassify a VUS in the CYBB gene as (likely) pathogenic. This is due to the possibility of a different, unidentified variant in the same gene or another variant in a different gene being responsible for the observed phenotype. To use functional analyses for reclassification purposes, it is essential to unequivocally demonstrate through the analysis that the variant under investigation distinctly leads to an altered function of the gene or the gene product.

4.2 Challenges in analysing gain-of-function (GoF) variants

The phenotypic expression of many genetic variants can vary significantly, especially in IEI, exhibiting variable expressivity, and the development of disease may not occur with 100% certainty (reduced penetrance). Furthermore, in recent years, there has been a significant increase in the identification of variants that result in a hypermorphic or neomorphic gain of function (GoF) effect. These variants lead to an enhanced or entirely new protein function. The identification and classification of GoF variants remains a challenge, even for geneticist, because prediction algorithms for determining pathogenicity of GoF variants are not reliably usable and ACMG criteria do not apply well. Therefore, regardless of the prediction algorithms used, both the phenotype and pedigree of the patient, as well as the function of a gene, play crucial roles in interpretation of variants. A practical guide for WES analysis is given by Vorsteveld et al. (14).

In some genes, both disease causing loss of function (LoF) and GoF variants have been reported. In the *STAT3* gene, GoF variants lead to its hyperactivation, causing immune dysregulation, early-onset lymphoproliferation and autoimmunity (68) whereas LoF variants result in impaired *STAT3* function, leading to a hyper-IgE recurrent infection syndrome-1 (HIES1) (69, 70). Another puzzling feature that seems to be particularly frequent in

genes associated with IEI is the observation that pathogenic variants of the same gene can follow different modes of inheritance. For example, both an autosomal recessive as well as an autosomal dominant inheritance is known to be causative in the genes such as *MEFV* (71, 72), *STING1* (73, 74) and *AICDA* (75, 76).

4.3 Somatic variants and mosaicism

IEI are most often caused by germline variants—genetic alterations that are present in every cell of the body. However, recent research has uncovered a significant role for somatic or post-zygotic variants—those that arise in a cell during the course of an individual's life and are not present in every cell—in these disorders. Somatic variants can lead to a mosaic pattern of disease, where some cells in the body carry the variant and others do not. These mosaic disorders can often present with atypical or milder phenotypes compared to their germline counterparts due to the presence of a population of normal cells. Several immune disorders have been associated with somatic variants.

Phenocopies refers to a category of disorders that exhibit clinical manifestations similar to IEI. However, in the case of phenocopies, the observed clinical features mimic those of IEIs without an underlying genetic defect. Instead, these disorders may be caused by somatic variants or other non-genetic factors (e.g., autoantibodies against various cytokines), leading to a phenotypic similarity to IEIs (45, 77). These disorders do not adhere to a Mendelian pattern of inheritance and the IUIS has designated phenocopies of IEIs as a distinct classification.

The identification of somatic variants using NGS demands specialized filters and algorithms due to the occurrence of these variants at exceptionally low allele frequencies (AF). The AF represents the proportion of mutated alleles in the sample. The AF for a somatic variant is influenced by the heterogeneity of the chosen tissue or sample for sequencing (78).

For example, somatic variants in the FAS-pathway cause autoimmune lymphoproliferative syndrome (ALPS). Other examples are autoinflammatory diseases such as AIFEC (autoinflammation with infantile enterocolitis) or NOMID (neonatal onset multisystem inflammatory disease) due to mosaicism in *NLRC4* in young children (79) or VEXAS (Vacuoles, E1 enzyme, X-linked, Autoinflammatory, Somatic syndrome) due to *UBA1*-variants in the elderly (80). Detecting these somatic variants requires sensitive techniques as the "mutated" cells may be a small proportion of the total blood cells in the body. The recognition of somatic variants in IEI has important implications for diagnosis and treatment, as well as for genetic counselling of affected individuals and their families.

5 Early genetic diagnosis is crucial for optimal treatment

An early molecular diagnosis of IEI is associated with improved health outcomes, decreased healthcare costs, and mitigates psychological stress for affected families (81–83). According to the ESID data, there is a 1.7% increase in the risk of mortality for each year of delay in diagnosis (84). Moreover, a genetic diagnosis paves the way for fundamental therapies in 34% (85), specifically for Hematopoietic Stem Cell Transplantation (HSCT), which is most effective when initiated early in the disease course before significant damage to the affected organs ensues (81). However, available therapies have been progressively expanding to include small molecule inhibitors, biologicals, gene therapy, and the use of adoptive transfer of virus-specific T cells to combat viral infections in immunocompromised patients (86). The rarity of the individual immunological diseases makes it difficult to conduct controlled studies, highlighting the necessity of thoroughly understanding the immunologic aetiology and possibly the underlying genetic causes to develop feasible hypotheses about how regulation of the immune response would affect the clinical course of the disease. As a result, genetic testing has become an indispensable tool for diagnosing and managing children afflicted with IEI (48).

6 Concluding remarks

In recent years, there has been an exponential increase in knowledge in human genetics, primarily driven by the development of new investigative techniques. This advancement has benefited many other disciplines dealing with the treatment of rare diseases. Although most IEIs are monogenic, many exhibit variable expressivity and penetrance, and reliable genotype-phenotype correlations are lacking. This emphasizes the importance of genetic diagnostics, which is becoming increasingly crucial in facilitating the diagnosis of these disorders.

Therefore, standardization is required in clinical practice by considering the clinical and laboratory characteristics of the patient when deciding on the genetic testing method. Close collaborations between physicians and geneticists are required to ensure on the one hand an efficient workflow to identify potential pathogenic variants that may have a significant impact on further therapies, especially for severely affected patients. On the other hand, even though prompt decision-making is essential, a prudent examination of variants should be pursued, and there may arise situations where it becomes necessary to engage specialized laboratories to conduct functional analyses, which, in turn, consume additional time. Finding the balance between efficient and rational use of all the modern testing methods is one of the main tasks for physicians and geneticists. Although various AI-driven tools are now available supporting the decision whether genetic testing is helpful, interpreting genetic data is far from straightforward and bears the risk of misinterpretation. Therefore, it is advisable to determine which genetic laboratory is specialized in IEI before initiating genetic diagnostics.

Author contributions

SH: Conceptualization, Supervision, Writing – original draft. IK: Writing – original draft. DS: Writing – review & editing. ND: Writing – review & editing. UB: Writing – review & editing. BA:

Writing – review & editing. CK: Conceptualization, Supervision, Writing – review & editing.

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Conflict of interest

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Inborn errors of immunity with susceptibility to *S. aureus* infections

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Staphylococcus aureus (*S. aureus*) is a significant human pathogen, in particular in patients with an underlying medical condition. It is equipped with a large variety of virulence factors enabling both colonization and invasive disease. The spectrum of manifestation is broad, ranging from superficial skin infections to life-threatening conditions like pneumonia and sepsis. As a major cause of healthcare-associated infections, there is a great need in understanding staphylococcal immunity and defense mechanisms. Patients with inborn errors of immunity (IEI) frequently present with pathological infection susceptibility, however, not all of them are prone to *S. aureus* infection. Thus, enhanced frequency or severity of *S. aureus* infections can serve as a clinical indicator of a specific underlying immunological impairment. In addition, the analysis of immunological functions in patients with susceptibility to *S. aureus* provides a unique opportunity of understanding the complex interplay between staphylococcal virulence and host immune predisposition. While the importance of quantitatively and qualitatively normal neutrophils is widely known, less awareness exists about the role of specific cytokines such as functional interleukin (IL)-6 signaling. This review categorizes well-known IEI in light of their susceptibility to *S. aureus* and discusses the relevant associated pathomechanisms. Understanding host-pathogen-interactions in *S. aureus* infections in susceptible individuals can pave the way for more effective management and preventive treatment options. Moreover, these insights might help to identify patients who should be screened for an underlying IEI. Ultimately, enhanced understanding of

Abbreviations

AD, autosomal-dominant; AIN, autoimmune neutropenia; AR, autosomal-recessive; C2, C3, complement component 2, complement component 3; CARD9, caspase recruitment domain family member 9; CD40, cluster of differentiation 40; CD40l, cluster of differentiation 40 ligand; CGD, chronic granulomatous disease; CID, combined immunodeficiency; CRP, C-reactive protein; DOCK, dedicator of cytokinesis; G-CSF, granulocyte colony-stimulating factor; GOF, gain of function; HAX1, HCLS1 associated protein X-1; HIES, hyper IgE syndrome; HIV, human immunodeficiency virus; HSCT, hematopoietic stem cell transplantation; IEI, inborn errors of immunity; Ig, immunoglobulin; IL, interleukin; IL-17RA, IL-17 receptor A; IL12B, IL12RB1, IL-12 subunit beta, IL-12 receptor beta 1; IL6ST, IL-6 signal transducer; IRAK-4, IL-1 receptor-associated kinase 4; IVIG, intravenous immunoglobulin; JAK, Janus kinase; LAD, leukocyte adhesion deficiency; LOF, loss of function; MAPK, mitogen-activated protein kinase; MRSA, methicillin-resistant *Staphylococcus aureus*; MyD88, myeloid differentiation primary response 88; NADPH, nicotinamide adenine dinucleotide phosphate; NEMO, NF-κB essential modulator; NF-kB, nuclear factor kappa B; NGS, next generation sequencing; Nox2, NADPH oxidase 2; OTULIN, OTU deubiquitinase with linear linkage specificity; PGM, phosphoglucomutase; PVL, panton-valentine leucocidin; RAC2, ras-related C3 botulinum toxin substrate 2; ROS, reactive oxygen species; *S. aureus, Staphylococcus aureus;* SCID, severe combined immunodeficiency; SCN, severe congenital neutropenia; SpA, staphylococcal protein A; spp, species pluralis; STAT3, signal transducer and activator of transcription 3; STAT1, signal transducer and activator of transcription 1; Th17, T helper 17; TLR, toll-like receptor; TMP/SMX, trimethoprim/sulfamethoxazole; TRIF, TIR-domain-containing adapter-inducing interferon-β; TSST-1, toxic shock syndrome toxin-1; TYK2, tyrosine kinase 2; ZNF341, zinc finger protein 341.

pathogenesis and immune responses in *S. aureus* infections may also be of relevance for the general population.

KEYWORDS

S. aureus, inborn errors of immunity (IEI), immunodeficiency, STAT3 deficiency, neutrophil dysfunction, chronic granulomatous disease (CGD), neutropenia, IL-6 deficiency

Introduction

The current list of inborn errors of immunity (IEI) comprises more than 485 monogenetic gene defects (1). Enhanced susceptibility to a specific pathogen such as *Staphylococcus aureus* (*S. aureus)* may raise suspicion of a certain type of immunological impairment. *Staphylococcus aureus* is a great challenge to our health care systems (2). Despite being considered a commensal, with a colonization rate of 20%–30% in the healthy population (3), it can also cause a wide variety of different infections. It is a leading cause of skin and soft tissue infections and abscesses, but may also lead to lung infections, osteomyelitis or endocarditis, in particular in patients with underlying conditions (2). The ability to colonize but also to cause harm to the host, emerges from a complex interaction between the pathogen and its host (4). *Staphylococcus aureus* is a specialist in adapting to the human host by evading almost every aspect of the immune system (5). In the last decades, changes in strains have led to an increase of *S. aureus* infections in otherwise healthy individuals (6). Thus, staphylococcal defense in the individual is shaped by both pathogen virulence factors as well as the patient's immune predisposition (4). Recurrent or severe *S. aureus* infections may both be an indicator of certain IEI and specific IEI can teach us about essential immune functions for staphylococcal defense.

S. aureus immune evasion and host immune response

Staphylococcal infections often arise from asymptomatic colonization and breaches through skin and mucosal barriers (7) (Figure 1). Immune evasion strategies of *S. aureus* are abundant and tackle particularly innate immunity (8, 9). Examples include inhibition of immune recognition, prevention of complement

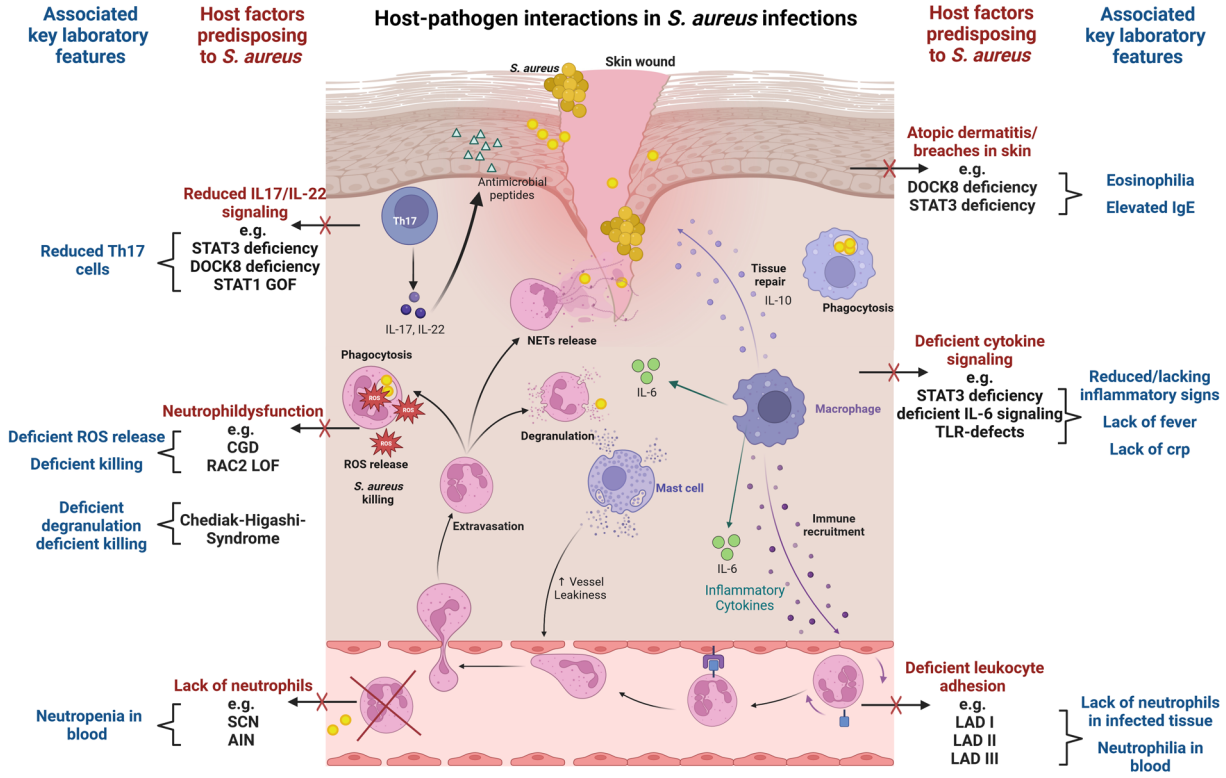

FIGURE 1
Host-pathogen interactions in *S. aureus* infections. The figure visualizes key immunological defense mechanisms and highlights host factors predisposing to *S. aureus* infection in case of deficiency. Commonly associated laboratory findings in the respective setting are also displayed. Selective examples of IEI with susceptibility to *S. aureus* infection are provided. The figure provides a simplified overview, and displayed host factors and interactions do not claim to be complete. The figure was created with BioRender.com.

activation (10), resistance to phagosomal killing (5) and direct killing of immune cells through different leucocidins (7). In addition, presence of peptidoglycan layer, polysaccharide capsule and surface proteins hamper opsonization (7). The most important players in *S. aureus* defense are phagocytes. In particular neutrophils, along with tissue-resident or monocyte-derived macrophages, are instrumental in identifying, engulfing, and eliminating staphylococci (11). As the first line of innate cellular defense, they also orchestrate subsequent immune responses. The crucial role of neutrophils is clearly evidenced by the enhanced staphylococcal susceptibility of patients with numeric or functional neutrophil defects (12, 13). *Staphylococcus aureus* has developed numerous mechanisms to reduce neutrophil extravasation, activation, and chemotaxis (9), and may also evade neutrophil extracellular traps using nucleases and proteases (14). Secretion of exopolysaccharides and biofilm formation inhibit phagocytosis (7). When internalized by phagocytes, *S. aureus* may neutralize reactive oxygen species and employ enzymes for survival (8). Through intracellular survival both in phagocytic and non-phagocytic cells, *S. aureus* may evade antibiotic killing and facilitate subsequent dissemination (15). Induction of IL-10 by *S. aureus* may lead to a phenotypic switch in the immune response during persistent staphylococcal infection allowing its persistence as commensal (16). Toxins like Panton–Valentine leucocidin (PVL), which are harbored by some more virulent strains, destroy immune cells and may lead to treatment failure and severe infections even in immunocompetent patients (17, 18). While most virulence factors address innate immunity, *S. aureus* may also interfere with the adaptive immune response, using proteins like SpA to bind immunoglobulins (19) and superantigens like TSST-1 to induce cytokine release and toxic shock syndrome (20).

The evasion strategies of *S. aureus* challenge infection management, prevention and vaccine development (8). We provide an overview of IEI that render individuals susceptible to *S. aureus* infections (Table 1 and Supplementary Table S1), highlighting key immunological defense mechanism involved in staphylococcal immunity.

IEI with low neutrophil numbers and susceptibility to *S. aureus* infections

Severe congenital neutropenia (SCN) is usually characterized by severe neutropenia (<500/µl) due to myeloid maturation arrest in the bone marrow. Over 20 different genes have been identified (21). Lack of mature neutrophils leads to a severe infectious phenotype with potentially life-threatening disease in the first months of life. Infections are caused not only by *S. aureus* but also by gram negative bacteria, and blood stream infections are common. Depending on the underlying gene defect there may be additional somatic features (Supplementary Table S1) (22).

Primary **autoimmune neutropenia (AIN)** of infancy, which is the most common type of neutropenia in childhood and may also present with nearly absent neutrophils and susceptibility to staphylococcal skin infections (abscesses, furunculosis), needs to be separated from SCN. AIN is typically detected in infancy, frequently as an incidental finding, and shows spontaneous remission in early childhood (23). Neutrophils mature normally in the bone marrow but peripheral numbers may be very low due to the presence of anti-neutrophilic antibodies. Infections are less severe compared to SCN. While the detection of anti-neutrophilic antibodies is suggestive of AIN it does not fully exclude additional SCN. Thus, in cases with severe infections or persistent neutropenia bone marrow evaluation and genetic testing may be indicated. If detected in older children or adults, AIN is more likely to be an immune phenomenon related to another IEI/autoimmune disorders requiring further diagnostic workup (24).

IEI with neutrophil function defects and susceptibility to *S. aureus* infections

Chronic granulomatous disease (CGD) represents the most common hereditary phagocyte dysfunction with an estimated prevalence of around 1:200,000 (25, 26). CGD leads to deficient reactive oxygen species (ROS) generation due to loss-of-function mutations affecting different aspects of the multicomponent enzyme NADPH oxidase in phagocytes (Nox2) (27). CGD patients experience severe infections accompanied by granuloma and abscess formation. *Staphylococcus aureus* is the most common pathogen isolated from skin infections/abscesses, liver abscesses and lymphadenitis, but it may also lead to pulmonary infections or sepsis. Patients are also very susceptible to *Aspergillus spp.* (26). Other characteristic pathogens in CGD include gram negative bacteria (e.g., *Salmonella*) and catalase positive bacteria (e.g., *Burkholderia*, *Serratia* and *Nocardia)* (12, 28). Additionally, CGD is associated with inflammatory complications like colitis, which might be related to defective T-cell regulation but also hyperactivation of NF-kB and inflammasome pathways (27, 29).

Leukocyte adhesion deficiency (LAD) is characterized by functional defects in neutrophil adhesion, integrin activation or rolling, leading to an inability to migrate effectively to infection sites (30). This results in a striking discrepancy with lack of pus formation at infection sites despite significant leukocytosis with neutrophilia in the blood. LAD patients typically experience recurrent bacterial and fungal infections, delayed wound healing, and other associated features (31). Three different genetic defects affecting neutrophils are known. Associated features are omphalitis and gingivitis (LAD I), developmental impairment and short statue (LAD II), and bleeding tendency (LAD III) (30, 32).

Combined IEI which frequently cause neutropenia or neutrophil dysfunction

Neutropenia has also been described in certain combined immunodeficiencies. Typical examples are **CD40Ligand (CD40l)** and **CD40 deficiency**, which are characterized by abnormal serum immunoglobulin levels due to impaired interaction between CD40l

TABLE 1 IEI with recurrent or severe *S. aureus* infections.

Immune dysfunction	Underlying defect of immune dysfunction	Selected examples	Typical type of *S. aureus* infection	Severity of *S. aureus* infection	Additional typical infections, suggestive signs	Prophylaxis options	General treatment options
IEI with neutropenia	Severe congenital neutropenia (SCN)	Neutrophil elastase defects, HAX1 def., X-linked neutropenia (WAS), Shwachman-Diamond syndrome, etc.	Blood stream, organ infections, abscesses	Severe, invasive, rapidly progressing	Fungal infections (*Candida, Aspergillus*), gram negative bacterial infections, gingivitis, oral ulcers	G-CSF, TMP/SMX, antifungal	Rapid empiric antibiotic treatment, increase G-CSF during infections, HSCT in some SCN
	Autoimmune neutropenia (AIN)	"benign" AIN (infancy), AIN associated to underlying IEI/ autoimmune diseases	Skin infections/folliculitis, rarely invasive infections	Mild-moderate	AIN of infancy: rarely other signs, AIN >5 years investigate for underlying IEI/ Autoimmune disease	Rarely needed	Antibiotic treatment as needed, G-CSF only in selected cases
	Combined IEI with Neutropenia	e.g., CD40l or CD40deficiency, RAC2 GOF, PGM3 deficiency	Blood stream, organ infections	Severe, invasive, rapidly progressing	Opportunistic infections (*PJP, Aspergillus*). Depending on underlying IEI systemic features	TMP/SMX, antifungal, IVIG/ScIG	Rapid empiric antibiotic treatment, G-CSF, HSCT
IEI with neutrophil dysfunction	Deficient release of ROS to all stimuli/ deficient killing	Chronic granulomatous disease (CGD): different traits	Abscesses (skin, organ), pneumonia, blood stream infections	Moderate to severe	Fungal infections (*Candida, Aspergillus*), gram negative, Catalase pos (e.g., *Nocardia, Burkholderia, Serratia*). Granuloma, colitis	TMP/SMX, antifungal	Rapid empiric antibiotic treatment, INF-y, HSCT, gene therapy
	Deficient ROS release/neutrophil chemotaxis (fMLP)	RAC2 LOF	Abscesses with lack of pus	Moderate to severe	Delayed wound healing and omphalitis	TMP/SMX	Antibiotic treatment, otherwise not well defined, depending on severity
	Deficient rolling, adhesion, extravasation	3 different types: LAD I, LAD II, LAD III	Skin and soft tissue infections with lack of pus	Moderate to severe in LAD I and III, milder in LAD II	Delayed wound healing and omphalitis in LAD I and LAD III. Neurodevelopmental impairment in LAD II. Bleeding in LAD III	TMP/SMX	Antibiotic treatment, fucose-based therapy for LAD II, HSCT (LAD I and LAD III)
	Deficient degranulation, reduced bactericidal activity	Chediak-Higashi-Syndrome	Skin infections	Moderate to severe (in particular if neutropenic)	Recurrent pyogenic infections. Systemic features: oculocutaneous albinism, neurological features, HLH.	G-CSF if neutropenic	HSCT, in particular if profound defects in cytotoxicity
IEI with defective cytokine signaling/TLR-defects	Reduced STAT3 signaling (incl. low IL-6 signaling)	STAT3-deficient HIES, AR ZNF341	Skin infections/cold abscesses, pneumonia	Severe tissue destruction possible, there may be an inadequate inflammatory response related to the degree of defective IL-6 signaling (potential lack of fever/low or absent CRP)	CMC. Multisystemic features, eczema, eosinophilia, IgE elevation, low Th17, encapsulated bacteria.	TMP/SMX, antifungal, IVIG/ScIG (STAT3-def.)	Treat with antibiotics if infection is suspected independent of CRP/fever/ general conditions. Surgical abscess drainage may be required
	Reduced IL-6 family signaling	AR partial LOF IL6ST or AD DN IL6ST			Skeletal abnormalities, eczema, eosinophilia, IgE elevation, variable Th17 cells	Consider TMP/SMX	
	Reduced IL-6 signaling only	IL6 receptor deficiency (AR IL6R def (IL6R)			Atopic dermatitis, eosinophilia, IgE elevation, normal Th17		
		IL-6 autoantibodies	Skin and soft tissue infections, sepsis in 1 case reported		Lack of multisystemic features no eczema, no CMC		
	TLR-signaling defects	IRAK-4 def, MyD88 def, EDA-ID (XR NEMO-def. and AD IKBA GOF)	Severe, invasive pyogenic infections (meningitis, sepsis, osteomyelitis,..). Skin infections	Rapidly progressing, severe infection, but lack of inflammation	Severe infection susceptibility to *S. pneumoniae. P. aeruginosa* also frequent. Additional viral/mycobact. infections, colitis and ectodermal dysplasia in EDA-ID	TMP/SMX+penicillin, Vaccination, in particular against encapsulated bacteria, IVIG/ScIG	Rapid empiric parenteral antibiotic treatment independent from CRP, fever or general conditions. Consider HSCT

The table lists the most prominent examples of IEI with susceptibility to *S. aureus* infections. IEI are grouped according to their most relevant underlying immune dysfunction associated to staphylococcal susceptibility. Descriptions of different IEI are kept to a minimum, and only key findings suggestive of the respective IEI are highlighted (for more details and references also see Supplementary Table S1). The table also displays potential prophylaxis and treatment options. In general, TMP/SMX may be used in patients with significant disease as antibiotic prophylaxis. However, local epidemiology and individual antimicrobial resistance needs to be considered. In IEI with deficient cytokine signaling, but also in patients with neutropenia, there is an urgent need to treat with empiric antibiotics upon clinical suspicion of infection regardless laboratory inflammation markers. HSCT can be a curative option in some diseases. Still, the need to treat may be variable for different genetic variants and also depends on the clinical severity.

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on T cells and CD40 on antigen-presenting cells (33, 34). These conditions lead to both impaired cellular and humoral immunity, which results in a broad infection phenotype. Patients frequently present with opportunistic infections (e.g., *pneumocystis jirovecii*, *cryptosporidium*, *aspergillus spp.*) (35). IgM may be elevated concomitantly to low IgA and IgG, which lead to bacterial respiratory and gastrointestinal infections (33). Intermittent or permanent neutropenia might be related to deficient release of growth factors important for granulopoiesis due to impaired CD40-CD40l-interaction (36). Furthermore, functional defects in neutrophils have been described in CD40l deficiency (37).

Mutations in Ras-related C3 botulinum toxin substrate 2 (RAC2) are also typically affecting neutrophil function. RAC2 is an essential regulator of neutrophil chemotaxis and contributes to NADPH oxidase function (38). Autosomal-dominant (AD) **RAC2 loss of function (LOF)** mutations cause LAD-like disease with neutrophilia and functional neutrophil defects (e.g., deficient chemotaxis and ROS generation) (39). In contrast, AD **RAC2 gain of function (GOF)** mutations lead to (severe) combined immunodeficiencies with lymphopenia and low immunoglobulins, frequent neutropenia and functional neutrophil abnormalities (38, 40).

Neutropenia has also been reported in some patients with deficiency in phosphoglucomutase 3 (PGM3), a disorder of glycosylation which is currently classified as autosomal-recessive Hyper IgE syndrome (1). **PGM3 deficiency** presents with eczema, eosinophilia, elevated IgE, but may also display a CID/SCID phenotype, facial dysmorphism and neurocognitive impairment (41).

Patients with autosomal-recessive deficiency of dedicator of cytokines (DOCK) 8 display severe atopic dermatitis with *S. aureus* colonization and skin infections (**DOCK8 deficiency**). Osteomyelitis has also been reported (42). DOCK8 plays a crucial role in lymphocyte proliferation, migration of dendritic cells, and generation of long-term memory in B- and T cells, thus predisposing patients to a mostly severe phenotype regarding viral and mycobacterial infections (43). Dysfunction of regulatory T-cells together with *S. aureus* exposure have been suggested to drive severe eczema in DOCK8 deficiency (44) and DOCK8-deficient murine neutrophils were prone to undergo *S. aureus*-induced cell death (45). In addition, reduced signal transducer and activator of transcription 3 (STAT3) signaling and low T helper 17 (Th17) cells have also been reported (46).

IEI with staphylococcal susceptibility associated to defective cytokine signaling

Autosomal-dominant Hyper-IgE syndrome due to dominant-negative mutations in STAT3 (**STAT3-HIES**) is one of the key IEI associated with a specific susceptibility to *S. aureus* infections, particularly in the skin and lung (47). Recurrent "cold" abscesses with lacking systemic signs of infections are typical. STAT3 functions as a transcription factor downstream of the tyrosine kinases janus activated kinase (JAK)1, JAK2, and tyrosine kinase 2 (TYK2) and enables signal transduction through various cytokines, such as interleukin-6 (IL-6), IL-10, IL-11, IL-21, and IL-23 (48). STAT3 deficiency results in failure of Th17 cell differentiation (49). Th17 function has been shown to be pivotal in Candida defense (50), explaining the patients' predisposition to mucocutaneous candidiasis. Th17 cells aid epithelial cells to produce neutrophil-recruiting chemokines and antimicrobial factors such as ß-defensins, which may be relevant for staphylococcal defense (51). STAT3-deficient neutrophils display normal functions (52), but are prone to undergo *S. aureus*-induced cell death (53). Furthermore, STAT3-HIES patients display variable antibody responses and low numbers of memory B cells, which likely contributes to enhanced incidence of respiratory infections with *H. influenzae* and *S. pneumoniae* (52). STAT3 is ubiquitously expressed and multisystemic features are present. Thus, deficient epithelial STAT3 signaling may contribute to aberrant staphylococcal control by cytokine dysregulation and aberrant tissue remodeling (54, 55). STAT3 is involved in both pro- and anti-inflammatory signaling which complicates our understanding of single factors for the overall phenotype.

Autosomal-recessive **ZNF341 deficiency** leads to reduced cytokine signaling via STAT3 and resembles STAT3-HIES by displaying similar multisystemic features (e.g., bone fractures, retention of primary teeth, facial dysmorphism) but also staphylococcal infections (56).

IEI affecting single cytokines may teach us about their individual contribution. Lack of functional IL-6 cytokine family signaling reduces typical local inflammatory reaction, leads to low CRP and reduced systemic symptoms although tissue damage may be considerable. Defective IL-6 signaling either by **IL-6 receptor deficiency** (57) or by partial **IL-6 signal transducer deficiency** (IL6ST) (58) also leads to pyogenic infections, cold abscesses and pulmonary *S. aureus* infections. Additionally, phenocopies of IEI such as autoantibodies against IL-6 show increased susceptibility to *S. aureus* infection lacking CRP response (59). *Staphylococcus aureus* infections are also described in ERBIN deficiency which recapitulates some features of STAT3 deficiency (60).

Frequent *S. aureus* skin infections have also been reported in patients with **STAT1GOF** who are very susceptible to fungal infections, have low Th17 cells, and display a high rate of autoimmune features (61, 62).

IEI with defects in toll-like receptor (TLR)-signaling and susceptibility to *S. aureus*

Autosomal-recessive **IRAK-4** and **MyD88 deficiencies** affect TLR and IL-1R induced activation of NF-κB and MAPKs through the classical pathway (63). They disrupt key pathways in the innate immune response and usually present with bacterial pyogenic infections early in life (<2years of age). Most common pathogens are *S. pneumoniae*, *S. aureus and Pseudomonas aeruginosa* (64). Lack of TLR-induced signaling affects particularly the production of IL-6 and IL-8, and may lead to severe invasive infections (e.g., meningitis, sepsis, osteomyelitis, arthritis and abscesses), but also localized skin infections, lymphadenitis and ENT infections, usually without marked fever or increase of CRP (64). Still, pus is seen at the site of infection,

which underlines that pus formation is not dependent on TLR-related cytokine signaling. As signs of infections may be absent but invasive infection may be rapidly progressing, it is vital to initiate empirical antibiotic treatment as soon as infection is suspected (64).

NEMO deficiency and **IκBα GOF**, which affect both NF-κB and TRIF-dependent signaling, result in a broad spectrum of immune dysfunctions and present also typically with colitis and ectodermal dysplasia. Apart from pyogenic bacterial infections, patients may also display mycobacterial infections, severe viral infections and opportunistic infections (64). Recently, more rare genetic defects associated to TLR-signaling have been reported, with variable phenotype depending on the protein involved.

Other diseases with susceptibility to *S. aureus*

Apart from classical IEI, increased susceptibility to *S. aureus* infections has also been reported in diseases such as **cystic fibrosis**, **HIV infection** and/or **diabetes mellitus** (65–68). In addition to aberrant host immune response, susceptibility to *S. aureus* may also be enhanced by colonization of multi-resistant strains (MRSA) carrying specific virulence factors.

Discussion: controversies, current knowledge gaps and future perspectives

While the key role of innate immunity for staphylococcal defense is well-established, the contribution of adaptive immunity is less clear.

In regards to B-cell immunity, evidence for a protective role of *S. aureus* antibodies is scarce. In fact, it has lately been suggested that *S. aureus* may induce non-protective antibodies, which then interfere with protective immune responses (69) facilitating commensalism and recurrent infections. Furthermore, patients with antibody deficiency do not display a specifically enhanced susceptibility to *S. aureus,* while they are clearly susceptible to other bacteria with a polysaccharide capsule (e.g., *S. pneumoniae, H. influenzae*). In contrast to the successful vaccine development for other encapsulated bacteria, there is still no available vaccine against *S. aureus*, and even adequate antibody induction to relevant *S. aureus* virulence factors did not lead to protection (70). The ability of anti-TSST-1 antibodies to provide protective immunity against superantigen-driven toxic shock syndrome appears to be an exception to the above, with IVIG being used as potential adjunctive therapy to ameliorate the symptoms (71).

Regarding the relevance of T cells, Th17 cells are often suggested to contribute to anti-staphylococcal-response, particularly at mucosa and skin sites (51). In mice, several studies document the importance of functional IL-17 signaling for the protection against mucocutaneous *S. aureus* infections (72, 73). Patients with IL-17RA deficiency are very prone to mucocutaneous candidiasis but do also display staphylococcal skin infections (74, 75). The initial hypothesis regarding the relevance of Th17 cells to prevent staphylococcal skin infection is closely related to the observed lack of Th17 in STAT3 deficiency (51). While the role of Th17 for candida defense is supported by other IEI with specifically deficient IL-17 signaling such as IL-17 autoantibodies (75), their relevance for *S. aureus* infections appears less significant. In the context of STAT3-HIES, the abundant changes in different cytokine signaling pathways and the contribution of ubiquitously deficient STAT3 needs to be considered. Of note, deficient IL-6 cytokine signaling is sufficient to predispose to staphylococcal infection even in the setting of normal Th17 cells (58, 76), and mere lack of Th17 cells does not induce susceptibility to *S. aureus* infection as evidenced in patients with IL12B/IL12RB1 deficiency (77) or CARD9 deficiency (78). Notably, STAT3-deficient patients with somatic mosaicism and normal Th17 compartment may still present with boils and pneumonia (79). Thus, lack of IL-17 signaling alone is likely insufficient in explaining enhanced susceptibility to *S. aureus,* even though patients may be more prone to folliculitis (74).

IEI with impairments in TLR and NF-κB signaling pathways such as in IRAK-4 or MyD88 deficiency, underline the significance of these pathways in recognizing and responding to *S. aureus* (80). Patients with STAT3-HIES, ZNF341 deficiency, partial IL6ST deficiency and IL-6 receptor deficiency all share deficient IL-6 signaling and enhanced frequency of "cold" staphylococcal abscesses and lung infections (1). IL-6 is a pleiotropic cytokine that is vital for acute-phase responses, defense against bacterial infections and tissue regeneration (81). The shared phenotype argues for an essential role of IL-6 in staphylococcal defense (82). Still, the precise molecular mechanism behind this particular predisposition and the contribution of other pathways is unknown.

Complement deficiencies might serve as additional risk factors in the context of *S. aureus* infections due to the crucial role of the complement system in opsonizing pathogens and facilitating their clearance by phagocytes. Susceptibility to *S. aureus* infections has been described in patients with C2 and C3 deficiencies (83) and complement activation was found to reduce persistent intracellular *S. aureus* burden in keratinocytes (84). Still, the role of complement in the defense against this pathogen appears less pronounced compared to its critical function in combating other encapsulated bacteria.

More recently, it has been proposed that specific genes may predispose to more severe infections via impairment of selective immune defense mechanism such as the altered response of non-leukocytic cells to staphylococcal alpha-toxin in OTULIN haploinsufficiency (85). With the growing use of NGS our understanding of specific factors in staphylococcal immunity will likely expand further. Still, the rareness of single IEI may hamper reliability of certain genotype-phenotype associations. An example is TYK2 deficiency, where the originally identified patient with susceptibility to *S. aureus* and hyper-IgE phenotype (86) was later judged to display deficient IL-6 signaling unrelated to TYK2 deficiency (87).

Last, the ability of *S. aureus* to survive intracellularly, notably within neutrophils, macrophages and as small colony variants in

epithelial cells, complicates the immune response and treatment strategies and might facilitate recurrent infections (88). Together with the multiple other evasion strategies this poses significant challenges in vaccine development against *S. aureus*. In the light of growing rates of MRSA, it therefore remains essential to continue to assess host-pathogen interactions on a functional level and further enhance our understanding about crucial immune defense mechanisms.

Conclusion and diagnostic suggestions

- Basic immunological workup in patients with recurrent or severe *S. aureus* infections should include a differential blood count and IgG, IgA, IgM, IgE
- Specific testing for CGD, HIES, complement deficiency, LAD, TLR deficiency, exclusion of secondary immunodeficiencies and assessment for phenocopies of IEI as well as genetic analysis may be warranted
- Inconclusive immunological investigation should be complemented by assessment of staphylococcal colonization

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Conflict of interest

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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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2024.1389650/full#supplementary-material

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IKAROS—how many feathers have you lost: mild and severe phenotypes in *IKZF1* deficiency

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Heterozygous germline variants in human *IKZF1* encoding for IKAROS define an inborn error of immunity with immunodeficiency, immune dysregulation and risk of malignancy with a broad phenotypic spectrum. Growing evidence of underlying pathophysiological genotype-phenotype correlations helps to improve our understanding of IKAROS-associated diseases. We describe 6 patients from 4 kindreds with two novel *IKZF1* variants leading to haploinsufficiency from 3 centers in Germany. We also provide an overview of first symptoms to a final diagnosis including data from the literature.

KEYWORDS

IKZF1, IKAROS, transcription factor, haploinsufficiency, immunodeficiency

1 Introduction

The protein IKAROS, encoded by *IKZF1*, is part of the zinc finger transcription factor family which plays a central role in lymphocyte, erythroid, myeloid and megakaryocyte differentiation and development (1–3). Germline heterozygous variants in human *IKFZ1* result in inborn errors of immunity (IEI) with infection susceptibility, immune dysregulation and risk of malignancy. The broad clinical spectrum includes bacterial, viral or fungal infections, autoimmunity, atopy, lymphoproliferative disorders and hematologic malignancies (4–7). To date, 4 different mechanisms impairing the function of IKAROS proteins have been described: haploinsufficiency (HI), dimerization defect (DD), dominant negative (DN) and gain-of-function (GOF) (8). Interestingly, regardless of the affected domain (and its corresponding functional defect), patients with IKAROS-associated diseases present with a phenotype of increased infection susceptibility and signs of immune dysregulation. Most patients with IKAROS

haploinsufficiency (HI) suffer from bacterial infections and symptoms of immune dysregulation (8). Around 5% of patients develop malignancies including B-cell acute leukemia (B-ALL) (9). The median age of symptom onset is 10 years but first symptoms may present up to the age of 60 (10, 11). Some carriers may be clinically asymptomatic (12–14). Immunological characterization of IKAROS-HI usually reveals an incomplete B-cell arrest with low B-cell numbers and serum immunoglobulin levels. Reported patients were treated with corticosteroids, immunoglobulin replacement therapy (IgRT), prophylactic and/or therapeutic antibiotics and hematopoietic stem cell transplantation (HSCT) depending on the severity of their clinical presentation (8).

Variants located in the dimerization domain or nonsense variants upstream of the dimerization domain so called "**dimerization defect**" (DD), are usually characterized by hypogammaglobulinemia and hematological manifestations, including autoimmune cytopenias, lymphoproliferative disorders and a variety of hematologic malignancies. In comparison to patients with HI variants, those with DD variants show moderate B-cell lymphopenia and hypogammaglobulinemia resulting in less frequent and severe bacterial infections (5). Most children manifest before the age of 10 and are treated with immunosuppressants and IgRT (10).

Patients with **dominant negative (DN)** *IKZF1* variants present with severe and invasive infections [esp. *Pneumocystis jirovecii* pneumonia (PCP)], usually before 2 years of age. Immunologically they are characterized by a combined immunodeficiency (CID) with agammaglobulinemia, severe B-cell lymphopenia, absence of plasma cells and an abnormal T-cell compartment with increased naïve and reduced memory cells. Patients are often managed with antimicrobial therapy including PCP prophylaxis, IgRT, and, due to the severity of the disease, HSCT (4, 15).

While HI, DD and DN variants cause an IKAROS loss-of-function (LOF), **gain-of-function variants** in *IKZF1* lead to an increased IKAROS DNA binding (16). The clinical phenotype is dominated by signs of immune dysregulation and atopy. Multiple autoimmune phenomena like gastrointestinal, endocrinological and hematologic manifestations have been reported. Individuals with GOF variants manifest with incomplete penetrance between 1 and 40 years of age and exhibit normal B-cell numbers and normal to slightly elevated immunoglobulin levels.

In summary, diagnosis and treatment of IKAROS-associated phenotypes can be challenging due to the broad spectrum of clinical signs and symptoms (Figure 1). We subsequently describe 6 affected patients from 4 kindreds with *IKZF1* variants and functional IKAROS-HI, and provide a clinical overview from first symptoms to final diagnosis.

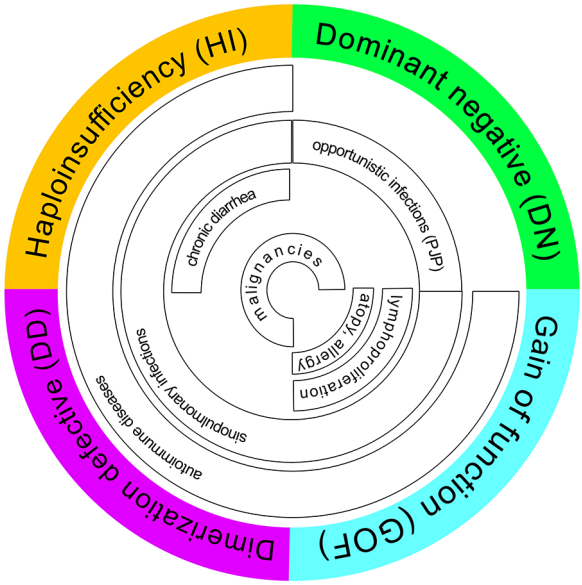

FIGURE 1
Phenotype-genotype correlation including overlapping features (review of the literature). PCP, pneumocystis jirovecii pneumonia.

2 Clinical vignettes and functional testing

2.1 Family A

Patient 1 is a 14-year-old boy presenting with osteomyelitis and streptococcal bacteremia at age 7. He has a history of recurrent otitis media, chronic purulent rhinitis and cough for 2 years as well as recurrent and long-lasting infections starting in childhood. His family reported frequent exhaustion upon physical activity. Between the age of 6 and 9 years, the child had intermittent diarrhea, without any causative pathogen ever detected. Intolerance for fructose or lactose was excluded, endoscopic investigation was not performed. Imaging at age 9 showed mild bronchiectasis and hepatomegaly. Further diagnostic workup revealed severe hypogammaglobulinemia and a lack of vaccination responses to tetanus, diphtheria and *Haemophilus influenz*ae. Further immunologic workup showed severely reduced B cells with a predominance of naïve B cells, absent class-switch, a reduced CD4/CD8-T-cell ratio due to expanded CD8+ T cells, as well as severely reduced naïve T cells (summarized in Table 1, details in Supplementary Table S1). T-cell proliferation *in vitro* was reduced to specific antigens such as tetanus and cytomegalovirus (CMV) (positive tetanus vaccination status, unknown status of CMV exposure). A *de novo IKZF1* missense variant [c.448T>C; p.(Cys150Arg)] was identified in whole exome sequencing (WES) (Figure 2). Considering the cumulative infectious burden with possible organ damage and risk for hematological malignancies led us to reconsider curative treatment options. HSCT from an HLA-identical sister was performed at 11.5 years of age following myeloablative conditioning with treosulfan, fludarabine and thiotepa. Three years post HSCT, the patient is well with full donor chimerism including a normal T- and B-cell compartment.

2.2 Family B

Patient 2 is a 19-year-old young adult, presenting at age 2 with epistaxis and hematomas. Persistently low platelet counts, and the presence of thrombocyte auto-antibodies (anti-GPIIb/IIIa) confirmed the diagnosis of immune thrombocytopenia (ITP) treated with

TABLE 1 Summary of clinical, immunological and genetic characteristics of *IKZF1* HI and treatment of the 6 affected individuals.

	Age at symptom onset	First point of contact	Clinical symptoms	Immunological workup	Management	*IKZF1* variant
Patient 1	7	Infectious diseases	- Osteomyelitis - Streptococcal bacteriemia - Recurrent upper airway infections - Chronic diarrhea	Lymphocyte count = B-cell count ↓ CD4/CD8 ratio ↓ Immunoglobulins ↓	IgRT, HSCT	c.448T>C, p.Cys150Arg (ACMG class 4, likely pathogenic)
Patient 2	2	Hematology	- Bleeding diathesis - Chronic immune thrombocytopenia	Lymphocyte count ↓ B-cell count ↓ CD4/CD8 ratio ↓ Immunoglobulins↓	Prednisolone, IgRT, Thrombopoietin-receptor-agonists	c.563T>C, p.Leu188Pro (ACMG class 3, uncertain)
Patient 3	18	Immunology	- Recurrent upper airway infections - Esophageal strictures - Epilepsy - Sepsis, pulmonary failure requiring ECMO	Lymphocyte count = B-cell count ↓↓ CD4/CD8 ratio ↓ Immunoglobulins ↓	IgRT, cotrimoxazole	p.141_239del del Exon4 + 5
Patient 4	3	Immunology	- Recurrent upper airway infections	Lymphocyte count = B-cell count progressively ↓ CD4/CD8 ratio ↓ Immunoglobulins ↓	IgRT	p.141_239del del Exon4+5
Patient 5	55	Immunology	- Recurrent infections of upper airways - Arthritis - Eczema - Chronic diarrhea - Hypothyroidism	Lymphocyte count = B-cell count = CD4/CD8 ratio ↓ Immunoglobulins ↓	IgRT	c.530T>C, p.Leu177Pro (ACMG class 4, likely pathogenic)
Patient 6	33	Infectious diseases	- Recurrent infections of upper airways	Lymphocyte count = B-cell count = CD4/CD8 ratio ↓ Immunoglobulins =	–	c.530T>C, p.Leu177Pro (ACMG class 4, likely pathogenic)

ECMO, extracorporeal membrane oxygenation; IgRT, immunoglobulin replacement therapy; HI, haploinsufficiency.

prednisolone and intravenous immunoglobulins. In 2023, he was started on thrombopoietin-receptor-agonists (*Revolade, NPlate*) due to recurrent episodes of symptomatic thrombocytopenia. At that time, his laboratory workup showed mild hypogammaglobulinemia, reduced IgA and IgM, and the patient was started on subcutaneous immunoglobulin (SCIG) treatment. The patient's father died from thrombosis and pulmonary embolism. The patient's half-brother (same father) also suffers from chronic ITP (Figure 2). Further immunophenotyping of the patient revealed reduced B cells, a reduced CD4/CD8 ratio due to decreased CD4+ T cells, and impaired B- and T-cell maturation with increased naïve B cells, decreased class-switched B cells, as well as reduced naïve CD4+ T cells. Vaccine responses to a variety of vaccines were undetectable (Table 1 and Supplementary Table S1). A novel heterozygous missense variant *IKZF1* [c.563T>C, p.(Leu188Pro)] was detected in WES. To date, the father and the brother have not been tested genetically.

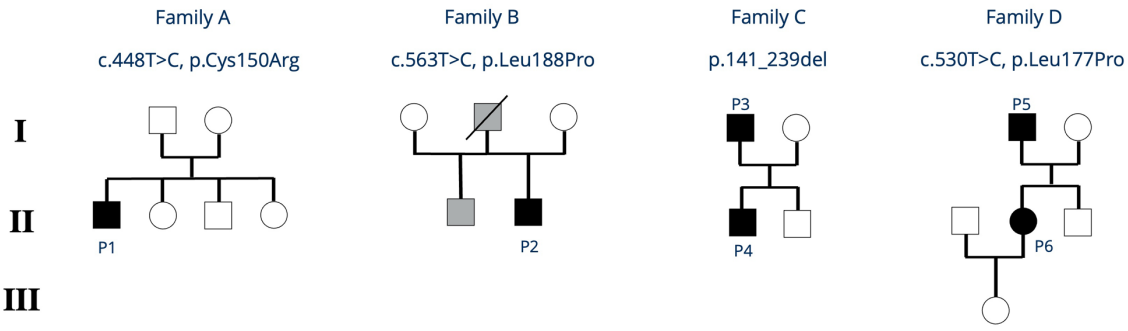

FIGURE 2
Family trees of all reported families with *IKZF1* HI. Black symbols indicate clinically affected index patients, grey symbols indicate individuals who are clinically affected but not genetically tested. P, patient.

2.3 Family C

Patient 3 is a 48-year-old male with a history of recurrent bacterial upper airway infections first presenting in adolescence. He reported recurrent and severe pneumonias, otitis and chronic pansinusitis (*S. aureus, H. influenzae*) requiring repeated antibiotic treatments. CT chest imaging showed bipulmonary micronodular lesions, but no bronchiectasis. At the age of 30 years, agammaglobulinemia was diagnosed. The patient was subsequently started on IgRT which improved the infection susceptibility. Of note, the patient requires 80 g IgG monthly to maintain IgG levels ≥10 g/L. Symptoms of sinusitis and productive cough further decreased when prophylaxis with cotrimoxazol was initiated. Immunophenotyping showed absent B cells, as well as a reduced CD4/CD8 ratio due to expanded CD8+ T-cells and borderline low naïve CD4+ T-cells. T cell proliferation to stimulation with mitogens and recall antigens was normal (Table 1 and Supplementary Table S2). At the age of 32 years, the patient was hospitalized with severe shingles [varicella zoster virus (VZV)]. At age 40, he started reporting recurrent episodes of diarrhea with weight loss. A previous episode of diarrhea could be explained by Salmonella infection, but the chronic intermittent course could not be attributed to any infectious agent. Histologically there were no signs of inflammation. To date the patient reports rare episodes of watery stools. In addition, he started complaining about swallowing difficulties. Endoscopy showed esophageal strictures, histology however did not confirm a suspected eosinophilic esophagitis. In his medical history, the patient reports of epilepsy with a first afebrile seizure at the age of 19, currently well-controlled with valproic acid and ethosuximide treatment. At present, the patient is obese and has both arterial hypertension and steatosis hepatis. At age 47 years, the patient was admitted to intensive care with pneumonia, pulmonary failure and fulminant sepsis. Following a very critical condition requiring extracorporeal membrane oxygenation, he has fortunately fully recovered and could be discharged from the hospital.

His now 10-year-old son (Patient 4, Figure 2) became symptomatic with recurrent upper airway infections (rhinitis, pharyngitis) starting from age 3. Due to the positive family history, clinical findings, and low IgG levels, he was started on IgRT at the age of 3 years. No major infections have occurred so far, but he also requires high doses of IgG (>0.9 g/kg body weight/month) to maintain IgG levels >6 g/L. His immunophenotyping shows progressive decline of B cells with reduced class-switch, but also a reduced CD4/CD8 T-cell ratio due to an expansion of CD8+ T-cells. Vaccination titers were low (tetanus) or undetectable (pneumococcus, measles) prior to IgRT despite respective vaccinations (summarized in Table 1, details in Supplementary Table S2).

Genetic testing of father and son revealed a deletion of exons 4 and 5 in the *IKZF1* gene of father and son.

2.4 Family D

Patient 5 is a now 61-year-old male with a recent history of recurrent upper airway infections starting from the age of 55. He reports 4 hospital admissions due to recurrent and prolonged pneumonias revealing mild hypogammaglobulinemia with low IgG and absent IgA levels. Chest imaging showed bronchiectasis and pulmonary granuloma. He was started on IgRT which led to reduced infectious complications. Apart from infections, he also reports arthritis in his metacarpophalangeal and proximal interphalangeal joints, eczema, hypothyroidism and several episodes of non-infectious intermittent diarrhea for over one year's duration. At present he is being re-evaluated for exacerbation of non-bloody watery diarrhea, with negative results for stool pathogens including protozoa, and normal calprotectin. He is scheduled for colonoscopy. Other co-morbidities include metabolic syndrome with arterial hypertension, coronary sclerosis, Type 2 diabetes mellitus, hepatic steatosis and sleep apnea syndrome. Immunophenotyping in this patient shows normal B-cell counts with mildly reduced class-switched B-cells, and a reduced CD4/CD8 T-cell ratio due to expanded CD8+ T-cells. Vaccination titers to tetanus toxoid and diphtheria toxoid were low prior to IgRT (summarized in Table 1, details in Supplementary Table S3).

Patient 6 is the now 34-year-old daughter of patient 5 (Figure 2). She reports long lasting respiratory infections starting in childhood without increased need for antibiotic treatment and no need for hospitalization. Her medical history is almost unremarkable without the need for prophylaxes to prevent infections. Immunophenotyping revealed normal B-cell counts, mildly impaired B-cell class-switch, mildly reduced IgA levels, normal IgG and normal vaccination titers, and increased gamma-delta T-cells (summarized in Table 1, details in Supplementary Table S3). She was found to have elevated anti-nuclear antibody (ANA)-titers 1:640 (AC-21), antimitochondrial antibody (AMA) 1:1,280 (M2 15 U/ml) so far without clinical evidence of autoimmune disease. Abdominal ultrasound revealed mild hepatosplenomegaly. Genetic testing of patient 5 and 6 revealed a novel heterozygous, pathogenic missense variant in *IKZF1* [c.530T>C, p.(Leu177Pro)].

2.5 Functional validation

Two of the herein reported four *IKZF1* heterozygous germline variants are novel. To assess their functional consequences, the 3 missense variants were subjected to *in-vitro* functional testing by immunofluorescence and electrophoretic mobility shift assay (EMSA). Immunofluorescence data showed that while NIH3T3 cells expressing WT IKAROS protein exhibit a punctate staining pattern, characteristic of pericentromeric heterochromatin localization, all three mutant proteins display diffuse nuclear staining (Figure 3A top). However, when the mutant was co-expressed with WT IKAROS protein to mimic a heterozygous condition, the normal punctate staining pattern was again observed. These data suggest that while the three mutant *IKZF1* variants lost their pericentromeric targeting, they did not exert dominant negative effects, as they did not abolish the pericentromeric localization of the WT protein under heterozygous conditions (Figure 3A bottom). Furthermore, EMSA data revealed that the ability of mutant IKAROS proteins to bind

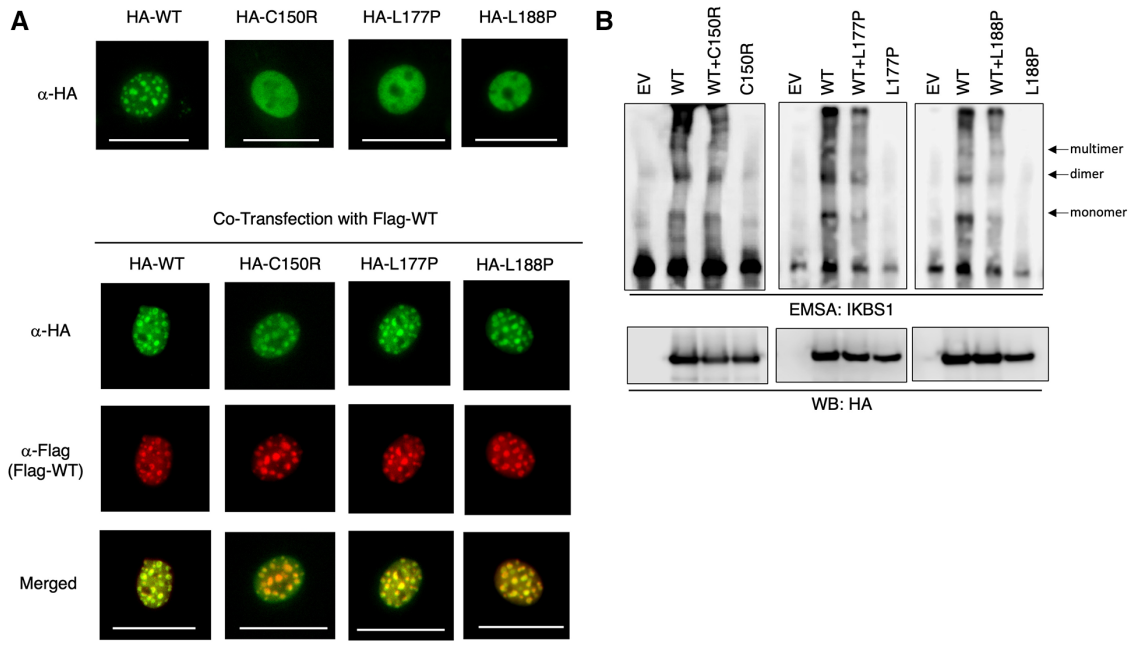

FIGURE 3
IKAROS mutants failed DNA binding and pericentromeric targeting. (**A**) NIH3T3 cells transfected with HA-tagged WT *IKZF1* and mutant *IKZF1* alone or together with Flag-tagged WT *IKZF1*. Pericentromeric heterochromatin localization of wild type and indicated mutant IKAROS proteins is revealed by fluorescence microscopy using tag specific antibodies. The scale bars indicate 25 µm. (**B**) Binding capacity of IKAROS mutants to corresponding DNA elements using an electrophoretic mobility shift assay (EMSA). Nuclear extracts of HEK293T cells transfected with HA-tagged WT *IKZF1* and/or mutant *IKZF1* were incubated with a IKBS1 DNA probe and separated by electrophoresis. Arrows indicate the monomer, dimer and multimer forms of IKAROS protein. Protein Expression of ectopic WT or mutant IKFZ1 protein was confirmed by western blot (WB) using a HA-specific antibody. EV, empty vector. Data shown are representative of three independent experiments.

to corresponding DNA elements was completely abrogated. However, the mutants did not interfere with the binding of the WT protein under heterozygous conditions (Figure 3B). For these 3 missense variants, our data suggest that our patients with these IKAROS variants can be characterized as having IKAROS-HI. As for the patient with deletion of exons 4 and 5, the exact breaking points could not be detected and were presumably located outside of the sequenced regions. A similar deletion (chr7:50.435.843–50.452.713, 16.8 kb) was detected in a Norwegian patient with hypogammaglobulinemia and low B cells (12).

3 Discussion

The herein described patients all carry germline *IKZF1* variants with functional HI and have a history of recurrent bacterial or viral infections, immune thrombocytopenia and inflammatory signs like arthritis and eczema, but no history of malignant disease.

According to previous review articles, *IKZF1* deficiency may present with a broad variety of clinical symptoms (Figures 1, 4) (5). In this IEI, a genotype-phenotype correlation has been described, i.e., signs and symptoms differ between *IKZF1* genetic variants according to their functional impact (8).

In the cohort with LOF variants presented here, the age at symptom onset ranges from early childhood until late adulthood with incomplete clinical penetrance. In agreement with the literature, the clinical spectrum spans from severe invasive infections to clinically oligosymptomatic individuals (Figure 4). Three of the 6 patients of our cohort report a history of chronic or intermittent diarrhea of infectious or undefined origin; the latter with a self-limiting course. Previous reports describe IKAROS patients with common variable immunodeficiency (CVID)-like phenotypes, of whom a minority (3 of 29) individuals had chronic or recurrent diarrhea of infectious or unknown cause (12). As in any IEI, a history of persistent or chronic diarrhea requires histological work-up based on the severity and duration of symptoms, as infectious causes may be treatable, and e.g., celiac-like disease is not a rare occurence in other IEIs, and may impact quality of life.

The immunophenotype in most of our patients showed an incomplete B-cell maturation arrest leading to a decline of B-cell numbers and serum immunoglobulin levels. These findings confirm the importance of IKAROS function in B-cell development. The most severe reduction of B-cell counts is expected in patients with dominant negative mutations, followed by haploinsufficiency and dimerization defective variants. The decline of B-cell numbers can be progressive, and the severity of B-cell deficiency may vary within one kindred (8, 14). Especially in LOF missense variants, patients additionally have abnormalities in their T-cell compartment with T-cell lymphopenia, elevated CD8+ T-cells and a decreased CD4/CD8 ratio (8). This is in line with the immunophenotyping of our patients, including one family harboring a large genetic deletion.

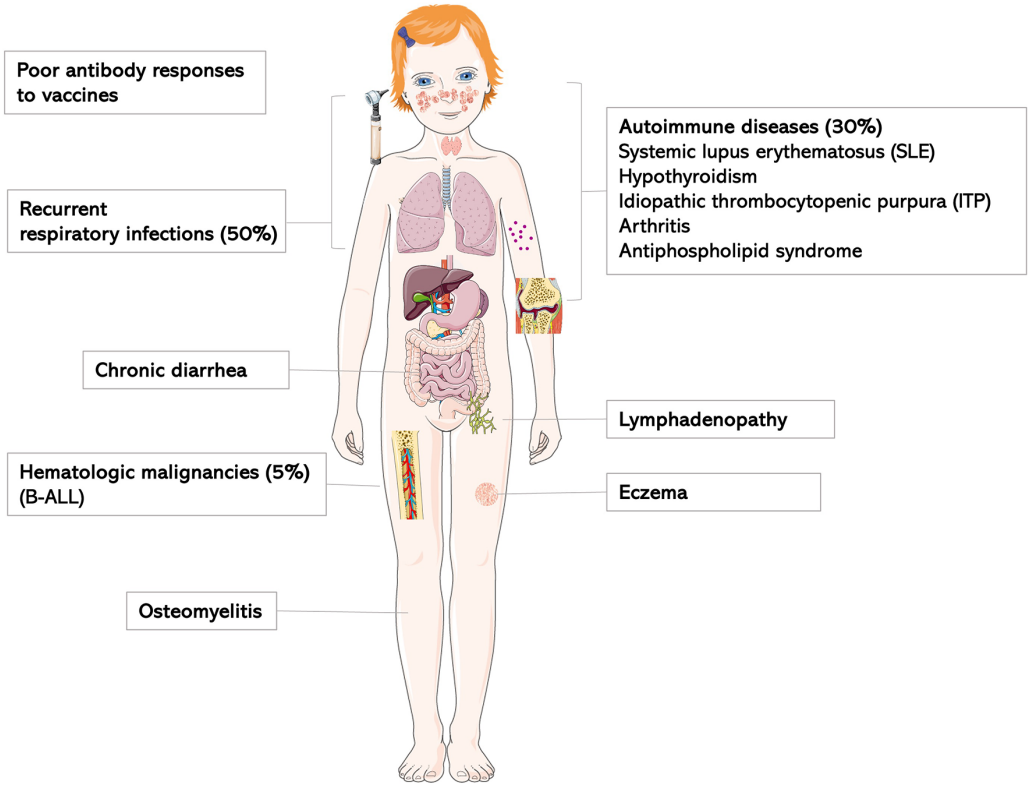

FIGURE 4
Clinical characteristics of *IKZF1*-haploinsufficiency patients as previously reviewed by PMID: 36433803.

In contrast to former reports, we also identified reduced CD4+ and CD8+ naïve T-cell numbers in some of our IKAROS-HI patients regardless of the severity of their clinical phenotype. Of note, newborns with *IKZF1* variants have been detected through severly reduced T-cell receptor excision circles (TREC) in newborn screening with severe combined immunodeficiency (SCID)-like phenotypes. Interestingly, some of these newborns had either functional T-cell abnormalities and/or partial immune-recovery on follow-up. The authors hypothesize an importance of fully functioning IKAROS during intrauterine lymphocyte development (17). Murine studies indeed confirm the importance of *IKZF1* for T-cell development (18). The biology of abnormalities in T-cell differentiation needs more scientific attention. Lastly, abnormalities of the dendritic cell (DC) compartment have also been described for IKAROS-HI patients (11). Heterozygous variants in humans have been shown to reduce peripheral DCs and to expand conventional DC1 numbers. This may indicate a regulatory effect of *IKZF1* in human DC development (19).

All variants described in our manuscript—including the two novel *IKZF1* germline missense variants p.Leu188Pro (Family B) and p.Leu177Pro (Family D)—lead to the clinical phenotype of IKAROS-HI. The number of functionally proven variants is consistently growing (Figures 5A,B), with genetic heterogeneity (Figure 5A) affecting Zinc finger domains 2 and 3, which are essential for DNA binding. In our cohort, functional testing indeed confirmed the lack of DNA binding capacity and PC-HC targeting in our reported *IKZF1* variants without having dominant negative effect (Figure 3). The clinical and immunological phenotypes mostly depend on the localization of the mutation within IKAROS protein. This has been elucidated by Kuehn et al., who have dissected protein impairment due to haploinsufficiency, dominant negative, dimerization vs. GOF (8). Our cohort who has functionally validated IKAROS-HI, however presented with a much broader range and severity of symptoms from mild upper airway infections to severe invasive infectious complications. Therefore with the here presented cohort, we contribute to the already reported clinical spectrum of IKAROS-HI with respect to genetic variants, immunophenotype, age at manifestation, the variety of symptoms, severity of clinical presentation and therapeutic management. Nevertheless, the here reported patients show symptom overlaps with other disease phenotypes associated with *IKZF1* genetic variants such as IKAROS-DD (e.g., immune cytopenia), which is illustrated in Figure 1. This challenges a strict genotype-phenotype correlation and illustrates the importance of the interplay between genetics, clinical symptoms, functional immunological workup as well as interprofessional care to make the correct diagnosis and patient management.

Most patients reported in this manuscript have a sustained clinical response to IgRT. The patients with ITP were treated with corticosteroids and thrombopoietin-receptor-agonists. This is in in line with the successful conservative management of most

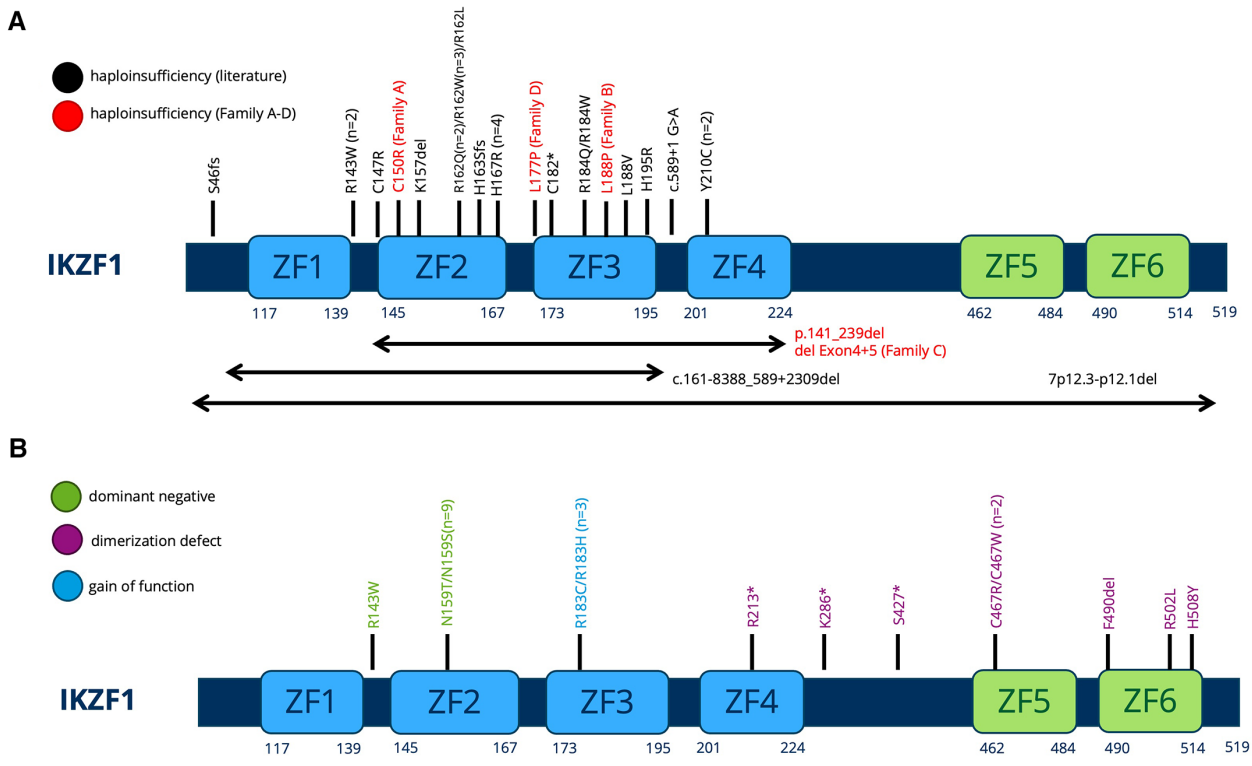

FIGURE 5
(A,B) Schematic of the IKAROS protein with indicated locations of the mutations of all patients previously described. The protein consists of 6 major Zinc finger (ZF) domains important for DNA binding and dimerization. (A) Variants leading to HI. Previously described variants are printed in black (PMID: 36433803). The families described in our manuscript are printed in red. (B) Dominant negative, Dimerization defect, Gain-of-function variants as previously described in PMID: 36433803.

IKAROS-HI patients in the literature: IgRT and prophylactic antibiotics for infection susceptibility, corticosteroids, high dose IgRT, rituximab for ITP and corticosteroids, immunosuppressants and anticoagulation for SLE and antiphospholipid syndrome (8, 12, 20). One of our patients received HSCT from his HLA-identical sister and shows persistent donor chimerism with cure from his IEI. This adds to the two previously reported patients with IKAROS-HI who underwent HSCT (8, 12, 20). As *IKZF1* is expressed in hematologic cells only, HSCT is a potentially curative treatment option. As in other IEI, HSCT outcomes depend on patient age and pre-transplant organ damage (21). Hence, the decision for or against HSCT should be discussed on a case-by-case basis, and be a shared decision between patients, families and treating physicians.

Alongside with the lifelong risk of severe infectious complications, one of the most decisive arguments in favour of early HSCT might be the risk of developing hematologic malignancies. *IKZF1* somatic variants are part of most oncological gene panels and a risk factor for poor outcome in hematological malignancies, mostly B-ALL (including pediatric B-cell precursor ALL) and entail more intense treatment protocols. Identification of somatic variants is relevant as these are of relevance in current protocols to guide tailoring of treatment intensity (22–24). *IKZF1* is located on chromosome 7p12.2, and comprises 8 exons. The most frequent somatic variations in B-ALL are deletions of one or more exons (25), whereas missense variants are rather rare (26, 27). Additionally, *IKZF1* copy-number variations have also been described especially in *BCR-ABL1*-positive ALL (25, 28).

Associations with hematologic malignancies are not restricted to somatic variants and have been reported in 2009 for germline *IKZF1* variants and ALL (29). The association of these variants with hematologic malignancies was revealed through identification of IKAROS-deficient patients with CVID who also developed leukemia: the here reported variants were heterozygous, and the suspected mechanism of dominance was haploinsufficiency (12). An infant diagnosed with CID and a germline *IKZF1* variant (attributed to DN IKAROS deficiency) later developed T cell leukaemia at the age of 13. In her T-ALL blasts, an additional *NOTCH1* variant could be detected (30). This further illustrates hematological malignancies on the basis of germline *IKZF1* variants, and sometimes additional somatic variants identified in hematological progenitors. Of note, hematological malignancies were reported in patients carrying variants leading to either HI, DN or DD (Figure 1).

Genetic screening of pediatric ALL cohorts, has revealed an accumulation of *IKZF1* germline variants (missense, nonsense, frameshift) (31). This raises the question if germline genetic testing should be performed in all leukemia patients, especially when they report a history of severe infections,

hypogammaglobulinemia or other IKAROS-related symptoms, or in case of a positive family history (32, 33).

Pediatricians of any specialization should be vigilant when looking after patients with recurrent infections and signs of autoinflammation or autoimmunity, and/or malignancies. Taking a careful family history and initiating basic immunological workup is at the reach of most professional caretakers around the globe. In case of clinical manifestations, a combination of warning signs and/or abnormal immunophenotyping, suggestive of possible IEI genetic testing should be initiated without delay, in order not to miss variants in genes such as *IKZF1*. This may influence prophylactic and potentially curative treatment decisions like HSCT. Additionally, the risk to develop malignant disease has an impact on medical management. With this report we have provided further insight into the genotype-phenotype-correlation of IKAROS-HI and its astonishing clinical variability and lifethreatening complications, as in one individual of our cohort. The growing number of variants reported will help to broaden our understanding of gene dosage effects and understand additional mechanisms impairing IKAROS function.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

Ethical approval was not required for the study involving human samples in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin. Written informed consent was obtained from the individual(s), and minor(s)' legal guardian/next of kin, for the publication of any potentially identifiable images or data included in this article.

Author contributions

TS: Conceptualization, Data curation, Visualization, Writing – original draft, Writing – review & editing, Investigation. JK: Conceptualization, Data curation, Visualization, Writing – original draft, Writing – review & editing, Investigation. HK: Data curation, Investigation, Methodology, Writing – review & editing. AG: Data curation, Writing – review & editing. FT: Writing – review & editing. KT-G: Writing – review & editing. AS: Methodology, Writing – review & editing. LP: Visualization, Writing – review & editing. RW: Writing – review & editing, Methodology. VW: Writing – review & editing. HB: Writing – review & editing. SR: Methodology, Writing – review & editing. MF: Writing – original draft, Writing – review & editing, Investigation. RK: Writing – original draft, Writing – review & editing, Investigation. CS: Conceptualization, Data curation, Project administration, Supervision, Writing – original draft, Writing – review & editing.

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Conflict of interest

Author AS and HB were employed by the company Labor Berlin Charité-Vivantes GmbH and author MF was employed by the company Hospital St. Georg GGmbH Leipzig.

The remaining 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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The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2024.1345730/full#supplementary-material

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Monogenic Inborn Errors of Immunity with impaired IgG response to polysaccharide antigens but normal IgG levels and normal IgG response to protein antigens

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In patients with severe and recurrent infections, minimal diagnostic workup to test for Inborn Errors of Immunity (IEI) includes a full blood count, IgG, IgA and IgM. Vaccine antibodies against tetanus toxoid are also frequently measured, whereas testing for anti-polysaccharide IgG antibodies and IgG subclasses is not routinely performed by primary care physicians. This basic approach may cause a significant delay in diagnosing monogenic IEI that can present with an impaired IgG response to polysaccharide antigens with or without IgG subclass deficiency at an early stage. Our article reviews genetically defined IEI, that may initially present with an impaired IgG response to polysaccharide antigens, but normal or only slightly decreased IgG levels and normal responses to protein or conjugate vaccine antigens. We summarize clinical, genetic, and immunological findings characteristic for these IEI. This review may help clinicians to identify patients that require extended immunologic and genetic evaluations despite unremarkable basic immunologic findings. We recommend the inclusion of anti-polysaccharide IgG antibodies as part of the initial routine work-up for possible IEI.

KEYWORDS

IEI, primary immunodeficiency, polysaccharide, vaccines, pneumococcal infections, vaccination

Abbreviations

ACTH, adrenocorticotropic hormone; AFP, alpha-fetoprotein; APDS, activated phosphoinositide 3-kinase δ syndrome; A-T, ataxia telangiectasia; BTK, Bruton's tyrosine kinase; CVID, common variable immunodeficiency; DAVID, deficit in anterior pituitary function and variable immune deficiency; DGS, DiGeorge syndrome; ED, ectodermal dysplasia; HLH, hemophagocytic lymphohistiocytosis; HSCT, hematopoietic stem cell transplantation; ID, immunodeficiency; IEI, Inborn Errors of Immunity; IgRT, immunoglobulin replacement therapy; ITP, immune thrombocytopenia; LIG4, DNA Ligase 4; LPS, lipopolysaccharides; NEMO, nuclear factor-kappa B essential modulator; NBS, Nijmegen Breakage syndrome; NGS, next generation sequencing; NFKB, nuclear factor KappaB; pDGS, partial DiGeorge syndrome; PHA, phytohemagglutinin; PS, polysaccharide; SAD, specific antibody deficiency; SCID, severe combined immunodeficiency; VUS, variant of unknown significance; WAS, Wiskott-Aldrich syndrome; XLA, X-linked agammaglobulinemia.

Introduction

Inborn Errors of Immunity (IEI), formerly termed primary immunodeficiency diseases (PID), are a heterogeneous group of mainly monogenic diseases characterized by infections and/or immune dysregulation. The absence or impaired function of any immunologic component can determine the clinical presentation of an IEI. The heterogeneity of these diseases makes it difficult to direct routine laboratory work up (1, 2). When IEI with antibody deficiency are suspected (e.g., in patients with recurrent infections of the airways, the ears, the meninges, or the skin), the basic immunologic evaluation recommended by the German AWMF guideline (dating back to 2011), comprises a full blood count and measurement of IgG, IgA, and IgM levels (3). Once B-cell defects are suspected and serum immunoglobulins are detectable, evaluation of the IgG response to protein as well as polysaccharide antigens is recommended (4). To date, most primary care physicians do not routinely determine anti-polysaccharide (e.g., anti-pneumococcal) IgG/IgG2 antibodies or IgG subclasses when an IEI with antibody deficiency is suspected. A growing number of monogenic IEI has been reported, which may initially present with an impaired IgG response to polysaccharide antigens, whereas total IgG levels and response to protein (e.g., anti-tetanus toxoid IgG antibodies) and polysaccharide conjugate vaccines may be normal. Since polysaccharide-specific IgG antibodies fall within the IgG2 subclass fraction, IgG2 levels may also be decreased in these patients (5–7). Without the determination of anti-polysaccharide IgG antibodies and IgG subclasses, the diagnosis of several rare IEI may be missed or delayed. Delayed therapeutic interventions may then result in further infections and irreversible organ damage (e.g., bronchiectasis).

Patients with an impaired IgG response to polysaccharide antigens often suffer from recurrent or severe sinopulmonary infections with encapsulated bacteria that express abundant polysaccharide antigens on their surfaces e.g., *Streptococcus (S.) pneumoniae, Haemophilus (H.) influenzae* serotype b, and *Neisseria (N.) meningitidis* (6, 8). Severe invasive infections like meningitis, septicemia, or osteoarticular infections can also occur (7, 9, 10). An impaired IgG response to polysaccharide antigens can be diagnosed if wild-type infections with *S. pneumoniae* or vaccination with a pure polysaccharide vaccine (e.g., Pneumovax™) do not result in a significant increase of pneumococcal-specific IgG antibodies (11–13). Commercially available tests to assess IgM and IgA pneumococcal polysaccharide specific antibodies before and after pneumococcal polysaccharide based vaccinations are not routinely used but may be helpful in testing patients with IEI under IgG replacement therapy (14–16).

Humoral immune responses to proteins and polysaccharides

Based mainly on studies in mice, Figures 1, 2 illustrate the differences between immune responses against proteins and polysaccharides. Knowledge of these mechanisms is exploited for routine vaccination (17). The main immunological differences between the two mechanisms are summarized in Table 1. It explains the impaired IgG response to polysaccharide antigens described in some patients after polysaccharide vaccinations, usually with a 23-valent pneumococcal polysaccharide vaccine. In natural infections, for instance with pneumococci, protein and polysaccharide responses occur in parallel, in contrast to vaccine responses. The review by Gingerich and Mousa summarizes the structure of the bacteria and the targets of antibody formation (18).

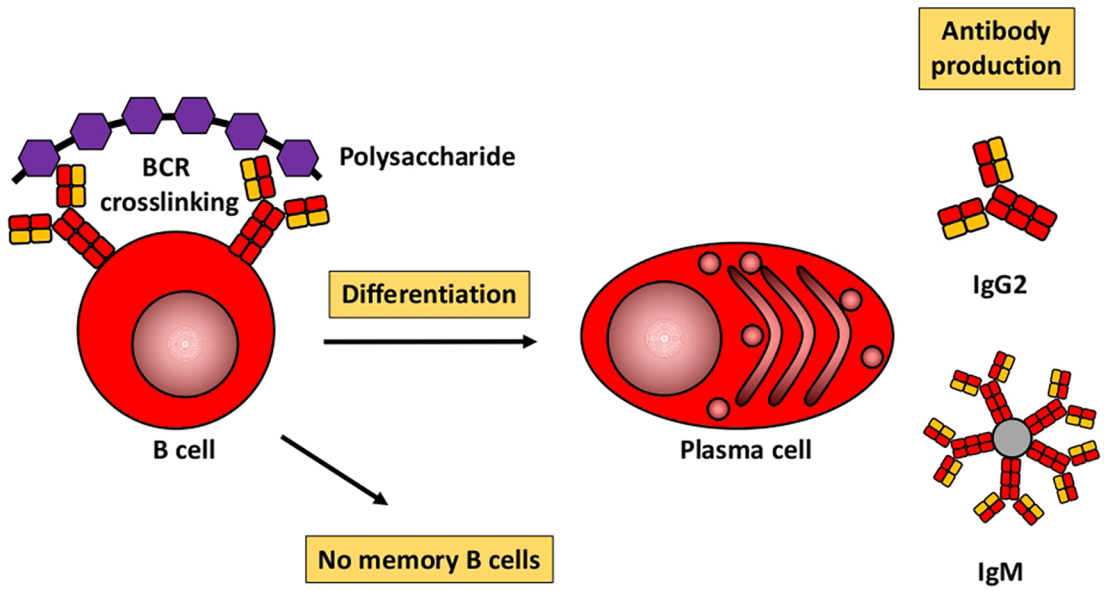

FIGURE 1
Humoral immune response to polysaccharides. Modified from Pollard et al. (17).

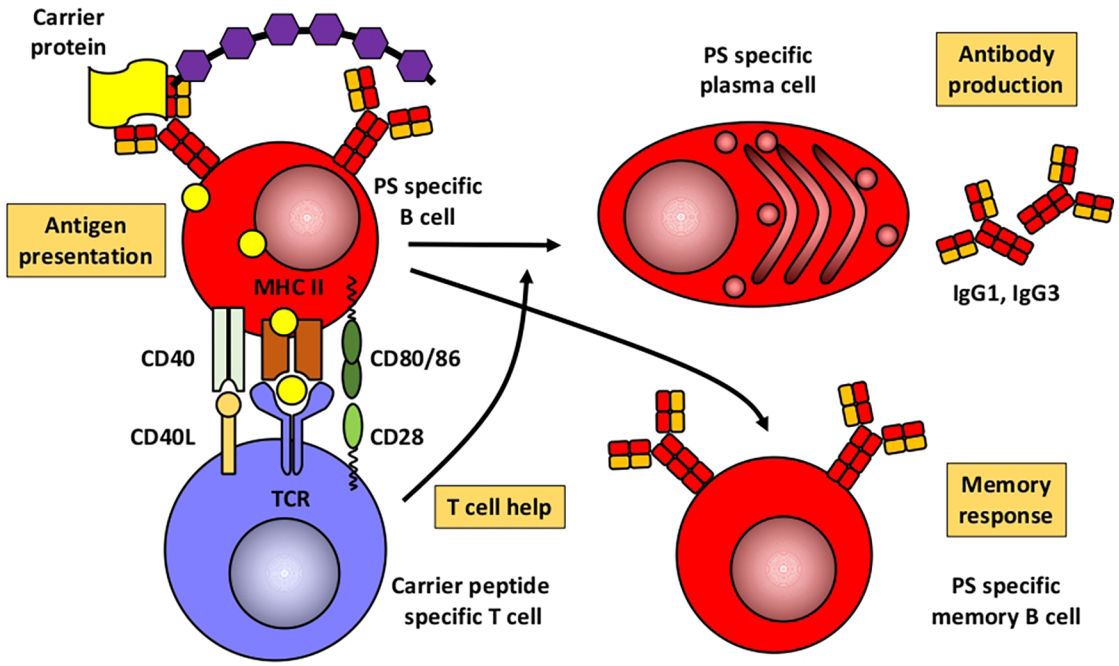

FIGURE 2
Humoral immune response to conjugated polysaccharides. Modified from Pollard et al. (17). PS, polysaccharide.

Recent literature has focused on the question as to which polysaccharides response is "normal", the missing standardization of measuring anti-polysaccharide antibodies, the differences in assessment specifically in conjugate-primed and non-primed individuals, excluding other underlying diseases, or the need to verify patient-reported respiratory tract infections in patients with an impaired response to polysaccharide antigens. This shows the importance of measuring anti-polysaccharide antibodies and evaluating an immune response to polysaccharide antigens, as it further enlightens immune response to certain microbes (19, 20). Over the last decade, next-generation sequencing (NGS) for detecting genetic variants in IEI has identified an ever-increasing number of genetic causes and led to more specific diagnoses (21). Some of the monogenic IEI lack anti-polysaccharide antibodies due to an impaired immune response to polysaccharide antigens. However, IgG in these IEI can be normal or slightly decreased and the response to protein (e.g., tetanus toxoid) or conjugate (e.g., polysaccharide-protein) vaccines can also be unimpaired. These IEI -presented below- mostly show other immunologic abnormalities in addition to an impaired IgG response to polysaccharide antigens. Our review is therefore not restricted to IEI with specific antibody deficiency (SAD), since SAD is strictly defined as an IEI with an impaired response to polysaccharide antigens as the only abnormal immunologic finding (13, 20, 22). Following these basic considerations, we will now discuss specific aspects of respective monogenic IEI. Common clinical, genetic, and immunologic findings are also summarized in Table 2.

TABLE 1 From Pollard et al. (17).

Polysaccharides	Polysaccharide conjugates
Induction by BCR crosslinking	T cells required
T cells not required	Carrier protein presented by MHC II
B cells short lived	Long-term immunity
No memory induced	Memory established
B cell memory pool depleted	New memory B cells
Hyporesponsiveness?	Boosted upon reexposure
Predominantly IgM and IgG2	Predominantly IgG1 and IgG3
Low immunogenicity in young children	Immunogenicity in young children

BCR, B cell receptor; MHC, major histocompatibility complex.

Hypomorphic mutations in *BTK*

X-linked agammaglobulinemia (XLA; M. Bruton) was first described in 1952 (23). It is an IEI with an estimated incidence of 1:200,000–700,000 live births. More than 600 pathogenic variants within *BTK* (encoding Bruton's Tyrosine Kinase) on chromosome Xq22.1 have been described to date (24). XLA patients have low or absent peripheral B cells and most patients have absent or very low IgG, IgA and IgM. Prior to IgG replacement therapy (IgRT) neutropenia is frequently observed (25–27). Without IgRT patients present with recurrent bacterial and viral infections beyond 3–6 months of age, once maternal IgG levels wane. Otitis media and pneumonia are the most frequent infections, severe invasive infections (meningitis, septic arthritis) as well as skin and gastrointestinal infections can also occur. Airway and invasive infections are mostly caused by *S. pneumoniae, H. influenzae* and *S. aureus. Pseudomonas* and *Helicobacter species* have been

TABLE 2 Monogenic IEI with impaired IgG response to polysaccharide antigens but normal IgG levels: Common clinical, genetic, and immunologic findings.

IEI[a]	Gene/ chromosome	Inheritance pattern	Incidence/ prevalence	Age at presentation	Clinical phenotype	Infections	Common pathogens	Immunological phenotype[b]
BTK/XLA	BTK; -Xq22.1	XL	1:200,000 – 700,000	6–18 months	Infections when maternal IgG wanes, absent/small tonsils, chronic lung disease, Crohn's like disease, arthritis, slight increased risk of malignancies	Respiratory, ENT, skin, CNS, osteoarticular, intestinal	*S. pneumoniae*, *H. influenzae*, *S. aureus*, *Helicobacter* spp. (skin), *Pseudomonas* spp., Enteroviruses (CNS), *Giardia lamblia*, *Pneumocystis jirovecii* (rarely)	Low/absent IgG, IgM, IgA, IgE, low/absent B cells, neutropenia (prior to IgRT)
Wiskott-Aldrich syndrome	*WAS;* Xp11.23	XL	1:50,000–250,000	Early childhood	Bleeding/bloody diarrhea, eczema, vasculitis, inflammatory bowel disease, IgA nephropathy, malignancies	Respiratory, ENT, meningitis, sepsis	*S. pneumoniae, H. influenzae, Neisseria meningitis* spp., HSV, *Molluscum contagiosum, Candida albicans, Pneumocystis jirovecii,*	Thrombocytopenia, low platelet volume, high IgA and IgE with normal IgG and/or decreased IgM, low isohemagglutinin levels, lymphopenia with low CD8+ T cells, increased proportion of effector memory T lymphocytes, normal T-cell proliferation upon PHA but reduced T-cell proliferation upon anti-CD3 stimulation, autoimmune cytopenias
NEMO deficiency	*IKBKG;* Xq28	XL	1:250,000	<1 year	Very variable, thickened skin, eczematous rashes, conical teeth, absent sweat glands, thin, sparse hair (often with unusual twirling), frontal bossing, failure to thrive, chronic diarrhea, autoimmune phenomena (AIHA, arthritis, inflammatory bowel disease-like colitis)	Respiratory, ENT, gastrointestinal, skin, soft tissues and bones, CNS	*S. pneumoniae, S. aureus, H. influenzae, P. aeruginosa,* Mycobacteria/BCG, CMV, HSV, ADV, HPV, *Pneumocystis jirovecii*	low IgG, low IgG2, low IL-10 response upon TNF-stimulation, NK cell abnormalities
pDGS	*TBX1*; 22q11.2	Mostly *de novo*, AD	1:4,000	Neonatal	Cardiac anomalies, hypoparathyreoidism, facial, palatal or ear nose throat dysmorphism, gastrointestinal anomalies, autoimmunity, allergy	Respiratory, ENT	*S. pneumoniae, S. aureus, H. influenzae, M. catarrhalis, P. aeruginosa, Respiratory viruses*	Low CD3+ T cells, low TRECs and naïve CD4 and CD8, low IgG, IgA, IgM, low switched and non-switched memory B cells
MECP2-Duplication	*MECP2*; Xq28	XL	1:150,000 (Australia)	Neonatal, infancy	Muscle hypotonia, mental retardation, impaired speech development, epilepsy, recurrent, severe pneumonia	Respiratory, sepsis	*S. pneumoniae, H. influenzae*	Low IgG2/IgG4, very high CrP during infections
NFKB1 deficiency	*NFKB1;* 4q24	AD, rarely AR	*1:10,000*	School age to adulthood	Bronchiectasis, interstitial lung disease gastrointestinal inflammation, chronic diarrhea, liver cirrhosis, hepatic hemangioma and hepatitis, skin disease, arthritis, thyroiditis, vasculitis, diabetes, Addison disease, lymphoproliferation with splenomegaly, lymphadenopathy and hepatomegaly, lymphomas and solid-organ cancer	Respiratory, ENT, gastrointestinal, skin	*H. influenzae, Streptococcus* spp. *M. catarrhalis, Pseudomonas* spp., *C. difficile, Salmonella* spp. *Campylobacter jejuni, E. coli, E. faecalis, S. epidermidis,* Influenza virus, RSV, rhinovirus, norovirus, rotavirus, adenovirus, EBV, CMV, John Cunningham virus *C. albicans, Aspergillus sp., P. jirovecii*	Low B cells, reduced switched memory B cells, expansion of CD21low B cells, low NK cells, low IgA, IgG and/or IgM, autoimmune cytopenias
NFKB2 deficiency	*NFKB2;* 10q24.32	AD	>55 patients described	School age to adulthood	Aspetic meningitis, opticus neuritis, lymphoproliferation, malignancy, asthma, ACTH deficiency, growth hormone deficiency, hypothyreoidism, prolactemia, trachyonychia, alopecia, eczema gastrointestinal disease, arthritis, lymphocytic organ infiltration (CNS, lung)	Respiratory, ENT, skin, eyes, CNS	*Salmonella* spp., EBV, CMV, *Toxoplasma gondii, Giardia lamblia*, *Candida* spp., *P. jirovecii*	Low marginal zone and switched memory B cells, total B cells can be normal, low IgG, IgA, IgM, autoantibodies (ANA, thyroperoxidase, anti-tyrosin-phosphatase, anti-Glutamat-Decarboxylase, anti-cytokine autoantibodies), autoimmune cytopenias

(*Continued*)

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TABLE 2 Continued

IEI[a]	Gene/ chromosome	Inheritance pattern	Incidence/ prevalence	Age at presentation	Clinical phenotype	Infections	Common pathogens	Immunological phenotype[b]
APDS	*PIK3CD;* 1p36.22 *PI3KR1;* 5q13.1	AD	<400 patients described	1–2 years	Lymphoproliferation, autoimmunity, malignancies, Infections, bronchiectasis	Mainly respiratory	*S. pneumoniae, H. influenzae, P. aeruginosa, S. aureus,* EBV, CMV, VZV, HSV, HPV, *Candida albicans*	High IgM, normal IgG/IgA, B-cell lymphopenia, increased transitional B cells, reduced memory B cells, CD4 lymphopenia (naïve CD4+ T cells)
CTLA4 deficiency	*CTLA4;* 2q33.2	AD	<200 patients described	School age	Lymphoproliferation, autoimmunity, malignancies, infections	Respiratory, gastrointestinal	*H. influenzae, S. pneumoniae, Salmonella enteridis, S. aureus,* EBV, CMV, HSV	Low IgG, T-cell lymphopenia (especially CD4+ T cells), B-cell lymphopenia (decreased B cell maturation), NK-lymphopenia
Ataxia–telangiectasia (A-T)	*ATM*; 11q22.3	AR	<1–9: 100,000	Early childhood	Progressive cerebellar ataxia, oculocutaneous telangiectasia, high incidence of malignancy (leukemia/ lymphoma), endocrine abnormalities, immune dysregulation, autoimmunity, granulomas	Respiratory, invasive infections, warts, granulomas due to vaccine-strain rubella virus	*H. influenzae, H. parainfluenza, S. aureus, S. pneumoniae, E. coli, S. viridians, P. aeruginosa, C. albicans,* RSV, VZV, EBV, HHV6, Rubella vaccine virus	Increased AFP level, defects in T cell receptor (TCR) and B cell receptor (BCR) rearrangement causing (T and/ or B cell) lymphopenia; (specific) antibody deficiency, 10%–20% hyper-IgM phenotype
Nijmegen breakage syndrome (NBS)	*NBS1;* 8q21-24	AR	1:100,000	Neonatal	Syndromic features: progressive microcephaly, prominent midface, sloping forehead, retrognathia, prominent nasal bridge and nose, large ears, café au lait spots, clino- and/or syndactyly, developmental delay, increased risk of malignancies (lymphomas and solid tumors), recurrent sinopulmonary infections females: primary ovarian failure	Respiratory, skin	*S. pneumoniae, H. influenzae,* Mycobacteria, recurrent HSV, severe or chronic EBV, CMV, HBV, HCV recurrent VZV, Rubella vaccine virus, *C. albicans*	(Combined) immunodeficiency—variable
DNA Ligase-4 syndrome	*LIG4*; 13q33.3	AR	>50 patients described	Neonatal	Microcephaly, facial features (resembling NBS), short stature, developmental delay, malignancy (lymphoma/ leukemia)	Respiratory, ENT, diarrhea	*S. pneumoniae, H. influenzae, Salmonella* spp., *Acinetobacter* spp., *C. albicans*, EBV, CMV, *Parainfluenza virus*	(Combined) Immunodeficiency, pancytopenia, myelodysplastic syndrome
Hyper IgE Syndrome (*STAT3*)	*STAT3;* 17q21.2	AD	1:11,000–1:100, 000	Neonatal, infancy	Severe eczema, bone brittleness (fractures), retention of decidual and delayed dentition of permanent teeth, scoliosis, coarse face, newborns: "prolonged exanthema toxicum infantum", adults: aortic aneurysm, increased risk for lymphoma	Skin infections/ abscesses, otitis, pneumonia	*S. aureus* *S. pneumoniae* *C. albicans*	IgE elevated, Eosinophils elevated, low IgG2, low memory B cells, low follicular helper cells

ACTH, adrenocorticotropic hormone; AD, autosomal dominant; AFP, Alpha Feto Protein; AIHA, autoimmune hemolytic anemia; ANA, antinuclear antibodies; AR, autosomal recessive; BCG, Bacille Calmette-Guérin, C. albicans, Candida albicans; CMV, cytomegaly virus; CNS, central nervous system; EBV, epstein barr virus; ENT, ear nose and throat; E. coli, Escherichia coli; E. faecalis, Enterococcus faecalis; ENT, ear nose and throat; HBV, Hepatitis B virus; HCV, Hepatitis B virus; HHV6, human herpes virus 6; HSV, Herpes simplex virus; H. influenzae, Haemophilus influenzae; NBS, Nijmegen breakage syndrome; RSV, respiratory syncytial virus; S. aureus, Staphylococcus aureus; sp., species; S. pneumoniae, Streptococcus pneumoniae; S. viridans, Streptococcus viridans; VZV, Varizella zoster virus; XL, X-linked.

[a]For references see text.

[b]In addition to an impaired IgG response to polysaccharide antigens.

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reported in chronic skin infections (28, 29). XLA patients are also susceptible to enteroviruses (e.g., poliovirus, coxsackievirus, echovirus) (30), that should be suspected in XLA patients with meningoencephalitis. *Giardia lamblia* can cause chronic diarrhea. In older children and adult XLA patients, chronic lung disease and autoimmunity (arthritis, enteropathy) is frequently reported, despite adequate IgG levels under IgRT (31, 32). Case studies have described patients with hypomorphic mutations in *BTK* who have no or only slightly decreased IgG levels, respond well to tetanus-toxoid vaccinations, but cannot mount an IgG response to polysaccharide antigens (33, 34).

Wiskott-Aldrich syndrome

The X-linked Wiskott-Aldrich syndrome (WAS) was first described in 1937 (35), and further delineated by R. Aldrich in 1954 (36). Positional cloning in the 1990s identified mutations in the gene encoding the Wiskott-Aldrich syndrome protein (WASp) as the cause of the disease (37). WASp is a crucial regulator of various functions in hematopoietic and immune cells, including cytoskeletal reorganization, immune synapse formation, and intracellular signaling (38, 39). Genotype-phenotype correlations for more than 150 mutations are sparse. WAS has an estimated incidence of about 4 per million live male births and presents a wide spectrum of clinical phenotypes (40). The disease commonly manifests as a bleeding disorder coupled with increased susceptibility to infections (encapsulated pathogens, viruses, opportunistic pathogens), autoimmunity (e.g., eczema, autoimmune cytopenias, vasculitis, inflammatory bowel disease, IgA nephropathy), and an increased risk of hematological malignancies (41). Typically, both cellular and humoral immunity are affected. Common laboratory findings include thrombocytopenia with reduced platelet volume and lymphopenia, often due to T-cell loss, while B-cell counts usually remain stable. Platelet volume may normalize in patients post splenectomy, so normal platelet volume does not exclude WAS in patient post splenectomy (42). Decreased CD8+ T cell count and function, reduced natural killer (NK) cell cytotoxicity, and impaired regulatory T cell (Treg) cell function contribute to the immunodeficiency and autoimmunity (43). WASp gene mutations disrupt actin polymerization in WAS-deficient T cells, affecting T- and B-cell interactions that are crucial for memory cell development and isotype switching (44). As a result, immunoglobulin levels are often altered (typically elevated IgA and IgE, normal IgG, and decreased IgM), isohemagglutinin titers tend to be low, and the specific IgG antibody response to various antigens is often insufficient (e.g., pneumococcal polysaccharide and tetanus toxoid) (45).

Nuclear factor-kappa B essential modulator (NEMO) deficiency

NEMO deficiency syndrome is a rare, combined immunodeficiency that was concurrently elucidated in three seminal publications in the early 2000s (46–48). It is caused by hypomorphic mutations in the X-linked *IKBKG* (also known as inhibitor of nuclear factor kappa-B kinase subunit gamma, NEMO) gene. Genetic analysis is complicated through homologous pseudogene sequences. NEMO is a key player in the NF-κB pathway, consequently, crucial for the development and function of the ectoderm, the immune system, and bones. The prevalence of NEMO deficiency is about 1:250,000. The phenotype spectrum in NEMO deficiency is exceedingly broad. 80% of patients exhibit ectodermal dysplasia (EDA) characterized by thickened skin, eczema, conical teeth, absent sweat glands, and hypotrichosis (49). Immune deficiency in NEMO patients renders them susceptible to recurrent respiratory tract infections, encapsulated bacteria, severe viral (CMV) and opportunistic infections (pneumocystis, environmental mycobacteria) (50, 51). Typically, patients present with severe pneumococcal infections despite vaccination, early episodes of meningitis, or deep tissue infections (51). Besides EDA and immune deficiency, a subset of patients also suffers from osteopetrosis, lymphedema, and/or autoimmunity. Characteristic laboratory findings are normal or low IgG (especially low IgG2) levels with elevated or decreased IgM or IgA levels. Immune response to stimulation with LPS, IL-1ß and TNF may be decreased, and IgG response to pneumococcal polysaccharide vaccination is in most cases insufficient. Class-switched B cells tend to be reduced, and/or NK cell activity abnormal (49–54).

DiGeorge syndrome/22q11.2 deletion syndrome

Neonatal co-occurrence of thymic aplasia and hypoparathyroidism was initially described by DiGeorge in 1965 (55) and later called DiGeorge syndrome (DGS). The 22q11.2 microdeletion is both the most common cause of DGS and the most common human chromosomal microdeletion syndrome in general, with an incidence of 1:4000 live births. Over 90% of the cases are *de novo* mutations causing haploinsufficiency of *TBX1*, a transcription factor involved in patterning of the third pharyngeal pouch. The genotype-phenotype correlation is weak and there is great clinical variability. While less than 1% of DGS patients are athymic (complete DGS), the majority presents with less severe thymic hypofunction (partial DGS, pDGS). pDGS is defined as <1,500/mm^3 CD3+ T cells and at least one of the following: (1) cardiac malformation, which is the main cause of death, (2) hypocalcemia, or (3) facial or palatal dysmorphism. Gastrointestinal anomalies, autoimmunity, mostly cytopenias and thyroid disease, and allergic manifestations are frequent. Patients present with recurrent infections including sinusitis, otitis media, bronchitis, and pneumonia (56). T-cell lymphopenia of varying severity is the most common feature, though 20% of patients have normal T-cell counts (57). Over time, T cells meet levels of age-matched controls due to homeostatic expansion, resulting in decreased T-cell receptor repertoire (56, 58). Lymphoproliferation upon stimulation with mitogens is mostly adequate. Delayed B-cell maturation, reduced numbers of naïve and unswitched memory B cells and peripheral class switched memory B cells, as well as hypogammaglobulinemia and isolated IgA or IgM deficiency may

occur (58). Impaired IgG response to polysaccharide antigens was reported in 40 to 55% of patients, most likely due to impaired assistance of thymus-derived T cells to the humoral immune response (56, 59).

Methyl-CpG-Binding protein 2 (MECP2) duplication syndrome

MECP2 duplication syndrome is an X-linked disorder first described in 2005 (60, 61). For a recent review, see (62). In Australia, incidence is estimated at 1: 150,000 live births, respective estimates from Europe are not available. More than 500 patients have been reported to date (reviewed in (62). Penetrance is 100%, affected males present with neonatal muscular hypotonia, severe intellectual disabilities, recurrent and frequent seizures, low sensitivity to pain, as well as severe infections of the lower airways. During infections, hyperinflammation with very high levels of inflammatory markers [e.g., C-reactive protein (CrP)] can be observed. The majority of affected patients die from severe pneumonia in childhood or as young adults. Most patients have low IgG2 and IgG4 subclasses and an impaired response to polysaccharide antigens (60, 63–71).

Nuclear factor-kappa B1 (NFKB1) deficiency

NFKB deficiency is an IEI with immune dysregulation. *NFKB1* loss-of-function or hypomorphic variants, first reported in 2015 (72), are inherited autosomal dominant resulting in haploinsufficiency (73). Recently, in 2021, the first autosomal recessive variant leading to NFKB1 deficiency was identified (74). NFKB1 deficiency affects up to 1 in 10,000 individuals. Three quarters of the patients fulfill the criteria of CVID. NFKB1 deficiency accounts for 4 to 5% of genetically resolved CVID cases, thus representing the most common monogenetic etiology of CVID (73, 75). Incomplete penetrance and age-depended disease progression were reported (73). Patients present with respiratory, gastrointestinal, skin, and opportunistic infections, mostly bacterial. Lymphoproliferation and autoimmunity, particularly cytopenias, are characteristic. There is a high frequency of multiorgan autoinflammation, noninfectious enteropathy, hepatopathy, and malignancy. Hypogammaglobulinemia and low IgA and/or IgM are common findings. A relative increase of $CD21^{low}$ B cells was associated with autoimmune and lymphoproliferative phenotypes. CD4 T-cell defects and a T_H1 and proinflammatory cytokine predominance are possible. Decreased numbers of circulating NK cells are frequent (75). An impaired IgG response to polysaccharide antigens was described in 53%–65% of patients with NFKB1 deficiency. NF-κB regulates the expression of activation-induced cytidine deaminase, an enzyme mediating class switch recombination, thereby possibly impairing humoral immunity if mutated (76, 77).

NFKB2 deficiency

In 2013, the first patients with *NFKB2* mutations were reported with a phenotype of CVID and adrenocorticotropic hormone (ACTH) insufficiency, a syndrome called DAVID (deficient anterior pituitary with CVID) (78). *NFKB2* encodes the precursor p100 and is located at 10q24.32. The reported damaging mutations follow an autosomal dominant pattern. To date, 27 cases of DAVID syndrome and a total of at least 50 cases of CVID with and without DAVID syndrome with confirmed pathological variants in the *NFKB2* gene have been published (79). Clinical expressivity and penetrance of *NFKB2*-related diseases are heterogeneous. Of note, inflammatory, autoimmune, and malignant manifestations often go beyond the common spectrum seen in CVID (79). A key feature, often preceding other symptoms, is autoimmune alopecia. Patients may also present initially with respiratory infections, diarrhea, and arthritis. Over time, about 40% of mutation carriers develop ACTH deficiency, sometimes accompanied by growth hormone and thyroid stimulating hormone deficiencies (80). Recurrent and severe infections with Herpesviridae, candida, and opportunists such as *Pneumocysits (P.) jivorecii* were reported. Most cases present with hypogammaglobulinemia and reduced marginal zone and switched memory B cells. Total B-cell, T-cell and NK-cell counts are often unimpaired. An impaired IgG response to polysaccharide antigens is present in almost half of the patients. The NFKB2 pathway is indispensable for both B-cell maturation, survival and function and T follicular helper cell generation, explaining humoral immunodeficiency (79).

Activated phosphoinositide 3-kinase δ syndrome (APDS)

APDS is caused by either pathogenic gain-of-function variants in *PIK3CD* (APDS1) or loss-of-function variants in *PIK3R1* (APSD2) (81). First described in 2013, APDS results in hyperreactive mTOR signaling and subsequent immune dysregulation (82). Inheritance is typically autosomal dominant, with onset of symptoms in the second year of life. Patients suffer from sinopulmonary infections resulting in bronchiectasis, B-cell lymphoproliferation (non-malignant or malignant), and autoimmune/inflammatory conditions [e.g., autoimmune hemolytic anemia, enteropathy, and immune thrombocytopenia (ITP)]. They are at increased risk of lymphoma, hemophagocytic lymphohistiocytosis (HLH), enteropathy, and occasionally developmental delay. Typical pathogens causing bacterial infections include *S. pneumoniae, H. influenza, Pseudomonas (P.) aeruginosa and S. aureus.* Viral infections are commonly caused by Herpesviridae (EBV, CMV, VZV, HSV) or HPV, and fungal infections by *C. albicans.* The immunological phenotype in APDS is characterized by a diminished antibody response to polysaccharide antigen, elevated IgM, normal IgG and IgA, B-cell lymphopenia, increased transitional B cells, reduced

memory B cells, and CD4 lymphopenia, especially naïve CD4+ T cells (83). The CD4/CD8 ratio is decreased (81, 84, 85).

Cytotoxic T lymphocyte antigen 4 (CTLA4) deficiency

Heterozygous pathogenic variants in *CTLA4* were initially reported in 2014. They lead to CTLA4 deficiency through haploinsufficiency, inadequate dimerization, or insufficient effector binding (86). The dominant inheritance pattern is accompanied by incomplete penetrance. Since CTLA4 plays a vital role in Tregs function and suppression of T-cell response, its deficiency results in immune dysregulation. Symptom onset typically occurs around 11 years of age, with a highly variable presentation. The immunological phenotype of CTLA4 deficiency primarily exhibits hypogammaglobulinemia, with lack of anti-polysaccharide antibodies, accompanied by significant reductions in T-cell lymphopenia, particularly evident in CD4+ T cells. B-cell lymphopenia with impaired B-cell maturation, and reduced NK cells are also notable features. Clinical manifestations encompass lymphoproliferation, autoimmune conditions (predominantly cytopenia or gastrointestinal involvement), increased susceptibility to infections, particularly of the respiratory tract [viral (EBV, CMV, HSV), bacterial (*H. influenzae*, *S. pneumoniae*, *Salmonella enteritidis*, *S. aureus*) and fungal (Candida species, Aspergillus species)], as well as gastrointestinal and neurological features. Additionally, patients face an increased risk of malignancies such as lymphoma and gastric cancer, often associated with EBV (87).

Ataxia–telangiectasia (A-T)

Ataxia–telangiectasia (A-T; Louis-Bar Syndrome) was first described in 1941 (88) and further characterized by Boder and Segwick 1958 (89). A-T is caused by mutations in the gene *ATM* (Ataxia Telangiectasia, Mutated) on human chromosome 11 (11q22.3) (90). The estimated incidence ranges from 1:40,000 to 1:300,000 live births with higher incidences in countries with high rates of consanguinity (91, 92). Several hundred pathogenic and likely pathogenic variants as well as >>1,000 variants of unknown significance (VUS) have been documented in the large gene comprising 60 exons, without any hotspots for mutations (see https://www.LOVD.nl/ATM). ATM protein has a crucial role in repair of DNA double strand breaks caused by radiation, oxidative or other genotoxic stress (90, 93). A-T is inherited in an autosomal recessive order. In non-consanguineous families, most A-T patients are compound heterozygote (94). Clinical features can vary but progressive ataxia noted once children start walking and oculocutaneous telangiectasia (in patients ages 5 years and older) are most prominent findings. Frequent sinopulmonary infections due to immunodeficiency, malignancies (in 1/4 of patients) as well as chronic lung and liver disease, gonadal insufficiency/sterility and premature aging are also reported. Life expectancy is limited mostly due to lung disease and malignancies [for review see (95, 96)]. Approximately 95% of individuals with A-T have marked elevated serum alpha-fetoprotein (AFP) levels (97). Some degree of immunodeficiency is present in approximately 2/3 of A-T patients, with lymphopenia, decreased levels of IgG, IgA, IgM, IgE and/or IgG subclasses and/or an impaired IgG response following vaccinations or infections being observed (98, 99). A-T patients may also may present with an hyper-IgM phenotype (100). A hyper-IgM phenotype or IgA deficiency indicate a poor prognosis (101). Of note, approximately 50% of A-T patients are detected by the newborn screening for severe combined immunodeficiency (SCID) based on low numbers of naïve T cells (102). Immunological impairment in most A-T patients sustains over time, thus frequent reevaluations of immunologic parameters are mostly not indicated (103). Immune dysregulation and autoimmunity in A-T such as immune thrombocytopenia (ITP), arthritis, and vitiligo is also observed [reviewed in (95)]. Chronic granuloma caused by live rubella vaccine virus have been described predominantly in patients with A-T and other DNA repair disorders [reviewed in (104, 105)].

Nijmegen breakage syndrome (NBS)

NBS is a rare autosomal recessive disease that was first described by researchers at the University of Nijmegen (106, 107). NBS is caused by mutations in *NBN* (mapped to human chromosome 8q21.3) that encodes the protein Nibrin, also termed NBS1 or p95 (108, 109). Nibrin plays an important role in DNA damage response and DNA repair (110). The majority of patients affected by NBS are of Slavic origin due to a founder mutation which leads to protein truncation (109). Prevalence is estimated to approximate 1:100,000 live births (110, 111). The main features of NBS include microcephaly, usually since birth, typical facial appearance with prominent midface, sloping forehead, retrognathia, prominent nasal bridge and nose, large ears and mild growth retardation. Some patients may also have café au lait spots, clinodactyly and syndactyly. Psychomotor development may initially be normal or mildly impaired, but intellectual abilities often decline. Female NBS patients are characterized by primary ovarian failure. Patients may suffer from recurrent respiratory and urinary tract infections, gastroenterocolitis, autoimmune diseases, and have a pronounced predisposition to malignancy due to chromosomal instability and radiation hypersensitivity, with more than 40% of NBS patients developing a malignancy (mainly lymphomas) by the age of 20 years (111–113). Immunological abnormalities found in most patients but are highly variable: T-cell lymphopenia (CD4+ T cells and/or CD8+ T cells) and elevated number of NK cells have been reported. IgG and IgA deficiency can occur, an impaired IgG response to pneumococcal polysaccharide antigens is observed in the majority of patients (75%), often combined with decreased levels of IgG2 and IgG4 subclasses (114). In contrast to A-T, immunodeficiency in NBS patients may progress over time (111, 112). Despite confirmed immunodeficiency, some NBS-patients do not suffer from frequent infections and do not require prophylactic antibiotics or IgRT (111, 112, 114).

DNA ligase 4 (LIG4) syndrome

DNA Ligase IV (LIG4) syndrome, also known as DNA Ligase IV deficiency or Ligase 4 syndrome, is a very rare autosomal recessive disorder that also belongs to the group of hereditary diseases associated with defects in cellular responses to DNA damage. The syndrome results from pathogenic variants in the gene encoding DNA ligase IV (*LIG4*) that was mapped to chromosome 13q33.3. LIG4 syndrome is caused by homozygous or, more often, compound heterozygous hypomorphic mutations in the *LIG4* gene. A genotype–phenotype correlation is suspected for single truncating mutations (115). The presentation of LIG4 syndrome is very heterogenous. Some individuals carrying a deleterious variant were reported to be asymptomatic (116). Common clinical findings are microcephaly, severe growth retardation, developmental delay and dysmorphic facial features, chronic liver disease and malignancy predisposition due to pronounced radiosensitivity (117, 118). Patients with hip dysplasia and other skeletal malformations were also reported. Immunologic findings comprise low IgG, variable immunodeficiency, and pancytopenia. Manifestation early in life has been reported for some patients, who presented with the above-mentioned clinical complex as well as severe combined immunodeficiency, radiosensitivity, chronic liver disease, and progressive bone marrow failure. Immunological findings include hypogammaglobulinemia and very low B cells with primarily IgG deficiencies causing sinopulmonary infections. Hypogammaglobulinemia can be accompanied by a decrease in IgA and IgM levels as well as T-cell abnormalities (117, 118). Patients with distal truncating pathogenic variants typically have a milder phenotype and may present later in life with hematological neoplasia with poor response to chemo- and radiotherapy (116, 117). Live expectancy is limited due to hematologic neoplasia, bone marrow failure, and chronic liver disease (117, 118).

Autosomal dominant hyper IgE syndrome (HIES) caused by dominant negative mutations in *STAT3*

First described as "Job's Syndrome", the term "Hyper-IgE Syndrome" (HIES) was later introduced because of high IgE-levels (119, 120). Several genetic variants associated with Hyper-IgE have been identified. Autosomal dominant HIES (AD-HIES) is characterized by (1) pulmonary infections, mainly due to *S. aureus* and *S. pneumoniae*, (2) bone brittleness and retention of decidual and delayed dentition of permanent teeth, (3) severe eczematous dermatitis and skin infections with *S. aureus* and *Candida* spp. (121). The identification of heterozygous, almost exclusively dominant-negative mutations in the gene that encodes the Signal transducer and activator of transcription 3 (*STAT3*), as genetic cause for AD-HIES explains the syndromic nature of HIES, affecting not only the immune system, but multiple further tissues (122). The reported prevalence among populations varies between 1 and 9: 100,000. (https://www.orpha.net/).

As in atopic dermatitis, the predominant immunological feature of AD-HIES is marked hypereosinophilia and elevated IgE (121, 123). It was shown early and repeatedly thereafter that IgG-responses against pneumococci and levels of IgG subclasses may be low, too (124–127). Thus, in patients with severe eczema normal levels of IgG subclasses and normal responses against pneumococci do not exclude AD-HIES, but low levels of IgG subclasses and/or impaired responses against polysaccharides are compatible with AD-HIES. Low IgG subclasses and/ or impaired humoral responses against pneumococci are probably caused by combined effects of less follicular helper T cells (producers of IL-21), impaired IL-6 signalling (and consequently low IL-21 production) and impaired responses to IL-21, that finally lead to less class-switched memory B cells in many patients with HIES caused by dominant negative mutations in *STAT3* (128–134). IgG2 subclass deficiency was described very early in AD-STAT3-HIES (125) and may contribute to the increased susceptibility to pneumococcal infections.

Other rare IEI with an impaired IgG response against polysaccharide antigens

Other rare IEI may present with an impaired IgG response to polysaccharide antigens as a predominant finding (135), e.g., Syk (136) and MAGT1-deficiency (137); but larger case series or cohort studies are lacking.

Discussion

Severe or recurrent infections with encapsulated bacteria (e.g., *S. pneumoniae, H. influenzae, N. meningitis species*) should prompt clinicians to evaluate patients for IEI, in particular when affected patients had been vaccinated against respective bacterial antigens. Apart from antibody deficiency, differential diagnostic considerations should include complement deficiency, asplenia or splenic impairment as in sickle cell disease, and defects in innate immunity such as Toll-like/IL1R defects e.g., IRAK4/MyD88 deficiency (12, 13). The majority of patients with recurrent or severe infections due to antibody deficiency (e.g., CVID, XLA) will be detected with the basic immunologic tests recommended by German AWMF guidelines (3). However, IgG, IgA, IgM, and even IgG responses to protein-based vaccines may (initially) be normal in patients with monogenic IEI with an impaired response to polysaccharide antigens. Thus, we would like to challenge current German guidelines because these IEI may be missed or the diagnosis delayed. An impaired response to polysaccharide antigens can be diagnosed once polysaccharide-specific antibodies do not increase following polysaccharide-based vaccinations or wild-type infections. In wild-type infections with *S. pneumoniae* protein as well as polysaccharide-specific antibodies to respective strains should be detectable in high/ normal concentrations. Thus, low or absent pneumococcal

specific IgG after microbiology-proven pneumococcal infection should prompt for further immunological and genetic workup. Commercially available tests for polysaccharide-specific total and IgG2 antibodies are mostly based on pneumococcal antigens. Patients with recurrent or severe bacterial infections and low specific pneumococcal antibodies should receive a "diagnostic" polysaccharide-based vaccination (e.g., Pneumomvax^{TM}) followed by measurement of the IgG vaccine response ≥4 weeks. Unfortunately, various serological and opsonophagocytic assays exist with limited comparability of the results. An international consensus for a standardized approach would be highly desirable. Determination of IgG subclasses may also be helpful, since polysaccharide-specific IgG antibodies are found within the IgG2 subclass fraction, thus IgG2 levels are often below age dependent normal ranges in patients with an impaired response to polysaccharide antigens (138). Of note, children below the age of 2 years respond poorly to polysaccharide antigens, therefore (diagnostic) vaccination with Pneumovax^{TM} is not approved in children <2 years of age. Also, IgG2 and IgG4 levels are frequently low in infancy and are likely increase to normal levels within the first two years of live (138, 139). Diagnosing an impaired IgG response to polysaccharide antigens is therefore more challenging in children younger than 2 years than in older children and adults.

In this review, we listed respective IEI to be considered once an impaired IgG response to polysaccharide antigens has been diagnosed. The clinical characteristics shown in Table 2 imply that

(a) syndromic aspects as in DGS, *MECP2*-Duplication syndrome, A-T, NBS and Lig4-Deficiency,
(b) male gender (XLA, WAS, *MECP2* Duplication syndrome),
(c) a positive family history for infections or autoimmunity, and
(d) consanguinity

in the context of recurrent infections should alert physicians to perform immunologic (and genetic) testing beyond basic evaluations for IEI. In most listed IEI, phenotypic presentations can vary significantly within a pedigree, thus a selectively impaired response to polysaccharide antigens may be present in relatives from patients with more pronounced immunologic findings.

Genetic testing has become more available and less expensive over the last years. Therefore, Whole Exome or Whole Genome Sequencing (WES, WGS) has become a standard diagnostic procedure when IEI is suspected based on clinical and/or immunological findings in high income countries (140). We assume that a growing number of patients with an impaired IgG response to polysaccharide antigens or strictly defined specific antibody deficiency (SAD, see below) will show pathogenic variants in one of the listed genes. Family members of patients with respective IEI should be offered genetic testing to allow close follow-up and early therapeutic interventions when pathogenic variants are identified. Genetic diagnosis of IEI in young children also permits genetic counseling of parents with a desire to have more children.

Delayed diagnosis of IEI with antibody deficiency is common (141), often patients show permanent organ damage due to recurrent sinopulmonary infections when diagnosed: hearing impairment following recurrent otitis, sinus surgery in patients with chronic or recurrent rhinosinusitis, bronchiectasis/chronic lung disease due to recurrent pneumonia (142).

Early diagnosis and treatment of IEI with an impaired IgG response to polysaccharide antigens listed in this review cannot only prevent further infections and organ damage, but can also prevent specific short and long term complications and risks in specific IEI [i.e., bleeding in WAS, risk of malignancy due to exposure to ionizing radiation as well as risk of rubella-induced granuloma following live-rubella vaccination in IEI with DNA repair disorders (A-T, NBS, LIG4 deficiency)] (104, 143).

Treatment of patients with an impaired IgG response to polysaccharide antigens depends on clinical symptoms, however, patients with recurrent bacterial infections will benefit from antibiotic treatment/prophylaxis and/or IgRT. Of note, in patients with normal IgG levels and an impaired IgG response to polysaccharide antigens, IgG dosing and intervals should not be guided by through levels of total IgG but by (a) the clinical response and (b) pneumococcal specific antibodies and/or IgG2 levels (if decreased prior IgRT). In patients with a normal IgG response to protein-based vaccines (tetanus toxoid), non-live protein-based vaccinations are advisable irrespective of IgRT when protective antibody titers cannot be guaranteed in IgG preparations (in particular: Meningococci A, B, C, W, Y). The add-on effect of such vaccinations, however, has not been scientifically evaluated.

Inhalant antibiotics may be an option in individual patients with recurrent pneumonia and bronchiectasis despite IgRT and systemic antibiotic prophylaxis. It has been successfully applied in a patient with *MECP2*-Duplication syndrome (144) as well as in CVID patients (145). However, there are no controlled trials on inhalant antibiotics in pediatric IEI patients.

HSCT has become an option for a growing number of patients with IEI, the outcome is generally better when patients are transplanted before chronic infections occur, thus, patients with a clear indication for a curative HSCT (e.g., patients with WAS) will benefit from early diagnosis (146). HSCT has been successfully performed in selected cases with DNA repair disorders, mainly because of malignancies (147, 148). Early diagnosis of a DNA repair disorder prior to HSCT enables adjustment of conditioning regimen and minimization of radiation exposure.

More specific treatment options in order to prevent disease progression, organ damage and/or to improve quality of life that are available for distinct IEI mentioned in the article: Recent clinical observations show impressive improvement of dermatitis and almost complete control of skin infections in patients with HIES on Dupilumab [subcutaneously (s.c.)]—an antibody that blocks the IL4-receptor alpha chain (149–155). Abatacept (s.c.), a CTLA4-immunoglobulin fusion drug, has shown excellent treatment results with a good tolerability and safety profile in patients with CTLA4 and LRBA deficiency (156, 157). This has also been shown for the orally available Leniolisib, a small molecule selective inhibitor of PI3Kδ, in patients with APDS (81, 84, 85). In A-T, antisense oligonucleotide treatment has shown amelioration of neurological disease progression in patients with selected *ATM* mutations (158, 159). This therapy has to be initiated at an early disease state, underscoring the importance of

timely diagnosis. Gene therapy may become an option for some of the listed IEI in the future (160, 161).

In this article, we purposely discussed patients that do not fulfill the strict criteria for SAD with a diminished antibody response to polysaccharide antigens following vaccination or wild-type infection as the only immunologic pathology (normal levels of IgG, IgA, IgM and IgG subclasses) (12, 13, 20, 22) for the following reasons: SAD is not a genetically distinct IEI. Indeed, genetic testing of patients with SAD may reveal mutations in various IEI genes described in this article. Also, IgG subclasses are not routinely tested in all countries, it is therefore unclear if patients with unknown IgG subclass levels do have strictly defined SAD. Furthermore, all patients with an impaired IgG response to polysaccharide antigens and frequent or severe bacterial infections will benefit from therapy, irrespective of additional immunologic abnormalities.

Lastly, this review underscores the importance of microbiology testing in patients with recurrent bacterial infections prior to antibiotic therapy. Once encapsulated bacteria are repeatedly detected, immunodeficiency with an impaired response to polysaccharide antigens may be suspected and diagnosed. Microbiology testing also helps to avoid non-effective antibiotic regimens due to resistances in patients that had multiple courses of antibiotics and select an effective prophylactic regimen if required.

Apart from monogenic IES listed in this manuscript, testing for polysaccharide specific antibodies is also helpful in patients with CVID and unclassified antibody deficiencies without an underlying genetic defect identified. Absent or low specific antibodies with no increase following a polysaccharide based vaccination should prompt clinicians to consider antibiotic prophylaxis or IgRT.

Conclusions

In patients with suspected IEI due to severe or recurrent bacterial infections who have normal IgG levels and a normal IgG response to protein-based vaccines, testing for polysaccharide-specific antibodies and IgG subclasses can help to identify rare monogenic IEI early, initiate treatment earlier and, thus, prevent morbidity, mortality, and organ damage. In our opinion, new guidelines on diagnostic measures in IEI should include the determination of specific IgG antibodies and IgG subclasses.

Author contributions

MF: Writing – original draft, Writing – review & editing. SD: Writing – original draft, Writing – review & editing. OS: Writing – original draft, Writing – review & editing. MV: Writing – original draft, Writing – review & editing. AS: Writing – original draft, Writing – review & editing. HvB: Writing – original draft, Writing – review & editing. VW: Conceptualization, Writing – original draft, Writing – review & editing. RK: Conceptualization, Writing – original draft, Writing – review & editing.

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Conflict of interest

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Clinical presentation, diagnosis, and treatment of chronic granulomatous disease

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Chronic granulomatous disease (CGD) is caused by an impaired respiratory burst reaction in phagocytes. CGD is an X-linked (XL) (caused by pathogenic variants in *CYBB*) or autosomal recessive inborn error of immunity (caused by pathogenic variants in *CYBA*, *NCF1*, *NCF2*, or *CYBC1*). Female carriers of XL-CGD and unfavorable lyonization may present with the partial or full picture of CGD. Patients with CGD are at increased risk for invasive bacterial and fungal infections of potentially any organ, but especially the lymph nodes, liver, and lungs. Pathogens most frequently isolated are *S. aureus* and *Aspergillus* spp. Autoinflammation is difficult to control with immunosuppression, and patients frequently remain dependent on steroids. To diagnose CGD, reactive oxygen intermediates (O_2^- or H_2O_2) generated by the NADPH oxidase in peripheral blood phagocytes are measured upon *in vitro* activation with either phorbol-12-myristate-13-acetate (PMA) and/or TLR4 ligands (*E. coli* or LPS). Conservative treatment requires strict hygienic conduct and adherence to antibiotic prophylaxis against bacteria and fungi, comprising cotrimoxazole and triazoles. The prognosis of patients treated conservatively is impaired: for the majority of patients, recurrent and/or persistent infections, autoinflammation, and failure to thrive remain lifelong challenges. In contrast, cellular therapies (allogeneic stem cell transplantation or gene therapy) can cure CGD. Optimal outcomes in cellular therapies are observed in individuals without ongoing infections or inflammation. Yet cellular therapies are the only curative option for patients with persistent fungal infections or autoinflammation.

KEYWORDS

chronic granolumatous disease, diagnosis, clinical presentation, HSCT, hematopoietic stem cell transplantation, therapy

Historical perspectives

The first clinical accounts of CGD date back to 1954 and 1957, when children with increased susceptibility to bacterial infections in the lungs, lymph nodes, and skin and granulomatous lesions were described. These patients showed pigmented lipid histiocytes in granulomas of visceral tissues and hypergammaglobulinemia in peripheral blood. The disease was named "fatal granulomatous disease of childhood," later changed to its current name, "chronic granulomatous disease" (CGD) (1–3). Antibiotic treatment and surgical drainage of abscesses improved life expectancy from less than 10 years to less than 20 years. Until the late 1960s, the presence of chronic granulomas

with typical lipid-laden histiocytes remained the only way to diagnose CGD. In 1967 Robert Baehner and David Nathan described how "intact leucocytes of two children with chronic granulomatous disease fail to reduce nitroblue tetrazolium (NBT) during phagocytosis." They also established this failure of phagocytes to reduce NBT as the first screening test for CGD (4, 5). At the same time, clinical observations discovered CGD not only in boys but also in girls, suggesting X-linked as well as autosomal recessive inheritance for CGD (6, 7). In the 1970s it became evident that phagocytes from patients with CGD lack a functional NADPH oxidase and that these phagocytes cannot form superoxide upon activation (8–10). Aspergillosis, BCGitis upon vaccination with BCG, and non-infectious (auto) inflammatory manifestations of CGD were clinically relevant issues that were first addressed 20 years after the initial descriptions of mainly bacterial infections (11, 12). Life expectancy improved further upon the introduction of antibiotic prophylaxis directed against bacteria with cotrimoxazole (13), and against aspergillosis with itraconazole (14, 15). Early observations indicated that CGD can be cured through allogeneic bone marrow transplantation. However, initial results were not considered sufficiently encouraging to offer this option upfront to every individual with CGD (16–18).

Molecular genetics of chronic granulomatous disease

Pathogenic variants in genes encoding subunits or regulatory proteins of the phagosomal NADPH oxidase complex cause CGD. The NADPH oxidase core complex consists of six proteins: the membrane-bound catalytic subunit gp91^{phox} (coded by *CYBB*), the membrane-bound activator p22^{phox} (coded by *CYBA*), the regulatory subunits p47^{phox} (coded by *NFC1*), p67^{phox} (coded by *NCF2*), p40^{phox} (coded by *NCF4*), and the GTPase RAC (in 96% of cases RAC2, RAC1 is also possible) (19). EROS (coded by *CYBC1*) is a chaperone protein for the dimerization of gp91^{phox} and p22^{phox} (20, 21). Upon activation, the phagosomal NADPH oxidase catalyzes the reaction of NADPH to form a cationic $NADP^+$, a proton (H^+), and two electrons. The electrons then enter the phagolysosome. There, oxygen molecules are reduced to two superoxide anions (O_2^-) ($NADPH + 2O_2 \Leftrightarrow NADP^+ + 2O_2^- + H^+$). A subsequent reaction catalyzed by a superoxide dismutase (SOD) [or alternatively myeloperoxidase (MPO)] produces superoxide peroxide (H_2O_2). The superoxide peroxide then either spontaneously decomposes into two superoxide ions (OH^-) or, in a second reaction catalyzed by MPO, combines with a chloride molecule to form hypochlorite (ClO^-). Both reactive oxygen species (ROS) not only lyse bacteria but also cause a pH-dependent influx of K^+, which subsequently activates proteases, crucial for the destruction of bacteria (Figure 1) (22). X-linked pathogenic variants in *CYBB* or autosomal recessive pathogenic variants in *CYBA, NCF1, NCF2*, and *CYBC1* lead to absent or severely reduced production of superoxide in all phagocytes (neutrophils as well as monocytes, macrophages, and eosinophils) (23–25).

Due to a large deletion in the X chromosome, patients with the X-linked form of the disease may display a contiguous gene defect causing the McLeod phenotype in erythrocytes, as well as Duchenne muscular dystrophy or retinitis pigmentosa. Patients with the McLeod phenotype must not receive repeated transfusions with erythrocytes expressing K20 and XK1. Acanthocytosis of

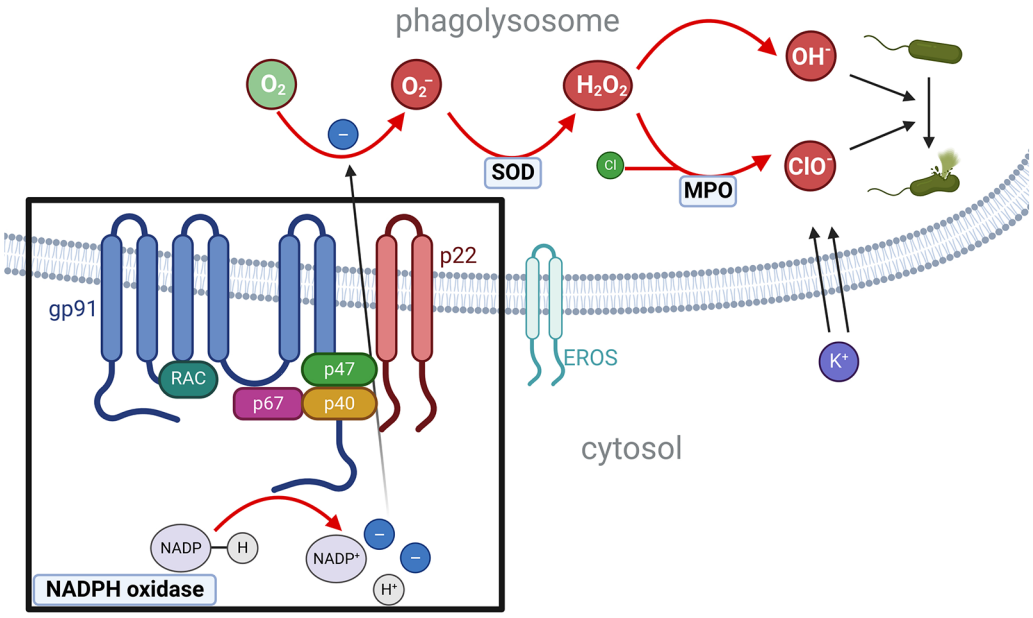

FIGURE 1
NADPH oxidase complex and the production of ROS.

erythrocytes in the peripheral blood may be indicative of the McLeod phenotype, but its absence does not rule it out (26–28).

ROS serve immunoregulatory functions in addition to their antimicrobial effect. The activation of ataxia-telangiectasia-mutated (ATM) kinase, for instance, requires NADPH oxidase (29). NADPH-deficient phagocytes show autophagic dysfunction, increased production of IL-1β upon activation with LPS, and an altered interferon signature. CGD patients show impaired apoptosis of peripheral blood neutrophils, yet not of monocytes (30–33).

$P40^{phox}$ deficiency, caused by autosomal recessive pathogenic variants in *NCF4*, is a similar but distinct disease. Neutrophils with $p40^{phox}$ deficiency exhibit impaired burst activity, while macrophages are less affected and demonstrate a greater capacity for producing reactive oxygen intermediates than in patients with bona fide CGD. As a result, patients with $p40^{phox}$ deficiency exhibit a far milder clinical phenotype. To date, no patients with $p40^{phox}$ deficiency have been described as suffering from invasive bacterial and fungal infections (34–36). Dominant negative mutations in *RAC2* result in a phenotype resembling leukocyte adhesion deficiency with an impaired oxidative burst, as RAC2 is not only crucial for NADPH oxidase but also controls cytoskeleton formation and cell adhesion (37).

Clinical presentation—infections and autoinflammation in CGD

Patients with CGD are at increased risk for invasive bacterial and fungal infections. Symptoms usually start in infancy, with a median age at diagnosis of CGD between 2.5 and 3 years. However, some patients are not identified until adolescence or adulthood (38–40). The infectious phenotype may differ significantly depending on local climate and antimicrobial resistance patterns. The most common pathogens are (from most to least common) *Staphylococcus aureus* (*S. aureus*), *Aspergillus* spp., *Burkholderia cepacia*, *Serratia* spp., *Nocardiae*, and *Salmonella* spp (38, 39, 41–45). Staphylococcal infections mostly affect the skin, lymph nodes, rectum, and brain. *Burkholderia cepacia* manifests in the lungs and can cause the life-threatening Cepacia syndrome, a condition similar to a cytokine storm in macrophage activation syndrome. *Serratia* and *Proteus* spp. cause liver abscess, and infections from *Nocardia* most often affect the lungs (38, 39, 41, 44–46). Molds, such as e.g., *Histoplasma* spp., *Phellinus* spp., *Rasamsonia* spp., *Rhizopus* spp., and *Trichosporon* spp., and, far less commonly, *Mucormucosis*, pose particular threats (47–52). Invasive mold infections mainly affect the lungs, brain, bones (as osteomyelitis), and nasal cavities (Figure 2) (53, 54). BCGitis is another common presenting symptom in regions where infants are routinely vaccinated with BCG (55, 56). Yet neither mycobacteria other than tuberculosis (MOTT) nor bona fide tuberculosis infections are common in CGD (38, 39, 45, 57, 58).

The eponymous granulomas of CGD are often found in the urinary tract, intestines, or lungs. They can lead to stenosis in hollow organs, or fibrosis. Granuloma formation can be seen as a mechanism by which the immune system contains infections, yet it is noteworthy that many granulomas found in patients with CGD are sterile and of autoinflammatory origin (59–61). About half the patients with CGD not only suffer from infections but also develop autoinflammation or immune dysregulation. Inflammatory bowel disease is the most common manifestation and may resemble Crohn's disease (62). Histology, however, may differ from Crohn's disease by pigmented lipid histiocytes, microgranulomas, and eosinophilic abscesses (63, 64). Further organs potentially affected by autoinflammation are the urinary tract/bladder and the lungs, but also sites like the brain, joints, and retina. Incident patients with CGD and systemic conditions resembling systemic lupus erythematosus, vasculitis, sarcoidosis, and thrombocytopenia were also described (59, 65–68) (Figure 3).

It is important to consider CGD in patients presenting with infections at any of the above sites. But in countries with endemic tuberculosis, the presence of difficult-to-treat or disseminated tuberculosis should prompt an evaluation for CGD (69). As Crohn's-like disease is common in CGD and may develop before the onset of invasive fungal or bacterial infections, CGD must be ruled out in any patient with Crohn's disease (64, 70–72). Similarly, CGD should be ruled out in patients with sterile granuloma formation (73–75).

Important differential diagnoses of CGD are autosomal dominant hyper-IgE syndrome caused by dominant negative mutations in *STAT3* ("DN-STAT3-HIES") and MyD88/IRAK4 deficiency. DN-STAT3-HIES is often associated with abscesses and pneumonia caused by *S. aureus*. Infections with *Aspergillus* spp. are much less common, and invasive aspergillosis is extremely rare in DN-STAT3-HIES, but aspergillomas may occur in the context of characteristic pneumatoceles, which are not typically seen in CGD (76). In contrast, chronic mucocutaneous candidiasis is common. In addition, DN-STAT3-HIES is associated with skeletal abnormalities and severe dermatitis, both of which are not features of CGD (77–79). On the other hand, patients with MyD88/IRAK4 deficiency often experience invasive infections with *Streptococcus pneumoniae*, *S. aureus*, and *Pseudomonas aeruginosa*, but fungal infections are rare (80–82). In contrast to patients with CGD, patients with DN-STAT3-HIES and MyD88/IRAK4 deficiency have impaired IL-6 signaling or production. This leads to cold abscesses in DN-STAT3-HIES and to low CrP despite invasive infections in MyD88/IRAK4 deficiency (77, 82–85).

Diagnostic workup and pitfalls

To diagnose CGD, ROS (O_2^- or H_2O_2) generated by the NADPH oxidase in phagocytes of peripheral blood are measured upon *in vitro* activation with either phorbol-12-myristate-13-acetate (PMA) and/ or TLR4-ligands (*E. coli* or LPS). We suggest that the assay be repeated at least once and that two different stimuli for the induction of the respiratory burst be used. ROS can be detected by different assays. O_2^- can be measured by chemiluminescence or the seminal NBT assay and H_2O_2 by the FACS-based DiHydroRhodamine (DHR) assay (Figure 4) (86, 87). While it was once considered the gold standard for the diagnosis of CGD, NBT has since been surpassed by the flow-

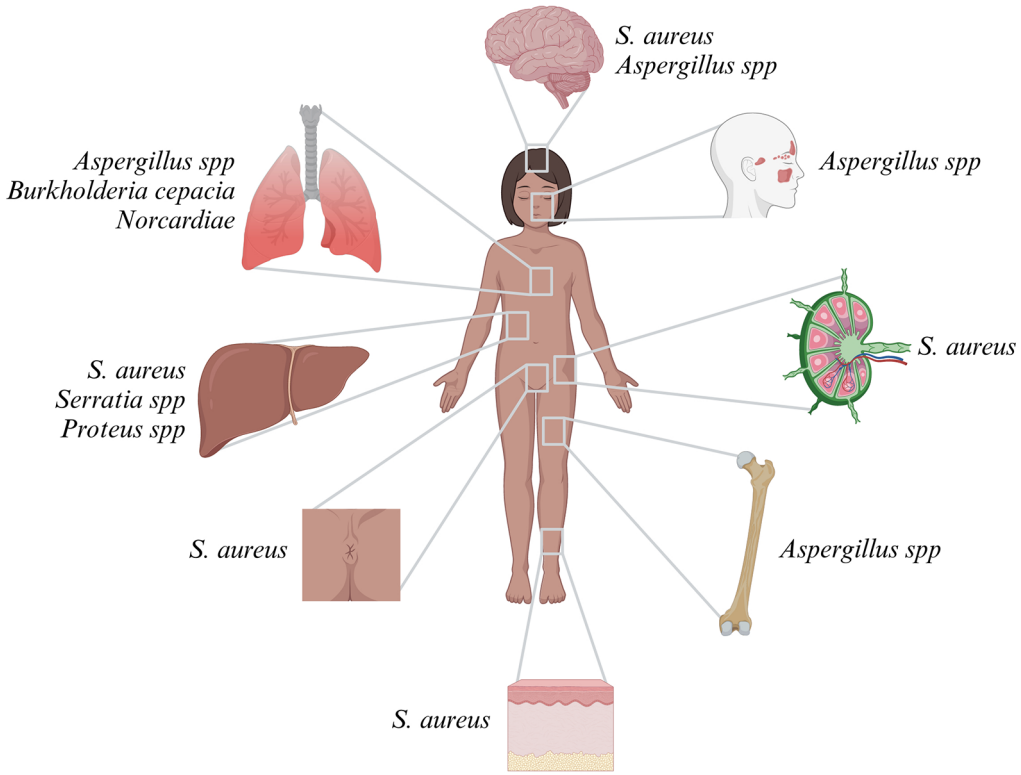

FIGURE 2
Infection sites and most common pathogens in CGD.

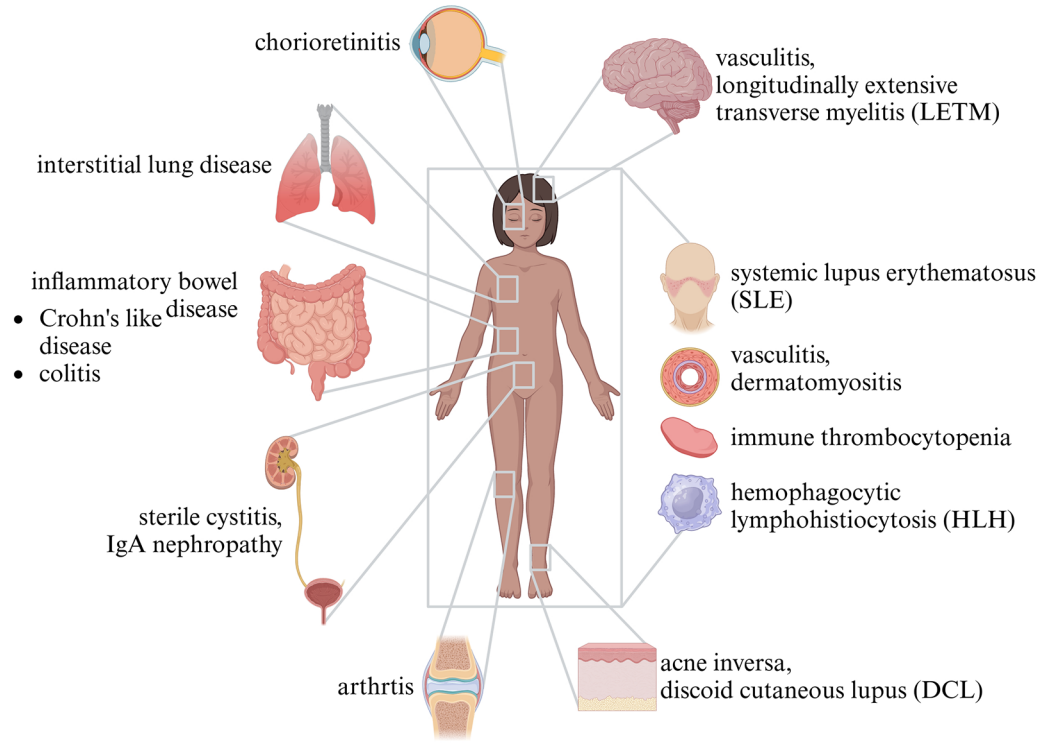

FIGURE 3
Autoinflammation in CGD.

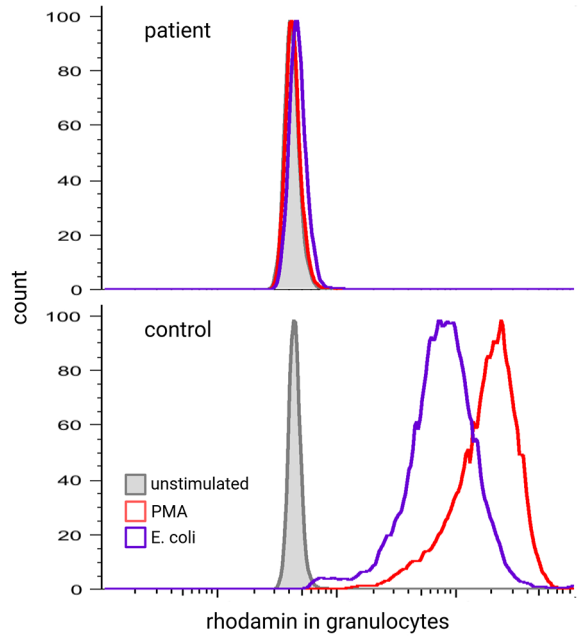

FIGURE 4
Respiratory burst of a healthy control and patient with CGD measured by DHR assay.

cytometry-based DHR assay in terms of time effectiveness, sensitivity, and quantification. However, the NBT assay remains a cost-effective and relatively easy-to-perform option, requiring only a microscope, a stimulant, and NBT. Especially in low-resource settings, the NBT assay is still a highly effective diagnostic tool (88–90).

Functional assays that measure the respiratory burst in phagocytes may be false positive in patients treated with paracetamol/acetaminophen, metamizole, or mesalazine (5-ASA) (91–93). Furthermore, CGD must be distinguished from MPO deficiency, which is not considered an inborn error of immunity. In CGD, the oxygen burst reaction is impaired in all phagocytes, whereas in MPO deficiency, the oxygen burst reaction is only impaired in neutrophils (94).

In patients with a repeatedly impaired respiratory burst, at least the seven genes *CYBA*, *CYBB, NCF1*, *NCF2*, *NCF4 CYBC1*, and *RAC2* should be analyzed for causative mutations.

Conservative vs. curative treatment of CGD

Upon diagnosis, all patients with CGD should receive conservative treatment with antibacterial and antifungal prophylaxis with cotrimoxazole and triazoles. Cotrimoxazole significantly reduces the incidence of bacterial infections while being relatively inexpensive (95). Itraconazole is still the most widely used agent to prevent invasive *Aspergillus* and other mold infections, but posaconazole may be a choice to consider depending on local resistance patterns (15). Female carriers with unfavorable lyonization of pathogenic variants in the X-linked *CYBB* may develop partial or complete clinical manifestation of CGD (38, 96–98). Given the documented occurrence of serious bacterial infections in patients with ROS below 10%, we propose considering prophylactic treatment against bacteria when ROS levels are below 20% and against molds when ROS levels are below 5% (38, 96, 98). We recommend regularly assessing the respiratory burst of carriers, with a suggested interval of 5 years (96).

Especially in North America, but less so in Europe, a significant number of patients are treated with IFN-γ (99). It has been shown to improve the splicing efficiency of *CYBB* (100). A recent meta-analysis showed a significant reduction in the likelihood of infection, but to date there is insufficient evidence of clinical improvement in patients with CGD on IFN-γ and data on long-term effects are lacking (101). The question of whether there is a general benefit of IFN-γ for all patients with CGD remains unanswered. Treatment is costly and not without side effects (mainly fever, but also mental impairment). In our opinion, IFN-γ can be considered in patients with particular pathogenic variants of *CYBB*.

Hygienic conduct includes avoiding exposure to molds. Patients with CGD should therefore abstain from doing agricultural work and gardening (including composting, mucking out stables, working in barns, etc.) as well as demolition of moist walls. Alternatively, patients must wear an FFP3 mask to prevent mold from entering their airways (102). We do not advise people with CGD to keep pets, although the risk may be manageable if there is strict adherence to hygiene (103). Furthermore, patients undergoing conservative treatment derive substantial benefits from regular follow-up by physicians experienced in CGD care (102). In our European setting, we strive for intervals of 3 months between visits. Although anti-infective prophylaxis greatly reduces mortality, infections still occur at a rate of 0.26–0.64 per patient-year with a cumulative lifetime risk of 20%–40% for aspergillosis, which remains the leading cause of death (39, 43).

Immunosuppressive therapy can become necessary to control autoinflammation and immune dysregulation (59, 104). For mild IBD in CGD, sulfasalazine or alternative aminosalicylates are commonly used as initial treatments (105). Recently, monoclonal antibodies targeting pro-inflammatory cytokines (such as infliximab, anakinra, adalimumab, and ustekinumab) have been explored (106–109). However, the available evidence is limited and ustekinumab is the only treatment that has shown somewhat favorable outcomes (109). Despite their known adverse effects, patients often remain dependent on corticosteroids to control IBD.

In retrospective studies, the median life expectancy of conservatively treated CGD patients is between 30 and 40 years. Additionally, quality of life and academic and professional achievements are severely impaired on conservative treatment (39, 110, 111). In contrast, allogenic hematopoietic stem cell transplantation (HSCT) can potentially cure CGD. Because transplantation-related mortality was originally at 15%–50%, HSCT was historically considered a salvage therapy only for patients with recurrent infections or refractory inflammation (112, 113). Improved HLA matching, fludarabine-based reduced-toxicity conditioning, and the accumulation of clinical experience

in guiding patients with CGD through allogenic HSCT have reduced treatment-related morbidity and mortality (Table 1) (115–121). Over the past decade, transplant series have reported survival rates of 83%–96% in CGD patients following matched donor transplantation (115, 116). Several studies have compared the prognosis of CGD patients treated conservatively with those treated with HSCT. A Swedish study of 41 patients reported a superior outcome with HSCT (93% vs. 74% survival) (114). Other studies, including our own retrospective European study, still failed to describe a clearly better survival after HSCT, with survival rates ranging from 76%–90% in both cohorts (41, 43, 45, 115, 118). However, significant reductions in infectious episodes and catch-up growth after HSCT are clearly evident in data from the UK, US, and Europe (43, 45, 115). In particular, patients between the ages of 5 and 14 without active complications at the time of HSCT have excellent outcomes (45, 115, 119, 120). Therefore, all young CGD patients with a ≥9/10 HLA-matched available donor should be considered for HSCT early in life, before chronic sequelae caused by infections and/or autoinflammation occur. Patients for whom no ≥9/10 HLA-matched donor is available can be offered haploidentical HSCT from an unaffected parent [published experience reviewed in (122)]. The idea of early transplantation also holds true for symptomatic X-linked carriers of mutations in CYBB and an unfavorable lyonization (96, 121). HSCT is also possible in older patients after recurrent or persistent infection or autoinflammation and may be the only life-saving option (96, 116, 117).

Gene therapy for CGD remains limited to trials in only a few highly specialized centers around the world. It is often mentioned as a future alternative cellular therapy "for patients without a suitable donor" (123, 124). But at least most children can be treated by HSCT from a haploidentical parent (122, 125–127). Gene therapy is also promoted for patients with a "high disease burden," as conditioning is milder than with HSCT (123). However, in the most recent trials, the mortality rates for gene therapy are still higher than those for HSCT. This is presumably due to the disease burden of the patients enrolled, but it may also be because of a high rate of graft rejection in gene therapy (120, 124, 128). To date, there is a lack of long-term data and head-to-head trials, or at least meta-analyses, comparing gene therapy with haploidentical HSCT in patients for whom a matched donor is not available (129). Nevertheless, gene therapy may emerge as an alternative to HSCT in selected situations (124, 130).

TABLE 1 Survival rates in CGD patients treated with HSCT vs. patients on conservative treatment (non-HSCT).

Author (year)	Survival (%)		Patients (*n*)
	HSCT	Non-HSCT	HSCT/ non-HSCT
van der Berg et al. (2009) (41)	81	74	24/307
Åhlin et al. (2013) (114)	93	46	14/27
Cole et al. (2013) (43)	90	90	30/32
Yonkof et al. (2019) (115)	88	85	50/457
Dedieu et al. (2021) (45)	88	87	50/54
Horwitz et al. (2001) (112)	70	–	10/–
Seger et al. (2002) (113)	85	–	27/–
Güngör et al. (2014) (116)	93	–	56/–
Morillo-Gutierrez et al. (2016) (117)	91	–	70/–
Parta et al. (2017) (118)	82.5	–	40/–
Lum et al. (2019) (119)	89	–	55/–
Chiesa et al. (2020) (120)	86	–	712/–
Tsilifis et al. (2023) (121)	71	–	7/–

Summary

Chronic granulomatous disease (CGD) is caused by an impaired respiratory burst reaction of all phagocytes rather than an impaired burst in neutrophilic granulocytes only. CGD is an X-linked (caused by pathogenic variants in *CYBB*) or autosomal recessive inborn error of immunity (caused by pathogenic variants in *CYBA*, *NCF1*, *NCF2*, or *CYBC1*). Patients with CGD are at increased risk for bacterial and/or fungal invasive infections of any organ, but mainly the lymph nodes, liver, and lungs. The leading pathogens isolated are *S. aureus* and *Aspergillus* spp. But *Serratia*, *Proteus* spp., *Burkholderia cepacia*, *Nocardia* spp., and *Salmonella* spp. are still often isolated, and infections with almost any intracellular bacteria and fungi are possible. Infections with *Aspergillus* spp. and *Burkholderia cepacia* remain the major cause of morbidity and mortality in patients on conservative treatment. Patients often develop skin infections by *S. aureus*. Autoinflammation, and inflammatory bowel disease in particular, is difficult to control by immunosuppression, and patients frequently remain dependent on steroids. Female carriers of pathogenic variants in *CYBB* and unfavorable lyonization may present with the partial or even full picture of CGD. For the diagnosis of CGD, reactive oxygen intermediates (O_2^- or H_2O_2) generated by the NADPH oxidase in phagocytes of peripheral blood are measured upon *in vitro* activation with either PMA and/ or TLR4 ligands (*E. coli* or LPS). These assays may be false positives in patients treated with paracetamol, metamizol or mesalazine (5-ASA). Conservative treatment must adhere to a strict hygienic conduct and antibiotic prophylaxis against bacteria and fungi. Cotrimoxazole and triazoles that work against intracellular bacteria and *Aspergillus* spp. are the mainstay of the latter. With ongoing advancements in diagnostics, prophylaxis, and therapeutic modalities, it is plausible that life expectancy may surpass the age range of 30–40 years in conservatively treated patients. Most patients with CGD who receive conservative treatment, however, face lifelong challenges such as recurrent and/or persistent infections as well as steroid-dependent autoinflammation and subsequently failure to thrive. Overall, this leads to an unfavorable psychosocial prognosis. In contrast, cellular therapies (allogenic HSCT from a healthy donor or autologous gene therapy-modified cells) can cure CGD. HSCT in individuals without ongoing infections or inflammation offers a fair, yet unfortunately still far from completely event-free prognosis and chance for overall survival. But neither persistent infections nor refractory autoinflammation are a contraindication against HSCT; rather, they are an indication to proceed to a definite cure through cellular therapy.

If no HLA-matched donor is available, most infants and children can be transplanted from a haploidentical parent. If no such donor is available, gene therapy may be an alternative option.

Author contributions

OS: Visualization, Writing – review & editing. HB: Conceptualization, Writing – original draft, Writing – review & editing, Supervision.

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Conflict of interest

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.

Publisher's note

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Infant with diffuse large B-cell lymphoma identified postmortem with homozygous founder Slavic *RAG1* variant: a case report and literature review

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Background and aims: There is an increased risk of lymphomas in inborn errors of immunity (IEI); however, germline genetic testing is rarely used in oncological patients, even in those with early onset of cancer. Our study focuses on a child with a recombination-activating gene 1 (*RAG1*) deficiency who was identified through a screening program for Slavic founder genetic variants among patients who died with malignancy at an early age in Belarus.

Results: We identified one homozygous founder *RAG1* variant out of 24 available DNA samples from 71 patients who developed lymphoma aged <3 years from the Belarusian cancer registry between 1986 and 2023. Our patient had an episode of pneumonia at 3 months of age and was hospitalized for respiratory distress, candida-positive lung disease, and lymphadenopathy at 14 months of age. The diagnosis of Epstein–Barr virus (EBV)-positive diffuse large B-cell lymphoma (DLBCL) was established. The patient had a normal lymphocyte count that decreased over time. One month after chemotherapy initiation, the patient died due to sepsis and multiple organ failure without a genetic diagnosis. In a retrospective analysis, T-cell receptor excision circles (TRECs) and kappa-deleting recombination excision circles (KRECs) were undetectable in peripheral blood.

Conclusions: A targeted screening program designed to detect a Slavic founder variant in the *RAG1* gene among children revealed a 14-month-old Belarusian male infant with low TREC levels who died of EBV-driven DLBCL and complications of chemotherapy including infections. This case highlights how patients with IEI and recurrent infections may develop serious non-infectious complications, such as fatal malignancy. It also emphasizes the importance of early identification, such as newborn

screening for severe combined immune deficiency. Earlier diagnosis of RAG deficiency could have prompted hematopoietic stem cell transplant well before the DLBCL occurrence. This likely would impact the onset and/or management strategies for the cancer.

KEYWORDS

RAG deficiency, lymphoma, malignancy in SCID, infant, case report

1 Introduction

Patients with inborn errors of immunity (IEI) have a much higher risk of developing cancer than the general population, in the range of 4%–25% (1, 2). There is some variability regarding the type of malignancy and its association with specific IEI. In patients with IEI, malignancies may occur at any age, including childhood, but on average they tend to manifest earlier than in the general population. In addition, there is a narrower age range with higher incidence of hematological malignancies than in the general population (3). Increased susceptibility to malignant neoplasms in IEI patients is mediated by a combination of intrinsic and extrinsic factors. Genetic disorders associated with impaired cell differentiation or apoptosis, cytoskeleton function, lymphocyte co-signaling, metabolism, cytotoxicity, and disorders leading to increased genotoxicity, such as chromosome instability, defective telomere maintenance and DNA repair, along with extrinsic causes, such as transforming viral infections and chronic tissue inflammation, contribute to the malignancy development (4, 5).

The most common IEI associated with increased cancer predisposition include DNA repair defects and/or IEI with susceptibility to oncogenic herpes group virus infection. Ataxia telangiectasia, Nijmegen breakage syndrome, and Bloom syndrome are the most frequently encountered DNA repair defects. Beside malignancy predisposition, these patients also have distinctive syndromic features that may expedite the diagnosis. In patients with combined immunodeficiencies (CIDs) with Epstein–Barr virus (EBV) susceptibility, infection may be one of the main triggers for the development of malignancy. These disorders include autoimmune lymphoproliferative syndromes, X-linked lymphoproliferative syndrome type I and regulatory T-cell disorders, and malignancy, mostly lymphoma (6–10).

Lymphoma is the most common tumor type in patients with IEI, with the risk of lymphoid malignancies being overall 8–10-fold higher (11). Non-Hodgkin lymphomas [diffuse large B-cell lymphoma (DLBCL), marginal zone, and Burkitt lymphomas] account for the vast majority of lymphomas observed in patients with IEI (12), whereas leukemia, Hodgkin's lymphoma, and solid tumors are less common in this group of patients (13).

We previously described 18 Slavic patients who were homozygous for the deleterious *RAG1* p.K86VfsX33 (c.256_257delAA) variant. This study is the largest report in the literature of *RAG* variants within a geographically restricted population (14).

Here we report the case of a 14-month-old male infant affected by EBV-positive DLBCL in whom homozygosity for the same *RAG1* Slavic founder variant was identified postmortem after targeted Sanger sequencing of 24 available DNA samples out of 71 patients who developed lymphoma aged <3 years from the Belarusian cancer registry between 1986 and 2023. In addition, we performed a literature search of malignancy occurrence in *RAG* deficiency.

2 Results

2.1 Cohort analysis

We analyzed the data of the Belarusian cancer registry and found 71 patients (48 boys, 23 girls) who had been diagnosed with lymphoma before the age of 3 years in the period between 1986 and 2023. There were 48 non-Hodgkin's lymphomas, 9 Hodgkin's lymphomas, and 14 unspecified lymphomas (diagnosed before 1995). The age at clinical diagnosis was in the range of 1 month to 2 years 10 months (median 2 years 2 months). Of these patients, 38% were reported to be alive in 2024. Genetic material for DNA extraction was available in only 24 patients who comprised the study cohort. The cohort for sequencing included 17 boys and 7 girls, of whom 62.5% were alive at the time of the study (median age 11 years). The age at clinical manifestation of lymphoma in this group of patients ranged from 7 months to 2 years 10 months (median 2 years).

Homozygosity for the founder Slavic *RAG1* p.K86VfsX33 variant was found in 1 of 24 samples of available DNA.

Previous studies in the same cohort of 24 patients had identified one homozygous patient for the founder Slavic *UNC13D* variant [p.Arg782SerfsTer12] and two patients had disease-associated deleterious variants [p.Pro465ArgfsTer82 and p.His321Tyr] in the *FOXN1* gene. In total, 4 of the 24 patients were diagnosed with IEI.

2.2 Clinical case

A male infant was born full-term from non-consanguineous parents who originated from Brest (Western Belarus), after a second pregnancy and second delivery. The patient's family history was unrevealing. He had received hepatitis B virus (HBV) (first dose) and bacille Calmette-Guérin (BCG) vaccine without adverse effects.

From the age of 3 months, the patient presented with a history of recurrent upper and lower (pneumonias) respiratory tract infections. At the age of 1 year 1 month, the patient was hospitalized because of fever, cough, dyspnea, lymphadenopathy, weakness, and refusal to eat. At physical examination, muscular hypotrophy of the second degree (weight 7.5 kg and height 75 cm), flabby skin, cyanosis of the nasolabial triangle, and

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respiratory distress were found. Chest radiograph showed bilateral polysegmental pneumonia (Figure 1B). A microbiological analysis of sputum found Candida.

An ultrasound examination showed hepato-(+3 cm)-spleno-(+3 cm)-megaly and lymphadenopathy. A lymph node biopsy was suggestive of DLBCL. Angiocentric lymphoid infiltrates of large, atypical cells and Hodgkin-like cells were accompanied by foci of coagulative necrosis. Large cells showed LCA, CD20, and patchy CD30 positivity. Immunostaining for EBV latent membrane protein was positive (Figure 1C). The lymphoma involved multiple lymph nodes (cervical, retroperitoneal, mediastinal, and abdominal) and multiple organs (liver, lungs,

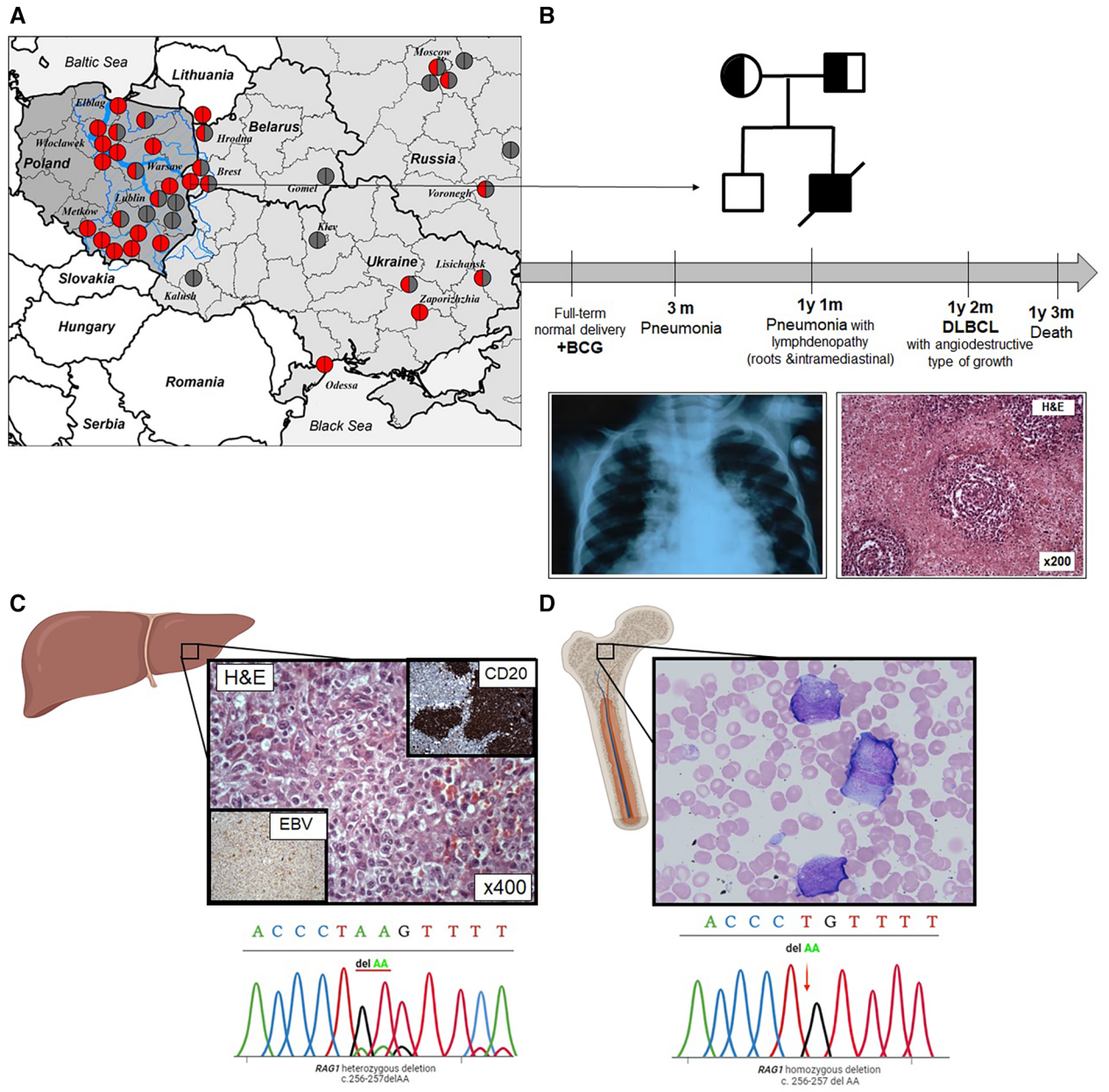

FIGURE 1
(A) Distribution map of Western and Eastern Slavic families with the *RAG1* p.K86VfsX33 variant in Slavic countries; the Vistula river basin is overlaid on the map of Poland. The blue line is the Vistula River basin and its largest tributaries (Bug, Narev, San, Wieprz, Pilica, and Muchowiec), the geographic area coincides with the region of the largest concentration of families where patients with p.K86VfsX33 homozygous variants were born. The birthplace of the patients was indicated by the location of the circles; homozygous p.K86VfsTer33 variant is represented by red circles; heterozygous p.K86VfsTer33 variant is half red/half gray; and other variants are gray. (B) Pedigree of the proband; the mother and father are carriers; deceased (line through). Timeline summarizing main turning point of patient's clinical history. Chest radiograph at the age of 3 months: pneumonia; liver biopsy at the age of 1 year 2 months: lymphoma with angiodestructive type of growth. (C) Liver biopsy: solid shut of lymphoma cells, some of them resemble Reed–Sternberg cells and electropherograms of Sanger sequencing of *RAG1* p.K86VfsX33 variant from lymphoma tissue. (D) Bone marrow smear: dissociation of neutrophil maturation cells and electropherograms of Sanger sequencing of *RAG1* p.K86VfsX33 variant in the bone marrow.

large and small intestines). Bone marrow investigation revealed dissociation of neutrophil maturation, hypochromia, and microanisocytosis of erythrocytes (Figure 1D).

Treatment was started according to the NHL-BFM-95 protocol with a dose reduction of one-third.

Laboratory investigation at the age of 1 year 1 month revealed progressive leukopenia (from 3,100 to 200 cells/μl), an imbalance in the ratio of stab and segmented neutrophils, and decreased hemoglobin (96 g/L). Lymphopenia (400 cells/μl), decreased T cells (300 cells/μl), and elevated activated T cells (41%) were detected. The percentage of B cells was elevated (33%), but the absolute number was slightly decreased (100 cells/μl). T-cell receptor excision circles (TRECs) and kappa-deleting recombination excision circles (KRECs) were undetectable (0 copies/10^6 leukocytes for both) and DNA was isolated from archived patient peripheral blood samples obtained at the time of hospitalization (1 year 3 months).

During the course of the lymphoma treatment, the patient's condition worsened. He developed sepsis (*Pseudomonas aeruginosa* and *Enterococcus faecium*), fibrinous-purulent peritonitis, and intestinal perforation. Resection of the ileocecal angle was performed. During surgery, biopsies of the liver, ileum, cecum, and ascending colon were taken. Extensive necrosis in the center and multiple nodular proliferations of diffuse large B-cell lymphoma were found in the tissues of the liver and intestines.

The patient died at the age of 1 year 3 months due to septic shock and multiple organ failure, 1 month after starting chemotherapy.

At 18 years after his death, targeted Sanger sequencing revealed a homozygous deletion in *RAG1* gene (NM_000448.3) c.256_257del (p.Lys86ValfsTer33).

3 Discussion

The recombination-activating gene 1 (*RAG1*) and 2 (*RAG2*) encode lymphoid-specific proteins that are expressed during the early stages of T-cell and B-cell development and initiate the process of V(D)J recombination by introducing DNA double-strand breaks (DSBs). The process of V(D)J recombination generates diverse T-cell and B-cell receptors capable of recognizing millions of possible antigens (15). Genotype–phenotype correlation is strong, as null variants of *RAG1* and *RAG2* genes result in the T-B-severe combined immune deficiency (SCID) phenotype, whereas hypomorphic *RAG* variants have been associated with distinct clinical entities, including Omenn syndrome (OS) and combined immunodeficiency with granuloma and/or autoimmunity (CID/G-AI) with herpesvirus infections and lymphoproliferation (16).

However, in RAG1/RAG2-deficient patients, as well as in overall SCID patients, hematopoietic neoplasms are extremely rare. This is presumably explained by the high frequency of life-threatening infections that require hematopoietic stem cell transplantation (HSCT) early in life. Currently, there are only six published cases of malignancy in five patients with *RAG1* deficiency (Table 1) (17–21).

These patients had only two types of neoplasms: DLBCL (three cases) and cutaneous lymphoproliferative disease (CLPD) (three cases). CLPD manifested at a later age (2.5, 5, and 15 years) in patients with milder phenotype, which can be described as combined immunodeficiency or "leaky" SCID (20, 21). In contrast, DLBCL manifested earlier: in two cases, the diagnosis was established in the first year of life and occurred even in patients with typical SCID phenotype (17, 19). In only one patient with "leaky" SCID phenotype was DLBCL diagnosed at the age of 5 years (18). In the current case, DLBCL was established at the age of 1 year 2 months. Even though the patient had a history of severe infectious episodes and lymphopenia, a diagnosis of SCID was contemplated, but was not formally established during the patient's lifetime. EBV was detected in peripheral blood and tumor tissue in all four patients with DLBCL, including the current case. Rituximab therapy was initiated to rapidly control the EBV load. Patients with CLPD were treated with a combination of prednisone, methotrexate, and local radiation therapy (patient 5). All patients underwent HSCT except patient 4 who was being prepared for transplantation at the time of publication. Patient 5 did not recover owing to severe infection after HSCT.

Numerous lymphoma sequencing studies show that somatic mutations are often found in IEI genes. The most recurrently mutated genes in Hodgkin's lymphoma and DLBCL include *TNFAIP3*, *SOCS1*, *ITPKB*, *B2M*, *KMT2D*, *ATM*, *TP53*, *ACTB*, and *IRF4* (22–24).

Many studies showed that RAG endonucleases are involved in the pathogenesis of lymphomas and leukemias. Dysregulation of RAG expression may cause chromosomal translocations, which are a hallmark of hematopoietic neoplasms (25). RAG endonucleases are able to bind to nonamer-like sequences (cryptic nonamer) and cleave at adjacent mismatches resulting from activation-induced cytidine deaminase (AID)-mediated deamination of methylated CpG sites (26), thereby generating genomic instability. Whole genome sequencing of cutaneous T-cell lymphomas (CTCLs) revealed that RAG binding sites flanked a significant number of deletion breakpoints (27). Since CTCLs occur in older adults, this suggests that dysregulated re-expression of the *RAG* genes may occur in mature CD4+ T cells, leading to tumorigenesis. Complex genomic rearrangements such as chromothripsis, in which DSBs are widespread, have also been found in CTCLs (27).

Somatic mutations in the *RAG1* and *RAG2* genes are rare in lymphogenesis and leukemogenesis. According to data from the catalogue of somatic mutations in cancer (COSMIC), out of 8,381 samples of hematopoietic and lymphoid tissue studied, only 24 (0.29%) had point mutations in the *RAG1* gene [https://cancer.sanger.ac.uk/cosmic, accessed May 2024]. These mutations have been found in acute myeloid leukemia, acute lymphoblastic B-cell leukemia, adult T-cell lymphoma/leukemia, plasma cell myeloma, diffuse large B-cell lymphoma, chronic lymphocytic leukemia-small lymphocytic lymphoma, mycosis fungoides-Sezary syndrome, and other cancers. A similar pattern is observed for the *RAG2* gene: *RAG2* somatic mutations have been reported in patients with Burkitt lymphoma, mycosis

TABLE 1 Clinical data of patients with *RAG* deficiency and malignancies.

Patient No._gender_ethnicity	*RAG*				Age		Malignancy			Treatment	Follow-up	Reference
	Gene	Mutation	*RAG1* activity (% of WT), mean ± SE (16)	Phenotype	At onset	*RAG1* deficiency/ malignancy	Type	Localization	Virus (blood/ blocks)			
P1_F_Turkish_Germany	*RAG1*	p.R561H	2.0 ± 0.6	T−B− SCID	4 months	~10 months/ 10 months	DLBCL	Lung, mastoid sinus	CMV tissue, EBV+tissue and blood	Anti-CD20 mAb, HSCT	Alive, 6 months at time of publication	(17)
		p.R561H	2.0 ± 0.6									
P2_F_Germany	*RAG1*	p.R314W	24.3 ± 5.2	$T^{low}B^{low}$ CID	1 year 5 months	3 years/2.5 years	Clonal T-cell lymphoproliferative lesion, DLBCL	Right tonsil	EBV+tissue and blood	Chemotherapy, anti-CD20 mAb, HSCT	Alive, 3 years at the time of publication	(18)
		p.R507W/ p.R737H	15.9 ± 0.8/ 0.2 ± 0.0									
P3_M_Slavic_Romania/ Austria	*RAG1*	p.K86VfsX33	2.7 ± 0.3	T−B− SCID	~4 months	4 months/ ~4 months	DLBCL	Liver	CMV blood, EBV+tissue and blood	Anti-CD20 mAb, modified CHOP, HSCT	Alive at the time of publication	(19)
		p.K86VfsX33	2.7 ± 0.3									
P4_M_China	*RAG1*	c.813T>A/ c.870G>A	n.d.	$T^{low}B^{low}$ SCID	2 years	~5 years/5 years	CLPD	Plantar skin	EBV− blood	Prednisone and methotrexate, HSCT	Alive, preparing for HSCT at the time of publication	(20)
		p.A740V	n.d.									
P5_F_Israel	*RAG1*	p.C335R	n.d.	T+B+CID	13 years	15 years/15 years	CLPD	Right cheek skin	EBV− tissue	Prednisone and methotrexate, local radiotherapy, HSCT	Died after HSCT	(21)
		p.C335R	n.d.									
P6_M_Slavic_Belarus	*RAG1*	p.K86VfsX33	2.7 ± 0.3	$T^{low}B^{low}$ CID	3 months	Postmortem/ 1 year 2 months	DLBCL	LN, liver, intestine	EBV+tissue and blood	Chemotherapy	Died on 2nd month of chemotherapy	Current case
		p.K86VfsX33	2.7 ± 0.3									

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fungoides-Sezary syndrome, acute lymphoblastic B-cell leukemia, DLBCL, chronic lymphocytic leukemia, small lymphocytic lymphoma, and so on. Somatic point mutations in the *RAG2* gene were detected in 18 (0.21%) of 8,393 hematopoietic and lymphoid tissue samples studied.

Approximately 15% of human cancers worldwide are caused by oncoviruses. Human oncogenic viruses include EBV, HBV, hepatitis C virus (HCV), high-risk human papillomaviruses (HPVs), human T-cell lymphotropic virus-1 (HTLV-1), Kaposi sarcoma-associated herpesvirus [KSHV; also known as human herpesvirus 8 (HHV-8), and Merkel cell polyomavirus (MCPyV)] (28). However, oncovirus infection alone is not sufficient to cause cancer. Within the context of multistep carcinogenesis, viral infection provides only a subset of the required oncogenic hits (29).

To successfully evade the immune response, oncoviruses have evolved powerful anti-apoptotic and proliferative programs. There are several basic oncogenic mechanisms. First, viruses encode proteins that are able to subvert, in a dominant manner, host-signaling mechanisms that regulate cell growth and survival. Second, recognition of viral genomes or replicative intermediates by the host leads to induction of the DNA damage response (DDR), which many oncoviruses need for their replication. Third, chronic inflammatory responses to persistent viral infection cause the formation of reactive oxygen species (ROS) that promote the acquisition of mutations (29).

Sheng et al. demonstrate that in a group of 329 patients with DLBCL, only 2.4% were EBV positive (23). According to our data, all patients with RAG1 deficiency had EBV-associated DLBCL. Evidence of the association between EBV positivity and mutation burden is mixed. Some studies have shown a strikingly lower number of somatic mutations in EBV-positive Hodgkin's lymphomas (30).

A targeted screening program for searching the Slavic founder variant in *RAG1* gene among Belarusian children who developed lymphoma aged <3 years revealed a 14-month-old Belarusian boy with low TREC levels who died of EBV-driven DLBCL and complications of chemotherapy including infections. This case highlights how patients with IEI and recurrent infections may develop serious non-infectious complications, such as fatal malignancy. It also stresses the importance of early identification, such as newborn screening for SCID, especially in the regions with domination of founder mutations. In addition, our case underlines the importance of resequencing the genomic DNA in patients with malignancy and somatic mutations in IEI genes.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by this study has been approved by the ethics committee of the Belarusian Research Center for Pediatric Oncology, Hematology and Immunology, Minsk, Belarus. Biological material was obtained on informed consent in accordance with the Declaration of Helsinki (IRB0012/2022). The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

TV: Investigation, Writing – original draft, Writing – review & editing. EP: Investigation, Writing – original draft, Writing – review & editing. TM: Investigation, Writing – original draft, Writing – review & editing. IS: Writing – original draft, Writing – review & editing. AK: Writing – original draft, Writing – review & editing. AD: Investigation, Writing – original draft, Writing – review & editing. MB: Resources, Writing – original draft, Writing – review & editing. JD: Writing – original draft, Writing – review & editing. DV: Visualization, Writing – original draft, Writing – review & editing. LN: Writing – original draft, Writing – review & editing. JW: Writing – original draft, Writing – review & editing. SS: Conceptualization, Supervision, Visualization, Writing – original draft, Writing – review & editing.

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Conflict of Interest

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The riddle of recurrent fever: a clinical approach to pediatric autoinflammatory diseases

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Autoinflammatory diseases (AIDs) are a group of immunodysregulatory disorders resulting in the increased release or signaling of pro-inflammatory cytokines. Patients with AIDs present systemic inflammation in sterile conditions, which are mainly caused by defects in the innate immune system. Fever is one of the typical symptoms of this derailed immune signaling. In addition, autoinflammatory diseases manifest with varying other symptoms during flare-ups and interphasic periods. The diagnosis of these rare diseases poses numerous challenges. This paper provides an overview of AIDs that arise in childhood and in which fever commonly presents as a symptom. It outlines clinical signs, pathophysiology, diagnosis, and management for each syndrome. Additionally, we discuss a comprehensive diagnostic approach for children where an AID is suspected.

KEYWORDS

inborn errors of immunity, monogenic, recurrent fever, periodic fever, autoinflammatory diseases, autoinflammation, inflammasome, pediatric

1 Introduction

Fever is one of the most common reasons to seek medical care. This is particularly the case for children, as fever makes up 30% of all pediatric ambulatory visits (1). Fever serves as a physiological immune-mediated response to infection, tissue damage or neoplasia. This response leads to inflammation to combat and remove the eliciting agents (2–4). Counter-regulatory signals make that inflammatory episodes ought to be well-controlled. Likewise, immune dysregulation can lead to derailed systemic inflammation and detrimental outcomes. Over 200 clinical conditions are now characterized by persistent or recurrent inflammation and are collectively termed as immune-mediated inflammatory disorders. These disorders can be classified in three main categories: autoinflammatory, autoimmune, and hyperinflammatory diseases (5). AIDs commonly present with signs of systemic inflammation during flare-ups. In addition, subclinical inflammation can be detected during the intercritical phase. While flares typically occur spontaneously, they can be triggered by physical or emotional stress, infection, trauma, hormonal fluctuation, cold, and vaccination. Many autoinflammatory diseases manifest in childhood. They often remain a diagnostic challenge, leading to significant diagnostic delay. Indeed,

while underlying diagnoses might be entirely different, presenting symptoms and routine blood examinations can be very similar. This paper will focus on the pediatric autoinflammatory diseases that commonly present with recurrent fever and highlight common and differentiating clinical features between diseases.

1.1 Autoimmunity vs. autoinflammation

The first monogenetic defects linked to autoinflammation were described in 1999: familial Mediterranean fever (FMF) and tumor necrosis factor (TNF)-receptor-associated periodic syndrome (TRAPS) (6–8). Autoinflammatory diseases are defined as "clinical disorders caused by defect(s) or dysregulation of the innate immune system, characterized by recurrent or continuous inflammation [elevated acute phase reactants (APR)] and by the lack of a primary pathogenic role of the adaptive immune system (autoreactive T-cells or autoantibody production)". More than 60 monogenic autoinflammatory syndromes have been described to date (9–11). An overview of the discussed AIDs in this paper is presented in the Supplementary Table 1. In contrast to aberrant activation of the innate immune response in AID, autoimmune diseases reveal a dysregulated adaptive immune response. Paul Ehrlich, who first introduced the concept "autoimmunity", pointed out the risk of the immune system recognizing self, which he referred to as "horror autotoxicus" (12). The hallmarks of autoimmunity comprise the overactivation of lymphocytes (T and/or B lymphocytes) and the formation of autoantibodies directed against autologous tissue (13). Autoimmune diseases have a female predominance and affect more than 3% of the total population. They can affect a single organ (e.g., diabetes mellitus type I) or they can be systemic (e.g., rheumatoid arthritis (RA) and systemic sclerosis (SSc)). The distinction between dysregulated innate and adaptive immune response, linked to autoinflammation and autoimmunity respectively, serves as a simplified framework for understanding the intricate underlying pathophysiology. Nowadays, this division is considered less clear-cut than previously believed, with overlapping features between both types of inflammatory disorders in various disease states. For example, Systemic Lupus Erythematosus (SLE) was first allocated as a classical autoimmune disease featuring complexes of autoantibodies and self-reactive B- and T lymphocytes, causing arthritis, nephritis and mucocutaneous symptoms. But recent insights in pathophysiology identified complement activation and type 1 interferons as the main drivers of the disease. This indicates that, alongside the adaptive immune system, innate immune signals likewise trigger autoimmune diseases (14). Furthermore, mutations in the NOD-like receptor (NLR), an innate immune sensor forming inflammasome and subsequent activation of IL-1β and IL-18, are strongly linked to AIDs. However, IL-1β also plays a major role in T cell survival and B cell proliferation. These observations highlight the complex interplay between innate and adaptive immunity (15).

1.2 Pathophysiology of autoinflammatory diseases

The 2022 International Union of Immunological Societies (IUIS) Phenotypical Classification defines three groups of autoinflammatory disorders, categorized according to their underlying pathophysiology: (1) type I Interferonopathies, (2) defects affecting the Inflammasome and (3) Non-Inflammasome related conditions. An overview of the different AIDs, including their pathophysiology, clinical presentation, and diagnostic work-up, can be found in the Supplementary Table 1. The subsequent sections will delve into each of these subgroups.

1.2.1 Type I interferonopathies

Pathogen recognition receptors (PRRs) in innate immune cells can detect and respond to pathogen associated molecular patterns (PAMPs) or self-derived molecules from damaged cells (DAMPs). PRRs, like toll-like receptors (TLRs) and NOD-like receptors (NLRs), induce downstream inflammatory cascades in an attempt to respond to pathogen invasion and/or damage of cells (16).

PRRs can have a transmembrane localization on the cell surface, like toll-like receptors (TLRs), or can be situated in the cytosol, like RIG-I-like receptors (RLR) or NOD-like receptors (NLRs). An important example of a cytosolic receptor is the cyclic guanosine monophosphate-adenosine monophosphate (cGAMP) synthase (cGAS), which is a central receptor of nucleic acids, derived from pathogenic or endogenous DNA or RNA (see Figure 1) (17).

The messenger of cGAS, cGAMP activates the adaptor protein STING, which results in the production of type 1 interferons (IFNs) (18, 19). The production of type I IFNs in response to recognition of DNA or RNA is an indispensable step in the antiviral response. But inappropriate production of these potent cytokines can have detrimental effects on the host (17). The genetic spectrum of type I interferonopathies extends beyond the cGAS-STING pathway and includes defects in nucleases, innate immune sensors, or adaptor molecules downstream of these sensors and defective feedback of the interferon pathway (20). The common trait among this group of diseases is the upregulation of type I IFN signaling in sterile conditions. As a consequence of the commonalities in the pathophysiological pathways, common clinical signs of different type I interferonopathies include early-onset encephalopathy, brain calcifications—mimicking the sequelae of congenital infections (TORCHES)—and skin vasculitis (exhibiting chilblains). Recurrent fever, however, is not commonly present in patients with type I interferonopathies. Hence, type I interferonopathies will not be discussed in detail, although two prototypical diseases will briefly be mentioned.

Aicardi-Goutières syndrome (AGS) is a prototypical type I interferonopathy (21). Seven different inborn errors of nucleic acid metabolism or sensing have been associated with the clinical phenotype of AGS: *TREX1*, *RNASEH2A*, *-B*, *-C*, *SAMHD1*, *ADAR1* and *IFIH1* (see Supplementary Table 1). The disorder typically presents with neurologic regression during infancy (22, 23). Intracranial calcifications on brain imaging are

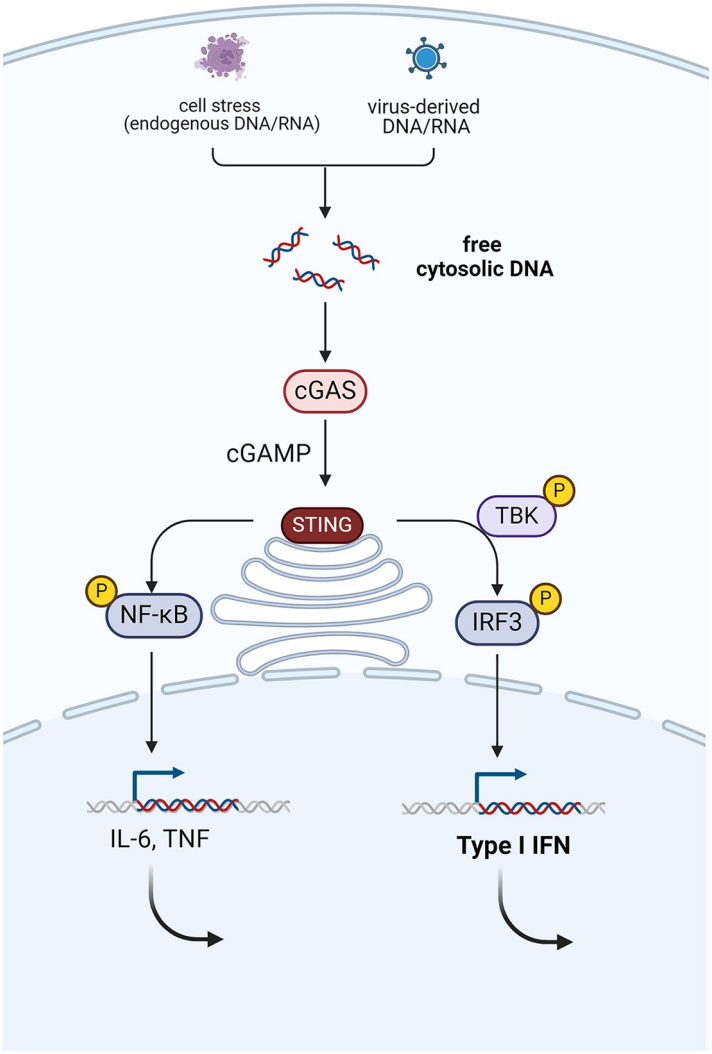

FIGURE 1
Activation of the cGAS-STING pathway via the detection of pathogenic or endogenous nucleic acids (DNA or RNA). This leads to production of type I interferons via phosphorylation of TANK-binding kinase 1 (TBK1) and interferon regulatory factor 3 (IRF3). Simultaneously, the NF-κB pathway is activated, which mediates the induction of pro-inflammatory cytokines, such as TNF and IL-6. Created with Biorender.com.

pathognomonic for AGS. Other neurological symptoms involve epileptic seizures and motor disorders like dystonia, spasticity and paraparesis. More recently, the phenotype of AGS has diversified with many non-neurological features, including chilblains, vasculitis, bowel-inflammation and lupus-like disease (24, 25).

STING-associated vasculopathy with onset in infancy (SAVI) is another monogenic type I Interferonopathy. Due to gain of function mutations in the STING protein, the STING pathway is constitutively activated independently of the production of cGAMP (26). Early onset vasculopathy causes chilblains, distal ischemia of the limbs and arthritis. There is a high mortality rate in this patient group due to interstitial lung disease, evolving to lung fibrosis and respiratory failure (24, 27). To date, no definitive treatment options have been established for these diseases. However, the use of Janus kinase (JAK) inhibitors as immunomodulator in SAVI patients has demonstrated promising outcomes (27).

1.2.2 Defects affecting the inflammasome

Inflammasomes are multiprotein complexes that consist of sensor proteins (or PRRs), apoptosis associated speck-like protein containing a CARD domain (ASC) and protease enzymes such as caspase-1 (28). Upon activation, caspase-1 cleaves pro-IL-1β and pro-IL-18 into their active forms IL-1β and IL-18. Caspase-1 also induces cell death by Gasdermin D (GSDMD)-mediated pore assembly and releases intracellular pro-inflammatory content, called pyroptosis (16, 29).

The family of inflammasome-forming proteins consists of the NOD-like receptor (NLR) proteins NLRP1, NLRP3, NLRP6 and NLR family CARD domain containing 4 (NLRC4), the protein absent in melanoma 2 (AIM2) and pyrin. NLRP1, NLRP3, NLRP6, NLRC4, AIM2 and pyrin undergo oligomerization to form inflammasomes (see Figure 2). Inflammasomopathies are caused by gain-of-function mutations of components involved in inflammasome activation pathways (31, 32). Familial Mediterranean Fever (FMF) is the most common pyrin-associated autoinflammatory disease (PAAD). Pyrin (*Greek for fever*), an intracellular PRR, forms an inflammasome in response to bacterial toxins. Pyrin senses pathogen induced modifications of Rho guanosine triphosphatase (Rho GTPases). The inactivation of RhoA through bacterial toxins causes reduced levels of phosphorylated pyrin via a decreased activity of PKN1 and PKN2 (Figure 2). This in turn releases pyrin of its inhibitory chaperone (14-3-3) protein and induces the formation of an active pyrin inflammasome. Consequently, this leads to the activation and release of IL-1β and IL-18, along with the release of other potent initiators and enhancers of innate immune responses via the process of pyroptosis. Gain-of-function mutations in the *MEFV* gene, which encodes pyrin, cause poor affinity to the regulatory proteins (14-3-3 and PKN1/2) and lead to constitutive activation of the pyrin inflammasome (33, 34). Compared to FMF, mutations in NLRP1, NLRP3 and NLRC4 or their regulatory proteins cause excessive formation and activation of other inflammasomes but equally lead to increases in IL-1b and IL-18 (29).

1.2.3 Non-inflammasome related conditions

The pathophysiology of non-inflammasome related conditions is very heterogenous. The two main and closely intertwined players of this third category of autoinflammatory disorders are TNF and nuclear factor-κB (NF-κB). Activation of TNF family receptors via TNF leads to activation of MAPK and NF-kB pathways. Downstream signaling of NF-kB in turn leads to TNF production (29).

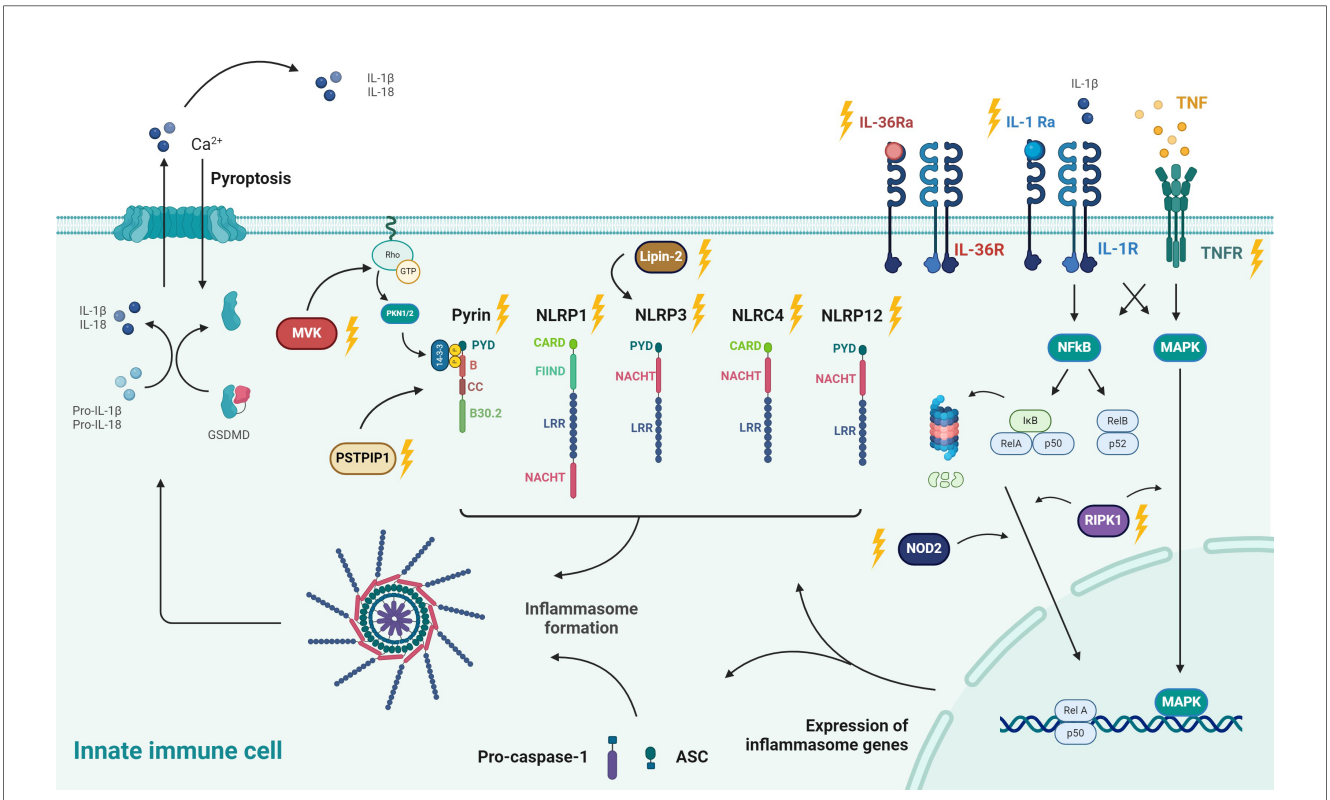

FIGURE 2
Pathophysiology of autoinflammatory diseases: pathways leading to inflammasome formation and their downstream effects, including pore formation (Gasdermin D; GSDMD), induction of pyroptosis, and secretion of IL-1β and IL-18. Different stimuli for specific inflammasomes (NLRP1, NLRP3, AIM,...) are shown. The proteins associated with heritable autoinflammatory conditions are denoted with yellow lightning bolts. These include MVK (associated with HIDS) and PSTPIP1 (PAPA syndrome) of which the pathways are reported to culminate in derailed Pyrin activity. Pyrin is regulated through Rho-GTPase via PNK1/PNK2. FMF is the most common Pyrin-associated autoinflammatory disease. Besides Pyrin, NLRP1, NLRP3, NLRP12 and NLRC4 are other inflammasomes, respectively associated with NLRP1 deficiency, CAPS, FCAS2, and NLRC4-MAS. Impaired function of Lipin-2, an indirect regulator of NLRP3, leads to Majeed syndrome. Deleterious IL1RN mutations underlie IL-1R antagonist deficiency (DIRA), which is the natural antagonist of the IL-1 receptor. Likewise, mutations in IL36N lead to a defective function of the IL-36 receptor antagonist and cause deficiency of IL-36R antagonist (DITRA). TRAPS is caused by mutations in the TNF receptor (TNFRSF1A) which affects NFκB as a master regulator of transcription of inflammasome-related proteins. Deficiency of RIPK1, a regulator of the NF-κB and MAPK pathways, lead to AIEFL. NOD2 is another protein that activates NFκB and of which deleterious variants underlie Blau syndrome. Created with Biorender.com. Adapted with permission from "Exploring the immune horizon: systemic inflammatory diseases in the era of SARS-CoV-2 and beyond." by Hoste L. (30). http://hdl.handle.net/1854/LU-01HFQA9EK0NY3S6HEKN4YD1C7P. The PhD thesis of Levi Hoste was published online by Ghent University – Department of Internal Medicine and Pediatrics under the license CC-BY. https://biblio.ugent.be/publication/01HFQA9EK0NY3S6HEKN4YD1C7P.

In TNF receptor–associated periodic syndrome (TRAPS), aberrant signaling of the mutant TNF receptor 1 leads to increased activation of MAPK and NF-κB pathways. Furthermore, defective autophagy of TNFR1 also triggers NLRP3 inflammasome activation (35, 36).

Another mechanism of action that calls for a detailed description are the "NFkBopathies" or relopathies, named after the central proteins RelA and RelB in the NF-κB pathway. The NF-kB complex is a central regulator of innate immune activation and is closely linked with the ubiquitination system (37). Activation of NF-κB is complex and can occur via multiple pathways, including the cytosolic NOD2 and the transmembrane IL-1 and TNF receptors. But also, ubiquitination, a post-translational modification process, has an important role in the positive regulation by stabilizing molecular complexes. Activation of NF-κB induces two major signaling pathways: the canonical pathway, which mainly consists of NF-κB1 p50 and RelA, and the non-canonical pathway, which contains NF-κB2 p52 and RelB (see Figure 2). After binding of TNF or IL-1 to their respective cell surface receptors TNFR1 and IL-1R, the canonical NF-κB pathway is activated by proteasomal degradation of the inhibitor of κB (IκB). After release of IκB, p50 and RelA translocate to the nucleus and induce transcription of inflammasome genes (38, 39).

Blau syndrome is an example of a relopathy. It is caused by heterozygous gain-of-function mutations in the NOD2 receptor, and characterized by arthritis, uveitis, and granulomatous dermatitis.

Upstream of the NF-κB pathway, deficiency of the IL-1-receptor antagonist (DIRA) leads to IL-1 hyperactivity and decreased NF-κB inhibition. The same mechanism can be observed in deficiency of the IL-36-receptor antagonist (DITRA), a member of the IL-1 family. Beyond signs of systemic inflammation, disorders of TNF/NF-κB activity exhibit a very diverse phenotype: oral and genital ulcers in haploinsufficiency of A20 (HA20), periorbital edema in TRAPS and granuloma formation in Blau syndrome. Since TNF plays a central role in pathophysiology, TNF inhibitors are the mainstay of the current therapy (13).

1.2.4 Undefined or non-Mendelian AID

Besides the monogenic AIDs outlined earlier, a significant number of patients exhibit non-Mendelian AID. They resemble AID but they lack any known monogenic defect so far. Examples are Behçet disease, systemic juvenile idiopathic arthritis (sJIA), periodic fever with aphthous stomatitis, pharyngitis, and adenitis syndrome (PFAPA), and syndrome of undifferentiated recurrent fever (SURF) (17, 33). PFAPA and SURF will be discussed in section 2.3.

2 Overview of pediatric autoinflammatory diseases

In this section, we will discuss autoinflammatory diseases that have an onset during childhood and typically manifest with recurrent episodes of fever. The systemic inflammation during flare-ups can manifest in many different organ systems, such as the serosa (pleural, pericardial or peritoneal), joints, skin or eyes. Our aim is to provide an overview and comparison of the characteristics of these diseases (see Tables 1, 2).

2.1 Defects affecting the inflammasome

2.1.1 Familial Mediterranean fever (FMF)

Familial Mediterranean Fever (FMF) is usually caused by homozygous or compound heterozygous mutations in the *MEFV* gene, with 30% of cases showing an autosomal dominant

TABLE 1 Overview of the demographic and main characteristics of recurrent fever syndromes.

Class	Disease	Typical age of onset	Sex ratio % (M/F)	Duration of episode	Time between episodes	Triggers
Defects affecting the inflammasome	FMF	1–7 years	60/40	1–3 days	2–4 weeks	Unspecific
	MKD	0–1 year	50/50	3–7 days	2–8 weeks, irregular, decreasing with age	Vaccination
	CAPS	0.1–5 years	50/50			
	NOMID	0–1 month		Chronically	Chronically	No trigger identified
	FCAS	0–5 years		<24 h	Chronically with diurnal pattern	Cold exposure
	MWS	0–5 years		1–3 days		No trigger identified
	NLRC4-MAS	1–2 month	50/50	>7 days	Variable	No trigger identified
	AIADK	1–50 years	55/45	2–10 days	3–6 weeks	Cold exposure
	AIADK	4–5 month	50/50	3–4 days		Unknown
	AIEFL	0–3 month		1–7 days	2–4 weeks	Unknown
Non-inflammasome related conditions	TRAPS	0–13 years	50/50	5–25 days	4 weeks	Unspecific
	PAPA	1–16 years	50/50	Variable		Minor trauma
	Majeed sy	1–2 years	50/50	1–4 days	2–4 weeks	No trigger identified
	DIRA	0–1 month	50/50	Chronically		Unknown
	DITRA	0–60 year	45/55	Chronically		Unspecific
Undef	PFAPA	0.5–5 year	60/40	2–7 days	2–8 weeks with "clockwork regularity"	No trigger identified
	SURF	1–35 years	40/60	3–5 days	4–6 weeks	No trigger identified

Undef, undefined; SURF, syndrome of undifferentiated recurrent fever.

TABLE 2 Overview of the clinical characteristics of recurrent fever syndromes.

	Disease	Oromucosal	Lnn	HSM	Serositis	Skin	Musculosketal	Gastro-intestinal	Eye	FTT	Others
Defects affecting the inflammasome	FMF	No	No	SM	Peritonitis, pleuritis, pericarditis	Erysipelas like rash	Monoarhritis, myalgia	Abdominal pain	Keratoconus	No	Vasculitis (HSP, PAN)
	MKD	Oral/genital ulcers, pharyngitis	Yes	HSM	No	Maculopapular rash	Arhtritis, arhtralgia, myalgia	Abdominal pain, diarrhea, vomiting	RP, cataract, conjunctivitis	No	
	CAPS										
	NOMID	No	Yes	No	No	Urticaria	Arthralgia, myalgia	No	OA, keratitis, uveitis, conjunctivitis	Yes	DD, seizures, SNHL
	FCAS									No	
	MWS									No	SNHL
	NLRC4 MAS	No		SM	No	Maculopapular, urticarial rashes	Arthralgia, myalgia	Abdominal pain, diarrhea	No	Yes	
	FCAS2	No	Yes	SM	No	Urticaria	Arthralgia, myalgia	Abdominal pain	Conjunctivitis	No	Headache, SNHL
	AIADK	No	No	HSM	No	Dyskeratosis	Oligoarthritis	No	No	Yes	
	AIEFL	Oral ulcers, tonsillitis	Yes	HSM	No	No	Arthralgia	Abdominal pain	No		Headache, hallucination
Non-inflammasome related conditions	TRAPS	No	Yes	yes	Peritonitis, pleuritis	Maculopapular, migratory rash	Arhralgia, myalgia, fasciitis	Abdominal pain, vomiting, diarrhea	conjunctivitis, PE	No	
	PAPA	No	No	No	No	Cystic acne, pyoderma gangrenosum	Arthritis	No	No	No	
	Majeed sy	No	No	HSM	No	Neutrophilic dermatosis	Bone pain and limb swelling	No	No	Yes	CRMO, flexion contractures
	DIRA	No	No	HSM	No	Pustulosis and pathergy	Bone pain and limb swelling	No	No	Yes	CRMO, skeletal abnormalities
	DITRA	No	No	No	No	Pustular psoriasis, acrodermatitis	No	No	No	No	
Undef	PFAPA	Pharyngitis, aphtous stomatitis	Yes	No	No	No	No	No	No	No	
	SURF	Oral ulcers, pharyngitis	Yes	HSM	No	Erythema	Arthralgia, arthritis, myalgia	Abdominal pain, vomiting, diarrhea	Periorbital edema, corneal erythema	No	Headache, malaise

CRMO, chronic recurrent multifocal osteomyelitis; DD, developmental delay; FTT, failure to thrive HM hepatomegaly HSM hepatosplenomegaly; HSP, Henoch Schönlein purpura; Lnn, lymphadenopathies; PAN, poly-arteritis nodosa; PE, periorbital edema; RP, retinitis pigmentose; SM, splenomegaly; SNHL, sensorineural hearing loss; SURF, syndrome of undifferentiated recurrent fever; Undef, undefined.

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inheritance pattern with incomplete penetrance (34). The *MEFV* gene encodes a protein called pyrin, which is mainly expressed in myeloid cells. As mentioned in section 1.2.2., gain-of-function of the pyrin inflammasome leads to increased IL-1 and IL-18 production, which in turn leads to systemic inflammation. The disease mainly affects people from the Mediterranean basin of Jewish, Turkish, Armenian or Arabic ancestry. However, the Mediterranean epidemiology of the disease is changing due to massive migration, making it more common in Europe, Japan, and North America (40). The prevalence in the general population is 1–5/10,000. In 90% of patients, disease manifestations start before the age of 20 (41). The typical inflammatory attack lasts less than 3 days and can be accompanied by high-grade fever, monoarthritis and serositis, causing abdominal pain (peritonitis) or thoracal pain (pleuritis or rarer pericarditis). However, children often present with subfebrile temperatures or no increase in temperature at all during the episodes (42). It has been reported that many FMF patients underwent a laparotomy or appendectomy because of severe abdominal pain mistakenly interpreted as an acute abdomen (43). Erysipelas-like erythema located on the lower extremities is a very common characteristic of FMF attacks. Furthermore, FMF is associated with a higher risk of vasculitis, including Henoch Schönlein purpura (HSP) and poly-arteritis nodosa (PAN) (40).

During attacks, an elevation of acute phase reactants in the blood (serum amyloid A (SAA), C-reactive protein (CRP) and leukocytosis) can be detected (40, 42). Urine analysis is usually normal but can show proteinuria (42%) or hematuria (13%) (44). The Tel-Hashomer diagnostic classification stipulates that 2 major criteria (recurrent fever and serositis, favorable response of colchicine, amyloidosis AA) or 1 major and 2 minor criteria (recurrent attacks of fever, erysipelas-like erythema, first-degree relative with FMF) must be present for diagnosis (45). For a diagnosis of FMF in children, the presence of two out of the following five criteria is sufficient: fever, abdominal pain, chest pain, arthritis, and a family history of FMF (46). These Yalçınkaya-Ozen criteria have been shown to be more sensitive for the pediatric population (47).

Daily colchicine is the mainstay of FMF management, usually resulting in complete remission or notable reduction in the severity and frequency of the attacks. In case of colchicine-resistance or intolerance, IL-1 inhibitors offer an effective add-on therapy. According to the European Alliance of Associations for Rheumatology (EULAR) the goal of the treatment of FMF is to reach complete control of unprovoked attacks and to minimize subclinical inflammation in between flares. During attacks, non-steroidal anti-inflammatory drugs (NSAIDs) can relieve symptoms. Colchicine has also been proven to be efficient in preventing renal amyloidosis, a life-threatening long-term complication of FMF (48).

2.1.2 Mevalonate kinase deficiency (MKD)

Mevalonate kinase deficiency (MKD) is a very rare autosomal recessive disease caused by mutations in the *MVK* gene. This gene encodes mevalonate kinase, mutations cause a decreased function of the enzyme. In the autoinflammatory disease, Hyper IgD Syndrome (HIDS), approximately 10% residual activity ought to be preserved, whereas in the rarer and more severe metabolic phenotype, mevalonic aciduria (MA), enzyme activity is less than 1%. Because of the scope of this review, we will focus on HIDS.

The majority of HIDS patients are of European ancestry. Disease flares are accompanied by a variety of symptoms. Almost all patients suffer from cervical lymphadenopathy and gastrointestinal complaints such as abdominal pain, diarrhea and vomiting. Mucocutaneous symptoms include aphthous ulcers (60%), maculopapular rash (40%) and pharyngitis (30%). Other frequent manifestations are arthritis or arthralgia of the large peripheral joints (70%), myalgia (40%), splenomegaly (30%) and eye inflammation (15%). In a few cases, an association with macrophage activation syndrome (MAS) has been observed (49, 50). The classification criteria as formulated by the EULAR are based on the confirmatory MVK genotype and include at least one among the following: gastrointestinal symptoms, cervical lymphadenitis or aphthous stomatitis (51). These classification criteria are used to differentiate similar AIDs, but are not validated for diagnostic use. Historically, increased serum IgD was part of the diagnostic criteria for MKD. Because of the lack in sensitivity and specificity, the use of serum IgD as a diagnostic marker is no longer encouraged (49, 52). An alternative diagnostic test is the measurement of urinary mevalonic acid during disease flares (50). Jeyaratnam and colleagues established that urinary increase of mevalonic acid had a 92% sensitivity and 90% specificity in their retrospective cohort. They conclude that MKD appears unlikely in patients with normal mevalonic acid excretion, but it cannot be entirely ruled out (11). Therapeutic management is focused on targeting the downstream cytokine pathways by blocking IL-1 signaling with biologicals, such as anakinra, an IL-1 receptor antagonist and canakinumab, a monoclonal antibody against IL-1β (49, 53).

2.1.3 Cryopyrin-associated periodic syndrome (CAPS)

Cryopyrin-Associated Periodic Syndrome (CAPS), now referred to as NLRP3-associated autoinflammatory disease (NLRP3-AID), was historically described in terms of three distinct clinical entities: Familial Cold Autoinflammatory Syndrome (FCAS), Muckle-Wells Syndrome (MWS) and Chronic Infantile Neurologic Cutaneous Articular syndrome (CINCA), alternatively called Neonatal Onset Multisystem Inflammatory Disease (NOMID). These different presentations of cryopyrin or NLRP3-inflammasomopathies represent a clinical spectrum with multiple shared features but increasing severity (11). Their combined prevalence is estimated at around 1/360,000 (54). NLRP3-AID is caused by heterozygous, gain-of-function mutations in the *NLRP3* gene and results in the overactivation of the cryopyrin inflammasome. NLRP3-AID generally present with low-grade fever, urticarial rash and conjunctivitis. Shared symptoms furthermore include lymphadenopathy, arthralgia and myalgia. Fever is a common clinical sign, but as an isolated feature is usually less debilitating to patients, because measured body temperature does not always

reach 38.3°C. Daily symptoms of flu-like malaise and chronic severe fatigue are reported by patients affected by FCAS and MWS. Although symptoms among patients can differ, they tend to be consistent in the same individual (55). Common complications of MWS and NOMID are neurosensorial hearing loss and AA amyloidosis (30%). Central nervous system symptoms, such as developmental delay and seizures, seen in many CINCA/NOMID patients, are a result of chronic sterile meningitis and elevated intracranial pressure (54, 56). There is a clear influence of the circadian rhythm with worsening of symptoms in the afternoon and evening. Generalized cold exposure is a pathognomonic trigger of complaints in FCAS. The diagnosis of NLRP3-AID can be made if raised acute phase reactants (CRP/SAA) can be detected in combination with at least two of the following clinical symptoms: urticarial-like rash (neutrophilic dermatitis), cold/stress-triggered episodes, sensorineural hearing loss, musculoskeletal symptoms (arthritis, arthralgia or myalgia), chronic aseptic meningitis and skeletal abnormalities (epiphyseal overgrowth, frontal bossing), even in absence of genetic confirmation (57). More recently, Gattorno et al. proposed classification criteria to differentiate NLRP3-AID. In the presence of a pathogenic or likely pathogenic *NLRP3* genetic variant, only one of the following symptoms are needed for NLRP3-AID classification: urticarial rash, red eye (conjunctivitis, episcleritis, uveitis) or neurosensorial hearing loss (51). Genetic evaluation is often inconclusive in NLRP3-AID. The presence of variants of uncertain significance (VUS) in the NLRP3 gene, such as V198M, R488K, and Q703K, cannot confirm the diagnosis of NLRP3-AID. These variants, also known as low-penetrance variants, have been described in asymptomatic individuals but may contribute to an NLRP3-AID phenotype in affected carriers, exhibiting both typical and atypical symptoms, such as gastrointestinal symptoms (58). Standard of care for patients with NLRP3-AID consists of IL-1 blockade, and most patients respond well when medication is dosed adequately. In some cases, higher dosages are required for optimal effect (13, 49, 59). Recently, Cosson et al. proposed a new classification system based on the measurement of cytokines release and cell death in a broad range of different mutant *NLRP3* variants. Functional studies revealed a phenotype-genotype correlation with possible specific therapeutic targets in the different groups (60).

2.1.4 NLRC4—macrophage activating syndrome (MAS)

The NLRC4 inflammasome-related pathology is caused by a gain-of-function mutation in the gene encoding for NLR-family CARD domain-containing protein 4 (NLRC4). Patients display recurrent, life-threatening episodes of autoinflammation and infantile enterocolitis (AIFEC) (61, 62). The phenotype of AIFEC includes fever and severe enterocolitis, frequently leading to failure-to-thrive and requiring parenteral nutrition. In some instances, a maculopapular or urticarial rash can be observed. Inflammatory episodes feature macrophage activation and cytotoxic T-cell dysfunction, resembling macrophage activation syndrome (MAS), often presenting early in life. Blood levels of IL-1β and IFNγ are typically upregulated, and an extremely high IL-18 can be measured, differentiating AIFEC from hemophagocytic lymphohistiocytosis (HLH). The trigger for flares is unknown so far. AIFEC can be considered a chronic inflammatory disease. This explains why elevated levels of acute phase reactants (e.g., IL-18) can also be observed in between episodes (63). Treatment with anakinra has been shown to reduce MAS episodes, induce biochemical resolution and lessen the need for systemic corticosteroids (64).

2.1.5 Familial cold autoinflammatory syndrome-2 (FCAS2)

Familial cold autoinflammatory syndrome-2 (FCAS2) is an autosomal dominant disease caused by mutations in the NLRP12 protein, a regulator of NF-κB and caspase-1 activity (65). Besides fever, the most prominent clinical features include urticaria, myalgia, arthritis, abdominal pain and headache. These symptoms are highly similar to the previously described FCAS, an NLRP3-inflammasomopathy. The following features only present in FCAS2, but are rarely observed: sensorineural hearing loss, lymphadenopathies, splenomegaly and thoracic pain. Onset of symptoms in FCAS2 occurs mainly during childhood (70%) and is induced by cold exposure (66). Corticosteroids can shorten fever intensity and duration but are unable to completely control disease manifestations. IL-1 blockade has been shown to be a successful treatment in a few cases (67).

2.1.6 Autoinflammation with arthritis and dyskeratosis (AIADK)

Only three cases have been described in literature of patients with autoinflammation with arthritis and dyskeratosis (AIADK). These patients showed early onset recurrent fever and disseminated dyskeratosis of the skin. During childhood, patients developed oligoarthritis, and signs of autoimmunity with positive antinuclear antibodies (2/3) and autoimmune hemolytic anemia (1/3). Vitamin A deficiency was documented in all patients, although supplementation did not affect disease evolution. Mutations within the *NLRP1* gene have been identified as the etiological basis for the observed clinical phenotype. The exact pathophysiology remains to be unraveled, but increased caspase-1, IL-18 and IL-1β secretion clearly showed enhanced inflammasome activation (68, 69). A good response to anakinra has been reported in one patient (69).

2.1.7 Autoinflammation with episodic fever and lymphadenopathy (AIEFL)

Autoinflammation with episodic fever and lymphadenopathy (AIEFL) is an autosomal dominant disorder caused by RIPK1 deficiency, an important regulator of necroptosis and the NF-κB and MAPK inflammatory pathways. Episodes of high grade fever recur every 2–4 weeks and have an onset in early infancy. The flares are accompanied by painful lymphadenopathy in the cervical, axillary, inguinal, and/or periaortic region. Additional symptoms can include hepatosplenomegaly, tonsillitis and aphthous ulcers. Patients furthermore report headaches, and/or hallucinations that coincided with their fevers. AIEFL shares many characteristics with another clinical syndrome, periodic

fever, aphthous stomatitis, pharyngitis, and cervical adenitis (PFAPA), but differs in that AIEFL patients have an earlier age of onset, lymphadenopathy that extends beyond the cervical region, and often experience hepatosplenomegaly or arthralgia (70, 71). Patients showed clinical improvement after treatment initiation of tocilizumab, a monoclonal antibody against the IL-6 receptor (71, 72).

2.2 Non-inflammasome related conditions

2.2.1 TNF receptor-associated periodic syndrome (TRAPS)

The TNF receptor-associated periodic syndrome (TRAPS), first named Familial Hibernian Fever (FHF), is an autosomal dominant disease caused by heterozygous, usually missense, mutations in the *TNFRSF1A* gene with poor genotype-phenotype (8). This condition is uncommon, affecting approximately 1 or 2 individuals per million. It typically manifests in childhood median onset at 4.3 years (0–13 years) and affects both sexes equally (73). TRAPS exhibits a range of symptoms, including fever, signs of serositis such as peritonitis and pleuritis, and a migrating maculopapular rash. Although fever is a typical sign, patients exhibit only subfebrile temperatures in 30% of disease flares. Patients typically complain of myalgia located beneath the areas affected by the rash. Eye symptoms may involve conjunctivitis and periorbital edema. Headaches and lymphadenopathies occur less frequently. The duration of disease flares varies greatly between individuals, but tends to be longer compared to other diseases discussed in this paper. This causes a significant disease burden, with an average of 70 symptomatic days per year (74). Many different diagnostic and classification criteria have been proposed. Most recently, Gattorno and colleagues combined molecular and clinical features in their classification criteria, i.e., presence of a confirmatory TNFRSF1A mutation and at least one among the following: duration of episodes ≥7 days, myalgia, migratory rash, periorbital oedema or relatives affected. In the absence of the identification of a pathogenic (or likely pathogenic) variant at least two items need to be present (51).

NSAIDs alleviate symptoms and can be used as supportive medication, but corticosteroids are needed to achieve complete remission of a flare. Unfortunately, the effect of corticosteroids tends to decline over time. Other therapeutic options include IL-1 blockade (canakinumab) and a TNF inhibitor (etanercept) (49, 59, 75).

2.2.2 Pyogenic arthritis, pyoderma gangrenosum and acne (PAPA) syndrome

The Pyogenic Arthritis, Pyoderma gangrenosum and Acne (PAPA) syndrome is an autosomal dominant disorder with incomplete penetrance caused by mutations in *PSTPIP1* (76). Although it has been stipulated that PSTPIP1 directly interacts with pyrin, the exact disease mechanism has not been clarified. The classical clinical triad consists of recurrent episodes of purulent but sterile arthritis, cystic acne and difficult treated pyoderma gangrenosum. Fever occurs inconsistently. The PAPA syndrome has a variable expression; affected patients may show only one or two of the cardinal features. PAPA manifests in patients aged 1 to 16-years-old, featuring recurrent episodes of oligoarticular arthritis, typically triggered by minor trauma and signs of systemic inflammation (77). PASH, characterized by pyoderma gangrenosum, acne, and suppurative hidradenitis, is a related condition (78).

The management of this uncommon AID relies on empirical approaches, tailored to each individual case. High doses of corticosteroids are often inadequate for symptom control, especially to treat skin manifestations. A recent review showed promising outcomes in PAPA patients treated with anakinra and canakinumab (79).

2.2.3 Majeed syndrome

Majeed syndrome is an autosomal recessive autoinflammatory disease caused by mutations in *LPIN2*, which encodes for Lipin-2. Lipin-2 is a regulatory protein and indirectly limits the activation of the NLRP3 inflammasome (80). Patients present with chronic recurrent multifocal osteomyelitis (CRMO), congenital dyserythropoietic anemia, and sometimes neutrophilic dermatosis (81). The recurrent episodes of bone pain caused by CRMO of the long bones and often close to the joints can be accompanied by hepatosplenomegaly in 30% and fever in half of the patients. In severe cases, failure to thrive, growth delay and flexion contractures can occur. The complete blood count shows microcytic anemia and sometimes neutropenia (13%). The severity of the anemia can differ, with around 25% requiring regular erythrocyte transfusions. Patients respond well to IL-1 blockade (82).

2.2.4 Deficiency of the IL-1 receptor antagonist (DIRA)

Deficiency of the IL-1 receptor antagonist (DIRA) is a potentially life-threatening autoinflammatory disease that results from autosomal recessive mutations in het *IL1RN* gene, leading to absence or defective production of the IL-1 receptor antagonist (IL-1RA). This causes unopposed amplification of the proinflammatory effect of IL-1β and IL-1α (83). Affected children exhibit symptoms within the first weeks of life characterized by CRMO, cutaneous pustulosis, and signs of systemic inflammation. Fever may not always be present. The sterile osteomyelitis typically involves the long bones, like in Majeed syndrome, but also vertebrae, ribs and clavicles. The phenotype is variable and can include failure to thrive, hepatosplenomegaly, lung disease, nail changes, diverse skeletal abnormalities (widening of ribs, periosteal reaction, vertebral fusion), eye symptoms (episcleritis, conjunctivitis), vasculitis and venous thrombosis (84). Patients respond extremely well to treatment with anakinra, the synthetic form of IL-1RA) (85).

2.2.5 Deficiency of the IL-36-receptor antagonist (DITRA)

Deficiency of the IL-36-receptor antagonist (DITRA) is an autosomal recessive disorder characterized by episodes of high-grade fever, generalized rash, and disseminated pustules. The

pathophysiology of DITRA shows similarities with DIRA (section 2.2.4): biallelic mutations in the *IL36N* gene lead to a defective IL-36 receptor antagonist (IL-36RA), a member of the IL-1 family, and causes uncontrolled action of the proinflammatory IL-36 family members (IL-36α, IL36β and IL36γ) (86). The age of onset varies greatly, from 1 month to 83 years old. The patients typically show generalized pustular psoriasis (GPP) or acrodermatitis continua of Hallopeau (ACH), two subtypes of pustular psoriasis, during the recurrent episodes of systemic inflammation (87).

Management of DITRA is complex, and often involves multiple immunosuppressants. Biologicals such as TNFα, IL-17 and IL-12/23 inhibitors have been shown to be superior to IL-1 inhibitors. Recently, a targeted treatment against the IL-36 receptor (spesolimab) led to promising results in a few cases (88, 89).

2.3 Undefined or non-Mendelian AID

2.3.1 Periodic fever with aphthous stomatitis, pharyngitis, and adenitis (PFAPA) syndrome

PFAPA, first described in 1986 by Marshall et al., was initially defined as episodic attacks of fever involving one of the three main symptoms: pharyngitis, cervical adenitis and aphthous stomatitis (90). In regions where FMF is not endemic, PFAPA is considered to be the most common periodic fever syndrome in children. While it was first hypothesized to be a monogenic disease, further research showed that the development of the disease is determined by a complex interplay of polygenetic predisposition and environmental factors. *IL10*, *STAT4*, *CCR1-3* and especially *IL12A* were identified as risk variants for developing PFAPA (91). Serum caspase-1 levels and IL-1 have been found to be increased during disease flares, which is consistent with the presumed inflammasome mediated origin of disease. However, many studies have shown inconsistent or conflicting findings for these and other cytokines (92). The episodes have an onset during the first years of life and usually disappear in adolescence. They typically present with a clockwork regularity every 4–6 weeks (93, 94). During the intercritical period, there is a general well-being with normal growth and development (70). PFAPA is diagnosed through exclusion, as it shares many symptoms with other AIDs. Eurofever/PRINTO clinical criteria require at least seven out of the following eight criteria to be fulfilled for classification: presence of pharyngotonsillitis, 3–6 days' duration of episodes, cervical lymphadenitis, periodicity and absence of diarrhea, chest pain, skin rash, arthritis (51, 70). Although no monogenic defect was found underlying this AID, heterozygous *MEFV* gene variants have been shown to act as disease modifiers. PFAPA patients with *MEFV* variants present with shorter recurrent fever episodes and a later age of onset (95). An important differential diagnosis to consider is cyclic neutropenia. Because, just like PFAPA, cyclic neutropenia presents with recurrent episodes of fever, aphthous stomatitis, and sometimes cervical adenopathies. Unlike in the case of PFAPA though, the neutrophile counts drop to below 500/μl for 3–5 days.

NSAIDs can be used for symptomatic relief but corticosteroids are the mainstay of treatment during attacks. Because of the rapid effect of a single dose of Prednisolone on the fever, this treatment can be considered as a diagnostic criterium. Only a minority of patients require a second dose. Corticosteroids may have the side effect of increasing the frequency of episodes (96). The interplay between PFAPA and FMF is also evident in their treatment, as colchicine has proven to be effective in reducing the number of attacks in patients with PFAPA. Colchicine is mainly used as prophylaxis in patients with frequent flares. Cimetidine, an H2-receptorantagonist with mild immunomodulating properties, is a second, less frequently used option for prophylaxis (97). The role of tonsillectomy in treating PFAPA remains controversial (91, 98). It has been reported to prevent recurrences in two-thirds of patients, although a recent Cochrane review concluded that the evidence, based on studies with small sample sizes, is of moderate quality. Given the risks associated with surgery, no firm conclusions can be drawn about its place in PFAPA treatment (97, 99, 100). In order to reduce the variation in treatment strategies, the Childhood Arthritis and Rheumatology Research Alliance (CARRA) published the Consensus treatment plan (CTP) for PFAPA in 2020 (100). The CTP defines the four different treatment approaches discussed above: (1) antipyretics, (2) corticosteroids, (3) prophylaxis (colchicine/cimetidine), and (4) surgery. Their aim to use this four-arm CTP to streamline future studies.

2.3.2 Syndrome of undifferentiated recurrent fever (SURF)

A significant number of patients under follow-up for recurrent fever do not fit the profile of the previously described monogenic autoinflammatory diseases, nor do they fulfill the diagnostic criteria of PFAPA syndrome. This heterogenous group is characterized by self-limiting episodes of systemic inflammation with diverse clinical presentations in absence of a confirmed molecular diagnosis and it has been variably referred to as atypical PFAPA, undefined systemic autoinflammatory disease (uSAID), or syndrome of undifferentiated recurrent fever (SURF) (101, 102). In what follows, we will refer to this group as SURF.

The most commonly reported symptoms during disease flares of SURF are fever, malaise, abdominal pain, arthralgia, myalgia and eye manifestations (periorbital edema and/or corneal erythema) (103, 104). Papa and colleagues proposed a preliminary case definition for patients with a clinical suspicion of SURF: they define as mandatory features recurrent fever with elevated inflammatory markers (at least three similar periods in 6 months) and negative PFAPA criteria, and genotype for other SAIDs. Additional supporting features are monthly attacks lasting 3–5 days accompanied by fatigue/malaise, abdominal pain, arthralgia/myalgia, eye manifestations and a favorable response to treatment with colchicine/anti-IL1 (103). A recent study by Vyzgha and colleagues illustrates the diagnostic challenges clinicians face, demonstrating a potential overlap between PFAPA and SURF. Data of patients with PFAPA, SURF and uSAID were analyzed from three international registries (Eurofever, JIRcohort and AID-net). Part of the population diagnosed as SURF (26%–37%) met the

criteria for PFAPA. Likewise 8%–17% of PFAPA patients fulfilled the preliminary case definition for SURF. This clinical heterogeneity highlights the complexity of classifying patients with recurrent fevers and underscores the need for updated definitions to differentiate SAIDs, in order to optimize treatment strategies (105).

In contrast to PFAPA, SURF patients will rarely show a favorable response to tonsillectomy or on-demand steroid therapy (105). The majority of SURF patients exhibit a complete and consistent response to colchicine. In more severe or therapy resistant cases, anti-interleukin (IL)-1 treatment (mainly anakinra) is the most effective and frequently used alternative (103, 106).

3 Discussion: diagnostic work-up

As for other rare diseases, the diagnostic process of autoinflammatory diseases (AIDs) can be difficult. First and foremost, it is key that autoinflammation is considered in the differential diagnosis of children presenting with fever of unknown origin, particularly in patients with recurrent episodes of systemic inflammation at an early age with atypical associated symptoms. Because the diagnosis relies to a great extent on clinical suspicion, earlier studies have shown that there is a median diagnostic delay of 7.3 years. And, although there has been a noticeable overall trend towards improvement in recent years, patients generally still have to wait for a median time of 5 years before a diagnosis is established (107, 108). Flare-ups significantly impact patients' quality of life, while chronic subclinical inflammation can also contribute to substantial morbidity and mortality. In most autoinflammatory syndromes an adequate treatment is available to control the severity and frequency of attacks and to prevent long-term sequelae (like AA amyloidosis). For these reasons, it is necessary to improve early recognition and diagnosis in this patient population. Table 3 provides an overview of red flags indicating when to suspect autoinflammation in a pediatric patient.

To guide the clinician in this diagnostic challenge, we designed a diagnostic algorithm (see Figure 3). In a first step, more common causes for systemic inflammation, such as infectious, autoimmune diseases or malignancies have to be ruled out (109). Subsequently, AID episodes (duration, interval) and the accompanying symptoms should be listed in a diary to identify a pattern. In combination with the age of onset and possible triggers of the emergence of fever, the number of potential diagnoses can be narrowed down. An overview of those demographical and clinical parameters can be found in Tables 1, 2. It is necessary to always consider family history and ethnicity. As autoinflammatory diseases present with systemic symptoms, a multidisciplinary work-up is necessary, including consultations with the ophthalmologist to screen for (asymptomatic) uveitis, conjunctivitis, periorbital edema, or retinitis pigmentosa, and the dermatologist to evaluate skin lesions, and if necessary, perform a skin biopsy.

In the following step, documentation of inflammation is needed. To objectively assess systemic inflammation, laboratory measurements can be used during disease flares. These include complete blood count (CBC), white blood cell differentiation and the measurement of acute-phase reactants such as C-reactive protein (CRP), ferritin, serum amyloid A (SAA), and erythrocyte sedimentation rate (ESR), or other biomarkers associated with complement, coagulation, and fibrinolysis systems. Urine analysis can be helpful for the detection of mevalonic acid and repeated urine sampling is necessary during a febrile episode. To evaluate subclinical, chronic inflammation, an assessment is required during the asymptomatic period. This includes repeated blood sampling, evaluation for hepato- and/or splenomegaly by ultrasound, as well as a urine analysis, to screen for proteinuria to detect renal AA amyloidosis.

Immunomodulatory therapy can also serve as a diagnostic criterium, with corticosteroids being administered in suspected cases of PFAPA or colchicine in suspicion of FMF or SURF.

Routine lab tests are usually significantly up- or downregulated during the acute episodes, but they have the disadvantage of lacking specificity. Therefore, comprehensive testing modalities, illustrated in step 4, have been developed in research settings in order to enhance the recognition of underlying diagnoses. One of the methods that holds the promise of making additional distinctions to differentiate the AIDs, where routine lab measures fail to do so, is cytokine profiling. The most common technique to quantify cytokines are enzyme-linked immunosorbent assay (ELISA)-based methods (110). The cytokines that are best known in the context of autoinflammatory diseases are IL-1, IL-18 and TNF (see Figure 2). Although cytokines are signaling molecules that can have many different functions depending on the context, IL-1, IL-18 and TNF are relatively specific markers for autoinflammation. Since the cytokine release in inflammasome activation is one of the key drivers of disease, it can also be a target for therapy and disease monitoring. An example of this targeted therapy can be seen in the management of NLRP3-AID. Indeed, IL-1 blockade, via canakinumab, a monoclonal antibody against IL-1β, and anakinra, a recombinant IL-1 receptor antagonist (IL-1Ra), have proven to be very effective (111). The type I interferonopathies are linked with an increased serum level of IFNα/β, which are also examples of potent pro-inflammatory cytokines. Because the direct detection of type I interferons is difficult to measure, quantification of IP-10 can be a valuable

TABLE 3 Red flags: when to suspect autoinflammation in a pediatric patient?

Red flags
• Recurrent fevers without apparent infectious causes
• Periodic fevers with a predictable pattern
• Poor response to antibiotics
• Mouth or genital ulcers
• Lymphadenopathies
• Signs of serositis or arthritis
• Unexplained rashes, especially in association with fever episodes
• Elevated inflammatory markers (e.g., CRP, ESR) during and between episodes
• Family history of autoinflammatory disorders
• Ethnic background associated with certain autoinflammatory diseases (e.g., Mediterranean descent for Familial Mediterranean Fever)
• Young age at the onset of disease flares
• Failure to thrive

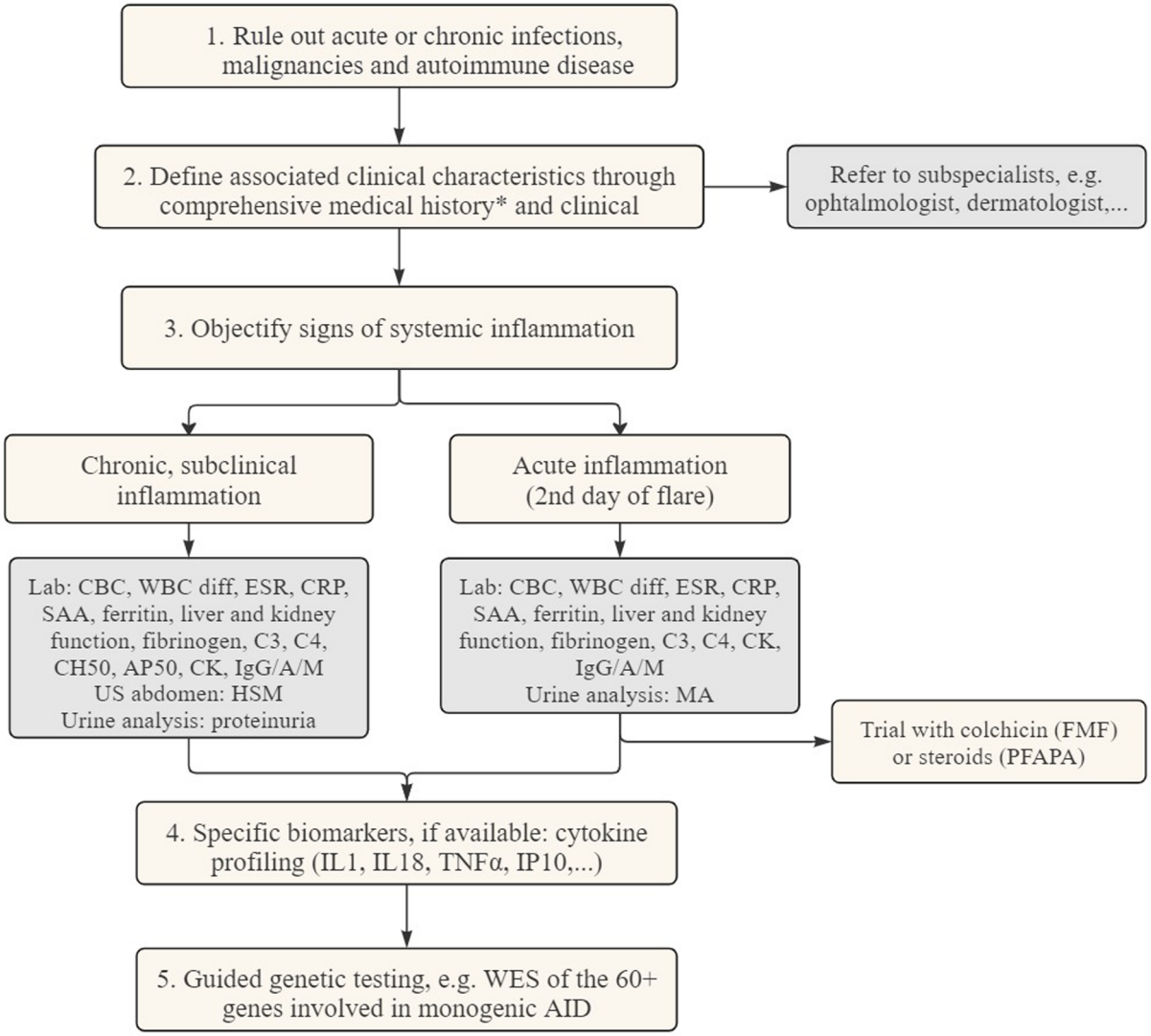

FIGURE 3
Diagnostic flowchart for patients suspected of autoinflammatory diseases. CBC complete blood count CK creatine kinase CRP c-reactive protein ESR erythrocyte sedimentation rate HSM hepatosplenomegaly MA mevalonic acid SAA serum amyloid A WBC diff white blood cell differentiation WES whole exome sequencing. *Medical history: duration of flares, time between episodes, possible triggers, ethnicity, associated symptoms, failure to thrive.

alternative. IFN-γ-inducible protein 10 (IP-10) is both induced by type I and type II interferons. Measuring cytokines can be challenging for different reasons: they are present in low concentrations (range of pg/ml), have short half-lives and are very sensitive to pre-analytical variations. Indirect methods to uncover cytokine signatures have been developed, of which the interferon stimulated genes (ISG) scores is best validated. The ISG score consists of a qPCR test that evaluates the expression of specific genes targeted by type I IFNs. Although ISG score testing may lead to false positives due to viral infections (especially in the pediatric population), experience clearly demonstrates that they are helpful in the further elaboration of type I interferonopathies (19, 112). Sönmez HE and colleagues developed a clinical score that allows clinicians to identify patients with a high suspicion of interferonopathy. These patients may benefit from a further work-up, including the performance of ISG score and cytokine profiling (113).

A final important question to address is the role of genetic testing in the diagnostic work-up. There is a consensus that patients with suggestive clinical symptoms should be genetically investigated (114). Although each single disease has a very low occurrence rate, the diagnostic yield of combined gene panels increases year by year. A study in 2022 by Le Goueff et al. demonstrated a diagnostic rate of 23% with a gene panel consisting of 502 genes (115). Next-generation sequencing (NGS) is now broadly used and has replaced the "gene by gene" method of Sanger sequencing (75). The *Infevers* database is a valuable source of information about all sequenced variants associated with AIDs that can be used by geneticists and clinicians (116). The European Molecular Genetics Quality Network Guidelines put forward a genetic diagnosis of

monogenic autoinflammatory diseases in 2020 (117). The interpretation of a genetic variant is full of possible pitfalls due to polymorphisms and incomplete penetrance. Evaluating variants of unknown significance (VUS) and estimating their clinical implications can be challenging. Therefore, it is essential to call upon an experienced team of genetics and clinicians to evaluate the results of genetic testing (114).

4 Conclusion

Pediatric autoinflammatory diseases are characterized by recurrent flares of symptomatic systemic inflammation and chronical subclinical inflammation. In this review we summarize the pathophysiology, clinical features, diagnosis and treatment of the most common autoinflammatory diseases with an onset in childhood. We focus in particular on the diagnostic work-up of recurrent fever in children with suspicion of autoinflammation. Even though AIDs are rare, the number of diseases has been rapidly expanding during the past 20 years. By unraveling the pathophysiology of these immune disorders, additional insights can be gained into innate immune regulation and the complex interplay between innate and adaptive immunity, which in turn can improve the treatment of inborn errors of immunity in general. Even in an era of high throughput genetic screening, there is still significant diagnostic delay for patients living with an AID, having major impacts on quality of life and leading to additional morbidity and mortality. After exclusion of infection, autoimmunity and malignancies, it is therefore important to consider autoinflammation in the differential diagnosis of patients presenting with recurrent fever. This overview of AIDs with an onset in childhood can be a useful tool for clinicians and facilitate early diagnosis and treatment of these rare diseases.

Author contributions

BM: Conceptualization, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. LH: Conceptualization, Investigation, Methodology, Supervision, Visualization, Writing – review & editing. ST: Investigation, Methodology, Supervision, Writing – review & editing. FH: Conceptualization, Investigation, Methodology, Project administration, Supervision, Visualization, Writing – review & editing.

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Conflict of interest

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Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2024.1448176/full#supplementary-material

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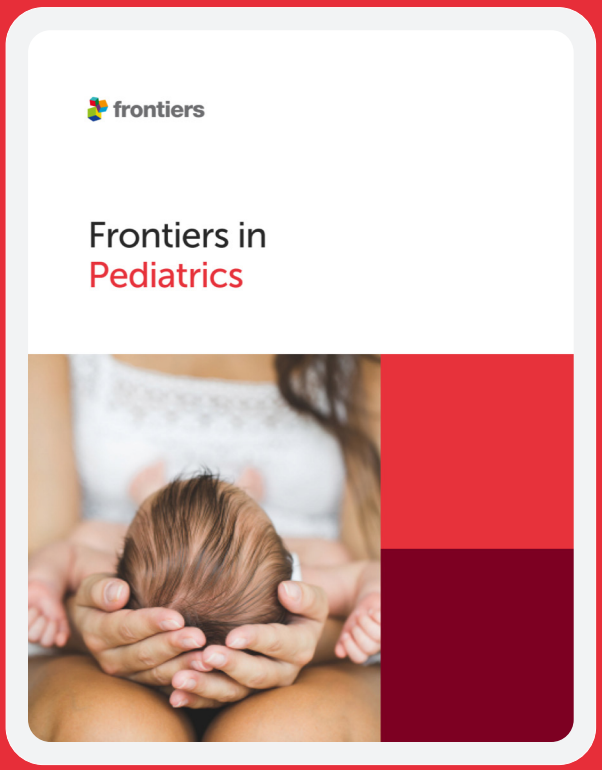

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