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Advances in primary immunodeficiencies (inborn errors of immunity) in central-eastern europe volume II

Edited by
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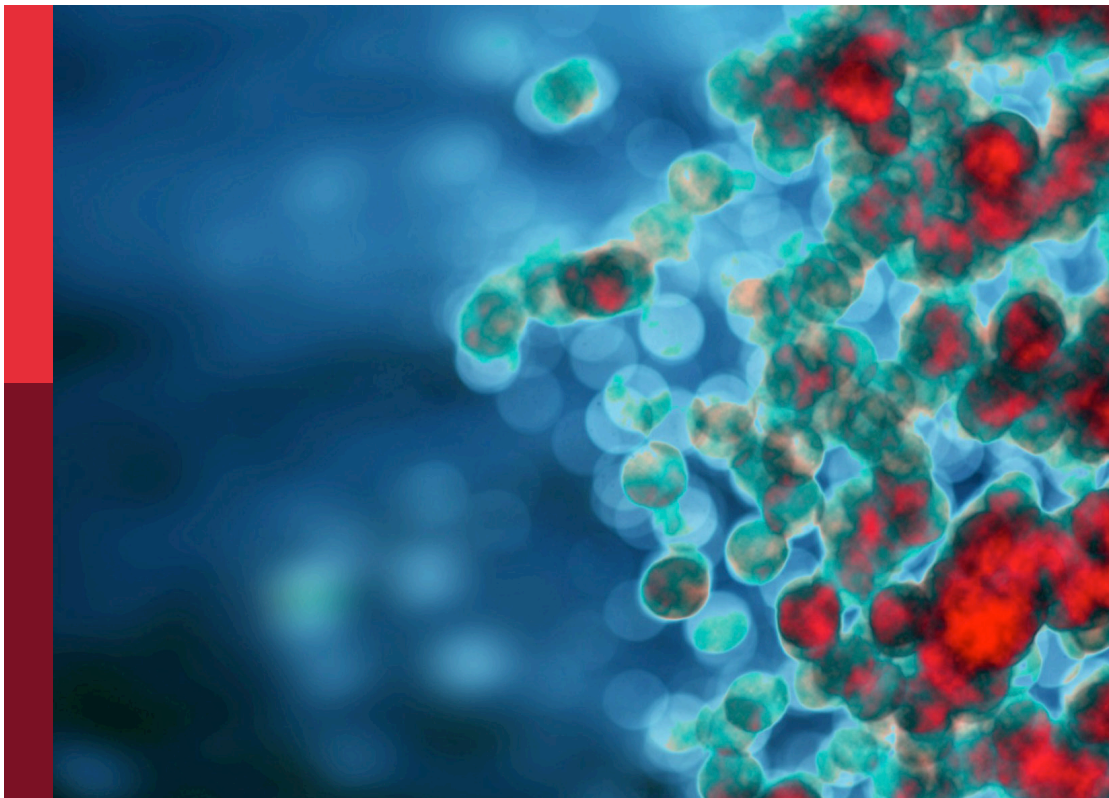

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Advances in primary immunodeficiencies (inborn errors of immunity) in central-eastern europe: Volume II

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Editorial: Advances in primary immunodeficiencies (inborn errors of immunity) in Central-Eastern Europe, volume II

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KEYWORDS

SCID (severe combined immunodeficiency), inborn error immunity, primary immunodeficiencies, antibody deficiency, newborn screen (NBS), JProject, education, awareness

Editorial on the Research Topic
Advances in primary immunodeficiencies (inborn errors of immunity) in Central-Eastern Europe, volume II

Knowledge of Primary Immunodeficiencies (PIDs) or Inborn Errors of Immunity (IEI) has expanded exponentially during the last 20 years. New and improved diagnostic tools, including new generation sequencing (NGS), and gene product activity or functional tests have led to profound advances in clinical immunology. These advances have made it easier to understand the mechanisms and pathogenesis of different types of IEI, and subsequently to introduce targeted therapies. They help define and recognize the increasing number of IEIs. It is worth noting the tremendous role of the International Union of Immunological Societies (IUIS) and its Expert Committee in updating the categorization of IEI approximately every 2 years. According to the last report in 2022, 485 different genetic defects causing IEI are known (55 novel compared to the 2019 report), previously categorized into 10 groups and published as phenotypical classification as well. Taking into account that number of all IEIs remains underestimated, the efforts of enthusiastic clinicians and scientists to improve diagnostics, recognition, and treatment are of great value (1–3). Much of this progress in the field of clinical and experimental immunology has been made in recent decades, mainly in western Europe, the USA, Japan, and Australia (1–5), with significant contributions also coming from Latin America, Africa, the Middle East, and both South and East Asia. Eastern and Central European (ECE) countries were isolated for many years, until the fall of the iron curtain in 1989, with limited access to the newest scientific achievements, diagnostic tools, and therapeutic methods. Only personal

connections, and direct collaboration with clinical and research centers in Western Europe and the US, made some progress possible in that region. Within the last 30 years, there has been a great deal of effort to overcome the gap between ECE and Western Europe in terms of IEI diagnostics, including molecular tests, treatment, and education. One of the most important initiatives was the J Project, initiated in 2004 by clinicians and scientists in Eastern and Central Europe. The goal of the JProject (JP) was to increase awareness, facilitate diagnosis including genetic tests, and improve therapy according to the latest knowledge in the area of the ECE region. In subsequent years, collaboration expanded to include "daughter J Projects" in countries such as Turkey, Iran, Egypt, Russia, and others (5–10).

Between the end of 2019 and 2020, we successfully published 11 manuscripts as an e-book dedicated to "*Advances in Primary Immunodeficiencies (Inborn Errors of Immunity) in Central-Eastern Europe*", covering the results of clinical and scientific work in the separate countries in the region as well the effects of the JP network (11). One year later we decided to edit the second volume of a special Research Topic on "*Advances in Primary Immunodeficiencies (Inborn Errors of Immunity) in Central-Eastern Europe*" to expose the successful efforts of single immunological centers or countries as well as the effects of scientific collaboration within the ECE region and western Europe and/or the US in the field of IEI. An invitation was sent by the Editors of Frontiers in Immunology to our colleagues from ECE and JProject collaborative immunological centers to submit original research articles, commentaries, opinion, and review articles resulting from the mentioned collaboration and documenting experiences covering areas such as the molecular defects of PIDs, achievements in diagnostics, the clinical characteristics of different PIDs, region-specific PIDs, the current treatment of different PIDs with immunoglobulin replacement therapy (IgRT), hematopoietic stem cell transplantation (HSCT), and biological treatment of autoinflammatory diseases.

After the review and editorial process, this Research Topic includes 19 articles reflecting new diagnostic tools, their influence on the recognition of IEI, country-related registries, and analysis of the clinical course of known and novel IEI and mutations as well as which treatments were selected for publications.

In March 2020, the World Health Organization (WHO) declared a COVID-19 pandemic, which has affected people all over the world regardless of age, sex, or comorbidities, with worse prognosis for older people with type I interferon autoantibodies or concomitant diseases. Patients with IEI also appeared to be at higher risk of developing COVID-19 at the start of the pandemic. However, further observations from different centers have shown that only certain types of IEI are associated with poor prognosis (12–19). It was not surprising to receive reports on the course of COVID-19 and the role of vaccination against SARS-CoV-2 in IEI patients in ECE. A Polish study on a group of 150 patients (adults, adolescents, and children) published by Koltan et al. shows that in severe humoral defects (CVID and XLA), the risk for a severe course of COVID-19 increases significantly with age and comorbidities. It also indicated the need for vaccination against SARS-CoV-2 within that group. Despite earlier reports on humoral and cellular response to immunizations against COVID-19 in inborn defects of immunity (20, 21). Another Polish group assessed SARS-CoV-2 vaccination coverage and hesitancy in adults with primary immunodeficiencies and autoinflammatory and rheumatic diseases on biologic therapy (Więsik-Szewczyk et al.). They showed a higher percentage of vaccinated individuals in each group compared to the general population in Poland.

Some papers stress the relevant role of awareness of IEI across countries as well as collaboration between immunological centers of the J Project. Naumova et al. demonstrated a higher number of newly diagnosed IEI in Bulgaria as a result of awareness, as well as the establishment of Expert Centers for Rare Diseases and the creation of national registries. Eldeniz et al. suggests adding the history of parental consanguinity and tuberculosis in the family to the list of warning signs of primary immunodeficiencies originally developed by the Jeffrey Modell Foundation. Taking into account the high percentage of consanguineous marriages in some nations or communities as well as the risk of tuberculosis is noteworthy. An accurate and significant conclusion on the tremendous role of cooperation between medical professionals in JP countries on the development of diagnostics, treatment, education, and awareness in the field of IEI is presented by Abolhassani et al.

In a mini review, Nikolouli et al. provide an analysis of the currently available *in vitro* models used to study IEI and its role in new therapeutic approaches. Following the world trend to encourage the timely diagnosis of severe combined immunodeficiencies as well as other severe T or B lymphopenia, pilot study results from Ukraine were published. TREC and KREC analyses were undertaken for almost 10 500 newborn children for severe combined immunodeficiency (SCID) and other severe IEI, with one case of CID detected. The DNA samples from known IEI (SCID, CID, XLA) were used as controls. The study proved that newborn screening for SCID and other severe IEI and proper treatment procedures can be introduced, such as HSCT and IgRT before the first symptoms and complications occurr. Select papers describe the new genetic causes of different inborn errors of immunity, such as STIM1 GOF mutation, and GATA2 defect. Other reports concern rare and not typical manifestations of known gene defects, including LIG1 deficiency and Omenn-like syndrome, SRP54 deficiency, and cyclic neutropenia or *Pneumocystis jirovecii* pneumonia in Aicardi-Goutieres syndrome. Different clinical aspects, such as pulmonary lesions or sleep quality or fatigue assessment in adult patients with humoral defects and other primary immunodeficiencies have been discussed by Polish authors. The essential issues of advancement in early diagnostics and treatment of ADA-SCID patients are presented in a multicenter report from Poland, which showed the limitations of enzyme replacement therapy due to its high costs and lack of approval in the EU, and restricted access to gene therapy. This indicates that the implementation of a newborn children screening program for SCID in Poland could improve early recognition and treatment of all SCID, including ADA-SCID. There was also scope to discuss experimental aspects of human hepatocyte transplantation for liver disease.

It should be emphasized that the papers published in this Research Topic document a substantial improvement in IEI awareness and research in Central and Eastern Europe. This

increased awareness of IEI has led to better and quicker recognition, including new gene mutations in different IEI, introducing new diagnostic tools and treatment approaches, as well as a better understanding of IEI-related complications or mechanisms, as shown in the articles included in this Research Topic.

The Editors hope that the second edition of this Research Topic on "*Advances in Primary Immunodeficiencies (Inborn Errors of Immunity) in Central-Eastern Europe*" will allow readers to learn more about the remarkable developments in the ECE region in terms of IEI-related specific diseases, their molecular background, and novel mutations in different phenotypes. It should also stimulate further research and cooperation within the J Project in ECE countries and elsewhere in this rapidly developing field of molecular medicine. Finally, we are grateful to the Editors of Frontiers in Immunology for their invitation to bring this collection together.

Author contributions

MP, J-LC, and LM contributed to the work equally. MP, J-LC, LM, and IT read and approved it for publication.

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Conflict of interest

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

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References

1. Casanova JL, Abel L. From rare disorders of immunity to common determinants of infection: following the mechanistic thread. *Cell* (2022) 185(17):3086–103. doi: 10.1016/j.cell.2022.07.004

2. Tangye SG, Al-Herz W, Bousfiha A, Chatila T, Cunningham-Rundles C, Etzioni A, et al. Human inborn errors of immunity: 2022 update on the classification from the international union of immunological societies expert committee. *J Clin Immunol* (2022) 42(7):1473–507. doi: 10.1007/s10875-022-01289-3

3. Bousfiha A, Jeddane L, Picard C, Al-Herz W, Ailal F, Chatila T, et al. Human inborn errors of immunity:2022 update on the IUIS phenotypical classification. *J Clin Immunol* (2022) 42(7):1508–20. doi: 10.1007/s10875-022-01352-z

4. Casanova JL, Abel L. Lethal infectious diseases as inborn errors of immunity: toward a synthesis of the germ and genetic theories. *Annu Rev Pathol* (2021) 16:23–50. doi: 10.1146/annurev-pathol-031920-101429

5. Notarangelo LD, Bacchetta R, Casanova JL, Su HC. Human inborn errors of immunity: an expanding universe. *Sci Immunol* (2020) 5(49):eabb1662. doi: 10.1126/sciimmunol.abb1662

6. Maródi L, The J Project Study Group. Fifteen years of the J project. *J Clin Immunol* (2019) 39(4):363–9. doi: 10.1007/s10875-019-00633

7. Maródi L, The J Project Study Group. The konya declaration for patients with primary immunodeficiencies. *J Clin Immunol* (2020) 40(5):770–3. doi: 10.1007/s10875-020-00797-4

8. Tuzankina I, Bolkov M, Nabieva U, Lázár I, Maródi L. The J daughter Siberia project. *J Clin Immunol* (2021) 41(1):262–5. doi: 10.1007/s10875-020-00894-4

9. Available at: https://www.Thejpnetwork.com.

10. Marodi L, The J Project Study Group. A rose amongst the thorns: the mission of the J project. *J Clin Immunol* (2022) 42:1151–5. doi: 10.1007/s10875-022-01285-7

11. Pac M, Reisli I, Casanova J-L, Maródi L. Advances in primary immunodeficiency in central-Eastern Europe. *Lausanne: Front Media SA* (2021). doi: 10.3389/978-2-88966-992-9

12. Bucciol G, Tangye SG, Meyts I. Coronavirus disease 2019 in patients with inborn errors of immunity: lessons learned. *CurrOpinPediatr* (2021) 33(6):648–56. doi: 10.1097/MOP.0000000000001062

13. Li J, Huang DQ, Zou B, Yang H, Hui WZ, Rui F, et al. Epidemiology of COVID-19: a systematic review and meta-analysis of clinical characteristics, risk factors, and outcomes. *J Med Virol* (2021) 93(3):1449–58. doi: 10.1002/jmv.26424

14. Delavari S, Abolhassani H, Abolnezhadian F, Babaha F, Iranparast S, Ahanchian H, et al. Impact of SARS-CoV-2 pandemic on patients with primary immunodeficiency. *J Clin Immunol* (2021) 41(2):345–55. doi: 10.1007/s10875-020-00928-x

15. Meyts I, Bucciol G, Quinti I, Neven B, Fischer A, Seoane E, et al. Coronavirus disease 2019 in patients with inborn errors of immunity: an international study. *J Allergy Clin Immunol* (2021) 147(2):520–31. doi: 10.1016/j.jaci.2020.09.010

16. Quinti I, Lougaris V, Milito C, Cinetto F, Pecoraro A, Mezzaroma I, et al. A possible role for b cells in COVID-19? lesson from patients with agammaglobulinemia. *J Allergy Clin Immunol* (2020) 146(1):211–3. doi: 10.1016/j.jaci.2020.04.013

17. Zhang Q, Bastard P, Liu Z, Le Pen J, Moncada-Velez M, Chen J, et al. Nborn errors of type I IFN immunity in patients with life-threatening COVID-19. *Science* (2020) 370(6515):eabd4570. doi: 10.1126/science.abd4570

18. Zhang Q, Bastard PCOVID Human Genetic Effort, , Cobat A, Casanova JL. Human genetic and immunological determinants of critical COVID-19 pneumonia. *Nature* (2022) 603(7902):587–98. doi: 10.1038/s41586-022-04447-0

19. Manry J, Bastard P, Gervais A, Le Voyer T, Rosain J, Philippot Q, et al. The risk of COVID-19 death is much greater and age dependent with type I IFN autoantibodies. *Proc Natl Acad Sci USA* (2022) 119(21):e2200413119. doi: 10.1073/pnas.2200413119

20. van Leeuwen LPM, GeurtsvanKessel CH, Ellerbroek PM, de Bree GJ, Potjewijd J, Rutgers A, et al. Immunogenicity of the mRNA-1273 COVID-19 vaccine in adult patients with inborn errors of immunity. *J Allergy Clin Immunol* (2022) 149(6):1949–57. doi: 10.1016/j.jaci.2022.04.002

21. Shields AM, Faustini SE, Hill HJ, Al-Taei S, Tanner C, Ashford F, et al. SARS-CoV-2 vaccine responses in individuals with antibody deficiency: findings from the COV-AD study. *Clin Immunol* (2022) 42(5):923–34. doi: 10.1007/s10875-022-01231-7

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Evaluation of the 10 Warning Signs in Primary and Secondary Immunodeficient Patients

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Objectives: Ten warning signs of primary immunodeficiency (PID) were suggested by the Jeffrey Modell Foundation (JMF), to increase physician awareness of PID. These warning signs have not yet been evaluated for patients with secondary immunodeficiency (SID). This study investigated whether the 10 warning signs used for the diagnosis of PID were also sufficient for the diagnosis of SID, and explored the possibility of additional signs.

Methods: This prospective study was conducted between June and December 2020. The mothers of 162 patients with PID and SID, and mothers of 200 healthy children, were asked to complete a questionnaire about family and personal history in addition to the warning signs of PID developed by the JMF. A JMF score was created by giving one point for each "Yes" answer for the 10 warning signs of PID. Medical records of the patients were evaluated for possible additional warning signs for PID and SID.

Results: The JMF scores of the PID (3.36 ± 1.65) and SID (3.72 ± 1.12) groups were significantly higher than the scores of the control group (0.34 ± 0.61) ($p < 0.05$). A sign for immunological evaluation in two patients without warning signs in the PID group was found to be chronic diarrhea. In addition to the 10 JMF warning signs, we found that consanguinity and a family history of tuberculosis were statistically significant in our PID group, compared with the SID and control groups.

Conclusions: The JMF warning signs are important for early diagnosis of PID. Our study showed that these signs may also be used for the early diagnosis of SID in patients and, according to our results, in addition to the 10 JMF signs for PID, parental consanguinity, chronic diarrhea, and a family history of tuberculosis may also be considered warning signs for the early diagnosis of PID.

Keywords: primary immunodeficiency, secondary immunodeficiency, combined immunodeficiency, 10 warning signs, childhood

INTRODUCTION

Primary immunodeficiencies (PIDs) are a group of diseases comprised of more than 450 innate errors of immunity (IEI), and they are becoming more prevalent (1). Although PIDs are rare diseases, they are more common than previously thought, following the use of modern diagnostic methods (2). A recent evaluation indicated that at least 1–2% of the world's population are affected by PIDs (3). Studies from our country also reveal a high incidence of PIDs in children. In this regard, training family physicians, pediatricians and, in particular, infectious disease specialists about PIDs will allow early diagnosis of these patients; early and effective treatment may allow them to reach adulthood (4, 5).

Several warning signs have been developed to increase physician awareness about the early diagnosis of PIDs. Warning signs of PID were developed by an institution called the Jeffrey Modell Foundation (JMF), which tries to increase awareness of this issue (http://www.info4pi.org/library/educational-materials/10-warning-signs). A detailed history should be taken for children admitted with a history of frequent infections, and the 10 warning signs of PID described by the JMF should be evaluated in addition to a full physical examination. This approach will allow early diagnosis of PID patients and, hence, the possibility of early and effective treatment before the development of organ damage (6, 7).These warning signs have not yet been evaluated for patients with secondary immunodeficiency (SID).

The objective of this study was to investigate whether the 10 warning signs used for the diagnosis of PID are sufficient for the diagnosis of SID, and to explore the possibility of additional signs.

MATERIALS AND METHODS

This prospective study was conducted between June and December 2020. This research was conducted using data obtained for clinical purposes. The study was approved by Necmettin Erbakan University Meram Medical School Ethics Committee (Date: 06.26.2020/No: 2020/2599).

The mothers of 162 patients diagnosed with PID and SID were asked to complete a questionnaire about family and personal history, in addition to the 10 warning signs of PID developed by the JMF. The same survey was completed by the mothers of 200 children without any defined primary/secondary immunodeficiency (i.e., healthy), and they formed the control group.

The study group was divided into two groups based upon clinical and laboratory findings to form the PID and SID groups. In addition to the questionnaire, the medical records of patients in the study groups were evaluated; age at diagnosis, treatment of the immunodeficiency, duration of diagnostic delay, and patient characteristics during the follow-up period were recorded. The SID was defined as using ESID criteria and the patients with chromosomal anomaly (50%), anti-epileptic drug use (30%), malnutrition 10%, uremia (5%), metabolic disease (5%) were classified as SID group. The questionnaire was provided to mothers of the study and control groups and the responses were recorded by the same research scientist. A JMF score was created by giving one point for each "Yes" answer for the 10 warning signs of PID.

Statistical Analyses

Analysis of the study data was performed by using the SPSS 25 program (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY, USA). Frequency, ratio, mean, and standard deviation (SD) of different variables of the individuals were analyzed by descriptive statistics. The Independent Samples T-test was used for dual comparisons, whereas triple comparisons were performed using one-way analysis of variance. Mean ± SD values of the groups were reported for evaluation of distribution rates of different variables by the groups using chi-square analysis. Crosstabs were made, and numbers and ratios were reported in the crosstabs. For variables found to be significant as a result of chi-square analyses performed for triple groups, further dual comparisons were performed to find out the reason for this difference. The level of significance was determined to be $p < 0.05$.

RESULTS

Of the patients included in the study, there were 98 (27.1%) in the PID group, and 64 (17.7%) in the SID group; the control group consisted of 200 (55.2%) healthy children. Overall, there were 200 (55.2%) males and 162 (44.8%) females. In the PID group, 48 (49%) were female and 50 (51%) were male, whereas in the SID group, 16 (25%) were female and 48 (75%) were male. In the control group, 98 (49%) were female and 102 (51%) were male. When sex distribution ratios were analyzed by group, the ratio of males in the SID group was significantly higher, compared with the PID and control groups ($p < 0.001$).

Ages of the participants varied between 1–216 months (mean ± SD: 74.93 ± 62.59). The mean age was 98.87 ± 66.37 months in the PID group, 67.89 ± 40.19 months in the SID group, and 65.46 ± 63.71 months in the control group. The demographic data of the patients are shown in **Table 1**. The mean age of the PID group was significantly higher compared with that of the control and SID groups ($p < 0.001$). There was no statistical difference between the control and SID groups.

When the PID and SID groups were compared, age at initiation of therapy in the PID group was older ($p < 0.05$), and the duration of therapy and follow-up period were longer ($p < 0.05$) in the PID group compared with those of the SID group, with statistically significant differences.

JMF scores of the PID (3.36 ± 1.65) and SID (3.72 ± 1.12) groups were significantly higher than those of the control group (0.34 ± 0.61) ($p < 0.001$). The distribution of JMF scores of the PID, SID, and control groups is shown in **Table 1E**. The JMF score was zero in two patients in the PID group; these two

TABLE 1 | The demographic characteristics of the patients with PID and SID, and the control group.

	Control		PID		SID		p
	Mean±SD	Median (min-max)	Mean±SD	Median (min-max)	Mean±SD	Median (min-max)	
Age (Month)	65.46±63.71	42 (1-204)	98.87±66.37	84 (7-216)	67.89±40.19	57 (3-204)	0.001
Age at first hospitalization (Month)	19.30±23.29	12.0 (1-120)	14.62±26.66	3.0 (1-162)	7.87 ±13.80	2.0 (1-60)	0.001
Age at diagnosis of immunodeficiency (Month)			48.58±53.74	21.00 (1-192)	36.64±38.87	24.00 (2-174)	0.127
Duration of diagnostic delay (Month)			21.04±32.71	10.50 (1-186)	21.67±25.79	12.0 (1-108)	0.897
Age at initiation of treatment (Month)			55.06±58.36	27.00 (1-210)	37.73±38.92	24.00 (2-174)	0.025
Duration of treatment (Month)			39.22±32.42	30.00 (1-162)	28.89±21.03	24.00 (1-120)	0.015
Age at discontinuation of treatment (Month)			97.50±60.57	84.00 (36-204)	39.00± 4.24	39.00 (36-42)	0.228
Duration of follow-up (Month)			49.57±41.76	36.00 (5-192)	34.00±22.95	30.00 (1-120)	0.007

PID, primary immunodeficieny; SID, secondary immunodeficiency.

TABLE 1E | Distribution of JMF scores of the PID, SID and Control Groups.

JMF Score	PID Group (n/%)	SID Group (n/%)	Control Group (n/%)	Total (n/%)
0 point	2 (2.0%)	0	146 (73.0%)	148 (40.9%)
1 point	10 (10.2%)	1 (1.6%)	41 (20.5%)	52 (14.4%)
2 points	23 (23.5%)	8 (12.5%)	12 (6.0%)	43 (11.9%)
3 points	17 (17.3%)	18 (28.1%)	1 (0.5%)	36 (9.9%)
4 points	22 (22.4%)	21 (32.8%)	0	43 (11.9%)
5 points	14 (14.3%)	13 (20.3%)	0	27 (7.5%)
6 points	6 (6.1%)	3 (4.7%)	0	9 (2.5%)
7 points	4 (4.1%)	0	0	4 (1.1%)
8 points	0	0	0	0
9 points	0	0	0	0
10 points	0	0	0	0

JMF, Jeffrey Modell Foundation.

patients had been screened for PID due to chronic diarrhea. The IUIS classification (2020) (8) of patients with PID, and the distribution of JMF scores of the PID subgroups are shown in **Table 2E**.

After evaluation of overall JMF scores, answers given to the questions were also compared separately. Regarding having ≥ 4 episodes of otitis in one year, ≥ 2 episodes of sinusitis in one year, and a family history of PID, the rates in the PID group were significantly higher than the control and SID groups ($p < 0.001$). No difference was found between the control and SID groups. Regarding oral antibiotic use for ≥ 2 months with little effect, recurrent deep tissue infections or organ abscesses, persistent thrush or cutaneous fungal infections, and ≥ 2 deep tissue infections including septicemia, the rates in the PID and SID groups were significantly higher compared with the control group ($p < 0.001$). No difference was found between the PID and SID groups. Regarding failure to thrive, the need for IV antibiotics to clear infections, and having ≥ 2 lower respiratory tract infections in one year, the rates in the SID group were higher than the PID group. The rate in the PID group was significantly higher than the control group ($p < 0.001$). Distribution of the 10 warning signs by groups is shown in **Table 2**.

Answers of the PID group to questions regarding the 10 warning signs of PID were estimated as observed and expected values. The criteria that exhibited a significant difference included the rates of recurrent deep cutaneous or organ abscesses, failure to thrive, need for IV antibiotics, ≥ 2 deep tissue infections including septicemia, a family history of PID, ≥ 4 episodes of otitis in one year, ≥ 2 episodes of sinusitis in one year, and ≥ 2 lower respiratory tract infections in one year. The criteria that exhibited the least differences between expected and observed values included the rates of being on oral antibiotics for ≥ 2 months with little effect, and a history of persistent thrush or cutaneous fungal infections. Among the warning signs of PID developed by the JMF, the criteria with the most statistically significant differences included the rates of ≥ 4 episodes of otitis in one year, recurrent deep cutaneous or organ abscesses, ≥ 2 episodes of sinusitis in one year, and ≥ 2 deep tissue infections including septicemia. These four warning signs seem to be less indicative of PID according to the statistics.

Odds ratios (ORs) for the 10 warning signs were estimated for both PID and SID groups versus the control group. Being on oral antibiotics was increased by 20.37-fold in the PID group compared with the control group, with a sensitivity of 45.91% and specificity of 96.00%. In the SID group, being on oral antibiotics was increased by 22.54-fold, with a sensitivity of 48.43% and specificity of 96.00%. OR, sensitivity, and specificity of the 10 warning signs are shown in **Table 3**.

According to the receiver operating characteristic (ROC) results, the predictive area under the ROC curve (AUC) value for the JMF score in the PID and SID groups was determined to be 0.974 (95% confidence interval (CI), 0.959–0.989), which was statistically significant ($p < 0.001$). The ROC curve for the JMF

TABLE 2 | Distribution of the warning signs of the groups according to Jeffrey Modell Foundation.

Criteria			PID		SID		Control Group		*P*
			N	%	n	%	n	%	
Oral antibiotic	Two or more months on antibiotics with little effect.	No	53	54.1	33	51.6	192	96	<0.001
		Yes	45	45.9	31	48.4	8	4	
Abscess	Recurrent, deep skin or organ abscesses	No	89	90.8	60	93.8	199	99.5	<0.001
		Yes	9	9.2	4	6.3	1	0.5	
Growing	Failure of an infant to gain weight or grow normally	No	61	62.2	29	45.3	197	98.5	<0.001
		Yes	37	37.8	35	54.7	3	1.5	
Thrush	Persistent thrush in mouth or fungal infection on skin	No	55	56.1	34	53.1	188	94	<0.001
		Yes	43	43.9	30	46.9	12	6	
IV antibiotic	Need for intravenous antibiotics to clear infections	No	17	17.3	0	0	170	85	<0.001
		Yes	81	82.7	64	100	30	15	
Septicemia	Two or more deep-seated infections including septicemia	No	88	89.8	55	85.9	200	100	<0.001
		Yes	10	10.2	9	14.1	0	0	
PI in family	A family history of PI	No	73	74.5	60	93.8	196	98	<0.001
		yes	25	25.5	4	6.3	4	2	
Ear infection	Four or more new ear infections within 1 year	No	90	91.8	63	98.4	199	99.5	<0.001
		Yes	8	8.2	1	1.6	1	0.5	
Sinus infection	Two or more serious sinus infections within 1 year	No	89	90.8	63	98.4	198	99	<0.001
		Yes	9	9.2	1	1.6	2	1	
Pneumonia	Two or more pneumonias within 1 year	No	35	35.7	5	7.8	193	96.5	<0.001
		Yes	63	64.3	59	92.2	7	3.5	

TABLE 2E | IUIS classification of the patients with PID (2020) and JMF scores of the PID subgroups.

Diagnoses	n	%	JMF scoreMean±SD	JMF scoreMedian (Min-Max)
Immunodeficiencies affecting cellular and humoral immunity	18	18.4	3.28±1.60	3.00 (1-7)
Combined immunodeficiencies with associated syndromic features	21	21.4	3.90±1.44	4.00 (1-6)
Immunodeficiencies due to Antibody Deficiencies	39	39.8	2.95±1.58	2.00 (0-6)
Diseases of immune dysregulation	7	7.1	2.14±1.57	2.00 (0-5)
Congenital defects of phagocyte number or function or both	8	8.2	4.50±1.69	4.00 (2-7)
Defects in Intrinsic and Innate Immunity	1	1	7.00±0.00	7.00 (7-7)
Auto-inflammatory disorders	3	3.1	4.00±0.00	4.00 (4-4)
Complement deficiencies	1	1	3.00±0.00	3.00 (3-3)
Bone marrow failure	0	0.0	.	.
Phenocopies of PID	0	0.0	.	.
Total	98	100.0		

IUIS, International Association of Immunology Societies.

score in the PID and SID groups is shown in **Figure 1**. The cut-off for the JMF score in the PID and SID groups was determined to be 1.5. In accordance with the determined cut-off, sensitivity was found to be 0.920% and specificity was 0.935%.

When parental consanguinity was examined between the groups, it was revealed that 41 patients (41.8%) in the PID group, 16 patients (25%) in the SID group, and 18 patients (9%) in the control group had consanguineous parents. The distribution of family history features of the patients who participated in the study by groups is shown in **Table 3E**. A statistically significant difference was determined between the groups ($p < 0.001$). The rate of parental consanguinity of the PID group was higher than both SID and control groups. The rate of parental consanguinity in the PID group was 7.27-fold higher than that of the control group (OR: 7.27; 95% CI: 3.87–13.64). This rate was 3.37-fold higher in the SID group (95% CI: 1.60–7.09).

In both the PID and SID groups, the rates of a family history of early death, rheumatic diseases, and malignancies were higher, compared with the control group ($p < 0.05$). The rate of a family history of tuberculosis was significantly higher in the PID group, compared with the SID and control groups, whereas the rate of a family history of allergic diseases was significantly higher in the control group compared with the PID and SID groups.

The most common group of diseases in our PID patients was that of immunodeficiencies due to antibody deficiency, as shown in **Table 2E**. Clinical characteristics of the patients with SID are shown in **Figure 2** and **Table 4**.

DISCUSSION

Currently, the awareness level of physicians and healthcare professionals about PID, and their experience with a clinical approach to a patient with PID are still insufficient. For this reason, the history, features, and physical examination findings from patients, as well as expert opinions were combined, and the "10 Warning Signs of Primary Immunodeficiency Diseases",

TABLE 3 | OR, sensitivity and specificity of the 10 warning signs developed by JMF.

		OR (95% CI)	Sensitivity	Specificity	PPV	NPV	Accuracy
Oral antibiotic	**PID**	20.37 (9.05-45.86)	45.91	96.00	84.90	78.36	79.53
	SID	22.54 (9.53-53.30)	48.43	96.00	79.48	85.33	84.47
Abscess	**PID**	20.12 (2.51-61.24)	4.32	98.88	90.00	30.90	32.88
	SID	13.26 (1.45-120.96)	6.25	99.50	80.00	76.83	76.89
Growing	**PID**	39.83 (11.86-133.71)	37.75	98.50	92.50	76.35	78.52
	SID	79.25 (22.89-274.37)	54.68	98.50	92.10	87.16	87.87
Thrush	**PID**	12.24 (6.04-24.83)	43.87	94.00	78.18	77.36	77.51
	SID	13.82 (6.44-29.63)	46.87	94.00	71.42	84.68	82.57
IV antibiotic	**PID**	27.00 (14.07-51.78)	82.65	85.00	72.97	90.90	84.22
	SID	31.13 (2.33-42.21)	100.00	85.00	68.08	100.00	88.63
Septicemia	**PID**	47.57 (2.75-820.92)	10.10	100.00	100.00	69.20	70.23
	SID	68.63 (3.93-197.78)	14.06	100.00	100.00	78.43	79.16
PI in family	**PID**	16.78 (5.64-49.86)	25.51	98.00	86.20	72.86	74.16
	SID	3.26 (0.79-13.45)	6.25	98.00	50.00	76.56	75.75
Ear infection	**PID**	17.68 (2.18-143.54)	8.16	99.50	88.88	68.85	69.46
	SID	3.15 (0.19-51.23)	1.56	99.50	50.00	75.95	75.75
Sinus infection	**PID**	10.01 (2.12-47.28)	9.18	99.00	81.81	68.99	69.46
	SID	1.57 (0.14-17.62)	1.56	99.00	33.33	75.86	75.37
Pneumonia	**PID**	49.62 (21.00-117.26)	64.28	75.39	50.00	84.64	72.31
	SID	325.34 (99.56-1063.13)	92.18	96.50	89.39	97.47	95.45

OR, Odds ratio; PPV, Positive predictive value; NPV, Negative predictive value; IV, Intravenous.

TABLE 3E | Distribution of family history features of the patients and the controls by the groups.

	Control Group		PID		SID		Total		P
	N	**%**	**n**	**%**	**n**	**%**	**n**	**%**	
Consanguinity	18	24	41	54.6	16	21.4	75	37.5	<0.001
First Degree	14	77.8	33	80.5	10	62.5	57	76	
Second Degree	1	5.6	3	7.3	1	6.3	5	6.7	
Third Degree	1	5.6	3	7.3	4	25	8	10.7	
Fourth Degree	2	11.1	2	4.9	1	6.3	5	6.7	
Family history of premature death (Yes/No)	11/189	5.5/94.5	66/32	67.3/32.7	38/26	59.4/40.6	115		<0.001
Family history of tuberculosis (Yes/No)	5/195	2.5/97.5	16/82	4.7/95.3	33/61	4.7/95.3	54		<0.001
Family history of rheumatic diseases (Yes/No)	23/177	11.5/88.5	28/70	28.6/71.4	22/42	34.4/65.6	73		<0.001
Family history of allergic diseases (Yes/No)	98/102	49/51	31/67	31.6/68.4	19/45	29.7/70.3	148		<0.001
Family history of Malignancy (Yes/No)	24/176	12/88	28/70	28.6/71.4	19/45	29.7/70.3	71		<0.001

which has significantly contributed to the diagnosis of PID, was defined by the JMF (9). In our study, the JMF scores of PID and SID patients calculated *via* these warning signs were statistically significantly higher compared with the control group. In addition, we found that parental consanguinity and a family history of tuberculosis, chronic diarrhea may also be warning signs of PID.

Every patient with suspected PID should be asked in detail for information related to the "10 warning signs" checklist during history taking. It has been proposed that PID should be investigated when ≥ 2 warning signs are present (10). In our study, the JMF scores of both the PID and SID groups were significantly higher than the control group. The cut-off for JMF score in terms of PID and SID was determined to be 1.5, with a sensitivity of 92% and specificity of 93.5%. According to our study results, we hypothesize that the JMF criteria are a guiding tool not only for PID patients but also for SID patients. In addition, in a study by Reda et al., at least one of the 10 warning signs was observed in all PID patients, whereas 28% of patients without PID had no warning sign (11). In our study, all patients in the SID group had warning signs of immunodeficiency, whereas two of our patients in the PID group had no warning sign. The reason for these two patients with a JMF score of zero being investigated was a history of chronic diarrhea. Considering this, we propose that a history of chronic diarrhea should be included in the warning signs of PID. We attribute the presence of JMF warning signs in all patients in the SID group to the fact that that they all had a more severe course that required IV immunoglobulin therapy.

Training programs aimed at increasing awareness of PID should target physicians who may discover a family history of PID, parental consanguinity, and a family history of early sibling deaths in societies where consanguineous marriages are common (11). In a study from Egypt by Reda et al., 60% of PID patients had consanguineous parents (11). In our country, the rate of parental consanguinity in PID patients has been found to be 14.3–37.5% (4). In our study, the rate of parental consanguinity in PID cases was 41.8%. This rate was significantly higher compared with the

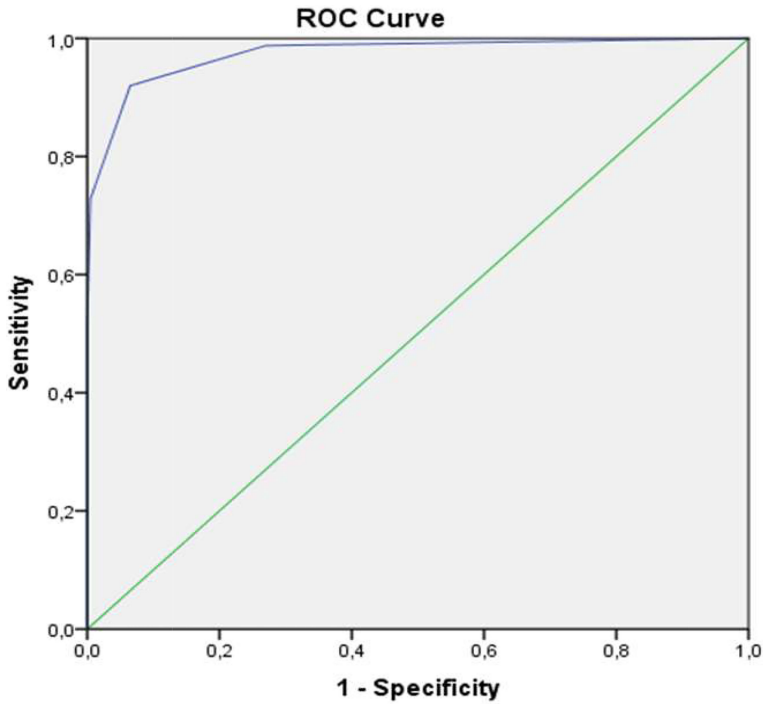

FIGURE 1 | The ROC curve for JMF score in the PID and SID groups.

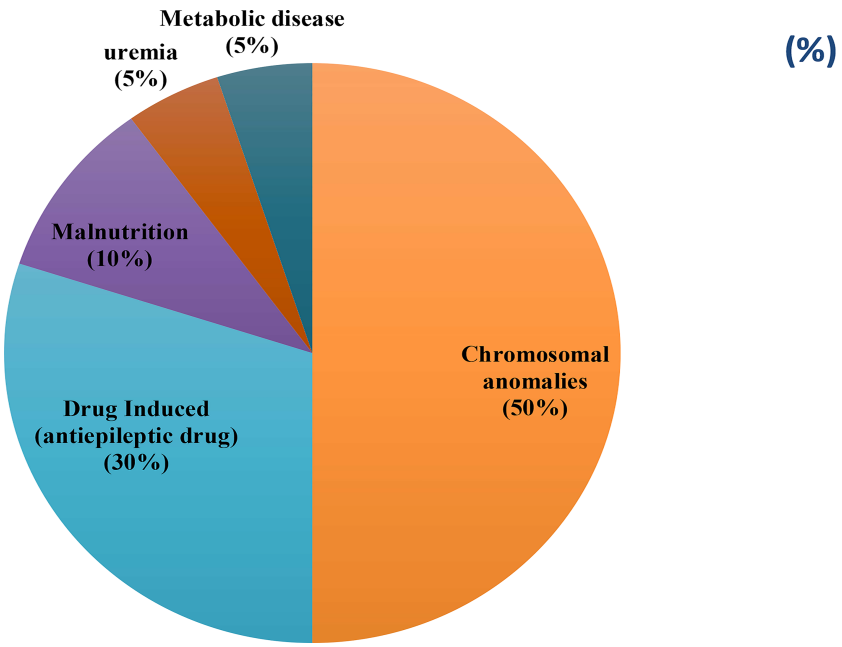

FIGURE 2 | The diagnosis of the patients with SID.

TABLE 4 | The diagnosis of the patients with SID.

	N	(%)
Chromosomal Anomalies	32	50
Trisomy 21	17	
Unspecified syndromes	5	
46 XX ınv9. XP11 duplıcatıon	1	
Brugada syndrome	1	
47 XYY	1	
Wıdemann steıner syndrome	1	
Klınefelter syndrome	1	
Noonan syndrome type 5	1	
West syndrome	1	
Swyer james syndrome	1	
Dravet syndrome	1	
Joubert syndrome	1	
Drug Induced	19	30
Anti-epileptic drug	19	
Malnutrition	7	10
Metabolic disease	3	5
Krabbe disease	1	
Nieman pick c disease	1	
Metachromatic leukodystrophy	1	
Uremia	3	5

control and SID groups, and we propose that parental consanguinity may be a warning sign of autosomal recessive-inherited PID for our region. According to a study conducted by Subbarayan et al. (6), one of the strongest identifiers of PID was a family history of immunodeficiency. In general, such a family history is 18 times more common in children with PID, compared to those without any identifiable PID. In our study, we determined a family history was 16.78 times more common in the PID patients compared with the control group. We propose that screening for PID would be important in the presence of a family history of PID, even when it exists alone.

In the Subbarayan et al. study, the most common of the 10 warning signs was the need for IV antibiotics. The second most common warning sign was a family history of PID (34%), followed by failure to thrive (31%) (6). Similarly, in the study by Reda et al., the most common warning sign was the need for IV antibiotics (92%). In our study, as in these two studies, the most common warning sign was the need for IV antibiotics (82.9%). The second most common warning sign was having ≥ 2 lower respiratory tract infections in one year (64.3%), and the third was being on oral antibiotics for longer than two months with little effect (45.9%). Our findings confirm that the 10 warning signs may be used for the diagnosis of PID, although in a different order of frequency, and that different frequencies may be reported in different studies.

Frequent infections, a more severe course than expected, long-lasting infections, the occurrence of unexpected or severe complications due to infections, incomplete recovery with antibiotic treatment, the need for prolonged use of antibiotics, chronic courses of infectious diseases, and the occurrence of infections with unusual agents may also be associated with PID diseases (12). Infections usually recover rapidly and without complications in children with a healthy immune system and no other risk factors (13). In our study, among the JMF warning signs, frequent recurrent upper respiratory tract infections were significant in the PID group, and frequent recurrent lower respiratory tract infections and failure to thrive were significant in the SID group. This may be due to the high rate of antibody deficiency, and the presence of accompanying conditions (tracheostomy, epilepsy) in our SID patients.

Comprehensive evaluation of family history and clinical features may be helpful for the early diagnosis of PID disease (14, 15). However, absence of a family history of immunodeficiency does not exclude the presence of PID. Since the majority of PID diseases are inherited, the presence of a similar disease, as well as the age and sex of affected individuals are important. In the study by Yorulmaz et al., 3.8% of PID patients were found to have a family member with PID (4). This was higher in the PID and SID groups in our study, with rates of 25.5% and 6.3%, respectively. These higher rates in our study may be due to asking not only about siblings and parents but also about the siblings of the parents and their children. In our study, the rate in the PID group was significantly higher than both the control and SID groups. A patient with a history of frequent infections and a family history of PID should be evaluated for PID.

In a study conducted by Yorulmaz et al. in Konya, the rate of sibling death among patients with combined immunodeficiency (CID) was 7.5% (4). However, in the Reda study from Egypt, 21.7% of the patients had sibling deaths. This 3-fold higher rate in Egypt may be related to a higher rate of consanguineous marriages and the level of community and economic development. The highest rate of sibling death was determined to be 50% in those with CID (11). Rates of early death in the family history were also evaluated in our study. According to the results, 66 (67.3%) of PID cases, 38 (59.4%) of SID cases, and 11 (5.5%) in the control group had a family history of early death. In our study, the reason for the high rate of a family history of early death may be the inclusion of questions about siblings of the parents and their children. Therefore, we think that an extended family history of early sibling death may be an important warning sign for the diagnosis of immunodeficient patients.

Worldwide, the mean duration of diagnostic delay between the onset of symptoms and diagnosis in PID diseases is 4.08 years. The biggest factor in an 8–10-year delay in the diagnosis of PID diseases after the onset of symptoms was the low level of physician awareness of these diseases (9). In our study, the mean duration of diagnostic delay for the PID and SID groups was 21 months, with no significant difference between the groups. Given that a delay in diagnosis can significantly increase morbidity and mortality, we may conclude that the index of suspicion for PID on the part of physicians in our region is similar to that in other centers.

In the literature, PID diseases have been reported to be more common in males than in females (6). The predominance of males results not from PIDs inherited in an autosomal recessive manner, but from X-linked PIDs. In our study of PID patients, 49% were female and 51% were male, with no statistically significant difference. We consider that this result was due to the high rate of consanguineous marriages in our study. In addition, the predominance of male gender in SID group could be due to the characteristics of the patients involved in our study.

Antibody deficiencies are the most common subtype of PID (16); they were also the most common PID group in our study. This is consistent with the European Society for Immunodeficiencies and the JMF databases, and our results are consistent with previous study results. However, given that the incidence of allergic, autoimmune, and hematological diseases, as well as the incidence of malignancies are high among PID patients, the medical history should be scrutinized in this respect (17). Studies suggest the need for some additional warning signs to facilitate early diagnosis in such patients (18, 19). In our study, the rates of the rheumatic diseases and malignancy in the PID and SID groups were significantly higher than in the control group. Patients with immunodeficiencies may present with infectious diseases, and also with immune dysregulation diseases and malignancies; we consider that these diseases should also be considered as warning signs of immunodeficiency.

The limitation of this study is that some of our patients are a few months old and the JMF warning signs are not to be highly specific in this population naturally. We comment that it should be considered the warning signs are specific after infancy.

In conclusion, early diagnosis of PID will allow effective treatment of these diseases. We agree that the 10 warning signs of PID diseases defined by the JMF are important for the early diagnosis of PID. From our study results, a family history of parental consanguinity or tuberculosis may also be warning signs of PID, and a history of chronic diarrhea should be included. Studies from different immunology centers may clarify these additions. This approach will allow early diagnosis of PID and, thus, early and effective treatment, which will allow patients to reach adulthood before the development of organ injury.

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 human participants were reviewed and approved by Necmettin Erbakan University Meram Medical School Ethics Committee. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

F-CE, YG, IR, SK, SG, and AY implemented the study and collected the data. F-CE, IR, S-NG, and AY wrote the manuscript. SK and S-NG analyzed the data. All authors participated in the design and interpretation of the studies, analysis of the data and review of the manuscript. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Tangye SG, Al-Herz W, Bousfiha A, Chatila T, Cunningham-Rundles C, Etzioni A, et al. Human Inborn Errors of Immunity: 2019 Update on the Classification From the International Union of Immunological Societies Expert Committee. *J Clin Immunol* (2020) 40:24–64. doi: 10.1007/s10875-019-00737-x
2. Bousfiha AA, Jeddane L, Ailal F, Benhsaien I, Mahlaoui N, Casanova JL, et al. Primary Immunodeficiency Diseases Worldwide: More Common Than Generally Thought. *J Clin Immunol* (2013) 33:1–7. doi: 10.1007/s10875-012-9751-7
3. Modell V, Orange JS, Quinn J, Modell F. Global Report on Primary Immunodeficiencies: Update From the Jeffrey Modell Centers Network on Disease Classification, Regional Trends, Treatment Modalities, and Physician Reported Outcomes. *Immunol Res* (2018) 66:367–80. doi: 10.1007/s12026-018-8996-5
4. Yorulmaz A, Artaç H, Kara R, Keleş S, Reisli İ. Retrospective Evaluation of 1054 Patients With Primary Immunodeficiency. *Asthma Allergy Immunol* (2008) 6:127–34.
5. Kilic SS, Ozel M, Hafizoglu D, Karaca NE, Aksu G, Kutukculer N. The Prevalances and Patient Characteristics of Primary Immunodeficiency Diseases in Turkey-Two Centers Study. *J Clin Immunol* (2013) 33:74–83. doi: 10.1007/s10875-012-9763-3
6. Subbarayan A, Colarusso G, Hughes SM, Gennery AR, Slatter M, Cant AJ, et al. Clinical Features That Identify Children With Primary Immunodeficiency Diseases. *Pediatrics* (2011) 127:810–16. doi: 10.1542/peds.2010-3680
7. Tavakol M, Jamee M, Azizi G, Sadri H, Bagheri Y, Zaki-Dizaji M, et al. Diagnostic Approach to the Patients With Suspected Primary Immunodeficiency. *Endocr Metab Immune Disord Drug Targets* (2020) 20:157–71. doi: 10.2174/1871530319666190828125316
8. Bousfiha A, Jeddane L, Picard C, Al-Herz W, Ailal F, Chatila T, et al. Human Inborn Errors of Immunity: 2019 Update of the IUIS Phenotypical Classification. *J Clin Immunol* (2020) 40:66–81. doi: 10.1007/s10875-020-00758-x
9. Modell V, Quinn J, Ginsberg G, Gladue R, Orange J, Modell F. Modeling Strategy to Identify Patients With Primary Immunodeficiency Utilizing Risk Management and Outcome Measurement. *Immunol Res* (2017) 65:713–20. doi: 10.1007/s12026-017-8907-1
10. O'Sullivan MD, Cant AJ. The 10 Warning Signs: Time for a Change? *Curr Opin Allergy Clin Immunol* (2012) 12:588–94. doi: 10.1097/ACI.0b013e3283591534
11. Reda SM, El-Ghoneimy DH, Afifi HM. Clinical Predictors of Primary Immunodeficiency Diseases in Children. *Allergy Asthma Immunol Res* (2013) 5:88–95. doi: 10.4168/aair.2013.5.2.88
12. Woroniecka M, Ballow M. Office Evaluation of Children With Recurrent Infection. *Pediatr Clinics North America* (2000) 47:1211–24. doi: 10.1016/S0031-3955(05)70268-6
13. Ballow M. Approach to the Patient With Recurrent Infections. *Clin Rev Allergy Immunol* (2008) 34:129–40. doi: 10.1007/s12016-007-8041-2
14. Karakoc-Aydiner E, Ozen A, Baris S, Ercan H, Ozdemir C, Barlan I. Alteration in Humoral Immunity Is Common Among Family Members of Patients With Common Variable Immunodeficiency. *J Investig Allergol Clin Immunol* (2014) 24:346–51.
15. Aytekin G, Budh DP. X-Linked Immunodeficiency. In: *StatPearls*. Treasure Island (FL): StatPearls Publishing (2021).
16. Modell V, Gee B, Lewis DB, Orange JS, Roifman CM, Routes JM, et al. Global Study of Primary Immunodeficiency Diseases (PI)–Diagnosis, Treatment, and Economic Impact: An Updated Report From the Jeffrey Modell Foundation. *Immunol Res* (2011) 51:61–70. doi: 10.1007/s12026-011-8241-y
17. Aghamohammadi A, Moghaddam ZG, Abolhassani H, Hallaji Z, Mortazavi H, Pourhamdi S, et al. Investigation of Underlying Primary

Immunodeficiencies in Patients With Severe Atopic Dermatitis. *Allergol Immunopathol* (2014) 42:336–41. doi: 10.1016/j.aller.2013.02.004
18. Arkwright PD, Gennery AR. Ten Warning Signs of Primary Immunodeficiency: A New Paradigm Is Needed for the 21st Century. *Ann N Y Acad Sci* (2011) 1238:7–14. doi: 10.1111/j.1749-6632.2011.06206.x
19. Bahrami A, Sayyahfar S, Soltani Z, Khodadost M, Moazzami B, Rezaei N. Evaluation of the Frequency and Diagnostic Delay of Primary Immunodeficiency Disorders Among Suspected Patients Based on the 10 Warning Sign Criteria: A Cross-Sectional Study in Iran. *Allergol Immunopathol* (2020) 48:711–19. doi: 10.1016/j.aller.2020.03.005

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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Case Report: Novel STIM1 Gain-of-Function Mutation in a Patient With TAM/STRMK and Immunological Involvement

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Gain-of-function (GOF) mutations in *STIM1* are responsible for tubular aggregate myopathy and Stormorken syndrome (TAM/STRMK), a clinically overlapping multisystemic disease characterised by muscle weakness, miosis, thrombocytopaenia, hyposplenism, ichthyosis, dyslexia, and short stature. Several mutations have been reported as responsible for the disease. Herein, we describe a patient with TAM/STRMK due to a novel L303P STIM1 mutation, who not only presented clinical manifestations characteristic of TAM/STRMK but also manifested immunological involvement with respiratory infections since childhood, with chronic cough and chronic bronchiectasis. Despite the seemingly normal main immunological parameters, immune cells revealed GOF in calcium signalling compared with healthy donors. The calcium flux dysregulation in the immune cells could be responsible for our patient's immune involvement. The patient's mother carried the mutation but did not exhibit TAM/STRMK, manifesting an incomplete penetrance of the mutation. More cases and evidence are necessary to clarify the dual role of STIM1 in immune system dysregulation and myopathy.

Keywords: STIM1, infection, gain of function, myopathy, calcium signalling

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INTRODUCTION

Calcium (Ca^{2+}) signalling, in which Ca^{2+} acts as a second messenger, controls numerous cellular functions, such as proliferation, apoptosis, exocytosis, differentiation, neurotransmission, hormone secretion, blood coagulation, and muscle contraction. Store-operated Ca2+ entry (SOCE) is a ubiquitous mechanism for Ca2+ entry into eukaryotic cells mediated by STIM1 and ORAI1

proteins. Human disorders have been related to SOCE, including autosomal recessive STIM1 and ORAI1 loss-of-function (LOF) mutations, resulting in insufficient SOCE and subsequently altering Ca^{2+} release-activated calcium (CRAC) channels (1). The alteration of CRAC channels generates a severe combined immunodeficiency, which includes recurrent and chronic infections, autoimmunity, muscular hypotonia, ectodermal dysplasia, anhidrosis, and mydriasis. Most STIM1 and ORAI1 LOF mutations do not express protein; however, mutations have been described that disrupt the STIM1 function and interfere with the STIM1-ORAI1 interaction (such as R426C and R429C mutations) or generate an obstructed ORAI1 channel, such as the R91W mutation (2). In contrast, gain-of-function (GOF) STIM1 and ORAI1 mutations are autosomal dominant forms that induce overactivation due to excessive Ca^{2+} entry through SOCE. These patients experience tubular aggregate myopathy and Stormorken syndrome (TAM/STRMK), with progressive muscle weakness, myalgia, miosis, ichthyosis, short stature, hyposplenism, thrombocytopaenia (3), and dyslexia (2). All GOF mutations share missense mutations that affect highly conserved amino acids in the EF-hand Ca^{2+}-binding motif (H72Q, N80T, G81D, D84G, D84E, S88G, L92V, L96V, Y98C, K104N, F108I, F108L, H109N, H109R, H109Y, I115F), in the sterile alpha motif domain (V138I), in the luminal coiled-coil domains of STIM1 (4) (CC1: R304W and R304Q, and CC2: K365N), between the S/P and K domains (S630F and H632fs*), in the K domain (R749H), and in the ORAI1 transmembrane domains forming the channel pore or concentric rings surrounding the pore (G97C, G98S, V107M, L138F, T184M and P245L) (2, 5–8). Missense mutations in the muscle-specific sarcoplasmic reticulum Ca^{2+} buffer calsequestrin-1 (CASQ1) have been also reported in patients with late-onset muscle weakness and myalgia, forming the mild end of the TAM/STRMK spectrum (9, 10).

CASE DESCRIPTION

We examined a 52-year-old European man from Spain with non-consanguineous parents. Written informed consent was obtained from the patient for the publication of any potentially identifiable data included in this article. The patient had a clear case of TAM/STRMK, with marked myopathy, defective dental enamel, numerous dental caries and root canals, brittle nails, no dystrophy, congenital pes cavus of the right foot, congenital hammer toes (fourth and fifth digits), arthrosis, generalised myalgia, muscle atrophy with myoclonus, and incapacitating fatigue after physical exercise.

The patient also experienced photosensitivity, with erythema and desquamation after sun exposure (doubtful association with drugs); however, there was no report of oral aphthous ulcers, cold sores, arthritis, or Raynaud's disease. The patient presented skin rashes that worsened in the summer, as well as solar urticaria and dermographism (erythema). He reported that each episode was accompanied by digestive symptoms, with a tendency to diarrhoea and with clinical worsening of his myopathy.

The patient experienced myalgia in the mornings, which decreased with exercise, as well as muscle stiffness, muscle fatigue, myalgia in the peripheral forearms and legs, and muscle contractures that had progressed in the past year. The patient had experienced muscle mass loss even while performing exercise (walking 1 h daily, Pilates), as well as intense post-exercise myalgia. He has always been in good physical shape; however, after 1 hour of exercise, the patient presented fatigue with extreme exhaustion. He woke at night because of muscle pain, which decreased with short walks. The patient experienced myoclonus in the arms with exercise, as well as pain in both wrists, with functional disability and spontaneous resolution.

The patient's skeletal muscle has preserved architecture but without increased endomysial connective tissue or adipose infiltration. A skeletal muscle biopsy showed discrete variability in muscle fibre size, with the presence of fibres with multiple internalised nuclei (approximately 8%). We observed no necrotic or regenerative fibres and no structural disorders (vacuoles or inclusions). Through ATPase techniques, we determined that the fibre type distribution was normal. With oxidative techniques, we observed a few COX-negative fibres with sorbitol dehydrogenase overexpression (approximately 3%), as well as NADH-TR alterations. We observed no inflammatory infiltrates and no alterations using the histochemical technique for phosphorylase and myoadenylate deaminase. Although the patient's phosphofructokinase level was not assessable, he presented high creatine kinase levels (**Table 1**) and pseudomyotonia, which was observed on an electromyogram.

Beyond the TAM/STRMK disease pattern, the patient presented immunological involvement, with respiratory infections since childhood, chronic cough, and chronic bronchiectasis. At the immunological level, a lymphocyte subpopulation study showed normal T, B, and natural killer cell counts (**Table 1**). The patient had normal activity of complement factors C3, C4, and CH50, as well as normal immunoglobulin levels (**Table 1**).

The patient presented adequate specific antibody response against protein and polysaccharide immunisation. The results of a study of lymphocyte proliferation to mitogens with CD3-CD28 and phytohaemagglutinin were within normal limits. The antibody studies were negative for antinuclear antibodies, antineutrophil cytoplasmic antibodies, celiac antibodies, and *Helicobacter pylori* antibodies.

The acid phosphatase test, and immunohistochemistry for human leukocyte antigen showed no abnormalities. The immunohistochemistry did show preserved membrane proteins (dystrophins 1 and 2, alpha and gamma sarcoglycans, caveolin, and merosin).

The patient showed a good response to the salmonella and tetanus toxoid vaccine. Functional respiratory tests showed reduced baseline spirometry: 11/19 forced vital capacity (FVC) of 4130 mL (77%); forced expiratory volume in 1 second (FEV1) of 3620 mL (91%); and a FEV1/FVC ratio of 88%.

The patient also presented various allergies (pollen, olive trees, reeds, banana, and grasses) and dyslipidaemia, which is under treatment. His allergic symptoms have worsened at the

TABLE 1 | Analytical studies.

		Patient	Healthy controls (range)[d]
Haemogram[a] (without alterations)	Haemoglobin (g/dL)	14.9	13.1–17.2
	Haematocrit (%)	45.5	39–50
	MCV (fl)	93.5	81–101
	Platelets	227,000	150–450,000
	Leukocytes	4,700	4000–10,000
	Neutrophils	2,000	2000–7000
	Lymphocytes	2,000	1000–3000
Biochemistry	Fibrinogen (mg/dL)	379	150–400
	Protein (g/dL)	6.7	6.6–8.3
	Albumin (g/dL)	4.4	3.5–5.3
	Glucose (mg/dL)	100	74–106
	LDH (U/L)	351	208–378
	Creatine kinase (U/L)	736	10–171
	Cholesterol (mg/dL)	275	25–200
Thyroid profile	TSH (μU/mL)	1.9	0.3–5.3
	Free T4 (pg/mL)	8.08	5.8–16.4
Liver profile	ALT (U/L)	28	3–50
	AST (U/L)	26	3–50
	GGT (U/L)	33	1–55
	AP (U/L)	83	33–120
	Total bilirubin (mg/dL)	0.4	0.3–1.3
Renal profile	Creatinine (mg/dL)	1	0.67–1.17
	Estimated glomerular filtration rate (mL/min)	84.9	>60
Vitamins	Folic acid (ng/mL)	16.37	3.1–20
	B12 (pg/mL)	254.00	180–914
	Vitamin D (ng/mL)	43.9	30–50
Complement	C3 (mg/dL)	122.3	70–140
	C4 (mg/dL)	18.8	15–30
Immunoglobulins[b]	IgA (mg/dL)	171	80–400
	IgE (kU/L)	60	0–100
	IgG (mg/dL)	1,084	600–1600
	IgG1 (mg/dL)	612.0	382–930
	IgG2 (mg/dL)	306.1	240–700
	IgM (mg/dL)	172	50–200
Lymphocyte subpopulations[c]	CD3 (%)	70.4	60–83.5
	(cells/μL)	1,198.3	714–2266
	CD4 (%)	51.71	32–62
	(cells/μL)	879.1	359–1565
	CD8 (%)	18.45	11–35
	(cells/μL)	313.7	178–853
	CD19 (%)	9.4	3–19
	(cells/μL)	160.4	61–321
	CD16+ CD56+ (%)	17.1	4-18
	(cells/μL)	291.2	149–283

[a]Distribution of the patient's immune cell populations in peripheral blood. Absolute counts $\times 10^9$ per litre of blood. [b] Immunoglobulin levels (IgG, IgG1, IgG2, IgA, IgM and IgE) measured by nephelometry. [c] Distribution of lymphocyte subpopulations in the patient's peripheral blood. [d] Internal range.

ALT, alanine aminotransferase; ANAs, antinuclear antibodies; ANCAs, antineutrophil cytoplasmic antibodies; AST, aspartate aminotransferase; AP, alkaline phosphatase; GGT, gamma-glutamyl transferase; Ig, immunoglobulin; LDH, lactate dehydrogenase; MCV, mean corpuscular volume; TSH, thyroid-stimulating hormone.

dermatological level, with particular involvement of the chest, back, and legs.

The patient takes eslicarbazepine (800 mg; 0-0-0.5), ezetimibe (10 mg; 0-0-1), chondroitin sulphate (400 mg; 2 doses every 24 h), bilastine (20 mg; 0-1-0), rupatadine (10 mg; 0-0-0-1), and budesonide/formoterol (1-0-1).

DIAGNOSTIC ASSESSMENT

The experimental protocol was approved by the ethics committee of Clinico San Carlos University Hospital (Madrid, Spain) and La Paz University Hospital (Madrid, Spain), and written informed consent was obtained from the family for participation in this study.

The patient underwent a next-generation sequencing gene panel for the diagnosis of primary immunodeficiencies. We found a variant of the *STIM1* gene on chromosome 11, a heterozygous missense mutation (T/C) affecting the nucleotide position g.4095848 (GRCh37.p13) of exon 7 of the gene encoding STIM1 in the genomic DNA extracted from the leukocytes (g.4095848T>C). This mutation affects leucine at position 303, generating a missense mutation by a proline (c.1477T>C, p.L303P, transcript ID ENST00000300737.4), a previously unreported variant, which we validated by Sanger

sequencing of genomic DNA from peripheral leukocytes (**Figure 1A**). His mother, who has cardiomyopathy, has the same *STIM1* mutation (L303P) (data not shown). No more data could be obtained from the mother, the father could not be tested, and the patient has no offspring. No other mutations were found in the *STIM1* coding region. Alignment of the human STIM1 protein sequence with sequences from the 7 animal species in which STIM1 has been sequenced showed L303 to be highly conserved throughout evolution (**Figure 1B**). Protein structure modelling showed how the nonpolar lateral chain of leucine 303 is oriented inside the interaction of the 2 alpha chains of CC1-IH STIM1 (4). The L303P mutation modifies the position of the lateral chain, affecting the proper conformation of the CC1-IH region of STIM1 (**Figure 1C**) (4). A mutation significance cut-off study (http://pec630.rockefeller.edu:8080/MSC/) predicted this variant as likely to be damaging (**Figure 1D**). These data suggest that a heterozygous germline missense *STIM1* mutation (g.4095848T>C; p.L303P) might be responsible for the novel autosomal dominant form of the patient's GOF STIM1 mutation.

Numerous studies have described the role of *STIM1* mutations in the cells implicated in TAM/STRMK, such as myoblasts and myotubes (5). Given that the patient showed immunological involvement, we wanted to test the protein expression and calcium signalling in immune cells. We then assessed STIM1 protein levels in the patient's peripheral blood mononuclear cells (PBMCs), detecting normal STIM1 protein levels compared with healthy donors (**Figure 1D**). Given that STIM1 GOF mutations induce excessive Ca^{2+} entry in muscle cells, thereby causing myopathy (10), we wanted to test whether the L303P STIM1 mutation affects calcium homeostasis in PBMCs. We stimulated PBMCs with the calcium ionophore ionomycin, measuring calcium mobilisation by changes in fluorescence of the Fluo4 dye loaded into the cells. Higher calcium flux in response to ionomycin was detected in the patient compared with the 3 healthy donors (**Figures 1E–G**), confirming that the L303P mutation generates a GOF in PBMCs that affects calcium homeostasis. A study of lymphocyte proliferation response to mitogens with CD3-CD28 and phytohaemagglutinin showed normal results (data not shown).

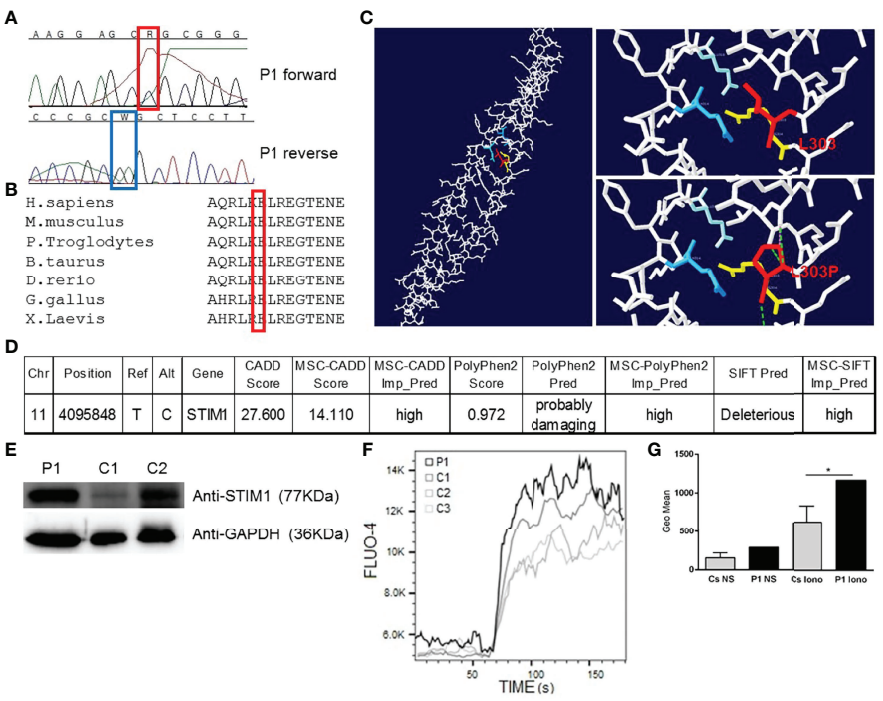

FIGURE 1 | Heterozygous *STIM1* mutation in a patient with myopathy. **(A)** The sequence of the PCR products of genomic DNA from the patient's leukocytes is shown. g.4095848T>C (c.1477T>C, p.L303P). This mutation has not been previously reported. **(B)** Multiple alignment of the sequences from humans and 6 other species, showing that L303 is a conserved amino acid in 7 analysed species. **(C)** The left panel shows the structure of human CC1-IH STIM1 (4). The top right panel shows the interactions between 2 alpha helices of the CC1-IH region of STIM1. Blue is Q314 and light blue is E318 of one alpha helix, and L303 and R304 are the red and yellow residues, respectively, of the other alpha helix (4). The bottom right panel shows the L303P mutation. The figure was produced using Swiss-PdbViewer. **(D)** Mutation significance cut-off (http://pec630.rockefeller.edu:8080/MSC/) of STIM1 L303P mutation. **(E)** Immunoblot analysis of STIM1 protein from the patient's (P1) peripheral blood mononuclear cells (PBMCs) and from 2 healthy donors (C1 and C2). We employed GAPDH as a loading control. The panels illustrate the results from a single experiment, representative of 3. **(F)** Calcium flux analysis in the PBMCs of P1 and 3 healthy donors (C1, C2, and C3) in response to ionomycin. The panels illustrate the results from a single experiment, representative of 3. **(G)** Calcium flux geometric mean (Geo Mean) is represented for C1, C2, and C3. ± SD and P1 in non-stimulated PBMCs (NS) or ionomycin-stimulated PBMCs (Iono). $p < 0.05$ (*).

DISCUSSION

The present case describes a patient with an autosomal dominant GOF *STIM1* mutation responsible for TAM/STRMK. The L303P mutation in the *STIM1* gene showed an incomplete penetrance in the mother, who carried the mutation without TAM/STRMK symptoms. *STIM1* mutations can be divided into those with GOF that manifest TAM/STRMK (2) and those with LOF that have an immune effect responsible for severe combined immunodeficiency, involving recurrent and chronic infections, muscular hypotonia, autoimmunity, ectodermal dysplasia, anhidrosis, and mydriasis (2). However, our patient has a GOF *STIM1* mutation responsible for TAM/STRMK and has had respiratory infections since childhood, including chronic cough and chronic bronchiectasis, despite the main immunological features in terms of immune response and levels of immune cells and other immune parameters being within normal limits. The analysis of immune cells (PBMCs) of the Ca^{2+} signalling revealed a GOF with higher levels of calcium flux compared with healthy donors (**Figures 1E, F**), revealing calcium flux misregulation in the immune cells that could be responsible for the patient's immune involvement. Worth to mention that the very close residue R304, the only mutated residue described in luminal coiled-coil CC1 domain, in mice harboring GOF R304W mutation presenting TAM/STRMK and abnormal immune cell counts as well as skin abnormalities (11) as shown by our patient.

Despite the study's limitations resulting from the lack of access to information and studies of the patient's relatives (incomplete penetrance of the mutation in the mother being the only datum), this is an important clinical case that warrants careful examination to clarify the dual role of STIM1 in immune system dysregulation and myopathy.

PATIENT PERSPECTIVE

It has been widely reported that *STIM1* GOF mutations are responsible for TAM/STRMK and that *STIM1* LOF mutations cause severe combined immunodeficiency. However, it is plausible that a *STIM1* GOF mutation in a patient with TAM/STRMK can also cause immune involvement, given that STIM1 is a protein involved in the immune system and that a misfunction (either by an excess or lack of calcium signalling) would affect the proper functioning of the immune system. Patients with TAM/STRMK should be followed up by clinical immunology departments to find more candidates with whom to study this preliminary finding in more depth. We cannot rule-out the possibility that this immunological involvement would be due to a digenic or polygenic condition by other genes involved in the immune system; however, excess calcium signalling in immune cells highlights a misfunction in the immune system. More cases need to be reported to accumulate sufficient evidence.

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

Study approval: The experimental protocol was approved by the ethics committee of Clinico San Carlos University Hospital (Madrid, Spain) and La Paz University Hospital (Madrid, Spain), and written informed consent was obtained from the family for participation in this study. Written informed consent was obtained from the patient for the publication of any potentially identifiable data included in this article.

AUTHOR CONTRIBUTIONS

EF: Physicians in charge of the patient's care. Clinical report and analytical studies. AR: Calcium signalling and Sanger sequencing. BG-S: Protein expression, Sanger sequencing and protein modelling. JO, KG-H, and MF-A: Clinical study, analytical parameters, manuscript editing. LG: Physician in charge of the patient's study and care. JM-G: Physician in charge of the patient's study and care. SS-R: Physician in charge of the patient's study and care, has read and revised the manuscript. RP: Laboratory head, experiment design, manuscript drafting and editing, Corresponding author. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Feske S. Immunodeficiency Due to Defects in Store-Operated Calcium Entry. *Ann N Y Acad Sci* (2011) 1238:74–90. doi: 10.1111/j.1749-6632.2011.06240.x

2. Silva-Rojas R, Laporte J, Böhm J. STIM1/ORAI1 Loss-Of-Function and Gain-Of-Function Mutations Inversely Impact on SOCE and Calcium Homeostasis and Cause Multi-Systemic Mirror Diseases. *Front Physiol* (2020) 11:604941. doi: 10.3389/fphys.2020.604941

3. Markello T, Chen D, Kwan JY, Horkayne-Szakaly I, Morrison A, Simakova O, et al. York Platelet Syndrome Is a CRAC Channelopathy Due to Gain-of-Function Mutations in STIM1. *Mol Genet Metab* (2015) 114(3):474–82. doi: 10.1016/j.ymgme.2014.12.307
4. Cui B, Yang X, Li S, Lin Z, Wang Z, Dong C, et al. The Inhibitory Helix Controls the Intramolecular Conformational Switching of the C-Terminus of STIM1. *PLoS One* (2013) 8:e74735. doi: 10.1371/journal.pone.0074735
5. Conte E, Pannunzio A, Imbrici P, Camerino GM, Maggi L, Mora M, et al. Gain-Of-Function STIM1 L96V Mutation Causes Myogenesis Alteration in Muscle Cells From a Patient Affected by Tubular Aggregate Myopathy. *Front Cell Dev Biol* (2021) 9:635063. doi: 10.3389/fcell.2021.635063
6. Morin G, Bruechle NO, Singh AR, Knopp C, Jedraszak G, Elbracht M, et al. Gain-Of-Function Mutation in STIM1 (P.R304W) Is Associated With Stormorken Syndrome. *Hum Mutat* (2014) 35(10):1221–32. doi: 10.1002/humu.22621
7. Ticci C, Cassandrini D, Rubegni A, Riva B, Vattemi G, Matà S, et al. Expanding the Clinical and Genetic Spectrum of Pathogenic Variants in STIM1. *Muscle Nerve* (2021) 64:567–75. doi: 10.1002/mus.27391
8. Jiang L-J, Zhao X, Dou Z-Y, Su Q-X, Rong Z-H. Stormorken Syndrome Caused by a Novel STIM1 Mutation: A Case Report. *Front Neurol* (2021) 12:522513. doi: 10.3389/fneur.2021.522513
9. Michelucci A, García-Castañeda M, Boncompagni S, Dirksen RT. Role of STIM1/ORAI1-Mediated Store-Operated Ca2+ Entry in Skeletal Muscle Physiology and Disease. *Cell Calcium* (2018) 76:101–15. doi: 10.1016/j.ceca.2018.10.004
10. Böhm J, Laporte J. Gain-Of-Function Mutations in STIM1 and ORAI1 Causing Tubular Aggregate Myopathy and Stormorken Syndrome. *Cell Calcium* (2018) 76:1–9. doi: 10.1016/j.ceca.2018.07.008
11. Silva-Rojas R, Treves S, Jacobs H, Kessler P, Messaddeq N, Laporte J, et al. STIM1 Over-Activation Generates a Multi-Systemic Phenotype Affecting the Skeletal Muscle, Spleen, Eye, Skin, Bones and Immune System in Mice. *Hum Mol Genet* (2019) 28:1579–93. doi: 10.1093/hmg/ddy446

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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Case report: Successful allogeneic stem cell transplantation in a child with novel GATA2 defect associated B-cell acute lymphoblastic leukemia

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GATA-binding protein 2 (*GATA2*) is a transcription factor responsible for the regulation of blood cell proliferation, differentiation, and maintenance in hematopoietic stem cells. Here, we describe successful bone marrow transplantation in a carrier of a novel GATA2 pathogenic variant who was diagnosed with immunodeficiency a few years after completion of B-cell precursor acute lymphoblastic leukemia (BCP-ALL) treatment. At the age of 4 years, the patient was diagnosed with and treated for BCP-ALL. Antileukemic therapy was complicated by pulmonary cryptococcosis. Two years after completion of the maintenance therapy, the child was consulted by an immunologist because of recurrent respiratory tract infections and an episode of sepsis. Flow cytometry revealed deep monocytopenia, lymphopenia, absence of B lymphocytes, considerably reduced NK cells, poor thymic T lymphocyte production, minor defects in T cell maturation, and absence of TCRγδ+ T cells. The presence of the likely pathogenic, heterozygous missense variant within exon 5 of *GATA2* (NM_032638.5: c.1047T>G, Cys349Trp) was identified in the proband and confirmed in the father of the patient, who underwent allogeneic hematopoietic stem cell transplantation (HSCT) from a matched unrelated donor due to myelodysplastic syndrome with excess blasts at the age of 22 years. An

allogeneic hematopoietic stem cell transplantation with a reduced toxicity conditioning protocol was performed using a matched sibling donor. Pre-transplant conditioning included fludarabine (5 × 30 mg/m2), treosulfan (3 × 14 g/m2), and thiotepa (10 mg/kg). Complete donor chimerism was achieved on post-transplant day 17. During the 12 months of the posttransplant observation period, she remained free from symptoms of acute or chronic graft-versus-host disease, and immunosuppressive treatment was therefore stopped. This is the second reported case of BCP-ALL in a patient with *GATA2* deficiency, and the first successfully treated with a reduced-toxicity conditioning HSCT protocol. The co-occurrence of lymphoid malignancies and primary immunodeficiencies points to the role of genetic counseling and family screening for possible cancer predisposition syndromes prior to the selection of related HSCT donors.

KEYWORDS

GATA2, acute lymphoblastic leukemia, immunodeficiency, hematopoietic stem cell transplantation, treosulfan

Introduction

GATA-binding protein 2 (*GATA2*) is a transcription factor responsible for the regulation of blood cell proliferation and differentiation as well as the maintenance of hematopoietic stem cells (1, 2). Germline mutations that cause *GATA2* haploinsufficiency can lead to a wide spectrum of clinical symptoms. The main features include cytopenia, which can affect several cell lineages, including B cells, natural killer (NK) cells, CD4+ T cells, and monocytes. Impaired multi-lineage hematopoiesis may lead to clonal selection and evolution into myeloid neoplasms. *GATA2* induced immunodeficiency is associated with an increased risk of atypical mycobacterial, herpes virus infection, and fungal disease (3–6). Moreover, about half of the patients develop pulmonary complications, mainly pulmonary alveolar proteinosis (PAP), caused by the abnormal accumulation of surfactant, which worsens gas exchange and can lead to pulmonary arterial hypertension (7). Other complications include lymphedema, chronic warts that are irresponsive to treatment, an increased risk of skin cancers, hypothyroidism, and hearing impairment (8). *GATA2* deficiency is an inherited autosomal dominant trait or caused due to *de novo* mutations and is characterized by high penetrance (approximately 90% by the age of 60 years) (4, 8).

Although the influence of *GATA2* deficiency on the development of myeloid neoplasms is well documented, B-cell precursor acute lymphoblastic leukemia (BCP-ALL) has been reported in only one patient with monocytopenia and mycobacterial infection (MonoMAC) syndrome (9, 10). Therefore, we describe the phenotype of GATA2 deficiency during the first year of life in carriers of the novel familial *GATA2* mutation who developed immunodeficiency after ALL treatment. This case illustrates the importance of collecting the family history of children diagnosed with ALL and immunological testing of ALL patients who show recurrent infections a few years after the completion of oncological treatment.

Methods

Peripheral blood sample was immunophenotyped by multicolor flow cytometry and fluorochrome-conjugated mouse monoclonal antibodies. The BD Multitest TM 6-color TBNK with Trucount tubes (Becton Dickinson, cat no. 337166) (CD3-FITC (clone SK7), CD16-PE (clone B73.1), CD56-PE (clone NCAM16.2), CD45-PerCP-Cy5.5 (clone 2D1), CD4-PE-Cy7 (clone SK3), CD19-APC (clone SJ25C1), CD8-APC-Cy7 (clone SK1)) was used as prescribed by the manufacturer to evaluate basic lymphocyte subsets. Maturation of the T helper and T suppressor/cytotoxic lymphocyte subsets was based on the differential expression of CD3PerCP (clone SK7, Becton Dickinson cat no. 345766), CD4-APC (clone SK3, Becton Dickinson cat no. 345771), CD8-APC-Cy7 (clone SK1, BD Pharmingen, cat no. 557834, CD27-APC (clone L128, Becton Dickinson cat. no. 337169), CD31-PE (clone WM59, BD Pharmingen cat. No 555446), CD45RA-FITC (clone HI100, BD Pharmingen cat. no. 555488), and CD45RO-FITC (clone UCHL1, BD Pharmingen cat. no 555492). Additionally, T regulatory cells and the distribution of TCRαβ and TCRγδ receptors were also evaluated (CD3-PerCP (clone SK7, Becton Dickinson cat no.

345766) CD4-APC (clone SK3, Becton Dickinson cat no 345771), CD25-FITC (clone 2A3, Becton Dickinson cat. No 340907), CD127-PE (clone HIL-7R-M21, BD Pharmingen, cat no. 557938), TCRαβ-FITC (clone WT31, BD Pharmingen 333140), and TCRγδ-PE (clone 11F2, Becton Dickinson, cat no. 333141). Briefly, 50 µl of the full blood sample was incubated with monoclonal antibodies for 15 min at room temperature. Erythrocytes were lysed for 15 min with a BD Lysing Solution. The samples were then washed with PBS+0.1% sodium azide. At least 2000 cells from the analyzed gate were acquired using an appropriately calibrated FACS Canto II flow cytometer (Becton Dickinson). The results were analyzed using the BD FACS Diva v.8 software (Becton Dickinson).

Bone marrow sample (BM) was subjected to flow cytometric immunophenotyping using a 4-tube, 8-color panel of fluorochrome-conjugated monoclonal antibodies against lymphoid and myeloid antigens (CD19-PE-Cy7, CD10-APC, CD22-PE, CD45-V500, CD34-PerCP-Cy5.5, CD38-APC-H7, CD117-PE, CD33-PE-Cy7, CD13-PE, CD15-FITC, CD11b-APC, CD64-PE, CD36-APC, CD4-APC, CD14-APC-H7 – Becton Dickinson, San Jose, CA, USA; HLADR-Pacific Blue, CD16-Pacific Blue, CD20-PacificBlue – Biolegend, San Diego, CA, USA; CD56-PE-Cy7 – Beckman Coulter, Brea, CA, USA). The samples were incubated with monoclonal antibodies for 15 min at room temperature. Erythrocytes were lysed by incubation for 10 min with an appropriately diluted BD Lysing Solution (Becton Dickinson). After erythrocyte lysis, the samples were washed with Cell Wash solution (Becton Dickinson) and analyzed using a FACS Canto II flow cytometer (Becton Dickinson). 300,000 cells per tube were recorded. The Infinicyt software was used for data analysis (Cytognos, Salamanca, Spain).

DNA isolation

Genomic DNA was extracted from peripheral blood samples using the QIAamp DNA Blood Mini Kit (QIAGEN, Hilden, Germany) or from buccal swabs using the Sherlock AX Kit (A&A Biotechnology, Poland). The concentration and quality of the isolates were determined by ultraviolet spectrophotometry (NanoDrop 8000, Thermo Scientific, Waltham, USA).

Direct sequencing

The presence of a likely pathogenic missense variant within exon 5 of *GATA2* (NM_032638.5: c.1047T>G, Cys349Trp) was identified by direct sequencing. Standard PCR conditions were applied with primers specifically designed to cover the *GATA2* coding region using NetPrimer software (Supplementary Table 1). Products were sequenced on an ABI3130 4-capillary sequencer (Thermo Fisher Scientific, Waltham, MA, USA), and the results were analyzed using Sequencher v. 5.0 (Gene Codes, Ann Arbor, USA).

Case report

A 4-year-old girl was referred to the oncology department for diagnosis and treatment of leukopenia, neutropenia, and anemia. The timeline of case report is shown in Figure 1. In the past, she had experienced several episodes of bronchitis and pneumonia. Leukopenia and anemia were discovered accidentally when she was treated with bilateral tympanostomy due to recurrent otitis media. A complete blood count with differential revealed profound neutropenia, thrombocytopenia, and lymphocytosis. Clinically, the patient presented with progressive weakness, and multiple sites of subcutaneous hemorrhage and cervical lymphadenopathy without hepatosplenomegaly were observed. Due to suspected malignancy the patient underwent hematological work-up with BM biopsy.

At referral, lymphoblasts expressing CD34/CD9/CD10/CD19/CD20/CD22/CD24/CD33/CD38/CD66c/CD79a/CD81/TdT comprised 85% of the nucleated cells in the BM but were not found in the peripheral blood of the patient. Acute lymphoblastic leukemia of B-cell progenitor origin was diagnosed based on the results of 8-color flow cytometry performed with a standard Euroflow panel of fluorochrome-conjugated monoclonal antibodies (11). The patient responded

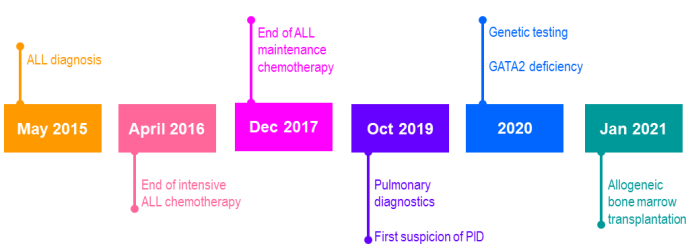

FIGURE 1
Case report timeline. ALL, acute lymphoblastic leukemia; PID, primary immunodeficiency.

well to the treatment according to the ALL IC-BFM 2009 protocol and showed 1.56% blast cells at day +15 and 0.08% blast cells at day +33 in the BM (12). Despite adequate trimethoprim prophylaxis, the patient developed symptoms resembling an upper respiratory tract infection. Although she received aggressive treatment with meropenem, vancomycin, amphothericin B, teikoplanin, ciprofloxacin, and clarithromycin, she developed an abscess in her right lung, which was positive for *Cryptococcus* antigen. Although mercaptopurin hypersensitivity was suspected because the patient demonstrated long periods of BM aplasia; however, thiopurin S-methyltransferase deficiency was excluded. Inflammatory lesions caused by *Streptococcus pneumoniae* MLSB were identified during bronchoscopy and effectively treated with antibiotics and antifungal agents accompanying long-term oncologic treatment. Pneumonia, probably of atypical bacterial origin, and reactivation of enteric *Clostridium* infection were also observed. Due to infectious complications, intensive phase of ALL chemotherapy was extended from 6 to 11 months. Consolidation and maintenance treatment were continued for 13 months, leading to mild leukopenia and neutropenia with normal monocyte counts after the completion of 32 months of chemotherapy. After the end of 2 years of oncologic treatment, the patient was referred by an outpatient immunologist for recurrent respiratory tract infections and a recent episode of sepsis. Fatigue, poor wound healing, and ecchymosis were also observed. Flow cytometry revealed deep monocytopenia, lymphopenia, absence of B lymphocytes, significantly reduced NK cells, poor thymic T lymphocyte production, minor defects in T cell maturation, and absence of TCRγδ+ T-cells (Figure 2). The values of the lymphocyte subpopulations with age-adjusted normal ranges are shown in Table 1.

The patient was consulted by a geneticist, and her family history was thoroughly examined. The father of the proband underwent allogeneic hematopoietic stem cell transplantation (HSCT) from a matched unrelated donor due to myelodysplastic syndrome (MDS) with excess blasts (EB) at the age of 22 years. The mother of the proband was diagnosed with melanoma *in situ* at the age of 35 years. Two paternal aunts died of breast and colon cancer, and one maternal aunt developed breast cancer (Figure 3A). The diagnosis of proband was based on immunophenotyping and cytogenetics, whereas the other oncological diagnoses in her family were based on self-reporting by the parents of the proband.

Because specific immunological abnormalities and BCP-ALL occurred in the proband whose father was treated for MDS and flow cytometry results were typical for *GATA2* deficiency, direct sequencing of the coding region of *GATA2* was performed. We identified a likely pathogenic heterozygous missense variant within exon 5 of *GATA2* (NM_032638.5; c.1047T > G, Cys349Trp). The mutation changed the structure of the 4 amino acids, forming a hinge between *GATA2* zinc finger domains ZF1 and ZF2. This mutation was also found in the symptomatic father, but was absent in healthy mother and sisters (Figure 3B).

The child qualified for allogeneic HSCT from an HLA-matched sibling donor (older sister). Due to the constitutional defect in the family, a sister was tested for *GATA2* deficiency and confirmed to have a wild-type *GATA2* sequence. Before transplantation, the child underwent diagnostic BM biopsy, and the sample was analyzed with flow cytometry for minimal residual disease (MRD) to exclude the presence of leukemic cells and to investigate maturation abnormalities in the hematopoietic compartment. Bone marrow smear showed normal did not present morphologic abnormalities (Supplementary Figure 1), and all cell lineages were represented and their proportions were appropriate for age (Supplementary Table 2). BM MRD immunophenotyping confirmed BCP-ALL remission with a balanced distribution of myeloid cells (46.8% of total cells, mostly neutrophils at different stages of maturation) and erythroid cells (total of 41.4%). Lymphoid, myeloid, and immature precursor cell subsets comprised 8.4%, 0.49%, and 0.58% of the total cells, respectively (Figure 4). Lymphoid cell subsets were represented by T cells and NK cells (94.8% and 5.2% of total lymphocytes, respectively), while no mature B cells or precursor B cells were present. The myeloid lineage constituted 0.49% of total BM cells, mostly of the mature phenotype (CD14+, CD64++, CD33++, CD11b+, CD36+, CD13+, CD117-); 8% of monocytes exhibited expression of CD56.

After confirming complete remission, the patient was administered megatherapy. Pre-transplant conditioning included fludarabine (5×30 mg/m^2), treosulfan (3×14 g/m^2), and thiotepa (10 mg/kg). No serotherapy was administered, and posttransplant graft-versus-host disease (GvHD) prophylaxis consisted of methotrexate on posttransplant days 1, 3, and 6, along with cyclosporine A. The grafting material was obtained from bone marrow with a CD34+ cell dose of 1.27 x 10^6 cells/kg of the recipient's body weight. The posttransplant period was complicated by grade 4 mucositis and asymptomatic *S. haemolyticus* bacteremia treated with targeted antibiotic therapy (vancomycin). The girl showed neutrophil engraftment on post-transplant day +17, and mononuclear cell chimerism studies confirmed full donor engraftment. Until discharge on post-transplant day +40, the patient presented with nausea and drug swallowing problems that were not associated with demonstrable organic findings. During 12 months of posttransplant observation, the patient showed both BM recovery and immune reconstitution (Supplementary Figure 2), and did not manifest any infectious or non-infectious complications; in particular, she remained free from symptoms of acute or chronic graft-versus-host disease, and thus immunosuppressive treatment was successfully stopped.

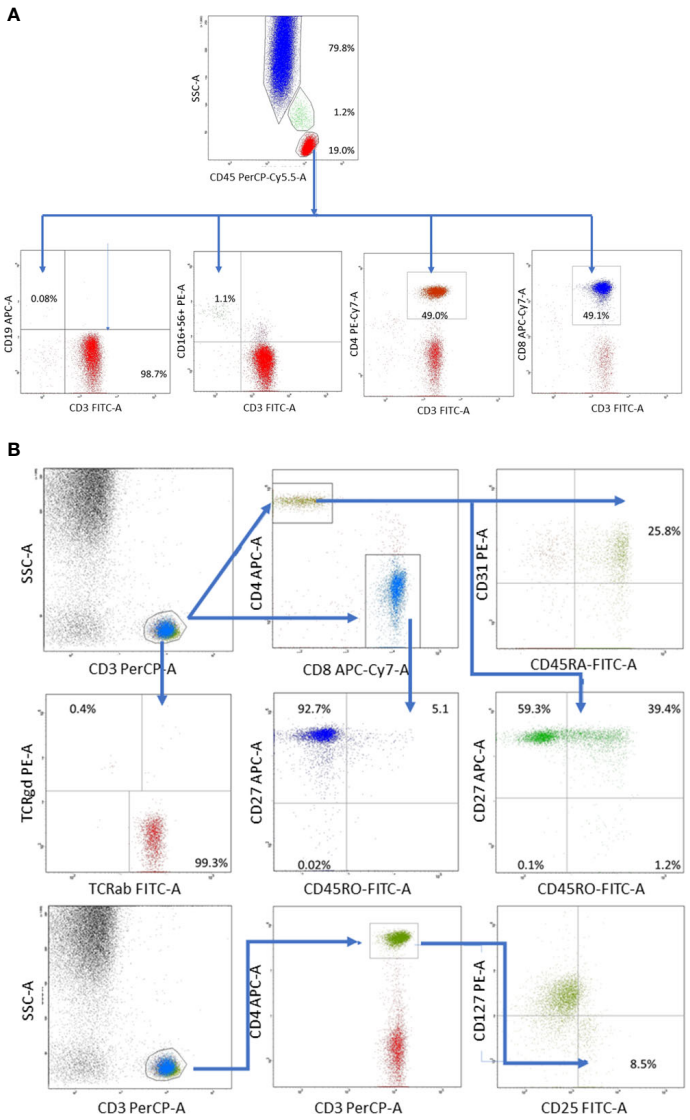

FIGURE 2
(A) Distribution of whole blood leukocyte subsets was based on differential expression of CD45 and side scatter characteristics: lymphocytes (low scatter and high CD45 expression), monocytes (medium side scatter and CD45 expression), and polymorphonuclears (high scatter and low CD45 expression) composed 19.0, 1.2% and CD79.8% of whole blood leukocytes, respectively. T lymphocytes were defined as CD3+CD19- cells, B lymphocytes as CD19+CD3- cells and composed 98.7% and 0.08% of total lymphocyte population, respectively. NK cells defined as CD3-CD16.CD56+ cells composed 1.1% of whole lymphocyte population.**(B)** T lymphocyte gate was set up based on high CD3 expression and low side scatter. Within T cell gate two subsets were identified: T helper (CD3+CD4+) and T suppressor (CD3+CD8+) cells. Recent thymic emigrants were identified as CD31+CD45RA+, while regulatory T cells as CD25+CD127- within T helper cell population. Remaining T lymphocyte maturation stages: naïve (CD27+CD45RO-), memory (CD27+CD45RO+), effector memory (CD27-CD45RO+) and effector (CD27-CD45RO-) were defined in the similar way within T helper (CD3+CD4+) and T suppressor (CD3+CD8+) gates. Distribution of T cell receptor variants was defined based on TCRαβ and TCRγδ expression within T cell gate).

Discussion

GATA2 was discovered in 2011 as an MDS/AML predisposition gene. Hahn et al. in their study found two different mutations in *GATA2* in two families with a predisposition to MDS/AML (3). Further research has shown that the mutation spectrum is broad and includes missense, nonsense, frameshift, complex mutations, splice defects, and even whole-gene deletions (13). The molecular landscape of *GATA2* deficiency is more complicated than initially suggested. According to Kozyra et al. some *GATA2* synonymous mutations do not alter protein function or stability but can lead to splicing errors or late-stage RNA loss without splicing disruption (14). Nevertheless, new mutations are still being identified, as in the

TABLE 1 Results of peripheral blood lymphocyte subpopulations.

Parameter:			Absolute counts	Normal range (age 5 y - 10 y)	
		%	(cells/ul)	(%)	(cells/ul)
Lymphocytes		19.0↓		29.6-49.8	
Monocytes		1.2↓		6.1-12.5	
Polymorphonuclears		79.8↑		41.6-64.1	
Lymphocytes			787↓		1700-3600
T	CD3+	98.7↑	777↓	52.4-77.9	1000-2600
T CD8+	CD3+CD8+	49.1↑	387	15.0-35.4	300-1000
T CD4+	CD3+CD4+	49.0	386↓	26.7-46.2	500-1500
NK cells	CD16+56+CD3-	1.1↓	9↓	6.2-29.8	140-690
B lymphocytes	CD19+	0.08↓	1↓	9.7-23.7	300-600
CD4:CD8		1.0		0.8-2.5	
Recent thymic emigrants	CD31+CD45RA+ (%CD3+CD4+)	25.8↓		>40	
Naïve T CD4+	CD27+CD45RO-(%CD3+CD4+)	59.3		55.6-75.8	
Memory T CD4+	CD27+CD45RO+ (%CD3+CD4+)	39.4↑		22.5-37.0	
Effector memory CD4+	CD27-CD45RO+ (%CD3+CD4+)	1.2↓		1.5-9.7	
Effector CD4+	CD27-CD45RA-(%CD3+CD4+)	0.1		0.1- 0.3	
Treg	CD25+CD127- (%CD4)	8.5↑		1.8-7.4	
Naïve T CD8+	CD27+CD45RO-(%CD3+CD8+)	92.7↑		57.0-83.7	
Memory T CD8+	CD27+CD45RO+ (%CD3+CD8+)	5.1↓		9.2-22.6	
Effector memory CD8+	CD27-CD45RO+ (%CD3+CD8+)	0.2↓		0,7-14.0	
Effector CD8+	CD27-CD45RA-(%CD3+CD8+)	1.93		0.9-17.9	
TCRαβ	TCRαβ+ (%CD3)	99.3↑		78.5-93.8	
TCRγδ	TCRγδ+ (%CD3)	0.4↓		6.0-21.4	

↑, above normal range; ↓, below normal range.

case of reported patient. Moreover, in contrast to adult mutation carriers, who manifest a complex GATA2 deficiency phenotype, initial clinical manifestation can be scarce and diagnosis challenging at a young age. Monocytopenia, deemed to be a hallmark of *GATA2* deficiency, may be absent at an early age or even an elevated monocyte count can be observed in these patients (15).

It has been postulated that *GATA*2 mutations are the most common germline defects that predispose to pediatric MDS, but importantly, they do not worsen the prognosis (15). However, the identification of such inherited predisposition genes is crucial for family screening, faster diagnosis, bone marrow transplant therapy, and selection of potential related donors (3).

Nonetheless, the diagnosis in our patient was difficult not only due to a new, so far unknown mutation, but also due to the reduced vigilance resulting from an atypical neoplasm. The influence of *GATA2* deficiency on AML/MDS development has been well described; however, the relationship between mutations in this gene and lymphoid neoplasms has not been widely documented. In 2016, Koegel et al. were the first to describe a case of B-ALL in an 11-year-old girl who was later diagnosed with MonoMAC syndrome and, therefore, with *GATA2* haploinsufficiency. After ALL relapse, she underwent HSCT but eventually died 3 months later due to neurologic injury resulting from leukemic brain infiltrates (10). Two years later, in 2018, Esparza et al. reported the first case of T-cell ALL in an 8-year-old girl with the *GATA2* mutation. Interestingly, this patient did not undergo HSCT because of her good clinical condition, with monthly immunoglobulin substitution and constant prophylaxis with azithromycin (9). In the same year, Donadieu et al. revealed one other case of T-cell ALL and one case of juvenile myelomonocytic leukemia among 79 surveyed patients with *GATA2* haploinsufficiency from French and Belgian populations (13). Our patient is the second ever reported patient with *GATA2* deficiency who developed BCP-ALL, but the first one showing long-term survival and with a

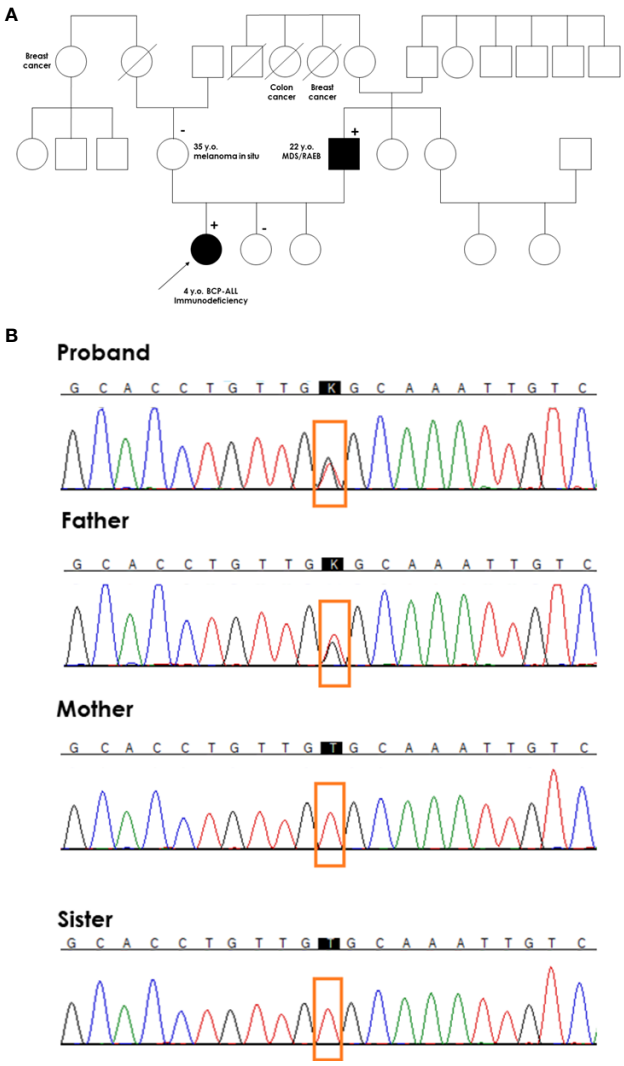

FIGURE 3
Genetic testing of the proband's family. **(A)** A pedigree of the proband's family. The proband with BCP-ALL had a germline mutation in exon 5 of the *GATA2* gene (c.1047T>G, Cys349Trp) inherited from her father with MDS. (+) denotes heterozygous mutation carrier in the germline; (-) denotes wild-type in the germline. A diagonal line through a symbol indicates that the person is deceased **(B)** Chromatograms of germline *GATA2* variant in the proband, and proband's parents and sister.

familial history of *GATA2* myeloid malignancy. One of the most intriguing conundrums in the observed patients is the fact that the reported *GATA2* variant associated with leukemogenesis was not lineage specific. Neither the case described by us nor those presented by other investigators explains the relationship between *GATA2* haploinsufficiency and the development of lymphoblastic neoplasms. Several authors have attempted to find a deleterious *GATA2* mutation in familial and sporadic cases of lymphoblastic tumors (3, 16). Collin et al. suggested that the *GATA2* transcription factor plays not only an important role in early hematopoiesis, but it can also affect B cell differentiation (17). It would be interesting to investigate somatic aberrations present in the leukemic sample of our patient. This could potentially shed light on the acquisition of cooperative gene lesions that promote leukemic evolution. Although *GATA2* activation has been observed in BCP-ALL cells, the role of *GATA2* haploinsufficiency in leukemogenesis has not been addressed (18).

Cell subpopulation studies in the peripheral blood and BM showed maturation abnormalities in the patient. In patients who developed *GATA2*-related MDS, monocytosis was observed at the initial stage (19, 20). Loss of B cells and their precursors is the most constant feature of *GATA2* deficiency, especially in patients who develop MDS, when monocytopenia might be masked by progenitor cell expansion and total lymphocyte counts maintained by expanding memory T cells (21). In the reported

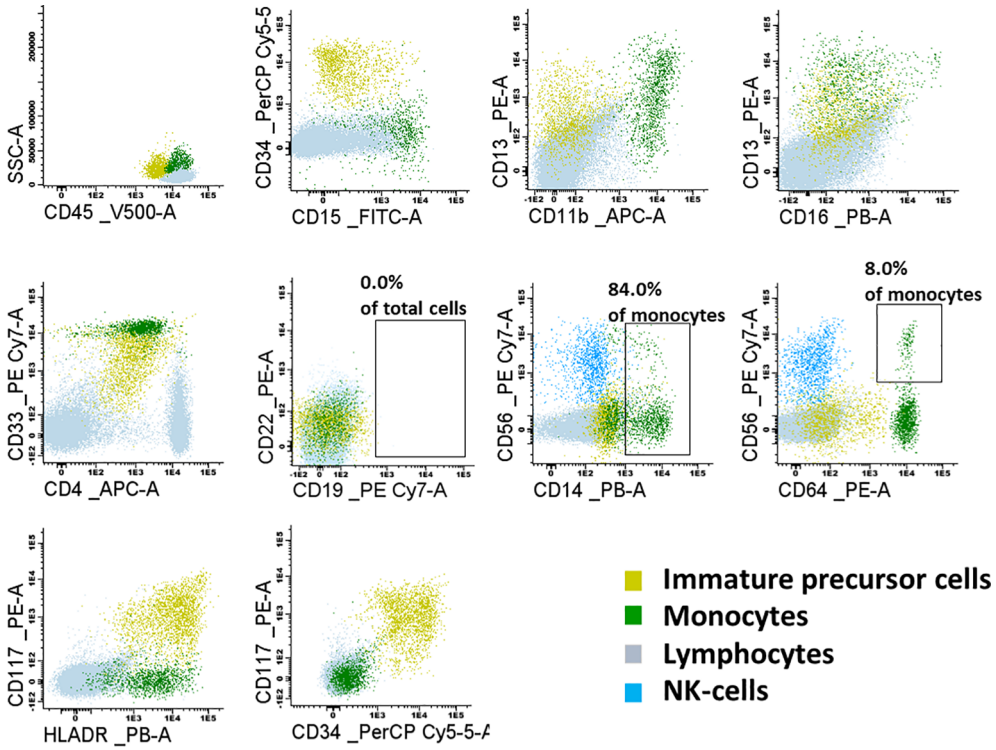

FIGURE 4
Flow cytometry dot-plots representing the lymphoid, monoid and immature precursor cell subsets in the examined bone marrow sample. Lymphoid subset was represented mostly by T-cells and NK-cells. No mature B-cells nor precursor B-cells were present. Monoid lineage was represented mainly by mature monocytes (84.0% of CD14+); 8% of monocytes were CD56+.

case, we did not observe an abundance of CD8+ T cells expressing CD45RA (TEMRA phenotype) or loss of CD27, in contrast to an earlier report (8).

The curative role of HSCT in *GATA2* deficiency has been firmly established in patients with myeloid malignancies; however, in earlier cohorts, only a minority of *GATA2* deficient patients were referred for transplantation (13). Recent worldwide experience with patients having *GATA2* deficiency suggests the need for HSCT in as many as 80% of these cases (22). The best timing of HSCT has not yet been established, because some patients with *GATA2* deficiency show long-term survival, and genetic reversal due to somatic rescue mutations has been reported (23). A reasonable recommendation is to perform HSCT before *GATA2* deficient patients develop malignancies or severe or recurrent infections that lead to organ failure (24). At the time of preparing this manuscript, there are no recommendations on the choice of conditioning protocol for *GATA2* deficiency. In children with myelodysplastic syndromes, HSCT outcomes were independent of *GATA2* germline mutations, but the cytogenetic profile and BM blast count were associated with different prognoses and megatherapy protocols (25). Hoffman et al. analyzed the outcomes of HSCT after myeloablative conditioning and reported an elevated risk of neurologic toxicities and post-HSCT thrombotic events in the *GATA2* cohort (26). However, these findings were not confirmed by Bortnick et al (25). In contrast, in a recently published paper, a busulfan-based myeloablative conditioning protocol was associated with typical complications and resulted in an 85.1% OS after 4 years (27). The conditioning protocol for our patient was chosen based on the fact that she was in complete remission, and BCP-ALL itself was not considered an unfavorable risk factor or an indication for HSCT alone. The leading indication for HSCT in this case was immunodeficiency, and the patient was prepared with a treosulfan-fludarabine-based reduced toxicity conditioning (RTC) regimen with the addition of thiotepa, similar to the ESID guidelines (28, 29). RTC protocols are associated with a lower incidence of late sequelae, such as endocrinopathy, which is important in children with long-term survival expectation (30).

In conclusion, we report a rare occurrence of BCP-ALL in *GATA2* deficiency patients successfully treated with a reduced toxicity conditioning HSCT protocol. The medical history of patient draws attention to the possible co-occurrence of lymphoid malignancies with primary immunodeficiencies and cancer predisposition syndromes. Genetic diagnosis of inherited bone marrow dysfunction was essential for the appropriate treatment of the patient and screening of her family.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to Marek Ussowicz, marek.ussowicz@umw.edu.pl.

Ethics statement

Written informed consent was obtained from the individual(s) and/or minor(s)' legal guardian/next of kin for the publication of any potentially identifiable images or data included in this article.

Author contributions

Concept, data collection, analysis, manuscript preparation and acceptance: EH-P, MU. Data collection, molecular studies, manuscript acceptance: KM-W, AP, WM. Data collection, immunological studies, manuscript acceptance: BP, ŁS, TS. Data collection, patient care, manuscript acceptance: AS-B, KK. All authors have read and approved the final manuscript. 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.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2022.928529/full#supplementary-material

References

1. Tsai FY, Orkin SH. Transcription factor GATA-2 is required for proliferation/survival of early hematopoietic cells and mast cell formation, but not for erythroid and myeloid terminal differentiation. *Blood* (1997) 89(10):3636–43. doi: 10.1182/blood.V89.10.3636

2. Orkin SH. GATA-binding transcription factors in hematopoietic cells. *Blood* (1992) 80(3):575–81. doi: 10.1182/blood.V80.3.575.575

3. Hahn CN, Chong C-E, Carmichael CL, Wilkins EJ, Brautigan PJ, Li X-C, et al. Heritable GATA2 mutations associated with familial myelodysplastic syndrome and acute myeloid leukemia. *Nat Genet* (2011) 43(10):1012–7. doi: 10.1038/ng.913

4. Hsu AP, Sampaio EP, Khan J, Calvo KR, Lemieux JE, Patel SY, et al. Mutations in GATA2 a re associated with the autosomal dominant and sporadic monocytopenia and mycobacterial infection (MonoMAC) syndrome. *Blood* (2011) 118(10):2653–5. doi: 10.1182/blood-2011-05-356352

5. Dickinson RE, Milne P, Jardine L, Zandi S, Swierczek SI, McGovern N, et al. The evolution of cellular deficiency in GATA2 mutation. *Blood* (2014) 123(6):863–74. doi: 10.1182/blood-2013-07-517151

6. Mir MA, Kochuparambil ST, Abraham RS, Rodriguez V, Howard M, Hsu AP, et al. Spectrum of myeloid neoplasms and immune deficiency associated with germline GATA 2 mutations. *Cancer Med* (2015) 4(4):490–9. doi: 10.1002/cam4.384

7. Ishii H, Tazawa R, Kaneko C, Saraya T, Inoue Y, Hamano E, et al. Clinical features of secondary pulmonary alveolar proteinosis: pre-mortem cases in Japan. *Eur Respir J* (2011) 37(2):465–8. doi: 10.1183/09031936.00092910

8. Spinner MA, Sanchez LA, Hsu AP, Shaw PA, Zerbe CS, Calvo KR, et al. GATA2 deficiency: a protean disorder of hematopoiesis, lymphatics, and immunity. *Blood* (2014) 123(6):809–21. doi: 10.1182/blood-2013-07-515528

9. Esparza O, Xavier AC, Atkinson TP, Hill BC, Whelan K. A unique phenotype of T-cell acute lymphoblastic leukemia in a patient with GATA2 haploinsufficiency. *Pediatr Blood Cancer.* (2019) 66(6):e27649. doi: 10.1002/pbc.27649

10. Koegel AK, Hofmann I, Moffitt K, Degar B, Duncan C, Tubman VN. Acute lymphoblastic leukemia in a patient with MonoMAC syndrome/GATA2 haploinsufficiency. *Pediatr Blood Cancer.* (2016) 63(10):1844–7. doi: 10.1002/pbc.26084

11. van Dongen JJM, Lhermitte L, Böttcher S, Almeida J, van der Velden VHJ, Flores-Montero J, et al. EuroFlow antibody panels for standardized n-dimensional flow cytometric immunophenotyping of normal, reactive and malignant leukocytes. *Leukemia* (2012) 26(9):1908–75. doi: 10.1038/leu.2012.120

12. *ALL IC-BFM 2009 a randomized trial of the I-BFM-SG for the management of childhood non-b acute lymphoblastic leukemia. final version of therapy protocol from august-14-2009* (2009). Available at: https://www.bialaczka.org/wp-content/uploads/2016/10/ALLIC_BFM_2009.pdf.

13. Donadieu J, Lamant M, Fieschi C, de Fontbrune FS, Caye A, Ouachee M, et al. Natural history of GATA2 deficiency in a survey of 79 French and Belgian patients. *Haematologica* (2018) 103(8):1278–87. doi: 10.3324/haematol.2017.181909

14. Kozyra EJ, Pastor VB, Lefkopoulos S, Sahoo SS, Busch H, Voss RK, et al. Synonymous GATA2 mutations result in selective loss of mutated RNA and are common in patients with GATA2 deficiency. *Leukemia* (2020) 34(10):2673–87. doi: 10.1038/s41375-020-0899-5

15. Wlodarski MW, Hirabayashi S, Pastor V, Starý J, Hasle H, Masetti R, et al. Prevalence, clinical characteristics, and prognosis of GATA2-related myelodysplastic syndromes in children and adolescents. *Blood* (2016) 127(11):1387–97. doi: 10.1182/blood-2015-09-669937

16. Hamadou WS, Mani R, Besbes S, Bourdon V, Youssef Ben Eisinger Y F, et al. GATA2 gene analysis in several forms of hematological malignancies including familial aggregations. *Ann Hematol* (2017) 96(10):1635–9. doi: 10.1007/s00277-017-3076-9

17. Collin M, Dickinson R, Bigley V. Haematopoietic and immune defects associated with GATA2 mutation. *Br J Haematol* (2015) 169(2):173–87. doi: 10.1111/bjh.13317

18. Wang H, Cui B, Sun H, Zhang F, Rao J, Wang R, et al. Aberrant GATA2 activation in pediatric b-cell acute lymphoblastic leukemia. *Front Pediatr* (2022) 9:795529. doi: 10.3389/fped.2021.795529

19. Wlodarski MW, Collin M, Horwitz MS. GATA2 deficiency and related myeloid neoplasms. *Semin Hematol* (2017) 54(2):81–6. doi: 10.1053/j.seminhematol.2017.05.002

20. Cada M, Lara-Corrales I, Dror Y, Feanny S, Hong-Diep Kim V, Grunebaum E. Monocytosis in a patient with a novel GATA2 mutation. *LymphoSign J* (2015) 2 (2):85–90. doi: 10.14785/lpsn-2014-0022

21. Novakova M, Aliova M, Sukova M, Wlodarski M, Janda A, Fro kova E, et al. Loss of b cells and their precursors is the most constant feature of GATA-2 deficiency in childhood myelodysplastic syndrome. *Haematologica* (2016) 101 (6):707–16. doi: 10.3324/haematol.2015.137711

22. Jørgensen SF, Buechner J, Myhre AE, Galteland E, Spetalen S, Kulseth MA, et al. A nationwide study of GATA2 deficiency in Norway–the majority of patients have undergone allo-HSCT. *J Clin Immunol* (2022) 42(2):404–20. doi: 10.1007/s10875-021-01189-y

23. Catto LFB, Borges G, Pinto AL, Clé D V., Chahud F, Santana BA, et al. Somatic genetic rescue in hematopoietic cells in GATA2 deficiency. *Blood* (2020) 136(8):1002–5. doi: 10.1182/blood.2020005538

24. Baliakas P, Tesi B, Wartiovaara-Kautto U, Stray-Pedersen A, Friis LS, Dybedal I, et al. Nordic Guidelines for germline predisposition to myeloid neoplasms in adults: Recommendations for genetic diagnosis, clinical management and follow-up. *HemaSphere* (2019) 3(6):e321. doi: 10.1097/HS9.0000000000000321

25. Bortnick R, Wlodarski M, de Haas V, De Moerloose B, Dworzak M, Hasle H, et al. Hematopoietic stem cell transplantation in children and adolescents with GATA2-related myelodysplastic syndrome. *Bone Marrow Transplant.* (2021) 56 (11):2732–41. doi: 10.1038/s41409-021-01374-y

26. Hofmann I, Avagyan S, Stetson A, Guo D, Al-Sayegh H, London WB, et al. Comparison of outcomes of myeloablative allogeneic stem cell transplantation for pediatric patients with bone marrow failure, myelodysplastic syndrome and acute myeloid leukemia with and without germline GATA2 mutations. *Biol Blood Marrow Transplant.* (2020) 26(6):1124–30. doi: 10.1016/j.bbmt.2020.02.015

27. Nichols-Vinueza DX, Parta M, Shah NN, Cuellar-Rodriguez JM, Bauer TR, West RR, et al. Donor source and post-transplantation cyclophosphamide influence outcome in allogeneic stem cell transplantation for GATA2 deficiency. *Br J Haematol* (2022) 196(1):169–78. doi: 10.1111/bjh.17840

28. Ussowicz M. Treosulfan in combination with fludarabine as part of conditioning treatment prior to allogeneic hematopoietic stem cell transplantation. *Drugs Today* (2020) 56(6):389. doi: 10.1358/dot.2020.56.6.3135200

29. *ESID EBMT HSCT guidelines 2017* (2017). Available at: https://esid.org/layout/set/print/content/download/15402/422689/file/ESID EBMT HSCT Guidelines 2017.pdf.

30. Faraci M, Diesch T, Labopin M, Dalissier A, Lankester A, Gennery A, et al. Gonadal function after busulfan compared with treosulfan in children and adolescents undergoing allogeneic hematopoietic stem cell transplant. *Biol Blood Marrow Transplant.* (2019) 25(9):1786–91. doi: 10.1016/j.bbmt.2019.05.005

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Case report: Cellular therapy for hydroa vacciniforme-like lymphoproliferative disorder in pediatric common variable immunodeficiency with chronic active Epstein-Barr virus infection

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Hydroa vacciniforme-like lymphoproliferative disorder (HV-LPD) is a cutaneous form of chronic active Epstein-Barrvirus (EBV) infection, which can develop into the extremely rare systemic lymphoma. Patients with Inborn errors of immunity (IEI), such as common variable immunodeficiency (CVID), are at higher risk of developing a severe course of infections especially viral and malignancies than the general population. The aim of the study was to present complex diagnostic and therapeutic management of HV-LPD. The clinical diagnosis was confirmed at the histological and molecular level with next generation sequencing. HV-LPD was diagnosed in a patient with CVID and chronic active Epstein–Barr virus (CAEBV) infection. The patient was refractory to CHOP chemotherapy and immunosuppressive treatment in combination with antiviral drugs (prednisone, bortezomib, gancyclovir). The third-party donor EBV-specific cytotoxic T cells (EBV-CTL, tabelecleucel) were used, which stabilised the disease course. Finally, matched unrelated donor hematopoietic cell transplantation (MUD-HCT) was performed followed by another cycle of EBV-CTL.

KEYWORDS

EBV-CTLs, EBV-specific cytotoxic T cells, CVID - common variable immunodeficiency disorders, Allo-HCT, allogeneic hematopoietic stem cell transplantation, NGS - next generation sequencing, HV-LPD

Introduction

Common variable immunodeficiency (CVID) is a primary humoral immunodeficiency, characterized by hypogammaglobulinemia and recurrent, severe infections and increased risk of developing antibody mediated-autoimmune diseases, granulomatous lesions, lymphoid and other types of neoplasms with frequency of 1.5-20.7% in CVID patients (1, 2). The most frequent malignancy is a non-Hodgkin lymphoma (1–3). Additionally, common epithelial tumors of stomach, breast, bladder and cervix can also occur. Pathological mechanisms for development of malignancy in CVID include impaired immune regulation and genetic predisposition. The body's inability to remove viral and bacterial factors, contributing to the formation of neoplasms, and other iatrogenic causes that increase susceptibility to neoplasia are important as well. Persistent chronic active Epstein-Barr virus (CAEBV) infection is another significant risk factor for lymphoma (4–7).

It is estimated that about 95% of the population aged 20-25 is infected with Epstein-Barr virus (EBV) (3, 7). B cells are classic target cells for EBV, however, T cells, natural killers (NK) and epithelial cells may be infected as well (8).

In immunocompetent individuals, primary infection is usually asymptomatic or in the form of infectious mononucleosis. Subsequently, the infection becomes latent. Its rare form is a chronic active EBV infection (CAEBV), which can manifest itself as fever, lymphadenopathy, splenomegaly, hepatitis, or pancytopenia. Other forms of acute infection, such as hemophagocytic lymphohistiocytosis and chronic EBV infections usually affect people with immunodeficiencies (9–11).

EBV-associated T/NK cell lymphoproliferative diseases (EBV-T/NK-LPD) are a group of heterogeneous and rare diseases resulting from the clonal proliferation of EBV-infected T or NK cells (1, 10, 12). They are more often diagnosed in patients with inborn errors of immunity or secondary immunodeficiency disorders (10, 12–14). Hydroa vacciniforme-like lymphoproliferative disorder (HV-LPD) is one of the very rare forms of EBV-associated diseases (4, 15). Since 2016, HV-LPD has been included in the classification of lymphomas, and since 2018, other forms of lymphomas and mucocutaneous lesions in the course of chronic active EBV infection have been added (16–18). Commonly in HV-LPD cases the hypersensitivity to insect bite was seen (19).

The classic form of HV-LPD with no systemic symptoms or hematological disorders and with high levels of EBV DNA in the blood may be self-limiting. This form is usually diagnosed in patients without documented immunodeficiency. In patients with immune dysfunction, HV-LPD is much more often progressive, with systemic changes, an increased number of T cells with γδTCR in peripheral blood, pancytopenia, lymphadenopathy and organomegaly, uveitis, coronary aneurysms, interstitial pneumonia. Ultimately, it leads to the development of lymphomas (4, 5). In addition, there were also described cases of HV-LPD exacerbation and hemophagocytic lymphohistiocytosis (HLH) (4, 10, 20, 21). Spontaneous elimination of EBV-infected T and NK cells in people with systemic HV-LPD is not possible (10, 17). However, no standard treatment has been established so far. Anti-CD20 monoclonal antibodies are ineffective because they eliminate only EBV-infected B cells. Attempts have been made to remove EBV-infected T or NK cells by the use of immunosuppressants or chemotherapy, but their efficacy was unsatisfactory. Currently, it is assumed that immunosuppressive treatment and/or chemotherapy will reduce the load of EBV-infected T or NK cells, and also minimize the number of EBV copies detected in peripheral blood to <200 IU/ml. The next stage of treatment should be allogeneic hematopoietic cell transplantation (allo-HCT). However, it brings an approximately 10% risk of death due to complications, and a further 10% of patients are likely to have a relapse. An additional problem is the choice of a conditioning regimen administered before HCT, as no clear recommendations have been established so far (9, 10, 22, 23). A solution to the issue of preparing patients with the systemic form of HV-LPD for transplantation, as well as the prevention of recurrence in the early period after HCT, could be the use of immunotherapy with EBV-specific allogeneic cytotoxic T lymphocytes (EBV-CTL) (20). Such attempts have been made with regards to the treatment of EBV-induced post-transplantation lymphoproliferative disorder (PTLD). So far, no information has been found in the available literature to support the validity of this therapeutic concept in the treatment of HV-LPD.

The objective of this report is to present a case of successful treatment of a 14-year-old boy with CVID who was diagnosed with HV-LPD. and successfully treated with immunosuppressants, standard chemotherapy, immunotherapy with EBV-specific allogeneic cytotoxic T cells and HCT. To our knowledge, this is the first clinical description showing the importance of specific immunotherapy in preparation for allo-HCT and in the period of immune reconstruction after transplantation in children.

Case description

A 14-year-old boy without burdened family, pregnancy and perinatal history. At 3 months of age, he was diagnosed with cytomegalovirus (CMV) and EBV infection with severe hepatitis, which was confirmed by PCR. The boy did not meet diagnostic criteria for HLH. The parameters of humoral and cellular immunity assessed at that time were normal. At the age of 5, he had an episode of diarrhea, followed by pneumonia complicated by a pleural empyema, which required decortication of the left lung. The patient was also highly hypersensitive to insect bites [Figure 1A]. In repeated immunology tests at the age of 5, agammaglobulinemia was observed (IgG 0.5 g/l; IgA <0.06 g/l; IgM 0.12 g/l), the

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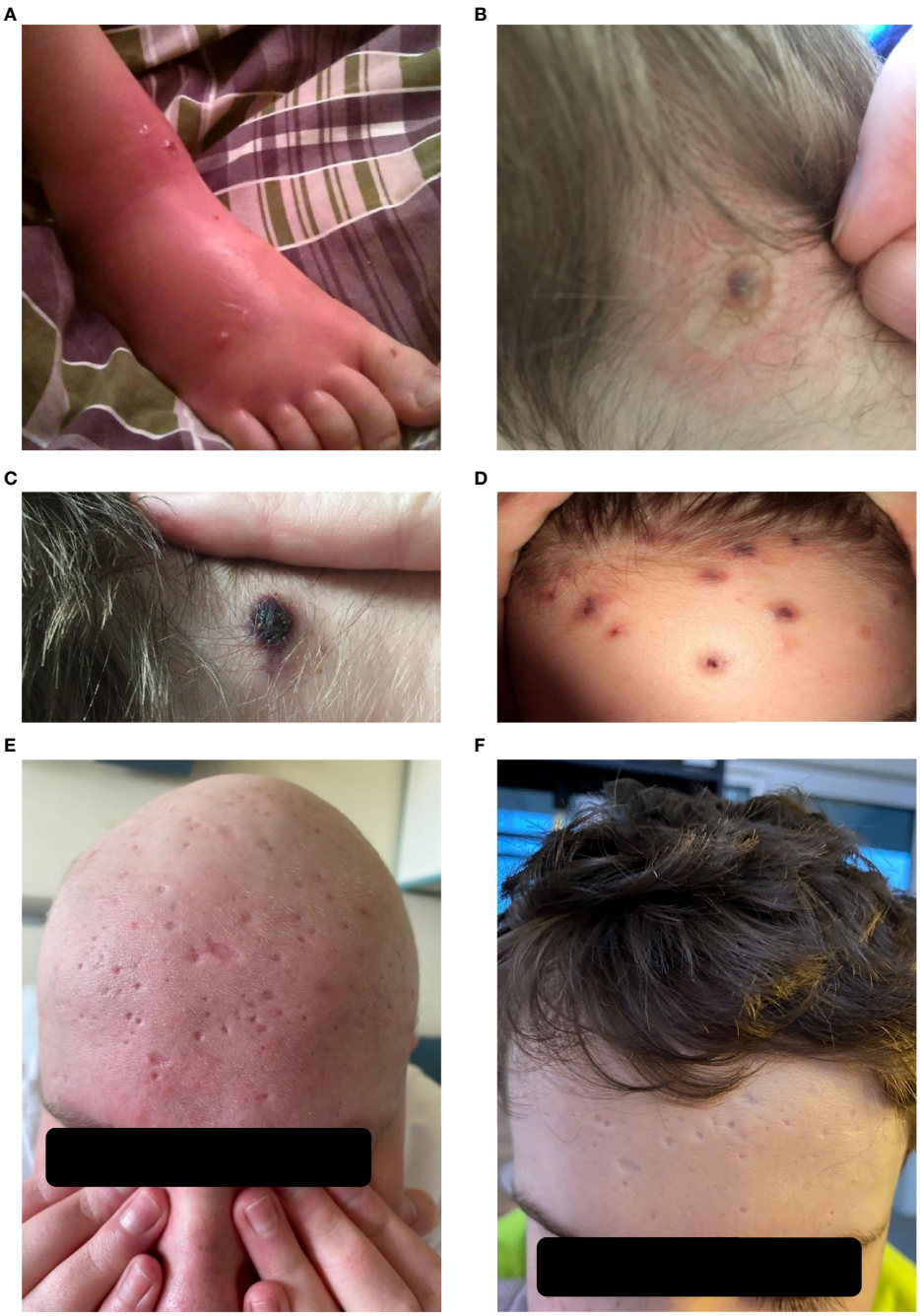

FIGURE 1
HV lesions.**(A–F)**, **(A)** Insect bite hypersensitivity, **(B)** Vesicles on the neck, **(C)** Bulla and healing erosions, **(D)** Papulopustules on the forehead, **(E)** Typical round punched out varioliform scarring after healing of HV lesions on the forehead before allo-HCT, **(F)** Typical round punched out varioliform scarring after healing of HV lesions on the forehead after allo-HCT.

percentage and absolute number of CD19+ cells were normal for the primary lymphocyte subpopulations, the CD3+CD4+/CD3+CD8+ ratio was inverted. In our case NK studies were not performed. In addition, a decreased percentage of memory switched B cells was demonstrated. Genetic tests using the next-generation sequencing method (NGS) were carried out at the age of 13. A mutation in the TNFRSF13B gene was detected, which resulted in defective production of the TACI protein. Thus, the diagnosis of the heterozygous variant of CVID was confirmed and a heterozygous variant in the STX11 gene was also demonstrated.

At the age of 5, substitution therapy with intravenous human immunoglobulin was implemented. After one year, subcutaneous infusions were introduced and carried out for 7 consecutive years, without complications. EBV and CMV viremia was not determined at that time.

At the age of 13, an erythematous papular rash occurred on lower limbs and then papulopustular skin lesions were observed, which receded leaving deep scars. Moreover, periorbital edema was also periodically observed. A relapse of new skin lesions was accompanied by high fever. Laboratory tests revealed thrombocytopenia, neutropenia and hypertransaminasemia. After three months, serous-filled vesicles began to appear around the papular erythematous changes [Figures 1B, C]. The skin lesions occurred with periods of exacerbation and remission [Figure 1D]. HHV6, Adenovirus, Parvovirus B 19, VZV, HSV 1, HSV 2, HIV, Aspergillus spp, influenza A, influenza B, RSV, SARS-Cov-2, as well as tuberculosis and other mycobacteriosis were excluded. Staphylococcus epidermidis was cultured from swabs of the vesicles on the scalp. Targeted treatment was applied but with no improvement. At the same time, a rapid increase in EBV viremia (>5X106 IU/ml) was observed. In therapy, 4 doses of anti-CD 20 monoclonal antibodies (rituximab) were used. Rituximab was used before the final diagnosis of T-cell involvement was obtained. A transient reduction in EBV viremia was achieved, but with no clinical improvement. The treatment was followed by a complete depletion of B cells. Additionally, a significantly increased percentage and absolute number of CD3+CD8+ cells (cytotoxic T cells) were noticed. A skin biopsy was performed, and histopathological examination showed features of vasculitis. Therefore, prednisone 1 mg/kg body weight was used in the treatment, however with no clinical improvement. Due to the above, histopathological examination of skin tissue samples was performed in a reference center. The consulting pathologist recognized EBER+ CD8. The images of the histopathology results and their description are presented in Figure 2 [Figure 2]. The child was assessed for T-cell receptor (TCR) clonality in peripheral blood T lymphocytes. The result was normal. PET-CT scanning showed features of scalp involvement, with no changes in the lymph nodes, liver and spleen [Figure 3]. Based on the above clinical presentation and hystopatologic findings the systemic form of HV-LPD from mature cytotoxic T cells infected with EBV was diagnosed. According to the WHO classification fulfilled criteria were presence of general symptoms, hypersensitivity to insect bites [Figure 1A] and no signs of organ involvement (besides skin). The literature analysis showed that the best treatment effects of systemic forms of HV-LPD were achieved in patients in whom

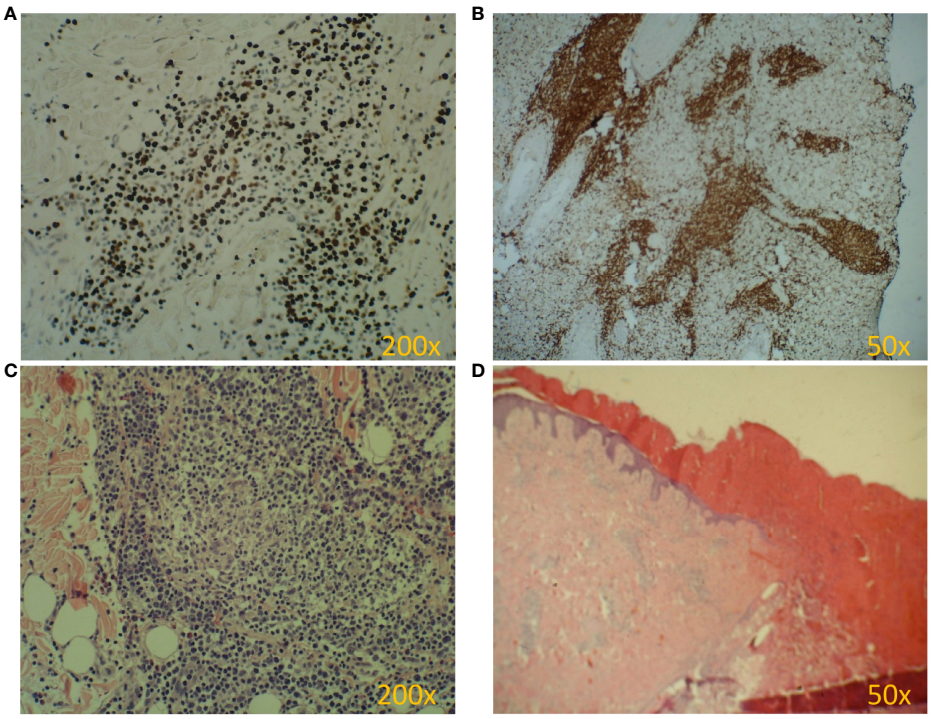

FIGURE 2
Pathology of HV biopsy specimens. **(A-D)**. **(A)** EBV antigen (EBER) is present in almost every cell. **(B)** Dominant cytotoxic T lymphocytes (CD8+) in the infiltrate. **(C)** Lymphocytic infiltrate in the dermis (H&E). **(D)** Skin ulceration (H&E).

the EBV load in peripheral blood was reduced to <200 IU/ml, and then allogeneic hematopoietic cells were transplanted (9). Based on individual reports, different therapeutic regimens were used in the treatment preparing for HCT, as shown in Figure 4. None of them resulted in the expected decrease in viral load. Additionally, the disqualification of an unrelated donor made it necessary to postpone the planned HCT for another month. For this reason, it was decided to conduct an experimental cell therapy with the use of T cells sensitized by EBV. Before HCT, the boy underwent 2 cycles of immunotherapy with Tabelecleucel (TabCel) (*i.v.* $2x10^6$/Kg, partially HLA matched) by Atara Biotherapeutics Inc. (Thousand Oaks, CA, USA). TabCel was administered three times in each cycle: on days 1, 8 and 15. The patient tolerated the treatment well, fever appeared on the 6th day after the first administration of the drug. Massive inflammation occurred on the scalp (erythema with subcutaneous swelling, petechiae and dark scabs), which disappeared after one day. Papular eruptions healed quickly with no tendency to deep scar [Figures 1E, F]. These changes were treated as the effect of immunotherapy. No such reactions were observed after subsequent intravenous administrations of the TabCel. Periodically, new HV skin lesions appeared that healed quickly leaving scars, but the general symptoms (fever, swelling) subsided. The EBV viremia did not decrease to the expected value of <200 IU/ml. In the first cycle of immunotherapy, the patient was infected with SARS-CoV-2, which was associated with a short-term fever, cough and increased fatigue that lasted for about 4 weeks. Pneumonia with the involvement of about 10% of the lung parenchyma was diagnosed. The treatment included remdesivir, convalescent plasma and empirical antibiotic therapy, which resulted in stabilization of lung lesions. Complications of COVID-19 were mild myocarditis and arterial hypertension. It was necessary to implement dual antihypertensive therapy (enarenal and metoprolol). COVID-19 and its treatment did not interfere with the implementation of specific immunotherapy. In the 8th week after the end of the 2nd cycle of HV-LPD therapy, conditioning according to Sawada (9) was started and then allogeneic hematopoietic cell transplantation was performed from a 10/10 HLA matched unrelated donor, ABO and Rh incompatible with the recipient (5). Cyclosporine and methotrexate were used to prevent graft-versus-host disease (GvHD). On the +5 th day after allo-HCT, a single dose of anti-CD20 antibodies was administered. Due to the positive CMV infection status in the recipient and negative in the donor (high risk of CMV reactivation), letermovir was administered prophylactically. Engraftment was achieved on day +24. The early post-transplant period was complicated by gastrointestinal mucositis. No new skin lesions were formed since HCT. In addition, CMV infection was not reactivated and there were no reports of other infections or fever. Since the administration of the conditioning regimen, a gradual decrease in EBV viremia was observed until undetectable levels on day +17. Due to viremia (10^3 IU/ml) on day +24, it was decided to administer monoclonal anti-CD20 antibodies single dose as pre-emptive therapy. Symptoms of GvHD were not observed. From day +31, 2 consecutive cycles of EBV immunotherapy with specific cytotoxic T cells were administered on days 1, 8 and 15 of each cycle. Immunosuppressive treatment was completed on day + 134 after allo-HCT.

From day +38 to day +300, no reactivation of EBV and CMV was observed. At the time of preparing the paper (approx. +300 after HCT) the patient's condition was good. No clinical signs of HV-LPD recurrence occured but there still were elevated levels of transaminases and gamma-glutamyl transferase (GGT). Hematological parameters were normal, except for a slightly reduced number of platelets (in the range of 70-150 K/µl). The boy required systematic human immunoglobulin supplementation, which was carried out from day +180 by the

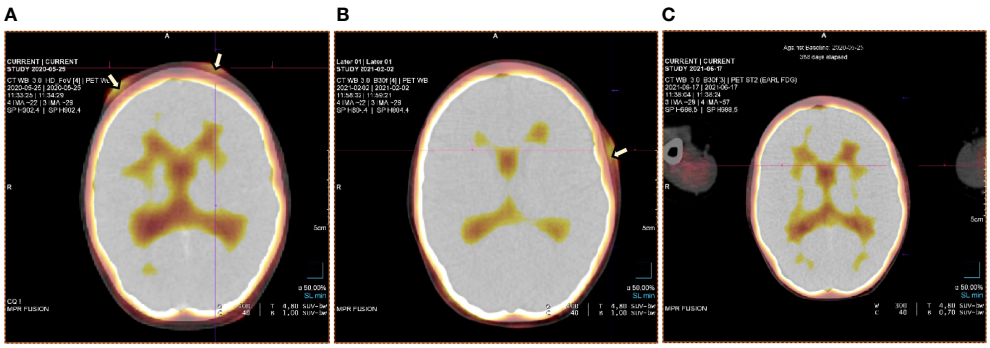

FIGURE 3
PET test at staging and check-ups at various stages of the therapy **(A-C)**. **(A)** PET test at the time of staging – lesions on the forehead involving the skin and subcutaneous tissue. **(B)** Primary lesions on the forehead subsided. A new leasion occured on the temple - february 2021. **(C)** Complete regression of lesions – june 2021.

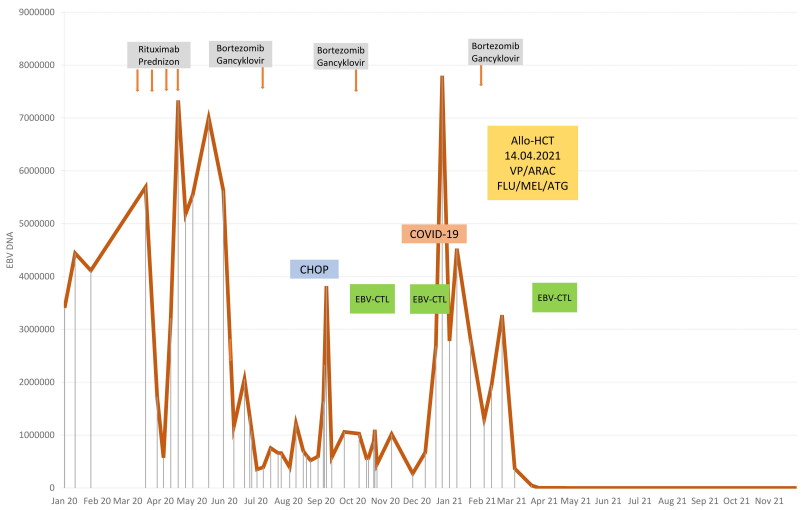

FIGURE 4
Therapy stages depending on EBV viremia.

subcutaneous route. In cyclic determinations of immunoglobulin levels around day +280, an increase in IgM concentration was observed, which may be an indication of reconstruction of humoral immunity. There was also an improvement in the analyzed lymphocyte subpopulations. The percentage of CD19+ cells decreased (3.4%), but their absolute number increased to normal (224/μl).

Discussion

HV-LPD most often affects the population living in Asia and South America (17, 22). Since this disorder is extremely rare in the European population, it can be assumed that in Europeans HV-LPD occurs as a complication of immunodeficiency (2). Infections in CVID are most often of bacterial etiology. Serious viral infections, including CMV and EBV, are less characteristic (8, 24). It cannot be ruled out that in the presented patient the cause of the severe course of EBV and CMV infection in infancy may be a genetic predisposition resulting from the coexistence of STX11 and TACI mutations. The patient did not develop full-blown HLH syndrome, the occurrence of which (in congenital form) requires mutations in both alleles of the STX11 gene. However, perhaps in a patient with a congenital defect in the humoral response, a mutation in allele 1 of the STX11 gene is enough to make the course of acute EBV infection more severe and then to develop into CAEBV. No similar observations were found in the available literature, therefore further research is needed to support this hypothesis. The patient's case shows that early genetic diagnosis and systematic molecular monitoring of EBV infections in patients with primary humoral deficiency are justified, as they allow for rapid implementation of pre-emptive therapy against CAEBV or EBV-LPD. The genetic tests are basis of EBV diagnostics (25). Only the TACI mutation resulting in the diagnosis of CVID was confirmed in our patient. This mutation is not associated with CAEBV and HV-LPD. The presented case confirmed that skin biopsy plays an important role in the diagnosis of atypical skin lesions in patients with IEI. It is essential that an experienced pathologist performs the histopathological assessment. Due to the rarity of skin lesions typical for HV-LPD, many pathologists will not consider a diagnosis of viral origin. It was similar in the presented case – the first pathologist failed to specify the final diagnosis. Due to the clinical and histopathological similarities of HV and HV-LPD, it is also important to analyze the clonality of the gamma T cell receptor in the section of affected skin (4). Such analysis was not performed in the presented patient. Only the clonality of the TCR VbCD3+ and TCR VbCD3+ CD4+ receptor was assessed demonstrating its polyclonality which, however, does not exclude the diagnosis of HV-LPD.

No standard treatment of HV-LPD has been established so far (4, 10, 17, 21, 22). Previously, thalidomide and chloroquine were used, and now the treatment includes steroid therapy, cyclosporin A, interferon alfa and chemotherapy (4, 10, 17, 21, 22). The treatment of our patient was personalized and adequate both to the disease development and the results of medical examinations. The application of anti-CD20 antibodies is usually effective in the treatment of CAEBV, however, it turned out to have no effect on the patient. In the case of CAEBV-infected B cells, their destruction effectively reduces the EBV load and brings clinical improvement (6). In HV-LPD other cells become infected – these were CD3+ CD8+ T cells in

our patient. Therefore, it is not surprising that there was no therapeutic effect after the administration of monoclonal antibodies directed against B cells. Drugs that act on T cells are required for the treatment of HV-LPD. Based on the treatment regimen of T cell lymphomas, an attempt was made to administer cyclophosphamide, doxorubicin, vincristine and prednisone (CHOP) chemotherapy, but with no effect. A similar decision was made by Bollard et al. in a patient with CAEBV (5, 9). Other authors also reported low, only 30% partial effectiveness of chemotherapy in HV-LPD treatment (21). Conventional chemotherapy also turned out to be ineffective in the progression of HV-LPD to systemic lymphoma, hence, there were attempts to implement immunosuppressive therapy in combination with bortezomib and ganciclovir as a bridge to hematopoietic cell transplantation (5, 9, 23, 26). There was an attempt to implement a similar treatment in the presented patient. Steroids, bortezomib and ganciclovir slightly and temporarily reduced the EBV viremia, eliminated general symptoms, but did not cause regression of skin lesions. For the above reasons, other options for optimal preparation of the patient for the HCT procedure were considered. Guided by the experience in the prevention and treatment of PTLD, a decision was made to institute an innovative therapy using EBV-specific allogeneic cytotoxic T cells (tabelecleucel), which resulted in stabilization of the disease, with no evident reduction of EBV viral load, but with virtually no side effects of immunotherapy. The lack of control of EBV viremia may have been caused by long gaps between the EBV-CTL treatment steps. The patient was treated as in PTLD, and it seems that a different strategy should have been implemented, namely: treatment every week until transplantation, and up to approximately 100 days after transplantation, until complete immune reconstruction occurred, causing the immune system to cope with possible reactivation of EBV.

HCT was the next step in the therapy. Hematopoietic cell transplantation (HCT) performed before the disease progression in the stage of irreversible organ damage is considered the most effective method of treatment (5). There are no official recommendations regarding the conditioning for HV-LPD. The diagnosis of refractory CAEBV HV-PLD was an indication for allo-HCT. However, the presence of these presentation of EBV infection in a patient with congenital IEI error was an additional argument in favor of a transplant. In the described case, based on scant literature available, it included early administration of low-dose rabbit antithymocyte globulin (to reduce recipient T cell immunity and enforce donor cell engraftment as well as to decrease the number of EBV-infected T/NK cells for better disease control (9, 10)) and reduced intensity conditioning with thiotepa, cyclophosphamide and fludarabine (20). The use of HCT in the patient confirmed the effectiveness of this therapy. Shortly after HCT, EBV viral load was undetectable and HV-LPD skin lesions subsided. In the post-transplant period, the patient received 2 more TabCell cycles, the assumption was to strengthen anti-EBV effect of transplantation, to reduce the risk of reactivation and further reduction of clinical symptoms of EBV infection. This is an extremely important aspect of treatment as increasing EBV viremia is a risk factor for early relapse and poor prognosis in patients with HV-LPD (19).

Conclusions

Molecular monitoring is important especially in IEI with T-cell defect, but testing of patients with humoral defects should also be considered.

In case of unclear skin lesions and/or symptoms of lymphoproliferation, it is imperative to consider viral infection as the cause.

Histopathological examination that involves viral pathogens, is an indispensable tool in the diagnostics of HV-LPD in patients with IEI. The preparations should be assessed by an experienced pathologist.

Innovative specific immunotherapy of EBV-CTL (tabelecleucel), used at various stages of treatment, and allo-HCT might be a curative option for patients with HV-LPD in the course of CVID.

Genetic and molecular tests allow for quick and accurate diagnosis, which is of great importance in patients with IEI to avoid complications. The use of combined therapy with tabelecleucel, followed by allo-HCT from an EBV-seropositive donor, allows for clinical and laboratory improvement.

Patient perspective

The patient is currently 11 months after transplantation of peripheral blood hematopoietic cells from a 10/10 HLA matched unrelated male donor, with ABO and RH incompatibility. The boy remains in complete remission. There was no recurrence of EBV or CMV infection. Complete hematological reconstruction was obtained. Subcutaneous infusions of immunoglobulins are used due to hypogammaglobulinemia. Anti-infective prophylaxis in accordance with guidelines for transplant patients was recommended.

The greatest doubt in the near term remains the fact that scleroderma cGvHD appeared 280 days after HCT, but regressed spectacularly after treatment with steroids, methotrexate and two extracorporeal photopheresis (ECP) treatments.

Late grafts are rare. It is worth analyzing whether cGvHD results from the previously used immunotherapy or whether it is the result of the interaction of two foreign immune systems - the donor and the recipient? Or maybe both of the therapies used?

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

Written informed consent was obtained from the minor(s)' legal guardian/next of kin for the publication of any potentially identifiable images or data included in this article.

Author contributions

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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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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References

1. TakManesh A, Azizi G, Heydari A, Kiaee F, Shaghaghi M, Hossein-Khannazer N, et al. Epidemiology and pathophysiology of malignancy in common variable immunodeficiency? *AllergolImmunopathol (Madr)* (2017) 45 (6):602–15. doi: 10.1016/j.aller.2017.01.006

2. Kimura H, Ito Y, Kawabe S, Gotoh K, Takahashi Y, Kojima S, et al. EBV-associated T/NK-celllymphoproliferativediseases in nonimmunocompromisedhosts: prospectiveanalysis of 108 cases. *Blood* (2012) 119(3):673–86. doi: 10.1182/blood-2011-10-381921

3. Chen CC, Chang KC, Medeiros LJ, Lee JY. Hydroa vacciniforme and hydroa vacciniforme-like T-cell lymphoma: an uncommon event for transformation. *J Cutan Pathol* (2016) 43(12):1102–11. doi: 10.1111/cup.12801

4. Quintanilla-Martinez L, Ridaura C, Nagl F, Sáez-de-Ocariz M, Durán-McKinster C, Ruiz-Maldonado R, et al. Hydroavacciniforme-likelymphoma: a chronic EBV+ lymphoproliferativedisorder with risk to develop a systemiclymphoma. *Blood* (2013) 122(18):3101–10. doi: 10.1182/blood-2013-05-502203

5. Bollard CM, Cohen JI. How I treat T-cell chronic active Epstein-Barr virus disease. *Blood* (2018) 131(26):2899–905. doi: 10.1182/blood-2018-03-785931

6. Szczawińska-Popłonyk A, Grześk E, Schwartzmann E, Materna-Kiryluk A, Małdyk J. Case report: Autoimmune lymphoproliferative syndrome vs. chronic active Epstein-Barr virus infection in children: A diagnostic challenge. *Front Pediatr* (2021) 9:798959. doi: 10.3389/fped.2021.798959

7. Chen CC, Chang KC, Medeiros LJ, Lee JY. Hydroa vacciniforme and hydroa vacciniforme-like lymphoproliferative disorder: A spectrum of disease phenotypes associated with ultraviolet irradiation and chronic Epstein-Barr virus infection. *Int J Mol Sci* (2020) 21(23):9314. doi: 10.3390/ijms21239314

8. Więsik-Szewczyk E, Ziętkiewicz M, Matyja-Bednarczyk A, Napiórkowska-Baran K, Suchanek H, Jahnz-Różyk K. The first polish cohort of adult patients with common variable immunodeficiency from 4 specialized centers: do we provide standards of care? *Pol Arch Intern Med* (2018) 128(9):563–6. doi: 10.20452/pamw.4315

9. Sawada A, Inoue M. Hematopoietic stem cell transplantation for the treatment of Epstein-Barr virus-associated T- or NK-cell lymphoproliferative diseases and associated disorders. *Front Pediatr* (2018) 6:334. doi: 10.3389/fped.2018.00334

10. Kimura H, Fujiwara S. Overview of EBV-associated T/NK-cell lymphoproliferative diseases. *Front Pediatr* (2019) 6:417. doi: 10.3389/fped.2018.00417

11. Kuri A, Jacobs BM, Vickaryous N, Pakpoor J, Middeldorp J, Giovannoni G, et al. Epidemiology of Epstein-Barr virus infection and infectious mononucleosis in the United Kingdom. *BMC Public Health* (2020) 20:912. doi: 10.1186/s12889-020-09049-x

12. Gratzinger D, de Jong D, Jaffe ES, Chadburn A, Chan JK, Goodlad JR, et al. T And NK-cell lymphomas and systemic lymphoproliferative disorders and the immunodeficiency setting: 2015 SH/EAHP workshop report-part 4. *Am J Clin Pathol* (2017) 147(2):188–203. doi: 10.1093/ajcp/aqw213

13. Natkunam Y, Gratzinger D, Chadburn A, Goodlad JR, Chan JKC, Said J, et al. Immunodeficiency-associated lymphoproliferative disorders: time for reappraisal? *Blood* (2018) 132(18):1871–8. doi: 10.1182/blood-2018-04-842559

14. Satou A, Tsuzuki T, Nakamura S. Other iatrogenic immunodeficiency-associated lymphoproliferative disorders with a T- or NK-cell phenotype. *J Clin Exp Hematop* (2019) 59(2):56–63. doi: 10.3960/jslrt.19013

15. Cohen JI, Manoli I, Dowdell K, Krogmann TA, Tamura D, Radecki P, et al. Hydroavacciniforme-like lymphoproliferative disorder: an EBV disease with a low risk of systemic illness in whites. *Blood* (2019) 133(26):2753–64. doi: 10.1182/blood.2018893750

16. Willemze R, Cerroni L, Kempf W, Berti E, Facchetti F, Swerdlow SH, et al. The 2018 update of the WHO-EORTC classification for primary cutaneous lymphomas. *Blood* (2019) 133(16):1703–14. doi: 10.1182/blood-2018-11-881268

17. Guo N, Chen Y, Wang Y, Huang Y, Feng Y, Li M, et al. Clinicopathological categorization of hydroavacciniforme-like lymphoproliferative disorder: an analysis of prognostic implications and treatment based on 19 cases. *Diagn Pathol* (2019) 14(1):82. doi: 10.1186/s13000-019-0859-4

18. Xu Z, Lian S. Epstein-Barr Virus-associated hydroavacciniforme-like cutaneous lymphoma in seven Chinese children. *Pediatr Dermatol* (2010) 27 (5):463–9. doi: 10.1111/j.1525-1470.2010.01094.x

19. Miyake T, Yamamoto T, Hirai Y, Otsuka M, Hamada T, Tsuji K, et al. Survival rates and prognostic factors of Epstein-Barr virus-associated hydroavacciniforme and hypersensitivity to mosquito bites. *Br J Dermatol* (2015) 172(1):56–63. doi: 10.1111/bjd.13411

20. El-Mallawany NK, Geller L, Bollard CM, Wistinghausen B, Mussai F, Wayne AS, et al. Long-term remission in a child with refractory EBV(+) hydroavacciniforme-like T-cell lymphoma through sequential matched EBV (+)-related allogeneic hematopoietic SCT followed by donor-derived EBV-specific cytotoxic T-lymphocyte immunotherapy. *Bone Marrow Transplant* (2011) 46(5):759–61. doi: 10.1038/bmt.2010.176

21. Yang YQ, Fan L, Wang L, Xu J, Zhang R, Ge Z, et al. Systemic lymphoma arising from hydroavacciniforme-like lymphoma: report of two cases with review of literature. *Int J Clin Exp Pathol* (2014) 7(9):6403–8.

22. Liu Y, Ma C, Wang G, Wang L. Hydroavacciniforme-like lymphoproliferative disorder: Clinicopathologic study of 41 cases. *J Am Acad Dermatol* (2019) 81(2):534–40. doi: 10.1016/j.jaad.2019.01.011

23. Kwong YL, Chan TSY, Tan D, Kim SJ, Poon LM, Mow B, et al. PD1 blockade with pembrolizumab is highly effective in relapsed or refractory NK/T-cell lymphoma failing l-asparaginase. *Blood* (2017) 129(17):2437–42. doi: 10.1182/blood-2016-12-756841

24. Więsik-Szewczyk E, Rutkowska E, Kwiecień I, Korzeniowska M, Sołdacki D, Jahnz-Różyk K. Patients with common variable immunodeficiency complicated by autoimmune phenomena have lymphopenia and reduced treg, Th17, and NK cells. *J Clin Med* (2021) 10(15):3356. doi: 10.3390/jcm10153356

25. Tangye SG. Genetic susceptibility to EBV infection: insights from inborn errors of immunity. *Hum Genet* (2020) 139(6-7):885–901. doi: 10.1007/s00439-020-02145-3

26. Pagano JS, Whitehurst CB, Andrei G. Antiviral drugs for EBV. *Cancers (Basel)* (2018) 10(6):197. doi: 10.3390/cancers10060197

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Development of *RAG2*$^{-/-}$*IL2Rγ*$^{-/Y}$ immune deficient FAH-knockout miniature pig

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Human hepatocyte transplantation for liver disease treatment have been hampered by the lack of quality human hepatocytes. Pigs with their large body size, longevity and physiological similarities with human are appropriate animal models for the *in vivo* expansion of human hepatocytes. Here we report on the generation of RAG2$^{-/-}$IL2Rγ$^{-/Y}$FAH$^{-/-}$ (RGFKO) pigs *via* CRISPR/Cas9 system and somatic cell nuclear transfer. We showed that thymic and splenic development in RGFKO pigs was impaired. V(D)J recombination processes were also inactivated. Consequently, RGFKO pigs had significantly reduced numbers of porcine T, B and NK cells. Moreover, due to the loss of FAH, porcine hepatocytes continuously undergo apoptosis and consequently suffer hepatic damage. Thus, RGFKO pigs are both immune deficient and constantly suffer liver injury in the absence of NTBC supplementation. These results suggest that RGFKO pigs have the potential to be engrafted with human hepatocytes without immune rejection, thereby allowing for large scale expansion of human hepatocytes.

KEYWORDS

immunodeficient, pig, liver damage, RAG2, IL2Rγ, FAH

Introduction

Orthotopic liver transplantation (OLT) (1) is currently the treatment of choice for patients with end-stage liver disease or liver failure. However, limited availability of donor organs and lifelong need for immunosuppression have limited the number of patients that can benefit from OLT (2, 3). Hepatocyte transplantation (HT) is an alternative to OLT, where donor hepatocytes are engrafted into the recipient's liver. Compared to OLT, HT is less invasive and cryopreserved hepatocytes can be thawed for use as required. Hepatocytes can also be genetically engineered to correct metabolic diseases or prevent immune rejection, thereby allowing for allogeneic transplantation (1). Nonetheless, clinical adoption of HT is low due to the limited supply of high quality hepatocytes (3). It is estimated that 5-20 billion hepatocytes are required to treat a single case of acute liver failure (4).

To address the availability of hepatocytes for HT, various *in vitro* methods of primary human hepatocytes (PHH) expansion had been developed. Though 2D *in vitro* cultures comprising of supporting nonparenchymal liver cells supported hepatocyte growth, these 2D-cultured hepatocytes are considerably dissimilar to hepatocytes *in vivo* (5, 6). Advances in 3D organoid culture techniques have improved hepatocyte culture allowing for their use in the study of liver diseases, drug metabolism, and gene therapy studies (7). Notably, when grown in 3D organoids, adult PHH can even be expanded (8, 9). Nonetheless, due to the costs and complexity of these systems, it is not feasible to scale-up such technologies to generate the billions of hepatocytes required to repopulate a human liver (5, 10).

An alternative strategy involved the use of immunodeficient mice for *in vivo* expansion of human hepatocytes (11). One of the earliest mouse models to demonstrate a human-mouse chimeric liver is the urokinase-type plasminogen activator (uPA) - recombination activation gene 2 (RAG2) knockout (uPA-Rag2$^{-/-}$) mouse model (12). Continuous hepatic injury from the transgenic expression of uPA driven by the murine albumin promoter, coupled with the absence of mature murine B and T cells, allowed for up 15% of the murine liver to be repopulated by human hepatocytes (12, 13). Further improvement in engraftment efficiency was observed in uPA-Rag2$^{-/-}$Il2r$\gamma^{-/Y}$ mice, where knockout of the interleukin 2 receptor subunit gamma (IL2Rγ) further impaired the functions of murine natural killer (NK) cells (14). To control the extent of liver injury, fumarylacetoacetate hydrolase (FAH) knockout mice were generated. FAH knockout leads to the toxic accumulation of fumarylacetoacetate in hepatocytes leading to liver damage. However, these mice can be rescued with 2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione (NTBC), which blocks tyrosine catabolism upstream of FAH. By cycling NTBC administration, which causes gradual FAH$^{-/-}$ hepatocytes death, the hepatic niche can be opened for rapid repopulation by transplanted human hepatocytes. Utilizing Fah$^{-/-}$Rag2$^{-/-}$Il2r$\gamma^{-/Y}$ (FRG) mice, it was demonstrated up to 90% human chimerism in murine liver and showed that human hepatocytes can be serially transplanted from a humanized-liver FRG mice to another FRG mouse (15).

Even though high-quality human hepatocytes can be expanded in FRG mice, the small size of mice still limit the scalability of this strategy. In contrast, in terms of genetics, anatomy, physiology, size and lifespan, pigs are closer to humans than small mammals (16, 17). The porcine immune system and its development are also more human-like (18, 19). Therefore, pigs can be excellent animal models for biomedical research, such as the development of humanized tissues and organs for transplantation. Already, pigs that lacked various porcine antigens, such as α-galactose-1,3-galactose, had been genetically engineered to reduce hyperacute xenograft rejection upon human xenotransplantation (20, 21). Pigs that have been genetically engineered to prevent transmission of porcine endogenous retroviruses have also been developed (22, 23). Nonetheless, delayed xenograft rejections and antibody mediated rejections ultimately sets in (21).

An alternative to xenograft transplantation, is the expansion of human cells in immunodeficient pigs. For example, thymectomized and partial hepatectomized mini-pigs had been shown to accommodate transplantation of human hepatocytes for 2-3 weeks (24). Similarly, thymectomized and splenectomized mini-pigs tolerated engraftment of human vascular grafts for up to three months without rejection (25). Though these models demonstrated that surgically produced immunocompromized pigs (SPIP) can potentially support human xenografts, considerable costs and surgical expertise is required, which limit the numbers of SPIP that can be produced. Moreover, rejection by residual functional porcine immune cells prevent achievement of high levels of human chimerism. In contrast, genetically engineered immunodeficient pigs once created, require less expertise in handling, maintenance, and propagation. Various RAG2$^{-/-}$ pigs have been created and they had been shown to tolerate human induced pluripotent stem cells engraftment (26, 27). Similarly, low percentages of human leukocytes can be detected in the peripheral blood and various organs of ART$^{-/-}$ IL2R$\gamma^{-/Y}$ SCID pigs engrafted with human CD34^{+} cord blood (28).

Herein, we report on the successful generation of RAG2$^{-/-}$ IL2R$\gamma^{-/Y}$FAH$^{-/-}$ (RGFKO) pigs through CRISPR/Cas9 editing of fetal fibroblasts followed by somatic cell nuclear transfer (SCNT). The RGFKO pigs had a severely defective immune system companioned with progressive liver damage. The immunodeficient pigs with liver injury will be a good large animal model amenable to human hepatocyte engraftment in future.

Results

Generation of RAG2$^{-/-}$IL2R$\gamma^{-/Y}$FAH$^{-/-}$ (RGFKO) pigs by somatic cell nuclear transfer

RAG2$^{-/-}$IL2R$\gamma^{-/Y}$FAH$^{-/-}$ (RGFKO) pigs were generated through sequential mutation of the Rag2 gene to generate RAG2$^{-/-}$fetuses

followed by mutations of IL2Rγ and FAH genes (Figure 1A). To mutate the RAG2 gene, we first designed a single guide RNA (sgRNA) targeting the coding sequence of RAG2 (Figure 1B). RAG2-sgRNA and Cas9 were then co-transfected into day 33 fetal fibroblasts by electroporation. After drug selection, nine single-cell colonies were obtained. PCR products were used to amplify the RAG2 for Sanger sequencing (Figure 1C). Genomic sequencing of colony C9 showed that a 1 bp insertion mutation and a 4 bp deletion mutation were detected in RAG2 (RAG2^{+}1/-4) (Figure 1D). Both mutations were sufficient to cause frame shift mutations in the RAG2 gene, resulting in the production of an inactive RAG2. As such, C9 was used as donor cell for somatic cell nuclear transfer (SCNT). SCNT embryos were then transferred into seven surrogate sows, resulting in six fetuses (F1-F6) (Table 1, Figure 1E). Fetuses were then harvested for fetal fibroblast isolation. T7 endonuclease I (T7EI) assays performed on the genomic DNA (gDNA) of F1- F5 demonstrated heteroduplexes of mutated DNA (Figure 1F). Consequently, F1 fetal fibroblast was used for subsequent mutation of IL2Rγ and FAH genes.

To mutate IL2Rγ and FAH, Cas9 protein together with sgRNA targeting the fifth exon of IL2Rγ and sgRNA targeting the second exon of FAH (Figure 2A), were used to transduce RAG2$^{-/-}$ fetal fibroblasts. After selection, 16 single-cell colonies (C24-C39) were obtained, and the target fragments of IL2Rγ and FAH were amplified by PCR for Sanger sequencing (Figure 2B). Colony C25 was shown to carry mutations of both IL2Rγ and FAH genes. Sanger sequencing of C25 revealed that the mutations in the IL2Rγ gene included a 16bp deletion, a 137bp deletion and a 1bp insertion, while those on the FAH gene included deletions of 1bp, 31bp, 297bp and 415bp (Figure 2C). All these mutations were verified to result in loss of function frameshift mutations of the respective genes. As such, triply edited colony C25 was used as donor cell for SCNT. SCNT embryos were then transferred into 24 surrogate sows and split into two groups – group A with 12 sows received NTBC while group B with another 12 sows received NTBC supplemented feed (Figure 1A). Ultrasonography confirmed pregnancy in seven sows from Group A (pregnancy rate of 58.3%; Table 2) and nine sows from Group B (pregnancy rate of 75%; Table 3).

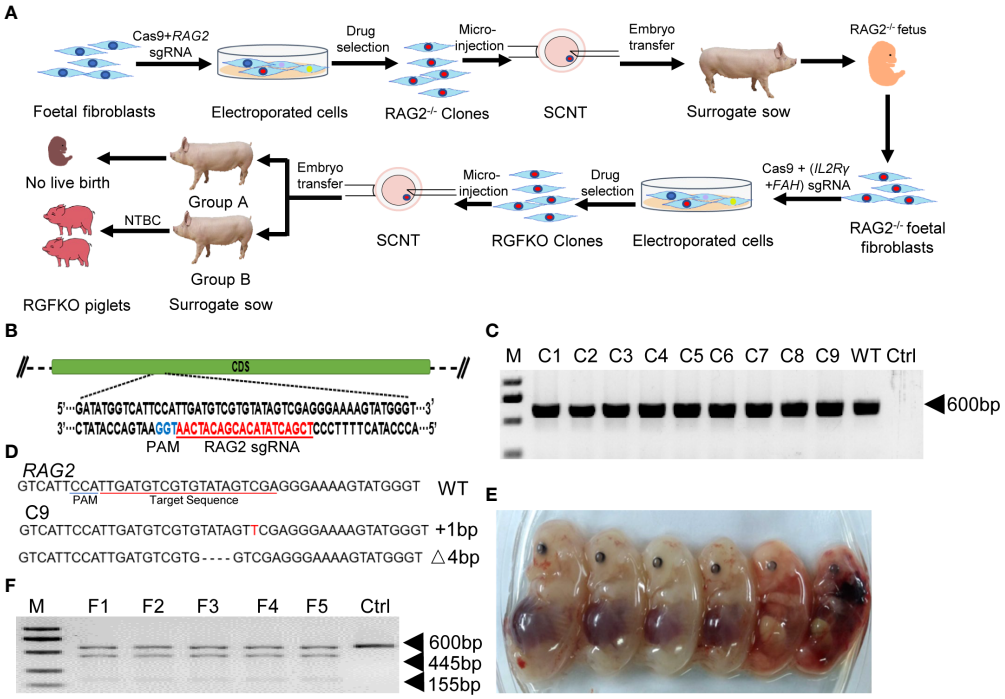

FIGURE 1
Targeted disruption of RAG2 by CRISPR/Cas9. **(A)** Schematic representation of the workflow to generate RGFKO pigs. RAG2$^{-/-}$IL2Rγ$^{-/Y}$FAH$^{-/-}$ (RGFKO) pigs were generated through sequential mutation of the RAG2 gene to generate RAG2$^{-/-}$ fetus first followed by mutations of IL2Rγ and FAH genes to generate triple knockout RGFKO pigs. To mutate the targeted gene(s), single guide RNA (sgRNA) targeting the respective gene(s) was first designed. Day 33 fetal fibroblasts were then electroporated with sgRNA and Cas9 plasmids, followed by drug selection. Positive clones were used as donor cell for somatic cell nuclear transfer (SCNT). SCNT embryos were then transferred into surrogate sows to establish pregnancy. **(B)** Endogenous RAG2 locus and the sgRNA targeting site are shown. **(C)** Genomic DNA was obtained from nine clones (C1-C9) after puromycin selection. RAG2 was amplified by PCR for further Sanger sequencing. Ctrl: no template control. **(D)** Alignment of Sanger sequencing results of clone C9 with wildtype RAG2 sequence demonstrating a one nucleotide insertion ($^{+}$1bp) and a four nucleotides deletion (Δ4bp) mutation. **(E)** Six fetuses were obtained from surrogate sows transplanted with SCNT embryos using clone C9 as donor nuclei. **(F)** Representative results of T7-endonuclease I assays, and gel shift assays performed on the genomic DNA of fetuses F1- F5. Wildtype genomic DNA was used as control (Ctrl).

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TABLE 1 Summary of the generation of RAG2$^{-/-}$ fetuses by somatic cell nuclear transfer.

Recipients	Donor Cells	Pregnancy[1] (%)	Duration of pregnancy (d)	No. of fetuses
1	C9 (RAG2$^{-/-}$)	–	–	–
2		–	–	–
3		–	–	–
4		+	35	6[a]
5		–	–	–
6		+	<29	0[b]
7		–	–	–
Total		28.6%		6

[1]: Pregnancy was confirmed using ultrasound scan on day 23.
[a]: fetuses were harvested for fetal fibroblasts.
[b]: Pregnancy was aborted before ultrasound scan on day 29.

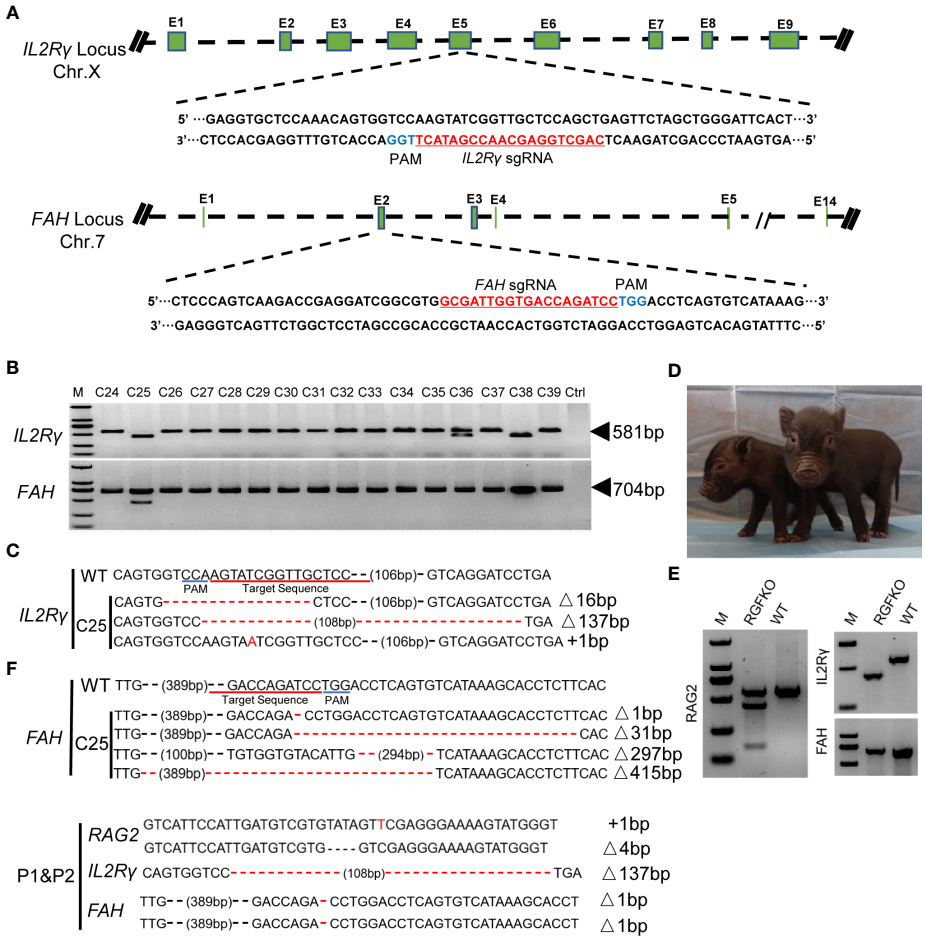

FIGURE 2
Targeted disruption of IL2Rγ and FAH by CRISPR/Cas9. **(A)** Endogenous IL2Rγ and FAH loci and their sgRNA targeting site are shown. **(B)** Genomic DNA was obtained from 16 clones (C24-C39) after puromycin selection. IL2Rγ and FAH were amplified by PCR for further Sanger sequencing. Ctrl: no template control. **(C)** Alignment of Sanger sequencing results of clone C25 with wildtype IL2Rγ and FAH sequences. 16 and 137 nucleotide deletion (Δ16bp, Δ137bp), and one nucleotide insertion ($^{+}$1bp) mutations were observed in IL2Rγ. Deletion mutations of 1, 31, 297 and 415 nucleotides were observed in FAH (Δ1bp, Δ31bp, Δ297bp, Δ415bp). **(D)** Two live RGFKO piglets (P1 and P2) were obtained after 113 days of pregnancy from surrogate sows transplanted with SCNT embryos using clone C25 as donor nuclei. **(E)** Representative results of T7-endonuclease I assays and gel shift assays performed on the genomic DNA of RGFKO piglet (P1). WT: Wildtype. **(F)** Alignment of Sanger sequencing results of RGFKO piglets (P1 and P2) in the target fragments in RAG2, IL2Rγ, and FAH genes. One nucleotide insertion ($^{+}$1bp) and 4 nucleotide deletional (Δ4bp) mutations were observed in RAG2. 137 nucleotide deletion mutation (Δ137bp) was observed in IL2Rγ. 1 nucleotide deletion mutation (Δ1bp) was observed in FAH.

TABLE 2 Summary of the generation of RGFKO pigs by somatic cell nuclear transfer in sows without NTBC supplementation (Group A).

Recipients	Donor Cells	NTBC status	Pregnancy[1] (%)	Duration of pregnancy (d)	No. of fetuses	No. of still-born	No. of live birth
1	RGFKO-C25	Without NTBC	–	–	–	–	–
2			–	–	–	–	–
3			+	<29	0	–	–
4			+	<29	0	–	–
5			+	<29	0	–	–
6			–	–	–	–	–
7			+	32	4[a]	0	0
8			+	29	5[a]	0	0
9			+	34	2[a]	0	0
10			–	–	–	–	–
11			–	–	–	–	–
12			+	32	5[a]	0	0
Total			58.3%		16	0	0

[1]: Pregnancy was confirmed using ultrasound on day 23.
[a]: Fetuses were harvested by surgery.

TABLE 3 Summary of the generation of RGFKO pigs by somatic cell nuclear transfer in sows with NTBC supplementation (Group B).

Recipient	Donor Cells	NTBC status	Pregnancy[1] (%)	Duration of pregnancy (d)	No. of still-born	No. of mummified fetuses	No. of live birth
1	RGFKO-C25	With NTBC	–	–	–	–	–
2			+	<29	–	–	–
3			+	<29	–	–	–
4			+	113	0	6	2
5			+	99	4	0	0
6			–	–	–	–	–
7			+	<29	–	–	–
8			+	<29	–	–	–
9			+	96	3	0	0
10			+	93-95	11	0	0
11			–	–	–	–	–
12			+	<29	–	–	–
Total			75.0%		18	6	2

[1]: Pregnancy was confirmed using ultrasound on day 23.

Although FAH deficiency in humans and mice were not observed to result in *in utero* fetal death (29–31), knockouts of FAH in pigs have been shown to affect fetal development (32). To verify if FAH knockout similarly affects fetal development in RGFKO pigs, four pregnant sows from Group A were euthanized at days 29-32 of gestation to determine fetal developmental status. All 16 fetuses were observed to have died and showed signs of calcification (Table 2; Supplemental Figure S1A). Sanger sequencing of PCR products confirmed the presence of the IL2Rγ 137bp deletion mutation and the FAH 1bp deletion mutation (Supplemental Figures S1B, C). This showed that loss of function mutation of FAH leads to *in utero* fetal developmental defects in RGFKO pigs.

In contrast to Group A, when pregnant sows were supplemented with NTBC (Group B), fetal development was not arrested at day 32. Out of the seven pregnant surrogate sows, four sows progressed till late gestation. Of these, three sows suffered miscarriages between days 93 to 99 of gestation and 18 stillbirths were produced (Table 3; Supplemental Figure S2). One sow successfully delivered two live births on day 113 of gestation and 6 mummified fetuses (Figure 2D; Table 3). gDNA of RGFKO piglets were used to obtain PCR amplicons of RAG2, IL2Rγ and FAH for T7EI assay. As shown in Figure 2E, gel shift assay demonstrated heteroduplexes and homoduplexes of mutated DNA. Sanger sequencing further confirmed the presence of the RAG2^{+}1/-4 mutations, the IL2Rγ 137bp deletion mutation and the FAH 1bp deletion mutation (Figure 2F).

To verify that there were no off-targeting issues in RGFKO pigs, we performed in silico off-target prediction using Cas-OFFinder (33). A total of 4, 2 and 9 off-target sequences (OTS)

were predicted for RAG2, IL2Rγ and FAH respectively (Supplemental Table S2). Using PCR amplification of the predicted sites and Sanger sequencing, we did not detect off-targeting issues in RGFKO pigs.

These results showed that we have successfully generated triple gene knockout RGFKO piglets.

RGFKO pigs have defective immune system

RGFKO piglets were supplemented with NTBC for the first three days and raised in standard conditions, which cause significant health stresses to immunodeficient RGFKO piglets. For the first two weeks after birth, milk intake by the piglets were normal. However, from day 29 onwards, growth retardation and systemic weakness in RGFKO piglets were apparent. A RGFKO piglet survived to 29 days and another one survived to 29 days, but the reason of death was unknown.

Necropsy of RGFKO piglets and age-matched WT piglets revealed that RGFKO piglets had under-developed thymus compared to age-matched WT piglets (Figure 3A). Spleen of RGFKO piglet was also smaller and thinner compared to WT piglets (the weight of RGFKO piglet's spleen was 4.04 g, while of WT piglet was 5.56 g; Figure 3B). Haematoxylin and eosin (H&E) staining of the spleen revealed that RGFKO spleen was hypocellular, lacked lymphoid follicles and germinal centers, and had reduced lymphoid aggregation in the white pulp (Figure 3C).

To evaluate the changes in lymphocyte populations, peripheral blood mononuclear cells (PBMCs) from RGFKO piglet were harvested. Flow cytometry analysis revealed that the proportion of non-monocyte/granulocyte (M/G) in peripheral blood was reduced from 78% in WT piglet to about 34% in RGFKO piglet. Percentages of $CD3^+CD16^-$ T cells in peripheral blood were reduced from 27.5% in WT to 18.8% in RGFKO piglets. NK cells ($M/G^-CD3^-CD16^+$) were almost non-detectable in RGFKO piglets (0.1%) compared to WT piglets (18.5%) (Figure 3D). $CD3^-/CD45RA^+$ is a well-established marker for porcine mature B cells, which has already been reported by other studies (34, 35). B cells ($CD3^-CD45RA^+$) in the peripheral blood of RGFKO piglets were also dramatically reduced to 0.3% compared to WT (37.8%). As shown in Figure 3D, although T cells could be detected in the peripheral blood of RGFKO piglets, no $CD3^+CD45RA^+$ T cells were detectable in RGFKO piglets (Figure 3E). This suggested that these T cells were immature, as $CD45RA^+$ is highly expressed in mature single positive T cells.

We also investigated the splenic lymphocyte composition. We observed that the percentage of $CD3^-CD45RA^+$ B cells in the spleens of WT and RGFKO piglets were comparable (Figure 3F). Given the absence of peripheral B cells, this suggested that a block in B cell maturation in the spleen had occurred. In addition, the percentage of $CD3^+CD45RA^-$ T cells in the spleen of RGFKO piglet were higher (21.0%) than those in WT piglets (12.2%). This most likely represented a population of immature T cells that had escaped thymic clearance and were entrapped in the spleen.

Transcriptional analysis of splenocytes showed that compared to WT, RGFKO piglets have lower expression of CD8 and IL2Rγ mRNA compared to WT. There was no difference in CD4 mRNA expression levels between RGFKO and WT piglets (Figure 3G). Western blot analysis using anti-IL2Rγ antibodies performed on splenocytes further demonstrated significant reduction in IL2Rγ expression in RGFKO piglets (Figure 3H).

Loss of functional mutation in RAG2 is known to impair V(D)J gene arrangement. As such, we analyzed the degree of V(D)J rearrangements in the T-cell receptor (TCR) and B-cell receptor (34). DNA extracted from spleen were used to detect the rearrangements of loci of TCRβ (TRB), TCRδ (TRD) and immunoglobulin heavy chain (IGH). Although TRB variable (TRB-V) and TRD variable (TRD-V) fragments were detected in both WT and RGFKO pigs but the rearrangement of TRB (TRB-VDJ) and TRD (TRD-VDJ) fragments was reduced in RGFKO pigs (Figure 3I). Similarly, the IGH variable fragment (IGH-V) was also detected in both WT and RGFKO pigs but the rearrangement of IGH fragment (IGH-VDJ) was also reduced in RGFKO pigs (Figure 3I).

Collectively, the data above showed that RAG2 and IL2Rγ were successfully edited in RGFKO immunodeficient pigs.

RGFKO pigs have progressive liver damage

Next, we assessed the impact of FAH mutations on RGFKO pigs. To ensure *in utero* development of RGFKO fetuses, surrogate sows were fed feed supplemented with NTBC (5 g/l) at a dose of 10 ml NTBC per 100 kg body weight from embryo transfer up till gestation day 94. Thereafter, NTBC dose was increased to 12 ml NTBC per 100 kg body weight. RGFKO piglets were also supplemented with NTBC at a dose of 12 ml NTBC per 100 kg body weight for the first three days after birth (Figure 4A).

Morphologically, the livers of RGFKO pigs did not show obvious pathologies such as tumors or nodules. Next, we analyzed the histology of livers from RGFKO pigs. We first confirmed the absence of FAH in RGFKO livers by immunohistochemistry (IHC) (Figure 4B). Western blot analysis using anti-FAH antibodies performed on liver homogenates demonstrated loss in FAH expression, thereby corroborating with the IHC observations (Figure 4C). Similarly, loss in FAH expression in the testes of RGFKO piglets was also observed (Figure 4D). H&E staining of RGFKO livers showed diffuse hepatocellular injury and cytoplasmic ballooning degeneration. Liver architecture was also disrupted with no clear demarcation of liver lobules

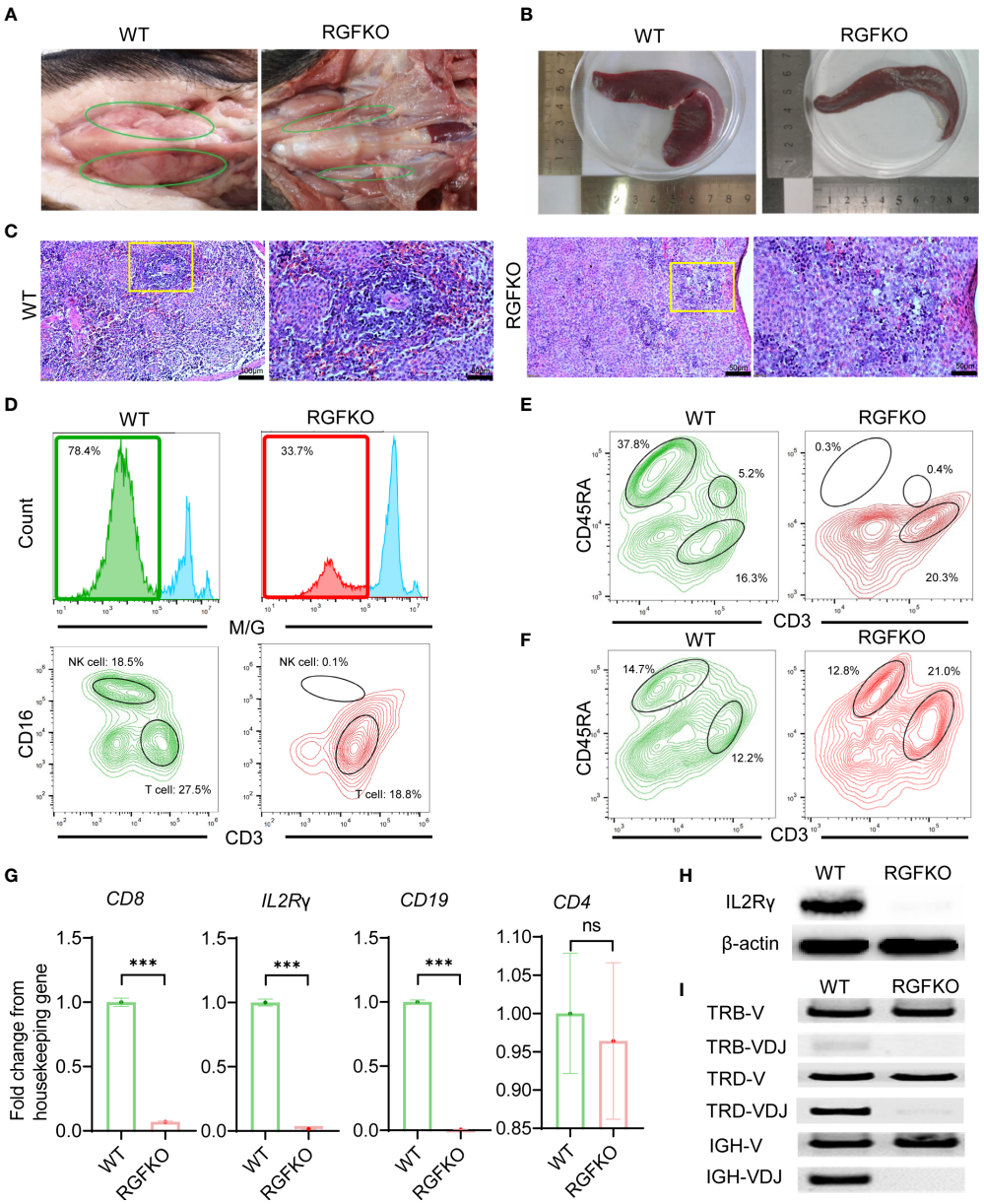

FIGURE 3
Immunological characterization of RGFKO pigs. **(A)** Representative images of the thymus of wildtype (WT) and RGFKO pigs. **(B)** Representative images of the spleen of WT and RGFKO piglets. **(C)** Representative spleen sections of WT and RGFKO piglets stained with hematoxylin and eosin. Left: normal spleen architecture in WT pig; Right: lacked lymphoid follicles and germinal centers, as well as reduced lymphoid aggregation in the white pulp in RGFKO pig. Boxed region indicating the white pulp region was further magnified. **(D–F)** Flow cytometric analysis of the peripheral blood **(D, E)** and splenocytes **(F)** of WT and RGFKO piglets. **(D)** Shown are representative images of histograms of macrophage/granulocyte (M/G) marker staining. The boxed region, representing M/G negative population, is further gated upon for T cells (M/G-CD3$^+$CD16$^-$) and NK cells (M/G-CD3-CD16$^+$) analysis. **(E, F)** Shown are representative plots of CD45RA against CD3. B cells are identified as CD3$^-$CD45RA$^+$. Numbers indicate the proportion of the indicated population as a percentage of total peripheral blood mononuclear cells **(D, E)** or splenocytes **(F)**. **(G)** qPCR analysis of the expression of the indicated genes in splenocytes of RGFKO and WT piglets. Shown are fold change of the indicated genes over housekeeping gene (GAPDH). Data shown are mean ± standard error (n = 2 for RGFKO, n = 3 for WT). ns: not significant. ***$p < 0.001$. **(H)** Western blot analysis of the expression of IL2Rγ in splenocytes of RGFKO and WT piglets. β-actin was used as a loading control. **(I)** PCR analysis of germline TRB-V, TRD-V and IGH-V genes, and V(D)J-recombined TRB-VDJ, TRD-VDJ and IGH-VDJ genes in splenocytes of RGFKO and WT pigs. Shown are representative images of PCR amplification products visualized by DNA gel electrophoresis.

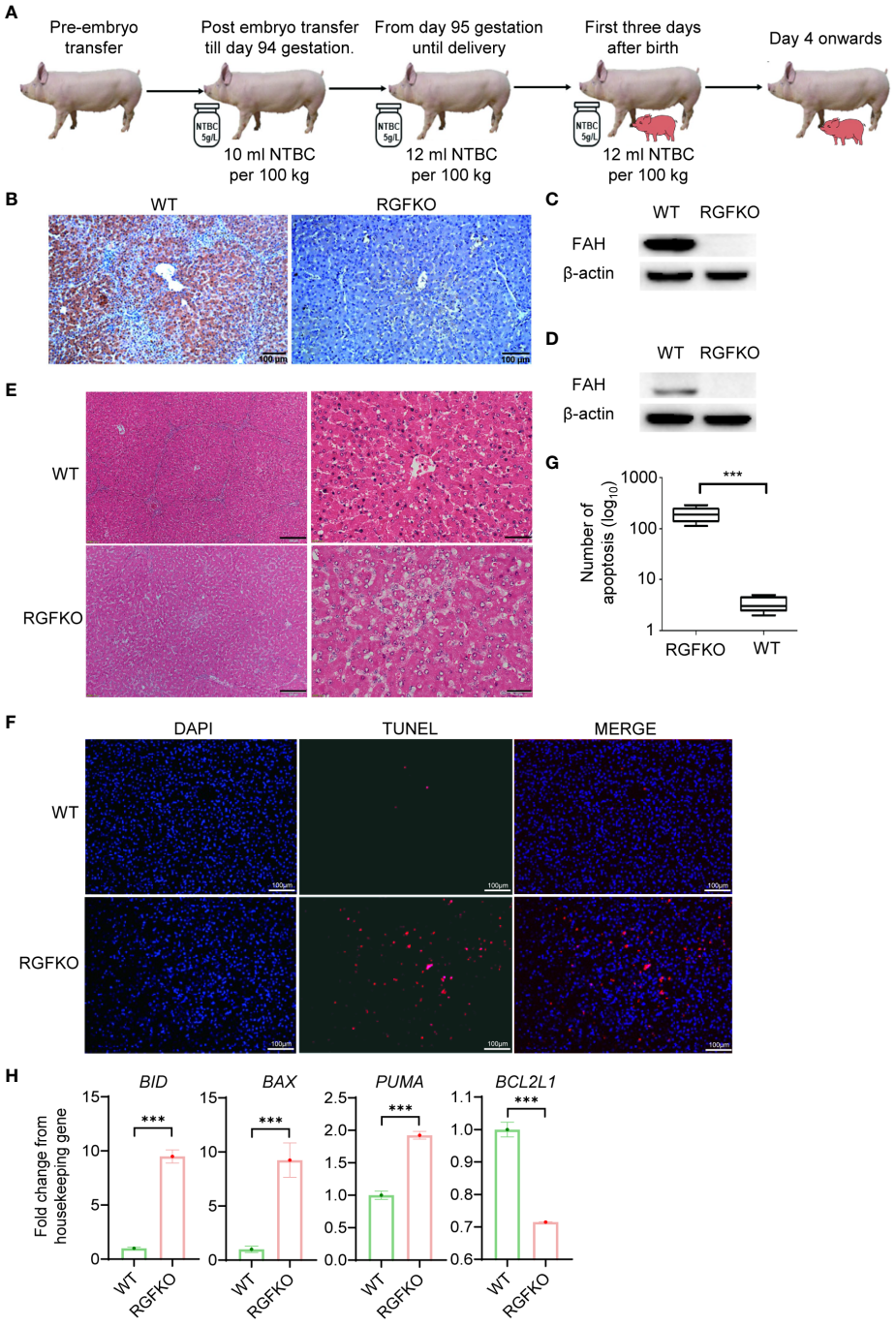

FIGURE 4
RGFKO pigs suffer hepatic damage when taken off NTBC. **(A)** Schematic of the NTBC dosing regimen for RGFKO pigs. Surrogate sows were supplemented with NTBC (5g/L) at a dose of 10 ml NTBC per 100 kg body weight for the first 94 days of gestation. NBTC dose was increased to 12 ml NTBC per 100 kg body weight thereafter until delivery of RGFKO piglets. RGFKO piglets were supplemented with 12 ml NTBC per 100 kg body weight for the first 3 days after birth. NTBC was then withdrew after that. **(B)** FAH immunohistochemistry analysis of the liver of RGFKO and wildtype (WT) pigs using anti-FAH. **(C, D)** Western blot analysis of the expression of FAH in hepatocytes **(C)** and testes **(D)** of RGFKO and WT piglets. β-actin was used as a loading control. **(E)** Representative liver sections of WT and RGFKO piglets stained with haematoxylin and eosin. Boxed region was magnified to show diffuse hepatocellular injury and cytoplasmic ballooning degeneration. **(F, G)** Evaluation of hepatocyte apoptosis *via* TUNEL assay was performed on liver sections of RGFKO and WT pigs. DAPI (blue) was used to stain the nuclei of hepatocytes, while TUNEL positive cells were stained red **(F)**. TUNEL positive cells were quantified through image analysis **(G)**. **(H)** qPCR analysis of the expression of the indicated genes in hepatocytes of RGFKO and WT piglets. Shown are fold change of the indicated genes over housekeeping genes (GAPDH). Data shown are mean ± standard error (n = 2 for RGFKO, n = 3 for WT). ns, not significant. ***$p < 0.001$.

(Figure 4E). TUNEL assay performed on RGFKO liver sections indicated diffuse staining of numerous apoptotic cells, which were absent in wildtype liver (Figures 4F, G). Transcriptional analysis of hepatocytes demonstrated upregulation of apoptotic genes, such as BID and downregulation of BCL2L1, an anti-apoptotic gene (Figure 4H). Liver function analysis of pig blood showed that alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (TBIL), direct bilirubin (DBIL) in RGFKO piglet were higher than those in WT piglets, while the alkaline phosphatase (ALP), total protein (TP) and albumin (ALB) were lower than WT (Table S6). In sum, the above data showed that the loss of function mutation in FAH resulted in the apoptosis of hepatocytes, which subsequently led to hepatocellular damage.

Discussion

In this report, we successfully generated RAG2$^{-/-}$IL2Rγ$^{-/Y}$FAH$^{-/-}$ (RGFKO) triple knockout *Diannan* miniature pig by SCNT through a two-step sequential mutation of RAG2 followed by IL2Rγ and FAH by CRISPR/Cas9. Consistent with previous reports of RAG2$^{-/-}$ (27, 36, 37), IL2Rγ$^{-/Y}$ (35, 37), RAG2$^{-/-}$Il2rγ$^{-/Y}$ in Landrace cross large white pigs (38), and RAG1$^{-/-}$ IL2Rγ$^{-/Y}$ FAH$^{-/-}$ in Landrace cross large white pigs (39), we observed that RGFKO pigs had hypotrophied or absent thymus, hypocellular spleen, reduced numbers of T, B and NK cells, as well as defective V(D)J recombination. In contrast to single RAG2$^{-/-}$ or IL2Rγ$^{-/Y}$ knockout pigs where no differences in spleen size was observed compared to wildtype (36, 37, 40), the spleens of RGFKO pigs were notably slimmer and smaller. Interestingly, we observed that at a transcriptional level, in contrast to CD8, CD19 and IL2Rγ whose expression were reduced, CD4 expression was not significantly different between RGFKO and wildtype pigs. This is consistent with the observation in RAG2$^{-/-}$ mice, where CD4 expression was not significantly different from wildtype mouse (41). Similarly, CD4 mRNA expression was also not reduced in RAG2$^{-/-}$ pigs compared to wildtype pigs (26).

In B cell development, CD45RA is expressed from pro-B stages onwards (42). VDJ recombination occurs at the pre-B stage, and successful VDJ recombination results in the progression of pre-B into immature B-cell. Immature B-cell then travel to the spleen where they undergo further maturation before entering the peripheral blood. We observed the presence of CD45RA^{+} B cells in the spleen but not in the periphery. This suggested that there is a block in B cell development from the pre-B stage onwards, which is consistent with the loss of VDJ rearrangement (43). Consequently, B cell maturation in the spleen is impaired, resulting in their sequestration in the spleen. In contrast to B cells, most thymocyte T cell populations are CD45RA^{-}. VDJ recombination occurs in DN3 stage and is required for thymocyte maturation into single positive T cells. CD45RA expression is only increased at the final step of T cell maturation in the thymus (44). We observed that T cell populations in both peripheral blood and spleen were CD45RA^{-}. This supports the notion that T cell maturation has been arrested in the immature thymocyte stages. These immunological changes along with the reduced rearrangements of VDJ TCR or IGH fragments strongly suggest the RAG2 inactivation. Coupled with the absence of NK cells and the significant reduction in IL2Rγ expression at both transcript and protein levels indicate the successful knockout of RAG2 and IL2Rγ.

In addition to the defects in the immune system, FAH expression in RGFKO pigs is dramatically reduced. We showed that consistent with the observations of (32), absence of NTBC supplementation during gestation led to *in utero* fetal death by day 30 of pregnancy, whereas gestational NTBC supplementation rescued RGFKO fetuses. When NTBC is withdrawn after birth, RGFKO suffered liver damage characterized by cytoplasmic ballooning degeneration and disruption of liver architecture.

However, by combining immunodeficiency with liver injury, the challenges involved in maintaining RGFKO pigs are also doubled. Firstly, the RAG2$^{-/-}$ genotype is associated with a high rate of still-births when housed in conventional living conditions (27, 36). Secondly, due to the absence of FAH, NTBC administration during gestation needs to be appropriately titrated to support *in utero* fetus development (32). As such, out of 26 RGFKO fetuses, only two live RGFKO piglets were successfully delivered. When housed under conventional living conditions, the lifespan of RAG2$^{-/-}$immunodeficient pigs were reported to be between 1-3 months (27, 36, 37). Here, we report that RGFKO pigs survived up till one month under conventional housing conditions. The shorter lifespan of RGFKO pigs was probably exacerbated by NTBC withdrawal after the third day of birth. As reported earlier that taking off NTBC FAH$^{-/-}$ succumbed of pigs within 20 days (32). With gnotobiotic living conditions (38) and continual supplementation of NTBC (32), survival of RGFKO pigs is expected to be vastly improved. Nonetheless, an important aspect of this study is that we have demonstrated the feasibility of producing an immunodeficient liver-damage pig model, which represents the foundational steps towards the future development of a humanized liver in pigs.

RGFKO pigs have the potential to be an important source of high quality human hepatocytes for hepatocyte transplant as well as *ex vivo* hepatocyte-directed gene therapy (45). *Ex vivo* hepatocyte-directed gene therapy to correct inborn errors of metabolism had been clinically evaluated in patients with familial hypercholesterolemia with modest results (46). This was mainly due to the poor engraftment rates from low-quality hepatocytes after *in vitro* culture and selection (46). To overcome the need for *in vitro* culture, transduction autologous

hepatocytes from FAH$^{-/-}$ pigs with lentivirus expressing FAH, and directly transplanted them back into their autologous host without further culture. They showed that these pigs can be taken off NTBC and thrive with minimal evidence of tumorigenicity and liver damage (47, 48). With the successful generation of our RGFKO pigs, human hepatocytes can now be engrafted into the livers of RGFKO pigs. This opens the possibility of using RGFKO pigs as a large animal model for evaluation of human hepatocyte-directed gene therapy, as well as evaluation of the efficacy of hepatocyte transplants using PHH and liver stem cells.

We have previously improved the safety of porcine xenografts through the inactivation of porcine endogenous retroviruses (22). To reduce the risk of human rejection of porcine xenograft, we have also developed pigs with knockout of porcine α-1,3-galactosyltransferase (20). By allowing the humanization of the porcine liver with human hepatocytes, RGFKO pigs can potentially reduce the amount of remaining porcine tissue in the porcine liver. Given the similarities in porcine and human liver, in terms of size, anatomy and vascular architecture, RGFKO pigs can not only serve as a source of human hepatocytes but also represent the next step forward towards pig-to-human liver transplantation.

In addition to human hepatocyte engraftment, FRG mice have also been used to study immune modulation of human liver disease *via* engraftment with human hematopoietic stem cells (HSC) (49). Such dual liver and immune humanized mice have contributed to the understanding of how the immune system mediate liver inflammation and fibrosis in non-alcoholic fatty liver disease (50) and hepatitis B virus infection (51, 52). In addition to the potential use of RGFKO pigs as human hepatocyte "cell factory" (45), RGFKO pigs can also be engrafted with human HSCs to generate dual humanized immune and liver pigs. Indeed, earlier works using RAG2$^{-/-}$ or IL2Rγ$^{-/Y}$ pigs have shown that immunodeficient pigs are amenable to human cell engraftment (27, 28). Similarities between human and porcine immune-related genes (53), as well as hematopoietic cytokines and immune signaling molecules (54), suggests that human immune lineages might differentiate better in pigs compared to mice. Given the role of the human immune system in various hepatic pathologies, such as hepatocarcinoma (55, 56), non-alcoholic steatohepatitis (50), and hepatitis (51, 52), such dual humanized pigs can thus see applications as preclinical models for evaluation of human-specific therapies, such as chimeric antigen receptor cell therapy and immune checkpoint therapy.

In conclusion, we successfully produced the RGFKO pigs by targeted disruption of the RAG2, IL2Rγ and FAH gene. The RGFKO pigs had a severely defective immune system companioned with progressive liver damage. These pigs will be value for establishing the platform of liver transplantation, stem cell therapies and immunotherapies.

Materials and methods

Experimental animals

Animals used in this study were *Diannan* miniature pigs. All animal experiments were approved by the Animal Care and Use Committee of Yunnan Agricultural University in China.

Chemicals

All chemicals used were obtained from Sigma Chemical Corp (Saint Louis, MO, USA).

In vitro maturation of oocytes

Porcine ovaries were collected from Hongteng abattoir (Chenggong Ruide Food Co., Ltd, Kunming, Yunnan Province, China), and cumulus-oocyte complexes (COCs) with at least three layers of compacted cumulus cells were collected from ovarian follicles of 3-6mm diameter. 50 oocytes were cultured in 200 μL microdrops of TCM199 medium supplemented with 0.1 mg/mL pyruvic acid, 0.1 mg/mL L-cysteine hydrochloride monohydrate, 10 ng/mL epidermal growth factor, 10% (v/v) porcine follicular fluid, 75 mg/mL potassium penicillin G, 50 mg/mL streptomycin sulfate, and 10 IU/mL equine chorionic gonadotropin (eCG) and human chorionic gonadotropin (hCG; Teikoku Zouki Co., Tokyo, Japan) and incubated at 38.5 °C with 5% CO_2 in 100% humidity for 42-44 h.

Somatic cell nuclear transfer and embryo transfer

SCNT was performed as previously described (57). After culturing for 42-44 h, oocytes with expanded cumulus cells were briefly treated with 0.1% (w/v) hyaluronidase and enucleated by gentle aspiration of the first polar body and adjacent cytoplasm using a bevelled pipette in Tyrode's lactate medium supplemented with 10 μM HEPES, 0.3% (w/v) polyvinylpyrrolidone, 10% FBS, 0.1 μg/mL demecolcine and 5 μg/mL cytochalasin B. A single RAG2$^{-/-}$ or RGFKO donor cell was inserted into the perivitelline space of an enucleated oocyte. Donor cell was fused with the recipient cytoplasts with a single direct current pulse of 200 V/mm for 20 μs using an embryonic cell fusion system (ET3, Fujihira Industry Co. Ltd., Tokyo, Japan) in fusion media [0.25 M D-sorbic alcohol, 0.05 mM, Mg $(C_2H_3O_2)_2$, 20 mg/mL BSA, and 0.5 mM HEPES (free acid)]. The reconstructed embryos were cultured for 2 h in PZM-3, activated with a single pulse of 150 V/mm for 100 ms, and then cultured in PZM-3 supplemented with 5 μg/mL cytochalasin B for 2 h at 38.5 °C with 5% CO_2, 5% O_2 and 90% N_2. Thereafter, reconstructed embryos

were maintained in PZM-3 under similar conditions. Reconstructed embryos cultured for 6-30 h after activation were surgically transferred to the oviducts of the estrous surrogate mother. Pregnancy was first confirmed at approximately 21-29 days after transfer using an ultrasound scanner.

Design of sgRNA

sgRNAs targeting the coding sequence of RAG2, fifth exon of IL2Rγ, or second exon of FAH, were designed using CRISPOR (http://crispor.tefor.net/) (Table S1) (58). sgRNA sequences were then cloned into pGL3-U6-sgRNA plasmids (Addgene no: 51133).

Transfection of porcine fetal fibroblast

Isolation of porcine fetal fibroblasts (PFFs) were as performed as previously. To generate $RAG2^{-/-}$ PFF and RGFKO PFF, 10 μg of pST1374-NLS-flag-linker-Cas9 plasmids (Addgene no: 44758) and 5 μg pGL3-U6-RAG2-sgRNA plasmids, and 10 μg of pST1374-NLS-flag-linker-Cas9 plasmids, 5 μg pGL3-U6-IL2Rγ-sgRNA and 5 μg pGL3-U6-FAH-sgRNA plasmids respectively were used to transfect 3 x 10^5 PFF using 4D-Nucleofector (Lonza) as per manufacturer's protocol. Transfected PFFs were recovered in DMEM supplemented with 10% FBS and incubated at 38 °C with 5% CO_2. After 48 h, 2 μg/ml of puromycin were added to the medium for 24–48 h to select successfully transfected cells. The survived cells were digested and about 100 cells were seeded into 100-mm-diameter culture dish for 8 days. After about 9 days, puromycin-resistant colonies were picked, and single-cell colonies were transferred to 96-well plates for expansion. When cell confluence reached 70-80%, a portion of the cells were harvested for PCR analysis.

Genomic sequence validation

DNA were extracted from cells, fetuses or ear tissues of piglets using TIANamp Genomic DNA Kit (TIANGEN, China, DP304). Touchdown PCR was used to amplify RAG2, IL2Rγ and FAH using primers shown in Table S3. A portion of PCR amplicons were used for T7 endonuclease I assay (Vazyme, China). Remaining PCR amplicons were cloned into pMD19T (Takara, Japan) *via* TA-cloning and sent for Sanger sequencing. DNA sequences were analyzed using SnapGene software (GSL Biotech, version number: v3.2.1.0).

CRISPR/Cas9 off-target analysis

Potential off-target sites were predicted using Cas-OFFinder (http://www.rgenome.net/cas-offinder/) (Table S2) (33). PCR was used to amplify the predicted regions using DNA obtained from RGFKO ear tissues. PCR amplicons were sent for Sanger sequencing to determine if there were off-target issues.

Histology, immunohistochemical staining and tunel assay

Liver and spleens of RGFKO pigs and age-matched wildtypes were harvested, fixed in 4% paraformaldehyde for 48 h, and embedded in paraffin. 3-5 mm thick sections were prepared for hematoxylin and eosin and immunohistochemical staining. Anti-FAH antibodies (ABClonal, China) were used to stain for FAH in liver sections at 1:100 dilutions. Images were acquired using BX53 biological microscope (Olympus, Japan). Tunel assay was performed using Tunel BrightRed Apoptosis Detection Kit (Vazyme, China, A113-03) as per manufacturer's protocol.

Flow cytometry

Peripheral blood mononuclear cells (PBMCs) were obtained from RGFKO pigs and stained with CD45RA-PE (Abd Serotec, USA, MCA1751PE), CD16-AF647 (Abd Serotec, USA, MCA1971A647), CD3-FITC (Abd Serotec, USA, MCA5951F) and Monocyte/Granulocyte panel-PE (ThermoFisher, USA, MA5-28824) in FACS buffer (PBS supplemented with 0.5% BSA). Data acquisition and analysis was performed using Beckman CytoFlex flow cytometer (Beckman Coulter, USA). Isotype-matched control antibodies were used for all fluorochrome-isotype combinations.

Reverse transcriptase-quantitative PCR (RT-qPCR)

For quantification of mRNA expression by RT-qPCR, RNA from splenocytes were extracted using TransZol Up (TransGen Biotech, China, ET111-01) according to manufacturer protocol. RNA was then reverse transcribed using PrimeScript™ RT reagent kit with gDNA Eraser (Takara, Japan, RR047A) according to manufacturer protocol. cDNA was diluted ten times and used for qPCR using TB Green Premix Ex Taq II (Takara, Japan, RR820). Primers against IL2Rγ, CD4, CD8, CD19 and GAPDH were used by (59) (Table S4). Primers against BID, BAX, PUMA and BCL2L1 were used by (27). The transcript abundance of the various markers was normalized to that of the housekeeping gene, GAPDH using the relative quantification method. The qPCR reaction was performed on the CFX96 Thermal Cycler (Bio-Rad, USA).

Western blot and protein visualization

Hepatocytes were homogenized in RIPA lysis buffer RIPA lysis buffer (Bestbio, China), separated by SDS-PAGE and

transferred to a polyvinylidene difluoride membrane by wet transfer. The proteins were blotted with primary antibodies against FAH (ABclonal, China, A13492, 1:1000 dilution), IL2Rγ (ABclonal, China, A1829, 1:1000 dilution) and β-actin (Zen BioScience, China, 200068-8F10, dilution 1:5000) and further probed with secondary antibodies conjugated with horseradish peroxide enzyme. The proteins were visualized with EasySee® Western Blot Kit (TransGen Biotech, China) on a ChemiDoc MP (Bio-Rad, USA).

Detection of V(D)J rearrangement

PCR amplifications of TRB-V, TRB-VDJ, TRD-V, TRD-VDJ, IGH-V and IGH-VDJ was performed on splenocyte DNA. The already described primers (37) were validated and used (Table S5).

Detection of liver function

The whole blood of RGFKO and WT pigs was collected and the liver function was detected in the first people's Hospital of Kunming, Yunnan Province (Table S6).

Statistical analysis

All statistical analysis was performed using Graphpad Prism 8.0.2. Unless otherwise stated, numerical data is presented as mean ± standard error of mean (SEM). For single comparison between two groups unpaired t-test was used.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

Ethics statement

The animal study was reviewed and approved by Animal Care and Use Committee of Yunnan Agricultural University in China.

Author contributions

Conceptualization, H-JW, QC, and H-YZ. Methodology, HZ, H-JW, and JG. Performance of experiments, HZ and JG. Results interpretation, HZ, WY, QC, and H-YZ. Writing, HZ, WY, KX and MAJ. Resources, H-JW and QC. 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.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2022.950194/full#supplementary-material

SUPPLEMENTARY FIGURE 1
FAH-deficiency leads to *in utero* fetal death without NTBC supplementation. Without NTBC supplementation during gestation, no RGFKO piglets were born. **(A)** Representative images of fetuses retrieved from pregnant sows implanted with RGFKO embryos at 29-35 days of gestation without NTBC supplementation. **(B, C)** Genomic DNA was obtained from 12 fetuses (F1-F12). RAG2, IL2Rγ and FAH were amplified by PCR **(B)** and amplicons of IL2Rγ and FAH were sent for Sanger sequencing. **(C)** Alignment of Sanger sequencing results of F3, F5, F8 and F12 with wildtype IL2Rγ and FAH sequences. 137 nucleotide deletion mutation (Δ137bp) was observed in IL2Rγ, while 1 nucleotide deletion mutation (Δ1bp) was observed in FAH.

SUPPLEMENTARY FIGURE 2
Stillbirths of RGFKO piglets. Images of stillbirths of RGFKO piglets from three surrogate sows implanted with RGFKO embryos at 93-99 days of gestation with NTBC supplementation.

SUPPLEMENTARY TABLE 1
sgRNA target sequences for RAG2, IL2Rγ and FAH.

SUPPLEMENTARY TABLE 2
Off-target sequences (OTS) predicted for RAG2, IL2Rγ and FAH.

SUPPLEMENTARY TABLE 3
Primer sequences used to amplify RAG2, IL2Rγ and FAH.

SUPPLEMENTARY TABLE 4
Primer sequences used for qPCR.

SUPPLEMENTARY TABLE 5
Primer sequences used for V(D)J rearrangement detection.

SUPPLEMENTARY TABLE 6
Biochemical function of liver.

References

1. Squires JE, Soltys KA, McKiernan P, Squires RH, Strom SC, Fox IJ, et al. Clinical hepatocyte transplantation: what is next? *Curr Transplant Rep* (2017) 4 (4):280–9. doi: 10.1007/s40472-017-0165-6

2. Fox IJ, Chowdhury JR. Hepatocyte transplantation. *Am J Transplant* (2004) 4 (s6):7–13. doi: 10.1111/j.1600-6135.2004.0340.x

3. Iansante V, Mitry R, Filippi C, Fitzpatrick E, Dhawan A. Human hepatocyte transplantation for liver disease: current status and future perspectives. *Pediatr Res* (2018) 83(1):232–40. doi: 10.1038/pr.2017.284

4. Fisher RA, Strom SC. Human hepatocyte transplantation: worldwide results. *Transplantation* (2006) 82(4):441–9.

5. Chen AX, Chhabra A, Fleming HE, Bhatia SN. Chapter 40 - hepatic tissue engineering. In: R Lanza, R Langer, JPV & A. Atala, editors. *Principles of tissue engineering, Fifth Edition*. London, United Kingdom: Academic Press (2020). doi: 10.1016/B978-0-12-818422-6.00041-1

6. Lin R-Z, Chang H-Y. Recent advances in three-dimensional multicellular spheroid culture for biomedical research. *Biotechnol J* (2008) 3(9-10):1172–84. doi: 10.1002/biot.200700228

7. Akbari S, Arslan N, Senturk S, Erdal E. Next-generation liver medicine using organoid models. *Front Cell Dev Biol* (2019) 7:345. doi: 10.3389/fcell.2019.00345

8. Garnier D, Li R, Delbos F, Fourrier A, Collet C, Guguen-Guillouzo C, et al. Expansion of human primary hepatocytes *in vitro* through their amplification as liver progenitors in a 3D organoid system. *Sci Rep* (2018) 8(1):8222. doi: 10.1038/s41598-018-26584-1

9. Zhang K, Zhang L, Liu W, Ma X, Cen J, Sun Z, et al. *In vitro* expansion of primary human hepatocytes with efficient liver repopulation capacity. *Cell Stem Cell* (2018) 23(6):806–19.e4. doi: 10.1016/j.stem.2018.10.018

10. Bianconi E, Piovesan A, Facchin F, Beraudi A, Casadei R, Frabetti F, et al. An estimation of the number of cells in the human body. *Ann Hum Biol* (2013) 40 (6):463–71.

11. Shafritz DA. A human hepatocyte factory. *Nat Biotechnol* (2007) 25(8):871–2. doi: 10.1038/nbt0807-871

12. Dandri M, Burda MR, Török E, Pollok JM, Iwanska A, Sommer G, et al. Repopulation of mouse liver with human hepatocytes and *in vivo* infection with hepatitis b virus. *Hepatology* (2001) 33(4):981–8. doi: 10.1053/jhep.2001.23314

13. Li Z, Wu J, Wang L, Han W, Yu J, Liu X, et al. Generation of qualified clinical-grade functional hepatocytes from human embryonic stem cells in chemically defined conditions. *Cell Death Dis* (2019) 10(10):763. doi: 10.1038/s41419-019-1967-5

14. Kawahara T, Douglas DN, Lewis J, Lund G, Addison W, Tyrrell DL, et al. Critical role of natural killer cells in the rejection of human hepatocytes after xenotransplantation into immunodeficient mice. *Transplant Int* (2010) 23(9):934–43. doi: 10.1111/j.1432-2277.2010.01063.x

15. Azuma H, Paulk N, Ranade A, Dorrell C, Al-Dhalimy M, Ellis E, et al. Robust expansion of human hepatocytes in fah-/-/Rag2-/-/Il2rg-/- mice. *Nat Biotechnol* (2007) 25(8):903–10. doi: 10.1038/nbt1326

16. Ekser B, Ezzelarab M, Hara H, van der Windt DJ, Wijkstrom M, Bottino R, et al. Clinical xenotransplantation: the next medical revolution? *Lancet* (2012) 379 (9816):672–83. doi: 10.1016/s0140-6736(11)61091-x

17. Yan S, Tu Z, Liu Z, Fan N, Yang H, Yang S, et al. A huntingtin knockin pig model recapitulates features of selective neurodegeneration in huntington's disease. *Cell* (2018) 173(4):989–1002.e13. doi: 10.1016/j.cell.2018.03.005

18. Facci MR, Auray G, Buchanan R, van Kessel J, Thompson DR, Mackenzie-Dyck S, et al. A comparison between isolated blood dendritic cells and monocyte-derived dendritic cells in pigs. *Immunology* (2010) 129(3):396–405. doi: 10.1111/j.1365-2567.2009.03192.x

19. Mair KH, Sedlak C, Käser T, Pasternak A, Levast B, Gerner W, et al. The porcine innate immune system: an update. *Dev Comp Immunol* (2014) 45(2):321–43. doi: 10.1016/j.dci.2014.03.022

20. Cheng W, Zhao H, Yu H, Xin J, Wang J, Zeng L, et al. Efficient generation of GGTA1-null diannan miniature pigs using TALENs combined with somatic cell nuclear transfer. *Reprod Biol Endocrinol* (2016) 14(1):77. doi: 10.1186/s12958-016-0212-7

21. Sykes M, Sachs DH. Transplanting organs from pigs to humans. *Sci Immunol* (2019) 4(41):eaau6298. doi: 10.1126/sciimmunol.aau6298

22. Niu D, Wei HJ, Lin L, George H, Wang T, Lee IH, et al. Inactivation of porcine endogenous retrovirus in pigs using CRISPR-Cas9. *Science* (2017) 357 (6357):1303–7. doi: 10.1126/science.aan4187

23. Yue Y, Xu W, Kan Y, Zhao H-Y, Zhou Y, Song X, et al. Extensive germline genome engineering in pigs. *Nat Biomed Eng* (2021) 5(2):134–43. doi: 10.1038/s41551-020-00613-9

24. Hsu HC, Enosawa S, Yamazaki T, Tohyama S, Fujita J, Fukuda K, et al. Enhancing survival of human hepatocytes by neonatal thymectomy and partial hepatectomy in micro-miniature pigs. *Transplant Proc* (2017) 49(1):153–8. doi: 10.1016/j.transproceed.2016.11.023

25. Itoh M, Mukae Y, Kitsuka T, Arai K, Nakamura A, Uchihashi K, et al. Kobayashi: Development of an immunodeficient pig model allowing long-term accommodation of artificial human vascular tubes. *Nat Commun* (2019) 10 (1):2244. doi: 10.1038/s41467-019-10107-1

26. Choi Y-J, Kim E, Reza AMMT, Hong K, Song H, Park C, et al. Recombination activating gene-2(null) severe combined immunodeficient pigs and mice engraft human induced pluripotent stem cells differently. *Oncotarget* (2017) 8(41):69398–407. doi: 10.18632/oncotarget.20626

27. Lee K, Kwon D-N, Ezashi T, Choi Y-J, Park C, Ericsson AC, et al. Engraftment of human iPS cells and allogeneic porcine cells into pigs with inactivated RAG2 and accompanying severe combined immunodeficiency. *Proc Natl Acad Sci* (2014) 111(20):7260–5. doi: 10.1073/pnas.1406376111

28. Boettcher AN, Li Y, Ahrens AP, Kiupel M, Byrne KA, Loving CL, et al. Novel engraftment and t cell differentiation of human hematopoietic cells in art–/–il2rg–/y scid pigs. *Front Immunol* (2020) 11:100(100). doi: 10.3389/fimmu.2020.00100

29. Ruppert S, Kelsey G, Schedl A, Schmid E, Thies E, Schütz G. Deficiency of an enzyme of tyrosine metabolism underlies altered gene expression in newborn liver of lethal albino mice. *Genes Dev* (1992) 6(8):1430–43. doi: 10.1101/gad.6.8.1430

30. Sniderman King L, Trahms C, Scott CR. Tyrosinemia type I. In MP Adam, GM Mirzaa, RA Pagon, SE Wallace, LJH Bean, KW Gripp, et al. editors. GeneReviews®[Internet] USA: University of Washington Press, Seattle (2017).

31. Grompe M. The pathophysiology and treatment of hereditary tyrosinemia type 1. *Semin Liver Dis* (2001) 21(4):563–71. doi: 10.1055/s-2001-19035

32. Hickey RD, Mao SA, Glorioso J, Lillegard JB, Fisher JE, Amiot B, et al. Fumarylacetoacetate hydrolase deficient pigs are a novel large animal model of metabolic liver disease. *Stem Cell Res* (2014) 13(1):144–53. doi: 10.1016/j.scr.2014.05.003

33. Bae S, Park J, Kim J-S. Cas-OFFinder: a fast and versatile algorithm that searches for potential off-target sites of Cas9 RNA-guided endonucleases. *Bioinformatics* (2014) 30(10):1473–5. doi: 10.1093/bioinformatics/btu048

34. Suzuki S, Iwamoto M, Saito Y, Fuchimoto D, Sembon S, Suzuki M, et al. Il2rg gene-targeted severe combined immunodeficiency pigs. *Cell Stem Cell* (2012) 10(6):753–8. doi: 10.1016/j.stem.2012.04.021

35. Watanabe M, Nakano K, Matsunari H, Matsuda T, Maehara M, Kanai T, et al. Generation of interleukin-2 receptor gamma gene knockout pigs from somatic cells genetically modified by zinc finger nuclease-encoding mRNA. *PLoS One* (2013) 8(10):e76478. doi: 10.1371/journal.pone.0076478

36. Huang J, Guo X, Fan N, Song J, Zhao B, Ouyang Z, et al. RAG1/2 knockout pigs with severe combined immunodeficiency. *J Immunol* (2014) 193(3):1496–503. doi: 10.4049/jimmunol.1400915

37. Suzuki S, Iwamoto M, Hashimoto M, Suzuki M, Nakai M, Fuchimoto D, et al. Generation and characterization of RAG2 knockout pigs as animal model for severe combined immunodeficiency. *Vet Immunol Immunopathol* (2016) 178:37–49. doi: 10.1016/j.vetimm.2016.06.011

38. Lei S, Ryu J, Wen K, Twitchell E, Bui T, Ramesh A, et al. Increased and prolonged human norovirus infection in RAG2/IL2RG deficient gnotobiotic pigs

with severe combined immunodeficiency. *Sci Rep* (2016) 6(1):25222. doi: 10.1038/srep25222

39. Ren J, Yu D, Wang J, Xu K, Xu Y, Sun R, et al. Generation of immunodeficient pig with hereditary tyrosinemia type 1 and their preliminary application for humanized liver. *Cell Biosci* (2022) 12(1):26. doi: 10.1186/s13578-022-00760-3

40. Kang J-T, Cho B, Ryu J, Ray C, Lee E-J, Yun Y-J, et al. Biallelic modification of IL2RG leads to severe combined immunodeficiency in pigs. *Reprod Biol Endocrinol* (2016) 14(1):74. doi: 10.1186/s12958-016-0206-5

41. Reza AMMT, Cho S-K, Choi Y-J, Hong K, Kim J-H. Microarray profiling of miRNA and mRNA expression in Rag2 knockout and wild-type mouse spleens. *Sci Data* (2018) 5(1):170199. doi: 10.1038/sdata.2017.199

42. Zuckermann FA, Peavey C, Schnitzlein WM, Schabacker D, Husmann RJ, Yang H, et al. Definition of the specificity of single-cell antibodies against porcine CD45 and CD45R: report from the CD45/CD45R and CD44 subgroup of the second international swine CD workshop. *Vet Immunol Immunopathol* (1998) 60 (3-4):367–87. doi: 10.1016/s0165-2427(97)00112-8

43. Ijspeert H, Wentink M, van Zessen D, Driessen GJ, Dalm VASH, van Hagen MP, et al. Strategies for b-cell receptor repertoire analysis in primary immunodeficiencies: from severe combined immunodeficiency to common variable immunodeficiency. *Front Immunol* (2015) 6:157. doi: 10.3389/fimmu.2015.00157

44. Fujii Y, Okumura M, Inada K, Nakahara K, Matsuda H. CD45 isoform expression during T cell development in the thymus. *Eur J Immunol* (1992) 22 (7):1843–50. doi: 10.1002/eji.1830220725

45. Haugabook SJ, Ferrer M, Ottinger EA. *In vitro* and *in vivo* translational models for rare liver diseases. *Biochim Biophys Acta (BBA) Mol Basis Dis* (2019) 1865(5):1003–18. doi: 10.1016/j.bbadis.2018.07.029

46. Grossman M, Rader DJ, Muller DWM, Kolansky DM, Kozarsky K, Clark BJ, et al. A pilot study of *ex vivo* gene therapy for homozygous familial hypercholesterolaemia. *Nat Med* (1995) 1(11):1148–54. doi: 10.1038/nm1195-1148

47. Hickey RD, Mao SA, Glorioso J, Elgilani F, Amiot B, Chen H, et al. Curative *ex vivo* liver-directed gene therapy in a pig model of hereditary tyrosinemia type 1. *Sci Transl Med* (2016) 8(349):349ra99. doi: 10.1126/scitranslmed.aaf3838

48. Hickey RD, Nicolas CT, Allen K, Mao S, Elgilani F, Glorioso J, et al. Autologous gene and cell therapy provides safe and long-term curative therapy in a large pig model of hereditary tyrosinemia type 1. *Cell Transplant* (2019) 28(1):79–88. doi: 10.1177/0963689718814188

49. Billerbeck E, Mommersteeg MC, Shlomai A, Xiao JW, Andrus L, Bhatta A, et al. Humanized mice efficiently engrafted with fetal hepatoblasts and syngeneic immune cells develop human monocytes and NK cells. *J Hepatol* (2016) 65(2):334–43. doi: 10.1016/j.jhep.2016.04.022

50. Her Z, Tan JHL, Lim Y-S, Tan SY, Chan XY, Tan WWS, et al. CD4(+) T cells mediate the development of liver fibrosis in high fat diet-induced nafld in humanized mice. *Front Immunol* (2020) 11:580968. doi: 10.3389/fimmu.2020.580968

51. Tan-Garcia A, Lai F, Sheng Yeong JP, Irac SE, Ng PY, Msallam R, et al. Liver fibrosis and CD206(+) macrophage accumulation are suppressed by anti-GM-CSF therapy. *JHEP Rep* (2020) 2(1):100062. doi: 10.1016/j.jhepr.2019.11.006

52. Tan-Garcia A, Wai L-E, Zheng D, Ceccarello E, Jo J, Banu N, et al. Intrahepatic CD206$^+$ macrophages contribute to inflammation in advanced viral-related liver disease. *J Hepatol* (2017) 67(3):490–500. doi: 10.1016/j.jhep.2017.04.023

53. Dawson HD, Loveland JE, Pascal G, Gilbert JGR, Uenishi H, Mann KM, et al. Structural and functional annotation of the porcine immunome. *BMC Genomics* (2013) 14(1):332. doi: 10.1186/1471-2164-14-332

54. Boettcher AN, Loving CL, Cunnick JE, Tuggle CK. Development of severe combined immunodeficient (scid) pig models for translational cancer modeling: future insights on how humanized scid pigs can improve preclinical cancer research. *Front Oncol* (2018) 8:559. doi: 10.3389/fonc.2018.00559

55. Zhao Y, Shuen TWH, Toh TB, Chan XY, Liu M, Tan SY, et al. Development of a new patient-derived xenograft humanised mouse model to study human-specific tumour microenvironment and immunotherapy. *Gut* (2018) 67(10):1845–54.

56. Zhao Y, Wang J, Liu WN, Fong SY, Shuen TWH, Liu M, et al. Analysis and validation of human targets and treatments using a hepatocellular carcinoma-immune humanized mouse model. *Hepatol n/a(n/a)* (2021). doi: 10.1002/hep.31812

57. Wei H, Qing Y, Pan W, Zhao H, Li H, Cheng W, et al. Comparison of the efficiency of banna miniature inbred pig somatic cell nuclear transfer among different donor cells. *PLoS One* (2013) 8(2):e57728. doi: 10.1371/journal.pone.0057728

58. Concordet J-P, Haeussler M. CRISPOR: intuitive guide selection for CRISPR/Cas9 genome editing experiments and screens. *Nucleic Acids Res* (2018) 46(W1):W242–5. doi: 10.1093/nar/gky354

59. Xie J, Ge W, Li N, Liu Q, Chen F, Yang X, et al. Efficient base editing for multiple genes and loci in pigs using base editors. *Nat Commun* (2019) 10(1):1–13.

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Hemizygous nonsense variant in the moesin gene *(MSN)* leads to a new autoimmune phenotype of Immunodeficiency 50

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Here, we present the findings of an investigation involving two male siblings with juvenile total tooth loss, early-onset chronic leg ulcers, and autoimmune thyroiditis, as well as focal segmental glomerulosclerosis with associated pulmonary emphysema in one and diabetes mellitus in the other. The clinical picture and lupus anticoagulant, cryoglobulin, and cold agglutinin positivity suggested the diagnosis of antiphospholipid syndrome. Flow cytometry analysis showed immunophenotypes consistent with immune dysregulation: a low number of naive T cells, elevated $CD4^+$ T cell counts, and decreased $CD8^+$ T-cell counts were detected, and more than half of the T-helper population was activated. Considering the siblings' almost identical clinical phenotype, the genetic alteration was suspected in the background of the immunodeficiency. Whole exome sequencing identified a previously not described hemizygous nonsense variant (c.650G>A, p.W217X) within exon 6 of the moesin (*MSN*) gene localized on chromosome X, resulting in significantly decreased *MSN* mRNA expression compared to healthy controls. We present a

putative new autoimmune phenotype of Immunodeficiency 50 (MIM300988) characterized by antiphospholipid syndrome, Hashimoto's thyroiditis, leg ulcers, and juvenile tooth loss, associated with W217X mutation of the MSN gene.

KEYWORDS

juvenile loss of teeth, chronic leg ulcer, autoimmunity, whole exome sequencing, moesin protein, antiphospholipid syndrome, thyroiditis

Introduction

Primary immunodeficiency diseases (PIDs) are a diverse group of mostly inherited genetic disorders characterized by loss of immune function and predisposition to recurrent infections or other immune diseases, such as autoimmunity and lymphoid malignancies. Loss-of-function mutations in genes encoding cytoskeletal proteins could result in PIDs (1).

Ezrin, radixin, and moesin (ERM) are three closely related proteins that link membrane-associated proteins directly to actin filaments in the cell cortex (2). ERM proteins organize the interface between the actin cytoskeleton and the plasma membrane and consequently play a central role in determining the structure and function of the plasma membrane and associated molecules, filopodia and microvilli, cellular morphology, adhesion, and epithelial integrity, while also affecting insulin and Rho signaling pathways (3). The ERM proteins are also known to play an important role in the formation of the immunological synapse (IS) (4). IS formation involves receptor–ligand pair clustering and intracellular signaling molecule recruitment, with coincident removal of other membrane proteins from the IS. MSN blocks inhibitory glycoproteins in the IS by concentrating these on distal poles, while EZR plays a role in the formation of signalosomes and the trafficking of signaling molecules (e.g., ZAP-70). Microfilament–membrane linkage is critical to this process. ERM proteins are also critical modulators of cortical architecture during highly dynamic cell behaviors, such as mitosis, migration, and junction remodeling (5). Indeed, ERM proteins regulate B- and T-cell activation through controlling B- and T-cell receptor dynamics, scaffolding protein assembly, and hence membrane-associated intracellular signaling (6). Two ERM members ezrin and moesin are highly expressed in lymphocytes (Supplementary Figures S1A, B), where chemokine activation or antigen presentation results in a rapid de- and rephosphorylation cycle, causing polarization and morphological changes. It was also described in a mouse model that excessive or deficient ERM-mediated crosslinking results in impaired lymphocyte homing (7). ERM proteins also play an important role in the regulation of B-cell receptor (BCR) signaling by undergoing antigen-induced conformational inactivation to facilitate lipid raft coalescence and BCR microclustering (8). Importantly, MSN has also been implicated in the IL-15–dependent proliferation of $CD8^+$ and $CD4^+$ Tregs, while MSN deficiency results in lymphocyte egress from lymphoid organs, causing persistent lymphopenia in peripheral blood. Recent studies suggest that ERM proteins are also responsible for aberrant cellular responses in the presence of viral, bacterial, and fungal pathogens. The fact that ERM proteins are positive regulators of X4-tropic human immunodeficiency virus-1 (HIV-1) infection corroborates this theorem (9).

The moesin protein-coding *MSN* gene is located on chromosome Xq12. Its pathogenic variants cause an X-linked recessive primary Immunodeficiency, termed Immunodeficiency 50 (IMD50). IMD50 is characterized by childhood recurrent bacterial respiratory, urinary, and gastrointestinal infections; complicated varicella-zoster; and extensive molluscum contagiosum virus infection (10). Typical laboratory findings are persistent lymphopenia, fluctuating neutropenia, decreased $CD4^+$ and $CD8^+$ cell and low $CD45RA^+$ T cell counts, increased senescent $CD8^+$ cell proportions, impaired T-cell proliferative responses and antibody formation, low levels of circulating NK and B cells, and hypogammaglobulinemia. The disorder does not affect overall survival. To the best of our knowledge, IMD50 has been described in nine cases so far (11, 12). Eight of them carried an identical missense mutation (c.511C>T, p.R171W). This missense variant causes MSN mRNA degradation and diminished protein expression, particularly in lymphocytes. One single individual carried a truncating variant caused by a premature stop codon (c.1657C>T, p.R533X), who did not suffer from the viral infections noted above. All patients were in satisfactory general condition at the examination; however, they required immunoglobulin therapy. No lethality has been reported in the literature.

Here, we present two male siblings with juvenile total tooth loss, early onset chronic leg ulcers and autoimmune thyroiditis, lupus anticoagulant, cryoglobulin, and cold agglutinin positivity, as well as immunophenotypes consistent with immune dysregulation, in whom whole exome sequencing identified a previously not described hemizygous nonsense variant

(c.650G>A, p.W217X) of the MSN gene. Based on detailed clinical, laboratory, and genetic analyses, we suggest a new autoimmune phenotype of IMD50.

Materials and methods

Laser Doppler flowmetry, toe pressure, and toe-brachial index

Laser Doppler flowmetry (LDF) is a noninvasive method based on the Doppler effect to detect blood flow in nutritive and thermoregulatory capillaries of the skin. The LDF instrument (PeriFlux System 5000, Perimed, Stockholm, Sweden) uses optical fibers to carry and detect laser light (wavelength, 780 nm). The LDF probe was attached to the skin by a double-sided adhesive tape. The detected flux signal is expressed as a perfusion unit (PU). Following the baseline skin perfusion detection at the temperature of the skin, the heatable probe can be set at 44°C. Due to the heat provocation, capillaries situated under the probe vasodilate, which is proportional to the increase of perfusion unit. The percent change of baseline and heat-provoked PU can indicate the local reserve capacity of related capillaries, which can be deteriorated in case of endothelial dysfunction or ischemia.

Transcutaneous partial oxygen pressure measurement

Transcutaneous partial oxygen pressure measurement ($tcpO_2$) is a noninvasive electrochemical method to detect the oxygen concentration of tissues. Precalibrated Clark electrode (Tina TCM 4000 oximeter, Radiometer, Denmark) was positioned on cleaned, hairless skin at the second intercostal space of the anterior chest wall as a reference probe, at the leg close to the ulcer, and at the dorsum of the foot with a self-adhesive fixation ring that was filled by contact liquid. The sensors are made of an oxygen-permeable membrane and a platinum–silver electrode with phosphate-buffered solution between them. A steady state in the supine position of the index limb was obtained for 15 min with the heating of probes to 44°C to achieve maximal vasodilation. The polarizing voltage generates an electrical potential difference that is proportional to the partial oxygen pressure of the tissue. This value is displayed in millimeters of mercury (mmHg). $TcpO_2$ value of >50 mmHg is considered physiologic, while $tcpO_2$ of <30 mmHg is viewed as a threshold for the diagnosis of severe ischemia.

Patient samples and cell preparations

Peripheral blood and/or skin samples from the two patients, their relatives, and healthy volunteers were harvested after the provision of written informed consent and in accordance with the Declaration of Helsinki. Peripheral blood mononuclear cells (PBMCs) were isolated using Ficoll-Paque Plus (GE Healthcare, Chicago, IL, USA) media for further analysis.

Whole exome sequencing

Genomic DNA was extracted from PBMCs by standard protocol. For exome sequencing, a total of 200 ng of genomic DNA was used for library preparation and sequenced with the exome kit Agilent SureSelectXT All Exon V6 (Agilent Technologies, Santa Clara, CA, USA) on the Genome Sequencer Illumina (HiSeq, Illumina, San Diego, CA, USA) platform (parameters: paired-end run type, read length: 2 × 150 bp, 60× average on target coverage). The 150-bp paired reads were aligned to the GRCh37.75 human reference genome by Burrows Wheel Aligner (BWA v0.7.9a) software (13). The variants were called by the Genome Analysis Toolkit Haplotype Caller (GATK v3.5) (Broad Institute, Cambridge, MA, USA) best practice and annotated by SnpEff (14) and VariantStudio (Illumina, San Diego, Ca, USA) software. Variants obtained by exome sequencing were filtered based on severity and frequency against public variant databases including dbSNP (https://www.ncbi.nlm.nih.gov/snp/) (15), ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) (16), NHLBI GO Exome Sequencing Project (ESP), (http://evs.gs.washington.edu/EVS/), GnomAD (http://gnomad.broadinstitute.org), and HGMD (http://www.hgmd.cf.ac.uk) (17) databases and an in-house clinical exome database of 300 unrelated Hungarian samples. Sanger sequencing was performed to confirm the moesin variant.

Quantitative RT-PCR

Total RNA was extracted from PBMC using the Direct-zol RNA Miniprep Kit (Zymo Research, Irvine, CA, USA). Reverse transcription was performed from 250 ng of total RNA using SuperScript RT enzyme with random hexamer primers (Thermo Fisher Scientific, Waltham, MA, USA). The expression level of moesin (Hs01085682_g1) was determined by RT-qPCR on QuantSudio™ 1 (Applied Biosystems, Waltham, MA, USA), and the samples were normalized to β-actin (Hs99999903_m1) mRNA level. The $_{\Delta\Delta}$CT method was used to quantify the differences.

Western blot

Total protein was extracted from PBMCs using M-PER™ buffer (Thermo Fisher Scientific, Waltham, MA, USA) supplemented with Halt™ Protease and Phosphatase Inhibitor Cocktail (Thermo Fisher Scientific, Waltham, MA, USA). Protein concentration was measured using Qubit™ Protein Assay Kit (Invitrogen, Waltham, MA, USA). A total of 10 µg of proteins per sample was separated on a 4%–15% precast gel (Bio-Rad, Hercules, CA, USA) and transferred to a nitrocellulose membrane. The staining was performed using the following antibodies: anti-moesin (NBP2-32875, Novus Biological), anti-β-actin (ab115777, Abcam) primary antibodies and HRP conjugated goat antimouse (12-349, Millipore), goat anti-rabbit (ab6721, Abcam) secondary antibodies, respectively. Detection of targeted protein was carried out with ECL (ImmunoCruz™, Santa Cruz Biotechnology, Dallas, TX, USA) using a chemiluminescence system (Syngene, Cambridge, UK).

Cell proliferation assay

PBMCs were cultured in RPMI 1640 medium containing 10% FBS, 1% L-glutamine, and 1% antibiotic/antimycotic solution at a density of 10^5 cells/well and were activated with 15 µg/ml phytohemagglutinin (PHA). For the control treatment, culture medium alone was added to the PBMC suspension. After, a 72-h incubation at 37°C in 5% CO_2 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) assay was performed. The absorbance was measured at 540 nm with the ELISA reader. All experiments were performed in triplicate. Data were expressed as a percentage change in the proliferation of PHA-activated PBMCs compared to the proliferation of untreated PBMCs in each group.

Flow cytometric detection of regulatory T-cell subpopulations

Regulatory T cells and their subgroups were determined with multiparametric flow cytometry using cell surface-specific anti-CD8-FITC (BD UCH-T4), anti-CD4-PerCP (BD SK3), and anti-CD25-APC (BD M-A251) antibodies, and for intracellular staining, PE-conjugated anti-Foxp3 (259D/C7, Becton Dickinson, Franklin Lakes, NJ, USA) antibody. Intracellular staining was performed using the Foxp3/Transcription Factor Staining Buffer Set (eBioscience), following the manufacturer's instructions. The fluorescence of labeled cells was recorded and analyzed using a FACS Calibur flow cytometer (Becton Dickinson). Lymphocytes were gated based on forward and sideward scatter (FSC and SSC). $CD25^+Foxp3^+$ conventional Treg cells were determined as the proportion of $CD4^+$ T cells. The ratio of $CD8^+$ Treg cells was determined as $CD25^+Foxp3^+$ cells in the $CD8^+$ lymphocyte gate. To determine the activated and naive/memory T-cell ratio, the following antihuman monoclonal antibodies were used: anti-CD3-FITC, anti-CD8-PE, anti-CD4-PerCP, anti-CD56-PECy5, anti-CD25-PE, anti-CD45RA-PE, and anti-CD45RO-PerCP (all from BD Biosciences). At least 10,000 cells were collected in the lymphocyte gate and analyzed. $CD3^+$, $CD4^+$, $CD8^+$ T cells, $CD3^+CD4^+CD25^-$ "resting T-helper cells," and $CD3^+CD4^+CD25low^+$ "activated" T cells were detected, and their absolute cell numbers were calculated. We tracked the percentages and absolute cell counts of naive $CD3^+CD45RA^+$ and $CD3^+CD45RO^+$ memory T cells.

Tests of the humoral immune responses

Disease-specific autoantibodies were measured using conventional ELISA tests or by immunoblotting. Systemic and organ-specific autoantibody screening tests (ANA screen Werfen) (ENA screen, anti-phospholipid autoantibody screen, ANCA screen, tTG screen Orgentec) and antigen-specific (TG, TPO, tTG, IA2, GAD65, ASCA, GBM) ELISA tests (Orgentec) were used to detect autoantibodies. Anti-measles antibody (IgG) measurements were performed using a self-developed ELISA assay, as previously reported (18). The anti-measles, anti-mumps, and anti-rubella indirect ELISA IgG ready-to-use kits (Euroimmun, Lübeck, Germany) were used to detect humoral antibody levels according to manufacturer's instructions, as long-lasting postvaccination immune response. The Mantoux tuberculin skin test was used to determine cell-mediated immunity to *Mycobacterium tuberculosis*. The anti-rubella antibody levels were measured using the previously described ELISA assay (19).

Statistical analysis

The differences were analyzed by two-way ANOVA with Graph-Pad Prism v8. Data were presented as the mean ± SEM. The difference was considered statistically significant at $p < 0.05$.

Results

Clinical characteristics of the patients

Two male siblings (P1: 36 years old and P2: 31 years old) were referred to the Department of Dermatology, Venerology, and Oncodermatology of the University of Pécs because of chronic ulcers on their legs (Figures 1A, B). In the case of P1, the ulcers appeared at 17 years of age on the left leg and at 24 years of age on the right leg, while in P2, the age of ulcer onset was 24 years on both

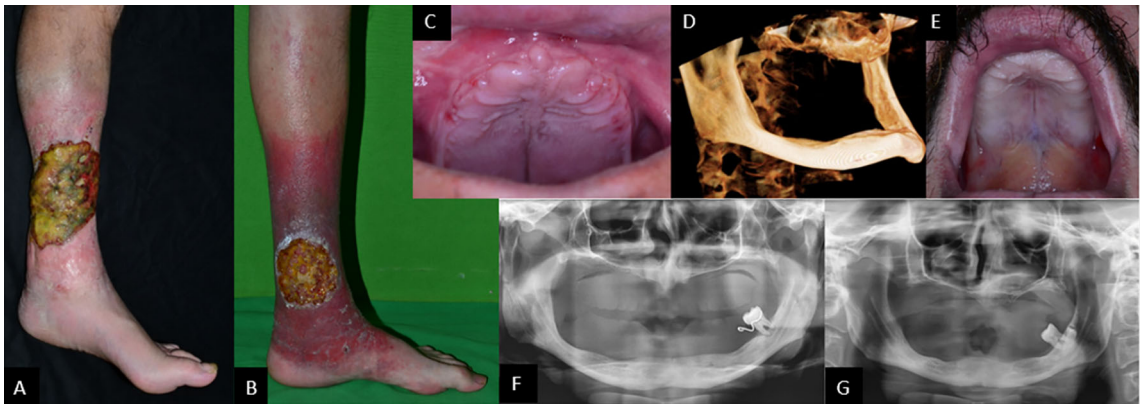

FIGURE 1
Biofilm-covered, 14-x-18-cm large ulcer in P1 **(A)** and 10-cm-diameter ulcer in P2 **(B)**, both on left leg. Complete tooth loss in P1 **(C)** and P2 **(E)**. Cone-beam CT scan showing severely atrophied jaws and missing processus alveolaris **(D)**. Panoramic radiographs showing highly atrophied jaws and a single remaining erupted tooth in both patients: P1 **(F)** and P2 **(G)**.

lower extremities. In both patients, cellulitis developed after the onset of ulcers. Their family history was negative. Perinatal anamnesis was without any documented alterations in both patients. P1 had recurrent cases of pneumonia between the ages of 4 and 10 years and suffered from thrombophlebitis of the left lower extremity in young adulthood. P2 had recurrent otitis media at age 4–5 years, resulting in tympanic perforation and permanent hearing loss of the right ear. Both siblings had chickenpox, but not herpes zoster. Both patients experienced recurrent swelling and bleeding of the gingiva from age 12. Without any visits to the general dental practitioner, there was no possibility to refer patients to a periodontist specialist. Gradually, periodontitis caused severe generalized alveolar bone loss and resulted in extremely mobile teeth when patients became ~19–20 years old (Figures 1C–G). At this stage, patients still had not visited a periodontist. The general dental practitioner made extractions, and the subtotal partially edentulous status was treated with removable prostheses. When further teeth became mobile, only extraction and prosthesis correction were performed. Parents and other known relatives of the patients did not present any of the above symptoms.

On examination, the patients were of normal height and weight (P1: 76 kg, 182 cm; P2: 64 kg, 183 cm). Routine laboratory tests indicated impaired liver functions and proteinuria in P1, diabetes mellitus in P2, as well as hypothyroidism in both (Table 1). A thyroid ultrasound (US) indicated signs of Hashimoto's thyroiditis (Figures 2A, B). In P1 bilateral pleural callus and pulmonary emphysema, enlargement and parenchymal thickening of both kidneys, low-grade hepatomegaly and multiple accessory spleen, and lack of thymus gland were detected by imaging examinations (chest X-ray, CT scans, and abdominal US) (Figure 2C).

Microscopic examination of kidney biopsy specimen PAS sections from P1 showed glomerular sclerotizing and scarring (Figure 2D). Immune fluorescence examination of a kidney biopsy did not detect immune complex deposition. Electron microscopy, however, showed fused and flattened podocyte foot processes in 85%–90% with properly fenestrated endothelium (Figure 2E). Together, these findings were consistent with the diagnosis of focal segmental glomerulosclerosis (FSGS). None of these symptoms were detected in P2.

Lower extremity arterial pulses were palpable in both individuals except for the left dorsal pedal artery of both patients and the tibial posterior artery of P2, probably due to ankle swelling distal to the wound. A handheld Doppler signal could be detected over distal arteries. Large arterial stenosis was ruled out by CT angiography. Angiological examinations showed satisfactory lower extremity venous circulation; however, laser Doppler flowmetry detected severely impaired microcirculation in both patients. Transcutaneous tissue oxygen pressure (tcpO2) after a 10-min resting period: P1: left leg 24 mmHg, left foot 26 mmHg; P2: left leg 18 mmHg, left foot: 2 mmHg. Laser Doppler flowmetry showed low values distal to the wound P1: 32°C: 22–26 PU, 44°C: 29–32 PU; P2: 32°C: 18–23 PU, 44°C: 120–140 PU. Polyneuropathy was not suspected by the normal result of the tuning fork vibration test in both patients.

Direct immunofluorescence examination of biopsies from the periphery of ulcers did not show specific immune-reactant deposition or vasculitis. A direct mutation analysis of the complement component 1 subcomponent R and subcomponent S genes (C1R and C1S) (20), responsible for the development of Ehlers–Danlos syndrome, periodontal type 1 (also known as Ehlers–Danlos syndrome type VIII) and Ehlers–Danlos syndrome, periodontal type, 2, respectively, was performed, and no mutation was found. Disease-specific collagen alterations were not detected by electron microscopy.

TABLE 1 Routine laboratory tests, immunohematology I (H, high; L, low).

	P1	P2	Units	Ref. interval
Glucose	5.64	**8.00H**	mmol/L	3.90–6.00
Fructosamine	*x*	**356H**	qmol/L	200–285
Haemoglobin a1c	5.30	**6.80H**	%	4.00–5.60
TSH	**22.59H**	**33.54H**	mU/L	0.270–4.20
Free T4 (fT4)	**8.1L**	**9.95L**	pmol/L	12.0–22.0
Anti-TG	**948.9H**	**337.1H**	IU/ml	<150
Anti-TPO	**214.2H**	**175.8H**	IU/ml	<75.0
Parathormon	3.40	3.90	pmol/L	1.60–6.10
Vitamin 25-OH D3	**10.0L**	**42.4L**	nmol/L	47.7–144
Calcium	**1.99L**	2.36	mmol/L	2.15–2.55
Anorg. phosphat	1.25	1.05	mmol/L	0.81–1.45
GOT	27	17	U/L	<44
GPT	20	16	U/L	<50
GGT	**197H**	23	U/L	<60
Total protein	**39.9L**	77.4	g/L	66.0–87.0
Albumin	**10.2L**	47.4	g/L	35.0–52.0
Carbamide	4.5	5.4	mmol/L	2.14–8.21
Creatinine	56	74	qmol/L	62–106
Urine total protein	**11.84H**	0.09	g/L	<0.10
Urine microalbumin	**8.328H**	9	mg/L	<20
IgA	3.02	4.0	g/L	0.70–4.00
IgM	0.4	0.94	g/L	0.40–2.30
IgG	17.40	14.30	g/L	7.00–16.00
IgG1	7.75	8.96	g/L	4.90–11.40
IgG2	5.51	3.36	g/L	1.50–6.40
IgG3	0.56	0.47	g/L	0.20–1.10
IgG4	0.55	0.52	g/L	0.08–1.40
Lupus anticoagulant test	+	+		
Lupus anticoagulant	**1.4H** **1.26H**	**1.3H** **1.29H**		0.8–1.2
Antithrombin activity	**150H**	**130H**	%	83–128
Protein C activity	**154H**	**159H**	%	70–130
Protein S activity	99	118	%	64–149
Cryoglobulin serum	**Mildly +**	Negative		
Cryoglobulin citrát	**Mildly +**	Negative		
Cryoglobulin heparin	**Mildly +**	+		
Red cell antibody	**Cold antibody**	**Cold antibody**		
Cold agglutinin	**At +4°C (+++)**	**At +4°C (+++)**		

Bold highlighting represents high (H) and low (L) values.

Immunoserological parameters

ANA screen, ENA screen, MPO (p-ANCA), Hs-PR3 (c-ANCA), cardiolipin IgG/IgM, B2-glikoprotein IgG/IgM, and prothrombin IgG/IgM were all negative in both patients. HIV-1/HIV-2 antibody, HIV-1 Ag, HBsAg, and HCV Ag were also negative. For P2: significant antibody titer (>100 IU/L, 10.6 mNe/ml) for hepatitis B virus (HBV) anti-HBs (surface) antigen with ELISA. Varicella-zoster (VZV/HH3) IgG was detected *via* ELISA. Complement activity was within reference values; prothrombin INR, thrombin time, D-dimer, fibrinogen, and protein S were also normal in both cases.

Identification of a hemizygous nonsense variant in the MSN gene through WES

To dissect the genetic background of the observed clinical alterations, bioinformatic analysis of the whole exome sequencing (WES) data generated using genomic DNA from

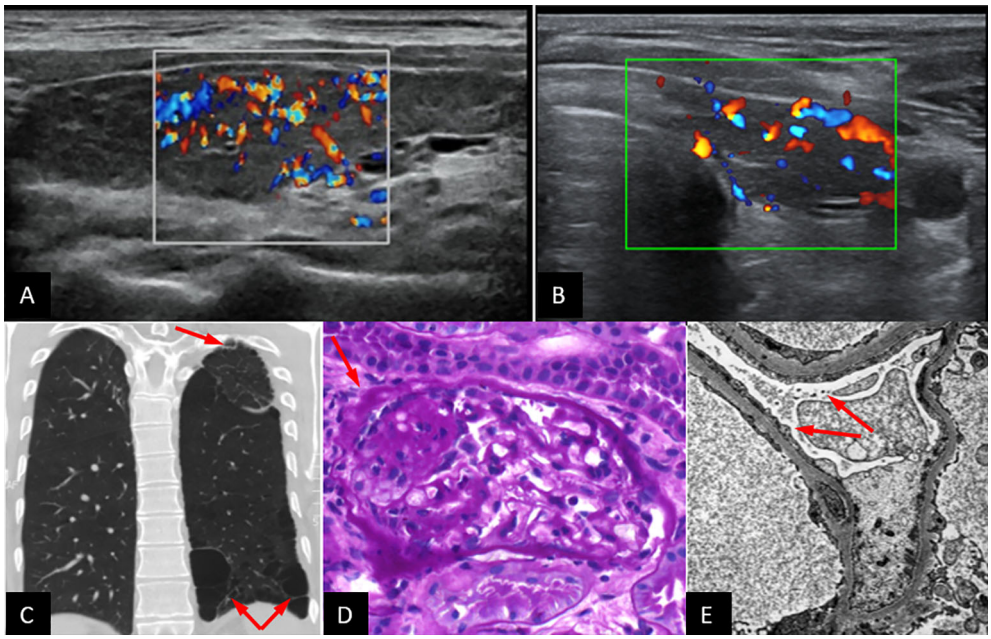

FIGURE 2
Thyroid ultrasound showing normal-sized, inhomogeneously structured, hypervascularized thyroid and connective tissue alterations, typical signs of Hashimoto's thyroiditis, in P1 **(A)** and P2 **(B)**. Chest CT of P1. Arrows indicate basal paraseptal bullae, paraseptal emphysema cranially, and dextrally. Apical callus and spread micronodules **(C)**. Kidney biopsy, PAS staining: a sclerotized region with scarring in the glomerulus (red arrow) **(D)**. Kidney biopsy, electron microscopy: podocyte foot processes fused (red arrows) and flattened in 85%–90%, endothelium properly fenestrated. Immune fluorescence did not detect immune complex deposition **(E)**. Diagnosis: focal segmental glomerulosclerosis.

P1 was carried out, and a novel hemizygous nonsense mutation, 650G>A in exon 6 of the moesin (*MSN*) gene on chromosome X, was identified. This mutation has not been documented before in the gnomAD, ClinVar, and the HGMD databases. Next, P2, the parents and the mother's two healthy brothers were directly tested for the identified moesin variant by Sanger sequencing. The same mutation was validated in P2, while in the patient's mother, heterozygous status was identified. None of the other screened family members carried the mutation (Figure 3).

This new mutation detected in the MSN gene is considered to be a variant of uncertain significance (VUS) due to the lack of literature data, but its pathogenicity was assumed with its predicted effect (premature stop codon formation) and the segregation results.

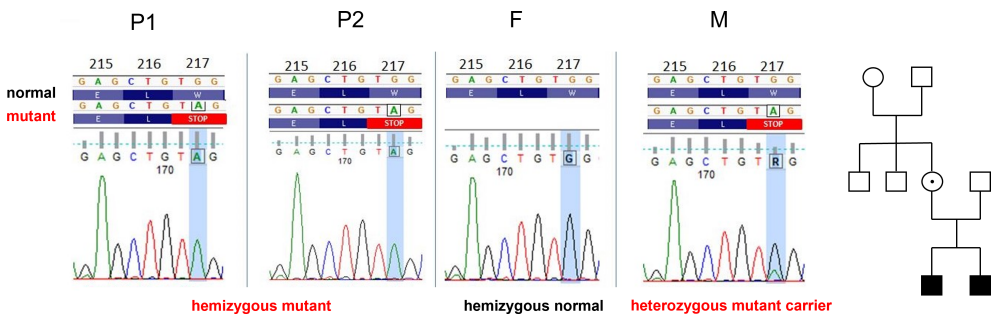

FIGURE 3
Identical *MSN* gene variants in P1 and P2: c.650G>A p.Trp217Ter, W217X (sixth exon). The father (F), maternal grandmother, and uncles carry wild-type alleles. The mother (M) is heterozygote. Family tree: white, wild-type; dotted, heterozygote, black, hemizygote.

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Dysregulated immunophenotype in patients carrying the novel hemizygous MSN mutation

Since mutations in the MSN gene are known to be associated with immunodeficiency, and since several episodes of recurrent infections occurred in both patients, we further investigated immune function abnormalities. To this end, immunologic profiling was performed using peripheral blood flow cytometry analysis at three different time points (in 2015, 2019, and 2020) (Table 2). Although mild anemia was detected in both patients, white blood cells, lymphocyte, neutrophil, eosinophil, and basophil counts were normal, and blood cell morphological abnormalities were not detected. $CD3^+$, $CD4^+$, $CD8^+$ T, and $CD19^+$ B-lymphocyte counts and ratios were mostly normal or occasionally slightly outside of the normal ranges. $CD56^+$ NK cell ratios, however, were markedly lower than normal in P1. Flow cytometry analysis showed immunophenotypes consistent

TABLE 2 Hematology, immunohematology II, and FOXP3 staining results (ND, not determined; H, high; L, low).

Hematology	P1			P2			Units	Ref. interval
Year of evaluation	**2015**	**2019**	**2020**	**2015**	**2019**	**2020**		
Hemoglobin	**111L**	**118L**	**121L**	**123L**	**116L**	**117L**	g/L	137–175
Hematocrit	**32.1L**	**36.7L**	**36.0L**	**35.5L**	**37.1L**	**36.0L**	%	40.1–51.0
Red cell count	**3.90L**	**4.06L**	**4.09L**	**4.13L**	**3.88L**	**3.85L**	T/L	4.50–6.00
White blood cell count	5.17	5.85	8.01	6.57	8.25	7.56	Giga/L	4.00–10.00
Neutrophils	2.47	2.7	4.65	3.70	5.03	3.70	Giga/L	1.78-5.38
Lymphocytes	1.57	2.08	1.71	1.62	2.12	2.43	Giga/L	1.32–3.57
Monocytes	0.620	**0.90H**	**1.29H**	0.670	**0.91H**	**1.17H**	Giga/L	0.30–0.82
Eosinophils	0.180	0.14	0.29	0.190	0.110	0.19	Giga/L	0.00–0.54
Basophils	0.020	0.01	0.02	0.020	0.020	2.02	Giga/L	0.00–0.08
Platelets	173.0	221.0	205.0	194.0	225.0	192	Giga/L	140.0–440.0
Σ Lymphocytes	1,570	2,080	1,710	1,620	2,120	**2,430H**	/ql	1,200–2,400
$CD3^+$ T-cell ratio	78.7	72.3	79.5	69.1	69.8	**61.1L**	%	64.0–82.0
Σ $CD3^+$ T cells	1,236	1,504	1,359	1,119	1,480	1,485	/ql	984–1,984
Σ $CD4^+$ T cells	**510L**	763	**593L**	748	1039	858	/ql	643–1,175
Σ $CD8^+$ T cells	595	666	576	**334L**	363	532	/ql	336–876
$CD4^+$ T-cell ratio	**32.5L**	36.7	**34.7L**	46.2	49.0	**35.3L**	%	36.0–54.0
$CD8^+$ T-cell ratio	**37.9H**	32.0	33.7	**20.6L**	**17.1L**	**21.9L**	%	22.0–36.0
CD4/CD8 ratio	**0.86L**	1.15	1.03	2.24	2.87	1.61		0.92–4.11
$CD19^+$ B lymphocytes	16.0	**21.5H**	13.8	10.6	11.5	14.2	%	7.2–16.4
Σ $CD19^+$ lymphocytes	251	**447H**	236	172	244	345	/ql	97–399
$CD5^+$ B lymphocytes	2.6	4.6	2.0	1.3	1.6	1.9	%	<5.0
Σ $CD5^+$ B lymphocytes	41	96	34	21	34	46	/ql	
$CD56^+$ NK cells	**4.2L**	**3.8L**	**5.6L**	16.1	15.3	25.4	%	9.6–27.0
$CD3^+$, $CD56^+$ NKT cells	18.1	1.7	2.2	4.0	1.5	4.9	%	
T-cell subpopulations								
CD3/$CD45RA^+$ (naive)	**26.5L**	**22.1L**	**27.8L**	**19,2L**	**18.7L**	**17.2L**	%	33.0–66.0
CD3/$CD45RO^+$ (memory)	55.9	56.4	52.2	53.3	49.2	44.5	%	24.0–57.0
CD3/$CD25^+$ (activated)	**21.5H**	**24.1H**	**28.5H**	**35.7H**	**30.1H**	**20.1H**	%	1.9–7.7
CD4+$CD25^+$ (activated) Th	**56.0H**	**53.7H**	**56.7H**	**60.4H**	**59.5H**	**51/1H**	%	4.0–11.0
HLA-DR/$CD3^+$ activated	6.6	**1.9L**	4.0	7.5	4.3	2.8	%	2.4–11.8
HLA-DR/$CD8^+$ activated	**2.6L**	**1.9L**	**3.0L**	5.7	**1.6L**	**1.1L**	%	5.0–25.0
$CD4^+$CD25high (Treg)	2.4	2.5	4.7	6.4	2.7	4.4	%	
$CD4^+CD25^+Foxp3^+$ (Treg)	ND	ND	3.1	ND	ND	5.7	%	
$CD8^+CD25^+Foxp3^+$ (Treg)	ND	ND	0.1	ND	ND	0.8	%	
Th1/Th2 intracellular cytokines								
Interleukin-4%				1.00	ND		%	
Interferon-gamma %				3.00	ND		%	

Bold highlighting represents high (H) and low (L) values.

with immune dysregulation. Naive ($CD45RA^+$) T-cell ratios were markedly lower than normal at every measurement in both patients ($CD3^+CD45RA^+$ ratio: P1: 22.1%–27.8%; P2: 17.2%–19.2%; normal range: 33.0%–66.0%). A large proportion of the total T-cell population was $CD25^+$, indicating an activated phenotype ($CD3^+CD25^+$ ratio: P1: 21.5%–28.5%; P2: 20.1%–35.7%; normal range: 1.9%–7.7%). Furthermore, more than half of the T-helper population was also activated ($CD4^+CD25^+$ ratio: P1: 53.7%–56.7%; P2: 51.1%–60.4%; normal range: 4.0%–11.0%). Interestingly, the ratio of cells expressing HLA-DR, another indicator of T-cell activation, was found normal within the total $CD3^+$ population in P1 on two of three occasions, and in P2 at all three measurements. On the other hand, the $CD8^+HLA\text{-}DR^+$ ratio was markedly reduced within the cytotoxic T-cell population ($CD8^+HLA\text{-}DR^+$: P1: 1.9%–3.0%; P2: 1.1%–5.7%; normal range: 5.0%–25.0%). The proportion of regulatory T cells, both within the $CD4^+$ and the $CD8^+$ population, was found normal. $CD4^+/CD25^+/Foxp3$ staining was carried out to identify Treg cell ratios. For P1, $CD4^+$ T-helper cells are $CD4^+/CD25^+/Foxp3^+$ in 3.1%, within the normal range, while $CD8^+$ T cytotoxic T cells are $CD8^+/CD25^+/Foxp3^+$ in 0.1%, similar as compared to healthy controls. For P2, the Treg ratio is 5.7% and $CD8^+$ cytotoxic T cells are $CD8^+/CD25^+/Foxp3^+$ in 0.8% (Table 2). Taken together, these results indicate that the novel *MSN* variant leads to impaired lymphocyte functions, primarily affecting T cells. A low $CD8^+$ cell ratio and absolute cell count indicate a disturbance in cell activation. This is accompanied by a low naive T-cell ratio, which supports a maturational defect in T cells, and may indicate congenital immunodeficiency.

The novel MSN variant leads to diminished moesin mRNA and protein expression

Moesin mRNA expression in PBMCs of the patients, the mother, and healthy controls was analyzed by qPCR. Compared to the controls, significantly decreased MSN transcript values were detected in the patients and lower mRNA expression in the mother. The decreased expression in the mother could be the consequence of the heterozygous mutation in the MSN gene (Figure 4). As the previously documented *MSN* mutations were associated with altered MSN protein expression, we hypothesized that the novel mutation might affect the expression of the MSN protein as well. A Western blot analysis was performed using an antibody directed against the full-length MSN protein (clone MSN/491). PMBCs of a healthy volunteer and the mother expressed the full-length moesin protein in a significant amount. The truncated 216 amino acid protein has a predicted 25-kDa molecular weight; however, this could not be detected in the patient's samples with the MSN antibody used (Figure 5). Transcripts with early stop codon are recognized and eliminated *via* nonsense-mediated mRNA decay (NMD) to prevent the accumulation of truncated

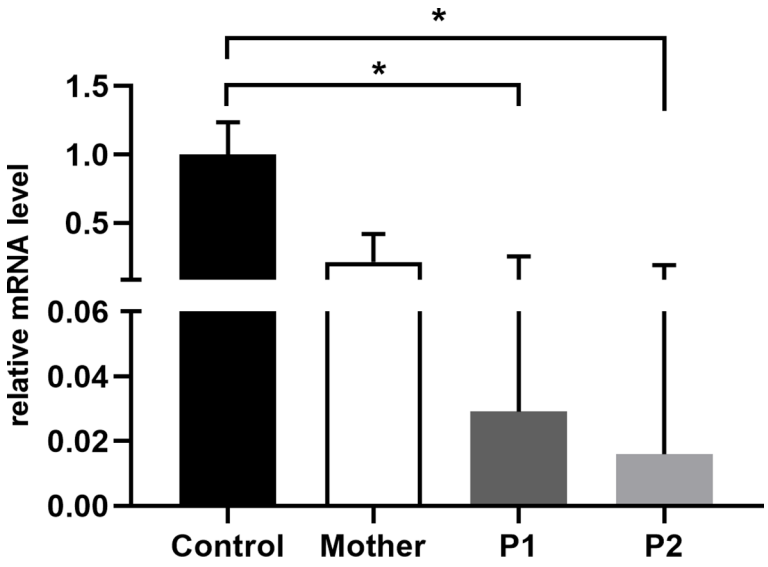

FIGURE 4
Relative quantity of MSN mRNA in PBMCs of healthy controls, mothers, and patients. Data are mean ± SEM for n = 4/healthy control and triplicates/mother, patients. *$p < 0.05$ control vs. mother, P1, and P2, based on one-way ANOVA followed by Bonferroni's *post-hoc* test.

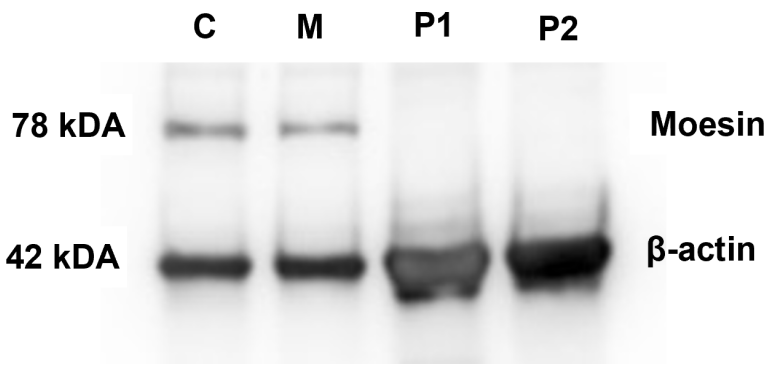

FIGURE 5
MSN protein detection. Western blot analysis showing the presence of moesin in PMBCs of healthy controls (C) and the patients' mothers (M). Beta-actin was used as the loading control.

proteins (21, 22). Further examinations are needed to confirm or exclude the NMD processes and to detect the presence of the truncated protein.

The novel MSN variant may lead to impaired T-cell proliferation

Since we detected profound immunophenotype alterations in our patients, we then wanted to test the functional capacity of T lymphocytes. The PHA-induced proliferative response of peripheral mononuclear cells was compared between the patients, their mother, and the healthy controls. Proliferative responses of P2 (53.1 ± 3.024) were significantly decreased compared to those of the healthy volunteers (123.7 ± 17.86) and the mother (140.1 ± 11.64). In the case of P1, the PHA-induced proliferation was somewhat lower, but the difference was not significant between healthy volunteers and the mother (Figure 6). The Tuberculin Skin Test (Mantoux test) showed hyperergic reaction in P1 (NB: as a result of compulsory BCG vaccination in Hungary, individuals with normal immune functions display hyperergic Mantoux test—a sign of type IV reaction against mycobacterial purified protein derivative). P2 refused the Mantoux test. The Quantiferon-TB test was negative in both P1 and P2.

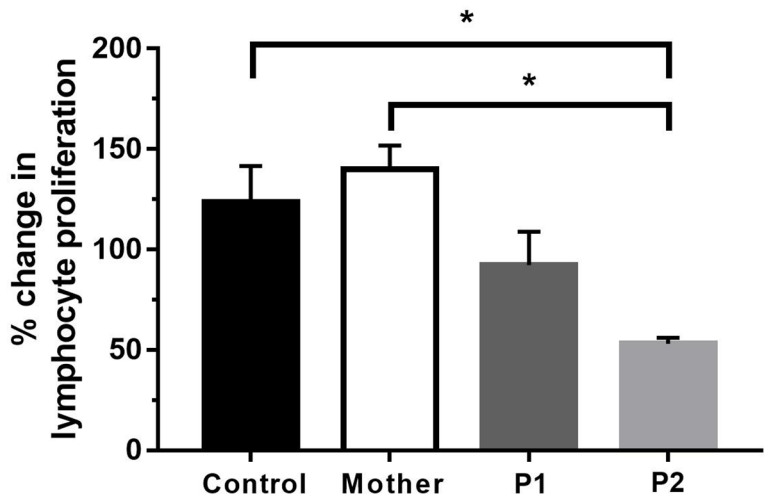

FIGURE 6
Proliferation of lymphocytes after 72 h of PHA activation. Data are the mean ± SEM of three independent experiments. Control represents the results of six healthy volunteers. *$p < 0.05$ healthy control and mother vs. P2, based on one-way ANOVA followed by Bonferroni's *post-hoc* test.

Dysregulated B-cell functions in patients with novel MSN mutation

Both patients had elevated antithyroglobulin (anti-TG) and antithyroperoxidase (anti-TPO) antibody titers and were positive for lupus anticoagulant, cryoglobulin (heparin), red cell antibody, and cold agglutinin (reacted at +4°C) and had elevated protein C and antithrombin activity (Table 1). Since live attenuated vaccines (LAVs) stimulate a strong, effective, and long-lasting immune response, we used anti-measles, anti-mumps, and anti-rubella (MMR LAV) postvaccination humoral antibody (IgG) titer testing as a model to investigate potentially impaired B-cell functioning. According to their vaccination certificates, P1 received primary and reminder measles vaccines at ages 14 months and 11 years, while P2 was vaccinated against measles and rubella at age 14 months and against measles, mumps, and rubella at 11 years (Table 3). Anti-measles, anti-mumps, and anti-rubella IgG antibody titers are presented in (Figure 7). In the case of P1, we detected insufficient circulating IgG antibody levels for measles and mumps (measured value/cutoff values were 132.18/200.0, 2.18/16.0, and 54.32/8.0 U/ml for measles, mumps, and rubella, respectively). In the case of P2, we detected sufficient antibody titers for all vaccines (measured value/cutoff values were 523.77/200.0, 35.42/16.0, and 10.76/8.0 U/ml for measles, mumps, and rubella, respectively, according to the International Notes, Hungary) (23).

Therapy and clinical outcome

There are currently no approved therapeutic recommendations in IMD50. The siblings did not require intravenous immunoglobulin therapy or granulocyte colony-stimulating factor. They were in satisfactory general health conditions at the time of the writing of the manuscript.

Angiological examinations detected severely impaired microcirculation in both patients. Due to the antiphospholipid syndrome, hematology suggested the introduction of anticoagulant vitamin K-antagonist therapy, which was not tolerated by P1 and refused by P2. Low molecular weight heparin therapy completely resolved ulcers in P1 in 6 months, while P2 refused this as well. In P1, noninvasive control studies in angiology showed a significant improvement in microcirculation. Transcutaneous tissue oxygen pressures ($tcPO_2$) on the left foot were measured after ulcer healing with the following tension values: 72 mmHg after 15 min resting period; 74 mmHg 5 min, 84 mmHg 10 min, and 89 mmHg 15 min after the walk test. The laser Doppler flowmetry measurements on the left big toe showed 135 PU on average and 122 PU after the walk test (pavement test at 10% gradient, 3.2 km/h, 5 min). P2 refused follow-up testing. No abnormalities suggestive of lower limb arterial circulatory dysfunction were detected at rest and were not provoked by exercise. Wound healing is explained by the microcirculation-improving effect of anticoagulant treatment. Local antiseptic treatment and antimicrobial foam dressing appropriate to the wound condition were applied, but no treatment modification was made.

Hormone replacement therapy in P1 leads to normalized thyroid hormone levels. P2 refused any thyroid hormone therapy. FSGS in P1 improved upon conservative and systemic glucocorticoid treatment, with less than 0.5 g/day proteinuria following 6 months of treatment, and upheld glomerular function throughout.

Both patients had first partial removable and later total removable dental prostheses. Implantation therapy was not a real alternative partly because of the family's financial circumstances and partly because of the highly questionable outcome.

Discussion

Here, we describe a pair of male siblings with a novel autoimmune phenotype, namely antiphospholipid syndrome, in connection with Immunodeficiency 50. We identified a hemizygous nonsense mutation of the *MSN* gene (p.W217X) as the genetic alteration responsible for the clinical findings. The newly identified mutation was different from the previously documented cases of *MSN* mutations (Table 4). In eight of those cases, an identical missense mutation (c.511C>T) was identified, leading to an arginine-to-tryptophan transition at an amino acid position of 171 (R171W). The remaining case carried a nonsense mutation (c.1657C>T), generating a premature stop codon at position 553 (R533X) (10–12). The newly identified mutation also leads to the formation of a premature stop codon at amino

TABLE 3 Patients' data and qualitative MMR ELISA (IgG) results.

	Date of birth	Immunization I.	Immunization II.	Quantitative ELISA results (Euroimmun, IgG)		
				Measles	Mumps	Rubella
P1	1,984.06.12	1,985.08.05. (Morbilli)	1,996.11.19. (Morbilli)	Negative	Negative	Positive
P2	1,989.04.18	1,990.06.18. (Morbilli-rubella)	2,001.10.16. (MMR)	Positive	Positive	Positive

Vaccination certificates registered immunization data of patients versus MMR vaccine-induced, measured qualitative results of humoral antibody (ELISA, IgG) levels.

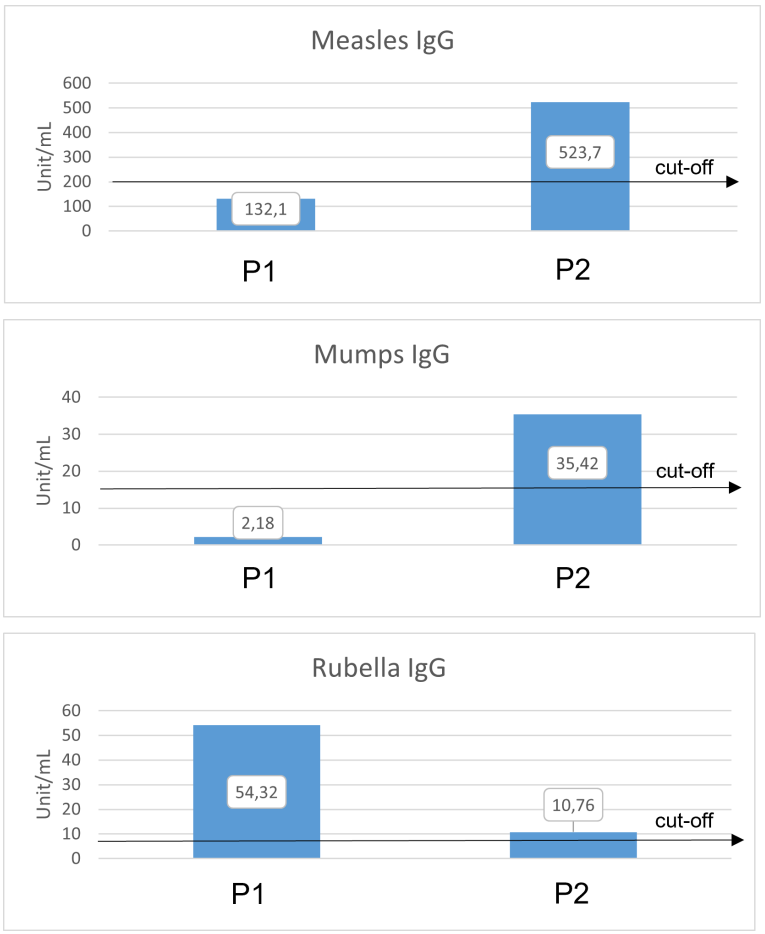

FIGURE 7
Anti-measles, anti-mumps, and anti-rubella antibody titers (Euroiummun, IgG) compared to the relative cutoff values. Anti-measles IgG cutoff = 200 U/ml. Anti-measles IgG P1 = 132.18 U/ml (negative), P2 = 523.77 U/ml (positive). Anti-mumps IgG cutoff = 16 U/ml. Anti-mumps IgG P1 = 2.18 U/ml (negative), P2 = 35.42 U/ml (positive). Anti-rubella IgG cutoff = 8 U/ml. Anti-rubella IgG P1 = 54.32 U/ml (positive), P2 = 10.76 U/ml (positive). Black arrows show cutoff values.

acid 216 (p.Trp217Ter) and a significantly truncated and likely dysfunctional MSN protein (the full-length moesin protein consists of 577 amino acids).

Since moesin has been shown to have a crucial role in lymphocyte homeostasis, lack or dysfunctionality of moesin protein could account for many or most of the immune abnormalities seen in both our patients and the previously reported cases with MSN mutations. Similar to the previously described R171W and R553X IMD50 variants, recurrent respiratory and ear infections were also observed in our patients with the novel W217X variant. There are, however, significant phenotypic differences between the previously reported cases and our patients, such as the presence of antiphospholipid antibodies, leg ulcers, and significant early tooth loss, or the lack of molluscum contagiosum or recurrent varicella-zoster virus infections in our cases. While the R171W mutation was reportedly associated with autoimmunity in the form of thrombotic thrombocytopenic purpura (TTP) and idiopathic thrombocytopenic purpura (ITP), our patients had organ-specific autoimmunity: Hashimoto's thyroiditis (HT) and antiphospholipid syndrome with cryoglobulinemia, red cell antibody, and cold agglutinin positivity. Based on the chronic microcirculatory dysfunction and the repeated lupus anticoagulant positivity, the clinical diagnosis of the antiphospholipid syndrome could be established. As moesin has been previously associated with self-tolerance, our clinical observation about this linkage may urge further investigations in this direction (13). To our knowledge, no monogenic cause has been found in connection with

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TABLE 4 Clinical and laboratory comparison of previously studied MSN deficiencies (PP1–PP9) vs. current subjects (P1 and P2).

Case	MSN mutation	Bacterial infections	Varicella-zoster	Eczema	Molluscum contagosum	Autoimmunity	Persistent lymphopenia	Fluctuating neutropenia	IgG therapy	Improvement with G-CSF	Alive and well
PP1	R171W	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	No G-CSF	Yes
PP2	R171W	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes
PP3	R171W	Yes	Yes	No	No	TTP	Yes	Yes	Yes	No G-CSF	Yes
PP4	R171W	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	Yes
PP5	R171W	Yes	No	Yes	No	No	Yes	Yes	Yes	No G-CSF	Yes
PP6	R171W	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	No-G-CSF	Yes
PP7	R553X	Yes	No	No	No	No	Yes	Yes	Yes	No-GCSF	Yes
PP8	R171W	Yes	Yes	No	No	ITP	Yes	Yes	Yes	Yes	Yes
PP9	R171W	No	No	Yes	No	No	Yes	Yes	Yes	No	Yes
P1	W217X	Yes	No	No	No	HT, LA	No	No	No	No	Yes
P2	W217X	Yes	No	No	No	HT, LA	No	No	No	No	Yes

PP9: neonatal case. Lymphopenia, neutropenia, varicella-zoster, and molluscum contagiosum did not occur in the studied patients. TTP, thrombotic thrombocytopenic purpura; ITP, idiopathic thrombocytopenic purpura; HT, Hashimoto's thyroiditis; LA, Lupus anticoagulant.

antiphospholipid syndrome, but evidence suggests that anti-moesin antibodies may play a role in the pathomechanism of antiphospholipid syndrome (14, 15). It is currently unclear why antiphospholipid antibodies were only observed in our patients and not in those with other MSN mutations. Furthermore, we suggest that the antiphospholipid syndrome-associated microcirculatory dysfunction was the primary causative factor in ulcer formation in our cases. However, it should also be noted that ERM proteins play a central role in endothelial homeostasis and may also be linked to diabetic angiopathy. MSN dysregulation may impair the endothelial barrier, resulting in lymphocyte recruitment and elevated fibronectin expression and deposition in the ECM, which might be a potential mechanism of ulcer formation.

Both panoramic radiographs and cone-beam CT showed severely atrophied jaws in our patients, and the extreme extent of bone loss was very unusual considering the age of the patients. Classical, known syndromes could not be accounted for the loss of teeth. Since dental status suggests severe periodontitis, and moesin is involved in several innate and adaptive immune functions, moesin dysfunction may be indirectly linked to tooth loss. Recognition of lipopolysaccharide (LPS) components of the outer membrane of gram-negative bacteria by monocytes/macrophages is an important step in the immediate and active adaptive immune response (24). It has been reported that LPS stimulation causes increased expression of moesin (25). Moesin, on one hand, can directly bind to LPS, while on the other hand, it can be associated with TLR4 and MD-2. This response involves the activation of inflammatory cytokines (TNF-α and IL-1β) through the activation of a complex network of cytoskeletal proteins, kinases, and transcription factors including CD14; TLR4; MD-2; MAPKs such as ERK1, ERK2, p38 MAPK, and c-Jun N-terminal kinases (JNK); and NF-κB (26, 27). In addition, inhibition of moesin interrupts LPS response pathways through a blockade of MyD88, IRAK, and TRAF6 (28). This hinders the activation of the MAP kinases p38 and ERK and the activation of NF-κB, which regulates TNF-α and IL-1β production (29). Thus, moesin may play an important role in innate immune responses and TLR4-mediated pattern recognition in periodontal diseases (25).

Secondary FSGS in P1 was ruled out by histology and clinical features, suggesting primary, immune-mediated FSGS (30). The effectiveness of glucocorticoids in our case and in many podocytopathies (kidney diseases in which direct or indirect podocyte injury drives proteinuria or nephrotic syndrome) suggests immune system-related pathogenesis of the kidney symptoms (31). This is further supported by the observation that the typical manifestations of podocytopathies on kidney biopsy are minimal change lesions or focal segmental glomerulosclerosis lesions (32, 33). Moreover, patients with podocytopathies may present with alterations in circulating T-cell subsets (e.g., reduced number of regulatory

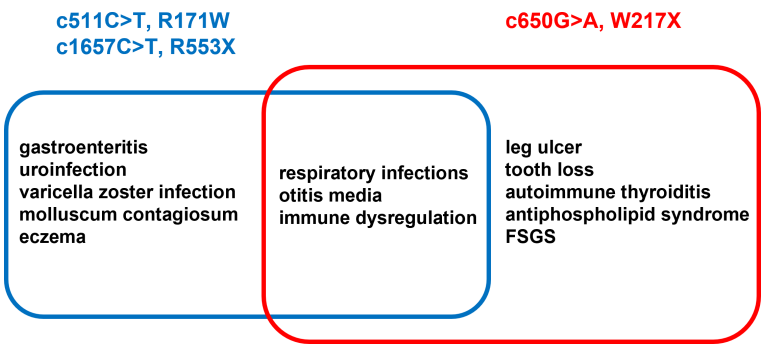

FIGURE 8
List of concomitant diseases described in eight previous reports (blue) vs. the siblings described in the current work (red).

T cells, with normalization when remission is achieved) (34), implying that the MSN mutation-associated immune abnormalities could have had a pathogenetic role in the development of the kidney symptoms in P1. Despite normal levels of immunoglobulins, a potential defect in specific antibody formation could not be ruled out. On the other hand, MSN deficiency-associated direct cytoskeletal function impairments could also have resulted in the aberrant podocyte morphology seen on electron microscopy.

Of note, MSN is also expressed at elevated levels in the lung (Supplementary Figures S1A, B), particularly in the distal epithelium (35). This is also suggestive of the role of MSN mutations in recurrent childhood pulmonary diseases and resulting emphysema, potentially through the modulation of alveolar structure and its effect on pulmonary inflammation.

In conclusion, here, we describe two siblings with a new mutation of the MSN gene, leading to a significantly truncated and likely dysfunctional moesin protein. As the MSN protein plays a wide range of functions in both immune and structural cells, precise identification of its role in symptomatology remains challenging. However, as the dominant factors are immune dysregulation and coupled organ-specific autoimmunity (Figure 8), the identified MSN mutation W217X is proposed as a novel autoimmune phenotype of IMD50. Further effort is needed to identify other patients with IMD50 and organ-based autoimmunity to ensure this clinical presentation is part of the spectrum of the disease. So we plan to launch a study on male patients with the autoimmune phenotype (e.g., antiphospholipid syndrome) to check their genetic background with WES and identify probable MSN pathogenic variants.

Data availability statement

The datasets presented in this study can be found in online repositories. The name of the repository and accession number can be found below: EMBL's European Bioinformatics Institute; PRJEB52893.

Ethics statement

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individuals for the publication of any potentially identifiable images or data included in this article.

Author contributions

AK and RG conceived and designed the research. AK, JK, VN, SH, and RG wrote the first draft of the manuscript. AK, ZP, GK, KK, ZN, JSz, TV, KM, TB, KB, CG, JSe, and ME performed functional experiments and analysis and drafted the clinical sections of the manuscript. AK and JK collected the individual clinical data. ZP, DC, and TK performed the sequencing, data analysis, and interpretation and drafted the exome sequencing method and analysis sections of the manuscript. KB, VN, and SH performed experiments and analyzed data. AK, KB, SH, VN, and PO performed the visualization. TK, TB, and RG edited and revised the manuscript. All the authors have read and agreed to the published version of the manuscript.

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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/fimmu.2022.919411/full#supplementary-material

References

1. Amaya-Uribe L, Rojas M, Azizi G, Anaya JM, Gershwin ME. Primary immunodeficiency and autoimmunity: A comprehensive review. *J Autoimmun* (2019) 99:52–72. doi: 10.1016/j.jaut.2019.01.011

2. McClatchey AI. ERM Proteins. *Curr Biol* (2012) 22:784–5. doi: 10.1016/j.cub.2012.07.057

3. Neisch AL, Fehon RG. Ezrin, radixin and moesin: Key regulators of membrane-cortex interactions and signaling. *Curr Opin Cell Biol* (2011) 23:377–82. doi: 10.1016/j.ceb.2011.04.011

4. Ilani T, Khanna C, Zhou M, Veenstra TD, Bretscher A. Immune synapse formation requires ZAP-70 recruitment by ezrin and CD43 removal by moesin. *J Cell Biol* (2007) 179:733–46. doi: 10.1083/jcb.200707199

5. García-Ortiz A, Serrador JM. Erm proteins at the crossroad of leukocyte polarization, migration and intercellular adhesion. *Int J Mol Sci* (2020) 21:1502–22. doi: 10.3390/ijms21041502

6. Parameswaran N, Gupta N. Re-defining ERM function in lymphocyte activation and migration. *Immunol Rev* (2013) 256:63–79. doi: 10.1111/imr.12104

7. Hirata T, Nomachi A, Tohya K, Miyasaka M, Tsukita S, Watanabe T, et al. Moesin-deficient mice reveal a non-redundant role for moesin in lymphocyte homeostasis. *Int Immunol* (2012) 24:705–17. doi: 10.1093/intimm/dxs077

8. Pore D, Gupta N. The ezrin-radixin-moesin family of proteins in the regulation of b-cell immune response. *Crit Rev Immunol* (2015) 35:15–31. doi: 10.1615/CritRevImmunol.2015012327

9. Barrero-Villar M, Cabrero JR, Gordón-Alonso M, Barroso-González J, Álvarez-Losada S, Muñoz-Fernández MÁ, et al. Moesin is required for HIV-1-induced CD4-CXCR4 interaction, f-actin redistribution, membrane fusion and viral infection in lymphocytes. *J Cell Sci* (2009) 122:103–13. doi: 10.1242/jcs.035873

10. Lagresle-Peyrou C, Luce S, Ouchani F, Soheili TS, Sadek H, Chouteau M, et al. X-Linked primary immunodeficiency associated with hemizygous mutations in the moesin (MSN) gene. *J Allergy Clin Immunol* (2016) 138:1681–1689.e8. doi: 10.1016/j.jaci.2016.04.032

11. Bradshaw G, Lualhati RR, Albury CL, Maksemous N, Roos-Araujo D, Smith RA, et al. Exome sequencing diagnoses X-linked moesin-associated immunodeficiency in a primary immunodeficiency case. *Front Immunol* (2018) 9:420. doi: 10.3389/fimmu.2018.00420

12. Delmonte OM, Biggs CM, Hayward A, Comeau AM, Kuehn HS, Rosenzweig SD, et al. First case of X-linked moesin deficiency identified after newborn screening for SCID. *J Clin Immunol* (2017) 37:336–8. doi: 10.1007/s10875-017-0391-9

13. Li H, Durbin R. Fast and accurate long-read alignment with burrows-wheeler transform. *Bioinformatics* (2010) 26:589–95. doi: 10.1093/bioinformatics/btp698

14. Cingolani P, Platts A, Wang LL, Coon M, Nguyen T, Wang L, et al. A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff. *Fly (Austin)* (2012) 6:80–92. doi: 10.4161/fly.19695

15. Smigielski EM, Sirotkin K, Ward M, Sherry ST. dbSNP: A database of single nucleotide polymorphisms. *Nucleic Acids Res* (2000) 28:352–5. doi: 10.1093/nar/28.1.352

16. Landrum MJ, Lee JM, Riley GR, Jang W, Rubinstein WS, Church DM, et al. ClinVar: Public archive of relationships among sequence variation and human phenotype. *Nucleic Acids Res* (2014) 42:980–5. doi: 10.1093/nar/gkt1113

17. Stenson PD, Ball EV, Mort M, Phillips AD, Shiel JA, Thomas NST, et al. Human gene mutation database (HGMD®): 2003 update. *Hum Mutat* (2003) 21:577–81. doi: 10.1002/humu.10212

18. Böröcz K, Csizmadia Z, Markovics Á, Mészáros V, Farkas K, Telek V, et al. Development of a robust and standardized immunoserological assay for detection of anti-measles IgG antibodies in human sera. *J Immunol Methods* (2019) 464:1–8. doi: 10.1016/j.jim.2018.07.009

19. Böröcz K, Csizmadia Z, Markovics A, Farkas N, Najbauer J, Berki T, et al. Application of a fast and cost-effective "three-in-one" MMR ELISA as a tool for surveying anti-MMR humoral immunity: The Hungarian experience. *Epidemiol Infect* (2020) 148:1–12. doi: 10.1017/S0950268819002280

20. Kapferer-Seebacher I, Heiss-Kisielewsky I, Pepin M, Dorschner M, Hale CJ, Hanna D, et al. Periodontal ehlers-danlos syndrome is caused by mutations in C1R and C1S, which encode subcomponents C1r and C1s of complement. *Am J Hum Genet* (2016) 99:1005–14. doi: 10.1016/j.ajhg.2016.08.019

21. Udy DB, Bradley RK. Nonsense-mediated mRNA decay uses complementary mechanisms to suppress mRNA and protein accumulation. *Life Sci Alliance* (2022) 5:1–13. doi: 10.26508/lsa.202101217

22. Karamyshev AL, Karamysheva ZN. Lost in translation: Ribosome-associated mRNA and protein quality controls. *Front Genet* (2018) 9:431. doi: 10.3389/fgene.2018.00431

23. International notes measles – *Hungary*. Available at: https://www.cdc.gov/mmwr/preview/mmwrhtml/00001472.htm.

24. Raetz CRH. Biochemistry of endotoxins. *Annu Rev Biochem* (1990) 59:129–70. doi: 10.1146/annurev.bi.59.070190.001021

25. Zawawi KH, Kantarci A, Schulze-Späte U, Fujita T, Batista EL, Amar S, et al. Moesin-induced signaling in response to lipopolysaccharide in macrophages. *J Periodontal Res* (2010) 45:589–601. doi: 10.1111/j.1600-0765.2010.01271.x

26. Beutler B, Krochin N, Milsark IW, Luedke C, Cerami A. Control of cachectin (tumor necrosis factor) synthesis: Mechanisms of endotoxin resistance. *Sci (80-)* (1986) 232:977–80. doi: 10.1126/science.3754653

27. Nathan CF. Secretory products of macrophages. *J Clin Invest* (1987) 79:319–26. doi: 10.1172/JCI112815

28. Weinstein SL, Sanghera JS, Lemke K, DeFranco AL, Pelech SL. Bacterial lipopolysaccharide induces tyrosine phosphorylation and activation of mitogen-activated protein kinases in macrophages. *J Biol Chem* (1992) 267:14955–62. doi: 10.1016/s0021-9258(18)42133-3

29. Han J, Lee JD, Bibbs L, Ulevitch RJ. A MAP kinase targeted by endotoxin and hyperosmolarity in mammalian cells. *Science* (1994) 265(5173):808–11. doi: 10.1126/science.7914033

30. Podestà MA, Ponticelli C. Autoimmunity in focal segmental glomerulosclerosis: A long-standing yet elusive association. *Front Med* (2020) 7:604961. doi: 10.3389/fmed.2020.604961

31. Yoo TH, Fornoni A. Nonimmunologic targets of immunosuppressive agents in podocytes. *Kidney Res Clin Pract* (2015) 34:69–75. doi: 10.1016/j.krcp.2015.03.003

32. Blaine J, Dylewski J. Regulation of the actin cytoskeleton in podocytes. *Cells* (2020) 9:1–27. doi: 10.3390/cells9071700

33. Kopp JB, Anders HJ, Susztak K, Podestà MA, Remuzzi G, Hildebrandt F, et al. Podocytopathies. *Nat Rev Dis Prim* (2020) 6:68–103. doi: 10.1038/s41572-020-0196-7

34. Tsuji S, Kimata T, Yamanouchi S, Kitao T, Kino J, Suruda C, et al. Regulatory T cells and CTLA-4 in idiopathic nephrotic syndrome. *Pediatr Int* (2017) 59:643–6. doi: 10.1111/ped.13255

35. Hashimoto S, Amaya F, Matsuyama H, Ueno H, Kikuchi S, Tanaka M, et al. Dysregulation of lung injury and repair in moesin-deficient mice treated with intratracheal bleomycin. *Am J Physiol - Lung Cell Mol Physiol* (2008) 295:566–74. doi: 10.1152/ajplung.90250.2008

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Case Report: Association between cyclic neutropenia and SRP54 deficiency

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Autosomal dominant mutations in the signal recognition particle (SRP) 54 gene were recently described in patients with severe congenital neutropenia (SCN). SRP54 deficiency cause a chronic and profound neutropenia with maturation arrest at the promyelocyte stage, occurring in the first months of life. Nearly all reported patients with SRP54 mutations had neutropenia without a cyclic pattern and showed a poor or no response to granulocyte colony-stimulating factor (G-CSF) therapy. We report here an 11-year-old female patient with cyclic neutropenia and recurrent heterozygous p.T117del (c.349_351del) in-frame deletion mutation in *SRP54*, who showed remarkable therapeutic response to G-CSF treatment. The diagnosis of cyclic pattern of neutropenia was established by acceptable standards. ELANE gene mutation was excluded by using various genetic approaches. The patient described here also had dolichocolon which has not been described before in association with SCN.

KEYWORDS

signal recognition particle, cyclic neutropenia, granulocyte - colony-stimulating factor (G-CSF), autosomal dominant disease, WES - whole-exome sequencing

Introduction

Severe congenital neutropenia (SCN) represents a heterogeneous group of genetic disorders characterized by an absolute neutrophil count (ANC) <500 per μL, recurrent, life-threatening bacterial infections and, in some cases, immunological or extra-hematopoietic abnormalities affecting the pancreas, central nervous system, heart, bone and skin (1–3). Patients with SCN have an extraordinarily high risk for leukemic transformation (4). To date, molecular abnormalities in more than twenty genes have been identified as a cause of SCN (5). The pathways linked to the genetic defects of SCN involve cellular stress mechanisms, like unfold response (*ELANE*) (6, 7), endoplasmic reticulum (ER) stress (*G6PC3*, *JAGN1*) (8, 9), defective endosome trafficking

(*VPS13B*, *VPS45*) (10, 11), impaired intracellular glucose homeostasis (*G6PC3*), and defective ribosome biogenesis (*SBDS*, *DNAJC21*, *EFL1*) (12–14). In about 25% of patients with a clinical history suggestive of SCN, the genetic defect remains unknown.

Cyclic neutropenia (CN) is characterized by periodical oscillation of ANC (15), The oscillation cycle of neutrophils is on average 21 days and can be combined with the oscillations of other blood cells including monocytes, lymphocytes and platelets. In the majority of cases CN is associated with mutation in *ELANE* gene, although the mechanism of ANC oscillating is not completely clear (15). There were reports of cases of cyclic pattern of neutropenia in patients with a *HAX1* mutation and biallelic *G6PC3* mutation (16, 17).

Recently, *de novo* dominantly inherited mutations in the signal recognition particle (SRP) 54 genes were described and found to represent the second most common cause of CN with maturation arrest (18). Only one case of cyclic neutropenia associated with *SRP54* mutation has been described (19). SRP54 is an evolutionarily conserved protein which is a key component of the ribonucleoprotein complex mediating the co-translational targeting of secretory and membrane proteins to the ER (18). Patients with SRP54 deficiency typically have chronic and profound neutropenia with maturation arrest at the promyelocyte stage, occurring early in life (18, 19). Bone marrow examination of patients with *SRP54* mutation revealed a major dysgranulopoiesis and features of cellular ER stress and autophagy. Neutropenia may associate with severe neurodevelopmental delay (autistic behavior) and an exocrine pancreatic insufficiency requiring enzyme supplementation. Patients may present with atypical phenotype with normal peripheral neutrophil counts and intermittent granulocyte maturation arrest. A recently published cohort analysis revealed variable immunological and clinical phenotypes in individuals with the same mutation, even in the same family (18). The influence of genetic modifiers in neutrophils may be a possible explanation.

Herein, we report an 11-year-old female patient with cyclic neutropenia and recurrent heterozygous p.T117del (c.349_351del) in-frame deletion in *SRP54*, who presented with dolichocolon and was successfully treated with G-CSF.

Methods

Clinical evaluation

The patient and her family members were interviewed, examined, treated and monitored at the Ternopil Regional Children's Hospital in Ukraine. Medical records were obtained from the electronic registry of the Ternopil University Clinic. The parents of the patient gave written informed consent to conduct the study and for publication of data. All procedures were performed in accordance with the ethical standards of the Institutional Research Committee.

Blood cells and immunological studies

Blood cell analysis was performed by routine hematological assays. Lymphocyte subsets of peripheral blood mononuclear cells were determined by immunofluorescent staining and flow cytometry. Cell surface markers were detected by using monoclonal antibodies to CD3, CD4, CD8, CD19, CD16, and CD56 cell surface antigens. Serum levels of IgG, IgA, IgM, C3 and C4 were measured by standard immunological assays.

Whole-exome sequencing *(WES)* and panel sequencing

Genomic DNA from the patient and her parents was isolated with the Gen Elute Blood Genomic DNA kit (Sigma-Aldrich, St. Louis, Missouri, USA)WES. WES was performed at the NY laboratory. At the New York Genome Center and the Rockefeller University an Illumina HiSeq 2500 machine and the Agilent 71 Mb SureSelect exome kit were used, in accordance with the manufacturer's instructions (20). *Panel sequencing*. A courtesy genetic analysis supported by the Jeffrey Modell Foundation was also performed at an Invitae Laboratory focusing on 407 primary immunodeficiency genes (21). Genomic DNA was enriched for targeted regions using a hybridization-based protocol, and sequenced using Illumina technology. All targeted regions were sequenced with ≥50x depth. Reads were aligned to a reference sequence. Clinically significant observations were confirmed by orthogonal technologies.

Targeted gene sequencing

Mutational analysis of *ELANE* was performed in the Laboratory of Immunopathology and Genetics at the University of Lodz, Poland. Sequences were analyzed by amplifying exons and flanking intronic regions of *ELANE* by PCR. The PCR primers and sequencing primers are available on request. Amplicons were sequenced with the Big Dye Terminator cycle sequencing kit (Applied Biosystems, Foster City, California, USA) and targeted regions were analyzed by an ABI 3130 capillary sequencer (Applied Biosystems). Sequence variants were determined by using the Sequencer v 5.0 software to identify the position of mutations.

Results

The 11-year-old female patient was the only child of non-consanguineous parents. She was born from a full-term pregnancy complicated with placental dysfunction, polyhydramnion and pyelonephritis. Her birth weight and length were 3,650 g and 55 cm, respectively. After birth, she developed purulent conjunctivitis which was successfully treated with local antibiotics. She was breastfed for up to 1 year of age and received all vaccines of the Ukrainian mandatory vaccination program, except for hepatitis B vaccine for unknown reasons. Her 27-year-old mother and the 38-year-old father had recurrent herpes labialis and herpetic keratitis, respectively. The family history was negative for dysmorphic features, hematological disease, immune deficiency, or neonatal deaths.

The first disease manifestations at 10 months of age include stomatitis, gingivitis and cervical lymphadenomegaly. During the second year of life she had recurrent stomatitis, impetigo and skin abscesses. At 2 years of age, she was evaluated for anemia, recurrent episodes of fever, mucositis and urinary tract infections. Ulcerative stomatitis recurred in every 2 to 4 weeks with fever, gingivitis, painful cervical lymphadenomegaly and angular cheilitis (Figure 1). Later on, exacerbations have been observed in every 3 weeks and lasted for 4-6 days. From the age of 3 years, the patient was treated with chronic constipation and radiology examination revealed dolichocolon (Figure 2). Later she developed steatorrhea and constipation with intermittent diarrhea. Abdominal ultrasound revealed an enlarged liver and enlarged, hyperechoic pancreas with homogeneous pancreatic parenchyma. She received pancreatic enzyme preparation (Creon, 30.000 IU/day) with moderate effectiveness. Pancreatic enzyme preparation was given to the patient because the ultrasound examination showed persistent changes in the pancreas, and the long-term constipation alternated periodically with episodes of diarrhea. She did not present with psychomotor developmental delay or autistic behavior, but the parents noted that she was irritable, often nervous and emotionally unstable. Neurological examination did not show any organic abnormalities.

Laboratory tests revealed neutropenia with compensatory monocytosis, anemia (lowest RBC: 3.16 T/L, lowest Hgb: 9.4 g/dL) with normal serum iron concentration and normal platelet count. Serial blood counts showed a cyclical pattern of neutropenia occurring in a 20 days period (Figure 3). Because of adherence issues we could perform counting of blood cells only in every 3 or four days (Figure 3). Serum amylase level was normal and stool analysis revealed normal elastase activity. Bone marrow cytology at 7 years of age revealed normocellularity, with polymorphic composition and no blast infiltration. Bone marrow aspirations also showed signs of a slight dysgranulopoiesis with eosinophia (11.6%) and myeloid maturation arrest at the promyelocyte stage. It was performed in the nadir phase (370 neutrophils/μL). Serum IgG, IgA, IgM,

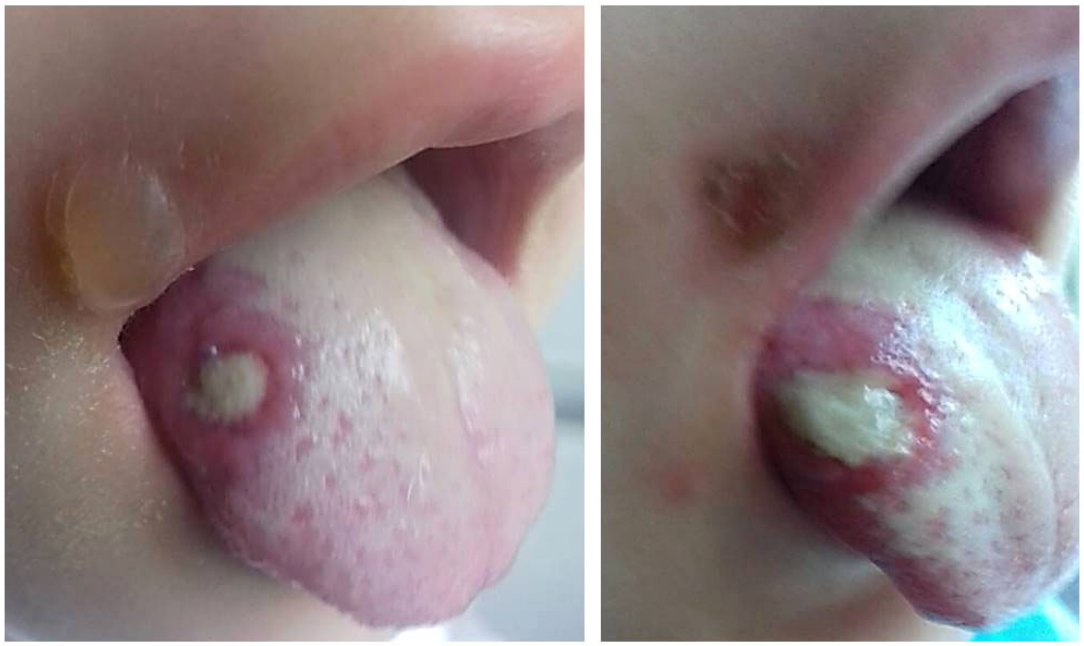

FIGURE 1
Deep ulcer on the right side of the tongue. Bullous lesion near the edge of the mouth is also visible. The pictures were taken three days apart.

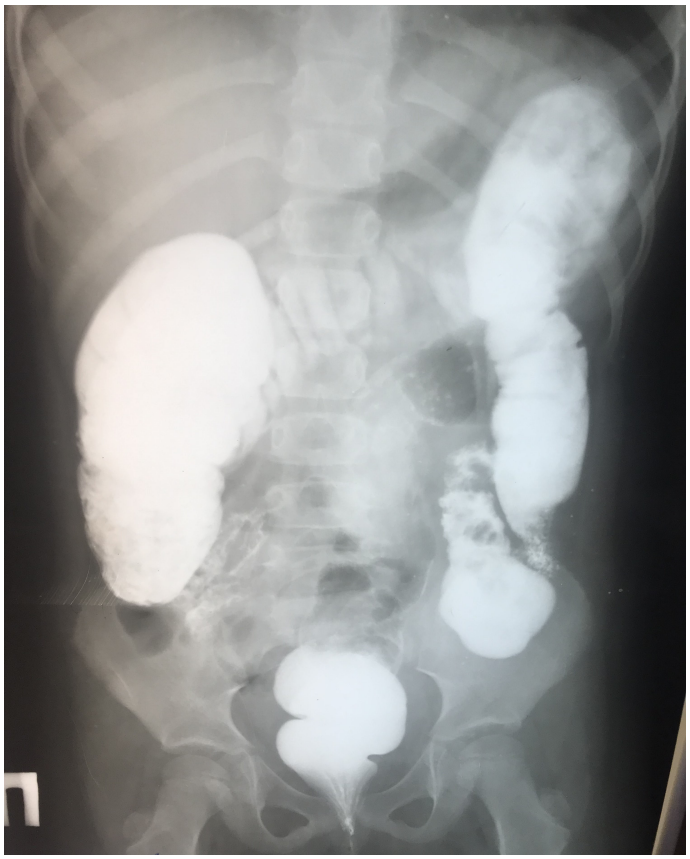

FIGURE 2
Irrigography by using contrast barium enema revealed dolichocolon at the age of 5 years.

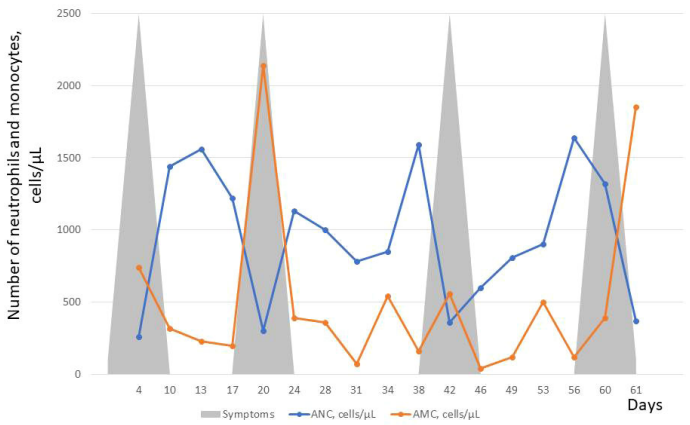

FIGURE 3
Absolute neutrophil count (ANC) and absolute monocyte count (AMC) over a period of 61 days before starting G-CSF therapy. About 20 day ANC cycles are presented by counting cell number at every 3 or 4 days. In contrast, AMC cycling was not observed over time. Rather, compensatory monocytosis were observed during the 2nd and 4th neutropenia cycles. Upper and lower respiratory tract infections were observed during each neutropenia cycles shown schematically by grey triangles.

and IgE levels and lymphocyte immunophenotypes were in the normal ranges.

Mutational analysis was first performed to search for possible *ELANE* sequence variant. Direct sequencing of all exons and exon-intron boundaries of *ELANE* NM_001972.3 did not reveal pathogenic variants that could predispose to cyclic neutropenia. Next, WES analysis was performed at the New York Genome Center and the Rockefeller University and revealed a heterozygous p.T117del (c.349_351del) in-frame deletion mutation in the *SRP54* gene, which was previously reported to be causal (18, 19, 22–24). The same sequence variant was found in an Invitae Laboratory by using different sample. None of these studies indicated *ELANE* mutation. Importantly, screening for genetic causes of neutropenia did not reveal mutations in other congenital neutropenia genes (the list of genes we have tested is available on request).

G-CSF treatment was initiated at the age of 10 years at the initial dose of 5 mcg/kg for at least 4 days in every 3 weeks. This treatment regimen resulted in ANC counts above 1.000/μL and reduced the frequency and severity of infections. The patient has remained on G-CSF treatment for the past year without any adverse events. This regimen was sufficient to maintain the patient's ANC above 1.000/μL. In every two months, mild aphthous ulcers appeared which healed without additional treatment in 1-2 days. Her growth parameters remained in the normal range for ages. The parents also noted that the girl became much calmer, and she had no episodes of behavior change and irritation. Due to concerns about side effects, the parents refused increase of the dose of G-CSF.

Discussion

SRP54 deficiency is a recently described cause of SCN. Mutations in *SRP54* cause syndromic neutropenia with Shwachman-Diamond syndrome-like features. Patients with SRP54 deficiency show a wide spectrum of immunological and clinical manifestations, ranging from mild asymptomatic neutropenia and febrile illnesses to severe neutropenia and life-threatening infection. Most patients with SCN receive long-term treatment with G-CSF and respond to this treatment. Lifetime treatment with G-CSF is indicated in patients responding to standard doses (5 mcg/kg per day). In those requiring higher doses of G-CSF or those who have transformed into myelodysplasia or acute myeloid leukemia (MDS/AML), hematopoietic stem cell transplantation should be considered, especially if an appropriate HLA-matched donor is available. In a large cohort of 23 patients with SRP54 deficiency, nearly all showed a poor or no response to G-CSF therapy (18). In contrast to ELANE deficiency, no development of AML was observed after a median follow-up for 15 years in this large cohort.

Up to date, 30 cases of patients with SRP54 deficiency were reported in the medical literature and all but one patient (19) had isolated neutropenia without a cyclic pattern (18, 19, 22–24). We report here the second patient with the p.T117del *SRP54* mutation who developed cyclic neutropenia showing cycles of approximately 20-days interval (Figure 3). The patient described by Carapito et al. was 8 years old, when he was started on G-CSF at 5 mcg/kg every other day, with improvement of neutrophil counts, mucositis, and infections (18). He continued to do well on G-CSF therapy and was 18 years of age, when his case was published. Like this patient, our patient showed good therapeutic response to G-CSF. We are not aware of more published data on remarkable therapeutic efficacy of G-CSF in patients with SRP54 deficiency but unpublished observation may exist. Currently, there is not convincing evidence for relationship between phenotype (cyclic pattern) of SRP54 deficiency and good response to G-CSF. Further studies and observations of more cases are needed for confirmation of such relationship.

Previous studies also suggest genotype-phenotype relationships (18, 19). SRP54 has three functional domains: N-terminal domain (N-domain), central GTPase domain (G domain), and C-terminal domain (M domain) (Figure 4). All the mutated residues in *SRP54* are located around the G domain which contains five specific G elements (G1-G5). G1 variants, like the p.T117del mutation, have been associated with a predominant hematological phenotype (Figure 4). However, subclinical pancreatic insufficiency appears widespread throughout the different variants. Further, patients with G variants residing outside the G1 element present with a severe neurodevelopmental disorder (extreme delayed speech, intellectual disability) and in some cases with exocrine pancreatic deficiency (18, 19). Published patients with p.T117del mutation were also observed as having a milder clinical phenotype with milder neutropenia both in quantitative terms and with respect to the age of first clinical manifestations with no apparent exocrine pancreatic deficiency or neurodevelopmental disorder. In the study of Bellanné-Chantelot et al, only 2 from 18 patients with *SRP54* mutations interacting directly with the G1 element had an extra-hematological phenotype including moderate exocrine pancreatic insufficiency in one case and severe intellectual disability with autistic traits in another (18). In the latter case the patient had a familial history of severe neurological disorders without known neutropenia, so it could not be excluded that his neurodevelopmental delay is a result of another cause. In contrast, our patient with the p.T117del mutation presented with gastrointestinal manifestations including steatorrhea, constipation and intermittent diarrhea. The association of dolichocolon with a SRP54 mutation is intriguing and the possible causal relationship remains to be elucidated. The patient presented here had hyperirritability and emotional instability but no neurological abnormalities were found. Patients with SCN, especially those with the ELANE mutation may often develop periodontitis (25). This dental anomaly was not observed in our patient and PubMed search did not reveal an

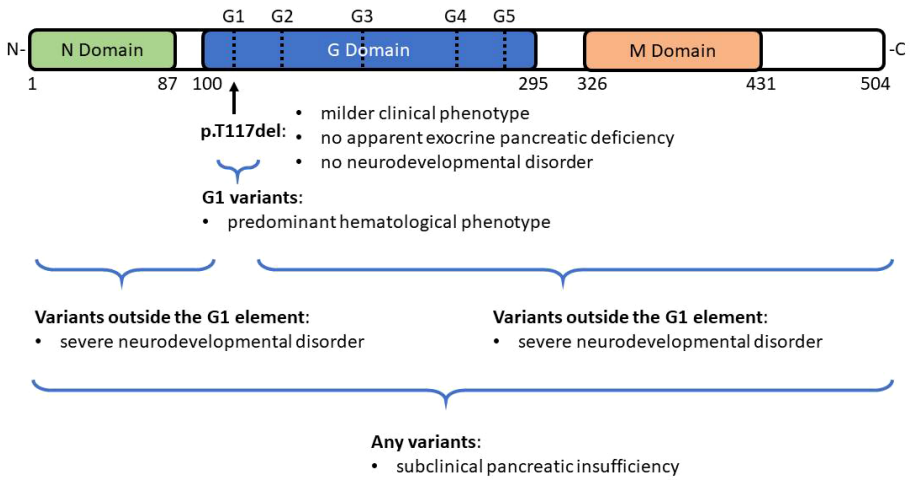

FIGURE 4
Domain structure of SRP54. SRP54 has three functional domains: N-terminal domain (N-domain), central GTPase domain (G domain), and C-terminal domain (M domain). All the mutated residues in *SRP54* are located around the G domain which contains five specific G elements (G1-G5). G1 variants, like the recurrent p.T117del mutation have been associated with a predominant hematological phenotype. In contrast, patients with G variants that reside outside the G1 element present with a severe neurodevelopmental disorder and in some cases with exocrine pancreatic deficiency. Subclinical pancreatic insufficiency appears widespread throughout the different variants.

association of SRP54 mutation with the development of periodontitis suggesting that this genetic form of SCN may be clinically milder than neutrophil elastase gene defect.

In summary, we present here a patient with cyclic neutropenia associated with heterozygous p.T117del (c.349_351del) in-frame deletion mutation in *SRP54*. Cyclic neutropenia is a rare hematological condition considered as an autosomal dominant disease caused primarily by *ELANE* gene mutations and characterized by regular fluctuations in blood neutrophil counts, leading to periodic neutropenia. Although in nearly all patients with SRP54 deficiency neutropenia present without a cyclic pattern, our case and the previously reported patient with p.T117del mutation suggest that SRP54 deficiency should also be considered as a possible genetic cause of cyclic neutropenia. We provided here data on successful treatment of an SRP54 deficient patient by administration of G-CSF, in contrast to nearly all previously reported patients with *SRP54* mutations who presented with a poor or no response to G-CSF therapy. Finally, for the general readers, we provide our proposed algorithm of to help the diagnosis of cyclic neutropenia (Figure 5).

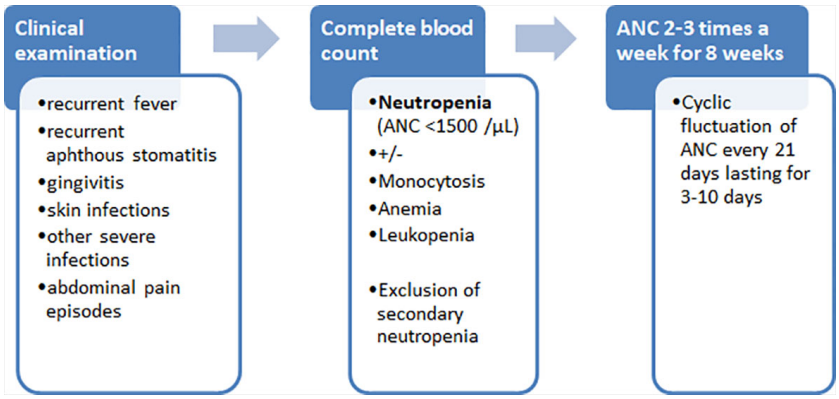

FIGURE 5
Diagnostic algorithm of cyclic neutropenia.

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 human participants were reviewed and approved by Ternopil University. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

ME: performing bioinformatics analysis and writing the initial draft. OB: conducting clinical research and patient care, editing the initial draft. LM: formulation of research goals, writing the final draft. 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.

Publisher's note

References

1. Tangye SG, Al-Herz W, Bousfiha A, et al. Human inborn errors of immunity: 2019 update on the classification from the international union of immunological societies expert committee. *J Clin Immunol* (2020) 40:24–64. doi: 10.1007/s10875-019-00737-x

2. Skokowa J, Dale DC, Touw IP, Zeidler C, Welte K. Severe congenital neutropenias. *Nat Rev Dis Primers.* (2017) 3:17032. doi: 10.1038/nrdp.2017.32

3. Cowland JB, Borregaard N. Granulopoiesis and granules of human neutrophils. *Immunol Rev* (2016) 273:11–28. doi: 10.1111/imr.12440

4. Olofsen PA, Fatrai Sz, van Strien PMH, et al. Malignant transformation involving CXXC4 mutations identified in a leukemic progression model of severe congenital neutropenia. *Cell Rep Med* (2020). doi: 10.1016/j.xcrm.2020.10007

5. Donadieu J, Beaupain B, Fenneteau O, Bellanné-Chantelot C. Congenital neutropenia in the era of genomics: Classification, diagnosis, and natural history. *Br J Haematol* (2017) 179:557–74. doi: 10.1111/bjh.14887

6. Köllner I, Sodeik B, Schreek S, et al. Mutations in neutrophil elastase causing congenital neutropenia lead to cytoplasmic protein accumulation and induction of the unfolded protein response. *Blood* (2006) 108:493–500. doi: 10.1182/blood-2005-11-4689

7. Grenda DS, Murakami M, Ghatak J, et al. Mutations of the ELA2 gene found in patients with severe congenital neutropenia induce the unfolded protein response and cellular apoptosis. *Blood* (2007) 110:4179–87. doi: 10.1182/blood-2006-11-057299

8. Boztug K, Appaswamy G, Ashikov A, et al. A syndrome with congenital neutropenia and mutations in G6PC3. *N Engl J Med* (2009) 360:32–43. doi: 10.1056/NEJMoa0805051

9. Boztug K, Järvinen PM, Salzer E, et al. JAGN1 deficiency causes aberrant myeloid cell homeostasis and congenital neutropenia. *Nat Genet* (2014) 46:1021–7. doi: 10.1038/ng.3069

10. Vilboux T, Lev A, Malicdan MC, et al. A congenital neutrophil defect syndrome associated with mutations in VPS45. *N Engl J Med* (2013) 369:54–65. doi: 10.1056/NEJMoa1301296

11. Kolehmainen J, Black GC, Saarinen A, et al. Cohen Syndrome is caused by mutations in a novel gene, COH1, encoding a transmembrane protein with a presumed role in vesicle-mediated sorting and intracellular protein transport. *Am J Hum Genet* (2003) 72:1359–69. doi: 10.1086/375454

12. Finch AJ, Hilcenko C, Basse N, et al. Uncoupling of GTP hydrolysis from eIF6 release on the ribosome causes shwachman-diamond syndrome. *Genes Dev* (2011) 25:917–29. doi: 10.1101/gad.623011

13. Tummala H, Walne AJ, Williams M, et al. DNAJC21 mutations link a cancer-prone bone marrow failure syndrome to corruption in 60S ribosome subunit maturation. *Am J Hum Genet* (2016) 99:115–24. doi: 10.1016/j.ajhg.2016.05.002

14. Stepensky P, Chacón-Flores M, Kim KH, et al. Mutations in EFL1, an SBDS partner, are associated with infantile pancytopenia, exocrine pancreatic insufficiency and skeletal anomalies in a Shwachman-Diamond like syndrome. *J Med Genet* (2017) 54:558–66. doi: 10.1136/jmedgenet-2016-104366

15. Mir P, Klimiankou M, Findik B, Hähnel K, Mellor-Heineke S, Zeidler C, et al. New insights into the pathomechanism of cyclic neutropenia. *Ann N Y Acad Sci* (2020) 1466:83–92. doi: 10.1111/nyas.14309

16. Cipe FE, Celiksoy MH, Erturk B, Aydogmus Ç. Cyclic manner of neutropenia in a patient with *HAX-1* mutation. *Pediatr Hematol Oncol* (2018) 35:181–5. doi: 10.1080/08880018.2018.1486489

17. Alangari AA, Alsultan A, Osman ME, Anazi S, Alkuraya FS. A novel homozygous mutation in G6PC3 presenting as cyclic neutropenia and severe congenital neutropenia in the same family. *J Clin Immunol* (2013) 33:1403–6. doi: 10.1007/s10875-013-9945-7

18. Bellanné-Chantelot C, Schmaltz-Panneau B, Marty C, Fenneteau O, Callebaut I, Clauin S, et al. Mutations in the SRP54 gene cause severe congenital neutropenia as well as shwachman-diamond-like syndrome. *Blood* (2018) 132:1318–31. doi: 10.1182/blood-2017-12-820308

19. Carapito R, Konantz M, Paillard C, et al. Mutations in signal recognition particle SRP54 cause syndromic neutropenia with shwachman-diamond-like features. *J Clin Invest* (2017) 127:4090–103. doi: 10.1172/JCI92876

20. Erdős M, Lányi Á, Balázs G, et al. Inherited TOP2B mutation: Possible confirmation of mutational hotspots in the TOPRIM domain. *J Clin Immunol* (2021) 41:817–9. doi: 10.1007/s10875-020-00963-8

21. Smedley D, Smith KR, et al. 100,000 genomes pilot on rare-disease diagnosis in health care - preliminary report.100,000 genomes project pilot investigators. *N Engl J Med* (2021) 385:1868–80. doi: 10.1056/NEJMoa2035790

22. Carden MA, Connelly JA, Weinzierl EP, Kobrynski LJ, Chandrakasan S. Severe congenital neutropenia associated with SRP54 mutation in 22q11.2 deletion syndrome: Hematopoietic stem cell transplantation results in correction of neutropenia with adequate immune reconstitution. *J Clin Immunol* (2018) 38:546–9. doi: 10.1007/s10875-018-0518-7

23. Saettini F, Cattoni A, D'Angio M, et al. Intermittent granulocyte maturation arrest, hypocellular bone marrow, and episodic normal neutrophil count can be associated with SRP54 mutations causing shwachman-diamond-like syndrome. *Br J Haematol* (2020) 189:e171–4. doi: 10.1111/bjh.16585

24. Goldberg L, Simon AJ, Rechavi G, et al. Congenital neutropenia with variable clinical presentation in novel mutation of the SRP54 gene. *Pediatr Blood Cancer* (2020) 67:e28237. doi: 10.1002/pbc.28237

25. Ye Y, Carlsson G, Wondimu B, et al. Mutation in the ELANE gene are associated with development of periodontitis in patients with severe congenital neutropenia. *J Clin Immunol* (2011) 31:936–45. doi: 10.1007/s10875-011-9572-0

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Newborn screening for severe combined immunodeficiency: The results of the first pilot TREC and KREC study in Ukraine with involving of 10,350 neonates

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Severe combined immunodeficiency (SCID) is a group of inborn errors of immunity (IEI) characterized by severe T- and/or B-lymphopenia. At birth, there are usually no clinical signs of the disease, but in the first year of life, often in the first months the disease manifests with severe infections. Timely diagnosis and treatment play a crucial role in patient survival. In Ukraine, the expansion of hemostatic stem cell transplantation and the development of a registry of bone marrow donors in the last few years have created opportunities for early correction of IEI and improving the quality and life expectancy of children with SCID. For the first time in Ukraine, we initiated a pilot study on newborn screening for severe combined immunodeficiency and T-cell lymphopenia by determining T cell receptor excision circles (TRECs) and kappa-deleting recombination excision circles (KRECs). The analysis of TREC and KREC was performed by real-time polymerase chain reaction (RT-PCR) followed by analysis of melting curves in neonatal dry blood spots (DBS). The DBS samples were collected between May 2020 and January 2022. In total, 10,350 newborns were screened. Sixty-five blood DNA samples were used for control: 25 from patients with ataxia-telangiectasia, 37 - from patients with Nijmegen breakage syndrome, 1 – with X-linked agammaglobulinemia, 2 – with SCID (JAK3 deficiency and DCLRE1C deficiency). Retest from the first DBS was provided in 5.8% of patients. New sample test was needed in 73 (0.7%) of newborns. Referral to confirm or rule out the diagnosis was used in 3 cases, including one urgent abnormal value. CID ($T^{low}B+NK+$) was confirmed in a patient with the urgent abnormal value. The results of a pilot study in Ukraine are compared to other studies (the referral rate 1: 3,450). Approbation of the

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method on DNA samples of children with ataxia-telangiectasia and Nijmegen syndrome showed a high sensitivity of TRECs (a total of 95.2% with cut-off 2000 copies per 10^6 cells) for the detection of these diseases. Thus, the tested method has shown its effectiveness for the detection of T- and B-lymphopenia and can be used for implementation of newborn screening for SCID in Ukraine.

KEYWORDS

newborn screening, TREC, KREC, severe combined immunodeficiency, inborn errors of immunity

Introduction

Severe Combined Immunodeficiency (SCID) is a genetically heterogeneous group of diseases that are accompanied by impaired T- and B-cell immune responses, leading to a combined dysregulation of cellular and humoral immunity (1). Patients with SCID are usually born with no clinical signs of the disease, but within the first year, and often within the first months of life in children with T-lymphocyte deficiency, and within the second half of the first year in children with certain defects in antibody production, the disease manifests as severe infections (2). Without proper diagnosis and timely immune-restoring treatments the children die in the first or second year of life. Hematopoietic stem cell transplantation (HSCT), enzyme replacement therapy for adenosine deaminase (ADA) deficiency, and gene therapy in some disease types are the only curative treatments today that allow not only to save life but also to ensure its quality (3). Studies of this disorders for the past 20 years show a considerably high chance of survival in those who underwent HSCT before 3.5 months of age, reaching up to 94% survival rate over 6 years (3, 4).

SCID affects approximately 1: 50,000-1: 100,000 newborns and only up to a third of the newly diagnosed cases have a family history (5, 6). Certain ethnic groups have a higher incidence of SCID because of founder mutations. For example, in Somalia ADA-SCID occurs 1: 5,000, while the DCLRE1C gene mutation (Artemis) in Navajo Americans is even more common, 1: 2,000 (6, 7). The rapid development of genetics contributes to the discovery of new mutations that cause inborn errors of immunity (IEI), including SCID (1).

Since SCID is a rare disease which is accompanied by life-threatening health problems, is not determined by standard clinical examinations, and has cutarive treatments, the effectiveness of which depends on the time of diagnosis, the need and appropriateness of screening has become evident (8, 9).

Neonatal screening, which was first introduced in the 1960s, now covers dozens of diseases and has reached great progress in Europe and worldwide in recent years (10). In 2008, newborn screening for severe combined immunodeficiency (SCID) was first introduced in Wisconsin, USA (11). Since then, newborn screening for SCID has implemented not only in all states of the USA, but also in many countries of Europe, the Middle East and Asia (12–18).

Of methods proposed for screening diagnosis of SCID, a DNA based technique has received prominence (8). It is a quantitative molecular genetic method of detecting T-cell receptor excision circles (TRECs), which are a by-product of T-cell differentiation in the thymus and thus can be used as a marker of T-lymphopenia (8, 11). The TREC analysis was developed by Douek et al., who demonstrated that TRECs are specific for naive T-cells and undergo decline either due to ageor infections such as HIV (19). In 2005, Puck and Chan proposed to use this technique for population-based SCID screening (20).

The TREC assay can only detect T-lymphopenia. However, there is another group of severe IEI associated with B-lymphocyte deficiency, including X-linked agammaglobulinemia (XLA, Bruton's disease) and autosomal recessive hypogammaglobulinemia, which also lead to life-threatening conditions. In 2007, a method was developed based on the detection of kappa-deleting recombination excision circles (KRECs), DNA fragments formed during the maturation of B-cells in the bone marrow, using polimerase chain reaction (PCR) (21). Combining these two techniques allows to detect not only congenital T-cell defects, but also other forms of IEI, which can be missed by analysis of TREC only, in particular, late onset of ADA deficiency, Nijmegen breakage syndrome (NBS) and other conditions (22). The issue of compliance of KREC assay with the general principles of neonatal screening is currently being discussed.

However, as the results of screening programs shown, low levels of TRECs/KRECs are detected not only in SCID, but also in other immunodeficiencies and diseases that are characterized by low levels of T- and/or B-cells (23–25).

Newborn screening for severe T- and/or B-cell deficiencies is an important tool for the timely diagnosis of IEI (26, 27). Diagnosis and treatment of congenital defects of the immune system have made significant strides in many countries, including Ukraine. However, in a significant proportion of children with severe immune deficiency in the first year of life, this diagnosis is made posthumously. These congenital defects

are underdiagnosed primarily because of the lack of opportunities for early diagnosis and low awareness of these pathologies in doctors and the other health care professionals (28, 29). In recent years in Ukraine, the expansion of HSCT and the development of a bone marrow donors registry have created opportunities for early correction of IEI and improving the quality and life expectancy of children with SCID. Therefore, the issue of early SCID detection using newborn screening becomes a pressing one.

Materials and methods

In 2020, we initiated the project "A pilot study on newborn screening for primary immunodeficiencies using TREC/KREC assay to identify T- and B-lymphopenia", which received support from the Ministry of Health of Ukraine. The study was performed according to the 1975 Declaration of Helsinki (as revised in 2000), and approved by the I.Horbachevsky Ternopil National Medical University Ethics Committee (Minutes № 55 from November 4, 2019).

In addition to the 4 diseases (phenylketonuria, cystic fibrosis, congenital adrenal hyperplasia, congenital hypothyroidism) of the newborn screening panel that are already being tested for in Ukraine, we included a screening for primary immunodeficiencies using TREC/KREC assay. The additional dried blood spot (DBS) for the SCID screening was collected between May 2020 and January 2022 (21 months in total). The pilot study covered the Ternopil region in western Ukraine. A total of 15 maternity hospitals in the region were involved in the study, including two maternity hospitals in the region capital, which had the largest numbers of births (5591 DBS). In addition, patients from the neonatal intensive care units of the neighboring Ivano-Frankivsk and Lviv regions were included the study. As of 01.01.2021, the population of Ternopil region was 1,030,600 inhabitants.

The TREC/KREC determination was performed at the Scientific Medical Genetic Center LeoGENE, LTD, Lviv, Ukraine. The other project partners were the Institute of Hereditary Pathology of the Ukrainian National Academy of Medical Sciences, Lviv, Ukraine; Western-Ukrainian Specialized Children's Medical Center, Lviv, Ukraine; and Ivano-Frankivsk Regional Children's Hospital, Ivano-Frankivsk, Ukraine.

We organized several information meetings for pediatricians, neonatologists, nurses, during which we provided information about the screening, its purpose and features. Neonatologists and nurses informed parents about the project and parental consent was obtained for each newborn.

Heel prick blood spot tests were performed mainly on the third day post-partum as part of the national NBS program. Blood samples were blotted on a blank disc of filter paper, which carries a unique number and information about the newborn. Blood spots were dried at a room temperature for at least 3 hours, protected from direct sunlight and stored at a temperature of +2 to +8°C.

Data on all DBS were entered into a computer database shared with the center where the analysis was performed. The database included key characteristics of the newborns, such as birth date, date of sample collection, sex, birth weight (BW), and gestational age (GA). According to the WHO definition, newborns with GA ≥38 weeks were defined as born at term; GA ≥32–37 weeks – as moderate preterm, GA ≥28–32 weeks - very preterm; <28 weeks - extremely preterm (30).

The analysis of TREC and KREC was performed by real-time polymerase chain reaction (RT-PCR) followed by analysis of melting curves in neonatal DBS. Due to the disruptions caused by the COVID-19 pandemic, it was not feasible to obtain commercial kits for screening. This compelled the Scientific Medical Genetic Center LeoGENE to adapt their methodology and develop a proprietary testing approach.

At the first stage of the pilot study, when logistical issues were agreed upon and the methodology was worked out, the time between the blood sampling and obtaining the newborn screening results was 3-5 weeks. At the second stage, we managed to reduce this time to 10 days.

Sample processing and real –time PCR

Isolation and purification of DNA from DBS on filter paper was carried out using a kit for the isolation of nucleic acids DNA-SorbB (AmplySens, RF). The DNA sample was dissolved in 75 µl of TE buffer and the concentration and optical characteristics were determined using the DENOVIX instrument.

The quantity of TREC and KREC molecules was analyzed by RT-PCR. Primer sequences used to amplify the sequences of TREC were (F: 5'-CCATGCTGACACCTCTGGT-3', R: 5'-TCGTGAGAACGGTGAATGAAG-3'), KREC (F: 5'-TCAGCGCCCATTACGTTTCT-3', R: 5` - GTGA GGGACAC GCAGCC-3') and the albumin gene as an internal control (F: 5'-TGAACAGGCGACCATGCTT-3', R: 5'-CTCTCCTTCTCAGAAAGTGTGCATAT-3') according to the protocol (31). RT-PCR reactions were prepared for each of the three primer pairs and contained 6 µl of dd water, 1.5 µl of 5xHOT FIREPol EvaGreen Mix Plus (Solis, BioDyne, Estonia), 0.3 µl of primers and 2 µl of DNA samples (10-25 ng/µl).

Plasmids with a known number of TREC and KREC copies were used as standards in RT-PCR. Deionized water was used as a no template control. DNA sample of patient with NBS and low number of TRECs and KRECs was used as a positive control. Real-time PCR was performed with the following parameters: polymerase activation (50°C, 2 min), initial denaturation (95°C, 10 min) and 50 cycles of following: denaturation (at 95°C, 15 sec), annealing and extension (at 60°C, 60 sec). The RT-PCR was

carried out on CFX96 Touch Real-Time PCR Detection System, Bio-Rad, USA. The Ct of positive control is 3-5 cycles above the majority of DNA samples we analyzed (Ct = 30 - 33). The RT-PCR amplification curves of DNA samples with low TRECs and/or KRECs were similar to NTC curves.

Due to the low copy number of TRECs and KRECs, compared to nuclear DNA genes, an additional analysis of PCR products by the melting method was performed to increase the specificity of the method. Analysis of melting curves was performed at a temperature from 50°C to 90°C with a step of 0.5°C (melting analysis). This technique is based on the dependence between the melting temperature of DNA fragments to their primary structure. This additional step was implemented to differentiate negative results from unspecific PCR product.

We used as a comparative method RT-PCR with fluorescent probes for three targets: albumin gene, TREC and KREC. RT-PCR reaction was prepared for three different primer pairs and contained 2.5 µl of dd water, 5µl of 2xMaxima Probe qPCR Master Mix (no ROX) (Thermo Scientific, USA), 0.5 µl of primer-probe mix (6 µl of 100nM forv. primer, 6 µl of 100nM rev. primer, 6 µl of probe and 100 µl of dd water), 1 µl of DNA samples. This method was used as an additional check of the tested patients with low levels of TRECs and/or KRECs. DNA sample with NBS was used as a positive control, DNA samples with normal level of TRECs/KRECs were used as a negative control, deionized water was used as a no template control. The results of 2 methods were quite similar.

The number of TREC and KREC copies per 10^6 cells was calculated using the following formula (31):

$$\frac{1,000,000\ x\ \text{mean SQ (TRECs or KRECs)}}{\text{Mean SQ (Albumin)}/2}$$

As a positive control, we used blood DNA samples of 65 patients with confirmed genetic and immunological diagnosis of IEI: 25 samples from the patients with ataxia-telangiectasia, 37 samples from patients with NBS (homozygous for the c.657del5 mutation of the NBN gene), one sample from a patient with XLA and 2 samples from the patients with SCID (JAK3 deficiency and DCLRE1C (Artemis) deficiency).

In cases where new sample test was needed, we collected additional information about the mother's history and treatment during pregnancy, the newborn's clinical condition, medicines taken (antibiotics, steroids), and laboratoty tests results.

Statistical analysis

Statistical analysis were performed using STATISTICA 10. To compare continuous variables between the groups we used the Mann–Whitney test and the Kruskal–Wallis test. Qualitative variables are shown as absolute frequencies and percentages. Quantitative variables were tested using Kolmogorov–Smirnov test or Shapiro–Wilk test for normal distribution and are expressed as median and interquartile range (IQR), when appropriate. For quantitative variables the Mann–Whitney test were performed. P-values of <0.05 were considered as statistically significant.

Definition and interpretation of the results

To avoid misunderstanding in terminology, we used published recommendations for uniform definitions in newborn screening for SCID (32). TREC copies above cut-off were determined as a *normal value*. Concordantly, TRECs below cut-off and without DNA ampflification failure were designated as an *abnormal value*. In the latter category, we distinguish an *urgent abnormal value* when TRECs were absent or very low (<100 copies per 10^6 cells). In the case of DNA amplification failure the test result was considered as *incomplete*. DNA amplification failure was defined when albumin gene amplification curve was the same as NTC or in the case of PCR inhibition (number of amplification cycles for albumin was over 29). Usually, number of amplification cycles for albumin was in range of 24-28 in all samples. Repeated RT-PCR-assay from the same newborn screening card was defined as a *retest*. If a new sample was requested for TREC analysis, it was defined as a *new sample test*. In the case of abnormal TRECs value after new sample testing, the newborn was recall for exmination to confirm or to rule out the diagnosis. We define this event as a *referral*. The same was applied to the evaluation of thr results of the KRECs analysis.

SCID newborn screening diagnostic decision algorithm is demonstrated in Figure 1. To decrease false positive results and reduce parental stress and anxiety (33), we did a retest in the case of an abnormal value or incomplete results. If the retest result was also abnormal, the mother with a child were invited by a phone call to come for a new sample test (second sample) within 10 days. When a retest result was urgent abnormal in two tests from the first sample, the child was immediately referred for diagnosis confirmation to the Regional Children's Hospital. The same algorithm was used with the new sample test. In the case of an abmormal value of the new sample test, the child was reffered to confirm the diagnosis. The patient underwent full clinical examination with an emphasis on detecting signs of immune deficiency, dysmorfic features and family history. Laboratory examination included complete blood count (CBC) with differential, lymphocyte subpopulation detected by cytometry assay, and immunoglobulins levels. Cytogenetic tests (karyotyping) and genetic testing (Next Generation Sequencing) were planned after clinical and immunological evaluation.

The main goal of this screening program was to identify newborns with SCID (defined as CD3 below 300 cells/µL). The

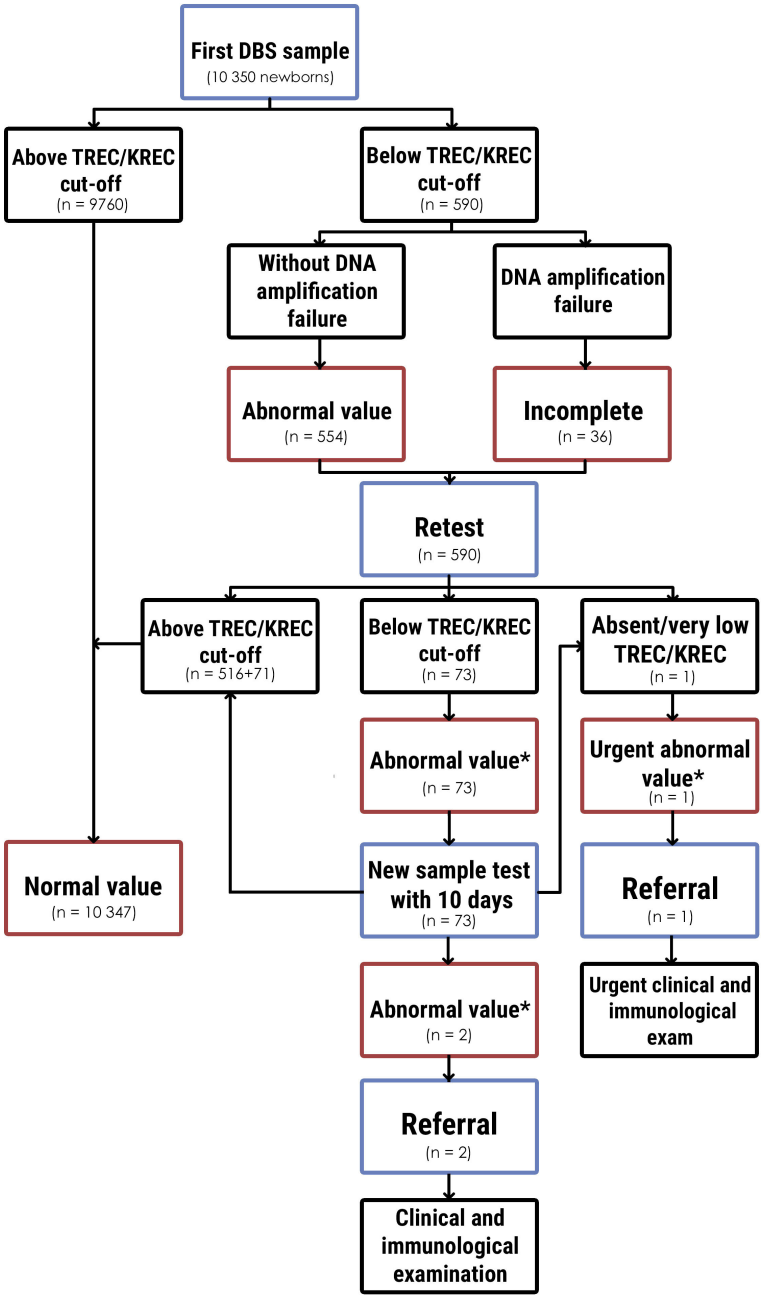

FIGURE 1
Newborn screening diagnostic algorithm for determination SCID and T- and B-lymphopenia and results. * - below TREC/KREC cut-off without DNA amplification failure.

second target was to identify newborns with non-SCID T-lympopenia and B-lympopenia, including combined immunodeficiency (CID), XLA, secondary immunodeficiencies, and other syndroms with T-cell impairment (15, 34, 35).

False-positive results were defined as abnormal TRECs and/or KRECs value in absence of SCID, XLA or other explanation of T- and/or B-lymphopenia.

Results

We analyzed 10,350 blood samples. The baseline characteristics of screened newborns are presented in Table 1. The gestational age of newborns ranged from 25 to 41 weeks, median – 38 weeks. There were 608 (5.87%) premature babies. The BW of the subjects ranged from 540

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TABLE 1 Baseline characteristics of the screened newborns.

Characteristic	n	%
Male/Female	5,279/5,071	51/49
Gestational age (GA)		
Extremely preterm (less than 28 weeks)	12	0.11
Very preterm (28 – 32 weeks)	30	0.29
Moderate preterm (32 – 37 weeks)	566	5.47
At term (GA ≥ 38 weeks)	9,742	94.13
Birth weight		
Less than 1,000 grams	8	0.08
1,000 – 1,499 grams	23	0.22
1,500 – 2,499 grams	340	3.29
≥ 2,500 grams	9,979	96.4

to 5,350 grams, median – 3,350 grams. The proportion of children with a BW of less than 2,500 grams was minor and amounted to 3.58% (371 newborns). TREC and KREC levels of screened newborns depending on GA and BW are presented in Figures 2, 3.

The Mann–Whitney test did not reveal significant differences between the median values of TREC levels in groups of children with different GA ($p>0.05$), whereas the median value of KRECs in extremely preterm newborn was significantly lower than in other groups with different GA ($p=0.02$, $p=0.01$, $p=0.01$, respectively). Meanwhile, the levels of both TRECs and KRECs was significantly lower in newborns weighing less than 1,000 grams compared to those weighing 1,500-2,499 grams ($p=0.04$). The level of KRECs was also significantly lower in the neonates weighing less than 1,000 grams compared to neonates weighing 1,000-1,499 grams and more than 2,500 grams ($p=0.009$, $p=0.07$, respectively).

Determination of cut-off values

One of the challenges of a pilot screening program is determination of cut-off values (34). Since we used TREC/KREC assay to identify SCID and other T- and B-lymphopenia for the first time, it was important for us not to miss any newborns with these conditions.

At the first stage, a cut-off level of below 5,000 copies per 10^6 cells for TREC and 5,000 copies per 10^6 cells for KREC was applied considering referral values proposed in the published protocol (31). In total, 4,833 newborns were screened at this stage. Among them 366 (7.6%) required a retest and 45 (0.9%) required a new sample test (Table 2). One urgent abnormal value after retest was detected (TRECs – 0). Two mothers refused to take a new sample test. One child had an abnormal value (only TRECs) in the new sample test and was referred for clinical and laboratory examination. IEI was not confirmed in this child, however possible other reasons of TRECs/KRECs below the cut-off was suggested (Table 3).

Taking into account a high rate of retests and new sample tests in addition to the results of our study of TREC/KREC levels in patient with ataxia-teleangiectasia (AT) (36), as well as the potential for high parental stress and anxiety, we reduced the cut-off threshshold to 2,000 copies per 10^6 cells for TRECs and KRECs. Another 5,517 newborns were screened during this stage. The rate of retest decreased to 4.1%, and proportion of abnormal value results declined to 0.5%. One child was referred to confim or rule out immunodeficiency (Table 2).

Characteristics of the patients with the urgent abnormal value and abnormal value in a new sample test results are presented in Table 3. Of this group, the first patient had an urgent abnormal value after retest (0 TREC twice). Determination of the lymphocyte subpopulations by flow

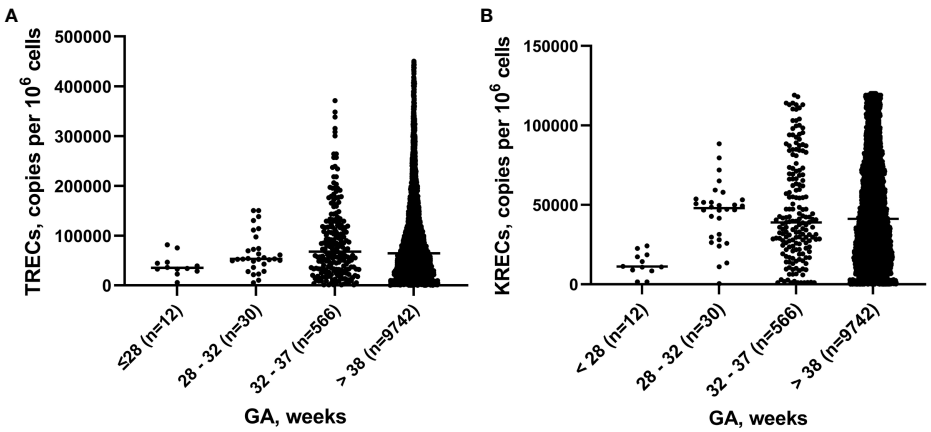

FIGURE 2
TRECs **(A)** and KRECs **(B)** (copies per 10^6 cells) in newborns depending on gestation age (GA).

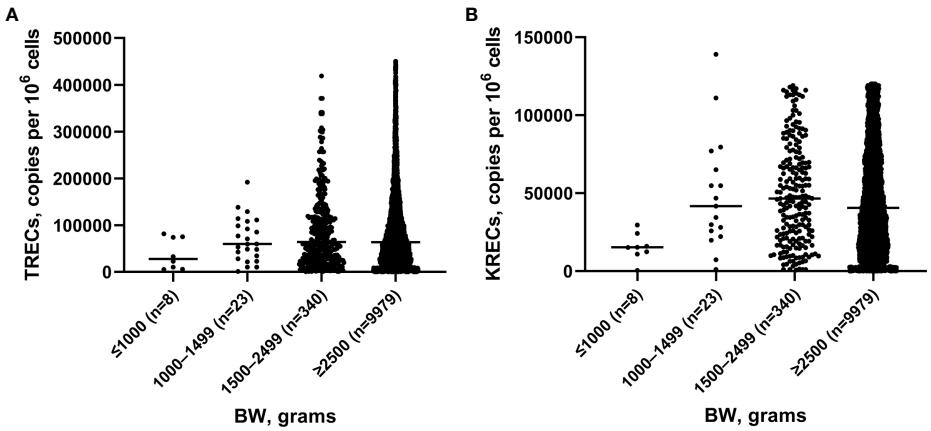

FIGURE 3
TRECs **(A)** and KRECs **(B)** (copies per 10^6 cells) in newborns depending on birth weight (BW).

cytometry confirmed the T-cell deficiency and the diagnosis of CID ($T^{low}B+NK+$). Unfortunately, the patient died at the age of 2 months due to COVID-19, complicated by pneumonia, venous thrombosis and progressive multiple organ failure. The amount of available biological material did not allow a further in-depth genetic research to determine the presence of specific mutations.

Samples from patients with known IEI (controls)

To assess the quality and sensitivity of TREC/KREC assay, we performed it on blood samples of the patients with confirmed immunodeficiencies. The control group consisted of 25 patients with AT, 37 patients with NBS, 1 patient with XLA and 2 patients with SCID, i.e. one with JAK3 deficiency (T-B+) and one with DCLRE1C (Artemis) deficiency ($T\text{-}B^{low}$). The average age of patients with AT at the time of examination was 8.45 ± 2.74 years, ranging from 3 to 14 years. The average age of patients with NBS was 4.5 years, ranging from 1 month to 13 years. TREC levels in all patients with AT and NBS were below 5,000 copies per 10^6 cells, whereas KRECs levels were below 10,000 copies per 10^6 cells in patients with AT (Figure 4). In the patients with NBS, 100% sensitivity of TREC and KREC values were 2,000 copies per 10^6 cells (Table 4). TREC levels in patients with SCID were less than 200 copies per 10^6 cells: 138 in the patient with JAK3 deficiency and 25 in the patient with DCLRE1C deficiency. Accordingly, KRECs were absent in the patient with XLA and were less than 1,000 copies per 10^6 cells in the patient with DCLRE1C (Artemis) deficiency. Albumin level was normal in all controls included to the study.

Discussion

Today, the most promising approach in Europe is to use the TREC/KREC/ACTB triplex assay for newborn screening to detect severe T- and B-cell lymphopenias. This method is included in the national programs of many European countries, and some of them have already launched pilot projects (6, 9). Meanwhile, the USA, Canada, Israel, Taiwan, Saudi Arabia, and a number of other countries, have implemented TREC assay for SCID screening (11–14, 17). Among the countries of Central and Eastern Europe, the first population-based screening for T- and B-lymphopenia began in 2017 in the Polish-German transborder area (15). We report

TABLE 2 Number of newborns, retests from the first DBS, and recalls in the study population.

	I period	II period	Total
	5,000 cut-off	2,000 cut-off	
Newborns	4,833	5,517	10,350
Retest	366 (7.6%)	224 (4.1%)	590 (5.8%)
Referral (urgent abnormal value)	1 (0.02%)	0	1 (0.01%)
New sample test	45 (0.9%)	28 (0.5%)	73 (0.7%)
Referral to confirm/rule out the diagnosis	1 (0.02%)	1 (0.02%)	2 (0.02%)

TABLE 3 Characteristics of patients with abnormal value (urgent abnormal value and abnormal value in new sample test).

N	TRECs/KRECs, copies per 10^6 cells	Diagnosis	Lymphocytes, cells/μL (%)	CD3, cells/μL (%)	CD4/CD8, cells/μL (%)	CD19, cells/μL (%)	IgA/ IgM/ IgG, g/l	Outcome
1.	0/31,200	CID ($T^{low}B+NK+$) Unknown genetic cause	700-1,300 (13-17)	520 (39.8)	260/250 (20/19)	620 (47)	<0.15 /0.62 /3.7	Died 2 mos (COVID-19, pneumonia, venous thrombosis)
2.	4,010/16,300	Prematurity (GA–33 weeks), BW – 2300g. Transient lymphopenia	1,680-3,880 (12-73)	2,285 (59)	1,369/861 (35/22)	1261 (32)	na	Alive
3.	129,000/723	Mother – threatened abortion, polyhydramnios, progesterone –long period, antibiotic.	5,390 (64)	4,086 (76)	2,317/1,488 (43/28)	1,003 (18.6)	0.22 /0.44 /5.17	Alive

CID, combined immunodeficiency; GA, gestational age; BW, birth weight.

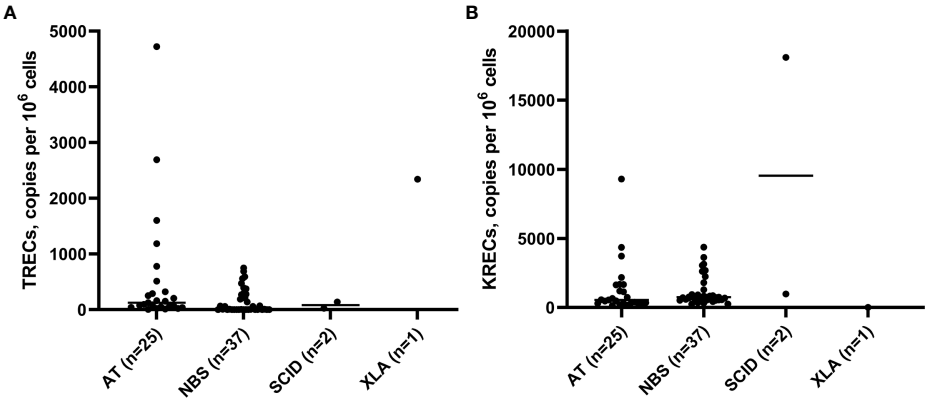

FIGURE 4
TRECs **(A)** and KRECs **(B)** (copies per 10^6 cells) in a control group (patients with AT, NBS, SCID (JAK3 deficiency and DCLRE1C deficiency), and XLA.

TABLE 4 Sensitivity of method depending on TREC/KREC cut-offs levels in patients of controls (AT and NBS patients).

Cut-off, copies per 10^6 cells	Sensitivity					
	TREC			KREC		
	AT (n=25)	NBS (n=37)	AT+NBS (n=62)	AT (n=25)	NBS (n=37)	AT+NBS (n=62)
10000	25 (100%)	37 (100%)	62 (100%)	25 (100%)	37 (100%)	62 (100%)
5000	25 (100%)	37 (100%)	62 (100%)	24 (96%)	37 (100%)	61(98.4%)
4000	24 (96%)	37 (100%)	61(98.4%)	23 (92%)	37 (100%)	60 (96.8%)
3000	24 (96%)	37 (100%)	61 (98.4%)	22 (88%)	37 (100%)	59 (95.2%)
2000	**22 (88%)**	**37 (100%)**	**59 (95.2%)**	**21 (84%)**	**37 (100%)**	**58 (93.5%)**
1000	21 (84%)	37 (100%)	58 (93.5%)	15 (60%)	28 (75.7%)	43 (69.4%)
500	19 (76%)	26 (70.3%)	45 (72.6%)	12 (48%)	5 (13.5%)	17 (27.4%)
100	11 (44%)	16 (43.2%)	27 (43.5%)	0	0	0

AT, ataxia-telangiectasia; NBS, Nijmegen breakage syndrome.
The sensitivity of TRECs and KRECs with cut-off 2000 copies per 106cells is highlighted in bold.

about the first pilot study of newborn screening for SCID in Ukraine using TREC/KREC assay.

It should be noted that currently two approaches are being used: DNA isolation followed by RT-qPCR (TREC/KREC/ACTB-assay) and commercial kits such as EnLite™ TREC kit for newborns from PerkinElmer (USA) and SCREEN newborn screening kit ID ImmunoIVD (Sweden) (Table 5). The EnLite Neonatal TREC test is intended for the semiquantitative multiplex determination of TREC and β-actin. It involves perforating DBS samples with a 1.5 mm punch head. The SCREEN-ID ImmunoIVD allows the quantification of TREC and/or KREC, β-actin as a quality control marker by quantitative real-time multiplex PCR (qPCR) using the ordinary 3.2 mm DBS. The kit includes all the necessary reagents pre-packaged in a set of elution and qPCR plates and requires only two pipetting steps. This technique allows to determine either only TRECs, or TRECs and KRECs simultaneously (18). Cut-off levels for these markers mostly depend on the chosen method (Table 4). The TRECs cut-off for the EnLite Neonatal TREC assay to discriminate screen-positive samples based on the

TABLE 5 Comparison of the SCID newborn screening results in different studies.

Study	Newborns, n	Cutoff TREC/ KREC	Sample processing	Retest	Referral (after 1st retest)	New sample test	Referral	Rate of the referral	SCID detected
Verbsky JW. et al. (Wisconsin, USA, 2012) (11)	207,696	25 TRECs/μl -1st year; 40 TRECs/μl- next year	RT-qPCR of TRECs and β-actin	na	63 (0.03%)	386 (0.19%) including abnormal in pretern and inconclusive)	9 (0.004%)	1: 2,884	5
Giżewska M. et al. (Poland-German, 2020) (15)	44,287	< 6/<4 copies/μL	Commercial kit (ImmunoIVD, Sweden)	321 (0.72%)	7 (0.02%)	68 (0.15%)	1 (0.002%)	1: 5,366	1 + 1(CID)
de Felipe B. et al. (Seville, Spaine, 2016) (16)	5,160	< 6/<4 copies/punch	RT-qPCR (TRECs/KRECs/ACTB-assay)	77 (1.5%)	na	10 (0.19%)	5 (0.1%)	1: 1,032	0
Argudo-Ramírez A. et al. (Catalonia, Spain, 2019) (24)	130,903	≤ 34 copies/μL	Commercial kit (PerkinElmer, Finland)	3108 (2.4%)	12 (0.01%)	304 (0.2%)	18 (0.01%)	1: 4,363	1
Barbaro M. et al. (Sweden, 2017) (35)	58,834	Last – 10/6 copies/3.2 mm punch	RT-qPCR (TRECs/KRECs/ACTB-assay)	572 (0.97%)	na	64 (0.11%)	3 (0.005%)	1: 20,000	1
Thomas C. et al. (French, 2019) (23)	190,517	≤ 34 copies/μL	Commercial kit (PerkinElmer, Finland)	na	139 (0.07%)	291 (0.15%)	26 (0.014%)	1: 1,154	3 + 3 leaky SCID
Chien YH. et al. (Taiwan, 2015) (14)	106,391	< 40 TRECs/μl	RT-qPCR of TRECs	na	5 (0.005%)	432 (0.4%)	19 (0.018%)	1: 4,433	2
Rechavi E. et al. (Israel, 2017) (34)	177,277	From 36 to 23 copies/blood sport	Commercial kit (PerkinElmer, Finland)	4.24% (for 36) 0.95% (for 23)	na	561 (0.3%)	46 (0.02%)	1: 3,853	8
Adams SP. et al. (UK, 2014) (37)	5,099	< 40 TRECs/μl	Commercial kit (PerkinElmer, Finland)	209 (4.10%)	na	51 (1.0%)	–	na	18 (control)
Al-Mousa H. et al. (Saudi Arabia, 2018) (17)	8,718	< 36 copies/μl	Commercial kit (PerkinElmer, Finland)	315 (3.6%)	16 (0.18%)	–	–	1: 545	3
Blom M. et al. (Netherland, 2018) (18)	1,272	30 copies/μL/ 6 copies/μL	Commercial kit (PerkinElmer, Finland/ Immuno IVD) Sweden) and	na	na	38 (3.0%)/ 5 (0.39%)	–	na	
This study	10,350	<5,000 copies/ per 10^6cells -1st stage; <2,000 copies/ per 10^6cells – next stage	RT-qPCR (TRECs/KRECs/albumin-assay)	590 (5.8%)	1 (0.01%)	73 (0.71%)	2 (0.02%)	1: 3,450	1 (CID)

manufacturer's recommendations is 30 copies/µl. For the SCREEN-ID kit, the TRECs cut-off according to manufacturer's recommendation is 6 copies/µl, while for KRECs it is 4 copies/µl. However, both manufacturers still recommend a pilot study with a large number of samples to establish a desired cut-off value based on a normal population distribution (18). Even though we have initially planned to use a commercial kit for this pilot project, this was hindered by the COVID-19 pandemic, the onset of which coincided with the start of the project. Since the restrictions caused difficulties with staff training and purchase of kits, we started to determine TRECs and KRECs in neonatal DBS using an RT-PCR adapted method, followed by the analysis of melting curves. Therefore, it was crucially important for us to establish the optimal cut-off values, which would, on the one hand, allow to capture all instances of SCID, XLA, and other diseases that present with severe T- and B-lymphopenia, and, on the other hand, to avoid a large number of false positive outcomes to reduce the potential for parental stress and anxiety.

Therefore, at the first stage, we decided on the cut-off of 5,000 copies per 10^6 cells in order not to miss cases of other immunodeficiency conditions that present with T- and B-lymphopenia. At this stage, we detected one case of CID with urgent ubnormal value of TRECs (twise 0) that was confirmed by immunological examination ($T^{low}B+NK+$). However, with this cut-off value, the retest proportion was high (7.6%) in comparison to other studies (Table 5) where percentage ranged from 0.72% (15) to 4.24% (34). New sample tests were needed for 45 (0.93%) newborns, which was also higher than reported in other studies (15, 16, 23, 24, 35), although it was comparable to the results of individual pilot studies (37). Only one child required a referral for further immunological examination based on the results of the new sample test. This was a premature baby (GA 34 weeks), with BW of 2,300 grams and TRECs level 4,010 copies per 10^6 cells. The child was diagnosed with transient lymphopenia and was followed up more than one year, during which period severe infections were not observed.

Since the parents expressed their concern in the instances of having to preforn a repeat blood stain, and taking into account a previous study on TRECs/KRECs in patients of the control group, the cut-off level at the second stage was reduced to 2,000 copies per 10^6 cells, which made it possible to reduce the number of retests and new sample tests almost two-fold and bring our outome indicators closer to the results of other published studies (17, 24, 34, 37).

As reported by other researchers, T- and/or B-cell lymphopenia in newborns is most often a result of maternal immunosuppression, prematurity, or congenital heart defects (15). Lower levels of TRECs in preterms were first pointed out to in a USA study (12). In Sweden, 40% of babies with lymphopenia were born prematurely (<37 weeks of gestation) (26), although no direct correlation with decreasing GA was observed (35), and the majority of results in preterms were above cut-off values. These results are comparable with our results, since we did not find a significant difference between the TREC median indicators in groups of children with different GA. Additionally, our study showed the correlation between the levels of TRECs and KRECs and the body weight of newborns; these levels were especially low in children with a weight of less than 1,000 grams. Other studies have shown low levels of TRECs/KRECs in twins and triplets (35). This may be related to both prematurity and low birth weight, a question which requires further investigation.

To reduce the need for new sample tests, researches use different strategies (33). While some screening programs set up different cut-off levels for full-term and premature babies (24), others contend that cut-off values require no change for preterm newborns despite a higher rate of retest in this cohort (23). Finally, some researches use three retests in the instances of abnormal TREC values and then take into account 2 out 3 results (24).

In our study, the referral rate, including caused by the urgent abnormal value was 0.03%, or 1 in 3,450 screened newborns. It is comparable with other published results (Table 5), where this number ranged from 1:545 in Saudi Arabia (17) to 1:20,000 in Sweden (35).

The results of TREC/KREC assay in patients of the control group showed that its sensitivity for detecting severe combined immunodeficiencies, in particular AT and NBS, is 95.2% for TRECs and 93.5% for KRECs, with the cut-off level of 2,000 copies per 10^6 cells. Meanwhile, for the patients with SCID, TREC levels were less than 1,000 copies per 10^6 cells, and as low as 138 in the patient with JAK3 deficiency and 25 in patient with DCLRE1C deficiency. KREC in the patient with XLA was 0.

Thus, TREC/KREC assay allows to detect both SCID and other CIDs that overlap with T- and B-lymphopenia. In the future, to reduce the anxiety of parents, cut down the number of retests, and therefore the cost of screening, the cut-off level can be lowered to 1,000, which will allow to capture SCID and effectively detect conditions accompanied by T- and B-lymphopenia.

Our study also showed a higher efficiency and sensitivity of TRECs detection, therefore, for the further implementation of screening in Ukraine, especially in the context of limited resources related to the war and COVID-19, it is possible to use only TREC assay to screen for SCID.

The RT-PCR technique used in the study allows establishing the number of TRECs and KRECs relative to the number of copies of the albumin gene, unlike commercial kits that provide data in copies per microliter or copies per punch. The use of commercial kits to determine TRECs and KRECs has the advantage of uniformity, but at the same time it is much more expensive.

Despite a number of advantages for the simultaneous use of TREC/KREC assay compared to the use of TRECs alone, in

particular for the detection of congenital B -cells defects, as well as late onset ADA deficiency (22), currently there is no consensus regarding the use KRECs assay in newborn screening and compliance of the IEI diagnosis associated with only B-lymphopenia with the general principles of newborn screening (6). In our study, only one child had a reduced level of KRECs together with a normal value of TRECs in a new sample test. The boy was born full-term (39 weeks) by cesarean section, weighing 3,750 g. During pregnancy, the mother had a threat of abortion, polyhydramnios and for a long time took progesterone drugs. The mother also recalled taking antibiotics during pregnancy. No other previously described causes of low KREC values were observed in the child. It is noted that B-lymphocytes are more sensitive to drug-induced apoptosis than T-lymphocytes, although, as described in the literature, these effects were associated with azathioprine (35). Further immunological examination of the boy did not reveal any pathological changes. Thus, the result was considered a false positive. The child was followed for 1,5 years during whih time he had 3 episodes of acute gastroenteritis. The Swedish study did not identify any patients with primary immunodeficiency based solely on low KREC levels during the three-year screening period, however the researchers still suggest that the triple assay is most suitable for newborn SCID screening to identify infants with NBS and XLA (26) that is especially relevant in patients of Eastern Slav origin (38).

It is worth underscoring that the newborn screening is only the first step on the way to a diagnosis, and in some instances a definitive diagnosis might be not achieved (6). A successful screening program has to include a system of measures, such as genetic counseling of the family, repeat testing, additional laboratory and instrumental studies, full support of the sick child and constant monitoring of their condition.

Strengths and limitation of the study

The strength of this pilot study is the determination of T- and B-lymphopenia using TREC and KREC assay by RT-PCR in neonatal dry blood spots. Another positive aspect is modification of the technique using melting curves analysis, which made it possible to avoid a large number of false negative results. In general, the obtained results were comparable to the data of other studies (Table 5). The selected cut-off levels for TREC and KREC were incrementally optimized to avoid unnecessary sampling and testing associated with unjustified additional costs. The authors also followed-up children with low TRECs and KRECs from 2 months to 2 years of age to determine the impact of lymphopenia at birth on subsequent morbidity in children.

A limitation of the study is a small group of screened newborns (10,350), covering only one region of Ukraine, which did not make it possible to detect a larger number of SCID and establish the prevalence of the disease, since its average frequency is 1 per 50-100 thousand newborns (5, 6). The TREC and KREC assay using proposed RT-PCR method for detecting T- and B-lymphopenia needs further standardization to successfully implement it in the newborn screening program for SCID. The small number of premature infants in this pilot study may have been the reason for no significant difference in the number of TRECs and KRECs in premature infants compared to full-term infants, as noted in other studies (34, 39). However, this number of screened infants is acceptable for a pilot study, which has the goal of testing the methodology and establish cut-offs, optimize the algorithm to avoid both false positive and false negative results.

Conclusions

The recognition of patient with CID in this pilot study with involving of 10,350 newborns and the results of 65 tested patients of control group with confirmed inborn errors of immunity has shown that the tested method for determination of TRECs and KRECs by RT-PCR followed by analysis of melting curves in neonatal dry blood spots is effective for the detection of severe T- and B-lymphopenia and can be used in newborn screening for SCID in Ukraine. Although determination of TREC in newborn screening for SCID has proven high sensitivity, it should be stressed that in order not to miss any other types of PID as agammaglobulinemia, Nijmegen breakage syndrome, AT or DiGeorge syndrome, as well as late-onset ADA SCID, newborn screening for inborn errors of immunity in the form of triple assay (including TREC and KREC) is optimal.

Data availability statement

The data analyzed in this study is subject to the following licenses/restrictions: The datasets of this study are available on request from the corresponding author. Requests to access these datasets should be directed to boyarchuk@tdmu.edu.ua.

Ethics statement

The study was approved by the I.Horbachevsky Ternopil National Medical University Ethics Committee (Minutes numero 55 from November 4, 2019). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Authors contributions

OB and HM designed and concept of the manuscript. OB, NY, and TH designed the concept of the pilot study project. NY, OS, IC, TH, LV, and MK were responsible for sample collection and logistic. VK, IS, and HM did the laboratory work. NY provided statistical analysis. OB, NY, VK, and HM analyzed and interpreted the data. OB, NY, and VK prepared figures and tables. OB, OS, and LV collected the relevant information and references. OB, OS, LV, and HM wrote the manuscript with contribution from all co-authors. All authors read, critically reviewed and approved the final version.

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Conflict of interest

Authors VK, IS, and HM were employed by Scientific Medical Genetic Center LeoGENE, LTD, Lviv, Ukraine.

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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References

1. Tangye SG, Al-Herz W, Bousfiha A, Chatila T, Cunningham-Rundles C, Etzioni A, et al. Human inborn errors of immunity: 2019 update on the classification from the international union of immunological societies expert committee. *J Clin Immunol* (2020) 40(1):24–64. doi: 10.1007/s10875-019-00737-x

2. Cirillo E, Giardino G, Gallo V, D'Assante R, Grasso F, Romano R, et al. Severe combined immunodeficiency-an update. *Ann N Y Acad Sci* (2015) 1356:90–106. doi: 10.1111/nyas.12849

3. Pai SY, Logan BR, Griffith LM, Buckley RH, Parrott RE, Dvorak CC, et al. Transplantation outcomes for severe combined immunodeficiency, 2000–2009. *N Engl J Med* (2014) 371(5):434–46. doi: 10.1056/NEJMoa1401177

4. Hardin O, Lokhnygina Y, Buckley RH. Long-term clinical outcomes of severe combined immunodeficiency patients given nonablative marrow transplants. *J Allergy Clin Immunol Pract* (2021) 10(4):1077–83. doi: 10.1016/j.jaip.2021.11.032

5. Aranda CS, Guimarães RR, de Gouveia-Pereira Pimentel M. Combined immunodeficiencies. *J Pediatr (Rio J)* (2021) 97 Suppl 1:S39–48. doi: 10.1016/j.jped.2020.10.014

6. Kwan A, Puck JM. History and current status of newborn screening for severe combined immunodeficiency. *Semin Perinatol* (2015) 39(3):194–205. doi: 10.1053/j.semperi.2015.03.004

7. Sanchez JJ, Monaghan G, Børsting C, Norbury G, Morling N, Gaspar HB. Carrier frequency of a nonsense mutation in the adenosine deaminase (ADA) gene implies a high incidence of ADA-deficient severe combined immunodeficiency (SCID) in Somalia and a single, common haplotype indicates common ancestry. *Ann Hum Genet* (2007) 71(Pt 3):336–47. doi: 10.1111/j.1469-1809.2006.00338.x

8. Puck JM. Laboratory technology for population-based screening for severe combined immunodeficiency in neonates: the winner is T-cell receptor excision circles. *J Allergy Clin Immunol* (2012) 129(3):607–16. doi: 10.1016/j.jaci.2012.01.032

9. King JR, Hammarström L. Newborn screening for primary immunodeficiency diseases. history, current and future practice. *J Clin Immunol* (2018) 38(1):56–66. doi: 10.1007/s10875-017-0455-x

10. Loeber JG, Platis D, Zetterström RH, Almashanu S, Boemer F, Bonham JR, et al. Neonatal screening in Europe revisited: An ISNS perspective on the current state and developments since 2010. *Int J Neonatal Screen* (2021) 7(1):15. doi: 10.3390/ijns7010015

11. Verbsky JW, Baker MW, Grossman WJ, Hintermeyer M, Dasu T, Bonacci B, et al. Newborn screening for severe combined immunodeficiency; the Wisconsin experience (2008–2011). *J Clin Immunol* (2012) 32:82–8. doi: 10.1007/s10875-011-9609-4

12. Kwan A, Abraham RS, Currier R, Brower A, Andruszewski K, Abbott JK, et al. Newborn screening for combined immunodeficiency in 11 screening programs in the united states. *JAMA* (2014) 312(7):729–38. doi: 10.1001/jama.2014.9132

13. Audrain M, Thomas C, Mirallie S, Bourgeois N, Sebille V, Rabetrano H, et al. Evaluation of the T-cell receptor excision circle assay performances for severe combined immunodeficiency neonatal screening on Guthrie cards in a French single centre study. *Clin Immunol* (2014) 150(2):137–9. doi: 10.1016/j.clim.2013.11.012

14. Chien YH, Chiang SC, Chang KL, Yu HH, Lee WI, Tsai LP, et al. Incidence of severe combined immunodeficiency through newborn screening in a Chinese population. *J Formos Med Ass* (2015) 114(1):12–6. doi: 10.1016/j.jfma.2012.10.020

15. Giżewska M, Durda K, Winter T, Ostrowska I, Ołtarzewski M, Klein J, et al. Newborn screening for SCID and other severe primary immunodeficiency in the polish-German transborder area: Experience from the first 14 months of collaboration. *Front Immunol* (2020) 11:1948. doi: 10.3389/fimmu.2020.01948

16. de Felipe B, Olbrich P, Lucenas JM, Delgado-Pecellin C, Pavon-Delgado A, Marquez J, et al. Prospective neonatal screening for severe T- and b-lymphocyte deficiencies in Seville. *Pediatr Allergy Immunol* (2016) 27(1):70–7. doi: 10.1111/pai.12501

17. Al-Mousa H, Al-Dakheel G, Jabr A, Elbadaoui F, Abouelhoda M, Baig M, et al. High incidence of severe combined immunodeficiency disease in Saudi Arabia detected through combined T cell receptor excision circle and next generation sequencing of newborn dried blood spots. *Front Immunol* (2018) 9:782. doi: 10.3389/fimmu.2018.00782

18. Blom M, Bredius RGM, Weijman G, Dekkers EHBM, Kemper EA, van den Akker-van Marle ME, et al. Introducing newborn screening for severe combined immunodeficiency (SCID) in the Dutch neonatal screening program. *Int J Neonatal Screen* (2018) 4(4):40. doi: 10.3390/ijns4040040

19. Douek DC, McFarland RD, Keiser PH, Gage EA, Massey JM, Haynes BF, et al. Changes in thymic function with age and during the treatment of HIV infection. *Nature* (1998) 396(6712):690–5. doi: 10.1038/25374

20. Chan K, Puck JM. Development of population-based newborn screening for severe combined immunodeficiency. *J Allergy Clin Immunol* (2005) 115(2):391–8. doi: 10.1016/j.jaci.2004.10.012

21. van Zelm MC, Szczepanski T, van der Burg M, van Dongen JJ. Replication history of b lymphocytes reveals homeostatic proliferation and extensive antigen-induced b cell expansion. *J Exp Med* (2007) 204(3):645–55. doi: 10.1084/jem.20060964

22. Borte S, von Döbeln U, Fasth A, Wang N, Janzi M, Winiarski J, et al. Neonatal screening for severe primary immunodeficiency diseases using high-throughput triplex real-time PCR. *Blood* (2012) 119(11):2552–5. doi: 10.1182/blood-2011-08-371021

23. Thomas C, Durand-Zaleski I, Frenkiel J, Mirallié S, Léger A, Cheillan D, et al. Clinical and economic aspects of newborn screening for severe combined immunodeficiency: DEPISTREC study results. *Clin Immunol* (2019) 202:33–9. doi: 10.1016/j.clim.2019.03.012

24. Argudo-Ramírez A, Martín-Nalda A, Marín-Soria JL, López-Galera RM, Pajares-García S, González de Aledo-Castillo JM, et al. First universal newborn screening program for severe combined immunodeficiency in europe. two-years' experience in Catalonia (Spain). *Front Immunol* (2019) 10:2406. doi: 10.3389/fimmu.2019.02406

25. Chien Y-H, Yu H-H, Lee N-C, Ho H-C, Kao S-M, Lu M-Y, et al. Newborn screening for severe combined immunodeficiency in Taiwan. *Int J Neonatal Screen* (2017) 3(3):16. doi: 10.3390/ijns3030016

26. Zetterström RH, Barbaro M, Ohlsson A, Borte S, Jonsson S, Winiarski J, et al. Newborn screening for primary immune deficiencies with a TREC/KREC/ACTB triplex assay–a three-year pilot study in Sweden. *Int J Neonatal Screen* (2017) 3(2):11. doi: 10.3390/ijns3020011

27. Boyarchuk O, Yarema N, Kinash M, Chornomydz I. Newborn screening for severe combined immunodeficiency: clinical and cost-effectiveness approaches. *Pol Merkur Lekarski* (2021) 49(289):80–3.

28. Hariyan T, Kinash M, Kovalenko R, Boyarchuk O. Evaluation of awareness about primary immunodeficiencies among physicians before and after implementation of the educational program: A longitudinal study. *PloS One* (2020) 15(5):e0233342. doi: 10.1371/journal.pone.0233342

29. Boyarchuk O, Lewandowicz-Uszyńska A, Kinash M, Haliyash N, Sahal I, Kovalchuk T. Physicians' awareness concerning primary immunodeficiencies in the ternopil region of Ukraine. *Pediatria Polska—Polish J Paediatrics* (2018) 93 (3):221–8. doi: 10.5114/polp.2018.77435

30. World Health Organization. Preterm Birth. (2018). Available at: https://www.who.int/news-room/fact-sheets/detail/preterm-birth (Accessed July 09, 2022).

31. Stegantseva MV, Guryanova IE, Sakovich IS, Polyakova EA, Belevtsev MV. Method of quantitative assay of circular molecules of T- and b-cell receptor, TREC and KREC, in peripheral blood by real-time PCR. *Eurasian J Oncol* (2017) 5 (3):449–56.

32. Blom M, Zetterström RH, Stray-Pedersen A, Gilmour K, Gennery AR, Puck JM, et al. Recommendations for uniform definitions used in newborn screening for severe combined immunodeficiency. *J Allergy Clin Immunol* (2022) 149(4):1428–36. doi: 10.1016/j.jaci.2021.08.026

33. Malvagia S, Forni G, Ombrone D, la Marca G. Development of strategies to decrease false positive results in newborn screening. *Int J Neonatal Screen* (2020) 6 (4):84. doi: 10.3390/ijns6040084

34. Rechavi E, Lev A, Simon AJ, Stauber T, Daas S, Saraf-Levy T, et al. First year of Israeli newborn screening for severe combined immunodeficiency - clinical achievements and insights. *Front Immunol* (2017) 8:1448. doi: 10.3389/fimmu.2017.01448

35. Barbaro M, Ohlsson A, Borte S, Jonsson S, Zetterström RH, King J, et al. Newborn screening for severe primary immunodeficiency diseases in Sweden–a 2-year pilot TREC and KREC screening study. *J Clin Immunol* (2017) 37:51–60. doi: 10.1007/s10875-016-0347-5

36. Boyarchuk O, Makukh H, Kostyuchenko L, Yarema N, Haiboniuk I, Kravets V, et al. TREC/KREC levels in children with ataxia-telangiectasia. *Immunol Res* (2021) 69(5):436–44. doi: 10.1007/s12026-021-09216-1

37. Adams SP, Rashid S, Premachandra T, Harvey K, Ifederu A, Wilson MC, et al. Screening of neonatal UK dried blood spots using a duplex TREC screening assay. *J Clin Immunol* (2014) 34:323–30. doi: 10.1007/s10875-014-0007-6

38. Sharapova SO, Pashchenko OE, Bondarenko AV, Vakhlyarskaya SS, Prokofjeva T, Fedorova AS, et al. Geographical distribution, incidence, malignancies, and outcome of 136 Eastern Slavic patients with nijmegen breakage syndrome and *NBN* founder variant c.657_661del5. *Front Immunol* (2021) 11:602482. doi: 10.3389/fimmu.2020.602482

39. Kanegae MP, Barreiros LA, Sousa JL, Brito MAS, de Oliveira Junior EB, Soares LP, et al. Newborn screening for severe combined immunodeficiencies using TRECs and KRECs: Second pilot study in Brazil. *Rev Paul Pediatr* (2017) 35:25–32. doi: 10.1590/1984-0462/;2017;35;1;00013

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Primary immunodeficiencies in Bulgaria - achievements and challenges of the PID National Expert Center

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Tremendous progress has been made in the recognition of primary immune deficiencies (PIDs) in Bulgaria since in 2005 we have joined the J Project Central-Eastern European collaborative program. Ten years later an Expert Centre (ExpC) for Rare Diseases - Primary Immune Deficiencies at the University Hospital "Alexandrovska"- Sofia was established. In May 2017 The National Register of Patients with Rare Diseases also became operational as a database containing clinical and genetic information for Bulgarian patients with PID. The transfer of data and information on Bulgarian PID patients to the European Primary Immunodeficiency Database, managed by the European Society for Primary Immunodeficiency (ESID) has started in 2020. The total number of registered patients now is 191 (100 men and 91 women), with more than half of them being children (106; 55.5%). Regular updating of the information in the register showed that 5.2% of patients are deceased and the majority (94.8%) is a subject to continuous monitoring as it has been reported for other European countries as well. With the establishment of the ExpC, the dynamics in the diagnosis and registration of patients with PID significantly intensified. For a period of 5 years (2016-2021) 101 patients were evaluated and registered in comparison with previous period - before ExpC establishment when only 89 patients were diagnosed. The most common pathology was humoral immune deficiency (85 patients; 44.5%). Ninety-six (50.3%) of the patients underwent genetic testing, and 66. 7% had genetically confirmed diagnosis. Three of the variants have not been reported in population databases. Following genetic investigation confirmation of the initial phenotypic diagnosis was achieved in 82.8% of cases and change in the diagnosis - in 17%. Sixty-two patients were on regular replacement or specific therapy, and the rest received symptomatic and supportive treatment. In summary, we present the first epidemiological report of PIDs in Bulgaria,

based on the National PID register. Data on the clinical, phenotypic and genetic characteristics of PID patients provided important information about the nature of primary immunodeficiency diseases in our country.

KEYWORDS

Primary immunodeficiency, Bulgarian PID Registry, epidemiology, genetic analysis, phenotypic characteristics

Introduction

Primary immune deficiencies (PIDs) are rare diseases. According to the latest update of the International Union of Immunological Societies (IUIS) classification (1), mutations in 430 genes cause 404 different phenotypes of immunological diseases, divided into 10 groups based on the type of immunological defect. Their prevalence among the world's population varies widely, from 1.51 (Germany) to 20.27 (Kuwait) per 100,000 (2, 3). In addition, the distribution of different PIDs also varies among populations. A number of factors are responsible for these variations and geographical location and structure of the populations, as well as the percentage of consanguinity marriages are among them. However, an important factor is the level of recognition of these rare diseases, and their registration in national and international registries (4, 5).

Bulgaria is a relatively small country with a population of 6,916,548 according to the National Statistical Institute data from 12.2020. The predominant ethnic population are Bulgarians who do not generally consanguinity relationships. However, there are also regions with closed communities (4% Roma ethnicity and 2% Turkish ethnicity), where the incidence of some PIDs such as Ataxia telangiectasia (AT) is increased. The first published case of PID in Bulgaria was described as dysgammaglobulinemia in 1965, and in 1997 our team diagnosed the first case of common variable immune deficiency (CVID) (6). However, more systematic work on the identification and registration of PID patients began in 2005 when Bulgarian immunologists became part of the Central-Eastern European collaborative program called J Project. The Expert Centre (ExpC) for Rare Diseases - Primary Immune Deficiencies at the University Hospital "Alexandrovska"-Sofia has been officially designated in April 2016 by order of the Minister of Health. In May 2017 the National Register of Patients with Rare Diseases, established and maintained by the National Centre for Public Health and Analysis (NCPHA), also became operational. The registry contains clinical and genetic information of PID patients. In 2020 the information on PID patients has also been included in the existing European Primary Immunodeficiency Database, managed by the European Society for Immunodeficiencies (ESID).

In this article we report for the first time the epidemiology of PIDs in Bulgaria, based on the National PID Registry, and analyze the factors which could improve the diagnosis and management of PID patients and their families. In addition, data summarized here will contribute to the improvement of our knowledge on these rare diseases of the immune system.

Material and methods

Work organization of the national register of patients with PID rare diseases

The software platform of the register has been developed by NCPHA, in accordance with the European legislation regarding personal data protection.

The register contains anonymous, clinical, laboratory and genetic data for subjects with PID and serves for epidemiological analysis in order to improve the management and treatment of PID patients. The structure of the database includes the following mandatory information: demographic data, family history, clinical and laboratory information on PID patients, genetic test results, age of onset of the disease and age of diagnosis. Treatment details were not obligatory part of the portfolio at the time of first patient registration, but were subsequently required. PID variants were grouped according to the IUIS classification from 2019 (1).

Patients with suspected PID were referred to the National PID ExpC for diagnostic conformation. The health structures from which patients were initially referred for diagnostic clarification were mainly university hospitals from all over the country, and more recently the practices of general practitioners (GPs). Data of patients with confirmed diagnoses were included into the registry by authorized persons working in the PID ExpC. Written informed consent form was signed by all registered patients or their legal guardians. The informed consent forms have been approved by the Ethics commission of the University Hospital "Alexandrovska" Sofia (protocol

number 100A/13th of May, 2020). The registry data was updated regularly.

Characteristics of patients

Patients were diagnosed according to the diagnostic criteria of ESID and IUIS (1, 7). Patients with secondary immune deficiencies were excluded. Epidemiological analysis included all registered patients until December, 2021.

Diagnostic algorithm and tests

The algorithm for the diagnosis of PID has been developed by the National PID expert group and included the following stages: characteristic symptoms for PID derived from the patient's medical history, screening tests, data from the patient's clinical examination and patient referral to PID centers for more specialized studies, including immune phenotyping, functional and genetic tests. Patient's course from symptoms' emergence through diagnosis and treatment was outlined in detail with the cooperation of GPs, pediatricians, internal disease specialists, neurologists est. Diagnostic tests included: complete blood count, differential count, flow cytometry immune phenotyping with a wide range of monoclonal antibodies, serum immunoglobulins and their subclasses, antibody response to vaccines (Diphtheria and Tetanus toxoid, Pneumococcal polysaccharide and Haemophilus influenzae type B), assessment of T lymphocyte function (Dynabeads Human T-Activator CD3/CD28 Assay and Phytohaemagglutinin Stimulation Assay). Testing for autoantibodies (antinuclear antibody screening extractable nuclear antigen panel, antibodies associated with organ-specific autoimmunity, autoimmune cytopenias, vasculitis, coagulopathies etc.), phagocytic activity (flow cytometry dihydrorhodamine test), complement hemolytic activity (Classical and Alternative pathway hemolytic assay - CH50, AH50) and specific components of the complement was performed as needed. Additionally, other functional tests were performed, such as STAT/JAK/MAPK pathways activity and cytokine profiling (ProcartaPlex multiplex cytokine panels, Invitrogen™). Genetic research has been performed using up-to-date techniques, including Sanger sequencing, Next-generation sequencing (NGS), Whole genome sequencing, Fluorescent *in situ* hybridization (FISH), according to standard protocols and in collaboration with INVITAE (Invitae Corp. San Francisco, California, U.S.), GRID (Genomics of Rare Immune Disorders, UK) and other PID centres within Jeffry Model Centres (JMC) Network and J Project.

Statistical methods

The data analysis was performed using descriptive statistics and the SPSSv16.0 program. A pair t-test was used to assess the difference in the age of onset of symptoms, age of diagnosis, delay in diagnosis, and gender in the group of patients with PID. P-value ≤0.05 was considered statistical significant.

Results

Characteristics of PID patients in the national registry

The Bulgarian National PID Registry includes 191 patients with PID, which represents a rate of 2.7 per 100,000. The last update is from December 2021. Until 2014 only 69 PID patients were diagnosed and registered in the National Registry (Figure 1). Since then, there has been an ascending tendency in the number of newly diagnosed patients, even more than doubled (177% increase).

The distribution of Bulgarian patients according to the updated IUIS classification from 2019 showed a predominance of antibody deficiencies (n=85, 44.5%) as those with CVID were 40 (20.9%), followed by selective IgA deficiency (n=22, 11.5%). Combined immune deficiencies (CID) with associated or syndromic features accounted for 17.3% (n=33), and individuals with auto-inflammatory diseases were 11% (n=21) of the patients. Seventeen patients (8.9%) remained without definitive diagnosis and represented a diagnostic challenge. Patients allocated to other PID categories make up less than 10% of all cases for the respective nosological group. Only 8 patients with hereditary angioedema (HAE) were included in the National Registry, classified to the group of complement deficiencies (n=11, 5.8%), as this entity is usually treated and monitored by specialists in allergic diseases. Diagnosed individuals with Immunodeficiency affecting cellular and humoral immunity were 10 (5.23%) and the same number of patients were registered with Congenital defects of phagocyte number, function or both. In the group of CID with associated or syndromic features, only 6 of 28 patients with AT from 4 ethnic families have been registered. The remaining 22 patients who have been clinically and genetically diagnosed with AT are currently pending to enter the registry so they were not included in this article.

Figure 2 shows the distribution of patients by gender and age groups. There is an almost equal ratio between males (n = 100) and females (n = 91). More than half of the patients are children (n = 106; 55.5%), 59 of whom are males and 47 - females. In the

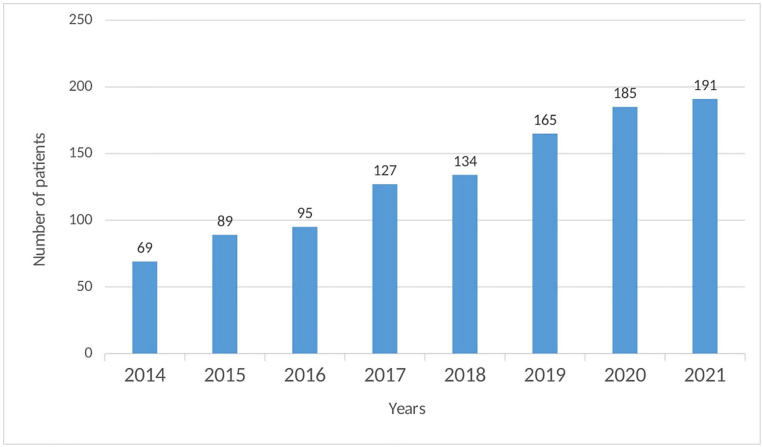

FIGURE 1
Distribution of the number of patients in the National PID Expert Center by years.

adult's group males predominated as well. An extensive research of the patient's medical history showed that in a large proportion of the adults (n = 40; 47%), the symptoms of primary immune deficiency dated back to childhood and adolescence. Ten (5.2%) of the registered patients were deceased and 181 (94.8%) were subject to long-term follow-up (Table 1). The distribution of patients by the age of onset of symptoms and the age of diagnosis showed that patients with severe combined immunodeficiencies (SCID) and CID with associated or syndromic features were diagnosed at 12 and 30 months (median age) after birth respectively, while the diagnosis of patients with antibody deficiencies was delayed with a median of 4 years ($p < 0.05$). Greater delay in the diagnosis has been observed in patients with immune dysregulation diseases (median - 8 years) and in undefined PID patients (median - 5 years). The youngest diagnosed patients was a newborn with Bruton's disease (genetic diagnosis was performed at birth due to positive family history of a brother with Bruton's disease) and an infant with Hyper IgE syndrome due to clinical manifestations of eczematous pustular rash on the face and neck with fast dissemination, elevated markers of inflammation and microbiological data for methicillin-susceptible staphylococcus aureus, development of occipital and liver abscesses, pulmonary consolidation in both lungs and sepsis (genetic diagnosis at 4 months of age revealed the presence of STAT3 mutation). We also analyzed the delay in diagnosis according to gender. We found that in the group of patients with phagocytic defects the mean delay in diagnosis for females was 12.75 years, while for males was significantly lower (2.12 years; p=0,016) which is due to X-linked inheritance.

Epidemiology of PID in Bulgaria

The geographical distribution of PID on the territory of Bulgaria is based on patients included in the register (Figure 3).

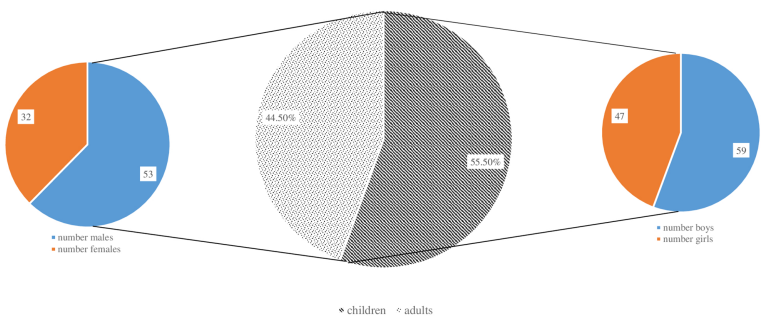

FIGURE 2
Distribution of patients in the National PID Expert Center by gender and age groups.

TABLE 1 Distribution of patients from the National PID Registry by main disease groups according to IUIS classification, sex, median age of onset and median age at diagnosis (diagnostic delay).

IUIS groups	Diagnosis	Patients (n)	Sex	Disease onset (median age; min-max)	Delay in diagnosis (median age; min-max)
1	Immunodeficiencies affected cellular and humoral immunity	10 (2ex)	M-5F-5	1m (0m -1y)	11m (0m-12y)
2	CID with associated or syndromic features	33 (3ex)	M-18F-15	2m (0m-10y)	1y (0m-46y)
3	Predominantly antibody deficiencies	85 (4ex)	M-39F-46	5y (8m-54)	4y (0m-52y)
4	Diseases of immune dysregulation	4	M-2F-2	5y (1y-22y)	8y (4y-10y)
5	Congenital defects of phagocyte number or function	10 (1ex)	M-6F-4	0m (0m-31y)	1y (0m-22y)
6	Defects in intrinsic and innate immunity	0			
7	Auto-inflammatory disorders	21	M-14F-7	3y (3m-45y)	1y (0m-27y)
8	Complement deficiencies	11	M-7F-4	9y (7m-3y)	3y (5m-13y)
9	Bone marrow failure disorders	0			
10	Undefined	17	M-9F-8	3y (0m-43y)	5y (1y-62y)

M, male; F, female; ex.-exitus letalis.

Patients were mainly concentrated in large cities such as Sofia with a population of 1,280,000 and Plovdiv with a population of 364 403 (North part of Bulgaria n=27/14.1%, South part of Bulgaria n=164/85.9%). The rest of the cases were distributed evenly in other districts of the country. It should be noted that in the municipality of Sarnitsa, Pazardzhik district, with a population of 3600 people, the majority of the population belongs to the so called Bulgarian Muslims ethnicity and the incidence of patients with AT (n = 28) is 0.7% of the population in the municipality, originating from 4 families.

Genetic diagnosis of patients with PID

Genetic testing was performed on 96 patients, representing 50.26% of the registered subjects (Figure 4). A mutation in one or more PID-related genes was found in 64 of them (66.7%), with the majority having autosomal recessive (AR) defects (50%). Autosomal dominant (AD) defects were established in 33.9% and X-linked defects in 16.1% of the cases. Figure 4 demonstrates that the highest number of genetically tested patients relative to the total number of patients assigned to a

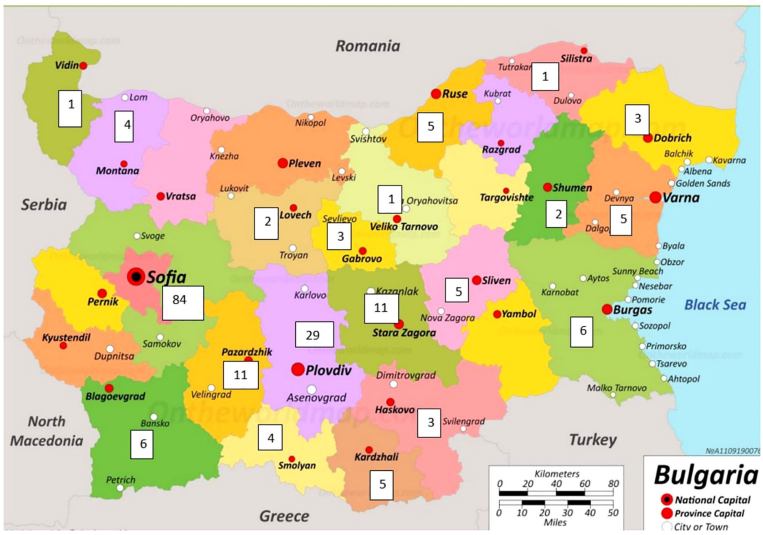

FIGURE 3
Geographical distribution of registered PID patients in Bulgaria (The map was adapted from https://ontheworldmap.com/bulgaria/).

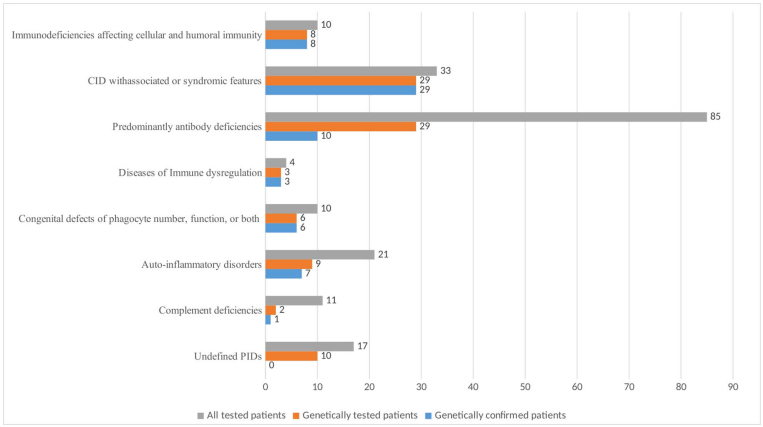

FIGURE 4
Number of PID patients by main classification groups with genetic testing and positive diagnostic confirmation.

PID category was in the group of CID with associated or syndromic features - 87.8%, followed by SCID - 80%, and the lowest number was in the group of patients with complement deficiencies (18%) and predominantly antibody deficiencies (34%). The analysis in different groups showed that pathogenic variants were entirely confirmed in all of the samples subject to testing if the patients had SCID, CID with associated or syndromic features, diseases of immune dysregulation and congenital defects of phagocyte number or function. In patients with antibody deficiency, the genetic diagnosis was positive in only 34.4% of those tested. Patients with undefined immune deficiency remained in this group due to the lack of molecular-phenotypic compliance, although over 58% of them were tested for pathogenic variants.

Detailed information referring pathogenic and likely pathogenic variants in selected patients included in the Bulgarian Registry with available exact molecular data is available as Supplementary material. Variants included missense mutations, duplications and deletion. Thanks to these data, a definitive diagnosis has been established. It is interesting to note that out of all patients diagnosed with CVID, only 4 had pathogenic variants related to CVID in a single gene TNFRSF13B in heterozygous state.

In 72 of all genetically tested patients with suspected PID, a targeted sequencing of a panel of genes associated with immune deficiencies (GRID program, INVITAE, TruSight One Sequencing Panels, Illumina) has been performed by next-generation sequencing technology, thus providing valuable information not only on the presence of pathogenic variants, but also on those of uncertain significance, some of which have certain clinical correlation.

This type of analysis gave us additional information on the carrier status of a total of 10 heterozygous pathogenic mutations associated with AR inherited diseases (Table 2). Three of the variants have not been reported in population databases (https://gnomad.broadinstitute.org/). The variant c.657_661del in the

TABLE 2 List of pathogenic variants responsible for AR PIDs in patients tested with NGS technology in heterozygous stage.

GENE	VARIANT	STATE	Variant classification	Inheritance
TNFRSF13B	*c.311G>A (p.Cys104Tyr)*	heterozygous	Likely Pathogenic	AR
NBN	*c.657_661del (p.Lys219Asnfs*16)*	heterozygous	PATHOGENIC	AR
TCIRG1	*c.1297C>T (p.Gln433*)*	heterozygous	PATHOGENIC	AR
PTPRC*	*c.308C>G (p.Ser103*)*	heterozygous	PATHOGENIC	AR
TNFRSF13B	*c.431C>G (p.Ser144*)*	heterozygous	PATHOGENIC	AR
ICOS*	*Deletion (Entire coding sequence)*	heterozygous	PATHOGENIC	AR
LIPA	*c.894G>A (Silent)*	heterozygous	PATHOGENIC	AR
AK2*	*c.597_599dup (p.Tyr200*)*	heterozygous	PATHOGENIC	AR
TNFRSF13B	*c.260T>A (p.Ile87Asn)*	heterozygous	PATHOGENIC	AR
HPS3	*NM_032383.3:c.1101C>A*	heterozygous	PATHOGENIC	AR

*newly established variants.

NBN gene was found by chance in three of the subjects who were not related to the patients with Nijmegen breakage syndrome included in the registry. This indicates a likely high frequency of carriage, requiring a larger number of tested individuals for accurate determination.

In two of the patients with suspected PID, we have identified pathogenic variants which significance for phenotypic expression remains to be clarified. In a girl with T cell lymphopenia, deafness, and mild developmental delay, a pathogenic variant has been detected in the FTCD gene (NM_006657.2: FTCD c.990dupG), in homozygous state with TruSight One Sequencing Panel, Illumina. Mutations in this gene are responsible for the so-called glutamate formiminotransferase deficiency, a pathology with autosomal recessive inheritance, outside the PID group. The second case presented a girl with multiple clinical manifestations in which we have identified a pathogenic mutation in the KMT2D gene (NM_003482.3: c.5627A> C) in a heterozygous state associated with AD Kabuki syndrome 1.

The results from target-sequencing analysis of PID-related genes showed that a total of 265 variants were classified as Variants of Uncertain Significance. The variants included missense, nonsense, deletion, insertion and frame shift mutations. Thirty-nine of them have not been described yet in population databases. Fifty-six of the variants were in genes responsible for diseases characterized by AD or X-linked inheritance. This required multidisciplinary discussion, search for clinical-laboratory correlation,and follow-up of patients. Active monitoring of the status of identified variants from Uncertain to Benign or Pathogenic/Likely Pathogenic was required as well. Functional-diagnostic confirmation of the significance of these changes had been also considered.

We have also analyzed the correlation between phenotypic and genetic diagnoses. Confirmation of the phenotypic diagnosis was achieved in 82.8% of cases, and change in the diagnosis in 11 patients (17.2%). Table 3 presents patients with non-compliance with the primary diagnosis based on phenotypic characteristics and genetic findings. Five of the patients remained in the same PID category, but with a different syndrome after genetic testing. Six patients were reclassified in another PID category. It is noteworthy that in patients with phenotypic hypogammaglobulinemia/CVID genetic testing often was the most useful and informative test for diagnostic clarification. Interestingly, in a child with a phenotypic characteristic of CVID and subsequent rapid development of non-Hodgkin's lymphoma, the genetic diagnosis showed X-linked lymphoproliferative syndrome caused by a deletion in the SH2D1A gene. Following genetically-based re-classification, the therapeutic algorithm was re-evaluated in this particular case as well as in the majority of patients with diagnostic shift.

Therapy in PID patients

PIDs are complex diseases with multiple clinical manifestations, which determine the need for a comprehensive approach towards their treatment. Because antibody deficiency was the most commonly diagnosed cause of PIDs, regular immunoglobulin replacement therapy (IRT) was the primary and most available therapy. In our country the treatment for PID patients who need IRT or C1 esterase inhibitors is completely reimbursed by the

TABLE 3 Shift in the diagnostic categorization of patients after genetic testing, based on the identification of affected gene.

Diagnosis before genetic testing	Gene, in which the pathogenic variant was identified	Diagnosis after genetic testing	Treatment
Periodic Fever, Aphthous Stomatitis, Pharyngitis, Adenitis (PFAPA)	*MVK*	Mevalonate kinase deficiency (Hyper IgD syndrome)	Etanercept
Periodic Fever, Aphthous Stomatitis, Pharyngitis, Adenitis (PFAPA)	*MEFV*	Familial Mediterranean Fever (FMF)	Colchicine
Combined immunodeficiency	*RAG1*	Leaky SCID caused by hypomorphic mutation	HSCT
Nijmegen breakage syndrome	*NHEJ1*	SCID (T-B-NK+) Cernunnos	IRT
SCID-Omenn syndrome	*IL2RG*	X-linked SCID	IRT, Corticosteroids, Rituximab, Chemotherapy, HSCT
Chronic granulomatous disease (CGD)-female carrier	*MPO*	MPO deficiency	Antibiotics
Hypogammaglobulinemia	*CD40L*	Hyper IgM syndrome (HIGM)	IRT
Auto-inflammatory disorders	*MASP2*	MASP2 deficiencies	Antibiotics, immune modulators
Chediak-Higashi syndrome	*TBX1*	22q11.2 deletion syndrome	Symptomatic therapy
Common variable immunodeficiency (CVID)	*SH2D1A*	X-linked lymphoproliferative syndrome	IRT, Chemotherapy, Rituximab
Common variable immunodeficiency (CVID)	*CTLA-4*	CTLA-4 haploinsufficiency	IRT, Corticosteroids

IRT, Immunoglobulin Replacement Therapy.

National Health Insurance Fund (NHIF) since 2013. Fifty-four (28%) of our patients received Intravenous immunoglobulin/ Subcutaneous Immunoglobulin (IVIg/SCIg), and in the last 5 years 91% of them received SCIg. One patient with CGD was treated with interferon gamma. Three patients received biological therapy for complications and two were treated with immunosuppressive therapy. Four patients were transplanted (SCID-2 and LAD-2) with good results, and in two others (Leaky SCID and Congenital neutropenia) the transplantation has been planned and forthcoming. The remaining patients were treated with long-term antimicrobial prophylaxis or anti-inflammatory therapy.

Discussion

More than 400 million individuals worldwide suffer from a rare disease, and about half of them are children. As part of rare diseases, the incidence of PIDs in different populations varies from 1:2,000 to 1: 100,000. For European countries it ranges between 1.51 (Germany), 4.2 (Switzerland), Norway 5.3/100,000, United Kingdom at 5.90, and 8.0/100,000 (France) (2, 8–11)

According to the National Bulgarian PID Registry, the total incidence of PID in Bulgaria was 2.7/100,000, of which patients with predominantly antibody deficiencies were approximately 1.2/100,000, and SCID were 0.11/100,000 - a clear indicator of insufficient identification of various forms of immune deficiency. This is due to under-diagnosis of persons who have died before they were identified or cases with mild course that were missed. In reference to this, the establishment of specialized PID centres and registration of patients in registries at the national level is an important approach to more effective identification of PID cases (4). Other strategies are being implemented to better detection of PID patients, such as the creation of web-based networks to facilitate collaboration between GPs and PID experts (12). Here we present for the first time epidemiological, clinical and genetic data on PIDs in Bulgaria, based on the National PID register. All districts in the country were covered, which made this study a representative one for Bulgaria. It was not surprising that the majority of patients came from big cities with large population, such as Sofia and Plovdiv. However, it should be noted that access to specialized medical care, including clinical immunology service, is extremely easy due to the operation of specialized PID centres. On the other hand, there are remote areas in the country, such as the Rhodope Mountains, inhabited by the Bulgarian Muslims ethnicity - a religious minority, where 28 patients have been identified with AT, genetically verified and belonging to 4 families. Interestingly, despite the classical mutation in the ATM gene, these patients exhibited a phenotypic profile of long-term AT survivors. In addition, some PID entities were probably under-reported because they were mainly monitored in departments with specialties other than immunology, such as hemophagocytic lymphohistiocytosis, which is treated mainly by hematologists or auto-inflammatory diseases that are very common in rheumatologists' practice. The data on the incidence of PID in Bulgaria should be interpreted carefully, due to the presence of prerequisites for under-diagnosis of PID patients. With the improvement of public awareness and collaboration between different medical specialists in the last 5 years, the detection of PID patients had improved significantly (almost twice). This was in line with the global trend in the PID community presented in the summary report from the JMC (12). Suspected patients were identified and referred to the PID ExpC in order to receive early and adequate diagnosis and treatment. The significant increase in the number of patients identified with immune deficiency was also due to the expansion of educational and awareness initiatives and the intensification of molecular diagnostics with current technologies. However, even more systematic steps are needed, including neonatal screening, prophylactic testing of immune competence at different stages of development of the children, adolescent and young adults, and improvement in the access to innovative treatment. We were already successful in using such approaches to identify patients with immune dysfunction. For example, our results from analysis of the post-vaccine immune response against protein antigens (tetanus and diphtheria) among a representative sample of the population showed (13) that individuals with insufficient titer of antibodies exhibited more frequent infectious and other symptoms and needed to be evaluated for the existence of primary or secondary immune disorders.

The distribution of PID diagnoses in Bulgaria was similar to that of the European Database (ESID), with a predominance of antibody deficiencies, followed by combined immune deficiencies with associated or syndromic features (14). Similar trend was also reported in the updated data for Russia (15) and Ukraine (16). Conversely, in Kuwait, the country with the highest incidence of PID (20.27/100,000), there was a predominance of severe forms of PID affecting cellular and humoral immunity followed by combined immune deficiencies with associated or syndromic features (3). According to the authors, this is due, on the one hand, to the improved patient identification due to the awareness of medical community, the well-organized referral of patients to clinical immunology service, and on the other hand - to the progress in the health system of the country. Similar factors were pointed at the base of the high incidence of PID in Iceland (18.8/100,000) - the country with the highest incidence of PID in Europe (17). Of course, in countries like Kuwait, the high incidence of consanguineous marriages must be taken into account, which was an explanation for the predominance of SCID (3).

On the other hand, data from the Consensus Middle East and North Africa Registry (18) showed that registered patients with inborn errors of immunity vary between 0.02 and 7.58 per 100,000 population. However, consanguinity in these areas are relatively high (60.5% of cases), and 27.3% of patients came from families with a confirmed previous family history of PID.

An important indicator is the delay between the onset of symptoms, diagnosis and initiation of treatment. Globally, it takes an average of 4.8 years to accurately diagnose a rare disease (12). Thirty percent of children with rare diseases will not survive until their 5th birthday, and thus serious steps are needed to improve this distressing statistic. In recent years, the delay in diagnosis has decreased significantly for the severe forms of PID in our country (under 12 months of age), thanks to the increased public awareness, significantly improved diagnostic capabilities, experience in PID and excellent collaboration between doctors from different medical specialties on national and international level. In comparison, the mean age of patients at baseline was 36 months and the mean delay in diagnosis was 41 months, according to the Middle East and North Africa Registry (18). It should be noted that the period from symptoms to diagnosis in patients with the most common clinically manifested immune deficiency – CVID, is the same to that in Germany (4 years) and shorter than in France and Sweden (6 years) (3, 8, 11).

At the same time, the diagnosis of the most severe form of immune deficiency, SCID, is extremely insufficient (0.11/100,000). This is a clear indicator of the need to implement a screening program for T- and B-cell deficiencies at a national level, for which there are already prerequisites in our country. A pilot study has been conducted to screen newborns with modern molecular technology in order to examine a dry blood spot taken at birth (Granted by Scientific Research Fund, Ministry of Education and Science). The data from the first stage of the study showed that 3 per 1,000 newborns were suspected of having T- and/or B-cell deficiency and were suitable for further clarification and follow-up (data not published). The results obtained on the prevalence of these diseases are a good basis for planning the necessary annual costs. Steps are to be taken for their inclusion in normative acts for mass genetic screening in the Republic of Bulgaria.

The entry of patient`s data from the Bulgarian Registry into the European database began in 2020. So far, 166 patients have been registered, which represents 86.9% of the patients in the National PID Registry. The process was slowed down by the fact that there were patients, mostly adults, who did not agree to consent to the registration of their personal data. Additionally, the necessary data and registration documents cannot be collected for some patients from remote areas of Bulgaria.

Our first analysis of Bulgarian patients with PID showed genetic diversity and a relatively high rate of confirmation of genetic diagnosis in 33.5% of all registered patients. The statistics was quite close to ESID Registry data, pointing genetic confirmation in approximately 36-43% of PID patients (French and German registries) (2, 11) and was significantly lower in comparison to the registered patients from the Russian population (49%) (15). The most common genes in which PID-related pathogenic variants were identified did not differ significantly from those reported for European populations. For example, 33% of the genetically tested patients diagnosed with CVID were carriers of a pathogenic mutation, data very similar to those reported by the German Registry (2). The highest percentage of genetically diagnosed patients had deletions in chromosome 22q11.2 - a total of 12 cases, followed by patients with a defect in the NBN gene and BTK gene - 5 (7.8 of the genetically proven). The observed tendncy for high frequency of heterozygous carriers of the so-called Slavic deletion in the NBN gene (c.657del5; rs587776650) - 4.16% of those tested, is not surprising for the Bulgarian population as this mutation is essentially characteristic for Slavic populations and therefore might be considered a Slavic founder mutation (18). High prevalence in the range of 0.5% to 1% of heterozygous carriers of c.657del5 was reported in population of Poland, Czech Republic/Slovakia, Ukraine and Germany (19, 20).

Despite the small number of detected and registered patients with SCID, the identified patients with defects in RAG and ADA prevailed over those with IL2R defect, a constellation different from the usual frequency of different forms of SCID. It is noteworthy that so far in our register there is no patient with a mutation in the WAS gene, given that the average incidence of Wiskott-Aldrich syndrome is between 1 and 10 cases per million boys. The aim of the diagnostic algorithm for the patients included in the registry was to use approaches based on NGS technologies for PID-related gene panels (75% of those tested were studied with this approach). This allowed us to detect and monitor patients with clinical and laboratory data corresponding to the so-called variants classified as “uncertain”, as well as follow-up the clinical manifestations in patients that are heterozygous carriers of pathogenic mutations responsible for AR diseases. The outstanding benefits of molecular diagnosis in patients were demonstrated with the change in the initial diagnosis in 11.4% of the tested patients, which also affected their therapy and prognosis. Patients with clinical and laboratory data of impaired immune response in whom the immune deficiency is classified as undefined are eligible for larger-scale genetic testing or phenocopies of PID should be suspected.

An extremely important issue is the treatment of PID. 95% of rare diseases lack FDA-approved treatment (12). With the introduction of IRT in the 1950s, the treatment of patients with predominantly antibody deficiency has been provided in a number of countries around the world. The NHIF in our country completely reimburses the treatment for PID patients who need IRT and C1 esterase inhibitor since 2013. Improving the access to immunoglobulin therapy is a persistent trend in all centres in Central and Eastern Europe. This is due to both the increase in the total number of patients and to a slight increase in

the percentage of patients treated with immunoglobulins that is 19 to 23%, in the cumulative review of all centres participating in the Jeffrey Modell Foundation Central East Euorope network (21). The percentage of our patients treated with IVIg/SCIg was slightly higher (28%), and in the last 5 years the use of SCIg had significantly increased (more than 90% receive their therapy at home). This tendency is in line with the global shift towards personalized patient care, enabling treatment adjustment in order to ensure the best possible lifestyle. The number of patients with access to biological therapy and stem cell transplantation is also increasing. Unfortunately, in Bulgaria there is still no experience in transplanting children with PID, so up to date the cases had been transplanted abroad but funded from the NHIF. Treating patients with specific immune dysregulation due to a known genetic defect and treating the clinical manifestations of each individual patient is also a challenge. However, we are still experiencing difficulties in the application of biological and other modern methods of treatment due to lack of experience or lack of access or authorization, especially when "off-label" drug use is required. In this regard, the collaboration between the centres is of great importance for the benefit of patients.

The contribution of the two international projects that supported the work in the field of PID in our country needs to be outlined. In 2005, we joined a program for cooperation in Central and Eastern Europe in the field of physician education and clinical trials and aimed to improve the diagnosis and clinical care of patients with PID diseases, known as the J Project (22). The second program was developed by the Jeffrey Modell Foundation. In 2015 The Functional High Expertise Centre was established for training, development and improvement of diagnosis, treatment and care of patients with primary immunodeficiency diseases in Sofia, part of an international network of JMC (23). As a result, public awareness and collaboration between individual specialists such as pediatricians, rheumatologists and immunologists have significantly improved. The path from symptoms to diagnosis and treatment has emerged. In addition, in 2017 JMF developed a point system for assessing the risk of immune deficiency (12). This strategy reduces the uncertainties associated with the primary risks of immune deficiency, as we can test, identify and treat undiagnosed patients. It also allows for better consideration of regional differences and the distribution, age, gender, terms and conditions of access to qualified medical care and treatment. Last but not least, there are socio-economic benefits. On the other hand, IPOPI developed a PID Patient Life Index (24) to measure the state of implementation of the six PID principles (diagnosis, treatment, universal health coverage, specialist centers, national patient organizations and PID registries). It was emphasized that in countries without immunologists, patients with PID are at risk of being undiagnosed or misdiagnosed, leading in health implications or even death.

Limitation of the study

This study has some limitations mainly related to the underdiagnosis of PID patients. Epidemiological and genetic analyzes were performed only on the basis of the patients diagnosed so far.

Concluding remarks

The Bulgarian PID Registry provided for the first time epidemiological, clinical and genetic data that contributed to the understanding of the history and nature of PID in Bulgaria. Moreover, the establishment of Expert Centers had led to a systemic interest in PIDs and advances in their management in our country. The progressive increase in the number of newly registered cases underlined the improvement of PID awareness not only among the medical community but also among the public community. Continuous updating of the registry with new data and maintenance/updating of existing data ensured access to systematic and detailed information in a timely manner. This opportunity contributed to better understanding of PID and ensured improved diagnostic and treatment protocols for Bulgarian patients. The Expert Centre for Rare Diseases - PID in the University Hospital "Alexandrovska" - Sofia had a significant contribution in this process, providing Bulgarian patients with PID with equal access to health care facilities. This was possible due to great energy and enthusiasm of scientists and medical doctors, as well as the introduction of new diagnostic and therapeutic tools. In addition, we need to offer easy access to updated PID information regarding PID for doctors. The role of the patient organization "Bulgarian Association of People with Primary Immune Deficiencies" was also important, as it was related to advocacy, education and patient support.

We believe that our efforts will contribute to the development of a long-term strategy by the health authorities in order to provide the necessary resources to improve outcomes in prevention, treatment and management of PID patients in Bulgaria and ultimately improve patient care.

Data availability statement

The data presented in the study are deposited in the ClinVar repository, submission numbers SCV002573411 – SCV002573436.

Ethics statement

Written informed consent form was signed by all registered patients or their legal guardians. The informed consent forms

have been approved by the Ethics commission of the University Hospital "Alexandrovska", Sofia.

Author contributions

EN conceived the original idea and wrote the manuscript with input from. EN, SM, SL, PY, and MM contributed to the diagnosis and clinical and immunological follow-up of the patients. VM collected and managed the data from the National PID Registry. MM contributed to the editing of the manuscript. 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.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2022.922752/full#supplementary-material

References

1. Bousfiha A, Jeddane L, Picard C, Al-Herz W, Ailal F, Chatila T, et al. Human inborn errors of immunity: 2019 update of the IUIS phenotypical classification. *J Clin Immunol* (2020) 40(1):66–81. doi: 10.1007/s10875-020-00758-x

2. El-Helou SM, Biegner A, Bode S, Ehl SR, Heeg M, Maccari ME, et al. The German national registry of primary immunodeficiencies (2012–2017). *Front Immunol* (2019) 10:1272. doi: 10.3389/fimmu.2019.01272

3. Al-Herz W, Al-Ahmad M, Al-Khabaz A, Husain A, Sadek A, Othman Y. The Kuwait national primary immunodeficiency registry 2004-2018. *Front Immunol* (2019) 10:1754. doi: 10.3389/fimmu.2019.01754

4. Lopes JP, Cunningham-Rundles CH. The importance of primary immune deficiency registries: The united states immunodeficiency network registry. *Immunol Allergy Clin North Am* (2020) 40(3):385–402. doi: 10.1016/j.iac.2020.03.002

5. Hassan A, Gholamreza A, Laleh S, Reza Y, Monireh M, Samaneh D, et al. Global systematic review of primary immunodeficiency registries. *Expert Rev Clin Immunol* (2020) 16(7):717–32. doi: 10.1080/1744666X.2020.1801422

6. Naumova E, Murdjeva M, Baleva M, Spasova M, Perenovska P. Bulgarian Association of clinical immunology - achievements and challenges in PID. In: *Year book of Bulgarian association for clinical immunology, 2014, vol.8*. Sofia: Publishing houseLitse (2015). p. p.16–22.

7. https://www.esid.org/…/Diagnostic-criteria-for-PID2.

8. Marschall K, Hoernes M, Bitzenhofer-Gruber M, Jandus P, Duppenthaler A, Wuillemin WA, et al. The Swiss national registry for primary immunodeficiencies: report on the first 6 years' activity from 2008 to 2014. *Clin Exp Immunol* (2015) 182:45–50. doi: 10.1111/cei.12661

9. Stray-Pedersen A, Abrahamsen TG, Frøland SS. Primary immunodeficiency diseases in Norway. *J Clin Immunol* (2000) 20(6):477–85. doi: 10.1023/a:1026416017763

10. Shillitoe B, Bangs C, Guzman D, Gennery AR, Longhurst HJ, Slatter M, et al. The united kingdom primary immune deficiency (UKPID) registry 2012 to 2017. *Clin Exp Immunol* (2018) . 192(3):284–91. doi: 10.1111/cei.13125

11. CEREDIH: The French PID study group. The French national registry of primary immunodeficiency diseases. *Clin Immunol* (2010) 135:264–72. doi: 10.1016/j.clim.2010.02.021

12. Modell V, Orange JS, Quinn J, Modell F. Global report on primary immunodeficiencies: 2018 update from the Jeffrey modell centers network on disease classification, regional trends, treatment modalities, and physician reported outcomes. *Immunol Res* (2018) 66(3):367–80. doi: 10.1007/s12026-018-8996-5

13. Lesichkova S, Mihailova S, Mihaylova A, Gesheva N, Yankova P, Naumova E. Age-related dynamics in post-vaccine antibody immune response to diphtheria and tetanus toxoid in Bulgarian subjects. *Acta Med Bulgarica* (2021) 48:46–52. doi: 10.2478/amb-2021-0007

14. Available at: https://esid.org/Working-Parties/Registry.

15. Mukhina AA, Kuzmenko NB, Rodina YA, Kondratenko IV, Bologov AA, Latysheva TV, et al. Primary immunodeficiencies in Russia: Data from the national registry. *Front Immunol* (2020) 11:1491. doi: 10.3389/fimmu.2020.01491

16. Volokha A, Bondarenko A, Chernyshova L, Hilfanova A, Stepanovskiy Y, Boyarchuk O, et al. Impact of the J project on progress of primary immunodeficiency care in Ukraine. *Cent Eur J Immunol* (2021) 46(2):250–7. doi: 10.5114/ceji.2021.108183

17. Ludviksson BR, Sigurdardottir ST, Johannsson JH, Haraldsson A, Hardarson TO. Epidemiology of primary immunodeficiency in Iceland. *J Clin Immunol* (2015) 35(1):75–9. doi: 10.1007/s10875-014-0107-3

18. Aghamohammadi A, Rezaei N, Yazdani R, Delavari S, Kutukculer N, Topyildiz E, et al. Consensus middle East and north Africa registry on inborn errors of immunity. *J Clin Immunol* (2021) 41(6):1339–51. doi: 10.1007/s10875-021-01053-z

19. Varon R, Seemanova E, Chrzanowska K, Hnateyko O, Piekutowska-Abramczuk D, Krajewska -Walasek M, et al. Clinical ascertainment of nijmegen breakage syndrome (NBS) and prevalence of the major mutation, 657del5, in three Slav populations. *Eur J Hum Genet* (2000) 8(11):900–2. doi: 10.1038/sj.ejhg.5200554

20. Maurer MH, Hoffmann K, Sperling K, Varon R. High prevalence of the NBN gene mutation c.657-661del5 in southeast Germany. *J Appl Genet* (2010) 51 (2):211–4. doi: 10.1007/BF03195730

21. Sediva A, Bataneant M, Belevtsev M, Blaziene A, Ciznar P, Förster-Waldl E, et al. Primary immunodeficiencies in central and Eastern Europe–the power of networking report on the activity of the Jeffrey modell foundation centers network in central and Eastern Europe. *Immunol Res* (2019) 67(4-5):358–67. doi: 10.1007/s12026-019-09093-9

22. Pac M, Casanova J-L, Reisli I, Marodi L. Editorial: Advances in primary immunodeficiency in central-Eastern Europe. *Front Immunol* (2021) 12. doi: 10.3389/fimmu.2021.667727

23. Quinn J, Modell V, Orange JS, Modell F. Growth in diagnosis and treatment of primary immunodeficiency within the global Jeffrey modell centers network. *Allergy Asthma Clin Immunol* (2022) 18:19. doi: 10.1186/s13223-022-00662-6

24. Nordin J, Solis L, Prévot J, Mahlaoui N, Chapel H, Sánchez-Ramón S, et al. The PID principles of care: Where are we now? a global status report based on the PID life index. *Front Immunol* (2021) 10. doi: 10.3389/fimmu.2021.780140

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COVID-19 in unvaccinated patients with inborn errors of immunity—polish experience

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At the beginning of the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) pandemic, patients with inborn errors of immunity (IEI) appeared to be particularly vulnerable to a severe course of the disease. It quickly turned out that only some IEI groups are associated with a high risk of severe infection. However, data on the course of Coronavirus Disease 2019 (COVID-19) in patients with IEI are still insufficient, especially in children; hence, further analyses are required. The retrospective study included 155 unvaccinated people with IEI: 105 children and 50 adults (67.7% and 32.3%, respectively). Male patients dominated in the study group (94 people, 60.6%). At least two comorbidities were found in 50 patients (32.3%), significantly more often in adults (56% vs. 21%). Adult patients presented significantly more COVID-19 symptoms. Asymptomatic and mildly symptomatic course of COVID-19 was demonstrated in 74.8% of the entire group, significantly more often in children (88.6% vs. 46%). Moderate and severe courses dominated in adults (54% vs. 11.4%). Systemic antibiotic therapy was used the most frequently, especially in adults (60% vs. 14.3%). COVID-19-specific therapy was used almost exclusively in adults. In the whole group, complications occurred in 14.2% of patients, significantly more often in adults (30% vs. 6.7%). In the pediatric group, there were two cases (1.9%) of multisystem inflammatory syndrome in children. Deaths were reported only in the adult population and accounted for 3.9% of the entire study group. The death rate for all adults was 12%, 15.4% for adults diagnosed with common variable immunodeficiency, 12.5% for those with X-linked agammaglobulinemia, and 21.4% for patients with comorbidity. The results of our study imply that vaccinations against COVID-19 should be recommended both for children and adults with IEI. Postexposure prophylaxis and early antiviral and anti-SARS-CoV-2 antibody-based therapies should be considered in adults with IEI, especially in those with severe humoral immune deficiencies and comorbidity.

KEYWORDS

inborn errors of immunity, adults, children, COVID-19 unvaccinated patients, COVID-19

Introduction

The first reports from China about the infection caused by the new SARS-CoV-2 virus came in November 2019. On 4 March 2020, the first case of COVID-19 was diagnosed in Poland. On March 11, 2020, The World Health Organization declared COVID-19 a pandemic. From that moment, infections caused by variants of the virus spread around the world, infecting over 527 million people and killing over 6.3 million people (1).

From the outset of the pandemic, patients with inborn errors of immunity (IEI) were identified as particularly vulnerable to SARS-CoV-2 infection and severe COVID-19. However, with emerging reports on the course of the infection in this particular group, it becomes clear that only certain IEI defects are associated with a poor prognosis (2–4). Similarly as in the entire population, the risk of death increases with age and the incidence of comorbidity diseases (5).

In the studies on patients with IEI presented so far, there is a significant difference in the demonstrated risk of a severe course and death due to COVID-19. This seems to be related to a different spectrum of diagnoses of IEI depending on geographic location and cultural differences (especially family relationship between parents) in certain countries (6, 7).

Preventive vaccinations have been and still are the hope for overcoming the pandemic and returning to normal life. The first vaccinations in Poland, in the so-called priority groups, began in December 2020. Patients with IEI were not included in the first place in the group eligible for vaccination. They received the opportunity to be vaccinated against SARS-CoV-2 only in May 2021.

Due to the still-limited data on the course of COVID-19 in children and adults with IEI, it is necessary to collect clinical data

to identify IEI-related risk factors for the serious course of COVID-19, as well as to define possible long-term or delayed complications of the infection. This knowledge should translate into the optimization of disease treatment in this specific group of patients. It will also be a reference point for assessing a possible change in the disease pattern in vaccinated patients with IEI. Therefore, the aim of the study was to conduct a clinical analysis of Polish patients diagnosed with IEI and COVID-19, who fell ill prior to the implementation of preventive vaccinations in this group.

Material

The retrospective study included 155 people: 105 children (67.7%) and 50 adults (32.3%) diagnosed with a disease/syndrome recognized as IEI according to the guidelines of the European Society for Immunodeficiencies (ESID). The characteristics of the study group, the type of IEI, treatment, and comorbidities are presented in the Results section and Table 1.

Methods

In order to identify IEI patients infected with SARS-CoV-2, a questionnaire was developed. It included questions about;

- Demographic data, the age of IEI, the type of deficiency, and its treatment and comorbidities;
- The age of SARS-CoV-2 infection and the reason and method of carrying out diagnostic tests to detect the infection;
- Symptoms, treatment, and complications;

The questionnaire was sent to clinical immunologists working in immunological centers in Poland. The mainstay for the diagnosis of COVID-19 was reverse transcription polymerase chain reaction (RT-PCR) tests, antigen tests, and/or the detection of IgM and/or IgG antibodies in people who had never been vaccinated against SARS-CoV-2. As the patients came from various centers in Poland, the tests were carried out with the use of the sets of different companies, but all of them had the required certifications.

The following analysis was made:

- Reason for testing for SARS-CoV-2 infection (before planned hospitalization, for epidemiological reasons—contact with the patient, clinical symptoms, other), month of infection (from 1 March 2020 to 30 April 2021);
- Clinical symptoms, especially fever, cough, the loss of smell and/or taste, pneumonia; the duration or recurrent nature of infection was analyzed (the diagnosis of a prolonged or relapsed form); the severity of the clinical course was defined;
- Acute complications, particularly respiratory failure, thromboembolic complications,
- "cytokine storm", death; delayed complications, including the development of a multisystem inflammatory syndrome temporarily associated with SARS-CoV-2 in children, as well as progressive or chronic complications;
- Treatment: symptomatic, antibiotic therapy, remdesivir, convalescent plasma, passive oxygen therapy, mechanical ventilation.

The severity of the clinical course was defined as follows:

- Asymptomatic—no clinical symptoms, a positive result of the PCR or antigen test or the detection of IgM and/or IgG antibodies;
- Mildly symptomatic—clinical symptoms that resolved spontaneously, possible symptomatic treatment, without acute complications, no indications for treatment typical for COVID-19;
- Moderate—hospitalization, COVID-19-specific treatment and antibiotic therapy required (bacterial superinfection suspected), pneumonia with normal saturation, without serious acute complications;
- Severe—hospitalization and COVID-19-specific treatment needed, acute complications requiring intervention, a prolonged or recurrent course of the infection, chronic complications.

All the above parameters were compared both in the pediatric and adult groups.

The normality of the observed values was tested using the Shapiro–Wilk test. For the continuous variables, with non-normal distributions, the median (minimum to maximum) was used. Continuous variables were analyzed using the Mann–Whitney U test. Categorical variables were analyzed using the chi-square test or Fisher exact test. For all data analyses, differences were considered statistically significant when $p < 0.05$. Statistical analysis was performed using the STATISTICA software (TIBCO Software Inc. Palo Alto, CA, USA), version 13.

The conducted research was approved by the Bioethics Committee (No. KB 327/2022).

Results

From March 2020 to the end of April 2021, COVID-19 was detected in 155 patients diagnosed with IEI. In the same period of time, 2,792,148 people (1) fell ill with COVID-19 in the general population in Poland.

Characteristics of the group

Predominantly antibody deficiency (PAD) dominated in the whole group (105/155; 67.7%) but significantly more often in adults compared to children (94% vs. 53.3%, respectively; p <0.001). In the group of adult patients, common variable immunodeficiency (CVID) and X-linked agammaglobulinemia (XLA) were the most frequently diagnosed (52% vs. 16%, respectively), whereas 39% of the pediatric population were diagnosed with antibody deficiencies other than CVID and XLA. Moreover, in the pediatric group, among other diagnoses, severe combined immunodeficiencies (SCIDs) and combined immunodeficiencies (CIDs), including syndromic ones, constituted 24.8%. All patients with SCID (four children) were at least 6 months after allogeneic–hematopoietic cell transplantation (HCT). IEI from the group of autoinflammatory syndromes were diagnosed in 12% of pediatric patients. The most common IEI groups are presented in Table 1. A detailed distribution of diagnoses is provided in the form of footnotes under Table 1.

The most common treatment of the underlying disease in the IEI group was human immunoglobulin replacement therapy (IgRT): in the entire group in 105 patients (67.7%), significantly more often in adults compared to children (86% vs. 59%, respectively; p < 0.001) (Table 1).

At least one comorbidity was diagnosed in 79 patients (51%) in the entire group and two or more in 50 patients (32.3%). The incidence of comorbidities in IEI patients was significantly more common in adults than in children (56% vs. 21%, respectively; p < 0.001) (Table 1). Among patients with comorbidities, 40 people (25.8%) had immune diseases, including immune cytopenia, other autoimmune diseases, bronchial asthma, and other allergies. Non-immunological diseases, including neurological and psychiatric problems, chronic infections, and non-immunological gastroenterological diseases and others were present in 60 patients (38.7%). Due to the observed multiple morbidity, one patient could have one or more immunological

TABLE 1 Characteristics of the study group.

Feature	Whole group N (%)	Children N (%)	Adults N (%)	p-value
	155 (100.0%)	**105 (67.7%)**	**50 (32.3%)**	
Sex:				
M/F	94(60.6%)/61(39.4%)	67(63.8%)/38(36.2%)	27(54%)/23(46.0%)	0.292
Age at diagnosis of IEI:				
Median [min–max]	6 [0.1–81]	3 [0.1–16]	37 [1–81]	**<0.001**
Observation period from IEI diagnosis to COVID-19 diagnosis:				
Median [min–max]	4 [0–34]	3 [0–14]	7 [0–34]	**<0.001**
Diagnosis:				
PAD*	103/155 (66.5%)	56/105 (53.3%)	47/50 (94.0%)	**<0.001**
CVID	39/155 (25.2%)	13/105 (12.4%)	26/50 (52.0%)	**<0.001**
XLA	10/155 (6.5%)	2/105 (1.9%)	8/50 (16.0%)	**0.002**
Other humoral	54/155 (34.8%)	41/105 (39.0%)	13/50 (26.0%)	0.149
CID *(including syndromic)* and SCID**	27/155 (17.4%)	26/105 (24.8%)	1/50 (2.0%)	**<0.001**
Autoinflammatory syndrome***	13/155 (8.4%)	13/105 (12.4%)	0/50 (0%)	**0.01**
Other IEI****	12/155 (7.7%)	10/105 (9.5%)	2/50 (4.0%)	0.339
Comorbidities:				
At least 1	79/155 (51.0%)	44/105 (41.9%)	35/50 (70.0%)	**0.001**
≥ 2	49/155 (31.6%)	21/105 (20.0%)	28/50 (56.0%)	**<0.001**
Treatment of IEI/comorbidities:				
IgRT	105/155 (67.7%)	62/105 (59.0%)	43/50 (86.0%)	**<0.001**
HCT	6/155 (3.9%)	6/105 (5.7%)	0/50 (0.0%)	0.178
Biologicals	6/155 (3.9%)	6/105 (5.7%)	0/50 (0.0%)	0.178
Immunosuppressants	21/155 (13.5%)	18/105 (17.1%)	3/50 (6.0%)	0.078

CVID, common variable immunodeficiency; CID, combined immunodeficiency; PAD, predominantly antibody deficiency; SCID, severe combined immunodeficiency; XLA, X-linked agammaglobulinemia; IgRT, immunoglobulin replacement therapy; HCT, hematopoietic cell transplantation.*PAD (in children and in adults, respectively): selective IgA deficiency (sIgAD): 6 (3 + 3), isolated IgG subclass deficiency with normal Ig levels and normal B cells: 10 (7 + 3), selective IgM deficiency: 2 (2 + 0), specific antibody deficiency with normal Ig levels and normal B cells: 1 (1 + 0), transient hypogammaglobulinemia of infancy: 3 (3 + 0), severe hypogammaglobulinemia with normal B cells and secondary causes excluded: 32 (25 + 7). **SCID (in children and in adults, respectively): 4 (4 + 0), all T-B+CID (in children and in adults, respectively): Nijmegen breakage syndrome: 6 (6 + 0), DiGeorge syndrome: 4 (4 + 0), Wiskott–Aldrich syndrome: 3 (2 + 1), ataxia–telangiectasia syndrome: 2 (2 + 0), other single cases: 5 (4 + 0), 3 under genetic diagnosis (3 + 0). ***Autoinflammatory disorders only in children: periodic fever aphthous stomatitis, pharyngitis, and adenopathy (PFAPA): 4 (4 + 0),TNF receptor-associated periodic syndrome, familial cold autoinflammatory syndrome, mevalonate kinase deficiency: one case eachUnclassified autoinflammatory disorders undergoing genetic diagnosis: 6. ****other IEI (in children and in adults, respectively):Congenital defects of phagocyte number or function (in children and in adults, respectively): 4 (3 + 1) (cyclic neutropenia: 2, CGD: 2)Diseases of immune dysregulation (in children and in adults, respectively): 7 (6 + 1) (autoimmune lymphoproliferative syndrome 5 (4 + 1), SAP deficiency 1 (1 + 0), familial hemophagocytic lymphohistiocytosis 1 (1 + 0).Congenital asplenia: 1 (1 + 0). Bold values indicate a statistically significant value.

and non-immunological comorbidities. A detailed list of comorbidities is presented in Table 2.

Diagnosis of COVID-19 in the group

In the study group, COVID-19 was occasionally diagnosed until August 2020 (3.2%). The largest number of patients with IEI became infected in October and November 2020 (22.6% and 27.8%, respectively), which was correlated with the incidence of infections in the general population in Poland. In February 2021, the increase in infections in patients with IEI was observed 1 month earlier than in the general population (Figure 1A). Unfortunately, in Poland, SARS-CoV-2 virus variants were not identified or reported as "different" in the first months of the pandemic. Single alpha variants were reported between October 2020 and January 2021. The analyses of SARS-CoV-2 variants began on a larger scale from February 2021, when the alpha variant was definitely dominant in Poland (8) (Figure 1B)

For the entire group, the age of patients with IEI at the time of SARS-CoV-2 infection ranged from 0.5 to 82 years, with a median of 13 years, in the pediatric population from 0.5 to 17 years, with a median of 8.5 years, and in adults from 18 to 82 years with a median of 42.5 (Table 3).

The reason for testing for SARS-CoV-2 infection in almost all adults (46/50; 92%) was the occurrence of the symptoms of the infection. In the pediatric group, the symptoms were a less frequent indication for testing than in adults (78/105; 74.3%). Approximately 25% of children and sporadic adults were tested due to epidemiological reasons (e.g., contact with SARS-CoV-2 in the school or home), including tests before or on admission to hospital (4/50; 8% and 27/105; 25.7%). In the presented group, COVID-19 was most frequently diagnosed using nasopharyngeal swab RT-PCR testing (67.7%), significantly more often in adults than in children (94% vs. 55.2%; $p < 0.001$). In children, the diagnosis was relatively frequently made based on anti-SARS-CoV-2 antibody titers (48.6%) (Table 3).

The course and treatment of COVID-19 in the group

The symptoms typical for SARS-CoV-2 that occurred significantly more often in adults compared to children were fever: 76% vs. 42.9%; $p < 0.001$, cough: 60% vs. 24.8%; $p < 0.001$, loss/disorders of smell: 20% vs. 4.8%; $p = 0.006$, and taste disorders: 22% vs. 8.6%; $p = 0.037$.

Other symptoms of infection (sore throat, runny nose, headache, abdominal pain, vomiting, diarrhea, chest pain, dyspnea, and enlarged lymph nodes) were also slightly more often reported by adult patients (66% vs. 58.1%; $p = 0.383$) (Table 3).

The asymptomatic course of COVID-19 was demonstrated in 20% of patients in the entire study group, significantly more often in children than in adults (25.7% vs. 8%; $p = 0.01$). The mildly symptomatic course was identified in 54.8% of the whole group, significantly more often in children compared to adults (62.9% vs. 38%; $p = 0.005$). The moderate course concerned 13.5% of all patients, significantly more often adults than children (22% vs. 9.5%; $p = 0.045$).

TABLE 2 Analysis of comorbidities in the presented group of patients with inborn errors of immunity (IEI), divided into immunological and non-immunological.

Feature	Whole group N (%)	Children N (%)	Adults N (%)	p-value
	155 (100%)	**105 (67.7%)**	**50 (32.3%)**	
Bronchial Asthma	15 (9.7 %)	11 (10.5 %)	4 (8.0 %)	0.626
Cytopenias	11 (7.1 %)	3 (2.9 %)	8 (16.0 %)	**0.003**
Other autoimmune	14 (9.0%)	8 (7.6%)	6 (12.0%)	0.27
Allergy	10 (6.5 %)	8 (7.6 %)	2 (4.0 %)	0.391
Cardiac defects	7 (4.5 %)	7 (6.7 %)	0 (0.0 %)	0.062
Gastrointestinal diseases	11 (7.1 %)	6 (5.7 %)	5 (10.0 %)	0.331
Obesity	7 (4.5 %)	2 (1.9 %)	5 (10.0 %)	**0.023**
Pulmonary (bronchiectasis, GLILD)	8 (5.2%)	4 (3.8%)	4 (8.0%)	0.232
Hypertension	7 (4.5 %)	0 (0.0 %)	7 (14.0 %)	**< 0.001**
Chronic infections	11 (7.1 %)	3 (2.9 %)	8 (16.0 %)	**0.003**
Prematurity	2 (1.3 %)	2 (1.9 %)	0 (0.0 %)	0.326
Diabetes	3 (1.9 %)	1 (1.0 %)	2 (4.0 %)	0.198
Cancers*	4 (2.6%)	0 (0.0%)	4 (8.0%)	**0.01**
Neurological/psychiatric diseases	14 (9.0 %)	8 (7.6 %)	6 (12.0 %)	0.374

*One newly diagnosed and three in anamnesis, without active anticancer treatment.
Bold values indicate a statisticallysignificant value.

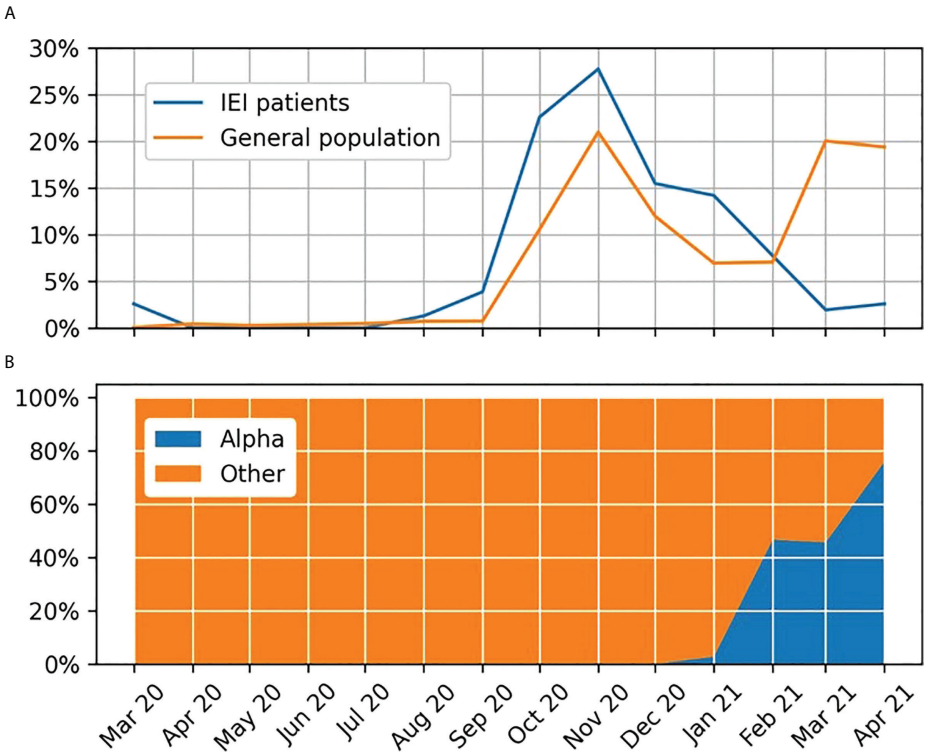

FIGURE 1
(A) Percentage of monthly COVID-19 diagnoses in the Polish population and among inborn errors of immunity patients. **(B)** Variants of the SARS-CoV-2 virus reported in the analyzed period in Poland.

Severe COVID-19 concerned 11.6% of patients, almost exclusively adults (32% vs. 1.9%; p<0.001) (Table 3).

Antibiotic therapy was the most common treatment of COVID-19 (in 29% of the entire group), significantly more often in adults than in children (60% vs. 14.3%; p<0.001). COVID-19-specific therapy was used almost exclusively in adults: remdesivir in 20% and convalescent plasma in 18%. These therapies were used in 1% and 1.9% of children, respectively. Similarly, passive oxygen therapy was used in 11% of adults and 1.9% of children. Mechanical ventilation was used only in 16% of adults (Table 3).

Complications of COVID-19

In the whole group, complications occurred in 14.2% of patients, significantly more often in adults than in children (30% vs. 6.7%; p <0.001). Regardless of age, among 22 patients with complications, as many as 9 (40.9%) were diagnosed with the coexistence of immunological and non-immunological comorbidities. Among 133 patients without the complications of COVID-19, only 18 (13.2%) had both immunological and non-immunological complications. The difference is statistically significant (p = 0.002). On the other hand, the lack of comorbidities significantly more often affected people without complications: 71/133 (53.4%) vs. 5/22 (22.7%) with complications (p = 0.008).

The following complications were observed in adults: respiratory failure in 14%, pulmonary complications (bacterial pneumonia as superinfection, pneumothorax, pulmonary fibrosis) in 12%, other complications in 8% (one case each: fever prolonged to 12 weeks, chronic urticaria, worsening of comorbidities of IEI, and sepsis).

Other complications occurred in individual children. The multisystem inflammatory syndrome in children (MIS-C) was diagnosed in two patients (2/105; 1.9%): in a 1-year-old girl with SCID within >100 days from HCT and in a 6-month-old girl with hypogammaglobulinemia that eventually turned out to be transient. Both girls were treated with high-dose immunoglobulins and steroids. The younger child developed giant aneurysms in the coronary arteries, requiring constant anticoagulation therapy. Decreased physical activity was observed in a 14-year-old boy with CVID. In the remaining five pediatric patients, sporadic complications such as behavioral disturbances and depression (two patients), one case each of diarrhea and long-lasting dysgeusia were reported. These complications were related to patients diagnosed with severe hypogammaglobulinemia with normal B cells and secondary causes excluded.

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TABLE 3 The results of research on COVID-19 infection in patients with IEI.

Feature	Whole group N (%)	Children N (%)	Adults N (%)	p-value
	155 (100%)	105 (67.7%)	50 (32.3%)	
Age at the diagnosis of COVID-19:				
Min/max/average/median/standard deviation	0.5/82/19.7/13/18.5	0.5/17/8.7/8.5/4.7	19/82/42.5/42.5/15.4	<0.001
Female n (%)	61/155 (39.4%)	38/105 (36.2%)	23/50 (46.0%)	0.294
Diagnosis of COVID-19:				
until 31/08/2020	6/155 (3.9%)	5/105 (4.8%)	1/50 (2.0%)	0.665
01/09/20–30/04/21	149/155 (96.1%)	100/105 (95.2%)	49/50 (98.0%)	0.665
Reason for testing for SARS-CoV-2:				
Symptoms	124/155 (80%)	78/105 (74.3%)	46/50 (92%)	**0.01**
Other	31/155 (20%)	27/105 (25,7%)	4/50 (8%)	
Diagnostic method:				
PCR	105/155 (67.7%)	58/105 (55.2%)	47/50 (94.0%)	**<0.001**
Ag	5/155 (3.2%)	5/105 (4.8%)	0/50 (0.0%)	0.176
Ab	54/155 (34.8%)	51/105 (48.6%)	3/50 (6.0%)	**<0.001**
Clinical symptoms:				
Fever	84/155 (54.2%)	45/105 (42.9%)	39/50 (78.0%)	**<0.001**
Cough	57/155 (36.8%)	26/105 (24.8%)	31/50 (62.0%)	**<0.001**
Smell impairment	16/155 (10.3%)	5/105 (4.8%)	11/50 (22.0%)	**0.003**
Taste impairment	21/155 (13.5%)	9/105 (8.6%)	12/50 (24.0%)	**0.012**
Other	94/155 (60.6%)	61/105 (58.1%)	33/50 (66.0%)	0.383
Course:				
Asymptomatic	31/155 (20.0%)	27/105 (25.7%)	4/50 (8.0%)	**0.01**
Mildly symptomatic	84/155 (54.2%)	66/105 (62.9%)	18/50 (36.0%)	**0.002**
Moderate	21/155 (13.5%)	10/105 (9.5%)	11/50 (22.0%)	**0.045**
Severe	19/155 (12.3%)	2/105 (1.9%)	17/50 (34.0%)	**<0.001**
Treatment:				
Antibiotic therapy	45/155 (29.0%)	15/105 (14.3%)	30/50 (60%)	**<0.001**
Remdesivir	12/155 (7.7%)	1/105 (1.0%)	11/50 (22.0%)	**<0.001**
Convalescent plasma	12/155 (7.7%)	2/105 (1.9%)	10/50 (20.0%)	**<0.001**
Passive oxygen therapy	14/155 (9.0%)	2/105 (1.9%)	12/50 (24.0%)	**<0.001**
Mechanical ventilation	9/155 (5.8%)	0/105 (0.0%)	9/50 (18.0%)	**<0.001**
Other	34/155 (21.9%)	22/105 (21.0%)	12/50 (24.0%)	0.682

Bold values indicate a statistically significant value.

There were no thromboembolic complications in the presented group.

In the entire group, six adult individuals (3.9%) died due to COVID-19 (6/50; 12%): three men aged 34, 39, and 66 and three women aged 51, 53, and 62. Four out of six patients who died were diagnosed with CVID (4/26; 15.4%), one with XLA (1/8; 12.5%) and one with selective immunoglobulin A deficiency (sIgAD). In the presented study, sIgAD is included in the "other humoral defects" group (1/13; 7.7%). All patients who died were diagnosed with two or more comorbidities of IEI (6/28; 21.4% of adults with at least two comorbidities) (Tables 3, 4). Four of the deceased patients were diagnosed with both immunological and non-immunological comorbidities. They were:

- Patient 1: bronchial asthma, chronic sensorimotor polyneuropathy, diabetes, arterial hypertension, obesity, hyperlipidemia, condition after lung abscess treatment;
- Patient 2: inflammatory bowel disease, malabsorption syndrome, cachexia;
- Patient 3: chronic urticaria, iron deficiency anemia, chronic esophagitis, pituitary tumor, hyperprolactinemia;
- Patient 4: autoimmune thyroiditis, sarcoidosis, hypertension, chronic sinusitis.

One patient deceased was diagnosed with immune-related comorbidities (bronchial asthma and Hashimoto thyroiditis). The last deceased patient suffered from non-immune accompanying diseases: chronic otitis media, audiological complications, hyperhomocysteinemia, and profound vitamin D deficiency (Table 2).

Discussion

Since the beginning of the pandemic, there have been many publications on COVID-19 in IEI patients, which include the

TABLE 4 Analysis of complications and deaths depending on selected diagnoses and comorbidity.

	Whole group	Children	Adults	p-value
Severe course (complications and/or death)				
All diagnoses/any event:	22/155 (14.2%)	7/105 (6.7%)	15/50 (30.0%)	**<0.001**
Diagnosis:				
Humoral defect	21/103 (20.4%)	6/56 (10.7%)	15/47 (31.9%)	**0.013**
XLA	4/10 (40.0%)	0/2 (0.0%)	4/8 (50.0%)	0.467
CVID	9/39 (23.1%)	1/13 (7.7%)	8/26 (30.8%)	0.225
≥2 comorbidities	14/49 (28.6%)	1/21 (4.8%)	13/28 (46.4%)	**0.001**
Type of complications:				
Respiratory failure	8/155 (5.2%)	0/105 (0.0%)	8/50 (16.0%)	<0.001
Pulmonary complications	6/155 (3.9%)	0/105 (0.0%)	6/50 (12.0%)	<0.001
Other	14/155 (9.0%)	7/105 (6.7%)	7/50 (14.0%)	0.146
Death				
All diagnoses:	6/155 (3.9%)	0/105 (0.0%)	6/50 (12.0%)	**<0.001**
Humoral defect	6/103 (5.8%)	0/56 (0.0%)	6/47 (12.8%)	**0.008**
XLA	1/10 (10.0%)	0/2 (0.0%)	1/8 (12.5%)	1.0
CVID	4/39 (10.3%)	0/13 (0.0%)	4/26 (15.4%)	0.281
≥2 comorbidities:	6/49 (12.2%)	0/21 (0.0%)	6/28 (21.4%)	**0.031**

Bold values indicate a statistically significant value.

descriptions of single or several cases, reports of SARS-CoV-2 infections identified in individual centers or countries, and presentations of data collected as part of international projects (6, 7, 9–11). Some of them were meta-analyses (4, 12). Much of the information was related to adult patients, while only individual publications exclusively concerned children (13–15). Our own analysis shows a relatively large group of patients with IEI (155 people) diagnosed with SARS-CoV-2 infection. The study group was dominated by children (67.7%). This, however, does not prove a higher incidence of SARS-CoV-2 infections in the pediatric population with IEI in Poland. In the authors' opinion, it is the result of more effective identification of children with IEI and COVID-19 than in the corresponding adult population. In Poland, the network of pediatric care centers providing care for patients with IEI is better developed compared to internal medicine centers. The lack of an effective, nationwide system of registration of patients with IEI does not make the task easier.

According to the summaries of the ESID registry from 2014, IEI with predominantly antibody defects were diagnosed in 56.7% of the reported people (16). The distribution of individual IEI diagnoses in the study group was typical for European populations, in which IEI with predominantly antibody defects prevail. However, the distribution may be different depending on the population (17–20).

The reason for testing for SARS-CoV-2 infection was slightly different in adults compared to children. In adults, the indication concerned almost exclusively the symptoms of the disease (92%), while in approximately one-fourth of children, the tests were conducted based on epidemiological premises or before planned admission to hospitals. This was partially due to the fact that most of the adult patients had the symptoms of infection, while in approximately 25% of children, the course of the infection was asymptomatic. In Poland, many patients from the pediatric group were tested at the request and expense of their parents. Therefore, in this population, cheaper serological tests were performed significantly more often than in adults (48.6% vs. 6%). In 94% of adults, the diagnosis was made on the basis of PCR tests, which are considered the gold standard in diagnostics, especially in patients with predominantly antibody defects (12).

In the presented group, all symptoms considered typical for COVID-19 occurred significantly more often in adults. The most persistent ones were fever and cough. Taste and smell disorders were rare in children. The other less characteristic symptoms of COVID-19 were also more common in adults than in children. Similar results were obtained in other studies, although the typical comparative analyses of clinical picture pediatric population and in adults were not found in the available literature (7, 12, 21).

At the beginning of the pandemic, IEI patients appeared to be at a high risk of developing COVID-19. However, it soon turned out to be entirely true. One of the largest meta-analyses of 649 patients with IEI and COVID-19 showed that the diagnosis of IEI is generally not a risk factor for a severe course of the disease. However, individual IEI groups may be associated with a higher risk of a severe course and death. These include combined immunodeficiencies, immune dysregulation syndromes, and certain defects of the innate immune system, especially those related to the type I interferon–associated immune response (4). Humoral immune defects definitely dominated in the group presented here. It is not surprising then that in 74.8% of patients the course of COVID-19 was asymptomatic or mildly symptomatic. In an interesting summary of publications on COVID-19 in patients with IEI by Quinti et al., the course was asymptomatic or mildly symptomatic in a smaller percentage of patients (48%), even that the most of the analyzed publications

concerned adults. Our data limited to adults only compared with Quinti's seem to be almost identical (46% vs. 48%) (12). Different results were obtained in a Czech study of 81 patients with IEI and COVID-19. In this group, the asymptomatic course concerned almost the same percentage of patients as in presented analysis (21% vs. 20%), but the risk of seriously severe COVID-19 was 2.3 times higher than in the general population. However, taking into account the fact that the mean age of the population was >42 years while in our study—19 years), it can be assumed that the vast majority of the analysis concerned elderly adults, which is itself a risk factor (21).

It was observed very early that the course of SARS-CoV-2 infection in adults is much more serious than in children (5). Own study confirmed that this trend also applies to IEI patients. The vast majority of IEI children were asymptomatic or mildly symptomatic (88.6%), while 56% of adults presented moderate-to-severe symptoms. Other researchers (7, 10, 11, 14, 22, 23) made similar observations. Delavari et al. observed a severe course of COVID-19 in children (6). However, it is worthy to note a different spectrum of IEI diagnoses in reported Iranian population. In total, 10 out of 19 described patients were children with combined immunodeficiencies before hematopoietic stem cell transplantation. Predominantly antibody defects occurred only in four patients. It is not surprising that more than 56% of patients in this group had a severe course of SARS-CoV-2 infection (6).

No generally accepted standard of treatment for patients with IEI and COVID-19 has been developed so far. Only single reports described the efficacy of convalescent plasma or specific monoclonal antibody cocktails against the viral spike protein in combination with antiviral drugs (remdesivir) at an early stage of SARS-CoV-2 infection. However, most often, patients with IEI and COVID-19 were treated in line with local guidelines (12, 21, 24). The treatment used in hospitalized patients is often the subject of analysis in the available literature, but there are usually no data on home therapy (6, 7, 12, 21). In the presented group, systemic antibiotic therapy was the most common therapeutic option used in symptomatic patients. In the pediatric group, apart from symptomatic treatment, it was almost the only therapy used and concerned 14.3% of patients. Antibiotic therapy was significantly more often used in adults (60%), which additionally emphasizes a more serious course of the disease in this population. The need for antibiotic therapy in some children and most adult patients with IEI and COVID-19 was demonstrated by other authors (6, 7, 12, 24). The remaining therapies were used almost exclusively in adults. At that time, Polish patients did not have access to monoclonal antibodies specifically blocking the SARS-CoV-2 spike protein.

One of the most important aspects of caring for patients with IEI and COVID-19 is the analysis of complications and deaths. It should constitute the basis for defining the risk factors of a serious course and an attempt to develop the principles of prophylaxis and treatment, especially at the beginning of the disease, which would prevent it from worsening. In the available literature, there are little unambiguous data indicating specific risk factors related to a type of immunodeficiency, the comorbidities of IEI, and the treatment used. IEI groups were defined, which are clearly associated with a high risk of a poor prognosis, as mentioned above.

Among people diagnosed with SCID, CID, immune dysregulation syndromes, and type I interferon defects, the risk of complications and death seems to be independent of age and may also affect children (4, 12). In the presented pediatric group, no deaths were reported despite the diagnosis of combined immunodeficiencies and autoinflammatory syndromes in 37% of patients. However, two patients (1.9%) were diagnosed with MIS-C. The obtained results may indicate a much-higher risk of this complication than in the general pediatric population. In the American analysis of the epidemiology of MIS-C in the population up to 21 years of age, among white people, it was estimated that this diagnosis concerns an average of 110 per 1 million people infected with SARS-CoV-2, which is 0.011% (25). In the early stage of the pandemic, there were reports that the risk of complications and death is higher in patients with CVID compared to patients with XLA (9). The studies conducted by Cohen in the group of patients with CVID did not confirm a higher risk of a serious course of COVID-19 in this population (26). The currently available meta-analyses show that the risk of a complicated course of COVID-19 is the same in the entire IEI group with predominantly antibody defects as in the separated subgroup of CVID or XLA (14% each), and the risk of death is very similar (8%, 9%, and 8%) (4). In the analysis of Bucciol et al., the pediatric and adult subgroups were not separated, which allows only for their comparison with our own results obtained for the entire cohort: for all IEI, the percentage of patients with complications and/or death was 14.2%, for CVID 23.1%, and for XLA 40%. The analysis of only deaths compared to the results presented by Bucciol et al. shows that in the entire analyzed group, the percentage was lower (3.9%), while it was very similar for patients with CVID (10.3%) and XLA (10%) (4). In the study by Sheidel et al., in the group of patients with both CVID and XLA, there were only adults; hence, the data were compared with our own results obtained in patients >18 years of age. Mortality associated with SARS-CoV-2 infection in patients with CVID in the British study was slightly higher than in our own study (18.3% and 15.4%, respectively) and significantly lower in patients with XLA (7.7% vs. 12.5%, respectively). However, in our own study, there were only 8 adult patients diagnosed with XLA and 26 in the British group (24).

Our study indicates a significant role of comorbidities in the severe course in COVID-19 and/or fatal outcome in IEI patients (complicated course including death—46.4%, death only—21.4%). This is consistent with the data on the general population, as well as many studies on IEI patients (5, 7, 24). The recently published study by Shields et al. showed that an additional risk

factor for serious complications and death from COVID-19 in people with IEI is lymphopenia diagnosed in patients before infection (24). This parameter was not analyzed in our study.

Patients with IEI, especially those predisposing to the severe course of COVID-19, as well as those with comorbidities, have indications for preventive vaccination with the use of a booster dose (in Poland, ≥5 years of age). It is also a population in which an inadequate vaccine response is possible. Therefore, in the periods of special epidemiological threat and special health situations, it is recommended to use pre-exposure prophylaxis with the use of long-acting monoclonal antibodies (≥12 years of age), as well as the early implementation of anti-SARS-CoV-2 treatment. Recommendations regarding postexposure prophylaxis change, depending on the effectiveness of the available drugs in relation to the dominant variants of the SARS-CoV-2 virus (27).

Summary

The present study confirms that in patients with severe deficits of humoral immunity (CVID and XLA), this risk for a severe course of COVID-19 increases significantly with age. An important aggravating factor is comorbidity, especially in people >18 years of age. In children, regardless of the type of IEI, the risk of a severe course of COVID-19 is very low. However, the risk of developing MIS-C might be higher than in the general population—that requires further testing in larger groups of patients vs. general population. Therefore, the vaccination of both adults and children should be promoted. What will be their effectiveness in prevention and the impact on the course of the disease remain to be seen. It seems that our study might justify the recommendation to use SARS-CoV-2-specific post-exposure prophylaxis and early treatment with antiviral drugs, SARS-CoV-2-specific monoclonal antibodies, or convalescent plasma in adults, especially those with diagnosed CVID and XLA and comorbidities.

Strengths and limitations of the study

The lack of Polish registry makes it impossible to accurately determine the number of patients with IEI. In addition, patients with COVID-19 were not referred directly to immunologists but to primary care physicians and hospitals treating COVID-19. Moreover, data were not obtained from every center treating patients with IEI.

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 The Bioethics Committee, Collegium Medicum Bydgoszcz, Nicolaus Copernicus University Toruń, No. KB 327/2022. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

SK, MZ, and MP contributed to conception and design of the study, supervised the study process, and critically analyzed the manuscript for important intellectual content. SK, MZ, EG, RB, EB-S, MC, ME, EH-P, NK, AL-U, MM-T, AM-B, MM, KN-B, KP-Ś, AP-N, JRo, JRe, IR, AS-G, MS, HS, JS-G, AS-P, ST, EW-S, BW-K, KZ, and MP organized the database. SK wrote the original version of the manuscript. SK and ME reviewed the literature and drafted the manuscript. All authors contributed to manuscript revision and read 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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References

1. Available at: www.worldometers.info/coronavirus/ (Accessed 2022-05-23).

2. Babaha F, Rezaei N. Primary immunodeficiency diseases in COVID-19 pandemic: Apredisposing or protective factor? *Am J Med Sci* (2020) 360(6):740–1. doi: 10.1016/j.amjms.2020.07.027

3. Cotsapas C, Saarela J, Farmer JR, Scaria V, Abraham RS. Do monogenic inborn errors of immunity cause susceptibility to severe COVID-19? *J Clin Invest* (2021) 131(14):e149459. doi: 10.1172/JCI149459

4. Bucciol G, Tangye SG, Meyts I. Coronavirus disease 2019 in patients with inborn errors of immunity: Lessons learned. *CurrOpinPediatr* (2021) 33(6):648–56. doi: 10.1097/MOP.0000000000001062

5. Li J, Huang DQ, Zou B, Yang H, Hui WZ, Rui F, et al. Epidemiology of COVID-19: A systematic review and meta-analysis of clinical characteristics, risk factors, and outcomes. *J Med Virol* (2021) 93(3):1449–58. doi: 10.1002/jmv.26424

6. Delavari S, Abolhassani H, Abolnezhadian F, Babaha F, Iranparast S, Ahanchian H, et al. Impact of SARS-CoV-2 pandemic on patients with primary immunodeficiency. *J Clin Immunol* (2021) 41(2):345–55. doi: 10.1007/s10875-020-00928-x

7. Meyts I, Bucciol G, Quinti I, Neven B, Fischer A, Seoane E, et al. Coronavirus disease 2019 in patients with inborn errors of immunity: An international study. *J Allergy Clin Immunol* (2021) 147(2):520–31. doi: 10.1016/j.jaci.2020.09.010

8. Available at: https://www.ecdc.europa.eu/en/publications-data/data-virus-variants-covid-19-eueea.

9. Quinti I, Lougaris V, Milito C, Cinetto F, Pecoraro A, Mezzaroma I, et al. A possible role for b cells in COVID-19? Lesson from patients with agammaglobulinemia. *J Allergy Clin Immunol* (2020) 146(1):211–3. doi: 10.1016/j.jaci.2020.04.013

10. Esenboga S, Ocak M, Akarsu A, Bildik HN, Cagdas D, Iskit AT, et al. COVID-19 in patients with primary immunodeficiency. *J Clin Immunol* (2021) 41(7):1515–22. doi: 10.1007/s10875-021-01065-9

11. Castano-Jaramillo LM, Yamazaki-Nakashimada MA, O'Farrill-Romanillos PM, MuzquizZermeño D, Scheffler Mendoza SC, VenegasMontoya E, et al. COVID-19 in the context of inborn errors of immunity: A case series of 31 patients from Mexico. *J Clin Immunol* (2021) 41(7):1463–78. doi: 10.1007/s10875-021-01077-5

12. Quinti I, Mezzaroma I, Milito C. Clinical management of patients with primary immunodeficiencies during the COVID-19 pandemic. *Expert Rev Clin Immunol* (2021) 17(2):163–8. doi: 10.1080/1744666X.2021.1873767

13. Moazzen N, Ahanchian H, Aelami MH, Asiyon H, Astaneh M, Naeimi AM, et al. COVID-19 in children with inborn errors of immunity: Clinical scenarios. *Am J Clin Exp Immunol* (2021) 10(3):77–85.

14. Deyà-Martínez A, García-García A, Gonzalez-Navarro EA, Yiyi L, Vlagea A, Jordan I, et al. COVID-19 in children and young adults with moderate/severe inborn errors of immunity in a high burden area in pre-vaccine era. *Clin Immunol* (2021) 230:108821. doi: 10.1016/j.clim.2021.108821

15. Yilmaz Topal O, Metin A, KulhasCelik İ, Metbulut AP, Alim Aydin S, KanikYuksek S, et al. Clinical characteristics of COVID-19 in children and young adolescents with inborn errors of immunity. *Pediatr Allergy Immunol* (2022) 33(1):e13661. doi: 10.1111/pai.13661

16. Haucka F, Bangola B, Rakhmanov M, Klein HG, Klein Ch. Rational laboratory diagnostics of primary immunodeficiency disorders. *J Lab Med* (2015) 39(5):343–54. doi: 10.1515/labmed-2015-0050

17. El-Helou SM, Biegner AK, Bode S, Ehl SR, Heeg M, Maccari ME, et al. The German national registry of primary immunodeficiencies (2012-2017). *Front Immunol* (2019) 19(10):1272. doi: 10.3389/fimmu.2019.01272

18. Marschall K, Hoernes M, Bitzenhofer-Grüber M, Jandus P, Duppenthaler A, Wuillemin WA, et al. The Swiss national registry for primary immunodeficiencies: Report on the first 6 years' activity from 2008 to 2014. *Clin Exp Immunol* (2015) 182(1):45–50. doi: 10.1111/cei.12661

19. Abolhassani H, Kiaee F, Tavakol M, Chavoshzadeh Z, Mahdaviani SA, Momen T, et al. Fourth update on theIranian national registry of primary immunodeficiencies: Integration ofMolecular diagnosis. *J Clin Immunol* (2018) 38(7):816–32. doi: 10.1007/s10875-018-0556-1

20. Shendi HM, Al Kuwaiti AA, Al Dhaheri AD, Al-Hammadi S. The spectrum of inborn errors of immunity in the united Arab Emirates: 5 year experience in a tertiary center. *Front Immunol* (2022) 13:837243. doi: 10.3389/fimmu.2022.837243

21. Milota T, Sobotkova M, Smetanova J, Bloomfield M, Vydlakova J, Chovancova Z, et al. Risk factors for severe COVID-19 and hospital admission in patients with inborn errors of immunity - results from a multicenter nationwide study. *Front Immunol* (2022) 13:835770. doi: 10.3389/fimmu.2022.835770

22. Goudouris ES, Pinto-Mariz F, Mendonça LO, Aranda CS, Guimarães RR, Kokron C, et al. Outcome of SARS-CoV-2 infection in 121 patients with inborn errors of immunity: A cross-sectional study. *J Clin Immunol* (2021) 41(7):1479–89. doi: 10.1007/s10875-021-01066-8

23. Pieniawska-Śmiech K, Kuraszewicz A, Sado J, Śmiech K, Lewandowicz-Uszyńska A. Assessment of COVID-19 incidence and the ability to synthesise anti-SARS-CoV-2 antibodies of paediatric patients with primary immunodeficiency. *J Clin Med* (2021) 10(21):5111. doi: 10.3390/jcm10215111

24. Shields A, Anantharachagan A, Arumugakani G, Baker K, Bahal S, Baxendale H, et al. Outcomes following SARS-CoV-2 infection in patients with primary and secondary immunodeficiency in the UK. *Clin Exp Immunol* (2022) uxac008. doi: 10.1093/cei/uxac008

25. Payne AB, Gilani Z, Godfred-Cato S, Belay ED, Feldstein LR, Patel MM, et al. Incidence of multisystem inflammatory syndrome in children among US persons infected with SARS-CoV-2. *JAMA Netw Open* (2021) 4(6):e2116420. doi: 10.1001/jamanetworkopen.2021.16420

26. Cohen B, Rubinstein R, Gans MD, Deng L, Rubinstein A, Eisenberg R. COVID-19 infection in 10 common variable immunodeficiency patients in new York city. *J Allergy Clin Immunol Pract* (2021) 9(1):504–507.E1. doi: 10.1016/j.jaip.2020.11.006

27. COVID-19 Treatment Guidelines Panel. *Coronavirus disease 2019 (COVID-19) treatment guidelines*. (National Institutes of Health) (2022). Available at: https://www.covid19treatmentguidelines.nih.gov/Accessed 06-08-2022.

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Coronavirus disease 2019 vaccination uptake and hesitancy among Polish patients with inborn errors of immunity, autoinflammatory syndromes, and rheumatic diseases: A multicenter survey

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Data regarding the willingness of patients affected by inborn errors of immunity to accept vaccination against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection are limited. Therefore, this study assessed SARS-CoV-2 vaccination coverage and hesitancy in immunodeficient patients by surveying adults with primary immune deficiencies and autoinflammatory and rheumatic diseases on biologic therapy. The study was conducted from September 20, 2021, to January 22, 2022, when the primary coronavirus disease 2019 (COVID-19) vaccinations were available to all adults in Poland. We included 207 participants consecutively recruited from five referral centers (57% female; median age: 42.6 [range: 18–76, standard deviation ± 14.70] years). Overall, 55% (n = 114), 17% (n = 36), and 28% (n = 57) of the patients had primary immune deficiencies, autoinflammatory diseases, and rheumatic diseases, respectively. Among the entire cohort, 168 patients (81%) were vaccinated, and 82% were willing to receive a booster dose. Patients with autoinflammatory diseases had the highest vaccination rate (94.4%). A strong conviction that it was the correct decision (72%), fear of getting COVID-19 (38%), and expert opinions (34%) influenced the decision to vaccinate. Among the unvaccinated patients, 33.3% had primary or vocational education ($p < 0.001$). Furthermore, only 33% believed they were at risk of a severe

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course of COVID-19 (p = 0.014), and 10% believed in vaccine efficacy (p <0.001). They also doubted the safety of the vaccine (p <0.001) and feared a post-vaccination flare of their disease (p <0.001). Half of the unvaccinated respondents declared that they would consider changing their decision. Vaccination coverage in immunodeficient patients was higher than in the general Polish population. However, the hesitant patients doubted the vaccine's safety, feared a post-vaccination disease flare, and had primary or vocational education. Therefore, vaccination promotion activities should stress personal safety and the low risk of disease flares due to vaccination. Furthermore, all evidence must be communicated in patient-friendly terms.

KEYWORDS

inborn errors of immunity (IEI), autoinflammatory syndromes, autoimmune inflammatory diseases, COVID-19 vaccination, biologic treatment

Introduction

Mass prophylactic vaccinations are currently the only effective strategy against a severe course of coronavirus disease 2019 (COVID-19). Regulatory COVID-19 vaccine trials generally excluded immunocompromised patients, enrolling only a few with cancer or autoimmune diseases (1). Therefore, this group of patients may have a conflicted attitude towards vaccination due to limited population-specific vaccine safety data. From the pandemic's outset, adult immunocompromised patients, including those with inborn errors of immunity (IEI), were identified as a vulnerable population with a high risk of developing severe COVID-19. Thus, they have been prioritized in the Polish vaccination program. Furthermore, in Poland, a negative attitude towards vaccinations has limited the possibility of achieving herd immunity (2–4). Between January and April 2021, the percentage of adult Poles who declared a negative attitude towards the COVID-19 vaccine and a lack of willingness to vaccinate against COVID-19 remained at a stable level of 31%, despite the implementation of educational programs, media campaigns, and vaccination promotions by public authorities and medical professionals (3). Data from October 2021 showed that only 53% of Poles were vaccinated with at least one dose, putting Poland in 23rd place for vaccination rates among countries in the European Union (5).

Studies have reported vaccine hesitancy in 13.4%, 19.4%, and 17.8% of patients with cancer, autoimmune diseases, and chronic lung diseases, respectively (6), despite expert opinions that the benefits of being vaccinated outweigh the limitations of available evidence for a specific high-risk group (7–10). Moreover, vaccine resistance appears to correlate with geographic location, country-specific regulations of vaccines availability, the proposed vaccination schedule, study methodologies, the study period in relation to the pandemic wave, and evolving vaccine safety and efficacy data (1, 11–16). For example, 54% of patients with autoimmune inflammatory rheumatic diseases (AIIRDs) initially accepted the idea of COVID-19 vaccinations (17). However, with time, self-reported vaccine acceptance among patients with AIIRDs increased from 62% in December 2020 to 94% in August 2021 (18). Finally, in 2022, 80% of patients with AIIRDs self-confirmed that they were vaccinated (19). However, the data based on online research reports may be overstated or biased due to an underrepresentation of persons with low income, lower education, or limited access to the internet, such as in rural areas. Moreover, vaccinated individuals have been more willing to complete vaccination surveys than unvaccinated individuals (19).

Emerging reports on vaccine safety and efficacy in the AIIRD and IEI populations should be sufficient to promote vaccination (20–23). However, nocebo-prone attitudes remain (24), and the daily vaccination pace has plateaued and even declined (25). Thus, understanding why those in high-risk groups continue to forego COVID-19 vaccinations is even more important.

In contrast to other chronic diseases and at-risk populations, vaccine hesitancy and willingness data is limited among those affected by IEI. For example, we only identified one Canadian study that used the SurveyMonkey Internet-based questionnaire platform conducted from April to May 2021 with a 40% response rate (26). Furthermore, the International Patient Organization for Primary Immunodeficiencies conducted a survey and presented the results during a November 2021 webinar organized by the European Society for Immunodeficiencies (ESID), but Polish patients were not represented (27).

Therefore, we addressed vaccine hesitancy among adult patients with IEI and autoinflammatory diseases (AIDs) in Poland, including those with AIIRDs treated with biologics, to clarify the

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attitude towards COVID-19 vaccination and explore differences between those with primary and secondary immunodeficiencies.

Materials and methods

Study population

We conducted an on-site survey on vaccination attitudes against COVID-19 among adult patients diagnosed with IEI and AIDs and patients with AIIRDs receiving targeted biologic therapy. All the included patients had scheduled in-person follow-up visits at reference centers. The study was performed from September 20, 2021, to January 22, 2022, primarily during the fourth COVID-19 wave of the pandemic. During this period, the number of reported daily deaths from COVID-19 in Poland was more than 500 (28). At the same time, the initial COVID-19 vaccination course was available to all adults in Poland, and in October 2021, the Polish Ministry of Health prioritized the third booster dose for select risk groups, including adult patients with significant immunodeficiencies independently of etiology (29).

Data collection

The survey included the following data: age, sex, clinical diagnosis, treatment, comorbidities (yes or no), COVID-19-related experiences, COVID-19 vaccination history, seasonal influenza vaccination history; COVID-19 vaccine booster intention (yes or no), and fears and expectations about the COVID-19 vaccination (Table 1). Also, the Brief Illness Perception Questionnaire was used to assess illness perception (30, 31). The supplementary materials contain the full-length questionnaire (Table S1).

Statistical analyses

The normality of the observed values was tested using the Shapiro-Wilk test. Continuous variables were analyzed using Mann-Whitney-U, Student's t, or Kruskal-Wallis tests. Categorical variables were analyzed using the Chi-square or Fisher's exact test. For all analyses, differences were considered statistically significant when the p-value was <0.05. Statistical analyses were performed using Statistica, version 13 (TIBCO Software Inc.).

Ethics statement

The Military Institute of Medicine Ethics Committee, Warsaw, Poland, approved this study (No. 31/WIM/2021). All patients provided written consent to collect and analyze their demographic and medical data.

Results

Population characterization

We invited 213 patients to participate; 6 patients (2%) refused (1 patient was diagnosed with primary immune deficiency, and 5 were diagnosed with chronic arthritis). Finally, we included 207 participants. The median age was 42.6 years (range: 18–76, mean 42 ± 14.70 years), and 118 were women (57%). The patients were recruited from five referral centers (four IEI centers and one rheumatology center with biologic treatments).

We included 114 patients (114/207, 55%) with IEI diagnosed based on the ESID guidelines, including common variable immunodeficiency (n = 53), agammaglobulinemia (n = 10), subclass deficiencies (n = 10), isolated immunoglobin A deficiency (n = 1), specific antibody deficiency (n = 1), unclassified hypogammaglobulinemia (n = 3), Wiskott-Aldrich syndrome (n = 1), autoimmune lymphoproliferative syndrome (n = 2), Nijmegen breakage syndrome (n = 1), Bloom syndrome (n = 2), and DiGeorge syndrome (n = 1). The most common treatment was human immunoglobulin replacement therapy (108/114 patients, 95%). In total, 98 (91%) and 10 (9%) of 108 patients underwent at-home subcutaneous immunoglobulin therapy and in-patient intravenous immunoglobulin, respectively. Only two brothers with X-linked agammaglobulinemia were familial cases among those with IEIs.

The AID group included 36 patients (36/207, 17%). The diagnoses included NLR family pyrin domain containing 3 (NLRP3)-related diseases (n = 12), tumor necrosis factor receptor-associated periodic syndrome (i.e., TRAPS, n = 9), familial Mediterranean fever (n = 3), mevalonate kinase deficiency (n = 2), undifferentiated systemic AIDs (n = 5), and Schnitzler syndrome (n = 5). Overall, 23 (61%) and 13 (39%) of 36 cases were sporadic and familial, respectively. The familial cases included five families: 1) a trio of two sisters and one of their daughters, 2) a trio of a mother and two daughters, 3) a trio of a mother with one son and one daughter, 4) a father and son pair, and 5) a mother and son pair. At the time of the survey, 32 of 36 patients with AIDs (89%) took anakinra, a short-lasting interleukin-1 inhibitor.

Finally, the AIIRD group included 57 patients (57/207, 28%), including confirmed rheumatoid arthritis (n = 18), ankylosis spondylitis (n = 30), psoriatic arthritis (n = 9), and juvenile idiopathic arthritis (n = 2). All were treated with biologic agents. The most common treatments were tumor necrosis factor-alpha inhibitors (n = 48, 84%) followed by interleukin-6 (n = 6, 10.5%) and interleukin-17 (n = 3, 5%) inhibitors.

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TABLE 1 Representative questions from the Vaccine Hesitancy Questionnaire.

Fear of SARS-Cov-2 infection was searched using the question
Do you think that you are at risk of severe COVID 19 course?
Yes
No
I do not know.
To address patients attitudes, opinions and hesitancy following questions were used:
What influenced your decision to vaccinate (multiple options can be chosen)?
My own opinion that it is correct decision
Fear of getting COVID-19
Expert opinion
Opinion of relatives and friends
Other
In your opinion vaccination against COVID-19 is safe?
Yes
Yes, but only for healthy people
I do not know
No.
In your opinion vaccination against COVID-19 is effective in persons with your disease?
Yes
I don't know
No
Are you afraid that vaccination against COVID-19 may flare/worsen your diseases?
Yes
No
I do not know
To address cocoon strategy we asked patients if in their opinion
Should COVID-19 vaccination be mandatory? (you can choose multiple answers)?
No.
Yes, for everyone
Yes, but only for selected professional groups
Yes, for people at risk of severe disease course

Overall (n = 207), 122 patients (60%) had other comorbidities or chronic conditions, but these did not differ among the groups (Table 2). Moreover, 53 patients (25%) lived in rural areas; 29 patients (14%) had primary and vocational education, 77 (37%) had secondary education, and 101 (49%) had higher-level education. Furthermore, 50% of the patients had been vaccinated against influenza, including 23.7% who were vaccinated annually; patients with AIIRDs had the lowest influenza vaccination rate (Table 2). Age, sex, education, living area, and COVID-19 history did not differ among the groups (Table 2), but their disease perception did; patients with AIIRDs anticipated the worst effects of the disease on their lives. Table 3 presents the Brief Illness Perception Questionnaire results and comparisons.

COVID-19 history and vaccination against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)

In total (n = 207), 62 patients (30%) suffered from COVID-19, including: 34 patients (29%) with IEIs, 10 (27%) with AIDs, and 20 (35%) with AIIRDs. Of these patients, 10 (17%) were hospitalized due to COVID-19 (8 with IEIs and 2 with AIIRDs). One patient with AIDs become SARS-CoV-2-positive during hospitalization due to a Schnitzler syndrome flare.

Moreover, 168 patients (81.2%) were vaccinated (IEI: n = 89 7,8.1%; AID: n = 34, 94.4%; and AIIRD: n = 45, 78.9%); the proportion of vaccinated patients did not differ among the groups (Table 2). The participants were vaccinated with BNT162b2 (n =126, 75.0%), mRNA-1273 (n = 16, 9.5%), AZD1222 (n = 15, 8.9%), and Ad26.COV2.S (n = 10, 6.0%). One response was missing. Side effects occurred in 59 patients (35.1%). Three severe events occurred in 3 patients, all of which had AIDs; 1 patient was hospitalized due to prolonged fever, 1 patient was observed in the emergency unit due to convulsions, and 1 had facial palsy requiring long-term rehabilitation. The other events were mild, moderate, and local-side reactions.

The decision to vaccinate was influenced by the strong conviction that receiving the vaccination was the correct decision (n = 121, 72.0%), a fear of getting COVID-19 (n = 78, 46.4%), expert opinions (n = 71, 42.3%), and the opinion of relatives (n = 15, 8.9%). The responders could provide multiple answers. Most vaccinated participants were willing to receive the booster dose (n = 132, 82%). Overall, 110 of 207 patients (53.1%) believed vaccination against COVID-19 should be obligatory.

Unvaccinated and vaccinated patient comparisons

We compared the unvaccinated (n = 39) and vaccinated (n = 168) patients. Among the unvaccinated individuals, 13 (33.3%), 12 (30.8%), and 14 (35.9%) patients had primary or vocational education, secondary education, and higher education, respectively. Among the vaccinated individuals, 16 (9.5%), 65 (38.7%), and 87 (51.8%) patients had primary or vocational education, secondary education, and higher education, respectively. The number of individuals with primary or vocational education significantly differed between the unvaccinated and vaccinated groups ($p < 0.001$).

Significantly fewer unvaccinated patients believed they were at risk for a severe course of COVID-19 (n = 13, 33.3% vs. n = 92, 56.7%, p = 0.014). Most unvaccinated patients did not believe that vaccine was effective given their condition (n = 35, 89.7%);

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TABLE 2 Comparisons of select features among the study groups.

	Total (N = 207)	Analyzed groups according to diagnosis				Vaccination status		
		AID (N = 36)	IEI (N = 114)	AIIRDs (N = 57)	p	NO (N = 39)	YES (N = 168)	P
Age Mean ± SD	42.6 ± 14.8	40.6 ± 14.2	41.4 ± 15.3	46.3 ± 13.6	0.064	38.6 ± 15.8	43.6 ± 14.4	0.053
Education								
Primary	9 (4.3 %)	2 (5.6 %)	6 (5.3 %)	1 (1.8 %)	0.061	6 (15.4 %)	3 (1.8 %)	**<0 .001**
Vocational	20 (9.7 %)	6 (16.7 %)	13 (11.4 %)	1 (1.8 %)		7 (17.9 %)	13 (7.7 %)	
Secondary	77 (37.2 %)	13 (36.1 %)	45 (39.5 %)	19 (33.3 %)		12 (30.8 %)	65 (38.7 %)	
Higher	101 (48.8 %)	15 (41.7 %)	50 (43.9 %)	36 (63.2 %)		14 (35.9 %)	87 (51.8 %)	
Sex								
Female	118 (57.0 %)	18 (50.0 %)	63 (55.3 %)	37 (64.9 %)	0.314	20 (51.3 %)	98 (58.3 %)	0.423
Male	89 (43.0 %)	18 (50.0 %)	51 (44.7 %)	20 (35.1 %)		19 (48.7 %)	70 (41.7 %)	
Residence								
City	154 (74.4 %)	25 (69.4 %)	84 (73.7 %)	45 (78.9 %)	0.573	28 (71.8 %)	126 (75.0 %)	0.679
Village	53 (25.6 %)	11 (30.6 %)	30 (26.3 %)	12 (21.1 %)		11 (28.2 %)	42 (25.0 %	
Co-morbidities								
Missing response	2 (1.0 %)	0 (0.0 %)	2 (1.8 %)	0 (0.0 %)	0.102	0 (0.0 %)	2 (1.2 %)	0.125
No	83 (40.1 %)	18 (50.0 %)	37 (32.5 %)	28 (49.1 %)		21 (53.8 %)	62 (36.9 %)	
Yes	122 (58.9 %)	18 (50.0 %)	75 (65.8 %)	29 (50.9 %)		18 (46.2 %)	104 (61.9 %)	
Do you think that you are at risk of severe COVID 19 course?								
Missing response	6 (2.9 %)	2 (5.6 %)	2 (1.8 %)	2 (3.5 %)	0.111	0 (0.0 %)	6 (3.6 %)	**0.017**
No	96 (46.4 %)	22 (61.1 %)	48 (42.1 %)	26 (45.6 %)		26 (66.7 %)	70 (41.7 %)	
Yes	105 (50.7 %)	12 (33.3 %)	64 (56.1 %)	29 (50.9 %)		13 (33.3 %)	92 (54.8 %)	
Did you suffer from COVID-19 disease?								
No	142 (68.6 %)	25 (69.4 %)	80 (70.2 %)	37 (64.9 %)	0.612	24 (61.5 %)	118 (70.2 %)	0.138
Yes, at home	51 (24.6 %)	9 (25.0 %)	24 (21.1 %)	18 (31.6 %)		10 (25.6 %)	41 (24.4 %)	
Yes, in hospital	11 (5.3 %)	1 (2.8 %)	8 (7.0 %)	2 (3.5 %)		3 (7.7 %)	8 (4.8 %)	
I do not know	3 (1.4 %)	1 (2.8 %)	2 (1.8 %)	0 (0.0 %)		2 (5.1 %)	1 (0.6 %)	
Have your family members/housemates suffered from COVID-19?								
No	121 (58.5 %)	18 (50.0 %)	69 (60.5 %)	34 (59.6 %)	0.288	19 (48.7 %)	102 (60.7 %)	0.274
Yes, at home	78 (37.7 %)	16 (44.4 %)	39 (34.2 %)	23 (40.4 %)		19 (48.7 %)	59 (35.1 %)	
Yes, in hospital	8 (3.9 %)	2 (5.6 %)	6 (5.3 %)	0 (0.0 %)		1 (2.6 %)	7 (4.2 %)	
Have you been vaccinated against influenza?								
Sometimes	56 (27.1 %)	12 (33.3 %)	35 (30.7 %)	9 (15.8 %)	**<0 .001**	9 (23.1 %)	47 (28.0 %)	**<0 .001**
Never	102 (49.3 %)	19 (52.8 %)	43 (37.7 %)	40 (70.2 %)		29 (74.4 %)	73 (43.5 %)	
Yes, yearly	49 (23.7 %)	5 (13.9 %)	36 (31.6 %)	8 (14.0 %)		1 (2.6 %)	48 (28.6 %)	

Bold means that they are statistically significant.

this attitude significantly differed from the vaccinated individuals (n = 119, 70.8%; p <0.001).

When asked if vaccination is safe, significantly more unvaccinated individuals answered "No or I don't know" (n = 29, 74.4%) than vaccinated individuals (n = 39, 23.2%; p <0.001). More unvaccinated than vaccinated individuals believed that the vaccine was only safe for healthy individuals (n = 8, 20.5% vs. n = 3, 1.8%; p <0.001). Furthermore, significantly more unvaccinated individuals feared a disease flare after vaccination than vaccinated individuals (n = 30, 76.9% vs. n = 13, 7.7%; p <0.001).

In the unvaccinated group, 44% of respondents did not answer the question about what drove their vaccination decision compared to 0.6% of vaccinated respondents. In addition, half of the unvaccinated patients declared they could change their minds in the future. Sex, age, residence, COVID-19 history, comorbidities, treatment regimen, and disease perception did not differ between the unvaccinated and vaccinated groups.

Discussion

In this study, 80% of patients with IEI, AIDs, and AIIRDs were vaccinated against COVID-19 compared to 54% in the general Polish population. The difference is even more striking when considering the mean age (32, 33). We confirmed that the COVID-19 vaccine acceptance rate was similar among the IEI,

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TABLE 3 Brief Illness Perception Questionnaire result comparisons among the study groups.

	Total (N = 207)	Analyzed groups according to diagnosis				Vaccination status		
		AID (N = 36)	IEI (N = 114)	AIIRDs (N = 57)	p	NO (N = 39)	YES (N = 168)	p
How much does your illness affect your life?	5.671 ± 2.65	5.889 ± 2.785	5.237 ± 2.594	6.404 ± 2.542	**0.029**	5.821 ± 3.025	5.637 ± 2.565	0.689
How long do you think your illness will continue?	9.541 ± 1.36	8.944 ± 2.216	9.746 ± 0.860	9.509 ± 1.390	0.157	9.436 ± 1.071	9.565 ± 1.421	0.075
How much control do you feel you have over your illness?	5.594 ± 2.79	6.167 ± 3.402	6.035 ± 2.566	4.351 ± 2.416	**<0 .001**	5.487 ± 2.470	5.619 ± 2.860	0.740
How much do you think your treatment can help your illness?	7.304 ± 2.18	7.889 ± 2.240	7.342 ± 2.173	6.860 ± 2.108	**0.024**	7.205 ± 2.142	7.327 ± 2.198	0.646
How much do you experience symptoms from your illness?	5.005 ± 2.53	4.639 ± 3.006	4.746 ± 2.551	5.754 ± 1.994	0.052	5.128 ± 2.697	4.976 ± 2.498	0.470
How concerned are you about your illness?	4.643 ± 2.98	5.250 ± 3.219	3.851 ± 2.810	5.842 ± 2.691	**<0 .001**	4.641 ± 2.969	4.643 ± 2.990	0.970
How well do you feel you understand your illness?	7.213 ± 2.43	6.833 ± 2.864	7.333 ± 2.349	7.211 ± 2.320	0.745	7.103 ± 2.521	7.238 ± 2.418	0.768
How much does your illness affect you emotionally? (e.g. does it make you angry, scared, upset or depressed)?	4.807 ± 2.82	4.667 ± 2.704	4.404 ± 2.793	5.702 ± 2.784	**0.020**	5.179 ± 3.292	4.720 ± 2.700	0.351

Bold means that they are statistically significant.

AID, and AIIRD groups. Furthermore, we found that vaccine hesitancy was primarily due to doubts about the vaccine's efficacy, safety, and flares of their underlying disease. Moreover, the decision not to vaccinate strongly correlated with primary or vocational education. However, half of the unvaccinated patients declared that they would consider changing their opinion on vaccinations.

Our results are similar to the Canadian study of IEI patients and a study that included patients with AIIRDs (15, 26). In our study, patients with AIDs had the highest vaccination rate (94%), but we could not identify a previous study targeting this patient population. Therefore, despite this group's limited data on prophylactic vaccination, a high COVID-19 vaccination acceptance rate among patients with AIDs occurred (34, 35). Moreover, flares of NLRP3-related AIDs were documented after the pneumococcal vaccine (35). We propose that familial aggregation of monogenic AIDs and similar decisions among familial cases contributed to the high vaccination rate in this group. This result may also be attributed to the policies and experience of the patient's referral centers, where the benefits and risks of vaccination were discussed in a personalized way and supported by the health care provider's expertise. In our study, only 50% of all patients received an influenza vaccine, with the lowest uptake among patients with AIIRDs (30%), consistent with other studies (36). However, in Poland, seasonal influenza vaccine administration is extremely low in the general population, with only 6% and 9% of individuals undergoing vaccination in the 2020/2021 and 2021/2022 seasons, respectively (37, 38).

In this study, 30% of patients answered that the experts' opinions were important for their decision to be vaccinated. This agrees with the study in patients with AIIRDs, where 34% of vaccinated individuals attributed their decision to advice from their physician (18), Trusting relationships with physicians can decreases vaccine hesitancy (39) by stressing the personal benefits (40). This finding emphasizes the importance of vaccination-specific counseling to improve COVID-19 vaccine coverage, which might also be relevant for other vaccines, such as influenza or pneumococcal (41). However, pharmacist-physician

coaching (42) might be ineffective among patients with ultrarare and rare diseases since they generally seek professional advice from a specialist before receiving any vaccines.

Our data on vaccine hesitancy also agrees with the Canadian study results that reported the primary reason for vaccine hesitancy was uncertainty of the benefits (26). However, other studies reported safety concerns among patients with IEI or AIIRDs. Some studies evaluated the specific patient concerns related to the COVID-19 vaccine, which included unknown long-term side effects, the newness of the COVID-19 vaccine, and the perception of rushed development and introduction with potential financial links to the pharmaceutical companies (9, 18, 24, 26). Those unvaccinated also feared that the vaccine would be harmful and could cause thrombosis (24), despite well-documented contrary evidence (43). Furthermore, a concern about potential side effects is a well-documented primary argument against COVID-19 vaccination among the general Polish population (3) and health care workers, especially nurses (4).

Flares of the existing disease due to prophylactic vaccination is another strong belief and stereotype among patients with systemic autoimmune diseases. Both patients and health care professionals present concerns about interactions with immunosuppressive treatment regimens or the underlying immune-mediated inflammatory disease. Consequently, these arguments become a more prominent reason for doubt or refusal over time (24, 41).

In our study, the decision not to vaccinate strongly correlated with primary or vocational education. This result is supported by studies performed in different countries (6, 17, 24). In our opinion, this result underscores the necessity to communicate the scientific results and arguments for vaccination in patient-friendly language.

In contrast to other studies on patients with IEI, half of the unvaccinated participants in this study declared the possibility of changing their choice (26). A study performed before introducing the vaccination program in the Netherlands presented a "watch and wait" strategy for patients unsure about vaccination. According to public health records, this approach possibly reduced vaccine hesitancy (44); thus, we agree with this strategy. However, patient opinions towards vaccination should be periodically reevaluated to address the risk of nocebo-prone attitudes.

This study has some limitations. For example, we included a small number of patients. However, the included patients had rare conditions, and their diagnoses and immunodeficiency states were confirmed by health care professionals based on accepted criteria and not self-reported. Nonetheless, we used a self-prepared questionnaire and did not include questions addressing a nocebo-prone attitude, which may be important in a vaccine hesitancy analysis. We also only included patients within referral centers that strongly promote prophylactic vaccination against COVID-19 for high-risk groups, which may have increased the number of vaccinated respondents.

Conclusions

To our knowledge, this is the first study on vaccine hesitancy in patients with IEI from central Europe, including a considerable proportion of AIDs patients. Also, our study is unique because we performed on-site surveys on consecutive patients. Thus, we obtained a very high response rate from patients with rare diseases.

The percentage of vaccinated persons in each subgroup was higher than that in the general Polish population. Unvaccinated patients doubted the efficacy and safety of the vaccine and were afraid of flares of their underlying disease after vaccination but did not fear a severe course of COVID-19; approximately one-third had only primary or vocational education. Nonetheless, despite their hesitancy, half of the unvaccinated respondents declared the possibility of changing their decision. Our findings suggest that vaccine promotion activities should stress personal safety. Furthermore, patients must be informed about the low risk of disease flares due to vaccination, and all evidence must be updated and communicated in patient-friendly language. Finally, these findings emphasize the importance of vaccination-specific counseling to improve COVID-19 vaccine coverage, which might also be relevant for influenza and pneumococcal vaccinations.

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 The Military Institute of Medicine Ethics Committee, Warsaw, Poland. The patients/participants provided their written informed consent to participate in this study.

Author contributions

EW-S, MZ, and AB designed the study. EW-S wrote the first draft of the manuscript. EW-S, AB, MZ, AM-B, KN-B, and AF-G collected data and performed literature searches. MZ performed the statistical analyzes. EW-S, MZ, and KJ-R performed a critical revision of the manuscript for intellectual content. All authors have read and agreed to the published version of the manuscript.

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/fimmu.2022.1010899/full#supplementary-material

References

1. Duly K, Farraye FA, Bhat S. COVID-19 vaccine use in immunocompromised patients: A commentary on evidence and recommendations. *Am J Health Syst Pharm* (2022) 79(2):63–71. doi: 10.1093/ajhp/zxab344

2. Sowa P, Kiszkiel Ł, Laskowski PP, Alimowski M, Szczerbiński Ł, Paniczko M, et al. COVID-19 vaccine hesitancy in Poland–multifactorial impact trajectories. *Vaccines* (2021) 9(8):876. doi: 10.3390/vaccines9080876

3. Raciborski F, Jankowski M, Gujski M, Pinkas J, Samel-Kowalik P. Changes in attitudes towards the COVID-19 vaccine and the willingness to get vaccinated among adults in Poland: Analysis of serial, cross-sectional, representative surveys, January-April 2021. *Vaccines (Basel)* (2021) 9(8):832. doi: 10.3390/vaccines9080832

4. Lindner-Pawłowicz K, Mydlikowska-Śmigórska A, Łampika K, Sobieszczańska M. COVID-19 vaccination acceptance among healthcare workers and general population at the very beginning of the national vaccination program in Poland: A cross-sectional, exploratory study. *Vaccines (Basel)* (2021) 10(1):66. doi: 10.3390/vaccines10010066

5. *What share of the population has been partly or fully vaccinated against COVID-19?* . Available at: www.ourworldindata.com (Accessed 26 October 2021).

6. Tsai R, Hervey J, Hoffman K, Wood J, Johnson J, Deighton D, et al. COVID-19 vaccine hesitancy and acceptance among individuals with cancer, autoimmune diseases, or other serious comorbid conditions: Cross-sectional, Internet-based survey. *JMIR Public Health Surveill* (2022) 8(1):e29872. doi: 10.2196/29872

7. Bellanti JA. COVID-19 vaccines and vaccine hesitancy: Role of the allergist/immunologist in promotion of vaccine acceptance. *Allergy Asthma Proc* (2021) 42(5):386–94. doi: 10.2500/aap.2021.42.210063

8. Arvanitis M, Opsasnick L, O'Conor R, Curtis LM, Vuyyuru C, Yoshino Benavente J, et al. Factors associated with COVID-19 vaccine trust and hesitancy among adults with chronic conditions. *Prev Med Rep* (2021) 24:101484. doi: 10.1016/j.pmedr.2021.101484

9. Ali Z, Sarwar M, Ansar S, Awan UA, Ahmed H, Aftab N, et al. COVID-19 vaccination hesitancy in patients with autoimmune diseases: A mystery that needs an immediate solution! *J Med Virol* (2021) 93(9):5216–8. doi: 10.1002/jmv.27014

10. Available at: https://esid.org/COVID-19/Joint-statement-on-the-current-epidemics-of-new-Coronavirus.Oct31COVID19_Joint_StatementUpdate.pdf.

11. Machingaidze S, Wiysonge CS. Understanding COVID-19 vaccine hesitancy. *Nat Med* (2021) 27(8):1338–9. doi: 10.1038/s41591-021-01459-7

12. Felten R, Dubois M, Ugarte-Gil MF, Chaudier A, Kawka L, Bergier H, et al. Vaccination against COVID-19: Expectations and concerns of patients with autoimmune and rheumatic diseases. *Lancet Rheumatol* (2021) 3(4):e243–5. doi: 10.1016/S2665-9913(21)00039-4

13. Bellanti JA, Settipane RA. Vaccine preventable diseases, vaccine hesitancy, and COVID-19: A role for the allergist/immunologist. *Allergy Asthma Proc* (2021) 42(5):365–7. doi: 10.2500/aap.2021.42.210070

14. Rzymski P, Poniedziałek B, Fal A. Willingness to receive the booster COVID-19 vaccine dose in Poland. *Vaccines (Basel)* (2021) 9(11):1286. doi: 10.3390/vaccines9111286

15. Ko T, Dendle C, Woolley I, Morand E, Antony A. SARS-COV-2 vaccine acceptance in patients with rheumatic diseases: a cross-sectional study. *Hum Vaccin Immunother* (2021) 17(11):4048–56. doi: 10.1080/21645515.2021.1958611

16. Priori R, Pellegrino G, Colafrancesco S, Alessandri C, Ceccarelli F, Di Franco M, et al. SARS-CoV-2 vaccine hesitancy among patients with rheumatic and musculoskeletal diseases: a message for rheumatologists. *Ann Rheum Dis* (2021) 80(7):953–4. doi: 10.1136/annrheumdis-2021-220059

17. Gaur P, Agrawat H, Shukla A. COVID-19 vaccine hesitancy in patients with systemic autoimmune rheumatic disease: an interview-based survey. *Rheumatol Int* (2021) 41(9):1601–5. doi: 10.1007/s00296-021-04938-9

18. Boekel L, Hooijberg F, Besten YR, Vogelzang EH, Steenhuis M, Leeuw M, et al. COVID-19 vaccine acceptance over time in patients with immune-mediated inflammatory rheumatic diseases. *Lancet Rheumatol* (2022) 18. E310-3. doi: 10.1016/S2665-9913(22)00009-1

19. Putman M, Kennedy K, Sirotich E, Liew JW, Sattui SE, Moni TT, et al. COVID-19 vaccine perceptions and uptake: results from the COVID-19 global rheumatology alliance vaccine survey. *Lancet Rheumatol* (2022) 4:e237–40. doi: 10.1016/S2665-9913(22)00001-7

20. Furer V, Eviatar T, Zisman D, Peleg H, Paran D, Levartovsky D, et al. Immunogenicity and safety of the BNT162b2 mRNA COVID-19 vaccine in adult patients with autoimmune inflammatory rheumatic diseases and in the general population: a multicentre study. *Ann Rheum Dis* (2021) 80(10):1330–8. doi: 10.1136/annrheumdis-2021-220647

21. Gao XS, Zhu FC. Distinct immune response to CoronaVac in SARS-CoV-2 seropositive and seronegative patients with autoimmune rheumatic disease. *Lancet Rheumatol* (2022) 4(2):e77–8. doi: 10.1016/S2665-9913(21)00392-1

22. Haberman RH, Um S, Axelrad JE, Blank RB, Uddin Z, Catron S, et al. Methotrexate and TNF inhibitors affect long-term immunogenicity to COVID-19 vaccination in patients with immune-mediated inflammatory disease. *Lancet Rheumatol* (2022) 6:e384–7. doi: 10.1016/S2665-9913(22)00069-8

23. Amodio D, Ruggiero A, Sgrulletti M, Pighi C, Cotugno N, Medri C, et al. Humoral and cellular response following vaccination with the BNT162b2 mRNA COVID-19 vaccine in patients affected by primary immunodeficiencies. *Front Immunol* (2021) 12:727850. doi: 10.3389/fimmu.2021.727850

24. Fragoulis GE, Bournia VK, Mavrea E, Evangelatos G, Fragiadaki K, Karamanakos A, et al. COVID-19 vaccine safety and nocebo-prone associated hesitancy in patients with systemic rheumatic diseases: a cross-sectional study. *Rheumatol Int* (2022) 42(1):31–9. doi: 10.1007/s00296-021-05039-3

25. Ratzan S, Schneider EC, Hatch H, Cacchione J. Missing the point - how primary care can overcome covid-19 vaccine 'Hesitancy'. *N Engl J Med* (2021) 384(25):e100. doi: 10.1056/NEJMp2106137

26. Aberumand B, Ayoub Goulstone W, Betschel S. Understanding attitudes and obstacles to vaccination against COVID-19 in patients with primary immunodeficiency. *Allergy Asthma Clin Immunol* (2022) 18(1):38. doi: 10.1186/s13223-022-00679-x

27. Available at: https://esid.org/Working-Parties/Clinical-Working-Party/Grand-Rounds-2021-2022/Nov-2021-COVID-vaccination-in-PID-patients.

28. Available at: https://ig.ft.com/coronavirus-chart/?areas=eur&areas=pol&areasRegional=usny&areasRegional=usnm&areasRegional=uspr&areasRegional=ushi&areasRegional=usfl&areasRegional=usco&cumulative=0&logScale=0&per100K=1&startDate=2021-06-01&values=deaths.

29. Available at: https://www.gov.pl/web/zdrowie/komunikat-nr-14-ministra-zdrowia-w-sprawie-szczepien-przeciw-covid-19-dawka-przypominajaca-oraz-dawka-dodatkowa-uzupelniajaca-schemat-podstawowy.

30. Broadbent E, Petrie KJ, Main J, Weinman J. The brief illness perception questionnaire. *J Psychosomatic Res* (2006) 60(6):631–7. doi: 10.1016/j.jpsychores.2005.10.020

31. Nowicka-Sauer K, Banaszkiewicz D, Staśkiewicz I, Kopczyński P, Hajduk A, Czuszyńska Z, et al. Illness perception in polish patients with chronic diseases: Psychometric properties of the brief illness perception questionnaire. *J Health Psychol* (2016) 21(8):1739–49. doi: 10.1177/1359105314565826

32. Available at: https://vaccinetracker.ecdc.europa.eu/public/extensions/COVID-19/vaccine-tracker.html#age-group-tab.

33. Available at: https://covid19-country-overviews.ecdc.europa.eu/vaccination.html.

34. Jeyaratnam J, ter Haar NM, Lachmann HJ, Kasapcopur O, Ombrello AK, Rigante D, et al. The safety of live-attenuated vaccines in patients using IL-1 or IL-6 blockade: an international survey. *Pediatr Rheumatol* (2018) 16(1):19. doi: 10.1186/s12969-018-0235-z

35. Jaeger VK, Hoffman HM, van der Poll T, Tilson H, Seibert J, Speziale A, et al. Safety of vaccinations in patients with cryopyrin-associated periodic syndromes: a prospective registry based study. *Rheumatology* (2017) 56(9):1484–91. doi: 10.1093/rheumatology/kex185

36. Lejri-El Euchi H, Chirpaz E, Foucher A, Sultan-Bichat N, Randrianjohany A, Poubeau P, et al. Vaccination against influenza and pneumococcal infections in patients with autoimmune disorders under biological therapy: Coverage and attitudes in patients and physicians. *Eur J Internal Med* (2019) 69:25–31. doi: 10.1016/j.ejim.2019.08.010

37. Available at: https://szczepienia.pzh.gov.pl/faq/jaki-jest-poziom-zaszczepienia-przeciw-grypie-w-polsce/https://www.ecdc.europa.eu/en/seasonal-influenza/prevention-and-control/vaccines/vaccination-coveragehttps://www.statista.com/statistics/1086732/poland-number-of-influenza-vaccinations/https://www.vaccinestoday.eu/stories/polands-vaccine-scepticism-a-european-problem/.

38. Available at: https://www.statista.com/statistics/1086732/poland-number-of-influenza-vaccinations/https://www.vaccinestoday.eu/stories/polands-vaccine-scepticism-a-european-problem/.

39. Abrams EM, Shaker M, Sinha I, Greenhawt M. COVID-19 vaccines: addressing hesitancy in young people with allergies. *Lancet Respir Med* (2021) 9 (10):1090–2. doi: 10.1016/S2213-2600(21)00370-2

40. Saul H, Gursul D. Stressing the personal benefits of the covid-19 vaccine might encourage more people to accept it. *BMJ* (2021) 375:n2923. doi: 10.1136/bmj.n2923

41. Smerilli G, Cipolletta E, Moscioni E, Francioso F, Risa AM, Maccarrone V, et al. Correspondence on 'SARS-CoV-2 vaccine hesitancy among patients with rheumatic and musculoskeletal diseases: a message for rheumatologists'. *Ann Rheum Dis* (2021) 80(10):e168. doi: 10.1136/annrheumdis-2021-220586

42. Abdel-Qader DH, Hayajneh W, Albassam A, Obeidat NM, Belbeisi AM, Al Mazrouei N, et al. Pharmacists-physicians collaborative intervention to reduce vaccine hesitancy and resistance: A randomized controlled trial. *Vaccine X* (2022) 10:100135. doi: 10.1016/j.jvacx.2021.100135

43. Sen P RN, Nune A, Lilleker JB, Agarwal V, Kardes S, Kim M. COVID-19 vaccination-related adverse events among autoimmune disease patients: results from the COVAD study. *Rheumatol (Oxford)* (2022) 17:keac305. doi: 10.1093/rheumatology/keac305

44. Mouter N, de Ruijter A, Ardine de Wit G, Lambooij MS, van Wijhe M, van Exel J, et al. "Please, you go first!" preferences for a COVID-19 vaccine among adults in the Netherlands. *Soc Sci Med* (2022) 292:114626. doi: 10.1016/j.socscimed.2021.114626

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Case report: Severe combined immunodeficiency with ligase 1 deficiency and Omenn-like manifestation

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DNA ligase I deficiency is an extremely rare primary immunodeficiency with only 6 patients reported in the literature. Most common manifestations include radiosensitivity, macrocytic anemia, lymphopenia with an increased percentage of gamma-delta T cells, and hypogammaglobulinemia requiring replacement therapy. Two-month-old girl with delayed development, T-B-NK+ SCID, and macrocytic anemia presented features of Omenn syndrome. Whole exome sequencing revealed two novel, heterozygous variants (c.2312 G>A, p.Arg771Gly and c.776+5G>T, p.Pro260*) in the LIG1 gene (NM_000234.1). Hematopoietic stem cell transplantation from a fully matched unrelated donor was performed at the age of 4 months using GEFA03 protocol. Mixed donor-recipient chimerism was observed, with 60-70% chimerism in the mononucleated cell compartment and over 90% in T-lymphocyte compartment, but autologous myeloid recovery. Stable CD4+ and CD8+ T-cell counts above 200/μL were achieved after 2 months, but the patient remained transfusion-dependent. Despite satisfactory immunological reconstitution, the second transplantation due to constitutional hemolytic defect has been considered. In light of possible re-transplantation, an issue of optimal conditioning protocol with sufficient myeloid engraftment is important. For the first time Omenn syndrome is described in a compound heterozygote carrying two the novel variants p.Arg771Gly and p.Pro260* in the LIG1 gene. Patients diagnosed with SCID and Omenn syndrome showing macrocytic anemia, should be screened for DNA ligase I deficiency.

KEYWORDS

ligase 1, immunodeficiency, Omenn-like, hematopoietic stem cell transplantation (HCST), Immunoglobilins

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Introduction

DNA ligase I deficiency is an extremely rare autosomal recessive primary immunodeficiency, caused by mutations in *LIG1* gene located on chromosome 19. As result, Okazaki fragments are improperly catalyzed during cell replication and single-strand DNA damage repair. The disease is associated with a diverse spectrum of clinical symptoms beginning in infancy or early childhood (1). An increased susceptibility to infections, macrocytic anemia, lymphopenia, increased percentage of γδ T cells, hypogammaglobulinemia requiring replacement therapy, and increased sensitivity to DNA damaging agents have been reported in all patients (2–4). Most of the only six reported cases demonstrated normal mental development. Growth retardation or failure to thrive and delayed or absent sexual maturation have been observed in two patients. Phenotype of one of the patients with delayed sexual maturation resembled Bloom syndrome and the patient died at the age of 19 years due to lymphoma (2). Fibroblasts from this patient showed an increased sensitivity to DNA damage caused by alkylating agents and ionizing and UV radiation (5, 6). Five remaining patients had normal physical and mental development (4). Severe combined immunodeficiency (SCID) was diagnosed in two cases and treated with allogeneic hematopoietic stem cell transplantation (HSCT) with reduced intensity conditioning regimen (3). Omenn syndrome was suspected in one patient, but skin biopsy did not confirm the diagnosis (3, 4).

Case description

A female infant was born at 35 weeks of gestation due to the first uncomplicated pregnancy of 28 y.o. mother by vaginal delivery with birth weight 1950 g and the 10 minutes Apgar score 6. Parents were healthy, non-consanguineous. After birth she demonstrated respiratory problems and hepatosplenomegaly. Blood tests revealed macrocytic anemia since the first day of life, with hemoglobin concentration 6.9 G/dL at birth date [normal range for the age, (N: 14.9-23.7 G/dL], mean corpuscular volume (MCV) 133 fL [N: 100-125 fL], thrombocytopenia in the first week of life (minimum platelet count $49x10^3$/μL), and lymphopenia since the third day of life (4-16%; 0.2 – $1.3x10^3$/μL). Due to respiratory failure probably of non-infectious cause, she was treated with a non-invasive positive pressure ventilation for one day, with nasal continuous positive airway pressure for the next 3 days. Infection markers and microbiological diagnostics were negative but newbborn was treated with empirical antibiotics (ampicillin and amikacin). Due to anemia, the child received multiple transfusions of irradiated, filtered, packed red blood cells and 10 doses of erythropoietin. Platelet concentrate was transfused once due to thrombocytopenia. Intravenous immunoglobulins were administered twice without serum immunoglobulins level tests (see the timeline in Figure 1). The girl was discharged from the neonatal unit at 32 days of age, but after a week, at the age of 5 weeks, she was re-admitted due to anemia (Hb 7.3 G/L) and lymphopenia $0.7x10^3$/μL) with erythematous papular rash not observed in neonatal period. Vitamin B12 and folate deficiency have been excluded. Flow cytometry revealed deep lymphopenia with T lymphocyte count 30 cells/μL, CD19+ B lymphocytes 93 cells/μL, and NK CD3-CD56.CD16+ 196 cells/μL. Hypercellular bone marrow with sparse erythroblastic and a reduced lymphocytic line, without signs of malignant proliferation, were found in bone marrow biopsy (Figure 2A). Based on the obtained laboratory results severe combined immunodeficiency was suspected.

The infant was admitted to the Immunology Department, Children's Memorial Health Institute, Warsaw, at the age of 8 weeks, in moderate general condition. The patient showed generalized erythematous-small lobular skin eruptions, with exfoliation located mainly on the face (Figure 2B), conjunctivitis of *Staphylococcus aureus* origin, and enlarged

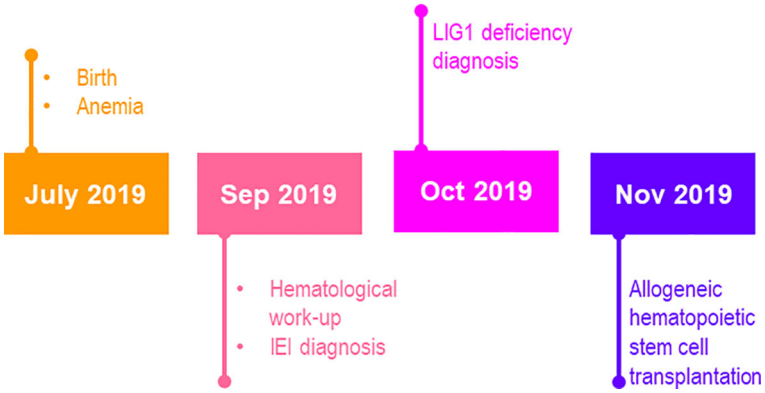

FIGURE 1
The timeline of case report.

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cervical and inguinal lymph nodes of up to 1 cm diameter. The size of spleen and liver were within the age-related normal ranges. The body length was below 3 percentile in relation to percentile growth charts for preterm infants at gestational age 32-37 weeks, but the body weight and head circumference were proportional to length. Systemic antibiotic therapy, palivizumab, and intravenous immunoglobulins (IVIG) have been introduced to treatment, while anti-viral, anti-fungal, anti-*Pneumocytis jiroveci* pneumonia (PJP) prophylaxis was continued. Due to anemia, the girl required red blood cell transfusions approximately every 2 weeks. During hospitalization, mild but chronic diarrhea and erosions around the anus were observed with a transient rotavirus positive stool result. Immunological tests were repeated and based on results severe combined immunodeficiency with decreased number of T, B and NK cells was diagnosed (Table 1).

Omenn syndrome was suspected due to erythematous-exfoliative skin lesions, peripheral lymphadenopathy, eosinophilia (7020/µl), despite low concentration of IgE (<2.00 kIU/L), and oligoclonal distribution of TCR Vβ chain (Figure 3A). Feto-maternal chimerism was excluded. Fibroblasts cultured from skin biopsy showed an intermediate sensitivity to ionizing radiation (Figure 3B) in comparison to fibroblasts from a patient with radiosensitive SCID due to Artemis deficiency. Histopathological evaluation of skin biopsy was not carried out due to small surface of typical skin lesions present mainly on head and face. Combined prednisone and cyclosporine A immunosuppressive treatment resulted in gradual improvement of skin condition and reduction of eosinophilia.

Whole exome sequencing of the patient's DNA was performed. The libraries were prepared using Twist Human Core Exome Plus Kit (Twist Bioscience) and sequenced on Illumina platform with a 100x mean mapped read depth. The results of genomic testing revealed presence of two rare heterozygous variants in the *LIG1* gene (NM_000234.1): a missense variant of uncertain significance c.2312 G>A, p.Arg771Gly with high pathogenicity scores, including DANN score (7) reaching 0.9995, and a likely pathogenic splice variant c.776+5G>T, p.Pro260*. In order to verify whether the c.776 +5G>T variant affected mRNA splicing, direct cDNA sequencing was executed. It was found that the splice-variant resulted in an insertion of 16 nucleotides in intron 9, leading to a premature protein synthesis termination at position 260 (Figure 3C). Direct sequencing of DNA of the child and her parents in search of the presence of both variants of *LIG1* was subsequently performed (Supplementary Table 1). It was found that each of the parents was a carrier of a single *LIG1* mutation, which confirmed that both *LIG1* variants are located in the proband on separate alleles. Datasets are submitted to the European Variation Archive (EVA) repository (https://www.ebi.ac.uk/eva/?eva-study=PRJEB*****) and are publicly accessible under project number PRJEB56316, accession numbers ERZ14199911 at EMBL-EBI.

Due to the immunological features of severe combined immunodeficiency, the girl was referred for HSCT from an

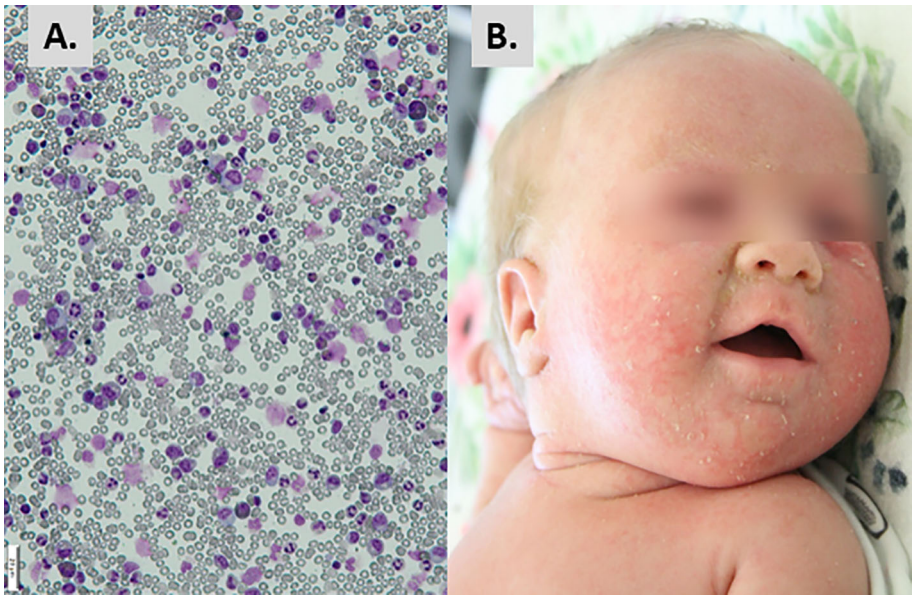

FIGURE 2
Initial presentation and diagnostic hallmarks. **(A)** Hypercellular bone marrow with suppressed erythroblastic lineage. **(B)** Erythematous-exfoliative skin lesions.

TABLE 1 Laboratory results at the time of diagnosis.

Parameter	Result	Normal range for the age
CD45+	313 cells/µl	(3800-8100 cells/µl)
CD3+	106 cells/µl	(2200-5500 cells/µl)
CD3+CD4+	87 cells/µl	1400-4200 cells/µl
CD3+CD8+	5 cells/µl	400-1400 cells/µl
CD19+	107 cells/µl	700-1800 cells/µl
CD56+	84 cells/µl	200-980 cells/µl
CD31+CD45RA+/CD3+CD4+	0%	50-74%
γδT%	18,9%	3,2-4,9%
IgG	5.96 g/l (1 day after IVIG)	3.36-10.5 g/l
IgM	<0.04 g/l	0.21-0.51 g/l
IgA	<0.07 g/l	<0.06-0.07 g/l
IgE	<2.00 kIU/L	
Mitogen response tests		
PHA	1705 ± 56 SI 11.0	>16000 cpm >65
anty-CD3	2494 ± 159 cpm, SI 16	>15000 cpm >60
Pansorbin	20646 ± 224 cpm SI 133	>2000 cpm >5
Microbiology		
Nasal swab	*Staphylococcus aureus MSSA, Klebsiella pneumoniae*	
Throat swab	Physiological flora	
Rectal swab	Physiological flora	
Fecal culture	Physiological flora, *Salmonella*, *Shigella* negative, Norovirus, adenovirus negative, rotavirus positive for 4 days,	
Conjunctival culture	*Staphylococcus aureus MSSA*	
Swab of the external auditory canal	*Staphylococcus aureus MSSA, Escherichia coli* ESBL/-/	
PCR in whole blood for CMV, EBV, HSV-1, HSV-2, Enterovirus, Human paraechovirus, HHV-6, HHV-7, Parvovirus B19	negative	
Serum Galactomannan, mannan	negative	

CMV, Cytomegalovirus; EBV, Epstein-Barr virus; HHV-6, Human herpesvirus 6; HHV-7, Human herpesvirus 7; HSV-1, Herpes simplex virus type 1; HSV-2 – Herpes simplex virus type 2; IgA – immunoglobulin A; IgE, immunoglobulin E; IgG, immunoglobulin G; IgM, immunoglobulin M; MSSA, methicillin-susceptible Staphylococcus aureus; PCR – polymerase chain reaction; PHA, phytohemagglutinin.

unrelated donor. At the age of 4 months, the girl was transplanted from a fully 10/10 HLA allele-matched unrelated donor using GEFA03 protocol, with intravenous busulfan at 0.5 mg/kg body weight (BW) given twice daily on days -4 and -3 (total dose 2 mg/kg), fludarabine i.v. at 30 mg/m2 daily from day -9 to day -4 (total dose 180 mg/m2), cyclophosphamide i.v. at 20 mg/kg daily on days -3 and -2 (total dose 40 mg/kg), and i.v. antithymocyte globulin (ATG)-Fresenius at 20 mg/kg BW daily from day -3 to day -1 (8).

Prophylaxis of a graft vs host disease (GVHD) was composed of cyclosporin A from -1 day and methotrexate on days +1, +3, +6. Peripheral blood progenitor cells in 14.67 x 10^6 CD34+ cells/kg BW dose were transplanted. Granulocytes recovered on +8 day after HSCT. From day +28, an isolated acute stage 2 skin GVHD in form of fine-grained rash on skin of the trunk and abdomen was diagnosed and treated with methylprednisolone. The patient developed mixed hematopoietic chimerism that stabilized at the level of 60-70% mononuclear cells of donor origin and more than 90% lymphocytes of donor origin (Supplemental Figure 1A). Immune reconstitution was regularly monitored (lymphocyte subset analysis results are shown on Supplemental Figure 1B), and the patient achieved stable CD4+ and CD8+ T-cell counts above 200 cells/mL after 2 months (Supplemental Figure 1C).

At the time of preparing of the manuscript, the girl has been followed-up for two years after HSCT. She is slightly delayed in physical and motor development. B-cell lymphopenia is observed permanently and the patient needs immunoglobulin therapy, as well as occasionally - red blood cells transfusions. The direct and indirect antiglobulin tests were negative, LDH

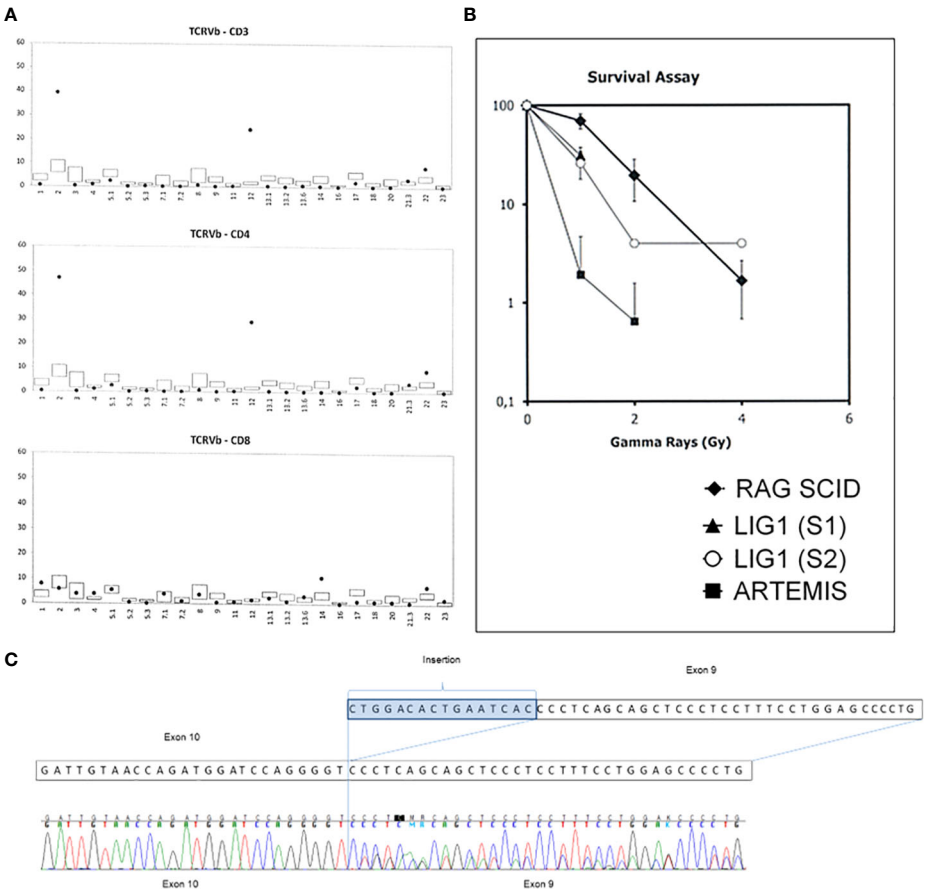

FIGURE 3
Diagnostic key-tests in the LIG1 deficiency patient. **(A)** Distribution of Vβ chain of the TCR receptor is oligoclonal with a significantly increased percentage of Vβ2 and Vβ12 chains among CD4 lymphocytes. **(B)** Clonogenic survival assay results. Fibroblasts cultured from skin biopsies of the patient and an Artemis-deficient SCID patient were exposed to increasing dose of irradiation and the percentage survival (axe Y) was determined after 8 days. S1- sample 1, S2- sample 2. RAG1 SCID patient sample was used as a control. **(C)** The cDNA sequencing results showing the splice site variant in the LIG1 which leads to an insertion of sixteen nucleotides in the exon 9 resulting in a shift of a reading frame and a premature protein termination at position 260.

activity, bilirubin direct and indirect results were within normal range. Due to anemia, the second transplantation has been considered, but the patient's parents did not consent to re-transplantation.

Discussion

The reported patient showed unique clinical features caused by presence of combined heterozygous mutation within the *LIG1* gene. The splice variant c.776+5G>T, p.Pro260* resulted in the insertion of 16 nucleotides in the intron 9 of *LIG1* leading to premature protein termination at position 260, and in consequence - to absence of functional domains of the protein. The missense variant (c. 2311G> T, p.Arg771Gly, R771G), which affected the structure of catalytic oligonucleotide/oligosaccharide binding-fold domain (OBD), was caused by defect located at the same position as reported in 4 other patients with *LIG1* deficiency (R771W), but associated with a different amino acid change (9, 10). However, both R771W and R641L variants decrease the activity of the DNA ligase (11, 12).

The biological function of ligase 1 in the replication and DNA repair is associated with sealing of Okazaki fragments during replication and catalyzing the ultimate ligation step of DNA repair (12). The cellular functions of LIG1 are mediated through its non-catalytic N-terminal domain (amino acids 1-261), that contains the nuclear localization signal and participates in protein–protein interactions (13).

DNA ligase I deficiency causes an extremely rare IEI with a diverse spectrum of clinical symptoms affecting not only immunity, but also growth, psychomotor development, and production of blood cells. The course of the disease in the

reported patient included severe anemia requiring multiple red blood cell transfusions since the first hours of life. It is unclear why mutations in the *LIG1* gene cause macrocytic anemia and other manifestations extending beyond immune deficiency, and whether any genotype-phenotype correlation exists. Macrocytic anemia probably results from an impaired DNA synthesis in hematopoietic precursor cells, yet in contrast to Fanconi anemia - it does not lead to bone marrow failure, but to hemolysis moderately compensated by reticulocyte production. Impaired DNA synthesis may also affect other rapidly dividing hematopoietic cells.

An IEI was for the first time suspected at the age of 8 weeks when absent T- and low B-lymphocyte counts were noted during work-up for constitutional hemolytic anemia. The diagnosis of T-B-NK+ SCID was similar to two other reported boys (3).

The presence of erythematous-exfoliating skin lesions suggestive of Omenn syndrome, due to uncontrolled proliferation of autologous T lymphocytes was a unique feature in the reported patient. The diagnosis was supported by clinical criteria of the ESID Registry, which state that probable diagnosis of Omenn's syndrome may be made when exfoliative erythroderma occurs in the first year of life and it is accompanied by at least one clinical symptom: lymphoproliferation, failure to thrive, chronic diarrhea, recurrent pneumonia, eosinophilia or elevated IgE with T-cell deficiency (low naive cells, reduced proliferation, oligoclonality), and with maternal engraftment and HIV infection excluded (14). Although there was no histopathological proof for such diagnosis, our patient was successfully treated as an Omenn syndrome with prednisone and cyclosporine: skin lesions, lymphadenopathy, and diarrhea disappeared, and the number of eosinophils normalized.

The essential consequence of *LIG1* defect is an impaired DNA repair mechanism. The reported patient's cells demonstrated an increased sensitivity to ionizing, UV radiation and DNA damaging agents *in vitro*, as in the first reported patient, or only to methyl methanosulphate, as in two SCID patients (4). Fibroblasts of our patient were sensitive to ionizing radiation and although sensitivity to other DNA damaging agents was not verified, it was considered as decisive information when HSCT procedure was planned. Based on the guidelines for HSCT in patients with radiosensitivity, and center experience - a reduced intensity conditioning protocol was administered. The peri-transplant period was relatively uneventful, mucosal toxicities were mild, and the patient was fed orally. However, the modified German Fanconi anemia protocol did not show sufficient myeloablative potential, as observed in ataxia-telangiectasia and Nijmegen breakage syndrome (15, 16).

Decreased pretransplant NK-cell counts can point out to exhaustion of common lymphoid progenitor compartment. At time of transplantation, the patient was profoundly lymphopenic and NK cell counts were not likely to affect the engraftment. It can not be ruled out, that suboptimal myelosuppression resulted in autologous recovery of NK cells and accelerated elimination of donor derived hematopoiesis. B- and NK-cell chimerism was not evaluated. The myeloid chimerism evaluation in CD15 positive cells was performed only once. All other chimerism measurements were carried up in mononuclear cells or in T-lymphocytes. It can be hypothesized, that vestigial donor hematopoiesis and myelopoesis (ca. 2% of all CD15 cells) are responsible for production of NK cells, and lack of competition from autologous lymphoid.

The reported experience with HSCT in *LIG1* deficiency suggested effectiveness of minimal intensity conditioning, but this was not confirmed in case of our patient. Both previously reported boys who underwent HSCT in Great Ormond Street Hospital (GOSH) from family donors,received minimal intensity conditioning: fludarabine 150 mg/m2, cyclophosphamide 1000 mg/m2, alemtuzumab 1 mg/kg and YTH 24/54 (anti-CD45 monoclonal antibodies) 800 µg/kg. Although the first patient received additionally YTH 24/54 anti-CD45 monoclonal antibodies in reduced dose due to adverse reaction during infusion, he achieved multilineage full donor chimerism. The second patient demonstrated low level B-cell engraftment, was transfusion-dependent, and remained on immunoglobulin replacement therapy. He also needed blood transfusions and chelation therapy (3).

Optimal conditioning protocol with sufficient myeloid engraftment is important in case of considered re-transplantation, but there is not enough data to determine the tolerance to chemotherapy regimens. In addition, despite limited long-term follow-up of patients with *LIG1* deficiency, an elevated risk of cancer incidence should be considered.

Conclusions

DNA ligase I deficiency should be considered in patients diagnosed with SCID associated with macrocytic anemia and features of Omenn syndrome. For the first time Omenn syndrome is described in a compound heterozygote carrying two novel variants p.Arg771Gly and p.Pro260* in the *LIG1* gene. HSCT is an option to cure LIG1 deficiency, however myeloid chimerism is required to control anemia and for sustained B-cell function, and an intensity of conditioning regimen remains questionable.

Data availability statement

The data presented in the study are deposited in the European Variation Archive (EVA) repository (https://www.ebi.ac.uk/eva/?eva-study=PRJEB56316), project number PRJEB56316, accession numbers ERZ14199911.

Ethics statement

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent to participate in this study was provided by the participants' legal guardian/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

ND-L, MP designed the concept of the manuscript. ND-L, AP, KB-P, BP, MU, MP wrote the manuscript with contribution from all co-authors. BP did the immunological studies AP, KB-P, MK did the genetic analysis. MB did the radiosensitivity of fibroblasts. ND-L, KB-S, EH-P, KG, KK, MU, MP contributed to clinical data collection and critical review. All authors contributed to the article and approved the submitted version.

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

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2022.1033338/full#supplementary-material

References

1. Online Mendelian Inheritance in Man (OMIM). **126391 Ligase I.* (1990). Available at: https://www.omim.org/entry/126391#contributors

2. Webster ADB, Barnes DE, Lindahl T, Arlett CF, Lehmann AR. Growth retardation and immunodeficiency in a patient with mutations in the DNA ligase I gene. *Lancet* (1992) 339(8808):1508–9. doi: 10.1016/0140-6736(92)91266-B

3. Silva JMF, Jones A, Sibson K, Bibi S, Jeggo P, Woodbine L, et al. Haematopoietic stem cell transplantation for DNA ligase 1 deficiency. *J Clin Immunol* (2021) 41(1):238–42. doi: 10.1007/s10875-020-00871-x

4. Maffucci P, Chavez J, Jurkiw TJ, O'Brien PJ, Abbott JK, Reynolds PR, et al. Biallelic mutations in DNA ligase 1 underlie a spectrum of immune deficiencies. *J Clin Invest.* (2018) 128(12):5489–504. doi: 10.1172/JCI99629

5. Teo IA, Arlett CF, Harcourt SA, Priestley A, Broughton BC. Multiple hypersensitivity to mutagens in a cell strain (46BR) derived from a patient with immuno-deficiencies. *Mutat Res Mol Mech Mutagen* (1983) 107(2):371–86. doi: 10.1016/0027-5107(83)90177-X

6. Teo IA, Broughton BC, Day RS, James MR, Karren P, Mayne LV, et al. A biochemical defect in the repair of alkylated DNA in cells from an immunodeficient patient (46BR). *Carcinogenesis* (1983) 4(5):559–64. doi: 10.1093/carcin/4.5.559

7. Quang D, Chen Y, Xie X. DANN: A deep learning approach for annotating the pathogenicity of genetic variants. *Bioinformatics* (2015) 31(5):761–3. doi: 10.1093/bioinformatics/btu703

8. Chao M, Ebell W, Bader P, Beier R, Burkhardt B, Feuchtinger T, et al. Consensus of German transplant centers on hematopoietic stem cell transplantation in fanconi anemia. *Klin Pädiatrie.* (2015) 227(03):157–65. doi: 10.1055/s-0035-1548841

9. Howes TRL, Tomkinson AE, DNA ligase I. The replicative DNA ligase. In: *Subcellular biochemistry*, Subcellular Biochemistry: Springer, Dordrecht (2012) 62:327–41. doi: 10.1007/978-94-007-4572-8_17

10. Pascal JM, O'Brien PJ, Tomkinson AE, Ellenberger T. Human DNA ligase I completely encircles and partially unwinds nicked DNA. *Nature* (2004) 432 (7016):473–8. doi: 10.1038/nature03082

11. Jurkiw TJ, Tumbale PP, Schellenberg MJ, Cunningham-Rundles C, Williams RS, O'Brien PJ. LIG1 syndrome mutations remodel a cooperative network of ligand binding interactions to compromise ligation efficiency. *Nucleic Acids Res* (2021) 49(3):1619–30. doi: 10.1093/nar/gkaa1297

12. Tang Q, Kamble P, Çağlayan M. DNA Ligase I variants fail in the ligation of mutagenic repair intermediates with mismatches and oxidative DNA damage. *Mutagenesis* (2020) 35(5):391–404. doi: 10.1093/mutage/geaa023

13. Tomkinson AE, Vijayakumar S, Pascal JM, Ellenberger T. DNA Ligases: Structure, reaction mechanism, and function. *Chem Rev* (2006) 106(2):687–99. doi: 10.1021/cr040498d

14. ESID Registry. Working definitions for clinical diagnosis of PID. *European Society for Immunodeficiencies Registry Working Party Diagnosis criteria* (2018). Available at: https://esid.org/Working-Parties/Registry-Working-Party/Diagnosis-criteria.

15. Ussowicz M, Wawrzyniak-Dzierżek E, Mielcarek-Siedziuk M, Salamonowicz M, Frączkiewicz J, Rybka B, et al. Allogeneic stem cell transplantation after fanconi anemia conditioning in children with ataxia-telangiectasia results in stable T cell engraftment and lack of infections despite mixed chimerism. *Biol Blood Marrow Transplant.* (2018) 24(11):2245–9. doi: 10.1016/j.bbmt.2018.07.001

16. Wolska-Kusnierz B, Pastorczak A, Fendler W, Wakulinska A, Dembowska-Baginska B, Heropolitanska-Pliszka E, et al. Hematopoietic stem cell transplantation positively affects the natural history of cancer in nijmegen breakage syndrome. *Clin Cancer Res* (2021) 27(2):575–84. doi: 10.1158/1078-0432.CCR-20-2574

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Comparison of pulmonary lesions using lung ultrasound and high-resolution computed tomography in adult patients with primary humoral immunodeficiencies

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Pulmonary involvement is the most common complication in patients with predominantly antibody deficiencies (PADs). Therefore, patients require repeated imaging tests. Unlike high-resolution computed tomography (HRCT), lung ultrasonography (LUS) does not expose patients to X-rays or contrast agents, and can be performed even at the bedside. This study aimed to evaluate lung lesions using simultaneous LUS and HRCT in a group of patients with PADs. Twenty-nine adult patients (13 women and 16 men) diagnosed with PADs according to the ESID criteria (23 Common variable immunodeficiency, 2 X-linked agammaglobulinemia, 2 IgG subclass deficiencies, and 2 Unspecified hypogammaglobulinemia) were included in the study. The mean age was 39.0 ± 11.9 years. The mean time elapsed between the first symptoms of PADs and the examination was 15.4 ± 10.1 years. Lung ultrasonography and high-resolution computed tomography were performed simultaneously according to a defined protocol during the clinic visits. In both examinations, lesions were compared in the same 12 regions: for each lung in the upper, middle, and lower parts, separately, front and back. A total of 435 lesions were described on LUS, whereas 209 lesions were described on HRCT. The frequencies of lesions in the lung regions were similar between LUS and HRCT. In both examinations, lesions in the lower parts of the lungs were most often reported (LUS 60.9% vs. HRCT 55.5%) and least often in the upper parts of the lungs (LUS 12.7% vs. HRCT 12.0%). The most frequently described lesions were LUS consolidations (99; 22.8%) and HRCT fibrosis (74; 16.5%). A statistically significant relationship was found in the detection of fibrosis in 11 of the 12 regions (phi = 0.4–1.0). Maximum values of the phi coefficient for the upper part of the left lung were recorded. Compared with

HRCT, LUS is an effective alternative for evaluating and monitoring pulmonary lesions in adult patients with PADs, especially for pulmonary fibrosis.

KEYWORDS

Lung ultrasonography, high-resolution computed tomography, chest sonography, antibody deficiencies, immunodeficiency, pulmonary fibrosis, interstitial lung disease

Introduction

According to the classification developed in 2019, the group of diseases defined as inborn errors of immunity (IEIs) includes over 400 entities (1). However, it should be emphasized that new diseases are described every year. IEIs represent a heterogeneous group of diseases with significantly different clinical presentations. The epidemiology of IEI is challenging to estimate, but in most registries, at least half of the cases are classified as predominantly antibody deficiencies (PADs) (2). Among others, this group includes diseases such as common variable immunodeficiency (CVID), X-linked agammaglobulinemia (XLA), or immunoglobulin G subclass deficiency.

Pulmonary complications are estimated to affect about 60% of patients with PADs and up to 90% patients with CVID (3). Recurrent bacterial respiratory infections are the leading symptoms in this patient group (4) and are often the main reason for the expansion of the diagnosis of primary immunodeficiencies. Frequent or severe respiratory infections can cause structural lung damage that may promote chronic pulmonary diseases such as bronchiectasis, atelectasis, or fibrosis. Early diagnosis and management with prophylactic antibiotics and Ig replacement therapy reduce the frequency of infections and their long-term effects (5).

Many patients with PAD also have pulmonary non-infectious complications such as the previously mentioned bronchiectasis or fibrosis, as well as asthma, interstitial disease, or malignancy. Depending on the population studied, the incidence of complications is various. It is estimated that asthma occurs in 31.2% of patients with CVID and 10.3% of patients with XLA (6). Bronchiectasis occurs in 25 to 79% of patients with PADs (7–9). Less common is interstitial lung disease (ILD), described mainly in patients with CVID (10-20%) (8). A particular form of ILD is granulomatous-lymphocytic interstitial lung disease (GLILD), which is described mainly in the course of CVID with a frequency of about 8-20% (10). The rarest non-infectious complication is malignancy. The most common are lymphomas, the incidence of which is estimated at less than 10% of patients (9).

Pulmonary complications in patients with PADs not only impair their quality of life, but also contribute to higher mortality. In patients with CVID, it has been estimated that the risk of death increases twofold if there are functional or structural changes in the lungs (11). A higher incidence of extrapulmonary complications such as splenomegaly, lymphomas, autoimmunity (especially autoimmune cytopenias), has been described among patients with GLILD (10).

Lung imaging studies should be performed to detect pulmonary complications of PADs. Such examinations are sometimes repeated multiple times during a patient's lifetime to monitor the disease. Currently, the gold standard for diagnosis is computed tomography (CT), especially high-resolution computed tomography (HRCT) (7, 12). Performing these tests is associated with patient exposure to X-rays or contrast agents. This limits their ability to perform tasks frequently. It is worth noting that increased radiosensitivity has also been demonstrated in patients with CVID compared with healthy individuals (13).

Lung ultrasonography (LUS) does not have these disadvantages. This method allows non-invasive diagnosis of the pleural cavities, pleura, and lungs. A disadvantage of LUS, and a prerequisite for imaging pulmonary lesions, is that they are in direct contact with the pleural line. It is essential that the examination is performed at the patient's bedside. This could be helpful when pulmonary imaging monitoring is necessary. The patient does not require any preparation, and the procedure can be repeated many times, even at short intervals.

Ultrasonography of the lungs has been an underestimated diagnostic method for many years. An appropriate air lung is a barrier to the propagation of ultrasonic waves. Initially, ultrasound was used for years only for the diagnosis of pathological lesions in the pleural cavity (fluid, neoplastic lesions of the chest wall) (14). As a result of lung disease, there is a loss of aeration (total or partial), and lesions appear on ultrasound, which are referred to as artifacts or consolidations. The artifacts do not correspond to anatomical structures but are formed when lung aeration is reduced (15). Artifacts are often accompanied by pleural lines (on the lung surface) and subpleural lesions. The constellation of individual artifacts, pleural lines, and subpleural lesions facilitates the differentiation of infectious interstitial lesions, cardiogenic pulmonary edema, and pulmonary fibrosis. The second type of lesion is a consolidation, that is, area of airless lung. Other ultrasound symptoms coexist with consolidations, allowing for

further differential diagnosis of inflammatory changes such as atelectasis, infarction in the course of pulmonary embolism, and metastatic changes or abscesses (16).

The last few decades have seen an increase in the number of original publications that show promise for the use of ultrasonography in the imaging diagnosis of pulmonary lesions. To date, well-developed criteria include lesions in which there is consolidation of the pulmonary parenchyma (pneumonia, atelectasis, lesions in the course of pulmonary embolism) and interstitial lesions (cardiogenic pulmonary edema, interstitial pneumonia, pulmonary fibrosis in the course of interstitial lung disease) (17–20). The results of numerous studies make it possible to consider LUS as a useful method for the diagnosis of lung lesions in examinations using X-rays (19, 20). Lung US is particularly well-established for lower respiratory tract infections in children (21, 22).

Despite numerous possible pulmonary complications in the course of IEI, we did not find data on the ultrasound images of the lungs in this group of patients in the available literature. The aim of our study was to characterize the lesions in the lungs that can be visualized using LUS in a group of patients with PADs. An additional goal was to compare the lung images obtained using ultrasound with those obtained using high-resolution tomography.

Material and methods

Study group

Twenty-nine patients (13 women and 16 men) with PADs (23 with CVID, 2 with XLA, 2 with IgG subclass deficiencies, and 2 with unspecified hypogammaglobulinemia) were included in this study. The mean age at the onset of the first symptoms was 23.6 ± 13.6 years. The mean diagnosis delay was 8.0 ± 9.0 years. At the time of testing, the mean age, was 39.0 ± 11.9 years. The mean time between the first symptoms of PADs and the examination was 15.4 ± 10.1 years. All patients received immunoglobulin replacement therapy (3 IVIG and 26 SCIG/fSCIG). The mean IgG concentration at the time of lung imaging studies was 8.4 ± 1.8 g/l. Most patients (26/29, 89.7%) declared that they had a history of recurrent lower respiratory tract infections. Twelve patients had infections only until the diagnosis of PADs was made and immunoglobulin replacement therapy introduced. While imaging studies were performed, we did not observe any clinical signs of respiratory tract infections. The most common noninfectious complication was polyclonal lymphoproliferation (n=16; 55.2%). followed by 51.7% autoimmunity, 44.8% pulmonary fibrosis, 34.5% bronchiectasis, 17.2% GLILD and 13.8% asthma. A detailed characteristic of the population is available in the Supplementary Material.

The inclusion criteria were as follows: age ≥18 years, diagnosis of PADs according to the diagnostic criteria of the European Society for Immunodeficiencies (23), and provision of written consent. The exclusion criteria were as follows: unfulfilled inclusion criteria, symptoms of acute respiratory tract infection, and in the case of women, pregnancy.

Lung ultrasound

Lung ultrasound was performed with a PHILIPS ultrasound scanner (year of manufacture 2016, WA, USA) using two probes: convex (2–6 MHz) and linear (4–12 MHz). Ultrasound examination of the lungs was performed according to a protocol involving scanning the entire lung surface available during the ultrasound examination bilaterally over the posterior, lateral, and anterior chest wall. We presented an ultrasound image of a normal lung in Figure 1.

The lesions observed in each lung field were anonymized in a dedicated form and submitted for statistical analysis. Ultrasound examinations were performed by a lung ultrasound specialist with 12 years of experience. Pulmonary fibrosis was assumed to be present in a region if the following criteria were met: pleural lesions (irregularity, fragmentation, blurred pleural line), vertical artifacts (B lines, Z lines, C lines), and subpleural consolidations. In Figure 2, we have shown examples of pathological changes in lung ultrasound images.

Chest high-resolution tomography

Chest HRCT scans were obtained using a 128-detector row Siemens Somatom Flash scanner (Siemens, Forchheim, Germany). Images were obtained in the craniocaudal direction during a single breath-hold with collimation 128 × 0.6 mm, rotation time 0.5 s, matrix 512 × 512 mm, and 0.6 mm reconstructed section thickness. Image analysis was performed using dedicated software (Syngo.via, Siemens) and an application (CT Chest in Syngo.via) with standard lung window settings (width,−50 HU; level, 1500 HU) and mediastinal window settings (width, 350 HU; level, 50 HU). HRCT was performed within 2 hours of lung ultrasound.

The lesions observed on HRCT were described in detail in a form dedicated to CT lesion descriptions and anonymized for further statistical analysis. Computed tomographic scans were reviewed by a radiologist with 18 years of experience. In both examinations, lesions were compared in the same 12 regions: for each lung in the upper, middle, and lower parts, separately, front and back.

Statistical methods

Statistical analyses were performed using the STATISTICA software (version 13; TIBCO Software Inc., Palo Alto, CA, USA). To determine the relationship between abnormalities detected

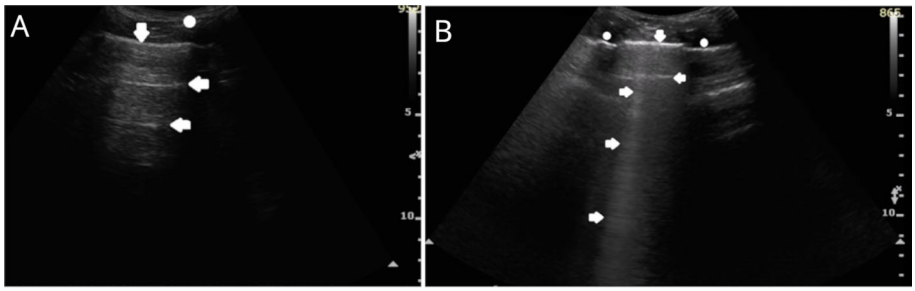

FIGURE 1
Image of normal lung on ultrasonography. **(A)** structures of chest wall (o), smooth and regular pleural line (↓), A lines, horizontal artifacts observed in properly aerated lung (←). Convex probe (1-6MHz). **(B)** ribs and anechoic shadow behind them (o), smooth and regular pleural line (↓), A line artifact (←), B line, vertical artifact of comet tail, in some objects visible in the last intercostal space as a normal variant (→). Convex probe (1-6MHz).

on HRCT and LUS, a chi-square test or Fisher's exact test (when the expected number was smaller than five) was employed with the phi coefficient as a measure of the power of correlation. Statistical significance was assumed at $p < 0.05$.

Bioethics committee

Studies involving human participants were reviewed and approved by the Ethics Committee of the Medical University of Gdansk, Gdańsk, Poland. All participants provided written informed consent to participate in the study.

Results

Lung ultrasonography

We described 435 lesions on lung ultrasonography in our study group of 29 patients with predominantly antibody

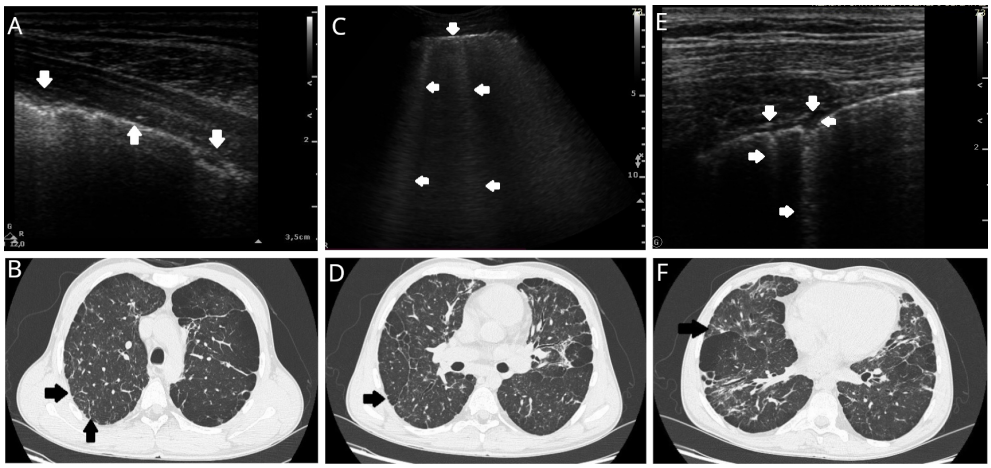

FIGURE 2
Examples of lung lesions in a patient with predominantly antibody deficiency on lung ultrasound (LUS) and high-resolution tomography (HRCT). **(A)** LUS: Irregular and infiltrated pleural line (↓), regular pleural line (↑). Linear probe. **(B)** HRCT (axial plane): Right lung - peripheral paraseptal emphysema with centrilobular emphysema inside the lung and fibrosis, thickening of the pleura between the arrows with subpleural fibrosis (arrowheads). Left lung combined centrilobular and panlobular emphysema with fibrosis. **(C)** LUS: Irregular pleural line (↓) and multiple B lines (←). Convex probe (1-6MHz). **(D)** HRCT (axial plane): Right lung - peripheral paraseptal emphysema with centrilobular emphysema inside the lung and fibrosis (arrowhead). Left lung combined paraseptal and centrilobular emphysema with fibrosis. **(E)** LUS: Irregular pleural line, and small subpleural consolidation (↓←) with vertical artifact C line (arising from subpleural lesion) (→). Linear probe. **(F)** HRCT (axial plane): Right lung – combined paraseptal, centrilobular and panlobular emphysema and fibrosis, thickening of the pleura with subpleural fibrosis (arrowhead). Left lung combined paraseptal, centrilobular and panlobular emphysema with fibrosis.

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deficiency. Most lesions were located in the lower regions of the lungs (265; 60.9%). The numbers of lesions in the middle and upper parts were 115 (26.4%) and 55 (12.7%), respectively. The most frequently described lesion was consolidation (n = 99; 22.8%). The frequencies of other lesions were as follows: C-lines (94, 21.6%), irregular pleural lines (93, 21.4%), B-lines (57, 13.1%), fragmented pleural line (45, 10.3%), blurred pleural lines (24, 5.5%), and Z-lines (23, 5.3%). Table 1 shows the number of lesions described in each of the 12 lung regions examined in this study. Pulmonary fibrosis, diagnosed according to the established definition, was diagnosed 79 times. Fibrosis was most common in the lower lung (48; 60.8%). In the middle and upper regions of the lungs, 20 (25.3%) and 11 (13.9%) lesions were suggestive of fibrosis, respectively (Table 2). In five patients (17.2%), no pathological lesions were detected on ultrasound.

High-sensitive computed tomography

Compared with LUS, the number of lesions described on HRCT was lower, amounting to 209. However, in this study, we also described most changes in the lower parts of the lungs (116; 55.5%). There were 25 (12.0%) and 68 (32.5%) lesions in the upper and middle lung regions, respectively (Table 1). The most frequently described lesion was fibrosis (n = 74, 16.5%). The frequencies of other lesions were as follows: tree-in-bud pattern (27, 6.0%), pleural thickening (23, 5.1%), pleural adhesions (23, 5.1%), bronchiectasis (22, 4.9%), thickening of the intertrabecular septum (16, 3.6%), emphysema bulls (9, 2%), consolidations < 5 mm (8, 1.8%), and calcifications (7, 1.6%). In three patients (10.3%), no pathological changes were observed on HRCT. Examples of pathological changes observed on HRCT are shown in Figure 2.

TABLE 1 Distribution of each lesion detected in lung ultrasound (LUS) and high-resolution computed tomography (HRCT) by lung region.

LUNG ULTRASONOGRAPHY					HIGH-RESOLUTION COMPUTED TOMOGRAPHY				
	Lesions	FRONT		BACK		Lesions	FRONT		BACK	
		LEFT	RIGHT	LEFT	RIGHT		LEFT	RIGHT	LEFT	RIGHT
TOP						Fibrosis	1 (3.4%)	5 (17.2%)	2 (6.9%)	5 (17.2%)
	Blurred PL	1 (3.4%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	PL thickening	0 (0.0%)	0 (0.0%)	2 (6.9%)	1 (3.4%)
	Irregular PL	2 (6.9%)	1 (3.4%)	2 (6.9%)	4 (13.8%)	Calcifications	0 (0.0%)	0 (0.0%)	1 (3.4%)	0 (0.0%)
	Fragmented PL	1 (3.4%)	0 (0.0%)	0 (0.0%)	3 (10.3%)	PL adhesions and clusters	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (3.4%)
	B-lines	2 (6.9%)	3 (10.3%)	3 (10.3%)	0 (0.0%)	Bronchiectasis	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (3.4%)
	C-lines	4 (13.8%)	4 (13.8%)	2 (6.9%)	4 (13.8%)	Emphysema bulls	0 (0.0%)	1 (3.4%)	0 (0.0%)	1 (3.4%)
	Z-lines	1 (3.4%)	2 (6.9%)	1 (3.4%)	1 (3.4%)	Thickening of the ILS	0 (0.0%)	0 (0.0%)	1 (3.4%)	1 (3.4%)
	Consolidations	4 (13.8%)	4 (13.8%)	2 (6.9%)	4 (13.8%)	Consolidations	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
						Tree-in-bud	0 (0.0%)	1 (3.4%)	0 (0.0%)	1 (3.4%)
MIDDLE						Fibrosis	4 (13.8%)	6 (20.7%)	5 (17.2%)	8 (27.6%)
	Blurred PL	1 (3.4%)	1 (3.4%)	0 (0.0%)	1 (3.4%)	PL thickening	1 (3.4%)	0 (0.0%)	5 (17.2%)	5 (17.2%)
	Irregular PL	5 (17.2%)	7 (24.1%)	6 (20.7%)	6 (20.7%)	Calcifications	0 (0.0%)	0 (0.0%)	1 (3.4%)	1 (3.4%)
	Fragmented PL	3 (10.3%)	4 (13.8%)	3 (10.3%)	3 (10.3%)	PL adhesions and clusters	2 (6.9%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
	B-lines	2 (6.9%)	2 (6.9%)	5 (17.2%)	6 (20.7%)	Bronchiectasis	1 (3.4%)	2 (6.9%)	1 (3.4%)	2 (6.9%)
	C-lines	9 (31.0%)	10 (34.5%)	2 (6.9%)	5 (17.2%)	Emphysema bulls	1 (3.4%)	0 (0.0%)	1 (3.4%)	1 (3.4%)
	Z-lines	1 (3.4%)	3 (10.3%)	2 (6.9%)	1 (3.4%)	Thickening of the ILS	1 (3.4%)	3 (10.3%)	1 (3.4%)	2 (6.9%)
	Consolidations	9 (31.0%)	10 (34.5%)	3 (10.3%)	5 (17.2%)	Consolidations	0 (0.0%)	1 (3.4%)	0 (0.0%)	3 (10.3%)
						Tree-in-bud	1 (3.4%)	2 (6.9%)	3 (10.3%)	4 (13.8%)
BOTTOM						Fibrosis	8 (27.6%)	9 (31.0%)	13 (44.8%)	8 (27.6%)
	Blurred PL	6 (20.7%)	5 (17.2%)	5 (17.2%)	4 (13.8%)	PL thickening	0 (0.0%)	1 (3.4%)	4 (13.8%)	4 (13.8%)
	Irregular PL	15 (51.7%)	15 (51.7%)	14 (48.3%)	16 (55.2%)	Calcifications	1 (3.4%)	2 (6.9%)	0 (0.0%)	1 (3.4%)
	Fragmented PL	6 (20.7%)	8 (27.6%)	8 (27.6%)	6 (20.7%)	PL adhesions and clusters	7 (24.1%)	5 (17.2%)	5 (17.2%)	3 (10.3%)
	B-lines	5 (17.2%)	9 (31.0%)	11 (37.9%)	9 (31.0%)	Bronchiectasis	3 (10.3%)	4 (13.8%)	6 (20.7%)	2 (6.9%)
	C-lines	15 (51.7%)	16 (55.2%)	10 (34.5%)	13 (44.8%)	Emphysema bulls	1 (3.4%)	1 (3.4%)	1 (3.4%)	1 (3.4%)
	Z-lines	5 (17.2%)	4 (13.8%)	0 (0.0%)	2 (6.9%)	Thickening of the ILS	1 (3.4%)	2 (6.9%)	3 (10.3%)	1 (3.4%)
	Consolidations	15 (51.7%)	16 (55.2%)	14 (48.3%)	13 (44.8%)	Consolidations	3 (10.3%)	1 (3.4%)	0 (0.0%)	0 (0.0%)
						Tree-in-bud	3 (10.3%)	4 (13.8%)	5 (17.2%)	3 (10.3%)

PL, pleural line; ILS, interlobular septum.
Frequency is shown using a color scale from lowest (green) to highest (red) separately for LUS and HRCT.

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TABLE 2 Analysis of the frequency of fibrosis with the coefficient phi for the correlations between findings detected in lung ultrasound and high-resolution computed tomography.

		FRONT																			
		LEFT					RIGHT					LEFT					RIGHT				
TOP	USG	HRCT				USG	HRCT				USG	HRCT				USG	HRCT				
		No		Yes			No		Yes			No		Yes			No		Yes		
		N	%	N	%		N	%	N	%		N	%	N	%		N	%	N	%	
	No	26	100.0 %	0	0.0 %	No	24	100.0 %	1	20.0 %	No	27	100.0 %	0	0.0 %	No	24	100.0 %	3	60.0 %	
	Yes	0	0.0 %	3	100.0 %	Yes	0	0.0 %	4	80.0 %	Yes	0	0.0 %	2	100.0 %	Yes	0	0.0 %	2	40.0 %	
		p	< 0.001	Phi	1.00		p	<0.001	Phi	0.876		p	0.002	Phi	1.00		p	0.025	Phi	0.596	
MIDDLE	USG	HRCT				USG	HRCT				USG	HRCT				USG	HRCT				
		No		Yes			No		Yes			No		Yes			No		Yes		
		N	%	N	%		N	%	N	%		N	%	N	%		N	%	N	%	
	No	22	100.0 %	4	57.1 %	No	22	95.7 %	0	0.0 %	No	22	100.0 %	2	28.6 %	No	17	94.4 %	7	63.6 %	
	Yes	0	0.0 %	3	42.9 %	Yes	1	4.3 %	6	100.0 %	Yes	0	0.0 %	5	71.4 %	Yes	1	5.6 %	4	36.4 %	
		p	0.010	Phi	0.602		p	< 0.001	Phi	0.905		p	< 0.001	Phi	0.809		p	0.054	Phi	0.396	
BOTTOM	USG	HRCT				USG	HRCT				USG	HRCT				USG	HRCT				
		No		Yes			No		Yes			No		Yes			No		Yes		
		N	%	N	%		N	%	N	%		N	%	N	%		N	%	N	%	
	No	13	86.7 %	6	42.9 %	No	11	78.6 %	4	26.7 %	No	10	83.3 %	6	35.3 %	No	13	86.7 %	5	35.7 %	
	Yes	2	13.3 %	8	57.1 %	Yes	3	21.4 %	11	73.3 %	Yes	2	16.7 %	11	64.7 %	Yes	2	13.3 %	9	64.3 %	
		p	0.021	Phi	0.461		p	0.009	Phi	0.519		p	0.022	Phi	0.476		p	0.008	Phi	0.525	

Comparison of pulmonary fibrosis in HRCT and LUS

We found no correlation between the occurrence of individual lesions on LUS and lesions observed on HRCT. However, it should be emphasized that the analysis of LUS results is based on the occurrence of certain combinations of symptoms rather than individual findings.

To compare the diagnostic capabilities of HRCT and LUS, we assessed the incidence of lesions indicative of pulmonary fibrosis in both studies. We chose fibrosis because it is the most frequently described lesion on computed tomography, and a set of ultrasound signs are known to identify this process in LUS. Table 2 summarizes the prevalence of fibrosis in the 12 examined regions of the lungs. A statistically significant relationship between the results of both imaging studies was found in 11 regions. The strength of the relationship was strong (phi = 0.40–0.69) or very strong (phi ≥ 0.70). The maximum values of the phi coefficient for the upper part of the left lung were recorded (phi = 1.0). Only the result for the back in the middle of the right lung was at the limit of statistical significance (p = 0.054). In the same region, the strength of the association between the results of the two examinations, assessed using the phi coefficient, was the weakest (phi = 0.396).

Discussion

Lung disease is a frequent complication of PADs with high morbidity and mortality rates. The spectrum of clinical manifestations is broad, and includes acute and chronic infections, structural abnormalities, and malignancies (3, 8). All these disorders have in common that diagnostic imaging is necessary to establish the diagnosis and monitor progression. Currently, we mainly use computed tomography for this purpose (7). In many groups of patients, the usefulness of lung ultrasound, which has been developing intensely in recent years, has been proven. To our knowledge, ultrasound lung lesions in patients with primary immunodeficiencies have not yet been described.

In our group of 29 patients with PADs, the lesions described on both LUS and HRCT were usually diffuse rather than focal. In most cases, the lesions closely resembled those described in interstitial lung disease. Twenty-four patients had multiple ultrasound abnormalities in the form of artifacts (B-, C-, and Z-line artifacts), pleural line lesions (irregular, fragmented, and blurred), and small subpleural consolidations (< 5 mm). Consolidations and accompanying pleural line lesions in the LUS were the most frequent, which may indicate lesions in the interstitial space and alveoli. These lesions may be secondary to atelectasis or post-inflammatory changes, which may be due to previous recurrent lower respiratory tract infections. It should be noted that vertical artifacts observed in large numbers upon LUS examination are an indirect parameter indicating a problem located in the interstitial space of the lungs or in the subpleural area.

The higher number of pleural lesions described on lung ultrasound than on HRCT may be due to technical differences

between these examinations. LUS allows for very accurate imaging of the pleural line and superficial parts of the lungs compared to CT. If interstitial lung lesions are predominant, LUS does not allow the assessment of deeper lung areas. On the other hand, computed tomography allows deep evaluation of the lung up to the mediastinum. Lung ultrasound and HRCT are complementary and used together may allow for improved diagnostic and monitoring capabilities for patients with PADs.

In our group, both LUS and HRCT showed that the lesions accumulated mainly in the lower and middle parts of the lungs. These observations are consistent with previously published lesion locations on HRCT in patients with PADs. Both Tanaka et al. (24) and Bondionii et al. (25) observed very few lesions in adult patients with CVID and XLA in the upper lung on HRCT. They were predominantly in the middle and lower parts. However, the accumulation of lesions in the lower lobes of the lungs, as observed in our study, has not been described.

In the CT scan performed up to 2 h after the LUS, numerous non-specific abnormalities were found in the studied patients. We found no correlation between individual lesions on LUS examination and lesions observed on HRCT. This is due to the fact that in LUS, it is not individual lesions but their co-occurrence in certain constellations that should be evaluated. This makes it impossible to directly compare the deviations described by the lung imaging techniques.

The most common lesions on HRCT are indicative of lung fibrosis. In case of LUS, we defined the features of the ultrasound image that indicated the presence of this pathology. We demonstrated a statistically significant and strong correlation between fibrotic images on LUS and HRCT. This supports the usefulness of ultrasonography in the diagnosis of pulmonary fibrosis, which is also described for idiopathic pulmonary fibrosis (26) or lesions in the course of systemic connective tissue diseases (19).

In the study group, no patient was found to have neoplastic disease; therefore, it is not possible to conclude on the diagnostic possibilities of neoplastic disease with lung ultrasonography.

Surprisingly, there were a low number of bronchiectasis cases in the study group. These lesions accounted for approximately 5% of all lesions described on HRCT. Bronchiectasis is a common complication of CVID. In the study group of patients with PADs, the majority had this immunodeficiency. According to various estimates, the percentage of patients with CVID diagnosed with bronchiectasis ranges from 25 to 79% (7). The low incidence of this complication in our group may have been due to well-managed immunoglobulin supplementation. Indeed, a close relationship between the incidence of bronchiectasis and IgG levels has been previously demonstrated (27).

Our study has a few limitations. We included a small group of patients; however, this population was well clinically characterized. This was a pilot study, and we performed the examinations only once. We did not analyze how the lung lesions changed during a longer follow-up period. Owing to the very high variability of the described abnormalities in both imaging studies and the different clinical presentations of PADs, it is necessary to conduct studies on a larger number of patients. To compare the usefulness of LUS and HRCT, it would be worthwhile to conduct a study on a group of patients with well-defined pulmonary complications. This will allow for a comparison of the two imaging studies in specific clinical situations. In future studies it would be worthwhile to correlate functional test results with lung ultrasound images.

Conclusions

In the study group of patients with predominantly antibody deficiencies, the diagnostic potential of ultrasonography for the evaluation of pulmonary lesions was evaluated. The lesions on LUS and HRCT were non-specific. The features of fibrosis found by both diagnostic methods correlated very well. Lung ultrasonography appears to be a promising method for imaging pulmonary lesions, especially fibrosis, in patients with primary immunodeficiencies. For lesions of a different nature, it is necessary to perform studies on a larger group of patients with strictly defined pulmonary complications.

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 Ethics Committee of the Medical University of Gdansk, Gdańsk, Poland. The patients/participants provided their written informed consent to participate in this study.

Author contributions

MZ and NB designed the study and wrote the first draft of the manuscript. This text was produced with equal contributions from both authors. MZ, NB, EW-S, and DG collected the data and performed the literature searches. NB performed the lung ultrasound examinations. MP described HRCT findings. MZ performed the statistical analyses. EW-S, ZZ, and KJ-R critically revised the manuscript for intellectual content. All the authors have read and agreed to the published version of the manuscript.

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 potential conflicts of interest.

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Supplementary material

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References

1. Bousfiha A, Jeddane L, Picard C, Al-Herz W, Ailal F, Chatila T, et al. Human inborn errors of immunity: 2019 update of the IUIS phenotypical classification. *J Clin Immunol* (2020) 40:66–81. doi: 10.1007/s10875-020-00758-x

2. Abolhassani H, Azizi G, Sharifi L, Yazdani R, Mohsenzadegan M, Delavari S, et al. Global systematic review of primary immunodeficiency registries. *Expert Rev Clin Immunol* (2020) 16:717–32. doi: 10.1080/1744666X.2020.1801422

3. Cinetto F, Scarpa R, Rattazzi M, Agostini C. The broad spectrum of lung diseases in primary antibody deficiencies. *Eur Respir Rev* (2018) 27. doi: 10.1183/16000617.0019-2018

4. Demirdag YY, Gupta S. Update on infections in primary antibody deficiencies. *Front Immunol* (2021) 12:634181/BIBTEX. doi: 10.3389/FIMMU.2021.634181/BIBTEX

5. Busse PJ, Razvi S, Cunningham-Rundles C. Efficacy of intravenous immunoglobulin in the prevention of pneumonia in patients with common variable immunodeficiency. *J Allergy Clin Immunol* (2002) 109:1001–4. doi: 10.1067/mai.2002.124999

6. Weinberger T, Fuleihan R, Cunningham-Rundles C, Maglione PJ. Factors beyond lack of antibody govern pulmonary complications in primary antibody deficiency. *J Clin Immunol* (2019) 39:440. doi: 10.1007/S10875-019-00640-5

7. Rodriguez JA, Bang TJ, Restrepo CS, Green DB, Browne LP, Vargas D. Imaging features of primary immunodeficiency disorders. *Radiol Cardiothorac Imaging* (2021) 3. doi: 10.1148/RYCT.2021200418

8. Casal A, Riveiro V, Suárez-Antelo J, Ferreiro L, Rodríguez-Núñez N, Lama A, et al. Pulmonary manifestations of primary humoral deficiencies. *Can Respir J* (2022) 2022:9. doi: 10.1155/2022/7140919

9. Movahedi M, Jamee M, Ghaffaripour H, Noori F, Ghaini M, Eskandarzadeh S, et al. Pulmonary manifestations in a cohort of patients with inborn errors of immunity: an 8-year follow-up study. *Allergol Immunopathol (Madr)* (2022) 50:80–4. doi: 10.15586/AEI.V50I1.388

10. Cinetto F, Scarpa R, Carrabba M, Firinu D, Lougaris V, Buso H, et al. Granulomatous lymphocytic interstitial lung disease (GLILD) in common variable immunodeficiency (CVID): A multicenter retrospective study of patients from Italian PID referral centers. *Front Immunol* (2021) 12:627423/BIBTEX. doi: 10.3389/FIMMU.2021.627423/BIBTEX

11. Resnick ES, Moshier EL, Godbold JH, Cunningham-Rundles C. Morbidity and mortality in common variable immune deficiency over 4 decades. *Blood* (2012) 119:1650–7. doi: 10.1182/BLOOD-2011-09-377945

12. Meerburg JJ, Hartmann IJC, Goldacker S, Baumann U, Uhlmann A, Andrinopoulou ER, et al. Analysis of granulomatous lymphocytic interstitial lung disease using two scoring systems for computed tomography scans–a retrospective cohort study. *Front Immunol* (2020) 11:589148/FULL. doi: 10.3389/FIMMU.2020.589148/FULL

13. Mahmoodi M, Abolhassani H, Mozdarani H, Rezaei N, Azizi G, Yazdani R, et al. In vitro chromosomal radiosensitivity in patients with common variable immunodeficiency. *Cent Eur J Immunol* (2018) 43:155. doi: 10.5114/CEJI.2018.77385

14. Mayo PH, Copetti R, Feller-Kopman D, Mathis G, Maury E, Mongodi S, et al. Thoracic ultrasonography: a narrative review. *Intensive Care Med* (2019) 45:1200–11. doi: 10.1007/S00134-019-05725-8/TABLES/2

15. Mongodi S, de Luca D, Colombo A, Stella A, Santangelo E, Corradi F, et al. Quantitative lung ultrasound: Technical aspects and clinical applications. *Anesthesiology* (2021) 134:949–65. doi: 10.1097/ALN.0000000000003757

16. Buda N, Kosiak W, Radzikowska E, Olszewski R, Jassem E, Grabczak EM, et al. Polish recommendations for lung ultrasound in internal medicine (POLLUS-IM). *J Ultrason* (2018) 18:198–206. doi: 10.15557/JOU.2018.0030

17. Buda N, Kosiak W, Wełnicki M, Skoczylas A, Olszewski R, Piotrkowski J, et al. Recommendations for lung ultrasound in internal medicine. *Diagnostics* (2020) 10:597. doi: 10.3390/diagnostics10080597

18. Volpicelli G, Elbarbary M, Blaivas M, Lichtenstein DA, Mathis G, Kirkpatrick AW, et al. International evidence-based recommendations for point-of-care lung ultrasound. *Intensive Care Med* (2012) 38:577–91. doi: 10.1007/S00134-012-2513-4/FIGURES/2

19. Buda N, Wojteczek A, Masiak A, Piskunowicz M, Batko W, Zdrojewski Z. Lung ultrasound in the screening of pulmonary interstitial involvement secondary to systemic connective tissue disease: A prospective pilot study involving 180 patients. *J Clin Med* (2021) 10:4114. doi: 10.3390/jcm10184114

20. Rodríguez-Contreras FJ, Calvo-Cebrián A, Díaz-Lázaro J, Cruz-Arnés M, León-Vázquez F, del Carmen Lobón-Agúndez M, et al. Lung ultrasound performed by primary care physicians for clinically suspected community-acquired pneumonia: A multicenter prospective study. *Ann Fam Med* (2022) 20:227–36. doi: 10.1370/AFM.2796

21. Jaworska J, Komorowska-Piotrowska A, Pomiećko A, Wiśniewski J, Woźniak M, Littwin B, et al. Consensus on the application of lung ultrasound in pneumonia and bronchiolitis in children. *Diagnostics* (2020) 10:935. doi: 10.3390/DIAGNOSTICS10110935

22. Dietrich CF, Buda N, Ciuca IM, Dong Y, Fang C, Feldkamp A, et al. Lung ultrasound in children, WFUMB review paper (part 2). *Med Ultrason* (2021) 23:443–52. doi: 10.11152/MU-3059

23. ESID - European Society for Immunodeficiencies . Available at: https://esid.org/Working-Parties/Registry-Working-Party/Diagnosis-criteria (Accessed August 27, 2022).

24. Tanaka N, Kim JS, Bates CA, Brown KK, Cool CD, Newell JD, et al. Lung diseases in patients with common variable immunodeficiency: Chest radiographic, and computed tomographic findings. *J Comput Assist Tomogr* (2006) 30:828–38. doi: 10.1097/01.rct.0000228163.08968.26

25. Bondioni MP, Duse M, Plebani A, Soresina A, Notarangelo LD, Berlucchi M, et al. Pulmonary and sinusal changes in 45 patients with primary immunodeficiencies: Computed tomography evaluation. *J Comput Assist Tomogr* (2007) 31:620–8. doi: 10.1097/RCT.0b013e31802e3c11

26. Manolescu D, Davidescu L, Traila D, Oancea C, Tudorache V. The reliability of lung ultrasound in assessment of idiopathic pulmonary fibrosis. *Clin Interv Aging* (2018) 13:437–49. doi: 10.2147/CIA.S156615

27. Wall LA, Wisner EL, Gipson KS, Sorensen RU. Bronchiectasis in primary antibody deficiencies: A multidisciplinary approach. *Front Immunol* (2020) 11:522. doi: 10.3389/fimmu.2020.00522

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In vitro systems to study inborn errors of immunity using human induced pluripotent stem cells

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In the last two decades, the exponential progress in the field of genetics could reveal the genetic impact on the onset and progression of several diseases affecting the immune system. This knowledge has led to the discovery of more than 400 monogenic germline mutations, also known as "inborn errors of immunity (IEI)". Given the rarity of various IEI and the clinical diversity as well as the limited available patients' material, the continuous development of novel cell-based *in vitro* models to elucidate the cellular and molecular mechanisms involved in the pathogenesis of these diseases is imperative. Focusing on stem cell technologies, this review aims to provide an overview of the current available *in vitro* models used to study IEI and which could lay the foundation for new therapeutic approaches. We elaborate in particular on the use of induced pluripotent stem cell-based systems and their broad application in studying IEI by establishing also novel infection culture models. The review will critically discuss the current limitations or gaps in the field of stem cell technology as well as the future perspectives from the use of these cell culture systems.

KEYWORDS

inborn errors of immunity, iPSCs, disease modeling, cell therapies, immune cells, macrophages

Introduction

In the last decades, the major progress in the field of genetics and the availability of high-throughput DNA sequencing techniques contributed to the discovery of more than 400 monogenic germline mutations affecting our immune system. These mutations are referred to as inborn errors of immunity (IEI) and can lead either to the loss of expression or loss/gain of function of the respective protein (1–3). The prevalence of IEI in the overall population is in the range of 1/10,000-1/50,000 (4). In most cases, IEI are identified early in life upon recurring

infections such as bronchitis or sinusitis and can be life-threatening if the patients do not receive proper treatment. The clinical phenotype of IEI shows a variety of disorders, including autoimmune or inflammatory diseases, allergies, cancer, and increased susceptibility to several pathogens.

According to the International Union of Immunological Societies, the IEI are classified into the following ten categories of conditions: Combined immunodeficiencies; Combined immunodeficiencies with syndromic features; Predominantly antibody deficiencies; Diseases of immune dysregulation; Congenital defects of phagocytes; Defects in intrinsic and innate immunity; Autoinflammatory diseases; Complement deficiencies, Bone marrow failure, and Phenocopies of IEI (2–5).

Due to the variable clinical features of IEI-related disorders, the medical care and treatment of these young patients is extremely challenging and requires a careful fine-tuning of the immune system. Children with IEI are usually treated with immunosuppressants, such as rapamycin or corticosteroids to decrease inflammation, however, this leads to a broad range of side effects. In addition, these types of treatment can only alleviate the symptoms but do not offer a curative solution for the patient. Other therapeutic strategies include the long-term usage of anti-fungal, anti-viral, or anti-bacterial agents, increasing the risk for the development of drug-resistant pathogens, which can cause life-threatening infections. In some cases, like in the severe combined immunodeficiency (SCID) syndrome, allogenic hematopoietic stem cell transplantation (HSCT) (or autologous HSC-gene therapy) is the only curative therapy (6, 7). However, HSCT always lurks the risk of immunological rejection or development of graft versus host disease with devastating consequences for the patient, pointing towards the need of suitable alternatives.

For these reasons, more targeted therapeutic approaches, which can directly modulate specific cell types or intracellular pathways, are preferred. These approaches include the use of specific inhibitors or biologics (antibodies or recombinant proteins). For the safe use of these emerging therapeutic agents, a detailed study of the pathophysiological mechanisms of the diseases is necessary. Given the rarity of IEI and the technical difficulties (obtaining sufficient samples from children or the low number of affected cells), the study of IEI-related diseases remains challenging. Thus, the development of novel systems to unravel the cellular and molecular mechanisms involved in the pathophysiology of the various IEI is of great importance.

Cell-based *in vitro* systems to study IEI

In the last years, the establishment of novel IEI *in vitro* systems has contributed enormously to the current understanding of the immunopathology involved in the various clinical features of different IEI-related diseases. As a consequence, these insights allowed for the development of new therapeutic approaches. The most appropriate *in vitro* models developed for these purposes are stem-cell based since stem cells have the capacity for self-renewal and differentiation into specialized cell types. The two main approaches used are based either on adult hematopoietic stem cells (HSCs) or induced pluripotent stem cells (iPSCs).

Adult HSCs are primary cells isolated from different sources such as peripheral blood, bone marrow, or umbilical cord. Their low number, inefficient long-term expansion, and heterogeneity however, impact their use in disease modeling and clinical applications.

As an alternative, iPSC-based *in vitro* models have proven to be one of the most successful options to adequately study IEI (8). Reprogramming of a few somatic cells, isolated from a patient, leads to the generation of stable, pluripotent, and patient-specific iPSC lines, which can give rise indefinitely to various cell types (Figure 1). Thus, iPSC technology becomes a promising tool to investigate the possible mechanisms involved in the pathophysiology of IEI using various cell types generated through specialized differentiation protocols from a single iPSC line. In addition, the fact that the generation of iPSCs is based on less-invasive methods for the patient renders the iPSC-derived cells preferable in comparison to primary specialized cells isolated consecutively from patients with more laborious and invasive procedures. Of note, this plays a particular role when children are affected.

Studying the immune system using animal models has given us insights into its function. However, in cases of IEI, which affect hematopoiesis and immune development, the inter-species differences within hematopoietic development are a considerable limitation for the use of animal models to adequately study IEI (9). In those cases, human iPSC-derived cells are better suited to clarify the role of specific IEI in the hematopoietic system.

Furthermore, the use of iPSC-derived cells is not only advantageous for studying IEI to unravel the pathomechanism of various diseases but also introduces alternative therapeutic strategies. The phenotypical and functional similarities of iPSC-derived immune cells such as granulocytes, macrophages, and dendritic cells with their respective primary counterparts (10) further support the use of iPSC-derived cells to study the onset of diseases and to develop novel cell therapy concepts. As a consequence, in the last years continuous optimization of the iPSC-based differentiation protocols improved both the quality and quantity of the derived cell types, aiming to fulfill the requirements for clinical application.

Hematopoietic differentiation protocols for the generation of iPSC-derived immune cells to study IEI

The fact that various tissues and cell types may be affected in different IEI underlines the complexity of IEI-related diseases

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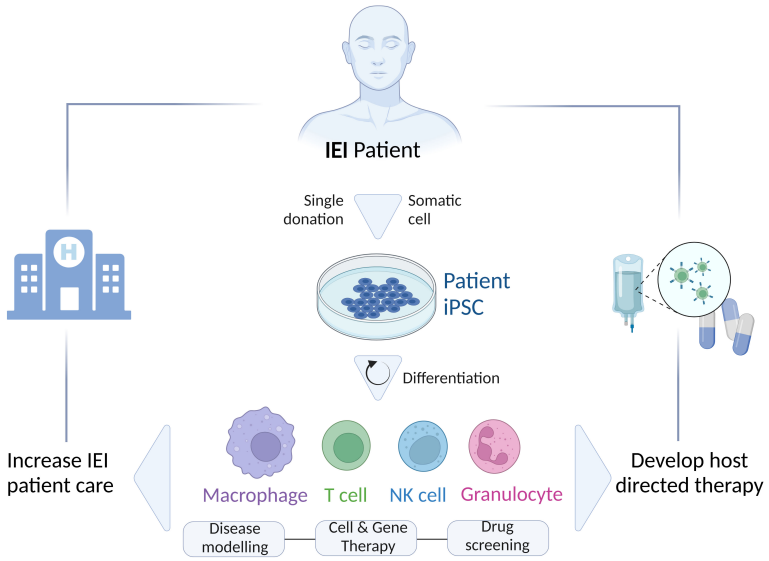

FIGURE 1
Schematic representation of the generation and differentiation of patient's-specific iPSCs to different cell types (Macrophages, T cells, NK cells, Granulocytes) for various applications (Disease modeling; Cell and Gene therapy; Drug screening) to study IEI and improve the patient's care by developing novel host directed therapies. IEI, inborn errors of immunity; iPSC, induced-pluripotent stem cells.

and the growing need for specialized and highly standardized immune cells to study the onset and progression of these diseases. The high demand for adequate numbers of the affected patient-specific immune cells led, upon the discovery of iPSCs in 2006, to the establishment of numerous hematopoietic differentiation protocols able to generate different lineages of the lympho-hematopoietic system. In recent years, several iPSC-based differentiation protocols have been established for the generation of for instance macrophages, granulocytes, dendritic cells (DCs), natural killer cells (NK), NKT cells, and T lymphocytes. In general, the iPSC differentiation protocols include as first step the differentiation of iPSCs to hematopoietic progenitors either by the support of stromal cells and the use of cytokines or by the formation of so-called embryonic bodies (EBs), which are aggregates containing cells of the three germ layers. As an example, in the context of macrophages, recent differentiation techniques result in the generation of cells using EBs. The differentiation to hematopoietic or myeloid progenitors within the cell aggregates happens either autonomously from factors produced by the cell aggregates (11–13) or by the addition of exogenous factors such as BMP4, VEGF, SCF, Flt3-ligand, and TPO (13–15). Of note, using modern differentiation media authentic macrophages can be generated from human iPSCs, which share phenotypical and functional hallmarks with their *in vivo* counterparts (16). Similarly, simplified two-step protocols have also been established for the generation of iPSC-derived NK cells. In these differentiation platforms, the cytokines IL-3, IL-7, SCF, IL-15 and Flt3-ligand are often used for the differentiation of iPSC-derived progenitor cells towards NK cells (17, 18). Although still quite challenging to generate from human iPSC, some progress has been made to produce iPSC-derived T lymphocytes (19–28). Some of the strategies that have been developed include the reprogramming of antigen-specific T cells to iPSCs and the subsequent differentiation to T cells with the respective antigen-specificity (20, 23, 27) or the generation of custom-made antigen-specific T cells using T-cell receptor (TCR)-transduced iPSCs (22, 26). Of note, the functional resemblance of the iPSC-derived lymphocytes to the *in vivo* lymphocytes raises hopes for the use of these cells for the treatment of several diseases.

Moving from the use of iPSC-derived immune cells for disease modelling towards cell-based therapies targeting IEI, required the development of differentiation protocols which allow a continuous and scalable production of iPSC-derived immune cells such as macrophages (12, 29), NK cells (30), and T lymphocytes (22). The successful use of iPSC-derived immune cells as a cell-based therapy requires authentic immune cells, which are functionally indistinguishable from their *in vivo* counterparts. For instance, iPSC-derived macrophages show typical morphological and phenotypical characteristics. When tested for their ability to secrete cytokines and to perform phagocytosis, iPSC-macrophages showed a similar cytokine secretion profile to the monocyte-derived macrophages and high phagocytic capacity, respectively (11, 12, 31). In addition, iPSC-macrophages have been shown to react highly similar to monocyte-derived macrophages to a variety of pathogens (12, 32–34). Similarly, iPSC-derived NK cells show typical NK

characteristics and full functionality, as proven in several studies to be able to eradicate HIV-infected $CD4^+$ T cells (17), myeloma or pancreatic tumor cells (18) as well as ovarian cancer cells (35). Likewise, iPSC-derived antigen-specific cytotoxic T cells (CTLs) directed against the melanoma epitope MART1 (25) or the WT1 antigen (20), showed antigen-specific reactivity upon stimulation with the respective antigen, proving their functional similarity to *in vivo* CTLs. These protocols are constantly adapted and pave the way for the generation of highly standardized, well-characterized cells from iPSCs, which are derived from healthy or diseased individuals and which can now be used to model IEI *in vitro*. Furthermore, the existence of such differentiation protocols makes these iPSC-derived cell products promising therapeutic agents for "bench to bedside" applications.

iPSC-based *in vitro* systems to study IEI

The role and importance of iPSC-derived immune cells for the field of IEI is constantly growing and opens new possibilities to study novel forms of treatment. The establishment of numerous disease models for the discovery of the responsible molecular and cellular factors for the clinical phenotype and, at the same time, establishment of promising alternative therapeutic strategies either through the discovery of potent drugs by drug screening approaches or through genetic manipulation of the cells for cell-based therapies, are of great importance. In the chapter below, we cite representative studies for most of the categories of IEI and for which iPSC-derived immune cells have been used to study IEI. A broader overview of the different studies published in this field in the last decade can also be seen in Table 1.

Combined immunodeficiencies

One of the most common diseases of this category is severe combined immunodeficiency (SCID), which is characterized by a lack of $CD3^+$ T cells. SCID is a life-threatening syndrome with a prevalence of 1/50.000-100.000 worldwide. *IL2RG*, *IL7R*, *JAK3*, *ADA*, *RAG1/2*, and *DCLRE1C* are the most common genes identified to be impaired in SCID patients, resulting in various clinical phenotypes. The current therapeutic approach for SCID patients, apart from antimicrobial drugs, is HSCT partially in combination with gene therapy. The first trial to generate iPSCs from a SCID-patient (adenosine deaminase; ADA deficient-SCID) was conducted in 2008 by Park et al. (83). Later, in 2015 Chang et al. used a patient-specific iPSC line with a mutation in the *JAK3* gene to generate T cells using a two-step OP9 and OP9-DL4 system (39). Studying these iPSC-derived JAK-deficient-T cells showed that JAK deficiency negatively impacts the differentiation of the cells into an early T cell progenitor stage, unraveling the mechanism of immunodeficiency in these patients (39). Correction of the *JAK3* mutation in iPSCs using CRISP/Cas9 technology restored normal T cell development (39). This highlights the importance of iPSC-based *in vitro* systems for studying human lymphopoiesis while developing novel gene correction strategies for human immunodeficiencies at the same time.

ADA deficiency causes abnormal differentiation and function of T cells leading to a severe combined immunodeficiency (84, 85). Recent data from ADA-deficient patients indicated that ADA deficiency impacts myeloid cells, such as neutrophils (43, 86). Given the difficulty in isolating neutrophils from ADA-deficient patients for follow-up studies, using patient-specific iPSCs for generating ADA-deficient neutrophils is very beneficial. Here Tsui et al. could show that ADA-deficient iPSCs generate lower numbers of neutrophils with increased frequency of hyper lobular neutrophils, characterized by decreased phagocytic capacity (43). Thus, the iPSCs technology was able to further associate the contributing mechanisms to the phenotype of ADA-deficient patients (43).

Combined immunodeficiencies with syndromic features

Ataxia telangiectasia (AT) is an inherited disease characterized by a severe neurological phenotype with a poor prognosis and a lack of efficient accessible treatment. AT is caused by a mutation in the ataxia-telangiectasia mutated gene (*ATM*), leading to a combined immunodeficiency in patients and an increased risk for the development of autoimmunity (87). Not differentiated towards immune cells, iPSCs generated from an AT patient were used as an *in vitro* model to study the cytotoxic effects of the potentially effective immunomodulators thioguanine, mercaptopurine, dexamethasone, mepacrine, thalidomide, and lenalidomide (44). In detail, AT iPSCs were more resistant to thioguanine compared to wild-type iPSCs and at the highest tested concentration of thalidomide and lenalidomide slightly higher cytotoxic effect was observed in AT iPSCs (44). Both AT and wild-type iPSCs were resistant to dexamethasone (44).

As another example, Wiskott-Aldrich syndrome (WAS) is an X-linked inherited immunodeficiency characterized by micro thrombocytopenia, autoimmunity, and hematological malignancies (28). The disease is caused by various mutations in the *WAS* protein gene. Generation of WAS-specific iPSCs and subsequent differentiation to megakaryocytes and platelets contributed to understanding the disease and identifying the responsible molecular and cellular players. More specifically, WAS-iPSC-derived megakaryocytes showed an abnormal pattern of F-actin distribution with abnormal pro-platelet processes, indicating dysregulated cytoskeletal protein

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TABLE 1 An overview of the latest studies using iPSC-derived cells to study IEI.

	Disease	Gene	Studied cell type (iPSC or iPSC-derived)	Application	Reference
Combined immunodeficiencies	SCID	*RAG2*	T cells	Gene Editing	(36)
		RAG2	T cells; NK cells	Disease Modeling	(37)
		RAG1	T cells	Disease Modeling	(38)
		JAK3	T cells	Disease Modeling/Gene editing	(39)
	SCID-X1	*IL-2RG*	NK cells	Gene Editing	(40)
	Reticular dysgenesis	*AK2*	Myeloid; erythroid precursors; myeloid cells	Disease Modeling	(41)
	XLF deficiency	*NHEJ1*	Hematopoietic progenitors	Disease Modeling	(42)
	ADA deficiency	*ADA*	Hematopoietic progenitors; neutrophils	Disease Modeling	(43)
Combined immunodeficiencies with syndromic features	AT	*ATM TREX1 RNASEH2B IFIH1*	iPSCs	Drug Screening	(44)
		ATM	iPSCs	Gene Editing	(45)
	WAS	*WAS*	Hematopoietic progenitors; T cells; NK cells	Disease Modeling/Gene Editing	(46)
		WAS	megakaryocytes	Disease Modeling	(47)
Predominantly antibody deficiencies	Hoffman syndrome	*TOP2B*	NK cells	Disease Modeling	(48)
Diseases of immune dysregulation	APECED	*AIRE*	iPSCs	Disease Modeling	(49)
	VEO-IBD	*IL10RA IL10RB STAT3*	Macrophages	Disease Modeling/Gene Editing/Drug Screening	(50)
Congenital defects of phagocytes	SDS	*SBDS*	Hemoangiogenic progenitors; neutrophils; endothelial cells	Disease Modeling	(51)
		SBDS	Pancreatic progenitors; mature pancreatic acinar cells; hematopoietic cells	Disease Modeling	(52)
	SCN	*G6PC3*	Granulocytes; neutrophils; monocytes/ macrophages	Disease Modeling/Gene Editing/Drug Screening	(53)
		ELANE	Granulocytes	Disease Modeling	(54)
		HAX1	Myeloid progenitors; neutrophils; monocytes	Disease Modeling/Gene Editing	(55)
	CF	*CFTR*	Lung progenitor cultures	Disease Modeling/Drug Screening	(56)
		CFTR	iPSCs	Disease Modeling/Gene Editing	(57)
		CFTR	Intestinal epithelia	Gene editing/Drug Screening	(58)
	GATA2 deficiency	*GATA2*	Hemogenic endothelial precursors; hematopoietic progenitors; NK cells	Disease Modeling	(59)
	PAP	*CSF2RA*	Macrophages	Gene Editing	(60)
		CSF2RA	Monocytes; macrophages	Gene Editing	(61)
	CGD	*NCF1*	Granulocytes; macrophages	Gene Editing	(62)
		CYBB	Granulocytes	Gene Editing	(63)
		CYBB	Neutrophils	Gene Editing	(64)
		CYBB	Monocytes; macrophages	Gene Editing	(65)
		CYBB	Granulocytes	Gene Editing	(66)
		CYBA NCF2	Neutrophils; macrophages	Disease Modeling	(67)
Defects in intrinsic and innate immunity	MYD88 deficiency	*MYD88*	Macrophages	Disease Modeling	(68)
	MSMD	*IFNGR2 IFNGR1 STAT1*	Macrophages	Disease Modeling	(69)

(Continued)

TABLE 1 Continued

	Disease	Gene	Studied cell type (iPSC or iPSC-derived)	Application	Reference
		IFNGR1	Macrophages	Disease Modeling	(70)
	TLR3 deficiency	*TLR3*	Trigeminal ganglion neurons	Disease Modeling	(71)
		TLR3 UNC93B	Neural stem cells; neurons; astrocytes; oligodendrocytes	Disease Modeling	(72)
Auto-inflammatory diseases	NOMID	*NLRP3*	Monocytes	Drug Screening	(73)
		NLRP3	Chondrocytes	Disease Modeling	(74)
	Blau syndrome	*NOD2*	Macrophages	Disease Modeling	(75)
		NOD2	Macrophages	Disease Modeling/Gene Editing	(76)
Bone marrow failure	FA	*FANCA*	iPSCs; hematopoietic progenitor cells	Disease Modeling	(77)
		FANCA	Hemoangiogenic progenitors	Disease Modeling	(78)
		FANCA	iPSCs; hematopoietic progenitor cells; mesenchymal stem cells	Disease Modeling/Drug Screening	(79)
	FA-like BMFS	*ADH5 ALDH2*	iPSCs	Disease Modeling	(80)
Phenocopies of IEI	NOMID-like disease	*NLRC4*	Macrophages	Disease Modeling	(81)
		NLRP3	Macrophages	Disease Modeling/Drug Screening	(82)

Diseases, affected genes, studied iPSC-derived cell types and application (Disease Modeling, Gene Editing, Drug Screening) are summarized. SCID, severe combined immunodeficiency; XLF, XRCC4-like factor; ADA, adenosine deaminase; AT, ataxia-telangiectasia; WAS, Wiskott-Aldrich syndrome; APECED, autoimmune polyendocrinopathy candidiasis ectodermal dystrophy; VEO-IBD, very early onset inflammatory bowel disease; SDS, Shwachman-Diamond syndrome; SCN, severe congenital neutropenia; CF, cystic fibrosis; PAP, pulmonary alveolar proteinosis; CGD, chronic granulomatous disease; MSMD, mendelian susceptibility to mycobacterial disease; NOMID, neonatal-onset multisystem inflammatory disease; FA, Fanconi anemia; FA-like BMFS, Fanconi anemia-like bone marrow failure syndrome.

rearrangement during pro-platelet formation (47). In this case, the use of patient-derived iPSCs could highlight the importance of the WAS protein for normal platelet production. Similar to the SCID studies, overexpression of the healthy WAS protein in patient- derived iPSCs could rescue the phenotype, paving the way for new therapeutic options. Similarly, Laskowski et al. restored the WAS protein function in patient-derived iPSCs using zinc finger nucleases technology and the differentiated hematopoietic lineages were restored (46). Of note, while differentiation of both healthy and WAS-iPSCs towards non-lymphoid cells was sufficient, a clear reduction in the generation of CD4/CD8 double positive T cells and NK cells was observed, which could be restored upon targeted correction of the *WAS* gene locus.

Diseases of immune dysregulation

Genetic forms of inflammatory bowel disease (IBD) are caused by mutations in genes that are involved in the IL-10 signaling pathway (88). IBD is characterized by severe bowel inflammation and is developed within the first 6 years of life (89). Many of the patients do not respond to anti-inflammatory and immunosuppressive treatments. To study the pathophysiology of IBD and contribute to novel therapeutic strategies, KO iPSC models for the genes *IL10R*, *IL10RB*, *STAT1*, and *STAT3* were generated using sgRNA-directed CRISPR-Cas9 lentiviral vectors (50). Using macrophages derived from these KO-iPSC lines these studies could show that defects in any of the IL10R chains or in STAT3 result in absence of BCL3 expression and reduced secretion of defined IL-10R-/STAT3-dependent cytokines (50). Of note, the phenotype of the KO-iPSC-derived macrophages could however be restored (reduced pro-inflammatory cytokines) using lentiviral vectors overexpressing the *IL-10R* gene. Using the same iPSC-macrophage system, small anti-inflammatory agents (SB202190 and Filgotinib) were tested and could confirm their anti-inflammatory effect by the reduction of TNF-α, IL-6, and CCL5, while no negative impact could be observed on iPSC-derived macrophages with respect to cell viability (50).

Congenital defects of phagocytes

Chronic granulomatous disease (CGD) is characterized by severe, recurrent, and life-threatening bacterial and fungal infections due to defects in the oxidative burst in phagocytes. Its prevalence is 1/250,000 individuals. CGD can be caused by mutations in any of the four components of the NADPH oxidase complex. The most common mutation is found in the *CYBB* gene, which encodes for the $gp91^{phox}$ subunit and is X-linked. To date, the only available treatment is allogeneic or autologous (genetic corrected) HSCT. To elaborate on new treatments for X-CGD patients, several studies have used X-CGD-patient-specific iPSCs to genetically modify the cells using

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zing finger nuclease-mediated gene targeting (90), transcription activator-like effector nucleases (TALENs) (66) or bacterial artificial chromosomes (BAC) transgenesis (64), respectively. In all iPSC-based studies, the *CYBB* function was successfully restored, leading to sufficient oxidative activity and ROS production in iPSC-derived granulocytes, proven the suitability of gene therapy to restore the anti-microbial function in immune cells. In a completely different approach, the NADPH oxidase activity of X-CGD iPSC-derived macrophages was restored using NOX2/p22^{phox} proteoliposomes, which were transported into the macrophages (91). The combination of patient-specific iPSC-derived cells with recombinant therapeutic proteoliposomes could in the future lead to the development of alternative antibacterial or antifungal therapies for patients with IEI. Similar to the aforementioned approaches, the iPSC system has also been used to establish and test gene correction of p47^{phox} deficiency. Introducing a functional *NCF1* minigene into the intron 1 of the *NCF1* gene using CRISPR/Cas9 (62) or targeted correction of the mutation (GT deletion in NCF1 pseudogenes) using zinc-finger nucleases (92) into p47-CGD iPSCs could restore oxidase function in iPSC-derived immune cells, highlighting the suitability of the iPSC system to test novel gene therapy concepts.

Defects in intrinsic and innate immunity

Mendelian susceptibility to mycobacterial disease (MSMD) is characterized by increased susceptibility to weakly virulent mycobacteria (e.g. *Mycobacterium bovis* Bacillus Calmette-Guerin; BCG). The genetic etiology of MSMD is complex, with a variety of genes and mutations involved, which all affect the sufficient breakdown of mycobacteria. Mutations can affect either T cells (e.g. *IL12RB1*, *IL12RB2*, *TYK2*) or macrophages (e.g. *IFNGR1*, *INFGR2*, *IRF8*, *CYBB*, *NEMO*), which lead to an impaired crosstalk of these two cell types. As an example, the clinical phenotype can be severe, as seen in patients with complete IFN-gamma receptor 1 or 2 deficiency (*IFNGR1/2*). In contrast, the clinical phenotype can also be mild to moderate, as seen in patients suffering from STAT1, IL-12/IL-23 receptor, or tyrosine kinase 2 deficiency. Of note, clinical symptoms and the impaired function of e.g. macrophages can be improved by treating patients with high dose IFNγ therapy. However, this kind of treatment is unsuitable for patients who suffer from complete *IFNGR1* or *IFNGR2* deficiency. The generation of iPSCs from patients harboring mutations in genes involved in the IFNγ signaling, such as *IFNGR1*, *IFNGR2*, and also *STAT1* were able to demonstrate in detail the impact of IFNγ on macrophages and the importance of this cell type in the onset and progression of mycobacterial susceptibility (69, 70). These studies revealed iPSC-derived macrophages with an impaired type II IFN system showing normal macrophage differentiation and phenotype but severely impaired intracellular killing activity for BCG (69, 70).

Autoinflammatory diseases

Neonatal-onset multisystem inflammatory disease (NOMID), also known as chronic infantile neurologic cutaneous articular syndrome (CINCA), is a rare genetic disease present from birth and caused by mutations mainly in the *NLRP3* locus. It is inherited in an autosomal dominant way, and the patients suffer from uncontrolled inflammation in several systems of the body, such as skin, joints, and central nervous system. The clinical phenotype varies and includes urticarial-like skin rash, arthritis, and chronic meningitis, which increases the risk of neurological problems. So far, anti-IL-1β treatment (e.g. Anakinra) using specific inhibitors is the preferable therapeutic option. However, its effectiveness is highly dependent on the severity of the disease phenotype. In addition, the complete IL-1β blockade involves the risk of uncontrolled immunosuppression. For this reason, selective NLRP3 inhibitors would be more beneficial as therapeutic option and several NLRP3 inhibitors have already entered clinical trials (93). However, given the different mutations observed in NOMID patients, it is possible that some NLRP3 mutants escape an efficient inhibition from already known inhibitors (94). Therefore, discovery of novel NLRP3 inhibitors is necessary. To test and screen for new therapeutic compounds, patient-specific NOMID-iPSCs could be used as a screening platform. Seki et al. generated iPSC-derived immortalized myeloid cell lines from wild-type and NLRP3-mutated iPSC clones and subsequently differentiated these into macrophages (73). Generated macrophages were further used for developing a high throughput system to identify compounds that show inhibitory effects specifically against the secretion of IL-1β and the activation of mutant NLRP3 (73). Out of almost 5,000 tested compounds, seven candidates were sufficiently blocking the IL-1β secretion. Interestingly those were already introduced in previous studies as NLRP3 inhibitors, indicating the effectiveness of the system (73).

Complement deficiencies

In addition to increased susceptibility and recurrent bacterial infections, deficiencies in the complement pathway have been linked to age-related macular degeneration (AMD). AMD is an ideal example underlining the interconnection of IEI with various tissues and organs of the body. Thus, using iPSCs generated from patients with AMD or from healthy individuals shed light on the mechanisms involved in the disease and showed the impact of an IEI in a complement protein on the progression of AMD. In this case, retinal pigment

epithelium derived from AMD-derived iPSCs was used to show impaired mitochondrial function under stress conditions and its link to the presence of the high-risk allele for the complement factor H (*CFH* locus) (95). More generated iPSC lines from three patients carrying the rare variants in the *CFH* locus and suffering from AMD are available tools for further cellular studies and the development of novel treatments (96).

Bone marrow failure

Fanconi anemia (FA) is an inherited condition diagnosed usually in children between the age of 3 and 14, and it is characterized, among other symptoms, by failure of bone marrow function. It is caused by mutations in at least 22 different genes, which are involved in the FA pathway, responsible for the DNA repair process, with the genes *FANCA*, *FANCC*, and *FANCG* to be utmost affected. The only curative treatment so far is HSCT, with gene therapy on the horizon (97). Given the difficulty that exists in recapitulating FA pathophysiology using mouse models, further understanding of the disease pathogenesis was accomplished using FA patient-specific iPSCs for disease modelling (77, 79). Marion et al. revealed that activation of the p53-p21 axis leads to accelerated erythroid differentiation in FANCA-deficient HPCs. Use of exogenous recombinant human GAS6 resulted in restored hematopoiesis, providing alternative options for improving therapy of FA in the future (77). In contrast, another study utilized the iPSC technology to screen and evaluate novel compounds, discovering that Tremulacin was able to rescue the hematopoietic defects of FA patient by suppressing the transcription of the inflammatory cytokine TNFα (79), which impressively shows the potential of the iPSC technology.

Current limitations and future perspectives

The numerous studies that have used iPSC-derived cells for disease modeling and drug screening for several IEI-related conditions could lay the foundation for developing novel therapies. The reason for this success is the unique potential of iPSCs to differentiate into almost all cells of the hematopoietic system and beyond. Although established protocols for the differentiation of a plethora of iPSC-derived immune cells exist, still the lack of protocols for a robust generation of T or B lymphocytes that resemble their *in vivo* counterparts as much as possible, is currently a limitation of the technology and must be confronted in the coming years.

While most of the aforementioned reports have used iPSCs as a disease modeling platform, the use of iPSC-based platforms for establishing drug screening, drug toxicology, and drug-drug interactions in the context of IEI is highly warranted and will accelerate the progress of personalized medicine further. Along this line, several studies used iPSC-derived cells for targeted drug testing or screening (44, 50, 53, 56, 58, 79). However, these attempts did not result in developing a widely accepted drug so far. Consequently, researchers have not fully exploited the full potential of iPSCs until now, and novel approaches are currently underway to solve this issue. Besides drug discovery in the context of IEI, the further improvement of existing differentiation protocols from feeder-based GMP-incompatible systems to xeno-free and GMP-compatible protocols, drove iPSC-derived cells to the first clinical trials. In 2021, 19 therapeutic clinical studies were globally ongoing (98). Interestingly, none of them was related to IEI, highlighting the effort that should be invested in the next years to bring the undoubtable benefits that we can gain from the iPSC technology closer to the clinic. One of the main challenges in using iPSC-derived cells as a cell-based therapeutic intervention are the immunohistocompatibility issues arising from the use of allogeneic cells. Most clinical trials currently use allogeneic cells since the generation of autologous iPSCs is time-consuming, which becomes a particular issue when the recipient urgently needs cell therapy. Therefore several studies have tried to elaborate alternative options, either by developing cell banks with homozygous iPSC lines or by generating immunocompatible iPSCs through genetic manipulation (99–101).

Given the very young age of most patients with IEI, the elimination of the possible tumorigenicity of iPSCs and iPSC-derived cells, should be highly warranted, before the use of iPSC-derived cells for the treatment of children suffering from IEI. In order to diminish the impact of the integrating vectors on the iPSCs genetic stability and to provide a reliable tool to generate novel therapies against IEI, different reprogramming strategies have been developed, such as the use of non-integrating vectors, synthetic mRNAs, or integrating vectors that can be excised. Establishing a universal and highly standardized procedure for confirming the genetic stability and purity of iPSCs to get approved for clinical use, could be very beneficial. Usage of Next Generation Sequencing analysis should also be considered to guarantee the detection of all possible genetic anomalies and to ensure the production of high-quality iPSCs with limited risk for tumorigenicity (102). To further minimize the tumorigenic risk, in the last two decades, several systems have been developed for the elimination of aberrant cells using suicide gene technology (103–108). Most recently, immunodepletion has also been used to selectively deplete contaminating iPSCs with the help of monoclonal antibodies (109, 110) or chimerized monoclonal antibodies (111). Further optimization of these tools will

significantly assist in facilitating the safe use of iPSC-derived cells in the clinical setting in the future.

While iPSC and thereof derived cells are used frequently for modeling IEI the clinical translation of cells to treat IEI is more in the future.

Authors contribution

EN, JR, GH, and NL designed, wrote and approved the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

NL filed and licensed patents in the field of iPSC-derived macrophages outside of the MS. NL is a consultant for CATALENT outside of the MS.

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.

Publisher's note

References

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

2. Bousfiha A, Moundir A, Tangye SG, Picard C, Jeddane L, Al-Herz W, et al. The 2022 update of IUIS phenotypical classification for human inborn errors of immunity. *J Clin Immunol* (2022). doi: 10.1007/s10875-022-01352-z

3. Tangye SG, Al-Herz W, Bousfiha A, Cunningham-Rundles C, Franco JL, Holland SM, et al. Human inborn errors of immunity: 2022 update on the classification from the international union of immunological societies expert committee. *J Clin Immunol* Published online June 2022, 1–35. doi: 10.1007/s10875-022-01289-3

4. Bousfiha A, Jeddane L, Picard C, Al-Herz W, Ailal F, Chatila T, et al. Human inborn errors of immunity: 2019 update of the IUIS phenotypical classification. *J Clin Immunol* (2020) 40(1):66–81. doi: 10.1007/s10875-020-00758-x

5. Tangye SG, Al-Herz W, Bousfiha A, Chatila T, Cunningham-Rundles C, Etzioni A, et al. Human inborn errors of immunity: 2019 update on the classification from the international union of immunological societies expert committee. *J Clin Immunol* (2020) 40(1):24–64. doi: 10.1007/s10875-019-00737-x

6. Pai S-Y, Logan BR, Griffith LM, Buckley RH, Parrott RE, Dvorak CC, et al. Transplantation outcomes for severe combined immunodeficiency, 2000-2009. *N Engl J Med* (2014) 371(5):434–46. doi: 10.1056/NEJMoa1401177

7. Speckmann C, Doerken S, Aiuti A, Albert MH, Al-Herz W, Allende LM, et al. A prospective study on the natural history of patients with profound combined immunodeficiency: An interim analysis. *J Allergy Clin Immunol* (2017) 139 (4):1302–10.e4. doi: 10.1016/j.jaci.2016.07.040

8. Ye Z, Chou B-K, Cheng L. Promise and challenges of human iPSC-based hematologic disease modeling and treatment. *Int J Hematol* (2012) 95(6):601–9. doi: 10.1007/s12185-012-1095-9

9. Parekh C, Crooks GM. Critical differences in hematopoiesis and lymphoid development between humans and mice. *J Clin Immunol* (2013) 33(4):711–5. doi: 10.1007/s10875-012-9844-3

10. Monkley S, Krishnaswamy JK, Göransson M, Clausen M, Meuller J, Thörn K, et al. Optimised generation of iPSC-derived macrophages and dendritic cells that are functionally and transcriptionally similar to their primary counterparts. *PloS One* (2020) 15(12):e0243807. doi: 10.1371/journal.pone.0243807

11. van Wilgenburg B, Browne C, Vowles J, Cowley SA. Efficient, long term production of monocyte-derived macrophages from human pluripotent stem cells under partly-defined and fully-defined conditions. *PloS One* (2013) 8(8):e71098. doi: 10.1371/journal.pone.0071098

12. Ackermann M, Kempf H, Hetzel M, Hesse C, Hashtchin AR, Brinkert K, et al. Bioreactor-based mass production of human iPSC-derived macrophages enables immunotherapies against bacterial airway infections. *Nat Commun* (2018) 9 (1):5088. doi: 10.1038/s41467-018-07570-7

13. Lyadova I, Gerasimova T, Nenasheva T. Macrophages derived from human induced pluripotent stem cells: The diversity of protocols, future prospects, and outstanding questions. *Front Cell Dev Biol* (2021) 9:640703. doi: 10.3389/fcell.2021.640703

14. Zhang H, Xue C, Shah R, Bermingham K, Hinkle CC, Li W, et al. Functional analysis and transcriptomic profiling of iPSC-derived macrophages and their application in modeling mendelian disease. *Circ Res* (2015) 117(1):17–28. doi: 10.1161/CIRCRESAHA.117.305860

15. Buchrieser J, James W, Moore MD. Human induced pluripotent stem cell-derived macrophages share ontogeny with MYB-independent tissue-resident macrophages. *Stem Cell Rep* (2017) 8(2):334–45. doi: 10.1016/j.stemcr.2016.12.020

16. Vaughan-Jackson A, Stodolak S, Ebrahimi KH, Browne C, Reardon PK, Pires E, et al. Differentiation of human induced pluripotent stem cells to authentic macrophages using a defined, serum-free, open-source medium. *Stem Cell Rep* (2021) 16(7):1735–48. doi: 10.1016/j.stemcr.2021.05.018

17. Ni Z, Knorr DA, Clouser CL, Hexum MK, Southern P, Mansky LM, et al. Human pluripotent stem cells produce natural killer cells that mediate anti-HIV-1 activity by utilizing diverse cellular mechanisms. *J Virol* (2011) 85(1):43–50. doi: 10.1128/JVI.01774-10

18. Knorr DA, Ni Z, Hermanson D, Hexum MK, Bendzick L, Cooper LJN, et al. Clinical-scale derivation of natural killer cells from human pluripotent stem cells for cancer therapy. *Stem Cells Transl Med* (2013) 2(4):274–83. doi: 10.5966/sctm.2012-0084

19. Flippe L, Gaignerie A, Sérazin C, Baron O, Saulquin X, Themeli M, et al. Rapid and reproducible differentiation of hematopoietic and T cell progenitors from pluripotent stem cells. *Front Cell Dev Biol* (2020) 8:577464. doi: 10.3389/fcell.2020.577464

20. Kawamoto H, Masuda K, Nagano S, Maeda T. Cloning and expansion of antigen-specific T cells using iPS cell technology: development of "off-the-shelf" T cells for the use in allogeneic transfusion settings. *Int J Hematol* (2018) 107(3):271–7. doi: 10.1007/s12185-018-2399-1

21. Iriguchi S, Yasui Y, Kawai Y, Arima S, Kunitomo M, Sato T, et al. A clinically applicable and scalable method to regenerate T-cells from iPSCs for off-the-shelf T-cell immunotherapy. *Nat Commun* (2021) 12(1):430. doi: 10.1038/s41467-020-20658-3

22. Nishimura T, Kaneko S, Kawana-Tachikawa A, Tajima Y, Goto H, Zhu D, et al. Generation of rejuvenated antigen-specific T cells by reprogramming to pluripotency and redifferentiation. *Cell Stem Cell* (2013) 12(1):114–26. doi: 10.1016/j.stem.2012.11.002

23. Wakao H, Yoshikiyo K, Koshimizu U, Furukawa T, Enomoto K, Matsunaga T, et al. Expansion of functional human mucosal-associated invariant T cells *via* reprogramming to pluripotency and redifferentiation. *Cell Stem Cell* (2013) 12(5):546–58. doi: 10.1016/j.stem.2013.03.001

24. Vizcardo R, Masuda K, Yamada D, Ikawa T, Shimizu K, Fujii SI, et al. Regeneration of human tumor antigen-specific T cells from iPSCs derived from mature CD8(+) T cells. *Cell Stem Cell* (2013) 12(1):31–6. doi: 10.1016/j.stem.2012.12.006

25. Themeli M, Kloss CC, Ciriello G, Fedorov VD, Perna F, Gonen M, et al. Generation of tumor-targeted human T lymphocytes from induced pluripotent stem cells for cancer therapy. *Nat Biotechnol* (2013) 31(10):928–33. doi: 10.1038/nbt.2678

26. Maeda T, Nagano S, Ichise H, Kataoka K, Yamada D, Ogawa S, et al. Regeneration of CD8αβ T cells from T-cell-Derived iPSC imparts potent tumor antigen-specific cytotoxicity. *Cancer Res* (2016) 76(23):6839–50. doi: 10.1158/0008-5472.CAN-16-1149

27. Minagawa A, Yoshikawa T, Yasukawa M, Hotta A, Kunitomo M, Iriguchi S, et al. Enhancing T cell receptor stability in rejuvenated iPSC-derived T cells improves their use in cancer immunotherapy. *Cell Stem Cell* (2018) 23(6):850–858.e4. doi: 10.1016/j.stem.2018.10.005

28. Orange JS, Stone KD, Turvey SE, Krzewski K. The wiskott-Aldrich syndrome. *Cell Mol Life Sci* (2004) 61(18):2361–85. doi: 10.1007/s00018-004-4086-z

29. Ackermann M, Rafiei Hashtchin A, Manstein F, Carvalho Oliveira M, Kempf H, Zweigerdt R, et al. Continuous human iPSC-macrophage mass production by suspension culture in stirred tank bioreactors. *Nat Protoc* (2022) 17(2):513–39. doi: 10.1038/s41596-021-00654-7

30. Zhu H, Kaufman DS. An improved method to produce clinical-scale natural killer cells from human pluripotent stem cells. *Methods Mol Biol* (2019) 2048:107–19. doi: 10.1007/978-1-4939-9728-2_12

31. Panicker LM, Miller D, Park TS, Patel B, Azevedo JL, Awad O, et al. Induced pluripotent stem cell model recapitulates pathologic hallmarks of gaucher disease. *Proc Natl Acad Sci U S A.* (2012) 109(44):18054–9. doi: 10.1073/pnas.1207889109

32. Rafiei Hashtchin A, Fehlhaber B, Hetzel M, Manstein F, Stalp JL, Glage S, et al. Human iPSC-derived macrophages for efficient staphylococcus aureus clearance in a murine pulmonary infection model. *Blood Adv* (2021) 5(23):5190–201. doi: 10.1182/bloodadvances.2021004853

33. Hale C, Yeung A, Goulding D, Pickard D, Alasoo K, Powrie F, et al. Induced pluripotent stem cell derived macrophages as a cellular system to study salmonella and other pathogens. *PloS One* (2015) 10(5):e0124307. doi: 10.1371/journal.pone.0124307

34. Nenasheva T, Gerasimova T, Serdyuk Y, Grigor'eva E, Kosmiadi G, Nikolaev A, et al. Macrophages derived from human induced pluripotent stem cells are low-activated "Naïve-like" cells capable of restricting mycobacteria growth. *Front Immunol* (2020) 11:1016. doi: 10.3389/fimmu.2020.01016

35. Hermanson DL, Bendzick L, Pribyl L, McCullar V, Vogel RI, Miller JS, et al. Induced pluripotent stem cell-derived natural killer cells for treatment of ovarian cancer. *Stem Cells* (2016) 34(1):93–101. doi: 10.1002/stem.2230

36. Gardner CL, Pavel-Dinu M, Dobbs K, Bosticardo M, Reardon PK, Lack J, et al. Gene editing rescues *In vitro* T cell development of RAG2-deficient induced pluripotent stem cells in an artificial thymic organoid system. *J Clin Immunol* (2021) 41(5):852–62. doi: 10.1007/s10875-021-00989-6

37. Themeli M, Chhatta A, Boersma H, Prins HJ, Cordes M, de Wilt E, et al. iPSC-based modeling of RAG2 severe combined immunodeficiency reveals multiple T cell developmental arrests. *Stem Cell Rep* (2020) 14(2):300–11. doi: 10.1016/j.stemcr.2019.12.010

38. Brauer PM, Pessach IM, Clarke E, Rowe JH, Ott de Bruin L, Lee YN, et al. Modeling altered T-cell development with induced pluripotent stem cells from patients with RAG1-dependent immune deficiencies. *Blood* (2016) 128(6):783–93. doi: 10.1182/blood-2015-10-676304

39. Chang C-W, Lai Y-S, Westin E, Khodadadi-Jamayran A, Pawlik KM, Lamb LSJ, et al. Modeling human severe combined immunodeficiency and correction by CRISPR/Cas9-enhanced gene targeting. *Cell Rep* (2015) 12(10):1668–77. doi: 10.1016/j.celrep.2015.08.013

40. Menon T, Firth AL, Scripture-Adams DD, Galic Z, Qualls SJ, Gilmore WB, et al. Lymphoid regeneration from gene-corrected SCID-X1 subject-derived iPSCs. *Cell Stem Cell* (2015) 16(4):367–72. doi: 10.1016/j.stem.2015.02.005

41. Rissone A, Weinacht KG, la Marca G, Bishop K, Giocaliere E, Jagadeesh J, et al. Reticular dysgenesis-associated AK2 protects hematopoietic stem and progenitor cell development from oxidative stress. *J Exp Med* (2015) 212(8):1185–202. doi: 10.1084/jem.20141286

42. Tilgner K, Neganova I, Singhapol C, Saretzki G, Al-Aama JY, Evans J, et al. Brief report: a human induced pluripotent stem cell model of cernunnos deficiency reveals an important role for XLF in the survival of the primitive hematopoietic progenitors. *Stem Cells* (2013) 31(9):2015–23. doi: 10.1002/stem.1456

43. Tsui M, Min W, Ng S, Dobbs K, Notarangelo LD, Dror Y, et al. The use of induced pluripotent stem cells to study the effects of adenosine deaminase deficiency on human neutrophil development. *Front Immunol* (2021) 12:748519. doi: 10.3389/fimmu.2021.748519

44. Genova E, Cavion F, Lucafò M, Pelin M, Lanzi G, Masneri S, et al. Biomarkers and precision therapy for primary immunodeficiencies: An *In vitro* study based on induced pluripotent stem cells from patients. *Clin Pharmacol Ther* (2020) 108(2):358–67. doi: 10.1002/cpt.1837

45. Ovchinnikov DA, Withey SL, Leeson HC, Lei UW, Sundarrajan A, Junday K, et al. Correction of ATM mutations in iPS cells from two ataxia-telangiectasia patients restores DNA damage and oxidative stress responses. *Hum Mol Genet* (2020) 29(6):990–1001. doi: 10.1093/hmg/ddaa023

46. Laskowski TJ, Van Caeneghem Y, Pourebrahim R, Ma C, Ni Z, Garate Z, et al. Gene correction of iPSCs from a wiskott-Aldrich syndrome patient normalizes the lymphoid developmental and functional defects. *Stem Cell Rep* (2016) 7(2):139–48. doi: 10.1016/j.stemcr.2016.06.003

47. Ingrungruanglert P, Amarinthnukrowh P, Rungsiwiwut R, Maneesri-le Grand S, Sosothikul D, Suphapeetiporn K, et al. Wiskott-Aldrich syndrome iPS cells produce megakaryocytes with defects in cytoskeletal rearrangement and proplatelet formation. *Thromb Haemost* (2015) 113(4):792–805. doi: 10.1160/TH14-06-0503

48. Broderick L, Clay GM, Blum RH, Liu Y, McVicar R, Papes F, et al. Disease-associated mutations in topoisomerase IIβ result in defective NK cells. *J Allergy Clin Immunol* (2022) 149(6):2171–6.e3. doi: 10.1016/j.jaci.2021.12.792

49. Karvonen E, Krohn KJE, Ranki A, Hau A. Generation and characterization of iPS cells derived from APECED patients for gene correction. *Front Endocrinol (Lausanne)* (2022) 13:794327. doi: 10.3389/fendo.2022.794327

50. Sens J, Hoffmann D, Lange L, Vollmer Barbosa P, Morgan M, Falk CS, et al. Knockout-induced pluripotent stem cells for disease and therapy modeling of IL-10-Associated primary immunodeficiencies. *Hum Gene Ther* (2021) 32(1–2):77–95. doi: 10.1089/hum.2020.235

51. Hamabata T, Umeda K, Kouzuki K, Tanaka T, Daifu T, Nodomi S, et al. Pluripotent stem cell model of shwachman-diamond syndrome reveals apoptotic predisposition of hemoangiogenic progenitors. *Sci Rep* (2020) 10(1):14859. doi: 10.1038/s41598-020-71844-8

52. Tulpule A, Kelley JM, Lensch MW, McPherson J, Park IH, Hartung O, et al. Pluripotent stem cell models of shwachman-diamond syndrome reveal a common mechanism for pancreatic and hematopoietic dysfunction. *Cell Stem Cell* (2013) 12(6):727–36. doi: 10.1016/j.stem.2013.04.002

53. Hoffmann D, Kuehle J, Lenz D, Philipp F, Zychlinski D, Lachmann N, et al. Lentiviral gene therapy and vitamin B3 treatment enable granulocytic differentiation of G6PC3-deficient induced pluripotent stem cells. *Gene Ther* (2020) 27(6):297–306. doi: 10.1038/s41434-020-0127-y

54. Nayak RC, Trump LR, Aronow BJ, Myers K, Mehta P, Kalfa T, et al. Pathogenesis of ELANE-mutant severe neutropenia revealed by induced pluripotent stem cells. *J Clin Invest* (2015) 125(8):3103–16. doi: 10.1172/JCI80924

55. Pittermann E, Lachmann N, MacLean G, Emmrich S, Ackermann M, G'hring G, et al. Gene correction of HAX1 reversed kostmann disease phenotype in patient-specific induced pluripotent stem cells. *Blood Adv* (2017) 1(14):903–14. doi: 10.1182/bloodadvances.2016003798

56. Jiang JX, Wellhauser L, Laselva O, Utkina I, Bozoky Z, Gunawardena T, et al. A new platform for high-throughput therapy testing on iPSC-derived lung

progenitor cells from cystic fibrosis patients. *Stem Cell Rep* (2021) 16(11):2825–37. doi: 10.1016/j.stemcr.2021.09.020

57. Smirnikhina SA, Kondrateva EV, Adilgereeva EP, Anuchina AA, Zaynitdinova MI, Slesarenko YS, et al. P.F508del editing in cells from cystic fibrosis patients. *PloS One* (2020) 15(11):e0242094. doi: 10.1371/journal.pone.0242094

58. Merkert S, Schubert M, Olmer R, Engels L, Radetzki S, Veltman M, et al. High-throughput screening for modulators of CFTR activity based on genetically engineered cystic fibrosis disease-specific iPSCs. *Stem Cell Rep* (2019) 12(6):1389–403. doi: 10.1016/j.stemcr.2019.04.014

59. Jung M, Cordes S, Zou J, Yu SJ, Guitart X, Hong SG, et al. GATA2 deficiency and human hematopoietic development modeled using induced pluripotent stem cells. *Blood Adv* (2018) 2(23):3553–65. doi: 10.1182/bloodadvances.2018017137

60. Kuhn A, Ackermann M, Mussolino C, Cathomen T, Lachmann N, Moritz T. TALEN-mediated functional correction of human iPSC-derived macrophages in context of hereditary pulmonary alveolar proteinosis. *Sci Rep* (2017) 7(1):15195. doi: 10.1038/s41598-017-14566-8

61. Lachmann N, Happle C, Ackermann M, Lüttge D, Wetzke M, Merkert S, et al. Gene correction of human induced pluripotent stem cells repairs the cellular phenotype in pulmonary alveolar proteinosis. *Am J Respir Crit Care Med* (2013) 189(2):131126070906004. doi: 10.1164/rccm.201306-1012OC

62. Klatt D, Cheng E, Philipp F, Selich A, Dahlke J, Schmidt RE, et al. Targeted repair of p47-CGD in iPSCs by CRISPR/Cas9: Functional correction without cleavage in the highly homologous pseudogenes. *Stem Cell Rep* (2019) 13(4):590–8. doi: 10.1016/j.stemcr.2019.08.008

63. Sweeney CL, Zou J, Choi U, Merling RK, Liu A, Bodansky A, et al. Targeted repair of CYBB in X-CGD iPSCs requires retention of intronic sequences for expression and functional correction. *Mol Ther* (2017) 25(2):321–30. doi: 10.1016/j.ymthe.2016.11.012

64. Laugsch M, Rostovskaya M, Velychko S, Richter C, Zimmer A, Klink B, et al. Functional restoration of gp91phox-oxidase activity by BAC transgenesis and gene targeting in X-linked chronic granulomatous disease iPSCs. *Mol Ther* (2016) 24 (4):812–22. doi: 10.1038/mt.2015.154

65. Flynn R, Grundmann A, Renz P, Hänseler W, James WS, Cowley SA, et al. CRISPR-mediated genotypic and phenotypic correction of a chronic granulomatous disease mutation in human iPS cells. *Exp Hematol* (2015) 43 (10):838–848.e3. doi: 10.1016/j.exphem.2015.06.002

66. Dreyer A-K, Hoffmann D, Lachmann N, Ackermann M, Steinemann D, Timm B, et al. TALEN-mediated functional correction of X-linked chronic granulomatous disease in patient-derived induced pluripotent stem cells. *Biomaterials* (2015) 69:191–200. doi: 10.1016/j.biomaterials.2015.07.057

67. Brault J, Goutagny E, Telugu N, Shao K, Baquié M, Satre V, et al. Optimized generation of functional neutrophils and macrophages from patient-specific induced pluripotent stem cells: Ex vivo models of X(0)-linked, AR22(0)- and AR47(0)- chronic granulomatous diseases. *Biores Open Access* (2014) 3(6):311–26. doi: 10.1089/biores.2014.0045

68. Craig-Mueller N, Hammad R, Elling R, Alzubi J, Timm B, Kolter J, et al. Modeling MyD88 deficiency *In vitro* provides new insights in its function. *Front Immunol* (2020) 11:608802. doi: 10.3389/fimmu.2020.608802

69. Haake K, Neehus A-L, Buchegger T, Kühnel MP, Blank P, Philipp F, et al. Patient iPSC-Derived Macrophages to Study Inborn Errors IFN-γ Responsive Pathway. *Cells* (2020) 9(2):483. doi: 10.3390/cells9020483

70. Neehus A-L, Lam J, Haake K, Merkert S, Schmidt N, Mucci A, et al. Impaired IFNγ-signaling and mycobacterial clearance in IFNγR1-deficient human iPSC-derived macrophages. *Stem Cell Rep* (2018) 10(1):7–16. doi: 10.1016/j.stemcr.2017.11.011

71. Zimmer B, Ewaleifoh O, Harschnitz O, Lee YS, Peneau C, McAlpine JL, et al. Human iPSC-derived trigeminal neurons lack constitutive TLR3-dependent immunity that protects cortical neurons from HSV-1 infection. *Proc Natl Acad Sci USA* (2018) 115(37):E8775–82. doi: 10.1073/pnas.1809853115

72. Lafaille FG, Pessach IM, Zhang S-Y, Ciancanelli MJ, Herman M, Abhyankar A, et al. Impaired intrinsic immunity to HSV-1 in human iPSC-derived TLR3-deficient CNS cells. *Nature* (2012) 491(7426):769–73. doi: 10.1038/nature11583

73. Seki R, Ohta A, Niwa A, Sugimine Y, Naito H, Nakahata T, et al. Induced pluripotent stem cell-derived monocytic cell lines from a NOMID patient serve as a screening platform for modulating NLRP3 inflammasome activity. *PloS One* (2020) 15(8):e0237030. doi: 10.1371/journal.pone.0237030

74. Yokoyama K, Ikeya M, Umeda K, Oda H, Nodomi S, Nasu A, et al. Enhanced chondrogenesis of induced pluripotent stem cells from patients with neonatal-onset multisystem inflammatory disease occurs *via* the caspase 1-independent cAMP/protein kinase A/CREB pathway. *Arthritis Rheumatol (Hoboken NJ).* (2015) 67(1):302–14. doi: 10.1002/art.38912

75. Kitagawa Y, Kawasaki Y, Yamasaki Y, Kambe N, Takei S, Saito MK. Anti-TNF treatment corrects IFN-γ-dependent proinflammatory signatures in blau syndrome patient-derived macrophages. *J Allergy Clin Immunol* (2022) 149 (1):176–188.e7. doi: 10.1016/j.jaci.2021.05.030

76. Takada S, Kambe N, Kawasaki Y, Niwa A, Honda-Ozaki F, Kobayashi K, et al. Pluripotent stem cell models of blau syndrome reveal an IFN-γ-dependent inflammatory response in macrophages. *J Allergy Clin Immunol* (2018) 141(1):339–49.e11. doi: 10.1016/j.jaci.2017.04.013

77. Marion W, Boettcher S, Ruiz-Torres S, Lummertz da Rocha E, Lundin V, Morris V, et al. An induced pluripotent stem cell model of fanconi anemia reveals mechanisms of p53-driven progenitor cell differentiation. *Blood Adv* (2020) 4 (19):4679–92. doi: 10.1182/bloodadvances.2020001593

78. Suzuki NM, Niwa A, Yabe M, Hira A, Okada C, Amano N, et al. Pluripotent cell models of fanconi anemia identify the early pathological defect in human hemoangiogenic progenitors. *Stem Cells Transl Med* (2015) 4(4):333–8. doi: 10.5966/sctm.2013-0172

79. Liu G-H, Suzuki K, Li M, Qu J, Montserrat N, Tarantino C, et al. Modelling fanconi anemia pathogenesis and therapeutics using integration-free patient-derived iPSCs. *Nat Commun* (2014) 5:4330. doi: 10.1038/ncomms5330

80. Mu A, Hira A, Niwa A, Osawa M, Yoshida K, Mori M, et al. Analysis of disease model iPSCs derived from patients with a novel fanconi anemia-like IBMFS ADH5/ALDH2 deficiency. *Blood* (2021) 137(15):2021–32. doi: 10.1182/blood.2020009111

81. Kawasaki Y, Oda H, Ito J, Niwa A, Tanaka T, Hijikata A, et al. Identification of a high-frequency somatic NLRC4 mutation as a cause of autoinflammation by pluripotent cell-based phenotype dissection. *Arthritis Rheumatol (Hoboken NJ).* (2017) 69(2):447–59. doi: 10.1002/art.39960

82. Tanaka T, Takahashi K, Yamane M, Tomida S, Nakamura S, Oshima K, et al. Induced pluripotent stem cells from CINCA syndrome patients as a model for dissecting somatic mosaicism and drug discovery. *Blood* (2012) 120(6):1299–308. doi: 10.1182/blood-2012-03-417881

83. Park I-H, Arora N, Huo H, Maherali N, Ahfeldt T, Shimamura A, et al. Disease-specific induced pluripotent stem cells. *Cell* (2008) 134(5):877–86. doi: 10.1016/j.cell.2008.07.041

84. Apasov SG, Blackburn MR, Kellems RE, Smith PT, Sitkovsky MV. Adenosine deaminase deficiency increases thymic apoptosis and causes defective T cell receptor signaling. *J Clin Invest* (2001) 108(1):131–41. doi: 10.1172/JCI10360

85. Carson DA, Kaye J, Seegmiller JE. Lymphospecific toxicity in adenosine deaminase deficiency and purine nucleoside phosphorylase deficiency: possible role of nucleoside kinase(s). *Proc Natl Acad Sci USA* (1977) 74(12):5677–81. doi: 10.1073/pnas.74.12.5677

86. Kim VHD, Roifman CR, Grunebaum E. Neutropenia in patients with adenosine deaminase deficiency. *Allergy Asthma Clin Immunol Off J Can Soc Allergy Clin Immunol* (2014) 10(Suppl 1):A41. doi: 10.1186/1710-1492-10-S1-A41

87. Zaki-Dizaji M, Akrami SM, Azizi G, Abolhassani H, Aghamohammadi A. Inflammation, a significant player of ataxia-telangiectasia pathogenesis? *Inflamm Res off J Eur Histamine Res Soc* (2018) 67(7):559–70. doi: 10.1007/s00011-018-1142-y

88. Glocker EO, Kotlarz D, Boztug K, Gertz EM, Schäffer AA, Noyan F, et al. Inflammatory bowel disease and mutations affecting the interleukin-10 receptor. *N Engl J Med* (2009) 361(21):2033–45. doi: 10.1056/NEJMoa0907206

89. Ruemmele FM, El Khoury MG, Talbotec C, Maurage C, Mougenot JF, Schmitz J, et al. Characteristics of inflammatory bowel disease with onset during the first year of life. *J Pediatr Gastroenterol Nutr* (2006) 43(5):603–9. doi: 10.1097/01.mpg.0000237938.12674.e3

90. Zou J, Sweeney CL, Chou BK, Choi U, Pan J, Wang H, et al. Oxidase-deficient neutrophils from X-linked chronic granulomatous disease iPS cells: functional correction by zinc finger nuclease-mediated safe harbor targeting. *Blood* (2011) 117(21):5561–72. doi: 10.1182/blood-2010-12-328161

91. Brault J, Vaganay G, Le Roy A, Lenormand J-L, Cortes S, Stasia MJ. Therapeutic effects of proteoliposomes on X-linked chronic granulomatous disease: proof of concept using macrophages differentiated from patient-specific induced pluripotent stem cells. *Int J Nanomedicine* (2017) 12:2161–77. doi: 10.2147/IJN.S128611

92. Merling RK, Kuhns DB, Sweeney CL, Wu X, Burkett S, Chu J, et al. Gene-edited pseudogene resurrection corrects p47(phox)-deficient chronic granulomatous disease. *Blood Adv* (2017) 1(4):270–8. doi: 10.1182/bloodadvances.2016001214

93. Schwaid AG, Spencer KB. Strategies for targeting the NLRP3 inflammasome in the clinical and preclinical space. *J Med Chem* (2021) 64(1):101–22. doi: 10.1021/acs.jmedchem.0c01307

94. Vande Walle L, Stowe IB, Šácha P, Lee Bl, Demon D, Fossoul A, et al. MCC950/CRID3 potently targets the NACHT domain of wild-type NLRP3 but not disease-associated mutants for inflammasome inhibition. *PloS Biol* (2019) 17(9): e3000354. doi: 10.1371/journal.pbio.3000354

95. Ebeling MC, Geng Z, Kapphahn RJ, Roehrich H, Montezuma SR, Dutton JR, et al. Impaired Mitochondrial Funct iPSC-Retinal Pigment Epithelium Complement Factor H Polymorphism Age-Related Macular Degeneration. *Cells* (2021) 10(4):789. doi: 10.3390/cells10040789

96. Koolen L, Gagliardi G, Ten Brink SCA, de Breuk A, Heesterbeek TJ, Hoyng CB, et al. Generation and characterization of human induced pluripotent stem cells (iPSCs) from three patients with age-related macular degeneration carrying rare variants in the CFH gene. *Stem Cell Res* (2022) 60:102669. doi: 10.1016/j.scr.2022.102669

97. Río P, Navarro S, Wang W, Sánchez-Domínguez R, Pujol RM, Segovia JC, et al. Successful engraftment of gene-corrected hematopoietic stem cells in non-conditioned patients with fanconi anemia. *Nat Med* (2019) 25(9):1396–401. doi: 10.1038/s41591-019-0550-z

98. Kim JY, Nam Y, Rim YA, Ju JH. Review of the current trends in clinical trials involving induced pluripotent stem cells. *Stem Cell Rev Rep* (2022) 18(1):142–54. doi: 10.1007/s12015-021-10262-3

99. Jang Y, Choi J, Park N, Kang J, Kim M, Kim Y, et al. Development of immunocompatible pluripotent stem cells *via* CRISPR-based human leukocyte antigen engineering. *Exp Mol Med* (2019) 51(1):1–11. doi: 10.1038/s12276-018-0190-2

100. Rim YA, Park N, Nam Y, Ham DS, Kim JW, Ha HY, et al. Recent progress of national banking project on homozygous HLA-typed induced pluripotent stem cells in south Korea. *J Tissue Eng Regener Med* (2018) 12(3):e1531–6. doi: 10.1002/term.2578

101. Lanza R, Russell DW, Nagy A. Engineering universal cells that evade immune detection. *Nat Rev Immunol* (2019) 19(12):723–33. doi: 10.1038/s41577-019-0200-1

102. Zhong C, Liu M, Pan X, Zhu H, et al. Tumorigenicity risk of iPSCs *in vivo*: nip it in the bud. *Precis Clin Med* (2022) 5(1):pbac004. doi: 10.1093/pcmedi/pbac004

103. Sułkowski M, Konieczny P, Chlebanowska P, Majka M. Introduction of exogenous HSV-TK suicide gene increases safety of keratinocyte-derived induced pluripotent stem cells by providing genetic "Emergency exit" switch. *Int J Mol Sci* (2018) 19(1):197. doi: 10.3390/ijms19010197

104. Liang Q, Monetti C, Shutova MV, Neely EJ, Hacibekiroglu S, Yang H. Linking a cell-division gene and a suicide gene to define and improve cell therapy safety. *Nature* (2018) 563(7733):701–4. doi: 10.1038/s41586-018-0733-7

105. Shi Z-D, Tchao J, Wu L, Carman AJ. Precision installation of a highly efficient suicide gene safety switch in human induced pluripotent stem cells. *Stem Cells Transl Med* (2020) 9(11):1378–88. doi: 10.1002/sctm.20-0007

106. Dahlke J, Schott JW, Vollmer Barbosa P, Klatt D, Selich A, Lachmann N, et al. Efficient genetic safety switches for future application of iPSC-derived cell transplants. *J Pers Med* (2021) 11(6):565. doi: 10.3390/jpm11060565

107. Lee M, Ha J, Son YS, Ahn H, Jung KB, Son MY, et al. Efficient exogenous DNA-free reprogramming with suicide gene vectors. *Exp Mol Med* (2019) 51(7):1–12. doi: 10.1038/s12276-019-0282-7

108. Zhou X, Naik S, Dakhova O, Dotti G, Heslop HE, Brenner MK. Serial activation of the inducible caspase 9 safety switch after human stem cell transplantation. *Mol Ther* (2016) 24(4):823–31. doi: 10.1038/mt.2015.234

109. Park J, Lee DG, Lee NG, Kwon MG, Son YS, Son MY, et al. Monoclonal antibody K312-based depletion of pluripotent cells from differentiated stem cell progeny prevents teratoma formation. *BMB Rep* (2022) 55(3):142–7. doi: 10.5483/BMBRep.2022.55.3.090

110. Tang C, Lee AS, Volkmer J-P, Sahoo D, Nag D, Mosley AR, et al. An antibody against SSEA-5 glycan on human pluripotent stem cells enables removal of teratoma-forming cells. *Nat Biotechnol* (2011) 29(9):829–34. doi: 10.1038/nbt.1947

111. Tan HL, Tan BZ, Goh WXT, Cua S, Choo A. In vivo surveillance and elimination of teratoma-forming human embryonic stem cells with monoclonal antibody 2448 targeting annexin A2. *Biotechnol Bioeng* (2019) 116(11):2996–3005. doi: 10.1002/bit.27135

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Care of patients with inborn errors of immunity in thirty J Project countries between 2004 and 2021

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Introduction: The J Project (JP) physician education and clinical research collaboration program was started in 2004 and includes by now 32 countries mostly in Eastern and Central Europe (ECE). Until the end of 2021, 344 inborn errors of immunity (IEI)-focused meetings were organized by the JP to raise awareness and facilitate the diagnosis and treatment of patients with IEI.

Results: In this study, meeting profiles and major diagnostic and treatment parameters were studied. JP center leaders reported patients' data from 30 countries representing a total population of 506 567 565. Two countries reported patients from JP centers (Konya, Turkey and Cairo University, Egypt). Diagnostic criteria were based on the 2020 update of classification by the IUIS Expert Committee on IEI. The number of JP meetings increased from 6 per year in 2004 and 2005 to 44 and 63 in 2020 and 2021, respectively. The cumulative number of meetings per country varied from 1 to 59 in various countries reflecting partly but not entirely the population of the respective countries. Altogether, 24,879 patients were reported giving an average prevalence of 4.9. Most of the patients had predominantly antibody deficiency (46,32%) followed by patients with combined immunodeficiencies (14.3%). The percentages of patients with bone marrow failure and phenocopies of IEI were less than 1 each. The number of patients was remarkably higher that those reported to the ESID Registry in 13 countries. Immunoglobulin (IgG) substitution was provided to 7,572 patients (5,693 intravenously) and 1,480 patients received hematopoietic stem cell therapy (HSCT). Searching for basic diagnostic parameters revealed the availability of immunochemistry and flow cytometry in 27 and 28 countries, respectively, and targeted gene sequencing and new generation sequencing was available in 21 and 18 countries. The number of IEI centers and experts in the field were 260 and 690, respectively. We found high correlation between the number of IEI centers and patients treated with intravenous IgG (IVIG) (correlation coefficient, cc, 0,916) and with those who were treated with HSCT (cc, 0,905). Similar correlation was found when the number of experts was compared with those treated with HSCT. However, the number of patients treated with subcutaneous Ig (SCIG) only slightly correlated with the number of experts (cc, 0,489) and no correlation was found between the number of centers and patients on SCIG (cc, 0,174).

Conclusions: 1) this is the first study describing major diagnostic and treatment parameters of IEI care in countries of the JP; 2) the data suggest that the JP had tremendous impact on the development of IEI care in ECE; 3) our data help to define major future targets of JP activity in various countries; 4) we suggest that the number of IEI centers and IEI experts closely correlate to the most

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important treatment parameters; 5) we propose that specialist education among medical professionals plays pivotal role in increasing levels of diagnostics and adequate care of this vulnerable and still highly neglected patient population; 6) this study also provides the basis for further analysis of more specific aspects of IEI care including genetic diagnostics, disease specific prevalence, newborn screening and professional collaboration in JP countries.

KEYWORDS

J Project, immunodeficiencies, Eastern and Central Europe, Asia, ESID, parameters

Introduction

Despite tremendous progress in the field over the past decades, primary immunodeficiency disorders (PIDs) also referred to as inborn errors of immunity (IEI) still represent a neglected area of medicine (1–3). Basic research into the field is concentrated in a small number of centers in the USA, Western Europe, Japan, China and Australia. Incidence and prevalence data are either not available or vary remarkably in different countries (4–6; this study). Legal restrictions and diagnostic difficulties in many countries make patient registries incomplete and unreliable (4). Immunology education at both graduate and postgraduate levels is focused mostly on autoimmunity and allergic diseases neglecting IEIs. Experts with the allergy-immunology license exam may have limited knowledge on IEIs especially their mechanistic and genetic dimensions.

More than 450 IEIs have been described but many of them only in a few patients and families and data were obtained mostly from mice studies (7–11). Limitations to publish rare IEI cases in medical journals hamper the accumulation of substantial amount of clinical data. Primarily IEI-focused medical journal, like the J Clin Immunol has only recently been established (12). Most hematology journals are reluctant to take IEI cases even with hematological phenotype of the patient (personal communication). IEI continues to receive negligible attention by governmental agencies and well-functioning, advanced IEI centers may suffer from brutal attack from unprofessional institutional leaders (JP Book 2015, pp. 56-57; www.thejpnetwork.com).

Despite the above listed difficulties and drawbacks, the field has been growing and progressing largely due to the development of molecular immunology and genetics (13–15). Since the characterization of the first IEI in the early fifties a large number of patients with new disease entities were described on immunological bases. Since the eighties, IEIs started to become widely known as a genetically defined group of diseases caused by single gene mutations (13). Very often, however, high tech genetic studies result in the discovery of new diseases only in one or a few individuals or families, and diagnosis of new cases are limited by the lack of available genetic assays (2, 16). The gap developing between advanced molecular genetic knowledge in leading centers and the limited accessibility of IEI diagnosis and treatment has to be closed despite the above listed obstacles. Novel educational and collaboration programs have been created to spread knowledge and to start IEI-focused medical care. One of these programs which can be considered as a prototype is the J Project (JP) started in 7 Eastern and Central European (ECE) countries in 2004 and covering now a large area of 32 countries in Eastern and Central Europe (ECE), Asia and Africa (16–23). In this paper we report major diagnostic and treatment parameters of IEI care that had been achieved by the end of 2021 in countries involved in the JP.

Results and discussion

JP meetings

At the turn of the millennium many countries in ECE reported less than 10 patients to the European Society for Immunodeficiency Registry (ESID-R) (24; Figure 1). Thus, taken 10 countries (with the exception of Bosnia & Herzegovina, Montenegro and Kosovo), the cumulative number of patients increased from max 100 to 5307 by the end of 2021 (Figure 1). A conceptual IEI-focused professional meeting series was started in 2004 and reached measurable results even in countries with low socioeconomic conditions including the Rep. of Moldova, Rep. of North Macedonia, Albania, and Kosovo (11, 25, 26). Over the past 18 years, 344 IEI-focused conferences were organized (Supplementary Table 1 and Figure 2A). These events have resulted in a remarkable progress in diagnosis and treatment of patients with IEI. More and more countries, first in Central Europe, next in Eastern Europe, and in 2009, Iran, Turkey and Egypt joined the JP and we have now 32 so-called JP countries (Figure 2B). The number of JP meetings increased from 6 per year in 2004 and 2005 to 44 and 63 per year in 2020 and 2021, respectively (Figure 2C and Supplementary Table 1). The cumulative number of meetings varied from 1 to 59 in various countries reflecting, at least in part, the population of the respective

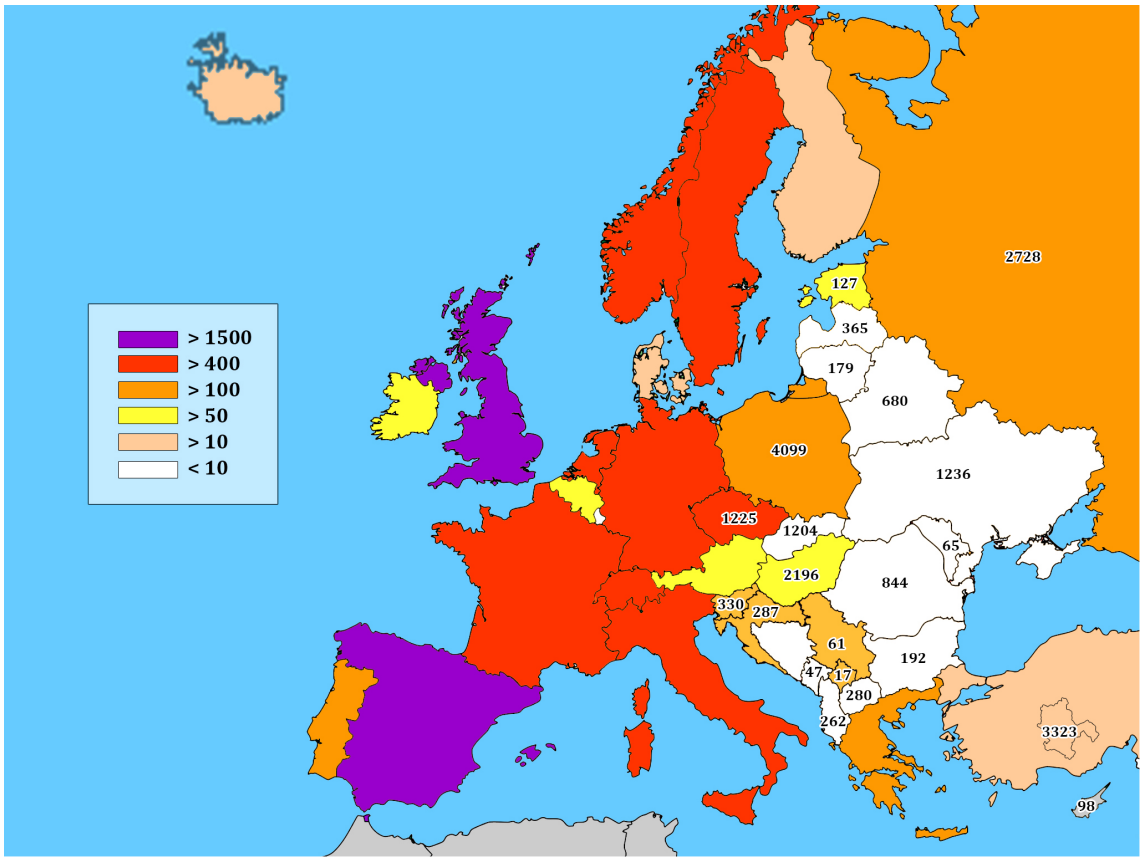

FIGURE 1
Number of patients with inborn errors of immunity reported from Eastern and Central Europe (ECE) to the ESID Registry in 2000 was less than 10 (see color coding and the scale on the left). Number of patients reported to this J Project survey at the end of 2021 is shown by numbers in ECE countries. For more details see Table 1.

countries (population and meeting number, correlation coefficient, cc, 0,72) (Figure 2D).

JP congresses

The vast majority of JP meetings were regional or national events organized by local opinion leaders. Three JP Congresses were also organized, traditionally in Antalya, Turkey by I Reisli, leader of the J Daughter Anatolia Project, in 2014, 2016, and 2019. In 2014, not only the first JP Congress but the 10th anniversary of the establishment of the JP and the 100th JP events that had been organized were celebrated. These JP Congresses with participation of the most prominent IEI researchers from all over the world were devoted to discuss newly published or unpublished novel primary immunodeficiencies in order to stimulate further clinical research in JP countries and promote collaboration. Importantly, these congresses provided excellent occasions to present novel clinical observations of colleagues from JP countries. In 2016 and 2019 we issued two declarations for patients with primary immunodeficiencies which we referred to as "Konya Declarations" (27).

The JP Steering Committee

To coordinate the activity of the JP, the JP Steering Committee (SC) was established in 2010; following this year SC meetings were regularly organized mostly in Budapest, Hungary, or at the time of ESID congresses (Edinburgh and Lisbon in 2017 and 2018, respectively) (Supplementary Figure 1). These SC meetings outlined previous achievements and future programs of the JP including joint clinical research which were published in reasonable international journals (28–33). An SC meeting to remember was the one in March 2019. By this time the JP was about to consider to become a new IEI society. This meeting was attended by leaders of the ESID (I Meyts, F Candotti, A Cant) and at the end of the long discussions

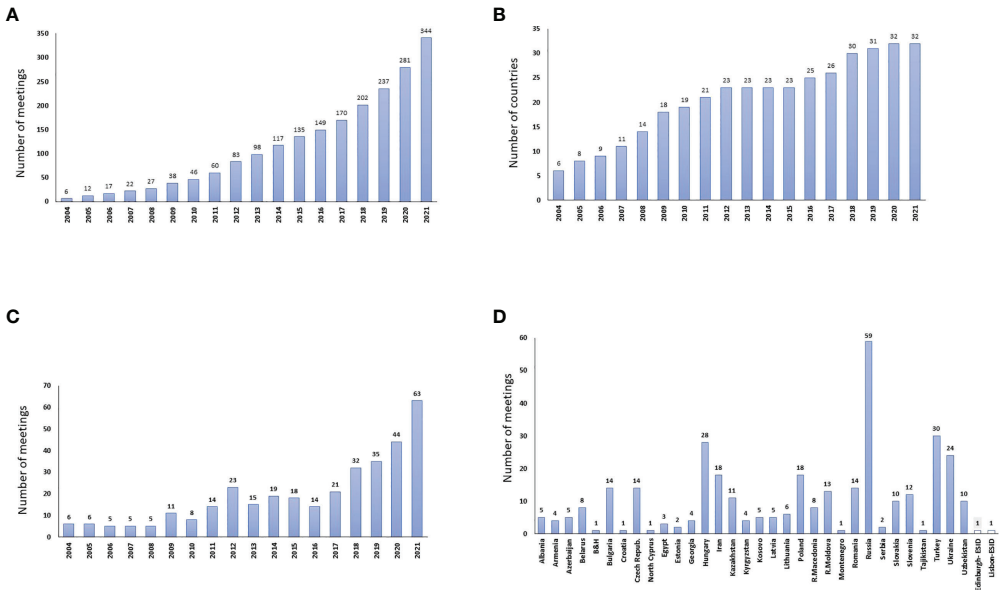

FIGURE 2
(A) shows the cumulative number of J Project (JP) meetings organized in Eastern and Central Europe (ECE), Asia and part of Africa. The average number of meetings per country was 10.75 in 2021. **(B)** shows the number of countries participating in the JP between 2004 and 2021 reaching 32 by 2020. **(C)** shows the number of JP meetings organized yearly over 18 years. A remarkable increase of meeting number occurred over the past 4 years which was not directly related to the number of participating countries. This is indicated by the difference between 2020 and 2021 when the JP country number was equal. **(D)** shows the cumulative number of JP meetings in participating countries. The largest number of meetings in Russia is in concert with the population estimated 138,000,000 in this country. On the other hand, the number of meetings in Hungary (estimated population, 9,600,000) was about half of that in Russia. Further, the same number of meetings were organized in Bulgaria and Czech Republic than in Romania with a population of 20,000,000 and Republic of Moldova (2,600,000) or Slovenia (2,200,000) suggesting that the activity and interest maybe more important than the size and population of the country. Unfortunately, there are countries with permanently low number of meetings including Croatia, Bosnia & Herzegovina, Estonia, Serbia) despite longer-term membership in the JP network (see also Supplementary Table 1).

of pros and contras we decided to go on further as a network and collaborate rather than compete with ESID.

Opening the scope of education and research: The COVID pandemic

The JP has been continually changing ever since its creation in 2004, recognizing the increasing need for physician education and clinical research collaboration initially in Central Europe, then in Eastern Europe, and subsequently elsewhere, most recently in Central Asia and Far-East Russia. This evolution based on the recognition of these needs has resulted in very sensitive changes and rearrangements of the JP program throughout Eurasia, as reflected in the annual editions of JP booklets and our regularly updated website (www.thejpnetwork.com). The prime focus of the collaboration has shifted from clinical education to genetics teaching.

Due to the dynamic progress and popularity of the JP, the area for which we hold responsibility had extended to the Pacific by 2000, and is bordered now by two oceans and eight seas (Supplementary Figure 2). This huge geographic dimension was never anticipated in our initial plans, when we established the Project in the Carpathian region of Europe, with no intentions to expand it elsewhere. In the JP book for 2019, we expressed our enthusiasm concerning the success with which knowledge of next-generation sequencing technologies had been disseminated, making it possible to define the genetic basis of more PIDs. However, in 2020 events took an unexpected turn, forcing the world to face new challenges. The very existence of humanity had been threatened from a viral disease caused by SARS-Cov-2. Most research laboratories, centers and institutions have changed direction and focused their research on studies of the mechanisms, prevention and treatment of COVID-19, the most severe form of coronavirus disease. The JP has joined forces with one of the most progressive PID research laboratories, led by J-L Casanova at the Rockefeller University and in Paris, and we have agreed to extend participation in the COVID-19 research of this laboratory to the whole area of the JP (34). The JP has been taking part in this research by establishing participating centers all over Eurasia and increasing awareness of unusual COVID-19 cases at JP meetings.

Organization of the JP was strongly supported from the beginning by the ESID and the Jeffrey Modell Foundation (JMF) as well as by grants from a few pharmaceutical companies

(18). Based on SC decision, the JP meeting organization has been coordinated by the Foundation for Children with Immunodeficiencies since 2014.

Patients diagnosed with IEI in JP countries

After 18 years of JP educational activity and published research we decided to put together basic parameters of patient care. To this end, questionnaires about diagnosis and treatment of patients were sent out to center leaders who were requested to fill them out and return with comments. This parameters survey was intended to be a kind of snapshot on what we had achieved and where we should be going to. Thus, specific questions about age, gender, disease duration and severity of illness and similar details were not included. We believe that JP centers should use primarily the ESID-R for entering patients data in more detail. Altogether, 24,879 patients with various IEIs were reported (Table 1, Figure 3 and Supplementary Figure 3). Classification was made according to

TABLE 1 Reported patients with inborn errors of immunity from J project countries/centers.

Country	Inborn errors of immunity according to the IUIS classification*											All	Estimated population	Patients /10^5
	1	2	3	4	5	6	7	8	9	10	UD			
1. Albania	6	17	73	5	134[§]	4	12	2	5	1	3	262	2 829 741	9.26
2. Armenia	0	0	4	0	4	0	(3151)	6	0	0	0	14	2 963 000	0.47
3. Azerbaijan	13	36	45	22	6	0	0	0	0	0	13	135	10 157 000	1.33
4. Belarus	47	173	198	51	34	3	4	85	24	3	58	680	9 600 000	7.08
5. Bosnia&H	No patients were reported because of government regulation													
6. Bulgaria	10	33	86	4	10	0	21	11	0	17	0	192	6 916 548	2.76
7. Croatia	18	70	115	6	25	10	1	28	4	1	9	287	3 888 529	7.38
8. Czech Rep	22	253	657	17	30	3	22	213	0	8	0	1225	10 700 000	11.45
9. N Cyprus	0	0	96	0	0	0	0	0	0	0	2	98	475 442	20.61
10. Egypt[@,a]	294	72	88	157	195	81	487	11	20	0	64	1469	18 000 000	8.16
11.Estonia	2	7	96	0	6	3	0	7	0	0	6	127	1 328 439	9.56
12. Georgia	1	0	8	0	1	0	92	0	0	0	20	122	3 979 765	3.07
13. Hungary	82	77	1304	69	59	26	49	360	13	4	153	2196	9 689 000	22.66
14. Iran	368	521	903	63	524	125	490	62	0	0	0	3056	84 000 000	3.64
15. Kazakhstan	9	7	142	5	8	0	3	24	0	6	0	204	19 135 477	1.07
16. Kyrgyzstan	0	0	0	0	0	0	0	0	0	0	9	9	6 592 000	0.14
17. Kosovo	1	4	8	0	4	0	0	0	0	0	0	17	1 935 000	0.88
18. Latvia	2	57	253	4	18	2	12	11	5	0	1	365	1 890 000	19.31
19. Lithuania	3	8	104	1	3	2	2	29	2	6	19	179	2 795 000	6.40
20. Macedonia	10	41	162	2	18	2	27	7	0	0	11	280	2 083 254	13.44
21. Moldova	3	11	50	1	0	0	0	0	0	0	0	65	2 597 000	2.50
22. Montenegro	1	6	4	1	28	0	1	4	0	2	0	47	621 718	7.56
23. Poland	169	879	2156	59	283	42	182	132	8	12	177	4099[#]	38 091 094	10.76
24. Romania	18	44	408	21	150	7	46	112	17	0	21	844	19 030 136	4.43
25. Russia	368	591	699	196	262	43	221	342	0	6	0	2728	145 478 097	1.87
26. Serbia	2	9	32	2	5	0	0	9	2	0	0	61	8 683 801	0.70
27. Slovakia	22	116	416	21	20	11	285	257	4	1	51	1204	5 449 270	22.09
28. Slovenia	17	82	54	37	39	6	18	74	3	0	0	330	2 080 000	15.87
29. Tajikistan	No patients were reported because of developmental issues													
30. Turkey[@,b]	133	166	2724	16	66	16	99	16	10	7	70	3323	8 000 000	41.54
31. Ukraine	46	276	631	47	91	10	26	46	6	0	57	1236	43 342 300	2.85
32. Uzbekistan	6	5	8	0	0	0	6	0	0	0	0	25	34 235 954	0.07
Summary	1673	3561	11524	807	2023	396	2106	1848	123	74	744	24 879	506 567 565	Average: 4.9
Percentage	6.72	14.31	46.32	3.24	8.13	1.59	8.46	7.43	0.49	0.30	2.99	100	–	–

*Tangye et al, JoCI, 2020; [§]These data include patients with hypo-IgA in the paripheral blood; [@]Data were reported from [a]Cairo and [b]Konya centers; [#]Malgorzata P, Bernatowska E. Eur J Pediatr 2016; 175(8):1099. UD, Unclassified disease; H, Herzegovina; N, North; IUIS, International Union of Immunological Societies.

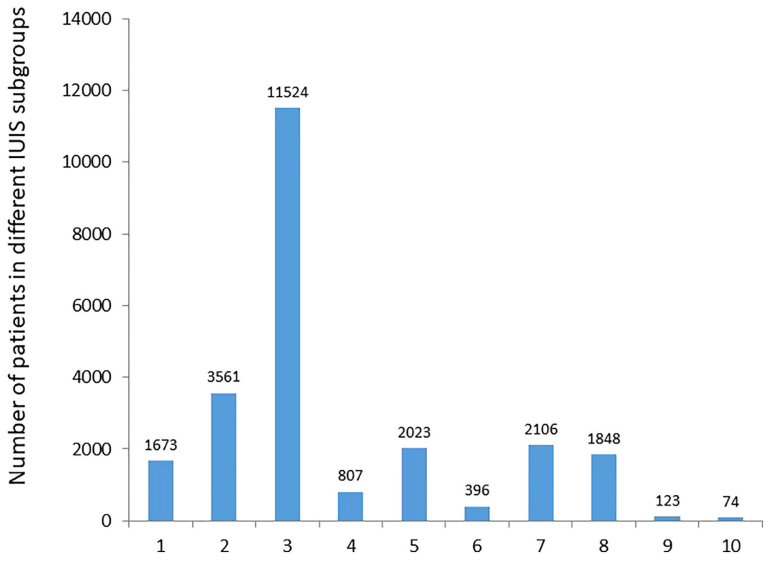

FIGURE 3
The total number of patients reported from 30 countries to various IUIS subgroups. The most common subgroups were predominantly antibody deficiency (11510) and combined immunodeficiencies with associated or syndromic feature (3557). The number of patients with periodic fever syndrome does not include patients reported from Armenia (3151 pts) to avoid unproportional presentation (see Table 1). The number of patients with no definitive IEI diagnosis was 734 representing 2,95% of the total number of 24,862 patients.

the IUIS committee of IEI: 1) Immunodeficiencies affecting cellular and humoral immunity; 2) Combined immunodeficiencies with associated or syndromic features; 3) Predominantly antibody deficiency; 4) Diseases of immune dysregulation; 5) Congenital defects of phagocyte number or function; 6) Defects in intrinsic and innate immunity; 7) Auto-inflammatory disorders; 8) Complement deficiencies; 9) Bone marrow failure; 10) Unclassified inborn error of immunity or IEI phenocopies. Most patients had predominantly antibody deficiency (11,524; 46,32%) followed by patients with combined immunodeficiencies with associated or syndromic feature (3,561; 14,31%). The percentages of patients with bone marrow failure and phenocopies of IEI were less than 1% each, respectively. These data were reported from 28 JP countries and two centers representing an estimated population of 506 567 565 (Table 1**).** Patients from Bosnia & Herzegovina and Tajikistan were not reported; the population of these two countries is estimated to be 12,42 million. The two countries that participated in the survey by reporting patients from JP centers were Turkey (Konya Center) and Egypt (Cairo University Center). Based on individual data, the prevalence of IEI in JP countries varied between 0.07 in Uzbekistan and 41.54 in Konya Center, Turkey, with an average prevalence of 4.9 (Table 1). The number of centers per country varied from 1 to 107 and correlated well with the population of the country (cc, 0,90) (Table 2).

We compared the percentage of patients in different IUIS subgroups reported to the ESID-R and the J Project. The data showed that the percentage of reported patients was comparable with only mild differences in subgroups III and IV, which were slightly higher in ESID-R, and in subgroups VII and VIII, which were somewhat higher among patients reported to the JP (Figure 4). These data are promising and suggest comparable attention to the wide range of IEI in the Western and Eastern parts of Europe. However, diagnosis of specific diseases, especially recently described IEIs may be completely different and should be analyzed in future. Importantly, the total number of patients reported to this survey was remarkably higher than those reported to the ESID-R in 13 countries (Figure 5). These data suggest that reporting activity to the ESID-R should be increased in JP centers to make the ESID-R a reliable database and a solid source of information about the widest range of IEI in both Western and Eastern Europe. Nineteen JP countries did not report at all to the ESID-R

In the beginning of JP activity, patients in centers were diagnosed primarily with antibody deficiencies. This is well exemplified by North Cyprus that joint the JP in 2019 and reported only patients with antibody deficiencies even in 2021 (Table 1). Also, Uzbekistan joining the JP in 2018 reported patients that fall only in 4 diseases' groups of IEI (Table 1). Countries with the highest prevalence value (Turkey, Hungary and Slovakia), however, reported a full spectrum of IEI patients suggesting a wide range of diagnosis. Similar data were observed in countries like Albania, Belarus, Lithuania and Poland with prevalence between 6.4 and 10,76 (Table 1).

Searching for basic diagnostic parameters revealed the availability of both immunochemistry and flow cytometry in 27 and 28 countries, respectively, but targeted gene sequencing

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TABLE 2 Diagnostic and treatment options in JP countries/centers.

Country	Available diagnostic parameters						Treatment options			
	Ct[1]	Exp[2]	ImmChem[3]	FlowCyt[4]	TGS[5]	NGS[6]	IVIG[7]	SCIG[8]	HSCT[9]	Other treatments
Albania	1	5	Y	Y	N	N	10	0	3	IS
Armenia	1	2	Y	Y	Y	Y	8	0	3	G-CSF
Azerbaijan	1	5	Y	Y	N	N	28	0	3	Thymus hormone
Belarus	2	8	Y	Y	Y	Y	37	29	33	G-CSF, IFN-γ, IS, biological therapy
Bosnia & H	No data were reported									
Bulgaria	1	15	Y	Y	Y	Y	5	49	4	Biological therapy
Croatia	2	6	Y	Y	Y	N	53	62	21	G-CSF, biological therapy
Czech Rep	17	42	Y	Y	Y	Y	168	301	86	Thymus transplantation, biological therapy
N Cyprus	2	2	Y	Y	N	N	12	0	0	IS, biological therapy
Cairo Center	1	10	Y	Y	Y	Y	62	0	25	G-CSF, IS, biological therapy
Estonia	2	6	Y	Y	Y	Y	49	9	5	IS, biological therapy
Georgia	1	1	Y	Y	N	N	0	0	0	IS, biological therapy
Hungary	7	17	Y	Y	Y	Y	430	160	70	G-CSF, IFN-γ, IS, virus specific T cell therapy
Iran	30	100	Y	Y	Y	Y	563	0	175	G-CSF, IFN-γ
Kazakhstan	2	12	Y	Y	Y	N	9	92	3	IFN-γ, G-CSF, anti-TNF-α
Kyrgyzstan	1	1	N	N	N	N	0	0	0	IS
Kosovo	1	2	N	N	N	N	2	1	1	IS
Latvia	2	7	Y	Y	Y	Y	14	20	15	IL-1RA, anti-TNF-α
Lithuania	2	11	Y	Y	Y	Y	4	50	2	anti-TNF-α, IFN-γ, G-CSF
N Macedonia	1	3	Y	Y	Y	Y	30	2	2	G-CSF, IS, biological therapy
R Moldova	1	5	Y	Y	Y	N	5	0	1	G-CSF
Montenegro	1	2	Y	Y	N	N	2	0	5	IS
Poland	19	160	Y	Y	Y	Y	331	510	320	IS
Romania	8	13	Y	Y	Y	Y	42	7	8	G-CSF
Russia	107	168	Y	Y	Y	Y	2101	11	492	IS, CSFs, ADA, biological therapy
Serbia	4	6	Y	Y	Y	Y	56	5	15	G-CSF, IS
Slovakia	3	12	Y	Y	Y	Y	60	200	39	IFN-γ, biological therapy, thymus transplantation
Slovenia	1	8	Y	Y	Y	Y	30	16	32	Thymus transplantation, virus specific T cell therapy
Tajikistan	No data were reported									
Turkey	28	44	Y	Y	Y	Y	1500	400	250	ADA, IFN-γ, gene therapy
Ukraine	7	16	Y	Y	N	N	160	84	47	G-CSF
Uzbekistan	2	4	N	Y	N	N	12	–	–	IS
Summary (Y)	260	690	(27)	(28)	(21)	(18)	5693	1879	1480	–
Mean ± SEM	9±3.8	23.9±8	–	–	–	–				–

[1]N° of centers; [2]N° of experts; [3]Immunochemistry (yes or no); [4]flow cytometry (yes or no); [5]targeted gene sequencing (yes or no); [6]new generation sequencing (yes or no); [7]N° of patients receiving intravenous immunoglobulin; [8]N° of patients receiving subcutaneous immunoglobulin; [9]N° patients who received hematopoietic stem cell therapy; IS, immunosuppression; G-CSF, granulocyte colony stimulation factor; IFN- γ, interferon-gamma; TNF-α, tumor necrosis factor alpha; IL1-RA, interleukin-1 receptor antagonist; ADA, adenosine deaminase.

and next generation sequencing was available only in 21 and 18 countries (Table 2 and Figure 6). These parameters are generally available in Central Europe but immunochemistry is still missing in 3 countries (Kosovo, Uzbekistan, Tajikistan) and flow cytometry in two countries (Kosovo and Kyrgyzstan). Genetic analysis is unavailable in Ukraine, a few South European countries, two Caucasian countries, and most Central Asian countries (Table 2 and Figure 6). No data are available from Bosnia & Herzegovina and Tajikistan. Together, these data suggest that further development of diagnostics is needed in Central Asia through more focused educational activity about the relevant genetic diagnosis in patients and families.

Treatment parameters

The number of experts ranged between 1 and 168 and there was also a strong correlation with the country populations (cc,

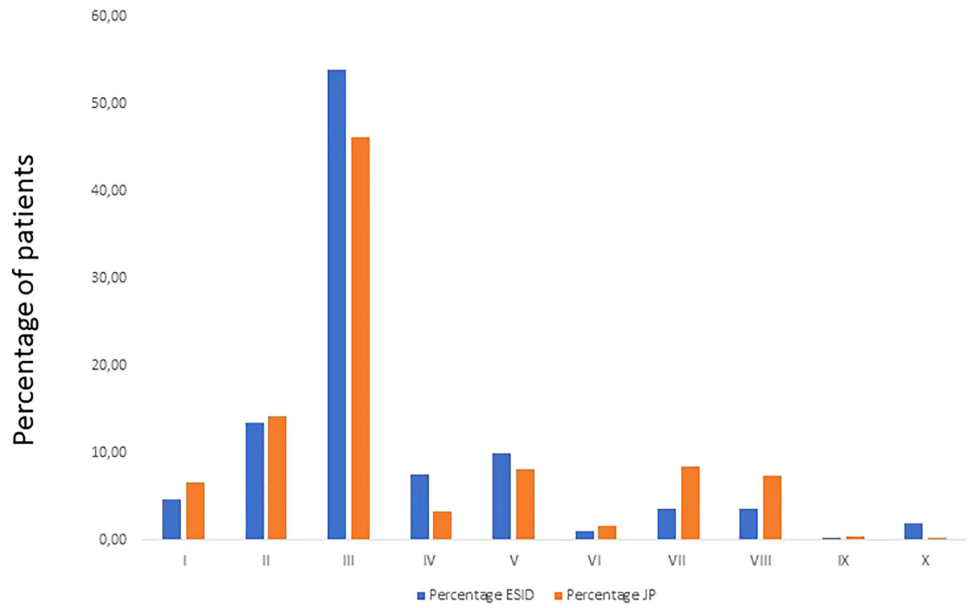

FIGURE 4
Comparable percentages of patients in different subgroups reported to the ESID-R (left columns) and the J Project (right columns). Despite slight differences in a few subgroups (subgroups 3, 4, 7 and 8), these data indicate similarly wide range of diagnosis of patients with different disease groups.

0,81; Table 2). Most patients diagnosed first suffered from antibody deficiency and were treated with intravenous immunoglobulins (IVIG) and later with subcutaneous immunoglobulin (SCIG) preparations. These treatment schedules were completed with hematopoietic stem cell transplantation (HSCT) in patients having both T cell and B cell immunodeficiencies even without precise genetic analysis. A wide range of other treatment options in IEI patients were also reported including a variety of medicines under the heading of biological therapy (Table 2).

By the end of 2021, immunoglobulin substitution had been provided to 7,572 patients (5693 intravenously) and 1480

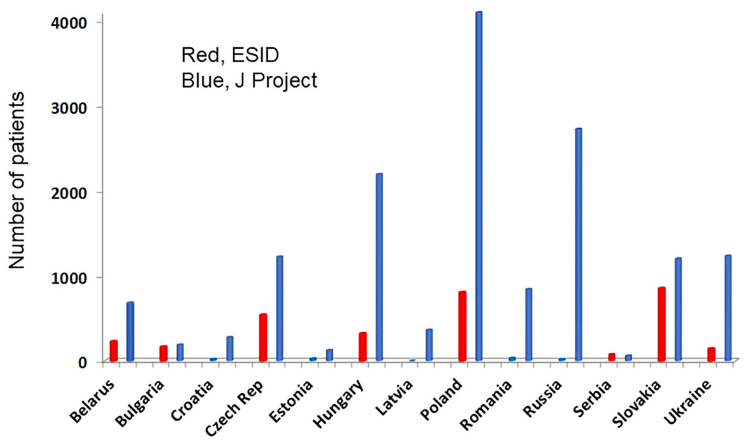

FIGURE 5
Total number of patients reported to the ESID-R (left columns, red) and the J Project (JP; right columns, blue) are shown. Such data were available only from 13 of the 32 JP countries indicating the lack of appropriate reporting activity. In addition, the total number of patients reported to the ESID-R was 3226 in contrast to the 15,234 patients reported in this survey.

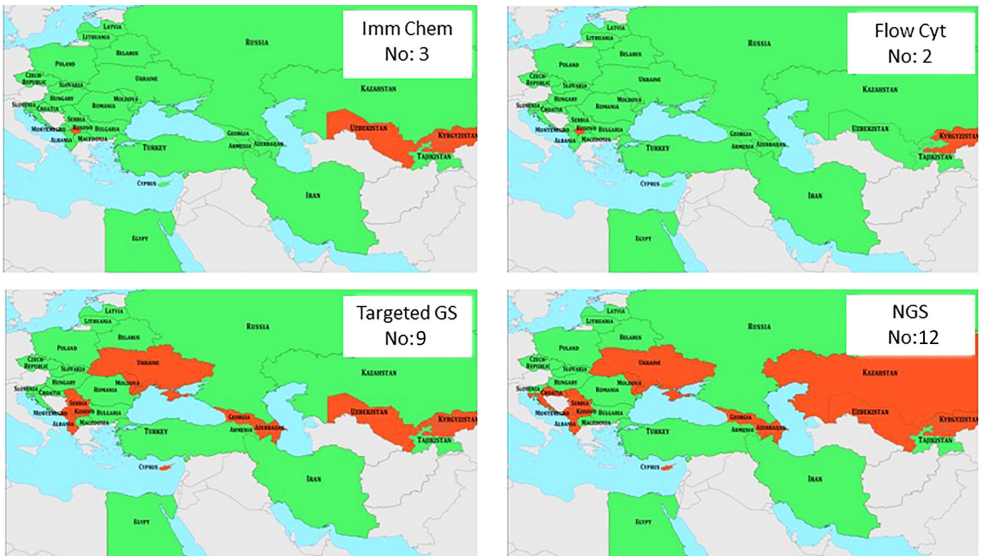

FIGURE 6
Availability of basic diagnostic parameters in J Project countries is shown in green. Red indicates the lack of various parameters. Numbers in insets indicate the number of countries that are missing various diagnostic measures. Imm Chem, immunochemistry; Flow Cyt, flow cytometry; GS, gene sequencing; NGS, new generation sequencing.

patients had received HSCT (Table 2). The number of IEI centers and experts were 260 and 690, respectively. We found high correlation between the number of PID centers and patients treated with IVIG (cc: 0,916) and with those who were treated with HSCT (cc: 0,905) (Figure 7). Similar correlation was found when the number of experts was compared with the number of patients treated with HSCT. However, the number of patients treated with SCIG only slightly correlated with the number of experts (cc: 0,489) and no correlation was found between the number of centers and patients on SCIG (cc: 0,174) (Figure 7). Although the total number of patients receiving IVIG was about three times higher, in eight countries more patients received SCIG (Table 2). In three countries (Lithuania, Bulgaria, and Kazakhstan) ten times more patients received SCIG compared to that of IVIG. In addition to several advantages of SCIG over IVIG, this could be due to the SARS-CoV-2 pandemic (35).

Conclusion

Several papers have been published before about the educational activity of the JP and the spread of the program in Eurasia (18, 30). In this study we first describe major diagnostic and treatment parameters of IEI care in countries of the JP after it had "grown up" and reached its 18 years in 2021 and outlined the progress we have made in a very important field of molecular and clinical medicine. We propose here that the JP has had remarkable impact on the development of IEI care and research in ECE and part of Asia. This ambitious project with the leadership of the JMF center established in 2004 at the Department of Infectiology and Pediatric Immunology in Debrecen, Hungary, was originally focused on a small area of Central Europe referred to as the Carpathian Euro region (Supplementary Figure 4). Due to outstanding ambition and support from international organizations and foundations, it has been permanently spreading across political, cultural and religious borders of Eurasia (24). We have come so far from our original plan and the progress we have made achieved the attention of the professional community worldwide. Similar successes were achieved and must be mentioned here in various parts of Asia, Africa, and South America (36, 37). Our data not only give hope to patients with PID but help to define major future targets of IEI awareness campaign and research in different continents. Importantly, the data presented here suggest that the number of IEI centers and IEI experts closely correlate to the most important treatment parameters, i.e. IVIG substitution and HSCT. We provide evidence that specialist education among medical professionals plays pivotal role in assuring diagnosis and adequate care in this vulnerable and still highly neglected patient population. This study also provides the basis for further analysis of more specific aspects of IEI care including genetic diagnostics, disease-specific prevalence and newborn screening as well as clinical research collaboration in J Project countries. Genetic testing of patients included in this study had not been requested because of the heterogenicity of diagnostic facilities in JP countries over the 18 years. This may represent a limitation of

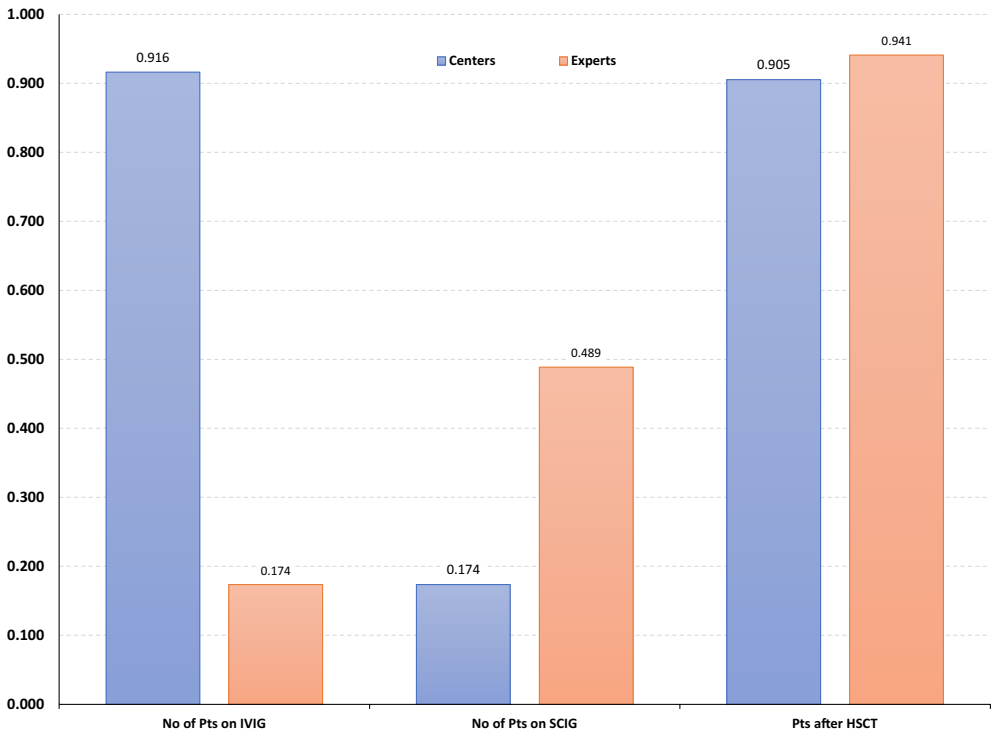

FIGURE 7
Correlations between the number of primary immunodeficiency (PID) centers and PID experts with the replacement of intravenous immunoglobulin (IVIG) or subcutaneous Ig (SCIG) or the number of hematopoietic stem cell transplantation (HSCT) performed in various countries. Data show that higher number of centers and experts favored the treatment with IVIG and HSCT but not with SCIG.

this study, but more importantly, it points to one of the focus of further research in the J Project.

The JP is an inspiring story for future immunologists worldwide and for the next generation of the Project in Eurasia. The secret sets of the success we described here is double: No 1: professional devotion, love of patients and research, and collaboration in any possible way we can; No 2: whatever we have achieved, there is so much more to do: more active participation at the advanced IEI communities like ESID, Latin American Society for Immunodeficiencies (LASID), African Society for Immunodeficiencies (ASID), and Clinical Immunology Society, improved quality of research publications, more organized national IEI patient care and data reporting to international databasis.

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

This study was approved by the Ethical Committee of the University School of Medicine, Debrecen, Hungary (ETT HRB 5975/2014/EHR and DE OEC RKEB/IKEB 3851-2013). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

IL performed all statistical analysis and holds the first authorship. GKi provided ESID Registry data. LM formulated the research goals and wrote the final draft. All other authors conducted clinical research and patient care and approved the submitted version. ASe, NG, NR, EB, MPa, ASh, PC, MJ, IR, ABo, TA, and LM share last authorship for their unique contribution to this 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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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2022.1032358/full#supplementary-material

SUPPLEMENTARY FIGURE 1
The first J Project (JP) Steering Committee meeting in 2010, chaired by (from L to R) I Reisli, M Serban, L Maródi (center), I Tuzankina, M Pac, and A Bondarenko. Delegates from all JP member countries, 19 at that time, attended the meeting in order to discuss previous achievements and future challenges of the JP.

SUPPLEMENTARY FIGURE 2
Geographic extension of the J Project by 2020 which included 32 countries and 6 J Daughter (JD) Project regions chaired by N Rezaei (JD Persia, 2009), I Reisli (JD Anatolia, 2009), A Elmarshafi and N Galal (JD Egypt, 2009), I Tuzankina (JD Siberia, 2010), E Kovzel (JD Central Asia, 2012), and E Tcyvkina (JD Far East Russia, 2019).

SUPPLEMENTARY FIGURE 3
Pie diagram showing the percentages of various inborn errors of immunity subgroups of patients reported to the J Project.

SUPPLEMENTARY FIGURE 4
The first 8 J Project meetings organized in Central Europe.

References

1. Bousfiha AA, Jeddane L, Ailal F, Benhsaien I, Mahlaoui N, Casanova J-L, et al. Primary immunodefciency diseases worldwide: More common than generally thought. *J Clin Immunol* (2013) 33:1–7. doi: 10.1007/s10875-012-9751-7

2. Maródi L, Casanova JL. Primary immunodeficiency diseases: The J project. *Lancet* (2009) 373:2179–81. doi: 10.1016/S0140-6736(09)61171-5

3. Casanova JL, Holland SM, Notarangelo LD. Inborn errors of human JAKs and STATs. *Immunity* (2012) 36:515–28. doi: 10.1016/j.immuni.2012.03.016

4. Grimbacher B. The European society for immunodefciencies (ESID) registry 2014. *Clin Exp Immunol* (2014) 178:18–20. doi: 10.1111/cei.12496

5. Lopes JP, Cunningham-Rundles Ch. The importance of primary immune deficiency registries: The united states immunodeficiency network registry. *Immunol Allergy Clin North Am* (2020) 40(3):385–402. doi: 10.1016/j.iac.2020.03.002

6. Abolhassani H, Azizi G, Sharifi L, Yazdani R, Mohsenzadegan M, Delavari S, et al. Global systematic review of primary immunodeficiency registries. *Expert Rev Clin Immunol* (2020) 16:717–32. doi: 10.1080/1744666X.2020.1801422

7. Tangye SG, Al-Herz W, Bousfha A, Chatila T, Cunningham-Rundles C, Etzioni A, et al. Human inborn errors of immunity: 2019 update on the classification from the international union of immunological societies expert committee. *J Clin Immunol* (2020) 40:24–64. doi: 10.1007/s10875-019-00737-x

8. Bousfiha A, Jeddane L, Picard C, Al-Herz W, Ailal F, Chatila T, et al. Human inborn errors of immunity: 2019 update of the IUIS phenotypical classifcation. *J Clin Immunol* (2020) 40:66–81. doi: 10.1007/s10875-020-00758-x

9. Tangye SG, Al-Herz W, Bousfiha A, Cunningham-Rundles C, Franco JL, Holland SM, et al. The ever-increasing array of novel inborn errors of immunity: an interim update by the IUIS committee. *J Clin Immunol* (2021) 18:1–14. doi: 10.1007/s10875-021-00980-1

10. Anzilotti C, Swan DJ, Boisson B, Deobagkar-Lele M, Oliveira C, Chabosseau P, et al. An essential role for the Zn^{2+} transporter ZIP7 in B cell development. *Nat Immunol* (2019) 20:350–61. doi: 10.1038/s41590-018-0295-8

11. Erdős M, Mironska K, Kareva L, et al. A novel mutation in SLC39A7 identified in a patient with autosomal recessive agammaglobulinemia: The impact of the J Project. *Pediatr Allergy Immunol* (2022) 33:e13805.

12. Bonagura VR, Casanova JL. Past, present, and future of the journal of clinical immunology, the international journal of inborn errors of immunity. *J Clin Immunol* (2020) 40:955–7. doi: 10.1007/s10875-020-00845-z

13. Casanova JL, Abel L. Lethal infectious diseases as inborn errors of immunity: Toward a synthesis of the germ and genetic theories. *Annu Rev Pathol* (2021) 16:23–50. doi: 10.1146/annurev-pathol-031920-101429

14. Ren A, Yin W, Miller H, Westerberg LS, Candotti F, Park CS, et al. Novel discoveries in immune dysregulation in inborn errors of immunity. *Front Immunol* (2021) 12:725587. doi: 10.3389/fimmu.2021.725587

15. Moens L, Meyts I. Recent human genetic errors of innate immunity leading to increased susceptibility to infection. *Curr Opin Immunol* (2020) 62:79–90. doi: 10.1016/j.coi.2019.12.002

16. Maródi L, the J Project Study Group. The 10th anniversary of the world primary immunodeficiency week: A J project celebration. *Eur J Immunol* (2021) 51:2364–6. doi: 10.1002/eji.202170105

17. Tuzankina I, Bolkov M, Nabieva U, Lázár I, Maródi L. The J daughter Siberia project. *J Clin Immunol* (2021) 41:262–5. doi: 10.1007/s10875-020-00894-4

18. Maródi L, the J Project Study Group. Fifteen Years J Project. *J Clin Immunol* (2019) 39:363–9.

19. Pac M, Casanova JL, Reisli I, Maródi L. Advances in primary immunodeficiency in central- Eastern Europe. *Front Immunol* (2021) 12:667727. doi: 10.3389/fimmu.2021.667727

20. Maródi L, the J Project Study Group. A rose amongst the thorns: the mission of the J project in a conflictual world. *J Clin Immunol* (2022) 42:1–5.

21. Mukhina AA, Kuzmenko NB, Rodina YA, Kondratenko IV, Bologov AA, Latysheva TV, et al. Primary immunodeficiencies in Russia: Data from the national registry. *Front Immunol* (2020) 11:1491. doi: 10.3389/fimmu.2020.01491

22. Volokha A, Bondarenko A, Chernyshova L, Hilfanova A, Stepanovskiy Y, Boyarchuk O, et al. Impact of the J project on progress of primary immunodeficiency care in Ukraine. *Cent Eur J Immunol* (2021) 46:250–7. doi: 10.5114/ceji.2021.108183

23. Pac M, Bernatowska E. Comprehensive activities to increase recognition of primary immunodeficiency and access to immunoglobulin replacement therapy in Poland. *Eur J Pediatr* (2016) 175:1099–105. doi: 10.1007/s00431-016-2746-2

24. Horváth Z, Rezaei N, Reisli I, Tuzankina I, Otarbayev N, Popandopulo P, et al. The spread of the J project. *J Clin Immunol* (2013) 33:1037–42. doi: 10.1007/s10875-013-9905-2

25. Sciuca S, Tomacinschii C, Selevestru R, Sacara V, Maródi L. Inborn errors of immunity in the Republic of Moldova: advances and hope. *J Clin Immunol* (2022).

26. Ismaili-Jaha V, Kuli-Lito G, Spahiu-Konjusha S, Baloku A, Maródi L. Through education and collaboration to better care for primary imunodeficiencies in Albania and Kosovo. *J Clin Immunol* (2022).

27. Maródi L, the J Project Study Group. The Konya declaration for patients with primary immunodeficiencies. *J Clin Immunol* (2020) 40:770–3. doi: 10.1007/s10875-020-00797-4

28. Gulácsy V, Soltész B, Petrescu C, Bataneant M, Gyimesi E, Serban M, et al. A novel large deletion and single nucleotide insertion in the wiskott-Aldrich syndrome protein gene. *Eur J Haematol* (2015) 95:93–8. doi: 10.1111/ejh.12424

29. Soltész B, Tóth B, Shabashova N, Bondarenko A, Okada S, Cypowyj S, et al. New and recurrent gain-of-function STAT1 mutations in patients with chronic mucocutaneous candidiasis from Eastern and central Europe. *J Med Genet* (2013) 50:567–78. doi: 10.1136/jmedgenet-2013-101570

30. Maródi L, the J Project Study Group. The creation and progress of the J project in Eastern and central Europe. *Ann N Y Acad Sci* (2011) 1238:65–73. doi: 10.1111/j.1749-6632.2011.06247.x

31. Gulácsy V, Freiberger T, Shcherbina A, Pac M, Chernyshova L, Avcin T, et al. Genetic characteristics of eighty-seven patients with the wiskott-Aldrich syndrome. *Mol Immunol* (2011) 48:788–92. doi: 10.1016/j.molimm.2010.11.013

32. Tóth B, Volokha A, Mihas A, Pac M, Bernatowska E, Kondratenko I, et al. Genetic and demographic features of X-linked agammaglobulinemia in Eastern and central Europe: a cohort study. *Mol Immunol* (2009) 46:2140–6. doi: 10.1016/j.molimm.2009.03.012

33. Wolska-Kuśnierz B, Gregorek H, Chrzanowska K, Piątosa B, Pietrucha B, Heropolitańska-Pliszka E, et al. Nijmegen breakage syndrome: Clinical and immunological features, long-term outcome and treatment options - a retrospective analysis. *J Clin Immunol* (2015) 35:538–49. doi: 10.1007/s10875-015-0186-9

34. Casanova JL, Su HC. A global effort to define the human genetics of protective immunity to SARS-CoV-2 infection. *Cell* (2020) 181:1194–9. COVID Human Genetic Effort.

35. Quinti I, Mezzaroma I, Milito C. Clinical management of patients with primary immunodeficiencies during the COVID-19 pandemic. *Expert Rev Clin Immunol* (2021) 17:163–8. doi: 10.1080/1744666X.2021.1873767

36. Aghamohammadi A, Rezaei R, Yazdani R, Delavari S, Kutukculer N, Topyildiz E, et al. Consensus middle East and north Africa registry on inborn errors of immunity. *J Clin Immunol* (2021) 41:1339–51. doi: 10.1007/s10875-021-01053-z

37. Condino-Neto A. The relevance of collaborative work: the Latin American society for immunodeficiencies (LASID) registry model. *Clin Exp Immunol* (2014) 178:16–7. doi: 10.1111/cei.12495

CITATION

Abolhassani H, Avcin T, Bahceciler N, Balashov D, Bata Z, Bataneant M, Belevtsev M, Bernatowska E, Bidló J, Blazsó P, Boisson B, Bolkov M, Bondarenko A, Boyarchuk O, Bundschu A, Casanova J-L, Chernishova L, Ciznar P, Csürke I, Erdős M, Farkas H, Fomina DS, Galal N, Goda V, Guner SN, Hauser P, Ilyina NI, Iremadze T, Iritsyan S, Ismaili-Jaha V, Jesenak M, Kelecic J, Keles S, Kindle G, Kondratenko IV, Kostyuchenko L, Kovzel E, Kriván G, Kuli-Lito G, Kumánovics G, Kurjane N, Latysheva EA, Latysheva TV, Lázár I, Markelj G, Markovic M, Maródi L, Mammadova V, Medvecz M, Miltner N, Mironska K, Modell F, Modell V, Mosdósi B, Mukhina AA, Murdjeva M, Műzes G, Nabieva U, Nasrullayeva G, Naumova E, Nagy K, Onozó B, Orozbekova B, Pac M, Pagava K, Pampura AN, Pasic S, Petrosyan M, Petrovic G, Pocek L, Prodeus AP, Reisli I, Ress K, Rezaei N, Rodina YA, Rumyantsev AG, Sciuca S, Sediva A, Serban M, Sharapova S, Shcherbina A, Sitkauskiene B, Snimshchikova I, Spahiu-Konjusha S, Szolnoky M, Szűcs G, Toplak N, Tóth B, Tsyvkina G, Tuzankina I, Vlasova E and Volokha A (2022) Care of patients with inborn errors of immunity in thirty J Project countries between 2004 and 2021.
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Case report: Challenges in immune reconstitution following hematopoietic stem cell transplantation for CTLA-4 insufficiency-like primary immune regulatory disorders

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Cytotoxic T-lymphocyte antigen-4 (CTLA-4) haploinsufficiency is a T-cell hyperactivation disorder that can manifest with both immunodeficiency and immune dysregulation. Approximately one-third of patients may present mild symptoms and remain stable under supportive care. The remaining patients may develop severe multiorgan autoimmunity requiring lifelong immunosuppressive treatment. Hematopoietic stem cell transplantation (HSCT) is potentially curable for patients with treatment-resistant immune dysregulation. Nevertheless, little experience is reported regarding the management of complications post-HSCT. We present case 1 (CTLA-4 haploinsufficiency) and case 2 (CTLA-4 insufficiency-like phenotype) manifesting with severe autoimmunity including cytopenia and involvement

of the central nervous system (CNS), lung, and gut and variable impairment of humoral responses. Both patients underwent HSCT for which the main complications were persistent mixed chimerism, infections, and immune-mediated complications [graft-versus-host disease (GVHD) and nodular lung disease]. Detailed management and outcomes of therapeutic interventions post-HSCT are discussed. Concretely, post-HSCT abatacept and human leukocyte antigen (HLA)-matched sibling donor lymphocyte infusions may be used to increase T-cell donor chimerism with the aim of correcting the immune phenotype of CTLA-4 haploinsufficiency.

KEYWORDS

CTLA-4, primary immunodeficiency, hematopoietic stem cell transplantation, abatacept, immune reconstitution, chimerism

Introduction

Regulatory T cell (Treg) defects are conditions included within the category of primary immune regulatory disorders (PIRDs) (1). They are defined by quantitative or qualitative impairment of the Treg compartment, predisposing to severe multiorgan autoimmunity with or without susceptibility to infections (2–4). Cytotoxic T-lymphocyte antigen-4 (CTLA-4) haploinsufficiency is included in this group, since the reduced surface availability of CTLA-4 ultimately results in Treg cell dysfunction (5). The three entities that negatively affect CTLA-4 function in human disease are CTLA-4, lipopolysaccharide-responsive and beige like anchor protein (LRBA), and DEF6 deficiencies (5). These three have been grouped under the term immune checkpoint defects (6). They share common features of dual symptoms of immune dysregulation and immune deficiency, with variable disease expressivity even in individuals with the same mutation (5, 7). Shared phenotypic manifestations of immune dysregulation include autoimmune cytopenia, enteropathy, and lymphoproliferation (5, 8, 9). This triad combination is not commonly seen in other PIRDs (5).

The long-term therapeutic approach for affected patients is challenging (5, 10). Approximately one-third of patients may present mild symptoms and remain stable under supportive care (8). The remaining patients may develop severe multiorgan autoimmunity requiring lifelong immunosuppressive treatment with Treg-sparing immunosuppression [mammalian target of rapamycin (mTOR) inhibitors] or targeted soluble CTLA-4-Ig (abatacept, belatacept) (10, 11). Currently, hematopoietic stem cell transplantation (HSCT) is offered to these patients with treatment-resistant immune dysregulation (1, 12). Nevertheless, little experience in HSCT in these conditions is reported (10), mostly in LRBA deficiency (13). Doubts regarding bridge or remission induction therapy (11, 13, 14), conditioning for the transplant (15), and post-HSCT chimerism goals remain.

Detailed clinical observations can provide insight into the challenges of HSCT management in this subgroup of PIRD patients and more so when they are diagnosed worldwide, including Eastern Europe (5), and the HSCT approach can be variable. Thus, we present case 1 (CTLA-4 haploinsufficiency) and case 2 (CTLA-4 insufficiency-like phenotype), both manifested with autoimmune cytopenia, enteropathy, and lymphoproliferation, with typical lung and central nervous system (CNS) involvement, and variable impairment of humoral responses. Both patients underwent HSCT, and the main complications were mixed chimerism, infections, and immune-mediated complications.

Clinical description

Case 1

We present an 18-year-old man with CTLA-4 haploinsufficiency [*CTLA4* frameshift mutation c.342_342delC, reported in Schwab et al. (8), subject 42] with low CTLA-4 expression in FoxP3+ CD4 T cell (Supplementary Figure S1) (16). Patient presented autoimmune and/or inflammatory disorders such as autoimmune hemolytic anemia (AIHA), idiopathic thrombocytopenic purpura (ITP), granulomatous-lymphocytic interstitial lung disease (GLILD), and autoimmune encephalitis, requiring multiple immunosuppressants as shown in Figure 1. HSCT was indicated due to partial response of these immune dysregulatory manifestations despite targeted treatment. His immune deficiency and dysregulation activity (IDDA) score (11) prior to HSCT was 17.6. Main characteristics of his baseline disease and HSCT are presented in Table 1. The patient received HSCT from his identical human leukocyte

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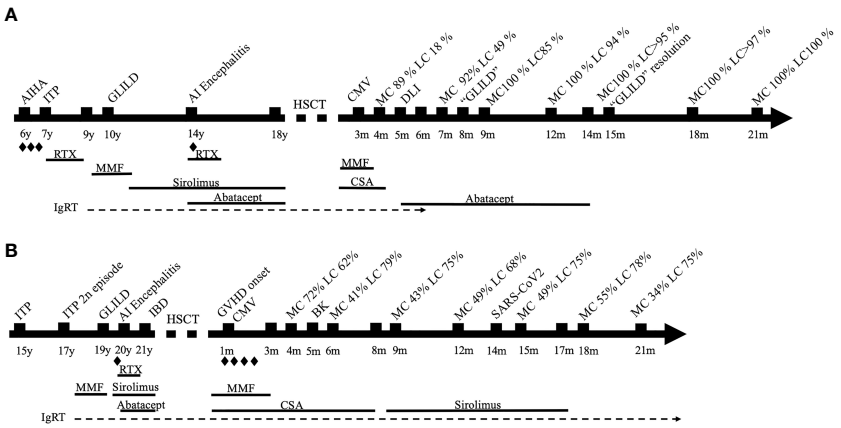

FIGURE 1
(A) Timeline of case 1. **(B)** Timeline of case 2. Steroids; AIHA, autoimmune hemolytic anemia; ITP, immune thrombocytopenic purpura; AI, autoimmune; HSCT, hematopoietic stem cell transplantation; CMV, cytomegalovirus; MC, myeloid chimerism; LC, lymphoid chimerism; CSA, cyclosporin; DLI, donor lymphocyte infusion; GLILD, granulomatous-lymphocytic interstitial lung disease; RTX, rituximab; IgRT, immunoglobulin replacement therapy; IBD, inflammatory bowel disease; GVHD, graft-versus-host disease.

antigen (HLA) sister, both sharing blood type and positivity for cytomegalovirus (CMV). The European Group for Blood and Marrow Transplantation (EBMT) guidelines were followed for HSCT using treosulfan, fludarabine, and thiotepa as conditioning treatment and cyclosporin and mycophenolate for graft-versus-host disease (GVHD) prophylaxis (17). The clinical course was complicated with low donor lymphoid chimerism and an episode of lung lesions that posed the differential diagnosis with GLILD. Both resolved with the combination of donor lymphocyte infusions (DLIs) from the identical HLA sister and expert management of immune modulation including abatacept introduction post-HSCT, as described below. The timeline is depicted in Figure 1.

Three months post-procedure, split chimerism showed myeloid lineage of 89% and lymphoid lineage of 18% from the donor. In order to improve the chimerism, cyclosporin was quickly withdrawn over 4 weeks and abatacept as a target therapy was started to control the dysregulated lymphocytes that could be left from the recipient. Three weeks later, chimerism remained very low in the lymphoid lineage (18%); therefore, the patient received three DLIs (total dose CD3 1.3×10^8) from his sister with no signs of GVHD after that. Seven months after transplant, 3 months after stopping cyclosporin and switching to abatacept, and 1.5 months after DLI, chimerism improved, showing 92% of myeloid and 49% of lymphoid from the donor.

Eight months after transplantation, the patient presented fever and diarrhea. Infectious screening was positive for *Campylobacter coli* in a stool culture. After 5 days of fever and on antibiotics, computed tomography (CT) scan showed multiple lung nodules suggesting an infection or lymphoproliferative disorder (Figure 2). The study was completed with brain magnetic resonance imaging, positron emission tomography (PET) scan, bronchoalveolar lavage (BAL), and bone marrow aspirate. Multiple adenopathies and splenomegaly were seen. Bone marrow aspirate was normal. Epstein–Barr virus (EBV) was positive in blood and in the BAL, although at a very low number of copies (<250 copies/ml), and the patient did not present other signs of posttransplant lymphoproliferative disorder (PTLD).

A lung wedge biopsy was performed, demonstrating non-necrotizing granulomatous inflammation concentrated bronchovasculocentrically and paraseptally, made up of histiocytes admixed with a lymphocytic component, predominantly T-cell CD4+. Microbiological tests ruled out infection. These findings were suggestive of a GLILD flare (18). To confirm which T cells (female donor vs. male patient) were driving this inflammation, fluorescence *in situ* hybridization (FISH) X/Y was performed in the lung tissue to assess the chimerism *in situ*. The same pattern as in the blood (Figure 2) was observed, ruling out enrichment of the patient's T cells in the granulomas. Lung lesions presented remission within 2 weeks without further intervention. The patient remained on abatacept as the sole immunosuppression.

Fifteen months post-HSCT, the patient presented good clinical evolution with an improvement in the CT scan and respiratory test. Chimerism presented sustained improvement, maintaining 100% and >95% in myeloid and lymphoid lineages, respectively. In this context, abatacept was discontinued.

Currently, 21 months posttransplantation, CT scan and pulmonary function tests are within normal range and the patient's chimerism is 100% in both lineages. He is off immunosuppression with good immune reconstitution and also off immunoglobulin replacement therapy (IgRT) (Table 1).

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TABLE 1 Table 1 Summary of the two cases.

Pre-HSCT	Case 1-♂	Case 2-♂
		\
Age at disease onset (years)	6	15
Age at CVID diagnosis (years)	10	15
Positive genetic diagnosis	*CTLA4* frameshift mutation c.342_342delC (13 years old)	No
Immune workup at CVID diagnosis		
Absolute lymphocytes (cells/mm^3)	1,100 (low)	1,200 (low)
CD19+ lymphocytes (%/abs)	13.9/153 (normal/normal)	11/132 (low/low)
CD4+ lymphocytes (%/abs)	43.9/473 (normal/normal)	32/384 (normal/low)
CD8+ lymphocytes (%/abs)	30.8/339 (normal/normal)	31/372 (normal/ normal)
CD19+ naive (IgM+IgD+CD27-) (%, abs)	97.3/149 (high/ normal)	78/102 (normal/normal)
CD19+ switched memory (IgD-CD27+)(%, abs)	0.5/1 (low/low)	12/16 (normal/low)
CD19+CD21^{low}CD38^{low} (%,abs)	Not determined	22.8/30 (high/normal)
Naive CD3+CD45RA+ (%)	54 (normal)	35.9 (normal)
Memory CD3+CD45RO+ (%)	31 (normal)	37.9 (normal)
IgG (mg/dl)	588 (low)	576 (low)
IgA (mg/dl)	18 (low)	44 (low)
IgM (mg/dl)	43 (normal)	34 (low)
Immune workup before HSCT		
Absolute lymphocytes (cells/mm^3)	900 (low)	700 (low)
CD19+ lymphocytes (%/abs)	22.3/200 (normal/normal)	0/0 (under RTX. low/low)
CD4+ lymphocytes (%/abs)	43.9/395 (normal/normal)	25.4/177 (low/low)
CD8+ lymphocytes (%/abs)	23.5/211 (normal/normal)	44/308 (high/normal)
CD19+ naive (IgM+IgD+CCD27-) (%/abs)	98.5/97 (high/ normal)	NA
CD19+ switched memory (IgD-CD27+) (%/abs)	0.4/0.8 (low/low)	NA
CD19+CD21^{low} CD38^{low} (%)	3.5 (normal)	NA
Naive CD4+ (CD4+ CD45RA+) (%)	52.9 (normal)	17.5 (low)
Eff.mem.CD4+(CD4+CD45RA-CCR7-) (%)	6.6 (normal)	55.4 (high)
Naive CD8+ (CD8+ CD45RA+) (%)	68 (normal)	10.8 (low)
Eff. mem.CD8+ (CD8+CD45RA-CCR7-)(%)	16.3 (normal)	75.5 (high)
IgG (mg/dl)	882 (normal, under IgRT)	1,381 (normal, under IgRT)
IgA (mg/dl)	12 (low)	24 (low)
IgM (mg/dl)	34 (low)	24 (low)
IgRT	Yes	Yes
Infections	CMV chronic infection	Periorbital infection
	Recurrent enteric infections (*Campylobacter* spp. and *Salmonella* spp.)	
Immune-dysregulatory phenotype	Evans syndrome	Immune thrombocytopenia
	Granulomatous-lymphocytic lung disease	Granulomatous-lymphocytic lung disease
	Autoimmune encephalomyelitis	Autoimmune encephalomyelitis
	Lymphoproliferation (splenomegaly, adenomegalies)	Inflammatory bowel disease
		Lymphoproliferation (splenomegaly)
Immunosuppressants prior to HSCT (see **Figure 1**)	Steroids, mycophenolate, sirolimus, rituximab, abatacept	Steroids, mycophenolate, sirolimus, rituximab, abatacept
IDDA score	17.6	21.6

(Continued)

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TABLE 1 Continued

Post-HSCT	Case 1-♂	Case 2-♂
Age at HSCT (years)	18	21
Karnofsky	100%	90%
Conditioning regimen	Flu-Treo-Thio	Flu-Treo-Thio
GVHD prophylaxis	Alemtuzumab, CSA, MMF	Alemtuzumab, CSA, MMF
Type of donor	BM-identical HLA MSD	BM-10/10 HLA MUD
	TNC 2.3 × 10e8/kg	TNC 1.63 × 10e8/kg
Graft failure	No	No
Slow engraftment at day 28	Yes	Yes
Infections	CMV, Herpes simplex	CMV, ADV, BK , SARS-CoV-2
GVHD	No	Yes, acute (CSA switched to sirolimus due to renal disease)
Mixed chimerism 3 months post-HSCT (both lineages)	Yes	Yes
Interventions for mixed chimerism	Switch CSA to abatacept DLI	No
Off IS	14 m post-HSCT (abatacept)	17 m post-HSCT (sirolimus)
Off IgRT	Yes, since 6 m post-HSCT	No
Immune workup 21 m post-HSCT		
Absolute lymphocytes (cells/mm^3)	2,500 (normal)	800 (low)
CD19+ lymphocytes (%/abs)	27/675 (high/high)	4.9/19 (low/low)
CD4+ lymphocytes (%/abs)	25/628 (low/normal)	25/100 (low/low)
CD8+ lymphocytes (%/abs)	36/900 (high/normal)	56/222 (high/low)
NK cells/mm^3 (%/abs)	8/200 (normal/normal)	8.2/32 (normal/low)
Naive CD4+ (CD4+ CD45RA+) (%/abs)	47.9/300.8 (normal/normal)	30.2/30.2 (low/low)
Eff.mem.CD4+(CD4+CD45RA-CCR7-)(%/abs)	26.8/168.3 (normal/normal)	40.1/40.1 (normal/low)
Naive CD8+ (CD8+ CD45RA+) (%/abs)	21.1/189.9 (normal/normal)	13.7/30.4 (low/low)
Eff.mem.CD8+ (CD8+CD45RA-CCR7-) (%/abs)	2.9/26.1 (low/low)	35.4/78.58 (normal/low)
CD19+ naive (%/ abs)	97.2/656.1 (high/high)	97.4/18.5 (high/low)
CD19+ switched memory (IgD –CD27+) (%/abs)	1.6/10.8 (low/low)	1/0.0 (low/low)
IgG (mg/dl)	1,497 (normal)	870 (normal, under IgRT)
IgA (mg/dl)	<3 (low)	<3 (low)
IgM (mg/dl)	137 (normal)	19 (low)

HSCT, hematopoietic stem cell transplantation; m, months; CVID, common variable immunodeficiency; NA, not applicable; Eff.mem., effector memory; IgRT, immunoglobulin replacement therapy; CMV, cytomegalovirus; IDDA, immune deficiency and dysregulation activity (ref 12); yo, years old; Flu, fludarabine; Treo, treosulfan; Thio, thiotepa; GVHD, graft-versus-host disease; CSA, cyclosporin; MMF, mycophenolate; BM, bone marrow; HLA, human leukocyte antigen; MSD, matched sibling donor; MUD, matched unrelated donor; TNC, total nucleated cell dose; ADV, adenovirus; DLI, donor lymphocyte infusion; IS, immunosuppressant; RTX, rituximab; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

Case 2

We present a 21-year-old man with common variable immunodeficiency (CVID) and a phenotype of immune dysregulation similar to CTLA-4 haploinsufficiency characterized by autoimmune cytopenia, inflammatory bowel disease (IBD), lymphoproliferation (GLILD), autoimmune encephalitis, and humoral deficiency requiring multiple immunosuppressants (Figure 1). No genetic defect was identified in a gene panel including inborn errors of immunity (IEIs) and PIRD-related genes (SureSelect Custom Constitutional Panel 17 Mb, Agilent), and an array comparative genomic hybridization (aCGH) was normal. The patient fulfilled the European Society for Immunodeficiencies (ESID) Registry Working Definitions for the Clinical Diagnosis for Common Variable Immunodeficiency (19) (Table 1). However, CTLA-4 expression in FoxP3+ CD4+ T cells resembled that of CTLA-4-deficient patients, with a marked decrease in the CTLA-4^{hi}FoxP3+CD4+ T cells (Supplementary Figure S1). HSCT was indicated due to a partial control of immune dysregulation despite sirolimus and abatacept. The IDDA score prior to HSCT was 21.6. The patient received

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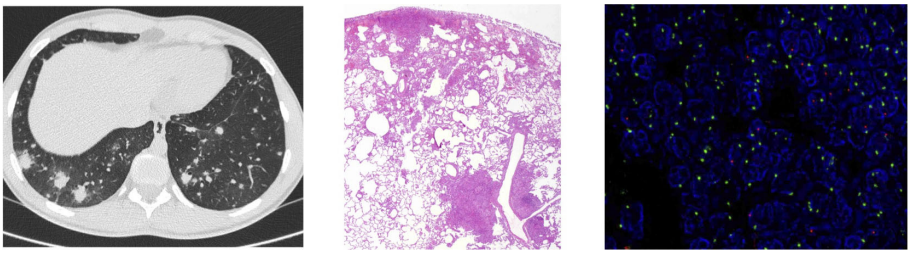

FIGURE 2
Study of lung lesions of case 1. First image computed tomography (CT) scan showing multiple lung nodules. Second image wedge luyng biopsy demonstrated a non-necrotizing granulomatous inflammation concentrated bronchovasculocentrically and paraseptally [hematoxylin and eosin (H&E), 2x]. Third image fluorescencein-situ hybridization (FISH) using locus specific indentifier (LSI) SRY. In the lymphocytic infiltrates, up to 27% of the nuclei showed a green dot (CEP X) and a red dot (SRY), and 73% of the nuclei showed two green dots (CEP X), thus indicating that the majority of the lymphocytic infiltrate corresponded to the female donor.

HSCT from an HLA-identical (10/10) unrelated donor with the same blood type. The patient was CMV-positive, while the donor was negative. The clinical course was complicated by GVHD, mixed chimerism, and infectious episodes, as described below. The timeline is depicted in Figure 1.

One month post-HSCT, he developed grade 2 GVHD requiring steroids and poor engraftment requiring granulocyte colony-stimulating factor and eltrombopag. He also presented CMV infection requiring preemptive treatment with foscarnet, cidofovir, and specific T-lymphocyte infusion. Thereafter, CMV infection was controlled, but he developed BK hemorrhagic cystitis, community-acquired pneumonia, and a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) upper respiratory tract infection.

Mixed chimerism persisted around 60%–70% in the lymphocytic lineage (Figure 1). Cyclosporin was switched to sirolimus for the treatment of GVHD at 8 months post-HSCT due to renal dysfunction. Lymphoid chimerism remained in similar range, with a slight increase to 75%. At 17 months post-HSCT, with controlled GVHD, immunosuppression was slowly discontinued with no complications. As the patient maintained a stable lymphoid chimerism above 70%–75% and persistent grade 2 GVHD, no further interventions were performed.

Currently, 21 months post-HSCT, the patient is off immunosuppression. He still presents mixed donor chimerism of 34% in granulocytes and 75% in T lymphocytes without autoimmune episodes. His immune cellular and humoral reconstitution is still incomplete, requiring IgRT and antimicrobial prophylaxis (Table 1).

Discussion

Currently, the management of CTLA-4 haploinsufficiency and similar diseases characterized by marked T-cell activation is still in discussion. Bridge or remission induction therapy (11, 13, 14), transplant conditioning (15), and post-HSCT chimerism goals need to be refined. The two cases reported here are representative of this group of patients who fail to respond to conservative immunosuppressant treatment and move to HSCT with high levels of immune dysregulation. Their post-HSCT outcome and management highlight the importance of an individualized approach to achieve maximum, if not full, lymphoid chimerism to ensure disease remission and complete immune reconstitution.

Over the last decade, allo-HSCT outcomes in IEI have improved significantly. The survival rate for conventional IEI transplants is now approaching 90% (17, 20, 21). HSCT is also being offered to young adults with high rates of success (22). This is due to improved donor selection, better management of HSCT complications, and optimized supportive care (20, 23, 24). Still, high levels of hyperinflammation prior to transplant may promote a greater incidence of alloreactivity disorders post-HSCT (25, 26), and has already been shown to worsen HSCT outcomes, with a greater risk of GVHD (as observed in case 2) and toxicity, and impaired immune reconstitution (25). Also, although the same conditioning and GVHD prophylaxis were given in both cases, they received different types of graft: case 1 received marrow from his HLA-identical sister and case 2 received marrow from a 10/10 HLA unrelated donor. Still, some immune disparities could be present and interfere in the immune reconstitution phase, as described before (25, 27). In addition, patient 2 presented a higher IDDA score and higher number of effector memory CD4+ cells prior to HSCT. All these factors may have contributed to the different post-HSCT outcome in both patients.

In our cases, HLA-matched donors (one related, one unrelated) and bone marrow source were used. In this sense, it is important to bear in mind that because of the genetic nature of most IEIs, genetic screening of family donors is warranted regardless of symptoms, since PIRDs can display a late or variable clinical onset, as typically described for CTLA-4 haploinsufficiency (8). In order to minimize the higher incidence of alloreactivity, the role of biologic modifiers or

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targeted therapies as a bridge to HSCT in PIRDs is an important field to explore (14). In our center, both patients received targeted immunosuppression including abatacept until 2 weeks prior to transplantation. Currently, one of the main questions that arise is the dichotomy between lifelong immune modulation vs. HSCT for IEI and PIRD (28).

Current recommendations do not identify patients with CTLA-4 haploinsufficiency who might benefit from long-term targeted immunomodulation vs. HSCT nor the optimal timing for HSCT (10). HSCT outcomes must be balanced with the risks of disease. Reports of HSCT for CTLA-4 haploinsufficiency are scarce. They illustrate that HSCT can be effective (8, 29). Schwab et al. (8) reported 12 transplanted patients among 90 symptomatic *CTLA4* mutation carriers undergoing allo-HSCT between 10 and 50 years of age. Main indications were uncontrollable cytopenia, enteropathy, and lymphoma with additional autoimmune disorders involving lymphoproliferative and infectious complications (8). Nine of the 12 patients (75%) are alive, three of them more than 5 years after HSCT and currently well without medication. Slatter et al. (29) described eight pediatric patients with CTLA-4 haploinsufficiency who underwent HSCT. All received transplants from 10/10 HLA-matched unrelated donors following a reduced-intensity conditioning regimen; the outcome was 50% GVHD, 25% autoimmune disorders, and an overall survival rate of 75% (29). In the report by Chan et al. (24), 13 of the 226 transplanted patients were CTLA-4 haploinsufficiencies, but no specific subgroup data analysis could be performed. The informed nature of decision-making for clinicians, patients, and families in these ill-defined situations is improved if clinical outcome data of defined patients with defined treatments are reported. For this purpose, a systematic description of patients with CTLA-4 haploinsufficiency undergoing HSCT is needed. This description should include IDDA score prior to HSCT. The IDDA score (11) can be used to compare semiquantitative values in one individual over time (clinical course, longitudinally) or between individuals or cohorts at a specific time point (cross-sectionally). The new IDDA version includes broader manifestations of immune dysregulation, factors that indicate the quality of life and need for supportive care, and the occurrence of malignancies (30). This is critical because the absence of ongoing medication and quality of life are important features that are rarely quantified, and these may be better in patients who have undergone transplantation (31–33). Also, a detailed description of immunosuppressants used prior to and post-HSCT and the dynamics of T-cell chimerism are necessary to enable comparisons of therapeutic approaches. In our patients, disease evolution prior to HSCT was 12 and 6 years, respectively; the patients had a high disease burden with IDDA scores >15 and had received multiple courses of immunosuppressants with only partial or transient responses. Despite the non-compelling abovementioned HSCT data, the transplant choice was made by the patients, mainly motivated by chronic disease fatigue and the low quality of life of two active young adults.

More and more adolescents and young adults (AYAs) with IEI are referred for HSCT (22, 34, 35). For these patients, aging leads to early end organ damage, reduced quality of life, and early death (36). Transplant decision is challenging, as they have survived childhood with conservative management, but HSCT needs to be considered before further deterioration. Both of our reported patients have received adult-stage HSCT with reduced toxicity regimen, and the decision to transplant was based on the lack of disease control despite long-term use of two targeted immunomodulators. For newly identified diseases with alternative targeted therapies (14, 37–39) such as CTLA-4 fusion proteins, careful follow-up of different treatment cohorts is necessary to determine the best treatment modalities in the long-term. The safety and efficacy of abatacept for adult patients with CTLA-4 insufficiency or LRBA deficiency are currently being evaluated in a phase 2 clinical trial (ABACHAI) (40).

For some IEIs, a certain level of mixed chimerism is sufficient to improve the patients' well-being. But is there a minimum level of donor T-cell chimerism required to correct the immune phenotype of PIRD whose immune pathology is T-cell activation? Chimerism was designed to monitor the percentage of the donor cells after the infusion and not to monitor the disease baseline or malignant relapse (41). Full donor T-cell chimerism in LRBA deficiency has been shown to be positively linked to the probability of remission, although data on the relevance of donor chimerism for cure are still limited (11, 13, 42, 43). In many IEIs, stable mixed donor chimerism does not lead to graft rejection, and it may suffice to correct the underlying immunodeficiency (44). However, it has been shown that donor myeloid chimerism is important for long-term immune recovery of T and B lymphocytes and adequate immune function after HSCT (45). In case 2, low mixed myeloid chimerism was a concern, although no DLIs were considered due to the high risk of GVHD and to donor availability. Some data suggest that mixed chimerism can cause persistent autoimmunity or autoinflammation in these patients (46–48). From the report by Slatter et al. (29), six of the eight patients are alive and well with donor chimerism ranging 85%–100%. So, for patients with CTLA-4 haploinsufficiency who present mixed chimerism post-HSCT, it seems reasonable to aim at high, or even full, T-cell chimerism. In cases of no active GVHD, like in case 1, weaning from the immunosuppressant drugs and performing DLI, especially in matched family donors, could be considered. In case 1, abatacept was also started to immunomodulate the patient's CTLA-4 haploinsufficient lymphocytes. The use of abatacept prior to HSCT is to modulate autologous activated T cells to control the underlying disease. Its use in the posttransplant phase could be beneficial both in controlling the remaining autologous T cells to reduce the risk of disease flare, but also to modulate allo-reactive donor T cells to reduce the risk of GVHD development (49, 50). On the other hand, if the patient presents GVHD, such as case 2, the approach to slowly withdraw the immunosuppressant drug, or even switch to a Treg-sparing regimen such as sirolimus, can be considered. It is difficult to ascertain what the main reasons are for such a different outcome. Our

hypothesis is that GVHD development in case 2 [linked to a matched unrelated donor (MUD) and higher levels of inflammation at the time of HSCT] was one of the main determinants in the different management of mixed chimerism and final outcome, since it obliged to a certain level of immunosuppression and interfered with immune reconstitution.

Beyond chimerism, during the early phase of PIRD patient transplant, the recipient's dysregulated lymphocytes are still a concern, especially in mixed chimerism. Therefore, close monitoring of inflammation and autoimmune complications is crucial for early flare recognition until chimerism and immune reconstitution are complete. In case 1, it is difficult to determine whether the lung nodular lesions were a flare of his GLILD in the context of persistent low chimerism and his being off abatacept (no infection or PTLD was demonstrated, and he was respiratory asymptomatic so suspicion of an immune reconstitution phenomenon was reduced). Also, no CT had been performed in the first 8 months post-HSCT to enable comparisons. The predominance of donor T cells in the nodules along with the improved chimerism thereafter might explain the quick resolution of the lung nodules in a GLILD. In patient 2, currently with 75% of lymphoid chimerism, no disease flare has been observed while off immunosuppression. Immunological biomarkers are still to be defined in the monitoring of PIRD patients' immune reconstitution and detection of immune dysregulation post-HSCT. Functional studies on the underlying genetic defect (i.e., transendocytosis test for checkpoint deficiencies) might be of interest in cases of persistent mixed lymphoid chimerism (6, 8, 51).

Conclusion

CTLA-4 haploinsufficiency encompasses a heterogeneous and often complex group of patients requiring an individualized therapeutic approach. Detailed descriptions of case reports and HSCT outcomes are crucial to identify strategies to improve allo-HSCT outcomes and help delimit the target T-cell chimerism and thereby avoid disease flares. These strategies may include the use of targeted immunomodulators not only prior to but also post-HSCT. Furthermore, we need to define post-HSCT-specific monitoring and evaluate improvements of disease burden with specific scores. All of the foregoing is necessary to enrich the informed nature of the decision-making in lifelong management of children and adults with these diseases.

Patient perspective and informed consent

Written informed consent was obtained from the individuals for the publication of any potentially identifiable images or data included in this article.

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 authors.

Ethics statement

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

AM-S and ÀD-M: These authors share first authorship; AM-S, ÀD-M, and JT: These authors contributed equally to this work; FF-A and LA: These authors share last authorship. All authors contributed to manuscript revision, read, 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

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References

1. Chan AY, Torgerson TR. Primary immune regulatory disorders: A growing universe of immune dysregulation. *Curr Opin Allergy Clin Immunol* (2020) 20 (6):582–90. doi: 10.1097/ACI.0000000000000689

2. Cepika AM, Sato Y, Liu JMH, Uyeda MJ, Bacchetta R, Roncarolo MG. Tregopathies: Monogenic diseases resulting in regulatory T-cell deficiency. *J Allergy Clin Immunol* (2018) 142(6):1679–95. doi: 10.1016/j.jaci.2018.10.026

3. Alkhairy OK, Abolhassani H, Rezaei N, Fang M, Andersen KK, Chavoshzadeh Z, et al. Spectrum of phenotypes associated with mutations in LRBA. *J Clin Immunol* (2016) 36(1):33–45. doi: 10.1007/s10875-015-0224-7

4. Egg D, Schwab C, Gabrysch A, Arkwright PD, Cheesman E, Giulino-Roth L, et al. Increased risk for malignancies in 131 affected CTLA4 mutation carriers. *Front Immunol* (2018) 9. doi: 10.3389/fimmu.2018.02012

5. Gámez-Díaz L, Seidel MG. Different apples, same tree: Visualizing current biological and clinical insights into CTLA-4 insufficiency and LRBA and DEF6 deficiencies. *Front Pediatr* (2021) 9. doi: 10.3389/fped.2021.662645

6. Gámez-Díaz L, Grimbacher B. Immune checkpoint deficiencies and autoimmune lymphoproliferative syndromes. *BioMed J* (2021) 44(4):400–11. doi: 10.1016/j.bj.2021.04.005

7. Lo B, Fritz JM, Su HC, Uzel G, Jordan MB, Lenardo MJ. CHAI and LATAIE: New genetic diseases of CTLA-4 checkpoint insufficiency. *Blood* (2016) 128 (8):1037–42. doi: 10.1182/blood-2016-04-712612

8. Schwab C, Gabrysch A, Olbrich P, Patiño V, Warnatz K, Wolff D, et al. Phenotype, penetrance, and treatment of 133 cytotoxic T-lymphocyte antigen 4–insufficient subjects. *J Allergy Clin Immunol* (2018) 142(6):1932–46. doi: 10.1016/j.jaci.2018.02.055

9. Kuehn HS, Ouyang W, Lo B, Deenick EK, Niemela JE, Avery DT, et al. Immune dysregulation in human subjects with heterozygous germline mutations in CTLA4. *Sci (1979)* (2014) 345(6204):1623–7. doi: 10.1126/science.1255904

10. Egg D, Rump IC, Mitsuiki N, Rojas-Restrepo J, Maccari ME, Schwab C, et al. Therapeutic options for CTLA-4 insufficiency. *J Allergy Clin Immunol* (2022) 149 (2):736–46. doi: 10.1016/j.jaci.2021.04.039

11. Tesch VK, Abolhassani H, Shadur B, Zobel J, Mareika Y, Sharapova S, et al. Long-term outcome of LRBA deficiency in 76 patients after various treatment modalities as evaluated by the immune deficiency and dysregulation activity (IDDA) score. *J Allergy Clin Immunol* (2020) 145(5):1452–63. doi: 10.1016/j.jaci.2019.12.896

12. Flinn AM, Gennery AR. Primary immune regulatory disorders: Undiagnosed needles in the haystack? *Orphanet J Rare Dis* (2022) 17(1):99. doi: 10.1186/s13023-022-02249-1

13. Seidel MG, Böhm K, Dogu F, Worth A, Thrasher A, Florkin B, et al. Treatment of severe forms of LPS-responsive beige-like anchor protein deficiency with allogeneic hematopoietic stem cell transplantation. *J Allergy Clin Immunol* (2018) 141(2):770–775.e1. doi: 10.1016/j.jaci.2017.04.023

14. Arnold DE, Chellapandian D, Leiding JW. The use of biologic modifiers as a bridge to hematopoietic cell transplantation in primary immune regulatory disorders. *Front Immunol* (2021) 12. doi: 10.3389/fimmu.2021.692219

15. Shaw P, Shizuru J, Hoenig M, Veys P. Conditioning perspectives for primary immunodeficiency stem cell transplants. *Front Pediatr* (2019) 7. doi: 10.3389/fped.2019.00434

16. Deyà-Martínez A, Esteve-Solé A, Vélez-Tirado N, Celis V, Costa J, Cols M, et al. Sirolimus as an alternative treatment in patients with granulomatous-lymphocytic lung disease and humoral immunodeficiency with impaired regulatory T cells. *Pediatr Allergy Immunol* (2018) 29(4):425–32. doi: 10.1111/pai.12890

17. Lankester AC, Albert MH, Booth C, Gennery AR, Güngör T, Hönig M, et al. EBMT/ESID inborn errors working party guidelines for hematopoietic stem cell transplantation for inborn errors of immunity. *Bone Marrow Transplant* (2021) 56 (9):2052–62. doi: 10.1038/s41409-021-01378-8

18. Dhalla F, Lochlainn DJm, Chapel H, Patel SY. Histology of interstitial lung disease in common variable immune deficiency. *Front Immunol* (2020) 11. doi: 10.3389/fimmu.2020.605187

19. Seidel MG, Kindle G, Gathmann B, Quinti I, Buckland M, van Montfrans J, et al. The European society for immunodeficiencies (ESID) registry working definitions for the clinical diagnosis of inborn errors of immunity. *J Allergy Clin Immunol Pract* (2019) 7(6):1763–70. doi: 10.1016/j.jaip.2019.02.004

20. Gennery AR, Slatter MA, Grandin L, Taupin P, Cant AJ, Veys P, et al. Transplantation of hematopoietic stem cells and long-term survival for primary immunodeficiencies in Europe: Entering a new century, do we do better? *J Allergy Clin Immunol* (2010) 126(3):602–610.e11. doi: 10.1016/j.jaci.2010.06.015

21. Neven B, Ferrua F. Hematopoietic stem cell transplantation for combined immunodeficiencies, on behalf of IEWP-EBMT. *Front Pediatr* (2020) 7. doi: 10.3389/fped.2019.00552

22. Marçais A, Mahlaoui N, Neven B, Lanternier F, Catherinot É, Salvator H, et al. Curative allogeneic hematopoietic stem cell transplantation following reduced toxicity conditioning in adults with primary immunodeficiency. *Bone Marrow Transplant* (2022) 57(10):1520–30. doi: 10.1038/s41409-022-01739-x

23. Pai SY, Logan BR, Griffith LM, Buckley RH, Parrott RE, Dvorak CC, et al. Transplantation outcomes for severe combined immunodeficiency, 2000–2009. *New Engl J Med* (2014) 371(5):434–46. doi: 10.1056/nejmoa1401177

24. Chan AY, Leiding JW, Liu X, Logan BR, Burroughs LM, Allenspach EJ, et al. Hematopoietic cell transplantation in patients with primary immune regulatory disorders (PIRD): A primary immune deficiency treatment consortium (PIDTC) survey. *Front Immunol* (2020) 11. doi: 10.3389/fimmu.2020.00239

25. Toubai T, Mathewson ND, Magenau J, Reddy P. Danger signals and graft-versus-host disease: Current understanding and future perspectives. *Front Immunol* (2016) 7. doi: 10.3389/fimmu.2016.00539

26. Ghimire S, Weber D, Mavin E, Wang Xn, Dickinson AM, Holler E. Pathophysiology of GvHD and other HSCT-related major complications. *Front Immunol* (2017) 8. doi: 10.3389/fimmu.2017.00079

27. Fürst D, Neuchel C, Tsamadou C, Schrezenmeier H, Mytilineos J. HLA matching in unrelated stem cell transplantation up to date. *Transfusion Med Hemotherapy* (2019) 46(5):326–36. doi: 10.1159/000502263

28. Cooper MA, Zimmerman O, Nataraj R, Wynn RF. Lifelong immune modulation versus hematopoietic cell therapy for inborn errors of immunity. *J Allergy Clin Immunol Pract* (2021) 9(2):628–39. doi: 10.1016/j.jaip.2020.11.055

29. Slatter MA, Engelhardt KR, Burroughs LM, Arkwright PD, Nademi Z, Skoda-Smith S, et al. Hematopoietic stem cell transplantation for CTLA4 deficiency. *J Allergy Clin Immunol* (2016) 138(2):615–619.e1. doi: 10.1016/j.jaci.2016.01.045

30. Seidel MG, Tesch VK, Yang L, Hauck F, Horn AL, Smolle MA, et al. The immune deficiency and dysregulation activity (IDDA2.1 'Kaleidoscope') score and other clinical measures in inborn errors of immunity. *J Clin Immunol* (2022) 42 (3):484–98. doi: 10.1007/s10875-021-01177-2

31. Moratto D, Giliani S, Bonfim C, Mazzolari E, Fischer A, Ochs HD, et al. Long-term outcome and lineage-specific chimerism in 194 patients with wiskott-Aldrich syndrome treated by hematopoietic cell transplantation in the period 1980-2009: an international collaborative study. *Blood* (2011) 118(6):1675–84. doi: 10.1182/blood-2010-11-319376

32. Booth C, Gaspar HB, Thrasher AJ. Gene therapy for primary immunodeficiency. *Curr Opin Pediatr* (2011) 23(6):659–66. doi: 10.1097/MOP.0b013e32834cd67a

33. Cole T, Pearce MS, Cant AJ, Cale CM, Goldblatt D, Gennery AR. Clinical outcome in children with chronic granulomatous disease managed conservatively or with hematopoietic stem cell transplantation. *J Allergy Clin Immunol* (2013) 132 (5):1150–5. doi: 10.1016/j.jaci.2013.05.031

34. Burns SO, Morris EC. How I use allogeneic HSCT for adults with inborn errors of immunity. *Blood* (2021) 138(18):1666–76. doi: 10.1182/blood.2020008187

35. Güngör T, Teira P, Slatter M, Stussi G, Stepensky P, Moshous D, et al. Reduced-intensity conditioning and HLA-matched haemopoietic stem-cell transplantation in patients with chronic granulomatous disease: A prospective multicentre study. *Lancet* (2014) 383(9915):436–48. doi: 10.1016/s0140-6736(13)62069-3

36. Albert MH, Hauck F, Wiebking V, Aydin S, Notheis G, Koletzko S, et al. Allogeneic stem cell transplantation in adolescents and young adults with primary immunodeficiencies. *J Allergy Clin Immunol Pract* (2018) 6(1):298–301.e2. doi: 10.1016/j.jaip.2017.07.045

37. Verma N, Burns SO, Walker LSK, Sansom DM. Immune deficiency and autoimmunity in patients with CTLA-4 (CD152) mutations. *Clin Exp Immunol* (2017) 190(1):1–7. doi: 10.1111/cei.12997

38. Yang L, Xue X, Chen X, Wu J, Yang X, Xu L, et al. Abatacept is effective in Chinese patients with LRBA and CTLA4 deficiency. *Genes Dis* (2021) 8(5):662–8. doi: 10.1016/j.gendis.2020.03.001

39. Lee S, Moon JS, Lee CR, Kim HE, Baek SM, Hwang S, et al. Abatacept alleviates severe autoimmune symptoms in a patient carrying a *de novo* variant in CTLA-4. *J Allergy Clin Immunol* (2016) 137(1):327–30. doi: 10.1016/j.jaci.2015.08.036

40. Krausz M, Uhlmann A, Rump IC, Ihorst G, Goldacker S, Sogkas G, et al. The ABACHAI clinical trial protocol: Safety and efficacy of abatacept (s.c.) in patients with CTLA-4 insufficiency or LRBA deficiency: A non controlled phase 2 clinical trial. *Contemp Clin Trials Commun* (2022) 30:101008. doi: 10.1016/j.conctc.2022.101008

41. Liesveld JL, Rothberg PG. Mixed chimerism in SCT: conflict or peaceful coexistence? *Bone marrow transplant.* (2008) 42(5):297–310. doi: 10.1038/bmt.2008.212

42. Bakhtiar S, Fekadu J, Seidel MG, Gambineri E. Allogeneic hematopoietic stem cell transplantation for congenital immune dysregulatory disorders. *Front Pediatr* (2019) 7. doi: 10.3389/fped.2019.00461

43. Laberko A, Gennery AR. Clinical considerations in the hematopoietic stem cell transplant management of primary immunodeficiencies. *Expert Rev Clin Immunol* (2018) 14(4):297–306. doi: 10.1080/1744666x.2018.1459189

44. Gennery AR, Lankester A. Long term outcome and immune function after hematopoietic stem cell transplantation for primary immunodeficiency. *Front Pediatrics Front Media S.A* (2019) 7. doi: 10.3389/fped.2019.00381

45. Cavazzana-Calvo M, Carlier F, le Deist F, Morillon E, Taupin P, Gautier D, et al. Long-term T-cell reconstitution after hematopoietic stem-cell transplantation in primary t-cell–immunodeficient patients is associated with myeloid chimerism and possibly the primary disease phenotype. *Blood* (2007) 109(10):4575–81. doi: 10.1182/blood-2006-07-029090

46. Nademi Z, Slatter MA, Dvorak CC, Neven B, Fischer A, Suarez F, et al. Hematopoietic stem cell transplant in patients with activated PI3K delta syndrome. *J Allergy Clin Immunol* (2017) 139(3):1046–9. doi: 10.1016/j.jaci.2016.09.040

47. Seidel MG, Böhm K, Dogu F, Worth A, Thrasher A, Florkin B, et al. Treatment of severe forms of LPS-responsive beige-like anchor protein deficiency with allogeneic hematopoietic stem cell transplantation. *J Allergy Clin Immunol* (2018) 141(2):770–775.e1. doi: 10.1016/j.jaci.2017.04.023

48. Leiding JW, Okada S, Hagin D, Abinun M, Shcherbina A, Balashov DN, et al. Hematopoietic stem cell transplantation in patients with gain-of-function signal transducer and activator of transcription 1 mutations. *J Allergy Clin Immunol* (2018) 141(2):704–717.e5. doi: 10.1016/j.jaci.2017.03.049

49. Hossain MS, Kunter GM, El-Najjar VF, Jaye DL, Al-Kadhimi Z, Taofeek OK, et al. PD-1 and CTLA-4 up regulation on donor T cells is insufficient to prevent GvHD in allo-HSCT recipients. *PloS One* (2017) 12(9):e0184254. doi: 10.1371/journal.pone.0184254

50. Zhu F, Zhong XM, Qiao J, Liu Q, Sun HY, Chen W, et al. Cytotoxic T lymphocyte antigen-4 down-regulates T helper 1 cells by increasing expression of signal transducer and activator of transcription 3 in acute graft-versus-Host disease. *Biol Blood Marrow Transplantation* (2016) 22(2):212–9. doi: 10.1016/j.bbmt.2015.11.003

51. Waters E, Williams C, Kennedy A, Sansom DM. *In vitro* analysis of CTLA-4-Mediated transendocytosis by regulatory T cells. *Methods Mol Biol* (2023) 2559:171–87. doi: 10.1007/978-1-0716-2647-4_12

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Case report: *Pneumocystis jirovecii* pneumonia in a severe case of Aicardi–Goutières syndrome with an *IFIH1* gain-of-function mutation mimicking combined immunodeficiency

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Aicardi–Goutières syndrome (AGS) is a genetically determined early-onset progressive encephalopathy caused by mutations leading to overexpression of type I interferon (IFN) and resulting in various clinical phenotypes. A gain-of-function (GOF) mutation in the *IFIH1* gene is associated with robust production of type I IFN and activation of the Janus kinase (JAK) signal transducer and activator of the transcription (STAT) pathway, which can cause AGS type 7. We detail the clinical case of an infant who initially presented with *Pneumocystis jirovecii* pneumonia (PCP), had recurrent respiratory infections, and was later treated with a JAK inhibitor, baricitinib, because of a genetically confirmed GOF mutation in the *IFIH1* gene. This spectrum of *IFIH1* GOF mutations with overlapping features of hyperinflammation and severe opportunistic infection, which mimics combined immunodeficiency (CID), has not been described before. In this case, therapy with baricitinib effectively blocked IFN-α activation and reduced STAT1 signaling but had no effect on the progression of the neurological disease.

KEYWORDS

Aicardi–Goutières syndrome (AGS), *IFIH1* gene, interferonopathy, Janus kinase inhibitor, combined immune deficiency

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Introduction

Aicardi–Goutières syndrome (AGS) is a genetically heterogeneous disorder originally defined as an early-onset progressive encephalopathy that is characterized by intracranial calcification, white matter abnormalities, cerebral atrophy, cerebrospinal fluid (CSF) lymphocytosis, and inappropriate induction of a type I interferon (IFN)-mediated immune response, belonging to the group of type I interferonopathies (1, 2). Although AGS particularly affects the brain, immune system, and skin in the first year of life, there is a wide spectrum of disease presentations, progression, and outcomes (3). CSF and serum analyses typically exhibit increased type I IFN activity and increased levels of expression of IFN-stimulated genes in peripheral blood, the so-called IFN signature (2, 4).

As more mutations have been identified in different causative genes for AGS, it has become clear that there are significant clinical differences between patients' phenotypes, and clinical variability has been observed even within the same genotype or family (4). In 2014, the pathogenic variant *IFIH1* (IFN induced with helicase C domain-containing protein 1 gene) was identified as the causative gene for AGS type 7 (2, 5) and accounts for only 4% of cases (6).

IFIH1 is an important intracellular sensor for various viruses; by recognizing viral double-stranded RNA, it triggers antiviral IFN responses (7). Mutations of innate immune sensor *IFIH1* are thought to be a predisposing factor for autoimmune diseases and can cause a monogenic form of systemic lupus erythematous similar to genetic overproduction of IFN- α, mutations in upstream components of the classical complement pathway, and apoptosis defects. By producing type I IFN, *IFIH1* enhances responses to its own RNA, thereby activating the adaptive immune system (7–9). The mutation in *IFIH1* may be gain of function (GOF) and can dramatically up-regulate the production of type I IFNs (5, 10, 11). Conversely, loss-of-function (LOF) variants in *IFIH1* result in a deficient antiviral defense. Therefore, it causes a primary immunodeficiency, leading to increased susceptibility to common respiratory RNA viruses (10).

The *IFIH1* GOF mutations are associated with the activation of the Janus kinase (JAK) signal transducer and activator of the transcription (STAT) pathway (5, 12). Recent evidence suggests that JAK inhibitors may be effective in blocking IFN-mediated inflammatory signaling by decreasing STAT1 phosphorylation in patients with AGS (12–15). In addition, there are some indications of possible effects on neuroinflammation and thus improvement in neurological function (12, 13). The IFN signature is an indicator of IFN signaling and might reflect disease activity (12, 13, 16). Furthermore, studies have shown that phosphorylated STAT1 (pSTAT) in T lymphocytes is greatly reduced during therapy (16).

Case report

A male infant was born to unrelated White parents at 38 weeks of gestation by induction of labor because of oligohydramnios and intrauterine growth restriction. Birth weight was 3,050 g, birth length was 50 cm, head circumference was 34.5 cm, and Apgar scores were 9 and 9 at 1 and 5 minutes, respectively. During pregnancy, the mother had confirmed infection with the SARS-CoV-2 virus.

At 13 days of age, the patient was hospitalized for lack of weight gain and dehydration. On admission, he weighed 2,920 g, was afebrile, and had a systolic murmur below the clavicle, respiratory distress, and abnormal neurological signs. A chest radiograph showed interstitial lung infiltrates, and an echocardiogram revealed a patent foramen ovale, ductus arteriosus, and a high level of pulmonary vascular resistance. Blood test analysis revealed leukopenia, neutropenia, thrombocytopenia, and normal inflammatory parameters. He had a negative hemoculture and a nasopharyngeal swab for respiratory viruses. Screenings for congenital infections and autoimmune thrombocytopenia were also negative. Polymerase chain reaction testing for the SARS-CoV-2 virus in the placenta, umbilical blood, and CSF was negative, and the newborn also had no antibodies against SARS-CoV-2.

He had abnormal neurological signs, with lethargy, hypotonia, and an abnormal spontaneous movement pattern. A cranial ultrasound showed subependymal germinolytic cysts and severe vasculopathy. Brain magnetic resonance imaging (MRI) showed diffusely slightly elevated white matter and basal ganglia signal in the T2-weighted sequences (Figure 1); susceptibility-weighted imaging (SWI) showed a hypointensive signal from slightly more prominent vessels in the basal ganglia areas.

Owing to the clinical presentation with abnormal neurological signs and neuroimaging findings, systemic inflammatory disease was suspected. Laboratory testing for interferonopathy was performed in the virology laboratory at the Hôpital Cochin, Paris, France, and showed increased IFN-α concentrations in serum (18 IU/ml) and in CSF (50 IU/ml). Whole-exome sequencing confirmed a heterozygous GOF mutation in exon 11, c.2159G>A (p.Arg720Gln), in the *IFIH1* gene (NM_022168.4). The mutation occurred *de novo* and had previously been reported in six individuals with AGS (5, 17, 18).

The patient had elevated serum immunoglobulins (Ig), with a level of IgG of 6.62 g/l, IgA of 1.32 g/l, and IgM of 1.75 g/l. He was found to have a markedly reduced concentration of B cells, T helper (Th) cells, T cytotoxic cells, and naïve CD4 + T cells. The percentages of Th1 and Th2 cells were also reduced. The percentages of activated T cells (HLA DR+) and Treg cells were markedly increased. The concentration of natural killer (NK) cells and recent thymic emigrants (RTEs)

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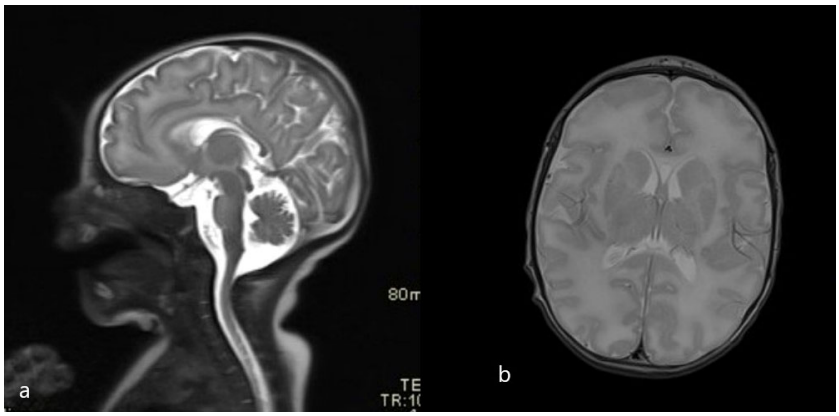

FIGURE 1
Initial brain MRI scans (T2 sagittal and axial plane). **(A)** Normal thickness of pons and midbrain. **(A, B)** Higher T2 signal of the frontal white matter.

(the marker of thymic T cell production) was normal. In addition, a T-cell proliferation test (CD3/CD28 stimulated T cells) was normal.

At 2.5 months of age, the patient underwent hernioplasty of the right inguinal hernia. After surgery, he presented shortness of breath. A chest radiograph showed interstitial infiltrates on the right side. Laboratory results showed mild elevation of inflammatory parameters, lactate dehydrogenases, and significant positive β-D-glucans. A nasopharyngeal swab was positive for rhinovirus and *Pneumocystis jirovecii*. *P. jirovecii* pneumonia (PCP) was confirmed by bronchoscopy, and antibiotic treatment with trimethoprim–sulfamethoxazole was initiated. Because of recurrent mucocutaneous candidiasis, he also received systemic antifungal therapy.

After the resolution of PCP at the age of 3.5 months, the patient was started on the JAK inhibitor baricitinib at a dosage of 0.1 mg/kg/day. Functional studies of STAT signaling (16) before initiation of baricitinib therapy revealed increased total STAT1 expression in monocytes and lymphocytes including CD4+ T cells, and increased levels of pSTAT after stimulation with IFN-α. Follow-up studies after 10 months of therapy with baricitinib revealed persistently increased total STAT1 protein levels, and there was a reduction in the level of pSTAT1 expression after stimulation with IFN-α (Figure 2).

At 4.5 months of age, the patient was treated for diarrhea caused by *Campylobacter jejuni* infection. At 5 months of age, he underwent an emergency right hemicolectomy owing to ischemia of the terminal ileum and ascending colon, and later

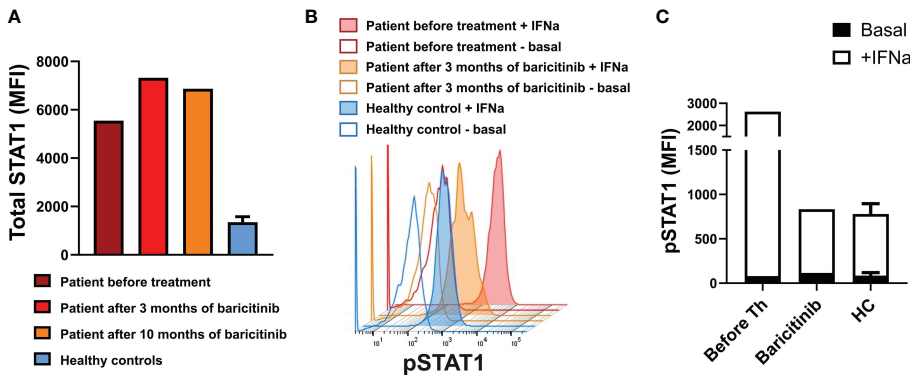

FIGURE 2
(A): Total STAT1 levels in CD4+ T lymphocytes from the patient on indicated time points and healthy controls. **(B)** Phosphorylated STAT1 (pSTAT1) levels in unstimulated whole-blood CD4+ T lymphocytes and on interferon (IFN)-α induction. **(C)** Basal (unstimulated) pSTAT1 levels and pSTAT1 levels on stimulation with IFN-α for 15 minutes in CD4+ T lymphocytes from the patient before treatment and after 3 months of baricitinib, and from healthy controls. Values in **(A)** and **(C)** for healthy controls (n=3) are the mean ± SD. MFI, median fluorescence intensity; HC, healthy controls.

underwent two relaparotomies owing to dehiscence of the ileotransverse anastomosis.

During the first year, he experienced recurrent viral respiratory infections: rhinovirus, bocavirus, coronavirus, enterovirus, and respiratory syncytial virus were detected. An increase in liver enzymes was noted during respiratory infections, which was partially attenuated by continuing baricitinib therapy during the infections.

After 1 year most immune parameters reached normal levels: concentrations of B cells, T helper cells, T cytotoxic cells, and naive CD4+ T cells and percentage of Th1 and Th2 cells. Only the percentage of Treg cells remained increased.

The neuroimaging results and abnormal neurological signs pointed us to an early diagnosis and initiation of treatment. At a neurological assessment at 3 months of age, the child presented with axial hypotonia and increased muscle tone of the extremities. Treatment with tiagabine and B vitamins was initiated, together with baricitinib. A neurological evaluation at 6 months of age showed decreased growth in head circumference, poor eye contact, and limb spasticity, with occasional dystonia. The patient was unable to hold up his head, and spontaneous movements were poorly expressed, without meaningful coordination. Further neurological follow-ups showed the developmental delay to be severe. A brain MRI scan at 12 months of age showed marked atrophy of the deep and subcortical white matter of both hemispheres, with enlarged ventricles, sulci, and subarachnoid spaces. The brainstem and cerebellar peduncles were also greatly reduced in volume. In the reduced basal nuclei, signs of calcifications were reported (Figure 3**).** At the age of 1.5 years, global developmental delay was identified, with intellectual disability and without any signs of speech development. Epileptic seizures with twitching in the left arm and short absences had occurred. An electroencephalogram (EEG) showed multifocal epileptiform discharges. Levetiracetam was introduced. Feeding problems became more frequent and, consequently, a percutaneous gastrostoma was introduced. The child and his family were also receiving follow-up treatment from the pediatric palliative care team.

Discussion

We have detailed the case of an infant with early-diagnosed AGS type 7 with genetically confirmed heterozygous GOF mutation in the *IFIH1* gene who initially presented neurological manifestations as well as *P. jirovecii* pneumonia (PCP) resembling combined immunodeficiency (CID). The patient was later treated with a Janus kinase (JAK) inhibitor, baricitinib, which reduced STAT1 signaling but had no effect on the progression of the neurological disease.

The initial clinical presentation of our patient was suggestive of prenatal onset of the disease and raised suspicion of interferonopathies. In a large case series of AGS patients, only 11.4% of infants had abnormal neurological signs at birth without obvious systemic signs, and others developed symptoms later in life, usually within the first year. Notably, individuals with an *IFIH1* mutation had normal development in the first year of life and often did not present until after 1 year (4). Rice et al. reported that clinical presentation at or shortly after birth is associated with a severe AGS phenotype, suggesting prenatal onset of the disease (17).

A heterozygous c.2159G>A (p.Arg720Gln) mutation in the *IFIH1* gene (NM_022168.4) has been previously identified in six individuals with a severe AGS phenotype and its functional effect has been demonstrated by IFN scoring (2, 5, 17, 18). Rice et al. reported two patients with intrauterine growth restriction and thrombocytopenia at birth who had severe developmental delay.

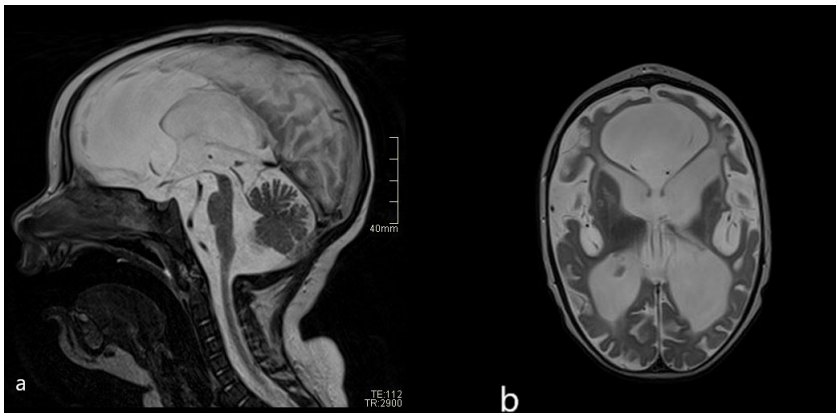

FIGURE 3
(A) Follow-up brain MRI scans (T2 sagittal and axial plane) after 1 year: severe atrophy of midbrain compared with initial MRI. **(B)** Severe reduction of brain volume and abnormal signal of the white matter—leucoencephalopathy.

MRI at 8 and 12 months of age showed cerebral atrophy with calcifications in the basal ganglia and white matter disease. The first patient developed seizures at 13 months of age and died of pneumonia at 2 years of age. The second patient had hypertrophic cardiomyopathy and nephrotic syndrome diagnosed at 7 and 10 months of age, respectively (5). Subsequently, Adang et al. described a male patient with the same mutation, who also presented in the neonatal period with hepatosplenomegaly and severe thrombocytopenia, had profound developmental delay, and developed severe pulmonary hypertension by the age of 2 years (18). Moreover, other studies have reported various amino acid substitutions in *IFIH1* mutation-positive patients (3, 11, 18–22). Recent studies suggest that IFN status assessment is a reliable disease marker (13, 17), and a positive correlation has been found between IFN activity in CSF measured within 1 year of disease onset and the degree of subsequent disability (4). In this case, analysis of CSF and serum showed increased IFN type I and an enhanced IFN signature.

P. jirovecii is an opportunistic microbial pathogen that is particularly threatening in immunocompromised individuals and can cause PCP (23). To our knowledge, PCP with a mutation in *IFIH1* has not previously been reported. Our patient also had persistent mucocutaneous candidiasis and numerous recurrent respiratory viral infections as clinical signs of immunodeficiency. The deficient antiviral defense has been described only in *IFIH1* LOF mutations (10), but even LOF mutations are not associated with opportunistic infections. CID-like presentation with PCP and severe T-cell lymphopenia was previously reported in two patients with stimulator of interferon genes (STING)-associated vasculopathy with onset in infancy (SAVI), which is also a type I interferonopathy and is caused by a GOF mutation in the transmembrane protein 173 gene (*TMEM173*) encoding STING (24, 25). Based on the disease course in our case, it appears that patients with AGS with the *IFIH1* GOF mutation may present similarly to patients with CID, with opportunistic infections associated with autoimmune and hyperinflammatory manifestations.

Previous studies have shown that JAK inhibitors can effectively block the activation of IFNs in patients with AGS (13), especially in patients with *IFIH1*-related disease (12). IFN signature and STAT1 phosphorylation in T lymphocytes could serve as indicators of response to treatment (12, 13, 16). In our patient, we started treatment with baricitinib, a JAK1 and JAK2 inhibitor, in the first months after birth, using the recommended dosage (13). During treatment, we noted a significant functional improvement, a significant reduction in the IFN signature, and a reduction in pSTAT in peripheral blood T lymphocytes. Studies of STAT signaling in our patient were performed as previously reported (16), including analysis of total STAT1 expression in T cells at different time points without technical repeats.

Some recent studies have reported improvement of neurological functions after JAK inhibitor therapy, even in patients with severe and prolonged disease (13, 26), suggesting that treatment in the earliest stages of the disease could lead to important clinical gains (27). By contrast, our patient experienced severe progression of encephalopathy in spite of early diagnosis and treatment with baricitinib, which was confirmed by neuroimaging at 12 months (28, 29). Our findings indicate that JAK inhibitors effectively blocked the systemic IFN-mediated inflammatory response but had little or no effect on the brain disease. Based on the functional studies of STAT signaling, our data suggest that the measurement of STAT1 phosphorylation can be used as a useful monitoring tool for adjusting the dosage of baricitinib. No common adverse effects were reported (16), and treatment was well tolerated by our patient.

Conclusion

Our case highlights the phenotypic heterogeneity of AGS. PCP and recurrent respiratory infections have not previously been described as part of the clinical spectrum associated with a GOF mutation in the *IFIH1* gene. This case shows that AGS with GOF mutation in the *IFIH1* gene could mimic CID with opportunistic infections associated with autoimmune and hyperinflammatory manifestations. In our experience, baricitinib effectively blocks IFN-α activation, but even early treatment with a JAK inhibitor failed to halt the progression of neurological manifestations.

Data availability statement

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

Ethics statement

Ethics review and approval were not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin. Written informed consent was obtained from the minor(s)' legal guardian/next of kin for the publication of any potentially identifiable images or data included in this article.

Author contributions

MŽ, ASŠ, NŠ, and TA contributed to patient care and treatment. MŽ, ASŠ, and TA wrote the manuscript and reviewed the literature. DK contributed to interpreting and

describing the imaging findings. MD performed genetic evaluation. AG and AI contributed to immunological evaluation and functional testing. All authors contributed to diagnostic procedure, manuscript revision, and read, 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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References

1. Aicardi J, Goutières F. A progressive familial encephalopathy in infancy with calcifications of the basal ganglia and chronic cerebrospinal fluid lymphocytosis. *Ann Neurol* (1984) 15:49–54. doi: 10.1002/ana.410150109

2. Oda H, Nakagawa K, Abe J, Awaya T, Funabiki M, Hijikata A, et al. Aicardi-goutières syndrome is caused by IFIH1 mutations. *Am J Hum Genet* (2014) 95 (1):121–5. doi: 10.1016/j.ajhg.2014.06.007

3. Crow YJ, Zaki MS, Abdel-Hamid M, Boesp O, Cordeiro NJV, Gleeson JG, et al. Mutations in ADAR1 , IFIH1 , and RNASEH2B presenting as spastic paraplegia. *Neuropediatrics* (2014) 45(6):386–93. doi: 10.1055/s-0034-1389161

4. Crow YJ, Chase DS, Lowenstein Schmidt J, Szynkiewicz M, Forte G, Gornall HL, et al. Characterization of human disease phenotypes associated with mutations in TREX1 , RNASEH2A, RNASEH2B, RNASEH2C, SAMHD1. ADAR, and IFIH1. *Am J Med Genet Part A* (2015) 167A(2):296–312. doi: 10.1002/ajmg.a.36887

5. Rice GI, Del Toro Duany Y, Jenkinson EM, Forte GMA, Anderson BH, Ariaudo G, et al. Gain-of-function mutations in IFIH1 cause a spectrum of human disease phenotypes associated with upregulated type i interferon signaling. *Nat Genet* (2014) 46(5):503–9. doi: 10.1038/ng.2933

6. Al Mutairi F, Alfadhel M, Nashabat M, El-Hattab AW, Ben-Omran T, Hertecant J, et al. Phenotypic and molecular spectrum of aicardi-goutières syndrome: A study of 24 patients. *Pediatr Neurol* (2018) 78:35–40. doi: 10.1016/j.pediatrneurol.2017.09.002

7. Funabiki M, Kato H, Miyachi Y, Toki H, Motegi H, Inoue M, et al. Autoimmune disorders associated with gain of function of the intracellular sensor MDA5. *Immunity* (2014) 40(2):199–212. doi: 10.1016/j.immuni.2013.12.014

8. Robinson T, Kariuki SN, Franek BS, Kumabe M, Kumar AA, Badaracco M, et al. Autoimmune disease risk variant of IFIH1 is associated with increased sensitivity to IFN-α and serologic autoimmunity in lupus patients. *J Immunol* (2011) 187(3):1298–303. doi: 10.4049/jimmunol.1100857

9. Gorman JA, Hundhausen C, Errett JS, Stone AE, Allenspach EJ, Ge Y, et al. The A946T variant of the RNA sensor IFIH1 mediates an interferon program that limits viral infection but increases the risk for autoimmunity. *Nat Immunol* (2017) 18(7):744–52. doi: 10.1038/ni.3766

10. Asgari S, Schlapbach LJ, Anchisi S, Hammer C, Bartha I, Junier T, et al. Severe viral respiratory infections in children with IFIH1 loss-of-function mutations. *Proc Natl Acad Sci U.S.A.* (2017) 114(31):8342–7. doi: 10.1073/pnas.1704259114

11. Amari S, Tsukamoto K, Ishiguro A, Yanagi K, Kaname T, Ito Y. An extremely severe case of aicardi-goutières syndrome 7 with a novel variant in IFIH1. *Eur J Med Genet* (2020) 63(2):103646. doi: 10.1016/j.ejmg.2019.04.003

12. Kothur K, Bandodkar S, Chu S, Wienholt L, Johnson A, Barclay P, et al. An open-label trial of JAK 1/2 blockade in progressive IFIH1-associated neuroinflammation. *Neurology* (2018) 90(6):289–91. doi: 10.1212/WNL.0000000000004921

13. Vanderver A, Adang L, Gavazzi F, McDonald K, Helman G. Janus kinase inhibition in the aicardi–goutières syndrome. *N Engl J Med* (2020) 383(10):986–9. doi: 10.1056/NEJMc2001362

14. Casas-Alba D, Darling A, Caballero E, Mensa-Vilaro A. Efficacy of baricitinib on chronic pericardial effusion in a patient with aicardi–goutieres syndrome. *Rheumatology* (2021) 61(4):e87–9. doi: 10.1093/rheumatology/keab860

15. Sanchez GAM, Reinhardt A, Ramsey S, Wittkowski H, PJ H, Berkun Y, et al. JAK1/2 inhibition with baricitinib in the treatment of autoinflammatory interferonopathies. *J Clin Invest* (2018) 128(7):3041–52. doi: 10.1172/JCI98814

16. Meesilpavikkai K, Dik WA, Schrijver B, van Helden-Meeuwsen CG, Versnel MA, van Hagen PM, et al. Efficacy of baricitinib in the treatment of chilblains associated with aicardi-goutières syndrome, a type I interferonopathy. *Arthritis Rheumatol* (2019) 71(5):829–31. doi: 10.1002/art.40805

17. Rice GI, Park S, Gavazzi F, Adang LA, Ayuk LA, Van Eyck L, et al. Genetic and phenotypic spectrum associated with IFIH1 gain-of-function. *Hum Mutat* (2020) 41(4):837–49. doi: 10.1002/humu.23975

18. Adang LA, Frank DB, Gilani A, Takanohashi A, Ulrick N, Collins A, et al. Aicardi goutières syndrome is associated with pulmonary hypertension. *Mol Genet Metab* (2018) 125(4):351–8. doi: 10.1016/j.ymgme.2018.09.004

19. Marguet F, Laquerrière A, Goldenberg A, Guerrot AM, Quenez O, Flahaut P, et al. Clinical and pathologic features of aicardi-goutières syndrome due to an IFIH1 mutation: A pediatric case report. *Am J Med Genet Part A* (2016) 170 (5):1317–24. doi: 10.1002/ajmg.a.37577

20. Zheng S, Lee PY, Wang J, Wang S, Huang Q, Huang Y, et al. Interstitial lung disease and psoriasis in a child with aicardi-goutières syndrome. *Front Immunol* (2020) 11:985. doi: 10.3389/fimmu.2020.00985

21. Van Eyck L, De Somer L, Pombal D, Bornschein S, de Zegher F, Liston A, et al. Gain-of-function mutation in IFIH1 can cause both aicardi-goutières syndrome and systemic lupus erythematosus with IgA-deficiency. *Pediatr Rheumatol Online J* (2014) 12(Supp1):P309. doi: 10.1186/1546-0096-12-S1-P309

22. Rutsch F, Macdougall M, Lu C, Buers I, Mamaeva O, Nitschke Y, et al. A specific IFIH1 gain-of-function mutation causes singleton-merten syndrome. *Am J Hum Genet* (2015) 96(2):275–82. doi: 10.1016/j.ajhg.2014.12.014

23. Boillat L, Angelini F, Crucis-Armengaud A, Asner SA, Rochat I. Pneumocystis jirovecii pneumonia in an infant: The tip of the iceberg. *Clin Pediatr (Phila)* (2019) 58(5):578–81. doi: 10.1177/0009922818825141

24. Saldanha RG, Balka KR, Davidson S, Wainstein BK, Wong M, Macintosh R, et al. A mutation outside the dimerization domain causing atypical STING-associated vasculopathy with onset in infancy. *Front Immunol* (2018) 9:1–8. doi: 10.3389/fimmu.2018.01535

25. Frémond ML, Hadchouel A, Berteloot L, Melki I, Bresson V, Barnabei L, et al. Overview of STING-associated vasculopathy with onset in infancy (SAVI) among 21 patients. *J Allergy Clin Immunol Pract* (2021) 9(2):803–18.e11. doi: 10.1016/j.jaip.2020.11.007

26. Tüngler V, Doebler-Neumann M, Salandin M, Kaufmann P, Wolf C, Lucas N, et al. Aicardi-goutières syndrome due to a paternal mosaic IFIH1 mutation. *Neurol Genet* (2020) 6(1):384. doi: 10.1212/NXG.0000000000000384

27. Crow YJ, Shetty J, Livingston JH. Treatments in aicardi–goutières syndrome. *Dev Med Child Neurol* (2020) 62(1):42–7. doi: 10.1111/dmcn.14268

28. La Piana R, Uggetti C, Roncarolo F, Vanderver A, Olivieri I, Tonduti D, et al. Neuroradiologic patterns and novel imaging findings in aicardi-goutières syndrome. *Neurology* (2016) 86(1):28–35. doi: 10.1212/WNL.0000000000002228

29. Vanderver A, Prust M, Kadom N, Demarest S, Crow YJ, Helman G, et al. Early-onset aicardi-goutières syndrome: Magnetic resonance imaging (MRI) pattern recognition. *J Child Neurol* (2015) 30(10):1343–8. doi: 10.1177/0883073814562252

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National experience with adenosine deaminase deficiency related SCID in Polish children

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Introduction: Deficiency of adenosine deaminase (ADA) manifests as severe combined immunodeficiency (SCID), caused by accumulation of toxic purine degradation by-products. Untreated patients develop immune and non-immune symptoms with fatal clinical course. According to ESID and EBMT recommendations enzyme replacement therapy (ERT) should be implemented as soon as possible to stabilize the patient's general condition, normalize transaminases, treat pulmonary proteinosis, bone dysplasia, and protect from neurological damage. Hematopoietic stem cell transplantation (HSCT) from a matched related donor (MRD) is a treatment of choice. In absence of such donor, gene therapy (GT) should be considered. HSCT from a matched unrelated donor (MUD) and haploidentical hematopoietic stem cell transplantation (hHSCT) are associated with worse prognosis.

Material and methods: We retrospectively evaluated the clinical course and results of biochemical, immunological and genetic tests of 7 patients diagnosed in Poland with ADA deficiency since 2010 to 2022.

Results: All patients demonstrated lymphopenia affecting of T, B and NK cells. Diagnosis was made on the basis of ADA activity in red blood cells and/or genetic testing. Patients manifested with various non-immunological symptoms including: lung proteinosis, skeletal dysplasia, liver dysfunction, atypical hemolytic-uremic syndrome, and psychomotor development disorders. Five patients underwent successful HSCT: 3 patients from matched unrelated donor, 2 from matched sibling donor, and 1 haploidentical from a parental donor. In 4

patients HSCT was preceded by enzyme therapy (lasting from 2 to 5 months). One patient with multiple organ failure died shortly after admission, before the diagnosis was confirmed. None of the patients had undergone gene therapy.

Conclusions: It is important to diagnose ADA SCID as early as possible, before irreversible multi-organ failure occurs. In Poland HSCT are performed according to international immunological societies recommendations, while ERT and GT are less accessible. Implementation of Newborn Screening (NBS) for SCID in Poland could enable recognition of SCID, including ADA-SCID.

KEYWORDS

adenosine deaminase (ADA) deficiency, SCID - severe combined immunodeficiency, ERT (enzyme replacement therapy), HSCT = hematopoietic stem cell transplant, lymphopenia

Introduction

Adenosine deaminase (ADA) deficiency is a rare inherited disorder of purine metabolism leading to life threatening severe combined immunodeficiency (SCID), otherwise known as ADA-SCID (1). The disease is inherited in an autosomal recessive manner, with incidence 1:200 000 - 1: 1 million births, and accounts for 10-20% of all SCID cases. The accumulation of deoxyadenosine, a metabolic substrate of ADA enzyme, causes abnormalities in immune cells development and functions and has systemic impact, mostly on skeletal development (costochondral abnormalities, skeletal dysplasia), cerebral (cognitive and behavioral defects), as well as hepatic and pulmonary functions (1, 2).

Most affected infants present with severe opportunistic infections, failure to thrive and developmental delay during their first 6 months of life. Undiagnosed or left untreated children die in their first year of life.

The hematopoietic stem cell transplantation (HSCT) from matched related donor (MRD) represents a successful treatment option with high survival rates and excellent immune reconstitution, and is an accepted treatment of choice. Two other therapeutic options are available: enzyme replacement therapy (ERT) and gene therapy (GT) (1).

This study reviews the diagnostic and therapeutic approach to ADA-SCID.

Materials and methods

This study included 7 Caucasian children 2 girls and 5 boys, now aged from 6 months to 13 years, diagnosed with ADA-SCID in Poland since 2010 to early 2022. Five patients were diagnosed in the Department of Immunology, Children's Memorial Health Institute (CMHI), Warsaw, two others – in the Department and Clinic of Pediatric Oncology, Haematology and Bone Marrow Transplantation, Wroclaw Medical University, Wroclaw, Poland and in the Department of Paediatrics, Haematology and Oncology, Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University in Torun, respectively. We retrospectively evaluated the clinical course and results of biochemical, immunological, and genetic tests leading to the diagnosis of ADA-SCID. Basic hematological, biochemistry, and immunological results, including immunoglobulin level, lymphocyte proliferation test, and flow cytometry results were recorded.

Adenosine deaminase (ADA) activity was measured in patients' erythrocyte lysate and expressed in nmol/h/mg Hb (3).

Molecular diagnostics involved whole gene direct Sanger sequencing in 3 cases, and next-generation sequencing (NGS) for primary immunodeficiency gene panel with confirmatory Sanger sequencing to targeted region in 4 patients.

Evaluation of lymphocyte subsets was performed using commercially available BD Multitest six color cocktails of antibodies and Trucount tubes (Becton Dickinson, cat. no. 644611). The assessed lymphocyte subset panel included T cells (CD3+/CD45+), cytotoxic T cells (CD3+CD8+/CD45+), helper T cells (CD3+CD4+/CD45+), NK cells (CD16+CD56+CD3-/CD45+), and B cells (CD19+/CD45+). Their distribution and absolute cell counts were determined using the lyse-no-wash approach according to manufacturer's instructions (4). The absolute number of individual subsets was calculated based on the proportion of the respective cell subpopulation and absolute lymphocyte count.

ERT was carried out in 4 patients in the Department of Immunology, Children's Memorial Health Institute, Warsaw. One patient received polyethylene glycol-conjugated bovine ADA, intramuscularly at a weekly dose of 20 U/kg and 3 patients were treated with elapegademase, at a dose 0.4 mg/kg a week in 1-2 doses. Six patients underwent HSCT in three Polish centers.

The study was performed in accordance with the Declaration of Helsinki on Biomedical Research involving Human subjects. The study was approved by the Bioethics Committee at the Children's Memorial Health Institute (Decision No. 21/KBE/2022).

Results

The diagnosis of ADA SCID was made in all 7 patients at the median age of 4.5 months (ranged 7 weeks to 27 months). None of the patients had positive family history or was detected in the Polish-German transborder area "RareScreen" newborn screening project (5). According to available data between 2010 and February 2022 SCID was diagnosed in 64 patients in Poland. The prevalence of ADA-SCID among all SCID was 10.9%. The first symptoms appeared during the first month of life in 4 patients (57%). Pneumonia was observed in all patients, in 4 of them during first months of life (2 weeks to 3 months of age), while in 3 others after the first year of life. Lymphopenia was diagnosed in 2 patients (28.5%) and failure to thrive in 2 other children (28.5%). Only 2 patients (P6, P7) demonstrated skeletal abnormalities on the chest X-ray as scapular spurring with flaring and cupping of rib ends (Figure 1).

Elevated transaminases were observed in total in 5 patients (71%), in 3 of them at diagnosis (43%). In patient P5, transaminase activity was slightly elevated already at the time of diagnosis, but significant increase in the value of transaminases (Alanine Aminotransferase - ALT 331 U/l, Aspartate Aminotransferase - AST 902 U/l) was observed while waiting for the ERT.

Delay in diagnosis ranged from 1.5 to 15 months (Table 1). Chronologically, first 3 patients were diagnosed after first year of life and the next 4 -in the first 6 months of life (Table 1)]. In patient P3, SCID was not suspected until at the age of 15 months due to severe nephrological symptoms i.e. atypical hemolytic uremic syndrome (aHUS) and arterial hypertension. At the time of the diagnosis the patient demonstrated multiple opportunistic infections (cytomegalovirus infection, *Pneumocystis jiroveci* and *Absidia* pneumonia) and advanced atrophic changes in the Central Nervous System (CNS) found in the brain magnetic resonance imaging. He died prior to establishing ADA-SCID diagnosis and was not treated with ERT or HSCT (6).

We confirmed viral infections in 4 patients (57%): 2 had cytomegaloviral infection, 1 respiratory syncytial virus (RSV), and 1 adenoviral (ADV) infection, 1 varicella zoster virus (VZV), and herpes simplex (HSV) (Table 1). Fungal infections were suspected in 4 more patients apart from patient 3. In 3 patients *Candida* infection was suspected due to the presence of fungal metabolites in serum (mannan or beta-D-glukan), in 1 patient *Aspergillius* (galactomannan in the serum), and in 2 patients

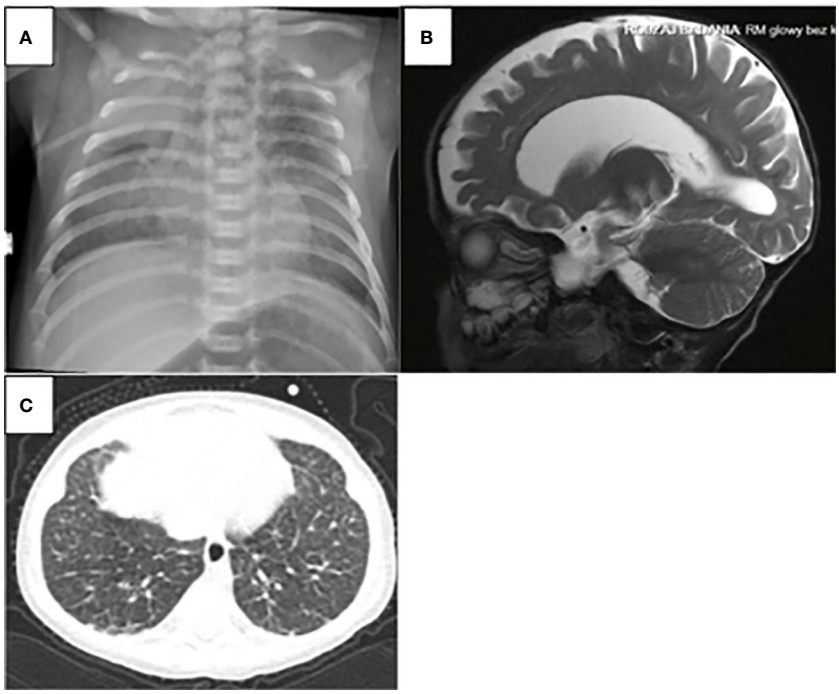

FIGURE 1
ADA-SCID sypmtoms: **(A)** Chest X-ray: scapular spurring with flaring and cupping of rid ends in the patient 6. **(B)** atrophic changes in the CNS were found in the brain magnetic in the patient 3 **(C)** computed tomography of the chest as ground-glass opacities with interlobular and intralobular interstitial thickening in patient 5.

TABLE 1 Age of onset of symptoms and diagnosis.

Pt	Sex	Lymphopenia	Neutropenia	Hypertransaminasemia	Pneumonia	Failure to thrive	Hepatomegaly	Bone dysplasia	Infections	Age at SCID diagnosis	Age at ADA diagnosis
P1	F	15 mo	13 mo	15 mo	6 mo	15 mo	No	No	No	13 mo	21 mo
P2	M	15 mo	15mo	15 mo	2x	x	16 mo	No	2.5y CMV, Asp	27 mo	27 mo
P3	M	14 mo	15 mo	15 mo	15 mo	since birth	15 mo	No	3 mo, VZV 9 mo HSV 15 mo CMV, PJP	15 mo	16 mo
P4	M	2.5 mo	2.5 mo	2,5 mo	3 mo	since birth	6 mo	No	No	3 mo	6 mo
P5	F	7 w	7 w	7 w	7 w	2 m	No	No	Local BCG 5 mo Can	2 mo	3 mo
P6	M	2 w	2 w	3 w	2 w	no	No	2 mo: X-chest	Local BCG 2 mo RSV, Can	7 w	8 w
P7	M	5 w	5 w	7 w	5w	no	No	2 mo: X-chest	5 w PJP, ADV Local BCG 3 mo, Can	6 w	7 w

ADA, adenosine deaminase; ADV, adenovirus; Asp, Aspergillus spp; BCG, Bacille Calmette-Guerin; Can, Candida spp; CMV, cytomegalovirus; F, female; HSV, herpes simplex virus; M, male; Mo, months; PJP, Pneumocystis jiroveci pneumonia; RSV, respiratory syncytial virus; Pt, patient; SCID, severe combined immunodeficiency; w, weeks; VZV, Varicella Zoster Virus.

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Pneumocystis jiroveci infection (positive specific IgG, chest x-ray image) (Table 1). Local Bacillus-Calmette-Guerin (BCG) complication was observed in 3 patients. In 2 of them local inflammation at the site of vaccination without lymphadenopathy developed between 3rd day and 4th week after initiation of ERT preliminary. In P5 the first signs of BCG complication were observed after 4 weeks of ERT and disappeared after starting treatment with two tuberculostatic drugs: isoniazid and rifampicin (Figure 2).

Laboratory tests showed lymphopenia in all patients, affecting subpopulations of T, B and NK cells (Table 2).

The diagnosis was made by measurement of ADA activity in 4 patients and in 6 patients confirmed by genetic testing. ADA activity in red blood cells (RBC) was very low in 3 patients (<1%) and low (4.9%) in one patient, who was tested 5 days after RBC transfusion (Table 2).

Genetic tests revealed the presence of 7 mutations in 6 patients. The most common variant (c.302G> A) was detected in 3 patients: in P3 and P4 as homozygous mutation while in P5 as heterozygous. The next common one (c.956_960delAAGAG) was found in 2 patients (P5, P7) in heterozygous genotype. In the rest of patients other variants were found (Table 2). In patient P2 no mutation was found, but testing was done only by Sanger.

ERT was administered to 4 patients for the period of 6 to 23 weeks with normalization of the lymphocyte count and transaminases activity (Figure 3).

HSCT was performed during the period of 2010-2022 in 6 patients: 2 MSD, 3 MUD, 1 haploidentical parental donor. All patients are alive at the time of preparing of this manuscript. The conditioning regimens in 5 cases were used in accordance with successive ESID recommendations. In two cases the regimen included treosulfan, fludarabine, and thiotepa.

None of the patients was referred for gene therapy (GT), which is available only in two centers across Europe. According to Polish regulations, the patient should obtain an agreement of the Ministry of Health for that treatment. Among five patients of CMHI one had a MSD, one died before any treatment and, only one agreed to undergo GT, however the parents eventually refused, due to complicated family situation. Two other patients decided to go for HSCT.

Discussion

The diagnosis of ADA SCID was established after typical symptoms appeared in the first months of life. The reported patients most frequently suffered from dyspnea with radiographical pneumonia, unresponsive to conventional treatment and leukopenia. Leukopenia and dependence on oxygen therapy stimulated the diagnostic work up for a primary immunodeficiency.

Pulmonary manifestation was demonstrated on imaging studies as ground-glass opacities with interlobular and intralobular interstitial thickening (Figure 1). These findings might be associated with infections, but they also may result from pulmonary alveolar proteinosis (PAP) frequently observed in the patients with ADA deficiency. Lung biopsy or bronchoalveolar lavage that could confirm pulmonary proteinosis were not performed, but improvement was observed with ERT treatment, with subsequent disappearance of dyspnea or weaning of oxygen therapy in two patients (P5, P6). According to the literature, reverse of PAP is observed after enzyme replacement therapy or after transplantation (7).

Although skeletal manifestations of ADA SCID, like scapular squaring and spurring and cupping of the costochondral junctions were not the first manifestation in Polish patients, they were observed in 2 patients after establishment of the diagnosis (8). Hypertransaminasemia was observed in the of patients. Patient P5 demonstrated significant increase in the value of transaminases during the period preceding initiation of ERT. Considering that hepatic failure can be rapid in patients with ADA-SCID, urgent treatment was

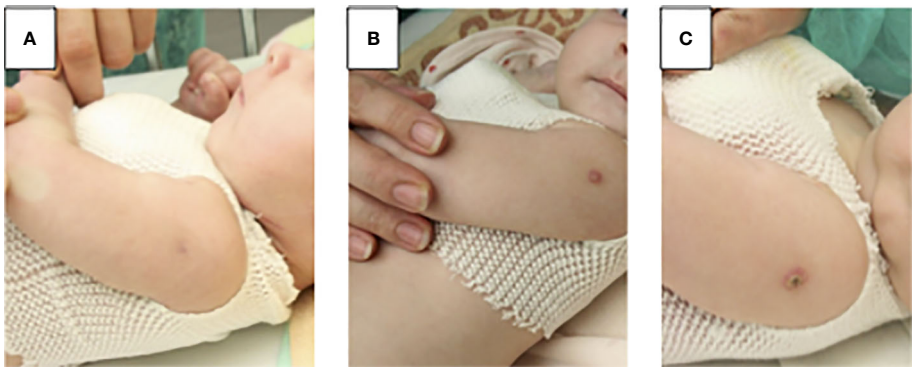

FIGURE 2
Local BCG disease in patient 5: **(A)** before enzyme replacement therapy (ERT) normal BCG vaccination scar **(B)** 4 weeks of ERT, at the site of the BCG injection small spot appeared and turned into a blister, treatment with two tuberculostatic drugs: isoniazid and rifampicin has started **(C)** 8 weeks of ERT, a crusty scrab formed, which healed into a small scar.

TABLE 2 Laboratory and clinical data at diagnosis.

Pt	WBC (K/µl)	ALC (K/µl)	AST (U/l)	ALT (U/l)	IgG (g/l)	IgA (g/l)	IgM (g/l)	CD3 (cells/ml)	CD4 (cells/µl)	CD8 (cells/µl)	CD19 (cells/µl)	CD16+56+CD3- (cells/µl)	RTE (%)	ADA activity (nmol/mg Hb/h)	Gene defect
P1	2,64 (L)	0.21 (L)	85 (H)	59 (H)	3.39 (N)	0.08 (N)	0.315 (N)	65 (L)	42 (L)	21 (L)	14 (L)	70 (L)	Nd	N	c.80T>A(;)646G>A p.Ile27Asn(;) Gly216Arg
P2	2 (L)	0.58 (L)	180 (H)	141 (H)	3.84 (L)	0.41 (N)	0.39 (N)	714 (L)	107 (L)	618 (L)	24 (L)	25 (L)	5.4 (L)	0.63 (L)	Not detected
P3	1.9 (L)	0.34 (L)	56 (H)	21 (N)	4.75 (N)	<0.06 (L)	<0.04 (L)	50 (L)	1 (L)	49 (L)	1 (L)	11 (L)	0 (L)	Nd	homozygous mutation c.302G>A p.Arg101Gln
P4	1.56 (L)	0.17 (L)	225 (H)	38 (N)	3.9 (N)	0.4 (N)	1.11 (N)	39 (L)	33 (L)	2 (L)	42 (L)	17 (L)		Nd	homozygous mutation c.302G>A p.Arg101Gln
P5	1.7 (L)	0.25 (L)	133 (H)	51 (N)	6.92 (H)	0.36 (N)	1.32 (H)	158 (L)	71 (L)	73 (L)	3 (L)	64 (L)	9.8 (L)	0.109 (L)	c.302G>A(;) 956_960delAAGAG p.Arg101Gln(;) Glu319Glyfs
P6	3.12 (L)	0.14 (L)	67 (N)	31 (N)	8.54 (4 days IVIG)	0.07 (N)	<0.04 (L)	35 (L)	31 (L)	4 (L)	2 (L)	5 (L)	0 (L)	4.9 (5 days after transfusion) (L)	c.363-3C>G(;) 778G>A p.(?)(;)Glu260Lys
P7	1.22 (L)	0.04 (L)	67 (N)	29 (N)	2.1 (L)	<0.07 (N)	0.08 (L)	27 (L)	20 (L)	7 (L)	3 (L)	22 (L)	0 (L)	0 (L)	c.956_960del(;)del ex 2: chr20: 44636227-44636288 p.Glu319Glyfs(;)

ADA, adenosine deaminase; ALC , absolute lymphocyte count; ALT, alanine transaminase; AST, aspartate aminotransferase; CD, cluster of differentiation; IgA, immunoglobulin A; IgG, immunoglobulin G; IgM, immunoglobulin M; L, below norm; N, normal; Nd, not done; H, above normal; RTE, Recent Thymic Emigrants: CD31+CD45RA+/CD3+CD4+; WBC, white blood cells.

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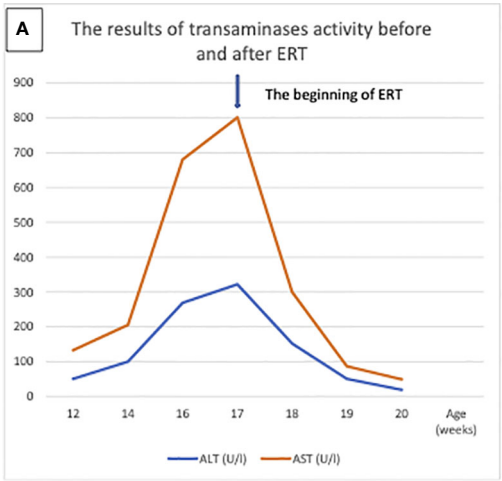

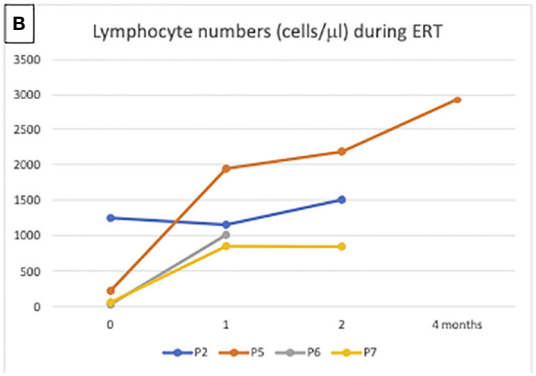

FIGURE 3
Enzyme replacement therapy (ERT): **(A)** Aminotransferases normalization in patient 5 after ERT, the arrow indicates the beginning of treatment, **(B)** Lymphocyte numbers in patient P2, P5, P6 and P7 after 1, 2 and 4 months ERT.

required (9). RBC transfusions, intended to provide the child temporarily with external source of ADA enzyme, and reduction of antibiotic prophylaxis were not effective in this patient. Significant improvement was achieved after initiation of ERT (Figure 3).

One of the reported patients (P2) showed mild phenotype with lung and liver function abnormalities but no life-threatening infections. Symptoms in this patient related to recurrent pneumonia showed milder course than in patients diagnosed with SCID, but hepatosplenomegaly, and hypertransaminasemia were present, and the child was consulted by pulmonologist and gastroenterologist before final diagnosis was established. Diagnosis of leaky ADA-SCID in patient P2 was based on criteria published by Shearer et al. (10), defined as lymphopenia CD3 T cells below 1000 cells/µl for the age up to 2 years, absence of maternal engraftment, and less than 30% of lower limit of normal T cell function (as measured by response to phytohemagglutinin (PHA). Delay in the diagnosis was 12 months from first symptoms, which emphasizes the need for multi-specialist teams taking care of children with rare diseases.

Another challenge in the reported group, was to initiate the right diagnostic approach in patients with atypical symptoms. In patient P3, recurrent diarrhea from the first month of life, failure to thrive and delayed psychomotor development were not sufficiently indicative of IEI. Even infections with VZV (at 3 months) and HSV (at 9 months) were not associated with immunodeficiency. Atypical hemolytic uremic syndrome (aHUS), respiratory failure, requirement for peritoneal dialysis and plasmapheresis, finally led to initiation of the diagnostic procedure for an inborn error of immunity. At the age of 15 months, when the diagnosis of SCID was finally established, the patient had already signs of multiple organ failure (MOF), advanced atrophic changes in the CNS, severe arterial hypertension, cytomegalovirus infection, and *Pneumocystis jiroveci* and *Absidia* pneumonia. Diagnosis of ADA-SCID was established *post mortem* (6).

Another issue that needs addressing here, is the post-BCG vaccination disease in an IEI patient (11). In Poland, vaccination against tuberculosis is obligatory in the first two days of life in all newborns, unless they have contraindications. For this purpose, locally-produced BCG Moreau strain vaccine is used, a descendant of the Brasilian BCG Moreau substrain with superior clinical safety profile. Serious complications in IEI patients are only observed in SCID, especially associated with low NK cell numbers, and patients with Mendelian susceptibility to mycobacterial diseases (MSMD). NK cells are thought to have a protective role probably due to IFN-γ production (11). However, none of our patients with ADA-SCID demonstrated a disseminated BCG disease, with local manifestations observed in total in 3 patients, one before ERT and two after initiation of ERT and with an increase in the number of lymphocytes, treated with antimycobacterial double therapy (isoniazid, rifampicin) (Figure 2; Table 3).

Currently, ADA-SCID diagnostics in Poland is quite easily accessible and not expensive. In cases when typical clinical manifestations are accompanied by poor flow cytometry results suggesting ADA-SCID diagnosis, the activity of the ADA enzyme in red blood cells is evaluated, and results confirmed by molecular methods. Before 2010, for various reasons the delay in the diagnostic process was far too long, leading frequently severe complications as in case of first patients from the described group, diagnosed at the age between 16 and 27 months, or fatal outcome without diagnosis. Therefore, the exact number of ADA SCID patients in Poland before 2010 remains unknown. The prevalence of ADA-SCID among all SCID diagnosed between 2010 and 2022 was 10.9%. According to the Statistics Poland reports during this period 50-88 infants' deaths were noted yearly due to different infections (Archive on the website: https://stat.gov.pl/en/topics/statistical-yearbooks/statistical-yearbooks/demographic-yearbook-of-poland-

TABLE 3 ERT and HSCT characteristics and outcomes.

Pt	Age at ERT onset	ERT duration (weeks)	Age at HSCT (mo)	Donor	Type of conditioning	CD34 +/kgx10^6	Clinical complications	Post-HSCT follow up (month)	Last lymphocyte value (K/ul)	Last engraftment (CD3, CD19, PMN)	BCG treatment
P1	n.a.	n.a.	16	MSD	none	6.55	None	140	2860	1897/261/4480	n.a.
P2	28 mo	12	33	MSD	Bu-Flu	4.28	hypertensive crisis, FUO, CMV	131	2350	1746/413/1450	n.a.
P3	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
P4	no	no	7	MUD	myeloablative, toxicity reduced (TREO+FLUD)	4.04	no	72	1900	1362/218/3750	n.a.
P5	4 mo	23	9	MUD	BuxFlu	4.6	mucositis III stage, hypertension	46	4600	chimerism 12-2021 - 88%	INH+RMP
P6	11 w	6	4	Hapl	Treo-flu-tiotepa	18.72	mucositis aGVHD st.2, autoimmune hemolytic anemia, patient retransplanted from the same donor (08.2022)	18		n/a	INH+RMP
P7	10 w	9	4,5	MUD	Treo-flu-tiotepa		aGVHD stage 2, autoimmune hemolytic anemia	3	600	475/0/2370	INH+RMP

aGVHD, acute graft versus host disease; BCG, Bacille Calmette-Guerin; CD, cluster of differentiation; CMV, cytomegalovirus; ERT, enzyme replacement therapy; FUO, fever of unknown origin; HSCT, hematopoietic stem cell transplantation; INH, isoniazid (isonicotinic acid hydrazide); mo, months; MSD, matched sibling donor; MUD, matched unrelated donor; n.a., not applicable; RMP, rifampicin; w, weeks.

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2021,3,15.html). It can't be excluded that some undiagnosed SCID patients were among them.

The newborn screening program for SCID could enable identification of such infants. In Polish-German transborder NBS study (TREC and KREC assays) among 101 012 tested newborns (37 025 from Poland and 63 987 in Germany) two cases of atypical SCID/CID (both in Germany), two cases of autosomal recessive agammaglobulinemia (homozygous mutations in IGLL1 gene – one in Germany, one in Poland), one case of Nijmegen breakage syndrome (in Poland) were diagnosed. No ADA-SCID patient was detected, probably because of small group of newborns.

Standard curative option of ADA-SCID treatment, HSCT from MSD, is offered in Poland for children with HLA-matched sibling as a first choice (12). Based on data on HSCT performed between 1981 and 2009 in 16 centers worldwide, an overall survival rate (OS) in ADA-SCID depends on type of HSCT, being 86% for MSD, 81% for MFD, 66% for MUD and 43 for Haplo (13). Recent studies on large groups of patients: 131 ADA-SCID diagnosed in 1982-2017 (14) and 152 patients with SCID, including 43 ADA-SCID treated in 2006-2014 (15), showed higher OS in patients transplanted from MSD/MFD under 3.5 months of age and without active infections. According to the European Society for Immunodeficiencies (ESID), the European Society for Blood and Marrow Transplantation (EBMT), and European Reference Network on Rare Primary Immunodeficiency Autoinflammatory Autoimmune diseases (RITA), gene therapy should be considered in absence of a matched family donor (16). This therapeutic modality does not require donor search and it is associated with lower incidence of complications, including absence of graft versus host disease (GvHD) and procedure-related mortality. Limited availability and lack of reimbursement are the major obstacles for GT in Poland - it is available in only very few centers in Europe, but not in Poland, and the cost is extreme. Besides, longer duration of the procedure and time to achieve immune reconstitution are important clinical drawbacks. Frequency of therapy failure is similar: 10-20% in MSD HSCT and 5-20% in GT, but procedure-related mortality is 5.6 and 0% respectively (12). Some authors recommend considering GT as a first-line treatment, even if MSD is available, as this procedure abrogates any risk of alloreactivity (17). However, since treosulfan-based conditioning was introduced results for MUD, and Haplo HSCT are comparable with MSD in some centers and therefore recommended when there is no access to GT (18). GT is not readily available in Poland due to lack of specialized treatment center, as well as high cost of the procedure and need to run ERT until GT is carried out.

ERT is an effective treatment that has been used for over 3 decades. It should be implemented as soon as possible to stabilize the patient's general condition, detoxify the organism from accumulating purine metabolites, normalize transaminases, as well as treat pulmonary alveolar proteinosis and bone dysplasia. ERT is recommended only as an interim therapy before curative treatment, such as GT or HSCT, even if such therapy is available to improve patient's condition and prevent ongoing neuro- and hepatotoxicity (9). Recommended ERT duration ranges from few months to around 2 years, as ERT is associated with several adverse effects, including development of malignancies (often EBV-related lymphomas, mostly after 8-10 years of treatment), and ultimate failure due induction of enzyme neutralizing antibodies in about 10% of patients (1). An increase in B cell numbers is evident within the first month of treatment, and in T lymphocytes after 2-4 months, as was seen in our patients (19).

Costs of the drug and lack of reimbursement due to lack of drug registration in Poland and the European Union are the major obstacles in conducting ERT. As a consequence shortages in availability of ERT have been recorded in the past resulting in limited number of patients treated for short time.

Conclusions

Despite low numbers of ADA-SCID patients diagnosed in Poland, an advancement in early diagnostics was observed due to an increase in physicians' experience and improved availability of molecular diagnostics. Diagnosis in younger patients gives a chance for more effective treatment and prevention of irreversible damage.

ERT was effective in the analyzed cases, but high costs greatly limit access to the drug. Patients have restricted access to novel methods such as gene therapy, due to high cost and limitation to only few treatment centers.

Implementation of NBS for SCID in Poland could enable recognition of SCID, including ADA-SCID.

Data availability statement

The original contributions presented in the study are included in the article/supplementary materials. Further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by Bioethics Committee at the Children's Memorial Health Institute. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

ND-L, MP designed the concept of the manuscript. ND-L, BP, NB, MU, MP wrote the manuscript with contribution from all co-authors. BP did the immunological studies. ES did the adenosine deaminase activity. ND-L, KB-S, NB, EB, BW-K, KK, SK, AD, JG, MU, MP contributed to clinical data collection and

critical review. All authors contributed to the article and approved the submitted version of the manuscript.

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

References

1. Gaspar HB, Aiuti A, Porta F, Candotti F, Hershfield MS, Notarangelo LD. How I treat ADA deficiency. *Blood* (2009) 114:3524–32. doi: 10.1182/blood-2009-06-189209

2. Whitmore KV, Gaspar HB. Adenosine deaminase deficiency – more than just an immunodeficiency. *Front Immunol* (2016) 7. doi: 10.3389/fimmu.2016.00314

3. Smolenski RT, Lachno DR, Ledingham SJ, Yacoub MH. Determination of sixteen nucleotides, nucleosides and bases using high-performance liquid chromatography and its application to the study of purine metabolism in hearts for transplantation. *J Chromatogr B Biomed Sci Appl* (1990) 527:414–20. doi: 10.1016/S0378-4347(00)82125-8

4. Piątosa B, Wolska-Kusnierz B, Siewiera K, Grzduk H, Gałkowska E BE, Bernatowska E. Distribution of leukocyte and lymphocyte subsets in peripheral blood. age related normal values for preliminary evaluation of the immune status in polish children. *Centr Eur J Immunol* (2010) 35:168–75.

5. Giżewska M, Durda K, Winter T, Ostrowska I, Ołtarzewski M , Klein J, et al. Newborn screening for SCID and other severe primary immunodeficiency in the polish-German transborder area: Experience from the first 14 months of collaboration. *Front Immunol* (2020) 11:1948. doi: 10.3389/fimmu.2020.01948

6. Bogdał A, Badeński A, Pac M, Wójcicka A, Badeńska M, Didyk A, et al. Atypical hemolytic uremic syndrome (aHUS) and adenosine deaminase (ADA)-deficient severe combined immunodeficiency (SCID)–two diseases that exacerbate each other: Case report. *Int J Mol Sci* (2021) 22:9479. doi: 10.3390/ijms22179479

7. Grunebaum E, Cutz E, Roifman CM. Pulmonary alveolar proteinosis in patients with adenosine deaminase deficiency. *J Allergy Clin Immunol* (2012) 129:1588–93. doi: 10.1016/j.jaci.2012.02.003

8. Verhagen MV, Trevisan V, Adu J, Owens CM, Booth C, Calder A. Chest radiographs for distinguishing ADA-SCID from other forms of SCID. *J Clin Immunol* (2020) 40:259–66. doi: 10.1007/s10875-019-00733-1

9. Kühl JS, Schwarz K, Münch A, Schmugge M, Pekrun A, Meisel C, et al. Hyperbilirubinemia and rapid fatal hepatic failure in severe combined immunodeficiency caused by adenosine deaminase deficiency (ADA-SCID). *Klin Padiatr.* (2011) 223(2):85–9. doi: 10.1055/s-0030-1269916

10. Shearer WT, Dunn E, Notarangelo LD, Dvorak CC, Puck JM, Logan BR, et al. Establishing diagnostic criteria for severe combined immunodeficiency disease (SCID), leaky SCID, and omenn syndrome: The primary immune deficiency treatment consortium experience. *J Allergy Clin Immunol* (2014) 133:1092–8. doi: 10.1016/j.jaci.2013.09.044

11. Bernatowska E, Pac M, Heropolitańska-Pliszka E, Pietrucha B, Dąbrowska-Leonik N, Skomska-Pawliszak M, et al. BCG Moreau polish substrain infections in patients with inborn errors of immunity: 40 years of experience in the department of immunology, children's memorial health institute, Warsaw. *Front Pediatr* (2022) 10. doi: 10.3389/fped.2022.839111

12. Kohn DB, Hershfield MS, Puck JM, Aiuti A, Blincoe A, Gaspar HB, et al. Consensus approach for the management of severe combined immune deficiency caused by adenosine deaminase deficiency. *J Allergy Clin Immunol* (2019) 143:852–63. doi: 10.1016/j.jaci.2018.08.024

13. Hassan A, Booth C, Brightwell A, Allwood Z, Veys P, Rao K, et al. Outcome of hematopoietic stem cell transplantation for adenosine deaminase–deficient severe combined immunodeficiency. *Blood* (2012) 120:3615–24. doi: 10.1182/blood-2011-12-396879

14. Cuvelier GDE, Logan BR, Prockop SE, Buckley RH, Kuo CY, Griffith LM, et al. Outcomes following treatment for ADA-deficient severe combined immunodeficiency: A report from the PIDTC. *Blood.* (2022) 140(7):685–705. doi: 10.1182/blood.2022016196

15. Lankester AC, Neven B, Mahlaoui N, von Asmuth EGJ, Courteille V, Alligon M, et al. Hematopoietic cell transplantation in severe combined immunodeficiency: The SCETIDE 2006-2014 European cohort. *J Allergy Clin Immunol* (2022) 149(5):1744–1754.e8. doi: 10.1016/j.jaci.2021.10.017

16. Lankester AC, Albert MH, Booth C, Gennery AR, Güngör T, Hönig M, et al. EBMT/ESID inborn errors working party guidelines for hematopoietic stem cell transplantation for inborn errors of immunity. *Bone Marrow Transplant.* (2021) 56:2052–62. doi: 10.1038/s41409-021-01378-8

17. Kreins AY, Velasco HF, Cheong KN, Rao K, Veys P, Worth A, et al. Long-term immune recovery after hematopoietic stem cell transplantation for ADA deficiency: a single-center experience. *J Clin Immunol* (2022) 42(1):94–107. doi: 10.1007/s10875-021-01145-w

18. Ghimenton E, Flinn A, Lum SH, Leahy TR, Nademi Z, Owens S, et al. Hematopoietic cell transplantation for adenosine deaminase severe combined immunodeficiency-improved outcomes in the modern era. *J Clin Immunol* (2022) 42(4):819–26. doi: 10.1007/s10875-022-01238-0

19. Weinberg K, Hershfield MS, Bastian J, Kohn D, Sender L, Parkman R, et al. T Lymphocyte ontogeny in adenosine deaminase-deficient severe combined immune deficiency after treatment with polyethylene glycol-modified adenosine deaminase. *J Clin Invest.* (1993) 92:596–602. doi: 10.1172/JCI116626

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Subjective sleep quality and fatigue assessment in Polish adult patients with primary immunodeficiencies: A pilot study

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Introduction: Primary immunodeficiencies (PIDs) are clinically heterogeneous disorders caused by abnormalities in the immune system. However, PIDs are genetically determined and may occur at any age from early childhood to elderly age. Due to chronic patterns, the risk of malignancy and organ damage in patients with PIDs may affect any aspect of life, including sleep patterns. To our knowledge, the prevalence of insomnia and subjective sleep quality have not been investigated in patients with PIDs. Therefore, this pilot study was conducted to investigate sleep quality, the prevalence of sleep disturbances, and fatigue in adult patients with PIDs in Poland.

Methods: All participants were surveyed using the Athens Insomnia Scale, Pittsburgh Sleep Quality Index, Fatigue Severity Scale, and a questionnaire concerning general health and demographic data. We included 92 participants: 48 women (52.2%) and 44 men (47.8%).

Results: Participants' mean age was 41.9 ± 13.9 years. The mean sleep duration was 7.0 ± 1.5 hours, and the mean sleep latency was 41.2 ± 53.1 minutes. Additionally, 44.6% of patients (n=41) had symptoms of insomnia and 44.6% (n=42) had poor sleep quality. Less than one-fourth (n=22; 23.9%) of the patients reported the use of sleeping pills; moreover, clinically significant fatigue was reported in 52.2% (n=48).

Discussion: Our investigation provides insight into the problem of sleep disturbances in patients with PIDs. Data have demonstrated that sleeping

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disorders with concomitant fatigue are common in patients with PID. Further studies are needed to determine the determinants of poor sleep quality in this specific group of patients.

KEYWORDS

sleep quality, fatigue assessment, primary immunodeficiencies, heterogeneous disorders, polish, inborn errors of immunity

1 Introduction

Primary immunodeficiencies (PIDs) are clinically heterogeneous group of disorders caused by abnormalities in the innate immune system. The onset of the disease occurs mostly in childhood; however, the initial symptoms may appear at any age (1). Based on national registers, the prevalence of symptomatic PIDs varies from 1:8500 to 1:100000 (2).

Clinical manifestations of PIDs include recurrent bacterial infections of the upper and lower respiratory and gastrointestinal tracts, as well as meningitis, arthritis, and skin and organ abscesses (3). Infections are characterized by a severe course and cannot always be treated with standard medications (4). As a consequence of recurrent respiratory system infections, patients develop bronchiectasis, chronic obstructive pulmonary disease (COPD), and interstitial lung disease (5).Viral infections of the respiratory tract, gastrointestinal tract, and skin are also common (6).

Furthermore, genetic defects lead to atopy, multi-organ autoimmunization, lymphoproliferation, and vulnerability to neoplastic and autoinflammatory diseases (1). The symptoms of autoimmunization may precede the occurrence of infection (7, 8). Due to chronic course, the risk of malignancy, and organ damage, PIDs may affect any aspect of patients' lives, including sleep patterns and quality of life.

Sleep is essential to humans. Sleep provides physical restoration (9), promotes memory consolidation (10), and maintains proper function of the immune system (11); however, its exact role remains unknown. The International Classification of Sleep Disorders has identified 7 major categories of sleep disorders: insomnia disorders, sleep-related breathing disorders, central disorders of hypersomnolence, circadian rhythm sleep-wake disorders, sleep-related movement disorders, parasomnias, and other sleep disorders (12).

Fatigue is tiredness or weakness experienced by healthy individuals in certain situations and resolve with resting. Aggravated fatigue that limits daily functioning is considered a deviation from the norm. When fatigue lasts more than 6 months, it is referred to as chronic fatigue, with prevalence in the general population varying from 13 to 30% (13, 14). It is more common in patients with chronic diseases, with the highest prevalence in those with autoimmune disorders (15). Although the association between various chronic diseases and fatigue has been highlighted in many studies, the underlying mechanism remains unclear.

Various sleep disorders have been investigated in chronic diseases, including rheumatic diseases (16), autoimmune diseases (15), lung diseases (17), and cardiovascular diseases (18). However, to the best of our knowledge, the prevalence of insomnia and subjective sleep quality have not been investigated in patients with PIDs. To fill this gap, we conducted a pilot study focusing on sleep quality and prevalence of insomnia in patients with PID.

2 Materials and methods

2.1 Study design

This pilot study investigated sleep characteristics, the prevalence of insomnia, subjective sleep quality, and fatigue in adult patients with PIDs in Poland. The study was conducted from February 2021 to February 2022 at 4 Polish clinical centers in Bydgoszcz, Gdańsk, Kraków, and Warszawa. The inclusion criteria were as follows: age ≥18 years, diagnosis of PIDs according to the diagnostic criteria of the European Society for Immunodeficiencies (19), and written consent. Patients who did not meet the inclusion criteria, did not agree to participate in the study, or did not complete their questionnaires were excluded from the study (Figure 1).

Data were collected using questionnaires. All participants were surveyed using the following scales and questionnaires: the Athens Insomnia Scale (AIS), Pittsburgh Sleep Quality Index (PSQI), and Fatigue Severity Scale (FSS). The survey included demographic questions to collect data on age, sex, work, residential status, comorbidities, PID-related factors, and type of immunoglobulin replacement therapy. Additionally, we assessed anxiety and depression using the Hospital Anxiety and Depression Scale (HADS).

2.2 Athens insomnia scale

The AIS is a self-report questionnaire used to assess the severity of insomnia based on the diagnostic criteria of the International Classification of Diseases (ICD), Tenth Revision. It comprises 8 items with a score of 0 to 3 for each item, where 0 indicates no problem at all and 3 indicates a very serious problem (20). According to the authors of the AIS, the cut-off value for insomnia is 6 points; however, some researchers use 8 points as the cut-off value. According to the validation of the Polish version of the AIS, we assumed that a global score ≥8 indicated insomnia. The psychometric properties of the Polish version of the AIS are highly satisfactory (Cronbach's alpha, 0.90) (21). In our study, Cronbach's alpha was 0.879.

2.3 Pittsburgh sleep quality index

The PSQI is used to measure self-reported sleep quality and sleep disturbances over the previous month. Nineteen items were evaluated with a score of 0–3, and they constituted 7 components: subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medication, and daytime dysfunction. The global score (range from 0 to 21), which constitutes the sum of the scores for the 7 components, indicates sleep quality. A global score ≤5 is associated with good sleep quality, whereas a global score >5 is associated with poor sleep quality (22). Internal consistency measured with Cronbach's alpha was 0.803.

2.4 Fatigue severity scale

The FSS is a self-reported 9-item questionnaire for measuring fatigue. Each item is evaluated with scores ranging from 1 to 7, where 1 corresponds to strong disagreement and 7 corresponds to strong agreement. The mean score of the items was used as the FSS score, with a score ≥4 indicating fatigue (23). Psychometric properties of the Polish version of the FSS were satisfactory (Cronbach's alpha, 0.915)

2.5 Hospital anxiety and depression scale

The HADS comprises 14 questions, 7 for each subscale, rated using a 0 to 3 response Likert scale (24). The maximum score for each subscale is 21 points. The cut-off value for moderate anxiety or depression is ≥8, while that for severe depressive or anxiety symptoms is ≥11 points. Scores below 8 indicate a normal result (25).

2.6 Statistical analysis

The normality of the observed values was tested using the Shapiro–Wilk test. Continuous variables were analyzed using the Student t-, Mann–Whitney U, and Kruskal–Wallis tests. Categorical variables were analyzed using the chi-square or Fisher exact test. Data were also assessed using Pearson or Spearman correlation analysis to estimate correlations between the variables. Multiple linear regression analyses were performed to investigate the predictors of AIS total score, FSS total score, and PSQI total score. For all data analyses, differences were considered statistically significant at $p<0.05$. Statistical analysis was performed using STATISTICA software (version 13; TIBCO Software Inc., Palo Alto, CA, USA).

3 Results

3.1 Study population characteristics

A total of 106 individuals took part in the baseline assessment. Eight participants refused to participate. Six individuals were excluded because they did not complete the questionnaire. Finally, the study included 92 participants: 48 women (52.2%) and 44 men (47.8%) (Figure 1). Participants' mean age was 41.9 ± 13.9 years. Most patients lived in cities (n=66; 71.7%). Almost half of the participants had higher education (n=43, 46.7%), 37.0% (n=34) had secondary education, and 16.3% (n=15) had primary or vocational education. Sixty patients (65.3%) had regular employment.

Common variable immunodeficiency (CVID) was the most frequent PID in our study group (n=47; 51.1%). Immunoglobulin G subclass deficiency affected 18.5% of patients (n=17), X-linked agammaglobulinemia affected 9.8% of patients (n=9), and other humoral immunodeficiencies affected 16.3% (n=15). We categorized the remaining 4.3% of patients (n=4) as having other PIDs. Most patients received immunoglobulin replacement therapy (n=87; 94.4%). In this group, subcutaneous immunoglobulins were administered to 85.1% of patients (n=74), and intravenous immunoglobulins were administered to 14.9% (n=13). The mean diagnostic delay of PIDs was 7.2 ± 10.8 years.

The majority of patients (66.3%; n=61) had comorbidities. Seventeen patients (27.9%) had one chronic disease, 15 (24.6%) had two chronic diseases, and 29 (47.5%) had three chronic diseases. Neoplastic disease affected 12 patients (13.0%). Two patients underwent ongoing cancer treatment. The remaining 10 patients were survivors of cancer. Among them, 7 patients had a history of lymphoma, and 3 other patients had histories of lung cancer, thyroid cancer, and carcinoid. We also assessed

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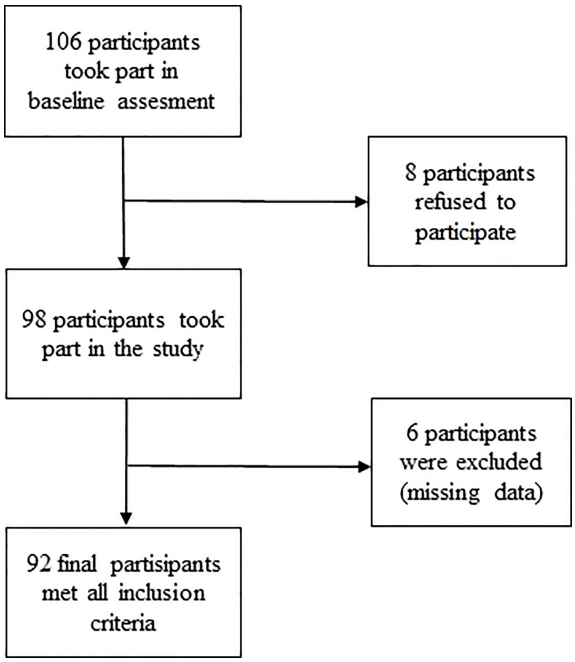

FIGURE 1
Flow chart of patients' selection.

additional factors that could have influenced participants' sleep patterns. Depression was declared as a comorbidity by 7.6% of participants (n=7). A stressful event in the last 3 months was declared by 54.3% (n=50) and was mainly negative (n=42; 84.0%). General pain was present in 75 patients (81.5%), with daily occurrence in the last 3 months in 29 of them (38.7%). Only 17 patients (18.5%) did not report pain in the last 3 months. Addictions were declared by 15.2% (n=14) of patients, of whom 12 had a nicotine addiction, 2 patients were addicted to medications, and none declared a narcotic addiction.

TABLE 1 Components of PSQI (0 – 3 p.).

Variable	Mean ± SD
Sleep efficiency	0.6 ± 0.9
Duration of sleep	0.6 ± 0.9
Sleep latency	1.4 ± 1.0
Sleep disturbance	1.3 ± 0.6
Overall sleep quality	1.3 ± 0.8
Need meds to sleep	0.5 ± 1.0
Day dysfunction due to sleepiness	1.0 ± 0.9
PSQI Total Score (0 – 21 p.)	**6.4 ± 4.1**
Sleep latency (min)	41.2 ± 53.1
Sleep duration (h)	7.0 ± 1.5

PSQI, Pittsburgh Sleep Quality Index; SD, standard deviation; min, minutes; h, hours; p, point.

3.2 Subjective sleep quality and symptoms of insomnia

The mean sleep duration declared by patients was 7.0 ± 1.5 hours (range, 3–11 hours), and approximately one-third of them slept <7 hours (n=30; 32.6%). Sleep latency lasted ≤30 minutes in

TABLE 2 Components of AIS (0 – 3 p.).

Variable	Mean ± SD
Sleep induction	1.4 ± 1.1
Awakenings during the night	1.4 ± 0.8
Final awakening earlier than desired	0.9 ± 0.9
Overall sleep duration	0.8 ± 0.9
Overall quality of sleep	0.9 ± 0.9
Sense of well-being during the day	0.81 ± 0.9
Functioning suring the day	0.7 ± 0.7
Sleepiness during the day	1.1 ± 0.7
AIS total score (0 – 24 p.)	**7.9 ± 5.2**

AIS, Athens Insomnia Scale; SD, Standard deviation; p, point.

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TABLE 3 Comparison of patients with good or poor sleep quality (PSQI), absence or presence of insomnia symptoms, and absence or presence of fatigue symptoms according to sociodemographic data.

	Sleep quality (PSQI)		Insomnia symptoms (AIS)		Fatigue (FSS)	
	good	poor	absence	presence	absence	presence
number (%)	50 (54.3%)	42 (45.7%)	51 (55.4%)	41 (44.6%)	44 (47.8%)	48 (52.2%)
Age (mean ± SD)	39.7 ± 12.8	44.6 ± 14.8	41.6 ± 13.4	42.3 ± 14.6	39.7 ± 13.5	43.9 ± 14.0
Sex, number (%)						
Female	20 (41.7)	28 (58.3)*	22 (45.8)	26 (54.2)	18 (37.5)	30 (62.5)
Male	30 (68.2)	14 (31.8)*	29 (65.9)	15 (34.1)	26 (59.1)	18 (40.9)
Education n (%)						
Primary or vocational	9 (60.0)	6 (40.0)	9 (60.0)	6 (40.0)	9 (60.0)	6 (40.0)
Higher	24 (55.8)	19 (44.2)	22 (51.2)	21 (48.8)	18 (41.9)	25 (58.1)
Secondary	17 (50.0)	17 (50.0)	20 (58.8)	14 (41.2)	17 (50.0)	17 (50.0)
Work status n (%)						
Unemployed	0 (0.0)	4 (100.0)	1 (25.0)	3 (75.0)	0 (0.0)	4 (100.0)
Retiree	2 (33.3)	4 (66.7)	2 (33.3)	4 (66.7)	2 (33.3)	4 (66.7)
Physical worker	8 (72.7)	3 (27.3)	7 (63.6)	4 (36.4)	9 (81.8)	2 (18.2)
Office-worker	22 (53.7)	19 (46.3)	24 (58.5)	17 (41.5)	20 (48.8)	21 (51.2)
Annuitant	13 (59.1)	9 (40.9)	13 (59.1)	9 (40.9)	9 (40.9)	13 (59.1)
Student	5 (62.5)	3 (37.5)	4 (50.0)	4 (50.0)	4 (50.0)	4 (50.0)
Residential status n (%)						
Village	18 (69.2)	8 (30.8)	18 (69.2)	8 (30.8)	14 (53.8)	12 (46.2)
City ≤ 50 000 habitants	6 (35.3)	11 (64.7)	6 (35.3)	11 (64.7)	6 (35.3)	11 (64.7)
City 50 000 -100 000 habitants	9 (69.2)	4 (30.8)	9 (69.2)	4 (30.8)	7 (53.8)	6 (46.2)
City ≥100 000 habitants	17 (47.2)	19 (52.8)	18 (50.0)	18 (50.0)	17 (47.2)	19 (52.8)

PSQI, Pittsburgh Sleep Quality Index; SD, standard deviation; AIS, Athens Insomnia Scale; FSS, Fatigue Severity Scale; BMI (kg/m^2), body mass index; *p <.05.

68 patients (73.0%) with a mean value of 41.2 ± 53.1 minutes (range, 2–300 minutes) (Table 1). Almost half of the patients had an AIS total score ≥8 (n=41; 44.6%) and PSQI total score >5 (n=42; 45.7%). The mean AIS was 7.9 ± 5.2 (Table 2), and the mean PSQI was 6.4 ± 4.1.

Participants' mean age was comparable between those with and without insomnia (42.3 ± 14.6 and 41.6 ± 13.4, p=0.827, respectively). Patients with poor sleep quality were older (44.6 ± 14.8) than those with good sleep quality (39.7 ± 12.8); however, the difference was not statistically significant (p=0.089).

Women had poor sleep quality more frequently (n=28; 58.3%) than men did (n=14; 31.8%, p=0.013). Patients with poor sleep quality had a higher body mass index (BMI) than those with good sleep quality (25.4 ± 4.9 vs. 23.2 ± 4.6, p=0.026) (Table 3). Symptoms of insomnia and subjective sleep quality were not associated with demographic characteristics, such as age, education, regular work status, or residential status (Table 4).

Patients who had poor sleep quality had a higher number of chronic diseases than those with good sleep quality (2.0 ± 2.9 vs. 1.0 ± 1.3, p<0.001). Likewise, patients with insomnia had more chronic diseases than those without symptoms of insomnia (2.0 ± 2.8 vs. 1.0 ± 1.9, p=0.046). The number of chronic diseases correlated with the AIS total score (r=0.48, p<0.001) and PSQI total score (r=0.45 p<0.001) (Table 5). Patients who experienced general pain almost every day for 3 months prior to the study had poor sleep quality (n=22, 75.9%; p<0.001) and symptoms of insomnia (n=20, 69.0%; p=0.006) (Table 3). Intensity of pain was correlated with PSQI total score (r=0.34, p<0.01) and AIS total score (r=0.36, p<0.01) (Table 5).

Patients with depression (p=0.044), autoimmune phenomena (p=0.012), cancer survivors (p=0.034), nicotine

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TABLE 4 Comparison of patients with good or poor sleep quality (PSQI), absence or presence of insomnia symptoms, and absence or presence of fatigue symptoms according to clinical data.

	Sleep quality (PSQI)		Insomnia symptoms (AIS)		Fatigue (FSS)	
	good	poor	absence	presence	absence	presence
number (%)	50 (54.3%)	42 (45.7%)	51 (55.4%)	41 (44.6%)	44 (47.8%)	48 (52.2%)
Body Mass Index (BMI, kg/m^2)						
(mean ± SD)	23.2 ± 4.6*	225.4 ± 4.9*	23.9 ± 4.7	24.7 ± 5.1	22.9 ± 4.9	25.4 ± 4.6*
Presence of chronic disease (other than PIDs) n(%)						
No	20 (66.7)	10 (33.3)	19 (63.3)	11 (36.7)	17 (56.7)	13 (43.3)
Yes	30 (48.4)	32 (51.6)	32 (51.6)	30 (48.4)	27 (43.5)	35 (56.5)
Number of chronic diseases						
(mean ± SD)	1.0 ± 1.3	2.0 ± 2.9***	1.0 ± 1.9	2.0 ± 2.8*	1.0 ± 2.0	2.0 ± 2.7*
Autoimmune phenomena n (%)						
No	41 (63.1)	24 (36.9)*	37 (56.9)	28 (43.1)	36 (55.4)	29 (44.6)*
Yes	9 (33.3)	18 (66.7)*	14 (51.9)	13 (48.1)	8 (29.6)	19 (70.4)*
Depression n (%)						
No	49 (57.6)	36 (42.4)*	48 (56.5)	37 (43.5)	43 (50.6)	42 (49.4)
Yes	1 (14.3)	6 (85.7)*	3 (42.9)	4 (57.1)	1 (14.3)	6 (85.7)
Neoplastic disease n (%)						
No	47 (58.8)	33 (41.2)*	47 (58.8)	33 (41.2)	42 (52.5)	38 (47.5)*
Yes	3 (25.0)	9 (75.0)*	4 (33.3)	8 (66.7)	2 (16.7)	10 (83.3)*
Addiction n (%)						
No	46 (59.0)	32 (41.0)*	46 (59.0)	32 (41.0)	38 (48.7)	40 (51.3)
Yes	4 (28.6)	10 (71.4)*	5 (35.7)	9 (64.3)	6 (42.9)	8 (57.1)
Active smoker n (%)						
No	47 (58.8)	33 (41.2)*	47 (58.8)	33 (41.2)	39 (48.8)	41 (51.2)
Yes	3 (25.0)	9 (75.0)*	4 (33.3)	8 (66.7)	5 (41.7)	7 (58.3)
Presence of pain in previous 3 months n (%)						
No	14 (82.4)	3 (17.6)*	13 (76.5)	4 (23.5)	12 (70.6)	5 (29.4)
Yes	36 (48.0)	39 (52.0)*	38 (50.7)	37 (49.3)	32 (42.7)	43 (57.3)
Frequency of general pain in previous 3 months n (%)						
Almost everyday	7 (24.1)	22 (75.9)***	9 (31.0)	20 (69.0)**	8 (27.6)	21 (72.4)**
For several days	27 (65.9)	14 (34.1)***	27 (65.9)	14 (34.1)**	24 (58.5)	17 (41.5)**
For more than 30 days	2 (40.0)	3 (60.0)***	2 (40.0)	3 (60.0)**	0 (0.0)	5 (100.0)**
Not at all	14 (82.4)	3 (17.6)***	13 (76.5)	4 (23.5)**	12 (70.6)	5 (29.4)**
The stressful event in the previous 3 months n (%)						
No	28 (66.7)	14 (33.3)*	28 (66.7)	14 (33.3)	23 (54.8)	19 (45.2)
Yes	22 (44.0)	28 (56.0)*	23 (46.0)	27 (54.0)	21 (42.0)	29 (58.0)

(Continued)

TABLE 4 Continued

	Sleep quality (PSQI)		Insomnia symptoms (AIS)		Fatigue (FSS)	
	good	poor	absence	presence	absence	presence
number (%)	50 (54.3%)	42 (45.7%)	51 (55.4%)	41 (44.6%)	44 (47.8%)	48 (52.2%)
The character of a stressful event n (%)						
Negative	15 (35.7)	27 (64.3)***	16 (38.1)	26 (61.9)**	18 (42.9)	24 (57.1)
Positive	8 (100.0)	0 (0.0)***	8 (100.0)	0 (0.0)**	4 (50.0)	4 (50.0)
Sleeping medications administration; n (%)						
No	47 (67.1)	23 (32.9)***	45 (64.3)	25 (35.7)**	37 (52.9)	33 (47.1)
Yes	3 (13.6)	19 (86.4)***	6 (27.3)	16 (72.7)**	7 (31.8)	15 (68.2)

PSQI, Pittsburgh Sleep Quality Index; SD, standard deviation; AIS, Athens Insomnia Scale; FSS, Fatigue Severity Scale; *p <.05. **p <.01. ***p <.001.

addicts (p=0.034), and those who experienced stressful events (p=0.037) had poorer sleep quality than their counterparts (Table 3), and the differences were statistically significant. However, these factors were not related to insomnia.

In the entire group, there was no association between the AIS total score or PSQI total score and the particular PID (p=0.177), diagnostic delay (p=0.846), type of immunoglobulin replacement therapy (p=0.079), location of PID treatment (p=0.229), or hospitalization in the previous 3 months (p=0.578) (Tables 6, 7). Patients with poor sleep quality had statistically more infections 3 months before the study than those with good sleep quality (1.1 ± 1.4 vs. 0.5 ± 0.9, p=0.03). The severity of infection did not affect sleep quality (p=0.534) or the symptoms of insomnia (p=0.055)

The regression model for AIS was statistically significant [F (13,78) = 5,32; p < 0,001], and the r-square value of 0.382 explained 38.2% of the variation. Significant predictors of AIS were the number of chronic diseases, anxiety disorders, and experiencing pain in the last 3 months (Table 8). If a patient declared more chronic diseases, the AIS score was higher (β = 0.23; p = 0.050). A higher AIS score was also obtained in patients with anxiety disorder (β = 0.71; p = 0.014) or borderline conditions (β = 0.49; p = 0.039) compared to those with no disorder. Patients who experienced pain for a few days in the past 3 months (β = -0.89; p < 0.001) or not at all (β = -0.73; p = 0.014) compared to those who experienced pain almost daily also had higher AIS values.

We also created multiple linear regression model for PSQI (Table 9). This model was a good fit to the data [F(13,78) = 3.80; p < 0.001] and explained a total of 28.6% of the PSQI variance (adj. R2 = 0.286). Significant predictors of the PSQI score were the number of chronic diseases, anxiety disorders, and experiencing pain in the past 3 months. The PSQI total score was higher in patients with more chronic diseases (β = 0.26; p = 0.041). In addition, the PSQI total score was higher in patients with anxiety disorders than in those without anxiety disorders (β = 0.87; p = 0.005). Patients who experienced pain almost daily compared to those who had pain for a few days over 3 months had a higher PSQI total score (β = -0.51; p = 0.044).

3.3 Fatigue

Fatigue was reported in 52.2% (n=48) of patients. Patients with fatigue had a higher BMI and more comorbidities than those without fatigue (2.0 ± 2.7 vs. 1.0 ± 2.0; p=0.044) (Table 4). The majority of patients with a diagnosis/history of neoplastic disease (n=10; 83.3%) experienced fatigue (p=0.029). Likewise, a large proportion of patients with autoimmune phenomena had fatigue (n=19, 70.4 % vs. n=8, 29.6%; p=0.038). Moreover, fatigue was frequent in patients who experienced general pain almost every day for 3 months prior to the study (n=21, 72.4%; p=0.002). Fatigue was also associated with insomnia (p=0.022) and poor sleep quality (p<0.001).

A higher percentage of fatigue was reported in women (n=30, 62.5%; p=0.06) and in patients with a longer diagnostic delay of PID than their counterparts (4.9 ± 6.9 vs. 9.3 ± 13.1 years; p=0.503); however, the differences were not statistically significant (Tables 4, 6).

The regression model created for FSS was a good fit to the data [F(13,78) = 4.14; p < 0.001] and explained a total of 31% of the FSS variance (adj. R2 = 0.310). Sex, anxiety and depressive disorders, and experiencing pain in the last 3 months were significant predictors in the model (Table 10). There were higher FSS scores in women (β = -0.46; p = 0.021), in those with borderline anxiety disorders compared to those without disorders (β = 0.56; p = 0.027), in those with depressive disorders compared to those without depressive disorders (β = 0.85; p = 0.037), and in those who had pain almost every day compared to those who had pain for a few days over a 3-month period (β = -0.61; p = 0.015).

TABLE 5 Comparison of patients with good or poor sleep quality (PSQI), absence or presence of insomnia symptoms, and absence or presence of fatigue symptoms according to primary immunodeficiencies (PIDs).

	Sleep quality (PSQI)		Insomnia symptoms (AIS)		Fatigue (FSS)	
	good	poor	absence	presence	absence	presence
number (%)	50 (54.3%)	42 (45.7%)	51 (55.4%)	41 (44.6%)	44 (47.8%)	48 (52.2%)
PIDs n (%)						
CVID	28 (59.6)	19 (40.4)	24 (51.1)	23 (48.9)	25 (53.2)	22 (46.8)
IgG subclasses deficiency	7 (41.2)	10 (58.8)	9 (52.9)	8 (47.1)	5 (29.4)	12 (70.6)
XLA	5 (55.6)	4 (44.4)	6 (66.7)	3 (33.3)	5 (55.6)	4 (44.4)
other immuno-deficiencies	4 (100.0)	0 (0.0)	2 (50.0)	2 (50.0)	3 (75.0)	1 (25.0)
other humoral immuno-deficiencies	6 (40.0)	9 (60.0)	10 (66.7)	5 (33.3)	6 (40.0)	9 (60.0)
PIDs treatment n (%)						
without treatment	3 (75.0)	1 (25.0)	1 (25.0)	3 (75.0)	3 (75.0)	1 (25.0)
other than IG replacement therapy	0 (0.0)	1 (100.0)	1 (100.0)	0 (0.0)	0 (0.0)	1 (100.0)
IVIG	4 (30.8)	9 (69.2)	7 (53.8)	6 (46.2)	6 (46.2)	7 (53.8)
SCIG	43 (58.1)	31 (41.9)	42 (56.8)	32 (43.2)	35 (47.3)	39 (52.7)
Age of first symptoms of PIDs (years)						
(mean ± SD)	21.3 ± 18.4	24.3 ± 18.7	22.4 ± 19.9	22.9 ± 16.9	20.9 ± 18.4	24.2 ± 18.7
Age of PIDs diagnosis (years)						
(mean ± SD)	27.3 ± 16.6	32.9 ± 16.7	29.6 ± 17.6	30.2 ± 15.9	25.8 ± 16.9	33.6 ± 15.9*
Diagnostic delay (years)						
(mean ± SD)	6 ± 8.8	8.6 ± 12.7	7.2 ± 10.3	7.2 ± 11.5	4.9 ± 6.9	9.3 ± 13.1
Immunoglobulin replacement therapy n (%)						
IVIg	4 (30.8)	9 (69.2)	7 (53.8)	6 (46.2)	6 (46.2)	7 (53.8)
SCIg	43 (58.1)	31 (41.9)	42 (56.8)	32 (43.2)	35 (47.3)	39 (52.7)
Place of immunoglobulin administration n (%)						
home	40 (57.1)	30 (42.9)	40 (57.1)	30 (42.9)	33 (47.1)	37 (52.9)
outpatient's clinic	0 (0.0)	2 (100.0)	1 (50.0)	1 (50.0)	1 (50.0)	1 (50.0)
hospital	7 (46.7)	8 (53.3)	8 (53.3)	7 (46.7)	7 (46.7)	8 (53.3)

PSQI, Pittsburgh Sleep Quality Index; SD, standard deviation; AIS, Athens Insomnia Scale; FSS, Fatigue Severity Scale; PIDs, primary immunodeficiencies; CVID, common variable immunodeficiency; XLA, X-linked agammaglobulinemia; Ig, immunoglobulin; IVIg, intravenous immunoglobin; SCIg, subcutaneous immunoglobulin; *p <.05.

3.4 Anxiety and depression

Among our patients, anxiety was more frequent than depression. Thirteen patients (14.1%) had a score ≥11, which indicates severe anxiety; 22 patients (23.9%) had ≥8 points, which indicates moderate anxiety; and 57 patients (62.0%) did not have anxiety. According to the HADS scale, 6 patients (6.5%) had severe depression with a score ≥11; these patients identified depression as a concomitant disease. Seventeen patients had moderate depression with a score ≥8 (18.5%), and 69 patients did not have depression (75%).

4 Discussion

To maintain good health, the recommended sleep duration is at least 7 hours per day (26). In our study approximately one-third of patients slept <7 hours, with the mean sleep duration of 6.99 ± 1.5 hours, compared to 7.7 hours in the general Polish population (27). Sleep latency is the length of time needed to fall asleep, and it is assumed to last <30 minutes (28). In our study, sleep latency was longer than half an hour in one-fourth of the patients (n=25; 27%).

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TABLE 6 Comparison of patients with good or poor sleep quality (PSQI), absence or presence of insomnia symptoms, and absence or presence of fatigue symptoms according to exacerbation of PID.

	Sleep quality (PSQI)		Insomnia symptoms (AIS)		Fatigue (FSS)	
	good	poor	absence	presence	absence	presence
number (%)	50 (54.3%)	42 (45.7%)	51 (55.4%)	41 (44.6%)	44 (47.8%)	48 (52.2%)
Presence of infections in previous 3 months n (%)						
No	34 (63.0)	20 (37.0)	33 (61.1)	21 (38.9)	27 (50.0)	27 (50.0)
Yes	16 (42.1)	22 (57.9)	18 (47.4)	20 (52.6)	17 (44.7)	21 (55.3)
Number of infections in previous 3 months (mean ± SD)						
	0.5 ± 0.9	1.1 ± 1.4**	0.6 ± 0.9	1.1 ± 1.4	0.7 ± 1.0	0.9 ± 1.3
The severity of infection in the previous 3 months n (%)						
severe	1 (25.0)	3 (75.0)	0 (0.0)	4 (100.0)	0 (0.0)	4 (100.0)
moderate	11 (52.4)	10 (47.6)	13 (61.9)	8 (38.1)	12 (57.1)	9 (42.9)
benign	4 (30.8)	9 (69.2)	5 (38.5)	8 (61.5)	5 (38.5)	8 (61.5)
Administration of antibiotics in previous 3 months n (%)						
No	38 (56.7)	29 (43.3)	38 (56.7)	29 (43.3)	32 (47.8)	35 (52.2)
Yes	12 (48.0)	13 (52.0)	13 (52.0)	12 (48.0)	12 (48.0)	13 (52.0)
Number of antibiotics administrated in previous 3 months (mean ± SD)						
	0.4 ± 0.8	0.5 ± 0.8	0.4 ± 0.7	0.5 ± 0.9	0.4 ± 0.7	0.5 ± 0.9
Hospitalization in previous 3 months (other reason than immunoglobulin administration) n (%)						
No	43 (55.8)	34 (44.2)	46 (59.7)	31 (40.3)	38 (49.4)	39 (50.6)
Yes	7 (46.7)	8 (53.3)	5 (33.3)	10 (66.7)	6 (40.0)	9 (60.0)

PSQI, Pittsburgh Sleep Quality Index; SD, standard deviation; AIS, Athens Insomnia Scale; FSS, Fatigue Severity Scale; PID, primary immunodeficiency; ** p <.01.

Insomnia has been a concern in various studies, and its prevalence varies depending on the study population. A study conducted on adult individuals in Poland (n=47,924) revealed insomnia in 28.1% of women and 18.1% of men (29). However, subjective sleep quality and prevalence of insomnia in patients with PIDs have not been reported. To our knowledge, this is the first study to assess subjective sleep quality and the prevalence of insomnia among patients with PIDs.

Among the patients in our study, neither a particular disease nor type of treatment was associated with insomnia symptoms or subjective sleep quality. However, there is no scale to assess the severity of PIDs, and physicians use the number and severity of infections to determine disease control and potential exacerbations. Herein, patients with poor sleep quality had more infections in the previous 3 months than those with good sleep quality. Nevertheless, sleep duration was not associated with the number of infections (p=0.110). We could not determine whether our patients were prone to infections due to poor sleep quality or if their sleep was altered by ongoing infection. Sleep impairment may be an important factor that increases susceptibility to infections in patients with PIDs who have a primarily impaired immune system. Sanjay et al. (30) investigated the association between sleep duration and susceptibility to pneumonia in a prospective study. The researchers included 56,953 women without relevant comorbidities and a prior history of pneumonia and assessed sleep deprivation. They revealed that sleep durations ≤5 hour and ≥9 hours were associated with a higher risk of pneumonia compared with a sleep duration of 8 hours. In their experimental study, Cohen et al. (31) observed sleep efficiency and sleep duration in 153 healthy volunteers over 14 days. Subsequently, the researchers administered nasal drops with rhinovirus to the participants and observed them for 5 days following the development of a clinical cold. The authors proved that reduced sleep efficiency and shorter sleep duration prior to exposure to the virus were associated with lower resistance to respiratory illness.

More than half of patients (67.4%) in our study had chronic diseases other than PID. Almost half of them had lung disease, representing a frequent comorbidity of PID (32). Other frequent comorbidities were rheumatological, gastroenterological, and cardiovascular diseases, with each group comprising approximately one-fourth of patients. In our study, patients

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TABLE 7 Correlation matrix (Pearson's r).

	age (years)	BMI (kg/m2)	diagnostic delay (years)	infections in previous 3 months (n)	antibiotics administration in previous 3 months (n)	comorbidities (n)	pain intensity (NRS)	total sleep duration (h)	PSQI total score	AIS total score	FSS total score
age (years)	—										
BMI (kg/m^2)	0.36***	—									
diagnostic delay (years)	0.14	0.11	—								
infections in previous 3 months (n)	0.02	0.21	0.23	—							
antibiotics administration in previous 3 months (n)	0.01	0.08	-0.01	0.58***	—						
comorbidities (n)	0.47***	0.22	0.19	0.05	0.12	—					
pain intensity (NRS)	0.36***	0.28	0.02	-0.002	0.05	0.36***	—				
total sleep duration (h)	0.04	0.20	0.14	-0.17	0.07	0.36***	0.27	—			
PSQI total score	0.21	0.12	0.12	0.21	0.06	0.45***	0.34***	-0.68*	—		
AIS total score	0.16	0.12	0.18	0.26	0.07	0.48***	0.36***	-0.48***	0.83***	—	
FSS total score	0.11	0.19	0.26	0.131	0.08	0.19	0.26	0.09	0.19	0.24	—

BMI, body mass index; PSQI, Pittsburgh Sleep Quality Index; AIS, Athens Insomnia Scale; FSS, Fatigue Severity Scale ***p <.001.

with poor sleep quality had significantly more chronic diseases than those with good sleep quality, as reported previously (18). Moreover, a higher number of comorbidities in our patients was related to poorer sleep quality. Basent et al. (33) recruited 5,878 individuals to investigate the association of common chronic diseases with sleep disorders and sleep quality. They found a significantly increased odds of poor sleep quality in patients with cardiac insufficiency, gallstone degenerative joint disease, and depression. A cohort study on individuals with COPD revealed poor subjective sleep quality in this group; interestingly, higher PSQI scores were associated with increased risk of COPD exacerbations during the follow-up (17). Matsuda et al. (34) assessed sleep quality using the PSQI during hospitalization for a broad spectrum of cardiovascular diseases. Almost half of the participants reported poor sleep quality.

In the regression model, we revealed that a higher number of comorbidities was a predictive factor for insomnia. Abad et al. (35) summarized that insomnia and unrefreshing sleep are common complaints in patients with rheumatologic disorders, such as, rheumatoid arthritis (RA), osteoarthritis, systemic lupus erythematosus (SLE), and Sjogren syndrome.

Momayyezi et al. (36) reported poor sleep quality in 69.3% of patients with cancer. In our study, 2 patients had ongoing cancer treatment, and both had poor sleep quality and insomnia. Among the cancer survivors, 7 (70.0%) had poor sleep quality, and 6 (60.0%) had insomnia. A study by Hammersen et al. (37) included 465 long-term lymphoma survivors in Germany and revealed poor sleep quality according to the PSQI in 224 (48.2%) patients. According to data obtained by Chen et al. (38), sleep quality measured by different methods, both subjective and objective, is affected in patients with cancers, particularly in those with lung, breast, gynecological, head, and neck cancers.

In our study, the incidence of autoimmune phenomena was 29.4% (n=27), which is comparable to that reported in other cohorts with PIDs (7). Patients with autoimmunity in our group had poorer sleep quality than those without autoimmune phenomena, and most of them had fatigue. Autoimmune diseases are also associated with sleep disorders (15). Many studies have investigated sleep quality in RA. They revealed that the vast majority of patients with RA have poor sleep quality, which may be associated with disease activity and ongoing inflammation (39). Likewise, patients with SLE report sleep disorders as frequent complaints with poorer sleep quality compared to the general population (40). Alterations in the immune system owing to sleep impairment may affect the course of autoimmune diseases. For instance, patients with Crohn's disease have an increased risk of disease flair subsequent to sleep disorders (41). Furthermore, many patients with autoimmune diseases experience chronic pain (42), neuropathic, somatic, or visceral, depending on the particular diseases, which is an independent factor that diminishes sleep quality.

We asked patients about stressful events 3 months before the study. Patients with poor sleep quality, insomnia symptoms, and

TABLE 8 Multiple linear regression model for AIS.

			95% CI				
Predictor	B	SE	LL	UL	t	p	β
Intercept	7.57	2.65	2.29	12.85	2.85	0.006	
Number of chronic diseases (n)	0.44	0.22	<-0.01	0.89	1.99	0.05	0.23
Diagnostic delay (years)	0	0.04	-0.09	0.09	-0.05	0.958	0
Infections in previous 3 months (n)	0.76	0.41	-0.04	1.57	1.89	0.063	0.18
Age (years)	0	0.04	-0.07	0.08	0.06	0.952	0.01
BMI (kg/m^2)	0	0.1	-0.2	0.2	-0.02	0.988	0
Sex							
man – woman	1.12	0.94	-0.75	2.98	1.19	0.238	0.22
HADS, anxiety							
severe anxiety – no anxiety	3.64	1.44	0.77	6.51	2.53	0.014*	0.71
moderate anxiety – no anxiety	2.54	1.21	0.14	4.94	2.1	0.039*	0.49
HADS, depression							
severe depression – no depression	-1.37	1.95	-5.26	2.52	-0.7	0.485	-0.27
moderate depression – no depression	-0.34	1.3	-2.92	2.25	-0.26	0.795	-0.07
Frequency of general pain in previous 3 months							
for several days – almost everyday	-4.57	1.19	-6.94	-2.2	-3.84	< .001***	-0.89
for more than 30 days – almost everyday	-1.61	2.23	-6.05	2.83	-0.72	0.473	-0.31
not at all – almost everyday	-3.75	1.5	-6.73	-0.77	-2.5	0.014*	-0.73

AIS, Athens Insomnia Scale; HADS, Hospital Anxiety and Insomnia Scale; BMI, body mass index; *p<.05 *** p<.001.

TABLE 9 Multiple linear regression model for PSQI.

			95% CI				
Predictor	B	SE	LL	UL	t	p	β
Intercept	4.6	2.24	0.13	9.06	2.05	0.044	
Number of chronic diseases (n)	0.39	0.19	0.02	0.77	2.08	0.041*	0.26
Diagnostic delay (years)	-0.02	0.04	-0.1	0.05	-0.54	0.589	-0.05
Infections in previous 3 months (n)	0.47	0.34	-0.22	1.15	1.36	0.177	0.14
Age (years)	0.03	0.03	-0.04	0.09	0.88	0.38	0.1
BMI (kg/m^2)	-0.02	0.08	-0.19	0.15	-0.21	0.833	-0.02
Sex							
man – woman	0.51	0.79	-1.07	2.09	0.65	0.52	0.13
HADS, anxiety							
severe anxiety – no anxiety	3.51	1.22	1.08	5.94	2.88	0.005**	0.87
moderate anxiety – no anxiety	1.57	1.02	-0.46	3.6	1.54	0.128	0.39

(Continued)

TABLE 9 Continued

			95% CI				
Predictor	B	SE	LL	UL	t	p	β
HADS, depression							
severe depression – no depression	-0.03	1.65	-3.32	3.26	-0.02	0.987	-0.01
moderate depression – no depression	0.47	1.1	-1.72	2.65	0.43	0.671	0.12
Frequency of general pain in previous 3 months							
for several days – almost everyday	-2.06	1.01	-4.06	-0.06	-2.05	0.044*	-0.51
for more than 30 days – almost everyday	-0.21	1.89	-3.97	3.54	-0.11	0.91	-0.05
not at all – almost everyday	-1.56	1.27	-4.08	0.96	-1.23	0.222	-0.39

PSQI, Pittsburgh Sleep Quality; HADS, Hospital Anxiety and Insomnia Scale; BMI, body mass index; * p <.05 **p <0.01.

fatigue experience stressful events more frequently than those without. However, the differences were statistically relevant only for the PSQI score. A study conducted by Otsuka et al. in a Japanese population revealed a significant positive association between sleep disorders and high levels of stress. Furthermore, individuals with high stress are more prone to develop insomnia symptoms than those without (43).

Herein, our patients who experienced general pain almost every day for 3 months prior to the study had poor sleep quality, symptoms of insomnia, and fatigue more frequently than those

TABLE 10 Multiple linear regression model for FSS.

			95% CI				
Predictor	B	SE	LL	UL	t	p	β
Intercept	30.19	7.17	15.91	44.47	4.21	< .001	
Number of chronic diseases (n)	-0.69	0.61	-1.9	0.52	-1.14	0.258	-0.14
Diagnostic delay (years)	0.12	0.12	-0.13	0.36	0.96	0.342	0.09
Infections in previous 3 months (n)	-0.89	1.1	-3.07	1.3	-0.81	0.422	-0.08
Age (years)	0.04	0.1	-0.16	0.25	0.42	0.675	0.05
BMI (kg/m^2)	0.36	0.27	-0.18	0.9	1.34	0.186	0.13
Sex							
man – woman	-6	2.54	-11.05	-0.95	-2.37	0.021*	-0.46
HADS. anxiety							
severe anxiety – no anxiety	6.97	3.9	-0.8	14.73	1.79	0.078	0.53
moderate anxiety – no anxiety	7.34	3.26	0.85	13.83	2.25	0.027*	0.56
HADS. depression							
severe depression – no depression	11.23	5.29	0.71	21.75	2.12	0.037*	0.85
moderate depression – no depression	6.82	3.51	-0.17	13.8	1.94	0.056	0.52
Frequency of general pain in previous 3 months							
for several days – almost everyday	-7.99	3.22	-14.4	-1.59	-2.48	>0.015*	-0.61
for more than 30 days – almost everyday	-3.51	6.04	-15.52	8.51	-0.58	0.563	-0.27
not at all – almost everyday	-5.11	4.05	-13.17	2.95	-1.26	0.211	-0.39

FSS, Fatigue Severity Scale; HADS, Hospital Anxiety and Insomnia Scale; BMI, body mass index; *p <.05.

without these disorders. The higher prevalence of pain was associated with poorer sleep quality and higher AIS total score (Tables 8, 9). The connection between pain, sleep quality, and fatigue has been reported in many chronic diseases, including rheumatic disorders (44). Studies on RA and fibromyalgia have revealed that pain increases sleep disturbances (35). Patients with chronic pain have diminished subjective sleep quality compared with those without pain (45). Other studies have indicated that sleep disorders may initiate pain (16). Disrupted sleep also increases sensitivity to pain (41). This relationship has been proven in many studies, but its direction remains unclear. Many authors have highlighted the bidirectional relationship between pain and sleep disturbances (46–48). In some comprehensive analyses, sleep disturbances were considered a stronger predictor of the incidence of pain than pain as an inductor of sleep disturbances (44, 49). Furthermore, sleep disorders can result in fatigue (48) and depression (47).

In our study, 40.2% of patients were overweight and obese. BMI was associated with subjective sleep quality and fatigue in our study. Patients with poor sleep quality and fatigue had a higher BMI than those with good sleep quality and no fatigue (Table 6). In a study conducted by Fatima et al. (50), a considerably higher percentage of overweight and obesity was reported in patients with poor sleep quality than in those without.

Fatigue is a nonspecific symptom characterized by tiredness or inability to function due to a lack of energy (51), which cannot be restored by resting (52). Although fatigue appears in healthy individuals, it is more frequent in patients with chronic diseases (14), including various rheumatological diseases (15), neurological disorders (53), COPD (54), and cancers (52). Recent studies have shown that fatigue is independent of disease severity and activity (14).

In our study, fatigue was reported in 52.2% of patients. Hajjar et al. reported that among 2,537 patients with PIDs, 25.9% had fatigue, with the highest prevalence among patients with CVID (55).

Our linear regression model revealed higher prevalence of fatigue in patients with borderline anxiety disorders compared to those without disorders, and in patients with depressive disorders compared to those without depressive disorders (Table 10). Likewise, Bansal et al. investigated the prevalence of fatigue in patients with primary antibody deficiencies (PAD) and revealed a high frequency of fatigue in patients with PAD, which was correlated with the presence of anxiety and depression (56). Among our patients, anxiety was a relevant predictor of insomnia and poor sleep quality. Individuals with an anxiety disorder or borderline condition had higher AIS scores compared to those with no such a disorder (Table 8) (57).

5 Conclusions

Sleep quality, fatigue, pain, and primary immune disorders may give an impression of distinct medical problems; however, they appear to be connected in complex relationships. Therefore, they must be considered in a holistic model. As aforementioned, many factors may affect sleep quality, resulting in complex consequences. Our study, which is the first to address this issue, provides insight into the prevalence of insomnia, fatigue, and subjective sleep quality assessment in patients with PIDs. The data demonstrated that decreased sleep quality, insomnia symptoms, and fatigue are common in this group. There is a need for further studies to explain the determinants of poor sleep quality in this specific group of patients.

6 Limitations

Although this study was carefully planned, it has some limitations. First, subjective sleep quality was measured, which may have been affected by some errors. Although an objective measurement of sleep quality provides undeniable data, its use in daily clinical practice is limited owing to its high cost and time requirement. Second, we did not investigate other sleep disorders, except for the symptoms of insomnia and sleep quality. Third, the sample size of this study was small. In the pilot study, we recruited 92 patients from 4 clinical centers. Fourth, data concerning general health and the administration of medication were provided by the patients, and they were not subject to verification. We could not provide ICD codes for chronic diseases. This observational nature of the study may have created bias. Another limitation is that the majority of our patients had primary antibody deficiencies (PAD). Patients with other PID were the minority (n=4, 4.35%), and we could not analyze those patients separately.

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 Independent Bioethics Commission for Research of the Medical University of Gdańsk approved the study (number: 422/

2017). The patients/participants provided their written informed consent to participate in this study.

Author contributions

KG and MZ designed the study with the support of KNS. KG and MZ prepared the first draft of the manuscript. This text was produced with equal contributions from both authors. KG, MZ, EW-S, AM-B, KN-B, and DS collected the data and performed literature searches. MZ and KG performed the statistical analyses. EW-S, AM-B, KN-B, ZZ, AH, and KN-S critically revised the manuscript for intellectual content. All authors contributed to the article and approved the submitted version.

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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References

1. Shields AM, Patel SY. The primary immunodeficiency disorders. *Med (United Kingdom)* (2017) 45:597–604. doi: 10.1016/j.mpmed.2017.07.011

2. Abolhassani H, Azizi G, Sharifi L, Yazdani R, Mohsenzadegan M, Delavari S, et al. Global systematic review of primary immunodeficiency registries. *Expert Rev Clin Immunol* (2020) 16:717–32. doi: 10.1080/1744666X.2020.1801422

3. Notarangelo LD. Primary immunodeficiencies. *J Allergy Clin Immunol* (2010) 125:S182–94. doi: 10.1016/j.jaci.2009.07.053

4. Heropolitańska-Pliszka E, Pietrucha B, Klaudel-Dreszler M, Wolska-Kuśnierz B, Matosek A, Bernatowska E. (2011) Wybrane zagadnienia z immunologii klinicznej – niedobory przeciwciał niedobory komórkowe (część I). *Pediatria Polska*. 86 (5) doi: 10.1016/S0031-3939(11)70532-7

5. Cinetto F, Scarpa R, Rattazzi M, Agostini C. The broad spectrum of lung diseases in primary antibody deficiencies. *Eur Respir Rev* (2018) 27:1–17. doi: 10.1183/16000617.0019-2018

6. Ruffner MA, Sullivan KE, Henrickson SE. Recurrent and sustained viral infections in primary immunodeficiencies. *Front Immunol* (2017) 8:665. doi: 10.3389/fimmu.2017.00665

7. Fischer A, Provot J, Jais JP, Alcais A, Mahlaoui N, Adoue D, et al. Autoimmune and inflammatory manifestations occur frequently in patients with primary immunodeficiencies. *J Allergy Clin Immunol* (2017) 140:1388–1393.e8. doi: 10.1016/j.jaci.2016.12.978

8. Raje N, Dinakar C. Overview of immunodeficiency DisoCivelek, mete lusis, aldons J genetics, molecular Angeles, losrders. *Immunol Allergy Clin North Am* (2015) 35:599–623. doi: 10.1038/nrg3575.Systems

9. Assefa SZ, Diaz-Abad M, Wickwire EM, Scharf SM. The functions of sleep. *AIMS Neurosci* (2015) 2:155–71. doi: 10.3934/Neuroscience.2015.3.155

10. Obal F, Krueger JM. *Front Biosci* (2003), 520–50. Department of Physiology, University of Szeged, A. Szent-Györgyi Medical Center, 6720 Szeged, Hungary, 2 Department of Veterinary and Comparative Anatomy, Pharmacology, and Physiology, Washington State University, Pullman, WA 99164-6520.

11. Krueger JM, Obal F Jr. Sleep function. *Front Biosci* (2003) 8:d511–9. doi: 10.2741/1031

12. Sateia MJ. International classification of sleep disorders-third edition highlights and modifications. *Chest* (2014) 146:1387–94. doi: 10.1378/chest.14-0970

13. Sunwoo JS, Kim D, Chu MK, Yun CH, Yang KI. Fatigue is associated with depression independent of excessive daytime sleepiness in the general population. *Sleep Breath* (2022) 26:933–40. doi: 10.1007/s11325-021-02448-3

14. Goërtz YMJ, Braamse AMJ, Spruit MA, Janssen DJA, Ebadi Z, Van Herck M, et al. Fatigue in patients with chronic disease: results from the population-based lifelines cohort study. *Sci Rep* (2021) 11:1–12. doi: 10.1038/s41598-021-00337-z

15. Zielinski MR, Systrom DM, Rose NR. Fatigue, sleep, and autoimmune and related disorders. *Front Immunol* (2019) 10:1827. doi: 10.3389/fimmu.2019.01827

16. Taylor-Gjevre R, Gjevre JA, Skomro R, Nair BV. Assessment of sleep health in patients with rheumatic disease. *Int J Clin Rheumtol* (2011) 6:207–18. doi: 10.2217/ijr.11.6

17. Shorofsky M, Bourbeau J, Kimoff J, Jen R, Malhotra A, Ayas N, et al. Impaired sleep quality in COPD is associated with exacerbations: The CanCOLD cohort study. *Chest* (2019) 156:852–63. doi: 10.1016/j.chest.2019.04.132

18. Hsu MF, Lee KY, Lin TC, Te LW, Ho SC. Subjective sleep quality and association with depression syndrome, chronic diseases and health-related physical fitness in the middle-aged and elderly. *BMC Public Health* (2021) 21:1–9. doi: 10.1186/s12889-021-10206-z

19. ESID - European Society for Immunodeficiencies. Available at: https://esid.org/Working-Parties/Registry-Working-Party/Diagnosis-criteria.

20. Fabbri M, Beracci A, Martoni M, Meneo D, Tonetti L, Natale V. Measuring subjective sleep quality: A review. *Int J Environ Res Public Health* (2021) 18:1–57. doi: 10.3390/ijerph18031082

21. Fornal-Pawłowska M, Wołyńczyk-Gmaj D, Szelenberger W. Validation of the polish version of the Athens insomnia scale | walidacja ateńskiej skali bezsenności. *Psychiatr Pol* (2011) 45:211–22.

22. Buysse DJ, Reynolds CF, Monk TH, Berman SR, Kupfer DJ. PSQI article.pdf. *Psychiatry Res* (1998) 28:193–213. doi: 10.1016/0165-1781(89)90047-4

23. Krupp LB, LaRocca NG, Muir-Nash J, Steinberg AD. The fatigue severity scale: Application to patients with multiple sclerosis and systemic lupus erythematosus. *Arch Neurol* (1989) 46:1121–3. doi: 10.1001/archneur.1989.00520460115022

24. Stern AF. The hospital anxiety and depression scale. *Occup Med (Chic Ill)* (2014) 64:393–4. doi: 10.1093/occmed/kqu024

25. Nowicka-Sauer K, Banaszkiewicz D, Staskiewicz I, Kopczynski P, Hajduk A, Czuszynska Z, et al. Illness perception in polish patients with chronic diseases: Psychometric properties of the brief illness perception questionnaire. *J Health Psychol* (2016) 21:1739–49. doi: 10.1177/1359105314565826

26. Garbarino S, Lanteri P, Bragazzi NL, Magnavita N, Scoditti E. Role of sleep deprivation in immune-related disease risk and outcomes. *Commun Biol* (2021) 4. doi: 10.1038/s42003-021-02825-4

27. Kiejna A, Rymaszewska J, Wojtyniak B, Stokwiszewski J. Characteristics of sleep disturbances in Poland - results of the national health interview survey. *Acta Neuropsychiatr* (2004) 16:124–9. doi: 10.1111/j.0924-2708.2004.00063.x

28. Chung S, Youn S, Kim C. Are you asking what time did your patients go to bed?: getting the short sleep onset latency. *Sleep Med Res* (2018) 9:58–62. doi: 10.17241/smr.2018.00150

29. Kiejna A, Wojtyniak B, Rymaszewska J, Stokwiszewski J. Prevalence of insomnia in Poland - results of the national health interview survey. *Acta Neuropsychiatr* (2003) 15:68–73. doi: 10.1034/j.1601-5215.2003.00011.x

30. Patel SR, Malhotra A, Gao X, Hu FB, Neuman MI, Fawzi WW. A prospective study of sleep duration and pneumonia risk in women. *Sleep* (2012) 35:97–101. doi: 10.5665/sleep.1594

31. Cohen S, Doyle WJ, Alper CM, Janicki-Deverts D, Turner RB. Sleep habits and susceptibility to the common cold. *Arch Intern Med* (2009) 169:62–7. doi: 10.1001/archinternmed.2008.505

32. Maglione PJ. Chronic lung disease in primary antibody deficiency: Diagnosis and management. *Immunol Allergy Clin North Am* (2020) 40:437–59. doi: 10.1016/j.iac.2020.03.003

33. Basnet S, Merikanto I, Lahti T, Männistö S, Laatikainen T, Vartiainen E, et al. Associations of common chronic non-communicable diseases and medical conditions with sleep-related problems in a population-based health examination study. *Sleep Sci* (2016) 9:249–54. doi: 10.1016/j.slsci.2016.11.003

34. Matsuda R, Kohno T, Kohsaka S, Fukuoka R, Maekawa Y, Sano M, et al. The prevalence of poor sleep quality and its association with depression and anxiety scores in patients admitted for cardiovascular disease: A cross-sectional designed study. *Int J Cardiol* (2017) 228:977–82. doi: 10.1016/j.ijcard.2016.11.091

35. Abad VC, Sarinas PSA, Guilleminault C. Sleep and rheumatologic disorders. *Sleep Med Rev* (2008) 12:211–28. doi: 10.1016/j.smrv.2007.09.001

36. Momayyezi M, Fallahzadeh H, Farzaneh F, Momayyezi M. Sleep quality and cancer-related fatigue in patients with cancer. *J Caring Sci* (2021) 10:145–52. doi: 10.34172/jcs.2021.021

37. Hammersen F, Lewin P, Gebauer J, Kreitschmann-Andermahr I, Brabant G, Katalinic A, et al. Sleep quality and health-related quality of life among long-term survivors of (non-) Hodgkin lymphoma in Germany. *PloS One* (2017) 12:1–13. doi: 10.1371/journal.pone.0187673

38. Chen D, Yin Z, Fang B. Measurements and status of sleep quality in patients with cancers. *Support Care Cancer* (2018) 26:405–14. doi: 10.1007/s00520-017-3927-x

39. Coskun Benlidayi I. Sleep impairment: an obstacle to achieve optimal quality of life in rheumatoid arthritis. *Rheumatol Int* (2018) 38:2183–92. doi: 10.1007/s00296-018-4155-5

40. Yin R, Li L, Xu L, Sui W, Niu M, Xu R, et al. Association between depression and sleep quality in patients with systemic lupus erythematosus: a systematic review and meta-analysis. *Sleep Breath* (2022) 26:429–41. doi: 10.1007/s11325-021-02405-0

41. Haack M, Simpson N, Sethna N, Kaur S, Mullington J. Sleep deficiency and chronic pain: potential underlying mechanisms and clinical implications. *Neuropsychopharmacology* (2020) 45:205–16. doi: 10.1038/s41386-019-0439-z

42. Mifflin KA, Kerr BJ. Pain in autoimmune disorders. *J Neurosci Res* (2017) 95:1282–94. doi: 10.1002/jnr.23844

43. Otsuka Y, Kaneita Y, Itani O, Nakagome S, Jike M, Ohida T. Relationship between stress coping and sleep disorders among the general Japanese population: a nationwide representative survey. *Sleep Med* (2017) 37:38–45. doi: 10.1016/j.sleep.2017.06.007

44. Moldofsky H. Rheumatic manifestations of sleep disorders. *Curr Opin Rheumatol* (2010) 22:59–63. doi: 10.1097/BOR.0b013e328333b9cc

45. Karaman S, Karaman T, Dogru S, Onder Y, Citil R, Bulut YE, et al. Prevalence of sleep disturbance in chronic pain. *Eur Rev Med Pharmacol Sci* (2014) 18:2475–81.

46. Lashley FR. A review of sleep in selected immune and autoimmune disorders. *Holist Nurs Pract.* (2003) 17(2):65–80. doi: 10.1097/00004650-200303000-00002

47. Boeselt T, Koczulla R, Nell C, Beutel B, Guenter K, Cassel W, et al. Sleep and rheumatic diseases. *Best Pract Res Clin Rheumatol* (2019) 33:101434. doi: 10.1016/j.berh.2019.101434

48. Irwin MR, Olmstead R, Carrillo C, Sadeghi N, FitzGerald JD, Ranganath VK, et al. Sleep loss exacerbates fatigue, depression, and pain in rheumatoid arthritis. *Sleep* (2012) 35:537–43. doi: 10.5665/sleep.1742

49. Finan PH, Goodin BR, Smith MT. The association of sleep and pain: An update and a path forward. *J Pain* (2013) 2013):1539–52. doi: 10.1016/j.jpain.2013.08.007.The

50. Fatima Y, Doi SAR, Mamun AA. Sleep quality and obesity in young subjects: a meta-analysis. *Obes Rev* (2016) 17:1154–66. doi: 10.1111/obr.12444

51. Adamowicz JL, Vélez-Bermúdez M, Thomas EBK. Fatigue severity and avoidance among individuals with chronic disease: A meta-analysis. *J Psychosom Res* (2022) 159. doi: 10.1016/j.jpsychores.2022.110951

52. Hinz A, Weis J, Brähler E, Härter M, Geue K, Ernst J. Fatigue in cancer patients: comparison with the general population and prognostic factors. *Support Care Cancer* (2020) 28:4517–26. doi: 10.1007/s00520-019-05260-8

53. Chaudhuri A, Behan PO. Fatigue in neurological disorders. *Lancet* (2004) 363:978–88. doi: 10.1016/S0140-6736(04)15794-2

54. Ebadi Z, Goërtz YMJ, Van Herck M, Janssen DJA, Spruit MA, Burtin C, et al. The prevalence and related factors of fatigue in patients with copd: A systematic review. *Eur Respir Rev* (2021) 30. doi: 10.1183/16000617.0298-2020

55. Hajjar J, Guffey D, Minard CG, Orange JS. Increased incidence of fatigue in patients with primary immunodeficiency disorders: Prevalence and associations within the US immunodeficiency network registry. *J Clin Immunol* (2017) 37:153–65. doi: 10.1007/s10875-016-0367-1

56. Bansal RA, Tadros S, Bansal AS. The presence of overlapping quality of life symptoms in primary antibody deficiency (PAD) and chronic fatigue syndrome (CFS). *Allergy Asthma Clin Immunol* (2020) 16:1–8. doi: 10.1186/s13223-020-0417-3

57. Yıldız E, Arslan Ş, Tuğan Yıldız B, Tuncel Berktaş D, Çölkesen F, Evcen R, et al. Poor sleep quality and associated factors in patients with common variable immunodeficiency. *J Turkish Sleep Med* (2022) 9:214–20. doi: 10.4274/jtsm.galenos.2022.53315

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