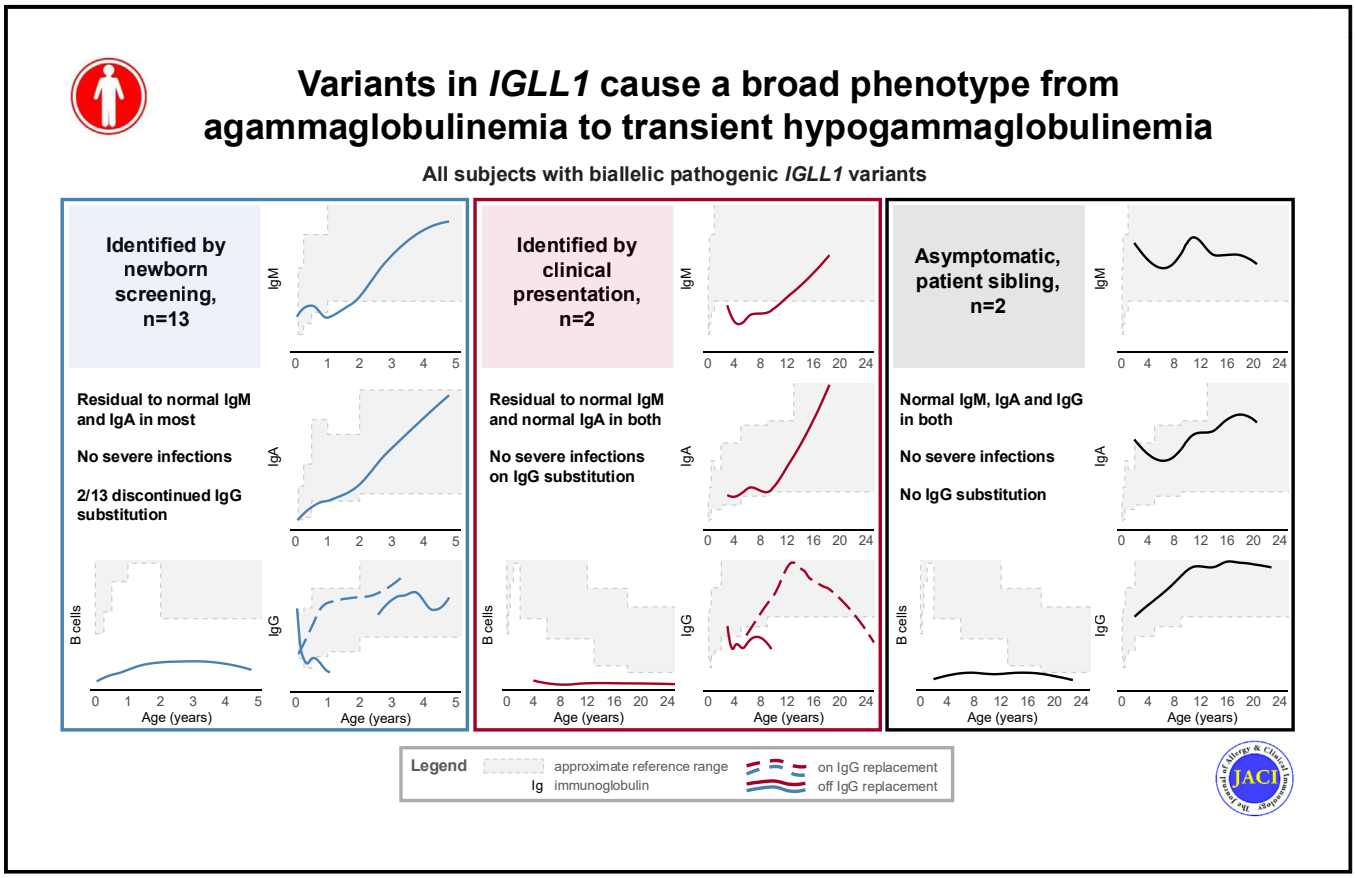
Variants in *IGLL1* cause a broad phenotype from agammaglobulinemia to transient hypogammaglobulinemia

Maarja Soomann, MD, Viktor Bily, MSc, Magdeldin Elgizouli, MBBS, PhD, Dennis Kraemer, MSc, Gülfirde Akgül, PhD, Horst von Bernuth, MD, PhD, et al

GRAPHICAL ABSTRACT



Capsule summary: Congenital B-cell deficiency, attributed to pathogenic *IGLL1* variants, is more prevalent than previously assumed. Despite exhibiting low B-cell counts, such patients manifest a milder clinical and immunologic phenotype than reported to date.

J Allergy Clin Immunol 2024;154:1313-24.

https://doi.org/10.1016/j.jaci.2024.08.002

Variants in *IGLL1* cause a broad phenotype from agammaglobulinemia to transient hypogammaglobulinemia

Maarja Soomann, MD,[a] Viktor Bily, MSc,[b] Magdeldin Elgizouli, MBBS, PhD,[c] Dennis Kraemer, MSc,[c] Gülfirde Akgül, PhD,[c] Horst von Bernuth, MD, PhD,[d,e,f,g] Markéta Bloomfield, MD, PhD,[h] Nicholas Brodszki, MD, PhD,[i] Fabio Candotti, MD,[j] Elisabeth Förster-Waldl, MD,[k] Tomas Freiberger, MD, PhD,[b] Maria Giżewska, MD, PhD,[l] Adam Klocperk, MD, PhD,[h] Uwe Kölsch, MD,[f] Kim E. Nichols, MD,[m] Renate Krüger, MD,[d] Ninad Oak, PhD,[m] Małgorzata Pac, MD, PhD,[n] Seraina Prader, MD,[a] Kjeld Schmiegelow, MD,[o,p] Anna Šedivá, MD, PhD,[h] Georgios Sogkas, MD, PhD,[q] Anna Stittrich, PhD,[r] Ulrik Kristoffer Stoltze, MD, PhD,[p] Katerina Theodoropoulou, MD, PhD,[s] Karin Wadt, MD, PhD,[o,t] Melanie Wong, MBBS, PhD,[u] Maximillian Zeyda, PhD,[v] Jana Pachlopnik Schmid, MD, PhD,[a] and Johannes Trück, MD, DPhil[a]

Berlin and Hannover, Germany; Brno and Prague, Czechia; Copenhagen, Denmark; Lausanne and Zurich, Switzerland; Lund, Sweden; Memphis, Tenn; Sydney, Australia; Szczecin and Warsaw, Poland; and Vienna, Austria

Background: Agammaglobulinemia due to variants in *IGLL1* has traditionally been considered an exceedingly rare form of severe B-cell deficiency, with only 8 documented cases in the literature. Surprisingly, the first agammaglobulinemic patient identified by newborn screening (NBS) through quantification of kappa-deleting recombination excision circles harbored variants in *IGLL1*.

Objective: We comprehensively reviewed clinical and immunologic findings of patients with B-cell deficiency attributed to variants in *IGLL1*.

Methods: NBS programs reporting the use of kappa-deleting recombination excision circle assays, the European Society for Immunodeficiencies Registry, and authors of published reports featuring patients with B-cell deficiency linked to *IGLL1* variants were contacted. Only patients with (likely) pathogenic variants, reduced $CD19^+$ counts, and no alternative diagnosis were included.

Results: The study included 13 patients identified through NBS, 2 clinically diagnosed patients, and 2 asymptomatic siblings. All had severely reduced $CD19^+$ B cells ($< 0.1 \times 10^9$/L) at first evaluation, yet subsequent follow-up assessments indicated residual immunoglobulin production. Specific antibody responses to vaccine antigens varied, with a predominant reduction observed during infancy. Clinical outcomes were favorable with IgG substitution. Two patients successfully discontinued substitution therapy without developing susceptibility to infections and while maintaining immunoglobulin levels. The pooled incidence of homozygous or compound heterozygous pathogenic *IGLL1* variants identified by NBS in Austria, Czechia, and Switzerland was 1.3:100,000, almost double of X-linked agammaglobulinemia.

Conclusion: B-cell deficiency resulting from *IGLL1* variants appears to be more prevalent than initially believed. Despite markedly low B-cell counts, the clinical course in some patients may be milder than reported in the literature so far. (J Allergy Clin Immunol 2024;154:1313-24.)

Key words: *Agammaglobulinemia,* IGLL1*, lamba5, B-cell deficiency, newborn screening, NBS, KREC, kappa-deleting recombination excision circles, predominantly antibody deficiencies, vaccine response*

From [a]the Division of Immunology and the Children's Research Center, University Children's Hospital Zurich, University of Zurich, Zurich; [b]the Molecular Genetics Laboratory, Centre for Cardiovascular Surgery and Transplantation Brno and Medical Faculty, Masaryk University, Brno; [c]the Institute of Medical Genetics, University of Zurich, Zurich; [d]Charité–Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, Department of Pediatric Respiratory Medicine, Immunology, and Critical Care Medicine, University Hospital Center, Berlin; [e]the Berlin Institute of Health at Charité–Universitätsmedizin Berlin, Berlin; [f]the Department of Immunology, Labor Berlin–Charité Vivantes GmbH, Berlin; [g]Charité–Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Berlin-Brandenburg Center for Regenerative Therapies, Berlin; [h]the Department of Immunology, 2nd Faculty of Medicine, Charles University and University Hospital in Motol, Prague; [i]the Childrens' Hospital, Skåne University Hospital, Lund; [j]the Division of Immunology and Allergy, Lausanne University Hospital and University of Lausanne, Lausanne; [k]the Department of Paediatrics and Adolescent Medicine, Division of Neonatology, Neuropaediatrics, and Paediatric Intensive Care and Center for Congenital Immunodeficiencies and Jeffrey Modell Diagnostic & Research Center, Medical University of Vienna, Vienna; [l]the Department of Pediatrics, Endocrinology, Diabetology, Metabolic Diseases, and Cardiology of the Developmental Age, Pomeranian Medical University in Szczecin, Szczecin; [m]the Department of Oncology, St Jude Children's Research Hospital, Memphis; [n]the Department of Immunology, The Children's Memorial Health Institute, Warsaw; [o]the Department of Clinical Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen; [p]the Department of Pediatrics and Adolescent Medicine, Rigshospitalet, Copenhagen; [q]the Department of Rheumatology and Immunology, Hannover Medical University, and Hannover Medical School, Hannover; [r]the Department of Human Genetics, Labor Berlin–Charité Vivantes GmbH, Berlin; [s]the Unit of Pediatric Immunology, Allergology and Rheumatology, Department of Woman, Mother, Child, Lausanne University Hospital and University of Lausanne, Lausanne; [t]the Department of Clinical Genetics, University Hospital Copenhagen, Copenhagen; [u]the Department of Allergy and Immunology, The Children's Hospital at Westmead, Sydney; and [v]the Department of Pediatrics and Adolescent Medicine, Austrian Newborn Screening, Clinical Division of Pediatric Pulmonology, Allergology and Endocrinology, Comprehensive Center for Pediatrics, Medical University of Vienna, Vienna.

Received for publication May 22, 2024; revised August 7, 2024; accepted for publication August 8, 2024.

Available online August 13, 2024.

Corresponding author: Maarja Soomann, MD, Division of Immunology, University Children's Hospital Zurich, Steinwiesstrasse 75, 8032 Zurich, Switzerland. E-mail: Maarja.Soomann@kispi.uzh.ch.

The CrossMark symbol notifies online readers when updates have been made to the article such as errata or minor corrections

0091-6749

https://doi.org/10.1016/j.jaci.2024.08.002

Abbreviations used

ALL: Acute lymphoblastic leukemia
B-ALL: Precursor B-cell ALL
CD: Cluster of differentiation
gnomAD: Genome Aggregation Database (https://gnomad.broadinstitute.org)
Hib: *Haemophilus influenzae* serotype b
ICOPE/STAGING: Interregional Childhood Oncology Precision medicine Exploration and Sequencing Tumor and Germline DNA—Implications for National Guidelines
IGLL1: Immunoglobulin lambda-like polypeptide 1
IgRT: Immunoglobulin replacement therapy
KREC: Kappa-deleting recombination excision circle
NBS: Newborn screening
PC: Pneumococcus
TdT: Terminal deoxynucleotidyl transferase
TREC: T-cell receptor excision circles
TT: Tetanus toxoid
XLA: X-linked agammaglobulinemia

Pathogenic variants in immunoglobulin lambda-like polypeptide 1 *(IGLL1)* were identified almost 25 years ago as causative in a rare form of autosomal recessive agammaglobulinemia.[1] In the first described patient, B cells constituted only 0.06% of peripheral blood lymphocytes. Notably, the bone marrow of this patient lacked mature, cluster of differentiation 19 (CD19), and surface IgM–positive cells. Analysis indicated a normal fraction of terminal deoxynucleotidyl transferase (TdT) positive and cytosolic or surface IgM-negative cells (TdT^+IgM^-), alongside low fractions of TdT^-IgM^+ cells. This was initially interpreted as indicative of a differentiation block between the pro-B and pre-B stages,[1] but current classification suggests a possible block between the pre–B I and pre–B II stages.[2] Despite this groundbreaking discovery, limited data exist on the exact immunologic phenotype of individuals with pathogenic variants in *IGLL1,* with only a handful of cases reported since its initial description in the literature.[1,3-8] Sequencing *IGLL1* is challenging because of its highly homologous pseudogenes *IGLL3P* and *IGLL5*. The most common known pathogenic variant, c.425C>T, is likely to have arisen as a result of a gene-conversion event between *IGLL3P* and *IGLL1* (see Fig E1, *A,* in this article's Online Repository available at www.jacionline.org).[1] Symptomatic patients often sought care for severe bacterial infections in infancy, and they universally had markedly low B-cell numbers. Reported serum immunoglobulin levels were either undetectable or severely diminished.[1,3-5] Treatment typically involved immunoglobulin replacement therapy (IgRT), but descriptions of the subsequent clinical course remain sparse.[1,3-5] A detailed understanding of the disease's immunologic implications and clinical consequences is crucial, emphasizing the need for continued research to refine therapeutic strategies and enhance patient outcomes.

The initial widely adopted newborn screening (NBS) test for inborn errors of immunity involved quantifying T-cell receptor excision circles (TREC) from dried blood spots to screen for severe combined immunodeficiency.[9] Subsequently, combined assays enabling concurrent detection of severe defects in the early stages of B-cell development by quantifying kappa-deleting recombination excision circle (KREC) levels have been developed and are commercially available.[10] Initially, these combined tests were only used in regional screening programs for brief periods.[11-14] However, these programs, involving relatively small screened populations, failed to identify any patients with agammaglobulinemia.[11-14] In recent years, combined TREC/KREC assays have been implemented in larger populations across various European countries, Japan, and Australia, resulting in the identification of patients with agammaglobulinemia.[6,15] The first patient reported to be identified with agammaglobulinemia through NBS did not have the expected common type, X-linked agammaglobulinemia (XLA),[16] but rather harbored known pathogenic variants in *IGLL1*.[6] Given the limited knowledge about this condition to date, managing these patients and providing counsel to affected families pose significant challenges.

Our objective was to compile a case series encompassing as many patients with *IGLL1*-associated B-cell deficiency as possible, aiming to deliver a comprehensive clinical, immunologic, and genetic description of this condition.

METHODS

We reached out to all NBS programs that used or reported the use of a combined TREC and KREC assay for NBS as of August 2022.[6,12,15,17] Additionally, we contacted the European Society for Immunodeficiencies Registry[18] to connect with clinicians caring for patients with *IGLL1* variants reported to the registry by August 2022; we also contacted the authors of all papers documenting *IGLL1*-related immunodeficiency up to August 2022.[1,3-8] We used custom forms to gather comprehensive information on individual patients, including screening results, clinical and laboratory findings, and prophylactic and therapeutic measures. All data were collected and transferred in anonymized form.

Inclusion criteria required patients to meet all the following: (1) biallelic or at least 2 different variants (if segregation analysis was not possible) in *IGLL1* classified as pathogenic according to the 2015 American College of Medical Genetics and Genomics;[19] (2) diminished $CD19^+$ count; and (3) no alternative diagnosis explaining the reduced $CD19^+$ count or the clinical presentation. Patients not meeting all 3 criteria were excluded.

Initial genetic analyses were predominantly conducted in certified local diagnostic laboratories by whole exome sequencing or targeted panel sequencing for inborn errors of immunity. For 3 patients (patients C1, C2, and S1), the primary analysis was research based, with details previously published.[4,8] Sequences were compared with GenBank reference sequences NM_020070.4 and NP_064455.1, and variant nomenclature followed Human Genome Variation Society guidelines. For compound heterozygous variants including c.425C>T, located in a highly homologous region of *IGLL1,* and pseudogenes *IGLL3P* and *IGLL5,* their location in *IGLL1* was confirmed through long-range PCR with primers designed to bind solely to *IGLL1* and Sanger sequencing, with one exception (patient N8).

Mitochondrial haplogroups were determined by HaploGrep 2.4.0[20] in patients with whole exome data available and at least one of the 2 recurrent variants; mtDNA Haplotree[21] was used to infer their countries of origin. Evolutionary age was estimated by the Genealogical Estimation of Variant Age method[22] using the Atlas of Variant Age of the Human Genome Dating database.[23]

In a subgroup of patients, we investigated somatic reversion of their initial *IGLL1* variants in peripheral blood $CD19^+$ B cells. $CD19^+$ and $CD3^+$ cells were separated from peripheral blood mononuclear cells by fluorescence-activated cell sorting. Subsequently, long-distance PCR and Sanger sequencing were conducted on the regions containing the original variants in all 3 cell populations.

Data were analyzed by R v4.2.2[24] incorporating the packages listed in Table E1 in the Online Repository available at www.jacionline.org. Continuous variables are presented with median, minimum, and maximum values.

The study adhered to the tenets outlined in the Declaration of Helsinki. Ethical approval was obtained from the relevant authorities in each country (Cantonal Ethics Commission of Zurich 2016-02280 and 2022-01029; Ethics Committee at the Pomeranian Medical University EA2/119/18; Ethics Committee of the University Medicine Charité EA2/119/18). Informed consent was obtained from patients or parents according to local protocols.

RESULTS

Twenty-five distinct patients were identified, and 17 patients from 15 families participated in the study (see Fig E2 in the Online Repository available at www.jacionline.org). Three patients did not meet the inclusion criteria (see Table E2 in the Online Repository), while outreach efforts to the respective physicians were unsuccessful in 4 cases despite multiple attempts over the course of 1 year. Additionally, one patient's family declined participation.

Among the participants, the majority (13/17, 76%) were diagnosed following NBS demonstrating low KREC levels. One patient was diagnosed as a result of a severe infection and another as a result of recurrent infections and bronchiectasis, and 2 asymptomatic individuals were identified via diagnosis of siblings.

Patients identified by NBS

The 13 patients diagnosed through NBS underwent follow-up for a median (range) duration of 16 (6-58) months. Notably, all had nonmeasurable or markedly low KREC levels shortly after birth, while their TREC levels were normal. These patients were born to healthy mothers in the 37th week of gestation or later after uneventful pregnancies, and they experienced uncomplicated perinatal periods. Importantly, none of the patients identified through NBS had any family history of inborn errors of immunity, and none was exposed to immunosuppressive drugs during pregnancy.

Immunophenotype

Confirmatory lymphocyte immunophenotyping was conducted at a median (range) age of 3 (2-18) weeks. All patients had either unmeasurable or very low $CD19^+$ B cells (median 0, range 0 to 0.06×10^9/L). In most patients, only a marginal increase in B-cell counts was observed during follow-up. However, B cells at least temporarily surpassed 0.1×10^9/L in 3 patients (Fig 1, *A*). Switched memory B cells ($CD27^+IgD^-$) were assessed during the follow-up period in 10 of 13 patients and were detectable in all but one, albeit with varying fractions (Fig 1, *D*).

Eleven of the 13 patients experienced transient neutropenia, mostly during the first 6 months of life (Fig 1, *E*). Concurrently, all patients displayed elevated platelet counts during the first 12 to 18 months of life (Fig 1, *F*).

In the initial investigations, all but one of the 13 patients had normal (maternally derived) IgG levels (Fig 1, *G,* and see Fig E2, *A*). Conversely, IgM levels were below local reference ranges in all patients except one (median 0.09 g/L, ranging from below the detection limit to 0.41 g/L), while IgA levels were consistently low in all patients (median below the detection limit, maximum 0.04 g/L) (Fig 1, *H* and *I,* and see Fig E2, *B* and *C,* in the Online Repository available at www.jacionline.org). In 2 patients, IgRT was initiated shortly after confirming the absence of peripheral blood B cells. For 2 other patients, this decision followed a clear trend in the reduction of IgG levels without them falling below the age-appropriate range. In the remaining 10 patients, IgG levels gradually declined with age, dropping below the local age-appropriate reference range at a median (range) age of 4 (2-6) months (see Fig E3, *A,* in the Online Repository). There was a positive correlation between IgG levels at 1 month of age and the age at which IgG fell below the age-appropriate reference range (Pearson $R = 0.74$, $P = .04$, Fig E3, *D*). Throughout the follow-up period, all patients demonstrated some IgA production, with levels reaching the age-appropriate reference range in all patients but one aged at least 18 months (Fig 1, *I*). All patients except one showed transient spikes of IgM production, but only one had persistently normal levels during the follow-up period (Fig 1, *H*).

Eight patients were vaccinated before initiating IgRT (Table I). For 5 children, specific antibody levels against tetanus toxoid (TT), *Haemophilus influenzae* serotype b (Hib), and/or pneumococcal (PC) polysaccharides were measured at multiple time points, while only single measurements were available for 2 children before commencing IgRT (Fig 1, *J* and *K*). In cases with longitudinal data, no evident and sustained specific antibody response was observed before initiating IgRT. One child showed a transient increase in anti-Hib IgG levels after the second dose of the primary vaccination series, but these levels quickly declined thereafter.

In 2 patients, IgRT was discontinued at ages 2.5 years (patient N7) and 3.5 years (patient N3). Subsequent immunization, according to local catch-up schedules, demonstrated robust specific antibody responses, with anti-TT IgG of 2540 IU/L and anti-PC IgG of 169.3 mg/L in patient N3 and anti-TT IgG of 1400 IU/L and levels >0.3 mg/L against 12 of 13 of the Prevnar 13 serotypes in patient N7. All vaccinations were tolerated well.

Immunophenotyping results from a bone marrow sample were available for one child (patient N4) at 2 years of age. The analysis revealed a normal fraction of the lymphoid line ($CD45^+$ SSClow 22.2%; reference range, 19.9% to 39.7%). Notably, there were increased fractions of T-cell and natural killer cell precursors, alongside a decreased fraction of B-cell precursors ($CD79^+$ 14.0%; reference range, 35.2% to 65.3%). Additionally, there was an elevated fraction of pre–B cells ($CD19^-TdT^+CD34^+$ 8.8%; reference range, 0.3% to 3.5%), an increased pre–B I fraction ($TdT^+CD34^+CD10^+/CD19^+$ 65.9%; reference range, 4.5% to 11.1%), and a decreased pre–B II fraction ($cIgM^+sIgM^-/CD19^+$ 9.5%; reference range, 21.8% to 30.1%), collectively indicating a partial differentiation block between the pre–B I and pre–B II differentiation stages.

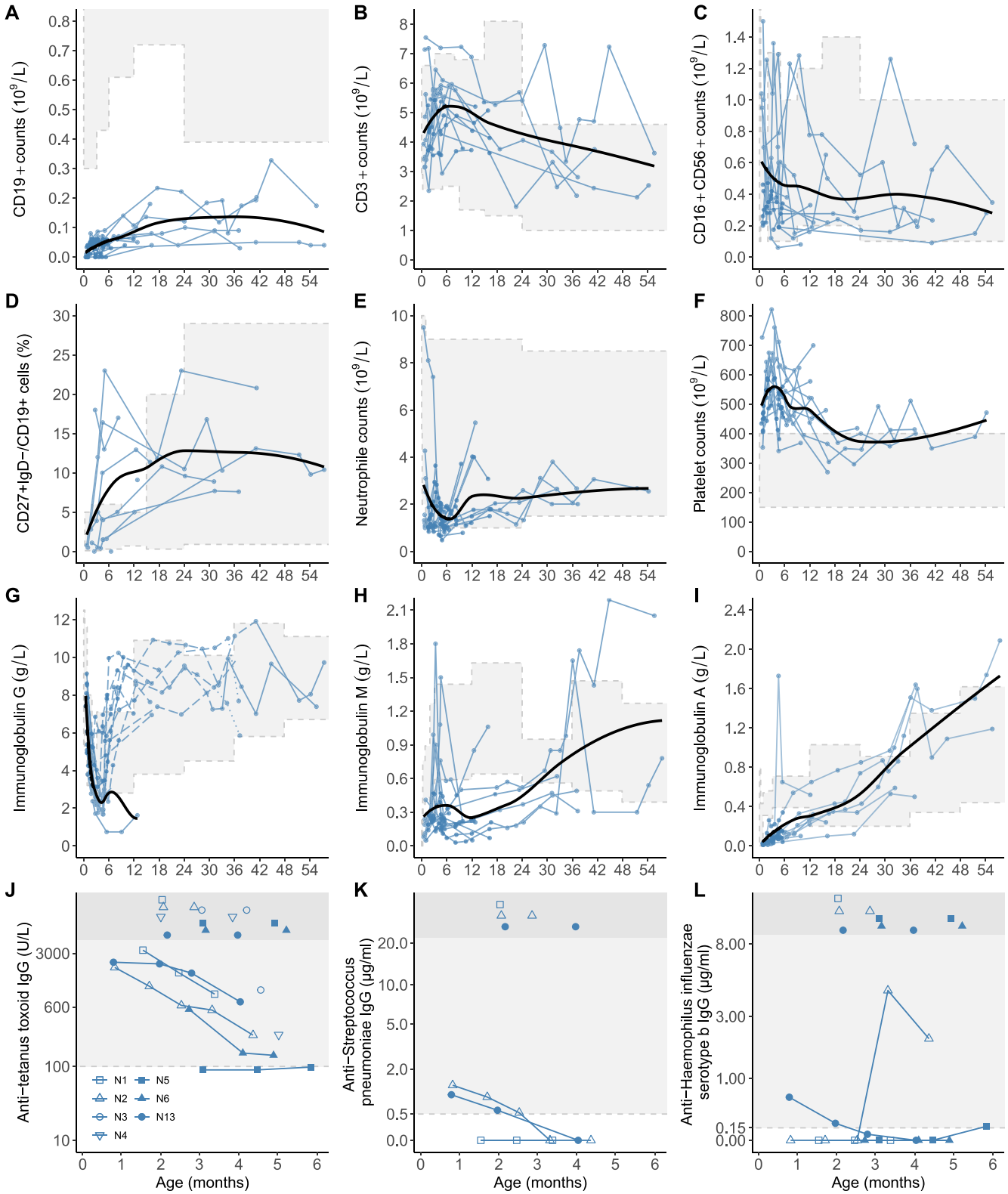


FIG 1. Immunologic phenotype of patients identified with NBS. Natural development of peripheral blood $CD19^+$ **(A)**, $CD3^+$ **(B)**, and $CD16^+CD56^+$ **(C)** lymphocyte counts, fraction of switched memory B ($CD27^+IgD^-/CD19^+$) cells **(D)**, neutrophilic granulocyte **(E)** and platelet **(F)** counts, IgG **(G)**, IgM **(H)**, and IgA **(I)** concentrations, and concentrations of specific antibodies to tetanus **(J)**, pneumococci **(K)**, and Hib **(L)**. *Light gray* areas represent age-appropriate reference ranges. IgG concentrations measured during immunoglobulin substitution are indicated by *dashed lines* and during dose reduction attempt by *dotted line.* Timing of individual vaccine doses is indicated in *dark gray area* of **(J)** to **(L)** with respective patient symbol. In **(J)** to **(L)**, logarithmic scale is used for "years" axis.

TABLE I. Characteristics of patients identified by NBS

Characteristic	Patient no.					
	N1	N2	N3	N4	N5	N6
General characteristics						
Age at immunologic diagnosis (weeks)	3	3	18	3	4	2
Gestational age (weeks), birth weight (g)	39-3/7, 3860	40-2/7, 2750	39-4/7, 2810	40, 3740	41-1/7, 4100	41-0/7, 3680
Sex	M	M	M	F	F	M
Clinical picture						
Infection	Mild URTI	Mild URTI	Mild GI and URTI	—	—	Mild GI and URTI
Atopy	Moderate AD, 3 months	Mild AD, 17 months	Mild AD, 3 years	—	Mild/moderate AD, 5 months	—
Autoimmunity	—	—	—	—	—	—
Malignancy	—	—	—	—	—	—
Syndromic features	—	—	Duplex kidney	—	—	—
Immunophenotype $CD19^+$; $CD3^+$; $CD16^+56^+$ cells ($\times 10^9$/L) at:						
Diagnosis	0.00; 4.76; 0.20	0.00; 3.06; 1.50	0.03; 4.17; 0.19	0.03; 3.97; NA	0.04; 3.97; 1.00	0.00; 3.83; 1.04
Age 5-8 months	0.06; 5.66; 0.12	0.07; 5.86; 1.23	NA	0.09; 5.99: 0.28	0.04; 4.47; 0.58	0.07; 3.59; 0.52
Age 12-18 months	0.08; 3.66; 0.24	0.08; 3.53; 0.50	NA	0.23; 5.17; 0.28	0.10; 4.55; 0.19	0.06, 3.63; 0.16
Age 30-42 months	0.03; 2.71; 0.23	0.09; 2.08; 0.72	0.05; 2.34; 0.09	0.12; 2.38; 0.32	NA	NA
Maximum B cells ($\times 10^9$/L), age	0.10, 2 0/12 years	0.09, 2 7/12 years	0.05, 3 5/12 years	0.23, 1 6/12 years	0.18, 1 4/12 months	0.07, 8/12 years
Genetics						
Age at genetic diagnosis (months)	2	1	6	3.5	1	3
Method	Trio-WES, Sanger	Trio-WES, Sanger	WES	Gene panel, Sanger	Gene panel, Sanger	Gene panel, Sanger
Zygosity	Compound heterozygous	Compound heterozygous	Homozygous	Homozygous	Compound heterozygous	Compound heterozygous
Variants (NM_020070.4)	c.258del pat; c.425C>T mat	c.258del mat; c.425C>T pat	c.425C>T	c.425C>T	c.258del mat; c.425C>T pat; del incl exons 1-2 pat	c.258del pat; c.425C>T mat
Protein	p.(Gln88Asnfs*7); p.(Pro142Leu)	p.(Gln88Asnfs*7); p.(Pro142Leu)	p.(Pro142Leu)	p.(Pro142Leu)	p.(Gln88Asnfs*7); p.0?	p.(Gln88Asnfs*7); p.(Pro142Leu)
Therapy						
Age at IgRT initiation (months)	3.5	4.5	5	6	6	5
Maximum dose (g/kg) (per 4 weeks)	0.6	0.6	0.5	0.3	0.4	0.4
Vaccinations before IgRT						
Schedule	National until IgRT	Adjusted until IgRT	National until IgRT	National until IgRT	Adjusted until IgRT	Adjusted until IgRT
Inactivated vaccines	1 DTaP-IPV-Hib-HBV, 1 PCV	2 DTaP-IPV-Hib-HBV, 2 PCV	2 DTaP-IPV-Hib-HBV	3 DTaP-IPV-Hib-HBV, 3 PCV	2 T, 2 Hib	2 T, 2 Hib
Live vaccines	—	—	Rotavirus	1 BCG	—	—
Follow-up						
Age at last follow-up (years)	3-1/12	3-1/12	4-10/12	3-5/12	1-4/12	1-0/12
Previously reported	No	No	Yes[6]	No	No	No

AD, Atopic dermatitis; *ASD II,* type II atrial septal defect; *DTaP,* diphtheria, tetanus, and acellular pertussis; *GI,* gastrointestinal; *HBV,* hepatitis B virus; *IPV,* inactivated polio vaccine; *mat,* maternal; *NA,* not applicable; *pat,* paternal; *PCV,* pneumococcal conjugate vaccine; *T,* tetanus; *URTI,* presumably viral upper respiratory tract infection; *WES,* whole exome sequencing.

Patient no.						
N7	**N8**	**N9**	**N10**	**N11**	**N12**	**N13**
4	3	4	2.5	3	8	3.5
37, 2940	40, 2500	40-5/7, 3225	40-3/7, 3630	37-2/7, 3650	36-6/7, 2070	38-2/7, 2990
F	F	M	M	F	M	F
Mild URTI	—	Mild URTI	Mild URTI	Mild URTI	Mild URTI	Mild URTI
—	Moderate AD, 2 months	—	—	—	—	—
—	—	—	—	—	—	—
—	B-ALL	—	—	—	—	—
—	—	—	—	ASD II	—	—
0.04; 7.45; 0.70	0.00; 3.40; 0.26	0.00; 5.52; 0.42	0.01; 3.32; 0.95	0.00; 7.04; 0.22	0.06; 5.35; 0.35	0.00; 3.06; 0.46
0.14; 7.13; 1.28	0.00; 4.80; 0.31	0.07; 5.13; 0.22	0.03; 5.18; 0.38	0.04; 3.68; 0.08	0.06; 5.44; 0.34	0.06, 5.27; 0.47
0.11; 6.79; 0.78	0.04; 4.04; 0.22	0.05; 4.28; 0.33	NA	NA	0.05; 5.1; 0.22	NA
0.17; 4.67; 0.19	NA	NA	NA	NA	NA	NA
0.33, 3 9/12 years	0.04, 1 4/12 years	0.07, 5 months	0.03, 7 months	0.08, 2 months	0.06, 2 months	0.06, 5 months
4	1	2	4.5	1	2.5	1.5
Gene panel	Gene panel	Trio-WES, WGS	Gene panel, Sanger	Trio-WES	Trio-WES	Trio-WES
Compound heterozygous	Allelic phase unknown	Homozygous	Compound heterozygous	Compound heterozygous	Homozygous	Homozygous
c.258del; c.425C>T	c.258del; c.425C>T	200 bp del of exon 3	c.258del pat; c.425C>T mat	c.425C>T mat; 61 kb del incl exons 1-2 pat	c.258del	c.258del
p.(Gln88Asnfs*7); p.(Pro142Leu)	p.(Gln88Asnfs*7); p.(Pro142Leu)	p.?	p.(Gln88Asnfs*7); p.(Pro142Leu)	p.(Pro142Leu)pp.?	p.(Gln88Asnfs*7);	p.(Gln88Asnfs*7);
1	1.5	2	3.5	4.5	refused by parents	4.5
0.4	0.4	0.6	0.4	0.4	NA	0.5
—	—	—	National until IgRT	—	—	National until IgRT
—	—	—	1 DTaP-IPV-Hib-HBV, 1 PCV	Refused by parents	Refused by parents	2 DTaP-IPV-Hib-HBV, 2 PCV
—	—	—	—	—	—	—
4-8/12	3-8/12	1-6/12	8/12	10/12	1-1/12	6/12
No	No	No	No	No	No	No

Genetic findings

Genetic analyses were conducted in all children after confirmation of the absence or near absence of peripheral blood B cells. Ten (77%) of 13 children carried either homozygous or compound heterozygous variants in *IGLL1* previously reported as pathogenic (Table I). One patient was compound heterozygous for a known pathogenic variant alongside a large deletion spanning exons 1 and 2. Additionally, another patient harbored a homozygous deletion of a 200 bp segment within exon 3, covering a region of a known pathogenic variant, and was thus considered likely causative. Furthermore, one patient (patient N8) had 2 previously described pathogenic variants, but segregation analysis assessing the allelic phase was declined by the patient's parents.

Clinical course

All patients except one (patient N12) received IgRT. No severe infections were reported before or during IgRT throughout the entire follow-up period. One family declined IgRT for their child, who, at last follow-up at 13 months of age, showed severe hypogammaglobulinemia (IgG 1.6 g/L) alongside persistently nearly absent B cells (0.05×10^9/L) but had only experienced mild, likely viral upper respiratory tract infections by then.

In the case of the child with the most significant B-cell recovery and persistent IgM normalization (patient N7), IgRT was stopped at the age of 2.5 years. Over the subsequent 2 years, this child maintained age-appropriate IgG levels and remained clinically stable. Another patient (patient N3), receiving 0.4 g/kg subcutaneous IgRT monthly resulting in very high IgG levels, had IgRT discontinued at age 3.5 years. After cessation, IgG levels initially decreased, but they remained within the age-appropriate range. Dose reduction of IgRT in patient N1 from 0.5 to 0.2 mg/kg per month led to a considerable drop in IgG levels (Fig 1, *G*).

One patient (patient N8) was diagnosed with precursor B-cell ALL (B-ALL) at 1.5 years of age after generalized petechiae were observed during a routine intravenous IgRT appointment. Cytogenetic analyses revealed 2 clones, one with partial monosomy 9p involving CDKN2A and partial monosomy 13q, and another with a partial loss of CDKN2A and monosomy 13. Treatment according to the 2017 AIEOP BFM ALL (Associazione Italiana di Ematologia e Oncologia Pediatrica Berlin–Frankfurt–Münster ALL) protocol led to remission at last follow-up, 26 months after B-ALL diagnosis.

Clinically diagnosed patients

Two patients (patients C1 and C2) underwent immunologic investigations prompted by infections—a severe varicella infection in patient C1, and recurrent respiratory and gastrointestinal infections in patient C2. Both had notably low B-cell counts and reduced IgG levels (Fig 2, *A* and *G-I*). Despite receiving vaccinations according to the local schedule, patient C1 experienced vaccine responses below the detection limit for Hib. In the initial measurement, there was a high level of anti-TT IgG, which rapidly declined, with no subsequent increase even after receipt of age-appropriate booster vaccination (Fig 2, *J-L*). IgRT was initiated at age 6. Apart from an episode of streptococcal tonsillitis at the age of 12 years, the patient has remained free of bacterial infections and has never required intravenous antibiotics. Retrospective dried blood spot analysis from the first week of life in patient C1 showed nearly undetectable KREC levels. In patient C2, vaccine responses were not tested. Despite initiating therapy with IgRT at 8 years of age, he developed bronchiectasis by age 13. Patient C2 also presented with additional clinical features (Table II). In addition *IGLL1* variants, patient C2 also harbored a heterozygous *GJB2* variant coding for connexin 26, associated with sensorineural hearing loss.

Asymptomatic siblings

Two patients (patients S1 and S2) were identified as a result of their siblings' diagnoses. Both had low B-cell numbers (Fig 2, *A*) but maintained age-appropriate levels of IgG, IgM, and IgA (Fig 2, *G-I*). Patient S1 had inadequately low levels of anti-TT IgG for age and vaccination history, but adequate specific antibody responses were recorded after Hib- and PC-containing booster vaccinations (Fig 2, *K* and *L*). Patient S1 had never experienced infections requiring antibiotic therapy. Patient S2 had only a single measurement available for anti-TT and anti-PC IgG, both adequate for age and vaccination history. Patient S2 experienced recurrent conjunctivitis from 1 to 3 months of age but had not required antibiotic therapy as of last follow-up at age 2. Retrospective analysis of dried blood spots obtained in the first week of life for both patients showed nearly undetectable KREC levels. Further details are listed in Table II.

Somatic reversion analysis

Somatic reversion analysis was conducted in 4 patients (patients N1, N3, N5, and N6), where peripheral blood $CD19^+$ B cells had reached at least 0.07×10^9/L. No reversions of the original variants were detected (Fig E1, *B*).

Haplotype analysis and relatedness

According to gnomAD v4.1.0 (Genome Aggregation Database),[25,26] the overall allele frequency of c.258del is 0.13% (0.16% in non-Finnish Europeans, 0.17% in Ashkenazi Jews, 0 to 0.03% in African/African American, East Asian, Finnish, Middle Eastern, and South Asian populations). For c.425C>T, the frequency is 0.09% (0.10% in non-Finnish Europeans, 0.30% in Ashkenazi Jews, with 0 to 0.02% in the other mentioned populations). Nine patients were included in a preliminary mitochondrial haplotype analysis, which suggested a potentially common origin for these 2 variants in Northern European populations (see Table E3 in the Online Repository available at www.jacionline.org), consistent with their frequencies in gnomAD. The evolutionary age of the c.425C>T variant can be estimated at approximately 8300 years, whereas the variant c.258del has not been annotated in the Atlas of Variant Age.

There was no family history suggesting relatedness between the families; nor was there evidence of maternal relatedness in the haplotype analysis (Table E3). Furthermore, there was no family history of parental consanguinity. In 11 patients with available whole exome data, no increased absence of heterozygosity was found.

Incidence

On the basis of data from national screening programs in Austria, Czechia, and Switzerland, the incidence of B-cell deficiency attributable to variants in *IGLL1* is conservatively estimated to be at least 1.3 per 100,000 births.

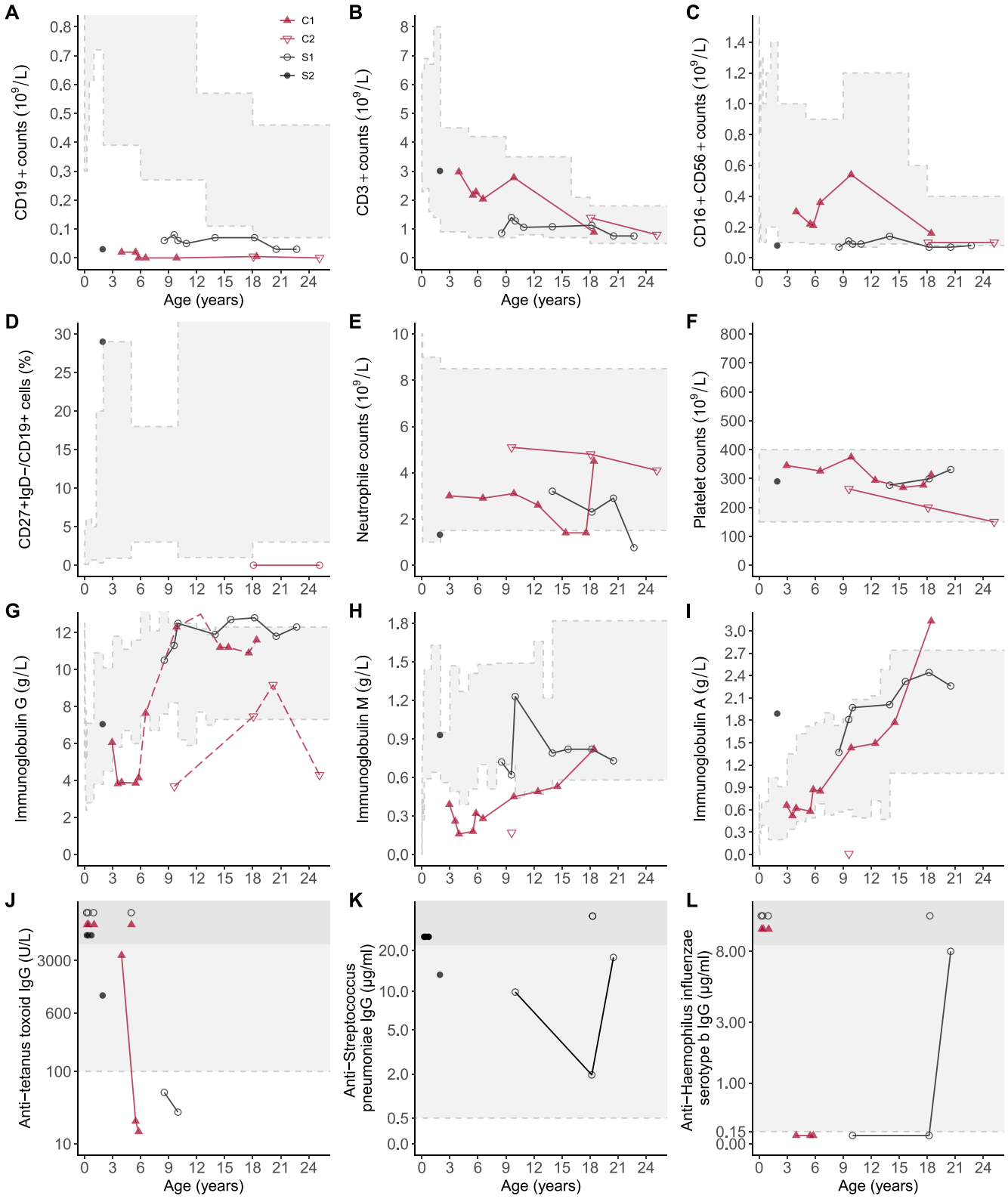


FIG 2. Immunologic phenotype of clinically diagnosed patients (patients C1 and C2, *red*) and asymptomatic but affected siblings (patients S1 and S2, *black*). Natural development of peripheral blood $CD19^+$ **(A)**, $CD3^+$ **(B)**, and $CD16^+CD56^+$ **(C)** lymphocyte counts, fraction of switched memory B ($CD27^+IgD^-/CD19^+$) cells **(D)**, neutrophilic granulocyte **(E)** and platelet **(F)** counts, IgG **(G)**, IgM **(H)**, and IgA **(I)** concentrations, and concentrations of specific antibodies to tetanus **(J)**, pneumococci **(K)**, and Hib **(L)**. *Light gray* areas represent age-appropriate reference ranges. Timing of individual vaccine doses is indicated in *dark gray area* of **(J)** to **(L)** with respective patient symbol. In **(J)** to **(L)**, logarithmic scale is used for "years" axis. In patient S1, cumulative concentration of anti-pneumococcal IgG against serotypes 6B, 19F, and 23F is provided. All vaccine antibody levels were measured in IgRT-naive patients.

TABLE II. Characteristics of clinically identified patients and asymptomatic siblings

Characteristic	Patient no. C1	C2	S1	S2
General characteristics				
Diagnosis pathway	Clinical	Clinical	Sibling of C1	Sibling of N10
Age (years) at immunologic diagnosis	4	8	8.5	2
Sex	F	M	F	M
Clinical picture				
Infections (age)	Prolonged varicella (2 years), tonsillitis (12 years)	Recurrent otitis media (1 years), gastroenteritis (3 years), bronchitis (4 years)	No bacterial infections	Conjunctivitis
Atopy	—	—	Moderate AD, 14 years	—
Autoimmunity				
Malignancy				
Complications	—	Bronchiectasis, conductive hearing loss	—	—
Other features	—	Exocrine pancreatic insufficiency, failure to thrive, unclear degenerative muscle disease and neuropathy	—	—
Genetics				
Age (years) at genetic diagnosis	6	15	10	2
Method	Gene panel	Gene panel	Gene panel	Gene panel, Sanger
Transcript	NA	NA	NA	NM_020070.4
Zygosity	Homozygous	Homozygous	Homozygous	Compound heterozygous
Variants (NM_020070.4)	c.258delG	c.258delG	c.258delG	c.258delG pat, c.425C>T mat
Protein	p.(Gln88Asnfs*7)§	p.(Gln88Asnfs*7)	p.(Gln88Asnfs*7)§	p.(Gln88Asnfs*7), p.(Pro142Leu)
Other findings		NM_004004.6(GJB2): c.487A>G p.(Met163Val) heterozygous		
Therapy				
Age (years) at initiation of IgRT	6	8	Not started	Not started
Maximum dose (g/kg) (per 4 weeks)	0.5	0.6	NA	NA
Vaccinations				
Schedule	National	No data	National	National
Inactivated vaccines	3 DTaP-IPV + Hib 2 DTaP-IPV 1 HPV yearly influenza	No data	3 DTaP-IPV + Hib 2 DTaP-IPV 1 PPSV23 and PCV13 1 Hib	3 DTaP-IPV + Hib 3 PCV13
Live vaccines	2 MMR	No data	2 MMR	1 MMR
Follow-up				
Age (years) at last follow-up	17	24	22	2.5
Previous report	Yes[4]	Yes[8]	Yes[4]	No

AD, Atopic dermatitis; *DTaP,* diphtheria, tetanus, and acellular pertussis; *HPV,* Human papillomavirus; *IPV,* inactivated polio vaccine; *mat,* maternal; *MMR,* measles, mumps, and rubella vaccine; *NA,* not applicable; *pat,* paternal; *PCV,* pneumococcal conjugate vaccine; *PPSV,* pneumococcal polysaccharide vaccine; *T,* tetanus.
§Current annotation; in first report, p.(Gly86fs*) was used.

Role in malignancy

Exploration of the ICOPE/STAGING (Interregional Childhood Oncology Precision medicine Exploration and Sequencing Tumor and Germline DNA—Implications for National Guidelines) and the Pediatric Cancer Genome Project/Genomes for Kids/St Jude Lifetime Cohort (SJLIFE) and Real-time Clinical Genomics databases revealed no cases of homozygous or compound heterozygous germline variants in *IGLL1* in about 380 and 2800 cases of pediatric ALL. There was a 11.0-fold enrichment for c.425C>T in the ICOPE/STAGING B-ALL cohort (see Table E4 in the Online Repository available at www.jacionline.org). No enrichment was observed for c.258delG or for all other rare, likely damaging *IGLL1* variants (gnomAD v4.1.0 allele frequency <0.5%, Combined Annotation Dependent Depletion score >20, and/or Rare Exome Variant Ensemble Learner score >0.5).

Conclusions

We present an extensive clinical and immunophenotypic characterization of the largest cohort to date of patients with B-cell deficiency attributable to *IGLL1* variants. Our cohort showed

a distinct cellular phenotype characterized by markedly low to absent peripheral blood $CD19^+$ B cells in early life—a crucial factor in their NBS-based diagnosis. Despite consistently low total B-cell counts, most patients experienced a progressive ability to produce IgM and IgA as they aged, with IgA levels reaching age-appropriate levels in most patients by 18 months, and almost all showing a trend toward normal IgM concentrations. Notably, 2 patients maintained age-appropriate IgG levels after discontinuation of IgRT, suggesting the potential for normal IgG production in some individuals.

The evaluation of vaccine responses was limited in our series, but it showed no clear increase in specific antibody concentrations against vaccine antigens in almost all patients with multiple pre-IgRT measurements in infancy. The lack of response underscores the presence of a humoral immunodeficiency in these children. However, the mechanism behind the variability of the immunologic phenotype remains unclear.

A notable finding in our cohort is the prevalence of mild to borderline neutropenia in the majority of patients, a feature not previously described in this population but reminiscent of a phenomenon observed in XLA,[27,28] affecting 4% to 26% of XLA patients.[27,29-32] Although the mechanisms remain unclear,[33] the resolution of neutropenia after IgRT initiation aligns with observations in XLA cohorts.[27,29,34]

Given the relatively short follow-up period, the long-term development of the immune system in these toddlers remains unknown. The apparent milder phenotype, coupled with the discrepancy between reported cases and estimated incidence, which is almost double that of XLA,[32,35] raises questions about the awareness, genetic testing practices, and potential underdiagnosis of B-cell deficiencies due to *IGLL1* variants. Notably, in a recent UK agammaglobulinemia cohort including 139 patients, 20% lacked a genetic diagnosis, and not a single case due to *IGLL1* variants was reported.[36] Moreover, in one of the largest genetic studies investigating causes of predominantly antibody deficiencies,[37] *IGLL1* was analyzed in only about 40% of the patients as a result of panel size restrictions (B. Grimbacher, personal communication).

The identification of a patient with B-ALL prompts further exploration of a potential pathogenetic link. Previous studies on B lymphocytic leukemia cells have shown changes in *IGLL1* expression in certain subtypes of the disease, suggesting a plausible connection. Notably, high *IGLL1* expression in tumor tissue has been correlated with a favorable prognosis.[38] Exploration of ICOPE/STAGING[39-41] and samples from available datasets at St Jude Children's Research Hospital for the variants present in the B-ALL patient showed conflicting results. There was enrichment for c.425C>T in the smaller dataset but not in the larger, but this was not the case for c.258del or for all likely damaging variants. Unfortunately, comprehensive somatic variant data were unavailable in these cohorts. The contribution of germline *IGLL1* variants to the development of malignancy in the patient with B-ALL in our cohort remains therefore uncertain, but the role of *IGLL1* in the pathogenesis of ALL merits further research in the future.

This study had some limitations. Despite its being the biggest cohort to date, it still included a limited number of patients with a relatively short follow-up period. Most patients initiated IgRT at a very early age, which might have avoided the development of clinical symptoms. In addition, because the study was conducted retrospectively, not all data could be obtained for all participants. This study also had several strengths. Because most of the patients were detected by NBS, the bias toward reporting more severe cases was likely quite low. In addition, despite the retrospective nature of this study, it was possible to obtain detailed genetic and longitudinal immunophenotyping data in most patients, allowing for a detailed evaluation.

Our findings challenge initial perceptions by showing a milder immunologic and clinical phenotype in patients with *IGLL1* variants than previously thought. Despite persistently low B-cell counts, many exhibited substantial immunoglobulin production and vaccine response as they aged. Ongoing follow-up is needed to understand the full extent and variability of this condition. Notably, clinicians should consider screening for *IGLL1* variants in older patients with predominantly antibody deficiencies, as the presentation may extend beyond early childhood.

DISCLOSURE STATEMENT

Funding was provided by the Georg and Bertha Schwyzer-Winiker Foundation, the Wolfermann-Nägeli Foundation, and the EMDO Foundation (to M.S. and J.T.). Funding was also provided by the Swiss National Science Foundation (320030_205097) and ITINERARE, a University Research Priority Program of the University of Zurich, Zurich, Switzerland (to J.P.S.). Funding was also received from the Jeffrey Modell Foundation and the Fund for the Support of Strategic Partnerships between Charles University in Prague, Czechia, and the University of Zurich (to M.B., A.K., and A.S.). Funding was also received from the Ministry of Health, Czechia (NU23-05-00097 to A.K.; and NU-007-00170 to V.B. and T.F.), and Masaryk University, Czechia (MUNI/A/1566/2023 to V.B. and T.F.). The funding agencies did not influence the study design; the collection, analysis, or interpretation of data; the writing of the report; or the decision to submit the report for publication.

Disclosure of potential conflict of interest: The authors declare that they have no relevant conflicts of interest.

We thank all patients and families for their participation. In addition, we thank the European Society for Immunodeficiencies Registry, particularly Gerhard Kindle for putting us in contact with physicians of patients listed in the registry. We thank Sara Şebnem Kiliç Gültekin from Bursa Uludağ University, Bursa, Turkey, and Maria Paula Martinez from Hospital de Niños Dr Ricardo Gutiérrez, Buenos Aires, Argentina, for discussing potential cases. We express our gratitude to Bodo Grimbacher and Andres Caballero Garcia de Oteyza from the Genetics and Genomics Unit of the Center for Chronic Immunodeficiency at the University Hospital Freiburg, Freiburg, Germany, for valuable discussions.

Clinical implication:

- **Patients with reduced B-cell counts resulting from pathogenic *IGLL1* variants should undergo a comprehensive immunologic examination before initiating IgG replacement therapy, with ongoing reassessment of therapeutic indications.**

REFERENCES

1. Minegishi Y, Coustan-Smith E, Wang YH, Cooper MD, Campana D, Conley ME. Mutations in the human λ5/14.1 gene result in B cell deficiency and agammaglobulinemia. J Exp Med 1998;187:71-7.
2. Wentink MWJ, Kalina T, Perez-Andres M, del Pino Molina L, IJspeert H, Kavelaars FG, et al. Delineating human B cell precursor development with genetically identified PID cases as a model. Front Immunol 2019;10:2680.

3. Conley ME, Dobbs AK, Farmer DM, Kilic S, Paris K, Grigoriadou S, et al. Primary B cell immunodeficiencies: comparisons and contrasts. Annu Rev Immunol 2009;27:199-227.
4. Moens LN, Falk-Sörqvist E, Asplund AC, Bernatowska E, Smith CIE, Nilsson M. Diagnostics of primary immunodeficiency diseases: a sequencing capture approach. PLoS One 2014;9:e114901.
5. Gemayel KT, Litman GW, Sriaroon P. Autosomal recessive agammaglobulinemia associated with an *IGLL1* gene missense mutation. Ann Allergy Asthma Immunol 2016;117:439-41.
6. 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.
7. Platt CD, Zaman F, Bainter W, Stafstrom K, Almutairi A, Reigle M, et al. Efficacy and economics of targeted panel versus whole-exome sequencing in 878 patients with suspected primary immunodeficiency. J Allergy Clin Immunol 2021;147:723-6.
8. Sogkas G, Dubrowinskaja N, Schütz K, Steinbrück L, Götting J, Schwerk N, et al. Diagnostic yield and therapeutic consequences of targeted next-generation sequencing in sporadic primary immunodeficiency. Int Arch Allergy Immunol 2022;183:337-49.
9. Routes JM, Grossman WJ, Verbsky J, Laessig RH, Hoffman GL, Brokopp CD, et al. Statewide newborn screening for severe T-cell lymphopenia. JAMA 2009;302:2465-70.
10. Nakagawa N, Imai K, Kanegane H, Sato H, Yamada M, Kondoh K, et al. Quantification of κ-deleting recombination excision circles in Guthrie cards for the identification of early B-cell maturation defects. J Allergy Clin Immunol 2011;128:223-5.e2.
11. 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;119:2552-5.
12. de Felipe B, Olbrich P, Lucenas JM, Delgado-Pecellin C, Pavon-Delgado A, Marquez J, et al. Prospective neonatal screening for severe T- and B-lymphocyte deficiencies in Seville. Pediatr Allergy Immunol 2016;27:70-7.
13. Son S, Kang JM, Kim JM, Sung S, Kim YS, Lee H, et al. The first newborn screening study of T-cell receptor excision circle and κ-deleting recombination excision circle for severe combined immunodeficiency in Korea: a pilot study. Pediatr Infect Vaccine 2017;24:134-40.
14. Nourizadeh M, Shakerian L, Borte S, Fazlollahi M, Badalzadeh M, Houshmand M, et al. Newborn screening using TREC/KREC assay for severe T and B cell lymphopenia in Iran. Scand J Immunol 2018;88:e12699.
15. Wakamatsu M, Kojima D, Muramatsu H, Okuno Y, Kataoka S, Nakamura F, et al. TREC/KREC newborn screening followed by next-generation sequencing for severe combined immunodeficiency in Japan. J Clin Immunol 2022;42:1696-707.
16. Cardenas-Morales M, Hernandez-Trujillo VP. Agammaglobulinemia: from X-linked to autosomal forms of disease. Clin Rev Allergy Immunol 2021;1:1.
17. 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;3:11.
18. Seidel MG, Kindle G, Gathmann B, Quinti I, Buckland M, Montfrans J van, 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;7:1763-70.
19. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 2015;17:405-24.
20. Weissensteiner H, Pacher D, Kloss-Brandstätter A, Forer L, Specht G, Bandelt HJ, et al. HaploGrep 2: mitochondrial haplogroup classification in the era of high-throughput sequencing. Nucleic Acids Res 2016;44:W58-63.
21. FamilyTreeDNA. Genetic testing for ancestry, family history and genealogy. Available at: https://www.familytreedna.com/public/mt-dna-haplotree/L. Accessed July 10, 2024.
22. Albers PK, McVean G. Dating genomic variants and shared ancestry in population-scale sequencing data. PLoS Biol 2020;18:e3000586.
23. University of Oxford Big Data Institute. Human genome dating. Available at: https://human.genome.dating/. Accessed July 10, 2024.
24. R Core Team. R: a language and environment for statistical computing. Vienna (Austria): R Foundation for Statistical Computing. 2022. Available at: https://www.R-project.org.
25. Chen S, Francioli LC, Goodrich JK, Collins RL, Kanai M, Wang Q, et al. A genomic mutational constraint map using variation in 76,156 human genomes. Nature 2024;625:92-100.
26. gnomAD (Genome Aggregation Database). Available at: https://gnomad.broadinstitute.org. Accessed July 7, 2024.
27. Kanegane H, Taneichi H, Nomura K, Futatani T, Miyawaki T. Severe neutropenia in Japanese patients with X-linked agammaglobulinemia. J Clin Immunol 2005;25:491-5.
28. Jacobs ZD, Guajardo JR, Anderson KM. XLA-associated neutropenia treatment: a case report and review of the literature. J Pediatr Hematol Oncol 2008;30:631.
29. Farrar JE, Rohrer J, Conley ME. Neutropenia in X-linked agammaglobulinemia. Clin Immunol and Immunopathol 1996;81:271-6.
30. Rezaei N, Farhoudi a, Pourpak Z, Aghamohammadi a, Moin M, Gharagozlou M, et al. Neutropenia in patients with primary antibody deficiency disorders. Iran J Allergy Asthma Immunol 2004;3:77-81.
31. Winkelstein JA, Marino MC, Lederman HM, Jones SM, Sullivan K, Burks AW, et al. X-linked agammaglobulinemia: report on a United States registry of 201 patients. Medicine 2006;85:193-202.
32. Rawat A, Jindal AK, Suri D, Vignesh P, Gupta A, Saikia B, et al. Clinical and genetic profile of X-linked agammaglobulinemia: a multicenter experience from India. Front Immunol 2021;11:612323.
33. Sullivan KE. Neutropenia as a sign of immunodeficiency. J Allergy Clin Immunol 2019;143:96-100.
34. Irga N, Wierzba J, Brozek J, Ochman K, Kanegane H, Miyawaki T, et al. [X-linked agammaglobulinemia (XLA) associated with agranulocytosis—case report]. Wiad Lek 2003;56:378-80.
35. Ryser O, Morell A, Hitzig WH. Primary immunodeficiencies in Switzerland: first report of the national registry in adults and children. J Clin Immunol 1988;8:479-85.
36. Stubbs A, Bangs C, Shillitoe B, Edgar JD, Burns SO, Thomas M, et al. Bronchiectasis and deteriorating lung function in agammaglobulinaemia despite immunoglobulin replacement therapy. Clin Exp Immunol 2018;191:212-9.
37. Rojas-Restrepo J, Caballero-Oteyza A, Huebscher K, Haberstroh H, Fliegauf M, Keller B, et al. Establishing the molecular diagnoses in a cohort of 291 patients with predominantly antibody deficiency by targeted next-generation sequencing: experience from a monocentric study. Front Immunol 2021;12:786516.
38. Chen D, Zheng J, Gerasimcik N, Lagerstedt K, Sjögren H, Abrahamsson J, et al. The expression pattern of the pre–B cell receptor components correlates with cellular stage and clinical outcome in acute lymphoblastic leukemia. PLoS One 2016;11:e0162638.
39. Stoltze UK, Foss-Skiftesvik J, van Overeem Hansen T, Byrjalsen A, Sehested A, Scheie D, et al. Genetic predisposition and evolutionary traces of pediatric cancer risk: a prospective 5-year population-based genome sequencing study of children with CNS tumors. Neuro Oncol 2023;25:761-73.
40. Foss-Skiftesvik J, Stoltze UK, van Overeem Hansen T, Ahlborn LB, Sørensen E, Ostrowski SR, et al. Redefining germline predisposition in children with molecularly characterized ependymoma: a population-based 20-year cohort. Acta Neuropathol Commun 2022;10:123.
41. Byrjalsen A, Hansen TVO, Stoltze UK, Mehrjouy MM, Barnkob NM, Hjalgrim LL, et al. Nationwide germline whole genome sequencing of 198 consecutive pediatric cancer patients reveals a high incidence of cancer prone syndromes. PLoS Genet 2020;16:e1009231.

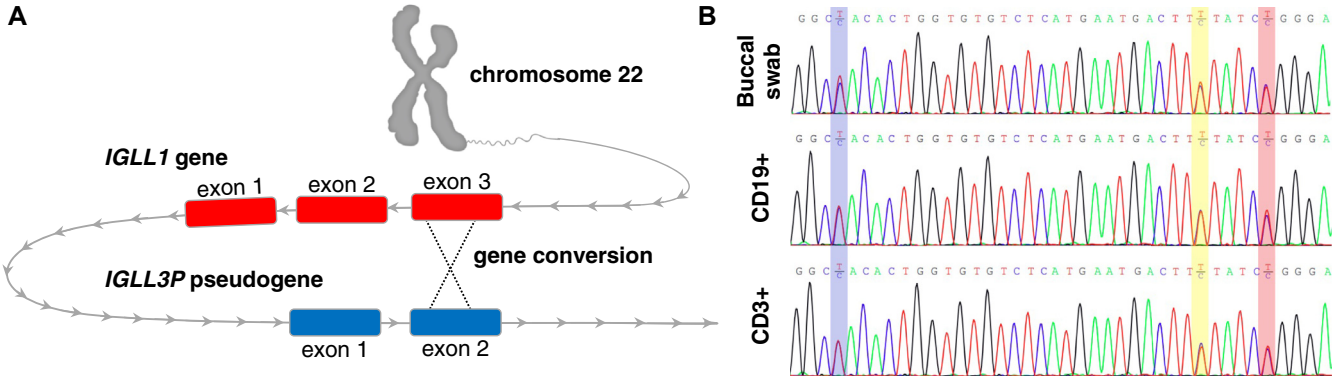


FIG E1. A, As proposed by Minegishi et al in 1998,[1] c.425C>T variant is likely to arise through gene conversion event between exon 3 of *IGLL1* and exon 2 of *IGLL3P*. **B,** Long-range PCR and Sanger sequencing of exon 3 of *IGLL1* revealed known pathogenic variant c.425C>T, p.(Pro142Leu) *(red),* and 2 additional synonymous variants, c.393T>C, p.(Ala131=) *(blue),* and c.420T>C, p.(Phe140=) *(yellow),* in patient N1. All 3 substitutions correspond to sequences in pseudogene *IGLL3P,* supporting these variants' having arisen by gene conversion. No somatic reversions could be detected in peripheral blood $CD19^{+}$ cells of patient N1.

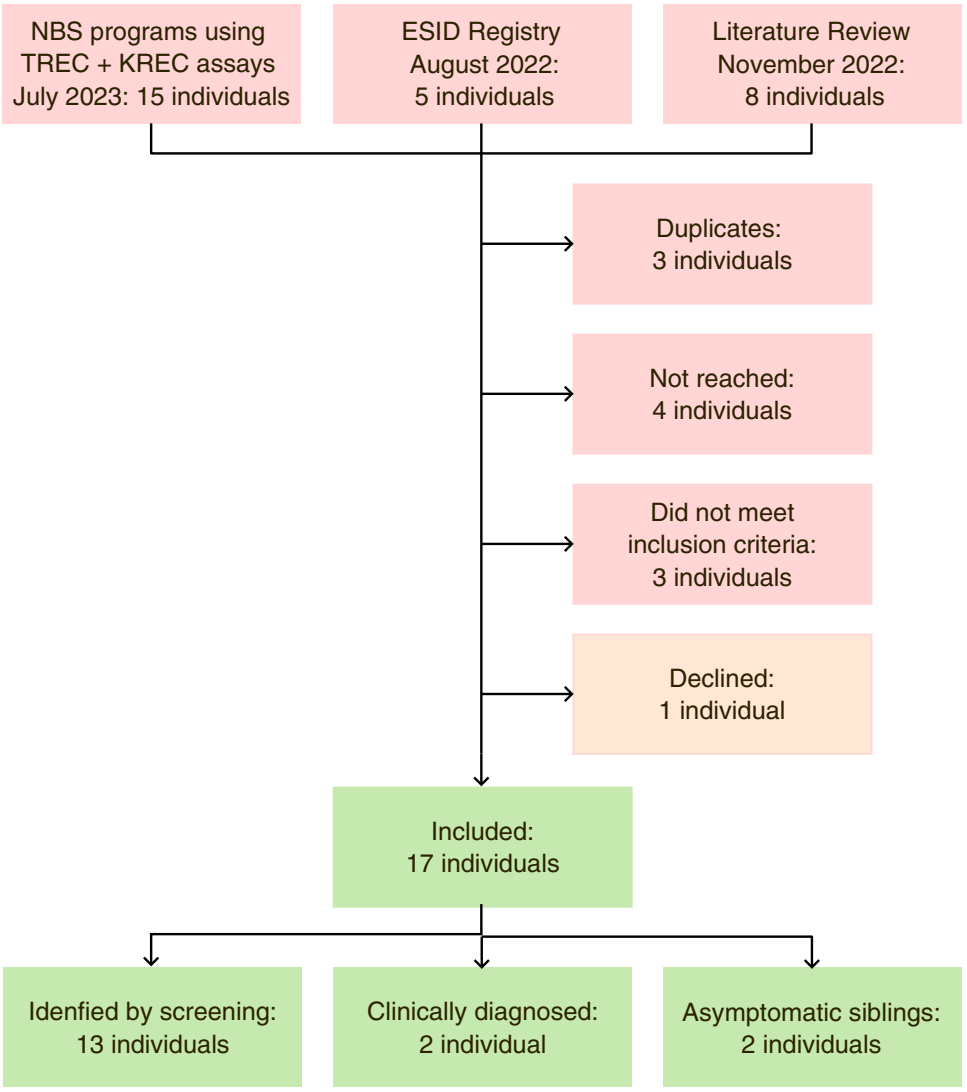


FIG E2. Patient flow. *ESID,* European Society for Primary Immunodeficiencies.

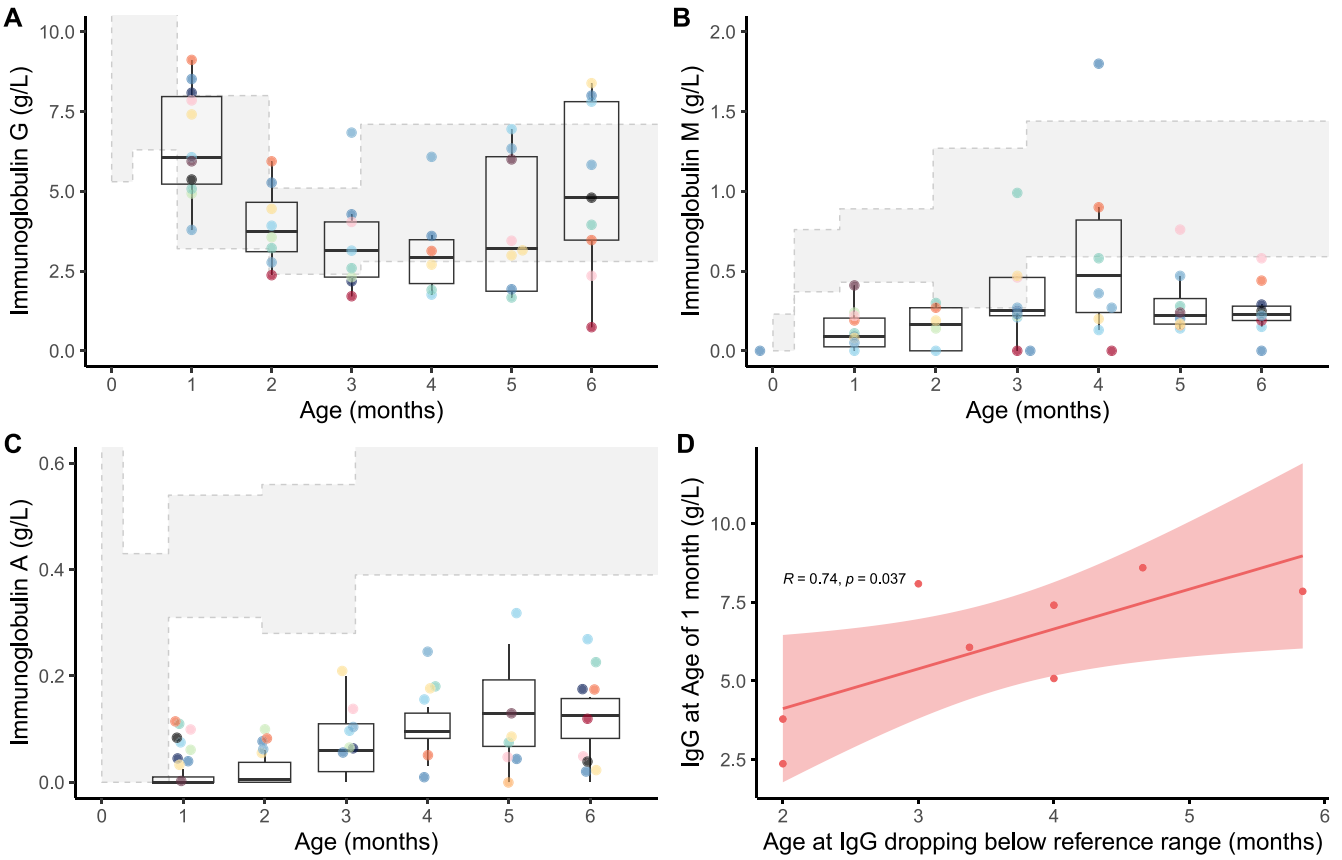


FIG E3. Course of peripheral blood concentrations of IgG **(A)**, IgM **(B)**, and IgA **(C)** in individual patients and of median and first and third quartiles presented by box plots during first 6 months of life in patients identified by NBS. Correlation between IgG levels at 1 month and age at which levels in sank below age-appropriate reference range in those not yet substituted **(D)**.

TABLE E1. Packages used for data analysis in R v4.2.2

Characteristic	Package name
Base packages	grid, stats, graphics, grDevices, utils, datasets, methods, base
Additional packages	reshape2 1.4.4, ggbeeswarm 0.7.1, ggbreak 0.1.2, deeptime 1.0.1, ggpubr 0.6.0, ggthemes 4.2.4, readxl 1.4.2, lubridate 1.9.2, forcats 1.0.0, stringr 1.5.1, dplyr 1.1.0, purrr 1.0.1, readr 2.1.4, tidyr 1.3.0, tibble 3.1.8, ggplot2 3.4.1, tidyverse 2.0.0

TABLE E2. Patients not meeting inclusion criteria

Patient no.	Patient source	Reasons for exclusion	Immunologic findings	Genetic findings
1	ESID registry	● Immunologic findings not compatible ● Genetic findings not compatible	● $CD19^+$ cells in age-appropriate reference range ● IgM reduced ● IgA reduced ● IgG reduced	One heterozygous previously undescribed *IGLL1* variant of unknown significance
2	ESID registry	● Immunologic findings not compatible ● Genetic findings not compatible	● $CD19^+$ cells above age-appropriate reference range ● IgM reduced ● IgA reduced ● IgG reduced	Two previously undescribed variants of unknown significance in *IGLL1* and genetically confirmed cystic fibrosis
3	Literature*	● Concomitant homozygous known pathogenic variants in TNFRSF13B	● Persistent low $CD19^+$ cells (0.013×10^9/L, <1% of total lymphocytes) ● IgM normal ● IgA normal ● IgG reduced	Homozygous c.258delG in *IGLL1* but also homozygous c.62-2A>G in TNFRSF13B

ESID, European Society for Primary Immunodeficiencies.
*From Platt et al.[7]

TABLE E3. Preliminary haplotype analysis

Patient no.	Genotype	Mitochondrial haplogroup	Haplogroup countries of origin
N1	c.425C>T mat/c.258del pat	V3c	Germany, United States, England, Finland
N2	c.258del mat/c.425C>T pat	H1c	Sweden, Poland, German, Irish
N3	c.425C>T hom	H4a1a	German, Swedish, Italian, England
N5	c.258del mat/c.425C>T pat, del including exons 1-2 pat	T1a1	United States, England, Ireland, Germany, Sweden, Finland
N6	c.425C>T mat/c.258del pat	H1b1	Sweden, Norway, Finland, Lithuania
N11	c.425C>T mat/61 kb del including exons 1-2 pat	H	Germany, United States, France, England
N13	c.258del hom	H1s1	Sweden, Germany, United States, England
C1	c.258del hom	U5b2a1a1	Germany, Norway, United States, England, Sweden
S1	c.258del hom	U5b2a1a1	Germany, Norway, United States, England, Sweden

hom, Homozygous; *mat,* maternal; *pat,* paternal.

TABLE E4. Enrichment analysis of *IGLL1* variants

Variant	Cancer cohort	Subpopulation	AF in cohort	AF in gnomAD v4.1.0	OR (95% CI) *P* value
c.425C>T	ICOPE/STAGING	ALL	3/760 (0.39%)	1,418/1,611,588 (0.09%)	9.5 (0.92 to 13.2) .03
	ICOPE/STAGING	B-ALL	3/310 (0.97%)		11.0 (2.25 to 32.5) .003
	St Jude	ALL	1/5642 (0.018%)		0.4 (0.01 to 1.12) .11
c.258delG	ICOPE/STAGING	ALL	1/760 (0.13%)	2,110/1,613,356 (0.13%)	1.01 (0.03 to 5.63) .63
	ICOPE/STAGING	B-ALL	0/310		0 (0.00 to 9.16) 1
	St Jude	ALL	5/5642 (0.09%)		0.68 (0.22 to 1.58) .58
All likely damaging variants*	ICOPE/STAGING	ALL	0/760	2,309/1,611,855 (0.14%)	0.00 (0.00 to 3.40) .63
	St Jude	ALL	9/4722 (0.19%)		1.33 (0.61 to 2.53) .34
	St Jude	B-ALL	7/3702 (0.19%)		1.32 (0.53 to 2.73) .38

AF, Allele frequency; *CADD,* combined annotation dependent depletion; *CI,* confidence interval; *OR,* odds ratio; *REVEL,* Rare Exome Variant Ensemble Learner.
*gnomAD v4.1.0 AF < 0.5%, CADD score >20, and/or REVEL score >0.5.