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Meaningful cases of primary immunodeficiencies, volume IV

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Meaningful cases of primary immunodeficiencies, volume IV

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Case report: Artemis deficiency and 3M syndrome—coexistence of two distinct genetic disorders

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The presence of two different genetic conditions in the same individual is possible, especially in populations with consanguinity. In this case report, we present the coexistence of Artemis deficiency (OMIM 602450) and Three M (3M) syndrome (OMIM 273750). A 10-months-old male patient with neuromotor developmental delay was evaluated for immunodeficiency due to recurrent respiratory infections diarrhea and oral moniliasis from the age of 1.5 months. He had facial dysmorphism with rotated ears, flat nose and hypertelorism. Neurological examination revealed generalized hypotonia and mental motor delay. Immunological screening of the patient demonstrated mild lymphopenia, hypogammaglobulinemia, reduced number of $CD3^+$ T cells (980 cells/mm^3) and $CD19^+$ B cells (35 cells/mm^3). He was diagnosed with leaky $T^-B^-NK^+$ SCID. Exome sequence analysis showed the presence of a homozygous pathogenic *DCLRE1C* variant [c.194C > T; p.T65I (NM_001033855)] and a homozygous pathogenic variant in *OBSL1*, a gene associated with 3M syndrome [c.3922C > T; p.R1308X (NM_001173431)]. Our proband died of sepsis and multiple organ failure. This case illustrates that different clinical findings in patients might not be explained with a single genetic defect, and consanguinity increases the change for coexistence of autosomal recessive diseases. Clinicians should consider exome sequencing to identify disease-causing mutations in patients with heterogeneity of clinical findings.

KEYWORDS

ARTEMIS deficiency, 3M syndrome, immunodeficiency, whole-exome sequencing, coexistence of genetic disorders

Introduction

Severe combined immunodeficiency (SCID) is a rare disorder characterized by profoundly defective T lymphocyte differentiation with or without abnormal development of B or NK lymphocytes or more rarely of the myeloid lineage and, presenting in infancy with life-threatening bacterial, fungal and viral infections infections (1, 2). SCID can be subdivided into T^+B^+, T^-B^+ or T^-B^- SCID according to the different genetic forms affecting T or B lymphocytes. Artemis deficiency is the most common form of radiosensitive SCID (3). Pathogenic variants in *DCLRE1C*, encoding Artemis, cause a block in T-and B-cell development and confer sensitivity to ionizing radiation (4). Artemis codes DNA Double-Strand Break Repair/Variable (V), Diversity (D), Joining (J) Recombination Protein of T-cell receptor genes and immunoglobulin in T and B cell development (5). Therefore loss of Artemis gene activity leads to impaired recombination

and causes T^-B^- SCID. Also, hypomorphic mutations in Artemis can cause atypical SCID, hyper IgM syndrome, Omenn syndrome and inflammatory bowel disease (3).

Three M (3M) syndrome [Online Mendelian Inheritance in Man (OMIM) 273750] is a rare autosomal recessive disorder and characterized by severe pre- and postnatal growth deficiency, facial dysmorphism, large head circumference and normal intelligence (6). 3M syndrome is caused by mutations in three genes (Obscurin-like 1 [OBSL1]; Cullin 7 [CUL7]; Protein 8-containing helix-coiled domain [CCDC8]). These genes interact with each other to form the 3M complex that maintains microtubule and genome integrity (7). Deletion or destruction of any 3M gene and disruption of the 3M complex cause severe microtubule damage, abnormal chromosome segregation and cell death (7). The treatment for 3M syndrome is supportive and based on the patient's symptoms.

Although rare, the presence of two different genetic conditions in the same individual is possible, especially in populations with consanguinity. Exome or whole genome sequencing is critical for correct diagnosis and optimal management of these diseases in cases whose clinical findings cannot be explained by a single cause. In this case report, we present the coexistence of Artemis deficiency (OMIM 602450) and Three M (3M) syndrome (OMIM 273750).

Case description

A 10-month-old male with neuromotor developmental delay was evaluated for immunodeficiency due to recurrent respiratory infections, diarrhea, and oral moniliasis from the age of 1.5 months. He was delivered at 34 weeks with a birth weight of 2,300 g, and he had no clinical history of hypoxia while in the neonatal intensive care unit. Neurological evaluation due to less interaction with surroundings and unable to sitting by 4 months was done. Hypotonia and exaggerated deep tendon reflexes on lower limbs were observed. His cranial MR imaging showed that non-specific hyperintensity in white matter. Metabolic screening (homosistein, amino acids in urine and blood samples, tandem mass spectrometry) was normal. He was the fourth child of consanguineous parents (**Figure 1A**). His sister died at the age of 8.5 months due to sepsis.

On physical examination, he had facial dysmorphism with posteriorly rotated ears, flat nose, and hypertelorism (**Figure 1B**). Neurological examination revealed generalized hypotonia and developmental motor delay. In immunological testing, mild lymphopenia, hypogammaglobulinemia, and reduced numbers of $CD3^+$ T cells (980 cells/mm^3) and $CD19^+$ B cells (35 cells/mm^3) were detected (**Table 1**). T lymphocyte activation with phytohemagglutinin was half of the control. Memory phenotype $CD4^+CD45RO^+$ T cells represented 58% of circulating $CD4^+$ T cells, but no evidence of maternal engraftment was observed upon HLA-typing of this patient and his mother. The diagnosis was felt to be consistent with leaky $T^-B^-NK^+$ severe combined immunodeficiency (SCID) (8). He was placed on trimethoprim-sulfomethoxazole (5 mg/kg/d, PO, daily) and fluconazole (6 mg/kg/d, PO, daily) for prophylaxis and on intravenous immunoglobulin replacement (0.5 g/kg/dose every 3–4 weeks). Still, the proband died from pneumonia, sepsis, and multiple organ failure at 21 months of age. Informed consent was obtained from the patient's parents for publication.

Exome sequencing of a premortem blood sample identified the presence of a homozygous *DCLRE1C* variant [c.194C > T; p.T65I (NM_001033855)] and a novel homozygous pathogenic variant in *OBSL1*, a gene associated with 3M syndrome [c.3922C > T; p.R1308X (NM_001173431)] (Supplementary Figure). The hypomorphic p.T65I mutation is known to result in reduced Artemis expression and either atypical or leaky SCID (9).

Discussion

Affected residual Artemis expression and/or function lead to a broad spectrum of phenotypes at the clinical, cellular and immunological level. The expression level and function of residual protein caused by a hypomorphic mutant is important in determining the prognosis and severity of immunodeficiency (10).

Classical SCID patients cannot live beyond infancy without hematopoietic stem cell transplantation (HSCT), but some children with Artemis deficiency can manage to recover from infections in the first years of life, and symptoms may delay until adulthood in some individuals with hypomorphic Artemis mutations (3). However, progressive degradation of immunity and increased organ damage cause impairment of survival. As a new gene therapy for Artemis-deficient SCID, Cowan et al. proposed the novel lentiviral construct AProArt, containing human *DCLRE1C* cDNA driven by the *DCLRE1C* promoter sequence in their study (11).

In the study of Ghadimi et al. in nine patients with *DCLRE1C* mutation, reported that there was consanguinity in 7 patients, and the most typical first presentation were pneumonia, otitis media, BCG lymphadenitis and gastroenteritis, respectively (12). Also, 33.3% of the patients had a family history of spontaneous abortion. Our case also had a family history of spontaneous abortion. The study conducted by Lee et al. including 2 patients with combined immunodeficiency (CID) and 12 patients with Artemis-deficient CID from previous other studies, reported that patients having hypomorphic mutations with residual Artemis expression, V(D)J recombination or double-stranded DNA repair capacity had significant morbidities such as autoimmunity, recurrent infections, EBV-related lymphoma and carcinoma (3). In their study, 9 patients underwent HSCT, 6 patients survived, and 4 patients who did not receive HSCT died. Early HSCT should be considered to prevent poor survival in Artemis deficiency. Inoue et al. reported in their study that 8 patients with Artemis-SCID had missense variants in 2 patients, large genomic deletions in 5 patients, and one patient with compound heterozygous for one missense variant and large genomic deletion (13). Eight patients underwent allogeneic HSCT and two patients died of complications after HSCT.

Physical examination features of 3M syndrome include short broad neck and thorax, deformed sternum, square shoulders,

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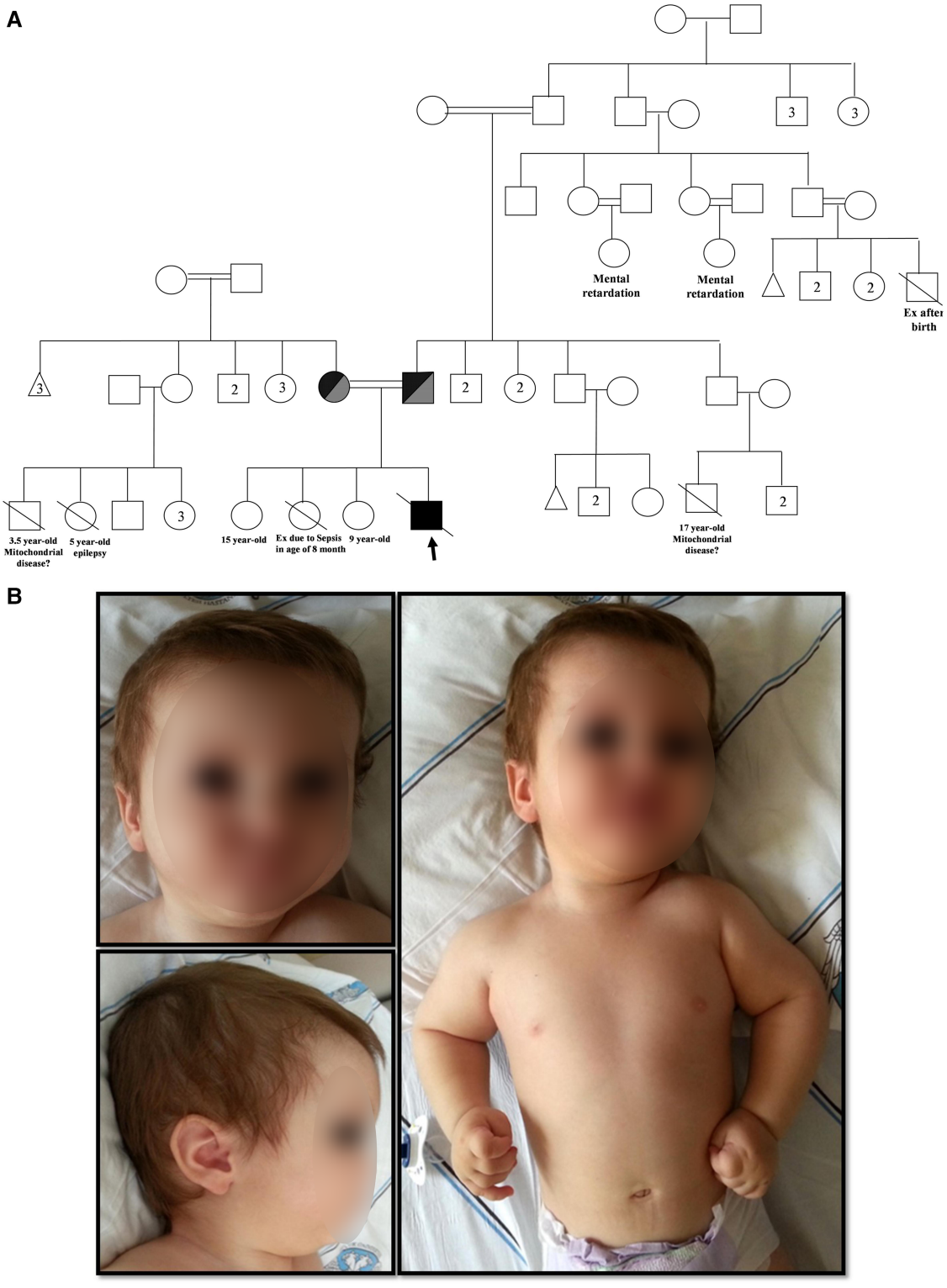

FIGURE 1
(A) Family pedigree chart. □ Male, ○ Female, △ Abortus, ⊠ ⊘ Deceased, ═══ Consanguineous marriage, ■ Homozygous *DCLRE1C + OBSL1* (Proband), ◓ ◓ Compound heterozygous *DCLRE1C + OBSL1*. Numbers inside symbols indicate number of individuals. (B) Physical appearance of the patient (posteriorly rotated ears, low nasal bridge, flat nose, retromicrognathia, hypertelorism).

TABLE 1 Immunological characteristics of the patient.

	10 months old	18 months old	Normal Value
Absolute lymphocyte count (/mm^3)	2,650	2,190	3,200–10,800
Lymphocyte subsets (% and absolute values) (/μl)			
CD3$^+$ T cell	37.1 (%)	33.6	51–79
	980	905	2,400–8,100
CD3$^+$CD4$^+$ T cell	13.6 (%)	13.4	31–54
	360	361	1,400–5,200
CD3$^+$CD8$^+$ T cell	16.1 (%)	13.4	10–31
	427	361	600–3,000
CD19$^+$ B cell	1.4 (%)	2.8	14–44
	35	75	500–3,600
CD16$^+$56$^+$ NK cell	33.2 (%)	-	5–23
HLA-DR$^+$	42.4 (%)	-	15–48
TCR-$\alpha\beta$/CD3$^+$	85.7	-	
TCR-$\gamma\delta$/CD3$^+$	13	-	
CD45RA/CD3$^+$	18.9	-	
CD45RO/CD3$^+$	69.8	-	
Serum Immunoglobulin levels			
IgG (mg/dl)	357	767 (with IVIG)	499.2–628.6
IgM (mg/dl)	22.8	35.3	67.7–94.4
IgA (mg/dl)	5.97	6.67	21–37.7
IgE (IU/ml)	18.1	5	<50
Anti-tetanus toxoid antibody levels (IU/ml)	0.40	-	>0.15 IU/ml
Anti-HBS (mIU/ml)	4.17	-	>10

prominent trapezii, hyperlordosis, prominent heels, short fifth fingers, loose joints, and skeletal changes including tall vertebral bodies and long slender tubular bones (6). This patient had dysmorphic features including frontal bossing, bulbous nose, broad forehead and triangular face. OSBL1 homozygous mutation lead to stop codon was detected. So, this additional mutation made confused to the clinician for accurate diagnosis before genetic analysis.

We detected a homozygous *DCLRE1C* variant (c.194C > T; p.T65I) and a homozygous pathogenic *OBSL1* variant in (c.3922C > T; p.R1308X). Volk et al. reported in three index patients with homozygous variants in *DCLRE1C* and nine patients from the same geographic region with homozygous or compound heterozygous *DCLRE1C* mutations in their study (9). There is no previously reported case with c.3922C > T; p.R1308X pathogenic variant.

Reports of multilocus variation causing blended phenotypes which are in 5% of the genetic diagnoses remain very limited (14). Chinn et al. reported an adult case with biallelic variations in *ZAP70* and *RNF168* having had a pediatric presentation (15). Another case report described developmental delay, and hearing loss in a patient with 3M syndrome due to co-existence variants in CUL7 and ILDR1 gene (16). Also, Amato et al. reported a case of Angelman syndrome (5,5 Mb deletion of 15q11.2–q13.1) with a coexisting intermediate junctional epidermolysis bullosa (COL17A1, c.3766 + 1G > A, homozygous) and autosomal recessive deafness type 57 (PDZD7, c.883C > T, homozygous) (17). This "double trouble" case illustrates the point that clinical findings in patients might not be explained by a single genetic defect, and consanguineous marriage increases the likelihood of coexistence of autosomal recessive diseases. Clinicians should consider exome or whole genome sequencing to identify disease-causing genetic defects in patients with potentially heterogenous clinical findings.

Data availability statement

The authors acknowledge that the data presented in this study must be deposited and made publicly available in an acceptable repository, prior to publication. Frontiers cannot accept a manuscript that does not adhere to our open data policies.

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

HA and IT: were involved in the diagnosis, treatment, and following of this patient and drafted the initial manuscript. AC: performed the immunological analyses and wrote the first draft of the manuscript. IC and JO: provided exome sequencing analysis and interpreted the relationship between genetic analysis and clinical findings. NK: contributed to interpreting exome sequencing analysis. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2023.1211254/full#supplementary-material

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Opinion paper: effectiveness of sirolimus in treating partial Di George syndrome with autoimmune lymphoproliferative syndrome-like features

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Di George syndrome is a disorder well known by pediatricians, caused by deletions in chromosome 22q11.2 and presenting with a wide range of clinical abnormalities, including immunodeficiency. Thymic function is diminished, leading to a decreased output of naive T cells (naïve T-helper cells, naïve T-regulatory cells, naïve cytotoxic T cells) as compared to healthy age matched controls. Immunedysregulation, such as autoimmunity and immunoproliferation are common in these patients, because the lack of a correctly functioning T cell repertoire. treatment of these complication are a challenge in many immunodeficiencies, including Di George syndrome. Gu et al. discuss a case of a patient with 22q11 deletion and lymphoproliferation and treated successfully with Sirolimus. This opinion paper highlights the need to collect information on the treatment of patients with immunoproliferation through sharing of information of individual cases and international cooperation.

KEYWORDS

Di George syndrome, ALPS, treatment, sirolimus, children

Introduction

Di George syndrome is a disorder well known by pediatricians, caused by deletions in chromosome 22q11.2 and presenting with a wide range of clinical abnormalities, including heart disease, endocrinological abnormalities (hypoparathyroidism), developmental problems (autism, ADHD, characteristic facial abnormalities and cleft lip, musculoskeletal abnormalities etc). Immunodeficiency is a consistent findings in these children, although the severity can vary depending on the remaining thymic tissue. The formation of the thymus is often impaired, leading to thymus hypo- or aplasia, which causes, in turn, a mild or severe T cell penia. Therefore, Di George Syndrome is one of the nearly 500 genetically known inborn errors of immunity (IEI). Thymic function is diminished, leading to a decreased output of naive T cells (naïve T-helper cells, naïve T-regulatory cells, naïve cytotoxic T cells) as compared to healthy age matched controls (1). Receptor repertoires showed skewed V-gene usage for naïve T-helper cells, whereas for naïve cytotoxic T cells, trend towards higher clonality were found.1 With age, T cell counts decrease even further, increasing the possibility of complications (2). This finding can be used to help detect 22q11.2 deletion syndrome patients during neonatal screening for IEI. Interestingly, 22q11 patients also show a perturbation of B-cell subsets, with larger proportions of naïve B cells and lower levels of memory B cells, including switched memory B cells, probably due to the failing T cell help. There may be hypogammaglobulinemia and poor responses to unconjugated Pneumococcal vaccination. For

patients with a total absence of the thymus, thymic transplantation is a feasible treatment, but in children with hypoplasia, long term follow up by an immunologist is warranted. Patients are at an increased risk of viral, candida and bacterial infections. As in other patients with diminished immunity, patients with Di George syndrome are also at risk to develop complications. Immunedysregulation, such as autoimmunity and immunoproliferation are common in these patients, because the lack of a correctly functioning T cell repertoire. Autoimmunity is seen in up to 10% of the patients with 22q11.2 (3). Lymphoproliferation has been described in several case reports and can be malignant as well as non-malignant. In review Autoimmune lymphoproliferative syndrome was described more recently. These patients present with chronic benign lymphoproliferation, auto immunity, especially involving the cells of the blood, and an increased risk of lymphoma. The presence of an increased number of a/b double negative T cells in the peripheral blood and lymphoid tissue are pathognomonic for this disease. Besides, hypergammaglobulinemia, increased vitamin B12, increased IL-10 levels and impaired Fas mediated apoptosis can help in the diagnostic process. Mutations in the FAS gene were described as a first cause of this disease, but later a growing list of mutations in other genes were proven to cause ALPS-like disease as well. Nowadays, these entities are referred to as Primary immune regulatory disorders (PIRD). The treatment options for ALPS and PIRD are numerous and depend on the severity of the symptoms. The authors describe a patient with Di George syndrome, lymphoproliferation (chronic adenopathies and splenomegaly) as well as autoimmunity (immune mediated thrombopenia, neutropenia). Further investigations revealed an increased IL-10 level and elevated DN T cells, but no genetic abnormalities associated with chronic lymphoproliferation were found in a whole exome screening. Based on the fact that the presentation of this child mimicked ALPS, they successfully treated the patient with Sirolimus for the cytopenia's and lymphoproliferative symptoms, according to earlier reports (4). Immunedysregulation is a well known complication seen in many types of IEI, and is often a challenge for clinicians treating these patients. Although important progress is being made in the identification of the genes involved in this process, and understanding the exact physiopathology, there are still many questions about the exact mechanisms. Searching for the genetic defect is crucial. Moreover, the "how to treat" question is often a tricky one, as the use of immunomodulatory drugs in patients that are already immunocompromised, can be a challenge. Therefor, it is important to continue to describe and study these patients, preferably using international cohorts, with complete phenotypical and genotypical data. The importance of international collaboration is stressed by the fact that these patients are usually rare. Clinicians should be aware to store material for further research in case biopsies are taken. These cohorts can also benefit the exploration of the different treatment strategies, as a fast growing list of new immunoregulatory drugs, like for example the JAK inhibitors, are added to the already existing, older treatment options, such as corticosteroids and Sirolimus. To investigate what drug to use in a certain individual with immunedysregulation, is one of the many tasks that lay ahead of us. In review References.

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

Written informed consent was not obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article because there are no patients in this paper.

Author contributions

The author confirms being the sole contributor of this work and has approved it for publication.

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A case report of a patient with recurrent and severe infections highlighting the importance of considering inborn errors of immunity

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Inborn errors of immunity (IEI) can often be misdiagnosed early in life due to their heterogenous clinical presentations. Interleukin-1 receptor-associated kinase 4 (IRAK-4) deficiency is one of the rare innate immunodeficiency disorders. We present the case of a patient who presented at the age of 15 days with meningitis and septic shock that responded to antibiotics. She was admitted again at the age of 45 days with *pseudomonas aeruginosa* bacteremia that was associated with increased inflammatory markers. Her third admission was at the age of 2.5 months due to left sided peri-orbital cellulitis that was again associated with elevated inflammatory markers. At 3.5 months, she experienced left orbital cellulitis, which was complicated by extensive sinus involvement, erosion, and abscess formation in the pterygopalatine fossa. Her condition progressed to septic shock and required multiple antibiotics and surgical interventions for drainage and control of the infection source. Both abscess and blood culture were positive for *pseudomonas aeruginosa*. An IEI was suspected but basic immunology testing was normal. Whole Exome Sequencing was performed and a novel mutation in IRAK4 was detected. In conclusion, we highlight the importance of raising awareness among pediatricians about the potentially lethal IEI and the need to consult specialists when these diseases are suspected. Among them is IRAK-4 deficiency which can be diagnosed by sophisticated functional assays and/or genetic testing.

KEYWORDS

inborn errors of immunity, immunodeficiency, IRAK-4, *pseudomonas aeruginosa*, FESS [Functional endoscopic sinus surgery]

Introduction

Inborn Errors of Immunity (IEI) are monogenic heterogeneous diseases that predispose patients to infections, immune dysregulation, and malignancy (1). Unfortunately, because the disease's manifestations are not specific to IEI and many pediatricians are unaware of them, patients experience significant delay in diagnosis (2–5). Subsequently, these patients are at increased risk of morbidity, tissue damage,

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and death (6). The Jeffery Modell Foundation has developed 10 warning signs to improve awareness about IEI and to facilitate earlier diagnosis (7). Among these signs are recurrent and severe infections, especially if they are caused by unusual organisms.

Pseudomonas sepsis is rare in healthy, non-hospitalized infants and the empirical antibiotic treatment for sepsis does not include agents against this microorganism (8). This gram-negative bacillus is an opportunistic environmental microorganism that can cause severe infections in children with impaired defense mechanisms and chronic illnesses. In this report, we present the case of an infant who presented with recurrent life-threatening pyogenic *Pseudomonas* infection in the head and neck region. Although the initial immunology workup was unremarkable, eventually the infant was diagnosed with Interleukin -1 Receptor-Associated Kinase 4 (IRAK-4) deficiency, an inborn error of immunity. We aim to highlight the importance of considering IEI in the differential diagnosis of patients with recurrent infections.

Case report

The patient was born at full term with an average birth weight and no history of perinatal complications to consanguineous parents. She received Hepatitis B vaccine at birth. Family history was not suggestive of immunodeficiency.

She was admitted at the age of 15 days with fever, convulsions, septic shock and evidence of bacterial meningitis—as suggested by cerebrospinal fluid (CSF) studies [WBC 991 cells (22% polymorphs) and increased protein at 2.8 g/L (0.15–1.3)] but negative gram stain and bacterial culture as she had been pre-treated with a few doses of IV ampicillin and cefotaxime. Her complete blood count (CBC) showed white blood cells (WBC) 7.52 (10^9/L) (32% neutrophils) and C-reactive protein (CRP) was elevated at 15.7 mg/dl (normal <0.5) which normalized to 0.16 mg/dl after treatment with ampicillin and cefotaxime. Antibiotics were changed to intravenous vancomycin and meropenem. The blood culture was sterile.

She was admitted again at the age of 45 days with fever, rhinorrhea, and decreased oral intake. Cerebrospinal fluid (CSF) sample showed WBC of 9 (10^9/L) (11% polymorphs) and protein of 0.4 g/L. CRP was elevated at 26.6 mg/dl, procalcitonin was elevated at 5.4 ng/ml (normal <0.05 ng/ml). Blood culture grew *pseudomonas aeruginosa*. Her condition improved and CRP normalized after receiving antibiotics.

At 2 and a half months, she was admitted for the third time with low-grade fever, decreased activity, rhinorrhea, decreased oral intake, and left-sided peri-orbital cellulitis that was associated with elevated CRP at 17.8 mg/dl. Cerebrospinal fluid studies were unremarkable. The blood and CSF cultures were sterile.

Her fourth admission was at the age of 3 and a half months with poor feeding, irritability, severe nasal congestion, left-sided facial swelling, redness with periorbital edema, and proptosis (Figure 1) consistent with a diagnosis of left orbital cellulitis. Computed tomography (CT) imaging of the head showed pterygopalatine fossa abscess with left cavernous sinus thrombosis. CBC showed a WBC count of 12.2 (10^9/L) (28% neutrophils) and CRP was elevated at 24 mg/dl. She was treated with IV Meropenem. After 2 days, her condition worsened with increased orbital swelling. She urgently underwent endoscopic sinus surgery to the left side for source control. Uncinectomy, anterior ethmoidectomy, and maxillary mega antrostomy with partial inferior turbinectomy were performed (Figure 2). There was purulent discharge involving the left maxillary sinus with bulging of the medial maxillary sinus into the septum. The posterior wall of the maxillary sinus was eroded with exposure of the contents of the pterygopalatine fossa (Figure 3). In addition, there was erosion of the bone secondary to extensive inflammation in the hard palate (Figure 4). Multiple biopsies were taken for histopathology and cultures grew *pseudomonas aeruginosa* similar to her blood culture. Intravenous ciprofloxacin was added. After about 48 h of improvement, she deteriorated again with septic shock and progressive buccal and left facial cellulitis extending to the left lateral neck. Urgent head and neck CT with contrast revealed extensive progression of the

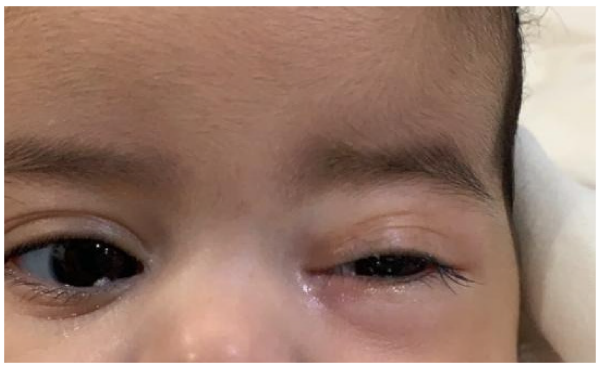

FIGURE 1
Left periorbital swelling with left facial swelling and proptosis.

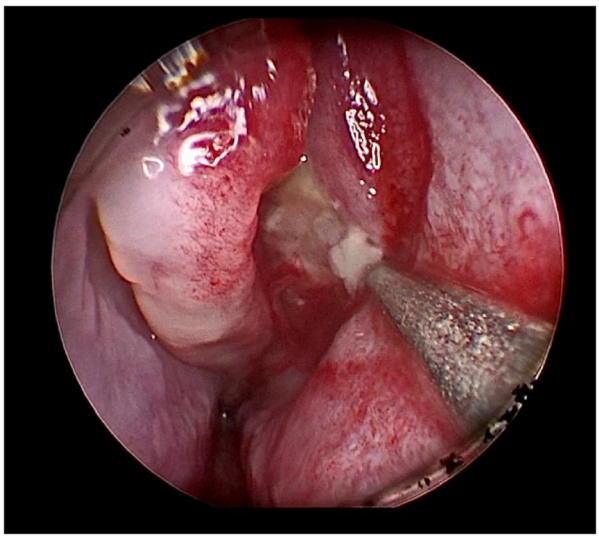

FIGURE 2
Gush of pus from left maxillary sinus upon initial cannulation.

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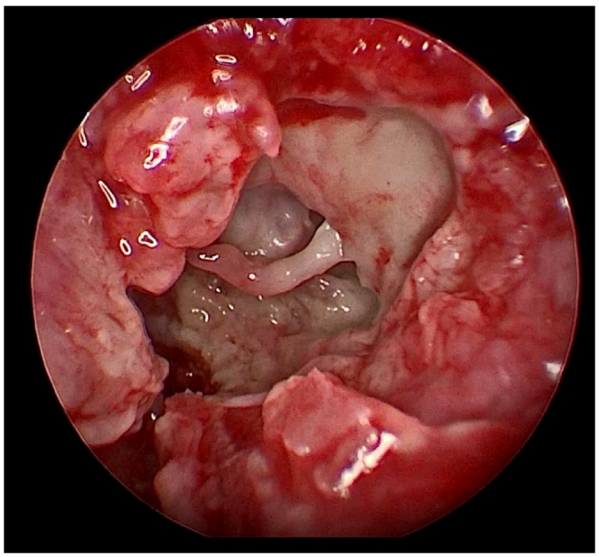

FIGURE 3
Eroded left posterior maxillary wall with exposure of pterygopalatine fossa content.

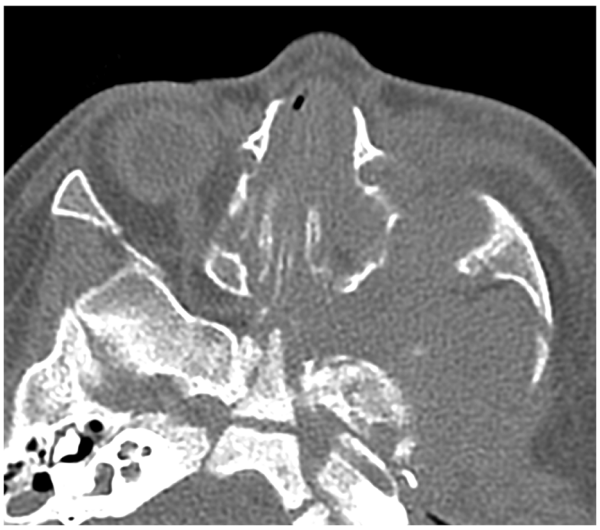

FIGURE 5
Axial CT scan of the paranasal sinuses shows a destructive inflammatory process of the left pterygopalatine fossa and widening of the foramen.

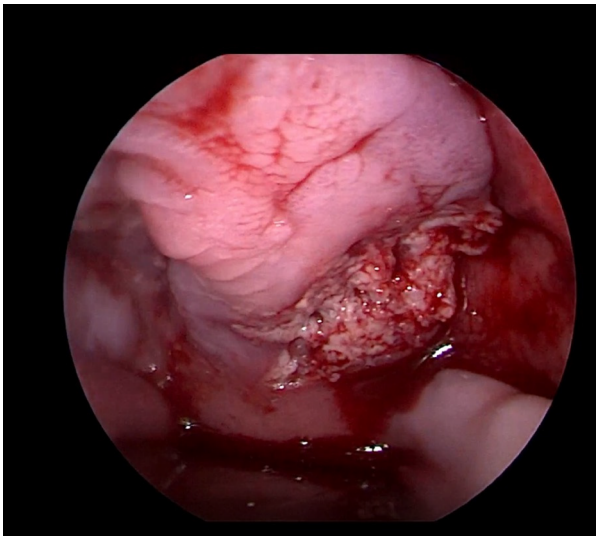

FIGURE 4
Second look intra oral left hard palate involvement.

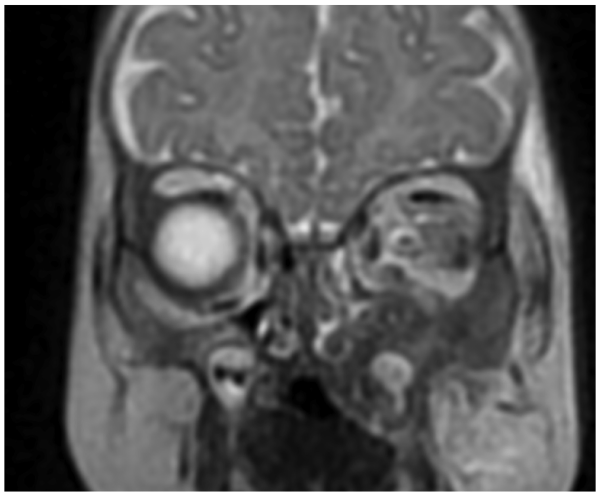

FIGURE 6
Coronal MRI of the paranasal sinuses shows an inflammatory process involving the left maxillary sinus, ethmoid sinus, floor of the orbit, and inferiorly into the sections of the hard palate.

pterygopalatine fossa abscess and thromboses of the left cavernous sinus, external and internal jugular veins (Figure 5). She was urgently taken to the operating room for source control including drainage of a hard palate collection that was bulging into the nasal floor (Figure 6). After about 2 weeks in the intensive care unit, she was successfully discharged to complete a prolonged IV antibiotic course.

An IEI was suspected because of recurrent and severe infections. Her basic immunology test results done at the age of 40 days and at the age of 4 months were normal (Table 1). Due to the high suspicion of IEI despite normal results of basic immunology testing, Whole Exome Sequencing was performed using a next-generation sequencing platform through Kuwait Medical Genetic Center.

Genetic methodology and result

Next-generation sequencing was carried out on an Ion Torrent S5 XL/Prime Machine, using Ion AmpliSeq Whole Exome sequencing Kit by Life Technologies to an average coverage depth of 70–100×. In-house bioinformatics analysis

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TABLE 1 Immunological tests.

	40 days old	4 months old
IgG[a]	306	583
IgM[a]	42	42
IgA[a]	<6	<6
CH100	241 (60–1,000 CAE)	473 (60–1,000 CAE)
CD3[b]	3,809	3,023
CD4[b]	2,773 (CD45RA: 77.61%, RO: 7.49%)	2,253 cels/ul (CD4 + CD31 + CD45RA+: 72%, 1,437)
CD8[b]	894	707
CD19[b]	1,037	2,485
CD56[b]	309	125
MHC I expression	99% on lymphocytes and monocytes	–
MHC II expression	84.5% on monocytes and 20% on lymphocytes	–
DHR[c]	Normal	NORMAL
CD11b+	–	Normal expression
CD18+	–	94.61%

[a]Mg/dl.
[b]cells/ul.
[c]dihydrorhodamine.

pipeline including signal processing, base calling, and variant annotation by alignment of reads to GRCh37/hg19 genome assembly. Primary filtering out of low-quality reads and probable artifacts were applied. Subsequent analysis and interpretation of the data were carried out for the sample. Identified variants and indels were filtered against external databases depending on their allele frequency focusing on rare variants with a minor allele frequency (MAF) of 1% or less. Evaluation was focused on coding exons along with flanking ±10 intronic bases. In Silico analysis of identified variants was performed using bioinformatics prediction programs. Classification of variants was performed based on ACMG guidelines (9).

Testing revealed that the proband was homozygous for a novel frameshift variant in IRAK4 ((NM_016123.4): c.274del; (p. Glu92AsnfsTer27). This variant was then confirmed by Sanger sequencing. The variant results in a 1-bp deletion in exon 3, generating a frameshift predicted to lead to a premature stop codon at position 27 downstream in the new reading frame. The variant has not been observed in gnomAD (Genome Aggregation Database) and was predicted to cause loss of normal protein function. Subsequent variant interpretation according to the ACMG guidelines (mutation type, predicted impact, absence in large control population, and the genotype–phenotype correlation), indicates that the variant can be classified as likely pathogenic with supporting evidence for pathogenicity criteria PVS1 (Very Strong strength level for pathogenicity in the ACMG) and PM2 (Moderate evidence of pathogenicity).

Discussion

We describe the case of a 4-month-old infant who presented with recurrent life-threatening infections including septicemia, meningitis, orbital cellulitis, and pyogenic head and neck infection caused by *pseudomonas* with an initial negative immunology workup. Typically, *pseudomonas aeruginosa* sepsis in children mainly occurs after prolonged hospitalization, and most commonly affects patients with chemotherapy-induced neutropenia or with IEI (10, 11).

IEI are genetic defects of the innate or adaptive immune system. The innate immune system is the first line of defense against pathogens and is critical to recognize microbes and start the inflammatory cascade (12). We described a novel frameshift variant in IRAK4 gene causing autosomal recessive immunodeficiency (OMIM # 607676). A review of the literature suggests that this variant creates a premature termination codon and is predicted to cause loss of normal protein function, resulting in severe IRAK4 deficiency (13). IRAK4 deficiency specifically abrogates Toll-like receptor TLR signaling. Defects in TLR signaling result in primary immune deficiency. Studies have described defects in components of the TLR signaling cascade, which includes MyD88, TIRAP, and the kinases IRAK1 and IRAK4 (14). Interleukin-1 receptor-associated kinase 4 (IRAK4) is the most upstream kinase in Toll/Interleukin-1 receptor (TIR) signaling (15) and is considered the master in the mammalian IRAK family. It plays a key role in the intracellular signal transduction from IL-1, IL-18, which are important mediators in the signal transduction of TLR and IL1R family members, collectively referred to as TIRs (16). The loss of IRAK4, or its intrinsic kinase activity, can entirely stop signaling through these pathways (17).

Due to defective activation of innate immune responses, IRAK-4-deficient patients are prone to recurrent pyogenic bacterial infections while preserving a normal resistance to common fungi, parasites, and viruses (11, 18, 19). *Pseudomonas* invasive infection has been reported in patients with such IEI but is less common than sepsis due to Gram-positive bacteria including *Streptococcus pneumonia* and *Staphylococcus aureus* (20–22). IRAK 4 deficient patients can present with meningitis, osteomyelitis, arthritis, abscesses, sepsis, and cellulitis. Immunological evaluation in IRAK4 deficiency usually reveals intact leukocyte populations, including T-, B-, NK-cell numbers, and T cells that exhibit normal proliferation. Serum immunoglobulin values and antibodies against specific vaccine antigens are usually within the normal range except for possible impaired responses to polysaccharide vaccines (23).

In this case report, we highlight the importance of raising awareness among pediatricians about the potentially lethal IEI and the need to consult specialists when these diseases are suspected. There are several inborn errors of immunity that can be severe but may not be detected in the initial immunologic workup like serum immunoglobulins and lymphocyte subsets testing. One example of such IEI is IRAK-4 deficiency which can be diagnosed by sophisticated functional assays then supported by genetic testing. It is recommended that IRAK-4 deficient patients receive prophylactic antibiotics including oral trimethoprim/sulfamethoxazole or penicillin. In addition, some patients may require monthly immunoglobulin infusions. It is imperative that such patients continue to receive regular vaccinations. Children who survive beyond childhood tend to

develop less frequent invasive diseases (24). Inborn errors of immunity should be also be considered in populations with high incidence of consanguineous marriages which increases the risk of autosomal recessive disorders (25).

Conclusion

Pseudomonas aeruginosa sepsis and invasive pyogenic infections in early childhood in a previously healthy child signify the possible presence of an IEI. This case report highlights the importance of performing a thorough genetic investigation especially when the pre-test probability of an IEI is high. Making such a diagnosis is lifesaving. Moreover, in addition to targeted/definitive antimicrobial therapy, the role of surgical intervention for source control is crucial in alleviating the pyogenic burden of this disease.

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

Ethical approval was not required for the study as this manuscript pertains only to a case report and specific ethics approval is not mandated in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians. Written informed consent was obtained from the individual(s), and minor(s)' legal guardian, for the publication of any potentially identifiable images or data included in this article.

Author contributions

FA: Writing – original draft, Writing – review & editing. MA: Writing – original draft. MA: Writing – original draft. AA: Writing – original draft, Writing – review & editing. RE: Software, Writing – original draft. WA: Writing – original draft, Writing – review & editing.

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Conflict of interest

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

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Case Report: Profound newborn leukopenia related to a novel RAC2 variant

Geoffrey Hall[1], Ágnes Donkó[2], Cristina Pratt[1], Julie J. Kim-Chang[1], Paul L. Martin[3], Amy P. Stallings[1], John W. Sleasman[1], Steven M. Holland[4], Amy P. Hsu[4], Thomas L. Leto[2] and Talal Mousallem[1]*

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We report the case of a 1-week-old male born full-term, who had two inconclusive severe combined immunodeficiency (SCID) newborn screens and developed scalp cellulitis and *Escherichia coli* bacteremia. He did not pass early confirmatory hearing screens. Initial blood counts and lymphocyte flow cytometry revealed profound neutropenia and lymphopenia with a T-/B-/NK- phenotype. Red blood cell adenosine deaminase 1 activity was within normal limits. A presumptive diagnosis of reticular dysgenesis was considered. Granulocyte colony-stimulating factor was started, but there was no improvement in neutrophil counts. Subsequent lymphocyte flow cytometry at around 4 weeks of age demonstrated an increase in T-, B- and NK-cell numbers, eliminating suspicion for SCID and raising concern for congenital neutropenia and bone marrow failure syndromes. Genetic testing revealed a novel variant in *RAC2* [c.181C>A (p.Gln61Lys)] (Q61K). RAC2, a Ras-related GTPase, is the dominant RAC protein expressed in hematopoietic cells and is involved with various downstream immune-mediated responses. Pathogenic *RAC2* variants show significant phenotypic heterogeneity (spanning from neutrophil defects to combined immunodeficiency) across dominant, constitutively activating, dominant activating, dominant negative, and autosomal recessive subtypes. Given the identification of a novel variant, functional testing was pursued to evaluate aberrant pathways described in other *RAC2* pathogenic variants. In comparison to wild-type RAC2, the Q61K variant supported elevated superoxide production under both basal and PMA-stimulated conditions, increased PAK1 binding, and enhanced plasma membrane ruffling, consistent with other dominant, constitutively active mutations. This case highlights the diagnostic challenge associated with genetic variants identified via next-generation sequencing panels and the importance of functional assays to confirm variant pathogenicity.

KEYWORDS

severe combined immunodeficiency, reticular dysgenesis, RAC2, inborn error of immunity, novel variant

Introduction

Severe combined immunodeficiency (SCID) is a rare, life-threatening immunologic genetic disorder resulting in profound T-cell deficiency with impaired T- and B-cell function. The phenotypic presentation of SCID can be variable, including additional B-cell and NK-cell deficiencies, but often exhibits strong genotype–phenotype correlations. One such phenotype, reticular dysgenesis (RD)—resulting from variants in *AK2*, includes additional physical and immunologic defects such as sensorineural hearing loss and neutropenia. Distinguishing RD from congenital neutropenia syndromes, bone marrow failure syndromes, and other immunodeficiencies based on phenotype may prove challenging, and confirmatory testing to identify a molecular cause is warranted.

Ras-related C3 botulinum toxin substrate (RAC) is a small GTP-binding protein within the Rho-GTPase family. There are three isoforms of RAC, with RAC2 being the dominant RAC protein expressed in hematopoietic cells. RAC2 is involved with various downstream cellular effector functions related to immune-mediated processes. Variants in *RAC2*, which result in immunodeficiency syndromes with wide phenotypic heterogeneity, have been increasingly recognized over the last decade. The total global incidence of *RAC2*-related immunodeficiency is unknown, and only 54 patients have been described (1).

In this report, we present a novel *RAC2* variant with an early RD-like phenotype and functional evidence of a constitutively active RAC2 protein. Our case is only the fifth patient (third identified variant) with a novel *RAC2* mutation to present with this unique phenotype (2, 3).

Case description

A 1-week-old male was born full-term and small for gestational age (weight: 2,610 g—5th percentile) to non-consanguineous parents, with a birth history notable for maternal pre-eclampsia. Initial newborn hearing screen results were unavailable. The remainder of the immediate newborn period was unremarkable. Upon presentation to his primary care pediatrician at 6 days of life, he was found to be febrile (101.6°F) with associated scalp cellulitis located at the site of a previous scalp electrode used during delivery. He was hospitalized and started on broad-spectrum antimicrobials, including ampicillin, gentamicin, acyclovir, and vancomycin. Blood cultures and scalp wound cultures were positive for *Escherichia coli*, and following antibiotic sensitivity results, he was transitioned to single therapy with cefepime. Urine culture and serum human immunodeficiency virus (HIV) 1/2 RNA were negative. Additional scalp wound cultures, including fungal cultures and HSV-PCR surface cultures, were negative. Cell counts, protein, and glucose from lumbar puncture were unremarkable. A BioFire® meningitis/encephalitis panel was negative. Brain MRI was suspicious for osteomyelitis; thus, the cefepime dose was escalated for meningitis coverage. Repeat imaging 10 days later demonstrated soft tissue inflammatory changes without evidence of osteomyelitis.

The initial and repeat newborn screen results were reported as unsatisfactory/inconclusive. Newborn screen results in North Carolina have three possible outcomes based on total quantifiable T-cell receptor excision circles (TRECs)—normal, abnormal, and borderline. Unsatisfactory/inconclusive results generally occur due to sample collection errors or failure of internal controls. State-guided follow-up protocols vary depending on the result, with recollection routinely recommended in cases of unsatisfactory/inconclusive results. His white blood cell (WBC) count was profoundly reduced at 500 cells/ml, with a complete absence of neutrophils, an absolute lymphocyte count (ALC) of 400 cells/ml (80%), and an absolute monocyte count of 100 cells/ml (20%). Granulocyte colony-stimulation factor (G-CSF) was initially administered at 10 µg/kg/day without improvement in neutrophil counts and discontinued after 10 days. The immunoglobulin G level was normal at 413 mg/dl, with undetectable IgA and IgM. He developed thrombocytopenia (nadir 39,000 platelets/ml) without anemia and subsequently showed improvement in platelet counts.

Initial lymphocyte flow cytometry at day of life 16 showed a T-cell ($CD3^+$) count of 320 cells/µl (78%), B-cell ($CD19^+$) count of 13 cells/µl (3%), and NK-cell count of 68 cells/µl (17%). The naïve T-cell ($CD3/CD45RA^+$) count was profoundly low at 26 cells/µl (15%), with an elevated $CD3/CD45RO^+$ percentage of 83% (Table 1). A primary immunodeficiency next-generation sequencing panel was sent but was unable to be processed due to insufficient DNA. The patient was then transferred to our institution, given suspicion for SCID with T-/B-/NK- phenotype.

Upon transfer, the patient was started on *Pneumocystis* and fungal prophylaxis with pentamidine and fluconazole, respectively. Mitogen proliferation to phytohemagglutinin (PHA) was normal. Maternal engraftment was not detected, and the red blood cell adenosine deaminase 1 (ADA) level was normal. Subsequent lymphocyte flow cytometry demonstrated an improvement in T- and NK-cell counts, with similarly low B-cell counts (Table 1). Multiple repeat newborn hearing screens were abnormal and thought to be associated with anatomical obstruction due to the scalp lesion (Figure 1). G-CSF was restarted and escalated to high-dose therapy (30 µg/kg/day) without notable improvement in neutrophil counts, so it was discontinued after 20 days. Bone marrow aspirate was obtained and showed no increased blasts, increased hematogones (29%), absence of maturing granulocytic elements (including neutrophil production), and active erythropoiesis without dysplasia. Bone marrow biopsy could not be obtained due to safety considerations related to the size of the patient.

Diagnostic assessment

A repeat immunodeficiency gene panel collected via buccal swab was sent and revealed a novel heterozygous variant in *RAC2* [c.181C>A (p.Gln61Lys)] (Q61K). Based on its location within Switch region 2, this missense variant was expected to impact

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TABLE 1 Immunophenotype of the patient.

Subset	DOL 16 cells/μl (%)	DOL 26 cells/μl (%)	DOL 110 cells/μl (%)	Reference cells/μl (%)
ALC	**410**	**1,288**	**288**	3,400–9,000
$CD3^+$	**320 (78%)**	**1,034 (80.3%)**	**201** (69.9%)	2,500–5,600 (51%–77%)
$CD4^+$	**197** (48%)	**739** (57.4%)	**156** (54.0%)	1,600–4,000 (35%–64%)
$CD8^+$	**121 (29%)**	**286** (22.2%)	**44** (15.2%)	560–1,700 (12%–28%)
$CD19^+$	**13 (3%)**	**46 (3.6%)**	**77** (26.9%)	300–3,000 (6%–41%)
NK cells	**68** (17%)	207 (16.1%)	**7 (2.6%)**	170–1,100 (4%–18%)
$CD3^+/CD45RA^+$	**26 (15%)**	**291 (28.1%)**	**58 (28.9%)**	1,200–3,700 (64%–95%)
$CD3^+/CD45RO^+$	150 **(83%)**	422 **(40.8%)**	**77 (38.3%)**	90–1,200 (3%–31%)
$CD4^+/CD45RA^+/CD62L^+$	NA	**146 (19.7%)**	**59 (38.0%)**	1,200–3,600 (61%–94%)

NA, not available.
Values outside the reference range (10th–90th percentile) are presented in bold (4).

several RAC2 effector pathways. Despite early non-normal hearing screen results, his hearing screen normalized at 1 month of age.

Given the severity of the immune deficiency, early sepsis, and an identified underlying molecular defect, a haploidentical (maternal, variant negative) hematopoietic stem cell transplant was pursued at 4.5 months of age. Paternal donation was not a viable option due to a known underlying hematologic abnormality (phenotypically inconsistent with *RAC2* variants). The conditioning regimen consisted of alemtuzumab, fludarabine, and melphalan, along with post-transplant cyclophosphamide. The post-transplant course was complicated by the development of veno-occlusive disease (VOD) requiring defibrotide. One month following the transplant, he achieved >98% donor engraftment in $CD3^+$, $CD15^+$, and whole blood.

Immune reconstitution has been complicated (Table 2) by ongoing immunosuppressive therapies for managing graft-vs.-host disease (oral/topical calcineurin inhibitors, oral/topical corticosteroids, oral ruxolitinib, abatacept) and two episodes of Epstein–Barr virus (EBV) infection (treated with 4 weekly doses of rituximab). His most recent T-cell count is 735 cells/μl at 19 months post-transplant.

He currently demonstrates mild developmental delays and is receiving speech and occupational therapy. A formal audiology assessment was completed at around 1 year of age and was normal. He receives nutrition via a gastrostomy tube. Growth has been stable but markedly below normal, with weight and height at the <1st and 2nd percentiles, respectively.

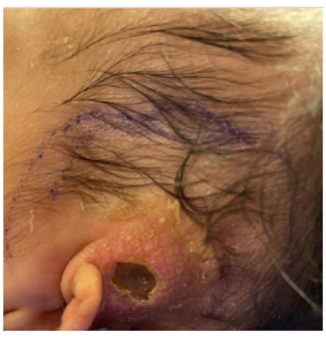

FIGURE 1
Scalp lesion. Evolving lesion located superior to the left ear of the patient notable for circumferential induration, absence of purulent material, and central necrosis/eschar formation.

Discussion

Ras-related C3 botulinum toxin substrate (RAC) is a small GTP-binding protein within the Rho-GTPase family (5). GTPases impact various downstream cellular functions by switching between active and inactive states induced through GTP/GDP binding (6). Within the RAC family, three homologous isoforms have been identified: RAC1, RAC2, and RAC3 (5, 7). RAC1 is ubiquitously expressed, and RAC3 is neuronally expressed, while RAC2 is the dominant RAC protein in hematopoietic cells. As such, it plays a fundamental role in immune-mediated cellular effector functions, including cell migration, cytoskeletal reorganization, and neutrophil superoxide production, although the specifics of these mechanisms are still being elucidated (8, 9). *RAC2* variants demonstrate phenotypic heterogeneity across dominant, constitutively activating, dominant activating, dominant negative, and autosomal recessive subtypes (2, 6, 10, 11). Thus, the clinical spectrum of disease can be quite variable, as described by Donkó et al. (1), including later-onset combined immunodeficiency (CVID) with normal or depressed neutrophil counts (10, 12–16), leukocyte-adhesion deficiency-like disease with normal or elevated neutrophil counts (11, 17), and severe combined immunodeficiency RD-like disease with neutropenia but lacking sensorineural hearing loss (2, 3).

TABLE 2 Post-hematopoietic transplantation immune reconstitution.

Subset	3.5 months cells/μl (%)	10 months cells/μl (%)	19 months cells/μl (%)
ANC	570	1,000	1,400
ALC	**1,903**	**1,541**	**921**
$CD3^+$	**944** (49.6%)	**1,415 (91.3%)**	**735 (80.2%)**
$CD4^+$	**676** (35.5%)	**887 (57.6%)**	**453** (49.1%)
$CD8^+$	**162 (8.5%)**	**444** (28.8%)	**254** (27.6%)
$CD19^+$	**523** (27.5%)	**0**[a]	**45 (4.9%)**[b]
NK-cells	403 **(21.2%)**	**103** (6.6%)	**119** (13.1%)
$CD3^+/CD45RA^+$	**365 (38.7%)**	NA	NA
$CD3^+/CD45RO^+$	404 **(42.8%)**	NA	NA
$CD4^+/CD45RA^+/CD62L^+$	**228 (33.7%)**	NA	NA

NA, not available.
Lymphocyte values outside the reference range (10th–90th percentile) (4) are presented in bold.
[a]1 month post-rituximab.
[b]3 months post-rituximab.

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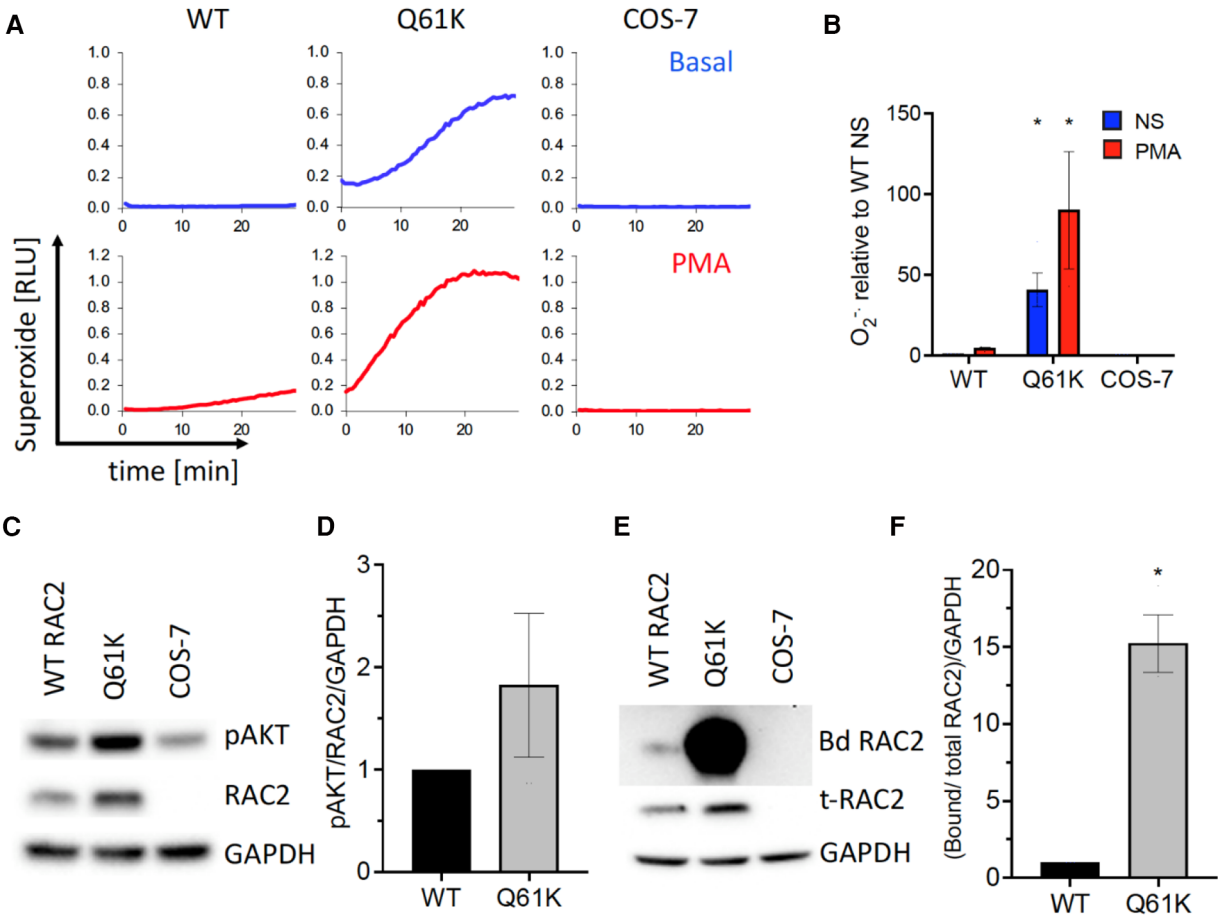

FIGURE 2
RAC2-based functional assays of superoxide production, AKT phosphorylation, and PAK1 binding. (**A**) Extracellular reactive oxygen species production for COS-7 cells transfected with NADPH oxidase components $gp91^{phox}$, $p67^{phox}$, $p47^{phox}$, and wild-type (WT), Q61K variant, or untransfected (COS-7) over 30 min under basal and PMA-stimulated conditions. (**B**) Summary bar graph of integrated kinetics of basal and PMA-stimulated superoxide production normalized to WT RAC2 non-stimulated, expressed as mean ± SEM from three independent experiments. (**C**) Q61K variant showing increased AKT phosphorylation consistent with enhanced constitutive RAC2 activation; representative Western blot. (**D**) Summary of AKT phosphorylation from three independent experiments analyzed by densitometry, normalized to WT and GAPDH levels, expressed as average ± SEM. (**E**) Q61K variant showing a significant increase in Western blot of PAK1-bound RAC2 (Bd RAC2) from wild-type or Q61K-transfected or non-transfected (COS-7) cells. (**F**) Quantification of the PAK1-bound RAC2 Q61K variant showing significantly higher bound RAC2 than wild-type RAC2, consistent with increased constitutive activity.

RD is caused by variants in *AK2*, which encodes adenylate kinase 2, a vital enzyme in the production of cellular energy through phosphorus transfer to produce adenosine triphosphate (ATP) (18). An obligatory, non-hematopoietic, clinical feature of RD is sensorineural hearing loss. However, the pathophysiologic mechanisms, either developmental or functional, related to hearing loss are not well understood. Importantly, despite the immunologic similarities of some *RAC2* activating mutations to RD, sensorineural hearing loss does not appear to be a phenotypic feature (2, 3). Thus, hearing evaluation should be considered an early priority to assist in differentiating between variants presenting as RD or RD-like.

Identification of a novel *RAC2* variant in this patient prompted research-based testing to assess RAC2 activity and the impact on cellular effects, including superoxide production and membrane ruffling, which have previously been described (1). Using a variant expression construct and heterologous expression system, the Q61K variant function was compared to wild-type RAC2. The methodological details are outlined by Donkó et al. (1). The Q16K variant supported elevated superoxide production by NOX2 in reconstituted cells under both basal (40-fold higher than wild-type) and PMA-stimulated (5-fold higher than wild-type) conditions (Figures 2A,B). In addition, PAK1 binding was assessed as a measure of downstream activity (GTP-bound RAC2) and demonstrated 15-fold higher binding (Figures 2C–F). Together, these findings were consistent with other dominant, constitutively activated *RAC2* mutations.

On initial presentation, our patient's clinical phenotype matched that of patients reported by Stern et al. and Lagresle-Peyrou et al. who had agranulocytosis with a T-/B-/NK- SCID phenotype associated with *RAC2* variants: [c.182 A>G (p.Gln61Arg)] (Q61R) and [c.34 G>A, (p.Gly12Arg)] (G12R) (2, 3). However, subsequently, T-cell counts increased to above 1,000 cells/μl, and mitogen proliferative response to PHA was

normal. Thus, this patient did not meet the Primary Immunodeficiency Treatment Consortium's (PIDTC) criteria for typical or leaky/atypical SCID (19). In addition to leukopenia with normal red cell and platelet counts noted shortly after birth, at the time of presentation to the outside tertiary care center, he was diagnosed with pancytopenia, consistent with sepsis. Prior to the initiation of his transplant preparation, leukopenia persisted with mild anemia and normal platelet counts. Altogether, these features raised concern for an alternative explanation for the phenotype of the patient, including syndromes associated with congenital neutropenia, bone marrow failure, or other causes of immune dysregulation. In retrospect, we speculate that the change in immunophenotype (improvement in lymphocyte counts) was reflective of high-dose G-CSF driving additional T-cell and NK-cell production at the time of flow cytometry evaluation. Randomized, placebo-controlled trials have demonstrated increased lymphocyte production in response to G-CSF among adult patients with SARS-CoV2 infection (ALC <800 cells/ml) as well as patients with HIV1 receiving antiretroviral therapy (ALC <350 cells/ml) (20, 21). In the HIV1 trial, increased $CD4^+$ and $CD8^+$ production was primarily of a memory ($CD45RO^+$) phenotype (20), which may have been representative of an oligoclonal expansion, although clonality testing was not explored. Interestingly, this patient demonstrated an increase in both the naïve ($CD45RA^+$) and memory T-cell compartment, although a higher proportion of memory T-cells overall was maintained. However, the total naïve T-cell percentage (28.1%) remained low for age (ref: 61%–94%) (4). Upon discontinuing G-CSF, T-cell counts ($CD3^+$) decreased to 201 cells/μl. Despite *RAC2* being classified as a SCID-related gene, the phenotype of this patient remained inconsistent with PIDTC criteria for typical and atypical/leaky SCID based on the following: $CD3^+$ count, naïve T cells >20% of total $CD4^+$ count, normal proliferative studies, no evidence of maternal engraftment, absence of T-cell clonality studies, and inconclusive SCID screen results (inhibiting TREC quantification) (19).

The SCID newborn screen results of the patient are particularly noteworthy given the challenge of interpreting unsatisfactory and inconclusive results. While there are clear guidelines for follow-up evaluation (or lack thereof) in cases of normal, borderline, and abnormal results, there is much more uncertainty in cases with unsatisfactory and inconclusive results. Unsatisfactory results occur when samples are unacceptable for processing at the state lab (e.g., uneven blood spotting on Guthrie cards), generally prompting recommendations to send a repeat sample. Inconclusive results are generated when there is a disruption in the internal RNase controls and may represent a technical or sample processing error. However, inconclusive results may be caused by severe cytopenia, reflecting an inability of the RNase primer to bind due to depressed or absent sample DNA. Identifying the basis for inconclusive results can be difficult, often requiring discussion with the state laboratory in cases of repetitive inconclusive reporting. Complete blood counts with differentials may represent an early and simple screening option to assess leukocyte counts and identify aberrations requiring evaluation.

Our case highlights the diagnostic challenges associated with genetic variants of inborn errors of immunity. Although our patient did not meet the recent 2022 PIDTC criteria for SCID, the immunologic impairment warranted HSCT. Constitutively activating *RAC2* mutations have been implicated in an RD-like SCID phenotype, and the initial presentation of our patient was consistent with this clinical picture. However, subsequent testing with improved (but still low) lymphocyte counts suggests that the patient had a combined immunodeficiency phenotype with a neutrophil defect. The details of our case reinforce the importance of confirmatory gene sequencing, especially among phenotypically variable immune defects, and the importance of functional assays to investigate aberrant pathways confirming variant pathogenicity. Furthermore, continuing reports on patients with *RAC2* variants will be paramount in characterizing disease phenotype and outcomes following definitive therapy.

Data availability statement

Anonymized data may be available upon request. Functional assays completed by co-authors at the NIH. Genetic results are provided through commercially available next-generation sequencing panels as part of the standard of care for such patients. Requests to access the datasets should be directed to talal.mousallem@duke.edu, tleto@niaid.nih.gov, or agnes.donko@nih.gov.

Ethics statement

The studies involving humans were approved by the institutional review board of Duke University with consent provided by the legal representatives of all patients for enrollment into the protocol, Genetic and Functional Analysis of Primary Immune Deficiencies (Pro00066839). The studies were conducted in accordance with the local legislation and institutional requirements. 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

GH: Conceptualization, Writing – original draft, Writing – review & editing. ÁD: Writing – original draft, Writing – review & editing, Data curation, Formal Analysis, Investigation, Methodology. CP: Writing – original draft, Writing – review & editing. JK-C: Writing – original draft, Writing – review & editing. PM: Formal Analysis, Writing – original draft, Writing – review & editing, Investigation. AS: Writing – original draft, Writing – review & editing. JS: Data curation, Writing – original draft, Writing – review & editing. SH: Writing – original draft, Writing – review & editing. AH: Formal Analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. TL: Formal Analysis, Investigation, Methodology,

Writing – original draft, Writing – review & editing. TM: Conceptualization, Investigation, Supervision, Writing – original draft, Writing – review & editing.

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Conflict of interest

TM received funding from Chiesi for a project entitled: A Single Arm, Open-Label, Multicenter, Registry Study of Revcovi Treatment in ADA-SCID Patients Requiring Enzyme Replacement Therapy. TM is the Duke Site PI for PIDTC [NIAID-University of California, San Francisco U54 AI082973 Puck (PI) 09/2019-08/2024]—Prospective Study of SCID Infants who receive Hematopoietic Cell Therapy. JS receives grant funding from Sumitomo Pharma America, Inc.

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

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Case Report: Early presentation of hereditary angioedema symptoms in a 2-year-old boy

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Hereditary angioedema (HAE) is a rare autosomal-dominant disease that is caused by a deficiency (type I) or dysfunction (type II) of the C1 inhibitor (C1-INH) due to a mutation in the *SERPING1* gene, which codes for C1-INH. HAE with quantitatively and qualitatively normal C1-INH (type III) is often caused by a mutation in the *F12* gene and no mutations in the *SERPING1* gene and is a group of very rare diseases. The C1 esterase inhibitor (C1-INH) is a major regulator of critical enzymes that are implicated in the cascades of bradykinin generation, which increases vascular permeability and allows the flow of fluids into the extracellular space, resulting in angioedema. HAE clinically manifests with intermittent attacks of swelling of the subcutaneous tissue or submucosal layers of the respiratory and gastrointestinal tract. Young children are typically asymptomatic, and those affected by HAE usually present with symptoms in their early 20s. This article describes the case of very early onset of hereditary angioedema caused by C1-INH deficiency in a 2-year-old boy who experienced recurrent episodes of hand and abdominal angioedema not associated with urticaria or pruritus. His father suffered from severe HAE due to a *de novo* mutation of the *SERPING1* gene. The same mutation of the *SERPING1* gene was detected in his son at the age of 9-months prior to the occurrence of angioedema symptoms, during genetic family counseling. This paper advances the understanding of HAE and highlights the importance of genetic counseling of families with HAE to avoid late or inaccurate diagnosis and to initiate treatment on time.

KEYWORDS

hereditary angioedema (HAE), C1-INH deficiency, early onset hereditary angioedema, C1-INH-HAE, *SERPING1*

1 Introduction

Hereditary angioedema (HAE) is a rare autosomal-dominant disease that is caused by a deficiency or dysfunction of the C1 inhibitor (C1-INH) and affects 1/50,000 people worldwide (1–3). Three main types of HAE exist. Type I of HAE is characterized by a low level of C1-INH and type II (dysfunction of C1-INH) is caused by a mutation in the *SERPING1* gene that codes for the C1 inhibitor protein. Type III (quantitatively and qualitatively normal C1-INH) is often caused by a mutation in the *F12* gene and no mutations in the *SERPING1* gene. In this rare type of HAE, clinical manifestations are similar to those of C1-INH deficiency HAE, except that there is a higher female predominance due to aggravation during pregnancy and estrogen dependency (1–8).

Currently, more than 700 different *SERPING1* variants are known to be linked to HAE type I and II (9). The pathophysiology of HAE is described as bradykinin-mediated. The C1 esterase inhibitor (C1-INH) is a regulator of the complement and contact systems and a major regulator of critical enzymes that are implicated in the cascades of bradykinin generation; such regulation increases vascular permeability and allows the flow of fluids into the extracellular space, resulting in angioedema. HAE clinically manifests with intermittent attacks of swelling of the subcutaneous tissue or submucosal layers of the respiratory or gastrointestinal tract (10). Attacks of swelling of tissues in the hands, feet, limbs, face, intestinal tract, or airway are triggered by precipitating factors such as emotional stress, trauma, hormonal influences, infections, and surgical or dental procedures (1–3). The severity and course of HAE may vary greatly even among family members harboring the same mutation (7, 8). The gene defect is already present at birth, but symptoms are uncommon in neonates and infants. Angioedema attacks can first occur at any age, but usually young children are asymptomatic, and those affected by HAE usually present with symptoms in their early 20s (3). In this study, we present the case of very early onset of hereditary angioedema caused by C1-INH deficiency in a 2-year-old boy who experienced recurrent episodes of angioedema of the extremities and face without urticaria and pruritus and an abdominal angioedema attack and also had a family history of HAE.

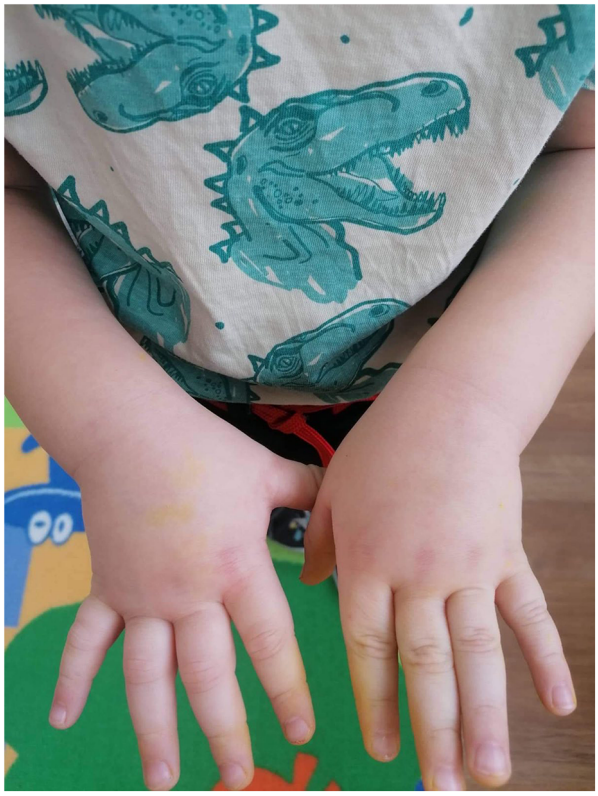

FIGURE 1
First attack of HAE angioedema of the hands (onset).

2 Case description

A 2-year-old boy started experiencing attacks of painful swelling in his face and upper extremities, which were triggered by a minor trauma and not associated with urticaria or pruritus. He was born normally from the first pregnancy and weighed 3,405 g at birth. The first episode of bilateral hand angioedema after the boy started playing with toys occurred at the age of 23 months and lasted 4 days (Figures 1, 2). The boy was immediately consulted by an allergologist-clinical immunologist and the serum complement component C4 and C1 inhibitor were evaluated, which revealed reduced levels of the following: C4 was 0.07 g/L (normal: 0.16–0.38), the C1 inhibitor was 0.05 g/L (normal: 0.15–0.35), and C3 in serum was 0.78 g/L (normal: 0.79–1.52) (Table 1). As the patient's father was suffering from HAE type I due to C1-INH deficiency and the same mutation of the *SERPING1* gene was detected in his son at the age of 9 months during genetic family counseling 1 year ago, the diagnosis of HAE type 1 due to C1-INH deficiency was established for this 2-year-old boy. HAE type I was diagnosed for the patient's father at the age of 23 years following recurrent peripheral and abdominal angioedema attacks with reduced C4 and C1-INH in serum. This diagnosis was made when he was consulted by an allergologist-clinical immunologist at the tertiary university hospital, and replacement therapy with C1-INH was initiated. For the father, it took 8 years from the onset of angioedema to confirm the diagnosis of HAE, before he was misdiagnosed as having systemic lupus, Crohn's disease, and appendicitis. Six years later when genetic testing was established in Lithuania, *de novo SERPING1* gene mutation was detected, and all of the boy's family members, except his father who died at the age of 63 years from heart attack, were tested. The boy's mother and sister had no mutations, but the same *SERPING1* gene heterozygous mutation (c.[473C>G]; [473=], p. [(Ser158Ter)]; [(Ser158=]) was detected in the boy, who was a 9-month-old asymptomatic child. At the time, this child was healthy with only a slightly reduced C1-INH level in serum (0.12 g/L, normal: 0.15–0.35 g/L). At the age of 2 years, the boy started experiencing angioedema attacks and treatment with plasma-derived C1 esterase inhibitor concentrate 500 IU (30 IU/kg) was initiated. At the follow-up of 2 years, it was found that the patient had experienced peripheral angioedema attacks only two times, but he had a history of one abdominal pain attack with vomiting that started 2 weeks after a common cold episode. In the emergency room, the abdominal ultrasound revealed terminal ileum wall thickness and a small amount of fluid in the pelvis and around the terminal ileum. Plasma-derived C1 esterase inhibitor concentrate 500 IU (30 IU/kg) was given intravenously for the angioedema attacks, following which symptoms resolved within 30 min. The patient was asked to continue the C1 inhibitor on-demand treatment.

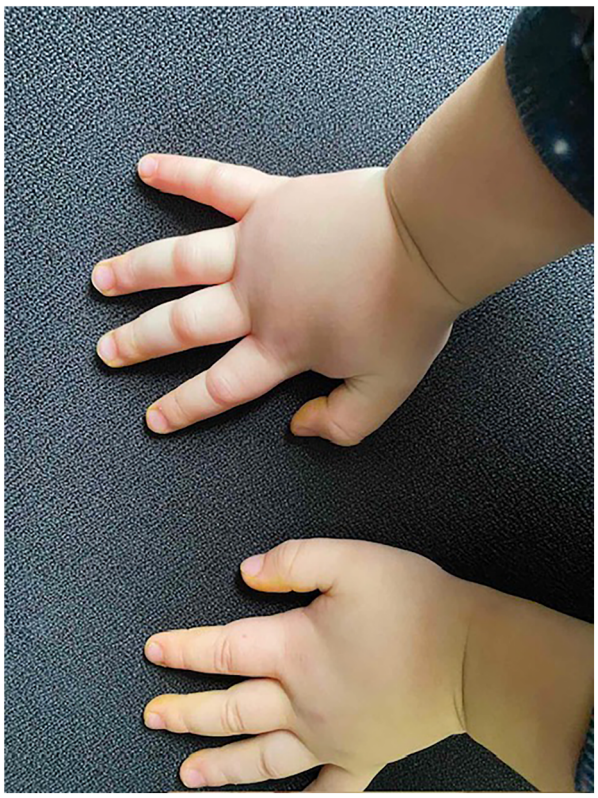

FIGURE 2.
First attack of HAE angioedema of the hands (2 h later).

TABLE 1 The complement system parameters of the patient and his father.

	Serum C1-INH level (g/L)	Serum C4 level (g/L)	Serum C3 level (g/L)
Before angioedema started (patient)	0.12	0.04	0.77
After the first angioedema attack (patient)	0.05	0.07	0.78
After angioedema attack (father)	0.09	0.04	0.83

Normal values in serum (g/L): C1-INH, 0.15–0.35; C4, 0.16–0.38; C3, 0.79–1.52.

3 Discussion

We present the case of very early onset of hereditary angioedema caused by C1-INH deficiency in a 2-year-old boy who experienced recurrent episodes of angioedema of the extremities and face without urticaria and pruritus and later an abdominal angioedema attack and also had a family history of HAE I. Our case is similar to the ones described in the literature, which shows that most attacks, and most first attacks, in children manifest with angioedema of the skin, and the earlier the onset of symptoms, the more severe the subsequent course of HAE I/II (1, 2). For C1-INH-HAE, the autosomal-dominant mode of inheritance has been described as that which occurs when one of the two alleles of the C1-INH gene (*SERPING1*) that encodes C1-INH is mutated. Mutations in *SERPING1* are responsible for the majority of cases of hereditary angioedema type I and II (6–9). The genotyping of subjects suffering from HAE has become diagnostically indispensable in clinical practice (11). In our presented case, the same *SERPING1* gene heterozygous mutation of one amino acid was confirmed for both the patient and his father, who had *de novo SERPING1* gene mutation. In the literature, it has been shown that only patients carrying missense mutations leading to a change in a single amino acid exhibited a less severe clinical phenotype; however, in our study, the boy started experiencing HAE symptoms at a relatively early age of 2 years when compared with his father, who had these symptoms when he was 15 years old. It is probable that there is no correlation between the same type of mutations and clinical phenotype. The involvement of epigenetic changes and environmental factors (i.e., temperature, pH, and oxidative stress) in the pathogenesis of HAE can also be postulated (6–8).

The detection of *SERPING1* mutation in a 9-month-old asymptomatic boy makes a strong argument for an early and timely diagnosis of HAE before peripheral angioedema attacks start at the age of 23 months, excluding other diseases and allergic angioedema. Apart from delayed diagnosis, misdiagnosis leads to inappropriate treatment, and by the time patients realize that they are misdiagnosed and want to make amends, it becomes too late, resulting in the denial of timely, , effective, and lifesaving treatment for them. However, in the presented case, early detection of *SERPING1* gene mutation and a family history of HAE prevented misdiagnosis and shortened the time to HAE diagnosis. From a large database of patients, HAE patients with family members diagnosed as having C1-INH-HAE were significantly less likely to be misdiagnosed than patients without a family history. Approximately 50% of patients with C1-INH-HAE type I or II have previously had their conditions misdiagnosed, most commonly as allergic angioedema or appendicitis (as was the case of our patient's father, when the HAE diagnosis was delayed for 8 years) (12–14). Therefore, we highly recommend that genetic family counseling be made standard care for patients affected by HAE.

Early diagnosis and appropriate treatment are essential for improving the lives of patients with this life-threatening disorder and disabling disease. HAE attacks of the upper airways can result in asphyxiation, abdominal attacks are painful and debilitating, and peripheral attacks of the hand and feet result in impaired function (1). The ultimate goals of treatment in HAE are to achieve total control of the occurrence of symptoms, which will reduce the frequency and severity of attacks, and to normalize patient lives (12). During the course of the Icatibant Outcome Survey (international, prospective, and observational registry collecting demographics and clinical outcomes in patients with HAE type I/II angioedema), triggers were identified in 56.0% of angioedema attacks, with the most common triggers being emotional distress, physical trauma, and infection (13). In the presented case, the 2-year-old boy experienced HAE attacks and these were associated with minor hand trauma while playing

with toys and infection in case of abdominal attacks. During the course of the above-mentioned survey, the most commonly reported prodromal symptoms associated with attacks were erythema marginatum (13.2%), nausea (9.3%), irritability (7.3%), and change in estrogen levels in women (7.1%) (12). Probably because of the boy's young age, prodromal symptoms were not observed in him.

Disease management for patients with HAE is currently achieved through the use of on-demand medications and short- and long-term prophylaxis. HAE attacks should be treated as early as possible and prophylaxis should be considered before known triggering events to reduce morbidity and mortality (1, 2). Acute treatment options include C1-INH replacement therapy [plasma-derived or recombinant human C1-INH (rhC1-INH) via intravenous administration], the kallikrein inhibitor ecallantide (subcutaneous administration, not approved for use in the EU including Lithuania, but approved in the United States), and the bradykinin B2 receptor antagonist icatibant (subcutaneous administration, not available in Lithuania). Short-term prophylaxis may be indicated before known triggers of swelling such as surgical or dental procedures. The available options include intravenous plasma-derived C1-INH (pdC1-INH), fresh frozen plasma, and attenuated androgens (e.g., danazol, oxandrolone). For long-term prophylaxis, there are options to include C1-INH (via intravenous or subcutaneous administration); subcutaneous lanadelumab, the fully human monoclonal antibody (mAb) against plasma kallikrein; attenuated androgens; and antifibrinolytics (tranexamic acid) (1, 2, 5, 14, 15). For children under the age of 12 years, the updated Lithuanian and international guidelines, including the World Allergy Organization (WAO)/European Academy of Allergy and Clinical Immunology (EAACI), recommend intravenous plasma-derived C1-INH and icatibant. Both are effective, well tolerated, and show a good safety profile (1, 16). The intravenous plasma-derived C1-INH concentrate given for the child in our study when he suffered acute angioedema attacks was the treatment of choice according to the guidelines, availability, and the fact of it being the only reimbursement option in Lithuania (16). For abdominal attacks, parenteral fluid replacement may be required as children are more susceptible to hypovolemia and dehydration, and extravasation into the peritoneal cavity and intestinal lumen can be substantial (1, 14, 15).

4 Conclusion

Our presented case is an original case report and strongly suggests that hereditary angioedema caused by C1-INH deficiency can occur very early in life. Early diagnosis and appropriate treatment are essential to improve the lives of patients with this disabling disease. As this disease is inherited in an autosomal-dominant way, the importance of genetic counseling of families with HAE is highly recommended to avoid late or inaccurate diagnosis of HAE and to initiate timely treatment to the affected members.

Data availability statement

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

Ethics statement

Ethical approval was not required for the study involving humans in accordance with the local legislation and institutional requirements. Written informed consent to participate in this study was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements. 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

JS-K: Conceptualization, Data curation, Methodology, Supervision, Writing – original draft, Writing – review & editing. JSt: Data curation, Methodology, Writing – original draft, Writing – review & editing. EG: Writing – original draft, Writing – review & editing. JSe: Conceptualization, Data curation, Writing – original draft, Writing – review & editing.

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Conflict of interest

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Case Report: Common variable immunodeficiency phenotype and granulomatous–lymphocytic interstitial lung disease with a novel SOCS1 variant

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Common variable immunodeficiency is a heterogeneous symptomatic group of inborn errors of immunity that mainly affects antibodies production and/or function, predisposing patients to recurrent and severe infections. More than half of them usually develop autoimmunity, lymphoproliferation, enteropathy, and malignancies. Among these conditions, chronic lung disease such as granulomatous–lymphocytic interstitial lung disease is one of the leading causes of death in these patients. Recently, many genes that play a key role in B and T cells' development, maintenance, and/or cytokines signaling pathways have been implicated in the pathogenesis of the disease. Here, we describe the first Argentinian patient presenting with common variable immunodeficiency and granulomatous–lymphocytic interstitial lung disease, harboring two *in cis* heterozygous variants in the *SOCS1* gene.

KEYWORDS

common variable immunodeficiency, granulomatous–lymphocytic interstitial lung disease, SOCS1, GLILD, inborn errors of immunity

Introduction

Common variable immunodeficiency (CVID) is a heterogeneous symptomatic group of inborn errors of immunity (IEI) that mainly affects antibodies' production and/or function, predisposing patients to recurrent and severe infections. Classically, CVID is defined in patients over 4 years of age with serum IgG <2 SD for the normal age range and markedly low IgA or IgM isotypes accompanied by a poor response to vaccines and/or absent isohemagglutinins, and in whom other causes of hypogammaglobulinemia have been excluded (1). Although it is frequently diagnosed in young adults, symptoms usually appear in early childhood (2). Moreover, more than half of them usually develop

Abbreviations
CVID, common variable immunodeficiency; GLILD, granulomatous–lymphocytic interstitial lung disease; IFN-γ, interferon gamma; IEI, inborn errors of immunity; IVIG, intravenous immunoglobulin; JAK, Janus kinase; STAT, signal transducer and activator of transcription; SOCS, suppressors of cytokine signaling; WBC, white blood cells.

autoimmunity, lymphoproliferation, enteropathy, and malignancies (3, 4). Among these conditions, chronic lung disease such as granulomatous–lymphocytic interstitial lung disease (GLILD) is one of the leading causes of death in these patients (5). Considering the wide range clinical picture of patients, CVID diagnostic criteria has evolved, and in 2019 the European Society for Immunodeficiencies (ESID) added to the published criteria the presence of autoimmunity, polyclonal lymphoproliferation, granulomatous disease, and increased susceptibility to infections (6). Despite being the most prevalent IEI, the genetic cause of CVID is only known in less than 35% of the patients, reinforcing the idea that in most cases there is only a clinical definition of CVID (7).

During recent years, many genes have been implicated in the pathogenesis of CVID, as follows: *ICOS*, *TNFRSF13B*, *TNFRSF13C*, *NFKB1*, *NFKB2*, *CD81*, *CD19*, *IL21R*, *PRKCD*, *CTLA4*, *STAT1*, *STAT3*, *IKZF1*, among others (8–12). These genes play a key role in B and T cells' development, maintenance, and/or cytokines signaling pathways, confirming the heterogeneous nature of this syndrome (13). If a specific mutation is identified, the patient is reclassified. One of the latest discovered genes associated with immune dysregulation is *SOCS1*. The suppressors of cytokine signaling (SOCS) are a family of essential downregulatory proteins of the Janus kinase and signal transducers and activators of transcription (JAK/STAT) activity. Their encoded protein SOCS1 can directly inhibit JAK kinase activity controlling immune responses to pro-inflammatory cytokines such as interferon gamma (IFN-γ). Moreover, *SOCS1*has the most potent ubiquitin ligase activity of this family that mediates protein's degradation (14, 15).

Recently, SOCS1 haploinsufficiency has been published as a new IEI in a small number of patients with a broad clinical phenotype spectrum, including immunological, rheumatological, and hematological symptoms (16–18). Here, we describe the first Argentinian patient presenting with CVID and GLILD due to a SOCS1 haploinsufficiency.

Case presentation

A 11-year-old boy, sixth child of non-consanguineous parents, from a rural area in the North of Argentina, with no relevant perinatal history and no vaccines complications, including BCG at birth, was the subject of our case study. His personal history includes vitiligo, an uneventful cat-scratch disease, and asthma as family background. At 8 years of age, he began with abdominal distension and pain. Splenomegaly is noted on physical examination in the absence of other palpable lymph nodes. His first laboratory evaluation revealed leukopenia and thrombocytopenia, mild elevated liver enzymes with normal bilirubin, hypogammaglobulinemia (IgG and IgM <2SD and IgA <1SD for age), normal serum proteins, albumin, and C3 and C4 proteins (Table 1). Stools study was also positive for *Giardia lamblia*. Then, he was referred to our hospital for further examination.

To complete the patient's evaluation, a bone marrow aspirate study was performed, excluding hematological malignancies. CMV, EBV, HIV were discharged by PCR. Abdominal CT scan showed homogeneous splenomegaly (Figure 1A). Regarding pneumological assessment, chest CT scan showed bilateral diffusely distributed nodules, including solid, subsolid, and ground-glass opacities, predominantly in the lung bases (Figure 1B); pulmonary function tests revealed a mixed ventilatory incapacity and low diffusing carbon monoxide (DLCO); and bronchoalveolar lavage showed an abnormal cytological pattern with increased lymphocytes infiltration. Furthermore, lung biopsy was performed showing the presence of a tuberculoid-like necrotizing granuloma, in the superior lobe, and GLILD in the middle lobe (Figure 2A), and immunohistochemistry informed: negative EBERs and CMV, and positive CD3 and CD20 (Figure 2B). Anatomopathological unit ruled out microorganisms with PAS, Ziehl–Neelsen, and Grocott staining. Moreover, cultures for common germs, fungi, and mycobacteria and PCR for *Mycobacterium* complex were done in lungs biopsy and bone marrow with negative results.

Immunological findings confirmed panhypogammaglobulinemia, abnormal polysaccharide response, and impaired response to mumps and measles vaccines, although tetanus toxoid, rubella, and varicella response were within normal range. The results were also normal for T, B, and NK cell subsets count. Low naive T cells with high activation markers expression (HLA-DR) and normal lymphoproliferative response were recorded. The B-cell compartment showed low switched B cells and high frequencies of $CD21^{low}$ (Table 1). Considering these findings, he was diagnosed with CVID and GLILD. He was started on intravenous immunoglobulin (IVIG) replacement and 2 mg/kg/day prednisolone. Considering that the upper lobe lung biopsy evidenced necrotizing granulomatous inflammation, we initiated anti-tuberculosis treatment in the patient based on the high prevalence of tuberculosis in Argentina and the fact that he was about to start immunosuppressive therapy for GLILD treatment, which can increase the risk of reactivation of latent tuberculosis infection, leading to active tuberculosis disease. We added four anti-tuberculous drugs (isoniazid, pyrazinamide, rifampin, and ethambutol) for 2 months followed by a continuation phase with two drugs during 6 months of his treatment.

Whole exome sequencing was performed using the Agilent SureSelect Human Exon Sequence Capture Kit XT v7, Santa Clara, USA. Sequencing libraries were prepared, followed by sequencing on the Illumina platform (Macrogen). Bioinformatic analysis was conducted following the Genome Analysis Toolkit (GATK) Best Practices Workflows, Broad Institute, Cambridge, USA. A total of 106,987 variants were obtained. Variants in genes associated with inborn errors of immunity, clinically overlapping with the proband, were prioritized (Supplementary Appendix S1). Two variants in SOCS1 were identified, NM_003745.2: c.[365G>A; 368C>A] p.[Gly122Glu; Pro123His], in the same allele, with no other clinically significant variants found (Figure 3A). The Gly122Glu variant is not found in gnomAD v4.0, has not been reported in the literature in SOCS1-related conditions, and bioinformatic tools do not predict a deleterious effect (REVEL). The Pro123His variant is also not found in gnomAD v4.0 or the literature. Interestingly, a pathogenic variant in the same codon but with a different amino acid change has been reported before (p.Pro123Arg) (18). Position P123 is highly conserved across vertebrates (philoP100 = 9.74) and deleteriousness of this variant is supported by bioinformatic

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TABLE 1 Laboratory findings.

		Patient (8 years)	Patient (9 years)	Patient (10 years)	Normal range
Initial results	Hemoglobin (g/dl)	12.3	16.3	14.2	11.5–15.5
	Hematocrit (%)	39.0	48.3	45.1	31.0–45.0
	WBC (cells/mm^3)	**3,000**	6,200	**4,000**	4,500–13,500
	Neutrophils % (mm^3)	27 (810)	41 (2,542)	40 (1,600)	32–54
	Lymphocytes % (mm^3)	65 (1,950)	47 (2,914)	40 (1,600)	28–48
	Monocytes (%)	5	11	9	3–6
	Platelets (10^3/µl)	**69**	181	**148**	150–450
	AST/ALT (UI/L)	**84/125**	28/21	27/15	≤40
	LDH (UI/L)	ND	210	ND	120–300
	Total proteins (g/dl)	6.6	6.8	6.8	6.0–8.0
	Albumin (g/dl)	4.6	4.6	3.9	3.8–5.4
	IgG (mg/dl)	**370**	821[b]	1,170[b]	969–1,485
	IgA (mg/dl)	**30**	**26**	**23**	87–239
	IgM (mg/dl)	**29**	**28**	**21**	68–182
	IgE (UI/ml)	ND	31	ND	≤90
	C3/C4 (mg/dl)	103/12	139/21	104/17	90–150/15–35
	Specific Ig antibodies	**Measles (−)/Mumps (−)** Rubella (+)/Varicella (+)	ND	ND	
	Tetanus toxoid (UI/ml)	0.18	ND	ND	≥0.1
	Pneumococcal (mg/L)	<30	ND	ND	
	Autoantibodies	(−)[a]	(−)[a]	(−)[a]	
	Isohemaglutinins	A (−)/B (1/64)	ND	ND	
Cellular response	CD3% (mm^3)	73 (2,127)	ND	80 (**1,280**)	55–78
	CD4% (mm^3)	30 (874)	ND	35 (**560**)	27–53
	CD8% (mm^3)	37 (1,078)	ND	41 (656)	19–34
	CD19% (mm^3)	14.6 (425)	ND	7.3 (117)	10–31
	CD56% (mm^3)	10.0 (291)	ND	10.5 (168)	4–26
	TCR αβ %	94.0	ND	95.1	54–66
	TCR γδ %	6.0	ND	4.7	5–8
	DN TCR αβ CD3$^+$ cells %	3.8	ND	3.4	<2.5
	Naive CD4$^+$ %	**35.6**	ND	**32.1**	37.8–69.6
	Central memory CD4$^+$ %	**53.3**	ND	**51.2**	21.4–40.3
	Effector memory CD4$^+$ %	**10.3**	ND	**14.2**	1.5–9.7
	Terminal effector CD4$^+$ %	**0.8**	ND	**2.5**	2.6–6.7
	HLA-DR CD4$^+$ %	**8.3**	ND	**10.0**	0.6–3.4
	Naive CD8$^+$ %	63.9	ND	**38.7**	49.2–82.7
	Central memory CD8$^+$ %	**7.0**	ND	**7.7**	11.5–30.7
	Effector memory CD8$^+$ %	1.3	ND	4.9	0.7–7.5
	Terminal effector CD8$^+$ %	**27.9**	ND	**41.0**	1.5–20.8
	HLA-DR CD8$^+$ %	**20.7**	ND	**25.0**	1.4–5.8
	Naive CD19$^+$ %	72.0	ND	77.6	
	IgM memory B cells %	24.4	ND	15.7	9.5–17.2
	Switched memory %	**3.3**	ND	**3.4**	5.5–14.9
	Transitional B cells %	**0.1**	ND	8.3	5.1–8.7
	CD21^{low} %	**23.9**	ND	**31.2**	5.6–12.6
	Plasmablast cells %	**0.0**	ND	0.1	0.1–0.2

WBC, white blood cells; ND, not done.

Bold numbers represent abnormal values for age-matched donors. ***T cells*** **(gated on CD3$^+$ CD4$^+$ or CD3$^+$CD8$^+$ cells)**: *Naive T cells* (CD45RA$^+$CD27$^+$); *Central memory T cells* (CD45RA$^-$CD27$^+$); *Effector memory T cells* (CD45RA$^-$CD27$^-$); *Terminal effector T cells* (CD45RA$^+$CD27$^-$); *Th1 memory* (CD45RA$^-$CXCR3$^+$ CCR6$^-$); *Th2 memory* (CD45RA$^-$ CXCR3$^-$ CCR6$^-$); *Th17 memory* (CD45RA$^-$CXCR3$^-$ CCR6$^-$); ***B cells*** **(gated on CD19$^+$ cells)**: *Naive B cells* (IgM$^+$IgD$^+$CD27$^-$); *IgM memory B cells* (IgM$^+$IgD$^+$ CD27$^+$); *Switched Memory* (IgD$^-$IgM$^-$CD38$^{+/-}$); *Transitional B cells* (CD38^{++}CD24^{++}); *CD21^{low} B cells* (CD21^{low}CD38$^{+/-}$); *Plasmablasts* (CD38^{++}CD27^{++}).

[a]Tested autoantibodies: anti-adrenal, anti-mitochondrial, anti-nuclear antibodies, anti-neutrophil cytoplasmic, anti-parietal cell, anti-*Saccharomyces cerevisiae*, anti-smooth muscle, anti-deaminated gliadin peptide, anti-transglutaminase, anti-glomerular basement membrane, anti-liver-kidney microsome.

[b]Under IVIG treatment.

prediction tools [(REVEL, University of California, Santa Cruz, USA), (CADD, University of Washington, USA)]. Sanger sequencing and familial segregation were performed confirming both variants in the patient, his mother, and two of his brothers (Figure 3B, C). Thereafter, we performed STAT-1 phosphorylation assay in the index case and his mother, which showed enhanced response of the JAK-STAT signaling pathway to IFN-γ stimulus (Figure 3D). The variants p.Gly122Glu and p.Pro123His were classified as variant of uncertain significance and likely pathogenic, respectively, according to the ACMG guidelines.

Following treatment initiation, he experienced improvement in cytopenia, splenomegaly, pulmonary function, and lungs images,

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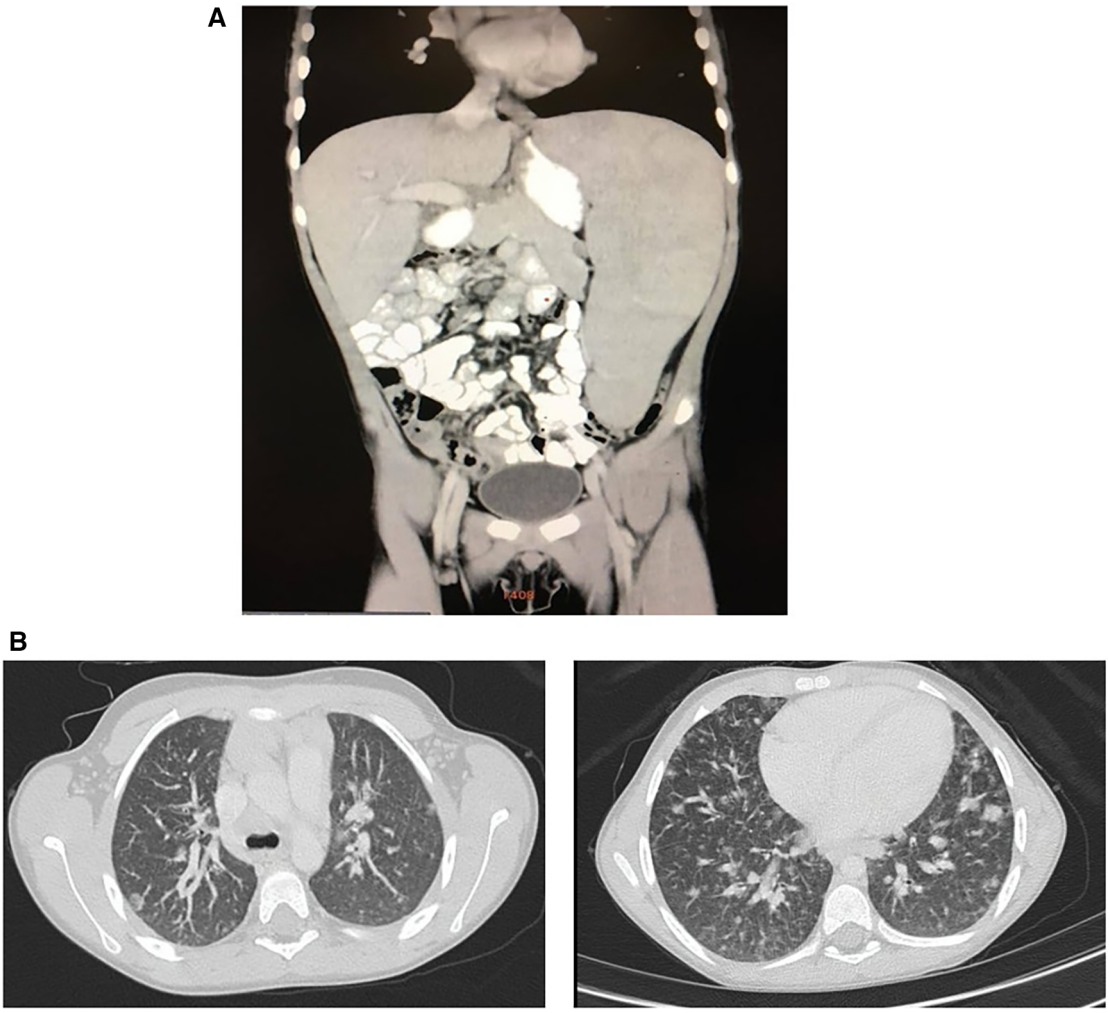

FIGURE 1
(A) Patient abdominal CT scan. Homogeneous craniocaudal splenomegaly measuring 176 mm associated with increased diameter of splenic vascular structures and increased hepatic hilar vasculature. No evidence of free fluid. (B) Patient chest CT scan. Bilateral diffusely distributed nodules, including solid, subsolid, and ground-glass opacities, predominantly in the lung bases with the largest measuring 12 mm.

and IgG levels. He completed 8 months of anti-tuberculosis treatment, followed by the transition from corticosteroids to mycophenolate mofetil (MMF). This last drug was replaced by rapamycin to address splenomegaly but only for four months as the patient presented severe neutropenia (570 cells/mm^3) and mild thrombocytopenia (127×10^3/µl). During his treatment, the patient developed a benign SARS-CoV-2 infection. Currently, the patient maintains stable condition with mild, fluctuating thrombocytopenia and moderate splenomegaly under IVIG and MMF. He continues follow-up for euthyroid thyroiditis and slightly elevated calprotectin levels. Notably, the patient reported no history of other remarkable infections besides the one mentioned previously.

Discussion

Common variable immunodeficiency can present with a variety of non-infectious symptoms that are often misdiagnosed, leading to significant delays in diagnosis and organ sequelae. The clinical course of these manifestations can be more severe than infections in certain instances (19). In the last few years, SOCS1 haploinsufficiency has been described as an autosomal dominant condition with variable expressivity phenotype and incomplete penetrance (16). Since its first description in 2020 in a cohort of 10 patients with early-onset autoimmunity [Evans syndrome, rheumatoid arthritis, systemic lupus erythematosus (SLE), Crohn's disease, psoriasis, type 1 diabetes], only a few studies have been published accounting for less than 20 patients around the globe (16, 18, 20, 21). Moreover, these patients suffered from bacterial infections (otitis media, pneumonia, abscesses), immunodeficiency, multisystem inflammatory syndrome, eczema, and Hodgkin lymphoma, among others. Here we describe the first Argentinian patient presenting with CVID phenotype due to SOCS1 haploinsufficiency.

SOCS1 variants may affect the function of the SOCS1 protein in various ways: some may affect the expression of the SOCS1 protein, while others may affect its function leading to dysregulation of cellular signaling, which may contribute to the development of

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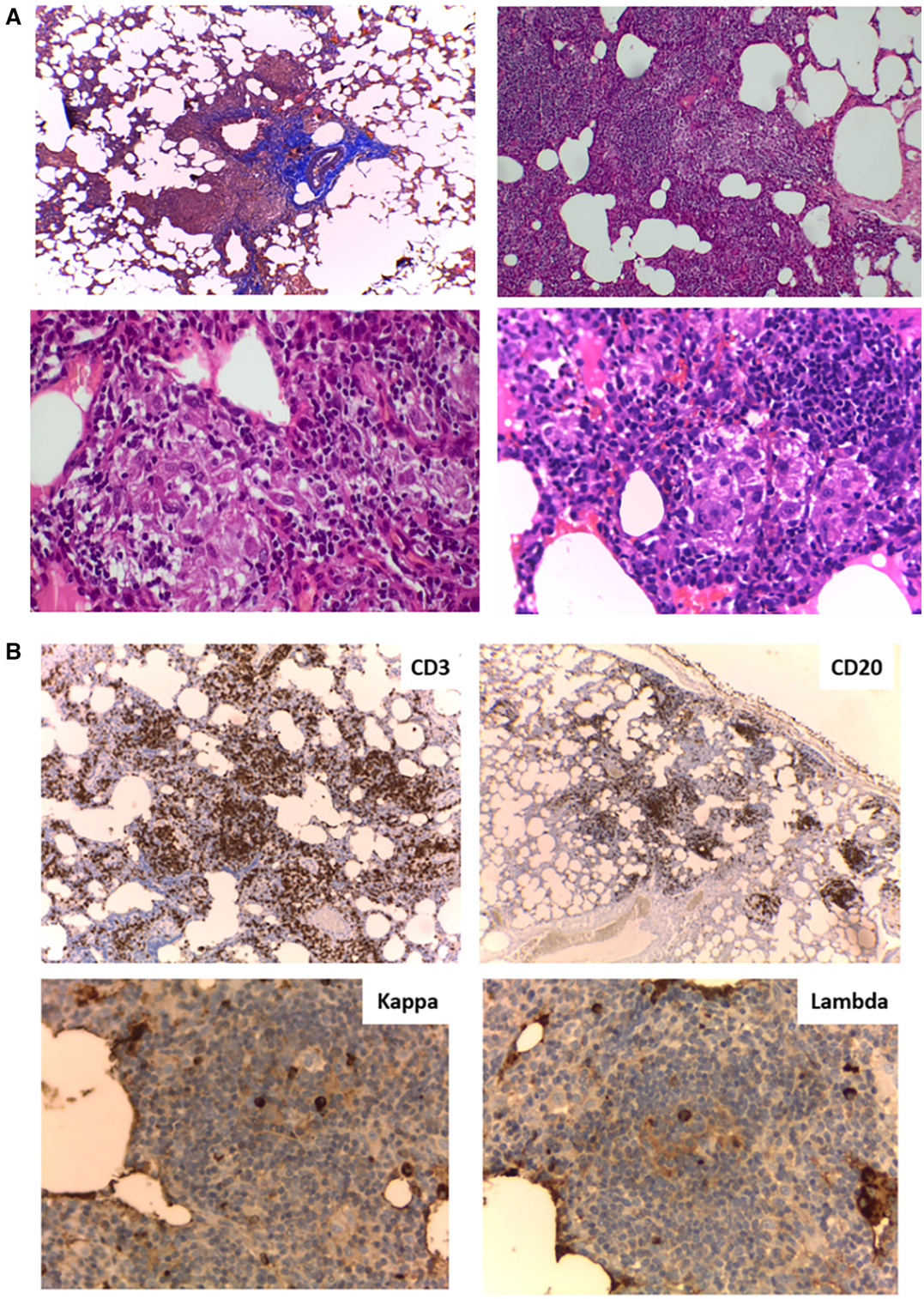

FIGURE 2
(**A**) Lung biopsy histology. (Upper left) Masson's trichrome stain (4×) peribronchiolar mononuclear inflammatory infiltrate; (Upper right) Hematoxylin and eosin (H&E) stain (10×) Interstitial expansion of lymphocytes and histiocytes; (Lower left and right) H&E stain (40×) Presence of poorly formed non-necrotizing granulomas. (**B**) Lung biopsy immunohistochemistry. (Upper left) (4×) $CD3^+$ lymphocytes at the interstitial level; (Upper right) (4×) Aggregates of $CD20^+$ lymphocytes; (Lower left and right) (40×) Kappa and lambda chains in similar proportions.

diseases. Hadjadj et al. reported two patients with heterozygous missense germline SOCS1 mutations in the same amino acidic residue as our patient but with a different amino acidic change. Both individuals experienced immune thrombocytopenic purpura (ITP) from very early onset, with one of them also presenting thyroiditis and polyarthritis (18). Nevertheless, none of them

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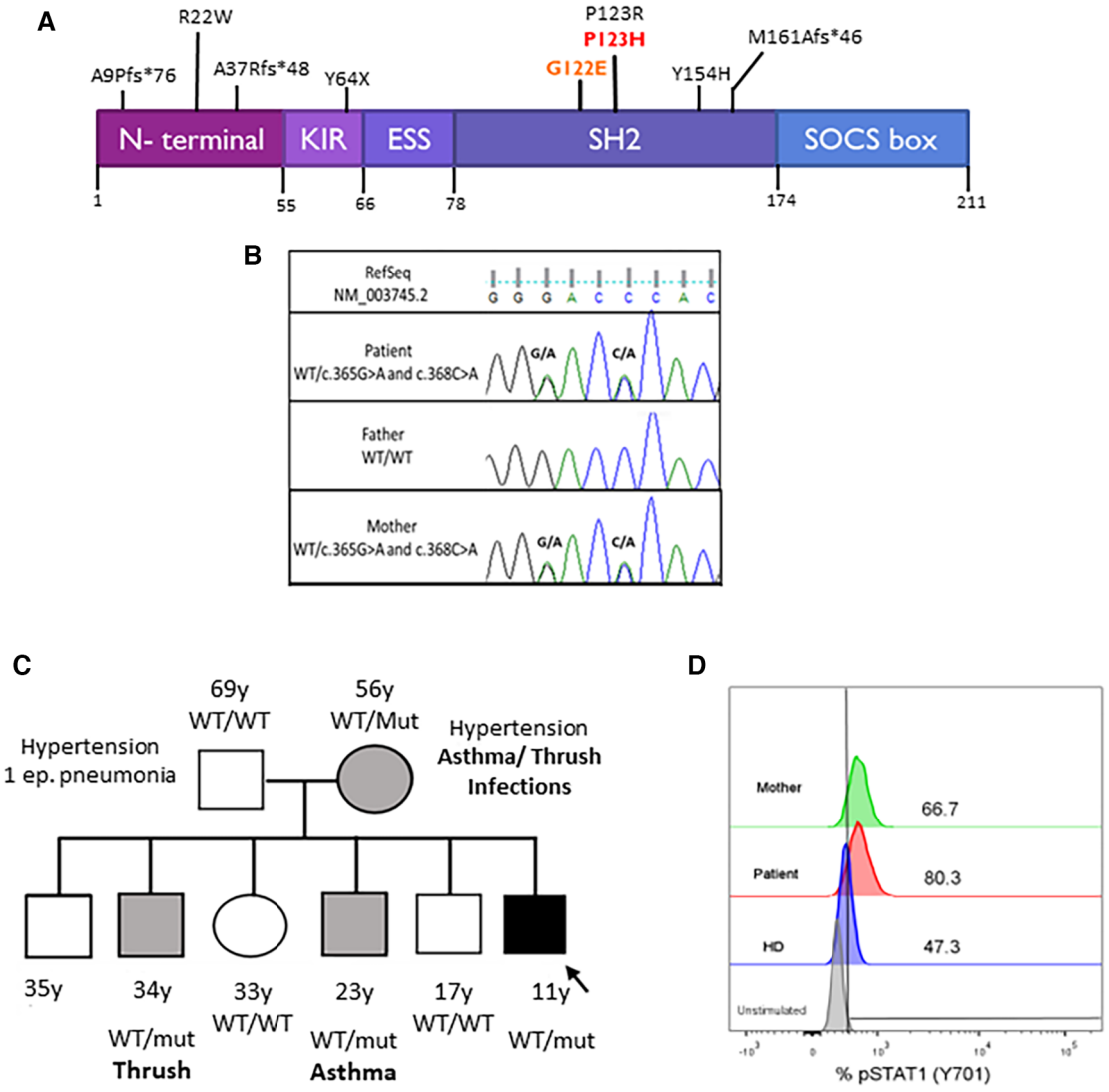

FIGURE 3
(A) SOCS1 protein domains and locations of the variants. The kinase inhibitory region (KIR) functions as a pseudo-substrate that can inhibit the tyrosine kinase activity of JAK proteins. The SRC-homology 2 (SH2) domain binds the activation loop of the JAK proteins' catalytic domain. The SOCS box recruits the ubiquitin-transferase system and initiates the proteasomal degradation of JAK proteins. Mutations are described in the literature. Our patient's mutations are in red. (B) Pedigrees of the family with SOCS1 variants. Squares: males; circles: females; black: affected mutation carriers; gray: unaffected mutation carriers. WT, wild-type *SOCS1* allele. (C) Sanger sequencing analysis. Sanger sequencing of the exon 1 of *SOCS1* in the proband and his parents confirmed the maternal origin of: c.365G>A (p.G122E) and c.368C>A (p.P123H) in the same allele of *SOCS1* gene. (D) STAT1 phosphorylation assay. Increased phosphorylation of monocytes ($CD14^+$) after 15 min with IFN-γ stimulation in the patient and his mother compared with a healthy control (HD).

presented with either a CVID phenotype or lymphoproliferation. To date, there are only two reported patients with hypogammaglobulinemia (21) and one patient with CVID with SOCS1 variants in the literature (20). Interestingly, this is a child who developed GLILD and ITP too, although she carries a different variant (p.Met161Alafs*46) that resulted in a SOCS1-truncating protein. Of note, our patient carries two cis variants in the SH2 domain located in contiguous amino acids (p.Gly122Glu and p.Pro123His, respectively). This domain acts as a regulatory checkpoint in intracellular signaling pathways of several cytokines (15). As noted in previous reports, our results also highlight the variable expressivity and incomplete penetrance nature of the disease, as the mother had abnormal laboratory markers compatible with immune dysregulation (Supplementary Table S1) and mild clinical symptoms, whereas both carrier brothers also have mild clinical symptoms. However, we cannot exclude the fact that he may develop other symptoms later in life, and appropriate genetic counseling should be provided. Unfortunately, we were unable to investigate each of them separately, but as mentioned previously, there is a previous report of a pathogenic variant in the same position. In this regard, although the P123H variant is likely to have a major impact on SOCS1 function, we cannot exclude a cis-interacting effect of the other variant, affecting the disease phenotype (22). Due to the limited number of identified cases, research on *SOCS1* variants requires further investigation with a larger patient population to fully elucidate their impact.

Although CVID is traditionally classified as a humoral immunodeficiency, recent years have seen the identification of additional cellular defects contributing to the disease. In this sense, our patient's $CD4^+$ and $CD8^+$ compartments exhibit a skew toward an activated/memory phenotype, similar to those we and others have described in other diseases with immune dysregulation (23–25). This altered phenotype may arise from the underlying mutation and the enhanced response to interferons as confirmed with STAT1 phosphorylation. In line with this, $CD21^{low}$ B cells express T-bet, a transcription factor that has originally been described as the master regulator of Th1 cell development (26). This population might contribute to the inflammatory symptoms and autoimmune manifestations observed in these patients. Of note, an increased prevalence of these cells has been observed in patients with CVID with autoimmune manifestations, splenomegaly, cytopenia, and other autoimmune diseases such as SLE, like our patient's phenotype (25, 27).

The introduction of Ig replacement therapy has significantly reduced infectious complications in CVID; however, its impact on inflammatory and autoimmune manifestations remains limited, becoming one of the most critical issues in CVID management. Thus, the fine-tuning regulation of cytokine signaling in patients with SOCS1 haploinsufficiency is essential for maintaining a balanced immune system. In this regard, treatment with several immunosuppressive therapies should be included. Steroids, mycophenolate mofetil, hydroxychloroquine, rapamycin, biological agents (rituximab, anti-TNFα), and JAK inhibitors (JAKinhibs) have been reported to control the hyper-inflammatory responses (28). Considering that autoimmune manifestations in patients with SOCS1 haploinsufficiency are frequently observed, compared with infectious presentation, the use of JAKinhibs may be a good option in these patients. They exert their therapeutic effect by targeting a key pathway in cytokine signaling competitively binding to the ATP-binding site of JAK kinases, thereby preventing their activation and subsequent STAT phosphorylation (29). It is well known that one main concern about the use of JAKinhibs is the potential development of severe infections as an adverse effect (30, 31). Nonetheless, as they may cause cytopenia by blocking hematopoiesis via JAK1/JAK2-dependent pathways, the use of these drugs should be under strict consideration and monitoring in each case (32).

In summary, we describe the first case of a patient from Argentina with CVID phenotype and immune dysregulation, due to novel SOCS1 mutation. This case emphasizes the clinical and laboratory heterogeneity in patients with CVID, highlighting the limitations of our current understanding of the underlying mechanisms. Molecular studies are needed to identify dysregulated pathways, paving the way for targeted therapies and improved patient outcomes.

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

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

Author contributions

MC: Conceptualization, Writing – original draft, Writing – review & editing, Visualization. ED: Writing – review & editing. ACG: Writing – review & editing. ALG: Data curation, Methodology, Writing – review & editing. AB: Data curation, Methodology, Software, Writing – review & editing. MM: Data curation, Methodology, Writing – review & editing. MG: Writing – review & editing. DD: Writing – review & editing.

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Conflict of interest

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

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2024.1423858/full#supplementary-material

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An efficient and successful outcome after haematopoietic stem cell transplantation in a patient with an LPS-responsive beige-like anchor gene mutation

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Lipopolysaccharide (LPS)-responsive beige ankyrin (LRBA) gene mutations were first reported as the cause of immunodeficiency syndromes and autoimmunity in 2012. The majority of LRBA patients have multiple organ system involvement and a complex clinical phenotype. Herein we present a comprehensive account on the disease progression and transplantation procedure in a patient with LRBA deficiency who exhibited progressive autoimmune disease symptoms along with recurrent pulmonary infections since the age of 6 years old. Despite receiving abatacept therapy and immunoglobulin replacement treatments to manage the symptoms, but the symptoms still progressed. Therefore, nine years after disease onset, patients were treated with allogeneic haematopoietic stem cell transplantation (allo-HSCT). The patient experienced acute and chronic graft-versus-host disease (GVHD) and recurrent infections after transplantation. During one and a half years of follow-up, we found that allogeneic haematopoietic stem cell transplantation can relieve the symptoms of autoimmune disease in patients with LRBA deficiency, and marked clinical improvement and recovery of immune function were observed following stem cell transplantation.

KEYWORDS

LRBA mutation, haematopoietic stem cell transplantation, neurological change, infection, graft-versus-host disease

1 Introduction

CTLA-4 is an immune checkpoint molecule predominantly expressed on the surface of *T* cells (1). LRBA proteins regulate the intracellular trafficking and storage of CTLA4, facilitating its rapid mobilization to the cell surface for effective suppression of hyperactivation (2). The main clinical manifestations of LRBA deficiency include recurrent respiratory infections, lymphoproliferation, and immune dysregulation characterized by inflammatory bowel disease, autoimmune cytopenia, and autoimmune hemolytic anemia (3). Additionally, patients with LRBA deficiency often develop bronchiectasis and interstitial lung diseases following recurrent infections (4, 5).

Currently, abatacept (a CTLA4-immunoglobulin fusion drug) has shown significant and sustained improvement in patients with LRBA deficiency (2, 6). While allogeneic hematopoietic stem cell transplantation (HSCT) is a standard treatment for various primary immunodeficiencies (PIDs), there are no established guidelines regarding HSCT in patients with autoimmune diseases. In this study, we present the clinical features and treatments of a patient diagnosed with LRBA deficiency. Hematopoietic stem cell transplantation has long-term efficacy in the treatment of patients with LRBA deficiency.

2 Method

2.1 Western blot

Isolated peripheral blood mononuclear cells (PBMCs) were stimulated with 10 ng/μl PHA for 48 h. Protein was extracted in RIPA and PMSF buffer. Protein extracts underwent electrophoresis on a 6% concentration polyacrylamide gel and were subsequently transferred to nitrocellulose membranes. The membranes were initially incubated in a blocking buffer followed by incubation with specific antibodies. For this study, LRBA antibody (1:1,000, Abcam, ab191174), DOCK8 antibody (1:5,000, Abcam, ab175208), and HRP-conjugated goat anti-mouse secondary antibody (1:1,000, Beyotime, A0216) were employed."

2.2 Flow cytometry

PBMCs were directly stained with the surface markers CD4-PE-Cy7, CD25-BV421, and CD45RA-FITC, followed by fixation/permeabilization (BD Biosciences) according to the manufacturer's instructions. Then, the cells were stained with fluorochrome-conjugated antibodies FOXP3-PE and CTIA4-APC. Analyses were performed using BD Celesta.

3 Results

3.1 Before transplantation

3.1.1 Clinical manifestations

A 6-year-old boy presented with autoimmune haemolytic anaemia (AIHA) and thrombocytopenia. Following treatment with immunosuppressive agents, no further episodes of AIHA have been observed. In addition to the manifestation of Evans syndrome, the patient also exhibited recurrent infection and hypogammaglobulinemia after reaching the age of 10 years, especially Invasive pulmonary fungal infection. The emergence of recurrent cough and expectoration suggested pulmonary infection. Despite receiving multiple courses of antibiotics without sustained improvement, sputum smear examination revealed the presence of a small number of spores and the (1,3)-β-D glucan test yielded positive results, further indicating potential fungal infection. The patient was initially treated with oral prednisolone and received regular monthly immunoglobulin replacement. The growth and development in patients suffering from severe malnutrition were found to be below 3 SDs for children of similar age and sex after reaching 10 years old. At 13 years of age, lung CT revealed fibrotic lesions in the lower lobes of both lungs. At the same stage, abdominal ultrasonography revealed hepatosplenomegaly suggesting severe liver fibrosis possibly due to autoimmune hepatitis. In addition, brain MRI showed abnormal signals in the bilateral temporal lobe, right parietal lobe and corpus callosum without any evidence of infection (Figures 1A–C). Other clinical manifestations included interstitial kidney injury and hypokalaemia.

Considering that LRBA deficiency may contribute to autoimmune disorders in patients, targeted therapy with subcutaneous injection of abatacept at a dosage of 125 mg/w was administered for 5 months prior to transplantation. However, despite the combination of abatacept and immunosuppressive therapy, complete prevention of disease progression was not achieved. Although examination results indicated reduced liver and spleen sizes in the patient, recurrent pulmonary infections persisted along with persistently low levels of immunoglobulins and B-cells (Figures 2A,B).

On the eve of transplantation, the child developed symptoms of septic shock due to sepsis. Following anti-shock treatment, improvements were observed in blood pressure and mental state before proceeding with immediate hematopoietic stem cell transplantation. Pre-transplantation laboratory findings are presented in Table 1.

3.1.2 Immunological manifestations

LRBA gene mutations were confirmed through whole exome sequencing. The patient harbors a compound heterozygous mutation, c.4801C>T (p.Arg1601*), located in exon 30, which constitutes a nonsense mutation inherited from the father. The other mutation, c.4239delT, represents a deletion variant originating from the mother. Functional validation was performed by western blotting, revealing an absence of LRBA expression and reduced CTLA4 expression in the patient (Figures 4A,C). Notably, there was a significant decrease in the proportion of naive CD4+ *T* cells, particularly CD4 CM cells; meanwhile, the proportion of CD8 CM cells among total CD8+ *T* cells increased. Due to their low abundance for testing purposes, B cell proportions could not be determined (Table 2). It is worth mentioning that the patient's immunoglobulin level remained consistently low throughout this study period (Figure 6E), necessitating regular administration of immunoglobulin.

3.2 HSCT

3.2.1 HSCT process

Due to severe clinical complications and the inefficacy of drugs, haematopoietic stem cell transplantation has become a

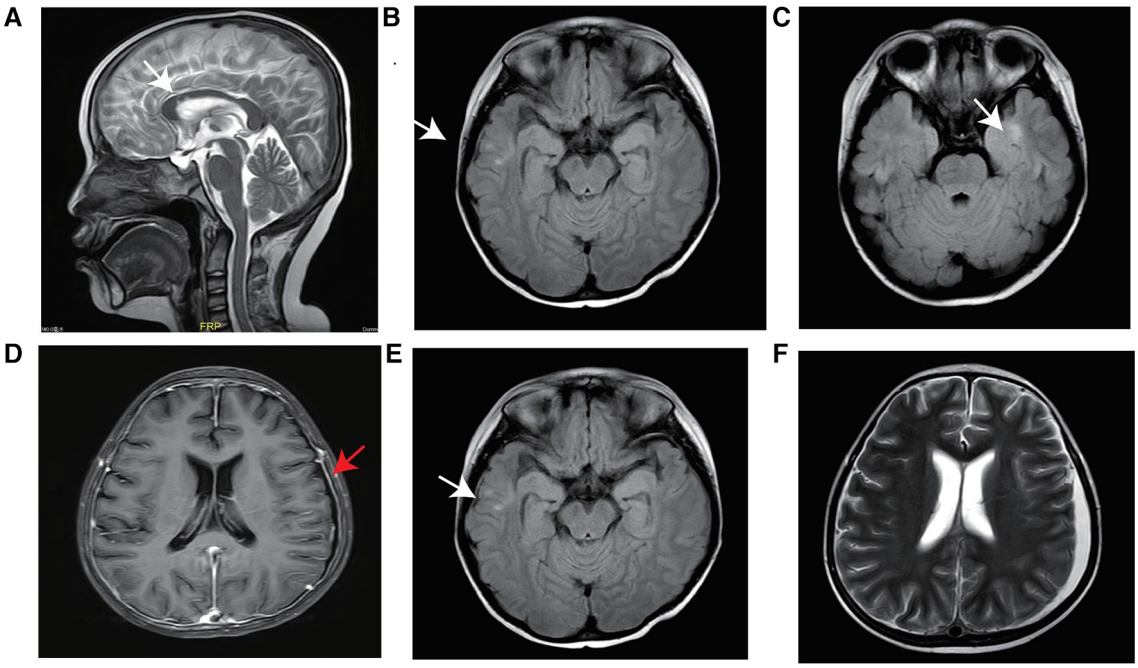

FIGURE 1
MRI of the brain. Abnormal signals from the corpus callosum body to the bilateral frontal ventricle and bilateral temporal lobe before transplantation (white arrow) (A–C), linear enhancement of the bilateral frontoparietal and occipital meninges after HSCT (red arrow) (D), abnormal signals (E), and a small amount of new subdural effusion in the left frontotemporal parietal region (F).

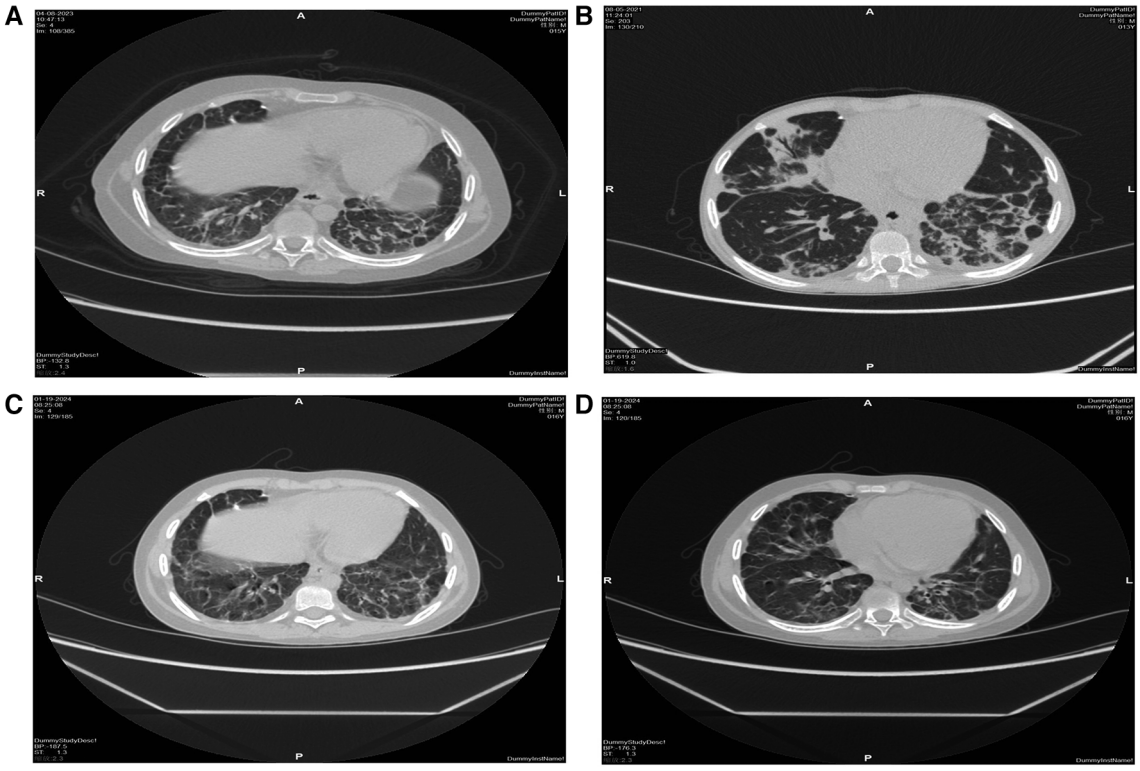

FIGURE 2
High-resolution CT scan of the lungs. Before transplantation, bilateral pulmonary fibrosis with segmental consolidation in the left lower lobe (A,B), consolidation was significantly absorbed, and fibrosis improved after transplantation (C,D).

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TABLE 1 Laboratory workup of the patient with lipopolysaccharide-responsive beige-like anchor deficiency.

Lab results	Pre-transplantation	Post-Transplantation (1Y6M)	Normal range
WBC	4.98	4.4	4.1–11.0 (10^9 /L)
RBC	5.21	4.19	4.6–6.0 (10^{12} cells/L)
PLT	189	178	100–407 (10^9 cells/L)
NE	57.7	56.9	37–77 (%)
LYM	33.5	38.2	17–54 (%)
Total bilirubin	6.8	8.8	2.68–35.26 (umol/L)
Direct bilirubin	0	0	0–10 (umol/L)
Indirect bilirubin	4	6.4	0–18.67 (umol/L)
Total protein	43↓	71.6	64.6–84.7 (g/L)
Albumin	22.6↓	41.4↓	42.5–54.8 (g/L)
Globulin	20.4	30.2	15.3–35.0 (g/L)
ALT	55↑	22	11.4–45.4 (U/L)
AST	96↑	30	13.9–35.3 (U/L)
ALP	175	198	62.0–334.5 (U/L)
Glutamyl transpeptidase	289↑	15	10.0–27.9 (U/L)
LDH	290↑	255	105–257 (U/L)
Cholinesterase	6,328	7,948	5,900–12,220 (U/L)
Urea	3.34	2.69	2.1–7.1 (mmol/L)
Creatinine	42	21↓	30.5–103.5 (umol/L)
Uric acid	312	245	185.9–465.7 (umol/L)

curative option. Fortunately, HLA (10/10)-matched unrelated donors were procured from the China Marrow Donor Program. The patient received mobilized peripheral blood cells containing 12.94×10^8 /kg MNC cells/kg and 7.47×10^6 CD34+ cells/kg following reduced-intensity conditioning regimens, including fludarabine 45 mg/m^2/d (D-7∼-4), BU 3 mg/kg/d (D-5∼-2), and ATG5 mg/kg/d (D-5∼-3) (Figure 5). The conditioning regimen was well tolerated without any signs of organ toxicity. Voriconazole was administered for fungal infection prophylaxis, Imipenem-Cilastatin Sodium combination drug for bacterial infection prevention, acyclovir for viral infection prevention, weekly gamma globulin infusion along with weekly monitoring of EBV and CMV-DNA quantification.

3.2.2 Engraftment

Granulocyte recovery for haematopoietic reconstitution was continuously observed on Day 13 post-HSCT. Megakaryocyte recovery occurred on Day 15 post-HSCT. Methylprednisolone was administered on day 15 after transplantation to treat implantation syndrome (Figure 5). Stable chimerism, with T, B cells and whole blood showing complete donor engraftment (100% of donor), was observed starting from day +28.

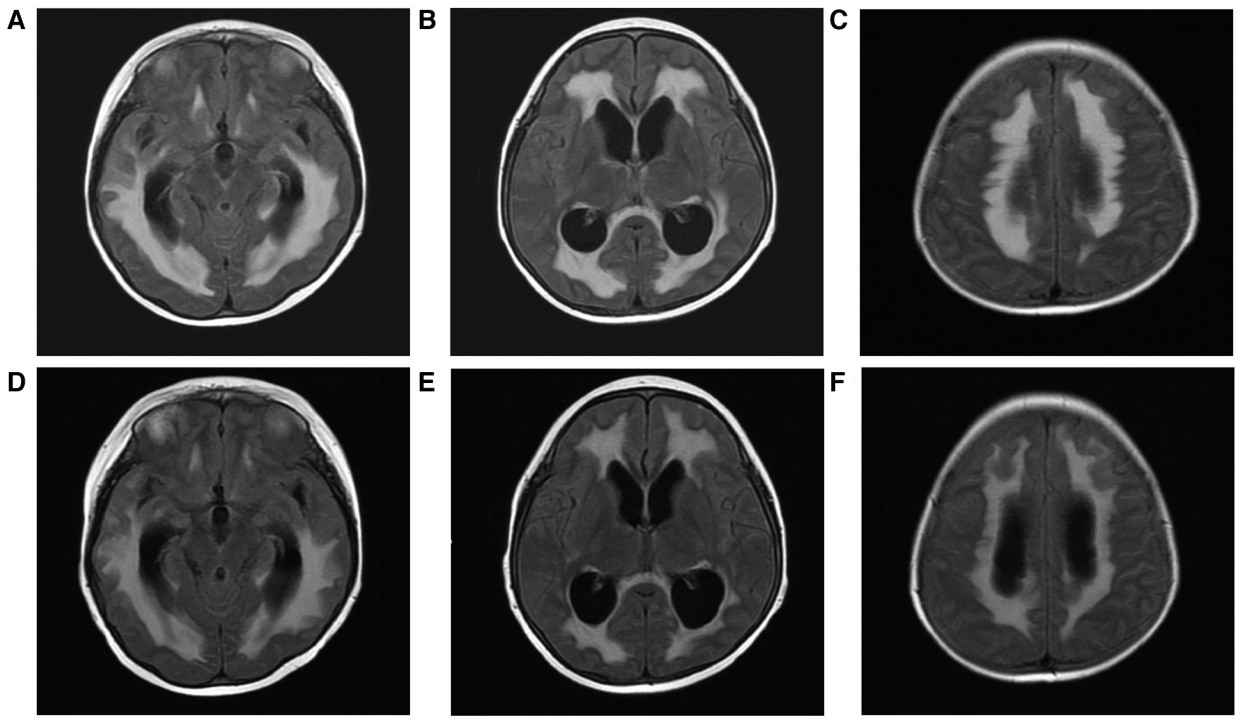

FIGURE 3
T2-FLAIR hyperintensity contrast-enhanced lesions, multiple abnormal signals in bilateral cerebral white matter after haematopoietic stem cell transplantation (A–C). Decreased area of white matter lesions after treatment (D–F).

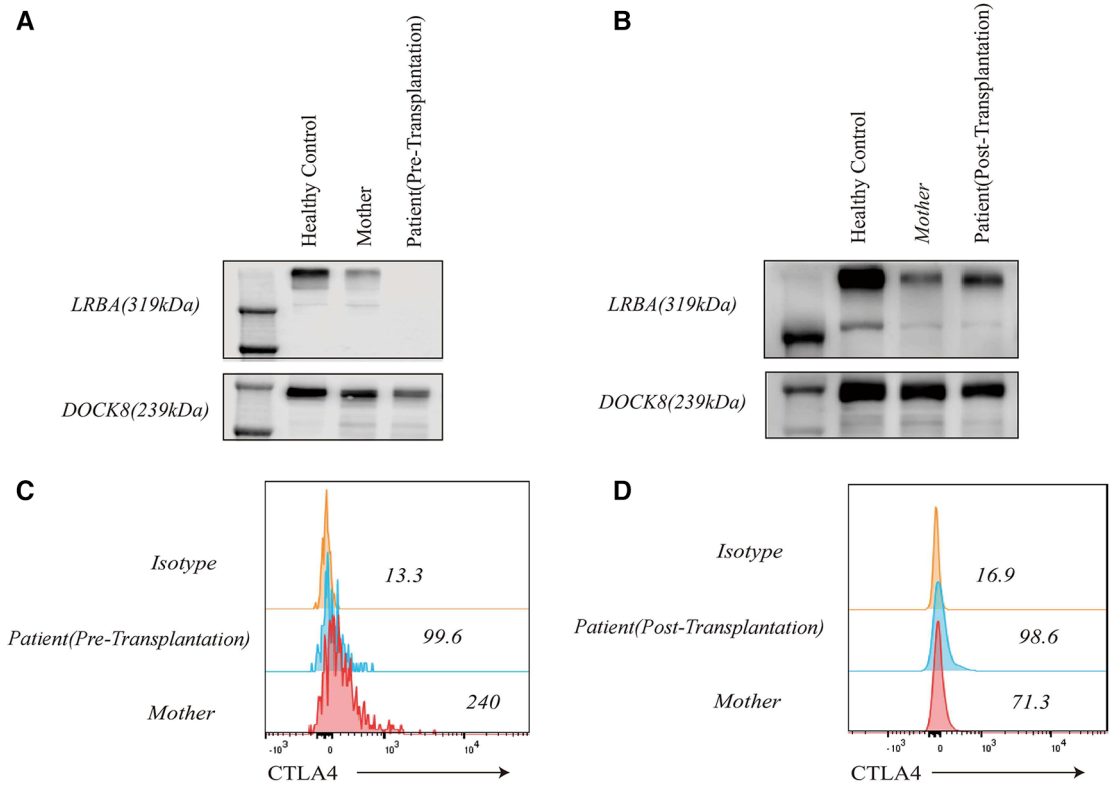

FIGURE 4
LRBA and CTLA4 expression levels in the patient, his clinically healthy mother and healthy control. LRBA expression was absent in the patient before transplantation (**A**), and CTLA4 expression was reduced (**C**) LRBA expression (**B**) and CTLA4 levels (**D**) normalized after haematopoietic stem cell transplantation.

3.3 After transplantation

3.3.1 GVHD

The patient developed grade II acute gastrointestinal tract graft-versus-host disease (GVHD) on 18D, presenting as watery diarrhoea. Stool culture results were negative, and the patient had a daily stool volume exceeding 500 ml during the day. By increasing methylprednisolone dosage to 2 mg/kg/day and enhancing intravenous nutrition, significant improvement in GVHD was observed. The patient was discharged from the laminar flow ward after 35 days. However, six months post-transplantation, the patient experienced loose paste-like stools with occasional blood-streaked mucus. Stool cultures remained negative throughout this period and liver function examination revealed alanine transaminase (ALT) levels of 224 U/L and aspartate transaminase (AST) levels of 134 U/L. Thus leading to a diagnosis of chronic gastrointestinal tract and liver GVHD. Oral corticosteroid and mycophenolate mofetil doses were increased along with glutathione supplementation for liver protection alongside other symptomatic and supportive treatments. The patient improved and was discharged (Table 3).

3.3.2 Infection

The patient experienced recurrent nervous system, gastrointestinal tract and respiratory tract infections after transplantation. Two months after transplantation, the patient developed persistent consciousness disturbance and fell into a mild coma. Brain MRI revealed a slight increase in subdural effusion in the left frontotemporal region compared to pre-transplantation levels, along with linear enhancement observed bilaterally in the frontoparietal and occipital meninges (Figures 1D–F). Adenovirus PCR testing on cerebrospinal fluid yielded positive results. Four months later, the patient experienced recurring symptoms including headache, vomiting, and fatigue. Brain MRI showed multiple abnormal signals in bilateral white matter as well as linear enhancement of bilateral frontotemporal meninges (Figures 3A–C). Posttransplant leukoencephalopathy was diagnosed. Despite a negative cerebrospinal fluid (CSF) culture, the patient improved after treatment with antibiotics. During the next month, abdominal pain recurred accompanied by mucus and bloody stools. Abdominal CTA revealed bowel wall thickening and stool culture was positive for *Clostridium difficile* toxin B. Remission was achieved through treatment with metronidazole and vancomycin.

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TABLE 2 Fine classification of immune cells.

Lab results	Relative number (%)			Absolute number (cells/μl)		
	Pre-transplantation	Post-transplantation (1Y6M)	Normal range	Pre-transplantation	Post-transplantation (1Y6M)	Normal range
T cell (CD3+)	91.2↑	80.7↑	56.84–75.02	11,541.6↑	1,354.9	1,184–2,144
CD8+ *T* cell (CD3 + CD8+)	30.06	53.8↑	21.91–36.80	508	904.0	489–1,009
CD8 Naïve (CD8 + CD45RA + CD27+)	53.1	18.5↓	35.34–72.32	269.8	167.2↓	231–568
CD8 TEMRA (CD8 + CD45RA + CD27−)	9.5	24.6	5.08–31.24	48.2	222.4	29–269
CD8 CM (CD8 + CD45RA −CD27+)	37.1↑	32.9↑	10.96–31.00	188.5	297.4↑	74–228
CD8 EM (CD8 + CD45RA −CD27−)	0.3↓	23.9↑	2.38–15.84	1.3↓	216.1↑	16–109
CD4+ *T* cell (CD3 + CD4+)	49.5↑	23.9	22.25–39.00	835.7	401.9	522–1,084
CD4 Naïve (CD4 + CD45RA + CD27+)	9.7↓	27.1	39.50–66.26	81.3↓	108.9↓	230–627
CD4 TEMRA (CD4 + CD45RA + CD27−)	5.2↑	5.3↑	0.00–1.54	43.8↑	21.3↑	0–12
CD4 CM (CD8 + CD45RA −CD27+)	77.2↑	34.9	25.34–49.90	645.2↑	140.2↓	182–403
CD4 EM (CD8 + CD45RA −CD27−)	7.9	32.7↑	4.68–15.70	65.9	131.4	29–117
TCRαβ + DNT/T	1.4	0.3↓	0.61–2.31	21.5	4.7↓	12–37
γδ *T* cell	15.3	4.7↓	6.55–20.28	235.9	63.7↓	81–343
B cell (CD19+)	–	13.2	8.84–17.76	–	222.1	203–476
Memory B (CD19 + CD27 + IgD−)		3.1↓	7.15–23.10		6.9↓	20–86
Naïve B (CD19 + CD27 −IgD+)		87.6↑	53.78–78.64		194.6	116–347
Transitional B (CD19 + CD24 + CD38+)		34.9↑	1.38–9.42		77.5↑	4–37
Plasmablasts B (CD19 + CD24–CD38+)		0.9	0.49–7.06		2.0	1–23.0

CM, central memory; EM, effector memory.

Prior to transplantation, pulmonary fibrosis was predominant with no abnormalities detected during pulmonary function tests. Following transplantation though early stages witnessed recurrent pulmonary infections; however lung CT scans indicated significant improvement compared to pre-transplantation conditions (Figures 1C,D). One year post-transplantation there were no apparent signs of infection observed in the child while neurological examination also displayed no abnormalities (Figures 3D–F).

3.3.3 Immunological recovery

LRBA protein and CTLA4 expression were detected at the ninth month after HSCT showing similarity to that in the patient's parents and healthy controls (Figures 4B,D). The patient's immune status was continuously monitored after transplantation. Although CD4+ *T*-cell counts were slightly low early after transplantation, they returned to normal levels. Reconstitution of CD8+ *T* cells began early after transplantation (Figures 6A,C) (Table 2). The patient's B cell counts remained low after transplantation, and gamma globulin was administered regularly. Fortunately, one and a half years after transplantation, the patient's B cells returned to normal levels, along with normalization of immunoglobulin levels. (Figures 6B,D) (Table 2). IVIG replacement therapy was discontinued at 1 year and 8 months after transplantation (Figure 6E).

4 Discussion

Lipopolysaccharide (LPS)-responsive vesicle trafficking and beach- and anchor-containing (LRBA) gene deficiency can lead to common variable immunodeficiency (CVID), which presents symptoms similar to autoimmune lymphoproliferative syndrome (ALPS) and manifestations reminiscent of X-linked immune dysregulation-nolvendocrinopathy, enteropathy (IPEX)-like syndrome. The specific treatment guidelines for CVID remain unknown, and symptomatic treatment is the main treatment. Current findings indicate that patients with LRBA deficiency exhibit dramatic and sustained improvement in response to abatacept, a CTLA4 (cytotoxic T lymphocyte antigen-4)–immunoglobulin fusion drug (1, 2). However, in this patient, abatacept did not respond well.

The indications for allogeneic haematopoietic stem cell transplantation (allo-HSCT) or gene therapy, such as severe combined immunodeficiency (SCID), are well-established. However, not all immunodeficient patients have clear indications for early allo-HSCT. Compared with novel

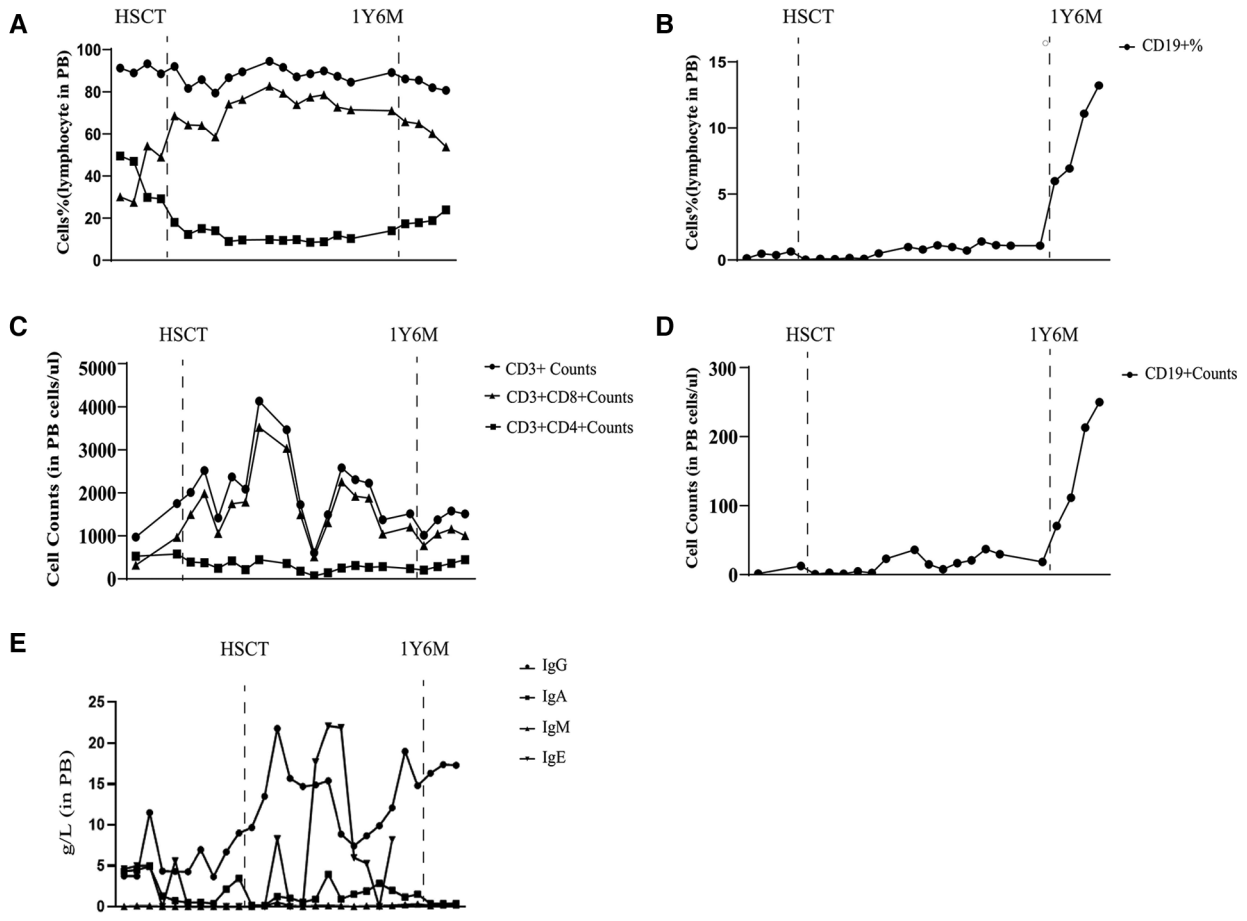

FIGURE 6
Laboratory values from diagnosis to one and a half years after transplantation. Proportion of CD3+, CD8+, and CD4+ T lymphocytes (A) Proportion of CD19+ B lymphocytes (B) Number of CD3+, CD8+, and CD4+ T lymphocytes (C) Number of CD19+ B lymphocytes (D) Immunoglobulin level (E).

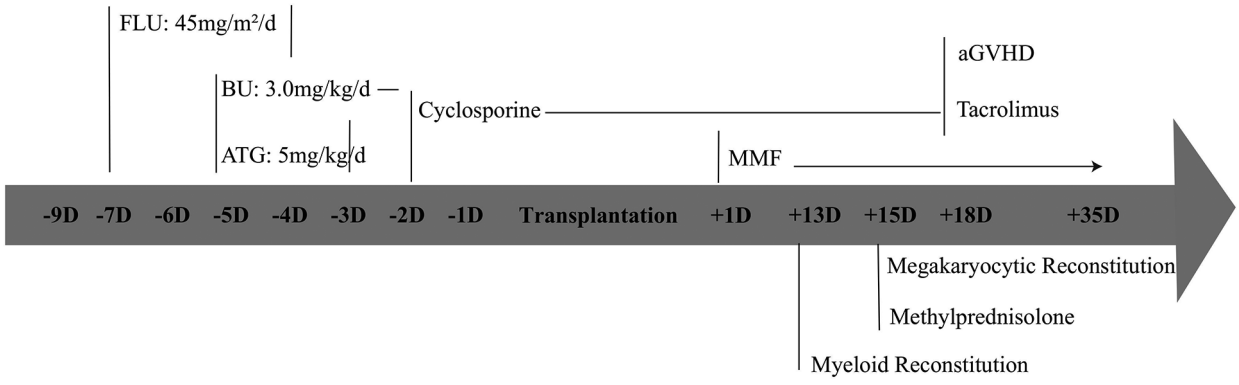

FIGURE 5
Elaboration on the transplantation procedure.

immune system-dysregulative PIDs, these PIDs exhibit heterogeneous disease manifestations and lack genotype–phenotype correlations.

A study has demonstrated that patients undergoing hematopoietic stem cell transplantation have comparable survival probabilities to those treated with abatacept. While almost all

TABLE 3 GVHD experienced by the patient after hematopoietic stem cell transplantation.

GVHD	Targeted organ	Score	Symptoms and clinical manifestations	Occurrence time	Therapy
aGVHD	Gastrointestinal tract	2	The patient presents with prominent diarrhea, characterized by profuse watery stools accompanied by scant fecal debris. Stool culture results are negative, and the daily stool volume exceeds 500 ml during the day.	18D	Refrain from administering cyclosporine and commence tacrolimus treatment, escalate the dosage of methylprednisolone to 2 mg/kg/day, incorporate omeprazole for acid suppression and gastric protection, administer parenteral nutrition support, and decrease oral food intake.
cGVHD	Gastrointestinal tract	1	The patient presents with 5–7 daily episodes of diarrhea, characterized by loose paste-like stools occasionally accompanied by blood-streaked mucus. Stool cultures yield negative results during this period, a noticeable improvement is observed after 12 days, with a reduction of bowel movements to 1–2 times per day.	6M	Intravenous administration of methylprednisolone at a dosage of 2 mg/kg/day, in combination with mycophenolate mofetil at a dosage of 45 mg/kg/day.
	Liver	2	ALT: 224 (U/L) AST: 134 (U/L)		

LRBA-deficient patients respond positively to abatacept therapy, the extent of response varies among individuals with different clinical presentations. The highest rates of complete remission are observed in those with lymphoproliferative disorders, followed by those with chronic diarrhea. Conversely, responses to abatacept treatment for immunodeficiency-related symptoms display greater heterogeneit (7). In our patient's case, although there was a notable improvement in lymphoproliferative manifestations, recurrent infections persisted as the most concerning aspect throughout the course of the illness and were unaffected by Abatacept therapy. Consequently, after identifying a suitable donor through the Chinese Bone Marrow Bank, the patient and their family decided to proceed with hematopoietic stem cell transplantation for further treatment.

Several reports have documented the diagnosis of approximately 12 children with LRBA deficiency who underwent haematopoietic stem cell transplantation. Typically, these patients exhibit remission of clinical symptoms post-transplantation or present with LRBA-associated manifestations, such as autoimmune thrombocytopenia, necessitating therapeutic intervention (8–13). In a recent multicentre follow-up of 76 patients with LRBA deficiency, 24 patients underwent HSCT. The overall survival rate after HSCT was 70.8%. Transplant-related mortality accounted for all nonspecific early deaths following the procedure. It is possible that HSCT is chosen as a last resort after many organ complications due to the early years of immature transplantation techniques and insufficient knowledge of LRBA defects, thus reducing the success rate of HSCT (4). In addition, a survey study from Turkey on 15 children with LRBA deficiency revealed that among the seven who underwent HSCT, they were alive and showed positive outcomes (median duration: 2 years; range: 1–3 years) (14).

In this study, the patient had persistent chronic inflammation and cumulative organ damage, leading to the selection of HSCT as a treatment option due to its potential effectiveness in suppressing the disease process. At present, after one and a half years post-transplantation, significant improvements have been observed in the patient's condition with no apparent signs of infection, restored liver function, disappearance of nervous system manifestations, and normalization of immune cell populations. These findings indicate the efficacy of transplantation treatment; however, it should be noted that early-stage clinical manifestations following transplantation can be complex. This complexity may not solely arise from the transplant procedure itself but also pose considerable challenges due to primary lesion accumulation.

Due to autoinflammation leading to multiple organ complications, the patient experienced recurrent pulmonary infection before transplantation, resulting in evident lung fibrosis that exacerbated the transplant procedure's difficulty. The patient continued to experience recurrent pulmonary infections during the early stages of transplantation, likely due to abnormal lung function caused by pulmonary fibrosis and incomplete immune recovery post-transplantation. During the preparation for transplantation, the patient developed septic shock due to sepsis and diarrhoea, which improved after gamma globulin and anti-infection therapy with advanced antibiotics, but it became more difficult to use the conditioning regimen before transplantation.

It is worth noting that neurological changes are a major challenge of transplantation for this patient. As previously reported, LRBA deficiency patients may experience neurological manifestations primarily due to autoinflammatory disease, characterized by granulomatous brain lesions, nerve demyelination and atrophy, as well as occasional intracranial haemorrhages (15). Prior to transplantation, although the patient did not exhibit any neurological symptoms, the patient's brain MRI showed abnormal signals in the bilateral temporal lobe, right parietal lobe, and corpus callosum. Subsequently after transplantation, the patient developed subdural effusion caused by adenovirus infection and linear enhancement of the bilateral frontoparietal and occipital meninges, and then brain MRI revealed multiple abnormal signals in the bilateral white matter. Posttransplant leukoencephalopathy was diagnosed, likely due to the process of transplantation itself, but it cannot be ruled out that this was a step related to LRBA deficiency. Fortunately thereafter no evident changes were observed on MRI scans and no significant neurological findings emerged.

Immune reconstitution after transplantation in immunodeficient patients is also a major concern. We found that the patient's LRBA protein and CTLA4 had recovered to normal levels one and a half

years after transplantation. However, regarding immune reconstitution, there was a relatively delayed recovery of B-cell function in the child following transplantation, possibly attributed to the utilization of a reduced-intensity conditioning regimen. However, regarding immune reconstitution, there was a relatively delayed recovery of B-cell function in the child following transplantation, possibly attributed to the utilization of a reduced-intensity conditioning regimen. Futhermore, glucocorticoids and immunosuppressants for the treatment of GVHD can inhibit the production of B lymphocytes as well. Previous studies have shown that B-cell reconstitution is faster in children with WAS syndrome than in children with CGD after transplantation (16). Therefore, whether LRBA deficiency itself has an effect on the reconstitution of B cells remains unknown and necessitates extensive research efforts. However, comparing pre- and post-transplantation B cell counts revealed that allogeneic haematopoietic stem cell transplantation played an important role in restoring lymphocyte levels among children with LRBA deficiency.

The use of HSCT for LRBA deficiency has demonstrated remarkable efficacy. However, the presence of complex disease activity, prolonged duration prior to HSCT, and irreversible organ damage are all associated with unfavorable outcomes. Seidel's team proposed (4) the IDDA scoring method, which was introduced by Seidel's team for evaluation and can further strengthen clinical management and be used to help doctors decide whether and when to perform HSCT in patients with LRBA deficiency.

Therefore, we believe that transplantation is an effective treatment for patients with multiorgan complications, but it is important to avoid a greater disease burden, longer duration before HSCT, and multiorgan involvement that increase the incidence of post-transplantation complications, which may reduce the chance of successful transplantation.

In conclusion, these data show that allogeneic haematopoietic stem cell transplantation could be considered a treatment option for individuals with LRBA deficiency. Nevertheless, more cases are needed to establish guidelines for the management of LRBA deficiency before and after transplantation.

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 humans were approved by Medical Ethics Committee of the Children's Hospital of Chongqing Medical University. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. 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

CS: Data curation, Investigation, Writing – original draft, Writing – review & editing. LZ: Investigation, Writing – review & editing. YM: Investigation, Writing – review & editing. LY: Data curation, Writing – review & editing. WH: Data curation, Writing – review & editing. XL: Investigation, Writing – review & editing. LZ: Data curation, Writing – review & editing. YA: Project administration, Investigation, Writing – review & editing. YD: Project administration, Data curation, Investigation, Writing – original draft, Writing – review & editing.

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Conflict of interest

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

Publisher's note

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Case Report: C3 deficiency in two siblings

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The complement system, a vital component of innate immunity, consists of various proteins and pathways crucial for the recognition and elimination of pathogens. In addition, it plays a major role in the initiation of adaptive response through the opsonization of antigens, contributing to B-cell activation and memory maintenance. Deficiencies in complement proteins, particularly C3, can lead to severe and recurrent infections as well as immune complex disorders. Here, we present a case report of two siblings with total C3 deficiency resulting from compound heterozygous mutations in C3 (NM_000064.4): c.305dup; [p.Asn103GlnfsTer66] and c.1269 + 5G>T, previously unreported in C3-related diseases. Both, the index case and her sister, presented a history of recurrent infections since early childhood and one of them developed hemolytic uremic syndrome (HUS). Immunological evaluation revealed absent plasma C3 levels, decreased memory B cells, hypogammaglobulinemia, and impaired response to polysaccharide antigens. The siblings showed partial responses to antimicrobial prophylaxis and vaccination, requiring intravenous immunoglobulin replacement therapy, resulting in clinical improvement. Genetic analysis identified additional risk polymorphisms associated with atypical HUS. This case highlights the importance of comprehensive genetic and immunological evaluations in complement deficiencies, along with the potential role of immunoglobulin replacement therapy in managing associated antibody defects.

KEYWORDS

C3 deficiency, recurrent infections, intravenous immunoglobulin, complement system, B-lymphocyte subsets

1 Introduction

The complement system, a highly conserved cascade, plays a major role in innate immunity. Comprising over 40 soluble and membrane-bound proteins, this system is activated through three initiation routes: classical, lectin, and alternative pathways. These pathways converge at the cleavage and activation of C3 and subsequently have common steps, highlighting the pivotal role of C3 in the system (1). The activation products of C3 execute multiple effector functions, including opsonization, recruitment, and activation of inflammatory cells that lead to the cytotoxic destruction of microbial pathogens. In addition, they facilitate the effective clearance of pathogens, apoptotic cells, cell debris, and immune complexes (ICs). Through its capacity to modulate B-cell response via co-stimulation, enhance antibody response, and support immunological memory, the complement system bridges the innate and adaptive immunity (2).

C3 deficiency represents a rare autosomal-recessive inherited inborn error of immunity (IEI), characterized by severe and/or recurrent infections and immune complex disorders like rheumatic and renal disease, particularly with childhood onset. Infections typically manifest early in life and are predominantly due to encapsulated bacteria such as

Streptococcus pneumoniae, *Haemophilus influenzae*, or *Neisseria meningitidis*. Conversely, autoimmune manifestations are less frequent with an older median age of onset (3). Impaired antigen opsonization in C3-deficient individuals is linked to impaired dendritic cell differentiation, memory B-cell responses, and regulatory T-cell development (4). Consequently, patients with C3 deficiency can exhibit a diminished antibody response to polysaccharide antigens and impaired B-cell differentiation; however, extensive immunological investigation has not been addressed in previous reports (5).

Here, we expand the clinical and immunological spectrum of total C3 deficiency by describing the first Argentinian family with a novel genetic variant causing total C3 deficiency.

2 Case description

The index case (P1) is a 15-year-old Argentinian girl, born to healthy, non-consanguineous parents. At the age of 6, she was referred to our immunology unit in Hospital de Niños "Ricardo Gutiérrez" due to a history of persistent and severe infections from early childhood onward. Since she was 4 months old, she has suffered multiple episodes of bronchial obstruction, acute gastroenteritis, sinusitis, and acute otitis media (AOM). At 2 years of age, she was hospitalized due to an episode of pneumonia, requiring intravenous antibiotic treatment. Two years later, she was admitted to the hospital because of an episode of osteomyelitis in the ankle with good response to clindamycin. Three months later she was hospitalized again with bloody diarrhea and acute kidney injury (initial assessment showed: 22% hematocrit; 13.000/mm^3 platelets; 1.4 mg/dl creatinine). It was assumed to be an episode of hemolytic uremic syndrome (HUS) with requirement of blood transfusion but with no need to start dialysis. Shiga-toxin-producing *Escherichia coli* was not investigated at that moment to determine if it was a typical or atypical HUS (Figure 1).

Initial laboratory tests were unremarkable except for an abnormal serum protein capillary electrophoresis showing an absent beta 2-globulin protein fraction. The immunological work-up for suspected inborn errors of immunity revealed normal lymphocyte subpopulations and Immunoglobulin levels; however, IgG4 levels were undetectable. Antibody response to tetanus toxoid and Rubella vaccine were normal. C3 serum protein levels and classical and alternative complement pathway activity were undetectable on more than one occasion, while C4 levels were near normal (Table 1). Her sister (P2), aged 12 years, also suffered from multiple respiratory tract infections since she was 2 months old, including four hospital admissions due to pneumonia, although no etiological agent was documented. At 5 years of age, immunological work-up revealed hypogammaglobulinemia with normal response to protein, normal lymphocyte subpopulations, absent beta 2-globulin protein fraction in capillary electrophoresis, and absent C3 serum levels as well as undetectable classical and alternative pathway activity. Since the age of eight, she suffered sporadic mild thrombocytopenia (range: 130,000–140,000/mm^3) without signs of splenomegaly and bleeding (Supplementary Table 1). Furthermore, when she was 11 years old, she developed leukopenia with an absolute neutrophil count of 1.100/mm^3, which was detected during a routine laboratory evaluation. Cytopenias were assumed to be autoimmune-mediated; however, no autoantibodies against platelets were measured to test this hypothesis. As these were slightly abnormal laboratory findings without accompanying clinical symptoms, no specific treatment has been initiated for these conditions.

Complement analysis of her parents and other family members revealed some individuals with low serum levels of C3; however, functional assays were normal or slightly diminished (Table 1), and only one family member experienced recurrent infections.

The siblings were suspected to have total C3 deficiency and were booster vaccinated with the conjugated 13-valent pneumococcal vaccine (PCV13) followed by the 23-valent pneumococcal vaccine (PSV23) after eight weeks to prevent

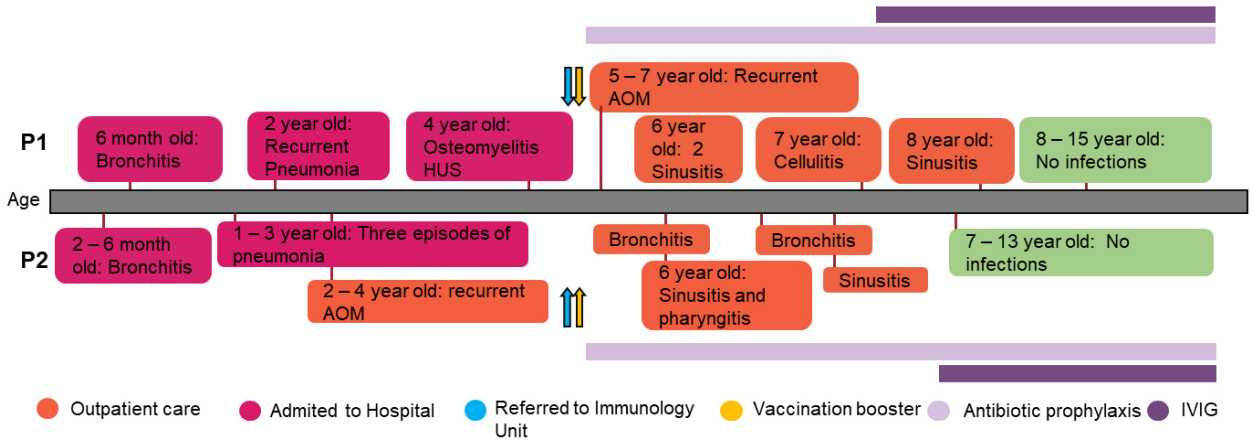

FIGURE 1
Patients' clinical records. Graphical display of the clinical course and the treatment administered in the patients.

TABLE 1 Immunological work-up.

	P1 (6 years old)	P1 (10 years old)	P2 (5 years old)	P2 (8 years old)	II.1	II.2	II.3	I.1
IgG (mg/dl)	1,040 (718–1,608)	1,150[a] (711–1,743)	578 (682–1,572)	872[a] (718–1,608)	—	—	—	—
IgA (mg/dl)	196 (78–223)	141 (11–315)	95 (52–150)	79 (78–223)	—	—	—	—
IgM (mg/dl)	243 (83–173)	190 (11–239)	199 (63–177)	232 (83–173)	—	—	—	—
IgE (IU/ml)	615 (<90)	—	112 (<90)	—	—	—	—	—
IgG subclasses (mg/dl)	IgG1 574 (300–840)	—	IgG1 411 (300–840)	—	—	—	—	—
	IgG2 126 (70–255)		IgG2 110 (70–255)					
	IgG3 174 (17–97)		IgG3 107 (17–97)					
	IgG4 ND (2–116)		IgG4 ND (2–116)					
Tetanus IgG (IU/ml)	3.4 (>0.1)	—	1.4 (>0.1)	—	—	—	—	—
Pneumococcal polysaccharide IgG (mg/ml)[b]	<30 (>147)	—	>270 (>147)	—	—	—	—	—
C3 (mg/dl)	<6 (90–150)	<6 (90–150)	<6 (90–150)	<6 (90–150)	47 (90–150)	54 (90–150)	63 (90–150)	72 (90–150)
C4 (mg/dl)	12 (15–35)	11 (15–35)	11 (15–35)	19 (15–35)	27 (15–35)	14 (15–35)	21 (15–35)	23 (15–35)
CH50 (UH50/ml)	<50 (180–280)	<50 (180–280)	<50 (180–280)	—	250 (180–280)	133 (180–280)	204 (180–280)	224 (180–280)
AH50 (minutes)	>60' (<12')	>60 (<12')	>60' (<12')	—	11' (<12')	12' (<12')	—	—
Lymphocytes (10^3/mm^3)	3.9 (1.5–6.5)	3.2 (1.5–6.5)	4.5 (1.5–6.5)	2.7 (1.5–6.5)	—	—	—	—
CD3+ T cells (%)	77 (65–72)	77 (65–72)	72 (67–75)	78 (67–75)	—	—	—	—
CD4+ T cells (%)	34 (33–43)	34 (33–43)	42 (32–48)	42 (32–48)	—	—	—	—
CD8+ T cells (%)	35 (25–32)	38 (25–32)	27 (22–29)	28 (22–29)	—	—	—	—
CD19+ B cells (%)	12 (10–16)	9 (10–16)	17 (11–18)	18 (11–18)	—	—	—	—
CD56+ NK cells (%)	11 (10–19)	12 (10–19)	5 (6–14)	3 (10–19)	—	—	—	—

Immunological laboratory studies in the index patient and her family members. Age-matched reference values are in parentheses. ND, not detectable.
[a]Under IVIG treatment.
[b]Pneumococcal vaccine response was measured using the overall response to all 23 serotypes present in the pneumococcal vaccine (PSV23) after 45 days of vaccination, using an enzyme immunoassay kit.

severe infections due to *S. pneumoniae*. Forty-five days later, pneumococcal response to polysaccharide antigens was measured. The index case was found to have an impaired antibody response (<30 mg/ml), while her sister had normal levels (>270 mg/ml). In addition, they were booster vaccinated with the quadrivalent meningococcal vaccine (ACYW), and started on antimicrobial prophylaxis with amoxicillin 25 mg/kg once a day. Despite these preventive measures, the patients continued to have recurrent infections, mostly AOM and sinusitis diagnosed by clinical criteria and imaging. These infections were treated orally with amoxicillin–clavulanic acid. However, the failure of antimicrobial prophylaxis to prevent recurrent sinopulmonary infections, as well as the evidence of impaired antibody synthesis or response, led to the decision of initiating intravenous immunoglobulin (IVIG) replacement therapy, leading to a marked decrease in the number of infections.

Subsequent immunological studies revealed normal or elevated plasma levels of alternative complement pathway proteins and regulators such as Factor I, Factor H, properdin, and Factor B. Moreover, the activity of activators of the alternative pathway of complement system such as nephritic factor autoantibody (C3nef), the quantification of C3 activation products (CAP), and soluble membrane attack complex (sC5b9) levels were normal. Taken together, these findings ruled out a possible secondary C3 deficiency due to dysregulation (Table 2). In addition, we detected low levels in C1q; however, subsequent laboratory studies over time revealed fluctuating levels, particularly in association with infectious events, reaching normal values (Supplementary Table 2).

TABLE 2 Complement evaluation.

	P1[a]	P2[a]	Age-matched RV
C1q (mg/L)	61	90	115–240
C2 (mg/L)	26	21	14–29
CFB (mg/L)	461	521	229–394
CFH (mg/L)	650	702	329–557
CFI (mg/L)	20	21	15–34
CAP	Negative	Negative	Negative
C3nef	Negative	Negative	Negative
sC5b9 (ng/ml)	266	328	136–385

Complement system evaluation in the index case and her sister. CAP; complement activation products. RV; reference values.
[a]The sample was taken during an infectious intercurrence.

Genetic analysis in the index case was performed by next-generation sequencing (NGS) using a gene panel [CFH, MCP (CD46), CFI, CFB, C3, THBD, DGKE, CFHR1, CFHR2, CFHR3, CFHR4, CFHR5, CFP, ADAMTS13] and copy number analysis of *CFH* and *CFHR1-5* by multiplex ligation probe amplification (MLPA). The NGS panel revealed two variants in *C3* (NM_000064.4):c.305dup; [p.Asn103GlnfsTer66] and c.1269 +5G>T, not previously reported in C3-related disease. Sanger sequencing of the family demonstrated the compound

heterozygous inheritance and the presence of both variants in P2 (Figure 2A). The c.305dup variant is observed at an extremely low frequency in gnomAD v4.0. It occurs in the exon 3 of the protein and is predicted to cause a frameshift leading to a premature stop codon. Therefore, it is predicted to disrupt normal protein functioning either by protein truncation or nonsense-mediated decay. The c.1269+5 G>A variant occurs in the 5′ splice site of intron 11; it is also extremely rare in public databases and is absent from the literature. The computational predictor SpliceAI lookup gives a score of 0.71, which is above the threshold of 0.2, evidence that correlates with an abnormal splicing and impairment of *C3* function. Both variants occur in the β chain of C3 protein and are predicted to undergo nonsense-mediated decay (Figure 2C). The variants were classified as pathogenic and likely pathogenic, respectively, according to the ACMG/AMP guidelines. In addition, other polymorphisms were identified in P1, who was found to be heterozygous for the *CFHR1/CFHR3* gene deletion and for the MCPggaac atypical HUS allele risk, also in the heterozygous state.

To further characterize the immunological phenotype of the patients, we analyzed the presence of autoantibodies and B-cell subpopulations. Antinuclear antibodies were negative in both; however, P1 had positive anti-FH (157 AU, cutoff 100 AU) and anti-C1q antibodies (40 AU, cutoff 20 AU). Flow cytometry analysis of B-cell subpopulations on several occasions showed persistent decreased total memory B cells due to reduced switched (IgD− IgM−) and non-switched memory B cells (CD27+ IgM+ IgD+) in one of the siblings and decreased non-switched memory B cells in her sister (Figure 2D).

Currently, both patients show good evolution and continue with antimicrobial prophylaxis and IVIG treatment, having sporadic bronchial obstruction of out-of-hospital management.

3 Discussion

IEI affecting the complement system are considered uncommon, comprising approximately 5% of all IEI (6). Primary total C3 deficiency is an extremely rare autosomal-recessive disease, with fewer than 50 cases reported worldwide since the first C3-deficient patient was described in 1972 (7, 8). Here, we described the clinical and molecular characterization of two siblings with total C3 deficiency, who presented with early-onset severe bacterial infections, and a severe episode of HUS in one of them. Despite antimicrobial prophylaxis, they continue to suffer from recurrent sinopulmonary infections. C3-deficient individuals typically suffer from recurrent pyogenic infections due to encapsulated bacteria such as *S. pneumoniae, H. influenzae, or N. meningitidis* manifesting early in life. IC-related diseases including systemic lupus erythematosus (SLE)-like illness and renal diseases are also observed in this condition.

In peripheral blood, naive and memory B cells can be differentiated by the expression of CD27, IgD, and IgM markers on their surface. Typically, switched memory B cells are IgD− IgM− and are generated in germinal centers by T-B collaboration. Non-switched memory B cells are CD27+ IgM+ IgD+ and their origin is proposed to be germinal center independent, participating in T-independent responses and in the protection against infections by encapsulated bacteria (9, 10). B-cell response is amplified through interaction of opsonizing C3-fragments with complement receptors CD21/CR2. This leads to increased B-cell receptor (BCR) signaling

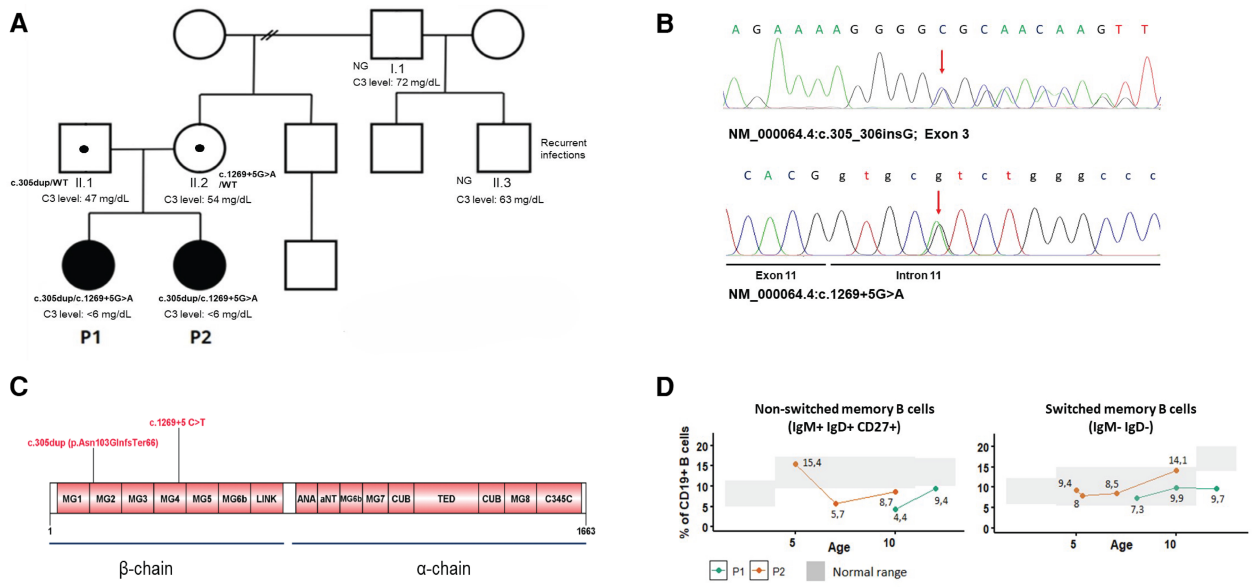

FIGURE 2
(A) Family pedigree and C3 levels of investigated individuals. Filled-in squares/circles represent affected individuals. NG, not genotyped. (B) Electropherograms of *C3* mutations in P1. (C) Graphical display of C3 protein domains and location of the mutations. (D) Level of switched and non-switched memory B cells over time.

when C3d-opsonized antigens are present on the B-cell surface, facilitating the differentiation from naive to antigen-experienced memory B cells (11).

This pivotal role is supported by the fact that C3-deficient patients have shown altered humoral homeostasis, compromising Immunoglobulins or B cells. Previous reports have described C3-deficient patients with low levels of total IgG or IgG2 and/or IgG4 deficiency (12–16). Moreover, C3 deficiency has also been associated with defective dendritic cell differentiation, altered memory B-cell responses to antigens, and decreased memory B cells (5, 17). Consistent with previous findings, we observed reduced memory B cells, hypogammaglobulinemia, impaired response to polysaccharide antigens, and undetectable levels of IgG4. Collectively, these observations reinforce the role of C3 on B-cell memory generation and potentially explain why these patients continued to have recurrent infections despite antimicrobial prophylaxis. Unfortunately, we were not able to confirm IgG4 deficiency, as we could not measure IgG subclasses on more than one occasion before IVIG therapy administration.

Molecular characterization of C3 deficiency has been described in a few families (3). Here, we expand the genetic spectrum of C3 mutations by reporting two deleterious variants not previously associated with C3-related diseases, resulting in absent plasma C3 levels, which was evidenced also by the absent beta 2-globulin protein fraction, and undetectable classical and alternative complement pathway activity. We also noted decreased levels of C1q and C4, alongside elevated levels of CFB and CFH, probably linked to underlying infectious or inflammatory events. Subsequent measurements ruled out a deficiency in C1q and C4.

Additional polymorphisms were found in heterozygous in P1 that are associated with atypical HUS: the deletion of *CFHR1/CFHR3* genes and the MCPggaac haplotype (18, 19) (Supplementary Table 3). It remains unclear whether these risk polymorphisms may have contributed to the development of renal manifestations in P1 as well as the development of a-FH autoantibodies. Considering that we only analyzed a subset of genes, we cannot rule out the presence of other clinically significant variants in the siblings. The potentially different repertoire of mutations and polymorphisms in complement proteins, the complotype, and their variable penetrance, might explain the difference in clinical observations between the sisters (20). With the increasing availability of next-generation sequencing technologies, investigating the complotype could aid in predicting individualized risk of different clinical manifestations.

As in most autosomal-recessive diseases, heterozygous carriers usually remain asymptomatic and are identified through a history of affected family members. Nevertheless, in some cases, carriers have developed clinical symptoms (21). In this case, further investigation of C3 levels in the family identified a family member with constantly decreased C3 levels similar to those observed in the parents, who are known carriers, and recurrent infections. Although we did not perform confirmatory genetic analysis, this underscores the importance of family testing in complement deficiencies, as the heterozygous state could increase the risk for infections or autoimmunity in certain individuals (22).

Regarding the management of complement deficiencies, clinical control, vaccination with protein-polysaccharides conjugate, and antimicrobial prophylaxis are considered cornerstones in treatment (23); however, in this case, antimicrobial prophylaxis and vaccination proved insufficient to ameliorate the number of infections and, given their antibody defects, IVIG treatment was initiated, resulting in a favorable clinical response.

In summary, to our knowledge, this is the first Argentinian reported case of complete C3 deficiency. We broaden the clinical and genetic spectrum of C3 deficiency by reporting two mutations previously unreported in C3-related diseases. This case highlights the importance of evaluating B-cell subpopulations, IgG subclasses, and IgG responses to polysaccharide antigens in C3-deficient patients to tailor appropriate management strategies. Prompt recognition of complement deficiencies that impair B-cell immunity allows early treatment installation when needed and prevents more severe infections.

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

AB: Data curation, Investigation, Writing – original draft, Writing – review & editing. AG: Conceptualization, Project administration, Supervision, Writing – original draft, Writing – review & editing. SR: Investigation, Writing – original draft, Writing – review & editing. MPT: Data curation, Resources, Writing – original draft, Writing – review & editing. DDG: Data curation, Resources, Writing – original draft, Writing – review & editing.

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Conflict of interest

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

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2024.1424380/full#supplementary-material

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Case Report: Aplastic anemia related to a novel *CTLA4* variant

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A 20-year-old male patient with a history of celiac disease came to medical attention after developing profound fatigue and pancytopenia. Evaluation demonstrated pan-hypogammaglobulinemia. There was no history of significant clinical infections. Bone marrow biopsy confirmed hypocellular marrow consistent with aplastic anemia. Oncologic and hematologic evaluations were unremarkable for iron deficiency, paroxysmal nocturnal hemoglobinuria, myelodysplastic syndromes, T-cell clonality, and leukemia. A next generation genetic sequencing immunodeficiency panel revealed a heterozygous variant of uncertain significance in *CTLA4* c.385T >A, p.Cys129Ser (C129S). Cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) is an inhibitory receptor important in maintaining immunologic homeostasis. To determine the functional significance of the C129S variant, additional testing was pursued to assess for diminished protein expression, as described in other pathogenic *CTLA4* variants. The results demonstrated severely impaired CTLA-4 expression and CD80 transendocytosis, consistent with other variants causing CTLA-4 haploinsufficiency. He was initially treated with IVIG and cyclosporine, and became transfusion independent for few months, but relapsed. Treatment with CTLA-4-Ig fusion protein (abatacept) was considered, however the patient opted for definitive therapy through reduced-intensity haploidentical hematopoietic stem cell transplant, which was curative.

KEYWORDS

Aplastic anemia, inborn error of immunity (IEI), novel variant, CTLA-4, haploinsufficiency, hematopoietic stem cell transplantation (HSCT)

Introduction

Inborn errors of immunity (IEIs) encompass a broad and heterogenous group of genetic disorders which disrupt immunologic homeostasis (1). Traditionally, these conditions have been characterized by which arm(s) of the immune system (e.g., innate, humoral, phagocytic, complement, etc.) are impacted resulting in increased susceptibility to infectious agents. However, coinciding autoimmune/autoinflammatory disease states are increasingly being recognized as a primary or secondary feature among IEIs (2). In particular, immune dysregulation is a primary feature of pathologic variants involving genes responsible for maintaining immunologic homeostasis.

Cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) is an inhibitory receptor present on T-cells and serves a fundamental role in regulation of immune responses. The process of T-cell ($CD4^+$) activation requires both primary and costimulatory signals via interaction with antigen presenting cells (APCs). One such costimulatory signal involves CD28 (present on T-cells) and CD80/86 (present on APCs). Once activated, T-cells upregulate expression of CTLA-4 on their surface which binds to CD80/86 resulting in transendocytosis of the receptors thereby limiting further activation (Figure 1) (3). This coinhibitory mechanism prevents unregulated T-cell activation. Additional immune regulation occurs via T-cell subpopulations such as T-regulatory-cells (T_{regs}). These cells constitutively express CTLA-4 providing an additional checkpoint in immune activation (4). In this way, CTLA-4 acts to maintain immunologic homeostasis, with disruptions caused by *CTLA4* variants leading to altered protein expression and clinical disease states.

To our knowledge, no cases of complete CTLA-4 deficiency have been reported in humans. Targeted genetic deletion leading to complete CTLA-4 loss of function in mice leads to fatal multiorgan lymphocytic infiltration primarily due to expansion of unregulated $CD4^+$ T-cells (5–8). Complete CTLA-4 deficiency may be incompatible with life in humans. However, pathogenic heterozygous variants in *CTLA4* can lead to disease phenotypes with variable clinical penetrance and expressivity. Phenotypic features reported in patients with CTLA-4 haploinsufficiency include: autoimmune cytopenias, hypogammaglobulinemia, lymphadenopathy, and organ dysfunction (enteropathy, splenomegaly, etc.) from lymphocytic infiltration (9). Additionally, polymorphisms in *CTLA4* have been associated with risk for type 1 diabetes mellitus, Graves' disease, multiple sclerosis, and malignancies (10–13).

In this report, we present a novel *CTLA4* variant manifesting as aplastic anemia and provide functional testing that confirms this novel variant is deleterious to CTLA-4 expression resulting in severely reduced transendocytosis.

Case description

A 20-year-old male of self-reported White race (ancestry unavailable) presented to medical care for 1 week of persistent and profound incapacitating fatigue. Additional symptoms included palpitations, lightheadedness and exertional dyspnea with ambulation, which all resulted in an inability to participate in collegiate athletics. His medical history was pertinent for celiac disease (confirmed via endoscopic biopsy with symptom resolution after implementing a gluten-free diet), pityriasis alba, and idiopathic wet macular degeneration status-post successful treatment with aflibercept. He did not have a history of other autoimmune disease, immune deficiency or severe/atypical infections. Paternal history was positive for rheumatologic/autoimmune disease including psoriasis, arthritis, and hypothyroidism. Maternal history was unremarkable. There is no history of consanguinity. The remaining family history was

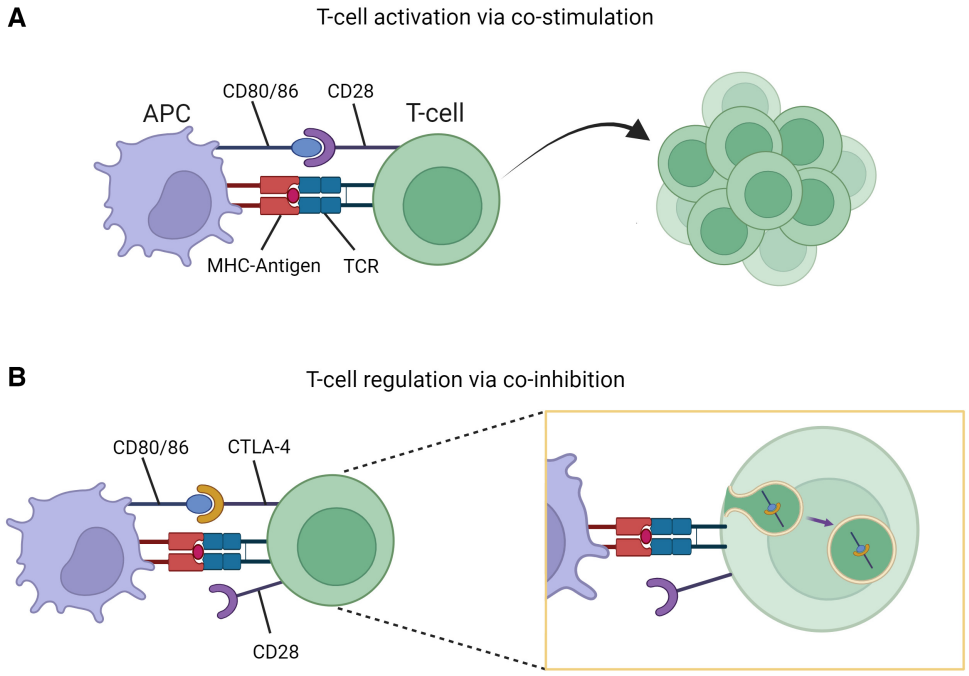

FIGURE 1
T-cell stimulation and regulation. **(A)** Co-stimulatory signaling through CD80/86-CD28 interaction resulting in T-cell activation and proliferation. **(B)** Co-inhibition through CD80/86-CTLA-4 interaction resulting T-cell regulation due to transendocytosis of CD80/86-CTLA-4 complex. Figure created with Biorender.com.

pertinent only for thyroid disease of unclear specificity in maternal and paternal grandparents.

Upon presentation, he was anemic (hemoglobin 8.4 mg/dl) and thrombocytopenic (platelet count 18,000/ml). The subsequent day, his white blood cell counts decreased from 4,400 cells/ml to 2,600 cells/ml, consistent with pancytopenia. Iron studies were consistent with mild iron overload and serum copper levels were slightly elevated. He had no prior history of blood transfusions and genetic testing for hemochromatosis demonstrated a heterozygous variant in *HFE* (c.187C >G, p.His63Asp) (H63D). The H63D carrier-status rarely results in clinically significant iron overload (14, 15). The mild elevation in serum copper based on internal lab reference ranges were not thought to be clinically significant and levels up to 158.9 µg/dl are considered normal (16). Additional evaluation including folate, B12, lactate dehydrogenase, and haptoglobin were unremarkable (Table 1). Bone marrow biopsy demonstrated hypocellularity with near absence of erythroid precursors and megakaryocytes, consistent with aplastic anemia. Further hematologic testing was unremarkable including: bone marrow chromosomal analysis, fluorescence *in situ* hybridization for BCR/ABL1 and chromosomal abnormalities (monosomy 5 and 7, trisomy 8, and 20q deletion), myelodysplastic syndrome mutation sequencing, telomere length studies, leukemia flow cytometry immunophenotyping, and T-cell clonality. PNH flow cytometry revealed a loss of GPI-anchored proteins on 0.04% and 0.6% of granulocytes and monocytes, respectively, suggesting that the aplastic anemia may be immune-mediated (18).

Blood count differential demonstrated profound lymphopenia with an absolute lymphocyte count of 268 cells/ml. Lymphocyte enumeration through flow cytometry revealed a T-cell ($CD3^+$) count of 222 cells/µl (83%), $CD4^+$ count of 107 cells/µl (40.1%), $CD8^+$ count of 88 cells/µl (32.9%), naïve T-cell ($CD45RA^+/CD4^+/CD62l^+$) count of 15 cells/µl (14.4%), B-cell ($CD19^+$) count of 3 cells/µl (1.3%), and NK-cell ($CD16/56^+$) count of 17 cells/µl (13.8%). T-cell proliferation to phytohemagglutinin (PHA) and tetanus was normal. Immunoglobulin evaluation demonstrated diffuse hypogammaglobulinemia with: IgG (122 mg/dl), IgM (7 mg/dl) and IgA (12 mg/dl). Of note, immunoglobulin levels collected 16-months prior demonstrated a similar pattern with an IgG of 176 mg/dl, an IgM <25 mg/dl, and IgA of 15.7 mg/dl. Antibody titers to tetanus and diphtheria toxoid were protective, and pneumococcal-23 antibody titers were protective to greater than 75% of serotypes tested.

TABLE 1 Diagnostic evaluation summary.

Lab	Result	Reference
Presenting labs		
WBC	4,400 cells/ml	3,200–9,800 cells/ml
Hemoglobin	**8.4 g/dl**	13.7–17.3 g/dl
Platelets	**18,000/ml**	150,000–450,000/ml
Reticulocyte count (%)	**17,200/ml (0.65)**	28,000–134,000/ml (0.70–2.0)
Folate	11.4 ng/ml	> 6.5 ng/ml
B12	404 pg/ml	123–730 pg/ml
Iron	**220 µg/dl**	50–160 µg/dl
Total Iron binding capacity	**248 µg/dl**	261–478 µg/dl
Copper	**131 µg/dl**	63–121 µg/dl
Lactate dehydrogenase	151 U/L	100–200 U/L
Haptoglobin	50 mg/dl	30–200 mg/dl
Immunoglobulin M	7 mg/dl	57–273 mg/dl
Immunoglobulin G	122 mg/dl	588–1,573 mg/dl
Immunoglobulin A	12 mg/dl	46–287 mg/dl
Flow cytometry[a]		
WBC	**2,000 cells/ml**	3,200–9,800 cells/ml
ALC (%)	**268 cells/ml** (13.4)	600–4,200 cells/ml (10–50)
$CD3^+$ (%)	**222 cells/µl (83.0)**	1,543–1,729 cells/ml (76.1–78.3)[b]
$CD4^+$ (%)	**107 cells/µl (40.1)**	942–1,066 (45.7–48.5)[b]
$CD8^+$ (%)	**88 cells/µl (32.9)**	544–637 (26.4–28.9)[b]
$CD19^+$ (%)	**3 cells/µl (1.3)**	232–281 (11.1–12.8)[b]
NK-cells (%)	**37 cells/µl (13.8)**	188–336 (9.0–10.8)[b]
$CD3/CD45RA^+$ (%)	**67 cells/µl** (30.3)	(20.3–44.1)
$CD3/CD45RO^+$ (%)	**78 cells/µl** (34.9)	(30.4–51.3)

Abnormal values are bolded.
[a]Flow cytometry results 2-weeks following presenting labs.
[b]Reference ranges from Valiathan et al. (17).

Diagnostic assessment

A primary immunodeficiency next generation sequencing panel was sent and revealed a novel heterozygous variant of uncertain significance in *CTLA4* (c.385T >A, p.Cys129Ser) (C129S). Paternal testing revealed the same variant. Maternal and sibling testing was negative. Given the patient's clinical phenotype, known risk for immune dysregulation with *CTLA4* variants, and without other identifiable etiologies for the aplastic anemia, a research-based functional assay was pursued.

A CTLA-4 functional assay was performed as previously described (19–21). pCMV6-CTLA4-MycDDK plasmid was obtained from Origene (#RC213631). Construct carrying C129S mutant allele was generated from the wild type (WT) plasmid by site-directed mutagenesis (QuikChange II XL; Agilent Technologies, #200523) according to manufacturer's instructions and validated by Sanger Sequencing. WT or C129S mutant *CTLA4* plasmids were transfected into CHO cells (ATCC # CCL-61) using Lipofectamine 2000 (ThermoFisher Scientific, #11668027), per manufacturer's protocol.

For transendocytosis experiments, transfected CHO cells were co-cultured 1:1 with CellTrace™ Violet (ThermoFisher Scientific #C34557) labelled CHO-$CD80^{GFP}$ cells (A gift of Bodo Grimbacher and David Sansom) for 16 h. CTLA-4 expression and transendocytosis of $CD80^{GFP}$ were measured by flow cytometry on the MACSQuant Analyzer 16 (Miltenyi Biotec). A known loss of function pathogenic variant in *CTLA4*, R51X, was used as a positive control and untransfected CHO cells were used as a negative control. Flow cytometry comparing CTLA-4 expression and CD80 uptake through transendocytosis were run in triplicate.

Results demonstrated that the C129S variant drastically impaired expression of CTLA-4 with a resulting decrease in transendocytosis as compared to wild type. These findings are similar to those seen with the causal allele R51X (Figure 2). Thus, the patient's C129S variant exhibited results consistent

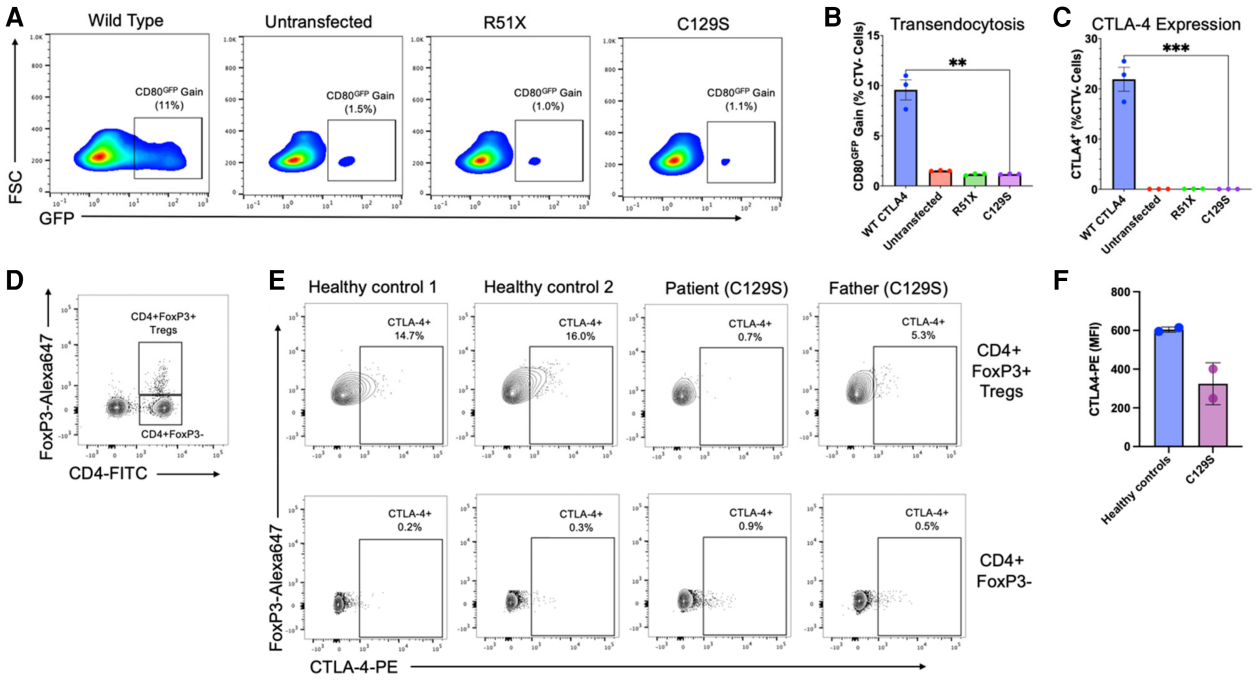

FIGURE 2
The C129S missense mutation dramatically impairs CTLA-4 expression resulting in decreased transendocytosis. **(A)** Representative example of CD80GFP transendocytosis assay results. These plots show cells previously gated on forward and side scatter profile, and negative for Cell Trace Violet. GFP signal represents percent transendocytosis of CD80. **(B)** Summary results from $n = 3$ independent experiments, performed as in A. **(C)** Percent CTLA-4+ CHO cells after transfection with WT or mutant CTLA-4 plasmids ($n = 3$),$^{**}p < 0.01$, unpaired t-tests. **(D)** Flow cytometry analysis of peripheral blood mononuclear cells (PBMCs) to identify CD4 + FoxP3 + regulatory T-cells (T¬¬regs) and CD4 + CD3- cells. **(E)** Analysis of CTLA-4 protein expression by Tregs (upper row) and CD4 + FoxP3 + cells (lower row), gated as shown in **(D)**. **(F)** Mean fluorescence intensity for CTLA-4 on Tregs from two healthy donors, and the patient and his father with the C129S variant.

with CTLA-4 haploinsufficiency. Moreover, analysis of peripheral blood mononuclear cells (PBMCs) from the patient and his father confirmed reduced CTLA-4 protein levels (Figures 2D,E).

Based on the results from the transendocytosis assay, and the patient's clinical history, the C129S variant was determined to be pathogenic, suggesting that the patient's phenotype was likely due to CTLA-4 haploinsufficiency. Initial therapy consisted of high-dose IVIG (1 g/kg × 2 doses), cyclosporine (maximum dose of 275 mg twice daily), and systemic corticosteroids (maximum dose of 30 mg daily). He responded well to these therapies with notable improvement in red blood cell production seen on bone marrow biopsy. Cyclosporine was then decreased to 175 mg twice weekly with a target trough level of 150–300 ng/ml, and systemic corticosteroids were discontinued. However, 6 months into therapy, he developed renal impairment and was transitioned to 5 mg daily of sirolimus with a trough goal of 5–15 ng/ml, but it was poorly tolerated. In the subsequent 2 months, his aplastic anemia relapsed. Off-label use of abatacept was considered given clinical reports showing positive responses in patients with CTLA-4 haploinsufficiency (22–24). However, given disease severity and the patient's preference, definitive therapy in the form of a haploidentical (sibling, variant negative) hematopoietic stem cell transplantation (HSCT) was pursued (25). Use of haploidentical donor marrow was favored to a matched-unrelated donor for the following reasons: earlier time to transplant, improved total stem cell dose which is critical in non-malignant disease such as aplastic anemia, reduced risk for graft-vs.-host disease due to lower T-cell concentration as compared to peripheral stem cell harvest, and comparable outcomes with use of post-transplant cyclophosphamide. The non-myeloablative conditioning regimen included standard anti-thymocyte globulin (0.5 mg/kg/day on day −9 and 2 mg/kg/day on day −8 and −7), fludarabine (30 mg/m^2/day on days −6 to −2), cyclophosphamide (14.5 mg/kg/day on days −6 and −5), and total body radiation (400 cGy) on day −1 as previously described (25). Post-transplant course was uncomplicated and graft-vs.-host disease prophylaxis included cyclophosphamide, tacrolimus and mycophenolate mofetil as previously described (25). He achieved >95% CD3$^+$ donor chimerism one-month post-transplant with subsequent increase to 100% at around 3-months post-transplant. His most recent chimerism study (1-year post HSCT) continues to show 100% donor chimerism in both peripheral blood and CD3$^+$ compartments. He acquired a primary Epstein-Barr infection at around 4-months post-transplantation with mild intermittent clinical symptoms of rash and pharyngitis. He now has resolution of clinical symptoms without intervention, and his most recent EBV DNA is below the threshold of assay detection. Now, 24-months post-transplant, infectious prophylaxes have been discontinued. Due to previous adverse reactions to immunoglobulin replacement, it was not administered

post-transplantation. Endogenous immunoglobulin levels normalized by 14-months post-transplant with an IgG of 823 mg/dl, IgM of 69 mg/dl, and IgA of 214 mg/dl. Platelet counts remain appropriate (>150,000/ml) and hemoglobin has been stable (12.5–15.4 g/dl). He remains transfusion independent. Tetanus and diphtheria toxoid titers remain protective. Inactivated vaccinations have been well-tolerated, with plans to administer live vaccines in the future. Growth has been appropriate with a body mass index of 19.7 kg/m^2. He has resumed normal activities.

Discussion

CTLA-4 haploinsufficiency is an autosomal dominant condition characterized by reduced CTLA-4 expression and/or function due to variants in *CTLA4* (26). Due to variability in expressivity and penetrance, the condition can be under recognized and underdiagnosed. This was well demonstrated in the case of our patient, where both he and his father shared a common variant in *CTLA4* and exhibited dissimilar phenotypes. While the patient developed severe aplastic anemia, his father has psoriasis, arthritis and hypothyroidism, likely due to CTLA-4 haploinsufficiency as well. In addition, the family history of thyroid dysfunction suggests this variant may be present across generations. Other inborn errors of immunity can also present with similar clinical features to CTLA-4 haploinsufficiency. An example is lipopolysaccharide-responsive beige-like anchor, or LRBA, deficiency. LRBA is a protein responsible for recycling cellular components, including CTLA-4, thereby preventing lysosomal degradation (27). Thus, deficiency of LRBA can lead to reduced CTLA-4 (28).

Therapeutic options for management of immune dysregulatory conditions can be challenging for the practicing immunologist. Immunosuppression to manage autoimmune and autoinflammatory symptoms must be balanced with heightened risk of infection and further marrow suppression. Targeted therapeutic options are limited in management of immune dysregulation, but sometimes can be tailored when the underlying mechanistic pathways are identified. Abatacept is a promising option for patients with CTLA-4 haploinsufficiency. Abatacept is a fusion protein consisting of CTLA-4 fused to the Fc region of human IgG (29). As such, it (at least partially) compensates for the insufficient endogenous expression and/or function of CTLA-4 and binds to CD80/CD86, thereby regulating T-cell stimulation. While abatacept has shown beneficial results in clinical reports, the absence of clinical trials renders it an off-label agent. Moreover, as a replacement therapy, it necessitates life-long treatment in managing CTLA-4 haploinsufficiency, with no available data regarding the long-term clinical implications. In this case, the patient presented with severe clinical disease. Severe or treatment refractory disease including cytopenias and aplastic anemia should prompt an early search for a potential stem cell donor. While abatacept could have been used, the only definitive cure is HSCT. Overall, his post-transplantation outcome has been excellent.

This report outlines a novel *CTLA4* variant with functional confirmation of a pathogenic aberration resulting in CTLA-4 haploinsufficiency. Furthermore, it highlights the diverse phenotypic spectrum increasingly recognized for inborn errors of immunity, especially those involved in immune regulation. Clinical immunologists need to maintain a high index of suspicion when evaluating patients presenting with autoimmunity, autoinflammation, and lymphoproliferation. Genetic sequencing should especially be considered in patients presenting with severe, non-malignant hematologic disease. Establishing a multidisciplinary collaboration is critical to early recognition, management, and occasionally curative intervention for these patients.

Data availability statement

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

Ethics statement

The studies involving humans were approved by Duke University Institutional Review Board. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article. The legal representatives for all participants provided written consent for enrollment into protocol, Genetic and Functional Analysis of Primary Immune Deficiencies (Pro00066839), which was approved by the institutional review board of Duke University.

Author contributions

GH: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Writing – original draft, Writing – review & editing, Software. JG-M: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Software, Writing – original draft, Writing – review & editing, Resources, Supervision, Validation. JM: Formal Analysis, Writing – review & editing, Data curation, Investigation, Methodology, Software, Validation, Writing – original draft. PM: Investigation, Writing – review & editing. JR: Investigation, Writing – review & editing. JS: Formal Analysis, Funding acquisition, Resources, Writing – review & editing. HL: Writing – review & editing. AA: Writing – review & editing. RB: Writing – review & editing. TM: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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Conflict of interest

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Publisher's note

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Case Report: p40^{phox} deficiency underlying pediatric-onset systemic lupus erythematosus

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Introduction: Systemic lupus erythematosus is a multi-faceted autoimmune disorder of complex etiology. Pre-pubertal onset of pediatric systemic lupus erythematosus (pSLE) is uncommon and should raise suspicion for a genetic driver of disease. Autosomal recessive p40^{phox} deficiency is a rare immunologic disorder characterized by defective but not abolished NADPH oxidase activity with residual production of reactive oxygen species (ROS) by phagocytic cells.
Case presentation: We report the case of a now 18-year-old female with pSLE onset at 7 years of age. She presented with recurrent fever and malar rash. Aspects of her immune dysregulation over time have included typical pSLE features including production of autoantibodies, hematologic manifestations, and hypocomplementemia, as well as chronic suppurative skin lesions and recurrent infections. Genetic analysis revealed biallelic pathogenic variants in *NCF4* resulting in p40^{phox} deficiency. Comprehensive NADPH oxidase activity studies confirmed significantly decreased production of reactive oxygen species, confirming the cellular phenotype seen in p40^{phox} deficient patients.
Conclusions: Here, we present a patient with pSLE harboring biallelic variants in *NCF4*. Our patient represents a unique clinical presentation of severe onset autoimmunity in the setting of a rare inborn error of immunity affecting NADPH oxidase activity. This case underscores the need to consider genetic causes of pSLE in cases of pre-pubertal onset and atypical disease.

KEYWORDS

p40^{phox} deficiency, NADPH oxidase complex, *NCF4*, reactive oxygen species, systemic lupus erythematosus, inborn error of immunity, pediatric SLE

Introduction

Phagocyte oxidase subunit p40 (p40^{phox}) deficiency is an inborn error of immunity characterized by impaired production of reactive oxygen species (ROS) by phagocytic cells. Unlike classic chronic granulomatous disease (CGD), individuals with p40^{phox} deficiency do not suffer from invasive bacterial and fungal infections, and their phagocytic cells exhibit residual ROS production (1). However, despite the residual ROS

production, p40^{phox} deficient patients can develop immune dysregulation, leading to various clinical manifestations, including superficial infections and inflammatory phenotypes, such as inflammatory bowel (IBD) disease and cutaneous lupus (1), and immune-mediated thrombocytopenia (ITP) (2). In adults, individuals with hypomorphic mutations in *NCF2* (p67^{phox}) and female carriers of *CYBB* (gp91^{phox}) mutations can develop multi-organ autoimmunity such as systemic lupus erythematosus (SLE) (3, 4).

SLE is a multisystem autoimmune disease with heterogeneous presentations including systemic and cutaneous inflammation, various organ system involvement such as arthritis, nephritis, and immune-mediated cytopenias, and elevated circulating autoantibodies. Pediatric SLE (pSLE) refers to cases wherein the onset of disease occurs under the age of 18 years, and such patients are at increased risk of disease-related organ damage and treatment-related morbidity over time (5, 6). While the underlying pathophysiology of SLE is complex, a small percentage of pSLE cases are the result of inborn errors of immunity associated with monogenic defects. Several genes, including ones resulting in complement deficiencies and interferonopathies, have been implicated in lupus predisposition (7, 8).

Here we report a patient with pSLE and autosomal recessive p40^{phox} deficiency, identified through exome sequencing (ES). This patient presented originally at 7 years old with malar rash and developed severe clinical manifestations, including refractory ITP and recurrent infections. Functional evaluation of NADPH oxidase activity through the dihydrorhodamine 1,2,3 (DHR) assay confirmed the cellular phenotype seen in p40^{phox} deficient patients with defective, but not abolished, ROS production. Our study expands the clinical spectrum of p40^{phox} deficiency and highlights the importance of considering this genetic disorder in the differential diagnosis of unresolved pSLE cases.

Case description

A now 18-year-old female was diagnosed with pSLE at the age of 7 years. Her initial presentation included sterile fevers, malar rash (Figure 1A), the presence of antinuclear antibodies (ANA), autoimmune hemolytic anemia, hypocomplementemia, and anti-phospholipid antibodies (lupus anticoagulant)—meeting classification criteria for SLE according to both the European League Against Rheumatism/American College of Rheumatology (EULAR/ACR) and the Systemic Lupus International Collaborating Clinics (SLICC) criteria (9, 10). She received high-dose intravenous methylprednisolone and was treated with hydroxychloroquine and mycophenolate mofetil.

Following her initial pSLE diagnosis, additional autoantibodies developed, including anti-Smith, and she developed recurrent and refractory ITP. B-cell phenotyping revealed normal proportions of naïve and memory B cells with no increased CD21^{low}CD38^{low} B cells. In the setting of positive autoantibodies, her ITP was managed with prednisone, azathioprine, and rituximab. Her

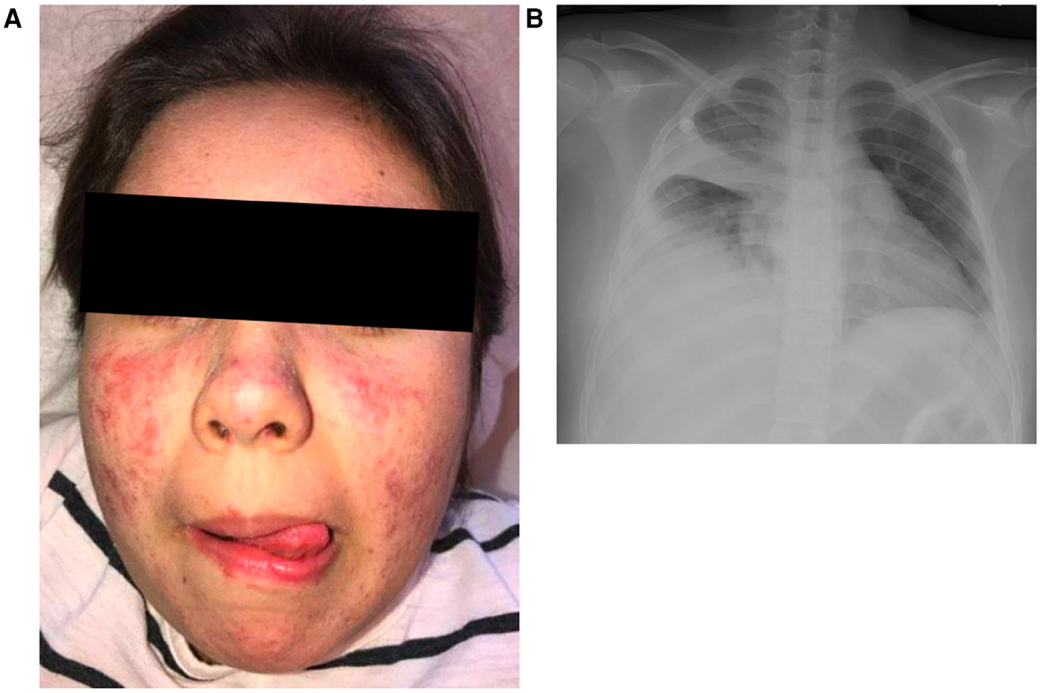

FIGURE 1
Clinical findings. **(A)** Photosensitive malar rash sparing the nasolabial folds. **(B)** Chest imaging showing right middle and lower lobe consolidation and parapneumonic effusion and right upper lobe atelectasis at the time of presentation with acute hypoxemic respiratory failure requiring management with bilevel positive airway pressure.

refractory ITP has required serial courses of rituximab over time for control. She has had no evidence of nephritis.

Additional relevant medical history post-pSLE diagnosis includes multiple bacterial urinary tract infections including one associated with presumed sepsis, acyclovir-responsive blepharitis, persistent right eyelid hordeolum, facial folliculitis, a consolidative pneumonia with parapneumonic effusion requiring drainage (Figure 1B), and fungal urinary tract infections secondary to *Candida* spp. Given her recurrent infections out of proportion to a typical pSLE course, additional immunologic studies were performed while she was already receiving immune suppression. She was found to have mildly elevated immunoglobulin levels and T cell and NK cell lymphopenia. At age 15 she developed skin abscesses on her neck, axillae, and chest that were successfully treated with oral antibiotics. She has never developed signs or symptoms suggestive of IBD.

Diagnostics

Five years after her pSLE diagnosis, she underwent research trio ES as part of an institutional genotype screening program for patients with pSLE. Variant analysis ruled out variants in known genes associated with monogenic SLE, including genes related to complement deficiencies, nucleic acid sensing, type I interferonopathies, and tolerance defects (8). However, the patient was found to have biallelic variants in *NCF4*, each inherited from a different parent (Figure 2A). The first variant identified, p.R58C, has previously been described as a pathogenic variant conferring p40^{phox} deficiency (1). The second variant, c.824+1G>A, is novel and was predicted to cause loss of the donor splice site. Confirmation of alternative splicing was assessed by RT-PCR using mRNA from patient peripheral blood mononuclear cells. The presence of an alternate transcript with shorter length was observed (Figure 2B); Sanger sequencing of the fragment confirmed an alternate splicing event resulting in in-frame skipping of all of exon 9. Exon 9 partially encodes the SH3 domain (Figure 2C) essential for p40^{phox} binding to other NADPH subunits, such as p67^{phox} (11).

To confirm p40^{phox} deficiency, the patient underwent comprehensive NAPDH oxidase studies. The DHR oxidation assay showed defective oxidation upon PMA stimulation in patient neutrophils, although all neutrophils and monocytes were capable of oxidizing DHR (Figure 3A). However, patient cells showed half the index of oxidation compared to control (Figure 3B). Further evaluation revealed a profound defect in H_2O_2 release upon zymosan and *S. aureus* stimulation, consistent with the cellular phenotype of p40^{phox} deficiency (Figure 3C). Moreover, protein electrophoresis of neutrophil lysate from the patient confirmed a reduction of approximately 50% in p40^{phox} expression compared to controls, while the other NADPH subunits (gp91^{phox}, p67^{phox}, p47^{phox}, and p22^{phox}) exhibited expression more similar to healthy individuals (Figure 4). These data confirm that this patient with pSLE exhibited the cellular phenotype of p40^{phox} deficient patients with affected p40^{phox} protein expression, impaired ROS production after stimuli, and reduced activity detected by DHR.

Conclusions and discussion

p40^{phox} deficiency is a rare inborn error of immunity resulting from impaired but not abolished ROS production by the NADPH oxidase complex in phagocytic cells. The residual ROS production

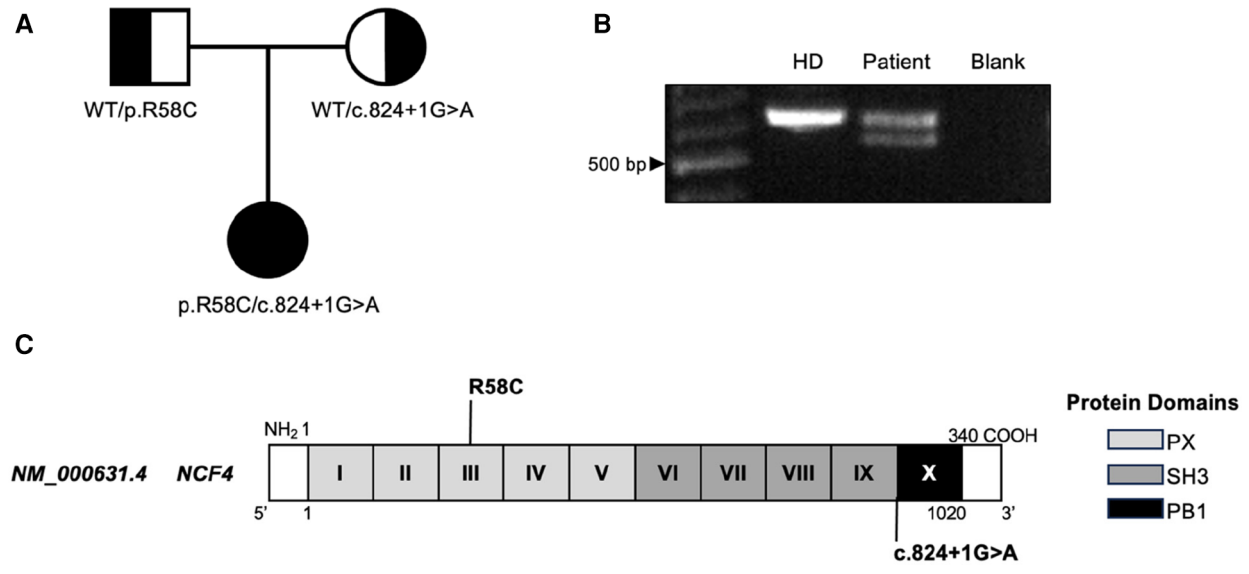

FIGURE 2
NCF4. **(A)** Familial segregation of *NCF4* variants. **(B)** RT-PCR of *NCF4* mRNA flanking exon 9 in the patient and a control. **(C)** Gene structure of *NCF4* and protein domains of p40^{phox} with patients' variants indicated. bp, base pair; HD, healthy donor.

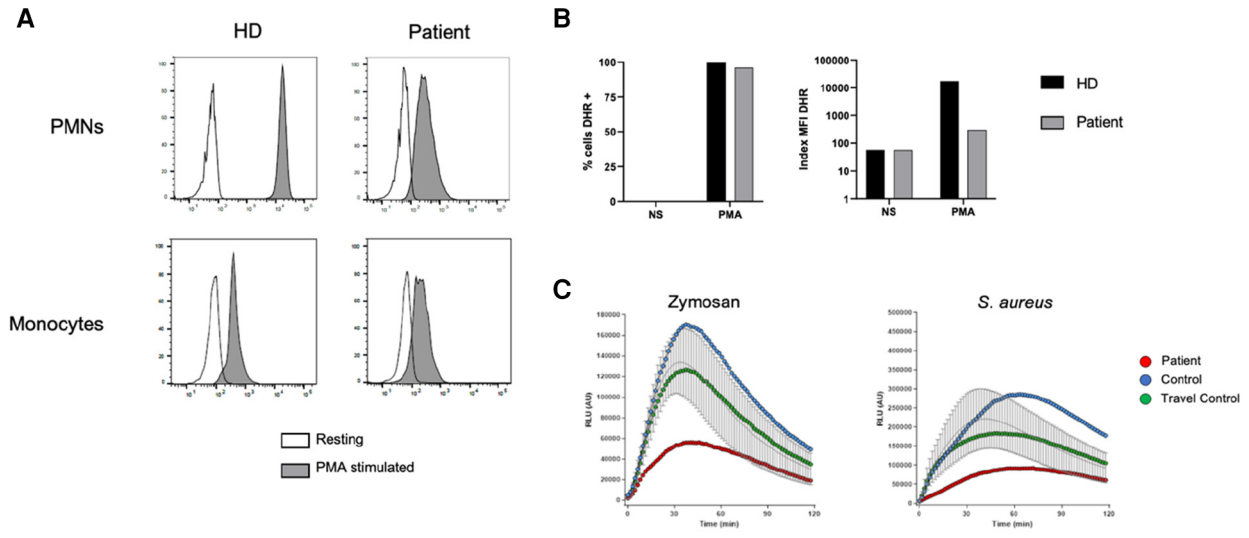

FIGURE 3
Functional studies. **(A)** DHR assay in neutrophils (top) and monocytes (bottom) upon PMA stimulation in the patient and a control. **(B)** Percentage (left) and Index MFI (right) of cells able to oxidize the DHR. **(C)** H_2O_2 release from patient and control neutrophils following different stimuli. DHR, dihydrorhodamine 1,2,3; HD, healthy donor; MFI, mean florescence intensity; NS, non-stimulated; PMA, phorbol 12-myristate 13-acetate; PMN, polymorphonuclear cell; RLU, relative light units.

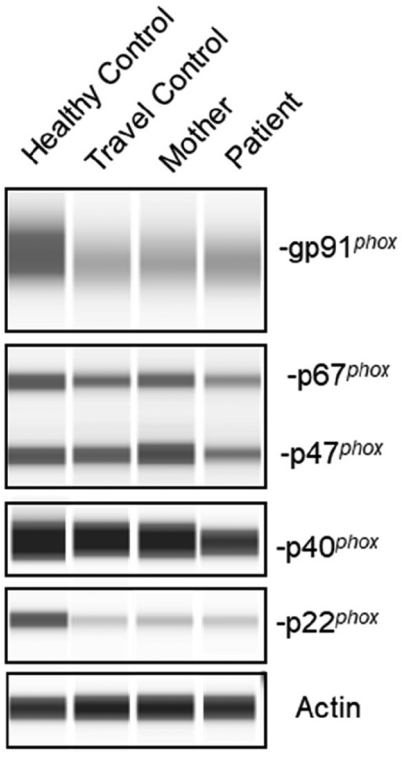

FIGURE 4
Protein expression of NADPH oxidase subunits. Protein expression of the NADPH subunits in neutrophil lysates from controls, the patient's mother (heterozygous for the *NCF4* c.824+1G>A variant) and the patient. Actin protein was used as loading control.

confers protection against invasive infections, but $p40^{phox}$ deficient patients can suffer from immune dysregulation. The most common inflammatory manifestations seen in $p40^{phox}$ patients are IBD and cutaneous inflammation consistent with lupus, including discoid lupus (1, 2). Defects in ROS production have been linked to autoimmune susceptibility, including lupus (3, 4).

pSLE is a severe early-onset autoimmune disease associated with positive autoantibodies and a broad clinical spectrum that can include fevers, rashes, arthritis, autoimmune cytopenias, and nephritis, among other features (6). Some patients with pSLE exhibit defects in genes associated with complement deficiencies, nucleic acid sensing, and B cell dysregulation, but previous studies have not confirmed defects in ROS production as a potential etiology of monogenic lupus (8).

To our knowledge this is the first case of pSLE associated with $p40^{phox}$ deficiency. While some $p40^{phox}$ deficient individuals have had lupus-like cutaneous lesions and ITP has been described (1, 2), our patient had an early-onset phenotype fulfilling classification criteria for SLE with multiple positive autoantibodies and no IBD manifestations to date.

Our patient's refractory ITP requires serial rituximab treatment for steroid-sparing purposes, which suggests that some of her autoimmunity is B-cell mediated. Epstein Barr virus transformed B cells from $p40^{phox}$ deficient patients exhibit severe impairments in ROS production (1). Little is known about the specific role of ROS in B cells and any contribution they may make to the development of autoimmunity. Further studies regarding defective ROS production in B cells are needed to decipher the pathophysiology of $p40^{phox}$ deficiency and the connection between ROS production and autoimmunity.

In summary, this case expands the clinical spectrum of p40^{phox} deficiency and highlights the importance of considering this genetic disorder in the differential diagnosis of unresolved pSLE cases, particularly those of pre-pubertal onset and/or associated with recurrent infections. It also suggests DHR assays with index quantification may be relevant during initial evaluations of such patients. Finally, this case confirms the pivotal role of ROS production in immune regulation.

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 humans were approved by Baylor College of Medicine Institutional Review Board (IRB) approved research protocol (H-29697). The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin. Written informed consent was obtained from the individual(s), and minor(s)' legal guardian/next of kin, for the publication of any potentially identifiable images or data included in this article.

Author contributions

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Conflict of interest

DK was employed by Leidos Biomedical Research Inc.

TV receives research support from AstraZeneca.

JRL has stock ownership in 23andMe and is a paid consultant for Genome International, Inc.

The remaining authors declare no commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2024.1425874/full#supplementary-material

SUPPLEMENTARY TABLE S1
Clinical course of p40^{phox} deficient patient with pSLE.

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A case report navigating CVID and sarcoidosis overlaps in pediatric nephritis

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Common variable immunodeficiency (CVID) can be complicated by granulomatous disease, often granulomatous lymphocytic interstitial lung disease (GLILD). Granulomatous interstitial nephritis represents an atypical presentation in pediatrics. Our patient is a previously healthy 13-year-old white male with a recent diagnosis of CVID. He presented with a rash and laboratory findings included pancytopenia (white blood cells 2.6 cells $\times 10^3/\mu l$, hemoglobin 11.8 g/dl, platelets $60 \times 10^3/\mu l$), hypercalcemia (14.9 mg/dl), elevated Vit D 1,25 OH level (>200 pg/ml), hyperuricemia (8.8 mg/dl), and acute kidney injury (AKI) (serum creatinine 1.1 mg/dl; baseline 0.64 mg/dl). A broad infectious workup was unremarkable. The rash improved with empiric doxycycline. Hypercalcemia and hyperuricemia were managed with fluid resuscitation, calcitonin, and zoledronic acid. Evaluation for malignancy including a positron emission tomography scan, revealed multiple mediastinal hypermetabolic lymph nodes and pulmonary ground glass opacities, later reported as small pulmonary nodules by computed tomography (CT). Splenomegaly was confirmed by ultrasound and CT. Peripheral smear, bone marrow biopsy, and genetic testing were non-revealing. His angiotensin-converting enzyme level was elevated (359 U/L), raising concerns for sarcoidosis. Given Stage 1 AKI, a renal biopsy was pursued and identified non-caseating granulomatous interstitial nephritis. Treatment with 60 mg of prednisone began for presumed sarcoidosis for 4 months, causing steroid-induced hypertension and mood changes. Zoledronic acid minimally reduced serum creatinine. *Pneumocystis jirovecii* pneumonia prophylaxis was initiated due to *T*-cell cytopenia. Chest CT findings showed a suboptimal response to steroids. A bronchoalveolar lavage demonstrated >50% lymphocytes (normal <10%) and the lung biopsy exhibited non-caseating granulomas, indicating GLILD. Rubella was identified by staining. Following a fever, he was found to have elevated liver enzymes and confirmed hepatitis with portal hypertension on CT. A liver biopsy revealed epithelioid non-caseating granuloma and HHV6 was detected by PCR. He was treated with four cycles of rituximab and granulocyte-colony stimulating factor for persistent neutropenia. Subsequent treatment with mycophenolate led to the resolution of the granulomatous lesions and cytopenias. The rare complication of granulomatous interstitial nephritis in CVID illustrates the intricate nature of diagnosis. This case

underscores the necessity for a holistic view of the patient's clinical and immune phenotype, including distinctive radiological presentations, for precise diagnoses and tailored management of CVID.

KEYWORDS

CVID, granulomatous disease, hypogammaglobulinemia, sarcoidosis, GLILD, granulomatous lymphocytic interstitial lung disease

Introduction

Common variable immunodeficiency (CVID) is among the most commonly diagnosed primary immunodeficiencies (1–3). The term "variable" reflects the heterogeneous clinical presentations of patients who present with hypogammaglobulinemia and increased susceptibility to infections. The vast majority of patients lack an identifiable underlying molecular defect (4) Granulomatous disease occurs in 8%–22% of CVID patients, often affecting the lungs, lymph nodes, and spleen (5–10). Granulomatous lesions appear on organ biopsies and may precede recognition of any underlying immune defects (6–11). Resultantly, a diagnosis of CVID is often delayed and occasionally patients might be mislabeled as having sarcoidosis due to clinical overlap and the greater prevalence of sarcoidosis (5, 12). These diagnostic delays can be significantly associated with increased morbidity and mortality contributions in CVID (13). The clinical impact of non-infectious manifestations, such as granulomas, is organ-specific, and the presence of granulomas and subsequent tissue damage in the lungs and liver have been demonstrated to lead to shorter patient survival (14).

Lymphocytic interstitial infiltrate, accompanying granuloma formation, contributes to organ failure and disease severity, as seen in "granulomatous lymphocytic interstitial lung disease" or GLILD (5, 9, 15). Granuloma presence, alongside autoimmune manifestations in CVID, can significantly elevate mortality risk, particularly in pediatric-onset cases (8, 13). The development of granulomas and autoimmunity in CVID each independently pose therapeutic challenges, necessitating a careful balance of immunosuppression in an already compromised immune system. Granulomatous interstitial nephritis in adult patients with CVID has been very sparsely reported in the literature (16–18). We aim to describe a unique and rare presentation of granulomatous interstitial nephritis in a pediatric patient with known CVID. We highlight the diagnostic challenge of distinguishing CVID-associated granulomatous disease from sarcoidosis and describe the management and clinical progression of our patient, in accordance with CARE Guidelines (19).

Narrative

Our patient was a previously healthy 13-year-old white male referred to the Allergy and Immunology service by his pediatrician following hypogammaglobulinemia identified on screening for celiac disease due to a known family history. Family history was remarkable for ulcerative colitis in the mother and an older brother with celiac disease. The patient lacked a history of recurrent or unusual infections. Immune phenotyping at the time of diagnosis (Table 1) included a complete blood count with pancytopenia [white blood cell (WBC) count 3.08×10^3/μl, hemoglobin 12.5 g/dl, platelets 71×10^3/μl]. He was also noted to have decreased lymphocyte subsets upon initial evaluation: CD3+ *T*-cell count 584×10^3/μl, CD3+CD4+ *T*-cell count 309×10^3/μl, CD3+CD8+ *T*-cell count 215×10^3/μl, CD19+ B-cell count 100×10^3/μl, and CD3−CD56+CD16+ NK cell count 27×10^3/μl. The B-cell subset had absent class-switched memory B cells, $CD19^+CD27^+IgM^-IgD^-$ at 0%, and elevated transitional B-cell percentage, CD19+CD38+Bright IgM at 17% with a normal count of 16×10^3/μl identified. $CD19+CD38^{-/low}$, $CD21^{-/low}$ autoreactive B cells were found to be 1×10^3/μl. He had diffuse hypogammaglobulinemia with IgG 246 mg/dl, IgA 18 mg/dl, and IgM 7 mg/dl. Pneumococcal IgG antibodies were positive in only 1 out of 23 serotypes, with a level >1.3 μg/ml. The patient was up to date on his childhood vaccines.

Shortly after the initial immune evaluation, our patient developed a diffuse non-blanching maculopapular rash and progression of pancytopenia (WBC 2.66×10^3/μl, Hemoglobin 11.8 g/dl, Platelet 60×10^3/μl) following a camping trip in the Ozarks, an area endemic for several tick-borne illnesses, which occurred 1 month before the rash onset. His rash was non-pruritic, noted to involve the palms and soles, and appeared petechial in nature around his thighs and axilla. Screening labs on admission displayed hypercalcemia (14.9 mg/dl), elevated Vitamin D 1,25 OH level (>200 pg/ml), hyperuricemia (8.8 mg/dl), and acute kidney injury (AKI) (serum creatinine 1.1 mg/dl up from baseline 0.64 mg/dl). Soluble IL-2 was elevated to a peak of 8,711 U/ml, with ratio of soluble IL-2 to WBC of 3.5.

The constellation of pancytopenia, hypercalcemia, and hyperuricemia triggered an evaluation for malignancy. The positron emission tomography (PET) (Figure 1a) scan showed multiple hypermetabolic lymph nodes within his mediastinum nodal enlargement and pulmonary ground glass opacities. Computed tomography (CT) (Figure 1b) later reported pulmonary changes as diffuse small pulmonary nodules. Splenomegaly was confirmed by ultrasound (US) and CT, and left kidney enlargement was seen on the US. Peripheral smear and bone marrow biopsy findings were normal. Concurrent broad evaluation for infection, including tick-borne illnesses, was pursued and was unremarkable. A critical trio whole-exome sequencing was also unrevealing. He was started on a 10-day empiric course of doxycycline with an improvement in the rash. Hypercalcemia and hyperuricemia were managed with fluid

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TABLE 1 Laboratory studies at diagnosis.

Complete blood count	Patient's value	Normal value
White blood cell count	**3.08×10^3/µl**	4.5–13.5 × 10^3/µl
Hemoglobin	**12.5 g/dl**	13.0–16.0 g/dl
Platelets	**71×10^3/µl**	150–450 × 10^3 µl
Immunophenotyping		
$CD3^+$ cells × 10^3/ml (%)	**584×10^3/µl** (82)	1,000–2,200 × 10^3/µl (60–76)
$CD4^+$ T cells × 10^3/ml (%)	**309×10^3/µl** (43)	530–1,300 × 10^3/µl (31–52)
$CD8^+$ T cells × 10^3/ml (%)	**215×10^3/µl** (30)	330–920 × 10^3 (18–35)
$CD4^+/CD8^+$ *T*-cell ratio	1.44	0.7–2.4
$CD19^+$ B cells × 10^3/ml (%)	**100×10^3/µl** (14)	110–570 × 10^3/µl (6–23)
$CD27^+$ Memory B cells × 10^3/ml (%)	3 × 10^3/µl (4)	2–122 × 10^3/µl (2–36)
$CD19^+CD27^+IgM^-IgD^-$ Class-Switched Memory B cells × 10^3/ml (%)	0 × 10^3/µl (0)	0–74 × 10^3/µl (0–22)
$CD19^+CD38^+$Bright IgM^+ Transitional B cells × 10^3/ml (%)	16 × 10^3/µl (17)	0–18 × 10^3/µl (0–6)
$CD56^+$ $CD16^+$ NK cells × 10^3/ml (%)	**27×10^3/µl** (4)	70–480 × 10^3/µl (3–22)
$CD19^+CD38^{-/low}$ $CD21^{-/low}$ autoreactive B cells	1 × 10^3 (1)	0–21 × 10^3/µl (0%–7%)
Mitogenic and antigenic induced lymphocyte proliferation		
Phytohemagglutinin 10 µg/ml	**40, 826 lymphocyte proliferation in counts per minute**	>163,507 Lymphocyte proliferation in counts per minute
Tetanus antigen	4,340 Lymphocyte proliferation in counts per minute	>2,000 lymphocyte proliferation in counts per minute
Cytokines		
Soluble IL-2 receptor (CD25)	**8,711 U/ml**	45–1,105 unit/ml
Immunoglobulins		
IgG	246 mg/dl	641–1,353 mg/dl
IgA	18 mg/dl	66–295 mg/dl
IgM	7 mg/dl	40–80 mg/dl
Vaccination antigen response		
Pneumococcal IgG	1/23 > 1.3 µg/dl	50%–70% > 1.3 µg/dl
Haemophilus influenzae B IgG	<0.15 µg/ml	≥1.00 µg/ml
Tetanus antitoxoid	0.10 IU/ml	≥0.10 IU/ml

Bold values represent those values outside of the listed reference ranges.

resuscitation, calcitonin, and zoledronic acid. He received intravenous IgG replacement (500 mg/kg) during admission and continued monthly replacement with a goal trough of 1,000 mg/ml.

The presence of hypercalcemia, elevated Vitamin D levels, hypermetabolic lymph nodes, and pulmonary manifestations prompted an assessment for lymphoproliferative disorder. A renal biopsy was obtained due to Stage 1 AKI and identified non-caseating granuloma on electron microscopy, raising concern for sarcoidosis. Hematoxylin and eosin staining revealed numerous well-formed epithelioid, non-caseating granulomas with focal extension into renal tubules, in addition to the presence of focal calcified bodies and Langerhans-type giant cells. His angiotensin-converting enzyme (ACE) level was found to be elevated at 359 U/L (reference range 13–100 U/L), and universal polymerase chain reaction (PCR) testing on the renal biopsy was found to be negative for acid-fast bacilli, bacterial, and fungal infection.

A course of prednisone 60 mg daily for presumed sarcoidosis was initiated for a 4-month period with a slow taper. While on steroids, he developed side effects including steroid-induced hypertension and mood changes, in addition to a suboptimal clinical response. He also remained cytopenic while on steroid therapy and required *Pneumocystis jirovecii* pneumonia prophylaxis for persistent *T*-cell lymphopenia. Chest CT findings also showed an almost negligible response to steroids; though some nodules were resolving, there was formation of new nodules (Figure 2). A bronchoalveolar lavage had greater than 50% lymphocytes (normal <10%). A lung biopsy was pursued to further characterize the lung nodules identified on CT to dictate the next therapeutic steps and it confirmed the presence of multi-organ granulomatous disease with the presence of non-caseating granulomas and lymphocytic inflammation, suggesting a diagnosis of GLILD.

Shortly after the biopsy, he spiked a fever and was found to have elevated liver enzymes. CT and ultrasound findings confirmed hepatitis with a suspicion of portal hypertension. A liver biopsy was performed and revealed epithelioid granulomas without necrotizing or caseating features, with the presence of focal areas of lobular and portal chronic inflammation. Histochemical staining identified focal glycogenated nuclei without copper or iron accumulation. Human herpes virus (HHV6) was detected in liver tissue by PCR.

He was treated with four cycles of rituximab, which normalized hemoglobin and platelet counts; however, he remained persistently neutropenic. Resultantly, he was started on granulocyte-colony stimulating factor (G-CSF) 5 µg/kg/day, which led to an improvement in absolute neutrophil counts. He subsequently received treatment with mycophenolate (MMF) for immune-mediated cytopenias, in addition to his multi-organ granulomatous disease, with good response, allowing discontinuation of G-CSF.

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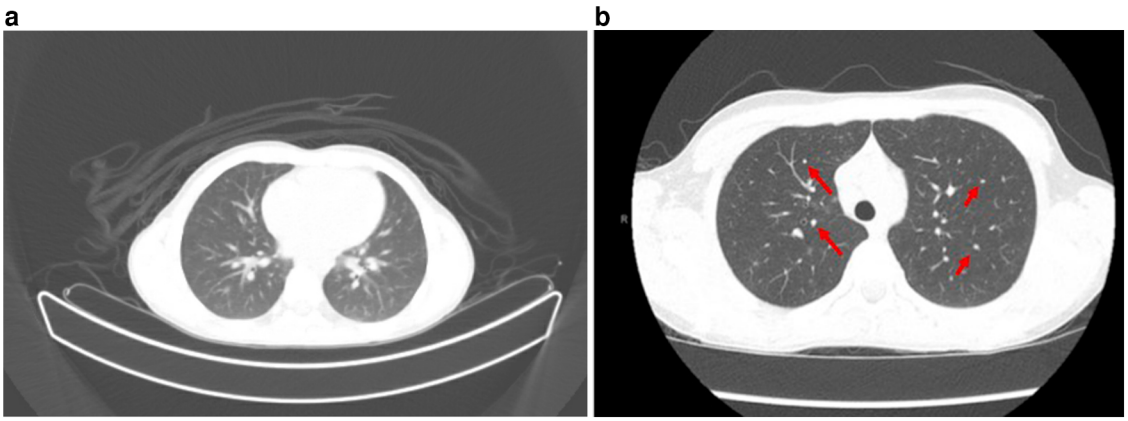

FIGURE 1
(a) PET scan with multiple mediastinal, axillary, and common hilar lymph nodes are appreciated, with representative lymph nodes. Multiple ground glass nodules within the bilateral lungs predominantly in a centrilobular distribution. Additional indistinct ground glass opacities along the posterior aspect of the bilateral lung. (b) CT scan with enlarged mediastinal lymph nodes demonstrated, correlating with hypermetabolic nodes from PET above. Multiple non-specific subcentimeter ground glass nodules (red arrows) throughout both lungs.

Due to a lack of steroids and the patient's intolerance to side effects, no further steroids were administered. Our patient demonstrated improvements in white blood cell, hemoglobin, and platelet counts. While he remained *T*-cell lymphopenic, he maintained normal lymphocyte proliferation, obviating the need for *Pneumocystis jirovecii* prophylaxis. Following 2 years of therapy, he experienced complete resolution of pulmonary ground glass opacities and nodules, and hepatosplenomegaly.

Discussion

Granulomatous findings in the tissue of patients with CVID have been noted to be mistaken for sarcoidosis (7, 12). Discriminating between these conditions can be complex due to overlapping clinical and diagnostic features, including multi-system non-caseating granulomas with hypercalcemia, lymphadenopathy, and propensity for pulmonary, liver, and lymph node involvement (20, 21). In some situations, arriving at an accurate diagnosis can be further complicated by an immune phenotype not yet identified.

Elucidating the differences between granulomatous disease in CVID and sarcoidosis is critically important to avoid inappropriate treatment and delays in targeted treatment (Table 2). Many patients with sarcoidosis in fact have normal ACE levels (7, 22, 23). ACE levels have also been reported to be abnormally elevated in CVID patients without granulomas (8). Further, ACE levels can be elevated in the setting of certain infections such as the human immunodeficiency virus (HIV) and other infections presenting with non-caseating granulomas such as histoplasmosis (24, 25). Both sarcoidosis and granulomatous disease in CVID include B-cell derangements; however, CVID can be delineated by the absence or the reduction of switched memory B cells, and

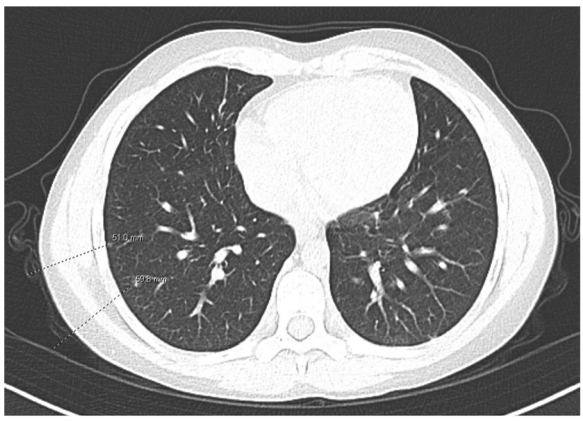

FIGURE 2
CT following the use of steroids. Mixed response in multiple pulmonary nodules, with some nodules having resolved and other nodules new.

TABLE 2 Key comparisons in CVID with GLILD and sarcoidosis.

Feature	CVID with GLILD	Sarcoidosis
B-cell subset	Absent or reduced memory switched B cells	Reduced memory B cells
Immunoglobulins	Hypogammaglobulinemia	Hypergammaglobulinemia or normal
Lung granulomas	Present	Present
Lymph node biopsy histologic findings	Ill-defined germinal centers, decreased plasma cells	Undisrupted architecture
Chest CT findings	Lower lung lobe with larger nodularity, ground glass opacities, and flame-shaped hemorrhages	Perilymphatic micronodular infiltrate in bronchovascular distribution
Bronchoalveolar lavage fluid	Normal CD4:CD8 (<3:1)	Elevated CD4:CD8 ratio (>3:5)
ACE levels	Normal or elevated	Normal or elevated
Spontaneous remission	Rare	Common in early stages

sarcoidosis favors reduced memory B cells alone (20). Sarcoidosis is typically characterized by the presence of hypergammaglobulinemia rather than hypogammaglobulinemia, a hallmark of CVID (26). Notably, patients with CVID and GLILD display markers reflecting *T*-cell activation and exhaustion, including soluble IL-2 receptor when compared to other patients with CVID with non-infectious complications (27).

From a histopathologic standpoint, lymphoid hyperplasia has been observed alongside granulomas in CVID and is absent in sarcoidosis. Specifically, patients with GLILD have exhibited CD4+ *T*-cell predominance in the lungs, variable CD8+ *T* cells and B cells were found in much smaller numbers. A notable absence of regulatory T cells in lung biopsies has also been reported (28). Follicular helper *T* cells have been noted within granuloma in CVID, whereas they are more weakly expressed in sarcoidosis (29). The presence of organizing pneumonias has been found in GLILD and a subgroup of patients with CVID and GLILD will progress to interstitial fibrosis with architectural remodeling (28).

Lymph node biopsy in CVID patients usually demonstrates disrupted architecture with ill-defined germinal centers and marked reductions in plasma cells, whereas plasma cells and germinal cells are more likely to be intact in sarcoidosis (20, 30). High-resolution CT and bronchoalveolar lavage findings can also help discriminate these conditions (12). GLILD on chest CT is more likely to include lower lobe disease with larger nodularity, ground glass opacities, and flame-shaped hemorrhages, while sarcoidosis is more often associated with perilymphatic micronodular infiltrate appearing in a bronchovascular distribution (31, 32). In the bronchoalveolar fluid, a CD4:CD8 ratio is typically normal in CVID and elevated (>3.5) in sarcoidosis (32).

It is estimated that 2% of patients with CVID have renal insufficiency (33). One study indicated that membranous glomerulonephropathy and tubulointerstitial nephritis were the predominant pathologic findings in renal biopsies from CVID patients with AKI and/or proteinuria (34).

Interstitial lymphocytic infiltration, in conjunction with granulomatous formations, indicates an increased risk of organ failure and disease severity (5). While up to 22% of patients with CVID have some variation of granulomatous disease, this prevalence is likely under-representative as most patients do not undergo routine biopsies (2, 35, 36). Tissue biopsy is not only crucial for diagnosis of granulomas but also to distinguish it from other conditions such as lymphoid hyperplasia and lymphomas (37, 38).

Our patient developed an exceedingly rare complication of granulomatous interstitial nephritis in CVID, in addition to GLILD, liver granulomas, and cytopenias. Initially, a diagnosis of sarcoidosis was made, however, his clinical presentation and features were more consistent with the granulomatous manifestations known to occur in CVID. An elevated soluble IL-2 receptor to white blood cell ratio, as was present in our patient, has been demonstrated to reflect granulomatous disease progression in CVID and may represent an important and readily available biomarker for risk stratification for this disease population (39). He clinically responded to a tailored treatment regimen with four cycles of rituximab, G-CSF, and mycophenolate for persistent neutropenia with resolution of the granulomas (Figure 3) and cytopenias.

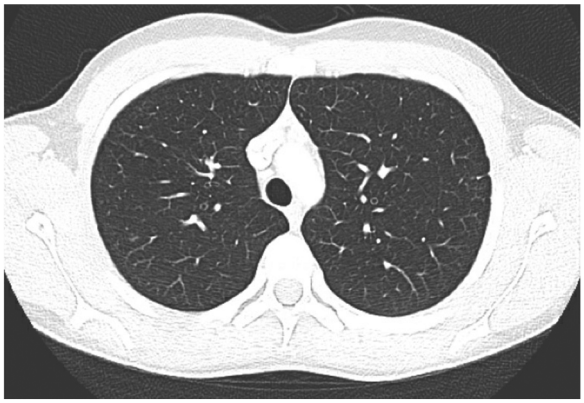

FIGURE 3
CT scan demonstrating interval near resolution of previously described punctate nodules throughout both lungs. Two isolated ground glass foci remain with the right lung. No new nodule or ground glass opacity.

Conclusion

This case underscores the diagnostic complexities inherent in differentiating CVID-related granulomatous disease from other similar conditions. The coexistence of hypogammaglobulinemia with specific chest CT findings steered the diagnosis toward GLILD rather than sarcoidosis. This case emphasizes the necessity of a thorough evaluation of a patient's clinical presentation and immune phenotyping when CVID-related granulomatous anomalies are suspected. Meticulous attention to the distinctive clinical and diagnostic features is paramount for achieving an accurate diagnosis, which is crucial for the effective management and targeted treatment of patients with CVID.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to amanda.salih@alumni.bcm.edu.

Ethics statement

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

AS: Writing – review & editing, Writing – original draft. AB: Writing – review & editing. AG: Writing – review & editing. SH: Writing – review & editing. MS-C: Writing – review & editing.

LT: Writing – review & editing. JH: Writing – review & editing, Formal Analysis, Conceptualization.

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Conflict of interest

JH serves on the advisory board and speaker bureau for Pharming, is an advisory board member and research grant recipient from the Immune Deficiency Foundation, a research grant recipient from the Jeffrey Modell Foundation and Takeda.

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

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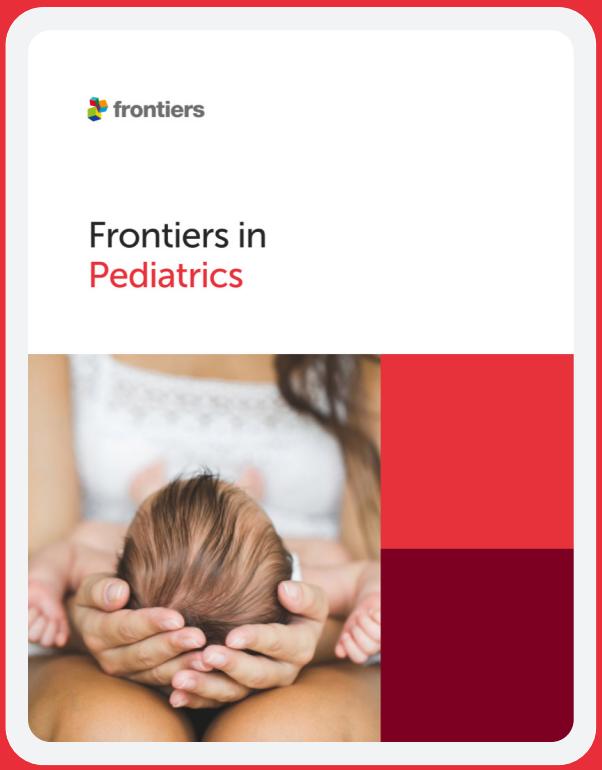

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