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Case reports in pediatric immunology 2022

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Case reports in pediatric immunology 2022

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Editorial on case reports in pediatric immunology 2022

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KEYWORDS

Kawasaki disease (KD), multisystem inflammation syndrome, DiGeorge S, primary immune deficiencies, papillon lefevre syndrome, HLH—hemophagocytic lymphohistiocytosis

Editorial on the Research Topic
Case reports in pediatric immunology 2022

Pediatric Immunology is a specialized field in pediatrics that deals with disorders of immune system. While most part of pediatric immunology is related to inborn errors of immunity [IEI, also known as primary immunodeficiency diseases (PIDs)], other diseases such as autoimmune rheumatic diseases, Kawasaki disease (KD) and multisystem inflammatory syndrome (MIS-C) associated with SARS-CoV-2 infection also forms a major part of this specialty. There have been several advances in the diagnosis and management of IEIs in last 2 decades. More than 450 different IEIs have been identified till date and every year new diseases are being discovered. The management of IEIs have evolved from immunoglobulin replacement to hematopoietic stem cell transplant, gene therapy and several novel targeted therapeutics. The research topic "Case Reports in Pediatric Immunology" is a collection of cases in Pediatric Immunology with diagnostic and therapeutic challenges.

Suzuki et al. reported a case of MIS-C that failed to respond to intravenous immunoglobulin (IVIg) and corticosteroids but showed a prompt response to cyclosporine. MIS-C has been reported to have several clinical and pathophysiological similarities with KD, a common childhood medium vessel vasculitis (1). The same group previously carried out a randomized controlled trial that reported the efficacy of cyclosporine in combination with IVIg in patients with KD who were predicted to be non-responsive to IVIg alone. The combination of IVIg and cyclosporine resulted in better coronary artery outcomes in these patients.

Basu et al. reported successful use of corticosteroids for liver abscess in patients with Papillon-Lefèvre syndrome. This disorder is characterized by a defect in neutrophil extracellular trap formation in response to reactive oxygen species. Although, the role of corticosteroids in management of liver abscess is well established for patients with chronic granulomatous disease (2), this is for the first time that its role has also been evaluated for management of liver abscess in Papillon-Lefèvre syndrome.

Poplonyk et al. reported a case of cardio-facio-cutaneous syndrome, which is a group of RASopathies (3), caused by a novel germline mutation in *MAP2K1* gene. PIDs with syndromic features is a special group disorders that are clinically and pathophysiologically heterogeneous. A high index of suspicion is needed to diagnose these disorders early. In children who have syndromic features and recurrent infections, the infections may commonly be attributed to neurological dysfunction, aspiration or to the cardiac defects.

However, it is important to evaluate for underlying immune abnormalities as this may change the management in these cases.

A proportion of patients with DiGeorge syndrome have an underlying immunodeficiency. Autoimmunity is being increasingly recognized in these cases (4). Gu et al. report a case of successful use of sirolimus in a patient with DiGeorge syndrome who presented with autoimmune lymphoproliferative syndrome (ALPS) like features. Autoimmune lymphoproliferative syndrome is a well-defined PID and role of sirolimus in these patients is established. However, several PIDs may also present with ALPS like phenotype. It is important to identify this phenotype so that more targeted therapies such as sirolimus can be used for these patients.

The newborn screening for severe combined immunodeficiency (SCID) has revolutionized this field and this potentially fatal disease is now being recognized early in life and curative treatment is being offered. Universal newborn screening for SCID is now being offered in all parts of the United States, several European and a few Asian countries. Vasco et al. report a case of GATA2 deficiency that was diagnosed using new born screening for SCID. GATA-2 deficiency has a wide spectrum of clinical phenotype and immunodeficiency is an important component of this syndrome.

Genetic sequencing has provided novel insights into the pathogenesis of PIDs (5). Several new genetic defects and novel variants are being identified in PIDs. Zhu et al. identified a case of Capping protein regulator and myosin 1 linker 2 (CARMIL2) deficiency using whole exome sequencing. CARMIL2 deficiency is a combined immunodeficiency with a broad clinical phenotype. Disseminated warts, recurrent respiratory infections and eczema are important clinical manifestations.

Familial hemophagocytic lymphohistiocytosis (HLH) is a potentially life-threatening disorder caused by defect in genes that are important for perforin synthesis and natural killer or cytotoxic T cell degranulation. Familial HLH may have varied clinical presentation. Central nervous system (CNS) involvement, especially when it is isolated, pose a diagnostic challenge. Yoshida et al. reported a case of familial HLH caused by *UNC13D* gene mutation who presented with unusual clinical presentation, i.e., cerebellar swelling and hydrocephalus.

Sgrulletti et al. report a case that shows the diagnostic conundrum between primary and secondary immunodeficiency. Patients with PIDs are predisposed to develop autoimmune manifestations and malignancy. At times, these may be the first and only clinical presentation. Often unaware of this aspect of PIDs, the treating physician may initiate immunosuppressive therapy including rituximab in a few cases of autoimmunity or malignancy. It may be extremely difficult to differentiate the hypogammaglobulinemia caused by use of immunosuppressant drugs from primary hypogammaglobulinemia. It is suggested to perform immunoglobulin levels in all patients before initiating rituximab. Genetic testing may be helpful in these cases.

Sun et al. report an adolescent girl with autoimmune polyglandular syndrome type III C + D who presented with Hashimoto's thyroiditis, vitiligo, anemia, pituitary hyperplasia, and lupus nephritis. This is an unusual presentation in a child. The pathophysiological mechanisms and immune basis are not clear.

Chimeric antigen receptor (CAR) T-cell therapy is an upcoming management strategy for several disorders especially malignancy. Hypogammaglobulinemia is a common complication of CAR T-cell therapy and this complication is more common in children. As a result, patients may be at risk of unusual infections following CAR T-cell therapy. Sanders et al. report a case of B-cell acute lymphoblastic leukemia (B-ALL) who received CAR T-cell therapy and developed hypogammaglobulinemia requiring immunoglobulin replacement therapy. He subsequently developed SARs-CoV-2 infection requiring a combination of antimicrobials.

Author contributions

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

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
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Case Report: Ciclosporin A for Refractory Multisystem Inflammatory Syndrome in Children

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Multisystem inflammatory syndrome in children (MIS-C) is a new syndrome involving the development of severe dysfunction in multiple organs after severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) infection. Because the pathophysiology of MIS-C remains unclear, a treatment strategy has not yet been established. We experienced a 12-year-old boy who developed MIS-C at 56 days after SARS-CoV-2 infection and for whom ciclosporin A (CsA) was effective as a third-line treatment. He had a high fever on day 1, and developed a rash on the trunk, swelling in the cervical region, and palmar erythema on day 2. On days 3, he developed conjunctivitis and lip redness, and fulfilled the criteria for classical Kawasaki disease (KD). Although intravenous immunoglobulin infusion (IVIG) was started on day 4, fever persisted and respiratory distress and severe abdominal pain developed. On day 5, because he fulfilled the criteria for MIS-C, methylprednisolone pulse was started for 3 days as a second-line treatment. However, he did not exhibit defervescence and the symptoms continued. Therefore, we selected CsA as a third-line treatment. CsA was so effective that he became defervescent and his symptoms disappeared. In order to clarify the relationship with treatment and the change of clinical conditions, we examined the kinetics of 71 serum cytokines to determine their relationships with his clinical course during the three successive treatments. We found that CsA suppressed macrophage-activating cytokines such as, IL-12(p40), and IL-18 with improvement of his clinical symptoms. CsA may be a useful option for additional treatment of patients with MIS-C refractory to IVIG + methylprednisolone pulse.

Keywords: MIS-C, cytokine, ciclosporin A, Kawasaki disease, treatment option, phlebitis

INTRODUCTION

Multisystem inflammatory syndrome in children (MIS-C) is a new syndrome that was first reported in Europe and the United States in 2020. Although the mechanism for onset of MIS-C remains unclear, recent manuscripts have suggested potential mechanisms from the viewpoint of various immunological angles (1–6). Based on the cytokine kinetics in MIS-C, a wide variety of cells related

to both innate immunity and adaptive immunity, such as macrophages, neutrophils, T cells, and B cells, may be activated (4–6).

Regarding treatment of MIS-C, no definite treatment has been established to date. However, based on the similarity with KD vasculitis in many clinical aspects, intravenous immunoglobulin infusion (IVIG) alone or IVIG plus steroid are recommended as the first line treatment (7). If patients with MIS-C are refractory to the first-line treatment, biological agents such as infliximab (IFX; anti-tumor necrosis factor-α antagonist) (8), anakinra (interleukin [IL]-1 blocker) (9, 10), and tocilizumab (IL-6 receptor inhibitor) (11) are recommended as a second-line treatment. However, these options are also not definitively established.

Here, we report a patient with MIS-C in whom ciclosporin A (CsA) was effective as a third-line treatment. We examined the kinetics of 71 serum cytokines and gave discussion to determine their changes in accordance with the treatments and clinical symptoms during the patient's clinical course.

CASE REPORT

A 12-year-old boy with no particular history of medical problems was diagnosed with severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) infection by polymerase chain reaction (PCR) analysis of a nasopharyngeal swab sample at 56 days before onset of MIS-C. He was admitted to his previous hospital because of low-grade fever and sore throat. His father was Caucasian, and his mother was Japanese.

At 56 days after the diagnosis of SARS-CoV-2 infection, he developed a fever (39.8°C) on day 1. On the next day (day 2), he had a rash on his trunk, swelling and pain in both sides of the cervical region, and palmar erythema. He was therefore re-admitted to the previous hospital, and ceftriaxone (50 mg/kg/day) was initiated. On day 3, he developed conjunctivitis and lip redness. On day 4, his fever persisted, and because he fulfilled the criteria for classical KD. Therefore, IVIG (2 g/kg) and aspirin (30 mg/kg) were started. However, the fever persisted, and abdominal pain and chest discomfort developed. On day 5, he was transferred to our hospital.

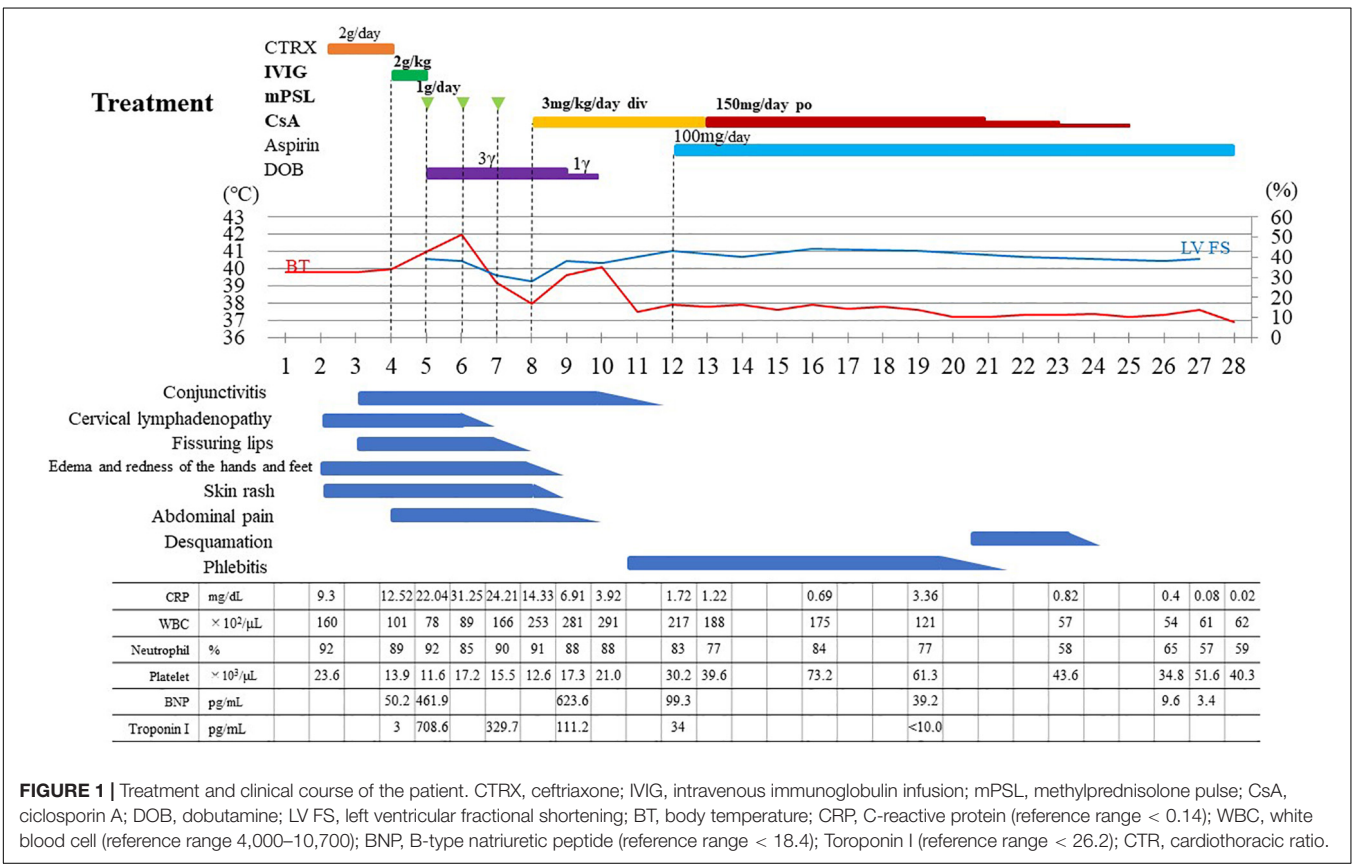
On admission, he had a fever of 41.0°C, severe pain throughout the abdomen, and bilateral cervical lymph node swelling with pain. Physical examination revealed multilobular cervical lymphadenopathy with a maximum diameter of 20 mm, and obvious redness of the lips and oral cavity. There were significant bilateral bulbar conjunctival congestions, and rashes on the face, trunk, and distal extremities. Although his blood pressure was 99/37 mmHg and within the reference range, he showed respiratory distress, including heart rate of 142 beats/min, respiratory rate of 50 breaths/min, and SpO_2 of 99% (nasal O_2: 2 L/min). Furthermore, cardiomegaly (cardiothoracic ratio: CTR; 54.6%) and pulmonary congestion were seen on both chest X-ray and computed tomography examinations, and intestinal gas showed prominent expansion on an abdominal X-ray (**Supplementary Figures 1A,B**). Electrocardiogram showed sinus tachycardia (heart rate: 142 beats/min), with flat and negative T waves on the V5 and V6 leads, suggesting myocardial damage (**Supplementary Figure 1C**). Although he had a negative test result for SARS-CoV-2, based on a TRC Ready (Tosoh Bioscience, Tokyo, Japan) analysis of a nasopharyngeal swab, SARS-CoV-2 Antibody Detection Kit (IgG/IgM) (Kurabo Industries Ltd., Osaka, Japan) was positive and negative, respectively.

Laboratory data on admission are shown in **Table 1**. Elevated neutrophil count ($7.137 \times 10^3/\mu L$) and percentage of leukocytes (90%), reduced lymphocyte count (469/μL) and platelet count ($116 \times 10^3/\mu L$), and elevated C-reactive protein level (22.04 mg/dL) were noted. In addition, elevated soluble IL-2 receptor level (7,044 U/mL, reference range 122–496 U/mL), normal ferritin level (173 ng/mL, reference range 13–277 ng/mL), elevated fibrin degradation product level (36.2 μg/mL, reference range 0–5 μg/mL), elevated brain natriuretic hormone level (461.9 pg/mL, reference range < 18.4 pg/mL), and elevated troponin I (708.6 pg/mL, reference range < 26.2 pg/mL) were found. The urinalysis did not show pyuria. Based on the clinical symptoms and laboratory data, he was diagnosed with MIS-C because he fulfilled the World Health Organization (WHO), Centers for Disease Control and Prevention (CDC) and Royal College of Paediatrics and Child Health (RCPCH) criteria (12–14). His clinical disease course following hospitalization is shown in **Figure 1**. We selected a methylprednisolone pulse (mPSL; 25 mg/kg/day) as a second-line treatment according to the guideline (7–9). Anticoagulation therapy (heparin: 10 IU/kg/h) was administered during the methylprednisolone pulse administration. Dobutamine was started simultaneously for the signs of cardiac failure such as cardiomegaly and pulmonary edema. Although his fever increased and decreased with the three consecutive mPSL pulses, he did not exhibit defervescence. In addition, his KD-like symptoms, decreased cardiac function, and abdominal pain persisted. Most guidelines recommend a biological agent as a second-line and/or third-line treatment after IVIG and steroid treatment. However, we discontinued glucocorticoid therapy and selected CsA as the third-line treatment because his clinical symptoms also fulfilled the six principal diagnostic criteria for classical KD (15–17). Initially, CsA was started by continuous intravenous injection (3 mg/kg/day) because he had severe abdominal pain at that time (day 8). The route of CsA treatment was changed from continuous intravenous injection to oral administration (3.75 mg/kg/day, divided by 2) on day 13 when his main symptoms, decreased cardiac function, and abdominal pain disappeared. He became defervescent and his symptoms, such as severe pain, disappeared within several days starting CsA treatment. We confirmed that no CAAs developed by both repeated transthoracic echocardiography and 3D computed tomography during his clinical course (**Supplementary Figure 2**). He was discharged after improvement of symptoms on day 28, and he has been doing well since discharge without any complications. Therefore, we judged CsA to be clearly effective for this patient with MIS-C refractory to IVIG + mPSL. Although various symptoms and signs disappeared after CsA treatment, his peripheral limbs (both hands and feet) turned purple and severe pain occurred when

TABLE 1 | Laboratory data on admission.

			(Reference range)				(Reference range)				(Reference range)
WBC	7930	/μL	(4,000–10,700)	Na	131	mEq/L	(138–144)	CRP	22.04	mg/dL	(<0.14)
Neutrophil	90.0	%		K	3.3	mEq/L	(3.6–4.7)	PCT	23.20	ng/mL	(<0.05)
Eosinophil	1.0	%		Cl	100	mEq/L	(102–109)	sIL-2r	7,044	U/mL	(122–496)
Basophil	1.0	%		Ca	9.4	mg/dL	(8.7–10.1)	Ferritin	173	ng/mL	(13–277)
Monocyte	1.0	%		Cr	0.62	mg/dL	(0.39–0.62)	U-β_2MG	125,826	μg/L	(<230)
Lymphocyte	6.0	%		UA	5.4	mg/dL	(3.0–7.0)				
RBC	412 × 10^4	/μL	(415–540 × 10^4)	BUN	23.3	mg/dL	(6.8–19.2)	BNP	461.9	pg/mL	(<18.4)
Hb	11.6	g/dL	(12.2–15.7)	TP	6.9	g/dL	(6.3–7.8)	TnI	708.6	pg/mL	(<26.2)
Ht	33.4	%	(35.8–45.0)	Alb	2.7	g/dL	(3.8–4.7)				
Plt	11.6 × 10^4	/μL	(18.0–44.0 × 10^4)	CK	61	IU/L	(51–270)	SARS-CoV-2	Antibody		
				CK-MB	<4	IU/L	(<12)		IgG (+)		
APTT	41.0	s	(25–35)	AST	48	IU/L	(15–31)		IgM (–)		
PTINR	1.42		(0.8–1.2)	ALT	32	IU/L	(9–32)				
Fib	511	mg/dL	(150–350)	LDH	283	IU/L	(145–270)				
FDP	32.6	μg/mL	(0–5)	TB	1.5	mg/dL	(0.3–1.1)				
				DB	0.8	mg/dL	(0.0–0.3)				

WBC, white blood cells; RBC, red blood cells; Hb, hemoglobin; Ht, hematocrit; Plt, platelets; APTT, activated partial thromboplastin time; PTINR, prothrombin time-international normalized ratio; Fib, fibrinogen; FDP, fibrin degradation product; Cr, creatinine; UA, uric acid; BUN, blood urea nitrogen; TP, total protein; Alb, albumin; CK, creatine kinase phosphokinase; CK-MB, creatine kinase MB type; AST, aspartate transaminase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase; TB, total bilirubin; DB, direct bilirubin; CRP, C-reactive protein; PCT, procalcitonin; sIL-2R, soluble interleukin-2 receptor; U-B2MG, urinary β2-microglobulin; BNP, brain natriuretic peptide; TnI, troponin I; SARS-CoV-2, severe acute respiratory syndrome-coronavirus-2.



CRP	mg/dL		9.3		12.52	22.04	31.25	24.21	14.33	6.91	3.92		1.72	1.22		0.69		3.36			0.82			0.4	0.08	0.02
WBC	×10^2/μL		160		101	78	89	166	253	281	291		217	188		175		121			57			54	61	62
Neutrophil	%		92		89	92	85	90	91	88	88		83	77		84		77			58			65	57	59
Platelet	×10^3/μL		23.6		13.9	11.6	17.2	15.5	12.6	17.3	21.0		30.2	39.6		73.2		61.3			43.6			34.8	51.6	40.3
BNP	pg/mL				50.2	461.9				623.6			99.3					39.2						9.6	3.4	
Troponin I	pg/mL				3	708.6		329.7		111.2			34					<10.0								

FIGURE 1 | Treatment and clinical course of the patient. CTRX, ceftriaxone; IVIG, intravenous immunoglobulin infusion; mPSL, methylprednisolone pulse; CsA, ciclosporin A; DOB, dobutamine; LV FS, left ventricular fractional shortening; BT, body temperature; CRP, C-reactive protein (reference range < 0.14); WBC, white blood cell (reference range 4,000–10,700); BNP, B-type natriuretic peptide (reference range < 18.4); Toroponin I (reference range < 26.2); CTR, cardiothoracic ratio.

they hung below the heart, such as in the standing and/or sitting position, on day 11. There were no significant findings such as thrombus on vascular echography and magnetic resonance imaging or right heart failure. We judged these phenomena to be venous stasis, because the pain and color changes to the skin rapidly improved on elevation of the peripheral limbs.

Elastic bandage use improved these symptoms, and thus exercise therapy was performed with an elastic bandage. Under exercise rehabilitation therapy, the symptoms improved in 10 days (18).

ANALYSIS OF CYTOKINE KINETICS

We evaluated his cytokine kinetics using archived serum samples collected intermittently from day 2 to day 166 of illness, in order to clarify the relationship with treatment and the change of clinical conditions. We performed comprehensive assays for cytokines using a BioPlex 3D system (Bio-Rad Laboratories Inc., Hercules, CA, United States) and BioPlex Human cytokine48/chemokine40 screening panel (Bio-Rad Laboratories Inc.). We also performed chemiluminescence enzyme immunoassay analyses using an HISCL-5000 system (Sysmex Asia Pacific Pte Ltd., Singapore) and ELISA assay (R&D Systems Inc., Minneapolis, MN, United States). Using these methods, we investigated the changes in 71 cytokines during the patient's disease course. We classified a treatment response when a cytokine decreased by 50% from its peak level, referenced by the day 166 level for that cytokine. IL-2 and CXCL2 decreased before the initiation of treatment. Although no cytokines were completely suppressed by IVIG alone, proinflammatory cytokines (IL-6, IL-1β), interferon (IFN)-γ and IFN-γ-related chemokines (CXCL9, CXCL10, and CXCL11), and many chemokines (CCL1, CCL2, CCL8, CCL19, CCL20, CCL22, and CCL27) were strongly suppressed by mPSL (**Table 2** and **Figure 2**). Although IL-6 was completely suppressed by mPSL, the patient did not become defervescent. As the third-line treatment, CsA mainly suppressed macrophage-activating cytokines such as IL-12(p40), and IL-18, and he became defervescent with disappearance of abdominal pain (**Supplementary Figures 3-1, 3-2**).

DISCUSSION

We reported a patient with MIS-C in whom CsA was effective as a third-line treatment. We examined the kinetics of 71 serum cytokines during the patient's clinical course. In the present case, three successive treatments (IVIG, mPSL, and CsA) were performed during the time course, and thus we could distinguish the relationship between each treatment and the cytokine changes. Few cytokines were suppressed by IVIG alone. However, IL-6, IFN-γ, and IFN-γ-induced chemokines (CXCL9, CXCL10, and CXCL11) (19) and chemokines (CCL1, CCL2, CCL8, CCL19, and CCL20) were strongly suppressed by mPSL. Although IL-6 was completely suppressed by mPSL, the patient did not become defervescent. Meanwhile, CsA suppressed cytokines related to innate immune responses such as IL-12(p40), IL-18, and growth factors such as vascular endothelial growth factor and hepatocyte growth factor. Thereafter, the patient became defervescent. In our previous study, we reported the kinetics of 71 cytokines in a 9-year-old girl with MIS-C during her entire clinical course (6). However, both IVIG and prednisolone were started simultaneously in that case, and we could not distinguish the relationship between each treatment and the cytokine changes. Nevertheless, after IVIG and prednisolone administration as the first-line treatment, IL-6, IL-10, IL-17, IL-8, and CCL20 rapidly decreased, and the patient became defervescent. Meanwhile, IFN-γ is an indicator of Th1 type immune responses, and IFN-γ-induced cytokines such as CXCL9, CXCL10 decreased gradually, rather than rapidly. There is a report that IFN-γ plays a central role in the pathophysiology of MIS-C, indicating that IFN-γ may be a notable cytokine for understanding the state of MIS-C patients (19, 20). Furthermore, the present case was considered to be a more severe case of MIS-C, with higher levels of IL-12(p40), IL-18, IFN-γ, and IFN-γ-induced chemokines such as CXCL9 and CXCL10 than in our previous report.

Because these cytokine kinetics were assessed for three separate treatments during the time course, the relationships between each of the treatments and the changes in cytokines seemed to be clear in this report. However, it is not definitive whether the cytokines kinetics tightly correspond to each treatment, and it remains possible that there may be synergetic effects with the prior treatment. Among the data, it is particularly interesting that the patient did not become defervescent after complete suppression of IL-6 by mPSL, and required a third-line treatment. Thus, it is possible that tocilizumab as an additional treatment may be an inappropriate choice, especially those who are refractory to previous steroid treatment.

MIS-C and KD are similar in many clinical presentations, and 25–65.5% of MIS-C patients also have symptoms that resemble classical KD (21). Because our case was not only definite MIS-C but also fulfilled the criteria for classical KD, we selected CsA as the third-line treatment when he showed resistance to both IVIG and mPSL (16, 17). We usually select oral administration for CsA, but chose continuous infusion (3 mg/kg/day) in the present patient between day 8 and day 13, because he had remarkable gastrointestinal symptoms such as severe pain. After his abdominal pain disappeared, we changed the administration route of CsA from infusion to per oral (150 mg/day, divided by 2). His plasma level of CsA was 321–372 ng/ml at infusion and his trough level was 87-97 ng/ml at oral administration. All symptoms and signs of MIS-C in our patient improved after CsA treatment and no CAAs developed.

Regarding the effect of CsA in the present case, it is possible that the suppressive effects of CsA on macrophages and dendritic cells may have contributed to the clinical improvements, because the levels of cytokines related to innate immune responses such as IL-12(p40) and IL-18 were decreased at defervescence.

Ciclosporin A was originally considered to suppress the immune system through inactivation of the nuclear factor of activated T-cells pathway. However, according to pathophysiology of MIS-C, both innate immunity and adaptive immunity are highly activated. If this hypothesis is true, the mechanism of action for CsA may not be sufficient to control the total immune activation in MIS-C. Therefore, CsA may not have been recommended as an additional option for treatment of MIS-C in the treatment guideline (7–9). However, recent studies have clarified that CsA may exert direct suppressive effects on not only T cells but also innate immune cells such as

TABLE 2 | Summary of cytokines which respond to each treatment ($n = 71$).

A peak before treatment initiation	mPSL response			CsA response			Unknown	
IL-2	IFN-γ	IL-6		IL-12 (p40)	IL-18		IL-4	CCL24
CXCL2	CXCL9	CXCL10	CXCL11	TNF-α	VEGF		IL-7	CXCL5
	CCL1	CCL2	CCL8	HGF			IL-9	CXCL6
	CCL19	CCL20					IL-12 (p70)	CXCL12
	IL-1β	CCL7	CXCL1	IL-1α	CCL23	LIF	IL-15	CXCL16
	IL-3	CCL17	G-CSF	IL-1ra	CCL25	GM-CSF	IL-16	FGF2
	IL-5	CCL21	IFN-λ3	IL-2Rα	CX3CL1	SCF	CCL3	M-CSF
	IL-8	CCL22		IL-13	CXCL13	NGF-β	CCL4	PDGF-BB
	IL-10	CCL26		IL-17	IFN-α2	IFN-λ2	CCL5	SCGF-b
		CCL27			MIF		CCL11	TNF-β
							CCL13	TRAIL
							CCL15	IFN-λ1

We classified the treatment response when the cytokine decreased by 50% from the peak levels, referenced to the day 166 levels for each cytokine. mPSL, methylprednisolone; CsA, ciclosporin A; IL, interleukin; CXCL, C-X-C motif chemokine ligand; IFN, interferon; CCL, C-C motif ligand; G-CSF, granulocyte colony stimulating factor; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor; HGF, hepatocyte growth factor; ra, receptor antagonist; Rα, receptor α; LIF, leukemia inhibitory factor; MIF, macrophage migration inhibitory factor; GM-CSF, granulocyte macrophage colony stimulating factor; SCF, stem cell factor; NGF, nerve growth factor; FGF, fibroblast growth factor; M-CSF, macrophage colony stimulating factor; PDGF, platelet derived growth factor; SCGF, stem cell growth factor; TRAIL, tumor necrosis factor related apoptosis-inducing ligand.

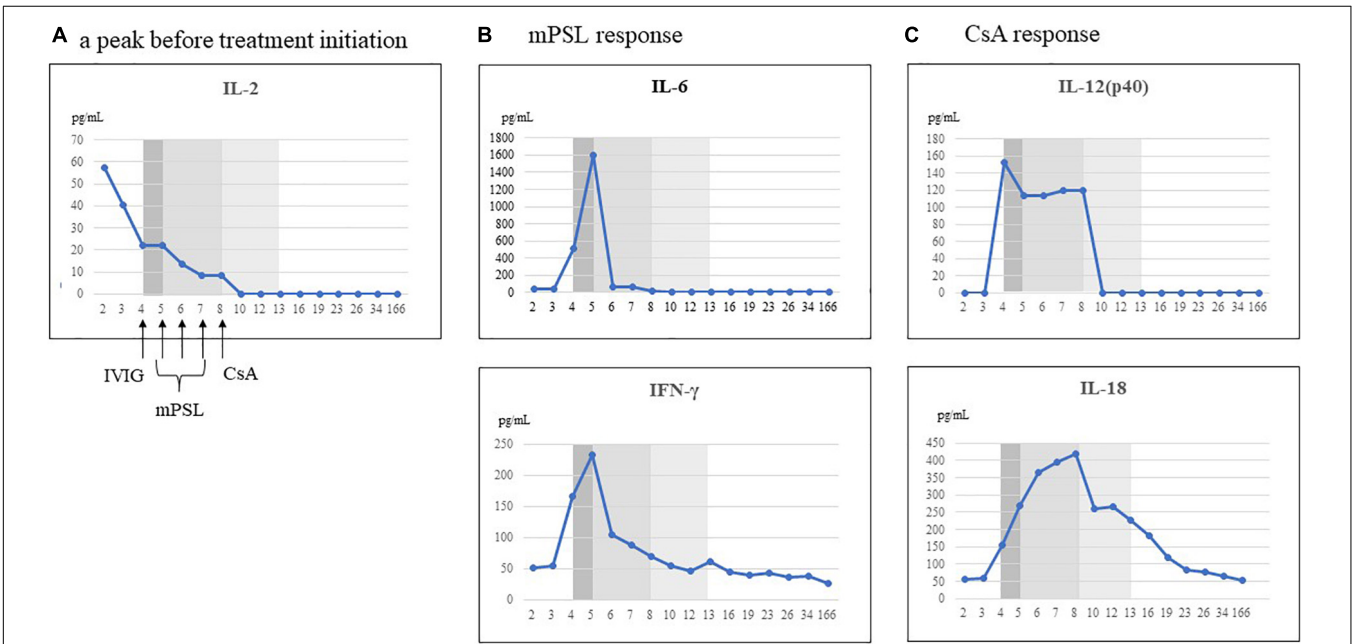


FIGURE 2 | Representative cytokines that responded to each treatment. We classified the treatment response when the cytokine decreased by 50% from the peak levels, referenced to the day 166 levels for each cytokine. **(A)** Cytokine decreased prior to initiation of treatment. **(B)** Cytokines that responded to methylprednisolone (mPSL) treatment. **(C)** Cytokines that responded to ciclosporin A (CsA) treatment. IL, interleukin; IFN, interferon; IVIG, intravenous immunoglobulin infusion.

dendritic cells, macrophages, and neutrophils (22–24). Indeed, cytokines related to innate immunity were suppressed by CsA in the present patient with MIS-C. These findings suggest that CsA may be a useful option for MIS-C refractory to IVIG + mPSL, which is known to involve activation of both innate immunity and adaptive immunity.

Although we have no evidence to explain why our patient developed venous stasis, we propose one possibility. The changes in skin color of his limbs were not chilblain-like skin lesions associated with SARS-CoV-2 infection (25), because the changes developed depending on the vertical position of the limbs and disappeared immediately in the horizontal position. Thus, we judged these phenomena to be venous stasis. Therefore, vasculitis caused by MIS-C may be not only arteritis but also phlebitis. Several reports have described that autoantibodies to autoantigens expressed in endothelial and cardiac tissues may trigger onset of MIS-C (1, 4, 26). Thus, it is possible that vasculitis may develop regardless of involvement of an artery or a vein. Therefore, the function of venous valves may be insufficient.

There are several limitations in the present case report. This is the first report to discuss the possibility of CsA treatment for MIS-C refractory to IVIG + mPSL. In addition, it is possible that the effect of CsA as a third-line treatment may have involved the summed results for IVIG and mPSL. Further studies are warranted to evaluate CsA treatment in refractory MIS-C patients.

CONCLUSION

The findings in the present case regarding the relationship between cytokine kinetics and treatment response suggest that CsA may be a useful option for patients with MIS-C who are refractory to IVIG + methylprednisolone pulse treatment.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of Wakayama Medical University (No: 3282) and National Center for Global Health and Medicine (NCGM-S-004245). Written informed consent was obtained from the patient and his parents for the publication of this case report. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

TaS performed the literature review and wrote the first draft of the manuscript. MU, YK, ToS, NK, TT, and HS assisted in the treatment of the patient. AS, MS, MM, AM, and ST contributed to the analysis and interpretation of cytokines and assisted in the preparation of the manuscript. HH and DT contributed to critically review of the manuscript. All authors have approved the final manuscript and have agreed to be accountable for all aspects of the work.

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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.2022.890755/full#supplementary-material

Supplementary Figure 1 | (A) Chest and abdominal X-ray image. Cardiothoracic ratio is 54.6%, pulmonary congestion is present, and intestinal gas shows prominent expansion. **(B)** Computed tomography image. Hair line is present. **(C)** Electrocardiogram. Heart rate is 142 beats/min, sinus rhythm is present, and T waves on the V5 and V6 leads are flat and negative, suggesting myocardial damage.

Supplementary Figure 2 | 3D-Computed Tomography (day 45).

Supplementary Figure 3-1 | Cytokines which responded to ciclosporin A (CsA). IL, interleukin; ra, receptor antagonist; Rα, receptor α; CCL, C–C motif ligand; CX3CL, C-X3-C motif ligand; IFN, interferon.

Supplementary Figure 3-2 | Cytokines which responded to ciclosporin A (CsA). TNF, tumor necrosis factor; LIF, leukemia inhibitory factor; HGF, hepatocyte growth factor; MIF, macrophage migration inhibitory factor; GM-CSF, granulocyte macrophage colony-stimulating factor; SCF, stem cell factor; VEGF, vascular endothelial growth factor; NGF, nerve growth factor; IFN, interferon.

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Case report: Corticosteroids as an adjunct treatment for the management of liver abscess in Papillon–Lefèvre syndrome: A report on two cases

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Papillon–Lefèvre syndrome (PLS) is a rare autosomal recessive disorder characterized clinically by palmoplantar keratoderma, periodontitis, and recurrent pyogenic infections. Liver abscess is rarely reported in patients. The use of corticosteroids for the treatment of liver abscess akin to chronic granulomatous disease (CGD) has not been reported previously. Here, we report 2 cases of liver abscess in PLS that responded to corticosteroids.

KEYWORDS

corticosteroids, Papillon–Lèfevre syndrome, liver abscess, surgical management, *Staphylococcus aureus*

Introduction

Papillon–Lefèvre syndrome (PLS) is a rare autosomal recessive disorder characterized clinically by palmoplantar keratoderma and periodontitis affecting both primary and permanent dentitions (1). Patients with PLS also have immune abnormalities, especially defects in neutrophils, and are predisposed to develop recurrent pyogenic infections. Liver abscess is increasingly being reported in patients with PLS (2). Majority of patients have been reported to respond to antimicrobials and surgical drainage. However, there are no reports on the use of corticosteroids for liver abscess in patients with PLS. Corticosteroids have been reported to be useful for the management of liver abscess in patients with chronic granulomatous disease (CGD). This suggests the presence of a hyperinflammatory response around the abscess. We report 2 patients with PLS who liver abscess was successfully resolved after administration of corticosteroids.

Case report

Case 1

A 5-year-old boy, born out of non-consanguineous marriage, presented with high-grade fever and right-sided upper abdominal pain. He developed liver abscess at the age of 3 years, and the pus culture grew methicillin-resistant *Staphylococcus aureus* (MRSA). He was admitted and received intravenous antibiotics for 14 days in a peripheral center.

Now at 5 years of age, he had hepatomegaly and palmoplantar keratoderma. There was no dental abnormality. Laboratory investigations showed anemia, neutrophilic leucocytosis, high erythrocyte sedimentation rate (ESR), elevated C- reactive protein (CRP), and high serum immunoglobulins (**Table 1**). Ultrasonography (USG) of the abdomen revealed a 10 × 8 cm abscess involving segments IV and VIII of the liver. Pigtail drainage of the abscess was conducted, and the pus grew methicillin-sensitive *Staphylococcus aureus* (MSSA). He was initiated on cloxacillin and vancomycin. However, he continued to have fever even after 14 days of antibiotics. Acute phase reactants were also elevated. Open surgical drainage of the abscess was planned. However, the intraoperative findings revealed no drainable collection, and only organized calcified pus was noticed. The pus culture was sterile, and the biopsy revealed a fibrocollagenous tissue. In view of the persistent fever, he was initiated on oral prednisolone (1 mg/kg/day). There was prompt defervescence and normalization of acute phase reactants. The size of liver abscess was reduced within 7 days of the initiation of prednisolone. Prednisolone was continued for 4 weeks and tapered off over the next 2 weeks. The follow-up ultrasound showed no liver abscess. The targeted next-generation sequencing (*invitae* primary immunodeficiency gene panel) showed a novel pathogenic homozygous complex rearrangement in the *CTSC* gene (resulting in the deletion of exon 1, which includes the initiator codon, and exon 3).

He is on follow-up for 18 months, is taking cotrimoxazole prophylaxis, and is doing well.

Case 2

A 6-year-old girl, born out of non-consanguineous marriage, presented with high-grade fever and right upper quadrant abdominal pain. At 4.5 years, she had liver abscess (3.4 cm × 3.5 cm × 3.1 cm) in segment III. The lesion was extending into the sub-capsule. She was managed in another healthcare facility, and a pig-tail catheter was inserted for drainage of the pus. The pus culture grew MRSA. She was treated with intravenous antimicrobials for 6 weeks. Five months later, she had recurrence of the liver abscess (pus grew MRSA) that was also managed in another healthcare facility.

On examination, she had thickened and keratotic skin over palms and soles, dental caries, and hepatomegaly (**Figure 1**). Laboratory investigations showed anemia, neutrophilic leucocytosis, and high ESR, CRP, and serum immunoglobulins (**Table 1**). Before presenting to us, she had undergone computed tomography (CT) of the abdomen that showed a heterogeneous area measuring 3.3 cm × 2.6 cm × 2.3 cm in size seen in segment III of the liver (**Figures 2A–C**).

The USG abdomen carried out in our institute showed multiple organized abscesses in the right lobe of the liver in segment VII/VIII (largest: 6.9 cm × 5.1 cm) and another abscess in segment VI (4.1 cm × 4.5 cm). The orthopantogram was normal. USG-guided fine-needle aspiration cytology for organized liver abscess was performed. The pus culture was sterile. She was empirically initiated on cloxacillin and vancomycin. For keratoderma, emollients, coconut oil, topical steroids, and calcitriol ointment were advised. However, she continued to be febrile after 2 weeks, and there was no decrease in abscess size. She was initiated on oral prednisolone (1 mg/kg/day) and continued on intravenous antimicrobials. She showed prompt recovery and became afebrile within 24 h. The USG abdomen showed a marked reduction in the size of the liver abscess (2.1 cm × 2.3 cm, 7 days after initiation of prednisolone). Prednisolone was continued for 6 weeks and tapered off in another 6 weeks. The whole exome sequencing showed a homozygous mutation in the *CTSC* gene resulting in deletion of exons 1–17.

The follow-up USG at 6 weeks showed 1 × 1 cm residual liver abscess (**Figure 2D**). She is being continued on cotrimoxazole prophylaxis (5 mg/kg/day of trimethoprim component) and has no recurrence of liver abscess in 2 months follow-up.

TABLE 1 Laboratory investigations.

Parameters	Case 1	Case 2
Hemoglobin (g/L)	92	89
White blood cell count ($\times 10^9$/L)	23.3	26.3
Neutrophils/lymphocytes/ monocytes/eosinophils (%)	81/16/2/1	70/20/6/4
Platelets ($\times 10^9$/L)	200	170
Erythrocyte sedimentation rate (mm in 1st h) (Normal < 20)	88	69
C reactive protein (mg/L) (normal < 6)	119	96
Nitroblue tetrazolium dye reduction test Dihydrorhodamine assay	Normal Normal	Normal Normal
% CD3 + T cell (N-56–75%;1.4–3.7/L) % CD19 + B cell (N-14–33%;0.39–12.4/L) % CD56 + NKcell (N-4–17%; 0.13–0.72/L)	77.42% (2.87/L) 14.74% (0.55/L) 7.81% (0.291/L)	68% (3.576/L) 17.7% (0.931/L) 6.9% (0.362/L)
Immunoglobulin G (g/L) (Normal 4.9–16.1) Immunoglobulin M (g/L) (Normal 0.5–2.0) Immunoglobulin A (g/L) (Normal 0.5–2.4)	22.2 3.27 4	20 4 3
Immunoglobulin E (Normal < 100 U/L)	66.4	64

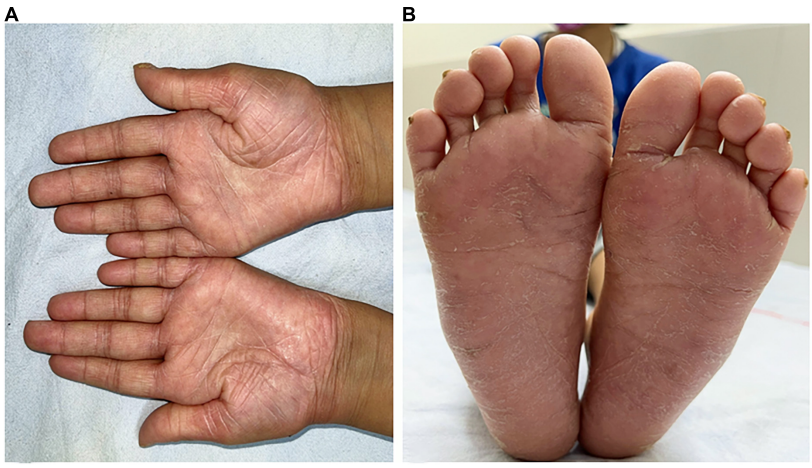

FIGURE 1
Diffuse erythematous hyperkeratotic scaling and fissuring in the **(A)** palm and **(B)** sole **(B)** of case 2.

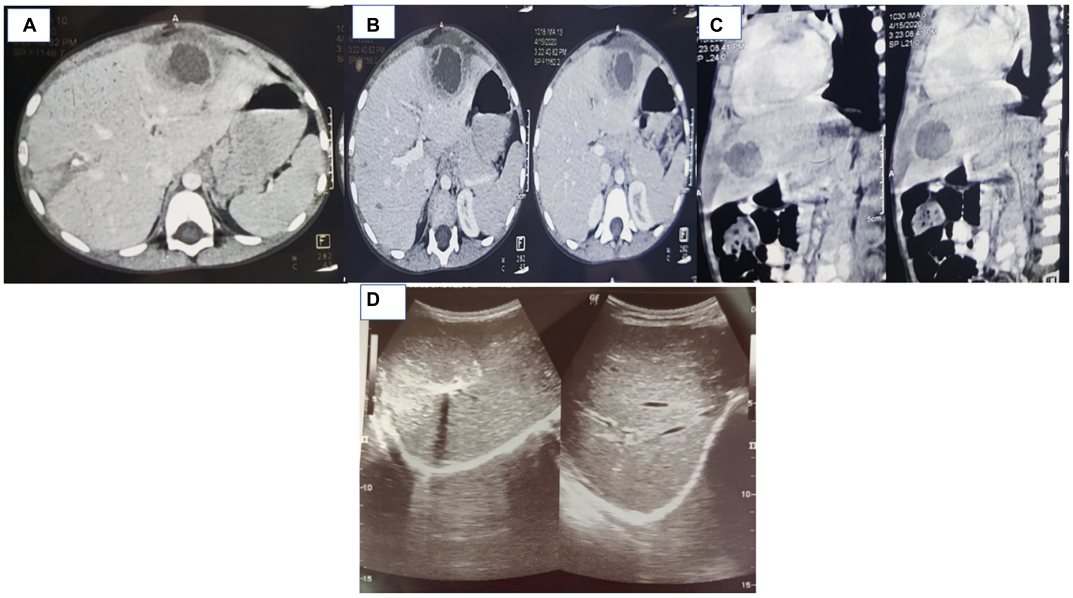

FIGURE 2
(A–C) Computed tomography of case 2 showing a hypoattenuating focal lesion with thick/shaggy walls with perilesional edema suggestive of abscess in the liver, arrow marks. **(D)** Ultrasound images of case 2 showing resolution of abscess following initiation of corticosteroids (after 6 weeks of initiation of therapy).

Herein, we report 2 patients with PLS with liver abscess that showed brisk improvement following the administration of corticosteroids.

Discussion

PLS is a rare autosomal recessive disorder characterized clinically by palmoplantar keratoderma, periodontitis, and recurrent pyogenic infections (1). PLS is caused by a homozygous or compound heterozygous mutation in the *cathepsin C* gene (CTSC). CTSC has a role in the activation of azurophilic granules in neutrophils and granzyme secretion in cytotoxic T lymphocytes. Patients with PLS lack active serine proteases in neutrophils and cytotoxic T lymphocytes and have decreased neutrophil extracellular trap formation in response to reactive oxygen species (2). This predisposes them to develop recurrent infections.

Progressive periodontitis leading to premature loss of teeth, gingival inflammation, and rapid destruction of periodontium

is the hallmark manifestation (3). Loss of teeth usually occurs during the teenage years, but the third molar may be spared (4). Periodontitis and loss of teeth were, however, not observed in both patients. Strict oral hygiene and careful management of infections may be required to prevent the development of this complication (4).

Palmoplantar keratoderma is another hallmark feature and usually develops by the age of 4. Hyperhidrosis may also be seen. Skin histopathology may reveal non-specific hyperkeratosis, focal parakeratosis, dilated tortuous capillaries in dermal papillae, and lymphocytic infiltration in the superficial layer (4). Palmoplantar keratoderma was observed in both patients in the present series for which topical corticosteroids, emollients, and calcitriol ointment were advised (5).

Immunological abnormalities include low T cells and increased NK cells and immunoglobulins (6). Hyperinflammation leads to defective neutrophil apoptosis, altered nuclear factor-κB signaling, and increased cytokines (7). Both cases in the present series had normal lymphocyte subsets but raised immunoglobulin levels. Deep seated abscesses involving the brain, liver, and kidneys have been reported in patients with PLS (2). Histopathological examination of the abscess has shown chronic granulomatous inflammation around the lesion similar to the histopathology seen in patients with CGD (8). This could be because of the impaired neutrophil function in patients with PLS similar to the neutrophil defect seen in patients with CGD, resulting in inflammatory granuloma (9). In immunocompetent patients, liver abscess usually has a thin rim with central, liquefied, non-enhancing contents, and responds to antibiotic therapy (9). Patients with neutrophil-killing defect and hyperinflammation may have atypical features such as dense, homogeneous with thick inspissated fluid in the liver abscess (9). Index cases had dense organized abscess with calcification. There may be a decreased response to antimicrobials in these cases, and co-administration of corticosteroids may be helpful. Corticosteroids decrease the activation, proliferation, and differentiation of inflammatory cells such as macrophages and lymphocytes (5). Corticosteroids also reduce immune infiltration and capillary leak of central venules, decrease porto-venous shunting, and restore pre-abscess immune milieu (10). This helps in better tissue penetration of antimicrobials because of less-inflamed milieu, particularly surrounding the pseudo-capsule of liver abscess (10). Liver abscess in patients with PLS has been reported to respond to antimicrobials and/or surgical drainage (**Supplementary Table 1**). In case 1, there was no response despite the surgical drainage and use of antimicrobials. Case 2 did not respond to the antimicrobials. Both cases showed a brisk response to corticosteroids in terms of improvement in fever, inflammatory parameters, and size of the abscess. Corticosteroids may also reduce the chances of recurrence of liver abscess. However, we need more data on these aspects.

Conclusion

Liver abscess in patients with PLS may need to be treated with corticosteroids along with antimicrobials. This may lead to rapid resolution and possibly reduce the chances of recurrence.

Data availability statement

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

Ethics statement

This study was performed in line with the principles of the Declaration of Helsinki. As this manuscript pertains only to case report and literature review, specific ethics approval is not mandated.

Author contributions

SB, RT, and AZB: data collection, writing the first draft, and editing the manuscript. AKJ: conceptualization, data collection, writing the first draft, editing the manuscript, and final approval of the manuscript. AM, AB, AS, RM, PV, and AR: writing of the initial draft and editing of the manuscript. All authors contributed to the article and approved the submitted version.

Conflict of interest

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

Publisher's note

Supplementary material

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Case report: The cardio-facio-cutaneous syndrome due to a novel germline mutation in *MAP2K1*: A multifaceted disease with immunodeficiency and short stature

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Cardio-facio-cutaneous syndrome (CFCS) belongs to the group of RASopathies, clinical disorders defined by disruptions in the RAS/MAPK signaling pathway. It is caused by heterozygous gain-of-function germline mutations in genes encoding protein kinases: *BRAF*, *MAP2K1* (*MEK1*), *MAP2K2* (*MEK2*), and in the GTPase-encoding gene *KRAS*. CFCS is characterized by craniofacial dysmorphic features, congenital heart defects, severe malnutrition, proportionate short stature, anomalies within the structure of skin and hair, and psychomotor disability. The pathophysiology of growth impairment is multifactorial with feeding difficulties, growth hormone deficiency, and insensitivity. Immunodeficiency has not been hitherto reported as an integral part of CFCS yet an increased activation of the RAS/MAPK signaling pathway may contribute to explaining the causal relationship between RASopathy and the dysfunctions within the B and T lymph cell compartments resulting in a deficiency in T cell costimulation and B cell maturation with impaired class switch recombination, somatic hypermutation, and high-affinity antibody production. We report on a boy born prematurely at 32 WGA, with the perinatal period complicated by pneumonia, respiratory distress syndrome, and valvular pulmonary stenosis. The boy suffered from recurrent pneumonia, obstructive bronchitis, sepsis, urinary tract infection, and recurrent fevers. He presented with severe hypotrophy, psychomotor disability, short stature, craniofacial dysmorphism, dental hypoplasia, sparse hair, and cryptorchidism. Whole genome sequencing showed a novel heterozygous pathogenic germline missense variant: c.364A > G; p.Asn122Asp in the *MAP2K1* gene, supporting the diagnosis of CFCS. The immunological workup revealed hypogammaglobulinemia, IgG subclass, and specific antibody deficiency accompanied by decreased numbers of T helper cells and naive and memory

B cells. Replacement immunoglobulin therapy with timely antibiotic prophylaxis were instituted. At the age of six years, growth hormone deficiency was diagnosed and the rGH therapy was started. The ever-increasing progress in genetic studies contributes to establishing the definitive CFCS diagnosis and sheds the light on the interrelated genotype-phenotype heterogeneity of RASopathies. Herein, we add new phenotypic features of predominating humoral immunodeficiency to the symptomatology of CFCS with a novel mutation in *MAP2K1*. While CFCS is a multifaceted disease, increased pediatricians' awareness is needed to prevent the delay in diagnostics and therapeutic interventions.

KEYWORDS

MAP2K1, rasopathy, immunodeficiency, short stature, craniofacial dysmorphism, immunoglobulins, growth hormone

Introduction

RAS genes constitute a multigene family including *HRAS*, *NRAS*, and *KRAS* encoding a group of small guanosine nucleotide-bound GTPases that act as an essential cellular signaling axis. These RAS-GTPases control activation of the downstream RAF-MEK-ERK pathway, constituting the mitogen-associated protein kinase (MAPK) cascade that is vital for a multiplicity of cellular processes in the nucleus and cytosol, including survival, proliferation, differentiation, motility, and apoptosis. They are activated by various extracellular stimuli, such as growth factors binding to receptor tyrosine kinase, cytokine receptors, and extracellular matrix receptors. Subsequently, activated RAS phosphorylate downstream transducers: RAF proteins (ARAF, BRAF, and CRAF), MEK1 and/or MEK2, and finally, ERK1 and/or ERK2 (1, 2). The complex nature of the RAS/MAPK signaling pathway with the multiplicity of mechanisms cumulated in the RAS/MAPK pathway dysregulated activations as their common pathogenetic denominator.

Cardio-facio-cutaneous syndrome (CFCS) belongs to the group of the RASopathies, a spectrum of clinical disorders caused by germline mutations in components or regulators of the RAS/MAPK pathway. With its numerous genes and overlapping regulatory mechanisms involved, interfering with other cellular pathways contribute to the complex genotype-phenotype correlations and the heterogeneity of phenotypic features (3, 4). This pathway-based, mechanistic approach to defining RASopathies makes these medical genetic syndromes unique as opposed to the isolated one gene-one syndrome approach (5–7). In CFCS, heterozygous gain-of-function mutations occur in genes encoding protein kinases: *BRAF*, accounting for 75% of the genetic background in the syndrome, *MAP2K1* (*MEK1*), *MAP2K2* (*MEK2*), both found in 25% of the mutation-positive patients, and in the GTPase-encoding gene *KRAS*, constituting the rarest genetic background, found in less than 2% of the CFC patients (5, 8). An activating YWHAZ variant in the RAF-ERK pathway has also been reported in individuals with clinical syndromic features consistent with CFCS thereby expanding the spectrum of deleterious gain-of-function mutations underlying the characteristic phenotype (9). Recently, 19p13.3 microdeletion including the *MAP2K2* gene in a newborn patient with CFCS and severe clinical phenotype has also been reported (10). Consequently, CFCS is a phenotypically heterogeneous disorder characterized by craniofacial dysmorphic features, congenital heart defects, severe malnutrition, proportionate short stature, anomalies within the structure of skin and hair, and psychomotor disability (5, 8, 9, 11, 12).

In a mathematical multifactorial correlation study, a degree of associations of clinical traits and their frequencies were verified to calculate the CFC index thereby proposing an objective method for the easier recognition and more accurate clinical diagnosis of CFCS (13). It is worth noting that the highest index has been attributed to developmental disability, neurocognitive and motor difficulties (14, 15), craniofacial dysmorphic features such as high cranial vault, macrocephaly, bitemporal narrowing, depressed nasal bridge, anteverted nostrils (16, 17), dysmorphologic, sparse hair (18), hyperkeratotic skin (19), as well as congenital heart defect and hypertrophic cardiomyopathy (20–22) and short stature (23, 24). The pathophysiology of growth impairment is multifactorial with feeding difficulties, growth hormone (GH) deficiency, and insensitivity have been postulated as possible contributors to short stature.

Finally, clinical manifestations of CFCS in individual patients may overlap and result from heterogeneous pathological mechanisms governing the ultimate genotype-phenotype relationship. Wide symptomatology associated with inflammatory and autoimmune disorders (25), energy metabolism disturbances, myeloproliferative disease (26), and tumorigenesis (27) may be, therefore, observable in affected patients at different stages of their development (28–30). Immunodeficiency associated with syndromic features has not been hitherto thoroughly studied and frequently reported as

an integral part of CFCS (31), yet an increased activation of the RAS/MAPK signaling pathway may contribute to explaining the causal relationship between RASopathy and the regulation of immune cells development and functions. It may be therefore assumed that dysregulation in cellular processes within the B and T lymphocyte compartments may result in deficiency in T cell costimulation and B cell dysfunctions with impaired class switch recombination (CSR), somatic hypermutation (SHM), and high-affinity antibody production.

Case presentation

The patient

We report on a case of a boy who was referred to our pediatric clinical hospital at the age of two years due to recurrent respiratory tract infections for immunodiagnostics. His antenatal history was remarkable for polyhydramnios and supported pregnancy due to the signs of life threat to the fetus since the 26 week gestational age (WGA). Due to polyhydramnios, repeated amniocentesis and drainage of the amniotic fluid were performed, and chorioamnionitis was an indication to terminate the pregnancy. He was born prematurely by cesarean section at 32 WGA, and the perinatal period was complicated by pneumonia, respiratory distress syndrome, and cardiac insufficiency due to valvular pulmonary stenosis. Since birth, he required combined antibiotic therapy and mechanical ventilation in the neonatal intensive care unit because of respiratory and circulatory insufficiency. He also presented with craniofacial dysmorphism and cryptorchidism raising the suspicion of Noonan syndrome yet sequencing of the *PTPN11* gene did not show any mutation. By the age of two years, the boy suffered from recurrent pneumonia and bronchitis, staphylococcal sepsis, urinary tract infection, and recurrent fevers with *Staphylococcus aureus* and *Pseudomonas aeruginosa* repeatedly cultured in tracheal aspirates. Since the age of four months, during the first two years of life, the number of pneumonia episodes was four every year, severe enough to require hospitalizations. In the first year of life, he underwent a multistep corrective surgery of valvular pulmonary stenosis. Due to the failure of thrive and feeding difficulties, gastrostomy was created to improve his nutritional status, yet poor weight gaining and recurrent fevers prompted the surgical team to remove it. The patient received a live Bacillus Calmette-Guerin (BCG) vaccine after birth and a measles-mumps-rubella (MMR) trivalent vaccine at the age of 13 months was not recommended due to recurrent infections. Inactive vaccines were administered without adverse effects and the diphtheria, tetanus, and acellular pertussis (DTaP) booster dose was given at the age of six years, 10 weeks before the immunological workup. On admission to our pediatric immunology unit, he presented with severe hypotrophy, psychomotor retardation, short stature, macrocephaly, facial dysmorphism with prominent forehead, depressed nasal bridge, and anteverted nostrils, macrostomia, dental hypoplasia, low-set ears, sparse hair with absent eyebrows and eyelashes, and bilateral cryptorchidism. The patient's phenotypic features are displayed in **Figure 1**. In-depth genetic studies including whole genome sequencing (WGS) showed a novel heterozygous pathogenic germline missense variant (NM_002755.3:[c.364A > G; p.Asn122Asp) in the *MAP2K1* gene and the diagnosis of the cardio-facio-cutaneous syndrome (OMIM #615279) was established. The detected missense substitution resulted in a change of asparagine into aspartic acid in a highly conserved amino acid residue 122. Furthermore, the variant occurred *de novo* as it has been shown in targeted parental studies. To follow the case history, see **Figure 2** (Timeline). The immunological workup revealed hypogammaglobulinemia, IgG subclass, and specific antibody deficiency accompanied by a decreased numbers of T helper cells and abnormalities within the B cell compartment with low numbers of naive and switched memory B cells (Data shown in **Table 1**). Replacement immunoglobulin therapy (IgRT) with intravenous (IVIg) followed by subcutaneous immunoglobulins (SCIg) along with timely antibiotic prophylaxis were instituted leading to significant improvement and reducing the infections rate. The regular IgRT has led to a remarkable alleviation of respiratory symptoms, and since the age of three years, he suffered from two episodes of bronchitis and episodic mild upper airway infections and, aged six years, required an admission to the hospital.

At the age of five years, the boy was referred to the department of pediatric endocrinology for hormonal assessment. On admission, his height was 86 cm corresponding with the standard deviation score (SDS) −8.3 and the body mass index (BMI) was corresponding with SDS −6.1. The evaluation of the endocrine system showed partial growth hormone deficiency

FIGURE 1
Phenotypical features of the patient with CFCS type 3 due to a novel pathogenic germline missense variant (NM_002755.3:[c.364A > G; p.Asn122Asp) in the *MAP2K1* gene.

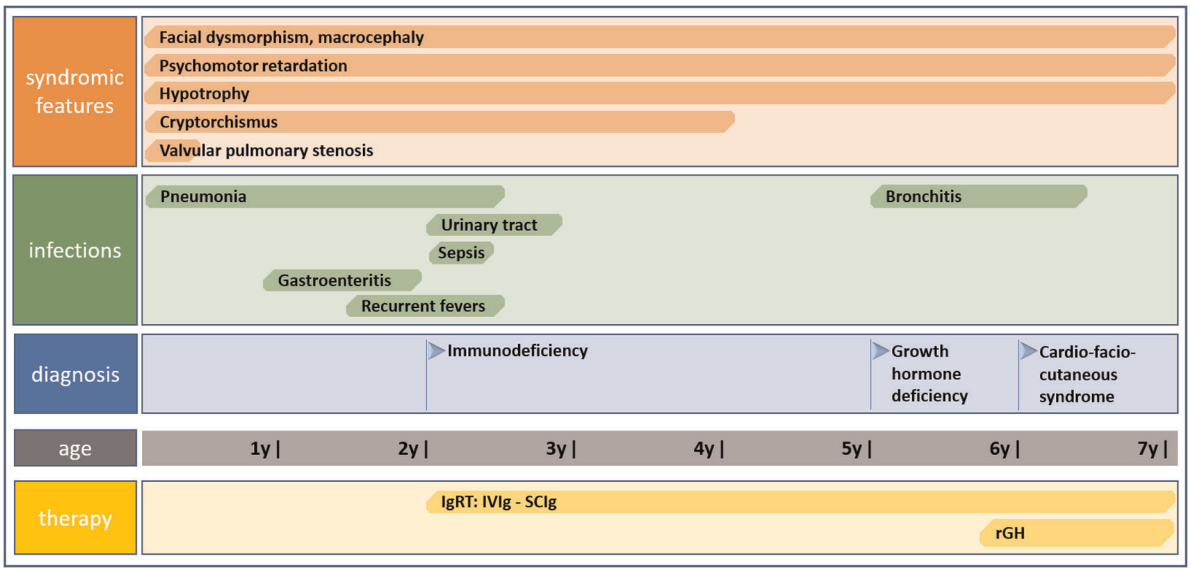

FIGURE 2
Timeline showing clinical case history including symptomatology, infectious history, diagnosis of CFCS, and therapy.

with remarkably low insulin growth factor 1 (IGF-1) concentration, and the bone age was estimated at 1.5 years. The recombinant growth hormone (rGH) therapy was started at the age of 6.5 years, reaching the height of −7.0 SDS, height velocity of 7 cm/year, the bone age of 4.5 years, and increasing the IGF-1 level after one year of the therapy. Data including hormonal laboratory parameters and a growth chart are displayed in **Table 2**.

Diagnostic assessment

Whole genome sequencing

Genomic DNA was extracted from peripheral blood (PB) leukocytes according to standard procedures. The sequencing library was prepared by Macrogen Inc. (Seul, Korea) using TruSeq DNA PCR-free kit (Illumina Inc, San Diego, California, USA) and 550 bp inserts. The library was sequenced on the Illumina Novaseq 6,000 platform using 150 bp paired-end reads following standard protocols. Bioinformatic analysis was performed as previously described (32). FastQC was used to confirm the quality of the sequenced reads which were mapped to the human reference genome GRCh38 using Speedseq framework v.0.1.2 (BWA MEM 0.7.10, Sambamba v0.5.9). Mapping coverage was calculated using Mosdepth 0.2.4. Sequence variants were detected using DeepVariant 0.8.0. and CNVnator v0.4, and annotated using Ensembl Variant Effect Predictor 97.3 (VEP). Variants with minor allele frequency below 0.5% or missing from gnomAD v3 database, and missing from an inhouse database of over 1,200 ethnically matched WGS samples (33) were selected for the analysis; variants in genes associated with RASopathies and immunodeficiency were prioritized. Variants in genes associated with immunodeficiency were reanalyzed using the minor allele frequency threshold below or equal to 3%. The clinical interpretation of detected mutations was performed based on various online databases of genomic variants including ClinVar2, GnomAD3, Human Gene Mutation Database (HGMD) Professional 2014.1. The pathogenicity of the identified variants was evaluated by multiple prediction tools integrated into WGS data analysis pipeline and VarSome Premium variant data analysis tool (34). The classification of the reported mutation was performed according to the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG/AMP) (35). The *de novo* occurrence of the pathogenic mutation in the proband was confirmed in parental testing employing targeted Sanger sequencing. A detailed summary of the pathogenicity prediction of the detected *MAP2K1* mutation including pathogenicity and conservation scores is shown in **Supplementary Table S2**.

Flow cytometric peripheral blood lymph cell immunophenotyping

Cells were labelled with the following murine fluorochrome-stained monoclonal antibodies: anti-CD45 FITC (fluorescein isothiocyanate), anti-CD14 PE (phycoerythrin), anti-CD19 PE,

TABLE 1 The immunological workup with antibody-mediated response and peripheral blood flow cytometric immunophenotyping in the CFCS patient.

Immunological workup	Age 2 years (1)	Reference values (1)	Age 6 years (2)	Reference values (2)
Antibody response				
Immunoglobulins				
IgG	184 mg/dl	520–1360 mg/dl	265 mg/dl	570–1410 mg/dl
IgA	19 mg/dl	45–135 mg/dl	73 mg/dl	65–240 mg/dl
IgM	28 mg/dl	46–190 mg/dl	5 mg/dl	55–210 mg/dl
IgG subclasses				
IgG1	95 mg/dl	315–945 mg/dl	193 mg/dl	306–945 mg/dl
IgG2	41 mg/dl	36–225 mg/dl	124 mg/dl	60–345 mg/dl
IgG3	7 mg/dl	17–68 mg/dl	18 mg/dl	99–122 mg/dl
IgG4	0.5 mg/dl	1–54 mg/dl	2 mg/dl	20–112 mg/dl
Antigen-specific antibodies				
Anti-diphtheria toxoid			<0.1 IU/ml	>1 IU/ml
Anti-tetanus toxoid			<0.1 IU/m	>1 IU/ml
PB lymphocyte immunophenotyping				
WBC	10210 cc		14020 cc	
Lymphocytes CD45+/SSC low	36.0%, 3676 cc	29.6–69.2%, 2300–6900 cc	28.0%, 3856 cc	29.6%–49.8%, 1700–3600 cc
B CD19+	11.0%, 209 cc	14.1–28.5,0%, 400–1700 cc	4.0%, 178 cc	9.7%–23.7%, 300–600 cc
Transitional B CD19 + CD38 + IgM++	23.7%, 99 cc	3.1%–12.3%, 20–200 cc	1.6%, 3 cc	4.6%–8.3%, 10–40 cc
Mature naïve B CD19 + CD27-IgD+	94,8%, 395 cc	54.0%–88.4%, 280–1330 cc	64.4%, 115 cc	47.3%–77.0%, 130–460 cc
Non-switched memory B (MZL) CD19 + CD27 + IgD+	2.0%, 4cc	2.7%–19.8%, 20–180 cc	12.3%, 22 cc	5.2%–20.4%, 20–100 cc
Switched memory B CD19 + CD27 + IgD-	2.1%, 5 cc	4.7%–21.2%, 20–220 cc	2.3%, 4 cc	10.9%–30.4%, 40–140 cc
Immature B CD19 + CD21lo	9.1%, 19 cc	4.1%–24.4%, 20–230 cc	4.9%, 9 cc	5.9%–25.8%, 20–120 cc
Activated B CD19 + CD38loCD21lo	4,6%, 11 cc	1.7%–5.4%, 10–60 cc	4.9%, 9 cc	2.3%–10.0%, 10–40 cc
Plasmablasts CD19 + CD38++IgM-	0.0%, 0 cc	0.6%–4.0%, 5–10 cc	0.0%, 0 cc	0.6%–5.3%, 0–3 cc
T CD3+	74.0%, 1491 cc	52.0%–92.0%, 850–4300 cc	47.0%, 1905 cc	55.0%–97.0%, 850–4300 cc
T helper CD3 + CD4+	34.0%, 1288 cc	25.0%–66.0%, 500–2700 cc	18.0%, 722 cc	26.0%–61.0%, 500–2700 cc
T suppressor/cytotoxic CD3 + CD8+	19.0%, 720 cc	9.0%–49.0%, 200–1800 cc	23.0%, 916 cc	13.0%–47.0%, 200–1800 cc
CD4+/CD8+	1.79	1.5–2.5	0.79	1.5–2.5
Recent thymic emigrants CD3 + CD4 + CD45RA + CD31+	47.0%, 607 cc	37,0%–100%, 190–2600 cc	33.8%, 244 cc	41.0%–81.0%, 190–2600 cc
Naïve T helper CD3 + CD4 + CD45RA + CD27+	66.1%, 851 cc	52.0%–92.0%, 300–2300 cc	47.9%, 345 cc	46.0%–99.0%, 300–2300 cc
Central memory T helper CD3 + CD4 + CD45RA-CD27+	2.9%, 307 cc	15.0%–56.0%, 160–660 cc	45.3% 327 cc	0.35%–100%, 160–660 cc
Effector memory T helper CD3 + CD4 + CD45RA-CD27-	9.6%, 123 cc	0.3%–9.0%, 3–89 cc	5.9%, 42 cc	0.3%–18.0%, 3–89 cc
Terminally differentiated memory T helper CD3 + CD4 + CD45RA + CD27-	0.4%, 5 cc	0.0%–1.2%, 0–16 cc	1.0%, 7 cc	0.0%–1,8%, 0–16 cc
Follicular CXCR5+ T helper CD3 + CD4 + CD45RO + CD185+	16.0%, 206 cc	6.0%–72.0%, 13–170 cc	11.2%, 29 cc	7.0%–85.0%, 13–170 cc
Regulatory T helper CD3 + CD4 + CD25++CD127-	0.5%, 6 cc	3.0%–17.0%, 39–150 cc	6.8%, 49 cc	4.0%–14.0%, 39–150 cc
Naïve T suppressor/cytotoxic CD3 + CD8 + CD27 + CD197+	27.7%, 199 cc	19.0%–100%, 53–1100 cc	26.1%, 239 cc	16.0%–100%, 53–1100 cc
Central memory T suppressor/cytotoxic CD3 + CD8 + CD45RA-CD27 + CD197+	1.2%, 8 cc	1.0%–9.0%, 4–64 cc	2.2%, 20 cc	1.0%–6.0%, 4–64 cc
Effector memory T suppressor/cytotoxic CD3 + CD8 + CD45RA-CD27-CD197-	7.4%, 53 cc	10.0%–55.0%, 24–590 cc	19.0%, 174 cc	5.0%–100%, 24–590 cc
Terminally differentiated T suppressor/cytotoxic CD3 + CD8 + CD45RA + CD27-CD197-	3.1%, 22 cc	6.0%–83.0%, 25–530 cc	12.8%, 117 cc	15.0%–41.0%, 25–530 cc
NK CD3-CD45 + CD16 + CD56+	22.0%, 808 cc	2%–25%, 61–510 cc	19.0%, 754 cc	2.0%–25.0%, 61–510 cc

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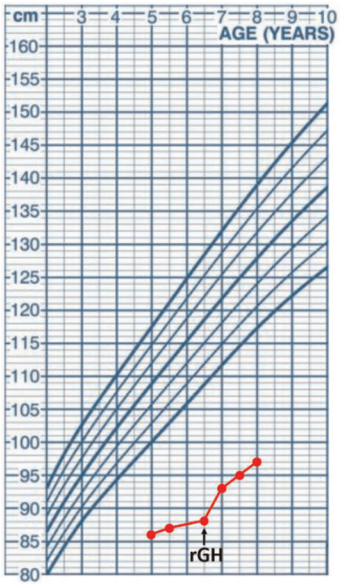
TABLE 2 Endocrinological workup of the presented CFCS patients before and one year after starting the rGH therapy, aged 6 and 7 years old, respectively.

Hormonal Parameters	Before starting rGH	One year after starting rGH	Reference values	Growth chart
Max GH after onset of sleep	7.1 ng/ml		>10.0 ng/ml	
Max GH after glucagon	9.4 ng/ml		>10.0 ng/ml	
Max GH after clonidine	7.2 ng/ml		>10.0 ng/ml	
IGF-1	12.0 ng/ml	90.0 ng/ml	115.0–249.0 ng/ml	
IGFBP-3	838.0 ng/ml	2462.0 ng/ml	2846.0–4462.0 ng/ml	
LH	0.2 mIU/ml		0.02–1.03 mIU/ml	
FSH	0.5 mIU/ml		0.25–1.92 mIU/ml	
Testosterone	0.45 nmol/L		0.1–1.0 nmol/L	
PRL	86.58 ng/ml		4.79–23.3 ng/ml	
PRL after precipitation with PEG	27.52 ng/ml		4.79–23.3 ng/ml	
Cortisol at 8:00 am	187.0 ng/ml		37.0–194.0 ng/ml	
Cortisol after glucagon	189.0 ng/ml		>180.0 ng/ml	
ACTH	109.6 pg/ml		10.0–60.0 pg/ml	
TSH	3.993 μIU/ml		0.700–4.170 μIU/ml	
FT3	1.54 pg/ml		0.86–1.37 pg/ml	
FT4	3.77 ng/dl		2.79–4.42 ng/dl	
anti-TPO Ab	7.9 IU/ml		<5.61 IU/ml	
anti-TG Ab	1.9 IU/ml		<4.11 IU/ml	
Insulin	2.2 μU/ml		<15.0 μU/ml	
PTH	57.6 pg/ml		10.0–60.0 pg/ml	
25-OH-D	36.8 ng/ml		30.0–50.0 ng/ml	



anti-CD19 PerCP (peridinin chlorophyll protein), anti-IgM FITC, anti-IgD FITC, anti-CD38 APC (allophycocyanin), anti-CD27 PE, anti-CD21 FITC, as well as anti-CD3 FITC, anti-CD4 FITC, CD45RA FITC, CD127 FITC, CD185 FITC, anti-CD8 PE, anti-CD16 + CD56 PE, CD25 PE, CD31 PE, CD45RO PE, anti-CD3 PerCP, CD197 PerCP, anti-CD4 APC and anti-CD8 APC (all Beckton-Dickinson Biosciences, USA). The acquisition of cells and analysis was carried out with the use of the flow cytometer FACSCanto and FACSDiva software (Beckton-Dickinson, USA). With sequential gating on biparametric scattering CD45 + CD14- lymphocytes, the following lymphocyte subpopulations were identified:

- CD19+ B cells, immature CD19 + CD21lo, immature activated CD19 + CD38loCD21lo, transitional CD19 + CD38hisIgMhi, non-switched memory CD19 + CD27 + sIgD+, switched memory CD19 + CD27 + IgD- B cells, and CD19 + CD38hisIgM- plasmablasts
- CD3+ T cells, CD3 + CD4+ T helper cells, CD3 + CD4 + CD31 + CD45RA+ recent thymic emigrants, naïve CD3 + CD4 + CD27 + CD45RA+, regulatory CD3 + CD4 + CD25+ +CD27-, central memory CD3 + CD4 + CD27 + CD45RO+, effector memory CD3 + CD4 + CD27-CD45RO+, terminally differentiated CD3 + CD4 + CD27-CD45RA+, follicular CD3 + CD4 + CD185 + CD45RO+, and regulatory CD3 + CD4 + CD45RO + CD127-CD25++ T helper cells. Among CD3 + CD8 + cytotoxic T cells, the following subsets were distinguished: naïve CD3 + CD8 + CD197 + CD27 + CD45RA+, central memory CD3 + CD8 + CD197 + CD27 + CD45RO+, effector memory CD3 + CD8 + CD197-CD27-CD45RO+, and terminally differentiated CD3 + CD8 + CD197-CD27-CD45RA+ cells.
- CD3-CD16 + CD56+ NK cells.

The relative values of PB lymphocytes, the B, T, and NK cells of the total lymphocyte population as well as B and T cell subsets were calculated. The absolute counts of all cell subsets were calculated from the PB leukocyte counts. A comparative analysis was done with the reference cut-off values of B (36) and T cell subsets (37) for pediatric populations at different age groups.

Auxology

The boys' height was measured in the lying position due to cerebral palsy using SECA measuring mat. The height and BMI SDS for chronological age were calculated using WHO references, and the bone age was estimated according to the Greulich and Pyle method for evaluation of the skeletal developments of the hand and wrist.

Discussion

Addressing the concerns on the heterogeneity of clinical features, the rarity of the syndrome, and complex genotype-phenotype relationships, it needs to be highlighted that the definitive diagnosis of CFCS is challenging for clinicians (38). Attempts have been made to better delineate the phenotypic perinatal (39) and childhood presentation (13) to facilitate the early diagnosis. Functional consequences of mutations in the CFCS-related RAS/MAPK pathway involving KRAS-BRAF-MAPK-ERK components show clinical variety as well, making the interrelated links between the causative pathogenic variant and the patient's phenotype difficult to predict. RAS/MAPK pathway plays pleiotropic roles at the crossroads of the development and homeostasis of endocrine and metabolic tissues. Directing the metabolism towards anabolic processes, such as macropinocytosis and autophagy as well as regulating the response to foods through neuroendocrine signals, the RAS/MAPK pathway acts as a modifier of the hormonal and metabolic balance (40) as well as bioenergetics related to mitochondrial physiology and high energy expenditure (41). The response to hormones, such as insulin, leptin, and GH, as well as the development of hormonally active organs, such as the hypothalamus, pancreas, and adipose tissue has been associated with the activation of the RAS/MAPK cascade.

Importantly, the RAS/MAPK pathway is mobilized downstream from the GH receptor and in RASopathies, increased activation of the RAS/MAPK signaling results in reduced IGF-1 generation in response to GH (39). The most widely proposed hypothesis of growth failure is a partial GH insensitivity due to a post-receptor signaling defect (42, 43). Nonetheless, the pathomechanism of short stature in RASopathies is complex and multifactorial, and besides GH deficiency, partial GH insensitivity, neurosecretory dysfunction, also neuromuscular, orodental and feeding disorders, and history of cardiac surgery have been proposed as contributory disorders (42–44). In our patient, severe hypotrophy, poor nutritional status, feeding disorders, muscular atrophy and hypotonia, as well as a history of valvular pulmonary stenosis in parallel with recurrent infections had a salient effect on his developmental impairment and growth failure. Moreover, failure to thrive may underpin immunodeficiency and, in turn, immunodeficiency may escalate failure to thrive and hormonal dysfunction, creating a vicious circle in pathomechanisms of immunity and development. In RASopathies, an increased RAS/MAPK pathway signaling in chondrocytes may impair growth plate development and longitudinal growth (45). MAPK activation is important in regulating the proliferation of pituitary somatotrophs and, therefore, proper GH secretion (46). The partial GH insensitivity in RASopathies has also been postulated, implying that the response to rGH in MAP2K1 deficiency-related CFCS may not be entirely satisfactory (42, 43, 47). In the presented patient, the GH secretion was just below the cut-off value and was accompanied by a very low IGF-1 and IGF-binding protein 3 (IGFBP-3) that might suggest a coexisting GH insensitivity. Interestingly, the elevated prolactin (PRL) concentration with nearly normal precipitation with polyethylene glycol (PEG) may be hypothesized to result from immunological disturbances and IgRT in the patient.

The pleiotropic effect of RAS-associated pathways on cellular growth, differentiation, and apoptosis may also be hypothesized as a potential background for the combined immunodeficiency in the patient studied. The Ras/MAPK cascade cumulating in ERK kinase underlie functional switching in lymph cells. Engagement of antigen receptors in lymph cells stimulates Ras proteins activation by guanine nucleotide exchange factors (GEFs): RasGRP1 acts downstream from antigen receptors in T cells, whereas RasGRP1 and RasGRP3 function in B cells (48–52). Biallelic loss-of-function (LOF) mutations in the *RASGRP1* gene have been described in several patients to develop a combined immunodeficiency (CID) and impaired cytoskeleton dynamics, susceptibility to severe viral, fungal, and bacterial infections, autoimmune cytopenias, and an Epstein-Barr virus (EBV)-driven lymphoproliferation. The immunodeficiency in RASGRP1 is characterized by impaired B and T cell activation and proliferation, decreased T cell numbers, and NK cell cytotoxic dysfunction (53). It has also been shown that in B cells, positive feedback-driven Ras activation is the proposed source of digital MAPK responses and signal amplification following antigen stimulation at the B cell receptor (54). Whereas the regulatory role of MAPK has been shown in crucial cellular processes, including driving proliferation and activation of dendritic cells, it has been hypothesized that the MAPK cascade promotes efficient adaptive immune response (55). While the *MAP2K1* variants have also been shown to activate the ERK-dependent cell cycle progression and autophagy (56), it has raised the question whether the autophagy-mediated altered MAP2K1 function contributes to a dysfunctional immunophenotype. Referring to the two hitherto reported CFCS patients with hypogammaglobulinemia and absent antigen-specific antibody response, both harbored the same c.389A > G; p.Tyr130Cys mutation in the *MAP2K1* gene (31). While in our patient, developmental disorders within the lymphocyte compartment have been found, it raises questions regarding the role of this novel pathogenic c.364A > G; p.Asn122Asp variant in *MAP2K1* in lymphocyte development and function, the degree of the immune response impairment, as well as immuno-endocrine correlations. A comparative analysis of the symptomatology and immunological workup of two CFCS patients with hypogammaglobulinemia and our patient is displayed in **Supplementary Table S2**. Noteworthy, different missense variants in the same gene that lead to increased activity of the mutated protein may have distinct activating potential resulting in a variable degree of dysregulation in downstream signaling pathways. It is therefore possible that an individual immunophenotype may be ascribed

to the different MAPK genotype (57). The presence of other potentially pathogenic variants that could contribute to the immunodeficiency has been excluded by careful bioinformatic analysis of the WGS data using an immunodeficiency gene panel and minor allele frequency threshold below or equal to 3%. Only two heterozygous variants, NM 000066.4:c.1282.C > T (p.Arg428Ter) in the *C8B* gene and NM 001083116.3:c.272C > T (p.Ala91Val) in the *PRF1* gene, were predicted as pathogenic. However, both variants were associated wit autosomal recessive disorders and therefore, their contribution to the immunophenotype of our patient seems unlikely. Further functional experimental and clinical studies are required for the precise delineation of the effect of both c.364 A > G; p.Asn122Asp and c.389A > G; p.Tyr130Cys missense substitutions on the MAP2K1 protein and the corresponding CFCS immunophenotype. Although the role of mutations in classical genes in components of the RAS/MAPK pathway has been elegantly studied (5, 8, 9, 58), the effect of disease-modifying altered mi-RNAs expression profiles has been revealed thereby highlighting a contribution of epigenetic regulation on *MAP2K1* and the phenotypic immuno-endocrine features in CFCS (59). It is also worth noting that genes in the RAS-MAPK pathway are among the most frequently deregulated genes in human cancer due to their regulatory role in cell proliferation, differentiation and survival. Interestingly, the genetic aberrations resulting in deregulated activation of the RAS-MAPK signaling pathway which have been recently reported in a spectrum of hematopoietic malignancies include the same N122D *MAP2K1* variant found in our CFCS patient (60). This N122D alteration occurs in the kinase domain of the *MAP2K1* gene, in the regulatory helix, while other mutations identified in CFCS are clustered.

The ever-increasing progress in genetic studies, contributing to establishing the definitive CFC diagnosis and shedding light on the interrelated genotype-phenotype heterogeneity of clinical syndromes belonging to the group of RASopathies needs to be highlighted. Herein, we add new phenotypic features of humoral immunodeficiency to the syndromic symptomatology of CFC with a novel mutation in *MAP2K1*. In this patient, multidisciplinary care of specialists in pediatric endocrinology, dermatology, cardiology, neurology, and physiotherapy is indicated, under the pediatric clinical immunologist's supervision.

Patient perspective

Finding a causative mutation and establishing a definitive genetic diagnosis means primarily an explanation for the multifaceted disease. Both for the family and leading physicians, it paves the way for future diagnostic and therapeutic interventions, shedding light on the expanded phenotype with immunodeficiency. Importantly, the everyday struggling with the child's intellectual and motor disabilities, failure to thrive, recurrent infections, increased risk of tumorigenesis, frequent medical consultations and treatments with SCIg and GH, is a disease burden for the patient's relatives. To cope with the multisystemic syndrome of CFC, medical and social support is needed for the family to enhance undertaking positive health-promoting stimulating activities. Therefore, the *de novo* nature of the pathogenic *MAP2K1* variant has an important informative role for the family.

Data availability statement

The datasets for this article are not publicly available due to concerns regarding participant/patient anonymity. Requests to access the datasets should be directed to the corresponding author.

Ethics statement

Ethical approval was not provided for this study on human participants because For a case report the ethical approval is not required. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

ASZP was responsible for the conception and design of the study, acquisition, and interpretation of data, and drafted the manuscript. NP helped in the acquisition and analysis of data and drafting the manuscript. MN analyzed clinical data and critically revised the manuscript. ASS, PS, and AJ contributed to the diagnosing the patient and interpretation of genetic studies. MOM contributed to the design of the study, acquisition, and interpretation of data, and participated in drafting the manuscript. AJ and MOM share senior authorship. All authors contributed to the article and approved the submitted version.

Conflict of interest

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

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2022.990111/full#supplementary-material.

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Case report: Cerebellar swelling and hydrocephalus in familial hemophagocytic lymphohistiocytosis

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Familial hemophagocytic lymphohistiocytosis (FHL) is a severe inborn error of immunity caused by a genetic defect that impairs the function of cytotoxic T and NK cells. There are only a few reported cases of FHL with diffuse swelling of the cerebellum and obstructive hydrocephalus. We report a case of FHL3 with neurological symptoms associated with cerebellar swelling and obstructive hydrocephalus. A male patient was hospitalized several times due to fever and decreased feeding, hepatosplenomegaly, and cytopenia since the first month of life. At 7 months of age, disturbance of consciousness was seen. Brain magnetic resonance imaging revealed signal intensity in the bilateral cerebellar hemispheres, diffusely increased periventricular white matter, and ventriculomegaly. Although he was treated with methylprednisolone pulse therapy, he was unresponsive to the treatment. He was then transferred to a local hospital after tracheotomy but died. Targeted clinical sequencing revealed a homozygous splice-site mutation in *UNC13D*. Pediatric hemophagocytic lymphohistiocytosis (HLH) includes some cases of central nervous symptom (CNS)-isolated HLH or CNS HLH preceding systemic lesions, which often do not initially meet the diagnostic criteria for FHL. Patients with FHL initiated by cerebellar symptoms may present with an atypical clinical course for HLH, leading to delayed diagnosis and poor outcomes. Despite the usefulness of a combination of a high percentage of lymphocytes in the peripheral leukocytes, a low lactate dehydrogenase level, and a high sIL-2R/ferritin ratio for identifying FHL, the diagnosis may be missed due to the absence of these results. Presymptomatic diagnosis of FHL by screening of newborns and subsequent early treatment of patients with a predicted poor prognosis may contribute to better outcomes.

KEYWORDS

cerebellar swelling, hydrocephalus, familial hemophagocytic lymphohistiocytosis, inborn errors of immunity, newborn screening (NS)

Introduction

Familial hemophagocytic lymphohistiocytosis (FHL) is a severe inborn error of immunity caused by a genetic defect that impairs the function of cytotoxic T and natural killer (NK) cells (1). The clinical symptoms of patients with FHL may vary widely. Patients with FHL may present with a variety of neurological symptoms prior

to diagnosis, which in some cases can be fatal (2). The prognosis of patients with FHL has improved recently, and they can be cured with immunochemotherapy and hematopoietic stem cell transplantation (3). The diagnosis is difficult in some cases because the diagnostic criteria are not fulfilled at the initial presentation in some cases (4). Nonspecific symptoms in the early phase may delay the diagnosis of FHL. Early diagnosis through awareness of neurological symptoms may contribute to appropriate treatment and improved prognosis. Although some cases of FHL with diffuse swelling of the cerebellum and associated progression of obstructive hydrocephalus have been reported (5–8), it is still not well recognized among clinicians. It is a rare but life-threatening condition in which obstructive hydrocephalus occurs as a result of cerebellar swelling. We report a case of FHL3 with neurological symptoms associated with diffuse cerebellar swelling and obstructive hydrocephalus.

Case descriptions

The patient was the second child of healthy, nonconsanguineous Japanese parents, and his sister was in good health at the time. He was born at 37 weeks of gestation, weighing 3,064 g. His perinatal course was uneventful. We show the clinical course of our case in **Figure 1**. At 1 month of age, he was brought to a local hospital due to a high fever and decreased feeding, which was managed with antimicrobials as clinicians suspected the presence of an infection. Although he developed transient bicytopenia, he was discharged. At 2 months of age, he was admitted for the second time due to fever, purpura, hepatosplenomegaly, and decreased hemoglobin level and platelet count. Laboratory findings showed elevated lactate dehydrogenase (LDH) and ferritin levels and increased atypical lymphocytes, while bone marrow examination showed no hemophagocytosis. He was treated with intravenous immunoglobulins and antimicrobials. At 4 months of age, the patient was admitted for the third time due to fever and hepatosplenomegaly and decreased hemoglobin level and platelet count. The results of a repeat bone marrow examination were normal. He was treated with cyclosporine and prednisolone. Oral sulphamethoxazole/trimethoprim was also initiated. The patient was admitted for the fourth time at 6 months of age due to fever and bulging of the anterior fontanel. Spinal fluid tests demonstrated normal findings; thus, he was discharged with antimicrobial treatment to relieve the fever.

At 7 months of age, he was seen for somnolence and irritability. Brain magnetic resonance imaging (MRI) revealed cerebellar swelling and hydrocephalus; thus, the patient was referred to a university hospital and was admitted for the fifth time. His weight and height were 7.3 kg [−1.3 standard deviation (SD)] and 71 cm (+0.2 SD), respectively. His anterior fontanel was bulging. His irritability was observed when his head was moved. He could not fix his eyes or perform light tracking. His head control was unstable. His direct light reflex on the left side was prompt, but that on his right side was sluggish. He showed facial nerve palsy on the left side. He was able to perform antigravity movements of his limbs, and his deep tendon reflexes were normal. **Table 1** shows the clinical and laboratory findings on admission. Patients fulfilled three out of eight of the HLH diagnostic criteria (hypofibrinogenemia, increased ferritin, and elevated sIL-2R). T2WI brain MRI at 10 days after onset showed the presence of signal intensity of the bilateral cerebellar hemispheres and diffusely increased periventricular white matter. Ventriculomegaly due to fourth ventricle obstruction was also noted. The diffuse cerebellar swelling caused the obstructive hydrocephalus (**Figure 2**). Cerebrospinal fluid examination was not performed. Other laboratory tests showed no remarkable cytopenia and scant elevated liver enzymes and LDH levels. Epstein–Barr virus (EBV) viral capsid antigen

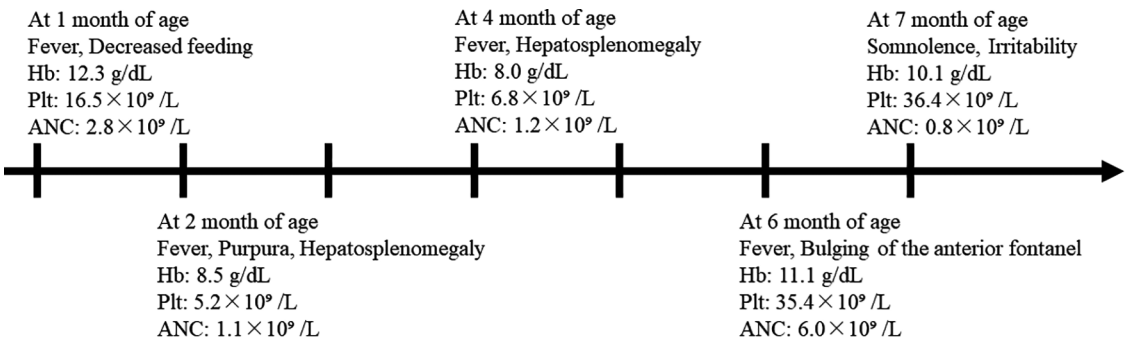

FIGURE 1
Clinical course and peripheral blood counts of our case. The horizontal axis shows the time course of this case, with one scale representing 1 month. Hb, hemoglobin; Plt, platelet count; ANC, absolute neutrophil count.

TABLE 1 Patient's clinical and laboratory findings and HLH diagnostic criteria.

	Patient findings	HLH-2004 diagnostic criteria (at least five out of eight main features)
Fever	36.9°C	≥38.5°C, more than 7 days
Hepatosplenomegaly	No	Radiographic or physical exam evidence
Cytopenia		2 or 3 hematopoietic lineages
Hemoglobin, g/dl	10.1	<9
Platelets, 10^9/L	364	<100
Neutrophils, 10^9/L	0.8	<1.0
AST, U/L	31	
ALT, U/L	10	
LDH, U/L	248	
Bilirubin, mg/dl	0.5	
Triglycerides, mg/dl	92	≥265 and/or
Fibrinogen, mg/dl	214	≤150
Ferritin, ng/ml	3,560	≥500
sIL-2R, U/ml	3,560	>2,400
Hemophagocytosis	No	Present in bone marrow or others
Decreased NK cell activity	ND	According to the local laboratory reference

HLH, hemophagocytic lymphohistiocytosis; AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase; sIL-2R, soluble interleukin-2 receptor; NK, natural killer; CSF, cerebrospinal fluid; ND, not described.

(VCA) IgG, VCA IgM, and EBV nuclear antigen were negative, and neither IgG nor IgM of human herpes virus 6 and 7 was found. Rapid viral antigen tests were negative for the respiratory syncytial virus, mycoplasma, and influenza. Blood cultures were also negative.

We consulted with the neurosurgeon and ruled out osteotomy of the posterior cranium; thus, we proceeded with conservative treatment. We administered osmotic diuretics and dexamethasone with the expectation of a cerebral pressure-reducing effect. On the first day of admission, as there were rhythmic movements of the right limbs and electroencephalography (EEG) showed diffuse slow waves, we judged that the patient must be having a seizure; thus, we administered fosphenytoin. On the third day of admission, the patient developed a fever and appeared to be mouthing; hence, we administered a continuous dose of midazolam, which resulted in a decrease in blood pressure, and dopamine was also administered. On day 4 of hospitalization, the patient suddenly had ventricular fibrillation and cardiac arrest; a computed tomography scan showed cerebral herniation. After resuscitation, the patient was treated with methylprednisolone pulse therapy based on the belief that the brain herniation was caused by cerebral edema due to high cytokine levels. His blood pressure was subsequently stabilized. We continued to administer prednisolone. The patient had central enuresis and hypothyroidism. EEG results and auditory brainstem response were negative, and after a discussion with his parents, he was transferred to a local hospital after a tracheotomy, where he subsequently died. Targeted clinical sequencing of 12 genes known to cause FHL (The Twist BioScience custom targeted panel, Illumina NextSeq) was performed on the blood samples, which revealed mutation c.754-1G > C at the homozygous status in *UNC13D*, leading to the diagnosis of FHL.

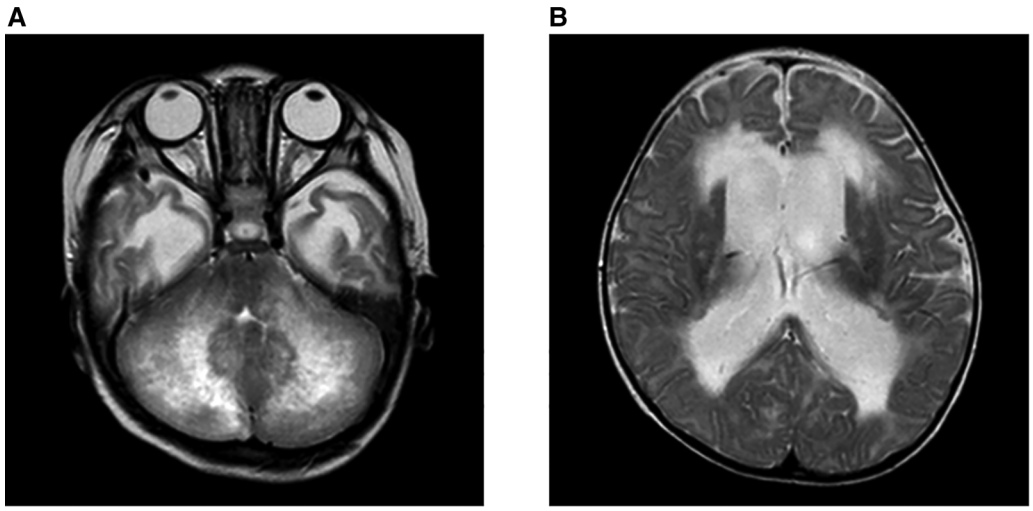

FIGURE 2
Brain MRI on admission at 7 months of age. Brain MRI on the 10 days after onset, on the T2WI. (**A**) Signal intensity of the bilateral cerebellar hemispheres and periventricular white matter were diffusely increased. (**B**) Ventriculomegaly due to the fourth ventricle obstruction. The diffuse cerebellar swelling caused the obstructive hydrocephalus.

TABLE 2 Clinical and laboratory findings in patients diagnosed with FHL complicated by cerebellar swelling.

	Case 1	Case 2	Case 3	Case 4	Our case
Ethnical origin	Unknown	Unknown	Unknown	Saudi	Japan
Familial disease	Two maternal uncles had died at ages 10 and 16 years	No	No	Unknown	No
Parental consanguinity	No	No	No	ND	No
Sex	Male	Female	Male	Male	Male
Type of FHL	2	2	3	4	3
Mutated gene	PRF1	PRF1	UNC13D	STX11	UNC13D
Allele 1	p. Leu215Ile	c.273C > T	p. Ile712_Gly713_delinsSer	p. Leu58Pro	c.754-1G > C
Allele 2	p. Ala262Asn	c.273C > T	p. Arg782Serfs * 12	p. Leu58Pro	c.754-1G > C
Age at diagnosis of HLH, months	36	156	84	31	7
Symptoms at diagnosis	Fever, hepatosplenomegaly	Headache, vomiting	Fever, hepatosplenomegaly	Fever, vomiting	Feeding disorder
Neurologic manifestations[a]	Ataxia, nystagmus, dysmetria, coma, epilepticus	Gait imbalance, double vision	Unsteady gait, ataxia, dysmetria, dysdiadochokinesia, dysarthria	Irritability, ataxic gait, somnolence	Somnolence, irritability
WBC, μl	ND	4,840	ND	5,320	3,980
Lymphocyte, %	ND	55	ND	ND	75
AST, U/L	60	39	ND	208	31
ALT, U/L	ND	38	ND	228	10
LDH, U/L	ND	ND	ND	ND	248
Triglycerides, mg/dl	213	ND	ND	319	92
Ferritin, ng/ml	179	ND	ND	2,578	11,866
sIL-2R, U/ml	ND	ND	ND	ND	3,560
Hemophagocytosis	Occasionally	rare	Yes	No	No
Findings of Cerebrospinal fluid	Pleocytosis (61 cell/mm^3)	High levels of IgG and IgM	ND	Normal	ND
Findings of brain MRI	Cerebellar swelling, downward herniation of cerebellar tonsils	Swollen white matter of the cerebellum extending into the axis in the T2-weighted image, resulting in tonsillar herniation	Hyperintense signal changes in cortex and white matter of cerebellar hemispheres and diffuse cerebellar edema in T2 weighted image	Diffuse enlargement of both cerebellar hemispheres, Mass effect on the fourth ventricle and brainstem, Mild herniation of cerebellar tonsils	Diffuse enlargement of both cerebellar hemispheres, hydrocephalus
Pre-transplant treatment	HLH-94	HLH-2004	Chemotherapy	HLH-2004	mPSL
Age at HSCT, months	48	459	ND	35	Not done
Conditioning regimen	Bu + CY + VP − 16	ND	ND	Flu + L − PAM + ATG	–
Outcome	Alive	Alive	Alive	Alive	Dead
Neurological complications	Seizure, behavior disturbances, hypoacusia, visual field defects	None	None	None	–
Observation period	16 months	18 months	ND	9 months	8 months
Reference	(7)	(8)	(9)	(10)	This study

FHL, familial hemophagocytic lymphohistiocytosis; HLH, hemophagocytic lymphohistiocytosis; WBC, white blood cell count; AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase; sIL-2R, soluble interleukin-2 receptor; HSCT, hematopoietic stem cell transplantation; Bu, busulfan; CY, cyclophosphamide; VP-16, etoposide; L-PAM, melphalan; ATG, anti-thymocyte globulin; ND, not described.
[a]Reported at some point during the course of the disease.

Discussion

This report presents a case of an infant who presented with CNS symptoms after multiple episodes of fever and cytopenia. Our patient presented with neurological symptoms due to cerebellar swelling and associated hydrocephalus. Pediatric FHL includes some cases of CNS-isolated HLH or CNS HLH preceding systemic lesions, which often do not initially meet the diagnostic criteria for FHL (9). Brain MRI findings in HLH are heterogeneous, with multiple white matter lesions being the most common (66%), followed by cerebellitis (19%) and brainstem dominant disease (15%) (9). Some HLH patients with cerebellitis have neurological symptoms preceding the onset of systemic HLH, and their diagnostic laboratory findings are negative. Patients with FHL initiated by cerebellar symptoms may present with an atypical clinical course for HLH, leading to delayed diagnosis and poor outcomes. We summarized a literature cohort of four cases of FHL with cerebellar swelling in **Table 2**. The cases included three boys and one girl: two with *PRF1* mutations and one each with other *UNC13D* and *STX11* mutations. In some cases, cerebellar swelling appears early and ahead of other cases, whereas, in others, as in our case, symptoms due to cerebellar swelling appear over time. Of the five cases, only two showed significant hemophagocytosis in the bone marrow. All cases except ours reached hematopoietic stem cell transplantation and survived, and three cases had no neurological complications.

Our patient died before we reached a definitive diagnosis, despite having had several opportunities for inpatient treatment. We have performed tests, including bone marrow examination, but have not obtained definitive evidence of disease. We had initially considered a possible diagnosis of autoimmune lymphoproliferative syndrome because of the patient's hepatosplenomegaly, a course of multiple cytopenias, and an elevated double negative T cell at 9.6% (10). Despite examinations for possible differential diseases as possible causes of cerebellar swelling, such as viral infections (rotavirus, herpesvirus, mycoplasma), congenital metabolic abnormalities, and drug-induced neurological disorders, all of these had negative findings. Patients had no family history of immunodeficiency and did not have an autoimmune disease phenotype. Contrarily, he had only two positive findings (ferritin and sIL-2R) according to the HLH criteria (11) when the patient presented for cerebellar symptoms. These two positive findings were determined using samples collected during resuscitation for cardiac arrest at 4 days after admission. The patient also met up to three of the HLH criteria at other admissions (fever, hepatosplenomegaly, and cytopenia) and did not fully meet the HLH criteria at any time point. Yasumi et al. have indicated the usefulness of a combination of a high percentage of lymphocytes in peripheral leukocytes (74.0% ± 14.4%), low levels of LDH (489 ± 163 IU/L), and a high ratio of sIL-2R to ferritin (13,500 ± 12,800 IU/μg) for identifying FHL (12). The patient had a high lymphocyte percentage and relatively low LDH levels (all data not shown) on all of his several admissions and a low sIL-2R/ferritin ratio on his first admission. The sIL-2R/ferritin ratio was not measured at any other admission. These markers may be useful in predicting FHL; however, the diagnosis may be missed due to some cases not being matched to them. Our case indicates that for patients presenting with unexplained multiple recurrent fevers and cytopenia, it is necessary to actively consider the possibility of inborn errors of immunity, including HLH, and perform functional tests, which can lead to an early diagnosis. The incidence of FHL was estimated as 1 in 50,000–300,000 live births in Japan (13). FHL is a candidate for newborn screening (NS) because it is a life-threatening condition after onset, and early diagnosis and curative treatment can improve its prognosis. Indeed, the polymerase chain reaction-based NS has been reported for FHL3 by *UNC13D* inversion (14). Although further prospective evaluation in NS for FHL may be needed, presymptomatic diagnosis of FHL by NS and subsequent early treatment of patients with a predicted poor prognosis may contribute to better outcomes.

Data availability statement

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

Ethics statement

This study was conducted in accordance with the Helsinki Declaration and approved by the Ethics Committee of Tohoku University (2019-1-561). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin. Written informed consent was obtained from the minor(s)' legal guardian/next of kin for the publication of any potentially identifiable images or data included in this article.

Author contributions

KO, SM, YA, ST, SK, and ME provided clinical information. YT, KM, and MA wrote the manuscript. KM and MA supervised the study and edited the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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

Publisher's note

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Case Report: Crossing a rugged road in a primary immune regulatory disorder

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Over the last decades, Inborn Errors of Immunity (IEI) characterized by an immune dysregulatory picture, isolated or combined with infections, have been increasingly identified and referred as Primary Immune Regulatory Disorders (PIRD). PIRD diagnosis may be difficult due to heterogeneity of time onset, sequence of clinical manifestations and laboratory abnormalities. Moreover, the dissection of a PIRD vs. a secondary immunodeficiency (SID) might be a real challenge since the same indications for immunosuppressant treatments might represent *per se* a PIRD clinical expression. Here we report a female patient with a history of recurrent respiratory and urinary tract infections since early infancy and a diagnosis of Rheumatoid Arthritis in adulthood. After poor response to several biologicals she was treated with Rituximab and sent to immunology referral for a severe hypogammaglobulinemia. Clinical and immunological features matched a diagnosis of common variable immunodeficiency and when IgG replacement therapy and antibiotic prophylaxis were added a good infectious control was obtained. Next generation sequencing analysis has revealed a novel heterozygous VUS in the *IKBKB* gene (c.1465A > G; *p*.Ser489Gly). Functional analysis has shown a reduced capacity of B lymphocytes and CD4 positive T cells in inducing IκBα degradation, with negative impact on NF-kB pathway. Due to recurrent infections attributed to a common condition in childhood and to an exclusive autoimmunity-centered approach in adulthood, both diagnosis and suitable treatment strategies have suffered a significant delay. To reduce the diagnostic delay, pediatricians, general practitioners and specialists should be aware of IEI and the challenges to differentiate them from SID. Furthermore, genetic characterization and functional analysis may contribute to a personalized approach, in a perspective of targeted or semi-targeted therapy.

KEYWORDS

inborn errors of immunity, primary immunodeficiency, secondary hypogammaglobulinemia, rheumatic disease, immune dysregulation

Introduction

Primary Immunodeficiencies, most recently termed as "Inborn errors of immunity" (IEI), refer to a wide group of inherited defects of one or more component of the innate and/or adaptive immune system (1). Susceptibility to recurrent and/or severe infectious diseases that take longer to resolve or need for hospitalization, use of intravenous antibiotics, atypical infections for localization or causing pathogen, historically represent warning signs for IEI (2). On the other hand, infections are common in childhood, so that accurate family and personal history together with anatomical and environmental factors should be considered before the suspicion of an IEI. Over the last decade, it has been increasingly acknowledged that immune-dysregulation, expressed with autoimmunity, hyper/auto-inflammation, granulomas, lymphoproliferative disorders and malignancies, might occur in patients with IEI (3). The term Primary Immune Regulatory Disorders (PIRD) coins a subset of IEI with non-infectious immune-mediated pathology stemming from cellular and molecular mechanisms leading to immune tolerance failure (4, 5). The recognition of molecular mechanisms underlying PIRDs is paving the way for targeted or semi-targeted therapeutic approaches (3). Approximately 20% of IEI patients might suffer from isolated immune dysregulation or combined with infectious recurrence (6). Diagnosis of a PIRD may be cumbersome due to their heterogeneity in terms of time onset, sequence of clinical manifestations and laboratory abnormalities. Not every infectious history or dysregulation is promptly associated with an IEI causing a significant diagnostic delay and a poor outcome. To make the history even more puzzling, some immunological treatments used for the same clinical indications that might be expressed in a IEI/PIRD condition may cause a iatrogenic secondary immunodeficiency (SID) (7). In fact, iatrogenic SIDs have been exponentially reported (8), in tandem with the progressive increase in the incidence of autoimmune diseases and the wider use of standard immunosuppressant drugs (i.e., corticosteroids and DMARDs) as well as biological therapies (bDMARDs) in several specialty settings (i.e., hematological, rheumatological, neurological, etc). These conditions mainly derive from B-cell targeting biologicals, with the anti-CD20 monoclonal antibody rituximab (RTX) as the cornerstone (9). Delayed manifestations of genetic immunological disorders and the increasing recognition of patients with previously undiagnosed IEI receiving biologics, renders the dissection of a IEI vs. a SID extremely challenging. Increasing awareness among the different subspecialties and appropriate investigation will favor early diagnosis as well as optimal treatment for a better outcome and quality of life.

Here we report the case of a currently 47-year-old female patient who suffered with recurrent and severe infections since early infancy and developed in adulthood a Rheumatoid Arthritis with poor response to several biological DMARDs. Immunology referral allowed a clinical diagnosis of Common Variable Immunodeficiency (CVID) and targeted Next Generation Sequencing (NGS) analysis identified a novel heterozygous variant in *IKBKB* gene. This study aims to reinforce the notion that pediatricians, general pratictioners (GPs) and different subspecialties should be aware of the diverse temporality and spectrum of IEI/PIRD disorders and enhance a multidisciplinary approach for continual improvement in the field.

Materials and methods

The work was conducted in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The patient gave her informed consent to perform immunological and genetic analysis. According to best general practice, immunological work-up included the evaluation of serum Ig level by nephelometry, serum IgG subclasses by radial immunodiffusion, T and B cell immunophenotype, specific IgG antibody response to pneumococcus vaccine, autoantibodies, complement C3 and C4 and specific serum IgE. Targeted Next-Generation sequencing.

DNA was extracted by QIAamp DNA Blood Mini Kit (Qiagen) and prepared, sequenced and analyzed according to manufacturer's protocol as previously reported (10). Our CVID custom Ion Torrent panel, including 62 known genes (**Supplementary Table S1**), was designed with Ampliseq Designer software using GRCh38 as references. Sanger sequencing on gDNA isolated from total PBMCs was performed to confirm the presence of mutations in *IKBKB* gene (ABI PRISM 3130, Applied Biosystems, Foster City, CA).

Peripheral blood immunophenotype

All flow cytometric analyses were performed on EDTA blood samples within 24 h of venipuncture. After red blood cell lysis with ammonium chloride, lymphocytes were washed, resuspended in PBS, and stained with the following mouse anti-human antibodies to identify T and B cell subsets: CD45RA (clone T6D11; Miltenyi Biotec), CD3 (clone BW264/56; Miltenyi Biotec), CCR7 (clone 3D12; eBioscience), CD4 (clone OKT4; Becton Dickinson), CD8 PE- (clone RPA-T8; Becton Dickinson), CD19 (clone SJ25C1; Becton Dickinson), CD16 (clone 3G8), CD56, CD27 (clone M-T271, Becton Dickinson), TCR α-beta (clone T10B9; Becton Dickinson), TCR gamma-delta (11F3; Miltenyi Biotec), CD21 (clone B-ly4; Becton Dickinson), CD24 (clone ML5; Becton Dickinson), IgD (clone IA6-2; Becton Dickinson), Goat F(ab)2 anti-Human IgM (μ) (Jackson ImmunoResearch), and CD38 (clone HIT2; Becton Dickinson). Cells were incubated with the appropriate

antibody cocktail for 30 min at 4 °C and then washed with PBS and resuspended in PBS for flow cytometric acquisition. At least 50,000 events were acquired within the lymphogate. Data were acquired on a FACSCanto II (Becton Dickinson) and analyzed with FlowJo software (Tree Star Inc, version 9.3.2).

FACS studies

PBMCs from patient and healthy controls were isolated by density gradient centrifugation on Ficoll-Paque PLUS (GE Healthcare), washed twice in PBS, and maintained in complete RPMI (Sigma-Aldrich) containing 10% FBS, 2 mM l-glutamine (Sigma-Aldrich), and 100 U/ml penicillin and streptomycin (Sigma-Aldrich).

p65 phosphorylation: Total PBMC (300.000 per tube) were stimulated with phorbol 12-myristate 13-acetate (PMA; Sigma, Cat# P1585) /Ionomycin (Ionomycin; Sigma Cat #I0634) at 37 °C for 0, 5, 10, 15 min. Stimulation was blocked and cells were fixed using Fix Buffer I (BD Biosciences, Cat #557870,) for 10 min at 37 °C then centrifuged, washed twice with FACS buffer (PBS supplemented with 2% FBS and 1 mM EDTA) and permeabilized for 10 min on ice with 1 ml Phosflow Perm Buffer III (BD Biosciences, Cat #558050). After two washes, the cells were stained for anti-CD4+, anti-CD19 + antibodies and with anti-phospho-p65 (BD Biosciences, Cat #558422) for 30 min at RT. Samples were washed three times and data were collected on FACSCanto II (Becton Dickinson).

IκBα degradation: Total PBMC (300.000 per tube) were stimulated with phorbol 12-myristate 13-acetate (PMA)/ Ionomycin (PMA 100 ng/ml, Ionomycin 1 μg/ml) at 37 °C for 0, 15, 30, 60 min.

Cells were washed with FACS buffer. Fc receptors (BD Biosciences, Cat#553141) were blocked for 5 min at RT followed by a 20 min of staining on ice with anti- CD4+, CD19 + antibodies and incubated for 30 min at 4 °C. After two washes, cells were permeabilized for 30 min at RT, according to manufacturer's protocol, centrifuged and washed twice with FACS buffer. Cells were stained with anti-monoclonal IκBα (H-4) (Santa Cruz Cat #sc-1643) for 30 min at RT then washed and incubated with anti-Mouse IgG (eBiosciences, Cat# 11-4011-85). After two washes, data were collected on FACSCanto II (Becton Dickinson). All Data were analyzed with FlowJo software (Tree Star Inc, version 9.3.2). Data were analyzed with Graph-Pad Prism, version 6.2 (Graph Pad Software, la Jolla, CA).

Results

Case presentation

A 47-year-old woman affected with Rheumatoid Arthritis (RA) was referred from the Rheumatology outpatient clinic to the Regional Center for Immunodeficiency Diseases at Tor Vergata University Hospital, Rome, Italy for hypogammaglobulinemia and recurrent infections.

Her family history was positive for Hashimoto thyroiditis, psoriasis, hepatocarcinoma and lymphoma. Past medical history revealed an intensive care unit admission at birth for respiratory distress, with non-invasive ventilation until the first month of life. Since early infancy, she suffered from one or two episodes per month of upper respiratory tract infections (URTI) that repeatedly required oral antibiotic treatment and the use of parenteral antibiotic therapy 2–3 times/year. In adolescenthood, in addition to recurrent URTI, she suffered from recurrent lower respiratory tract infections (LRTI) and recurrent urinary tract infections (UTI). At the age of seventeen, after a routine dental procedure, a mandibular osteitis occurred that required surgical curettage. This was complicated by *Staphylococcus aureus* infection with the need of parenteral antibiotic treatment for 3 months. Also, at the age of twenty-two, tonsillectomy offered no improvement of infections (**Figure 1A**).

The patient was diagnosed with RA at the age of 32, with main involvement of hands and feet small joints. After transient and poor response to oral corticosteroids and conventional Disease Modifying Antirheumatic Drugs (DMARDs) such as methotrexate (MTX), leflunomide and cyclosporine, she started anti-TNFα therapy with Etanercept, which was discontinued after 8 months for secondary inefficacy. Other biological- DMARDs were later administered, always in combination with MTX, with poor efficacy. During this time recurrent infections continued, including vaginal candidiasis and HSV1 infections (7–8 times/ year). At the age of 37 she started RTX (2 cycles/year), with good rheumatological response (**Figure 1B**). No immunological investigations had been performed except for a pre-RTX immunoglobulin dosage which showed low IgM (41 mg/dl) with normal IgG and IgA (793 and 170 mg/dl, respectively). Due to further worsening of infections and the observation of severe hypogammaglobulinemia (IgG 383 mg/dl, IgA 50 mg/dl, IgM 12 mg/dl mg/dl), she was referred to our Immunology Unit.

Immunological work-up and IEI diagnosis

As reported in **Table 1**, an extended immunological work-up confirmed the hypogammaglobulinemia (IgG 488 mg/dl, IgA 50 mg/dl, IgM 25 mg/dl) and detected low serum free light chains (kappa sFLC 6.58 mg/L, lambda sFLC 7.45 mg/L). The immunophenotype showed a moderate increase of central memory CD4+ T cells (CD3 + CD4 + CD27 + CD45RA + 60.5%), with a remarkable reduction of effector memory and EMRA CD4 T cells (CD3 + CD4 + CD27-CD45RA- 4% and CD3 + CD4 + CD27-CD45RA + 1.3%, respectively), low naïve CD8 T cells (CD3 + CD8 + CCR7 + CD45RA + 16.7%) with expansion of the effector and terminal memory compartment (CD3 + CD8 + CCR7-CD45RA- 49,8%, CD3 + CD8 + CCR7-CD45RA + 30%).

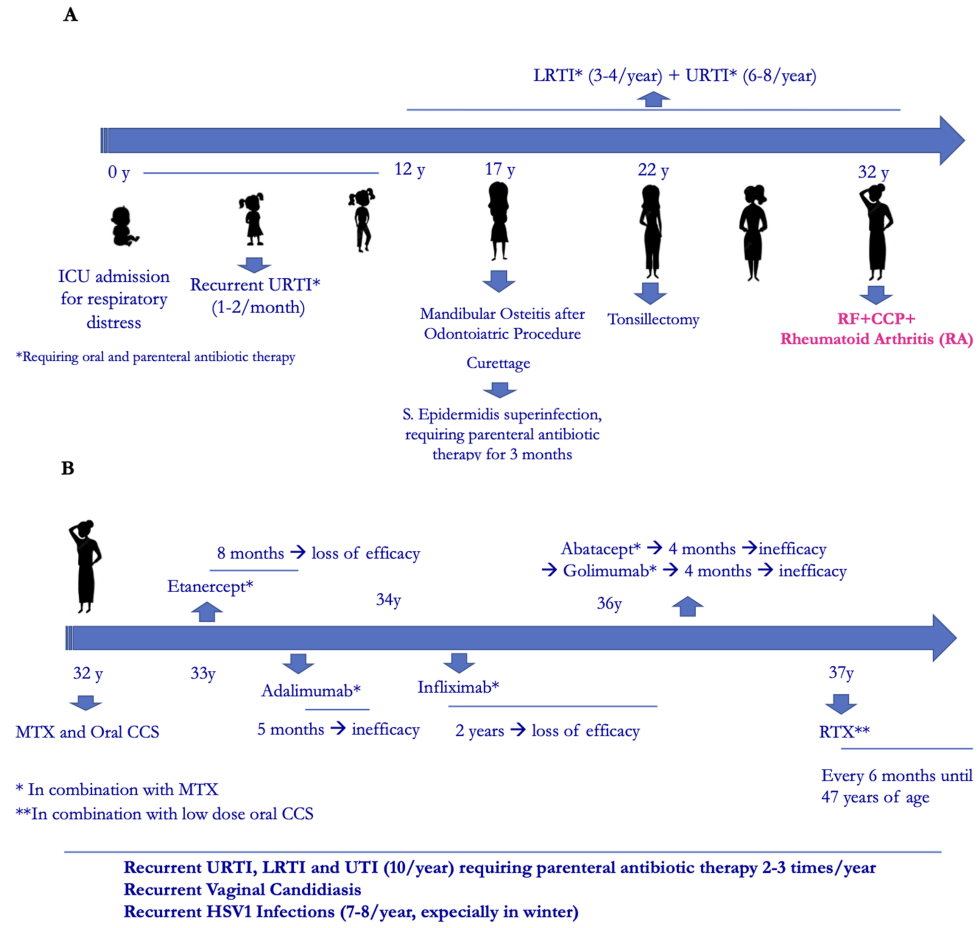

FIGURE 1
(A) Clinical history timeline. (B) Rheumatological treatment timeline.

Despite normal frequencies of B cells, dramatically low switched (CD27 + IgD-IgM- 0.08%) and low IgM memory B cells (CD27 + IgD + IgM + 3.5%) were identified. Based on clinical and immunological results a presumptive diagnosis of CVID was made. Specific antibody response could not be determined since a clinical worsening during an acute infection promptly required immunoglobulin replacement treatment. After the use of RTX, the patient started a treatment with Sulfasalazine and low dose oral corticosteroids in addition to regular IgG replacement therapy, with good control of infections and autoimmunity. She is now doing well with 500 mg/kg/4 weeks of intravenous immunoglobulin (IVIG) replacement therapy (obtaining an IgG level of approximately 10 g/L) and azithromycin prophylaxis.

Genetic characterization and functional tests

Targeting NGS analysis including 62 genes (**Supplementary Table S1**) causing primary antibody defects has been performed. We identified a novel heterozygous variant of uncertain significance (VUS) in *IKBKB* gene (c.1465A > G; *p*.Ser489Gly) leading to the substitution of the conserved serine in position 489 with glycine (SCV002758753 - https://submit.ncbi.nlm.nih.gov/clinvar/). This variant was not found in gnomAD exomes and genomes.

To evaluate its pathogenic role, we investigated the NF-kB pathway. The p65 phosphorylation was investigated in CD4+ T cells and CD19+ B cells at different time points after PMA/Ionomycin stimulation. We observed a delayed and reduced capacity to phosphorylate p65 overtime in CD4+ T cells (**Figure 2A**) and an almost absent response in CD19+ B cells (**Figure 2A**). Further, IκBα degradation was normally regulated in CD4+ T cells and impaired in CD19+ B cells where protein degradation was particularly reduced (**Figure 2B**).

Discussion

Here we present the case of a 47-year-old female patient who suffered with recurrent infections since early infancy, who received a diagnosis of Rheumatoid Arthritis at age 32

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TABLE 1 Immunological data.

	Pt	Range
Total Lymphocytes (cell/mcl)	2,765	1,600–2,400
CD3+ (%)	78.7	61–84
CD4+ (%)	62.9	32–60
CD8+ (%)	14.2	13–40
CD19+ (%)	11.7	10–31
CD16+ (%)	9.6	3–27
Naïve CD4 (CD3 + CD4 + CD27 + CD45RA+) (%)	34.3	31–57
Central Memory CD4 (CD3 + CD4 + CD27 + CD45RA+) (%)	60.5	10–27
Effector Memory CD4 (CD3 + CD4 + CD27- CD45RA-) (%)	4	12–44
EMRA CD4 (CD3 + CD4 + CD27-CD45RA+) (%)	1.3	4–12
Naïve CD8 (CD3 + CD8 + CCR7 + CD45RA+) (%)	16.7	18–61
Central Memory CD8 (CD3 + CD4+ CCR7 + CD45RA+) (%)	3.6	3–12
Effector Memory CD8 (CD3 + CD4+ CCR7- CD45RA-) (%)	49.8	25–58
EMRA CD8 (CD3 + CD4+ CCR7-CD45RA+) (%)	30	5–20
CD19 + CD27 + IgD + IgM + (%)	3.57	4–12
CD19 + CD27 + IgD−IgM− (%)	0.08	4–16
CD21low (%)	0.4	0–6
Transitional (%)	13.7	5–15
IgG (mg/dl)	488	604–1,909
IgA (mg/dl)	50	61–301
IgM (mg/dl)	25	59–297
Kappa sFLC (mg/L)	6.58	6.7–22.4
Lambda sFLC (mg/L)	7.45	8.3–27

but refractory to several biological DMARDs before she was treated with Rituximab. In the setting of a worsening recurrence of infections, hypogammaglobulinemia was observed, and an extensive immunological work-up allowed a clinical diagnosis of Common Variable Immunodeficiency (CVID) and genetic analysis identified a novel heterozygous variant in *IKBKB* gene.

Infectious recurrence, mainly involving the respiratory tract, represent an extremely common feature during pediatric age (11) with approximately 10%–20% of children <10 years of age suffering from recurrent respiratory tract infections, requiring antibiotic treatment and, in some cases, hospitalization (12). Many children with IEI conditions may remain undiagnosed or misdiagnosed for a longtime since infections might be underestimated and each infection treated as an isolated episode while the underlying cause and appropriate investigations are missed. IEI may present at any age and with a variable clinical presentation. Approximately 25%–30% of patients with IEI may suffer with manifestations of immune dysregulation, isolated or with coexisting infections (6). Autoimmune cytopenias, rheumatologic diseases and inflammatory bowel disease as more common. In some cases, the evolution of additional medical problems complicate matters and unveiling a PIRD condition may be a daunting task. PIRD patients require a multispecialistic approach but, since the autoimmune manifestation might appear as predominant (6), these patients are often at first evaluated by non-IEI addicted specialists who might tend to manage the immune dysregulation with no vigilance on a potential underlying PIRD condition. Therefore, it's not unusual that IEI diagnosis is delayed. Also, the infectious recurrence as a potential warning sign for an IEI, may be misinterpreted as a common complication of the extended use of a wide range of biologicals/immunosuppressant treatments. Additionally, prolonged B cell deficiency have been documented in 30%–56% of patients receiving B-cell targeting agents such as RTX for the treatment of B cell lymphoproliferative diseases and autoimmune disorders (8, 13–19). Although the precise role of RTX is not easily discernible due to the heterogeneity of previous treatments, a significant increase in the percentage of patients developing isolated or combined immunoglobulin deficiency over RTX treatment has been observed for all three isotypes. Some of these patients present symptomatic hypogammaglobulinemia and require immunoglobulin replacement to prevent infectious complications (20). In our patient, at pre-RTX use IgM deficiency was detected while some years post-RTX a severe hypogammaglobulinemia occurred. In a previous study we have reported that persistent hypogammaglobulinemia after RTX may occur in a subset of children with autoimmune cytopenia, but this should not always be interpreted as iatrogenic secondary hypogammaglobulinemia since it may unveil an IEI disorder (13). Several studies have evaluated the presence of post-RTX hypogammaglobulinemia in patients with autoimmune disorders, including rheumatologic disease (14, 21–23). An imbalance between naïve and memory B cells with low switched memory B cells may mimic a CVID condition and establishing the pathogenetic role of B cell perturbation beyond a pure iatrogenic effect may be challenging, however this is not only necessary but dutiful (23).

Rheumatologic diseases are more frequently observed in patients with IEI, especially in female adults with CVID (24). However, it is not unusual to make a diagnosis of rheumatologic disease before IEI diagnosis. In our patient, either during infancy and in adulthood, a comprehensive care of the patient missed evaluation and eventually reevaluation of the patient's immune system over time for a proper and timely management.

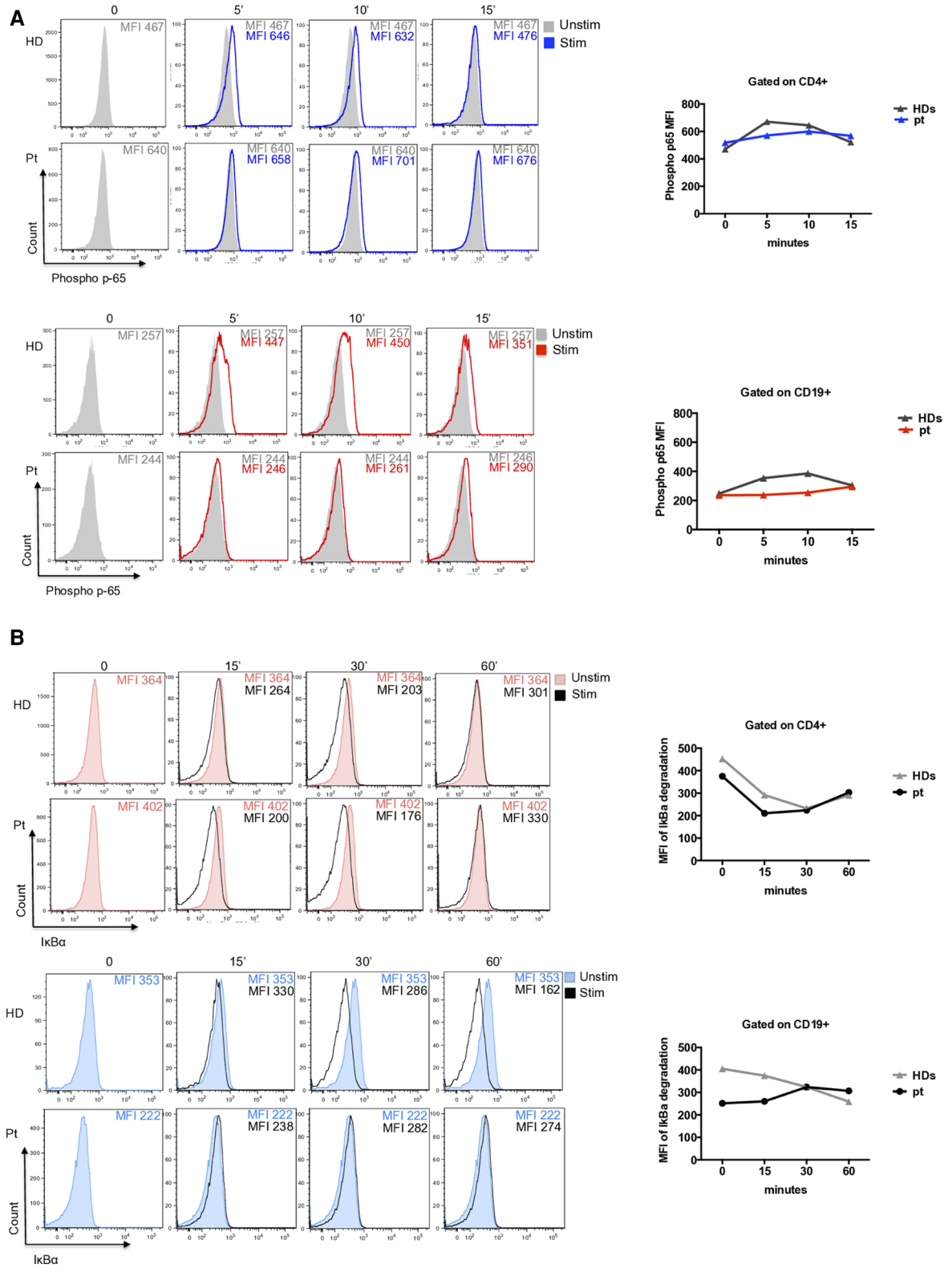

FIGURE 2
(A) Representative histograms of phospho-p65 expression in CD4+ T cells (blue histograms) and CD19+ B cells (red histograms) from patient and unrelated control. Graphs show the mean of two independent experiments. (B) Representative histograms of IκBα degradation (a reduction of IkBa expression is showed due to degradation) in CD4+ T cells (red filled) and CD19+ B cells (blue filled) from patient and an unrelated control. Graphs show the mean of two independent experiments.

Among PIRD-related genetic variants found in cohorts of rheumatologic patients with hypogammaglobulinemia, both monoallelic variants in NF-kB subunits deficiency and post-translational modification of NF-kB pathway proteins have been detected. These could be responsible for either immunodeficiency, autoimmune diseases (RA, SLE and inflammatory bowel diseases), or both. *IKBKB* gene is implicated in NF-κB transcription signaling and particularly in the activation of the canonical NF-κB pathway, which is relevant for lymphocyte activation, homeostasis and control of self-tolerance. Upon receptor ligation, signals induce activation of the IKK complex, which includes the kinases IKKalpha, IKKbeta and NEMO that are encoded by *IKBKA*, *IKBKB* and *IKBKG* genes, respectively. Then, phosphorylation of the inhibitory protein IκBα allows release of NF-κB molecules p65 (RelA), c-Rel, and p50 to the nucleus that act as transcription factors with induction of a multitude of pro-inflammatory cytokines (i.e., IL-1, IL-2, IL6, IL8, IL12 and TNFα), chemokines (i.e., CXCL1 and CXCL10) and other inflammatory mediators (i.e., adhesion molecules as ICAM-1, VCAM-1, ECAM-1, anti-apoptotic factors as Fas, BCL-2, Caspase, BFL-1 and cell cycle regulator as PAI2 and Cyclin) (25, 26). Thus, it is not surprising that IKK/NFKB aberrations can cause a variety of immune-related disorders.

In our patient, we identified a novel heterozygous variant of uncertain significance (VUS) in *IKBKB* gene (c.1465A > G; *p*.Ser489Gly), in a strong conserved serine in position 489. Functional analysis revealed a strongly reduced capacity to phosphorylate the p65 overtime in CD4+ T cells and an almost absent response to stimulation in CD19+ B cells. Further, IκBα degradation was normally regulated in CD4+ T cells and impaired in CD19+ B cells. Whether this patient's functional deficiency is due to the underlying genetic defect or, alternatively, directly induced by RTX and other immunosuppressive treatments is a matter of concern. The use of immunosuppressive drugs as CCS and RTX overtime, could affect NF-kB signaling and alter T and B cell homeostasis. Only *in vitro* experiments using transducing vectors might confirm the pathogenicity of this variant.

In this scenario, it goes without saying that increasing awareness of pediatricians and other specialists who take care of these patients is warranted for an early immunology referral to prompt diagnosis and timely treatment with significant prognostic and socio-economic implications for the patient, family and community. The wider use of new laboratory-based genetic technologies and functional immune studies are leading to a better understanding of IEI/PIRDs pathophysiology. In IEI-addicted immunology centers, molecular and functional characterization of patients are started simultaneously with PIRD suspicion and with the initiation of first-line treatment for immune dysregulation. Molecular characterization of each distinct PIRD patient may expedite the therapeutic approach, by the use of immune-modifying biologicals directly targeting the altered immune pathway. This is crucial to optimize treatment efficacy and minimize possible adverse events (21). Conversely, in non-IEI addicted setting, molecular and functional characterization are explored after first and second lines of treatment (27). Thus, patients receiving RTX and/or other immunosuppressants should not exclusively regarded as affected with a iatrogenic immunodeficiency since a higher degree of suspicion for a previously undiagnosed IEI is warranted when atypical clinical and immunological features occur. In these cases, a prompt immunological referral and work-up, i.e., extensive B cell immunophenotype, Ig isotypes, vaccine responses, free serum light chains as well as evaluation and function of candidate genes by targeted NGS or whole exome/genome sequencing is recommended (10, 20). Growing knowledge on the molecular mechanisms sustaining immune dysregulation will be extremely beneficial for applying precision medicine and for continual translational progress in the field (28).

Conclusions

In conclusion, this case highlights the importance of an early suspicion for IEI disorders since childhood and of clinical and immunological reevaluations over time for a correct interpretation of more complicated courses. Collaborations among pediatricians, expert clinicians, geneticists, are required to expand the spectrum of diagnosed IEI disorders and benefit those patients who present manifestations spanning multiple disease domains. Targeted or semi-targeted treatment options might control or balance altered immune signaling pathways, however further studies are needed to highlight the off-target effects of biologics.

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 below: https://www.ncbi.nlm.nih.gov/ - SCV002758753.

Ethics statement

The studies involving human participants were reviewed and approved by Policlinico Tor Vergata Ethics Committee, Viale Oxford 81, 00133 Rome, Italy. The patients/participants provided their written informed consent to participate in this study.

Author contributions

VM and GDM conceived and designed the study and revised the work critically for intellectual content. VM, MS, EDD, SG, BK provided care of the patient, clinical samples and clinical data. MS wrote the initial draft. VF and GDM performed tNGS experiments and contributed to data interpretation. MB provided genetic counselling. GDM, CC, SDC performed experiments and analyzed the data. MS, CC, SDC, SG contributed to the study design and data interpretation. VM, MS, GDM, CC, SDC contributed to writing the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2022.1055091/full#supplementary-material.

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GATA2 deficiency detected by newborn screening for SCID: A case report

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The early diagnosis and treatment of inborn errors of immunity (IEI) is crucial in reducing the morbidity and mortality due to these disorders. The institution of newborn screening (NBS) for the diagnosis of Severe Combined Immune Deficiency (SCID) has decreased the mortality of this disorder and led to the discovery of novel genetic defects that cause this disease. GATA2 deficiency is an autosomal dominant, pleiotropic disease with clinical manifestations that include bone marrow failure, monocyte and B cell deficiency, leukemia, pulmonary alveolar proteinosis and lymphedema. We present the case of an infant identified by newborn screening for SCID due to GATA2 deficiency.

KEYWORDS

GATA2, T cell receptor excision circles, severe combined immunodeficiency, hematopoietic stem cell transplant, inborn errors of immunity

Introduction

Severe combined immune deficiency (SCID) is a primary immune deficiency characterized by a profound deficiency in T cells and variable deficiencies in B and NK cells that is fatal if not detected and treated early in life (1). To date, more than 20 different genetic defects have been identified, thought to comprise approximately 90% of the causative defects in SCID (2). Early diagnosis and treatment of SCID before the acquisition of infections improves survival. Newborn screening for SCID using the T cell receptor excision circles (TRECs) assay has been implemented throughout the United States and several other countries. TRECs are a validated biomarker for newly formed naive T cells, which are decreased in all genetic causes of SCID and other causes of T cell lymphopenia. In numerous studies, the TREC assay has been shown to be a very sensitive assay to screen for SCID (3).

GATA2 deficiency syndrome has a wide phenotype including immunodeficiency, cytopenias, bone marrow failure and leukemia. The GATA family consist of 6 transcriptional factors that regulate gene expression by binding to the DNA motif GATA and other transcription factors *via* two zinc finger domains. Of the 6 GATA proteins, GATA1 and GATA2 play critical roles in hematopoiesis with GATA2 being a key transcriptional regulator required for the development and maintenance of a healthy stem cell pool (4, 5). Hematological manifestations of GATA2 deficiency include a range of peripheral cytopenias of which the most common are profound cytopenias of B-cells, NK cells, and monocytes, that tend to be progressive with loss of bone marrow progenitor populations over time and a tendency toward clonal hematopoiesis (6–8).

Evaluation of families with symptomatic relatives, have shown that the development of cytopenias is progressive and that there is partial penetrance with the identification of

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TABLE 1 Patient's complete blood counts with age specific normal values are given on parenthesis. Trend over time.

	7 days old	15 days old	1 month old	3 months old	4 months old	5 months old	6 months old
RBC (10*6/μl)	4.41 (4.1–6.7)	3.99 (4.1–6.7)	3.69 (3.8–5.4)	4.33 (3.8–5.4)	4.26 (3.8–5.4)	4.7 (3.8–5.4)	4.89 (3.8–5.4)
Hemoglobin (g/dl)	18.3 (15–24)	16 (15–24)	13.3 (10.5–14)	13.4 (10.5–14)	12.5 (10.5–14)	13.1 (10.5–14)	12.9 (10.5–14)
MCV (fl)	134 (99–115)	126 (99–115)	108 (72–88)	90 (72–88)	84 (72–88)	82 (72–88)	79 (72–88)
Platelets (10*3/μl)	84 (150–450)	150 (150–450)	610 (150–450)	608 (150–450)	572 (150–450)	540 (150–450)	562 (150–450)
WBC (10*3/μl)	5.6 (5–21)	5.0 (5–21)	3.4 (6–14)	2.0 (6–14)	4.1 (6–14)	3.6 (6–14)	5.2 (6–14)
Absolute neutrophils	3.2 (1.4–13.4)	2.1 (1.4–13.4)	1.8 (1.3–6.7)	1.3 (1.3–6.7)	3.2 (1.3–6.7)	2.8 (1.3–6.7)	4.4 (1.3–6.7)
Absolute lymphocytes	0.2 (1.8–9.7)	0.7 (1.8–9.7)	0.6 (2.5–9.9)	0.4 (2.5–9.9)	0.3 (2.5–9.9)	0.4 (2.5–9.9)	0.1 (2.5–9.9)
Absolute Monocytes	1.7 (0.7–1.9)	1.9 (0.7–1.9)	0.8 (0.7–1.9)	0.3 (0.7–1.9)	0.4 (0.7–1.9)	0.3 (0.7–1.9)	0.4 (0.7–1.9)

phenotypically normal patients amongst these family cohorts (7). The age of clinical onset is usually during childhood or early adolescence, and the penetrance is estimated at 90% by the age of 60 years (7). Herein, we describe a newborn infant presenting with undetectable TREC in her newborn screen that was found to have severe persistent T cell lymphopenia during the first 6 months of age secondary to GATA2 deficiency.

Case description

An 8-day old female is referred to immunology clinic with an abnormal newborn screen reporting undetectable TRECs. Complete blood count (CBC) reported red blood cell macrocytosis for age, thrombocytopenia, and severe lymphopenia (**Table 1**). Lymphocyte immunophenotyping by flow cytometry showed markedly reduced naïve T cells, low/absent B cells and decreased NK cells (Absent bright CD56 cells) consistent with SCID phenotype (**Table 2**). She was started on Ig replacement therapy (SCIG), antimicrobial prophylaxis with acyclovir, fluconazole, and Trimethoprim-Sulfamethoxazole at 1-month of age. Thrombocytopenia resolved by 2-weeks of age.

Her initial work up included molecular testing for SCID and T cell disorders (*ADA, CD3, CD45, DCLREIC, FOXN1, IL2RG, IL7R, JAK3, LIG4, NHEJ1, ORA1, RAG1/2, RMRP, STAT5B, STIM1, TBX1, and ZAP70*), telomere length measurement, and flow cytometry-based mitogen testing which were all normal. A limited exome that includes >4,800 genes (Trusight, Illumina) was performed and demonstrated a known heterozygous pathogenic variant in GATA2 (p.Thr354Met, c.1061C > T), which was confirmed by Sanger sequencing. Her pattern of lymphopenia of B cells and NK cells, followed by progressive monocytopenia for age, was consistent with GATA2 deficiency. Parental evaluation demonstrated that the mother harbored the same mutation, and even though, mother has not presented with any clinical manifestations of disease, she was noted to have 1.7% CD56 bright cells which is below published normal (9).

Complications during the first 8 months of life included medication induced elevated transaminases requiring discontinuation of fluconazole prophylaxis, chronic diarrhea with a negative infectious work up that self-resolved after 1 month, and an uncomplicated *Klebsiella oxytoca* urinary tract infection. Bone marrow aspirate and biopsy at 7 months of age revealed a mildly hypocellular marrow for age (80% cellularity) with no dysplasia, no excess blasts, negative FISH panel for MDS and normal cytogenetics. Serial monitoring of lymphocyte immunophenotyping continued to show severe T cell, B cell and NK cell cytopenias that fitted a phenotypic picture of severe combined immunodeficiency (**Table 2**). Since cytopenias did not recover and worsen over time (specifically T cells), the decision was made to proceed with hematopoietic stem cell transplant (HSCT) before she developed life threatening infections.

At 8 months of age, she received a matched unrelated donor bone marrow transplant (male, 34 y old, 10/10 HLA matched, CMV matched) following myeloablative conditioning with busulfan, fludarabine and equine anti-thymocyte globulin (ATG). She engrafted with 100% donor chimerism. She was able to be weaned off SCIG 7 months post-transplant and started vaccinations 2 months after SCIG was discontinued with evidence of seroconversion for vaccine induced antibodies for pneumococcus and *Haemophilus influenzae* type B. Immunosuppression was discontinued at 10 months post-transplant, and she was off prophylactic antimicrobials after 1 year.

Two and a half years after transplant she was diagnosed with Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph + ALL) that was found to be donor derived. She was treated with 4 drug induction chemotherapy per COG protocol AALL1131 and imatinib followed by consolidation with blinatumomab. She achieved complete remission. Two years and 9 months after her initial transplant, she received a second allogeneic HSCT, this time a haploidentical transplant using her father as a donor. The myeloablative conditioning regimen consisted of total body irradiation, thiotepa, cyclophosphamide, and ATG, with $\alpha\beta$ T-cell and CD19 cell depletion as graft vs. host disease (GVHD) prophylaxis. She engrafted with 100% donor chimerism. She has mild, chronic skin GVHD but is currently 13 months post-second transplant and doing well (**Figure 1**). The patient's donor derived leukemia case has been reported elsewhere (10).

Discussion

Since the recognition of GATA2 deficiency as the underlying cause of several clinical entities formerly known as MonoMAC syndrome, familial myelodysplastic syndrome (MDS)/acute myeloid leukemia (AML), DCML deficiency (dendritic cell, monocyte, B,

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TABLE 2 Patient's lymphocyte immunophenotyping given in absolute counts. Trend over time.

Age	ALC/mm^3	CD3/mm^3	CD4/mm^3	CD8/mm^3	CD19/mm^3	CD56/mm^3	CD45RA%	CD45RO%
7 days old	224	105	52	24	2	35	54	24
15 days old	450	270	90	171	0	31	6	0
1 months old	575	334	167	167	6	207	4	2
3 months old	380	304	171	137	4	57	64	0
4 months old	164	121	67	52	2	10	58.8	1.97
5 months old	180	148	81	67	2	18	52	1.01
6 months old	208	144	89	54	2	21	60.55	3.79

and NK lymphoid deficiency), and Emberger syndrome, a lot has been learned about this genetic defect, and currently it is recognized as a frequent genetic cause for bone marrow failure and immune disorders in children and young adults. Deletions, mutations in regulatory regions, frameshift mutations, and substitutions have been described within the GATA2 locus without clear evidence of genotype-phenotype correlation. The clinical phenotype is highly variable and include asymptomatic carriers to early development of clinically significant cytopenias that evolve to MDS. The lack of genotype-phenotype correlation is highlighted in our patient and her mother, both with the same mutation and significantly different phenotypes, with her mother being asymptomatic to date. However, based on population studies that have reported unaffected individuals carrying GATA2 mutation into their fifth and sixth decades of life with a lifetime risk of MDS of approximately 90%, close monitoring of her mother is warranted (5, 7, 11–13).

The evolution of cytopenias in GATA2 deficiency syndrome has been described using symptomatic patients with DCML deficiency, and even though T cell cytopenias have been described, the profound and persistent T cell lymphopenia from birth in our patient is unusual and to our knowledge this early finding has not been described in GATA2 deficiency before (3, 7, 8). It is unclear if this presentation is simply a variable presentation of GATA2 deficiency, or if there are other genetic modifiers that may have affected these results. There were no clear defects that affect T cell development in the sequencing panel, but this possibility cannot be ruled out.

Early identification of children with inborn error of immunity (IEI) and its molecular constitution has paved the way for pre-symptomatic treatment and improved quality of life. With this goal in mind the TRECs assay was added to the NBS since 2008 and implemented in all 50 states by 2018 (3). The TRECs assay detects not only SCID but any condition causing low naïve T-cell counts, as described on our patient. **Table 3** summarizes IEI other than SCID that have been diagnosed using NBS (3, 14). Several genetic panels are commercially available for the diagnosis of IEI that can usually apply to patients with certain clinical phenotypes or abnormal NBS. With a growing number of identifiable genetic mutations associated with specific phenotypes, panels may miss previously unidentified genes associated with disease. Next generation sequencing has become more affordable and available for clinical practice over the last several years, and whole-exome sequencing and whole-genome sequencing are becoming important diagnostic tools for identification of IEI. The ability to screen for genetic variants in many genes is important, as a variety of clinical and immunological phenotypes may result from mutations in a single gene (genetic pleiotropy), or mutations in multiple genes can underlie the same phenotype (genetic heterogeneity). In the case of our patient, initial negative molecular testing for SCID led to next generation sequencing and detection of the GATA2 variant. It was decided to use this diagnostic method to confirm the diagnosis, which proved to be a cost-effective approach for our group (15, 16).

The genetic diagnosis of our patient allowed the identification of carrier status of her mother. Even though she continues to be asymptomatic to date, close monitoring is warranted since prior

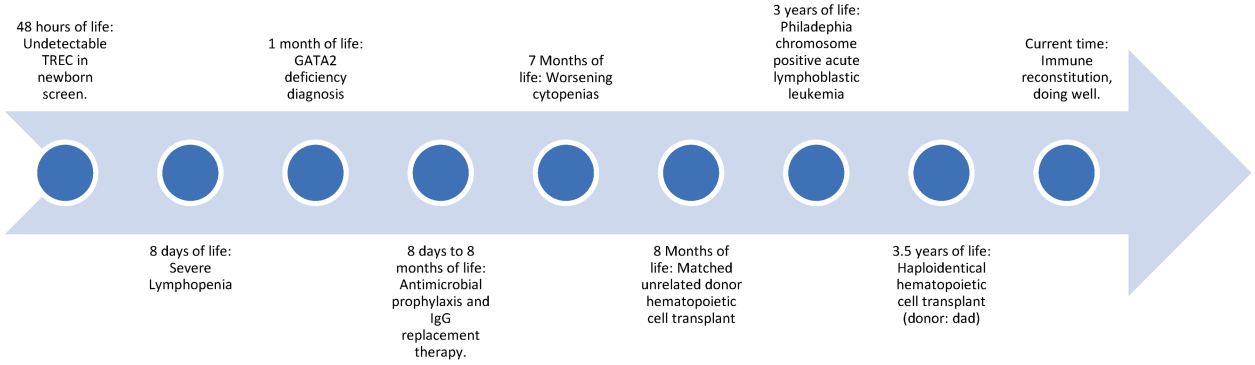

FIGURE 1
Timeline of events of patient described.

TABLE 3 Causes of low TREC detected on newborn screen.

Severe combined immunodeficiency
• Typical • Leaky (Including Omenn Syndrome)
Syndromes
• DiGeorge • Trisomy 21 • Ataxia-Telangiectasia • CHARGE • Jacobsen syndrome • Barth syndrome • GATA2 deficiency
Secondary
• Congenital heart disease • Congenital gastrointestinal malformations: gastroschisis, intestinal atresia, meconium ileus • Hydrops

Table adapted from Currier R, Puck JM. SCID newborn screening: What we've learned. *J Allergy Clin Immunol*. 2021 Feb;147(2):417–426.

studies have demonstrated a high penetrance by 60 years of age. Early manifestation of disease can be subtle and so specific testing for B and NK cell by flow cytometry may be warranted (16, 17).

The clinical presentation of our patient adds to the wide range of clinical manifestations of GATA2 deficiency, and suggests how a stepwise, multidisciplinary diagnostic approach can aid in timely diagnosis and management for infants with severe T-cell lymphopenia. There is much more to learn about GATA2 deficiency and its interaction with additional genetic or environmental factors, that predisposes them to severe complications even after curative therapies (10, 18–20).

Conclusion

The presence of undetectable TRECs is an excellent method to identify patient with inborn errors of immunity beyond SCID and genetic testing, tailored to the center clinical expertise level, should follow negative molecular testing for SCID to provide prompt diagnosis and management of 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/s.

Author contributions

JV, JR, JT, and LB: took part in patient care including analysing and interpreting patient data. AEV: had the primary responsibility of preparing the manuscript under the guidance of AR. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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

Publisher's note

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Case report: Primary immunodeficiency due to a novel mutation in CARMIL2 and its response to combined immunomodulatory therapy

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Capping protein regulator and myosin 1 linker 2 (CARMIL2) is necessary for invadopodia formation, cell polarity, lamellipodial assembly, membrane ruffling, acropinocytosis, and collective cell migration. CARMIL2 deficiency is a rare autosomal recessive disease characterized by dysfunction in naïve T-cell activation, proliferation, differentiation, and effector function and insufficient responses in T-cell memory. In this paper, we report a 9-year-old female patient with a novel pathogenic variant in CARMIL2 (c.2063C > G: p.Thr688Arg) who presented with various symptoms of primary immunodeficiencies including recurrent upper and lower respiratory infections, perioral and perineum papules, reddish impetiginized atopic dermatitis, oral ulcer, painful urination and vaginitis, otitis media, and failure to thrive. A missense mutation leading to insufficient CARMIL2 protein expression, reduced absolute T-cell and natural killer cell (NK cell) counts, and marked skewing to the naïve T-cell form was identified and indicated defective maturation of T cells and B cells. Following 1 year of multitargeted treatment with corticosteroids, hydroxychloroquine, mycophenolate mofetil, and thymosin, the patient presented with significant regression in rashes. CD4+ T-cell, CD8+ T-cell, and NK cell counts were significantly improved.

KEYWORDS

primary immunodeficiency, novel mutation, combined immunotherapy, whole exome sequencing (WES), CD4+/CD8+ lymphocytes

Introduction

Capping protein regulator and myosin 1 linker 2 (CARMIL2) belongs to the human CARMIL family and encodes a 1,435 amino acid protein. It has been reported that CARMIL2 is necessary for invadopodia formation, cell polarity, lamellipodial assembly, membrane ruffling, acropinocytosis, and cell migration (1–3). CARMIL2 acts as a molecular link between vimentin filaments and dynamic actin assembly (1, 4, 5) and promotes actin polymerization at the leading edge of migrating cells (1, 6). In addition, CARMIL2 is involved in CD28-mediated T-cell costimulation and activation (5, 7–11).

CARMIL2 deficiency is a rare autosomal recessive disease characterized by dysfunction in naïve T-cell activation, proliferation, differentiation, and effector function and insufficient responses in T-cell memory (5, 8, 11, 12). The clinical manifestations of CARMIL2 deficiency are recurrent infections (mainly respiratory). Skin features include skin warts, verrucous papules, eczematous dermatitis, psoriatic rash, seborrheic dermatitis, recurrent condyloma, solar urticaria, and spongiotic dermatitis. Patients with CARMIL2 deficiency are sometimes prone to Epstein–Barr virus (EBV)-related smooth muscle tumors. Other clinical features include lymphocytic esophagitis, dysphagia, Crohn's disease, and failure to thrive. The clinical presentations of CARMIL2 deficiency that have been previously reported are summarized in **Supplementary Table S1** (5, 10, 12–20).

In this paper, we report a 9-year-old female patient, born to Chinese consanguineous parents, who presented with recurrent respiratory infections, persistent dermatitis, recurrent skin abscess, oral ulcer, otitis media, and failure to thrive. Whole exome sequencing was performed in samples from the girl and her parents and revealed an unreported missense variant in the CARMIL2 gene (NM_001013838.2:c.2063C>G: p.Thr688Arg) that was predicted to be deleterious to the patient. A heterozygous mutation in CARMIL2 was also identified in each parent, who were asymptomatic carriers. By analyzing the clinical manifestations and immunological characteristics of this patient, recognition of CARMIL2 deficiency is expanded, underscoring the importance of consideration of molecular causes in patients with primary immunodeficiencies (PID).

Materials and methods

Clinical case

The patient and her family members were recruited from Shanghai Children's Medical Center. Gastroenterological endoscopy was performed in July 2020 to evaluate the patient's gastrointestinal inflammation in the Digestive Department, and a lung biopsy was performed in August 2020 in the General Surgery Department due to the patient's recurrent respiratory infections. The patient was diagnosed with PID based on genetic analysis and treated in the Rheumatology Department, and therapeutic efforts were observed for2 years, starting in 2020.

Genetic workup

Genomic DNA was extracted from peripheral blood isolated from the patient and her family members to perform exome sequencing, following standard instructions. The whole exome sequencing was performed at the Clinical Molecular Diagnostic Laboratory at Shanghai Children's Medical Center.

Western blotting

Proteins in blood samples taken from the patient and her family members were extracted and analyzed by Western blotting using standard protocols. An anti-CARMIL2 antibody (NBP2-62215; EM-53 NOVUS, United States) was used to detect CARMIL2 protein levels, and antibeta actin (mAbcam 8226; Abcam, United States) was used as a loading control. Results were analyzed using FlowJo V10.

3D modeling

A three-dimensional homology structure of the leucine-rich repeat (LRR) domain was modeled using SWISS-MODEL software.

Flow cytometry

Peripheral blood mononuclear cells (PBMCs) were incubated with directly labeled antibodies to detect cell surface proteins. 4′6-Diamidino-2-phenylindole (DAPI) was used to exclude nonviable cells. Positive staining was considered based on the negativity of isotype control. Antibodies used included antihuman CD4 FITC (BioLegend, United States), antihuman CD8 Percp-cy5.5 (BioLegend, United States), antihuman CD45 RA-PE (BioLegend, United States), antihuman CCR7 BV421 (BioLegend, United States), antihuman CD19 Percp-cy5.5 (BioLegend, United States), antihuman CD27 PE-cy7 (BioLegend, United States), and antihuman IgD FITC (BioLegend, United States).

Ethical considerations

Clinical information and biospecimens from the patient and her family members were obtained upon written consent.

Results

Clinical features of the patient

The patient was a 9-year-old girl, born to Chinese consanguineous parents, who presented with recurrent and intermittent upper and lower respiratory infections since early childhood. Her childhood respiratory infectious problems greatly improved with age. The patient complained of

recurrent perioral and perineum asymmetrical erythematous papules and oral ulcers since 2018, which were alleviated by usual treatment. In addition, she had recurrent symptoms of frequent, urgent, and painful urination along with vaginal discomfort, which was diagnosed as recurrent urethritis and vaginitis. In addition, she has erythematous impetiginized atopic dermatitis that was widespread on her hands, fingers, and feet, perionyxis on her fingers and toes, and plantar heel pain. The cutaneous and mucosal infections were associated with multiple symptoms, including a hoarse voice, otitis media, and short stature with a height of less than 95% since childhood.

Because of the recurrent skin and mucosal inflammation, immunodeficiency or autoinflammatory diseases and autoimmune diseases were considered possible diagnoses. Thus, impetiginized atopic dermatitis, contact dermatitis, autoimmune pemphigus, or other immunodeficiencies were considered. Behcet's disease was also considered, given the patient's history of recurrent oral ulcers. Immunophenotyping of the patient demonstrated decreased absolute T-cell, B-cell, and NK cell counts but normal regulatory T-cell counts initially. Allergen testing revealed no allergens. The immunologic evaluation included measurements of immunoglobulin levels, antinuclear antibodies, and anti-double-stranded DNA (anti-dsDNA), all of which were initially negative. In addition, EBV-DNA and cytomegalovirus (CMV)-DNA quantity was within normal limits, excluding EBV or CMV infection. Gastrointestinal endoscopy showed evidence of mild chronic colitis with a scattered accumulation of eosinophils. Interstitial lung vasodilation and congestion were observed with an accompanying infiltration of a large number of scattered and focal inflammatory cells in the lung biopsy sample taken in August 2020. Acute inflammatory cells and tissue cells were also observed in the bronchioles. The patient had an older sister that died at a young age due to a fatal respiratory infection and two healthy siblings (**Figure 1A**).

The patient was treated with corticosteroids, hydroxychloroquine, mycophenolate mofetil, and thymosin for 2 years. The initial dose of prednisone was 15 mg per day (0.6 mg/kg) in July 2020, and hydroxychloroquine (0.1 g per day) and thymosin (1.6 mg biw) were used at the same time. Under this multitargeted treatment, many of her clinical symptoms were relatively improved. The most significant changes included the regression of rashes, especially perioral and perineum rashes, and the improvement of otitis media. Dosages of prednisone were gradually reduced to 2.5 mg every other day in July 2021. However, the patient still suffered from an intermittent cough and recurrent oral ulcer. In addition, the alveolar septal fibrosis caused by recurrent lung infections was not significantly improved. Considering the frequent recurrence of oral ulcers and intermittent cough, the patient had to take 5 mg of prednisone every other day in August 2021, and the dose gradually increased to 10 mg per day in November 2021. Meanwhile, mycophenolate mofetil (250 mg bid) was also given. The patient has been treated with prednisone (7.5 mg per day) and hydroxychloroquine (0.1 g per day) to prevent the recurrence since August 2022.

Genetic workup

To exclude the possibility of genetic diseases, whole exome sequencing was performed on the patient and her parents, which revealed an unreported homozygous missense variant in CARMIL2 (NM_001013838.2:c.2063C>G:p.Thr688Arg) that was predicted to be deleterious in the patient. Both parents were asymptomatic carriers of the heterozygous variant of CARMIL2. The missense CARMIL2 variant found in the patient was located 1-bp downstream of an LRR16 domain spectrum and had not been previously reported in the general population variant databases (**Figure 1B**, **Supplementary Table S1**). The mutation was also highly conserved between species (**Figure 1C**).

Identification of the unreported CARMIL2 missense variant

To explore the effects of the missense variant in the CARMIL2 gene on mRNA and protein levels, PBMCs were isolated from the patient and her heterozygous family members.

Western blot results showed that serum CARMIL2 protein levels were significantly lower in the patient compared to protein levels in the patient's parents and younger sister (**Figure 2A**), indicating that the missense variation in the CARMIL2 gene results in insufficient expression of CARMIL2.

3D homology modeling of the structure of CARMIL2

The CARMIL2 protein consists of an N-terminal noncanonical pleckstrin homology (PH) domain, an LRR domain, a helical homodimerization domain (HD), an extended intrinsically disordered region that contains the capping protein-binding region (CBR), and a proline-rich domain (PRD) that interacts with the SH3 domains of class-I myosin (4, 21) (**Figure 1B**). The LRR domain is divided into 16 parts (6) that are necessary and sufficient for localization to vimentin. The LRR domain structure consists of repeating regions with a β-strand-turn-α-helix structure and a horseshoe sharp with a solvent-accessible concave interior surface made of parallel β-strands and a convex exterior surface made of an array of α-helices (8, 21, 22). The CBR is composed of two conserved motifs, the capping protein (CP) interaction (CPI) motif (23), which has the ability to decrease

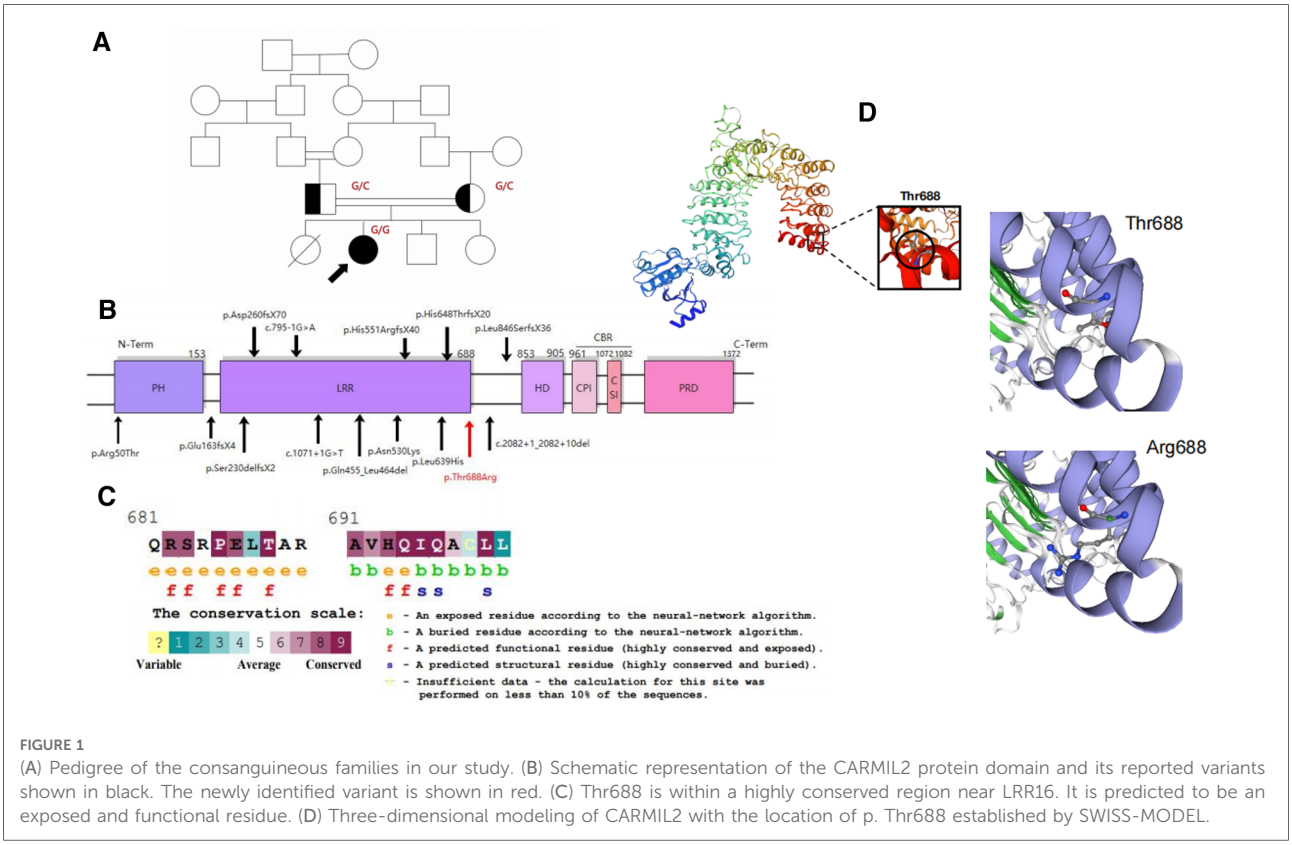

FIGURE 1
(A) Pedigree of the consanguineous families in our study. (B) Schematic representation of the CARMIL2 protein domain and its reported variants shown in black. The newly identified variant is shown in red. (C) Thr688 is within a highly conserved region near LRR16. It is predicted to be an exposed and functional residue. (D) Three-dimensional modeling of CARMIL2 with the location of p. Thr688 established by SWISS-MODEL.

the affinity of CP for actin filaments (8) and the CARMIL-specific interaction (CSI) motif (1), which plays a role in inhibiting actin capping *via* CP binding.

A homology model of the horseshoe sharp LRR region was constructed using the SWISS-MODEL template library (24), and the variant lies adjacent to LRR16 (total 16). Exchanging an uncharged amino acid for another considerably larger, positively charged side chain may destabilize the surrounding structure of the protein and hence disrupt the function of the protein (**Figure 1D**).

Immunological analyses

An immunologic investigation was performed on the patient, including detailed T-cell, regulatory T-cell (Treg), and B-cell immunophenotyping to evaluate the immune status before and during treatment. Initially, the patient's immunophenotype showed that absolute T-cell and NK cell counts were reduced, but regulatory T-cell counts were normal (**Table 1**). CD19+ B-cell counts and immunoglobulin levels did not exhibit significant deviation except IgA. Interestingly, the levels of interleukin-17α (IL-17α) and interferon-γ (IFN-γ) were significantly decreased.

Combined therapy was initiated and maintained for 2 years. Following the treatment, the patient's immune phenotypes were assessed again. As listed in **Table 2**, CD4+ T-cell, CD8+ T-cell, and NK cell counts were significantly improved. Certainly, the long-term clinical effects were still observed.

To access the immunological function of CARMIL2-deficient T cells, PBMCs were extracted from the patient and one age-matched control and then gated and stained with CD45RA/CCR7. As mentioned in previous research (5, 12, 13, 18), T-cell activation was impaired. CD4 T cells exhibited significantly larger differences in the percentage of cells compared to CD8 T cells. The marked skewing to the naïve form indicates defective maturation of T cells (**Figure 2B**). Meanwhile, marked decreases in $IgD^{-}CD27^{+}$ switched memory (B_{SM}) and $IgD^{+}CD27^{+}$ nonswitched memory (B_{NSM}) cells were observed and suggested impaired maturation of B cells in CARMIL2-deficient patients (**Figure 2C**). Further functional research like lymphocyte proliferation is warranted in the future.

Discussion

In this article, we reported a female patient from a consanguineous Chinese family with an unreported

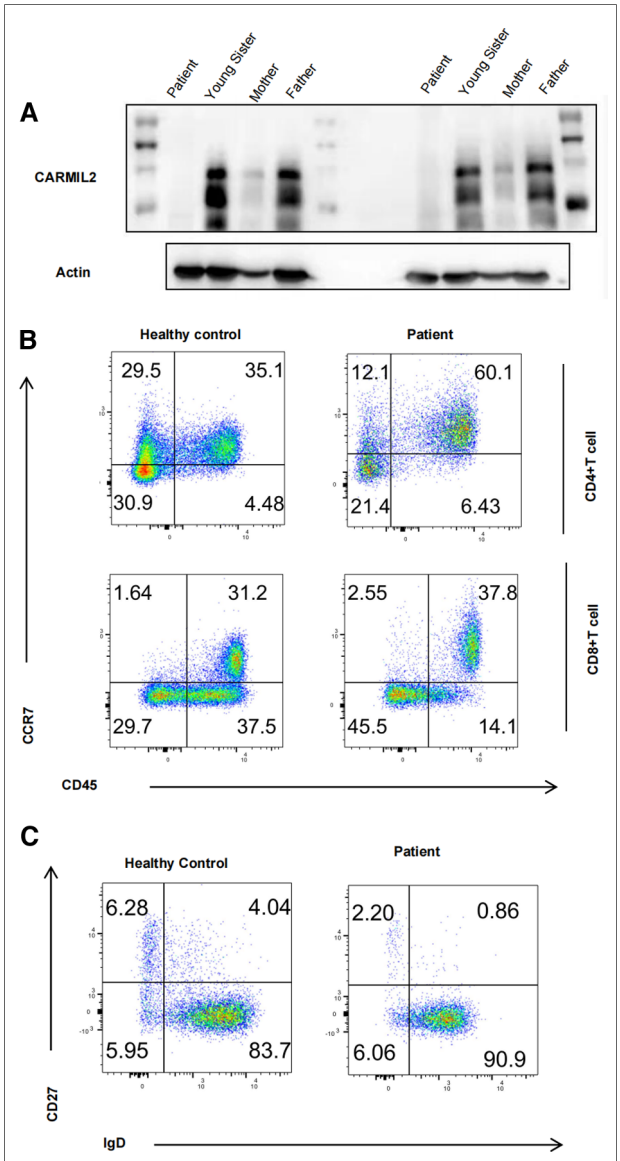

FIGURE 2
(A) Immunoblot analysis of our patient and her heterozygous family members. (B) Graphs show CD4/CD8 T-cell subtype populations. The contour plot shows that CD4 and CD8 T cells that are naïve (TN, $CD45RA^{+}CCR7^{+}$), central memory (T_{CM}, $CD45RA^{-}CCR7^{+}$), and effector memory (T_{EM}, $CD45RA^{-}CCR7^{-}$) T cells (corresponding percentages are indicated in each square). (C) Contour plot showing that B cells that are naïve (B_{N}, $IgD^{+}CD27^{-}$), switched memory (B_{SM}, $IgD^{-}CD27^{+}$), and nonswitched memory (B_{NSM}, $IgD^{+}CD27^{+}$) B cells (corresponding percentages are indicated in each square).

pathogenic variant in CARMIL2 (c.2063C>G:p.Thr688Arg) who presented with various symptoms of PID. These symptoms included recurrent upper and lower respiratory infections, perioral and perineum papules, erythematous impetiginized atopic dermatitis, oral ulcer, painful urination and vaginitis, otitis media, and failure to thrive. This missense mutation led to insufficient CARMIL2 protein expression,

TABLE 1 Immune workup of the patient.

Laboratory	Patient's initial value	Normal range
Lymphocyte subset quantification		
CD16+/CD56+ NK cells (cells/μl)	3.27	90–900
CD16+/CD56+ NK cells (%lymphocytes)	3.63%	4%–26%
CD3+/CD4+ T cells (cells/μl)	10.34	300–2,000
CD3+/CD4+ T cells (%lymphocytes)	11.49%	27%–53%
CD3+/CD8+ T cells (cells/μl)	49.96	300–1,800
CD3+/CD8+ T cells (%lymphocytes)	55.51%	19%–34%
CD19+/CD20+ B cells (cells/μl)	640.95	200–1,600
CD19+/CD20+ B cells (%lymphocytes)	15.9%	10%–31%
	Patient	Mean of 17 HCs
T-cell subsets		
CD4+CD45RA−CCR7+ (CD4+T CM)%	12	19.01
CD4+CD45RA+CCR7+ (CD4+T N)%	55.6	54.69
CD4+CD45RA−CCR7− (CD4+T EM)%	25.7	17.12
CD4+CD45RA+CCR7− (CD4+T E)%	6.7	9.18
CD8+CD45RA−CCR7+ (CD8+T CM)%	2.57	3.05
CD8+CD45RA+CCR7+ (CD8+T N)%	36.9	50.43
CD8+CD45RA−CCR7− (CD8+T EM)%	46.1	24.75
CD8+CD45RA+CCR7− (CD8+T E)%	14.4	21.79
B-cell subsets		
CD19+CD27+IgD− (switched memory B-cell)%	2.2	8.22
Regulatory T-cell quantification		
CD4+FOXP3+ cells	0.24/μl	—
CD4+FOXP3+ cells (%lymphocytes)	0.26%	—
B-cell subset phenotyping		
IgG (g/L)	11.6	6.7–15.3
IgM (g/L)	1.67	0.48–2.31
IgA (g/L)	3.5	0.52–2.74
IgE (IU/ml)	<4.23	<200
Complement C3 (g/L)	1.53	0.9–1.8
Complement C4 (g/L)	0.35	0.1–0.4
Others		
IL-2 (pg/ml)	2.18	0.64–8.84
IL-4 (pg/ml)	1.01	0.1–3.88
IL-6 (pg/ml)	1.43	1.05–15.8

(*continued*)

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TABLE 1 Continued

Laboratory	Patient's initial value	Normal range
IL-10 (pg/ml)	0.89	0.45–4.98
IL-17 (pg/ml)	8.29	16.67–65.76
TNF-α (pg/ml)	1.32	0.1–5.97
Interferon-γ (pg/ml)	0.00	0.44–16.2

TABLE 2 Immune workup before and after treatment.

Laboratory	Patient's initial value	Patient's post-treatment value	Normal range
Lymphocyte subset quantification			
CD16+/CD56+ NK cells (cells/μl)	3.27	112.38	90–900
CD16+/CD56+ NK cells (%lymphocytes)	3.63%	2.88%	4%–26%
CD3+/CD4+ T cells (cells/μl)	10.34	1,385.05	300–2,000
CD3+/CD4+ T cells (%lymphocytes)	11.49%	35.44	27%–53%
CD3+/CD8+ T cells (cells/μl)	49.96	1,270.70	300–1,800
CD3+/CD8+ T cells (%lymphocytes)	55.51%	32.52%	19%–34%
CD19+/CD20+ B cells (cells/μl)	640.95	732.45	200–1,600
CD19+/CD20+ B cells (%lymphocytes)	15.9%	18.74%	10%–31%
Others			
IL-2 (pg/ml)	2.18	4.43	0.64–8.84
IL-4 (pg/ml)	1.01	<2.44	0.1–3.88
IL-6 (pg/ml)	1.43	14.32	1.05–15.8
IL-10 (pg/ml)	0.89	5.39	0.45–4.98
IL-17 (pg/ml)	8.29	6.51	16.67–65.76
TNF-α (pg/ml)	1.32	13.00	0.1–5.97
Interferon-γ (pg/ml)	0.00	27.22	0.44–16.2

reduced absolute T-cell and NK cell counts, and marked skewing of the naïve T-cell form, indicating defective maturation of T cells and B cells. This is the first report of a new, unrecorded CARMIL2 variant of Chinese descent, expanding the clinical spectrum of CARMIL2 deficiency.

Summarizing previously published clinical characteristics associated with different CARMIL2 mutations showed that the clinical manifestations of the different mutations appear to be heterogeneous. The patient in the current report had a striking feature of cutaneous and respiratory infections, consistent with most of the previous findings associated with different CARMIL2 mutations (5, 12–17, 19, 20). Notably, as listed in **Supplementary Table S1**, several studies (5, 12, 13, 25) have mentioned that EBV infection or EBV-triggered lymphoproliferative disorders may be a prominent finding in CARMIL2-deficient patients. To explore this point, EBV and CMV quantification and gastrointestinal endoscopy were performed on the present patient, but there was no evidence of EBV/CMV infection or EBV+ smooth muscle tumor. More examinations, such as a positron emission tomography-computed tomography (PET-CT) scan, may provide more information regarding EBV/CMV infection or associated tumors. In addition, the current patient did not suffer from gastrointestinal problems, such as inflammatory bowel disease (IBD), chronic diarrhea, esophagitis, or dysphagia, that have often been observed in patients with other CARMIL2 mutations (12, 15–19). The mechanisms by which different CARMIL2 variants spread along the gene may lead to different immune phenotypes and remains poorly understood.

To estimate the pathogenic effects of the current CARMIL2 mutation, the bioinformatic SWISS-MODEL tool was used to predict the mutant CARMIL2 structure. The resulting 3D model revealed an exchange of the uncharged amino acid Thr for another considerably larger, positively charged side chain, potentially destabilizing the surrounding structure of the protein and disrupting the function of the protein. Additionally, the exchange of charge may generate perturbations in the electrostatic potential distribution (13), which has been shown in another study with a reversed exchange in the LRR1 domain of the same CARMIL2 gene (**Figure 1D**). Thus, the exchange of the amino acid was likely related to the decrease in CARMIL2 expression in the patient's PBMCs. More specific mechanisms should be addressed in future studies.

The mechanism by which the mutation at site 688 led to the downregulation of CARMIL2 expression was proposed according to the 3D model that was generated. There are two main hypotheses. On the one hand, the LRR domain has an essential role in CD28 costimulation by blocking colocalization with CARMA1 at the immune synapse (9). The initiation, formation, and maintenance of immunocyte synapses rely upon the polymerization and dynamic rearrangement of the cortical actin cytoskeleton (26). As a result, deficiency of CARMIL2 may lead to remodeling of the cortical actin cytoskeleton on T cells and subsequent activation by altering its colocalization with CARMA1. Thus, CARMIL2 is required for CD28 cosignaling in T cells for

subsequent maturation and function (7, 8). Regarding naive CD4+ T cells, CARMIL2 deficiency impairs the differentiation of naive CD4+ T cells, leading to the decrease of Th1 and Th17 production, IFN-γ, and IL-17α (2, 27).

On the other hand, the LRR domain is necessary for colocalization with the vimentin intermediate actin filament network (6). There is a list of PIDs associated with actin-related cytoskeletal defects (28). Vimentin, which is filled with dynamic actin filament networks nucleated by the Arp2/3 complex (29, 30), is important for collective cell migration based on wound healing. The current research indicated that the p.Thr688Arg variant is localized adjacent to LRR16, affecting the "horseshoe sharp" structure and changing the electrostatic potential distribution and interaction with vimentin. CARMIL2 may act as a critical link connecting vimentin to the migration, invasion, and wound healing of lymphocytes (31, 32). Interestingly, the vimentin network is functionally "upstream" to CARMIL2, whereas the actin network is functionally "downstream" (6). Lanier et al. (6) depleted CARMIL2 and found that vimentin filament networks were not affected, although F-actin was disrupted. CARMIL2 regulates CP, which is a critical determinant of actin assembly and actin-based cell motility (6). Actin polymerization plays a pivotal role in the formation of the immunological synapse, antigen recognition, signal transduction, and T-cell proliferation, migration, adhesion, and invasion into tissues during the immune response (33, 34). Hence, CARMIL2 deficiency inhibits Arp2/3-dependent actin network assembly by regulating CP at the leading edge and inhibiting barbed-end capping (35), affecting the immune response.

In addition, in the present study, the patient's B-cell maturation was impaired. Most research on B-cell maturation has focused on investigating the proliferation and differentiation of T cells. Wang et al. (7) described biallelic loss-of-function mutations in CARMIL2, affecting the CD28-responsive pathway and B-cell receptor (BCR)-responsive pathway in B cells. More experiments are needed to explore how B-cell function is affected.

Because no specific treatment directly targeting the impaired immune pathway has been established yet, the clinical treatment of PID due to CARMIL2 deficiency is extremely limited. At present, the patient still needs a small dose of prednisone (7.5 mg per day) to prevent the recurrence, which may be due to the mountainous environment and limited local medical care. We consider that the immune dysregulated phenotype is rescued by immunomodulation. Allogeneic hematopoietic stem cell transplant (Allo-HSCT) is a potential treatment for CARMIL2 deficiency (16, 36); however, the lack of long-term data makes it impossible to use Allo-HSCT as a routine treatment for CARMIL2 deficiency. Moreover, when immunophenotypes were assessed after treatment, CD4+ T cells, CD8+ T cells, and NK cells exhibited significant increases (**Table 2**). The mechanisms of this immunotherapy are still unclear, and further experiments are needed.

Conclusion

Here, we described a patient with primary immunodeficiency who presented with prominent cutaneous and respiratory infections and in which an unreported homozygous variant in CARMIL2 was identified. The mutation led to decreased protein expression and T-cell activation and proliferation, which were manifested clinically. The clinical characteristics of the patient broadened the spectrum of PID symptoms, highlighting the importance of genetic diagnosis in patients with PID. Because the initial phenotype of the patient could be rescued partially by immunotherapy, the present report provides clinical treatment for similar 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 human participants were reviewed and approved by the Ethics Committee of Shanghai Children's Medical Center. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin. Written informed consent was obtained from the minor(s)' legal guardian/next of kin for the publication of any potentially identifiable images or data included in this article.

Author contributions

YZ study design, patient treatment, data analysis, manuscript preparation, and 3D model structure. LLY immunological analysis and western blotting. HH and XMX patient treatment and manuscript assistance. YL: immunological analysis. JW genetic workup. YLJ study design, patient treatment, manuscript revision, and study supervision.

All authors contributed to the article and approved the submitted version.

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Conflict of interest

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

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2022.1042302/full#supplementary-material.

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Case report: Effectiveness of sirolimus in treating partial DiGeorge Syndrome with Autoimmune Lymphoproliferative Syndrome (ALPS)-like features

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Background: DiGeorge Syndrome (DGS) is a rare disease associated with 22q11.2 chromosomal microdeletion, also known as a velocardiofacial syndrome, based on the frequent involvements of the palate, facial, and heart problems. Hematologic autoimmunity is rare in DGS but presents with a refractory course and poor prognosis. Herein, we report a case of partial DGS in a patient with refractory immune cytopenia and autoimmune lymphoproliferative syndrome (ALPS)-like manifestations.

Case description: A 10-year-old boy with growth retardation presented initially with a ventricular septal defect at 7 months old, which had been repaired soon after. The patient suffered from thrombocytopenia and progressed into chronic refractory immune thrombocytopenia (ITP) at 30 months old. One year later, the patient developed multilineage cytopenias including thrombocytopenia, neutropenia, and anemia. First-line treatment of ITP, like high-dose dexamethasone and intravenous immunoglobulin, had little or short-term effect on controlling symptoms. Whole-exome sequencing revealed the presence of a *de novo* heterozygous 2.520 Mb deletion on chromosome 22q11.21. Moreover, decreased proportion of naive T cells and elevated double-negative T cells were found. The patient was given sirolimus therapy (1.5 mg/m^2, actual blood concentration range: 4.0–5.2 ng/ml) without adding other immunosuppressive agents. The whole blood cell count was gradually restored after a month, and the disease severity was soothed with less frequency of infections and bleeding events. Decreased spleen size and restrained lymph node expansion were achieved after 3-month sirolimus monotherapy.

Conclusions: This case is the first description on the efficacy of sirolimus monotherapy to treat refractory multilineage cytopenias of DGS presented with ALPS-like features.

KEYWORDS

DiGeorge syndrome, ALPS-like, DNTs, mTOR, sirolimus monotherapy

Abbreviations

ALPS, autoimmune lymphoproliferative syndrome; CM, central memory T cells; DGS, DiGeorge syndrome; DNTs, double-negative T cells; EM, effector memory T cells; ITP, immune thrombocytopenia; mTOR, mammalian target of rapamycin; MLPA, multiplex ligation-dependent probe amplification; TEMRA, terminally differentiated effector memory T cells; Tregs, regulatory T cells.

Introduction

DiGeorge Syndrome (DGS) is the most common chromosomal microdeletion disorder, caused by *de novo* nonhomologous meiotic recombination events and characterized by typical facial features. Based on their immunophenotype and degree of thymic hypoplasia, DGS is divided into a partial type and a complete type (1–3). Immunodeficiency, particularly impaired T-cell production, as a secondary consequence of diminished or lost thymic function affects up to 75% of pediatric DGS patients (4, 5). As one of the important features of immune dysregulation, increased $TCR\alpha\beta^{+}CD4^{-}CD8^{-}$ double-negative T (DNT) cells and immune cytopenias are frequently seen in disorders with autoimmune lymphoproliferative syndrome (ALPS)-like phenotypes (6). Sirolimus is considered as an effective and safe therapeutic option for multilineage immune cytopenias with ALPS-like phenotypes (6). In light of the ALPS-like features with augmented DNTs in the DGS patient of our study, we envisaged that sirolimus, an inhibitor for the mammalian target of the rapamycin (mTOR) pathway, could possibly be used to treat the immune dysregulation in DGS, at least temporarily constraining the adverse consequences from immunodeficiency and the autoimmune manifestations (7). Here, we reported the efficacy and safety of sirolimus for treating a partial DGS patient with refractory autoimmune manifestations concomitant with increased DNTs.

Case description

Patient presentation

The patient was a 10-year-old boy, born by a cesarean section at term to consanguinity-unrelated parents. The child was found to have a ventricular septal defect that was soon repaired by surgery at 7 months old. At age of 1.5 years old, thrombocytopenia was found (Plt 8×10^9/L). The anti-glycoprotein (GP) IIb/IIIa test was positive, indicating severe bleeding (8). The bone marrow biopsy was normal, excluding the possible myeloid or lymphocyte-derived deformation. Based on the clinical features and cellular characteristics, immune thrombocytopenia (ITP) diagnosis was made. The symptoms were mitigated in response to glucocorticoid therapy (2 mg/kg bodyweight daily for 4 weeks). The patient unfortunately relapsed at 33 months of age and progressed to chronic refractory ITP. He suffered from splenomegaly at 6 years of age (5 cm below the left costal margin), after which massive lymphadenopathy was noticed. At 7 years of age, the patient developed neutropenia (0.15×10^9/L) with low hemoglobin levels (99 g/L). The patient had a notable speech impediment and growth retardation. Computed tomography examination indicated a decreased thymus volume (**Figure 1A**). Serum IL-10 (8.28 pg/ml, reference range 1.2–4.55 pg/ml) was above the normal range, while parathyroid hormone was lower than normal (8.9 pg/ml, reference range 10.2–50.5 pg/ml). The ratio of lymphocyte subsets was abnormal (**Table 1**), with noticeably decreased naive T cells and elevated DNT cells (naive T cells, 9.5%, reference range 39.72%–65.59%; DNT cells, 4.4% of $CD3^{+}$ T cells, reference range 0.82%–2.91%). The patient initially received first-line treatment for ITP (high-dose dexamethasone and intravenous immunoglobulin) with a poor response that did not last long. After monotherapy with sirolimus, the platelets and neutrophils recovered to a relatively normal level along with the disappearance of other clinical manifestations such as enlarged spleen and expanded lymph nodes.

Genetic findings

Whole-exome sequencing (WES) revealed the presence of a *de novo* heterozygous 2.520 Mb deletion on 22q11.21 chromosome (18893867–21414817) (**Figure 1B**). To confirm the size of the missing area, multiplex ligation-dependent probe amplification (MLPA) analysis was performed with the standard MLPA kit (P250, MRC-Holland). The results revealed a 50% decrease relative to the reference bar height, indicating a heterozygous deletion (**Figures 1C,D**).

Laboratory findings

The proportion and the absolute number of naive CD4 T cells were markedly decreased (proportion, 9.5%, reference 39.72-69.59%; cell number, 32 cells/μl, reference 294-683 cells/μl, **Table 1**). Determination of T-cell receptor (TCR) repertoires by flow cytometry revealed that the TCR repertoires of the patient were diverse and normally distributed in CD4 and CD8 T cells (**Figure 1E**). We then assessed the *in vitro* proliferative response of CD4 and CD8 T cells of this patient. Upon TCR ligation with anti-CD3/anti-CD28 antibodies, CD4 and CD8 T cells exhibited a retained potentiality of T cell division (**Figure 1F**). The patient was followed up for 1 year after sirolimus treatment, and the platelet count was recovered without any other hematologic abnormality being found. Consistent with the clinical improvements, his DNT cells decreased (from 4.4% to 3.2%) along with an elevation of regulatory T cells (Tregs, from 3.7% to 5.2%).

Clinical course

Based on the medical history, clinical presentations (ventricular septal defect, thymus dysplasia, velopharyngeal hypoplasia, and delayed language development), genetic findings (chromosome 22q11.21 deletion), and laboratory data (decreased parathyroid hormone and reduction of naive T cells with a normal TCR Vβ repertoire), the patient was diagnosed with partial DGS.

The patient presented with elevated DNT cells (≥2.5% of $CD3^{+}$ cells) and chronic (>6 months), nonmalignant, infection-free lymphadenopathy and splenomegaly, which are commonly seen in other ALPS-like disorders. Indeed, the patient had autoimmune cytopenias (hemolytic anemia, thrombocytopenia, and neutropenia) and hypoparathyroidism. Considering his autoimmune and lymphoproliferative symptoms, ALPS-like diseases such as activated PI3K-kinase delta syndrome (APDS) or CTLA-4 haploinsufficiency with autoimmune infiltration (CHAI) were considered (9). However, the WES results did not reveal defined variations

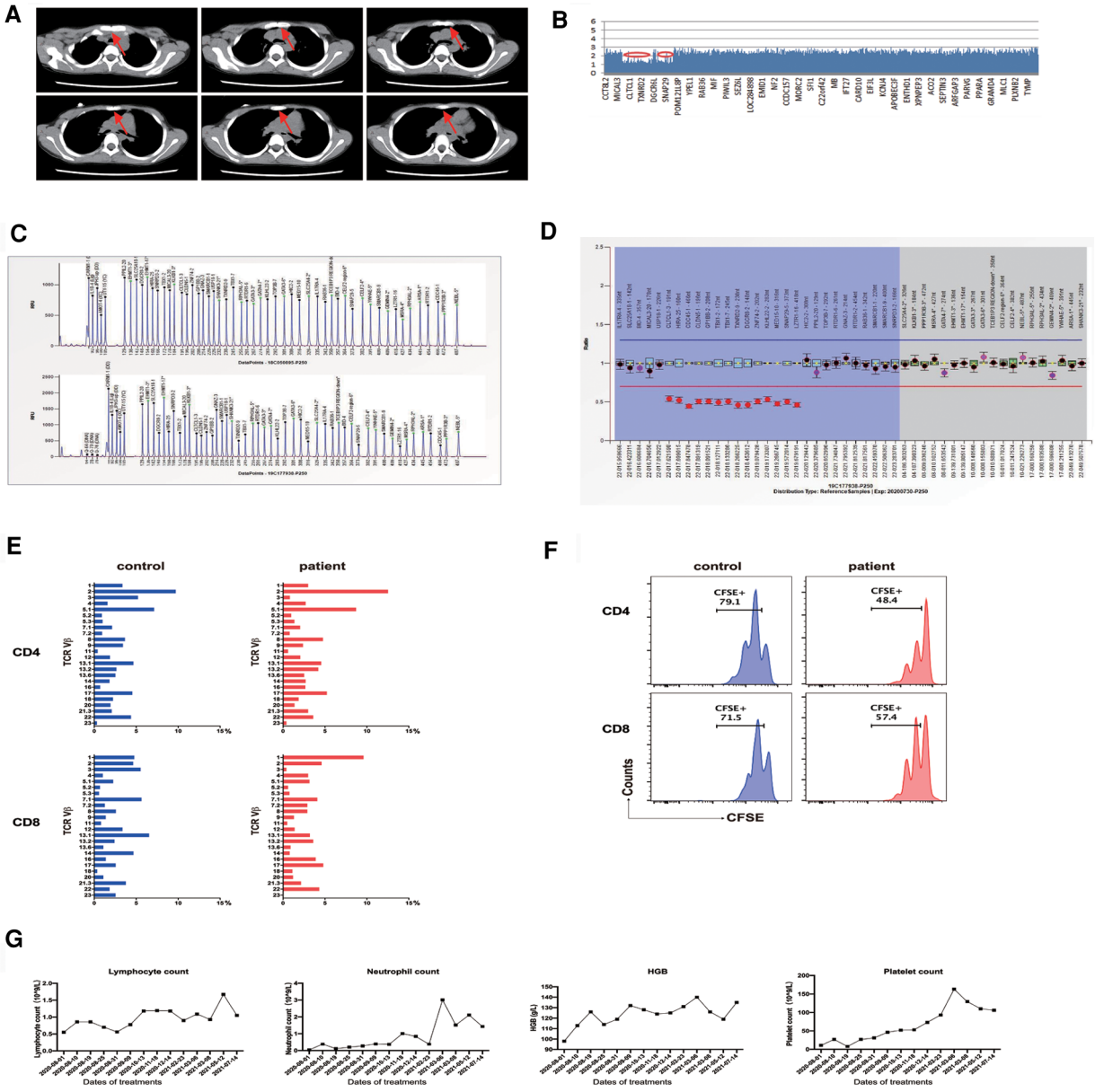

FIGURE 1
(A) Thymus volume is significantly reduced. (B) Whole-exome sequencing identifies a 2.520 Mb deletion on chromosome 22q11.21. (C,D) MLPA analysis reveals chromosome 22q11.21 deletion. (E) TCR Vβ repertoires analyzed by flow cytometry show a diverse and normal distribution of Vβ subfamilies. (F) Upon stimulation by anti-CD3/anti-CD28 for 4 days, CFSE-labeled CD4 and CD8 T cells from patient PBMCs show a retained proliferative response compared to a healthy control. (G) Changes in patient's lymphocyte, neutrophil, platelet, and hemoglobin (HGB) levels during sirolimus therapy. CFSE, carboxyfluorescein succinimidyl ester; MLPA, multiplex ligation-dependent probe amplification; PBMC, peripheral blood mononuclear cells; TCR, T-cell receptor.

associated with ALPS-like diseases. The WES results indicated that DGS, if not solely, would be the main contributor to ALPS-like phenotypes of the patient. In light of the clinical ALPS-like manifestations, sirolimus therapy was initiated (1.5 mg/m^2, actual blood concentration range: 4.0–5.2 ng/ml) without the addition of other immunosuppressive agents. After 1 month of sirolimus treatment, the whole blood cell counts were gradually restored (**Figure 1G**), with a decrease in the severity and frequency of infections and bleeding episodes. Lymphadenopathy and splenomegaly were rapidly alleviated in this patient. The enlarged spleen shrunk from 5 cm below the left costal margin to 1 cm, highlighting encouraging results as early as 3 months from the start of sirolimus monotherapy.

Discussion

Sirolimus has been increasingly recognized as an effective agent for ALPS patients and was reported to achieve a partial rescue of Tregs and suppression of DNT cells, which is consistent with our observations (10, 11). To some extent, applying sirolimus can achieve the sustainable recovery of immune cytopenias and

TABLE 1 Lymphocyte subsets of the patient.

	Patient		Reference	
	%	Abs # (/μl)	%	Abs # (/μl)
Lymphocytes				
T cells				
DNT/CD3$^+$	4.4	37	0.82–2.91	13–48
γδT	3	26	8.10–20.76	124–388
CD3$^+$	60.1	832	57.10–73.43	1325–2276
CD4$^+$	24.3	337	24.00–38.72	531–1110
CD4 CM	78.5	265	24.24–52.73	165–475
CD4 naive	9.5	32	39.72–69.59	294–683
CD4 EM	11.8	40	3.40–11.17	24–87
CD4 TEMRA	0.2	1	0.10–1.29	0–9
Treg/CD4$^+$	3.7	—	4.10–9.40	—
CD8$^+$	31.1	431	21.01–33.94	480–1112
CD8 CM	26.3	113	13.21–37.89	92–287
CD8 naive	24	103	41.41–73.04	245–657
CD8 EM	36.7	158	1.52–15.39	9–130
CD8 TEMRA	13	56	2.01–21.65	12–164
B cells				
B cell	24.9	368	9.19–19.48	216–536
Naive B	19	70	51.84–77.61	123–362
Memory B	6.4	24	8.96–24.09	28–89
Plasmablast	0.4	1	0.7–5.67	3–21
Transitional	5	18	2.5–9.07	7–37

CM, central memory T cells; DNT, double-negative T cell; EM, effector memory T cells; TEMRA, terminally differentiated effector memory T cells; Treg, regulatory T cell.

splenomegaly, and the rebalance of abnormal immunophenotype. These data further suggest that sirolimus monotherapy is highly effective and may be beneficial for treating partial DGS with immune dysregulation associated with multiple cytopenias.

The clinical manifestations of DGS include hypoparathyroidism, conotruncal cardiac malformation, velopharyngeal insufficiency, facial dysmorphism, and intellectual disability. The immune dysregulation manifestations described in DGS include impaired antibody immune response resulting in poor response to vaccines and IgA deficiency (12–14). Autoimmune diseases such as juvenile rheumatoid arthritis, ITP, autoimmune hemolytic anemia, and Hashimoto thyroiditis are collectively common in DGS patients (14–17). The patient in the present study was shown to have an ALPS-like phenotype in many aspects, including decreased Tregs, increased IL-10 levels, and elevated DNT cells (6).

Recent findings showed that DNT cells are present in various chronic inflammatory diseases, including systemic lupus erythematosus (SLE), Sjögren's syndrome, psoriasis, axial spondylarthritis, and other rheumatic diseases as well (18). A clinical trial of sirolimus in patients with active SLE showed that mTOR blockade corrected proinflammatory DNT cell differentiation and activation (19). Furthermore, various studies have suggested that sirolimus is effective in multilineage cytopenias characterized by ALPS-like features. It has been suggested that increased DNT cells in ALPS-like diseases possibly come from autoreactive CD8 T cells via losing CD8 expression (19). Even though sirolimus monotherapy induced a reduction in DNT cells, it is not clear whether this is a secondary response from the holistically diminished autoimmune symptom or a direct cellular transformation in response to sirolimus.

In partial DGS, the primary thymic defect leading to impaired central tolerance was suggested because of autoimmune signs. Indeed, the absence of an appropriate central tolerance in partial DGS patients could lead to the escape of autoreactive thymocytes and the reduced absolute number and frequency of FoxP3$^+$ thymocytes, consequently resulting in increased susceptibility to autoimmune manifestations (20). In recent works, increased FAS (APO-1/CD95) expression on lymphocytes and increased levels of FAS ligand (FASL) were found in patients with DGS (21). More efforts to understand the pathophysiology of partial DGS with ALPS-like symptom is expected.

Conclusion

We reported a patient with partial DGS associated with clinically ALPS-like features, whose condition of refractory multilineage cytopenias was successfully treated with sirolimus monotherapy.

This case report emphasized that comprehensive laboratory diagnostic work is required for the accurate diagnosis of partial DGS. Otherwise, it could be easily misled by the immune-related cytopenias presented in other ALPS-like disorders. This case also showcases that sirolimus, as an effective drug for treating other ALPS-like disorders, is a good candidate for partial DGS patients, especially with autoimmune cytopenias and elevated DNTs.

Data availability statement

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

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee of Beijing Children's Hospital, Capital Medical University. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin. Written informed consent was obtained from the individual(s), and minor(s)' legal guardian/next of kin for the publication of any potentially identifiable images or data included in this article.

Author contributions

HG, WM, JG, and RW designed most of the study. WM and HG carried out much of the work. ZC, JY, XX, RZ, and RW carried out the diagnosis and treatment and collected the data generated from clinical laboratories. HG, WM, JG, and RW drafted the manuscript. WM and JG revised the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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

Publisher's note

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Case report: Clinical course and treatment of SARS-CoV-2 in a pediatric CAR-T cell recipient with persistent hypogammaglobulinemia

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This report describes a pediatric patient who underwent chimeric antigen receptor (CAR) T-cell therapy for refractory B-cell acute lymphoblastic leukemia (B-ALL) four years prior, with resultant hypogammaglobulinemia for which he was receiving weekly subcutaneous immune globulin. He presented with persistent fever, dry cough, and a tingling sensation in his toes following a confirmed COVID-19 infection 3 weeks prior. His initial nasopharyngeal SARS-CoV-2 PCR was negative, leading to an extensive workup for other infections. He was ultimately diagnosed with persistent lower respiratory tract COVID-19 infection based on positive SARS-CoV-2 PCR from bronchoalveolar lavage (BAL) sampling. He was treated with a combination of remdesivir (antiviral) and casirivimab/imdevimab (combination monoclonal antibodies) with immediate improvement in fever, respiratory symptoms, and neurologic symptoms.

KEYWORDS

COVID-19, SARS-CoV-2, hypogammaglobulinemia, CAR-T cell therapy, virus neutralizing monoclonal antibody, remdesivir, pediatric

1. Introduction

Intact humoral immunity is critical to successful elimination of SARS-CoV-2 infection, as well as for prevention of re-infection (1). Prior studies have indicated that SARS-CoV-2 infected individuals who have recovered from infection produce immunoglobulin (Ig)G antibodies targeting the viral N (nucleocapsid) and S (spike) proteins, including the receptor-binding domain. Immunocompromised individuals with iatrogenic B cell depletion and humoral immunodeficiencies associated with dysregulation are at higher risk of severe COVID-19 infection (2).

Secondary hypogammaglobulinemia is a known sequela of chimeric antigen receptor (CAR) T-cell therapy, an immunotherapy used in the treatment of hematologic malignancies, including relapsed or refractory pediatric B-cell acute lymphoblastic leukemia (B-ALL). CAR-T therapy targets malignant B-cells by engineering anti-CD19 CAR T cells that are intended to destroy malignant cells but also commonly destroy normal B-cells (3). The resulting deficiency can persist for several years after therapy (4). Due to the increased risk for life-threatening

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infections, it is recommended that pediatric patients with hypogammaglobulinemia following CAR T-cell therapy receive routine intravenous or subcutaneous immunoglobulin G (5).

This case report describes the clinical course of persistent SARS-CoV-2 infection in a 13-year-old child with a history of B-ALL with secondary hypogammaglobulinemia following CAR-T therapy.

2. Case description

A 13-year-old male child with history of B-ALL who had been in remission for four years following CAR-T cell therapy presented with two weeks of malaise and fevers following COVID-19 infection 23 days earlier. At the time of initial COVID-19 diagnosis, he had nasal congestion, mild cough, and dysgeusia without fever. Multiple family members also tested positive for COVID-19 at that time. Diagnosis was made *via* nasopharyngeal (NP) swab which was positive for SARS-CoV-2 by polymerase chain reaction (PCR).

2.1. Prior malignancy, hypogammaglobulinemia treatment, and infection history

At time of his B-ALL diagnosis, initial cytogenetics were concerning for hypodiploidy (associated with a poor prognosis). He had refractory disease and was referred for hematopoietic stem cell transplant (HSCT), which he received 6 months after his initial diagnosis with a matched sibling donor. Unfortunately, he suffered relapse shortly after HSCT. He ultimately underwent CAR-T therapy 9 months later after which he entered remission. Bridging therapies he received prior to CAR-T included blinatumomab, which is a bispecific CD19-directed CD3 T-cell engaging immunotherapy. Following CAR-T therapy, he developed B cell aplasia and secondary hypogammaglobulinemia requiring immunoglobulin replacement.

Baseline immunological evaluation three years prior to the current admission noted a normal complete blood count with absolute lymphocyte count of 3800 cells/ul. IgG, IgA, and IgM were all two standard deviations below normal with a history of intermittent use of IgG supplementation due to low levels. Lymphocyte enumeration revealed elevated numbers of T cells (mostly due to increased CD8 cells), along with absent B cells. Mitogen proliferation was normal and T-cell receptor (TCR) spectratyping showed a normal distribution of the T cell repertoire. He was initiated on monthly IVIg (intravenous immune globulin) supplementation (20 grams) and eventually transitioned to weekly Cuvitru (5 grams) subcutaneous injections without an issue. Repeat lymphocyte subset testing 3 months prior to admission continued to demonstrate absence of B cells (**Table 1**).

He had normal pulmonary function testing (PFT) two years after his initial B-ALL diagnosis, and again three months prior to admission.

TABLE 1 Lymphocyte subset testing prior to presentation.

Labs	Normal value	Patient's value
Lymphocyte subsets		
CD3+ %	52%–90%	90
CD3 + absolute count	850–3200/μl	2763
CD3 + CD4+ %	20–65%	45
CD3 + CD4 + absolute count	400–2100/μl	1378
CD3 + CD8+ %	14%–40%	43
CD3 + CD8 + absolute count	300–1300/μl	1337
CD4/CD8 ratio	0.9–3.4	1.0
CD19+ %	7%–24%	<1
CD19 + absolute count	120–740/μl	<8
CD3-CD56+ %	4%–51%	10
CD3-CD56 + absolute count	92–1200/μl	295

Since his B-ALL diagnosis, he had a documented history of three viral infections: rhinovirus/enterovirus on two separate occasions, and influenza A once for which he was treated with a five day course of oseltamivir as an outpatient. He did not have a history of any significant bacterial or fungal infections.

2.2. Hospital course

Three weeks after initial COVID diagnosis, the patient presented to the emergency department with fevers for two weeks, which were initially intermittent, but had become constant for five days prior to admission to the hospital. He reported worsening cough, although he denied shortness of breath, wheezing, or chest pain. He noted ten pounds of unintentional weight loss since symptom onset. While in the emergency room, he started complaining of tingling and pain in his toes. There was no history of recent travel, animal exposures or other infectious exposures.

On initial physical exam, the patient had a temperature of 37.4°C, a pulse of 130 beats/minute, blood pressure 116/71 mm Hg, respiratory rate 26 breaths/minute, and oxygen saturation 98% on room air. Two hours later, he developed a fever to 38.2°C. He did not appear to be in distress and was alert and oriented. His work of breathing was normal and breath sounds were mildly diminished in the left lower and right middle lung fields. Capillary refill was less than two seconds, and there were no rashes, ecchymoses, or petechiae. Neurological assessment revealed normal cranial nerve exam, normal strength in all 4 extremities, normal deep tendon reflexes, and intact sensation. However, he was exquisitely tender to touch on both the dorsal and plantar aspects of his toes on the left foot. With reported tingling in his bilateral toes and hyperalgesia with palpation of the bilateral distal lower extremities in a stocking-glove distribution, a clinical diagnosis of peripheral neuropathy was made.

Initial laboratory testing revealed a normal complete blood count with elevated inflammatory markers (**Table 2**). Notably, point-of-care PCR testing for influenza A and B and SARS-CoV-2 from a NP swab were negative. A chest x-ray demonstrated bilateral patchy peripheral opacities, left greater than right. The patient was admitted to the pediatrics inpatient service for additional workup

TABLE 2 Laboratory findings at the time of presentation.

Labs	Normal value	Patient's value
White blood cell count, $\times 10^9$/L	4.5–13.5	8.9
Neutrophil count, $\times 10^9$/L	1.8–8.0	5.2
Lymphocyte count, $\times 10^9$/L	1.5–6.5	3.3
Hemoglobin (g/dl)	11.5–15.5	11.7
Platelet count, $\times 10^9$/L	130–400	193
ALT (U/L)	6–63	35
Albumin (g/dl)	3.8–5.4	2.9
Erythrocyte sedimentation rate (mm/hr)	<15	67
C-reactive protein (mg/dl)	<0.8	7.8
Lactate dehydrogenase (U/L)	90–200	418
Ferritin (ng/ml)	22–322	5,665
Troponin T (ng/L)	<6	<6
Brain natriuretic peptide (pg/ml)	<25	<25
aPTT (sec)	29–40	31.4
INR	0.87–1.18	1.20
D-dimer (ng/ml)	0–230	432
Fibrinogen (mg/dl)	154–448	609
IgG (mg/dl)	528–2190	671
IgA (mg/dl)	44–395	8
IgM (mg/dl	56–352	<25

Abbreviations: dl, deciliter; g, gram; hr, hour; Ig, immunoglobulin; L, liter; ml milliliter; mm, millimeter; ng, nanogram; pg, picogram; sec, second; U, unit.

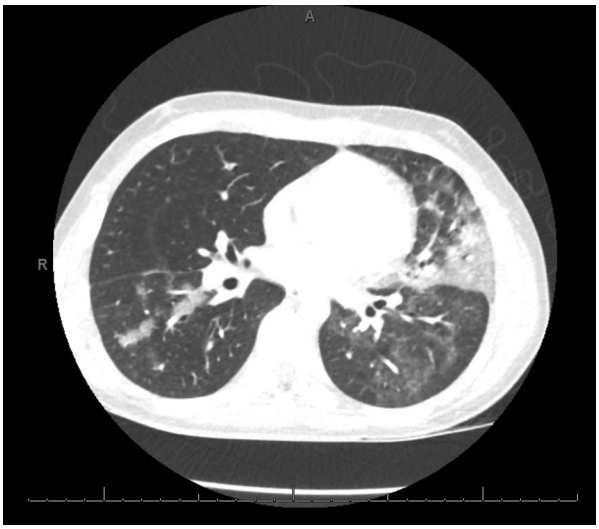

FIGURE 1
The patient underwent a computed tomography (CT) scan of the chest on hospital day (HD) 4 which revealed "bilateral patchy consolidations with adjacent, scattered ground glass opacities".

and management and was started on IV ceftriaxone for treatment of presumed community-acquired pneumonia following recent SARS-CoV-2 infection. A blood culture drawn on admission resulted as negative after 5 days incubation.

Following admission, he exhibited daily fevers ranging from 38.4–40.2°C as well as a persistent dry cough. Due to concern for multisystem inflammatory syndrome in children (MIS-C) following COVID-19 infection, a SARS-CoV-2 serology was obtained and was negative, with the caveat that antibody testing was not considered reliable given his known hypogammaglobulinemia. Daily labs were obtained which demonstrated persistently elevated C-reactive protein (CRP) and rising ferritin. A computed tomography (CT) scan of the chest was performed on hospital day (HD) 4 which revealed "bilateral patchy consolidations with adjacent, scattered ground glass opacities (**Figure 1**)." Antimicrobial coverage was broadened from ceftriaxone (50 mg/kg every 24 h) to vancomycin (15 mg/kg every 8 h, increased to every 6 h following the fourth dose based on trough level), cefepime (50 mg/kg every 8 h), and micafungin (150 mg every 24 h). Evaluation for other infectious etiologies was unrevealing, including common bacterial pathogens (*Streptococcus pneumoniae*, *Legionella pneumophila*), viral pathogens (EBV, CMV, parvovirus) and fungal pathogens (Cryptococcus, Aspergillus). Adenovirus blood PCR was positive at a low level that was not thought to be elevated enough to explain his symptomatology and rather reflected reactivation. Indirect testing for fungal pathogens returned with very elevated beta-D-glucan (>500) which was attributed to IVIg administration (known to falsely elevate this value). Subsequent labwork revealed decreasing CRP, however fevers persisted and ferritin levels continued to increase. Echocardiogram done on HD 4 showed low-normal left ventricular function and normal coronary arteries. He was changed to meropenem (700 mg every 8 h) and azithromycin (10 mg/kg every 24 h) on HD 5 after a sputum gram stain revealed gram negative bacilli. However, sputum culture later grew normal upper respiratory flora without a predominant organism. As he was due for his home dosing of weekly subcutaneous Ig, he was given a monthly dose of IVIg (20 g) on HD 5.

Due to persistent symptoms, bronchoscopy was performed on HD 11, during which turbid bronchioalveolar lavage (BAL) fluid was obtained for further testing. Cell count on BAL fluid revealed 1,850 WBC with a differential of 70% lymphocytes, 18% histiocytes, and 6%neutrophils. Infectious evaluation from samples obtained during the bronchoscopy was unrevealing—negative bacterial and fungal cultures, ova and parasites (O & *P*) examination, acid-fast bacilli (AFB) smear and culture, *Pneumocystis jirovecii* direct-fluorescent antibody, respiratory pathogen panel, and *Aspergillus* galactomannan. Flow cytometry was performed on BAL fluid and was not consistent with relapsed malignancy. SARS-CoV-2 PCR and viral cultures were not performed given the negative SARS-CoV-2 NP PCR done on admission.

Due to the patient's oncologic history, persistent fevers, and increasing ferritin, etiologies other than infection were considered, including relapsed leukemia and associated hemophagocytic lymphohistiocytosis (HLH). Lymphocyte enumeration was performed again and did not demonstrate the presence of B cells or blast forms. Bone marrow biopsy was performed on HD 18 and did not reveal a monotypic B cell population, increased blasts, or hemophagocytosis. Repeat TCR spectratyping did not reveal any changes in the normal distribution of T cell receptor families.

Chest x-ray on HD 17 demonstrated a new right upper lobe infiltrate; thus a second bronchoscopy was also performed on HD 18, with additional infectious studies sent and transbronchial

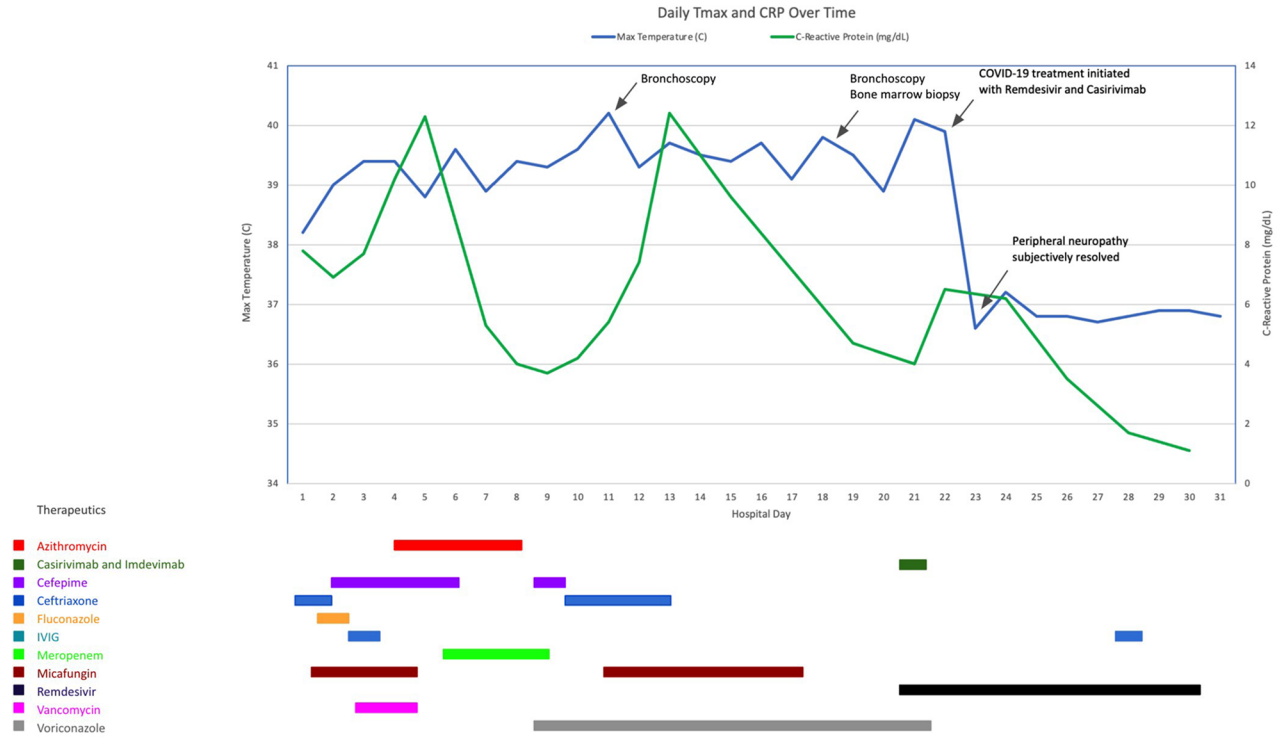

FIGURE 2
Clinical course and treatment of the patient including daily maximum temperature (Tmax) and C-reactive protein (CRP) during his hospitalization.

biopsy performed. SARS-CoV-2 PCR performed on BAL fluid from this bronchoscopy was positive after 20 cycles of PCR, indicative of a high viral load (6). Of note, a simultaneous SARS-CoV-2 PCR on an NP swab specimen resulted as negative. After multi-disciplinary discussion, the patient was treated with an infusion of combination virus-neutralizing monoclonal antibodies (mAb) against SARS-CoV-2 (casirivimab/imdevimab 1,200 mg/1,200 mg) that were active against the circulating strain of SARS-CoV-2 (delta variant), as well as a 10-day course of remdesivir (200 mg loading dose followed by 100 mg daily). The patient defervesced within hours of initiation of treatment with casirivimab/imdevimab and remdesivir. Dexamethasone was avoided due to concern that this could worsen infection by suppressing T cell activity. He improved subjectively, with self-reported increase in energy and resolution of his bilateral foot pain within 24 h. **Figure 2** demonstrates his fever curve and CRP levels in relation to treatment.

3. Discussion

Since the emergence of SARS-CoV-2 in 2019, most published studies suggest that the majority of healthy children who contract COVID-19 are asymptomatic or have a mild-to-moderate disease course compared to adults (7). Data regarding outcomes of immunocompromised children infected with SARS-CoV-2 are more limited, and experts suggest that treatment should be decided on a case-by-case basis depending on the underlying immune defect (8). Here we describe a unique case of prolonged COVID-19 lower respiratory tract infection in a pediatric patient who was immunocompromised due to prior CAR-T therapy for B-ALL with resultant B cell aplasia and hypogammaglobulinemia. Our patient also exhibited peripheral neuropathy as an acute clinical manifestation of COVID-19 infection which is a relatively unusual finding. Finally, our patient's clinical course was complicated by a negative SARS-CoV-2 nasopharyngeal PCR swab, which raised questions of alternative diagnoses (e.g., relapsed leukemia, hemophagocytic lymphohistiocytosis) and ultimately delayed the initiation of appropriate antiviral therapy. He was ultimately diagnosed with SARS-CoV-2 by PCR tests run on BAL fluid. Generally there is good concordance between NP and BAL samples, although discordant results have been described (9). It is also interesting to note that at his initial infection three weeks prior to admission, our patient tested positive by PCR testing *via* NP swab, indicating that it was previously possible to identify infection by this modality for him.

Chimeric antigen receptor (CAR) T-cell therapy is an immune-modulating treatment approved in the pediatric population for refractory and relapsed B-ALL. CAR T-cells are autologous T cells that are removed from a patient, engineered with anti-CD19 + chimeric antigen receptors that combine an antigen recognition domain with T-cell activation domains, and then re-infused to the patient. The intended result is destruction of malignant CD19+ B cells, although B cell aplasia and subsequent hypogammaglobulinemia secondary to destruction of normal B cells is a common side effect ("on-target, off-tumor"). Response

to CAR-T cell therapy in pediatric and young adult patients with refractory and relapsed B-ALL is generally favorable and durable, with 63% overall survival rate at 36 months, and an estimated 9.28 quality-adjusted life-years (QALYs) gained (10, 11). Survival in relapsed pediatric B-ALL patients with measurable residual disease (MRD) positivity following re-induction who are treated with CAR-T cell therapy prior to HSCT has been shown to improve survival compared to patients treated with chemotherapy prior to HSCT (12).

Patients who undergo CAR-T cell therapy are at increased risk of infection due to multiple factors including prior chemotherapy and cancer treatment, lymphodepleting chemotherapy during the CAR-T cell process, cytokine release syndrome in the immediate post CAR-T cell infusion time period, and hypogammaglobulinemia (13). In children, infections with bacteria predominate in the first 28 days after CAR-T therapy, following which respiratory viral infections are more common. The majority of these respiratory viral infections are considered mild-moderate and not life-threatening (14).

Hypogammaglobulinemia is a common complication after CAR-T cell therapy, with one study demonstrating that 29% of patients had low IgG levels at 63 days post-CAR T-cell infusion (14). Of note, hypogammaglobulinemia is more common and more severe in pediatric as compared to adult patients and can persist for >4 years post infusion (5, 15). Some experts suggest that pediatric CAR T cell recipients with IgG levels less than 400 mg/dl should receive regular supplementation of immune globulin.

Persistent SARS-CoV-2 infection in patients with B cell deficiency, including CAR-T cell recipients, has been described. In one report, 2 of 3 CAR-T cell recipients had prolonged infection lasting >5 months (16). In another case report, a 73-year-old patient with multiple myeloma presented 25 days after CAR-T therapy with cough and hypoxia, and was found to be positive for SARS-CoV-2 by RT-PCR testing of an NP swab with a low cycle threshold (20.1 for nucleocapsid protein, 21.5 for envelope protein) (17). He was treated with convalescent plasma and remdesivir and improved, but subsequently presented again at 41 days post CAR-T therapy with cough and dyspnea, progressing to hypotension and intubation on day 55. At that time, he was still positive for SARS-CoV-2 from NP swab with a low cycle threshold (13.3 for N1 gene; 16 for E gene). He was treated again with convalescent plasma as well as dexamethasone but died on day 74 due to respiratory failure. SARS-CoV-2 RNA was retrospectively detected in his blood plasma samples with an increase observed after the steroid course. Failure of convalescent plasma treatment was attributed to multiple factors including (1) concomitant T cell deficiency limiting immune response (2) possible low levels of effective anti- SARS-CoV-2 antibodies in the convalescent plasma (3) intra-host evolution of SARS-CoV-2 allowing for evasion from circulating antibodies and (4) steroid treatment which may have contributed to T cell dysfunction. In fact, SARS-CoV-2 intra-host evolution in patients with chronic infection has been well described and may account for emergence of new variants (18).

Larger studies of adults who have undergone CAR-T cell therapy report high mortality and morbidity secondary to COVID-19 infection. A multicenter study from Europe of 56 patients who had undergone CAR-T therapy at a median of 7.4 months prior to COVID-19 diagnosis (range 1 day to 25.3 months), reported a 41.1% attributable mortality rate. Additionally, 80% of patients required admission to the hospital for COVID-19 infection with 39.3% requiring admission to the ICU. Of note, there was only a single pediatric patient in that study (19). Factors associated with mortality included older age, not being in complete remission at the time of COVID-19 diagnosis, and having metabolic comorbidities, such as diabetes and cardiovascular disease. The median time to clinical resolution of COVID-19 infection was reported to be 20 days (range 0–157 days). Patients were treated with varying combinations of convalescent plasma, steroids, and remdesivir, although no significant impact of these therapies on clinical outcome was found.

Once the diagnosis of SARS-CoV-2 was made, our patient was treated with both casirivimab/imdevimab (virus-neutralizing monoclonal antibody) and remdesivir (antiviral), and symptoms completely resolved within approximately 24 h of initiation of treatment. Virus-neutralizing mAb products have typically been used for patients with mild-moderate SARS-CoV-2 infection who are at risk for progression to severe disease (20). However, use of mAb in combination with remdesivir for patients with severe B cell deficiency and protracted SARS-CoV-2 infection has been described previously in 3 adult patients. Combination mAb therapy is preferred over a single monoclonal antibody to prevent emergence of mutant virus, and remdesivir is used adjunctively to decrease viral burden (21). This therapeutic approach was also utilized in an adult patient with X-linked agammaglobulinemia on chronic immunoglobulin replacement who was reported to have a recurrent disease course, with initial admission for COVID-19 pneumonia with hypoxia and treatment with remdesivir, dexamethasone, IVIG, and antibiotics, followed by re-admission two weeks later for fever and diarrhea with negative SARS-CoV-2 RT-PCR testing by NP swab. He was ultimately diagnosed with persistent SARS-CoV-2 infection on day 30 of hospitalization (positive RT-PCR testing from sputum and NP swab) and treated with a 10-day course of remdesivir (days 31–40) with immediate improvement. He was also given monoclonal antibodies (casirivimab/imdevimab) on day 38 of hospitalization and tolerated the infusion well (22).

Our patient's clinical course was complicated by clinical symptoms consistent with a peripheral neuropathy which was initially quite perplexing. Given the complete resolution within one day of initiation of anti-SARS-CoV-2 therapies, we concluded that this symptom was secondary to COVID-19 infection as well. While there is limited literature describing Acute Neuropathy Associated with Covid-19 (or ANAC-19), it appears that the majority of affected patients develop neurologic symptoms within one month of infection (23). Common clinical symptoms include paraparesis, quadriparesis, cranial nerve involvement, and hyporeflexia, although sensory symptoms have also been reported.

Overall, our case demonstrates a prolonged and atypical course of COVID-19 in an immunocompromised pediatric patient with B

cell aplasia and hypogammaglobulinemia following CAR-T cell therapy. It additionally highlights unique and uncommon features of COVID-19 infection (e.g., acute neuropathy) and the consideration of a variety of treatment options in an immunocompromised host. The use of combination monoclonal antibody treatment with an antiviral medication was highly efficacious for our patient and has been used in similar clinical contexts in adult patients. We avoided the use of corticosteroid treatment due to concern that T cell function may be affected, as prior studies have indicated that the presence of both B and T cell dysfunction may portend a poorer prognosis as compared to patients with a pure B cell deficiency.

Data availability statement

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

Ethics statement

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

Author contributions

HS, CC, and HSA conceived of the manuscript. All authors contributed to the writing of the manuscript. JC, VD, and NN edited the manuscript. All authors contributed to the article and approved the submitted version.

Conflict of interest

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

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Hashimoto's thyroiditis, vitiligo, anemia, pituitary hyperplasia, and lupus nephritis—A case report of autoimmune polyglandular syndrome type III C + D and literature review

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Objective: This study aims to summarize the clinical characteristics of one teenager with autoimmune polyglandular syndrome (APS) type III C + D to improve the understanding of APS III C + D and its effect of thyroid function.
Methods: This article reported the clinical manifestations, laboratory examinations, treatment methods, and outcomes of an adolescent with anemia admitted to the Pediatrics Department of Tianjin Medical University General Hospital in July 2020 and reviewed the literature.
Results: A girl, aged 13 years and 1 month, was admitted to the hospital due to anemia for more than 4 years and episodic abdominal pain for 1 week. Four years ago, the girl went to a local hospital for "vitiligo", and a routine blood test revealed anemia. The lowest hemoglobin (HGB) was 61 g/L, and the blood test revealed iron deficiency anemia. She had no menstrual cramps for 2 months. Urine routine showed protein 3+~4+ and 258 red blood cells (RBCs)/high-power field. Urine protein was 3,380 mg/24 h. Free thyroxine was low, thyroid-stimulating hormone was >100 uIU/ml, thyroid peroxidase antibody was >1,000 IU/ml, and thyroglobulin antibody and thyrotropin receptor antibody were negative. Pituitary magnetic resonance imaging showed a mass in the sellar region with a uniform signal and a maximum height of about 15.8 mm. The result of the antinuclear antibody was 1:80 homogeneous type, and anti-dsDNA and anticardiolipin antibodies IgA and IgM were slightly higher. Thyroxine and iron were given for 1 month, menstruation resumed, and urine protein and RBC count decreased. After 5 months of treatment, free thyroid function, HGB, RBCs in urine, and pituitary returned to normal. Later, a renal biopsy showed changes in focal proliferative glomerulonephritis, and the girl was diagnosed with lupus glomerulonephritis type III. After 3 days of shock therapy with methylprednisolone, prednisone, mycophenolate mofetil, and other treatments were administrated for 1 year. At the time of writing, urine protein was 280 mg/24 h.
Conclusion: Co-occurrence of Hashimoto's thyroiditis, vitiligo, anemia, pituitary hyperplasia, and lupus nephritis is rare. It is very important to pay attention to the screening of thyroid function.

KEYWORDS

autoimmune polyglandular syndrome (APS) type III, Hashimoto's thyroiditis (HT), vitiligo, anemia, lupus nephritis (LN), pituitary hyperplasia

1. Introduction

Autoimmune polyglandular syndromes (APSs) refer to the dysfunction of two or more endocrine glands under the invasion of autoimmune inflammation; it can also be a syndrome of autoimmune diseases involving non-endocrine systems. APSs are currently divided into four types (1–3), APS I, –IV. The prominent feature of APS III is the absence of adrenal involvement, which is different from APS I, II, and IV. The clinical diagnosis of APS III is defined as autoimmune thyroid disease (AITD) combined with at least one other autoimmune disease. APS III is also divided into four types (4). Among them, those with diabetes or hypophysitis are APS IIIA, those with autoimmune digestive tract diseases or pernicious anemia are APS IIIB, those with vitiligo are APS IIIC, and those with systemic lupus erythematosus (SLE) are APS IIID.

Although autoimmune thyroid disease, vitiligo, and SLE often occur in pairs, it is rare for our patient to suffer from all three of the above diseases simultaneously, involving the kidneys and pituitary gland. Thus, the clinical characteristics of this APS III C + D case were analyzed, and the related literature was reviewed to improve the understanding of APS III C + D and the effect of thyroid function.

2. Case description

A girl, aged 13 years and 1 month, was admitted to the hospital in July 2020 due to anemia for more than 4 years and episodic abdominal pain for 1 week. More than 4 years before admission, the girl went to a local hospital for "vitiligo". A routine blood test revealed anemia, and the exact value of hemoglobin (HGB) was unknown. Her parents did not pay attention. She was treated with oral traditional Chinese medicine and physiotherapy to improve "vitiligo". In the past 2 years, she has not taken any related drugs, and there is no obvious progress. Since then, routine blood monitoring showed anemia, and HGB was once 80 g/L, but no intervention was given. The girl usually had no obvious uncomfortable complaint, no skin and gum bleeding, no repeated oral ulcers, no Raynaud's phenomenon, no joint swelling and pain, and no abnormal stools and urine. There was no obvious cause for pain in her lower right abdomen and around the umbilicus 1 week before admission. Abdominal ultrasound and CT in the local hospital showed an "enlarged appendix", and the routine blood examination showed that the HGB was as low as 65 g/L. The girl was hospitalized in the surgery department and preparing for surgery; 1 U of suspended red blood cells (+Dex) were transfused three times, Ceftriaxone was infused for 6 days, and HGB rose to 74 g/L. After her abdominal pain improved, she was referred to our department. She had no history of surgery, trauma, and poison exposure.

The girl was born at full term from a gravida 1 para 1 mother aged 37 years and had no history of birth injury or asphyxia. She had normal intellectual developmental milestones and poor academic performance. She had menarche at the age of 12 years: 4/30, regular menstruation, and low menstrual flow usually. However, she had no menstrual cramps in the past 2 months. Her father had coronary heart disease and hyperlipidemia. Her half-sister suffered from thyroid disease.

Physical examination results are as follows: pulse, 70 bpm; respiration, 17 bpm; blood pressure, 97/60 mmHg; weight, 47 kg (P50–75); height, 151 cm (P10–25). The girl had good nutritional status. Several depigmented spots of different shades and clear boundaries can be seen on the skin of the girl's face, abdomen, and feet, 0.5 cm × 0.5 cm–2 cm × 1 cm in size. It can be seen that the girl's left upper eyelid is red, swollen, and slightly prolapsed, and one small nodule with pain can be palpated on the eyelid. The palpebral conjunctiva, lips, and oral mucosa were slightly pale. The girl had no yellowing of hair, no swelling of superficial lymph nodes, less lingual papilla, cracked tongue, and no oral ulcers. The girl had no obvious enlargement of the bilateral thyroid. The girl's nail surface was of a slightly flat, rough texture. No pubic and armpit hair was seen. Other physical examinations showed no abnormality.

Laboratory examination results (see **Table 1**) such as red blood cell (RBC), HGB, hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) showed lower values. Reticulocyte (RET) was slightly higher. Peripheral blood smear showed morphological small red blood cells with an enlarged central pale area (**Figure 1**). Ferritin (Fer) and serum iron (SI) were lower, and folic acid and vitamin B12 were normal. The above results suggested iron deficiency anemia.

Further examination revealed that free thyroxine (FT4) was lower than the normal value and thyroid-stimulating hormone (TSH) and thyroid peroxidase antibody (TPOAb) were significantly elevated. Thyroid ultrasound showed mild enlargement of the bilobal thyroid, thickening of the isthmus, reduced echo heterogeneity, and multiple nodules in the bilobal thyroid. Pituitary magnetic resonance imaging (MRI) showed a mass in the sellar region with a uniform signal and a maximum height of about 15.8 mm. The pituitary stalk was still in the middle, without thickening. Growth hormone (GH) was normal at a low value. Insulin-like growth factor 1 (IGF1) and insulin-like growth factor binding protein 3 (IGFBP3) were normal. The bone age was 12.7 years (0.5 years behind the patient's actual age). Prolactin (PRL) was higher. Gynecological ultrasound indicated the development of the uterus and bilateral ovaries. Blood cortisol and adrenocorticotropic hormone (ACTH) rhythm were normal. Electrolytes and plasmatic and urinary osmolarity were normal, so diabetes insipidus was not supported. Parathyroid hormone (PTH) was normal.

Rroutine urine examination showed PRO3+∼4+, BLD3+, WBC1+∼2+, RBC 258/high-power field (HPF), and WBC45/HPF. The level of 24 h urinary protein was 71.9 mg/kg.24 h, which indicated massive proteinuria. Urine protein electrophoresis showed that albumin accounted for 80%. The levels of 24 h urinary creatinine and blood and urine β2-microglobulin were normal. Urine phase-contrast microscopy indicated 95% glomerular red blood cells. Antiglomerular basement membrane (GBM) antibody was negative. Antinuclear antibody (ANA) was 1:80 homogeneous type, and perinuclear

TABLE 1 Laboratory examination.

Blood routine test							
RBC (4.0–4.5)*10^{12}/L	HGB (110–150) g/L	HCT (37.0–43.0)%	MCV (82.0–95.0) fl	MCH (27–33) pg	MCHC (320–360) g/L	RET (0.5–1.5)%	EPO (5.4–31) mIU/ml
3.65	61	26.9	67.1	16.7	249	1.66	20.2
Ferric metabolism							
Fer (4.63- 204) ng/ml	SI (5.4–28.6) μmol/L	TIBC (40.8–76.6) μmol/L	UIBC (19.7–66.2) umol/L	Folic acid (3.10–20.5) ng/ml	Vitamin B12 (187.00–883.00) pg/ml		
<1.0	2.7	67.1	64.4	7.98	627.73		
Thyroid axis							
FT3 (2.63–5.70) pmol/L	FT4 (9.01–19.05) pmol/L	TSH (0.35–4.94) uIU/ml	TPOAb (0.00–35.00) IU/ml	TGAb (0.00–40.00) IU/ml	TRAb (0.00–1.75) IU/L		
2.89	<5.15	>100	>1,000	35.2	1.51		
Growth hormone (GH) axis							
GH (0.06–5.00) ng/ml	IGF-1 (111.00–996.00) ng/ml	IGFBP3 (2.4–10.00) μg/ml					
0.06	174	5.08					
Bone metabolism							
Ca (2.10–2.55) mmol/L	PHOS (0.80–1.45) mmol/L	ALP (40–150) U/L	25-OHD (17.5–133.00) nmol/L	PTH (1.10–7.30) pmol/L			
2.27	1.36	86	19.55	3			
Adrenocorticotropic hormone (ACTH) axis							
Blood cortisol (5–25) μg/dl	0AM	8AM	4PM	ACTH (0–46) pg/ml	0AM	8AM	4PM
	2.57	10.5	3.44		17	33.1	20.8
Gonadal axis							
FSH (follicular phase 3.03–8.08, ovulatory period 2.55–16.69, luteal phase 1.38–5.47) IU/L	LH (follicular phase 1.80–11.78, ovulatory period 7.59–89.08, luteal phase 0.56–14) IU/L	PRL (follicular phase 5.18–26.53, ovulatory period 5.18–26.53, luteal phase 5.18–26.53) ng/ml	E2 (follicular phase 21.00–251.00, ovulatory period 38.00–649.00, luteal phase 21.00–312.00) pg/ml	P (follicular phase 0.00–0.30, ovulatory period1.49–5.87, luteal phase 1.20–15.9) ng/ml	T (follicular phase 10.83–56.94, ovulatory period 10.83–56.94, luteal phase 10.83–56.94) (pg/dl)		
4.81	3.58	58.33	22	<0.10	13.07		
Electrolytes and osmolarity							
K (3.5–5.3) mmol/L	Na (135–150) mmol/L	CL (96–108) mmol/L	CO2CP (21–31) mmol/L	AG (4.00–20.00) mmol/L	Plasmatic osmolality (280–310) mOsm/kgH_2O	Urinary osmolality (600–1,000) mOsm/kgH_2O	
3.6	139	106	24	12.6	294	815	
Blood lipid, myocardial enzyme, and coagulation function							
TC (3.59–5.17) mmol/L	TG (0.57–1.71) mmol/L	HDL-C (0.80–2.20) mmol/L	LDL-C (1.33–3.36) mmol/L	CK (25–200) U/L	CK-MB (0–24) U/L	HDH (94–250) U/L	coagulation function (N)
4.09	1.97	0.95	2.52	40	8	202	N
Kidney function and related items							
Urea (1.7–8.3) mmol/L	CREA (44–115) mmol/L	UA (140–414) mmol/L	C-reactive protein activity (87.0–133.0)%	Free protein S content (89.3–112.5)%	Cystatin-C (0.58–1.03) mg/L	Serum β2-MG (0.80–2.90) mg/L	urine β2-MG (0.91–2.2) mg/L
2.9	44	276	106	67.2	0.74	1.74	1.85
Liver function							
TP (62–85) g/L	ALB (35–55) g/L	GLO (20–40) g/L	ALT (5–40) U/L	AST (8–40) U/L	GGT (7–49) U/L	TBIL (3.4–20.0) μmol/L	DBIL (0.1–6.8) umol/L
67	36	31	8	24	10	8.4	2

(*continued*)

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TABLE 1 Continued

Blood routine test							
Tumor items							
CA19-9 (0.00–37.00) U/ml	CA125 (0.00–35.00) U/ml	AFP (0.00–8.78) ng/ml	CEA (0.00–5.00) ng/ml	CA15-3 (0.00–31.3) U/ml	NSE (0.00–16.30) μg/L		
49.84	35.1	1.36	1.2	6.8	15.33		
Glucose metabolism							
HbA1c (4.00–6.00)%	Fasting BG (3.60–5.80) mmol/L	Fasting insulin (4.00–18.00) mU/L	ICA (NEG)	GADA (NEG)			
5.8	5.06	7.9	NEG	NEG			
Immunologic test							
ANA < 1:80	C3 (79.00–152.00) mg/dl	C4 (16.00–38.00)mg/dl	Anti-dsDNA Ab (<18) IU/ml	ACA-IgM (<61.1) U/ml	ACA-IgA (<62.5) U/ml	ACA-IgG (<66.7) U/ml	LA test (NEG)
Homogeneous type 1:80	91.20	27.70	30.4	119.5	62.9	31.9	NEG
ASMA (NEG)	IgG (751.00–1560.00) mg/dl	IgA (82.00–453.00) mg/dl	IgM (46.00–304.00) mg/dl	IgE < 165.00 IU/ml	pANCA (NEG)	C1q-Ab (<3.18) U/ml	ASO (<116.00) IU/ml
NEG	1280	223	292	16.6	Suspiciously positive	2.8	<25.0
RF (<20) IU/ml	EAN spectrum (NEG)	CRP (<8.00) mg/L	ESR (0–20) mm/h	T/B lymphocyte subsets	IL-6 (0.1–2.9) pg/ml	Ig G subclass 4 (NEG)	
<20	NEG	<0.49	20	N	6.8	NEG	
Etiological examination							
Anti-TP (NEG)	Anti-HIV (NEG)	TB.SPOT test (NEG)	MP-IgM (NEG)	Hepatitis virus-Ab (NEG)	EBV-Ab (NEG)	CMV-IgM (NEG)	
NEG	NEG	NEG	NEG	NEG	NEG	NEG	

TIBC, total iron binding capacity; UIBC, unsaturated iron binding capacity; FT3, free triiodothyronine; TGAb, thyroglobulin antibody; TRAb, thyrotropin receptor antibody; PHOS, phosphorus; ALP, alkaline phosphatase; 25-OHD, 25-hydroxyvitamin D; E2, estradiol; P, progesterone; T, testosterone; CO_2 CP, carbon dioxide combining power; AG, anion gap; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; CK, creatin-kinase; CK-MB, creative kinase-MB isoenzyme; LDH, lactic dehydrogenase; N, normal; CREA, creatinine; UA, uric acid; β2-MG, β2-microglobulin; TP, total protein; ALB, albumin; GLO, globulin; ALT, alanine transaminase; AST, aspartate transaminase; GGT, gamma-glutamyl transferase; TBIL, total bilirubin; DBIL, direct bilirubin; AFP, alpha fetoprotein; CEA, carcinoembryonic antigen; NSE, neuron specific enolase; HbA1c, hemoglobin A1c; BG, blood glucose; ICA, islet cell antibodies; NEG, negative; GADA, glutamic acid decarboxylase antibody; C, complement; LA, lupus anticoagulant; ASMA, antismooth muscle antibody; Ig, immunogloulin; ASO, antistreptolysin "O"; RF, rheumatoid factor; ENA, extractable nuclear antigen; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; Anti-TP, Anti-treponema pallidum; HIV, human immunodeficiency virus; TB, tuberculosis; MP, mycoplasma pneumoniae; EBV, Epstein–Barr virus; CMV, cytomegalovirus.

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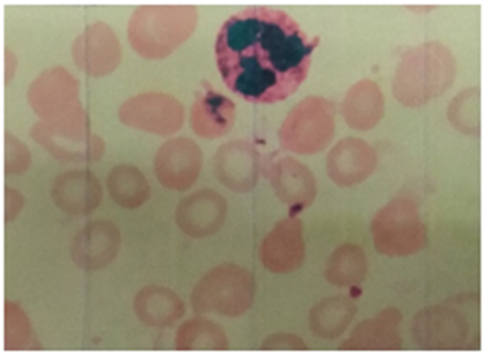

FIGURE 1
Peripheral blood smear showing small red blood cells with an enlarged central pale area.

antineutrophil cytoplasmic antibody (pANCA) was suspiciously positive. Antidouble-strand DNA antibody (anti-dsDNA Ab), anti-cardiolipin antibody (ACA)-IgM, and ACA-IgA were elevated.

Triglyceride, serum carbohydrate antigen (CA) 19–9, and CA125 were slightly increased. Chest ultrasound showed that pleural effusion was seen bilaterally (12.6 mm on the left and 11.6 mm on the right). Chest CT showed ground-glass density nodules and micronodules in the lower lobe of the right lung, and the density of the cardiac chambers decreased, suggesting anemia. Abdominal ultrasound indicated that the liver, gallbladder, pancreas, spleen, kidneys, and adrenal gland were normal. Abdominal CT indicated multiple and full lymph nodes at the root of the mesentery and a small amount of pelvic effusion. Orbital CT showed enlarged left lacrimal gland and increased surrounding fat density, which was consistent with inflammatory changes.

The clinical diagnosis was Hashimoto's thyroiditis (HT) (with pituitary hyperplasia), iron deficiency anemia, glomerulopathy, hyperlipidemia, and vitiligo. The family refused renal puncture. The patient was considered to be APS IIIC.

Initially, the girl was treated with levothyroxine sodium (50 μg/d) to supplement thyroxine, polysaccharide iron complex (0.15/d) to supplement iron, and captopril (12.5 mg, twice per day) to reduce urinary protein. Her condition (see **Figure 2E**) improved, and she was discharged from the hospital in 12 days. One month after discharge, her menstruation resumed. The hemoglobin was significantly increased, and TSH, urine protein, and red blood cells were significantly decreased (see **Figures 2C–E**). After 5 months of treatment, HGB and FT4 returned to the normal range (see **Figures 2A,B**); re-examination MRI showed that the pituitary decreased to 6.5 mm and returned to normal (**Figure 3**). Tumor markers returned to normal and did not support the tumor.

Until July 2021, the girl had been treated with mycophenolate mofetil (MMF) (0.75 g, twice a day) for 7 months; laboratory report showed urine occult blood ±–+, 800 mg–2,300 mg/24 h urine protein, and suspiciously positive anti-dsDNA and pANCA. Afterward, the girl was admitted to Peking Union Medical College Hospital for renal biopsy, and renal pathology showed focal proliferative glomerulonephritis (specific description: a total of 16 glomeruli can be seen in the whole film, including 1 spherical sclerosis and 1 staged sclerosis, with focal staged mesangial cell proliferation, increased mesangial matrix, and focal staged endothelial cell proliferation. A small number of capillary loops were compressed and narrowed. GBM was not significantly thickened. The subendothelial and mesangial areas were seen with erythrophilic deposition. The renal tubular epithelial cells were seen with opacity and vacuolar degeneration. There were many small focal, dense, mononuclear-dominated inflammatory cell infiltrations in the renal inerestitum). Combined with immunofluorescence, the clinical diagnosis was SLE and lupus nephritis (LN) type III. Sequential therapy include methylprednisolone 500 mg shock for 3 days and then changing to prednisone (30 mg per day). The hormone was gradually reduced to 10 mg per day for maintenance, MMF 0.75 g twice a day, tacrolimus 2 mg in the morning and 1 mg in the evening, and captopril 12.5 mg twice a day.

At the time of writing, the girl has been treated for 2 years; her height is 159.8 cm (P50), and anti-dsDNA, pANCA, and Anti-cardiolipin antibodies are all within normal ranges. The riglyceride level is normal. Urinary proteins are significantly reduced (**Figure 2E**).

3. Discussion

Autoimmune polyglandular syndrome (APS) was first reported by Schmidt in 1926 (5). APS type III is the most common type of APS in adults (3). Tian et al. (6) have well summarized the APS type III cases reported from 1989 to 2019, with a total of 64 relatively detailed cases. Our case of childhood APS type III C + D (HT, vitiligo, anemia, pituitary hyperplasia, SLE) has not been reported yet (3, 6, 7), and the specific mechanism is not fully elucidated.

3.1. Vitiligo and HT, SLE

A study showed that among 1,098 patients with vitiligo, nearly 20% had at least one comorbid autoimmune disease, of which 12.9% had thyroid disease and 0.3% had SLE (8). Single-nucleotide polymorphism of the tyrosine phosphatase nonreceptor 22 (PTPN22) gene is shared among patients with vitiligo and AITD (9). Studies have found that the PTPN22 1858T allele is associated with SLE and AITD (10). These findings suggest that the association observed between vitiligo, AITD, and SLE may be explained, at least in part, by the sharing of susceptibility genes.

(1) Vitiligo and HT: Vitiligo with thyroid disease is mainly vulgaris-type, and segmental-type is rare (4). This patient is consistent with literature reports (4, 11, 12).
(2) Vitiligo and SLE: The patient's condition involved multiple systems when she was admitted to the hospital; in addition to vitiligo, a disease of the immune system, there is anemia

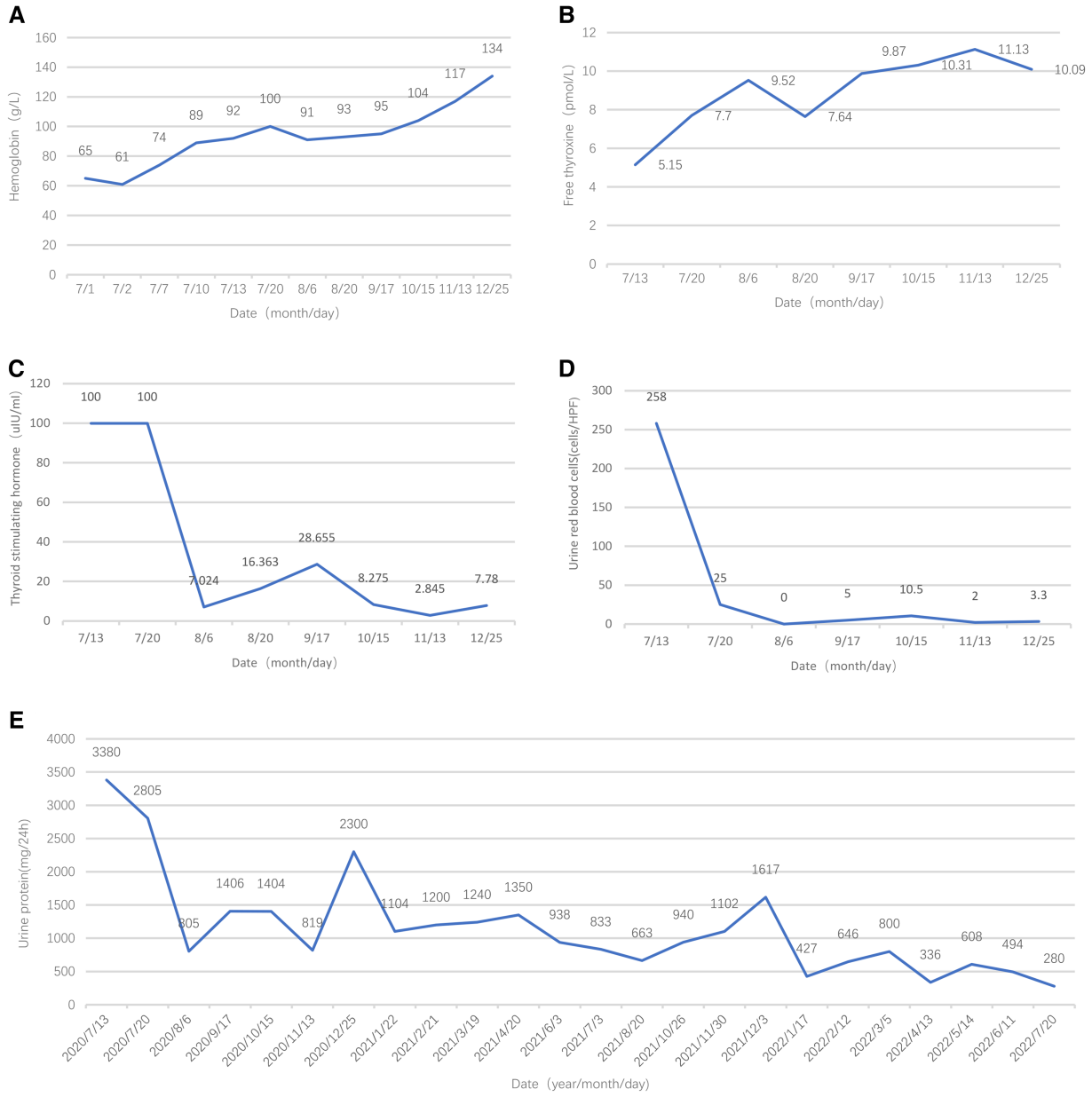

FIGURE 2
Follow-up results of hemoglobin, free thyroxine, thyroid-stimulating hormone, urine red blood cells, and urine protein.

and urinary system damage, which was complex. Considering that the patient is an adolescent girl with chronic onset, we first performed related examinations for SLE. The girl also had serous cavity effusion and abnormal immunological indicators: ANA titers of 1:80, positive anti-dsDNA antibodies, and elevated anti-cardiolipin antibodies, supporting SLE. However, fever, repeated rashes, oral ulcers, photosensitivity, and joint swelling or pain were not reported. ANA titers were low, complements were not low, erythrocyte sedimentation rate was not fast, and anemia was nonhemolytic anemia; all these were insufficient evidence to diagnose SLE. Finally, 1 year later, the family members agreed to the renal biopsy, and the diagnosis of SLE and lupus glomerulonephritis type III was confirmed.

3.2. HT and anemia

Thyroid hormones have a wide range of physiological functions and can affect the functions of multiple organs and systems, including the hematopoietic system (13–15). The onset of hypothyroidism-related anemia is insidious, and the clinical manifestations are not specific, so it is often misdiagnosed and missed (16).

Hypothyroidism can reduce the metabolism of the hematopoietic system, reduce erythropoietin, reduce bone marrow hematopoietic function, and cause anemia. Low metabolism of iron can cause iron deficiency anemia (IDA). There may be antibodies against gastric parietal cells, resulting in atrophic gastritis and intrinsic factor deficiency. At the same time, decreased gastric acid secretion and malabsorption of iron

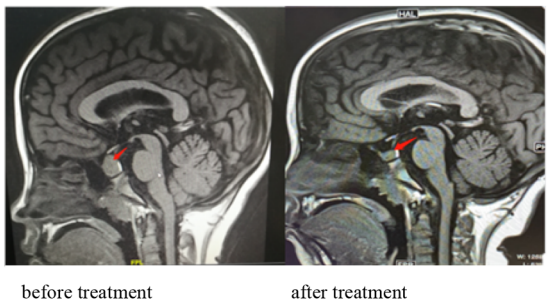

FIGURE 3
Pituitary MRI showing a mass in the sellar region with a uniform signal and a maximum height of about 15.8 mm. The pituitary stalk is still in the middle, without thickening. MRI showing that the pituitary decreased to 6.2 mm and returned to normal after treatment.

and vitamin B12 can cause anemia. This patient has microcytic hypochromic anemia, iron deficiency, and a relative lack of erythropoietin (EPO), which is consistent with the research (15, 17). We considered that it might be related to the interference of EPO generation by hypothyroidism. However, long-term urinary occult blood can also aggravate anemia. Also, the appearance of urinary protein results in insufficient hematopoietic raw materials for hemoglobin synthesis in the body. Combined with the presence of LN in the girl, secondary kidney damage results in reduced EPO production and interference with iron metabolism, affecting bone marrow hematopoiesis.

3.3. HT and kidney damage

Secondary nephritis of SLE was considered according to the renal biopsy. However, through levothyroxine replacement therapy, urinary occult blood was significantly improved and urinary protein decreased, which suggested that in addition to LN, it is also necessary to pay attention to the effect of HT on the kidneys.

(1) Currently, some studies (18) believe that AITD-related nephropathy may be caused by the deposition of thyroid peroxidase and thyroglobulin outside the glomerular basement membrane, resulting in the formation of *in situ* immune complexes or the formation of circulating immune complexes with antibodies in the glomerulus. In hypothyroidism, the glomerular filtration barrier is damaged, the glomerular capillary basement membrane is thickened, the permeability is enhanced, a large amount of urine protein is lost, and the plasma colloid osmotic pressure is reduced, further aggravating the edema. In addition, impaired immune tolerance to megalin, a thyroid-stimulating hormone-regulated glycoprotein expressed on thyroid cells, also contributes to its pathogenesis. Serum total thyroxine (TT4) and FT4 were negatively correlated with massive proteinuria.

At the same time, chronic kidney disease can also lead to changes in thyroid hormones and thyroid function (19–21). Patients with massive proteinuria have high levels of TSH, which is related to the loss of thyroid hormones and thyroid-binding globulin from the urine (21). Also, the conversion of T4 into triiodothyronine (T3) decreases, thyroid-binding globulin decreases, and blood T3 and T4 decrease. Correspondingly kidney damage can exacerbate Hashimoto's thyroiditis.

(2) HT and SLE: The coexistence of the two kinds of diseases may be related to both having the susceptibility gene 5q14.3–q15 (22) and the high expression of HLA-B8 and DR3 (23). Th1 predominance is also an immunological mechanism for the coexistence of SLE and AITD (24). The thyroid itself is also a part of the systemic organ damage in SLE, which may cause thyroid dysfunction. In terms of the severe complications of SLE, those with thyroid diseases carried higher risks for lupus nephritis involvement (25). There is a correlation between hypothyroidism and lupus activity (26). Also, decreased albumin and increased serum creatinine are associated with hypothyroidism (25–29). However, antithyroid antibodies (ATAs) were not associated with SLE activity. The positive rate of ATA and the incidence of abnormal thyroid function in children with LN were higher than those in the general population. When SLE is severely active, it affects the regulation of the hypothalamus -pituitary -thyroid (HPT) axis, and the level of T3 decreases, which is proportional to the severity and duration of the disease (30).

3.4. HT and pituitary hyperplasia, growth impairment, and disorder of reproductive function

3.4.1. HT and pituitary hyperplasia

Pituitary hyperplasia can be secondary to primary hypothyroidism (PPH) (31–33), and its degree correlates with the severity of hypothyroidism (34).

Pituitary tumor-like hyperplasia is due to primary hypothyroidism feedback activation of the HPT axis, resulting in increased thyrotropin-releasing hormone (TRH), stimulation of anterior pituitary TSH cell proliferation, pituitary enlargement (34), and even adenomas. Due to an insufficient understanding of primary hypothyroidism, there have been many clinical reports of surgical treatment of pituitary tumors. Correct and timely diagnosis can avoid unnecessary surgery or inappropriate drug treatment. Through thyroid hormone replacement therapy, with the recovery of thyroid function, the secondary pituitary hyperplasia or adenoma will gradually shrink until it disappears. The patient in this case was a girl with hyperplasia of the pituitary gland. After 5 and a half months of treatment, the MRI scan showed that the pituitary was significantly reduced to normal (**Figure 3**).

3.4.2. HT and growth impairment

The thyroid hormone mediates bone maturation and development of the skeleton *via* its direct and permissive effects on GH (35).

On the one hand, *via* specific membrane transporters, T3 enters the target cell nucleus where it binds and activates either

thyroid hormone receptor α or β (TRα, TRβ). TRα is the main receptor expressed in the skeleton and mediates T3 action in bone and cartilage. TRβ mediates negative feedback control of the HPT axis (36, 37). Thyroid hormone mediates the growth, development, and maturation of the skeleton by regulating chondrocyte proliferation, promoting differentiation of bone progenitor cells, mineralization, and angiogenesis.

In juvenile hypothyroidism, skeletal maturation is predominantly affected by delayed fusion of the epiphysis and delayed bone age. It leads to delayed skeletal development, linear growth retardation, and short stature.

On the other hand, the thyroid hormone also has a permissive role in the action of GH by promoting GH secretion from the pituitary, as well as GH-dependent IGF 1 production in the bone (35). GH secretion decreases when thyroxine level decreases, eventually leading to impaired height.

Prompt treatment of children with thyroid hormone replacement induces a period of fast growth in which skeletal maturation and bone age are also accelerated (36). However, whether the predicted adult height is attainable depends on the severity of hypothyroidism and its duration before thyroid hormone replacement begins. The girl reported in the present case had a deceleration of growth, delayed bone age (0.5 years behind the patient's actual age) as shown by imaging examination, a lower level of GH than the normal range, and a height increase of 8.8 cm after 2 years of thyroid hormone treatment, with an increase in height percentile from P10–25 to P50.

3.4.3. HT and disorder of reproductive function

HT can lead to a disorder of reproductive function through direct and indirect interactions with the hypothalamus–pituitary–ovarian axis and the reproductive organs (38). First, the synergistic effect between follicle-stimulating hormone (FSH) and T3 can directly stimulate the function of granulosa cells and the formation of luteinizing hormone (LH)/human chorionic gonadotropin receptors (38, 39). Thyroid receptors exist on oocytes, and thyroid antibodies exist in follicular fluid (38, 40–42).

Severe juvenile hypothyroidism can result in follicle dysplasia, ovulatory dysfunction, and insufficient corpus luteum development with low progesterone production. It also affects the function of the ovaries and leads to menstrual disorders and delayed sexual maturation.

Due to primary hypothyroidism feedback activation of the HPT axis, TRH increased. TRH leads to the proliferation of prolactin cells and the increase of PRL (43). Hyperprolactinemia is also a common cause of ovulatory dysfunction (44). It may impair the pulsatile secretion of gonadotropin-releasing hormone (GnRH) and result in ovarian dysplasia.

In overt thyroid dysfunction, rapid initiation of thyroid hormone therapy can make endocrine hormones return to normal. Also, menstruation can be restored (32, 43) and sexual development can be normalized. The girl in this case had stopped menstruation for 2 months, and prolactin was slightly higher. After 2 months of treatment, menstrual cramps began again and prolactin had returned to normal, which is consistent with reports.

3.5. Tips

This case suggests that (1) when there is multisystem immune damage, the possibility of thyroid involvement, APS, and SLE should be considered if it cannot be explained by common etiologies or if it cannot be cured for a long time. (2) When chronic anemia is inconsistent with renal function, or there is unexplained proteinuria or occult blood in urine, please pay attention to screening for hypothyroidism to reduce unnecessary blood transfusions. (3) The diseases in children with no obvious symptoms of abnormal thyroid function and mild thyroid enlargement are difficult to diagnose and can be misdiagnosed and mistreated easily. Thus, regardless of whether the thyroid is enlarged or not, in the presence of immune system diseases or the involvement of multiple systems, it is very important to screen free thyroid function and antibodies. (4) For pituitary hyperplasia, we should be wary of severe hypothyroidism and check free thyroid function in time to reduce unnecessary surgery.

In conclusion, the clinical manifestations of APS are complex and diverse. Patients diagnosed with APS should be followed up regularly to be alert to other comorbidities. Also, this patient should be alert to the occurrence of polyphospholipid syndrome in the follow-up process in the future. The pathogenic mechanism of APS type III C + D in children is still fully unclear, and the long-term effect of the therapy still needs further observation in larger sample sizes over longer time periods.

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

YS contributed to the conception, wrote, and revised the manuscript. XK contributed to the design of the study. RZ gave a total review. LH performed data curation. ZM reviewed the literature, wrote sections of the manuscript, and polished the paper. YJ did formal analysis. All authors contributed to the article and approved the submitted version.

Conflict of interest

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

Publisher's note

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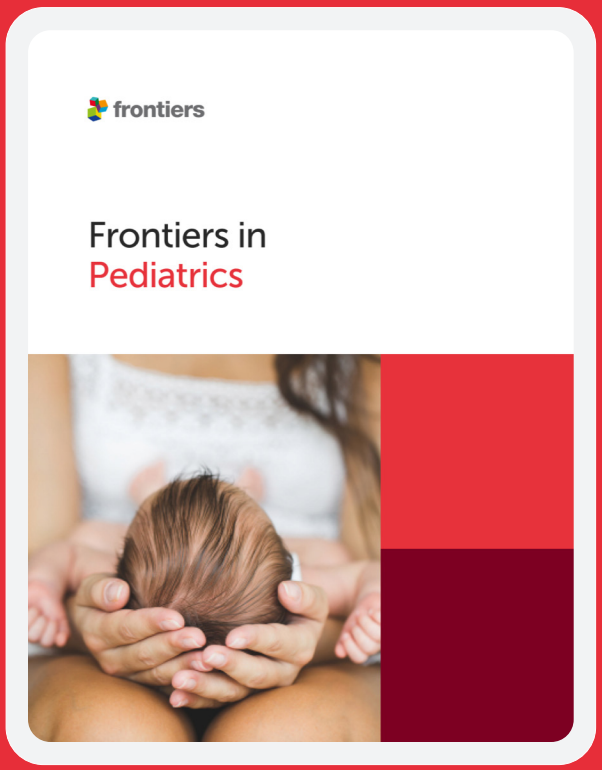

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