Journal of Clinical Immunology
https://doi.org/10.1007/s10875-021-01031-5

ORIGINAL ARTICLE

Genomic Spectrum and Phenotypic Heterogeneity of Human IL-21 Receptor Deficiency

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Received: 7 February 2021 / Accepted: 29 March 2021
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Abstract

Biallelic inactivating mutations in *IL21R* causes a combined immunodeficiency that is often complicated by cryptosporidium infections. While eight IL-21R-deficient patients have been reported previously, the natural course, immune characteristics of disease, and response to hematopoietic stem cell transplantation (HSCT) remain to be comprehensively examined. In our study, we have collected clinical histories of 13 patients with IL-21R deficiency from eight families across seven centers worldwide, including five novel patients identified by exome or NGS panel sequencing. Eight unique mutations in *IL21R* were identified in these patients, including two novel mutations. Median age at disease onset was 2.5 years (0.5–7 years). The main clinical manifestations were recurrent bacterial (84.6%), fungal (46.2%), and viral (38.5%) infections; cryptosporidiosis-associated cholangitis (46.2%); and asthma (23.1%). Inflammatory skin diseases (15.3%) and recurrent anaphylaxis (7.9%) constitute novel phenotypes of this combined immunodeficiency. Most patients exhibited hypogammaglobulinemia and reduced proportions of memory B cells, circulating T follicular helper cells, MAIT cells and terminally differentiated NK cells. However, IgE levels were elevated in 50% of IL-21R-deficient patients. Overall survival following HSCT (6 patients, mean follow-up 1.8 year) was 33.3%, with pre-existing organ damage constituting a negative prognostic factor. Mortality of non-transplanted patients ($n = 7$) was 57.1%. Our detailed analysis of the largest cohort of IL-21R-deficient patients to date provides in-depth clinical, immunological and immunophenotypic features of these patients, thereby establishing critical non-redundant functions of IL-21/IL-21R signaling in lymphocyte differentiation, humoral immunity and host defense against infection, and mechanisms of disease pathogenesis due to IL-21R deficiency. Outcome following HSCT depends on prior chronic infections and organ damage, which should thus be considered as early as possible following molecular diagnosis.

Keywords IL-21/IL-21R signaling · T follicular helper cells · B cell differentiation · STAT3

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Introduction

Interleukins (ILs) produced by hematopoietic and non-hematopoietic cells mediate the development, survival, activation, proliferation, and differentation of cells of the immune system [1, 2]. IL-21 is a type I cytokine, produced predominantly by $CD4^+$ T cells, including T follicular helper (Tfh) cells, Th17 and NKT cells [3–6]. On binding its cognate receptor, IL-21 activates JAK/STAT signaling pathways, inducing transcriptional networks that regulate immune cell fate and effector functions [7].

Published online: 30 April 2021

The receptor for IL-21 comprises IL-21R and the "common gamma chain" subunit (γc), which is shared by receptors for IL-2, IL-4, IL-7, IL-9, and IL-15 [8, 9]. Consistent with the broad expression of IL-21R, IL-21 has pleiotropic effects on many immune cell types, including promoting $CD8^+$ T cell activation and proliferation [10], NK cell maturation and cytotoxicity [11], and differentiation of $CD4^+$ T cells, including regulatory T cells [12, 13]. IL-21 appears to exert its most potent effects on B cells, inducing vigorous proliferation, memory B cell formation, isotype switching, and differentiation to plasma cells [14–18]. This parallels the enriched production of IL-21 by Tfh cells within germinal centers (GCs) of secondary lymphoid tissues [3, 19], and strong expression of IL-21R on GC B cells [17].

The non-redundant role of IL-21/IL-21R signaling has been revealed by the identification and characterization of patients with loss-of-function (LOF) biallelic variants in *IL21* [20] or *IL21R* [21–24] who develop an autosomal recessive combined immunodeficiency (CID) with variable presentations. Currently, eight patients from five families with unique homozygous mutations in *IL21R* have been reported [22–25]. The majority of patients suffered from recurrent respiratory bacterial and gastrointestinal infections, especially cryptosporidial infections associated with chronic cholangitis. Other features of IL-21/IL-21R deficiency are chronic diarrhea, failure to thrive, tinea corporis, herpes labialis, candida and mycobacterial infections, and hypogammaglobulinemia [22–25]. Detailed analysis of immune cells from these individuals revealed impaired T cell proliferation, cytokine production and cytotoxicity [22, 23, 25], reduced Tfh and innate T cell generation [23, 26, 27], impaired B cell differentiation [22–24, 28, 29], and reduced NK cell cytotoxicity [22].

Here, we have identified five novel patients with recessive *IL21R* mutations, and comprehensively define the clinical and laboratory features of IL-21R deficiency in 13 patients. Furthermore, we expand the clinical and laboratory spectrum of the disease, and report treatment outcomes including those following allogenic hematopoietic stem cell transplant (HSCT).

Material and Methods

Patients

We performed a retrospective analysis of 13 patients with IL-21R deficiency based on a standardized clinical questionnaire. Six patients (P1–P6) were enrolled from three centers in Turkey and an additional five patients came from French (P11), Israeli (P12, P13), and American centers (P9,10). Five patients (P2, P3, P4, P6, P13) were not reported previously. We provide longer-term and/or more detailed clinical follow-up information for six previously reported patients (P1, P5, P9, P10, P11, P12) using the same questionnaire. We additionally included two deceased patients (P7, P8) previously described in the literature [21, 22].

While five of the patients (P1, P4, P5, P6, P12) were identified by a targeted panel of inborn errors of immunity (IEI) genes or whole exome sequencing (WES), variants in P2, P3, P9, P10, P11, and P13 were detected with targeted capillary sequencing.

The study was approved by the respective ethics review boards of the participating institutes, including the Ethics commitees of the Hacettepe University (GO13-228); Marmara University (09.2018.624); Royal Prince Alfred Hospital, Camperdown, Australia (X16-0210/LNR/16/RPAH/257); and Medical University of Vienna, Austria (1796/2018). Written informed consent for genetic investigations was obtained from each family.

Immunophenotyping and Functional Analysis of IL-21R-Deficient Lymphocytes

PBMCs from IL-21R-deficient patients ($n = 5–10$) and healthy donors ($n = 11–40$) were analyzed by immunofluorescent staining and flow cytometry [30] to determine proportions of B- ($CD20^+$), T- ($CD3^+$), NK- ($CD3^-CD56^+$), and dendritic cells (DCs) ($CD19^-CD3^-CD56^-CD235a^-CD14^-CD20^-HLA\text{-}DR^+$). Subsets of T cells ($CD4^+$, $CD8^+$, iNKT (TCR $V\alpha24J\alpha Q^+$), MAIT (TCR $V\alpha7.2^+CD161^+$), Vγδ (TCR $V\gamma\delta^+$), Vαβ (TCR $V\alpha\beta^+$)) were also determined. Frequencies of naïve ($CD45RA^+CCR7^+$), central memory (T_{CM}, $CCR7^+CD45RA^-$), effector memory (T_{EM}, $CCR7^-CD45RA^-$), and T_{EMRA} ($CD45RA^+CCR7^-$) cells within the $CD4^+$ and $CD8^+$ T cell populations, and of Treg ($CD25^+$, $CD127^-$) and Tfh cells ($CD45RA^-CXCR5^+$) were enumerated as percentages of total $CD4^+$ or $CD8^+$ T cells. T follicular regulatory (Tfr) cells were defined as the proportion of $CXCR5^+CD45RA^-$ $CD4^+$ T cells within the Treg population [31]. Th17 and Th1-like phenotypes within memory T cells and Tfh subsets were defined by the differential expression of CXCR3 and CCR6 ($CXCR3^-CCR6^+$ and $CXCR3^+CCR6^-$, respectively) [26, 31]. Frequencies of transitional ($CD27^-CD10^+$), naïve ($CD27^-CD10^-$), total memory ($CD10^-CD27^+$), class-switched memory ($IgD^-IgM^-IgG^+IgA^+$), and $CD21^{low}$ ($CD19^+CD21^-$) B cells were also determined [30, 32]. Lastly, DCs were assessed by quantifying proportions of plasmacytoid DCs ($CD123^+CD11c^-$), myeloid DCs ($CD11c^+CD123^+$) and mDC subsets cDC1 ($CD141^+$), $CD16^+$ DC ($CD16^+$), cDC2 ($CD1c^+$). Data was acquired on a FACSymphony [30].

Data for B cell and $CD4^+$ T cell function was derived from previously published studies using well-established in vitro assays [26, 32–34]. Specifically, naïve or memory $CD4^+$ T cells sort-purified from PBMCs isolated from healthy donors

or IL-21R-deficient patients were cultured (30–40 × 10^3 cells/well/200 μl) with T cell activation and expansion beads (anti-CD2/CD3/CD28; Miltenyi Biotech) alone (Th0) or under Th17 (2.5 ng/mL TGFβ, 20 ng/mL IL-1 β, 50 ng/mL IL-6, 50 ng/mL IL-21, 100 ng/mL IL-23) or Tfh (50 ng/mL IL-12) polarizing conditions. After 5 days, supernatants were harvested and production of IL-4, IL-5, IL-13, IL-17A, IL-17F, or IL-22 was determined by cytometric bead arrays (Becton Dickinson). To quantify acquisition of expression of IL-21, activated naive $CD4^+$ T cells were re-stimulated with phorbol myristate acetate (PMA, 100 ng/ml)/ionomycin (750 ng/ml) for 6 h, with Brefeldin A (10 μg/ml) added after 2 h. Cells were then fixed, permeabilized, and stained with anti-IL-21 monoclonal antibody. Sort-purified naive B cells were cultured with CD40L alone or together with IL-21 (~5–10 × 10^3/200 μl/well) for 7 days; immunoglobulin (Ig) secretion was then determined by Ig heavy-chain specific ELISAs.

Genetic Evaluation

Target enriched sequencing was designed to cover 356 IEI genes as previously described [35] and used to diagnose P1 and P6. WES was perfomed for P4, P5, and P12 as described [24, 35]. For Sanger sequencing of *IL21R* in P2 and P3, genomic DNA was extracted from whole blood (QIamp DNA Blood Kit, Qiagen). Targeted Sanger sequencing of *IL21R* in P7–P10 has been reported [22, 24]. Primer sequences used to identify *IL21R* variants in P2, P3, P11, and P13 and validate NGS findings in P1, P4, P5, and P6 are described in Supplementary Table I. Pathogenicity of genetic variants was evaluated based on the allele frequency in public databases including ExAC and gnomAD and prediction tools such as Polyphen-2, SIFT and the combined annotation dependent depletion (CADD) score.

RT-PCR for Qualitative Detection of Rubella Virus

Skin biopsies of P4 were formalin-fixed and paraffin-embedded and RNA-isolated using Maxwell™ RSC DNA FFPE Kit (Promega Corporation) followed by real-time PCR kit for qualitative detection of rubella virus (SACACE Biotechnology).

Statistical Analysis

For single comparisons of independent groups, a Mann-Whitney test was performed. Differences in mean values were considered significant at $p < 0.05$. All data analysis and figure generation was performed using GraphPad Prism (v. 8.4.2, La Jolla, CA, USA).

Results

Genetic Diagnosis and Mutation Analysis

We identified two novel genetic variants in *IL21R* in our patient cohort (P4, P6). P4 carries a homozygous missense variant (c.473 T > C, p.L158P) in the extracellular domain, while P6 has a single nucleotide substitution (c.1421C > G), causing a premature termination codon at amino acid p.S474 in the cytoplasmic domain of IL-21R (Fig. 1). Six unique *IL21R* variants have been described previously: three missense mutations (three families; P7, P8 [R201L]; P11 [W138S]; P12, P13 [R201Q]), a single nucleotide frameshift deletion (1 family: P1, P2, P3 [D179Tfs*51]), a single in-frame codon deletion (1 family; P9, P10 [C81-H82del]), and a splice site mutation (P5 [c.153-1G > T]) (Fig. 1). We performed in silico analysis of the novel and previously reported *IL21R* variants and found that all were rare alleles (MAF < 0.01), located in highly conserved residues of IL-21R and predicted to be deleterious (SIFT, Polyphen-2, Splicefinder). We also identified a heterozygous variant in *CARD14* (Q1001*; CADD score 36) in P6 located at the extreme C-terminus of CARD14 in a region of undefined function. Variants in *CARD14* have been identified in individuals with defective skin barrier, such as atopic dermatitis and psoriasis. However, clinical penetrance of several of these variants is incomplete [36]. The impact of this variant on *CARD14* remains to be determined.

Impact of *IL21R* Variants on Protein Structure and Expression

We next assessed the effect of germline variants on the IL-21R structure by identifying the variants' positions on the crystal structure of the IL-21/IL-21R complex (Fig. 2) [37, 38]:

a) *p.C81-H82del (P9, P10)*

This variant removes one of three disulphide linkages within the N-terminal fibronectin domain (each shown in yellow). These deleted amino acids are in close proximity to a protein surface that interfaces with IL-21. Thus, it is likely that this

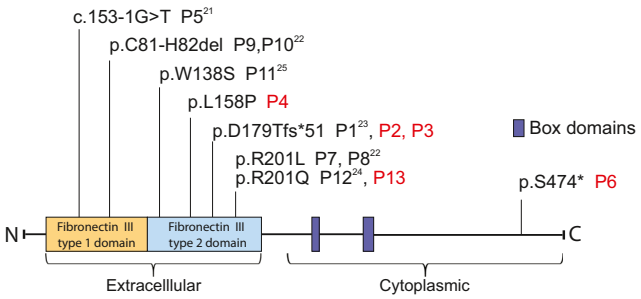


Fig. 1 IL-21R domain structure and patient mutations. Domain structure and positions of mutations identified in different patients are indicated. Newly identified patients are marked in red

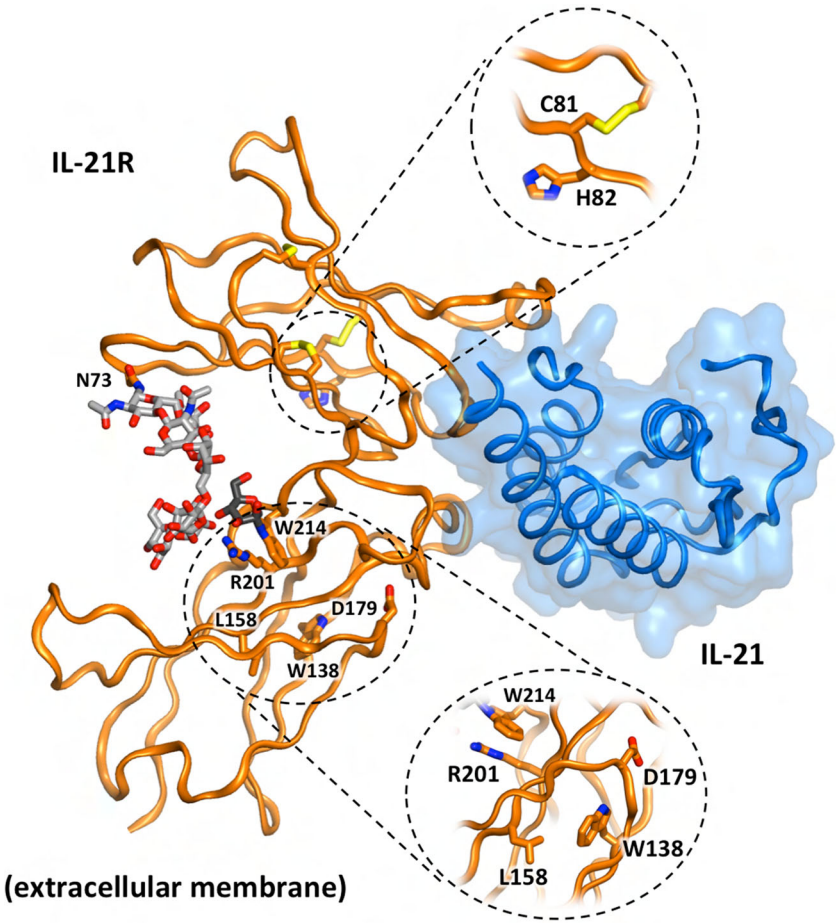


Fig. 2 Pathogenic variants mapped onto the crystal structure of the IL-21/IL-21R complex. IL-21R (orange cartoon) contains two external fibronectin domains, the N-terminal of which (upper) has three internal disulphides (yellow sticks), one involves residue C81 (exploded view). IL-21 (blue cartoon and transparent surface) binds the hinge between the two receptor fibronectin domains. A complex *N*-linked carbohydrate (gray sticks, extending from N73) bridges the two fibronectin domains and has been postulated to stabilize IL-21R [37, 38]. Residues W138, L158, D179, and R201 (orange sticks) are positioned within the C-terminal fibronectin domain proximal to the extracellular membrane. The side chains of W138 and L158 are buried within the hydrophobic interior of the domain. D179 sits on the exterior surface proximal to, but not directly interfacing, IL-21 while R201 is on an exterior surface distal to IL-21 binding. The side chain of R201 is positioned adjacent to the side chain of W214 (within the WSXWS motif) which is also glycolysylated (dark gray sticks) and may contribute to stability of the N73-projected carbohydrate bridge

variant affects the fold or interaction surface (i.e., binding), and/or stability of the fibronectin domain, and thus overall expression of IL-21R. Consistent with this latter possibility, we were unable to detect significant expression of IL-21R on EBV-transformed B cells established from P9 and P10 [29], while PBMCs from these patients failed to respond to IL-21, evidenced by a lack of phosphorylation of STAT1, STAT3, and STAT5 [22].

b) *W138S (P11), L158P (P4)*

The side chains of both W138 and L158 contribute to the hydrophobic core of the C-terminal fibronectin domain fold. In fact, these residues are likely to directly interact with each other. Consequently, the W138S and L158P variants may destabilize the integrity of the fibronectin fold, compromising expression of IL-21R. Indeed, IL-21R could not be detected on the surface of PBMCs from P11 (unpublished observations).

c) *R201L/Q (P7, P8, P12, P13)*

R201 is spatially adjacent to the first tryptophan residue (W214) in the canonical WSXWS motif present in the extracellular domain of all class I cytokine receptors. W214 undergoes post-translational mannosylation, while its side chain is in close proximity to a complex carbohydrate that bridges across from N73 in the N-terminal fibronectin domain [37, 38]. This carbohydrate bridge may stabilize the IL-21R fold. Notably, of all the possible N-terminal carbohydrate linkages, N73 is critical for IL-21R expression [37]. The R201L and R201Q variants will result in a loss of a positively charged

arginine, destabilizing the fibronectin domains of IL-21R, compromising expression and IL-21 binding. Consistent with this possibility, IL-21R was not detected on activated B cells from P12 [R201Q] [24], and IL-21 failed to induce STAT phosphorylation in lymphocytes harboring either the R201L [22] or R201Q [24] variants.

d) *D179Tfs*51 (P1, P2, P3)*

This variant causes a frameshift at D179, thereby removing the WSXWS motif (aa 214-218) and introducing a premature stop codon 51 amino acids downstream [23]. The carbohydrate bridge formed between N73 and the WSXWS motif is critical for transport of IL-21R from the Golgi to the plasma membrane [38]. Thus, this truncating variant would dramatically reduce, if not abolish, cell surface expression of IL-21R, consistent with a lack of phosphorylation of STAT3 in $CD4^+$ T cells from P1 in response to stimulation with IL-21, but not IL-10 [23].

e) *S474* (P6)*

This truncating variant deletes 65 amino acids from the C-terminal region of IL-21R. Although there are six conserved tyrosine residues in the cytoplasmic domain of IL-21R, Y510 has been found to be indispensable for IL-21-induced activation of STAT proteins [39]. Thus, while this variant version of IL-21R may be expressed at the cell surface, it is unlikely to be able to initiate IL-21-mediated signaling in immune cells.

Clinical Characteristics

Patient Demographics and Clinical Findings Median age of disease onset in our cohort was 2.5 years (0.5–7 years) (Supplementary Fig. 1), while the median age at diagnosis was 8.5 (1–19) years. All patients were born to consanguineous parents. Clinical characterics of all patients are shown in Table 1 and Fig. 3, including the family tree of P1 and P2, with a detailed clinical history of each patient provided in the Supplementary results.

Infectious Diseases Recurrent bacterial infections of the upper and lower respiratory tracts leading to hospitalization were the most common infectious diseases, affecting 11/13 (84.6%) patients and causing bronchiectasis in 6/13 (46.2%) patients. Respiratory bacterial pathogens included *Streptococcus pneumoniae* and *Pseudomonas aeruginosa*. *Mycobacterium tuberculosis* and *Mycobacterium massilense* were detected in P5 and P10, respectively, suggestive of a significant T cell defect [40]. Viral infections were observed in 5/13 (38.5%) patients. Notably, P5 experienced systemic CMV infection, involving the lung and eyes at age 18 years. P4 suffered granulomatous skin lesions that histologically resemble those reported in ataxia telangiectasia and other CIDs following MMR vaccination (Fig. 3b–d) [41]. In-tissue PCR of biopsied lesions from P4 confirmed long-term persistence of live rubella virus vaccine as the trigger for skin inflammation. Fungal pathogens were detected in 6/13 (46.2%) patients, with four suffering from pulmonary *Pneumocystis jirovecii* infections and five affected by esophageal, hepatosplenic, or systemic candidiasis.

Inflammatory Diseases Severe intestinal inflammation was noted for P1 and P3, carrying the same genetic variant, and presented as diarrhea of lethal severity in P3. Cryptosporidiosis-associated cholangitis was reported in 6/13 (46.2%) patients and resulted in hepatic fibrosis in P7 and P8 and lethal liver failure in P9 and P10 (Table 1). Erythematous urticarial plaques on the face and extremities were seen in P5 (Supplementary Fig. 2). A skin punch biopsy revealed basal vacuolar changes, mucin accumulation in the dermis, and superficial and deep perivascular lymphocytic infiltration reminiscent of lupus-like dermatitis. Recurrent attacks of bronchial asthma were a shared feature in P4 and P5, and particularly pronounced in P6 who also suffers from severe food allergy leading to recurrent anaphylactic crises and urticaria (Table 1).

Growth Retardation Growth retardation affected 5/13 of patients and was particularly severe in P1. Both weight (z-score −5.59) and height (z-score −5.62) were below the third percentile in P1 possibly due to sustained steroid treatment.

Laboratory and Immunological Features of IL-21R Deficiency

Results from laboratory tests for IL-21R-deficient patients are shown in Fig. 4a, b and Supplementary Table II. Serum IgG and IgA levels were reduced in 9/13 (70%) and 7/12 (58%) of patients, respectively. Serum IgM was variable, reduced in 5/12 (42%) but increased in 3/12 (25%) of patients. IgE was elevated in 5/12 (42%) of patients, being particularly high in P6 (up to 40,000 IU/ml) consistent with their asthma and severe allergies. Interestingly, we observed an expansion of $CD4^-CD8^-$ double negative T cells in 3/12 of patients. B cells, $CD4^+$ T cells, and $CD8^+$ T cells were dysregulated in 45, 55, and 45% of patients, respectively, while NK cell numbers were decreased in 40% of patients.

Immunological Profiling of 10 Patients with IL-21R Deficiency

We performed in-depth immunophenotypic analyses on five novel IL-21R-deficient patients (P2–P6). Where possible, this data was combined with data we previously generated from

Table 1 Clinical features of patients with IL-21R deficiency

Patient	Total *n* (%)	P1	P2	P3	P4	P5
First description		Erman 2015	Novel	Novel	Novel	Kotlarz 2014
Age		11	1	8*	9	19*
Sex		Male	Female	Male	Male	Male
Age of onset (years)		0.5	-***	1	1.5	4
Age at diagnosis (years)		5	1	2	6	19
Consanguinity		+	+	+	+	+
Bacterial infections						
Recurrent bacterial respiratory infections	11 (84.-6)	+		+	+	+
Bronchiectasis	6 (46.2)				+	+
Recurrent otitis media	6 (46.2)	+				+
Respiratory mycobacterial infections	2 (15.4)					*M. tuberculosis*
Fungal infections						
Fungal respratory infections	5 (38.5)			*P. jirovecii*		+
Candidiasis	5 (38.5)			Esophageal		Hepatosplenic
Parasitic infestations						
Cryptosporidiosis-associated cholangitis	6 (46.2)	+				
Cryptosporidiosis intestinal inflammation	5 (38.5)	+		+		
Viral infections					Rubella persistance	CMV penumonia, CMV retinitis,
Inflammatory complications						
Liver fibrosis	2 (18.2)					
Failure to thrive	5 (38.5)	+		+		
Asthma	3 (23.1)				+	+
Other				Polydactily, celiac-like findings, giardiasis	Recurrent bronchiolitis, hepatomegaly	Mediastinal and intestinal lymphadenopathy, lupus-like skin inflammation
Treatment		HSCT, Prophylactic antibacterial therapy and IVIG	Prophylactic antibacterial therapy and IVIG	Prophylactic antibacterial therapy and IVIG	Steroids, Prophylactic antibacterial therapy and IVIG	HSCT, prophylactic antibacterial therapy, Voriconazole and IVIG
Outcome		Died due to pulmonary aspergillosis day +61 post-HSCT	Alive	Died due to severe diarrhea	Died due to pulmonary complications	Died due to multiorgan failure day +55 post-HSCT

Patient	P6	P7	P8	P9	P10	P11	P12	P13
First description	Novel	Kotlarz 2013	Kotlarz 2013	Kotlarz 2013	Kotlarz 2013	Ives 2013	Stepensky 2015	Novel
Age	19	4*	10*	18*	9*	15*	15	6
Sex	Female	Male	Male	Male	Male	Female	Female	Female
Age of onset (years)	2	2	4	5	3	3	2	-***
Age at diagnosis (years)	19	n\a	n\a	13	8	13	8	0.3

Table 1 (continued)

Consanguinity	+	+	+	+	+	+	+	+
Bacterial infections								
Recurrent bacterial respiratory infections	+	+	+	+	+	+	+	
Bronchiectasis	+			+		+	+	
Recurrent otitis media				+	+	+	+	
Respiratory mycobacterial infections					*M. massilense*			
Fungal infections								
Fungal respratory infections				*P. jirovecii*	*P. jirovecii*		*P. jirovecii*	
Candidiasis			Esophageal	Esophageal	Systemic			
Parasitic infestations								
Cryptosporidiosis-associated cholangitis		+	+	+	+	+		
Cryptosporidiosis intestinal inflammation			+	+	+			
Viral infections				Chronic norovirus infection	Chronic norovirus infection	CMV infection		
Inflammatory complications								
Liver fibrosis		+	+					
Failure to thrive			+	+	+			
Asthma	+							
Other	Food allergies		Recurrent sinusitis, *H. pylori* associated gastritis, hepatomegaly		Bacteremia. Hematogenous spread of M. massilense	*H. pylori* gastritis		
Treatment	Omalizumab, Prophylactic antibacterial therapy and IVIG	Liver transplant at age 4 y	HSCT	Prophylactic antibacterial therapy and IVIG	Prophylactic antibacterial therapy and IVIG	HSCT, prophylatic antibacterial therapy, IVIG	HSCT	HSCT
Outcome	Alive	Died due to bacteremia, systemic CMV infection and multiorgan failure day +542	Died due to multiorgan failure and graft rejection day +84	Died due to liver and respiratory failure	Died due to infectious complications with liver and renal failure	Died due to severe digestive GvHD/CMV ARDS day +149 post-HSCT	Alive	Alive

n/p not published, *n/a* not applicable, * age at time of death, ** age at clinical description, ***asymptomatic, *CMV* cytomegaly virus, *HSCT* hematopoietic stem cell transplantation, *IVIG* intravenous immunoglobulin stubstitution, *ARDS* acute respiratory distress syndrome

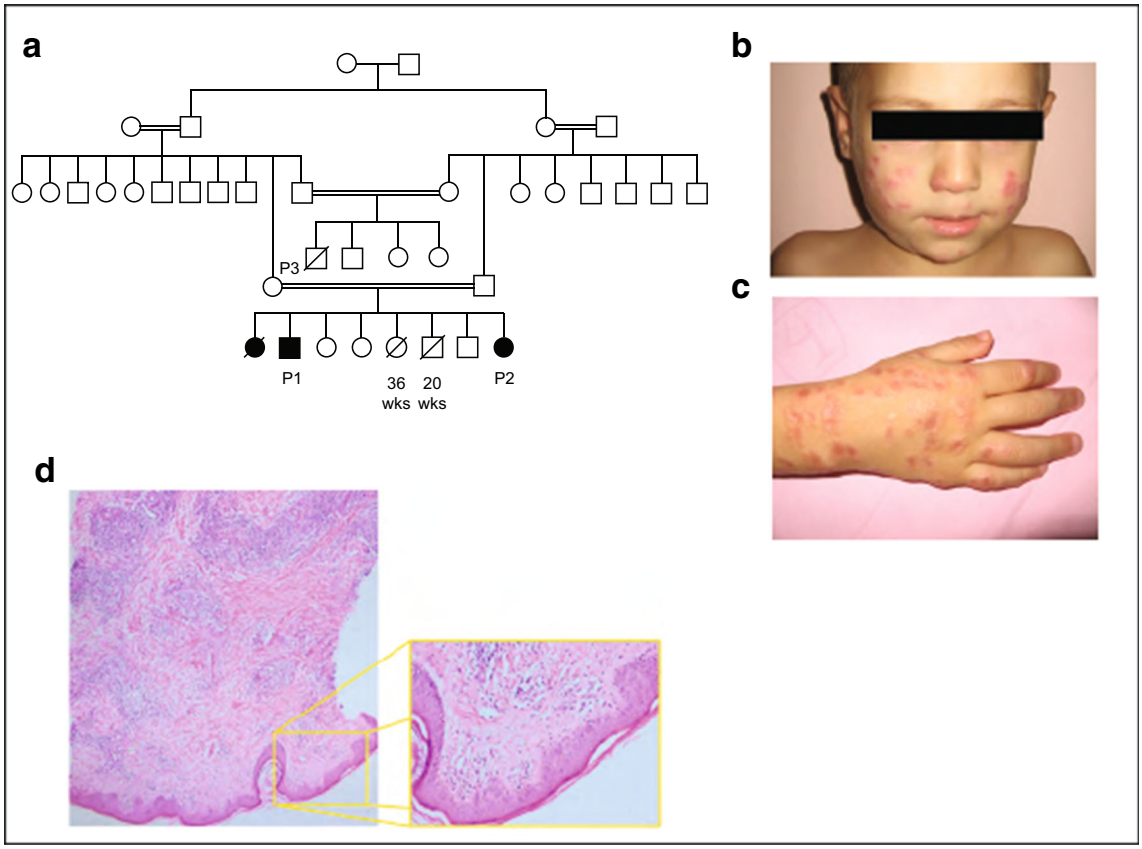


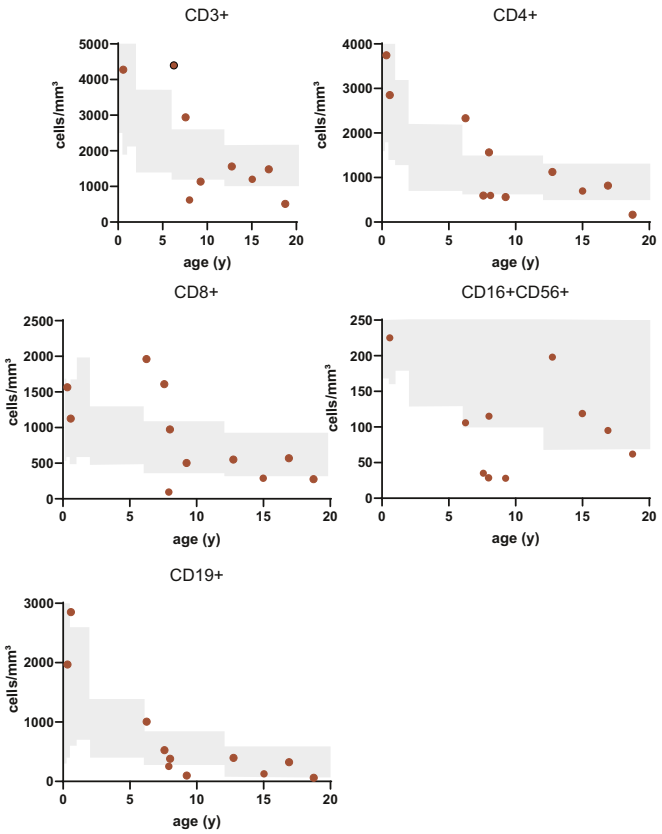
Fig. 3 Clinical features of IL-21R-deficient patients. **a** Pedigree of the family of P1, P2, and P3. **b**, **c** Cutaneous involvement in P4 (face and arm). **d** H&E stain of a skin biopsy of P4 showing lymphohistiocytic inflammation. Predominant infiltration of CD3+ cells of the superficial dermis invading underlying subcutaneous tissue

analyses of five other IL-21R-deficient patients (P1, P9, P10, P11, P12) [25–27, 33]. This enabled us to assess the effect of IL-21R deficiency on the development and differentiation of peripheral blood lymphocytes.

Compared with age-matched controls, proportions of T cells were decreased ($p < 0.01$) in IL-21R-deficient patients (Fig. 5a). However, within the population of total T cells, proportions of TCR$\alpha\beta$, TCR$\gamma\delta$, and iNKT cells were comparable in IL-21R-deficient patients and healthy donors (Fig. 5b–d). MAIT cells were significantly reduced in IL-21R-deficient patients (Fig. 5e). Consistent with $CD4^+$ T cell lymphopenia in one third of patients (Fig. 4b), proportions of $CD4^+$ T cells were also reduced in IL-21R-deficient patients (Fig. 5f). Within the $CD4^+$ T cell lineage, proportions of naïve, T_{CM}, and T_{EM} subsets were comparable in patients and healthy donors. We also quantified memory $CD4^+$ T cell subsets defined by differential expression of CXCR3 and CCR6 [26, 31]. This revealed a trend for fewer Th17-type ($CCR6^+CXCR3^-$) cells within the memory T cell compartment of IL-21R-deficient patients compared to healthy donors ($p = 0.054$, Fig. 5h). This is consistent with a partial reduction in production of Th17 cytokines by IL-21R-deficient memory $CD4^+$ T cells ex vivo compared to memory $CD4^+$ T cells from healthy donors (Fig. 7a), as well as a reduced but not abolished ability of IL-21R-deficient naïve $CD4^+$ T cells to differentiate into Th17-cytokine producing cells in vitro (Fig. 7a) [26, 34]. Other memory $CD4^+$ T cell subsets (e.g., $CXCR3^+CCR6^-$ Th1-type) were unaffected by abolished IL-21R signaling (Fig. 5h). However, from a functional perspective, production of Th2 cytokines IL-4, IL-5, and IL-13 was greatly enhanced for IL-21R-deficient memory $CD4^+$ T cells ex vivo compared to memory $CD4^+$ T cells from healthy donors (Fig. 7c) [26].

Further analysis of $CD4^+$ T cell subsets revealed normal proportions of regulatory T cells (Treg), as a percentage of all $CD4^+$ T cells, in the patients (Fig. 5i). However, circulating Tfh (cTfh) cells were significantly decreased compared to healthy donors (Fig. 5j). This was also observed when cTfh cells were enumerated as a proportion of memory $CD4^+$ T cells (Fig. 5k), indicating a bona fide cTfh deficit rather than being secondary to a general reduction in memory $CD4^+$ T cells. This is consistent with our previous finding of impaired differentiation of IL-21R-deficient naïve $CD4^+$ T cells into Tfh-type cells in vitro (Fig. 7d) [34]. Th17-type cTfh cells were also reduced, albeit not significantly ($p = 0.053$), in IL-21R-deficient patients relative to healthy donors (Fig. 5l). Similar to total Tregs, Tfr cells were comparable in IL-21R-deficient patients and healthy donors (data not shown). In contrast to $CD4^+$ T cells, proportions and absolute numbers

a



b

	Increased (no and % of patients)	Normal (no and % of patients)	Decreased (no and % of patients)	Unknown (no and % of patients)
Immunoglobulin serum levels				
IgG	1/13 (8%)	3/13 (23%)	9/13 (70%)	0
IgA	0	5/12 (42%)	7/12 (58%)	1
IgE	5/12 (42%)	7/12 (58%)	0	1
IgM	3/12 (25%)	4/12 (33%)	5/12 (42%)	1
Absolute lymphocyte subpopulations				
WBC	5/8 (63%)	2/8 (25%)	1/8 (13%)	5
ALC	3/11 (27%)	5/11 (45%)	3/11 (27%)	2
B cells	2/11 (18%)	6/11 (55%)	3/11 (27%)	2
T cells	2/9 (22%)	4/9 (44%)	3/9 (33%)	4
CD4+ cells	2/11 (18%)	5/11 (45%)	4/11 (36%)	2
CD8+ cells	2/11 (18%)	6/11 (55%)	3/11 (27%)	2
CD4-CD8- cells	3/11 (27%)	8/11 (73%)	0	2
CD16+CD56+ NK cells	0	6/10 (60%)	4/10 (40%)	3

WBC, white blood cell count; ALC, all lymphocyte count.

Fig. 4 Laboratory features of IL-21R-deficient patients. **a** Proportions of lymphocyte subsets in IL-21R-deficient patients. One timepoint is displayed per patient. Gray areas indicate healthy donor control ranges [61]. **b** Laboratory tests of IL-21R-deficient patients. *WBC* white blood cell count, *ALC* absolute lymphocyte count

of total $CD8^+$ T cells (Fig. 5f) as well as naïve, T_{CM}, T_{EM}, and T_{EMRA} subsets (Fig. 5m), and the activation status of $CD8^+$ T_{CM}, T_{EM}, and T_{EMRA} cells [determined by expression of CD57 (Fig. 5n)] were unremarkable in IL-21R-deficient patients.

IL-21 has also been reported to regulate murine NK cell maturation [42]. Thus, we assessed NK cells and subsets. Proportions of total as well as $CD56^{bright}$ and $CD56^{lo}$ NK cell subsets were unaffected by impaired IL-21/IL-21R signaling (Fig. 5o, p). However, the composition of the $CD56^{lo}$ NK cell subset was significantly perturbed by IL-21R-deficiency. Specifically, the $CD56^{lo}$ NK-subset in IL-21R-deficient patients was dominated by $CD57^-$ cells, while the corresponding subset in healthy donors is predominantly comprised of $CD57^+$ cells (Fig. 5p). $CD56^{lo}CD57^+$ NK cells exhibit greater cytotoxic potential than $CD56^{lo}CD57^-$ NK cells and thus represent a more mature subset of human NK cells [43].

Proportions of total B cells and naïve B cells were significantly increased, and memory B cells significantly reduced, in IL-21R-deficient patients (Fig. 6a, b). Naive B cells in healthy donors comprise two major populations: $IgM^{lo}IgD^{hi}$ (~75%) and IgM^-IgD^{hi} (~20–25%) cells. Interestingly, >90% of naïve B cells in IL-21R-deficient patients were $IgM^{lo}IgD^{hi}$ cells, resulting in significant reductions in proportions of IgM^-IgD^{hi} B cells (Fig. 6c). Within the contracted memory B cell population in IL-21R-deficient patients, proportions of IgG^+ or IgA^+ switched cells were significantly decreased (Fig. 6d). These flow cytometric data correlated with functional data inasmuch that naïve B cells from IL-21R-deficient patients exhibited no response to the stimulatory effect of IL-21, evidenced by undetectable levels of IgM as well as of the class-switched Ig isotypes IgG and IgA. This contrasts responses of naïve B cells from healthy donors which produce large amounts of IgM, and detectable amounts of IgG and IgA (Fig. 7e, f) [33, 34]. Assessment of myeloid cells revealed comparable proportions of total DCs and plasmacytoid DCs in IL-21R-deficient patients and healthy donors (Fig. 7e, f). However, proportions of myeloid DCs—particularly the $CD141^+$ subset—were lower in IL-21R-deficient patients (Fig. 6f, g).

Collectively, while impaired IL-21R signaling has limited effects on lymphocyte development, further differentiation of these cells is substantially compromised, with significant reductions in $CD4^+$ T cells, MAIT cells, cTfh cells, total and class-switched memory B cells, terminally differentiated NK cells and myeloid-derived DCs, and trends for reductions in Th17 cells.

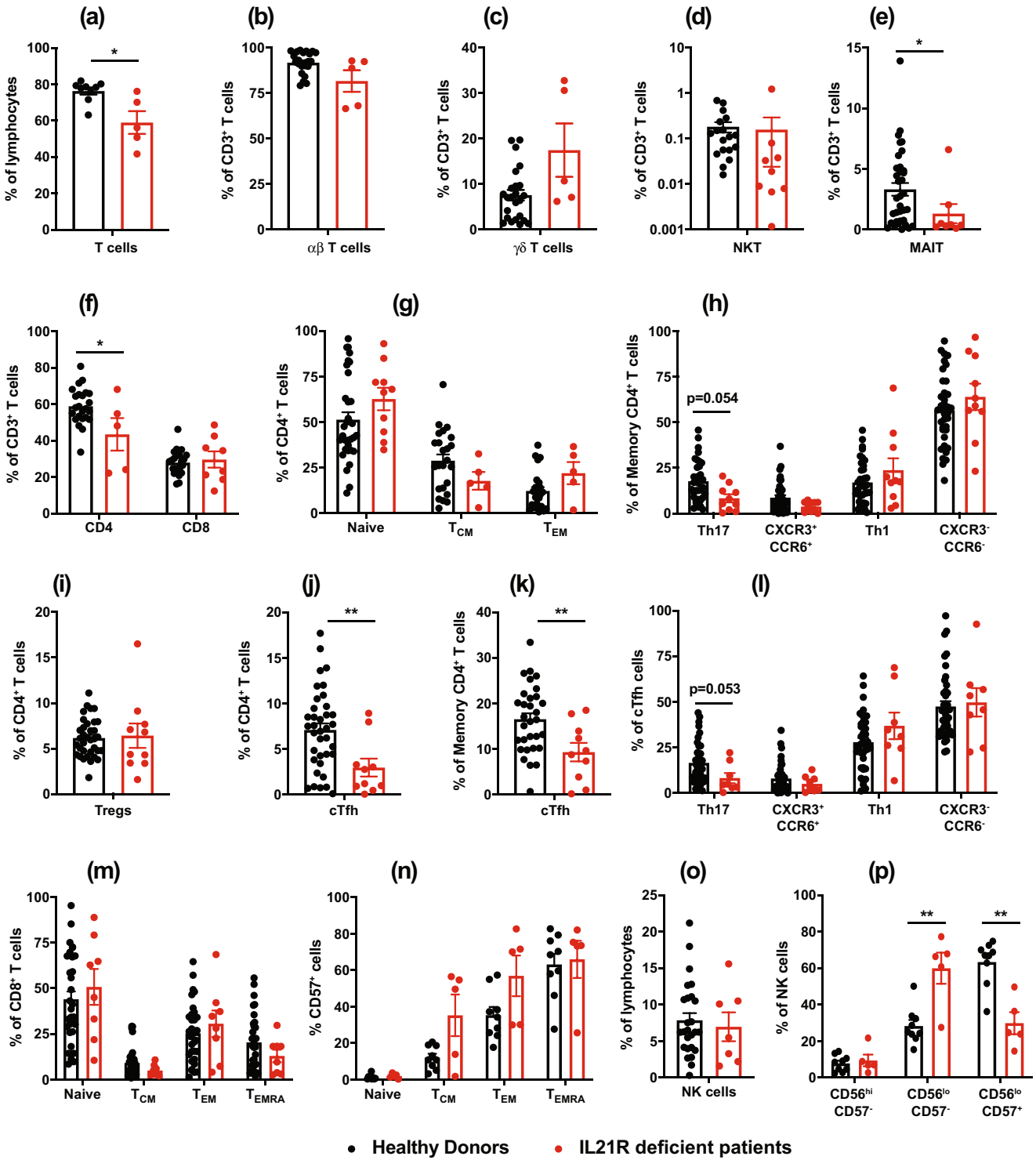


Fig. 5 *IL21R* mutations impact Tfh, Th17, MAIT cell formation and NK cell differentiation. PBMC from healthy controls or IL-21R-deficient ($n = 5$–10) patients were labeled with mAbs against CD3, CD4, CD8, CD56, CD161, TCR Vβ11, TCR Vα7-2, TCR Vα24, CCR7, and CD45RA. **a**–**f** Proportions of **a** total ($CD3^+$) T cells, **b** αβ T cells, **c** γδ T cells, **d** NKT cells, **e** MAIT cells, and **f** $CD4^+$ and $CD8^+$ T cells. **g**–**l** Within $CD4^+$ T cells, frequencies of **g** naïve, T_{CM}, or T_{EM} cells; **h** Th17 ($CXCR3^-CCR6^+$), $CXCR3^+CCR6^+$, Th1 ($CXCR3^+CCR6^-$), or $CXCR3^-CCR6^+$; **i** regulatory T cells; **j**, **k** cTfh cells, as well as subsets of **l** Th17, $CXCR3^+CCR6^+$, Th1, or $CXCR3^-CCR6^+$, cTfh cells. **m**, **n** Proportions of **m** naïve, T_{CM}, T_{EM}, or T_{EMRA} $CD8^+$ T cells, and **n** percentages of these subsets expressing CD57. **o**, **p** Proportions of **o** total NK cells within lymphocytes, and of **p** $CD56^{hi}$, $CD56^{lo}CD57^-$, and $CD56^{lo}CD57^+$ NK cell subsets. For all graphs, values represent mean ± SEM, with individual symbols corresponding to one healthy donor or IL-21R-deficient patient. Statistics performed using *t* tests with Mann-Whitney tests. $*p < 0.05$, $**p < 0.01$. FACS data for some of the patients has been published previously [25–27, 33] but is included here to provide an overview of all available patients

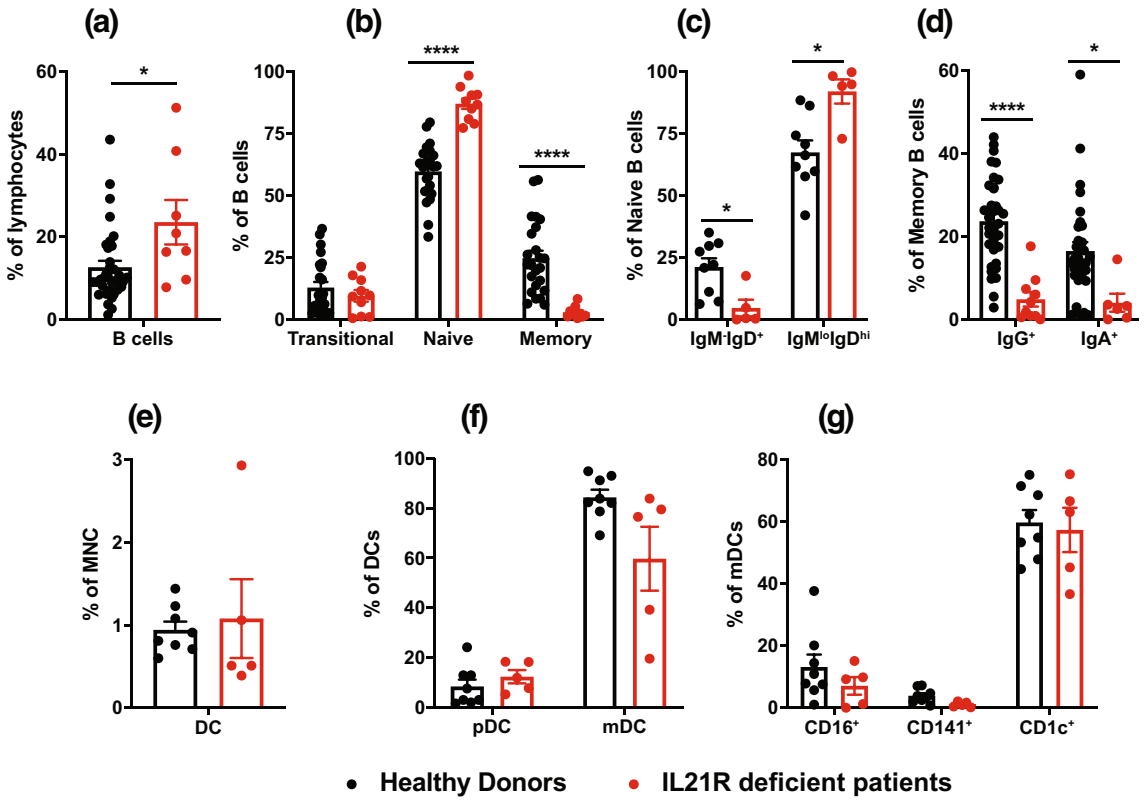


Fig. 6 Impaired generation of memory B cells and induction of class switching due to IL-21R deficiency. PBMC from healthy controls or IL-21R-deficient ($n = 5$–9) patients were labeled with mAbs against CD19, CD20, CD10, CD27, IgG, IgA, CD123, CD11c, CD141, CD16, and CD1c. **a**–**d** Proportions of **a** total B cells within lymphocytes; **b** transitional, naïve, and memory B cells within the total B cell population; **c** naive B cells with a $IgM^{-}IgD^{+}$ or $IgM^{lo}IgD^{hi}$ phenotype; and **d** memory B cells expressing IgG or IgA. **e**–**g** Proportions of **e** DC within all leukocytes, **f** plasmacytoid ($CD123^{+}CD11c^{-}$) and myeloid cells ($CD11c^{+}CD123^{+}$) within the total DC population, and **g** mDC subsets [cDC1 ($CD141^{+}$), $CD16^{+}$ DC ($CD16^{+}$), cDC2 ($CD1c^{+}$)]. For all graphs, values represent mean ± SEM, with individual symbols corresponding to one healthy donor or IL-21R-deficient patient. Statistics performed using *t* tests with Mann-Whitney tests. $^{*}P < 0.05$, $^{****}P < 0.0001$

Therapeutic Approach and Outcome

Prophylactic antibacterial therapy (TMP-SMX) and monthly intravenous immunoglobulins (IVIG) were given to all patients. CMV pneumonitis in P5 required ganciclovir; omalizumab treatment was given to P6 to treat their allergies. P1 and P3 received steroids, azathioprine, and 5-ASA for intestinal inflammation. Although all patients received extensive antimicrobial and supportive therapy, mortality of untransplanted patients was 42.8% (3/7) due to infectious complications. The liver disease in P1 necessitated a differential diagnosis with sclerosing cholangitis and autoimmune hepatitis. The progressive liver disease developed despite clarithromycin being administered to treat cryptosporidium infection.

Five IL-21R-deficient patients in our cohort (P1, P5, P8, P11, P12, P13) underwent HSCT, as outlined in Table 2. All of these patients had a molecular diagnosis of IL-21R deficiency prior to HSCT. We collected HSCT data of these five patients. P8 also underwent transplant, though prior to the initial report of IL-21R-deficiency [22]. Thus, we retrieved transplant information of P8 from the literature [22]. The median age at transplant was 11.5 years. Stem cell source was bone marrow ($n = 4$) or peripheral blood ($n = 1$). Fully matched related donors were used for five patients. All patients except for P13, who was asymptomatic, showed lung or GI tract damage prior to HSCT (Table 2). P1, P4, P5, and P11 presented with bronchiectasis, while P5 and P12 had PCR-confirmed CMV viremia pre-transplant. Cholangitis was observed in P1, P8, and P11. This necessitated a liver transplantation in P1 1 month pre-HSCT. P5 also suffered chronic norovirus enteritis at the time of HSCT (Table 2). All patients received pre-HSCT conditioning regimens comprised of either reduced toxicity conditioning (RTC; P1, P11, P12, P13) or reduced intensity conditioning (RIC, P5) to prepare for donor engraftment. Notably P12 and P13, who had the least complications clinically, received RTC consisting of fludarabine (30 mg/m^2 × 5 days), treosulfan (14 g/m^2 × 3 days), and thiotepa (10 mg/kg). All patients received antithymocyte globulin (ATG) for a minimum of 3 days. Cyclosporine A (CSA), methotrexate (MTX), or mycophenolate mofetile (MMF) were administered as GvHD prophylaxis (Table 2).

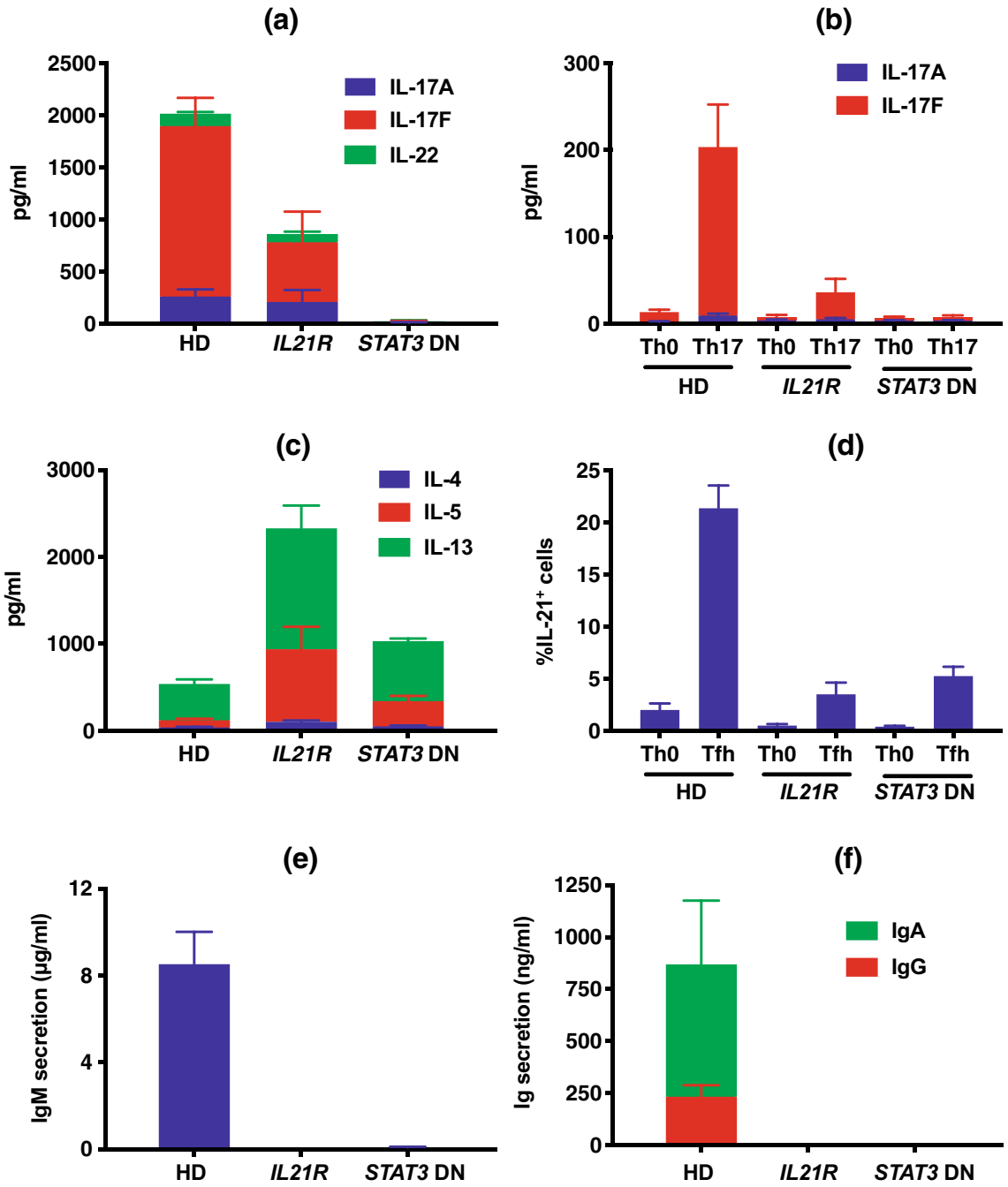


Fig. 7 Functional defects in B and T cells due to inactivating biallelic *IL21R* variants. **a**–**d** Memory (**a**, **c**) or naïve (**b**, **d**) $CD4^+$ T cells were sort-purified from healthy donors (HD, $n = 22$–24), or individuals with biallelic variants in *IL21R* (*IL21R*, $n = 6$) or DN variants in *STAT3* (*STAT3* DN, $n = 6$–9), and then cultured for 5 days in vitro under non-polarizing Th0 conditions (**a**, **c**), Th17 conditions (**b**) or Tfh-polarizing conditions (**d**). Secretion of the indicated Th17 (**a**, **b**; IL-17A, IL-17F, IL-22) or Th2 (**c**; IL-4, IL-5, IL-13) cytokines, or expression of the Tfh-cytokine IL-21 (**d**) was then determined. **e**, **f** Naive B cells were sort-purified from healthy donors (HD, $n = 35$), or individuals with biallelic variants in *IL21R* (*IL21R*, $n = 6$) or *STAT3* DN variants ($n = 10$), and then cultured for 7 days in vitro with CD40L and IL-21. Secretion of IgM (**e**) or IgG and IgA (**f**) was then determined. NB: the data depicted in these graphs has been presented in previous publications [26, 33, 34], but is reproduced here to enable comparison between flow cytometric and functional defects, as well as clinical features, resulting from IL-21R deficiency and *STAT3* DN mutations

Post-transplant complications occurred in 5 out of 6 patients: Four patients fully engrafted post-HSCT and 1 out of 5 patients experienced GvHD. However, four patients (P1, P5, P8, P11) succumbed post-HSCT. HSCT was complicated in P1 by engraftment failure and invasive pulmonary aspergillosis causing death 61 days post-transplant. P5 failed to engraft

Table 2 Details of HSCT and clinical course of transplanted IL-21R-deficient patients

Patient no.	Age at HSCT	Organ damage prior to HSCT	HSCT conditioning regime	GvHD prophylaxis	HSCT source		Complications			Clinical outcome, follow-up time
					Donor	cell source, cells/kg	Engraftment (neutrophil)	GvHD	Other	
1	12 years 11 months	Bronchiectasis, cholangitis, hepatic transplant	Fludarabine (150 mg/m2) Treosulfan (108 g/m2)	MTX	10/10 MSD	Unknown BMC	Failed	No	Failure to engraft, severe pulmonary aspergillosis,	Died on day 61 post-HSCT
5	20 years 2 months	Bronchiectasis	Fludarabine (150 mg/m2) Melphalan (100 mg/m2)	CSA, MTX	10/10 MFD	8×10^6 BMC	Failed	No	Failure to engraft, severe bact. Infections, PRES, CMV reactivation, ARDS	Died on day 55 post-HSCT
11	15 year 6 months	Bronchiectasis, lung resection, cholangitis	Fludarabine (180 mg/m2) Busulfan (AUC 12000 micro M.min)	CSA, MMF	10/10 MSD	6.5×10^6 PBSC	Yes, day +14	Acute GvHD, grade IV: skin, GI, liver (D+40)	CMV reactivation associated ARDS	Died on day 149 post-HSCT
12	9 years 3 months	Bronchiectasis	Fludarabine (150 mg/m2) Treosulfan (42 g/m2) Thiotepa (10 mg/kg)	CSA, MMF	12/12 MSD	2.5×10^6 BMC	Yes, day +14	No	CMV reactivation	Clinical remission, IVIG not needed
13	2 years 6 months	No	Fludarabine (150 mg/m2) Treosulfan (42 g/m2) Thiotepa (10 mg/kg)	CSA, MMF	12/12 MSD	1.04×10^6 BMC	Yes, Day +14	No	No	Clinical remission, IVIG not needed
8	10 years	Cholangitis, liver fibrosis	Not published	Not published	HLA matched	Not published	1st HSCT: yes 2nd HSCT: n/p	No	Increased cholangitis, CMV infection, graft rejection, required 2nd HSCT, multiorgan failure	Died on day 84 post-HSCT

HSCT hematopoietic stem cell transplantation, *AUC* area under the curve, *MTX* methotrexate, *CSA* cyclosporine A, *MMF* mycofenolate mofetil, *MSD* matched sibling donor, *MFD* matched family donor, *BMC* bone marrow cells, *GvHD* graft versus host disease, *GI* gastrointestinal, *PRES* posterior reversible encephalopathy syndrome, *CMV* cytomegalovirus, *ARDS* acute respiratory distress syndrome, *IVIG* intravenous immunoglobulin substitution

and presented with severe systemic bacterial infections, encephalopathy, CMV reactivation, and acute respiratory distress syndrome (ARDS), succumbing 55 days post-transplant. P8 developed increased cholanigits, CMV infection, and required a second HSCT after graft rejection (Table 2**)**. P8 died of multiorgan failure following a second transplant [22]. HSCT was complicated by acute grade 4 GvHD in P11 with skin, GI, and liver involvement starting from day 40 post-transplant, and treated with glucocorticoids, CSA, MMF, sirolimus, infliximab, and ATG. P11 developed CMV reactivation and succumbed to CMV-associated ARDS in the third month post-transplant. In total, four patients suffered from CMV reactivation post-HSCT, which was controlled only in P12 (Table 2**)**.

We report a 33% overall survival rate in our cohort at a mean follow-up of 1.8 years with three patients dying due to infectious complications and one patient succumbing to multiorgan failure of unreported etiology. Both P12 and P13 were successfully transplanted and remain free of IVIG substitution post-HSCT.

Discussion

CIDs are a heterogeneous group of primary immune disorders and exhibit defects in T cell development and function, along with compromised B cell responses [44, 45]. Germline biallelic LOF variants in *IL21* or *IL21R* cause a CID [40]. Recessive *IL21R* LOF variants were first identified in four patients from two unrelated families from distinct geographical locations (P7–P10) [22]. IL-21R deficiency predisposes to infections associated with CID and primary antibody deficiency (*Pneumococci* respiratory infections, recurrent otitis media, *Cryptosporidium* cholangitis), and infections common in patients with T or NK cell deficiencies (invasive/mucocutaneous fungal infections, *P. jirovecii* lung infections, CMV pneumonia). The newly described infections of giardiasis and systemic candidiasis in our cohort are reminiscent of the infectious phenotype of other CIDs such as CD40L deficiency [46].

Our in-depth dissection of circulating immune cell subsets illustrates obligate roles of IL-21R in differentiation and effector function of human lymphocyte subsets. IL-21/IL-21R signaling unequivocally has a non-redundant role in generating or maintaining Tfh-, memory B-, MAIT-, and $CD56^{dim}CD57^{+}$ terminally differentiated NK cells, and a likely role in Th17 cells. Our findings confirm, extend, and refine previous studies that delineated specific and unique functions of IL-21 in human and murine lymphocyte development [47]. Our findings also provide insight into mechanisms underlying some clinical features of IL-21R deficiency. Thus, the paucity of memory B cells and Tfh cells, together with an impaired ability of IL-21R-deficient naïve $CD4^{+}$ T cells to differentiate into IL-21-expressing Tfh cells in vitro and of IL-21R-deficient naïve B cells to differentiate into plasmablasts in response to IL-21 [26, 33, 34], explains hypogammaglobulinemia and impaired humoral immunity. While the in vitro analyses indicated these cellular deficiencies were intrinsic to B cells and T cells, they are also likely to be interdependent, as IL-21 is produced predominantly by Tfh cells, and Tfh-derived IL-21 potently induces human B cell proliferation, class switching, and plasmablast formation, and promotes plasma cell survival [47]. Thus, the B cell intrinsic defect in class switching due to the fundamental inability of IL-21R-deficient naïve B cells to respond to IL-21 could be exacerbated by dysfunctional and/or reduced Tfh cells due to B cell extrinsic defects affecting IL-21R-deficient $CD4^{+}$ T cells. The partial Th17 deficit in IL-21R-deficient patients likely contributes to frequent fungal infections in some, but not all, of these patients indicating the impact of IL-21R signaling on human Th17 cells.

IL-21 activates STAT1, STAT3, and STAT5 [48]. Prior analysis of individuals with inactivating or dominant negative (DN) variants in *STAT1* or *STAT3* revealed that the effects of IL-21 on human B and T cell subsets are predominantly, if not exclusively, mediated by STAT3 [25, 26, 29, 32–34]. Consequently, our findings define the contribution of defective IL-21R signaling to disease pathogenesis in autosomal-dominant hyper-IgE syndrome (AD-HIES) due to DN *STAT3* mutations, namely recurrent infections, impaired humoral immunity, and candidiasis [2, 9, 22]. Interestingly, recessive mutations in the transcription factor *ZNF341* phenocopies the clinical features of *STAT3* DN mutations, consistent with ZNF341 regulating IL-21-induced STAT3 activation [49, 50]. The current findings for this larger cohort of IL-21R-deficient patients, together with our observations for STAT3 DN [25, 26, 29, 32–34] and ZNF341 [28] deficiency, establish that IL-21 is a major STAT3-activating cytokine that regulates differentiation of T cells and B cells into key effector subsets (Tfh, Th17, MAIT cells; total and class-switched memory B cells), thereby explaining the overlap in some of the clinical features of individuals with pathogenic variants in *IL21R*, *STAT3*, or *ZNF341*. Interestingly, several clinical, laboratory, and cellular features of STAT3 DN and IL-21R- or ZNF341-deficient individuals also overlap with individuals with DN heterozygous variants in *IL6ST* [12]. These include reduced Th17, Tfh and memory B cells, Th2-skewed memory $CD4^{+}$ T cells, hyper-IgE and recurrent fungal infections [26, 28, 32–34, 40, 51]. This highlights the interplay between STAT3/ZNF341-mediated signaling downstream of receptors for IL-6 and IL-21. Importantly, our observation that fungal infections occur in fewer IL-21R-deficient patients (~50%) than STAT3- or ZNF341-deficient patients (>90%) also demonstrates partial redundancy for IL-21R signaling in generating human Th17 cells during host defense against fungi, whereas Th17 deficiency is highly penetrant in these other IEIs [28, 51]. Indeed, our functional analyses demonstrated a

partial defect in generating Th17 cells in IL-21R-deficient individuals, but complete abolition of Th17 cell differentiation by *STAT3* DN mutations [26, 34, 51, 52]. Our data also establish the Th17 defect is T cell intrinsic, evidenced by impaired differentiation of naïve IL-21R-deficient $CD4^+$ T cells into IL-17A/IL-17F-producing cells [34] (Fig. 6). This highlights important combinatorial contributions of additional STAT3-activating cytokines to Th17-cell generation, such as IL-6 and IL-23, evidenced by partial deficiencies in Th17 cells in individuals with biallelic variants in *IL23R* [53] or DN heterozygous *IL6ST* variants [12], and variability in the incidence of fungal infections in these individuals.

IL-21- and IL-21R-deficient patients [20–24], similar to corresponding gene-targeted mice [54], exhibit elevated levels of serum IgE. Increased IgE in some cases of IL-21R-deficiency may contribute to atopic disorders. Consistent with this observation, memory $CD4^+$ T cells from IL-21R-deficient individuals, as well as those with *STAT3* DN, *IL6ST* DN, or homozygous *ZNF341* variants, exhibit exaggerated production of Th2 cytokines IL-4, IL-5, and IL-13 (Fig. 6) [12, 26, 28], while IL-21 signaling negatively regulates class switching to IgE and abrogates anaphylaxis in mice [55, 56]. Thus, IL-21 potentially regulates IgE production directly by repressing Ig ε class switching and survival of IgE^+ B cells, and indirectly by restraining the generation of Th2 effector cells producing IgE-promoting cytokines such as IL-13. IL-21R-deficiency has not previously been associated with lymphohistiocytic infiltration of the skin. Prolonged persistence of live rubella virus vaccine in granulomatous skin lesions in P4 adds IL-21R deficiency to the growing list of immunodeficiencies associated with rubella-mediated skin inflammation [57]. Furthermore, it highlights that immunity to some viruses is impaired in patients with IL-21R deficiency [58] and suggests cautious consideration of live vaccinations—in particular against rubella—in IL-21R-deficient individuals.

P6 suffered recurrent anaphylaxis and severe atopy in addition to higher IgE levels than other IL-21R-deficient patients (Table 1, Supplementary Table 2). Two scenarios may contribute to these clinical findings. First, the *IL21R* variant in P6 is predicted to truncate the IL-21R protein at amino acid residue S474 in the cytoplasmic domain. This removes 65 C-terminal amino acids, including Y510 which acts as a phospho-tyrosine docking site for STAT proteins, enabling their activation following receptor engagement by IL-21 (Fig. 1) [39]. Thus, this variant may uniquely impact the structure/function of IL-21R and its interactions with STAT1 and/or STAT3. Second, the heterozygous *CARD14* variant identified in P6 may contribute to severe atopy, as has been reported for other individuals with *CARD14* variants [36]. These possibilities will be pursued in future studies.

Cryptosporidium-associated liver disease remains the most prominent cause of morbidity in IL-21R deficiency. Correspondingly, P1, P9, and P10 succumbed to cryptosporidiosis-associated sclerosing cholangitis, and P3 suffered severe and subsequently fatal diarrhea triggered by intestinal cryptosporidiosis. P1 and P7 underwent liver transplant after extensive cryptosporidiosis-mediated liver damage. Furthermore, increased cholangitis was observed in P8 as a post-HSCT complication. The cases of P4, P5, and P12 illustrate that even in the absence of cryptosporidiosis, IL-21R-deficient patients may develop severe organ damage necessitating HSCT. Even with antibacterial and supportive treatment, IL-21R deficiency is a potentially lethal disease. Thus, HSCT should ideally be considered prior to occurrence of cryptosporidiosis [59].

HSCT for IL-21R deficiency is difficult and should be considered carefully. Indeed, while some patients have a relatively high quality of life, IL-21R deficiency was associated with high mortality (57%) in non-transplanted patients. Primary and secondary graft failure were observed in 2/6 and 1/6 patients, respectively, ultimately resulting in death. Myeloablative conditioning (MAC) prior to HSCT is generally associated with improved engraftment and reduced risk for graft rejection [60]. Thus, MAC or MAC with RTC may be favorable to RIC protocols in establishing sustained engraftment in IL-21R deficiency. Notably, the two patients who survived HSCT—P12, P13—were the youngest in our cohort and also had the least organ damage. This suggests that early transplant prior to chronic infections is a positive prognostic factor for HSCT of IL-21R-deficient patients. Indeed, P13 was diagnosed prior to disease onset due to previous family history. These observations also suggest that organ damage secondary to opportunistic infections, rather than IL-21R deficiency itself, is responsible for the high mortality of HSCT in these individuals. Even larger patient cohorts of IL-21R-deficient patients need to be studied to provide clear treatment recommendations in the future.

Our comprehensive analysis of IL-21R-deficient patients has enabled us to characterize the critical function of IL-21/IL-21R signaling in human health and disease, and provide mechanisms underlying disease pathogenesis. In conclusion, our report provides additional awareness to IL-21R deficiency as a recently defined IEI, expands the spectrum of disease of affected individuals, and indicates the importance of early diagnosis of IL-21R deficiency to improve survival of the patients by providing early therapeutic options prior to cryptosporidium infection.

Abbreviations *AD-HIES,* Autosomal-dominant hyper-IgE syndrome; *ARDS,* Acute respiratory distress syndrome; *ATG,* Anti-thymocyte globuline; *CID,* Combined immunodeficiency disorder; *CSA,* Cyclosporine A; *cTfh,* Circulating T follicular helper; *DCs,* Dendritic cells; *DN,* Dominant negatve; *GC,* Germinal centers; *HSCT,* Hematopoietic stem cell transplant; *IEI,* Inborn error of immunity; *IL-*

21R, Interleukin-21 receptor; *Ils,* Interleukins; *IVIG,* Intravenous immunoglobulins; *LOF,* Loss-of-function; *MAC,* Myeloablative conditioning; *MMF,* Mycophenolate mofetile; *MTX,* Methotrexate; *RIC,* Reduced intensity conditioning; *RTC,* Reduced toxicity conditioning; *Tfh ,* T follicular helper; *Tfr,* T follicular regulatory; *WES,* Whole exome sequencing; *γc,* Common gamma chain

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10875-021-01031-5.

Authors' Contribution DC, DM, SB, KB, and SGT collected and analyzed clinical, laboratory, and/or experimental data; wrote the drafts of the manuscript; and prepared the figures and tables. LW performed immunophenotyping. DBL and DC performed structural analyses of *IL21R* variants. ESA and RA provided clinical, immunophenotyping data for P1, P2, and P4, and prepared the tables. AM and MT provided patient care, collected samples, and provided clinical, immunophenotyping, and genetic data for P3 and P6. DC, OS, and İT provided patient care, collected samples, and provided clinical and immunophenotyping data for P1, P2, and P4. NK, GK, SHT, EKA, and AO provided patient care, collected samples and clinical data of P5. BK and DUÇ provided HSCT data of P1. AU provided pathological data of P4. SKB provided clinical and technical input and support. JD and RJ-H analyzed NGS data of P1, P4, P5, and P6. FO and A-IS are pathologists who performed RT-PCR for qualitative detection of rubella virus in P4. GU provided clinical, immunophenotyping, and genetic data for P9 and P10. EKD performed and provided immunophenotyping data. BK, KW, and PS provided clinical, laboratory, and genetic data and outcomes following HSCT for P12 and P13. BN and AD provided clinical, laboratory, and genetic data and outcomes following HSCT for P11. CSM performed and provided immunophenotyping data. KB, CSM, and SGT supervised the project. All co-authors contributed to and edited drafts of the original and revised manuscripts and table, and approved the final submitted version.

Funding E.K.D. is the recipient of a Scientia Award from UNSW Sydney. C.S.M is supported by an Early-Mid Career Research Fellowship from the Department of Health of the New South Wales Government of Australia. S.G.T was a Principal Research Fellow (1042925) of the NHMRC, and is currently a recipient of an NHMRC Leadership 3 Investigator Grant (1176665) and NHMRC program grant (1113904). KB is supported by a European Research Council Consolidator Grant (iDysChart, ERC grant agreement number: 820074). S.B. is supported by the Scientific and Technological Research Council of Turkey (318S202). PS and KW received funding from the Deutsche Forschungsgemeinschaft (DECIDE, DFG WA 1597/4-1).

Compliance with Ethical Standards

Ethics Approval This work is a review of recently reported genetic variants that represent novel inborn errors of immunity. No human research studies were performed in order to produce this review. Thus, no approvals by appropriate institutional review boards or human research ethics committees were required to undertake the preparation of this report.

Consent to Participate Not applicable as this is a review of recently reported genetic variants.

Consent to Publish The authors consent to publish the content of this review. However, as noted above, as this is a review of recently reported genetic variants that represent novel inborn errors of immunity, we did not require consent to publish from participants.

Conflict of Interest The authors declare that they have no conflict of interest.

Availability of Data and Materials Not applicable

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