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Brief Communication

Severe combined immunodeficiency (SCID) presenting in childhood, with agammaglobulinemia, associated with novel compound heterozygous mutations in DCLRE1C

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ABSTRACT

Severe combined immunodeficiency (SCID) can be caused by deleterious mutations in *DCLRE1C*, leading to deficient non-homologous end joining by compromising the function of the Artemis protein. This impairs the process of V(D)J recombination of the T- and B-cell receptors and typically results in radiosensitive T^-, B^-, NK^+ SCID presenting during the first months of life. We present a case of a 3-year-old girl with two novel compound heterozygous variants in *DCLRE1C* (c.58G > C and c.374A > C) that were associated with marked reduced numbers of peripheral T- and B-cells and undetectable total serum IgG. Despite the severe laboratory phenotype, the patient had a normal development, albeit failure to thrive (−2.5 to −3 SD), during her first years of life including day-care attendance at preschool for 1.5 years. After being diagnosed with pneumonia the clinical picture of SCID was recognized and the girl successfully underwent hematopoietic stem-cell transplantation.

1. Introduction

The development of severe combined immunodeficiency (SCID) has been associated with genetic abnormalities in several different genes involved in lymphocyte development and function. *DCLRE1C* encodes Artemis, a protein with both *endo*- and exonuclease activity that is involved in the opening of DNA hairpins and the trimming of overhangs in the non-homologous end joining (NHEJ)-mediated DNA double-strand break repair pathway. Deleterious mutations, especially within the catalytically active β-lactamase and β-CASP domains usually leads to significantly impaired V(D)J recombination, resulting in defective generation of functional T- and B-cells and to increased radio-sensitivity [1]. The resulting radiosensitive T^-, B^-, NK^+ SCID usually presents during the first months of life and may also give rise to Omenn syndrome [2]. Mutations in *DCLRE1C* associated with milder immune deficiencies, presenting later in life, have also been previously described [3]. These can range from atypical SCID to mere antibody deficiencies and are characteristically associated with more benign appearing immunological laboratory parameters such as reduced peripheral naïve T-cells or reduction in a single IgG subclass. Our present case however combines later presentation with an immunological laboratory profile showing a typical severe SCID phenotype.

2. Case presentation

A 3-year-old girl presented at the local emergency room with a 2-week history of cough and fever. Pulmonary x-ray showed right middle lobe consolidation and the patient was treated with amoxicillin for a suspected bacterial pneumonia. After an initial improvement, the patient returned 5-days later with continuous fevers and clinical worsening, despite ongoing antibiotic therapy. The patient was transferred to a regional hospital and admitted for further therapy. Additional workup with a CT of the thorax displayed atelectasis of the right middle lobe, with consolidations stretching from the right lung hilum downwards into the right lower lobe and flexible bronchoscopy was performed. Therapy with intravenous cefotaxime was initiated. Analysis of bronchoalveolar lavage with PCR for *P. jiroveci* was positive, cotrimoxazole and fluconazole were added to the antimicrobial regime and IgG substitution was initiated. Additional laboratory diagnostics showed undetectable total serum IgG (< 0.07 g/L) which prompted a suspicion of an underlying primary immunodeficiency.

The patient was subsequently transferred to our university hospital

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Table 1
Laboratory parameters.

	Patient	Normal range
Peripheral cell numbers		
Total Leukocytes	2700 cells/μl	4000–12,000 cells/μl
CD3+ T-cells	250 cells/μl	900–4500 cells/μl
CD3+ CD4+ T-cells	140 cells/μl	500–2400 cells/μl
CD3+ CD8+ T-cells	100 cells/μl	300–1600 cells/μl
CD56+ NK-cells	610 cells/μl	100–1000 cells/μl
CD19+ B-cells	20 cells/μl	200–2100 cells/μl
IgD+ CD27- (Naïve)	75%	76–85%
IgD- CD27+ (Switched)	3%	5–12%
T-cell activation by FASCIA		
SEA CD4+ T-cells	1 blasts/μl	553–7743 blasts/μl
SEA CD8+ T-cells	0 blasts/μl	123–2365 blasts/μl
PWM CD4+ T-cells	2 blasts/μl	233–2189 blasts/μl
PWM CD8+ T-cells	2 blasts/μl	50–549 blasts/μl
ConA CD4+ T-cells	0 blasts/μl	620–3800 blasts/μl
ConA CD8+ T-cells	18 blasts/μl	180–1757 blasts/μl
PPD Tb CD4+ T-cells	0 blasts/μl	11–2022 blasts/μl
PPD Tb CD8+ T-cells	0 blasts/μl	0–29 blasts/μl
Pnc CD4+ T-cells	0 blasts/μl	0–269 blasts/μl
Pnc CD8+ T-cells	0 blasts/μl	0–13 blasts/μl
Serum immunoglobulins		
IgG	< 0.07 g/L	5.0–12.0 g/L
IgA	< 0.06 g/L	0.40–1.30 g/L
IgM	< 0.17 g/L	0.27–1.40 g/L

Laboratory investigations of the patient's peripheral B-cell sub-populations are expressed as % of CD19+ peripheral B-cells. FASCIA responses are expressed as number of CD4+ and CD8+ T-cell blasts/μl. SEA, Staphylococcal enterotoxin A, PWM, Pokeweed mitogen, ConA, Concanavalin A, PPD Tb, Purified protein derivative Tuberculin, Pnc, Pneumococcus. Reference ranges are age adjusted.

for clinical and immunological workup. She was the first child of non-consanguineous parents and had a healthy 4-month old sister. A cousin on the paternal side had died of leukaemia at 18 months of age but additional family history was unremarkable. She had received vaccinations in accordance with national recommendations without any noted adverse reactions. A review of previous medical records showed failure to thrive (i.e., length −2.5 SD and weight −3 SD) and a history of pneumonia at 20 months of age that was successfully treated with antibiotics, albeit associated with a brief period of candida stomatitis which responded to topical therapy. The immunological workup showed marked lymphopenia with severely diminished peripheral CD3+ T-cells and CD19+ B-cells in combination with normal numbers of peripheral CD16/CD56+ NK-cells (Table 1). Immunoglobulins, including IgA, IgM and IgG were undetectable in serum (Table 1). T-cell activation, as assessed by our clinical operational FASCIA method, was significantly impaired to mitogens, superantigens and selected microbial antigens within both the CD4+ and CD8+ T-cell populations (Table 1) [4]. Whole exome sequencing identified two novel missense variants in *DCLRE1C* (c.58C > G and c.374A > C) leading to two amino acid substitutions in Artemis (p.Asp20His and p.Gln125Pro). Both variants are uncommon (< 0.0001 in the ExAC database), reside in evolutionary conserved regions of the gene (as evaluated by PHAST, GERP and phyloP) and are predicted to be deleterious by several variant evaluation metrics (CADD score 33 and 28 respectively, both categorized as deleterious by Sift). The p.Asp20His and the p.Gln125Pro substitutions are both located in the catalytically active β-Lactamase domain of Artemis, which is important for V(D)J recombination [1]. Confirmatory Sanger sequencing of the patient and her parents showed the parents to be heterozygous for one variant each, thus confirming the compound heterozygous state of the patient (Fig. 1).

The patient's clinical status improved with above described therapy and allogeneic hematopoietic stem-cell transplantation (HSCT) was planned. The lack of suitable related donors prompted us to search for an unrelated HLA-matched donor and a well-matched donor was identified. The patient was conditioned with reduced intensity (fludarabine 150 mg/m^2, treosulfan 21 g/m^2 and antithymocyteglobulin 5 mg/kg) and transplanted 7 weeks after first presenting at our hospital. The bone-marrow graft with a TNC of 5.1×10^9 cells corresponded to a CD34+ cell dose of 3.6×10^6 cells/kg of body weight. She had an uneventful post-HCT course and is now in clinical good condition beyond the 2-year follow-up with 100% donor chimerism, however still in need of IgG substitution.

3. Discussion

Patients with SCID variants resulting in severely impaired T-cell responses and undetectable serum immunoglobulins (i.e., agammaglobulinemia) usually present early in infancy with opportunistic infections. The severely impaired antibody response also makes them particularly vulnerable to infections with encapsulated bacteria. These infections however, most commonly appear after 7–9 months of age as levels of maternal antibodies are diminishing [5]. In light of the immunological laboratory findings in our case, it is intriguing that our patient was able to survive three years with relatively minor infectious complications. The patient was furthermore able to attended preschool for over one year, where transmission of infectious agents among children is recognized to be quite intense. Little is known about the in vivo function of innate immunity in patients with SCID caused by variants in DCLRE1C. NK-cell function appears to be intact as indicated by animal studies and clinical experience with suspected NK-cell mediated rejection in the setting of HCT of Artemis associated SCID [6,7]. Reports also indicate the presence of innate lymphoid cells in patients with SCID caused by defects in V(D)J recombination even though the same publication postulates them to be redundant to human immunity, at least in the setting of functional T- and B-cells [8]. The later onset of clinical symptoms in the current case could also relate to residual function of ARTEMIS, which might preserve some T-cell function. We are naturally unable to tell weather our patient was able to avoid fatal infections due to functioning innate immunity, residual ARTEMIS function, lack of exposure to infectious agents or some other yet unknown mechanism. The history of preschool attendance however makes lack of exposure unlikely.

Early detection of SCID is important to prevent the development of life threatening infections and to allow for rapid curative stem-cell transplantation. Screening new-borns for levels of T-cell receptor excision circles (TREC) levels is an effective way of detecting new-borns with SCID, as shown by a recent pilot study [9]. Rapidly elucidating the underlying genetic lesions in cases of SCID is also clinically important. It not only influences the care of the affected patient in terms of choice of hematopoietic cell (graft) source and conditioning, but also offers the possibility to perform genetic diagnostics on relatives and even affect the outcome of future related individuals through preimplantation genetic diagnosis [10,11]. The identification of genetic variants with clinical significance for primary immunodeficiency is greatly assisted by the previous publication of genetically and immunologically well-characterized cases. The publication of such information not only adds to the basic understanding of human immunology but also assist in the further development of personalized sequencing based clinical medicine.

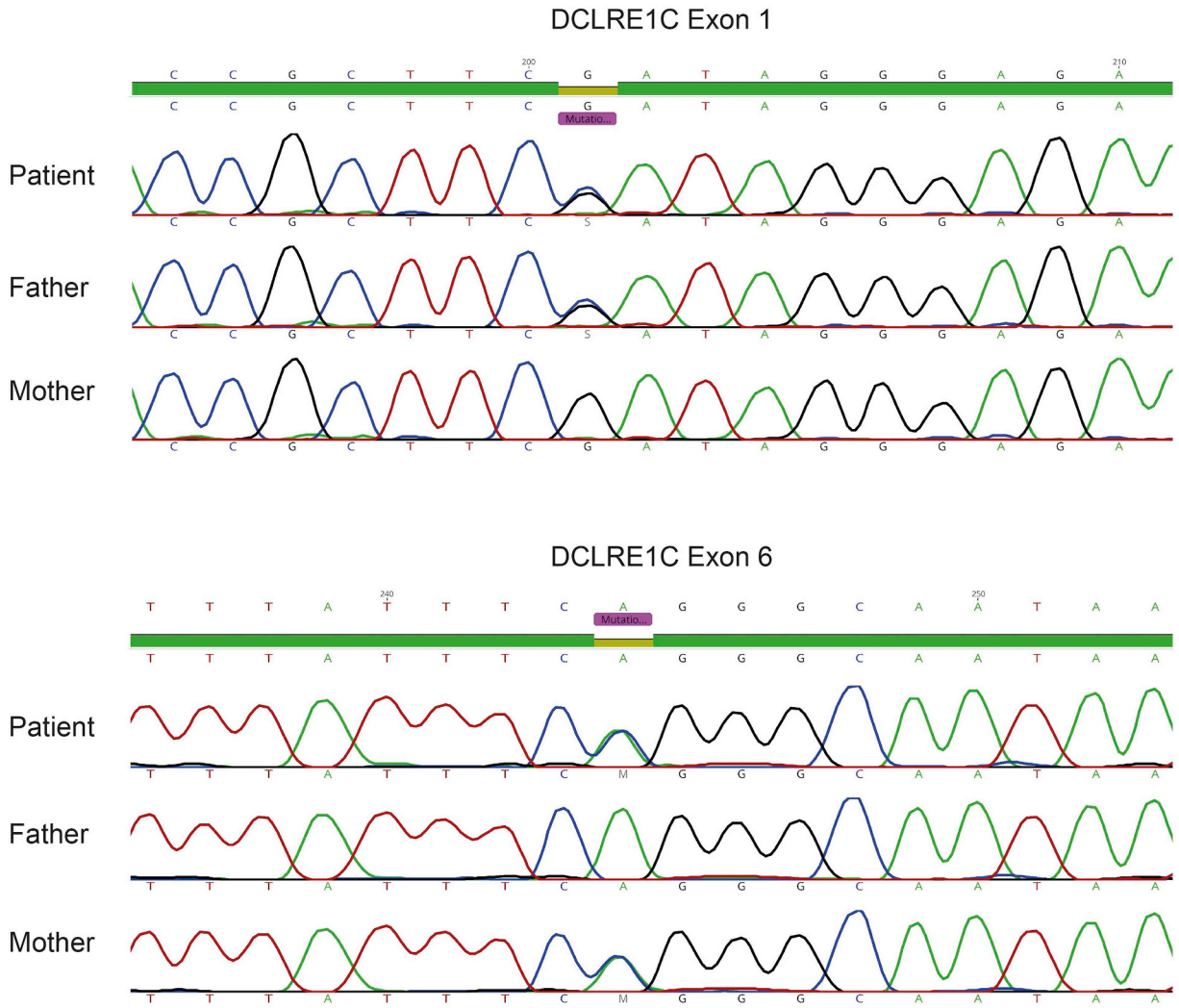

Fig. 1. Sanger sequencing was performed on the patient and her parents to confirm our initial findings. The patient's parents were found to be heterozygous carriers for the two DCLRE1C variants found in the patient, thus confirming the compound heterozygous state of the patient.

Conflict of interest

None of the authors has any potential financial conflict of interest related to this manuscript.

Ethical clearance

A regional ethical review board approved the study.

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