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Variable clinical presentation of hypomorphic *DCLRE1C* deficiency from childhood to adulthood

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Abstract

Background: In this study, we aimed to report long-term follow-up of our pediatric and adult patients with *DCLRE1C* (DNA cross-link repair 1C) hypomorphic mutation who were diagnosed leaky severe combined immunodeficiency (SCID).

Methods: Eighteen patients (13 children and five adults), aged between 6 and 29 years were included. Clinical and immunological features, including immunoglobulin levels, T and B cells, natural killer cell subsets, regulator T (Treg) cell ratios/markers, and cytokines, were assessed before and after hematopoietic stem cell transplantation (HSCT) and compared with healthy controls.

Results: Recurrent infections (78%) and skin manifestations (61%) such as granulomatous skin lesions, warts, and vitiligo were the most common clinical findings. Autoimmune diseases were observed in 33% and malignancy in 17%. Most patients had low serum IgA and B- and T-cell lymphopenia at the first admission. Recent thymic emigrants (RTE), T_{naive}, B_{naive}, $CD56^{dim}CD16^{+}$ cell ratios were significantly lower in the patients than in control; however, follicular helper T T_{FH} and Th1 [interferon gamma (IFN-γ)] cell ratios were significantly higher than the control. Although, Treg ratio and its functional receptors tend to be high but not significant. Eleven patients (61.1%) were treated with HSCT. Median follow-up times of transplant patients was 56 (9–67) months.

Conclusion: Patients with hypomorphic *DCLRE1C* mutations may present with variable clinical and laboratory findings at different ages. Our study showed a helper T (Th)1-dominant immune response before and after HSCT. Increased IFN-γ and T_{FH} cells ratio could be a reason of chronic inflammation and autoimmunity developing before and after HSCT. Long-term follow-up of these patients after HSCT will help to better understand the disease and its pathophysiology.

KEYWORDS
artemis, combined immune deficiency, *DCLRE1C*, leaky severe combined immunodeficiency, severe combined immune deficiency

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1 | INTRODUCTION

Artemis (*DCLRE1C*) is a complex protein that functions the Variable (V), Diversity (D) and/or Joining (J) recombination process and generates diversity in T-cell receptor and immunoglobulins (Ig). It has both endonuclease and exonuclease activity and encodes a V(D)J recombination/DNA repair factor that belongs to the metallobetalactamase superfamily.[1,2]

Most of the mutations in *DCLRE1C* destroy endonuclease activity and V(D)J recombination, which is a critical step in the development of both B and T lymphocytes, then results in severe combined immunodeficiency (SCID) in humans with a lack of T and B lymphocytes.[3] The patients' dermal fibroblasts and hematopoietic cells have been shown to be sensitive to ionizing radiation due to the general nonhomologous end joining (NHEJ) DNA double-strand break (DSB) repair defect.[3,4] On the other hand, hypomorphic mutations in this gene allow residual endonuclease activity and V(D)J recombination with reduced efficacy.[4] This not only results in a milder immunodeficiency but also leads to susceptibility to autoimmunity, lymphoproliferation, and malignancy.[5,6] Mechanism of increased autoimmunity and malignancy in these patients are not clear.

Patients with hypomorphic *DCLRE1C* mutation may present at different ages from infancy to adulthood with different clinical manifestations, ranging from recurrent infections, granulomatous skin lesions, autoimmunity to malignancy. To date, only 16 hypomorphic mutations in the *DCLRE1C* gene have been reported in 35 patients, causing a leaky SCID phenotype that most likely resembles the combined immunodeficiency (CID) phenotype (Table 1, Figure 1).[4,7–14] The mutations in the patients included in our study were first described as hypomorphic mutations in the *DCLRE1C* gene in 2015.[11] Only two of the patients with hypomorphic *DCLRE1C* mutation identified to date were adults, and malignancy was prominent in these cases. The remaining patients were pediatric and presented with recurrent infections or granulomatous skin lesions (Table 1, Figure S1).[4,7–14] Hematopoietic stem cell transplantation (HSCT) is known as only curative therapy for both SCID and leaky SCID. Despite management, immunological phenotypes, and treatment strategies of SCID are well-defined, diagnosis and treatment for patients with leaky SCID due to hypomorphic *DCLRE1C* mutations are not fully clear.[11,15–19] Only a few studies have evaluated the long-term follow-up and outcomes of HSCT in patients with leaky SCID with hypomorphic *DCLRE1C* mutation.[4,7,8,10,12] In this study, we aimed to report the long-term clinical and laboratory outcomes of our cohort with hypomorphic *DCLRE1C* mutation, including six new patients ranging in age from childhood to adulthood, and to share our long-term experience.

Key message

Our study shows the pre-transplant and post-transplant immunology evaluation of patients with hypomorphic Artemis mutation and the long-term follow-up of these patients. It also draws attention to the fact that these patients do not always present with infection in terms of clinical presentation. It also draws attention to the fact that these patients do not always present with infection.

2 | MATERIALS AND METHODS

2.1 | Study design and patients

The study was carried out between 2003 and 2023 at Necmettin Erbakan University, Department of Pediatric Immunology and Allergy. Eighteen patients with *DCLRE1C* mutation (16 patients: c.194C > T; two patients: c.194C > T, c.1669_1670insA) were included in the study. Demographic and laboratory data of the patients including gender, age, lymphocyte counts, serum immunoglobulin (Ig) levels (IgG, IgM, IgA), T, B and natural killer (NK) cell subsets, specific antibody responses, their therapies including immunoglobulin replacement therapy (IgRT), antibiotic prophylaxis, and HSCT results were retrospectively recorded. Five mililiters blood were obtained from patients and healthy controls for flow cytometric and cytokine analysis.

2.2 | Flow cytometry analysis

The following antibodies were used for evaluation of T, B, and NK cell subsets; CD3 (SK7), CD4 (SK3), CD8 (SK1), CD19 (SJ25C1), CD16-56 (3G8), CD57(NK1), CD27(L128), IgM(G20-127), IgD(IA6-2), CD38(HB-7), CD21(B-IJ4), CD45RA(HI100), CC-chemokine receptor 7 (CCR7) (150503), C-C chemokine receptor type (CXCR5) (RF8B2), CXCR3(1C6), CCR6(11A9), programmed cell death protein (PD1) (EH12.1), and forkhead box P3 (Foxp3) (236A/E7). When required, after surface staining, cells were fixed and permeabilized with a fixation/permeabilization kit (eBioscience) for intracellular staining. In T-cell subsets, CD4 + CD45RA + CD31+ recent thymic emigrant (RTE); CD45RA + CCR7+ naive T cell (Tnaive); CD45RA-/CCR7-; CD4+ and CD8+ effector memory T (T_{EM}) cell; CD45RA-/CCR7+, central memory T (T_{CM}) cell; CD45RA+/CCR7-, terminally differentiated effector memory (CD4 + TEMRA); follicular helper (T_{FH}), Th1 and CD4 + FoxP3+ regulator T (Treg) cell ratios were evaluated. In NK cell subset, $CD56^{bright}$, $CD56^{dim}CD16^{+}$ and $CD56^{+}CD57^{+}$ cell ratios were analyzed. In addition, expressions of cytotoxic T-lymphocyte-associated protein (CTLA)-4, Helios, inducible co-stimulatory molecule (ICOS) and glucocorticoid-induced TNFR-related protein (GITR) on Treg cells were also analyzed. Samples were analyzed with Becton Dickinson Canto II (BD Biosciences, Heidelberg, Germany) device and the results were analyzed with the Flow-Jo 10.1r5 software.

2.3 | Cytokine staining analysis

Cytokine staining was performed as previously described.[20] Briefly, peripheral blood mononuclear cell (PBMC) was cultured with interleukin

TABLE 1 Clinical, immunological and genetic features of the patients having hypomorphic *DCLRE1C* mutation.

Referance	Age onset	Clinical features	Immunological findings	Autoimmunity	Malignancy	HSCT	Mutation
Moshous et al.[4] (4 patients)	9 m 10 m 1year 4years	Candidiasis and Recurrent sinopulmonary infections Bronchiectasis Cerebral abscess Liver cirrhosis	T and B cell lymphopenia Hypogammaglobulinemia (IgG and IgA)	Yes (1/4) (AIHA, ITP)	Yes (2/4) (EBV related B cell lymphoma)	Yes (1/4) MUD	c.1353_1359 del/c.del Ex1–3 c.1290_1306 del
Rohr et al. 2009 (1 patient)	9 m	Inflammatory bowel disease Recurrent sinopulmonary infections Labial abscess	T and B cell lymphopenia Low CD4+ CD45RA+ High CD19 + CD27+ High CD19 + IgM-IgD-	No	No	Yes Haploidentic	c.461 + 1G > A
Volk et al.[11] (12 patients)	2–12years	Respiratory infections Viral skin lesions Severe Varicella Granulomatous skin Lesions	T and B cell lymphopenia Hypogammaglobulinemia (IgG and IgA) Low CD4 + CD45RA+ Low CD8 + CD45RA+	Yes (3/12) Vitiligo Juvenile idiopathic arthritis and Hashimoto's thyroiditis	No	No data	c.194C > T c.1669_1670insA
Fevang et al.[12] (1 patient)	23years	Granulomatous skin Lesions	T and B cell lymphopenia Hypogammaglobulinemia (IgG and IgA)	No	Yes (EBV related B cell lymphoma)	Yes Haploidentic	c.632G > T
Ijspeert et al.[8] (2 patients)	5years 4years	Respiratory infections Granulomatous skin lesions	T and B cell lymphopenia Hypogammaglobulinemia (IgG and IgA) Low CD4+ CD45RA+ Low CD4+ CD45RO+ Low CD8+ CD45RA+	No	No	Yes (2/2) MUD	c.464 + 1G > A splice site c.972 + 1997G > C 190-bp insertion
Bajin et al.[9] (1 patient)	5.5years	Recurrent oral ulcers Respiratory infections Intractable diarrhea Sclerosing cholangitis	Hypogammaglobulinemia (IgG and IgA) High IgM Low CD4, CD19, CD4+ CD45RA+	No	Yes (Large granular lymphocytic leukemia)	No	c.1464delG
Lee et al.[10] (2 patients)	5years 6years	Respiratory infections	Hypogammaglobulinemia (IgG and IgA) Absent specific antibody production Low CD8 and CD4+ CD45RA+ CD19 absent	Yes (2/2) (AIHA, neutropenia, ITP)	No	Yes (2/2) MRD	Compound heterozygous exon 1–3 deletion, p.Thr71Pro
Nahum et al.[13] (4 patients)	14years 17years 16years 14years	Recurent sinopulmonary infections	Hypogammaglobulinemia (IgG and IgA) Low CD3, CD4+, CD8+ Low Treg (2 patients) Low naive T cell (2 patients)	Yes (4/4) AIHA Celiac disease ITP	Yes (2/4) B cell lymphoma	No data	c.1299_1306dup, p.Cys436*

(Continues)

TABLE 1 (Continued)

Referance	Age onset	Clinical features	Immunological findings	Autoimmunity	Malignancy	HSCT	Mutation
Meric et al.[14] (8 patients)	6 months 8 months 12 months 1 months 2 months 15 months 2 months 8 months	Respiratory sinopulmonary infections Warts Diarrhea Monilia, axillary abscess	Hypogammaglobulinemia (IgG and IgA) Low CD3, CD4+, CD8+	Yes (1/8) AIHA	No	No	c.500C > T, p.Thr167Met c.632G > T, p.Gly211Val c.(1_195)_(248_332)del (Exon 1–3) c.194C > T c.560T > G, p.(Leu187*) c.(1_195)_(333_322) del(Exon 1–4)

Abbreviations: AIHA, autoimmune hemolytic anemia, EBV, Epstein–Barr virus; HSCT, hematopoietic stem cell transplantation; ITP, immune thrombocytopenic purpura; MRD, matched related donor; MUD, matched unrelated donor.

(IL)-2 and T-cell activation kits (Miltenyi Biotec) for 72 h at 37°C in 5% CO_2. Then, T cells were stimulated with phorbol 12-myristate 13-acetate (PMA) (10 ng/mL; Sigma-Aldrich) plus ionomycin (1 μg/mg; Sigma-Aldrich) in the presence of Golgi-Plug (BD) for 4–5 h. Cells were first stained with surface monoclonal antibody (mAbs) including CD3 and CD4, then fixed/permeabilized with the FoxP3 Staining Buffer S (eBioscience) and stained with intracellular antibodies including IL-4 (8D4-8), IL17A (TC11-18H10) and interferon (IFN)-γ (B27). Results were analyzed on a Becton Dickinson Canto II (BD Biosciences, Heidelberg, Germany) device and analyzed with the FlowJo software.

2.4 | Histological analysis and immunohistochemistry

The preparations and blocks of the cases were extracted from the archive. 3-micron sections were taken from the blocks on positively charged slides for immunohistochemical CD-68 and histochemical Ziehl–Neelsen (EZN) staining. Slides were transferred to the Ventana XT device for CD68 staining. Following to incubation with EDTA, EZN staining was applied manually. H&E, CD-68, and EZN stained slides were reevaluated under a light microscope (Olympus BX 46) by a single pathologist.

2.5 | Hematopoietic stem cell transplantation

A high-resolution genotyping method was employed to analyzed all patients and donors. All patients received Fludarabine 30 mg/m^2 for 5 days, and cyclophosphamide 10 mg/kg for 4 days for a non-myeloablative conditioning regimen including anti-thymocyte globulin (ATG Fresenius) at a total dose of 15–30 mg/kg. One patient's HSCT was rejected and transplanted again from another donor with a myeloablative conditioning regimen with the same agents, including Fludarabine and ATG, and replacing cyclophosphamide with treosulfan at a dose of 12 g/m^2. All patients were administered a calcineurin inhibitor for graft-versus-host disease (GVHD) prophylaxis in combination with short-course 6 mg/m^2 methotrexate. Neutrophil engraftment was considered to be the first of three consecutive days with an absolute neutrophil count (ANC) ≥0.5×10^9/L, and platelet recovery was defined as the first of three consecutive days with a platelet count greater than ≥20×10^9/L for seven consecutive days.[21] However, some patients' neutrophil or platelet levels did not decrease enough for transfusion support because the conditioning regimen was non-myeloablative; therefore, the day with the lowest count was recorded as engraftment day. Based on modified Glucksberg and NIH criteria, respectively, acute GVHD (aGVHD) and chronic GVHD (cGVHD) were diagnosed and graded. IgRT was administered whenever the IgG level was less than 400 mg/dL. In cases of an increased viral load of cytomegalovirus (CMV), preemptive ganciclovir treatment was administered for a minimum of 21 days. Event-free survival (EFS) was defined as the survival probability without evidence of rejection or death by any cause. Overall survival (OS) was defined as the time from the date of transplantation to the last visit or death by any cause.

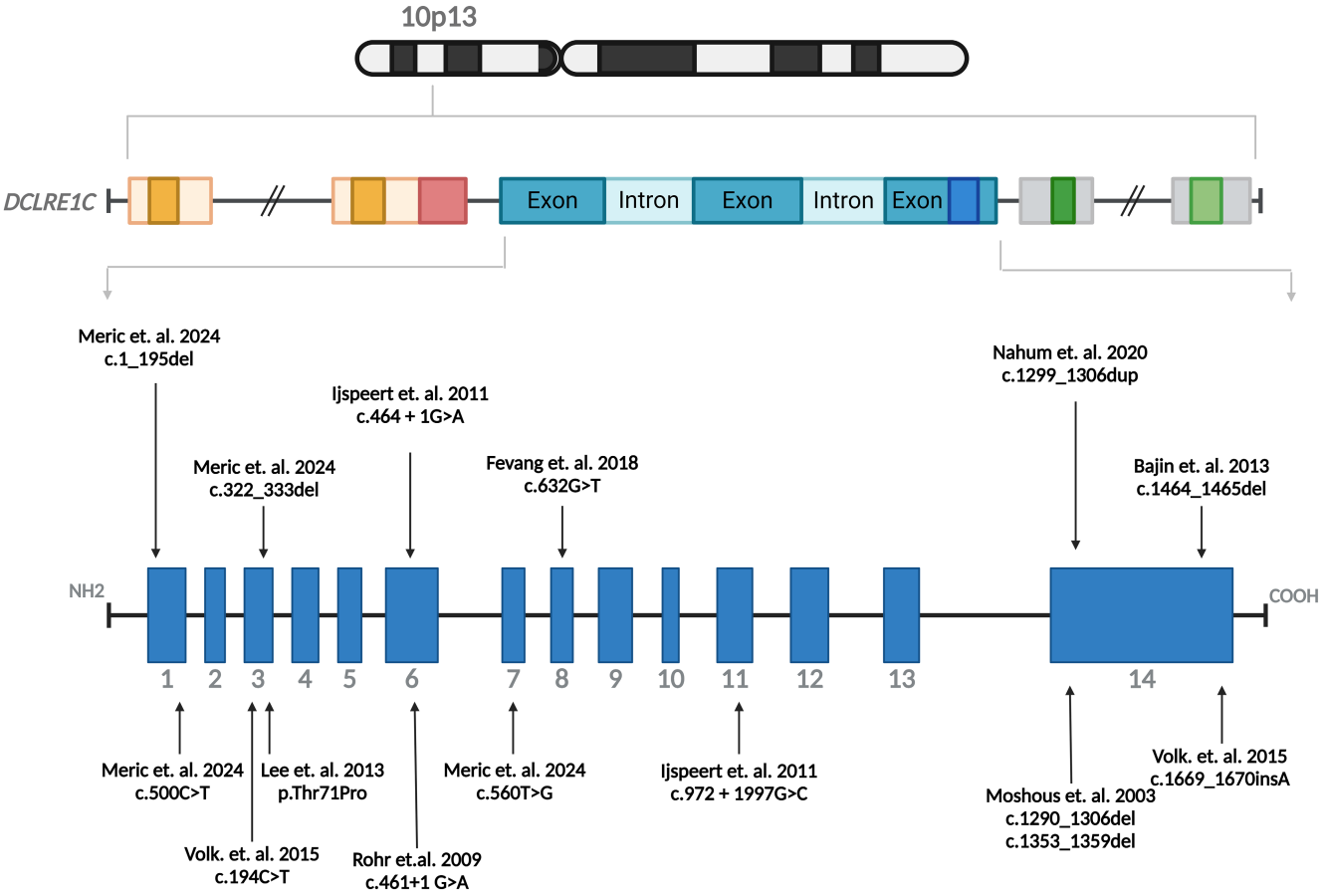

FIGURE 1 Identified hypomorphic mutations in the *DCLRE1C* gene to date.

2.6 | CD25 activation test

For CD25 activation, PBMCs were isolated by gardinet centrifugation. After PBMC isolation, they were stained with CD3 (PE), CD4 (FITCH), CD8 (PerCp), and CD25 (APC) mAb according to the surface staining protocol for basal CD25 expression and flow cytometric analysis was performed. Then, PBMCs were incubated with IL-2 and T-cell activation kits (Miltenyi Biotec) for 72 h at 37°C in 5% CO_2. After 72 h, staining was performed again and CD25 expressions were detected. CD25 expressions were evaluated separately in CD3 + CD25+, CD4 + CD25+, and CD8 + CD25+ cells. Samples were analyzed with Becton Dickinson Canto II (BD Biosciences, Heidelberg, Germany) device, and the results were analyzed with the Flow-Jo 10.1r5 software. After 72 h of incubation, CD25 expression percentage was compared with the basal CD25 expression percentage to determine CD25 expression change.

2.7 | In silico analysis of commonly seeing hymomorphic *DCLRE1C* mutation in our cohort

We used the CADD calculator (https://cadd.gs.washington.edu/snv) to compute the Combined Annotation Dependent Depletion (CADD) score for the variant identified in the *DCLRE1C* gene, c.194C > T.[22] We performed multiple sequence alignment (MSA) of the amino acid sequences obtained from Ensembl database for primates, rodents, and lagomorphs (Table S1) using the ClustalΩ method in the JalView tool.[23] We obtained the tertiary structure of the Artemis protein from the Protein Data Bank (6WO0). Using the PremPs tool, we calculated the stability change of the amino acid alteration resulting from the variant in the protein structure and obtained the mutated protein structure.[24]

2.8 | Statistical analysis

Mean and standard deviation were used for patients' ages, follow-up times, and delay in diagnosis. Demographic data were given as mean ± standard deviation. The GraphPad 6 software was used for statistical analysis. Mann–Whitney–*U*-tests were used to compare to the groups. Survival analyses were performed with the Kaplan–Meier test. $p < .05$ were evaluated as significant.

3 | RESULTS

3.1 | Summary of demographic, clinical, and laboratory features

Demographic and clinical findings of the patients are summarized in Table 2. Eighteen patients (13 female/five male) from 10 families were included in this study. Among the patients in our cohort, those

TABLE 2 Demographic characteristics of patients (GenBank: NM_001033855.3; "rs" numbers of c.194C>T; p.T65I and c.1669_1670insA; 77Nfs*21; rs41297016, rs886037924 respectively).

Family No/ Patients	Sex	Age	Age at diagnosis	Follow up (year)	HSCT	Clinical course	Prophylactic drugs before HSCT	Prophylactic drugs after HSCT	Variant genotype	Alive/death
1/P1	F	29 years	9 years	20	not done	Recurrent URTI, LRTI, bronchiectasis, oxygen dependent chronic lung disease	IgRT, ABP, inhaler steroids	Not transplanted	c.194C>T;p.T65I missense variant HO	Alive
1/P2	F	16 years	5 years	11	not done	Recurrent pneumonia, bronchiectasis	IgRT, ABP, inhaler steroids	Not transplanted	c.194C>T;p.T65I missense mutation HO	Deceased at 16 years (due to respiratory failure while suffering from pneumonia)
1/P3	F	7 years	3 years	4	not done	Recurrent pneumonia, granulomatous skin lesions, JIA, uveitis, hypothyroidism	IgRT, ABP	Not transplanted	c.194C>T;p.T65I missense variant HO	Deceased at 7 years (due to pneumonia)
2/P4	M	17 years	5 years 6 m	11.5	MUD	Recurrent URTI, LRTI, vitiligo	IgRT, ABP	No medication	c.194C>T;p.T65I missense variant c.1669_1670insA; p.T577Nfs*21 frameshift variant CH	Alive
2/P5	M	18 years	9 years 3 m	8.75	MUD	Recurrent URTI, LRTI, vitiligo, AIHA	IgRT, ABP	No medication	c.194C>T;p.T65I missense variant c.1669_1670insA; p.T577Nfs*21 frameshift variant CH	Alive
3/P6	M	14 years	3 years 6 m	11.5	MRD	Recurrent URTI, LRTI, warts, granulomatous skin lesions	IgRT, ABP	IgRT, ABP	c.194C>T;p.T65I missense variant HO	Alive
4/P7	F	19 years	4 years 2 m	14.9	MUD	Recurrent URTI, LRTI, gastroenteritis, recurrent lymphadenopathies, bronchiectasis, lymphoma (age 12)	IgRT, ABP	IgRT, ABP	c.194C>T;p.T65I missense variant HO	Alive
5/P8	F	18 years	7 years	11	MUD	Severe varicella, recurrent URTI, LRTI, brucella, warts, bronchiectasis	IgRT, ABP	IgRT	c.194C>T;p.T65I missense variant HO	Alive

TABLE 2 (Continued)

Family No/ Patients	Sex	Age	Age at diagnosis	Follow up (year)	HSCT	Clinical course	Prophylactic drugs before HSCT	Prophylactic drugs after HSCT	Variant genotype	Alive/death
5/P9	M	14 years	5 years	9	MRD	URTI, warts	IgRT, ABP	IgRT	c.194C > T;p.T65I missense variant HO	Alive
5/P10	F	29 years	20 years	9	not done	Recurrent URTI, LRTI, warts, bronchiectasis	IgRT, ABP, inhaler steroids	Not transplanted	c.194C > T;p.T65I missense variant HO	Alive
6/P11	F	17 years	8 years 6 m	8.5	MRD	Recurrent URTI, LRTI	IgRT, ABP	No medication	c.194C > T;p.T65I missense variant HO	Alive
6/P12	F	12 years	3 years 9 m	8.3	MUD	Recurrent URTI, LRTI, granulomatous skin lesions	IgRT, ABP	IgRT	c.194C > T;p.T65I missense variant HO	Alive
7/P13	M	12 years	4 years	8	MUD	Recurrent URTI, warts	IgRT, ABP	No medication	c.194C > T;p.T65I missense variant HO	Alive
7/P14	F	14 years	6 years	8	not done	Acute lymphocytic leukemia (age 5), granulomatous skin lesions, AIHA	IgRT, ABP, splenectomy, AFP	Not transplanteed	c.194C > T;p.T65I missense variant HO	Alive
8/P15	F	12 years	10 m	10.9	MUD	Recurrent URTI, LRTI, vitiligo, Hashimoto thyroiditis	IgRT, ABP	No medication	c.194C > T;p.T65I missense variant HO	Alive
9/P16	F	15 years	6 years	9	MUD	Immune thrombocytopenia, URTI	IgRT, ABP	IgRT, ABP, AFP, AVP	c.194C > T;p.T65I missense variant HO	Alive
5/P17	F	6 years	Prn	6	not done	Diagnosed prenatal, URTI	IgRT, ABP	Not transplanted	c.194C > T;p.T65I missense variant HO	Alive
10/P18	F	14 years	11	3	not done	Recurrent URTI, LRTI, warts, lymphoma (age 13), delayed radiation myelopathy	IgRT, ABP	Not transplanted	c.194C > T;p.T65I missense variant HO	Deceased at 14 years (due to myelopathy following radiotherapy for lymphoma treatment)

Abbreviations: ABF, antibiotic prophylaxis, AFP, antifungal prophylaxis, AIHA, autoimmune hemolytic anemia, AVP, antiviral prophylaxis; CH, compound heterozygous, HO, homozygous, HSCT, hematopoietic stem cell transplantation, IgRT, immunoglobulin replacement therapy, LRTI, lower respiratory tract infection, MRD, matched related donor, MUD, matched unrelated donor, Prn, prenatal period, URTI, upper respiratory tract infections.

aged ≥18 were considered adults, and patients aged <18 were pediatric (according to the World Health Organization). Thirteen of them were children (72%) and five were adults (28%).

The mean age at diagnosis and evaluation was 6.69 ± 4.36 years (median: 5.5 years) and 16.3 ± 5.5 (median: 15 years), respectively. The diagnostic delay was 3.04 ± 2.41 years (median: 2.25). The mean follow-up period was 8.6 ± 4.29 years (median: 8-years). P17 was diagnosed prenatally through genetic analysis of the cordocentesis sample due to the presence of hypomorphic mutation in her two siblings.[25]

Recurrent respiratory infections (78%) and skin manifestations (61%) such as granulomatous lesions, warts, and vitiligo were the main prominent presentations of the patients (Table 2, Figure S1). The onset of the skin manifestations was between three and 5 years of age. Autoimmunity was found in six patients (33%), One of the patients had juvenile idiopathic arthritis, autoimmune hypothyroidism, and three had vitiligo (Table 2). Two patients with vitiligo (P5, P15) developed autoimmunity at second and fourth years of HSCT including autoimmune hemolytic anemia (AIHA) and Haschimato thyroiditis. Three patients (17%) were diagnosed with malignancy, including non-Hodgkin lymphoma (NHL at 12 years of age), acute lymphoblastic leukemia (ALL at 5 years of age), and Hodgkin lymphoma (HL at 13 years of age). All patients received immunoglobulin replacement (IgRT) and antibiotic prophylaxis therapy, and 11 patients (61%) underwent to HSCT (eight patients had matched unrelated donors and three patients had matched related donors). Patients with a suitable donor within the family were referred directly to the transplant center. Patients without a suitable donor were referred to national and international stem cell and cord banks. Patients with a suitable donor from the bank were transferred to the transplant center after the procedures were completed. Patients without suitable donors were followed up with medical treatment by updating their screening intermittently. We were able to access detailed transplant data for eight of the 11 transplanted patients. All patients who underwent HSCT were alive, but three patients died in the overall cohort when searching for suitable donor. Four patients are still searching for suitable donor. The follow-up periods of surviving patients ($n = 15$) and deceased patients ($n = 3$) until death are summarized in Table 2. Median follow-up times of transplanted patients was 56 (9–67) months. Three patients died during the donor screening period for HSCT. One patient died at the age 15 due to respiratory failure resulting from pneumonia. Another patient died at the age of seven because of pneumonia, and another patient died due to myelopathy following radiotherapy for lymphoma treatment.

Result of immunological parameters are shown in Table 3. Ten patients (61%) had lymphopenia at initial presentation. The most striking laboratory feature was low serum IgA levels, which were present in almost all patients (94.4%). Serum IgA level was below 7 mg/dL in 14 (78%) patients. Low IgG and IgM were detected in 61% and 39% of patients, respectively. In our patients under 14 years of age ($n = 10$.), whom we followed for a long time (4–21 years) until HSCT, we found that lymphopenia became evident with age, and there was a significant decrease lymphocyte count, CD4 + Th, CD19 + B cells, and serum IgA levels (Figure 2A–D). Isohemagglutinin titer was evaluated in 16 and anti-HBs in 13 patients. These values were negative in nine (56%) and 12 patients (92%), respectively. Twelve patients (67%) were evaluated for serum Rubella IgM levels. Rubella IgM levels were positive in five patients with granulomatous skin lesions. The patients' anti-tetanus and anti-pneumococcal antibodies results could not be evaluated.

Skin biopsy of the patients revealed granuloma formation and necrosis in all patients with skin granulomas. No acid-fast bacteria were detected in the lesions with Ziehl–Neelsen stain (ZN stain) (Figure S2). These lesions did not respond to either antituberculosis treatment or local and systemic steroid therapy even when administered at different times. The size of the granulomatous skin lesions, warts, and vitiligo markedly decreased after HSCT; however, a patient developed new granulomatous lesions 1 year after HSCT. Rubella could not be in skin biopsy specimens using polymerase chain reaction (PCR).

3.2 | Hematopoietic stem cell transplantation

The characteristics of patient and transplant are summarized in Table 4. Detailed data were available for eight of the transplanted patients from a single center. The median age of patients who underwent HSCT from this center was 10 years, and all had a matched (9/10 or 10/10) unrelated donor. All donors were CMV IgG- and EBV IgG-positive, IgM-negative. All patients were successfully had either full or mixed chimerism at the last follow-up, except one patient who had primary graft rejection and was subsequently retransplanted with a myeloablative regimen, resulting in full chimerism. All patients were alive at the last follow-up, with a median survival of 56 months.

In the non-HSCT group, 42.8% of the patients (3/7) died, with a median survival of 11 years (95% CI for the median: 4–11) and a mean survival of 12.07 ± 3.06 years (mean ± SEM). There were no deaths in the HSCT (0/11) group, and the average survival was 14.9 ± 0 years. Overall (18) survival appears to be 16.83 ± 1.65 (mean ± SEM) years (Figure S5). The only GVHD complications were three cases of grade 2 acute GVHD and two cases of mild chronic GVHD, all of which had completely resolved at the most recent follow-up.

The most frequent complication was CMV viremia, followed by engraftment syndrome, autoimmunity, and adenovirus infection to a lesser extent (Table 4). IgRTwas administered to three of eight patients after HSCT due to low IgG levels or as prophylaxis for frequent infections. Six of all transplanted patients receive IgRT.

3.3 | Results of T, B and NK cell subsets

At the time of diagnosis, the majority of exhibited T-cell (83.3%) and B-cell (94.4%) lymphopenia. When we evaluated B-cell subsets, the B-cell ratio was significantly lower in patients before and after HSCT than in controls ($p < .05$). Although B-cell ratios increased after HSCT, this increase was not statistically significant ($p > .05$)

TABLE 3 Laboratory characteristics of patients (ne: Not evaluated, y: Years, m: Mouth).

Patients	Age at evaluation	ALC (count/mm^3) (normal range[26])	IgG (mg/dL)	IgM (mg/dL)	IgA (mg/dL)	CD3+ T cells (count/mm^3)	CD4+ T cells (count/mm^3)	CD8+ T cells (count/mm^3)	CD19+ B cells (count/mm^3)	AntiHBs	İsohemaglutinin
P1	9 years	3780 (1200–4700)	1110 (842–1943)	97 (54–392)	6.6 (62–390)	1689 (1000–4900)	695 (500–2700)	1557 (300–2100)	118 (200–2200)	ne	½
P2	5 years	3840 (1400–5500)	1710 (745–1004)	159 (78–261)	6.6 (57–282)	2940 (1900–3600)	1560 (600–2000)	1980 (300–1300)	480 (300–1200)	ne	1/8
P3	4 years	2200 (1400–5500)	4 (640–2010)	88 (52–297)	1 (44–244)	1562 (1900–3600)	836 (600–2000)	682 (300–1300)	102 (300–1200)	ne	½
P4	5 years 6 m	2170 (1400–5500)	1050 (745–1004)	100 (78–261)	6.6 (57–282)	1388 (1900–3600)	988 (600–2000)	477 (300–1300)	238 (300–1200)	2	1/64
P5	9 years 3 m	791 (1200–4700)	489 (842–1943)	132 (54–392)	64 (62–390)	490 (1000–4900)	245 (500–2700)	190 (300–2100)	31 (200–2200)	4.5	1/16
P6	3 years 6 m	800 (1400–5500)	240 (640–2010)	35 (52–297)	6.6 (44–244)	547 (1900–3600)	217 (600–2000)	267 (300–1300)	22 (300–1200)	0.58	1/1
P7	4 years 2 m	1400 (1400–5500)	363 (745–1004)	149 (78–261)	32.6 (57–282)	532 (1900–3600)	220 (600–2000)	308 (300–1300)	193 (300–1200)	0	1/8
P8	7 years	2440 (1200–4700)	1140 (764–2134)	86.5 (69–387)	6.6 (70–303)	2025 (1000–4900)	903 (500–2700)	1000 (300–2100)	24 (200–2200)	5	½
P9	5 years	900 (1200–4700)	560 (745–1004)	54 (78–261)	18 (57–282)	558 (1900–3600)	207 (600–2000)	243 (300–1300)	36 (300–1200)	3.5	1/16
P10	20 years	1220 (1200–4100)	1190 (913–1884)	86 (88–322)	4 (139–378)	683 (1100–4100)	268 (600–2400)	439 (400–1500)	24 (200–1400)	0.16	1/16
P11	8 years 6 m	1600 (1200–4700)	1040 (842–1943)	20 (54–392)	24 (62–390)	602 (1000–4900)	229 (500–2700)	396 (300–2100)	13 (200–2200)	1.35	¼
P12	3 years 9 m	2000 (1400–5500)	135 (640–2010)	15 (52–297)	6.6 (44–244)	540 (1900–3600)	160 (600–2000)	240 (300–1300)	17 (300–1200)	0.4	½
P13	3 years 8 m	1000 (1400–5500)	470 (640–2010)	420 (52–297)	6.6 (44–244)	490 (1900–3600)	180 (600–2000)	420 (300–1300)	90 (300–1200)	0.5	1/1
P14	6 years	600 (1200–4700)	240 (745–1004)	12 (78–261)	6.6 (57–282)	390 (1000–4900)	36 (500–2700)	312 (300–2100)	0 (200–2200)	33	1/1
P15	10 m	3030 (320012300)	65 (463–1006)	113 (46–159)	6.6 (17–69)	1424 (2400–8100)	606 (1400–5200)	1151 (600–3000)	272 (500–3600)	0	½
P16	6 years	700 (1800–18,700)	456 (745–1004)	179 (78–261)	6.6 (57–282)	532 (1000–4900)	399 (500–2700)	126 (300–2100)	126 (200–2200)	ne	1/32
P17	2 m	2020 (2900–11,400)	482 (376–685)	19 (36–77)	6.6 (9–30)	1353 (2400–8100)	178 (1400–5200)	202 (600–3000)	20 (500–3600)	ne	ne
P18	11 years 6 m	1146 (1400–4200)	1070 (835–2094)	163 (67–433)	6.1 (47–484)	561 (1100–4100)	229 (700–2500)	435 (400–1500)	189 (200–1400)	1.1	ne

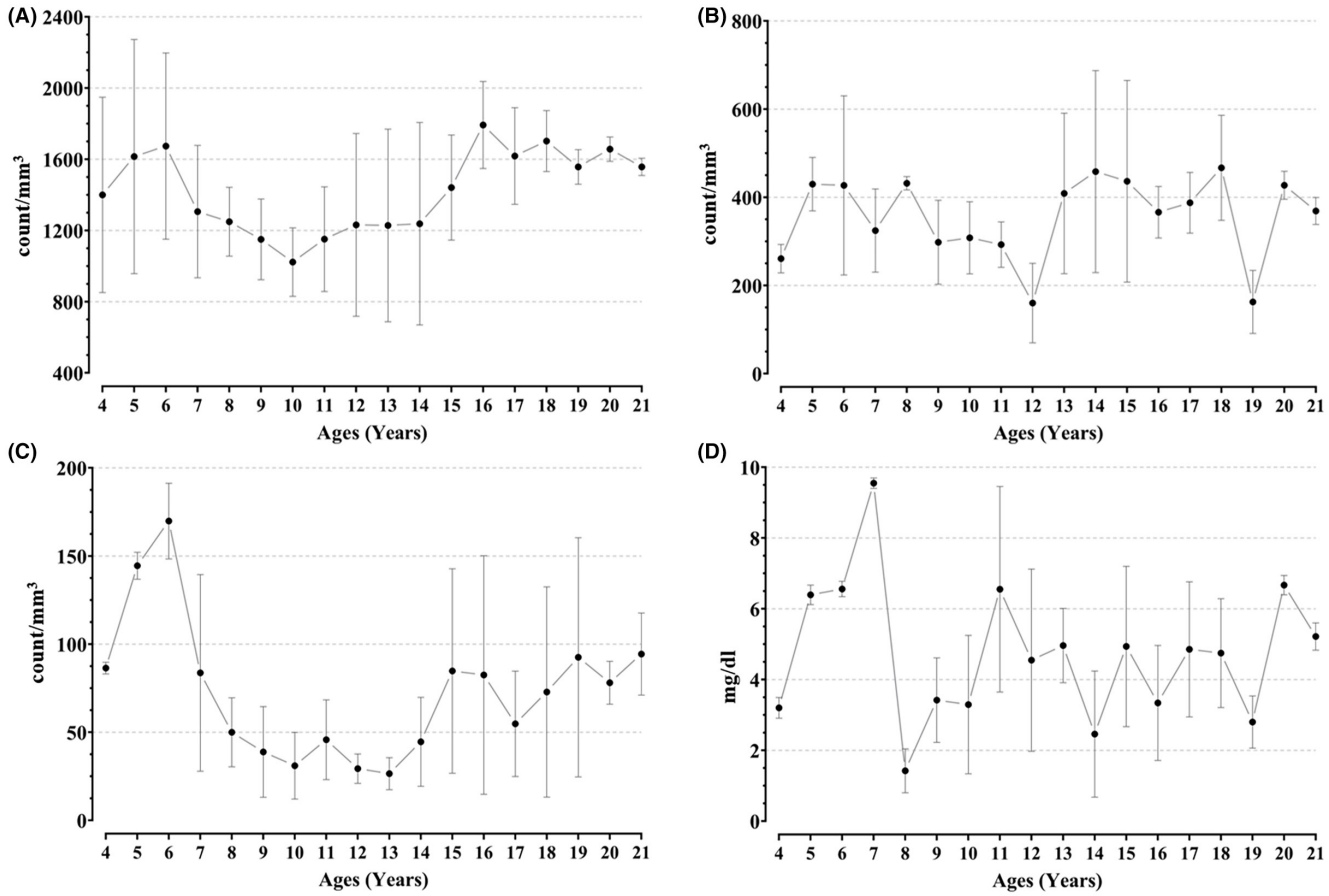

FIGURE 2 Changes in lymphocyte, CD4+ Th cell, CD19+ B cell and IgA levels depending on age of patients (The data presented are the mean and standard deviation of the values observed in patients). (Patients included for analysis: P1, P4, P5, P6, P7, P8, P9, P11, P14, and P16). (A) Lymphocyte; (B) CD4+ Th cell; (C) CD19+ B cell; (D) Serum IgA.

($p > .05$) (Figure 4A). B_{naive} cell ratio was lower in patients than in controls and post-transplant patients ($p < .05$). Although switched memory B-cells ratio slightly lower in patients than in controls, it was not significant ($p > .05$) (Figure 4B). Low lymphocyte count, T-cell and B-cell lymphopenia became more pronounced with age, especially toward adulthood. Regarding T-cell subsets, CD4+ and CD8+ T_{EM} cell ratios were higher in patients than in controls ($p < .05$). Although effector T_{EM} cell ratio decreased after HSCT, it remained higher than controls ($p > .05$). CD4+ and CD8+ T_{CM} cell ratios were higher compared to controls before HSCT, but these ratios were similar to controls after HSCT ($p > .05$). CD4 + TEMRA cell ratio was lower in patients before HSCT than in controls, but after HSCT, this ratio became like controls. In contrast, CD8 + TEMRA cell ratio was higher in patients than in controls before and after HSCT. The changes in CD4+ and CD8+ TEMRA cells were not statistically significant ($p > .05$) (Figure 3C,D).

When the patients evaluated in terms of other T-cell subsets and cytokines; T_{FH} and Th1 cell ratios (both IFN-γ and CXCR3+ T cells) were significantly higher in patients before and 1–2 years after HSCT than in controls ($p < .05$) (Figure 3E,F). RTE cell ratio (CD4 + CD45RA + CD31+) was significantly lower in patients than in controls ($p < .05$) before HSCT but returned to similar levels after HSCT ($p > .05$) (Figure 4G). However, Treg cell ratios and its functional markers including CTLA-4, Helios, ICOS, and GITR expression on the Treg cells were slightly higher in patients than in controls although this difference was not statistically significant (Figure S3a–e).

When comparing NK cell subsets between patients and controls, $CD56^{dim}CD16^{+}$ NK cells were very significantly lower in patients than in controls ($p < .05$). However, $CD56^{bright}$ NK cells, which are potent producers of cytokines, were slightly lower in patients than in controls, although this difference was not statistically significant ($p > .05$) (Figure 3H).

3.4 | CD25 activation results

CD25 activation assay was performed to evaluate T-cell functions. CD25 activations could be evaluated both before and after HSCT in six patients. A 3.05-fold, 2.23-fold, and 8.40-fold increase in CD25 expression was detected in pre-HSCT CD3, CD4, and CD8 cells, respectively. After HSCT, 181-fold, 86.4-fold, and 127-fold increases in expression were detected in CD3, CD4, and CD8 cells, respectively. In healthy controls, a 227-fold, 260-fold, and 212-fold

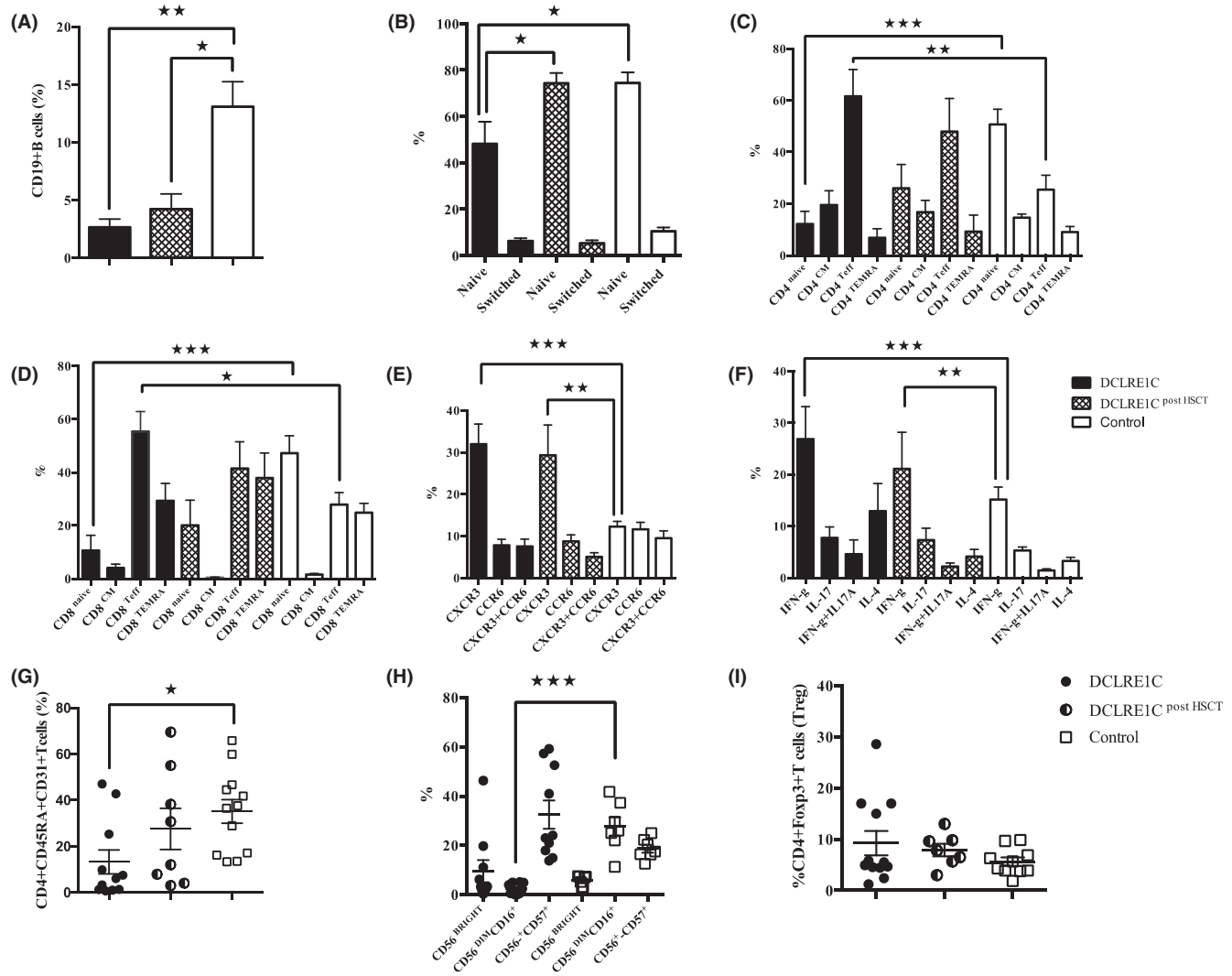

FIGURE 3 Comparison of ratios of B, T, NK cell subsets and cytokine expressions before and after HSCT in patients and healthy controls by flowcytometry. (A) CD19+ B cells, (B) B cell subsets, (C) CD4+ T memory cell subsets, (D) CD8+ T memory cell subsets, (E) Surface markers of T helper cell subsets, (F) Cytokines of T helper cells, (G) RTE, (H) NK cell subsets, (I) CD4 + Foxp3+ Treg cells. (HSCT: Hematopoietic stem cell transplantation, CM, Central memory; T_{eff}, Effector memory T cell; TEMRA, Terminally differentiated effector memory T cell; RTE, Recent thymic emigrant; Treg, Regulator T cell). $^{*}p < .05$, $^{**}p < .005$, $^{***}p < .001$.

increase in CD25 expression was detected in CD3, CD4, and CD8 cells, respectively (Figure S4).

3.5 | In silico analysis of artemis variant

The variant c.194C>T identified in the *DCLRE1C* gene causes a Thr165Ile amino acid change in the β-lactam domain of the Artemis protein (Figure 4A). The CADD score for the variant identified in the *DCLRE1C* gene was 26.5. After performing multiple sequence alignment (MSA) using the amino acid sequences of Artemis proteins from four primates, one lagomorph and one rodent, we observed that Thr65 was phylogenetically conserved (Table S1). We calculated the ΔΔG value as 1.02 (kcal/mol) based on the amino acid change in the post-variant protein structure and observed that the Artemis protein was destabilized. Changes in intramolecular hydrogen bonds occurred due to the amino acid change. In the wild-type condition, the Thr65 amino acid residue forms a hydrogen bond with Gly118 (2.98 Å) and two different hydrogen bonds with Ser62 (2.931 Å and 3.064 Å), while after the variant, the hydrogen bond with Gly118 and one of the hydrogen bonds with Ser62 are lost. The Ile65 amino acid formed a cryptic hydrogen bond with Cys64 after the variant (3.165 Å) (Figure 4A–C).

4 | DISCUSSION

DCLRE1C deficiency typically results in causes T-B- SCID within opportunistic infections during the first year of life. Hypomorphic mutations with residual protein activity led to milder clinical forms of inborn error of immunity (IEI) typically present later in life (Table 1).[4,7–14] However, information on the clinical, laboratory,

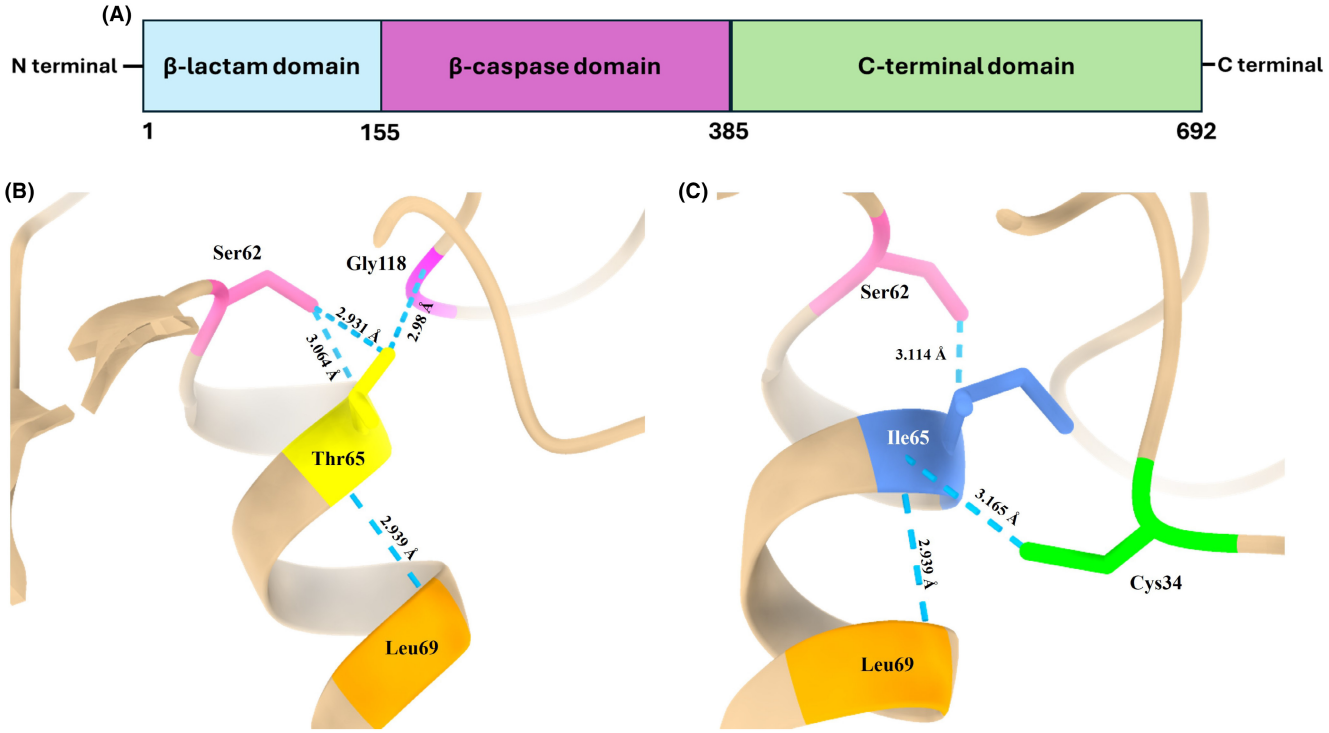

FIGURE 4 (A) Schematic representation of the structural regions of the Artemis protein. (B) Wild-type Artemis protein. (C) Mutant Artemis protein resulting from the c.194C > T variant and its interaction with other amino acids [Blue dashed lines represent hydrogen bonds, and numbers on blue dashed lines represent bond lengths in Angstrom units (Å)].

and demographic characteristics of diseases caused by hypomorphic mutations. Our study presents 20 years of clinical experience, documenting changes in clinical and laboratory findings, long-term follow-up, and post-transplant outcomes in *DCLRE1C* deficiency. Our study is also in terms of including both adult and pediatric patients.

Recurrent sinopulmonary infections, skin symptoms such as granulomas, warts, and vitiligo are the most prominent clinical findings in patients with hypomorphic *DCLRE1C* mutation and other IEIs including Ataxia Telangiectasia, X-linked agammaglobulinemia, CID, SCID (*RAG*, *DCLRE1C*, *PRKDC* deficiency), and chronic granulomatous disease.[8,11,13,15,16,27–31] Most of our patients had experienced recurrent sinopulmonary infections and skin involvement consistent with findings reported in the literature. The mechanism of granuloma formation in IEI is fully understood. However, recent reports have demonstrated that Rubella vaccine strain can chronically infect M2 macrophages and keratinocytes in patients with T-cell deficiency, leading to granuloma formationss.[32] All patients in this cohort received measles, mumps, and rubella (MMR) vaccines at 1 year of age according to the national immunization program. However, only four patients aged 2–6 had cutaneous granulomas. We were able to measure serum Rubella IgM level in only 12 patients with/without granuloma, and it was positive in all four patients with granulomas. However, serum Rubella IgM was positive in only one patient without cutaneous granulomas. We did not perform PCR for Rubella virus on the biopsy samples because this test was not available in our center. We were unable to detect acid-fast bacteria in skin biopsies taken from granulomas. However, all the patients with granulomatous skin lessions had decreased $CD56^{dim}CD16^{+}$ NK cell ratio. This may contribute to the development of granulomatous skin lesions in these patients.

Artemis, encoded by the *DCLRE1C* gene Artemis, plays a critical role in the V(D)J recombination, its main function as a component of the NHEJ pathway of DNA repair is to process specific DNA double-strand breaks that are repaired in a slow kinetic process.[33] In silico analyses we conducted in our study showed that the amino acid interactions in the Artemis protein formed as a result of the hypomorphic *DCLRE1C* gene variant change and an unstable protein is formed. This was the first time that it was shown that this resulted in destabilization of the protein. This can be explained by the fact that unlike the variants in the *DCLRE1C* gene that result in complete loss of function of the Artemis protein and cause SCID, hypomorphic variants do not change the protein level but affect the stability of the protein, causing a milder clinical course.

The frequency of autoimmunity and malignancy is 5 to 30 times higher in individuals with IEI than in the general population.[34] Similar to other IEIs, patients with SCID or hypomorphic mutations in the *DCLRE1C* gene have an increased susceptibility to autoimmunity and malignancy.[4,10–14,16,35,36] Similar to the literature, we found the frequency of autoimmunity and malignancy in our cohort to be 33% and 17%, respectively. A possible mechanism of malignancy and immune dysregulation is that mutation in *DCLRE1C* results with ineffective DNA repair with genomic instability, with a consequent clinical picture of SCID/leakySCID associated with radiosensitivity. Diffuse large B-cell lymphoma (NHL), large granular lymphocytic leukemia,

TABLE 4 HSCT characteristics and outcomes.

Characteristic	Value
Age at Tx (median, years)	10.7 (5.8–14.5)
Gender	
Male	2 (25%)
Female	6 (75%)
MUD HLA match	
10/10	3 (38%)
9/10	5 (62%)
Conditioning regimen	
Cy Flu ATG	7 (88%)
Treo Flu ATG	1 (12%)
GVHD prophylaxis	
Tacr+Mtx	5 (62%)
CsA + Mtx	3 (38%)
Stem cell source ($\times 10^6$/kg) (median, range)	
BM	2 (25%)
PBSC CD34 ($\times 10^6$/kg)	6 (75%) 6.4 (3.1–11.4)
Engraftment (median days, range)	
Neutrophil	9.5 (5–18)
Platelet	8 (5–17)
Chimerism (%)	
First	37 (2–96)
Last	98 (2–100)
Acute GvHD grade 2	3 (38%)
Chronic GVHD (mild)	2 (25%)
Complications	
CMV	5
Adenovirus	1
Engraftment syndrome	3
AIHA	1
Follow-up (months, median, range)	56 (9–67)
Survival ($n = 8$)	100%
EFS ($n = 8$)	88%

Abbreviation: AIHA, autoimmune hemolytic anemia.

in situ carcinoma, HL have been reported in the patients with hypomorphic *DCLRE1C* mutation. Malignancy was detected in 21% ($n = 6$) of all patients with the hypomorphic *DCLRE1C* gene variant reported in the literature.[9,12,14,37] The malignancy rate in our cohort was 17% ($n = 3$), which is consistent with the rates reported in the literature. During the follow-up of our patients, one was diagnosed with HL and another with NHL. Additionally, one patient was diagnosed with ALL during family screening. This shows importance of screening of family members regarding the mutation, if they have malignancy or autoimmunity even no history of infection. Moshous et al. described the presence of polyclonal T and B lymphocyte, and chromosomal instability in their patients with an EBV-associated lymphoma, which may responsible development of malignancy in these patients.[4] Jacobs et al. reported that dysfunctional Artemis activity combined with p53 inactivation predisposes to thymic lymphomas.[34] We only showed increased radiosensitivity in some of our patient in our previous report.[11] The average time to diagnosis of malignancy in patients with the hypomorphic *DCLRE1C* gene variant is 10.2 months (9–264), and all patients with malignancy generally died due to infections.[4,9,12,14] In our cohort, the average time to detect malignancy was 10 (60–156) months, and one patient died. While the mortality rate in our total cohort was 17%, the mortality rate following the development of malignancy was 33% and was found to increase approximately twofold. The literature data and data in our cohort show that the development of malignancy significantly increases the risk of mortality in these patients. Therefore, we believe that careful consideration of the risk of malignancy and prompt transplantation are crucial for the survival of patients.

The patients with the hypomorphic *DCLRE1C* variant have an approximately 37% risk of developing autoimmune disease.[4,10,11,14] In our cohort, the rate of autoimmune disease was 33%. Vitiligo, which was present at diagnosis in two patients and developed during follow-up in one patient, has not been previously reported in other patients with *DCLRE1C* deficiency in the literature (two of these patients were previously reported, Volk et al.[11]) In our cohort, two patients developed autoimmunity after HSCT. Nahum et al. could be evaluated Treg ratio in only two of their four patients with hypomorphic *DCLRE1C* mutation and was found to be lower than healthy control.[13] However, we found increased ratio of Treg cell and its receptors including CTLA4, Helios, ICOS, GITR, and CD25 on Treg cell in our patients compared with controls. We could not check Treg function by suppression assay and its repertoire. A decrease in the number and/or dysfunction of Treg cells leads to a loss of immune tolerance and the development of autoimmune diseases.[35] However, Treg cells are not the sole contributors to this condition; they interact with other Th subsets in the development of autoimmune diseases.[38,39] Increased IFN-γ expression originating from Th1 cell may contribute to the development of autoimmune diseases by suppressing Th17 responses.[40] High levels of INF-γ + T cells along with low levels of IL-17 + Th17 cells, detected in our study may affect Treg cell functions.T_{FH} cells are an essential part of humoral immunity by assisting B cells to produce high-affinity antibodies. The production of high-affinity pathogenic antibodies appears to be crucial for most autoimmune disorders.[41] T_{FH} cell ratio was significantly increased in our patients compared to controls. The increased count of T_{FH} did not return to normal levels despite HSCT. Increased TFH cell ratio in these patients may be related to autoimmunity. Further mechanistic studies will help to understand the role of the *DCLRE1C* gene in autoimmunity.

Lymphopenia, low serum IgA level, T/B cell lymphopenia, and low RTE ratio were the most important laboratory findings of our patients. Lymphopenia was present in 55.5% of patients at initial presentation, but similar to the literature, it developed in all patients during follow-up.[4,7–14] In the presence of the hypomorphic *DCLRE1C* variant, the Artemis protein cannot fully perform its function.[2] In

this case, V(D)J recombination does not work properly, and T-cell development is negatively affected. Failure of T cells to develop properly leads to decreased production of mature T cells in the thymus. There are few studies evaluating T-cell subset including CD4+ and CD8+ CD45RA+CD45RO+ in the patients with hypomorphic *DCLRE1C* mutation.[8,10,11] In these studies, the proportion of these cells was found to be lower than in controls.

In Artemis deficiency, unrepaired DNA fragments may leak into the cytosol and initiate type I IFN production, leading to chronic inflammation. We have previously shown higher levels of IFN- α 2a and IP-10 in patients than in controls.[42] In this study, we have shown that type II IFN (IFN-γ) levels are also increased in these patients as additional cytokines contributing to chronic inflammation such as granulomatous skin lesions and bronchiectasis. The increased ratio of Th1 cells did not return to normal levels despite HSCT. When we evaluated NK cell subsets, the $CD56^{dim}CD16^{+}$ NK cells, being active mediators of natural and antibody-dependent cellular cytotoxicity were significantly lower in patients than in controls. Five patients in our cohort had warts, which may be related to decreased NK cell subsets contributing to skin viral infections, including warts.

5 | CONCLUSIONS

As a results, the hypomorphic variant in the *DCLRE1C* (c.194C>T) gene causes the Artemis protein to become unstable. Depending on the type of hypomorphic variant, the degree of stabilization of the protein may vary, and accordingly, patients carrying the hypomorphic *DCLRE1C* variant may have variable clinical presentation and immunological findings. Our study showed a Th1-dominant immune response in patients before and after HSCT. Increased IFN-γ production, T_{FH} cell ratio and decreased NK subsets may be a reason for chronic inflammation, skin lesions, or autoimmunity. Although patients have CD4+ Th and CD19+ B cell lymphopenia and low serum IgA levels at the time of diagnosis, it is noteworthy that this low level continues after diagnosis. Long-term follow-up of these patients after HSCT will help to better understand the disease and its pathophysiology.

AUTHOR CONTRIBUTIONS

Esra Hazar: Conceptualization; methodology; writing – review and editing; writing – original draft; data curation. **Mehmet Ali Karaselek:** Data curation; conceptualization; writing – review and editing; writing – original draft. **Hasan Kapakli:** Data curation. **Oznur Dogar:** Data curation. **Serkan Kuccukturk:** Visualization; formal analysis. **Vedat Uygun:** Data curation; writing – review and editing. **Hasibe Artac:** Data curation. **Sıdıka Fındık:** Methodology; investigation. **Ali Sahin:** Formal analysis. **Sevket Arslan:** Data curation. **Sukru Guner:** Data curation. **Ismail Reisli:** Data curation. **Sevgi Keles:** Conceptualization; writing – review and editing; project administration; supervision; validation; methodology; formal analysis; data curation; resources; visualization; writing – original draft.

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CONFLICT OF INTEREST STATEMENT

There is no conflict of interest of any authors. 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.

ETHICS STATEMEMT

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Local Ethics Committee (approval number:2021/3417). All patients provided written informed consent before enrollment.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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