ORIGINAL ARTICLE

Immunologic Heterogeneity in 2 Cartilage-Hair Hypoplasia Patients With a Distinct Clinical Course

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Abstract

Introduction: Cartilage-hair hypoplasia (CHH) syndrome is a rare autosomal recessive syndrome associated with skeletal dysplasia, varying degrees of combined immunodeficiency (CID), short stature, hair hypoplasia, macrocytic anemia, increased risk of malignancies, and Hirschsprung disease.
Purpose: To provide clinical and immunological insights obtained from 2 unrelated patients who displayed clinical characteristics of CHH.
Methods: Two patients with suspected CHH syndrome due to skeletal dysplasia and immunodeficiency underwent an immunological and genetic work-up using flow cytometry, next-generation sequencing (NGS) of the immune repertoire, and Sanger sequencing to identify the underlying defects.
Results: Patient 1 presented with low birth weight and skeletal dysplasia. Newborn screening was suggestive of T-cell immunodeficiency, as T-cell receptor excision circle levels were undetectable. Both the T-cell receptor (TCR) Vß and TCR-γ (TRG) repertoires were restricted, with evidence of clonal expansion. Genetic analysis identified compound heterozygous *RMRP* variants inherited from both parents. Patient 2 presented with recurrent lung and gastrointestinal infections, skeletal dysplasia, failure to thrive, and hepatomegaly. The polyclonal pattern of the TCRß repertoire was normal, with only slight overexpression of TCR-ßV20 and restricted expression of Vßs. TRG expressed a normal diverse repertoire, similar to that of the healthy control sample. Genetic analysis identified biallelic novel regulatory variants in *RMRP*. Both parents are carriers of this mutation.
Conclusion: Our findings demonstrate how the immunological work-up, supported by genetic findings, can dramatically change treatment and future outcome in patients with the same clinical syndrome.

Key words: RMRP. Severe combined immunodeficiency (SCID). Cartilage-Hair Hypoplasia (CHH). NGS. TCR repertoire.

Resumen

Introducción: El síndrome de hipoplasia de cartílago-cabello (CHH) es un síndrome autosómico recesivo poco frecuente, asociado con displasia esquelética, diversos grados de inmunodeficiencia combinada (CID), baja estatura, hipoplasia del cabello, anemia macrocítica junto a un mayor riesgo desarrollar de tumores malignos y enfermedad de Hirschsprung.
Propósito: Proporcionar conocimientos clínicos e inmunológicos obtenidos de dos pacientes, sin relación familiar, que mostraron características clínicas de CHH.
Métodos: Dos pacientes con sospecha de síndrome CHH debido a la presencia de displasia esquelética e inmunodeficiencia, se sometieron a un estudio inmunológico y genético mediante citometría de flujo, secuenciación de próxima generación (NGS) del repertorio inmune y secuenciación de Sanger, para identificar los defectos subyacentes.
Resultados: Paciente 1: presenta al nacimiento bajo peso y displasia esquelética. El cribado de recién nacidos (NBS, por sus siglas en inglés) sugirió una inmunodeficiencia de células T, ya que los niveles del receptor de células T (TREC, por sus siglas en inglés) eran indetectables. El estudio posterior reveló anemia macrocítica, linfopenia grave e hipogammaglobulinemia. Tanto el repertorio del receptor de células T (TCR) - Vß como el repertorio del receptor de células T gamma (TRG) estaban restringidos y con evidencia de expansión clonal. El análisis genético identificó variantes heterocigóticas compuestas heredadas de ambos padres en RMRP. Paciente 2: presentaba una clínica de infecciones pulmonares y gastrointestinales recurrentes, displasia esquelética, retraso en el crecimiento y hepatomegalia. El estudio inmunológico, el hemograma completo (CSC) y los análisis de subpoblaciones de linfocitos fueron normales. El repertorio de TCRß reveló un patrón policlonal normal con solo una ligera sobreexpresión de TCR-ßV20 y varias expresiones restringidas de Vßs. TRG expresó un

repertorio diverso normal, similar a la muestra de controles sanos. El análisis genético identificó nuevas variantes reguladoras bialélicas en RMRP. Ambos padres son portadores de esta mutación.
Conclusión: Nuestros hallazgos demuestran cómo el estudio inmunológico, respaldado por hallazgos genéticos, puede cambiar drásticamente el tratamiento adecuado y el pronóstico clínico en pacientes con un mismo síndrome clínico.

Palabras clave: RMRP. Inmunodeficiencia combinada severa (SCID). Hipoplasia de cartílago-cabello (CHH). NGS. Repertorio TCR.

Summary box

- **What do we know about this topic?**
  We know that the syndrome mainly affects the skeletal and immune systems. Several descriptive papers cover the different mutations causing CHH.
- **How does this study impact our current understanding and/or clinical management of this topic?**
  Ours is the first study to show how the use of NGS for analysis of the T-cell receptor repertoire has the ability to influence decisions concerning patients' health, ie, whether the patient must undergo HSCT immediately or the decision can be postponed.

Introduction

Cartilage-hair hypoplasia (CHH) syndrome is a rare autosomal recessive syndrome characterized by chondrodysplasia, variable degrees of combined immunodeficiency (CID), short stature, hair hypoplasia, anemia, increased risk of malignancies, and Hirschsprung disease. The syndrome is caused by mutations in *RMRP*, a gene encoding the untranslated RNA component of mitochondrial RNA processing endoribonuclease. This ribonucleoprotein cleaves mitochondrial RNA at a priming site of mitochondrial DNA replication. *RMRP* also interacts with the telomerase reverse transcriptase (TERT) catalytic subunit in order to form a distinct ribonucleoprotein complex that has RNA-dependent RNA polymerase activity and produces double-stranded RNAs that can be processed into small interfering RNA [1]. The prevalence of CHH is exceptionally high among specific populations including the Finnish and the Amish [2], and more than 70 *RMRP* mutations have been identified to date. Mutations are found mainly in the transcribed region and the promoter region (from the TATA box to the transcription initiation site) of the *RMRP* gene. The most common mutation is the +70A>G point mutation [3-6]. Overall mortality is high in CHH, mostly from malignancies and lung disease related to immunodeficiency. Median age at death from immunodeficiency-related causes was found to be 40.9 years [7].

Clinical heterogeneity and variable degrees of immunodeficiency have been reported among patients harboring *RMRP* variants. Abnormalities of cellular immunity can range from severe impairment causing severe combined immunodeficiency (SCID), ie, T- and B-cell deficiency, to near normal with no clinical significance [6,8-10]. In addition, immune dysregulation leading to a wide spectrum of autoimmune manifestations is also frequent in the CHH patient [11]. For some CHH patients who display severe T-cell immunodeficiency, early diagnosis is possible through a newborn screening panel and is clinically important. This may prevent future infections and clinical deterioration, since adequate isolation and treatment can be initiated immediately. Newborn screening for severe T-cell immunodeficiency is widely performed using detection of T-cell receptor excision circles (TRECs) in dried blood spots collected on Guthrie cards. This assay is increasingly used for newborn screening for SCID worldwide, as well as in Israel [12]. Interestingly, the TREC assay can also identify newborns with syndromes (those associated with severe T-cell immunodeficiency), newborns with secondary causes of T-cell immunodeficiency, and newborns with T-cell lymphopenia. Thus, it is possible to identify some, but not all, CHH patients with profound T-cell lymphopenia [13]. Hematopoietic stem cell transplantation (HSCT) has profoundly changed the natural history of severe T-lymphocyte deficiencies by curing many patients with SCID and CID [14]. In contrast to SCID, not all CHH patients should be considered for HSCT. Only those with chronic or recurrent infections in association with autoimmunity or bone marrow hypoplasia are good candidates if a well-matched donor is available [15].

In this study, we describe the immune work-up of 2 unrelated patients who presented with clinical findings suggestive of CHH. One was diagnosed through newborn screening for SCID and, interestingly, displayed severe immunodeficiency including an abnormal T-cell receptor repertoire, while the other was diagnosed at the age of 1 year based mainly on skeletal dysplasia and recurrent lung and gastrointestinal infections. We used next-generation sequencing (NGS) to determine the T-cell receptor (TCR) repertoire. In both patients, Sanger sequencing identified distinct mutations in the *RMRP* gene. This research extends our current understanding of CHH in patients with suspected immunodeficiency and the correlation between their T-cell receptor γ (TRG) repertoire and their diseases.

Methods

Clinical Data

Clinical information was obtained from the patients' electronic medical records. All procedures were performed in accordance with the ethical standards of the institutional and/or national research committee and with the Declaration of Helsinki and its later amendments or comparable ethical standards.

Immune Function

Cell surface markers of peripheral blood mononuclear cells (PBMCs) were determined by immunofluorescent staining using flow cytometry, as previously described [16]. Lymphocyte proliferation was assessed in response to the mitogens phytohemagglutinin (PHA) and anti-CD3 using tritiated thymidine incorporation, as previously described [16]. The serum concentration of immunoglobulins was measured using nephelometry (BN-II, Siemens).

TREC analysis was performed using DNA extracted from the study patients' PBMCs, as previously described [16].

TCR-Vβ Repertoire

The expression of the representatives of specific TCR variable β families were detected and quantified using patients' PBMCs with flow cytometry (NAVIOS, Beckman Coulter) according to the manufacturer's instructions (Beta Mark TCR Vβ repertoire kit, Beckman Coulter), as previously described [16].

Determination of the TCR-γ Repertoire Using Next-Generation Sequencing

TCR libraries were generated from patient and control genomic DNA extracted from PBMCs using primers to the conserved regions of V and J genes in the TCR-γ locus according to the manufacturer's protocol (Lymphotrack, Invivoscribe Technologies). Quantified libraries were pooled and sequenced using Mi-Seq Illumina Technology. FASTA files from the filtered sequences were submitted to the ImMunoGeneTics (IMGT) HighV-QUEST webserver (http://www.imgt.org), further filtered for productive sequences only (no stop codons or frameshifts), and analyzed [3]. Analyses were performed on CDR3 amino acid sequences. V and J gene usage patterns were analyzed for TCR repertoires. Repertoire diversity was calculated using the Shannon and Gini-Simpson diversity indices [4].

Sanger Sequencing

Sanger sequencing (NCBI Reference Sequence: NR_003051.3) was used for identification of mutations and familial segregation of both nucleotide changes in the *RMRP* gene.

Bone Marrow Transplantation

Patients underwent transplantation in house, at our hematology-oncology unit. Standard prophylactic medications consisted of trimethoprim/sulfamethoxazole for *Pneumocystis jiroveci* (PCP), intravenous immunoglobulin (IVIG) to maintain IgG levels above 6 g/L, acyclovir in the case of donor–recipient serologic cytomegalovirus disparity, and cyclosporine A for graft-versus-host disease (GVHD). Granulocyte colony-stimulating factor was given from the day of transplant until neutrophil counts were $>1.0 \times 10^9$/L for 3 consecutive days. The HSCT infusion itself originated from bone marrow. Conditioning regimens were based on the European Group for Blood and Marrow Transplantation and European Society for Immunodeficiencies guidelines for HSCT in PID.

Engraftment and Immunologic Assessment

Neutrophil engraftment was confirmed after 3 consecutive days with an absolute count of over 0.5×10^9/L. Lymphocyte engraftment and chimerism were evaluated mainly by analysis of microsatellite variable numbers of tandem repeats, and fluorescence in situ hybridization studies using Y-specific probes in sex-mismatched couples. Absolute numbers and percentages of lymphocytes were quantified by assessment of cell surface markers using immunofluorescent staining and flow cytometry (Epics V, Beckman Coulter) with antibodies purchased from Beckman Coulter. Serum immunoglobulin concentrations (IgG, IgM, IgA) were measured using standard nephelometry.

Results

Clinical Description

We present 2 unrelated patients who were evaluated for suspected CHH. Patient 1 was born to a healthy nonconsanguineous Ashkenazi-Jewish couple after spontaneous pregnancy. Fetal ultrasound in the third trimester revealed short long bones. The mother gave birth to a girl at 34 weeks of gestation (birth weight of 1800 g and length of 35 cm ([–2 SD]). The postnatal period was uneventful. Newborn screening was suggestive of T-cell immune deficiency, as TREC levels were undetectable. At the fourth week of life, the blood work-up showed severe lymphopenia and anemia (Table).

Patient 2 was brought to our clinic at age 1 year, mainly to evaluate his recurrent infections. He was born to healthy nonconsanguineous Palestinian parents. His birth weight was 3.3 kg. After birth, the patient was noticed to have short limbs. At the second month of life, examination revealed hepatosplenomegaly. The patient later presented with poor weight gain. At the first year of life, he weighed only 7.6 kg and 4 months later 7.8 kg (–3 SD). Since birth, he had experienced recurrent pneumonia (once a month) and multiple episodes of gastroenteritis, some of which required hospitalization. Initial findings in our hospital were short stature (72 cm, –3 SD), short limbs, skeletal dysplasia, failure to thrive, and hepatomegaly.

Immunological Evaluation

The initial immunologic evaluation for patient 1 at the fourth week of life revealed severe lymphopenia with depleted T and B cells (Table). Lymphocyte proliferation

Table. Immune Profile at Presentation.

	P1 (4 wk)	P2 (16 mo)	Normal range
Lymphocytes/mm^3	870	2002	2900-11 400/1400-12 000
Lymphocyte subsets, cells/mm^3			
T cells	87	1211	1900-8400/700-8800
CD4	35	480	1500-6000/400-7200
CD8	139	360	300-2700/200-2800
B cells	165	320	180-3500/160-3700
NK	55	29	6-30
TREC	12		>400
Immunoglobulins, mg/dL			Normal range
IgG	496		590-1430
IgA	Undetectable		38-222
IgM	24		56-208
Proliferation capacity[a]			Healthy donor
No mitogen	1381		1097
PHA 6, µg/ml	2327		103 158
PHA 25, µg/ml	3822		13 5014
Anti-CD3 antibody	2379		36 154

Abbreviations: PHA, phytohemagglutinin; TREC, T-cell receptor excision circle.
[a]Counts per minute of H3-thymidine uptake in response to mitogens.

was markedly reduced following stimulation with PHA. In order to evaluate thymus activity and naïve T-cell production, TRECs in peripheral blood were quantified. The TREC level was remarkably low (Table). Hypogammaglobulinemia was detected based on the absence of IgA and low levels of IgM. IgG levels were borderline, as expected in the postnatal period. At the age of 3 months, IgG levels were 342 mg/dL (normal range, 470-1230 mg/dL), and monthly replacement therapy was started with IVIG.

The TCR-Vβ repertoire was assessed using the traditional TCR-Vβ assay with flow cytometry. Patient 1 had a monoclonal pattern with overexpression of TCR-Vβ12, compared with healthy controls (Figure 1). Taken together, the laboratory results were suggestive of SCID. The patient was also assessed for cell chimerism upon diagnosis owing to suspected transplacentally acquired maternal T cells (a pathognomonic feature of SCID) and showed no chimerism after birth. Patient 2's immunologic evaluation was performed at the age of 1 year. Findings for the complete blood count and lymphocyte subpopulations were normal (Table). The TREC level was low (Table). Immunoglobulin levels were normal for age (Table). The TCR-Vβ repertoire revealed a normal polyclonal pattern with only slight overexpression of TCR-Vβ20 and very restricted TCR-Vβ expression, compared with healthy controls (Figure 1).

TRG Repertoire Analyses

In order to further characterize the TCR repertoire, high-throughput immune sequencing of the T-cell receptor γ chain (TRG) repertoire was performed on PBMCs from both patients and a healthy age-matched control. Of the 4 chains of TCR (α, β, γ, δ), the TRG repertoire was specifically selected for sequencing because it is rearranged earlier in the development of T cells and is often used for sensitive and comprehensive detection of clonal expansions [4,5,17].

As shown in Figure 2A, the treemaps for the TRG repertoire determine that patient 1 expressed a restricted diverse repertoire with evidence of clonal expansion consistent with abnormal T-cell selection within the thymus. Patient 2, on the other hand, expressed a normal diverse repertoire, similar to that of the healthy control. We used the Shannon H and Simpson D indices [4] to measure the diversity of the repertoires. These

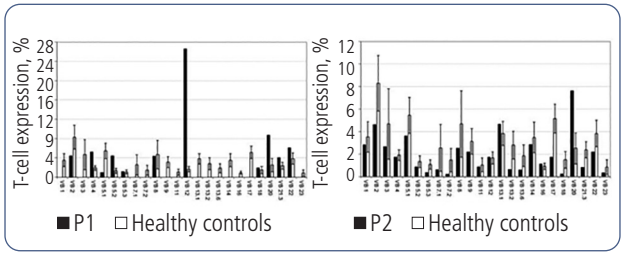

Figure 1. TCR-Vβ repertoire analysis. Twenty-four TCR-Vβ families were detected and quantified using flow cytometry, which was performed on total lymphocytes obtained from two *RMRP*-deficient patients. Levels of expression of the patient (black bars) were compared with those of normal control values (clear bars), which were obtained from the IOTest Beta Mark-Quick Reference Card.

are commonly used in ecology [18-20]. The diversity indices take into account the unique and total sequences and the evenness of the clonal size. Thus, the Shannon H index reflects the overall diversity of the repertoire, whereas the Simpson D index focuses on how unevenly the clones are distributed in a given repertoire due to the presence of clonal expansions, as previously shown [19,20]. The Shannon H diversity index for patient 1 repertoires was relatively low compared with patient 2 and the controls (Figure 2C). Calculation of the Simpson D index showed unevenness in the TRG repertoire for patient 1 compared with patient 2 and the controls (Figure 2B). Thus, in accordance with the treemaps, the diversity indices showed that patient 1 but not patient 2 had a restricted and clonally expanded TRG repertoire compared with the controls. Nonproductive sequences of the TRG were also analyzed, although the results did not differ from those of the productive sequences (Figure S1).

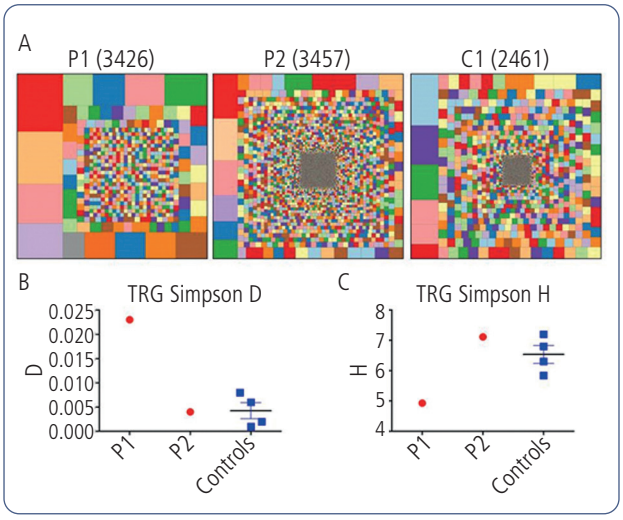

Figure 2. STRG repertoire determined by NGS. A, Treemap representation of T-cell receptor γ (TRG) repertoire in peripheral blood mononuclear cell samples from patients with *RMRP* deficiency and 1 representative healthy control. Each square represents a unique V to J joining and the size of the square represents the relative frequency within that sample. B and C, Quantification of the unevenness and the diversity of the TRG repertoire using the Simpson D index of unevenness (B) and the Shannon H index of diversity (C) in 4 healthy controls and in patients with *RMRP* deficiency. Controls are the same as those used elsewhere [30].

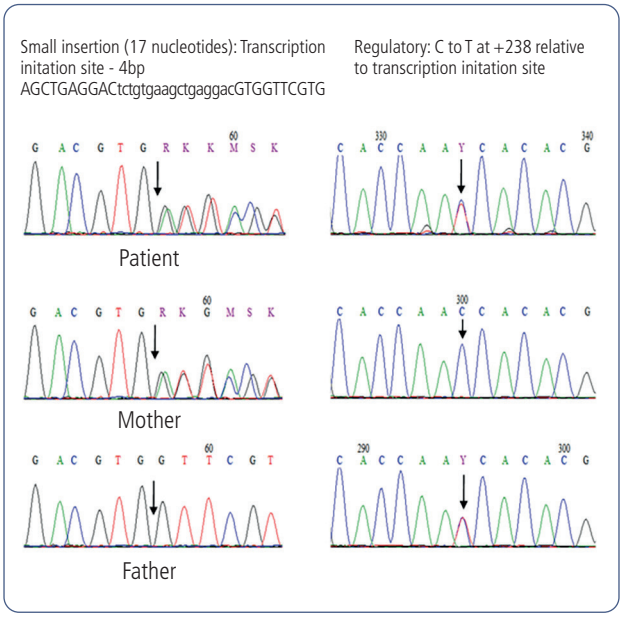

Figure 3. Sanger sequencing of the compound heterozygous *RMRP* variants in patient 1. A, The duplicated 17 nucleotides in the maternal allele are boxed. The variant in the paternal allele is marked by an arrow.

Genetic Analyses

Sanger sequencing revealed 3 distinct mutations in the *RMRP* gene of both patients. Two of the mutations are known [1,21] and 1 is novel. Familial segregations were elucidated (Figures 3 and 4). In the case of patient 1, biallelic compound heterozygous known mutations were found (Figure 3). The first mutation, which was maternally inherited, is a small 17-nucleotide duplication, 4 nucleotides upstream from the transcription initiation site (TIS) (1). The second mutation, which was paternally inherited, is a regulatory mutation of C to T at +238 relative to the TIS (20). In patient 2, a homozygous novel regulatory mutation was found at +222 relative to the TIS. Both parents are carriers of this mutation (Figure 4).

HSCT for Patient 1

Patient 1 was 10 months old when he underwent transplantation. Conditioning was with treosulfan/fludarabine. A brother was found to be a matched related donor. As described, GVHD prophylaxis, when used, was based mainly on cyclosporine A. Mycophenolate mofetil and corticosteroids were added when GVHD was suspected or diagnosed. Acute and chronic GVHD appeared only in conditioned patients, all of whom received adequate prophylaxis.

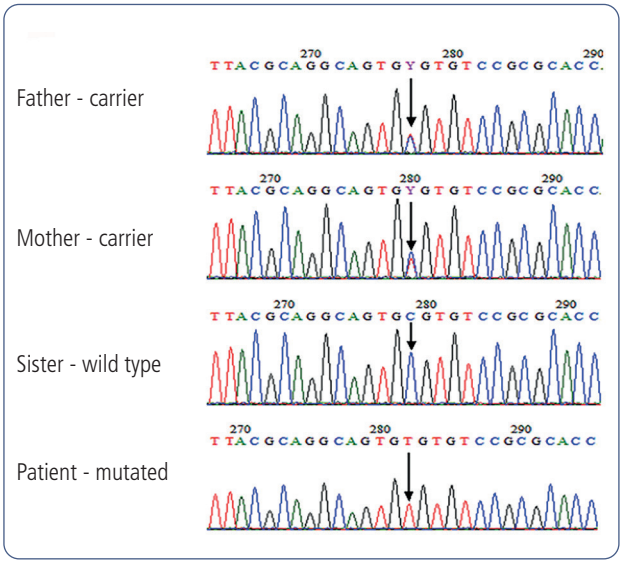

Figure 4. Sanger sequencing of the homozygous novel *RMRP* variant in patient 2. The variant is marked by an arrow.

Immune Reconstitution

Neutrophil engraftment was confirmed on day +12 after HSCT, lymphopenia (defined as an absolute lymphocyte count <2500) recovered 5 months after HSCT and remained stable until present. T-cell deficiency (CD3 <1000) recovered 6 months after HSCT, and immunoglobulin levels recovered 3 months after HSCT.

Normal TREC levels (>400 copies) were detected 6 months after HSCT.

Chimerism based on FISH studies using Y-specific probes in sex-mismatched couples was 100% 3 months after HSCT and remained stable at 60% 1 year after HSCT.

Discussion

CHH is a well-known syndromic PID with a wide spectrum of clinical presentations, specifically regarding the immune system. Patients can be diagnosed immediately after birth through newborn screening for SCID or later in life, based on clinical suspicion.

In this report, we describe 2 patients diagnosed with CHH based on clinical and molecular findings. Patient 1 was identified through the Israeli newborn screening program. CHH patients with absent TREC tend to have a greater immunodeficiency, thus making the diagnostic process much simpler. CHH patients with positive results through the newborn screening program are likely to follow a different route of treatment [13,22]. Recent studies summarizing the latest results of the newborn SCID screening programs revealed several outcomes. Besides the diagnosis of typical SCID, syndromes such as DiGeorge syndrome, trisomy 21, trisomy 18, and CHARGE syndrome accompanied by severe lymphopenia were also identified using the TREC test [23-25]. In addition, these studies detected 2 CHH patients. The first CHH patient, who was diagnosed with SCID and clinical manifestations of skeletal dysplasia and Hirschsprung disease, died at the age of 1 month. Her *RMRP* mutation was discovered postmortem. The second patient was initially diagnosed with leaky SCID and was characterized by intrauterine growth restriction and short limbs [23,24]. Scott et al [22] described 8 Amish CHH patients with abnormal TREC levels who tested positive in newborn screening. Four patients had absent TREC and tended to have a greater degree of immunodeficiency. Patient 2 had also presented with low TREC levels. Unfortunately, there is no newborn screening TREC program in the Palestinian territories, and the evaluation was based on clinical suspicion.

As often seen in patients with CHH who present with variable clinical symptoms and therefore undergo a completely different treatment with different outcomes, we detected such differences in the cases we report, specifically when evaluating immune function. Patient 1 had lymphopenia from birth, with severe T- and B-cell depletion, while patient 2 had lymphocytes within the normal range. Interestingly, patient 1 had a restricted TCR regardless of any underlying infection, which could potentially lead to abnormal T-cell selection or Omenn syndrome. In contrast, patient 2 had a diverse TCR, which was normal compared to a healthy control. The Shannon H diversity index for the repertoires of patient 1 was relatively low, compared with patient 2 and the controls. Similar results were also observed for the Simpson D index. Using advanced methods, such as NGS, we were able to show differences in the immunologic profile between the patients, eventually leading to different clinical outcomes.

Moreover, we observed a genotype-phenotype correlation, which is also supported by the literature [10,13,26,27]. In patient 1, the positions of the 2 known mutations—4 base pairs before the TIS (maternal) and at +238 (paternal)—can explain her severe clinical phenotype of immune deficiency and bone dysplasia. Thiel et al [10] suggested that insertions, duplications, and triplications within the conserved 24- to 26-bp region between the TATA box and TIS, as in the maternal mutation, cause reduced *RMRP* transcription, which resulted from impaired binding of the RNA polymerase III transcription factor complex, leading to a cellular phenotype of defects in rRNA cleavage and ribosome assembly and a clinical phenotype of bone dysplasia [10]. The other mutation lies within the P4 region of *RMRP*, which is responsible for the regulation of cell cycle progression at G2 through mRNA cleavage, leading to immune deficiency in the case of a mutation there [10,11]. Patient 2 harbored a novel homozygous regulatory mutation at position +222, which lies in the P1 and P2 regions of *RMRP* and is responsible for its rRNA cleavage activity and ribosome assembly, but not immune deficiency. A mutation at +220, ie, 2 nucleotides upstream from the mutation we describe, is associated with milder phenotypes [10]. Indeed, the clinical manifestations of milder bone dysplasia and very mild immune deficiency observed in patient 2, compared with patient 1, correlate well with the genetic findings. Although the TCR for patient 2 was diverse, we recorded low TREC levels at the age of 1 year. We cannot assume the TREC results for the newborn period, but we can learn about the high sensitivity of the test in T-cell immunodeficiency. One explanation could be the mild restrictions found in the TCR-Vβ examination.

Therapy in CHH is focused on immunodeficiency. Most of the other available therapies for CHH patients are mainly palliative. HSCT has profoundly changed the natural history of severe T-lymphocyte deficiency, including SCID and other syndromes associated with severe T-cell lymphopenia. Excellent outcomes have been reported for SCID and other moderate T-lymphocyte dysfunctions. After HSCT, patients have been reported to reach the age of 30 [14,28,29]. The current understanding is that not all CHH patients should be considered for HSCT, only those with chronic or recurrent infections and an available matched donor. Profound immunodeficiency associated with CHH can be cured by stem cell transplantation. Unfortunately, replacing hematopoietic stem cells does not seem to improve skeletal dysplasia [23,25]. In our report, patient 1 went through successful HSCT from a matched related donor. It has been 3 years since the procedure, and the patient is alive and well, does not require immunological therapy, and has experienced no significant complications. In contrast, patient 2 was advised to continue regular follow-up and receive symptomatic treatment.

In summary, we describe 2 unrelated CHH patients with distinct clinical presentations and immune work-up findings and different modes of diagnosis. In order to delineate the difference between these patients, which eventually led to

different treatment approaches, we included analyses of their TCR using advanced methodologies. We showed how this could help to choose the appropriate treatment for each patient. Detection of patients through the newborn screening program for severe T-cell immunodeficiency can help in the decision-making process when treating newborns with immunodeficiency, as in the CHH case we report, thus enabling the patient to benefit from early HSCT.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

References

1. Ridanpaa M, van Eenennaam H, Pelin K, Chadwick R, Johnson C, Yuan B, et al. Mutations in the RNA component of RNase MRP cause a pleiotropic human disease, cartilage-hair hypoplasia. Cell. 2001;104(2):195-203.
2. Makitie O. Cartilage-hair hypoplasia in Finland: epidemiological and genetic aspects of 107 patients. J Med Genet. 1992;29(9):652-5.
3. Alamyar E, Duroux P, Lefranc MP, Giudicelli V. IMGT((R)) tools for the nucleotide analysis of immunoglobulin (IG) and T cell receptor (TR) V-(D)-J repertoires, polymorphisms, and IG mutations: IMGT/V-QUEST and IMGT/HighV-QUEST for NGS. Methods Mol Biol. 2012;882:569-604.
4. Kansal R, Grody WW, Zhou J, Dong L, Li X. The Value of T-Cell Receptor gamma (TRG) Clonality Evaluation by Next-Generation Sequencing in Clinical Hematolymphoid Tissues. Am J Clin Pathol. 2018;150(3):193-223.
5. Mahe E, Pugh T, Kamel-Reid S. T cell clonality assessment: past, present and future. J Clin Pathol. 2018;71(3):195-200.
6. Makitie O, Kaitila I, Savilahti E. Susceptibility to infections and in vitro immune functions in cartilage-hair hypoplasia. Eur J Pediatr. 1998;157(10):816-20.
7. Vakkilainen S, Taskinen M, Klemetti P, Pukkala E, Makitie O. A 30-Year Prospective Follow-Up Study Reveals Risk Factors for Early Death in Cartilage-Hair Hypoplasia. Front Immunol. 2019;10:1581.
8. Hermanns P, Tran A, Munivez E, Carter S, Zabel B, Lee B, et al. RMRP mutations in cartilage-hair hypoplasia. Am J Med Genet Part A. 2006;140(19):2121-30.
9. Kavadas FD, Giliani S, Gu Y, Mazzolari E, Bates A, Pegoiani E, et al. Variability of clinical and laboratory features among patients with ribonuclease mitochondrial RNA processing endoribonuclease gene mutations. J Allergy Clin Immunol. 2008;122(6):1178-84.
10. Thiel CT, Mortier G, Kaitila I, Reis A, Rauch A. Type and level of RMRP functional impairment predicts phenotype in the cartilage hair hypoplasia-anauxetic dysplasia spectrum. Am J Hum Genet. 2007;81(3):519-29.
11. Vakkilainen S, Makitie R, Klemetti P, Valta H, Taskinen M, Husebye ES, et al. A Wide Spectrum of Autoimmune Manifestations and Other Symptoms Suggesting Immune Dysregulation in Patients With Cartilage-Hair Hypoplasia. Front Immunol. 2018;9:2468.
12. Rechavi E, Lev A, Simon AJ, Stauber T, Daas S, Saraf-Levy T, et al. First Year of Israeli Newborn Screening for Severe Combined Immunodeficiency-Clinical Achievements and Insights. Front Immunol. 2017;8:1448.
13. Jilkina O, Thompson JR, Kwan L, Van Caeseele P, Rockman-Greenberg C, Schroeder ML. Retrospective TREC testing of newborns with Severe Combined Immunodeficiency and other primary immunodeficiency diseases. Mol Genet Metab. 2014;1:324-33.
14. Antoine C, Muller S, Cant A, Cavazzana-Calvo M, Veys P, Vossen J, et al. Long-term survival and transplantation of haemopoietic stem cells for immunodeficiencies: report of the European experience 1968-99. Lancet. 2003;361(9357):553-60.
15. Bordon V, Gennery AR, Slatter MA, Vandecruys E, Laureys G, Veys P, et al. Clinical and immunologic outcome of patients with cartilage hair hypoplasia after hematopoietic stem cell transplantation. Blood. 2010;116(1):27-35.
16. Frizinsky S, Rechavi E, Barel O, Najeeb RH, Greenberger S, Lee YN, et al. Novel MALT1 Mutation Linked to Immunodeficiency, Immune Dysregulation, and an Abnormal T Cell Receptor Repertoire. J Clin Immunol. 2019;39(4):401-13.
17. Sufficool KE, Lockwood CM, Abel HJ, Hagemann IS, Schumacher JA, Kelley TW, et al. T-cell clonality assessment by next-generation sequencing improves detection sensitivity in mycosis fungoides. J Am Acad Dermatol. 2015;73(2):228-36 e2.
18. Johnston EL, Roberts DA. Contaminants reduce the richness and evenness of marine communities: a review and meta-analysis. Environ Pollut. 2009;157(6):1745-52.
19. Lee YN, Frugoni F, Dobbs K, Tirosh I, Du L, Ververs FA, et al. Characterization of T and B cell repertoire diversity in patients with RAG deficiency. Sci Immunol. 2016;1(6):eaah6109.
20. Rechavi E, Lev A, Lee YN, Simon AJ, Yinon Y, Lipitz S, et al. Timely and spatially regulated maturation of B and T cell repertoire during human fetal development. Sci Transl Med. 2015;7(276):276ra25.
21. Bonafe L, Schmitt K, Eich G, Giedion A, Superti-Furga A. RMRP gene sequence analysis confirms a cartilage-hair hypoplasia variant with only skeletal manifestations and reveals a high density of single-nucleotide polymorphisms. Clin Genet. 2002;61(2):146-51.
22. Scott EM, Chandra S, Li J, Robinette ED, Brown MF, Wenger OK. Abnormal Newborn Screening Follow-up for Severe Combined Immunodeficiency in an Amish Cohort with Cartilage-Hair Hypoplasia. J Clin Immunol. 2020;40(2):321-8.
23. Kwan A, Church JA, Cowan MJ, Agarwal R, Kapoor N, Kohn DB, et al. Newborn screening for severe combined immunodeficiency and T-cell lymphopenia in California: results of the first 2 years. J Allergy Clin Immunol. 2013;132(1):140-50.

24. Strand J, Gul KA, Erichsen HC, Lundman E, Berge MC, Tromborg AK, et al. Second-Tier Next Generation Sequencing Integrated in Nationwide Newborn Screening Provides Rapid Molecular Diagnostics of Severe Combined Immunodeficiency. Front Immunol. 2020;11:1417.
25. Vogel BH, Bonagura V, Weinberg GA, Ballow M, Isabelle J, DiAntonio L, et al. Newborn screening for SCID in New York State: experience from the first two years. J Clin Immunol. 2014;34(3):289-303.
26. Ip W, Gaspar HB, Kleta R, Chanudet E, Bacchelli C, Pitts A, et al. Variable phenotype of severe immunodeficiencies associated with RMRP gene mutations. J Clin Immunol. 2015;35(2):147-57.
27. Kostjukovits S, Klemetti P, Valta H, Martelius T, Notarangelo LD, Seppanen M, et al. Analysis of clinical and immunologic phenotype in a large cohort of children and adults with cartilage-hair hypoplasia. J Allergy Clin Immunol. 2017;140(2):612-4 e5.
28. Berthet F, Le Deist F, Duliege AM, Griscelli C, Fischer A. Clinical consequences and treatment of primary immunodeficiency syndromes characterized by functional T and B lymphocyte anomalies (combined immune deficiency). Pediatrics. 1994;93(2):265-70.
29. Gennery AR, Khawaja K, Veys P, Bredius RG, Notarangelo LD, Mazzolari E, et al. Treatment of CD40 ligand deficiency by hematopoietic stem cell transplantation: a survey of the European experience, 1993-2002. Blood. 2004;103(3):1152-7.
30. Somech R, Lev A, Lee YN, Simon AJ, Barel O, Schiby G, et al. Disruption of Thrombocyte and T Lymphocyte Development by a Mutation in ARPC1B. J Immunol. 2017;199(12):4036-45.

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