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CASE-BASED REVIEW
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A Novel Interleukin 17 Receptor A Mutation in a Child with Chronic Mucocutaneous Candidiasis and Staphylococcal Skin Infections

Nalan Yakıcı[1], Sevil Oskay Halaçlı[2], Çağman Tan[2], Pınar Gür Çetinkaya[2], Halil T. Akar[2], Büşra Çavdarlı[3], Begüm Özbek[2], Deniz Çağdaş[2], İlhan Tezcan[2]

[1]Department of Pediatric Immunology and Allergy, Karadeniz Technical University Faculty of Medicine, Trabzon, Turkey
[2]Department of Pediatric Immunology, Hacettepe University Faculty of Medicine, İhsan Doğramacı Children's Hospital, Ankara, Turkey
[3]Department of Medical Genetics, Ankara City Hospital, Ankara, Turkey

What is already known on this topic?

- *Pathologies in the IL17 pathway are known to cause chronic mucocutaneous candidiasis, eczema and staphylococcal infections. However, pathology may not be detected in basal immunological tests in these patients.*

What this study adds on this topic?

- *Here, we once again draw attention to the fact that patients suffering from the clinical findings mentioned should be investigated for pathologies in the IL17 pathway, even if their baseline immunological tests are normal.*

ABSTRACT

Objective: Chronic mucocutaneous candidiasis leads to persistent or recurrent fungal infections of the nail, skin, oral, and genital mucosa. Impaired interleukin 17-mediated immunity is a cause of chronic mucocutaneous candidiasis. We aimed to show the pathogenicity of a novel interleukin 17 receptor A mutation through functional studies.

Materials and Methods: After next-generation sequencing analysis showed the interleukin 17 receptor A variant, we confirmed the variant by Sanger sequencing and functional validation of the variant by flow cytometry.

Results: We present the case of a 6-year-old male patient who presented with recurrent oral and genital Candida infections and eczema. He had staphylococcal skin lesions, fungal susceptibility, and eczema. The patient carried a novel homozygous nonsense [(c.787C> T) (p.Arg263Ter)] mutation in the interleukin 17 receptor A gene. Sanger sequencing confirmed the variant and revealed the segregation of the variant in the family. We used flow cytometry to detect interleukin 17 receptor A protein expression in peripheral blood mononuclear cells from patients and measured Th17 cell percentage. We observed low interleukin 17 receptor A protein expression in patient peripheral blood mononuclear cells, decreased CD4+ interleukin 17+ cell percentage, and decreased interleukin 17F expression in CD4+ cells compared to healthy controls.

Conclusions: Innate immune defects may lead to chronic recurrent fungal and bacterial infections of the skin, mucosa, and nails. Generally, genetic and functional analysis is needed in addition to basic immunological tests.

Keywords: IL17RA deficiency, chronic mucocutaneous candidiasis, *Candida albicans*

INTRODUCTION

Chronic mucocutaneous candidiasis (CMC) is characterized by persistent and/or recurrent fungal infections involving the nails, skin, and oral and genital mucosa caused by Candida species, mainly *Candida albicans (C. albicans)*.[1] It is an infectious phenotype in patients with inherited or acquired T-cell deficiency.[1-4] Studies explaining the etiology of CMC have shown that Th17 and interleukin 17 (IL17) cells are particularly important in mucocutaneous host defense against Candida species.[5-7]

Isolated CMC is defined as a condition characterized by persistent or recurrent infections of the nails, skin, and oral and genital mucosa, caused typically by *C. albicans* and less often by *Staphylococcus aureus*, without other severe infectious or autoimmune findings.[1]

Corresponding author:
Nalan Yakıcı
✉ nalanyakici@hotmail.com
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The first sporadic cases of CMC were detected in 1967, whereas the first familial cases were reported in the 1970s.[8,9] Subsequent studies identified many inherited genetic defects that can cause isolated or syndromic CMC presentations.[10,11]

To date, 4 genetic etiologies have been identified in patients with chronic mucocutaneous candidiasis diseases (CMCD): AD IL17F, AR IL17 receptor A (RA), AR IL17 receptor C (RC), and AR TRAF3-interacting protein 2 [TRAF3IP2, encoding nuclear factor kappa-beta activator 1 (ACT1)].[10-12] The IL17 family consists of 6 cytokines: IL17A (IL17), IL17B, IL17C, IL17D, IL17E (IL-25), and IL17F. Interleukin-17 cytokines function by forming homo- or heterodimers.[13,14] The IL17R family includes the following 5 receptor subunits: IL17RA, IL17RB, IL17RC, IL17 receptor D (RD), and IL17 receptor E (RE). These receptors form various heterodimers, through which different IL17 cytokines signal in an ACT1-dependent manner, and IL17RA forms the most common subunit of all these receptor complexes.[13]

Interleukin (IL)-17RA deficiency was first reported in a 7-year-old boy who suffered from recurrent CMC resistant to local antifungal therapy. He also developed *S. aureus* skin abscess and folliculitis on the hip, a chronic candidal interdigital intertrigo. He was also treated for recurrent conjunctivitis, acute media otitis, bronchitis, and folliculitis. He developed severe atopic dermatitis and was diagnosed with chronic allergic rhinitis.[10]

Interleukin 17 receptor F deficiency was described in several members of a family suffering from CMC.[10]

Interleukin 17 receptor C was described in 3 patients.[10] Chronic intertrigo, CMC, aphthous stomatitis, and onychomycosis, and pustules on the skin and scalp were important findings in all the patients.[11]

Nuclear factor kappa-beta activator 1 deficiency was reported in 2 siblings[11] who had severe seborrheic dermatitis. Chronic mucocutaneous candidiasis, bilateral staphylococcal blepharitis, folliculitis, cicatricial alopecia, and onychomycosis had developed in the patients. Dental abnormalities, esophageal stricture possibly due to previous esophageal candidiasis, and hypergammaglobulinemia due to recurrent infections were seen in another patient.[15] Patients with ACT1 deficiency suffer from pustular lesions on the scalp resulting in alopecia that scarred over months and years. Pustular lesions may lead to scarring hair loss in certain areas of the body, particularly the lower extremities. These histological findings were suggestive of discoid lupus erythematosus.[16]

This article presents the case of a 6-year-old male patient with a clinical picture of CMC who was found to carry a homozygous [(c.787C> T) (p.Arg263Ter)] mutation in the *IL17RA* gene that has not been reported previously. In addition, we aimed to demonstrate the pathogenicity of this novel mutation through functional studies.

MATERIALS AND METHODS

After genetic analysis, we performed a detailed flow cytometric analysis including IL17RA expression. Written informed consent was obtained from the parents.

Genetic Analysis

Genomic DNA was isolated from the peripheral blood samples of the patient (EasyOne-Qiagen, Hilden, Germany). A total of 266 primary immunodeficiency (PID)-associated genes were sequenced to include exon-intron and splice-site regions, using targeted next-generation sequencing (NGS) on the ION Torrent platform.[17] The sequencing process detected 923 nucleotide changes in the patient, and these variants were analyzed using the Ionreporter 5.10 software (Thermo Fischer). Filtering was performed to identify pathogenic gene mutations among the variations detected by the analysis. Alleles with an allele frequency of <1% were included in the analysis for filtering.

Flow Cytometry

The analysis of peripheral blood lymphocyte populations was performed by 1 laser 3-color flow cytometry (BD Biosciences FACSCalibur, New Jersey, USA), using 100 µL of whole blood stained with 20 µL of the following monoclonal antibodies against lymphocyte subsets fluorescein isothiocyanate (FITC), phycoerythrin (PE) (obtained from Becton Dickinson, BD, USA) ((CD3 (FITC), CD4 (FITC), CD8 (PE), CD16+56 (PE), and CD19 (PE)) and incubated in the dark for 15 minutes at room temperature.

To detect IL17RA expression, peripheral blood mononuclear cells (PBMCs) were separated using the density gradient method. The cells were then washed and stained with anti-IL17RA antibody (BioLegend, New Jersey, USA). To identify CD4+ IL17A+ and CD4+ IL17F Th17 cells, PBMCs were stained with anti-CD4, anti-IL17A, and anti-IL17F antibodies (BD, USA). The stained cells were analyzed using a FACS CANTO II flow cytometer (BD Biosciences).

Following the separation of PBMCs, cells were washed with phosphate-buffered saline (PBS)) and stained with Carboxyfluorescein (CFSE) for 5 minutes to determine lymphocyte proliferation. After washing, the cells were fed with Roswell Park Memorial Institute (RPMI) containing L-glutamine (1%) and penicillin/streptomycin (1%) and incubated in a 37°C incubator with 5% CO_2 for 96 hours. The analysis was performed using a FACS CANTO II flow cytometer (BD Biosciences).

RESULTS

Patient Report

A 3.5-year-old male patient with consanguineous parents (Figure 1A) presented with eczema and recurrent oral and genital Candida infections.

His symptoms started at the age of 8 months, with watery, itchy, and eczematous lesions on the neck, back ear, and inner side of the arms and legs. He had oral Candida plaques and perineal candidiasis. The skin lesions were alleviated by local corticosteroid and oral antifungal therapy, but no improvement in oral Candida plaques or genital fungal infections was observed.

Physical examination revealed oral and genital Candida infections and dry skin. The scalp and nails were not affected. In addition, there was no history of bacterial respiratory tract infections, such as pneumonia, sinusitis, or otitis at the time of diagnosis, and the patient did not have bacterial skin infections, such as skin abscesses or folliculitis.

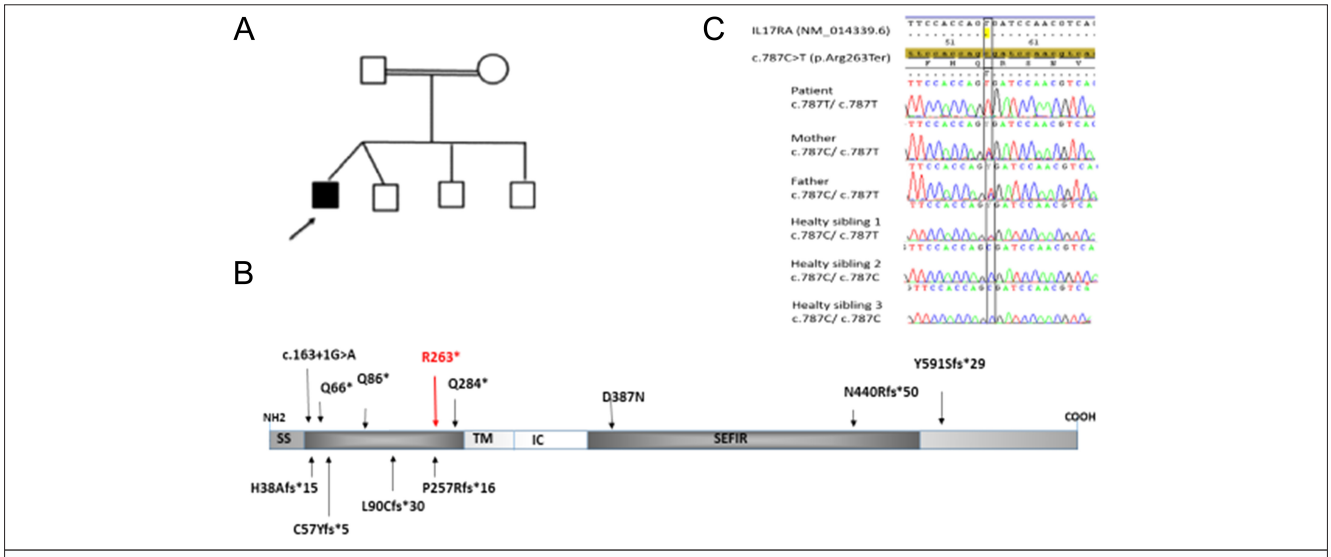

Figure 1. (A) Pedigree (fraternal twins). (B) Schematic diagram of the mutations of the IL17RA protein detected to date, including our patient. Our patient is shown in red. (C) Sanger sequence analysis of *IL-17RA* mutation in patients and family members. While the patient was homozygous for the IL-17RA mutation [(c.787C> T) (p.Arg263Ter)], his parents and one sibling (healthy sibling 1) were heterozygous for the same mutation. No mutation was found in the patient's twin (healthy sibling 3) and the other sibling (healthy sibling 2). *IL17RA*, interleukin 17 receptor A.

Whole blood count, immunoglobulin, lymphocyte subgroups, T and B cell subgroups, and lymphocyte proliferation values were normal (Table 1). Fluconazole prophylaxis was initiated. Oral and genital candida infections resolved after fluconazole treatment.

Table 1. Clinical and Laboratory Features of the Patient

Age (months)	42	72
Complete blood count		
Hemoglobin (g/dL)	12.9	13.9
Leukocyte (/mm³)	8900	5800
Platelet count (/mm³)	283 000	248 000
Absolute neutrophil count (/mm³)	3900	2400
Absolute lymphocyte count (/mm³)	4300	3100
Serum immunoglobulin levels		
IgG (mg/dL)	538 (451-2599)	612 (591-2768)
IgM (mg/dL)	85.4 (33-459)	85 (44-644)
IgA (mg/dL)	63.2 (28-376)	105 (49-437)
IgE (U/mL)	36.2 (15-150)	16 (15-150)
Lymphocyte subgroup (%/count) (/µL)		
CD3	72 (55-83)	68 (56-75)
	3096 (1656-3841)	3944 (991-2997)
CD4	44 (28-47)	43 (25-48)
	1360 (871-2379)	1695 (635-1620)
CD8	28 (16-32)	20 (16-43)
	866 (518- 1433)	788 (293-1221)
CD19	15 (13-31)	16 (11-28)
	464 (421-1397)	631 (249-865)
CD16+56	9 (3-19)	14 (5-21)
	278 (123-785)	552 (128-474)

The patient was followed up for 2.5 years with fluconazole treatment, without symptoms. However, in the last 6 months, purulent bullous lesions developed, especially on the fingertips and sometimes around the mouth (Figure 2). These lesions recurred 5-6 times within 6 months. *Staphylococcus aureus* grew in the microbial samples taken from the lesion.

Mutation in Interleukin 17 RA

We detected a homozygous mutation (c.787C> T (p.Arg263Ter]) in exon 8 of the *IL17RA* gene, which was identified as a mutation occurring in the extracellular (EC) domain of the protein, resulting in a premature stop codon using NGS-PID panel

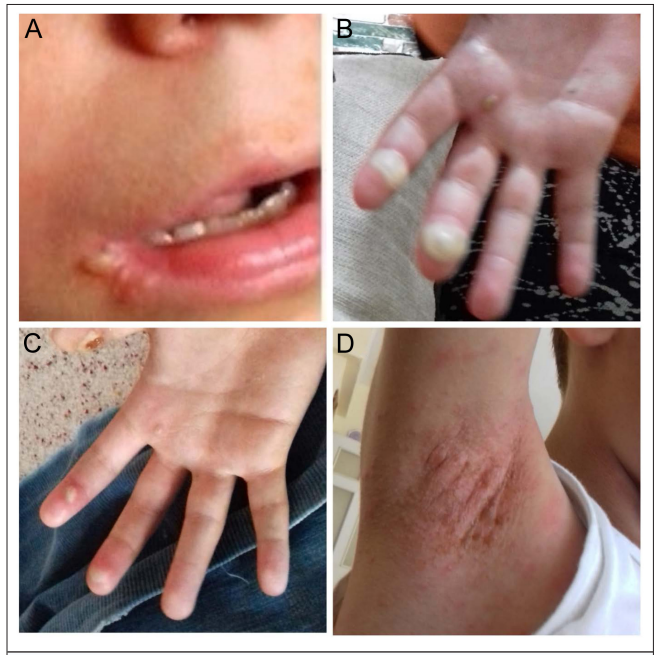

Figure 2. (A) Purulent lesion at the edge of the mouth. (B-C) Purulent lesions on the fingertips. (D) Folliculitis in the axillary area.

(Figure 1B). Next, we confirmed the mutation using Sanger sequencing (Figure 1C). This variant was not identified in the 400 individuals examined for an NGS-PID panel study in the Pediatric Immunology Laboratory of Hacettepe University and 1000 healthy Turkish individuals.

Detection of Interleukin 17 RA Expression and Th17 Cell Percentage

The expression of IL17RA was severely reduced in PBMCs from the patients. Furthermore, we detected a decreased CD4+IL17A+Th17 cell percentage as well as reduced IL17F expression in CD4+T lymphocytes. T-lymphocyte proliferation was evaluated as normal compared with that in healthy control samples (Figure 3).

DISCUSSION

Inherited deficiencies in IL17-associated immunity are known to cause CMC.[1] A direct IL17 signaling pathway disorder is present in diseases associated with the etiology of isolated CMC.[10-12] However, isolated CMC resulting from inborn errors of IL17 cytokines or receptors is relatively rare, and patients with 5 (IL17F),[10] 23 (IL17RA),[10,18,19] 3 (IL17RC),[11] and 7 (TRAF3IP2)[12,15,16] mutations have been reported to date.

The first genetic cause for isolated CMC was reported in 2011. Puel et al[10] identified IL17RA and IL17F deficiencies as 2 genetic etiological causes of isolated CMC.

The first case described was a 7-year-old male patient with recurrent cutaneous candidiasis, which was resistant to local antifungal therapy since the first month of life. He suffered from atopic eczema from the age of 1.5 years.[10] Also when the patient was 5 months old, he developed skin abscesses and folliculitis due to *S. aureus* infection.

In 2 siblings with recurrent mucocutaneous candidiasis, chronic skin infections, respiratory tract infections, and vasculitis, a large chromosomal deletion was detected (X Kell blood group 3) in all regions of the *IL17RA, CECR1* (encoding adenosine deaminase (ADA 2), and *XKR3* genes. Chronic mucocutaneous infections in these patients are due to IL17RA deficiency, but the chronic inflammation may be due to ADA2 deficiency.[19]

Subsequently, the largest case series with autosomal recessive mutations in IL17RA was reported in 21 patients from 12 families, 11 of whom were related, including the first case.[18] In all of these patients, the first episode of CMC occurred in the first 6 months of life, and 14 patients had various staphylococcal skin infections (skin abscesses, folliculitis, furuncles, and crusted pustular lesions on the face and scalp) in the same age. Chronic mucocutaneous candidiasis affects the skin (intertrigo), scalp, mucosal areas (oral thrush, anogenital candidiasis), and nails.

Interleukin 17 receptor A consists of EC, transmembrane (TM), intracellular (IC) (SEFIR similar expression to fibroblast growth factor genes), and IL17R domains.[13] Mutations in the EC domain were observed in this patient (Figure 1B). Including the first case identified, 8 different mutations in the EC domain were detected in 15 of the 21 cases described by Levy et al. Three of these mutations were detected in Turkish patients. Of these 15 cases, 14 had oral mucosal candidiasis, 5 had genital candidiasis, 10 had skin candidiasis, 4 had nail candidiasis, and 6

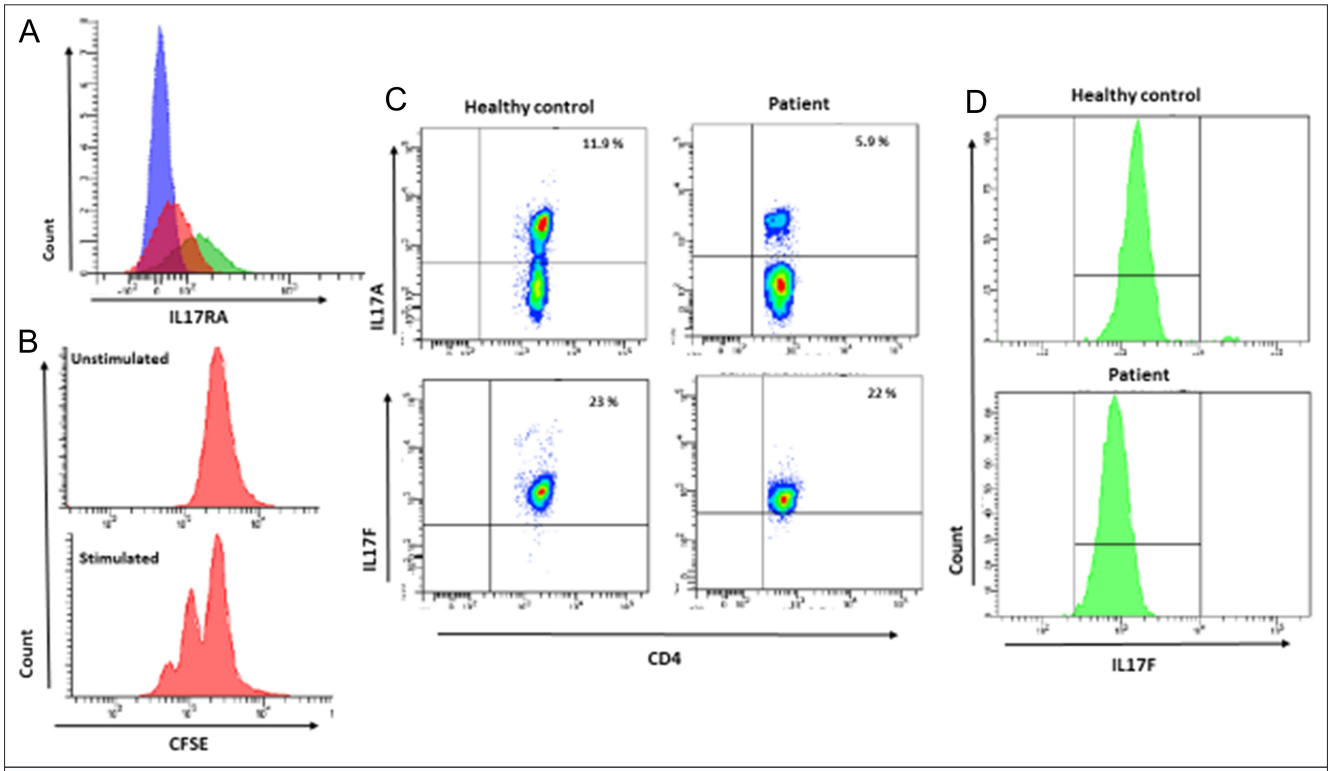

Figure 3. (A) Flow cytometric analysis of IL17RA expression in the patient (isotype control (blue), healthy control (green), patient (red)). (B) Proliferation analysis of the patients' unstimulated (up) and stimulated T cells (below). (C) Analysis of Th17 cells. (D) IL17F expression in CD4+ cells in the healthy control and the patient. IL17F, interleukin 17F; IL17RA, interleukin 17 receptor A.

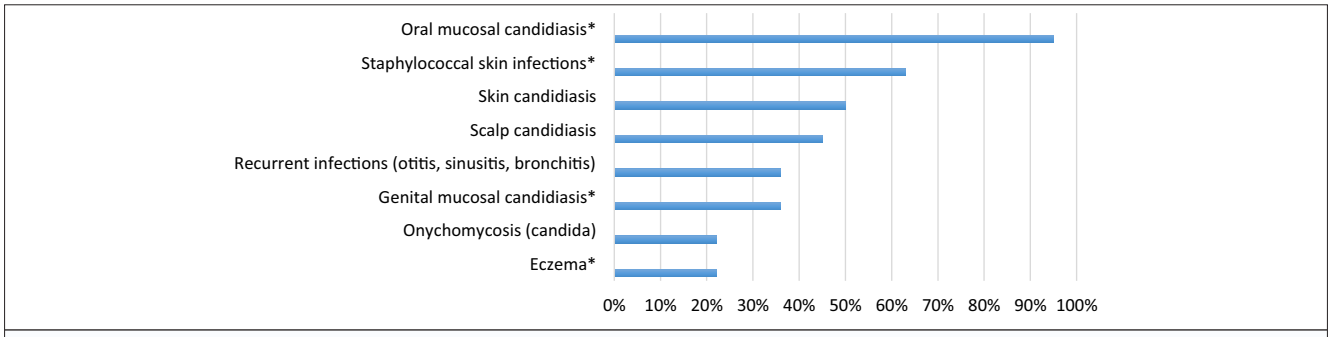

Figure 4. Clinical Presentation of 24 Patients with IL17RA Deficiency (*indicates our patient's clinical presentation)10,18

had scalp candidiasis. Eight patients had staphylococcal skin infections (skin abscesses, folliculitis, and furunculosis). Two patients had eczema. Six patients were treated for upper and lower respiratory tract infections (sinusitis, otitis, bronchitis, and lobar pneumonia), including 1 patient with suspected pulmonary tuberculosis.

Our patient had eczema, thrush, and genital candidiasis, which started at the age of 8 months. The scalp and nails were unaffected by fungal infections. There were no recurrent upper or lower respiratory tract infections. There was no history of any skin disease other than eczema at the time of diagnosis. However, in the last 6 months, purulent bullous lesions due to Staphylococcus have developed, especially on the fingertips. The clinical features of the 24 cases identified thus far, including those of our patient, are shown in Figure 4. We identified a novel homozygous mutation that caused a premature stop codon (Figure 1B). Detection of severely decreased IL17RA protein expression in patients' PBMCs in parallel with decreased CD4+IL17A+ cells and reduced expression of IL17F supported the pathogenicity of the mutation. Interleukin 17 A and IL17F, produced by Th17 cells, bind to IL17RA and activate cellular signaling to initiate an inflammatory response. In the absence and reduced expression of IL17RA, decreased Th17 cell numbers were observed in patients with IL17RA defects, as in our study.

Staphylococcal infections have been reported to date only in IL17RA and ACT1 deficiencies among isolated causes of CMC but have not been reported in patients with IL17F and IL17RC deficiencies.[10-12] Although the mechanism is unclear, it is thought that staphylococcal infections may result from the inability of IL17E.[18] Unlike patients with IL17RC and IL17F deficiency, PBMCs of patients with IL17RA and ACT1 deficiency did not respond to IL17E.[10-12] Interleukin 17 E exerts its function through a heterodimeric structure created by IL17RA and IL17RB receptors.[13] Nuclear factor kappa-beta activator 1 is an essential adapter protein involved in the signaling of all IL17 cytokines.[12] This mechanism may also explain the staphylococcal skin abscess that developed in this patient.

CONCLUSION

One limitation of this study is that we did not measure the IL-17 cytokine levels in tissue culture in the patient compared to normal control. Interleukin 17 was shown to be increased in patients with IL17-RA defect.

In conclusion, molecular genetic studies should be used to provide an early diagnosis in patients with CMC. Patients should be directed to centers where detailed functional immunological studies can be performed.

Availability of Data and MaterialThe data supporting the findings of this study are available from the corresponding author upon reasonable request.

Ethics Committee Approval: This study was approved by the Ethics Committee of Hacettepe University (TSA-2016-9087).

Informed Consent: Written informed consent was obtained from the parents, they signed informed consent to publish patient's data and photos.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – N.Y., D.C., I.T.; Design – N.Y., S.O.H., D.C.; Supervision – D.C., I.T.; Resources – N.Y., P.G.C., H.T.A., S.O.H., B.C., B.O., D.C.; Materials – N.Y., S.O.H., B.C., B.O., D.C.; Data Collection and/or Processing – N.Y., D.C.; Analysis and/or Interpretation – N.Y., D.C., I.T.; Literature Search – N.Y., D.C., I.T., S.O.H.; Writing Manuscript – N.Y., S.O.H., D.C., I.T.; Critical Review – D.C., I.T.

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Declaration of Interests: The authors declare that they have no conflict of interest.

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