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LETTER TO EDITOR

A Novel *TRAF3IP2* Mutation Causing Chronic Mucocutaneous Candidiasis

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Abstract

Inborn errors of the IL-17-mediated signaling have been associated with chronic mucocutaneous candidiasis (CMC). We describe a patient with CMC, atopic dermatitis, enamel dysplasia, and recurrent parotitis harboring a novel compound heterozygous mutation of *TRAF3IP2*, leading to autosomal recessive ACT1 deficiency and deficient IL-17 signaling.

Keywords ACT1 · *TRAF3IP2* · chronic mucocutaneous candidiasis · *Candida* spp. · IL-17

To the Editor,

Patients with chronic mucocutaneous candidiasis (CMC) present recurrent or persistent infections affecting the nails, skin, and oral and genital mucosa caused by *Candida* spp., mainly *Candida albicans* [1]. In the last decade, the genetic and functional mechanisms that underlie this condition have been elucidated. The identification of autosomal dominant (AD) IL-17F and JNK1 deficiencies and of autosomal recessive (AR) IL-17RA, IL-17RC, and ACT1 deficiencies, all impairing IL-17A and IL-17F signaling, in patients with CMC and, for some of them, *S. aureus* mucocutaneous diseases, has demonstrated the crucial role of the IL-17A/F dependent immunity for mucocutaneous protection against *C. albicans* and to a lesser extent *S. aureus*. In parallel, heterozygous gain-of-function (GOF) mutations of *STAT1*, impairing the production of IL-17A and IL-17F, have been identified and up to date described in about half of CMC patients [1, 2].

We describe an adolescent boy with CMC, recurrent parotitis, and dermatitis, who carries compound heterozygous private variants of *TRAF3IP2* encoding ACT1. These results highlight the importance of next-generation sequencing (NGS) in the diagnosis of inborn errors of immunity (IEI).

The patient, a 12-year-old Caucasian male born to non-consanguineous parents, has been presenting with a history of repeated episodes of oral thrush and persistent scalp infection caused by *Candida* spp. since 6-months old (Fig. 1a, b). A diagnosis of CMC was established at the age of 2 years-old and he has been on antifungal prophylaxis since then. Noteworthy, he also presented severe atopic dermatitis (Fig. 1c), seborrhea, and recurrent blepharitis, as well as enamel dysplasia and recurrent parotitis from the age of 5 years.

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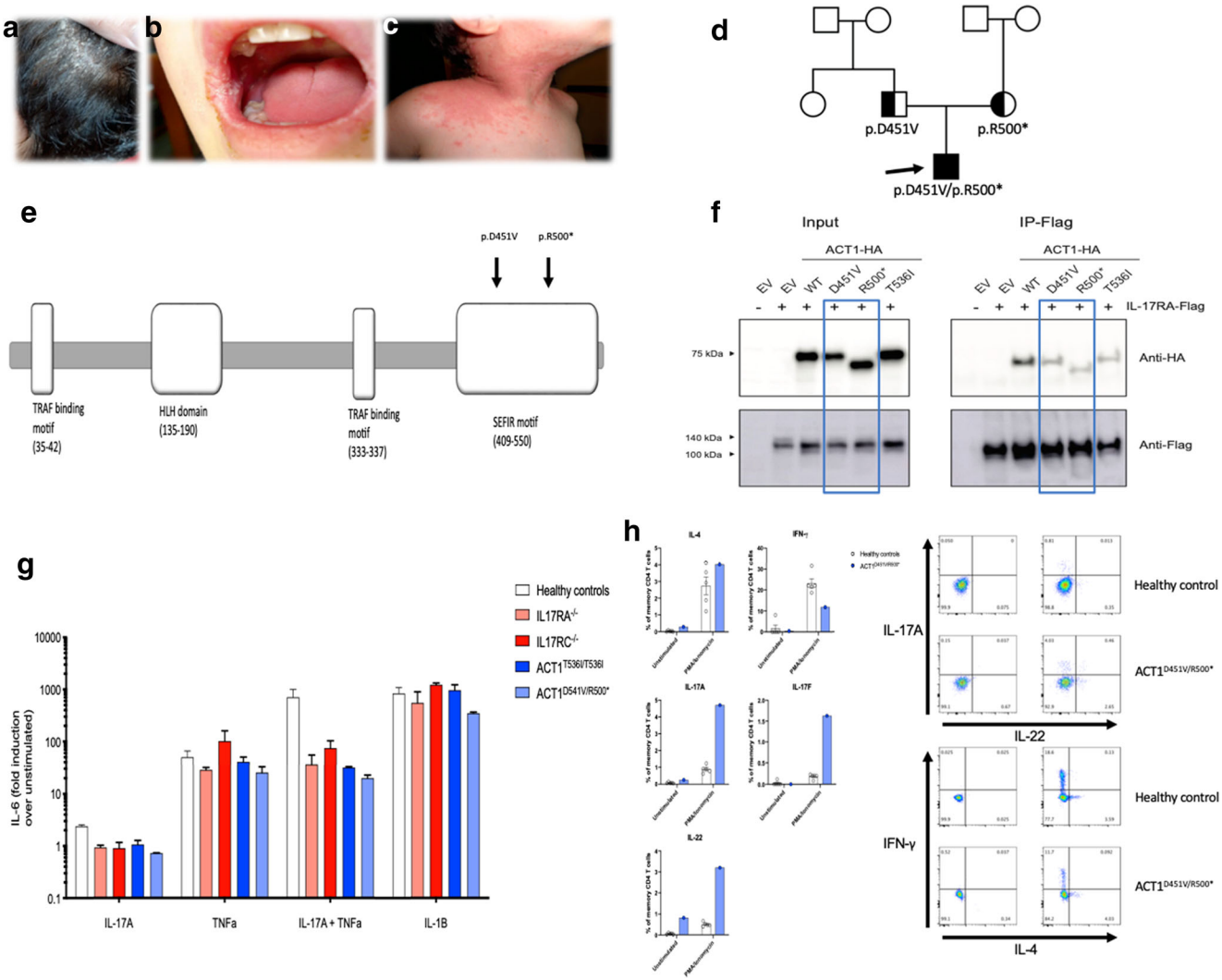

Fig. 1 **a**–**c** Clinical features of the patient with persistent scalp infection (**a**), oral thrush (**b**), and severe atopic dermatitis (**c**). **d** Pedigree of the kindred showing the familial segregation of the alleles. **e** Schematic representation of the ACT1 protein. **f** HEK-293T cells were cotransfected with plasmids containing Flag-tagged IL-17RA, and HA-tagged WT, D451V, R500*, or T536I ACT1. Cell lysates were immunoprecipitated with anti-Flag antibodies. Left panel shows the input and right panel the immunoprecipitation. Immunoblotting analysis performed with anti-HA or anti-Flag specific antibodies. **g** IL-6 production by SV40-fibroblasts from healthy individuals, patients with IL-17RA or IL-17RC deficiency, and the patient under study, after 18 h of stimulation with IL-17A, TNF-α, IL-17A+TNF-α, or IL-1β as measured by ELISA. **h** Cytokine measurement by intracellular flow staining of controls and patient's PBMCs after 5 h of stimulation with PMA/ionomycin and gated on memory CD4+ T cells

There was no family history of sibling deaths or recurrent candidiasis. At the age 10 years, he had normal lymphocyte subsets (including normal number of Th17 cells), immunoglobulin levels, and vaccine responses. Oxidative burst measured by flow cytometry was also normal. He had no signs of auto-immunity (see supplementary data — Table 1). Genetic sequencing of *AIRE*, *STAT1*, and *Dectin 1* cDNA failed to identify any rare variants in these genes. At the age of 12 years, a NGS panel of CMC associated genes (see supplementary data — Table 2) was performed and revealed the presence of private compound heterozygous variants of *TRAF3IP2*: a nonsense variant, c.1498C>T/p.R500*, inherited from the mother, and a missense variant, c.1352A>T/p.D451V inherited from the father (Fig. 1d). The R500* variant was classified as pathogenic (null variant, absent from healthy controls in Exac, and multiple lines of computational evidence support a deleterious effect: Mutation Taster — Disease Causing automatic, DANN — 0.99; CADD score of 38 (MSC, 3.313)). The D451V variant was classified as a variant of uncertain significance (missense variant, absent from healthy controls in Exac, and most bio-informatics tools support its deleterious effect: Mutation Taster — Disease Causing, DANN — 0.99; CADD 28.7, Polyphen B probably damaging 0.988). Interestingly, both variants are located in

the SEF/IL-17 receptor (SEFIR) domain (residues 409-550), which is critical for adequate interaction between ACT1 and the IL-17Rs (Fig. 1e).

In order to analyze the impact of these variants on ACT1 expression and its interaction with IL-17RA, HEK-293T cells were transfected with a plasmid encoding IL-17RA-Flag tagged together with a plasmid encoding wild type or mutant HA-tagged ACT1 variants (Supplementary Material). The previously reported CMC-causing T536I ACT1 variant [3] was used as an internal control. After 24 h, cells were lysed and an immunoprecipitation using Flag-tagged IL-17RA was performed. Total input showed that all variants are expressed at levels comparable to WT ACT1; however, as expected, p.R500* is associated with a lower MW protein (Fig. 1f, left panel). Moreover, both variants (p.R500* and p.D451V) show reduced binding to IL-17RA, as shown by a reduced amount of ACT1 being pulled down by IL-17RA, as compared to wild type ACT1, at levels similar to the T536I variant (Fig. 1f, right panel).

We then tested ACT1$^{D451V/R500*}$ patient's SV40-immortalized fibroblasts responses to IL-17A and TNF-α, separately or in combination, and IL-1β, together with that of fibroblasts from healthy individuals or patients with AR complete IL-17RA or IL-17RC deficiency [1]. Whereas the 24 h of stimulation with IL-17A alone induced a weak production of IL-6, even in controls' fibroblasts, IL-6 production was strongly enhanced in control fibroblasts in response to TNF-α and even more in response to TNF-α+IL-17A. In contrast, patient's fibroblasts, similarly to IL-17RA- or IL-17RC-deficient fibroblasts, when stimulated with TNF-α+IL-17A did not show any further enhancement in the presence of IL-17A as compared to the stimulation with TNF-α alone. All controls and patients' fibroblasts showed normal responses to IL-1β (Fig. 1g).

Finally, we measured the proportions of IL-17A-, IL-17F-, and IL-22-producing memory CD4$^+$ cells among peripheral blood mononuclear cells (PBMCs), after 5 h of stimulation with PMA/ionomycin. As previously reported [3], PBMCs from the ACT1$^{D451V/R500*}$ patient showed enhanced proportions of IL-17A-, IL-17F-, and IL-22-producing memory CD4$^+$ cells, as compared to healthy controls tested in parallel, whereas proportions of IL-4 and IFN-γ-producing memory CD4$^+$ cells were comparable or slightly reduced, respectively, as compared to healthy controls (Fig. 1h).

IL-17A (also known as IL-17) and its closest related member of the IL-17 family, IL-17F, form homo- or heterodimers (IL-17A/F). They signal through a heterodimeric receptor complex composed of IL-17RA and IL-17RC, which belongs to the SEFIR protein family [4]. IL-17A is a key signature cytokine of Th17 cells and has been suggested to play a critical role in the human pathogenesis of autoimmune or inflammatory diseases [5]. The role of IL-17A and IL-17F in protecting against mucocutaneous *C. albicans* infections has been demonstrated following the identification of AD IL-17F or JNK1 deficiency or AR IL-17RA, IL-17RC, or ACT1 deficiencies, in patients with CMC [1, 6]. AR ACT1 deficiency was first reported in 2013, with the identification of a private homozygous mutation, T536I, in the SEFIR domain of ACT1 [3]. ACT1 is an adaptor molecule that interacts with multiple partners, including members of the IL-17R family [5]. Upon stimulation, ACT1 is recruited to IL-17RA and IL-17RC by homotypic dimerization of two SEFIR domains, playing an essential role in signaling downstream of IL-17A and IL-17F and in mucocutaneous protection against *Candida* or *S. aureus* infections [1, 6]. In their original description, Boisson and colleagues described two siblings with CMC and blepharitis since early childhood, transient atopic dermatitis, and seborrhea [3]. Similarly, our patient suffered from CMC, atopic dermatitis, and blepharitis during early childhood. Dental abnormalities have also been reported [7], alike in our patient. Our patient presented recurrent parotitis, which has never been reported in ACT1-deficient patients. It probably results from impaired IL-17 immunity in the epithelial defense against *Staphylococcus aureus*. Alternatively, it may result from an autoimmune mechanism, as *Act1*-deficient mice developed systemic autoimmune disease with histological and serological features of human Sjogren's syndrome [8]. More recently, two patients presenting with CMC that harbor biallelic nonsense mutations of *TRAF3IP2* were described [9]. The boy, like our index case, also suffered from scalp and tooth abnormalities. Very recently, a putative role for TRAF3IP2 in the etiology of discoid lupus erythematosus was described in four siblings of a consanguineous Lebanese family, in which the hair follicle differentiation pathway was drastically suppressed whereas cytokine and inflammation responses were significantly upregulated [10].

In conclusion, we describe the 10th patient with AR ACT1 deficiency, with compound heterozygous (R500*/D451V) variants of *TRAF3IP2*. The variants, located in the SEFIR domain of ACT1, lead to an abnormal interaction of ACT1 with IL-17Rs and abolished signaling downstream of IL-17A and probably IL-17F, causing impaired anti-*Candida* immunity. Further studies are warranted to clarify the pathogenesis of recurrent parotitis noted in the index case.

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Author Contribution FM, medical doctor of the patient, wrote the paper. SJP reviewed the paper. JF designed the research study and wrote the paper. AIC reviewed the paper. CM designed the research study. JLC designed the research study. WTL designed the research study. AP designed the research study and wrote the paper. JFN, medical doctor of the patient, designed the research study and wrote the paper.

Availability of Data and Materials Not applicable for that section.

Declarations

Conflict of Interest The authors declare no competing interests.

Ethical Approval The case report was performed with accordance of the ethical standards.

Consent to Participate Freely given and informed consent to participate in the case report was obtained from patient parents.

Consent to Publish Patient parents have consented to the submission of the case report to the journal.

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