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ORIGINAL ARTICLE

Chronic Mucocutaneous Candidiasis in an Adolescent Boy Due to a Novel Mutation in TRAF3IP2

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Abstract

Background IL-17-mediated signaling is crucial in defense against fungi and bacteria. Defective Th17 immunity has been implicated in a group of disorders called chronic mucocutaneous candidiasis (CMC). TRAF3IP2 is an adaptor protein involved in downstream signaling for IL-17 receptors.

Case An 18-year-old boy, product of consanguineous wedlock, presented with history of repeated episodes of oral thrush and recurrent pneumonia from first year of life. On examination, he was wasted and had oral thrush and abnormal dentition; grade 2 clubbing and respiratory system examination revealed coarse crepitations. On evaluation, HIV status was negative and basic immunological screen was unrewarding. Genetic testing by next-generation sequencing showed a novel homozygous mutation in *TRAF3IP2* gene not reported to date. The defect is likely to cause ACT1 deficiency. He was started on antibiotic and antifungal prophylaxis and remains well on follow-up.

Conclusion We describe an adolescent boy with recurrent oral candidiasis and bronchiectasis due to a novel mutation in *TRAF3IP2* gene, not reported to date. This is also the only second report of CMC due to ACT1 deficiency.

Keywords Mucocutaneous candidiasis · TRAF3IP2 · ACT1 · immune deficiency

Introduction

Chronic mucocutaneous candidiasis (CMC) comprises a group of heterogeneous disorders characterized by recurrent or persistent candida infections affecting nails, skin, and mucus membranes [1]. Patients with CMC are not only predisposed to candidiasis but also suffer from recurrent staphylococcal infections [1, 2]. Diseases known to present with CMC include hyper-IgE syndrome (due to STAT3 defect), STAT1 gain of function defect [3], mutations in *IL12B* and *IL12RB1* (known to cause Mendelian susceptibility to mycobacterial disease), mutations in *IL-17A* and *IL-17F* [4], CARD9 deficiency, mutations in autoimmune regulator gene (AIRE) causing autoimmune polyendocrinopathy syndrome-1 (APS-1), and autoantibodies against IL-17 [5]. Reduced number of Th17 or defective IL-17 signaling noted in these diseases underlies the pathogenesis of CMC, highlighting the fact that IL-17 is a key cytokine for defense against candida [2, 6]. Studies performed in the last decade have confirmed the role of IL-17A and IL-17F as key players in mucocutaneous immunity to *Candida albicans*, and, to a lesser extent, *Staphylococcus aureus* [4].

TRAF3IP2, also referred to as ACT1, is an adaptor protein in the downstream signaling of IL-17 receptors and plays a pivotal role in normal immune defense against Candida infections [7]. We describe an 18-year-old boy with CMC and bronchiectasis caused due to a novel mutation in the *TRAF3IP2* gene. Written informed consent has been obtained from the patient and the family.

Case

An 18-year-old Indian boy, born to third-degree consanguineous parents presented with fever, cough, and tachypnea for

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the past 15 days. He had cough with copious amount of sputum production. There was no history of hemoptysis. He also complained of whitish lesions in the mouth with difficulty in swallowing solid foods, though he was comfortable while ingesting liquids. He had been unwell from the age of one and had been hospitalized on several occasions in the past. He had repeated episodes of pneumonia from first year of life, and records confirmed he had developed clubbing (grade 2) by the age of five. Most of these episodes were treated with intravenous antimicrobials elsewhere, and he had required oxygen therapy on three occasions so far. Parents also reported recurrent episodes of whitish lesions in the oral cavity from the age of one. He had been diagnosed to have recurrent oral candidiasis and advised fluconazole prophylaxis; however, he had been irregular on therapy. There was no family history of sibling deaths or recurrent candidiasis in any of the family members (Pedigree—Fig. 1b).

On examination, he was emaciated and looked pale. His weight was 30 kg, height 160 cm, BMI 11.75 kg/cm^2 (< 3SD, suggestive of severe thinness). He had grade 2 clubbing in all the fingers and was noted to have angular cheilitis, bald tongue, and oral thrush. Teeth were abnormal in shape and some incisors appeared peg-shaped (Fig. 1a). Respiratory examination revealed bilateral coarse crepitations. Other systems were normal.

Investigations revealed HB—120 g/L, TC—10.3×10^9/L, and PC—380×10^9/L. Renal and liver functions were within normal limits. HIV ELISA was negative. Chest radiograph showed evidence of bronchiectasis affecting lower zones in both the lungs. Blood culture was sterile. Sputum examination showed few gram-positive cocci; however, bacterial and fungal cultures were sterile. Sputum for acid-fast bacilli smear (modified ZN stain) and tuberculin skin test were negative. Barium swallow revealed long segment stricture at level of D4 measuring 3–3.5 cm long with a caliber of 2–3 mm, with dilatation of the esophagus proximal to stricture. Esophagogastroduodenoscopy (EGD) confirmed these findings; however, there was no evidence of esophageal candidiasis at this point. Under fluoroscopy, the stricture was dilated and he was advised for follow-up dilatation.

Immunological workup revealed hypergammaglobulinemia and normal lymphocyte subsets (Table 1). Flow cytometry was performed to enumerate Th17 cells after PMA/Ionomycin (10 ng/mL/1 μg/mL) stimulation in whole blood diluted in complete RPMI1640 medium, of patient as well as control, in presence of 10 μg/mL Brefeldin. Th17 cells were normal in the index case (Table 1, Fig. 1c).

He was further evaluated, and genetic testing was carried out by next-generation sequencing (NGS), using TruSight One panel (Illumina, San Diego, CA, USA) that includes coding exons and flanking intronic sequences > 4600 genes associated with inherited Mendelian diseases [8]. This included 196 genes associated with immunological disorders. The list of these 196 genes are provided as online supplementary information.

NGS analysis revealed a homozygous mutation in exon 3 in the transcript *NM_147686.3* of *TRAF3IP2* gene that results in substitution of arginine with a stop codon at position 283 of the protein (*p.Arg283**, *c.847C>T)*. No other clinically significant variant was detected in other genes associated with immunological conditions. There was no other clinically significant homozygosity identified in the 4620 genes tested on the clinical exome. Carrier status was confirmed in both the parents (Fig. 2a). He was diagnosed to have CMC due to *TRAF3IP2* mutation and was empirically treated with broad spectrum antibiotics and injectable fluconazole. He became afebrile, cough was passive, and oral thrush resolved over one week of hospital stay. He was discharged on cotrimoxazole and itraconazole prophylaxis. At 1.5 years of

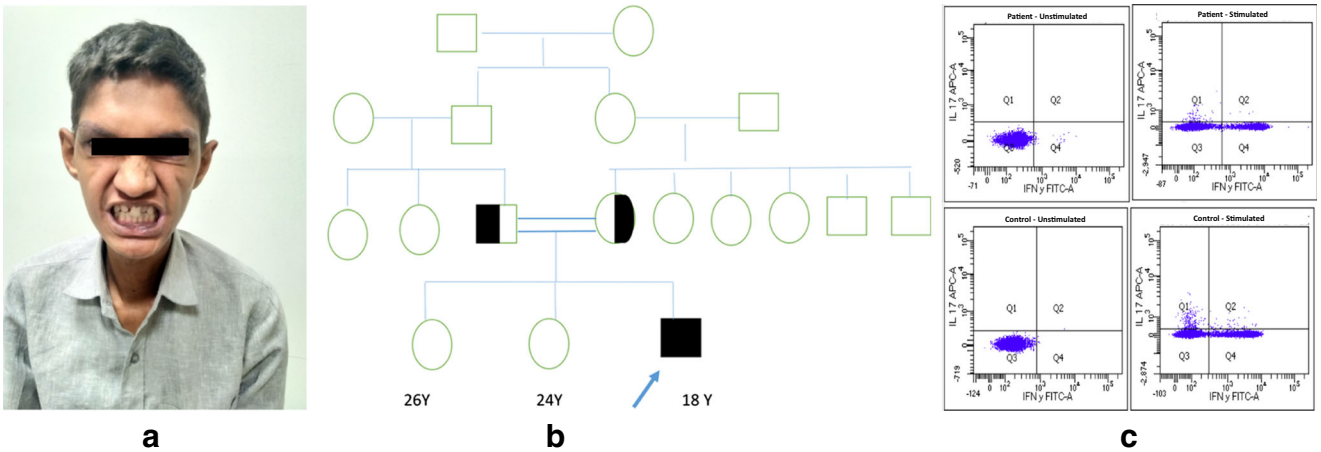

Fig. 1 **a** Abnormal dentition in the boy with CMC. **b** Pedigree: Proband has been highlighted. Genetic tests were carried out in the proband and the parents. **c** Th17 assay: patient—CD4+ IL-17+ cells = 0.6%, CD4+ IFN-Y+ cells = 25.1%; control—CD4+ IL-17+ cells = 0.7%, CD4+ IFN-Y+ cells = 40.2%. Th17% was normal in the index case

Table 1 Immunological investigations in the boy with CMC

Age	2 years	9 years	18 years
IgE (IU/mL)	540	278	225 (< 60 IU/mL)
IgG (mg/dL)	–	2820	3230 (639–1349)
IgA (mg/dL)	–	297	573 (70–312)
IgM (mg/dL)	–	235	300 (56–352)
CD3	–	–	1540 cells/mm^3 (700–2100)
CD19	–	–	556 cells/mm^3 (100–500)
CD56	–	–	553 cells/mm^3 (90–600)
NBT	–	–	Normal
Complement			
C3	113		
C4	41		
Th17 cells	–	–	0.6% (control 0.7%)

NBT nitro blue tetrazolium test)

follow-up, he remains well with no recurrence of oral thrush or pneumonia and has gained six kgs of weight and three cms of height.

Discussion

Chronic mucocutaneous candidiasis (CMC) is characterized with recurrent infections of the skin, nails, and mucosa with Candida, especially *Candida albicans*. Th17 cells are the most important source of IL-17, a cytokine crucial for defense against Candida. Defects associated with reduced number of Th17 or abnormal IL-17 signaling have been associated with CMC [1]. IL-17 cytokines act on their corresponding receptors and activate downstream pathways that include NFKB (nuclear factor kappa-light-chain-enhancer of activated B cells), MAPK (mitogen-activated protein kinases), and C/EBPs (CCAAT/enhancer-binding protein), resulting in the production of anti-microbial peptides [5]. The TRAF3IP2 adaptor protein is the common mediator during signaling for all IL-17 cytokines and is pivotal for antibacterial responses [7]. Germline pathogenic variations in the *TRAF3IP2* gene have been shown to be associated with familial candidiasis 8 (CANDF8) and susceptibility to psoriasis [9].

Gu and colleagues have reported TRAF3IP2 to be a client protein of the molecular chaperone, Hsp90. N-Terminus of TRAF3IP2 is necessary for Hsp90 interaction. Deletions and mutations in the N-terminus would impact binding to Hsp90 impairing IL-17-mediated signaling [10].

The index case had a homozygous nonsense mutation in *TRAF3IP2* (p.Arg283*), which was predicted to cause premature termination of the protein. The truncated protein is predicted to have a length of 282 amino acids (aa) as opposed to the original length of 565 aa. The resultant protein is likely to lack a functional SEFIR domain (residues 409–550) of the protein that is responsible for homotypic interaction with IL-

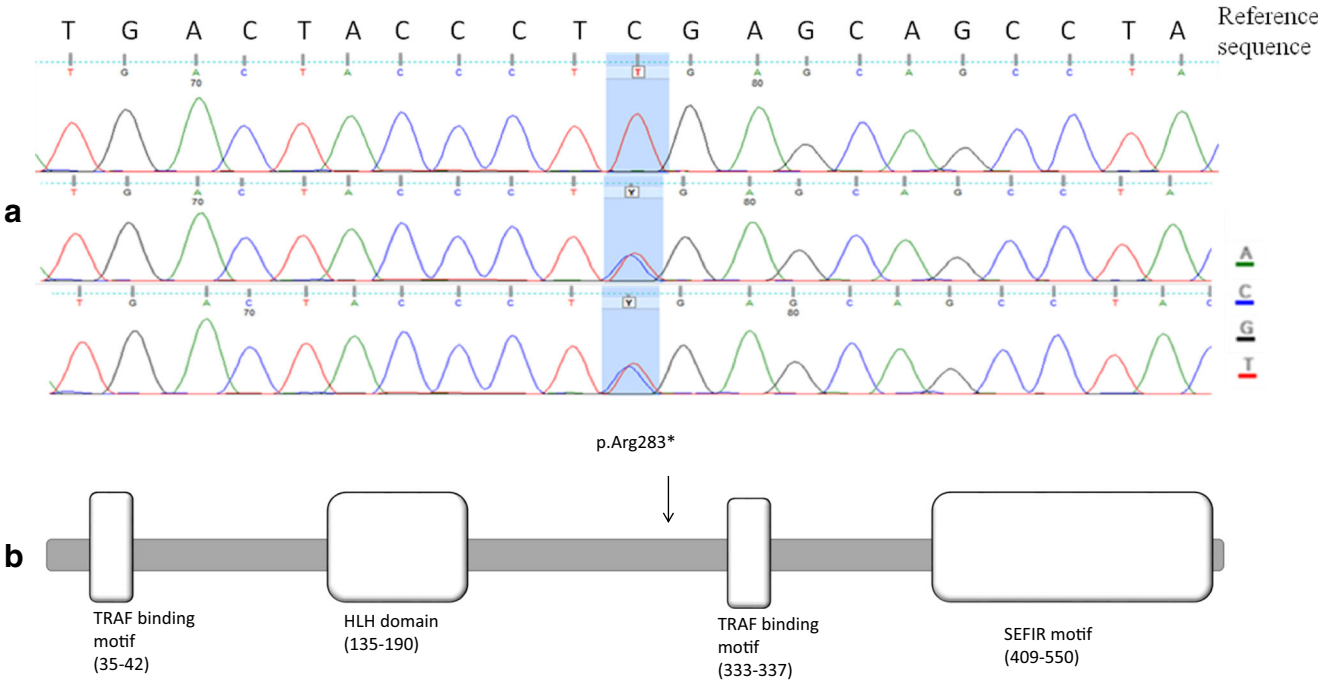

Fig. 2 **a** Electropherogram showing homozygous change in the proband and parents as heterozygous carriers. **b** Schematic representation of TRAF3IP2 domains highlighting the TRAF binding motifs, HLH domain, and SEFIR motif, depicting the identified mutation p.Arg283* with respect to the domains

17R [7]; this will likely result in loss-of-function. Moreover, due to introduction of a premature stop codon, this aberrant transcript will likely be targeted by nonsense mediated mRNA decay (NMD) mechanism [11]. Figure 2b shows a schematic representation of TRAF3IP2 domains [12] depicting the identified mutation p.Arg283* with respect to the domains. A study on a mouse model with a truncating mutation (p.Gln214*) in *TRAF3IP2* showed that the mouse lacking functional *Traf3ip2* protein had high levels of serum IgE and an atopic dermatitis (AD)–like skin disease. It was suggested that *Traf3ip2* causes skin inflammation through the IL-17-mediated pathway and causes hyper-IgE-emia through the CD40- and B cell–activating factor-mediated pathway [13].

Boisson and colleagues have previously reported a different biallelic mutation in *TRAF3IP2* in two siblings who had CMC [14]. These patients (30-year male and 28-year female) had recurrent oral candidiasis and blepharitis due to *Staphylococcus aureus* infection since early childhood. They were noted to have transient atopic dermatitis and seborrhea during early childhood. The mutations affected the SEFIR domain of *TRAF3IP2*. Index child neither reported blepharitis nor atopic dermatitis, though he had recurrent pneumonia since early childhood. We could not identify the pathogen that caused pneumonia in the index case. We noted hypergammaglobulinemia in our patient, not reported in the previous cases. This was likely a result of recurrent infections. Esophageal stricture was probably a sequelae of previous episodes of esophageal candidiasis. Dental abnormalities, esophageal stricture, and bronchiectasis have not been previously reported. Our case had a nonsense mutation in *TRAF3IP2*, while the cases reported by Boisson et al. had missense mutation [14]. As the mutation in the index case would result in loss of SEFIR domain of the protein, we predict this would result in failure of homotypic interaction with IL-17R; however, no functional studies to prove this aspect could be carried out in our patient. We could not perform a Western blot assay to study the ACT1 protein expression in the index case.

To the best of our knowledge, this is only the second case report in the world literature of a mutation in the gene *TRAF3IP2* causing CMC. In addition, our case had a novel mutation not reported to date. Further studies are warranted to shed more light on the pathogenesis of dental abnormalities and esophageal stricture noted in the index case.

Conclusion

Homozygous nonsense mutation (p.Arg283*) in *TRAF3IP2* gene is linked to CMC.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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