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Novel STAT1 gain-of-function mutation and suppurative infections

To the Editor,
Chronic mucocutaneous candidiasis (CMCC) is a heterogeneous group of disorders characterized by non-invasive persistent Candida species infections of the skin, nails, and mucous membranes. Heterozygous dominant gain-of-function (GOF) mutations in signal transducer and activator of transcription 1 (STAT1) have been described as causing impaired STAT1 dephosphorylation, diminished IL-17-producing T-cell numbers, and CMCC (1, 2). Here, we report on the case of a 17-year-old boy who presented to our Department for CMCC. He was born preterm (36 weeks) to healthy non-consanguineous parents from Italy, by a pregnancy complicated by threatened miscarriage and gestosis. Since childhood, he suffered from undocumented dermatologic alterations and, at 7 years of age, he was diagnosed as affected with mucocutaneous candidiasis. At 8 years of age, he suffered from a severe varicella infection, and since 11 years of age, the patient experienced recurrent herpetic infections of the genitals and limbs. Since the same period, he also suffered from recurrent suppurative eyelid infections (Fig. 1a) and cutaneous abscesses, unusual in this immunodeficiency, which developed on an otherwise healthy skin. The patient only experienced cutaneous abscess formation, while lymph nodes and inner organs were never involved. At 10 years of age, the patient presented with a prolonged (20 days) and severe gastroenteritis, which eventually led to severe dehydration. Familial history revealed no members with relevant fungal infectious diseases or immunodeficiencies. At the first evaluation, the patient showed oral thrush, onychomycosis (Fig. 1b), suppurative eyelid infection (Fig. 1a), furunculosis, and periodontitis. Cultures from the oral lesions, the nails, and the esophageal mucosa grew Candida albicans, sensitive to Azoles. Esophageal biopsy revealed the presence of fungal hyphae and chronic inflammatory infiltrate. Given the high susceptibility to Candida infection, a daily prophylactic treatment with fluconazole was started with a dramatic decrease in frequency and severity of fungal infections. Full-length sequencing of STAT1 genomic DNA identified a T387A STAT1 heterozygous mutation in the DNA-binding domain (DBD; Fig. 1c). This mutation has not been previously reported (3). None of the parents carried the mutation (Fig. 1d). To evaluate STAT1 phosphorylation, patient whole blood sample was stimulated with IFN-α (40,000 U/ml) or IFN-γ (1000 U/ml) and analyzed by flow cytometry. Both stimuli resulted in increased STAT1 phosphorylation in the patient $CD3^+$ T cells and $CD14^+$ monocytes, respectively, compared with control values (Fig. 1e). Routine laboratory evaluation revealed a normal or low–normal lymphocyte count and a normal T- and B-lymphocyte enumeration. The proliferative response to common mitogens (phytohaemagglutinin, PMA plus ionomycin, CD3 cross-linking) was normal. Total Ig and Ig subclasses levels and response to protein vaccines were normal. IgE levels were persistently elevated (684 kU/l). The study of the B-cell compartment revealed a number of $CD19^+$ cells within the normal range. The patient showed a normal representation of transitional ($CD3^-$ $CD19^+$ $CD24^+$ CD38hiCD27$^-$; 8.2%),

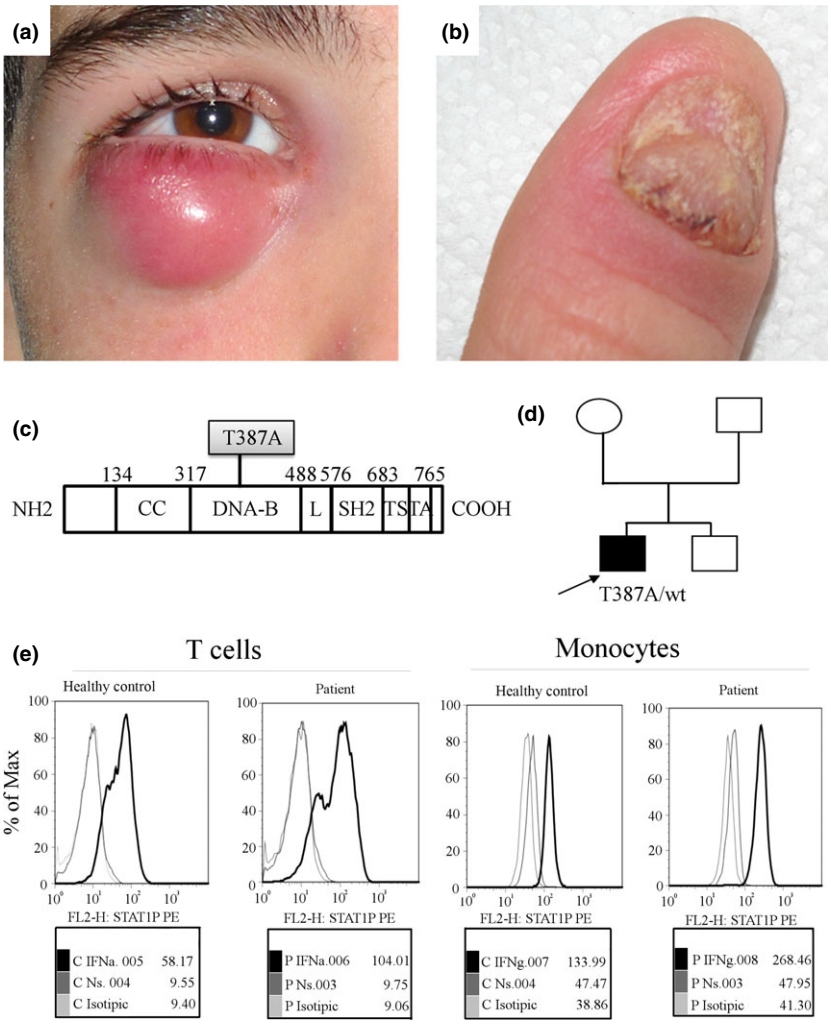

Figure 1 Clinical features. (a) Suppurative eyelid infection. (b) Onychomycosis. (c) Genomic sequence analysis of *STAT1* gene showing a T387A heterozygous mutation in the DNA-binding domain (DBD). (d) Family pedigree. The proband is indicated with an arrow. (e) Patient whole blood sample stimulated with INF-α (40,000 U/ml) or IFN-γ (1000 U/ml) and analyzed by flow cytometry. Both stimuli result in increased STAT1 phosphorylation in the patient $CD3^+$ T cells and $CD14^+$ monocytes, respectively, compared with control values.

mature ($CD3^-$ $CD19^+$ $CD24^-$ CD38dim/loCD27$^-$; 79.8%), and memory ($CD3^-$ $CD19^+$ $CD24^+$ IgM^+ $CD27^+$; 12%) B-cell subsets. However, memory B cells mostly included IgM and only a few cells were switched memory B cells (88% and 12% of the memory B cells, respectively). The function of B cells was studied *in vitro* by evaluating the response to the Toll-like receptor 9 ligand CpG. B cells from the patient carrying the *STAT1* mutation adequately proliferated in response to CpG, and $CD27^{bright}$ terminally differentiated plasma cells normally developed (Fig. S1). Accordingly, adequate levels of IgG and IgM were detected in the supernatants, even though only small amounts of IgA were secreted in the patient, differently from the control. The study of the T-cell compartment revealed normal representation of CD4 and CD8 naïve and memory T cells (CD4: 18.3% and 16.2%; CD8: 21.2% and 8.7%, respectively). We then evaluated the percentage of $CD4^+$ $IL\text{-}17A^+$ and $CD4^+$ $IFN\text{-}\gamma^+$ cells following PMA plus ionomycin stimulation for 6 h, to evaluate TH17 and TH1 development. The patient showed a lower number of $CD4^+$ $IL\text{-}17A^+$ cells than controls (0.25% vs. 1.66%) and increased percentage of $CD4^+$ $IFN\text{-}\gamma^+$ cells (34.15% vs. 20.70%; Fig. 2a,b). We also studied TH17 *in vitro* differentiation and found a reduced (2.97% vs. 6.59%), but not abolished TH17 development in the patient (Fig. 2c). Finally, we studied the transcription levels of some STAT1 target genes (CXCL9, CXCL10, CCL5, and ICAM-1). The levels of CXCL9, CXCL10, CCL5, and ICAM-1 were higher than in the control either in unstimulated PBMC or following IFN-γ stimulation (Fig. 2e). The patient also had increased surface expression on unstimulated monocytes of MHC class II, whose transcription is under STAT1 control (Fig. 2d).

In this study, we reported on a patient with CMCC, recurrent herpetic infections, and suppurative eyelid infections carrying a *de novo* heterozygous GOF mutation in exon 14 (p.T387A) of STAT1 in the DBD. This mutation has not been previously reported (3, 4). As previously described, the underlying pathogenic mechanism involves STAT1 gain of function due to impaired STAT1 dephosphorylation. Consistently with previous reports, laboratory evaluation revealed persistently elevated IgE levels (684 kU/l), normal

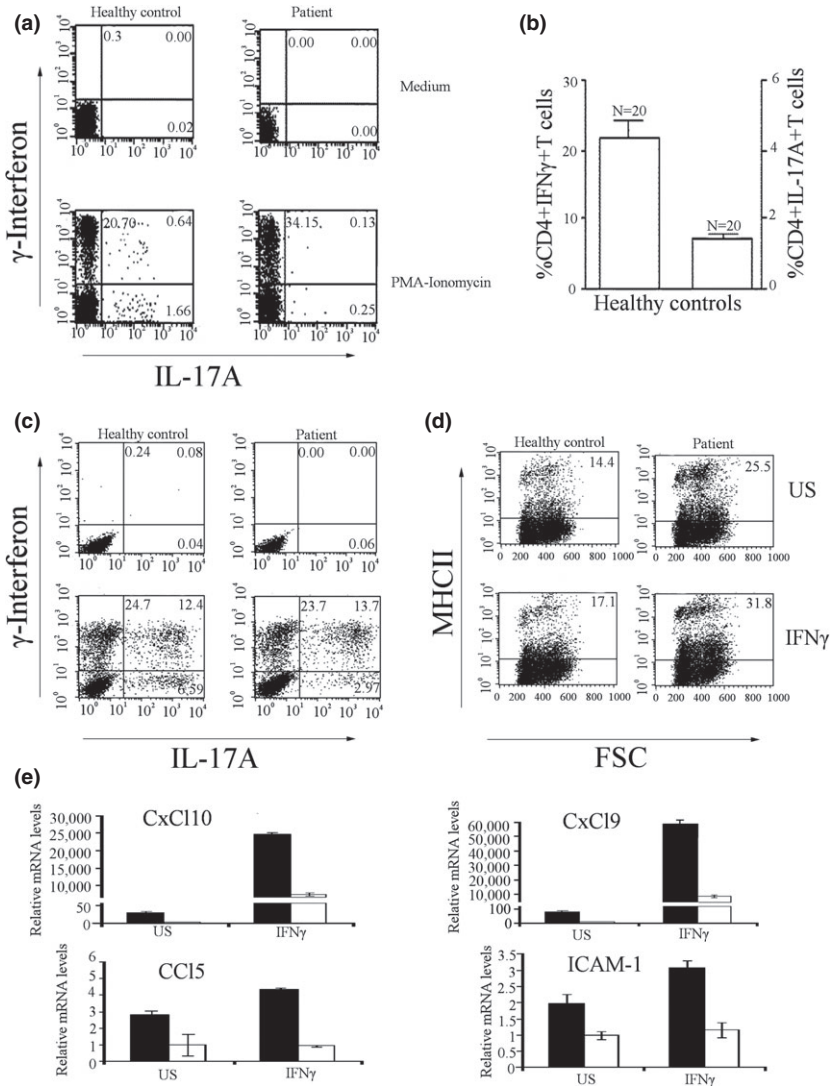

Figure 2 *STAT1* GOF mutation impairs TH17 development and increases the expression of STAT1-regulated genes. (a) Percentage of $CD4^+$ IL-$17A^+$ and $CD4^+$ IFN-γ^+ cells following PMA plus ionomycin stimulation for 6 h. The patient shows a lower number of $CD4^+$ IL-$17A^+$ cells than the control (0.25% vs. 1.66%) and increased percentage of $CD4^+$ IFN-γ^+ cells (34.15% vs. 20.70%). (b) Percentage of $CD4^+$ IFN-γ^+ and $CD4^+$ IL-$17A^+$ cells in 20 healthy controls (values expressed as mean ± SD). (c) $CD4^+$ IL-$17A^+$ and $CD4^+$ IFN-γ^+ cell development after stimulation of $CD4^+$ cells, separated by positive selection using human CD4 microbeads, with anti-CD28, anti-CD3 X-L, IL-6, IL-1β, TGF-β1, IL-23 for 6 days in the patient and a healthy control. After 6 days, cells were split and cultured for further 6 days with the addition of IL-2. The patient shows a reduced (2.97% vs. 6.59%), but not abolished $CD4^+$ IL-$17A^+$ development. $CD4^+$ IFN-γ^+ development is comparable in the patient and control (23.7% vs. 24.7%). (d) MHC class II surface expression on unstimulated monocytes or after stimulation with IFN-γ. The patient shows increased MHC class II surface expression on either resting cells and after stimulation with IFN-γ as compared to the healthy control. (e) Real-time PCR analysis of the mRNA extracted from the patient PBMCs showing higher levels of CXCL9, CXCL10, CCL5, and ICAM-1 than in the control either in unstimulated PBMC or following IFN-γ stimulation.

to low–normal lymphocyte cell counts, and reduced levels of switched memory B cells (5).

STAT1 GOF mutations are considered responsible for very complex and variable phenotypes, characterized by susceptibility to herpetic (6) and fungal infections (7), autoimmunity, enteropathy, cardiac and vascular alterations, bronchiectasis (8), parodontitis, and failure to thrive (5, 9). In our patient, the clinical phenotype is dominated by recurrent furunculosis, parodontitis, and suppurative eyelid infections, mostly caused by *Staphylococcus* infections (10). As the hallmark in the infectious history of GOF mutations of STAT1 is considered the *Candida* infection, the case herein described further extends the complexity of the phenotype observed in these patients. In this patient, we also found an

increased transcription of pro-inflammatory molecules, as CXCL9, CXCL10, CCL5, and ICAM-1, which could help explain the pathogenesis of some features of this complex phenotype.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:
Figure S1. PBMCs cultured with medium or CpG were stained with antibodies to CD27 and IgM at day 7.

Daily subcutaneous administration of human C1 inhibitor in a child with hereditary angioedema type 1

To the Editor,
Hereditary angioedema (HAE) is a rare autosomal-dominant inherited disorder, caused by local elevations of bradykinin due to a quantitative or qualitative deficiency of C1-INH resulting in recurrent mucosal or subcutaneous swelling attacks. Hereditary angioedema attacks can occur in all locations of the body and are potentially life-threatening if the face or larynx is affected. The diagnosis of HAE is based on clinical symptoms (e.g., severe abdominal pain or recurrent non-pruritic swelling of the skin or submucosal tissues lasting for 2–7 days) and laboratory screening with C4 (usually decreased in patients with HAE), C1-INH antigenic protein (decreased in HAE type 1) and C1-INH function (decreased in patients with HAE types 1 and 2). The majority of the patients benefit from an on-demand therapy (for review, see Ref. (1)). However, depending on the severity of disease, frequency of attacks, patient's quality of life, availability of resources, and failure to achieve adequate control by appropriate on-demand therapy, prophylactic treatment should be considered. Long-term prophylaxis with plasma-derived (pd)C1-INH concentrate requires frequent i.v. injections, in most cases twice per week (2, 3). S.c. infusions of pdC1-INH concentrate are thought to reduce this burden. First pre-clinical studies in adult patients with HAE reported on the safety and feasibility of s.c. administration of pdC1-INH concentrate with a bioavailability of functional C1-INH of 39.7% compared to i.v. administration (4). Recently,