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CORRESPONDENCE

A Novel Monoallelic Small Deletion Confers Gain-of-Function of STAT1 in a Child with Candidiasis and Mycobacterial Diseases

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To the Editor:

Signal transducer and activator of transcription 1 (STAT1) is a transcription factor that regulates cellular responses to type I, II, and III interferons (IFNs) and interleukin (IL)-27 [1, 2]. Inborn errors in STAT1 immunity are classified into four diseases: autosomal recessive (AR) complete, AR partial or autosomal dominant (AD) STAT1 deficiency, and AD STAT1 gain-of-function (GOF) [3]. Cells transfected with mutant STAT1 GOF alleles exhibit stronger responses to stimulation with IFN-γ, IFN-α, and IL-27 than cells transfected with wild-type (WT) alleles [3]. Patients with AD STAT1 GOF have various clinical conditions [1, 2], including recurrent and severe infections (bacterial, viral, or fungal), inflammatory and autoimmune diseases, aneurysms, and tumors [1, 2]. However, the precise molecular mechanism of the STAT1 GOF remains undetermined [3]. To date, 120 rare or private variants of *STAT1* GOF have been reported [1]. Almost all were missense, except for one deletion (L301del) [3]. Here, we present a novel GOF *STAT1* deletion (N297del) in the coiled-coil domain (CCD) in a child presenting with an adverse BCG reaction and disseminated tuberculosis.

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A 4-year-old boy from a rural area in central Mexico had three healthy older siblings (Fig. 1a) and was born at 35 weeks of gestation; he was hospitalized for one month due to respiratory distress syndrome (Fig. 1b). From the first few months of his life, he had chronic mucocutaneous candidiasis that was refractory to treatment. He received the BCG vaccine when he was 4 months old, but developed an ulcer at the injection site, which took 3 months to heal. At 6 months, right axillary adenitis started, and at 18 months, fever developed. The axillary lymph node was removed, and histopathology revealed histiocytes, lymphoplasmacytic infiltrates of neutrophils, and multinucleated cells. At 2 years and 6 months, he was hospitalized for 11 days for bilateral pneumonia and oral candidiasis, necessitating mechanical ventilation and treatment with ceftriaxone and fluconazole for 10 days. At 2 years and 10 months, he presented with fever. Myeloculture revealed *Staphylococcus aureus*, which was resolved with outpatient treatment with ciprofloxacin (25 mg/kg/day) for 10 days. At 3 years, he was readmitted for fever, cough, and dyspnea. Physical examination revealed oral candidiasis and right deltoid and axillary scars; pulmonary hypoventilation; low oxygen saturation (85%); and right axillary adenomegaly 4 × 3 cm. Chest radiography showed left basal consolidation, and thoracoabdominal computed tomography (CT) showed bronchiectasis and multiple enlarged axillary, lung hilum, mesenteric, retroperitoneal, and inguinal lymph nodes (Fig. 1c; online resource: Fig. S1a-S1c). Treatment with intravenous ceftriaxone (75 mg/kg/day) and fluconazole (12 mg/kg/day) was initiated. He underwent resection of several right axillary lymph nodes, and histopathology revealed chronic granulomatous caseous lymphadenitis and acid-fast bacteria (Ziehl–Neelsen staining). GeneXpert MTB/RIF testing of the lymph nodes and gastric juice was positive. Bacterial cultures of the lymph nodes and gastric juice revealed *Mycobacterium tuberculosis*

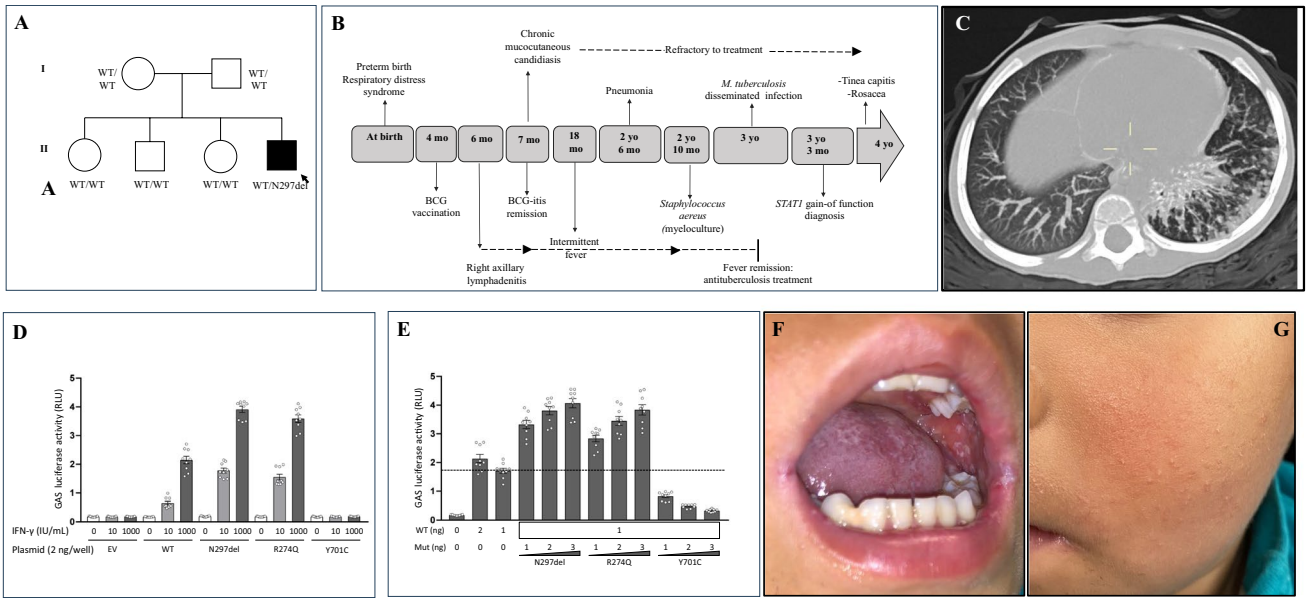

Fig. 1 Clinical, radiological, and genetic features of the patient. **a** Family pedigree; the patient is indicated by a black square. **b** Clinical evolution of the patient. **c** Computed tomography images of micronodular pattern in the lungs. **d** U3C cells, after transfection of plasmids carrying empty vector (EV), wild type (WT), N297del, Y701C (known LOF), or R274Q (known GOF) mutants (2 ng/ well), along with the reporter plasmids (Cignal GAS Reporter Assay Kit, QIAGEN), were stimulated with IFN-γ at 10 and 1,000 IU/mL for 16 h and subsequently underwent a luciferase reporter assay. **e** The total amount of expression vector containing WT (1 or 2 ng) and/or mutant (1, 2, or 3 ng) STAT1 was maintained at 5 ng by supplementing with an EV. The transfectants were stimulated with 1,000 IU/mL of IFN-γ for 16 h and then subjected to a luciferase reporter assay. The GAS luciferase activity was determined by normalizing the values against the Firefly/Renilla luciferase luminescence ratio. Three individual experiments were performed in triplicate. The results shown in the bar graphs represent the mean with SEM (Standard error of the mean). **e** Whitish plaques on oral mucosa (cheeks, tongue, and labial commissure). **f** Cheek rosacea

leading to the diagnosis of disseminated tuberculosis. The patient was placed on intensive treatment with rifampicin (15 mg/kg/day), isoniazid (15 mg/kg/day), pyrazinamide (15 mg/kg/day), and ethambutol (25 mg/kg/day) and showed a good therapeutic response.

Due to the recurrence and severity of infectious manifestations, an inborn error of immunity was suspected. Initial laboratory results were hemoglobin 10.6 (normal value [NV]: 11.5–12.3) g/dL, white blood cells $9{,}1 \times 10^9$/L (NV: 5.0–14.5), neutrophils $5{,}1 \times 10^9$/L (NV: 1.5–8.5), lymphocytes $2{,}3 \times 10^9$/L (NV: 2.0–8.0), monocytes $1{,}6 \times 10^9$/L (NV: 0.1–1.0), and platelets 632×10^9/L (NV: 200–450). The dihydrorhodamine test for neutrophils was similar to that of healthy controls upon ester phorbol activation. Other results were: IgG 786 (923 ± 256) mg/dL, IgM 54.9 (65 ± 25) mg/dL, IgA 38.5 (124 ± 45) mg/dL, IgE 3 (< 90) IU/mL, HIV antigen/antibody tests were negative, CD4 + 780 (560–2,700) cells/mL, CD3 + 2347 (1,200–4,100) cells/mL, CD8 + 1338 (330–1,300) cells/mL, CD19 + 1050 (220–1300) cells/mL, CD16/CD56 + 404 (48–540) cells/mL. Since we were unable to reach a diagnosis based on previous results, we ordered a targeted next-generation sequencing test for inborn errors of immunity. The patient harbored the c.891_893del (p.N297del) variant in *STAT1* at heterozygous state. Since this variant was novel, luciferase reporter assay was performed to evaluate transcriptional activation by GOF-STAT1 mutant (Fig. 1d). The N297del *STAT1* behaved as a GOF protein in response to stimulation with low concentrations of IFN-γ. The luciferase activity of the GAS was also measured by co-transfection with the expression vector containing the WT STAT1 and/or the mutant STAT1. The mutant N297del exerted a dose-dependent dominant-negative effect on WT-STAT1-mediated GAS induction after IFN-γ stimulation (Fig. 1e). For this variant, both parents and his siblings were WT/WT. The patient was discharged from the hospital with intensive antituberculosis treatment and prophylactic antifungal medication. During the most recent follow-up visit, the patient continued to have mucocutaneous candidiasis despite prophylactic treatment (Fig. 1f; Online Resource: Fig. S1e & S1f). He also presented with rosacea of the cheeks and chin (Fig. 1f), and scalp tinea (Online Resource: Fig. S1g).

In a patient with several mycobacterial diseases, including those caused by *M. tuberculosis*, we identified a novel pathogenic variant, N297del, in *STAT1* that exhibited GOF features. This variant results in the loss of three nucleotides; thus, the open reading frame of the protein is retained (in-frame deletion). Of 750 STAT1 residues, 80 were affected

by the 120 mutations described for the STAT1 GOF [3]. In the patient described here, residue 279 was affected, where no mutations have been described to date. Residue 279 is part of the CCD domain, in which 49% of the pathogenic variants of *STAT1* GOF are found [3]. The patient described here with a novel variant of *STAT1* had tuberculosis. Both loss-of-function and GOF variants can cause this manifestation, and functional studies are needed to define either [3]. A luciferase assay performed revealed increased STAT1 activity. Clinically, the patient was diagnosed with disseminated *M. tuberculosis* infection after 2 years of fever and adenitis, which did not improve with treatment. Although it has been described that mycobacterial infections are present in approximately 6% of patients with STAT1 GOF [2, 3], the frequency of tuberculosis may be higher in patients living in countries considered to have a high burden of tuberculosis, such as Mexico [4]. Other infections associated with the STAT1 GOF [3] in our patient were *Candida* (oral mucosa) and *S. aureus* (bone marrow) infections. Rosacea is a facial inflammatory disorder that shows an increasing incidence with age. Pediatric rosacea is rare [5]; however, because STAT1 GOF is a genetic disorder [3], patients may develop rosacea at a young age. Rosacea in STAT1 GOF has been described in some pediatric patients [1]; however, the percentage of STAT1 GOF patients with rosacea and the age of onset are unknown. The patient reported here was diagnosed with facial and ocular rosacea at 3 years of age, suggesting pediatric onset. The patient described in this study expands the genetic and clinical spectrum of patients with STAT1 GOF.

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Author Contributions HGT treated the patient. MT, UPB, CSF & VLT performed the molecular and genetic analysis. SEP, JB, SO & LBG drafted the manuscript. All authors reviewed the manuscript.

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Data Availability No datasets were generated or analysed during the current study.

Declarations

Ethics Approval Written informed consent was obtained in accordance with local regulations, with approval from the Institutional Review Board.

Consent to Participate Written informed consent for participation was obtained from the patient's parents.

Consent for Publication Consent for publication was obtained from the patient's parents. All authors approved the final version of the manuscript.

Competing interests The authors declare no competing interests.

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