

Letter to the Editor

Gain-of-function *STAT1* mutations are associated with PD-L1 overexpression and a defect in B-cell survival

To the Editor:

Heterozygous gain-of-function mutations in the coiled-coil domain of *STAT1* were recently identified as a cause of chronic mucocutaneous candidiasis (CMC).^{1,2} As with *STAT3* mutations in hyper-IgE syndrome, the candidal susceptibility associated with gain-of-function *STAT1* mutations appears secondary to T_H17-cell deficiency.³ The mechanistic link between constitutive *STAT1* activity and diminished T_H17 cells is yet to be clearly defined. Here, we present a kindred with a novel gain-of-function *STAT1* mutation associated with a complex clinical phenotype including candidiasis, humoral immunodeficiency, overexpression of programmed cell death protein ligand 1 (PD-L1), and increased B-cell apoptosis.

We identified 4 related individuals heterozygous for a novel E235A missense mutation in a highly conserved segment of the coiled-coil domain of *STAT1* (Fig 1, A). To our knowledge, this is the first mutation described in exon 9 of the *STAT1*α gene locus to be associated with CMC. The mutation is not present in

unaffected adult family members. The index patient (II.6) is a 60-year-old woman with CMC and progressive antibody deficiency. Beginning in infancy, the patient experienced candidal infections at sites including the oral cavity, esophagus, vagina, skin, and nails. Later, as a young woman, the patient was diagnosed with IgG2 subclass deficiency that progressed to frank hypogammaglobulinemia (IgG 514, IgA 7, IgM < 10 mg/dL) and B-cell lymphopenia (2 CD20⁺ cells/μL; normal range, 97–440) requiring intravenous immunoglobulin. Over her lifetime, the patient suffered recurrent pulmonary infections from *Pseudomonas aeruginosa*, *Streptococcus pneumoniae*, *Serratia* species, *Mycobacterium avium*, and respiratory syncytial virus that resulted in severe bronchiectasis requiring lobectomy. Consistent with published reports of patients with gain-of-function *STAT1* mutations, our index patient (II.6) has experienced human papillomavirus positive squamous cell carcinoma of palate, basal cell carcinoma, shingles, and fibromuscular dysplasia with carotid and celiac/splenic artery dissection.

The index patient's daughter (III.2) is a 30-year-old woman with CMC, B-cell lymphopenia (6 CD20⁺ cells/μL), and IgG2 subclass deficiency manifesting in adolescence. Despite antifungal therapy, the patient experienced candidal infections at sites

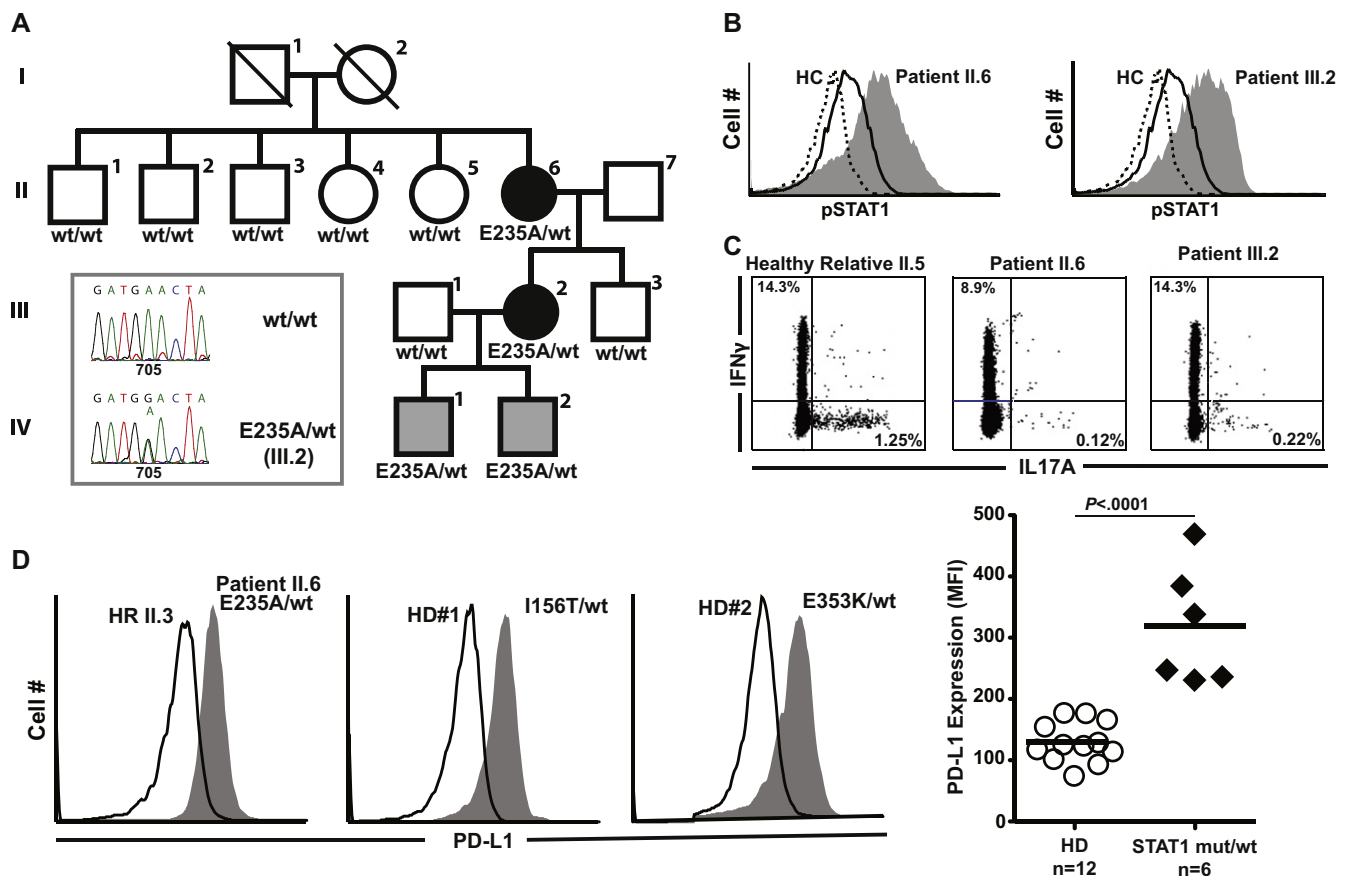


FIG 1. Gain-of-function *STAT1* mutations increase PD-L1 expression. **A**, Pedigree with patients (black) and carriers (gray) heterozygous at *STAT1* nucleotide 705. **B**, Increased *STAT1* phosphorylation in IL-21-treated T cells from patients (filled), compared with controls (solid) and unstimulated (dashed). **C**, Reduced T_H17-cell frequencies in E235A *STAT1* patients. **D**, PD-L1 overexpression on CD3⁺CD4⁺CD62L⁺CD45RO⁻ cells from subjects with *STAT1* mutations (filled) versus controls (solid). MFI, Mean fluorescence intensity.

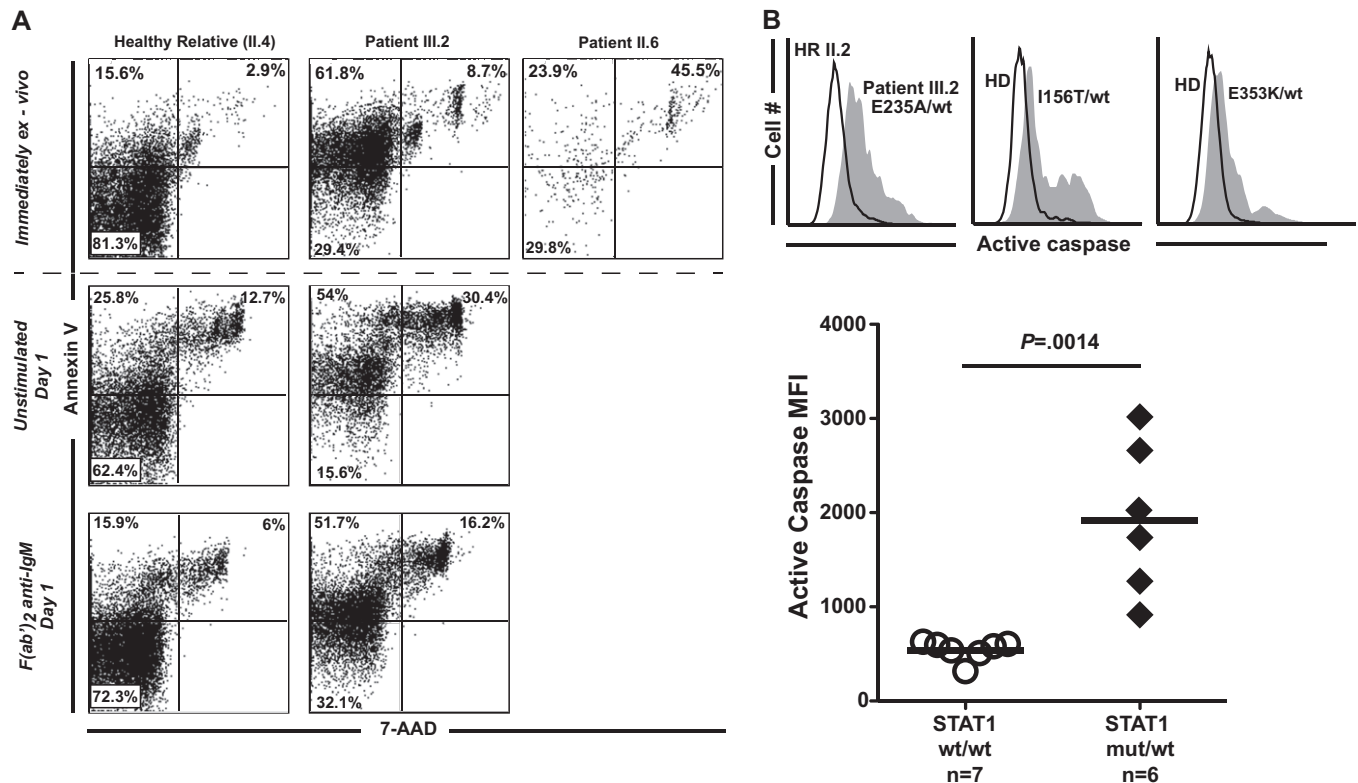


FIG 2. Gain-of-function *STAT1* mutations promote B-cell death. **A**, Annexin V and 7-aminoactinomycin D staining of CD19⁺ cells immediately *ex vivo* (top row) and after 24 hours in culture with (middle row) or without rescue with F(ab')₂-anti-IgM (bottom row). **B**, Active caspase staining of CD19⁺ cells in subjects heterozygous for the indicated *STAT1* mutations (filled) versus related and unrelated controls (solid). MFI, Mean fluorescence intensity.

including the vagina, skin, and nails. She has also experienced noncandidal infections including pneumonia, otitis media, sinusitis, and chronic bronchitis. The third and fourth persons carrying the E235A allele are children of patient III.2, 2 males aged 6 weeks and 24 months (IV.1 and IV.2). They have not yet manifested symptoms of immunodeficiency.

Stimulation of T cells from patients II.6 and III.2 with IL-21 significantly increased the phosphorylation of STAT1 compared with a healthy control (Fig 1, B). Furthermore, stimulation of patient PBMCs with phorbol 12-myristate 13-acetate/ionomycin demonstrated diminished IL-17-secreting CD4⁺ cells compared with a related healthy control (Fig 1, C). Hence, the E235A mutation confers to STAT1 a gain of function and is associated with T_H17-cell deficiency.

A remarkable feature of our kindred is the overexpression of PD-L1 on the surface of naive CD4⁺ T cells. All 4 family members carrying the E235A allele had higher PD-L1 staining compared with members without it (Fig 1, D). To investigate whether the overexpression of PD-L1 was a common feature to gain-of-function *STAT1* mutations, we obtained PBMCs from 2 additional patients carrying either the I156T or the E353K missense *STAT1* mutation. Both patients, unrelated to our kindred, revealed a similar increase in PD-L1 expression on their naive T cells (Fig 1, D). Recent data from mice demonstrate that the expression of PD-L1 on undifferentiated naive T cells prevents commitment to the T_H17 lineage through a PD-1/PD-L1 interaction. In this context, PD-L1 expression is dependent on IL27/IL27R binding and STAT1.⁴

Accordingly, constitutively active STAT1 molecules in subjects carrying gain-of-function *STAT1* mutations may be responsible for increased PD-L1 expression on naive T cells, thereby discouraging differentiation into T_H17 cells.

As previously described, the clinical phenotype of patients with gain-of-function *STAT1* mutations is quite broad and can include candidiasis, antithyroid autoimmunity, squamous cell carcinoma, and vascular anomalies. In this issue, 2 reports show broader phenotypes to gain-of-function *STAT1* mutations with an immunodysregulation polyendocrinopathy enteropathy X-linked syndrome (IPEX)-like autoimmune syndrome in one report and disseminated coccidioidomycosis and histoplasmosis in the other. Here, we report a not yet appreciated feature associated with gain-of-function *STAT1* mutations: humoral immunodeficiency.

STAT1's function as a key modulator of cell death is thoroughly described. Perhaps the best illustration of this is growth arrest of the STAT1-negative U3A fibroblast line upon transfection with wild-type STAT1 α and treatment with IFN- γ .⁵ The IFN- γ receptor requires STAT1 for intracellular signaling. Moreover, transfection of the same cell line with constitutively activated STAT1 initiates caspase-mediated apoptosis.⁶ Related experiments implicate STAT1 activation during apoptosis in B-cell lymphoma cells.⁷

Interestingly, progressive B-cell lymphopenia is a remarkable feature of patients (II.6 and III.2) in our kindred, suggesting a defect in cell survival. Indeed, CD19⁺ B cells from subjects carrying the E235A allele appear apoptotic with increased Annexin

V staining (Fig 2, A, top row) and elevated caspase activity (Fig 2, B). In culture for 24 hours, B cells from patient III.2 demonstrated even greater Annexin V and considerable 7-AAD staining, evidence of accelerated cell death (Fig 2, A, middle row). The patient's B cells were only partially rescued by the stimulation of their B-cell receptors (Fig 2, A, bottom row). We also found enhanced caspase activity in B cells from the 2 additional patients who were heterozygous for either the E353K or the I156T missense *STAT1* mutation (Fig 2, B). B-cell lymphopenia was a significant finding in the former patient (39 cells/ μ L) but not the latter (335 cells/ μ L). Altogether our data reveal that gain-of-function *STAT1* mutations increase B-cell apoptosis. Over time, this may result in B-cell lymphopenia and antibody deficiency.

In summary, we identified individuals heterozygous for gain-of-function *STAT1* mutation with 2 unappreciated features. The first is the overexpression of PD-L1 on naive T cells, which provides a general mechanism for how constitutively active STAT1 blocks the development of the T_H17 lineage. The second feature, accelerated B-cell apoptosis that may result in progressive B-cell lymphopenia and humoral immunodeficiency, further broadens the clinical phenotype associated with gain-of-function *STAT1* mutations.

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