Published in final edited form as:
J Allergy Clin Immunol. 2013 June ; 131(6): 1691–1693. doi:10.1016/j.jaci.2013.01.004.

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

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Keywords

STAT1; CMC; T_H17 cells; PD-L1; antibody deficiency; B cell lymphopenia

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 has 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 have identified four related individuals each 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.

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All patient samples were collected in accordance with IRB-approved protocols.

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, nml range 97–440) requiring IVIG. Over her lifetime, the patient suffered recurrent pulmonary infections from *P. aeruginosa*, *S. pneumonia, Serratia* species, *M. 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 HPV^+ 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 female 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 including the vagina, skin and nails. She has also experienced non-candidal infections including pneumonia, otitis media, sinusitis and chronic bronchitis. The third and forth persons carrying the E235A allele are children of patient III.2, two males ages 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 phosphorylation of STAT1 compared to a healthy control (Fig 1, B). Furthermore, stimulation of patient PBMCs with PMA/ionomycin demonstrated diminished IL-17 secreting $CD4^+$ cells compared to 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 overexpression of PD-L1 on the surface of naïve $CD4^+$ T cells. All four family members carrying the E235A allele had higher PD-L1 staining compared to members without it (Fig 1, D). To investigate if overexpression of PD-L1 was a common feature to gain-of-function *STAT1* mutations, we obtained PBMCs from two 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 naïve T cells (Fig 1, D). Recent data from mice demonstrate that the expression of PD-L1 on undifferentiated naïve 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 naïve 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, anti-thyroid autoimmunity, squamous cell carcinoma and vascular anomalies. In this issue two reports show broader phenotypes to gain-of-function *STAT1* mutations with an 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 STAT1α and treatment with interferon-γ (5). The interferon-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 stimulation of their B-cell receptors (Fig 2,A bottom row). We also found enhanced caspase activity in B cells from the two additional patients that 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/μl) but not the latter (335 cells/ul). 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 two unappreciated features. The first is the overexpression of PD-L1 on naïve 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.

Acknowledgments

Declaration of all sources of funding: This work was supported by grant number AI061093, AI071087, AI082713 and AI095848 from NIH-NIAID (to E. M.), K12HD0141401-10 from NIH-NICHD (to N.R.) and MO 2160/2-1 from DFG (to H.M.), and by the Division of Intramural Research, NIAID, NIH (to SMH and JDM).

We would like to acknowledge Dr. O'Shea of the NIH for sharing his unpublished manuscript with our group and to thank the patients and their family members for making this work possible.

Abbreviations

STAT1	Signal transducer and activator of transcription 1
PD-1	Programmed cell death protein 1
PD-L1	Programmed cell death protein ligand 1
CMC	Chronic mucocutaneous candidiasis

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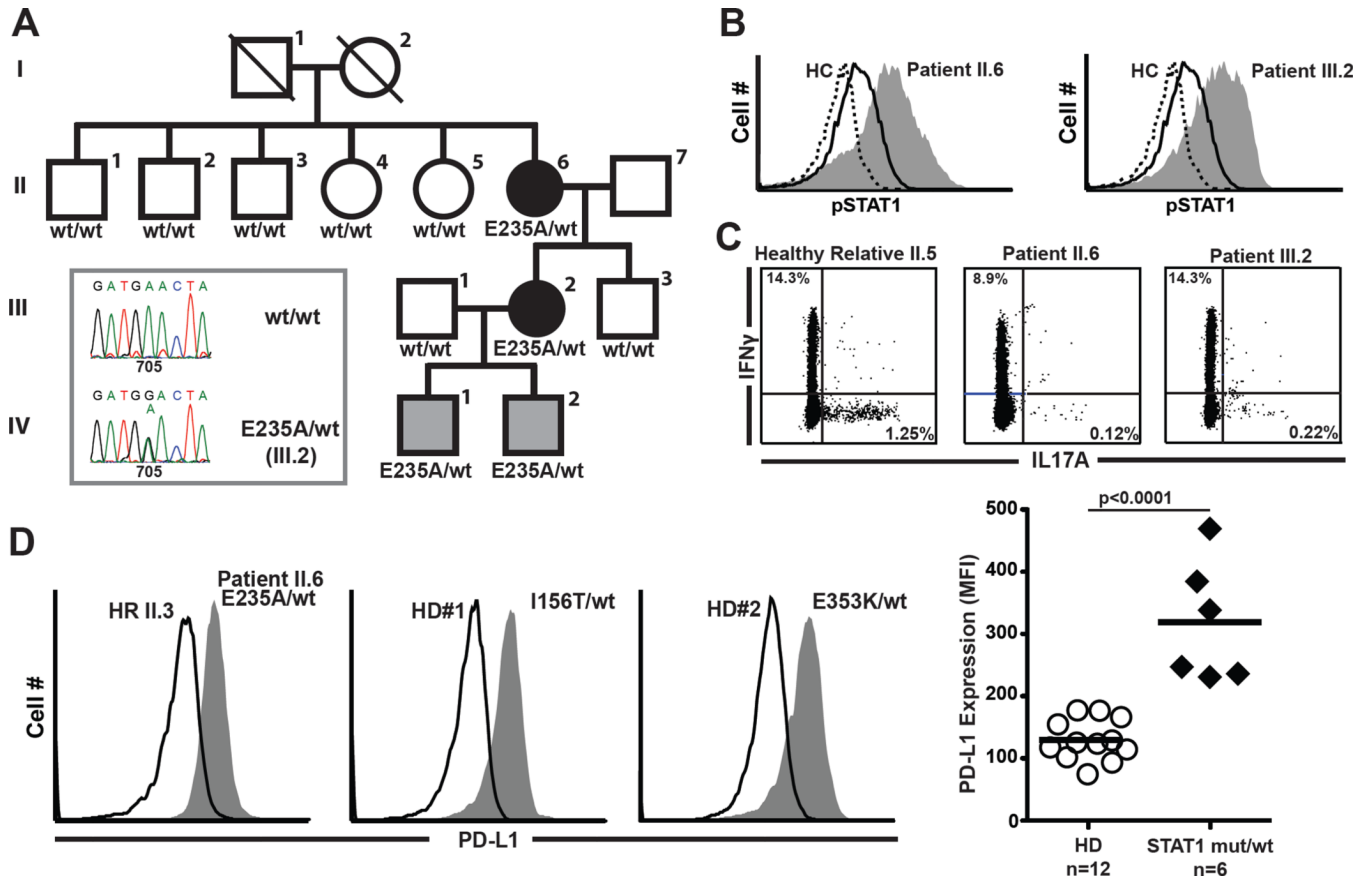

FIG 1.

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 to 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).

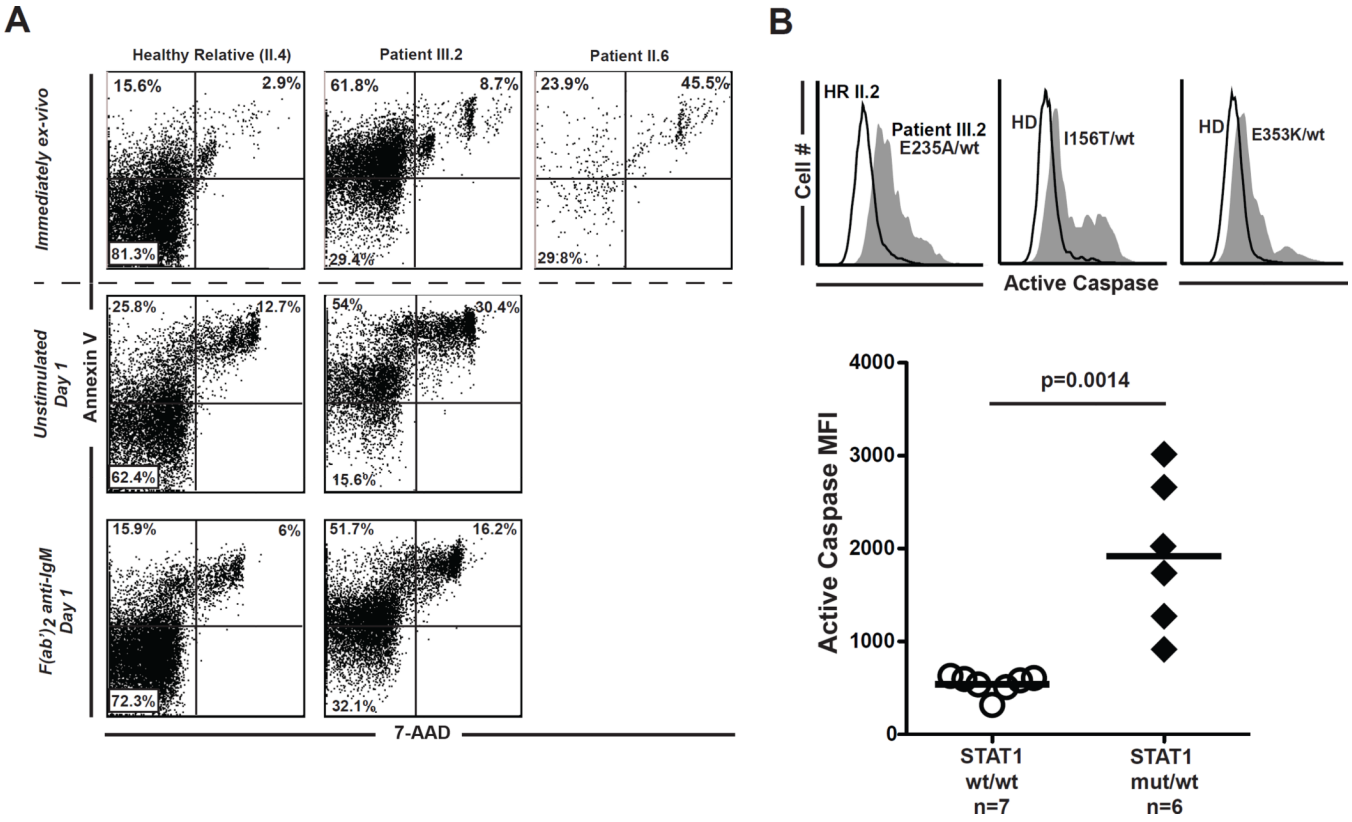

FIG 2.

Gain-of-function *STAT1* mutations promote B-cell death. **A,** Annexin V and 7-AAD 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) vs. related and unrelated controls (solid).