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JAK3 deficiency caused by a homozygous synonymous exonic mutation that creates a dominant splice site

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To the editor

Janus kinase 3 (JAK3) is a cytosolic tyrosine kinase that is primarily expressed in hematopoietic cells.¹ Null mutations in *JAK3* typically manifest as T⁻B⁺NK⁻ severe combined immunodeficiency (SCID), while hypomorphic mutations can present with a broader range of immune dysregulation.^{1,2} Here we report two siblings with a homozygous synonymous exonic mutation in *JAK3* that creates a dominant splice site and dramatically decreases expression of WT protein. This mutation resulted in T⁻B⁺NK^{low} SCID in one patient and in a combined immunodeficiency (CID) with granulomatous skin disease in her sibling.

Four children resulted from the consanguineous union of two parents from Saudi Arabia (Fig 1A). Given a family history of SCID in an older sibling, the family's fourth child (Patient 1) was screened shortly after birth by lymphocyte flow cytometry and diagnosed with T⁻B⁺NK^{low} SCID. Her 6-year-old brother (Patient 2) had a persistent violaceous, granulomatous dermatitis on his arms since 2 years of age (see Fig E1 in this article's Online Repository at www.jacionline.org) but no history of frequent or unusual infections. Skin biopsy revealed a prominent dermal nodular infiltrate and poorly formed granulomas extending to the deep dermis (see Fig E2 in this article's Online Repository at www.jacionline.org). Special stains for organisms performed with Periodic acid–Schiff, Gram, Grocott's methenamine silver, and Fite were negative (data not shown). As

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granulomatous skin disease has been associated with CID^{2,3} an immunologic evaluation of both siblings was performed.

Immunological workup of Patient 1 revealed nearly absent T cells (56 cells/ μ l), normal B cells (1600 cells/ μ l), low NK cells (54 cells/ μ l) and negligible T cell proliferation to PHA, ConA and anti-CD3 (Table 1). All detectable T cells were of maternal origin (data not shown). Patient 2 had low numbers of T cells (566 cells/ μ l), B cells (120 cells/ μ l) and NK cells (17 cells/ μ l), consistent with a CID. He also had reduced percentages of naïve T cells, and inversion of the CD4:CD8 ratio (Table 1). Proliferation to mitogens and antigens was below the lower limit of normal (Table 1). There was no evidence of maternal engraftment (data not shown). Patient 2 had normal TCR V β usage that did not suggest a restricted T cell repertoire (see Fig E3 in this article's Online Repository at www.jacionline.org), and normal immunoglobulins (Table 1), but poor responses to tetanus and pneumococcal vaccines (data not shown).

To determine the underlying genetic defect, genomic DNA from Patient 1 was evaluated in a commercial laboratory using massive parallel sequencing of 46 genes associated with SCID/CID that included *JAK3*. While no pathogenic variants were reported, the possibility of a non-coding mutation in *JAK3* that affects protein expression was pursued, as mutations in this gene typically present with T⁻B⁺NK⁻ SCID. Immunoblotting of lysates from Epstein Barr virus-transformed B-lymphoblastoid cell lines (BLCLs) revealed a dramatic decrease in JAK3 expression in both patients (Fig 1B). STAT5 phosphorylation in response to IL-2 and IL-15 stimulation of BLCLs was severely diminished in the patients, confirming a functional defect in JAK3 signaling (Fig 1C).

A comprehensive list of all exonic variants found by the commercial laboratory was requested. It included a single homozygous, synonymous variant in exon 19 of *JAK3* (c. 2652C>T; pV884V) that had been presumed benign as it did not alter the predicted amino acid sequence. However, *in silico* analysis using the independent splice site prediction tools MaxEntScan⁴ and Human Splicing Finder⁵ indicated that the variant likely created a donor splice site (see Table E1 in this article's Online Repository at www.jacionline.org). The variant was confirmed by Sanger sequencing to be homozygous in both patients, and heterozygous in the parents and healthy sibling (Fig 1D).

To evaluate whether the mutation impacted splicing of *JAK3* mRNA, a 218 bp cDNA fragment surrounding the mutation site was amplified by RT-PCR (Fig 1E, top panel). mRNA purified from control PBMCs produced a single band of the expected molecular weight (Fig 1E, bottom panel). In contrast, two bands were amplified from PBMC mRNA isolated from Patient 1: a barely detectable full-length band, and a much more abundant, lower molecular weight band (Fig 1E, bottom panel). Sanger sequencing revealed that the full-length band contained WT cDNA, while the lower band contained two cDNA species with deletions of either 29 or 30 bp at the 3' end of exon 19 (Fig 1F). The 29 bp deletion resulted in a frameshift with a premature stop codon (p.K884Pfs*73). Immunoblotting of patient BLCL lysates using a mAb specific for the N-terminus of JAK3 revealed no truncated protein at the predicted molecular weight of approximately 95 kDa, indicating that the mutant protein was not expressed or was rapidly degraded (Fig 1B). The 30 bp deletion

removes 10 terminal amino acids of exon 19 (p.V884_P893del), thereby encoding a mutant protein ~1.2 kDa smaller than WT JAK3. Due to the closeness of their predicted molecular weight (115 kDa versus 114 kDa), the WT and p.V884_P893del mutant protein would be expected to migrate as a single band of ~115 kDa on gel electrophoresis. The dramatic decrease in the intensity of this band strongly suggests that the p.V884_P893del mutation decreases protein stability.

It is notable that Patient 1 and Patient 2 had identical homozygous mutations in *JAK3* with similar reductions in protein expression but different phenotypes (see Table E2 in this article's Online Repository at www.jacionline.org for additional clinical details on all siblings). The relatively normal TCR V β repertoire of Patient 2, residual proliferation to mitogens, and lack of significant infections indicates that his T cells retain some function and are not merely expanded from a limited number of clones. Such phenotypic variability has been documented in a variety of other PID genes⁶. Modifier genes, epigenetic factors, or environmental exposures may all play a role in determining the clinical phenotype.⁶

Synonymous codon variants are generally thought to have little significance⁷ and are often filtered out during analysis of the vast amount of data generated through next generation sequencing.⁶ Nevertheless, synonymous mutations in genes such as *IL10RA* and *MRE11A* have previously been reported to cause disease through disruption of known splice sites.^{8,9} Our case illustrates the importance of evaluating synonymous mutations even when they are distant from known splice sites, as they may create new donor or acceptor splice sites.

The significance of a synonymous variant can be determined through a series of steps. First, splicing prediction tools can determine the probability that a variant is pathogenic. Next, cDNA amplification and sequencing can definitively demonstrate that splicing is affected. Finally, functional analysis is necessary to prove that that a change in splicing is responsible for the patient phenotype. As whole exome sequencing and targeted gene panels become an increasingly important part of the clinical immunologist's toolbox, the need to evaluate the biologic relevance of synonymous variants will continue to increase.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

BLCL	B-lymphoblastoid cell line
CID	combined immunodeficiency
JAK3	Janus kinase 3
SCID	severe combined immunodeficiency

HSCT hematopoietic stem cell transplantation

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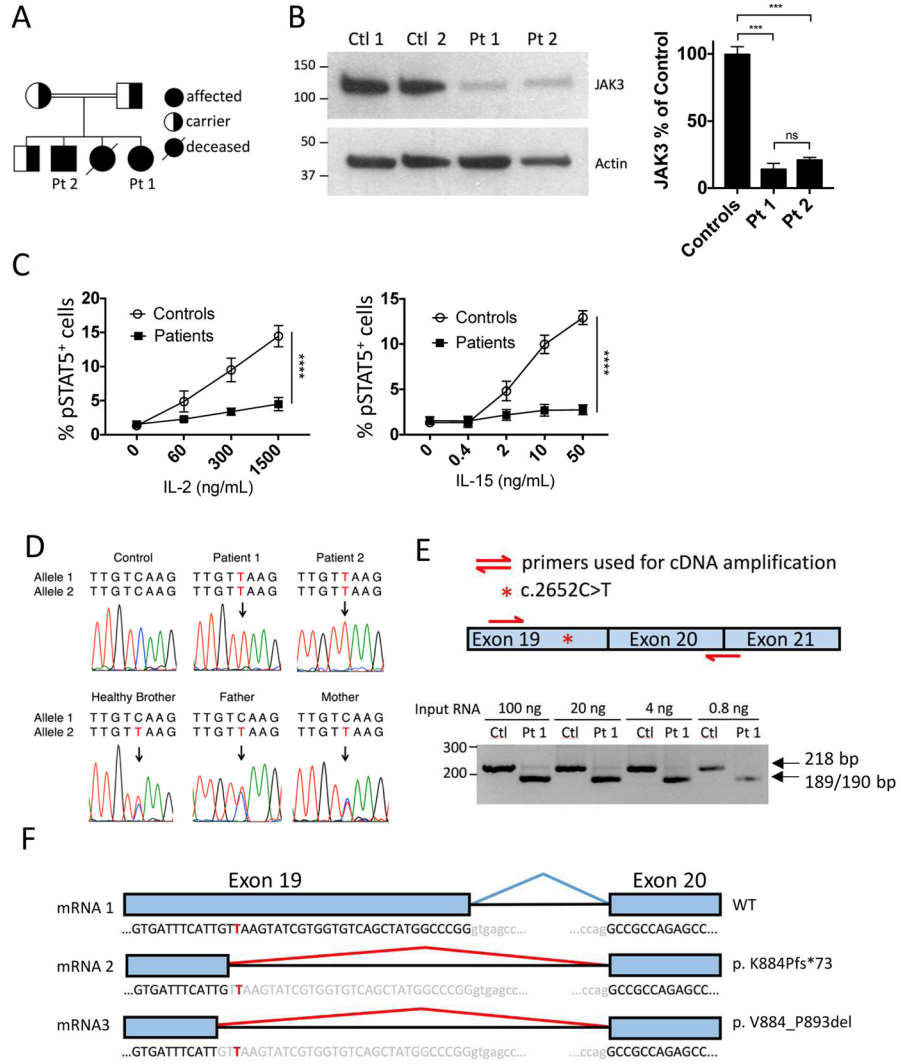


Fig 1. Characterization of the *JAK3* mutation

(A) Pedigree. (B) Immunoblot of lysates from BLCLs derived from patients and controls (left). *JAK3* expression relative to controls (right). *** $p < 0.001$, ns=not significant (one-way ANOVA). (C) Percentage of pSTAT5⁺ BLCLs after stimulation for 10 minutes with IL-2 or IL-15. **** $p < 0.0001$ (two-way ANOVA). (D) Sanger sequencing of c.2652C>T variant. (E) RT-PCR amplification of *JAK3* mRNA encompassing the patient mutation site. (F) Diagram of mRNA splice variants induced by mutation. For B and C $n=2$ for controls, $n=2$ for patients; data pooled from 2 independent experiments. Columns/lines and bars represent means and SEM.

Table 1

Immune profile of patients

	Patient 1		Patient 2	
	Age 3 months	Normal Values for Age ^a	Age 6 years	Normal Values for Age ^a
Lymphocyte subsets (cells per μl)				
CD3 ⁺	56	2500–5600	566	1000–2600
CD3 ⁺ CD4 ⁺	7	1600–4000	154	530–1500
CD4 ⁺ CD45RA ⁺ CCR7 ⁻ (TEMRA)	16.90%	0.1–1.9%	3.90%	0.4%–2.6%
CD4 ⁺ CD45RA ⁺ CCR7 ⁺ (naïve)	11.90%	76.7–91.4%	5.60%	57.1%–84.9%
CD4 ⁺ CD45RA ⁻ CCR7 ⁻ (effector memory)	54.20%	1.1–5.3%	38.50%	3.3%–15.2%
CD4 ⁺ CD45RA ⁻ CCR7 ⁺ (central memory)	16.90%	6.7–15.6%	52%	11.3%–26.7%
CD4 ⁺ CD31 ⁺ CD45RA ⁺ (recent thymic emigrants)	34.30%	59.7–82.4%	6.60%	45.3–63.6%
CD3 ⁺ CD8 ⁺	37	500–1700	344	330–1100
CD8 ⁺ CD45RA ⁺ CCR7 ⁻ (TEMRA)	0.90%	1.5–22.7%	15.40%	9.1–49.1%
CD8 ⁺ CD45RA ⁺ CCR7 ⁺ (naïve)	0.30%	62.1–94.0%	2.30%	28.4–80.6%
CD8 ⁺ CD45RA ⁻ CCR7 ⁻ (effector memory)	96.30%	1.3–19.5%	71.30%	6.2–29.3%
CD8 ⁺ CD45RA ⁻ CCR7 ⁺ (central memory)	2.50%	0.9–5.6%	11.00%	1.0–4.5%
CD19 ⁺	1600	300–2000	120	270–860
IgD ⁺ CD27 ⁻ (naïve)	n.d.		94.10%	47.30–77.00%
IgD ⁺ CD27 ⁺ (unswitched memory)	n.d.		3.00%	5.20–20.40%
IgD ⁻ CD27 ⁺ (switched memory)	n.d.		1.00%	10.90–30.40%
CD24 ^{hi} CD38 ^{hi} (transitional)	n.d.		19.90%	7.2–23.8%
CD24 ^{low} CD38 ^{hi} (plasmablasts)	n.d.		0.00%	0.4–5.2%
CD19 ⁺ CD21 ^{low} CD38 ^{low}	n.d.		5.60%	2.5–9.4%
CD24 ^{hi} CD38 ^{low} (marginal zone-like)	n.d.		9.80%	12.6–36.0%
CD16 ⁺ /CD56 ⁺	54	160–1100	17	70–480
Immunoglobulins				
IgG (mg/dL)	809 [*]	280–750	775	639–1344
IgA (mg/dL)	<7	6–50	104	70–312
IgM (mg/dL)	39	15–70	171	34–210
IgE (kU/L)	<1	0–30	5	0–200
T cell proliferation (counts per minute)				
Phytohemagglutinin	750	96,090–358,179	26,802	96,090–358,179
Concanavalin A	657	65,699–239,344	24,681	65,699–239,344
Anti-CD3	1966	62,927–217,761	34,629	62,927–217,761
Tetanus	n.d.		1239	8544–102,895
Candida	n.d.		17348	6231–197,940

	Patient 1		Patient 2	
	Age 3 months	Normal Values for Age ^a	Age 6 years	Normal Values for Age ^a
Background	1273	204–2104	322	204–2104

^a normal values from age-matched controls in the Boston Children's Hospital clinical immunology laboratory

* on IVIG

n.d. = not done

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