A novel *STAT3* splice-site variant in a kindred with autosomal dominant hyper IgE syndrome

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ABSTRACT

Background: Dominant negative STAT3 loss-of-function is the most common genetic cause of hyper-IgE syndrome (HIES). Patients may present with a host of both immune and non-immune manifestations, including connective tissue abnormalities, recurrent infections, malignant predisposition, and biochemical evidence of elevated serum IgE or eosinophilia.

Aim: To describe a novel splice-site variant in *STAT3* resulting in HIES.

Methods: Case report of two family members with HIES.

Results: A proband and his son presented with neonatal-onset pustular rash, recurrent skin and sinopulmonary infections and elevated serum IgE and were diagnosed with AD-HIES. They were identified to harbor a novel splice-site variant in the DNA-binding domain (DBD) of *STAT3*: c.1110-3C>G, predicted to result in defective splicing in exon 12. Interestingly, a number of other patients with AD-HIES have mutations affecting the same splice-site, suggesting this may be a hot-spot for mutagenesis.

Conclusion: Splice-site mutations in the DBD of *STAT3* are increasingly identified as a cause of AD-HIES. Future work is required to delineate whether patients with splice-site mutations have unique clinical characteristics, supporting efforts for genotype-phenotype correlation in this disease.

Statement of Novelty: We present a novel splice-site mutation in the DNA-binding domain of *STAT3* leading to autosomal dominant hyper-IgE syndrome.

Introduction

'Job syndrome' was defined in 1966 as a clinical entity comprising of eczematoid dermatitis, recurrent "cold" *Staphylococcal* skin abscesses and sinopulmonary infections (Davis et al. 1966). In 1972, Buckley et al. furthered the phenotype of this entity to include elevated serum IgE levels, terming the disorder 'Hyper IgE syndrome' (Buckley et al. 1972). In the 2 decades that followed, the phenotypic spectrum of the disease was expanded further. Additionally, scoring criteria were proposed for the diagnosis of HIES based on the presence of clinical features such as abnormal facial features and dentition, recurrent fractures, skin, sinopulmonary and skeletal abnormalities, infections, and biochemical serum findings (Grimbacher et al. 1999).

Heterozygous variants in *Signal Transducer and Activator of Transcription* (*STAT) 3* were the first identified genetic cause of HIES in 2007, leading to the definition of autosomal dominant (AD)-HIES (Holland et al. 2007; Minegishi et al. 2007). The variants were described to have a dominant negative effect, interfering with the normal activity of the wildtype *STAT3* allele (Minegishi et al. 2007). STAT3 is a member of the STAT family of transcription factors, with pivotal roles

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in the signaling of various cytokines and growth factors. Notably, STAT3 is essential for both pro-inflammatory (IL-6, IL-17) and anti-inflammatory (IL-10) signaling pathways, accounting for the perplexing phenotype of recurrent infections and abnormal inflammation in patients.

We hereby present a family (father and son) affected by AD-HIES. The patients were both diagnosed with a novel intronic variant in the DNA-binding domain (DBD) of *STAT3*, suspected to disrupt splicing.

Methods

Patient and blood samples

Data compiled prospectively and retrospectively from patient medical records were entered into the Canadian Centre for Primary Immunodeficiency Registry and Tissue Bank, which has been approved by the SickKids Research Ethics Board (protocol no. 1000005598). Patient and family members provided written informed consent.

T-cell proliferative responses

Lymphocyte proliferative responses to mitogens, including PHA, anti-CD3 and anti-CD28 antibodies, were determined by thymidine incorporation (Alsalamah et al. 2019). All assays were performed in triplicate and were compared with random normal controls.

Genetic diagnosis and sequencing confirmation

Genomic DNA was isolated from patient peripheral blood leukocytes using the Geneaid genomic DNA extraction kit (Geneaid Mini Kit; Sensi Capital Corp, Toronto, Ontario, Canada). Patient variant was identified via whole exome sequencing using Illumina HiSeq2500 GATK 1.1.28 sequencing platform (Merico 2016), and confirmed by Sanger sequencing using DTCS Quick Kit on an automated sequencer (Beckman-Coulter CEQ 8000). Variants were later confirmed by a clinical laboratory.

Clinical Cases

Proband (P-I)

A 12-year-old male was first referred to us for evaluation of a rash and recurrent infections. The first concern noted by his parents was a severe blistering rash since birth. This was present since the first day of life, leading to a neonatal intensive care unit admission where he was treated with intravenous (IV) antibiotics. Over the course of his life the rash persisted, starting as erythematous patches spanning his entire body which would evolve into large coalescing pustules, with no clear trigger. He had been treated continuously with steroid creams and had received many courses of oral antibiotics for secondary skin infections, eventually being prescribed prophylaxis with cephalexin. Additionally, since 6 months of age, he experienced various infections. These included multiple acute otitis media episodes necessitating myringotomy tubes, an episode of severe pneumonia requiring hospital admission, and multiple skin abscesses. At the age of 12, he had an episode of sacroiliac osteomyelitis with *Staphylococcus aureus* bacteremia, treated with 6 weeks of IV and then oral antibiotics. He additionally experienced 2 lower limb fractures with minimal trauma.

Review of the patient's past medical history revealed that he was born at term following an uncomplicated pregnancy. Parents confirmed delayed loss of two primary teeth which required extraction by a dentist. His medications included cephalexin for skin infection prophylaxis and vitamin D. He had received all childhood immunizations with no difficulty, and had no allergies.

Family history included a father with environmental allergies, and a healthy mother. Parents were Canadians of English/Scottish ancestry and non-consanguineous. He had 2 healthy male full siblings.

On initial physical examination, the patient showed areas of skin excoriation, as well as multiple boils on his arms and legs, most notably on his knees. Ear exam had evidence of bilateral middle ear effusions. The remainder of his exam was unremarkable, with no abnormal facial features, high-arched palate or scoliosis. A full panel of laboratory investigations was performed (Table 1). Overall, the patient had eosinophilia and elevated IgE, with mixed specific vaccine responses. He received a clinical diagnosis of hyper IgE syndrome, with a Grimbacher score of 48, highly suggestive of the disease (Grimbacher et al. 1999).

Proband's son (P-II)

The proband's only son presented to us for evaluation at the age of 6 months for a severe rash and recurrent infections. Similar to his father, he developed a rash

Table 1. **Patient Laboratory Investigations (normal values in parentheses).**

	P-I (Proband) – at 12 y of age	P-II (Proband's son) – at 2 y of age
White blood cells	9.9 (4–10 × 10^9 cells/L)	8.71 (5.14–13.38 × 10^9 cells/L)
Hemoglobin	136 (120–160 g/L)	91 (102–127 g/L)
Platelets	291 (150–400 × 10^9 cells/L)	**455** (202–403 × 10^9 cells/L)
Neutrophils	5.27 (2–7.5 × 10^9 cells/L)	2.58 (1.54–7.92 × 10^9 cells/L)
Eosinophils	**0.76** (0.02–0.5 × 10^9 cells/L)	0.23 (0.03–0.53 × 10^9 cells/L)
Basophils	0.02 (0–0.2 × 10^9 cells/L)	0.04 (0–0.2 × 10^9 cells/L)
Lymphocytes	3.07 (1.5–7 × 10^9 cells/L)	5.34 (1.13–5.52 × 10^9 cells/L)
Monocytes	0.75 (0.05–0.8 × 10^9 cells/L)	0.51 (0.19–0.94 × 10^9 cells/L)
CD19+	503 (200–600 cells/μL)	**1510** (434–1274 cells/μL)
CD3+	1492 (800–3500 cells/μL)	3474 (1578–3707 cells/μL)
CD3+CD4+	1048 (400–2100 cells/μL)	**2557** (870–2144 cells/μL)
CD3+CD8+	305 (200–1200 cells/μL)	734 (472–1107 cells/μL)
CD16+CD56+	**50** (70–1200 cells/μL)	267 (155–565 cells/μL)
IgG	9.5 (7–15.5 g/L)	8.7 (3.2–11.5 g/L)
IgA	0.5 (0.5–3.6 g/L)	0.6 (0–0.9 g/L)
IgM	0.4 (0.4–2.9 g/L)	1.2 (0.4–1.5 g/L)
IgE	**5122** (<179 IU/mL)	**1640** (<450 IU/mL)
Anti-tetanus toxoid IgG	**0.03 → 0.56 after booster** (>0.1 U/mL)	0.26 (>0.1 U/mL)
Anti-measles IgG	Reactive	N/D
Anti-mumps IgG	**Non-reactive**	N/D
Anti-rubella IgG	Reactive	N/D
T-cell PHA stimulation index	3868 (>300)	989 (>300)

Note: *N/D: not done.

starting on the second day of life, initially diagnosed as severe seborrheic dermatitis of his scalp, ears and neck, as well as a pustular rash affecting his trunk. He had received multiple courses of treatment with topical steroids, as well as topical and oral antibiotics. He also had 2 infantile episodes of thrush which resolved after treatment with nystatin. At the age of 6 months, he was admitted to hospital for treatment of a febrile pneumonia which progressed to culture-negative sepsis. He was treated successfully with IV antibiotics. No further past medical history was reported. Upon review in our clinic, he was noted to have extensive eczema/excoriation, with a few pustules noted, but again no abnormal facial features or high-arched palate. Laboratory investigations (Table 1) identified elevated IgE, as seen in the patient's father. His Grimbacher score at the time of presentation was 28.

Genetic Evaluation

The proband underwent whole exome sequencing on a research basis, revealing a heterozygous novel splice-site variant in *STAT3*: c.1110-3C>G, affecting intron 11, predicted to result in defective splicing of exon 12 in the DNA-binding domain (DBD). The variant was confirmed in a clinical laboratory, and later identified in the proband's son as well.

Outcome

The proband is currently 27 years old, while his son is 6 years old. Both have been prophylactically receiving trimethoprim-sulfamethoxazole as anti-*Staphylococcal* coverage, and have remained stable with no severe infections on this prophylactic treatment.

Discussion

STAT3 loss-of-function (LOF) caused by dominant negative pathogenic variants in *STAT3* is the most common genetic cause of HIES (Adam 1993). One area which remains unclear is what exactly is the mechanism of STAT3 LOF. In particular, it is unknown whether variants spanning various disease domains result in negative dominance by the same mechanism. We presented a family affected by AD-HIES, with a novel splice-site variant in the DBD of *STAT3*. Seven other pathogenic variants affecting the splicing of exon 12 have been previously documented in 9 patients, suggesting that this area may be a host-spot for mutagenesis. Of these 7 variants, 5 affected the same splice site as our patient, between intron 11 and exon 12 (Woellner et al. 2010; Sundin et al. 2014; Renner et al. 2008). This also included 1 mutation in the exact same nucleotide as our patient, but with a different substitution

(c.1110-3C>A) (Sundin et al. 2014). The outcome in the reported patients was skipping of exon 12, resulting in a truncated protein (Woellner et al. 2010; Sundin et al. 2014). This loss of 10 amino acids (371–380) within the DBD likely impaired the ability of mutant STAT3 to bind effectively to its cognate targets, impacting downstream gene expression.

In addition to our knowledge gap pertaining to STAT3 LOF mechanism, there is also an incomplete understanding of whether a genotype-phenotype correlation exists for this disorder. Indeed, to date there is no clear correlation between specific variants and disease severity (Adam 1993), although previously an association was made between variants in the Src-homology 2 (SH2) domain and certain connective-tissue features, such as a high-arched palate, inter-alar distance and scoliosis (Heimall et al. 2011). Our patients exhibited several characteristic features of the disease, but a less robust connective tissue phenotype including no characteristic facies, midline anomalies or scoliosis. Sundin et al. proposed that splice-site variants may have a differential impact on various cell types and tissues, given variability in splicing machinery (Sundin et al. 2014). It may therefore stand to reason that patients with such variants may have different tissue involvement than patients with variants in coding regions of *STAT3*. However, this hypothesis is not consistently supported when reviewing phenotypes reported in other patients with splice-site variants (Woellner et al. 2010; Sundin et al. 2014; Renner et al. 2008).

To summarize, we reported on a family with AD-HIES affected by a novel splice-site variant in the DBD of *STAT3*. This report contributes to expanding the genotypic spectrum of the disease, while raising questions regarding a possible genotype-phenotype correlation.

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