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A Novel Variant in the *STAT3* Gene Associated with Autoimmune Enteropathy in a Father–Son Duo

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ABSTRACT: Autoimmune enteropathy (AIE) is a rare clinical condition characterized by intractable diarrhea of early childhood.[1] While AIE can feature both simple Mendelian inheritance or a complex multifactorial pattern, the pathogenesis remains unclear in many cases.[2] This is important for providing an accurate recurrence risk as affected individuals survive until adulthood and have children. Here, we describe a father and son with AIE who had clinically distinct courses. Exome sequencing has revealed a novel missense variant, c.2147C>T; (p.Thr716Met) in the signal transducer and activator of transcription 3 gene (*STAT3*) in both the father and son, consistent with autosomal dominant inheritance. *STAT3* regulates immune reactions, and mutations in this gene have previously been associated with autoimmunity and Hyper IgE syndrome, but not AIE. Further studies are needed to assess the functional effect of this variant as it relates to the phenotype.

KEYWORDS: auto-immune enteropathy, novel mutation, *STAT3*, hypothyroidism, exome sequencing, pediatrics, inheritance

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Introduction

Autoimmune enteropathy (AIE) features intractable diarrhea, with onset often before 6 months of age, in addition to poor weight gain and autoimmune disease.[1,3] The majority of patients are male, and inflammation is generally limited to the small intestine.[4] Circulating IgG anti-enterocyte or anti-goblet cell antibodies are common, but whether they are pathologic or epiphenomena remains unclear.[4] Specific diagnosis can be difficult as some patients do not develop these auto-antibodies. Anti-goblet cell antibodies can also be present in inflammatory bowel disease (IBD), further obscuring diagnosis. Histological findings can mimic celiac disease, as AIE is also associated with intestinal villous blunting, and 30% of affected adults have auto-antibodies to tissue transglutaminase.[4] Additionally, AIE is often complicated by the development of other autoimmune diseases, such as diabetes mellitus, hypothyroidism, and thrombocytopenia, suggesting it may comprise a systemic autoimmune syndrome.[1]

A subset of AIE patients has causative mutations in the X-linked forkhead box P3 (*FOXP3*) gene, which encodes a protein necessary for regulatory T cell (CD4+CD25+) development.[2] These patients have a syndrome of immune dysfunction, polyendocrinopathy, and enteropathy with X-linked inheritance (IPEX, Online Mendelian Inheritance in Man (OMIM) 304790).[5] Other potential causes of AIE, such as CD3 gamma deficiency (MIM 186740), are still under investigation.[6]

Therapy for AIE consists of supportive care, immune suppression, and bone marrow transplantation.[4,7,8] Mortality is high with common causes of death being malnutrition and infection,[9] usually related to neutropenia from AIE or immune suppression from therapy.

In this case, we report the results of exome sequencing in a male infant with AIE who was admitted for poor weight gain.

Case Report

The patient was born at full term after an uneventful pregnancy and delivery. About five months of age, he developed one to two loose stools per day, intermittent emesis, and a slow rate of weight gain. He refused to take more than 25 ounces of formula per day and ate small amounts of table foods. He was admitted for evaluation, with weight less than the first percentile (6.5 kg) at 11 months.

The infant was initially diagnosed with celiac disease because of elevated anti-transglutaminase IgA and biopsy of the duodenum showing inflammation with villous atrophy (see Fig. 1A and B). In addition, he was found to have a low free T4, and levothyroxine therapy was started. Thyroid antibodies were absent, and brain MRI revealed a posterior pituitary lucency, consistent with central hypothyroidism. Sweat chloride was obtained and was normal. His weight improved with gluten-free scheduled feedings, and he was discharged home only to be re-admitted a month later for inadequate weight gain.

Auto-antibodies were obtained, but only anti-enterocyte antibodies were found at that time. Endoscopy showed worsening inflammation of the antrum, duodenum, and colon, consistent with a diagnosis of AIE (see Fig. 1C). Budesonide (9 mg/day) was started with oral feeds (elemental formula and table foods) and supplemented with parenteral nutrition (PN). Over several weeks, he had good weight gain and was eventually discharged home after transitioning from PN to nasogastric (NG) tube supplementation.

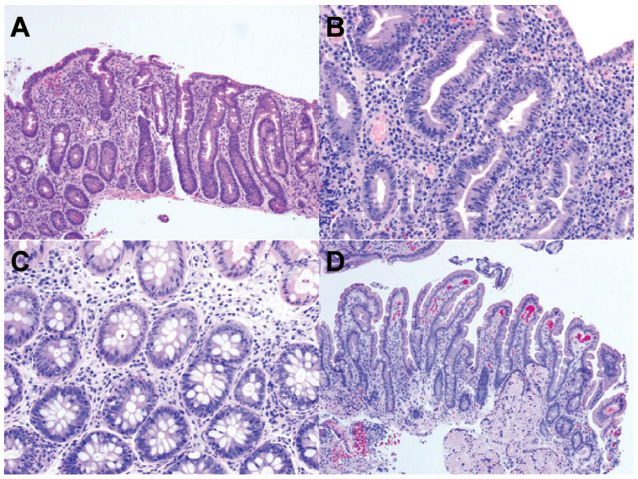

Figure 1. Biopsy of the duodenum (**A** and **B**) at the initial evaluation showed severe villous blunting with crypt hyperplasia and increased intraepithelial lymphocytes. The colon showed increased apoptoses and focal cryptitis (**C**). After treatment, the duodenum showed normal villous architecture (**D**).

Endoscopy was repeated at 22 months of age because of slow weight gain, but showed no gross or histologic diagnostic abnormalities (see Fig. 1D). Inadequate oral intake was suspected because of dysphagia with solid foods and refusal to take adequate formula. NG dietary supplementation was continued. Eventually, oral intake and weight gain improved. Currently, at three years of age, he is doing well on chronic budesonide (9 mg/day), with normal stools, no emesis, and no other gastrointestinal complaints. He is now at the 30th percentile of weight (see Fig. 2). The weight for height is at the 80th percentile. An attempt to decrease budesonide led to vomiting and weight loss, and his dose was increased with improvement in symptoms.

The family history was significant for AIE in the proband's father, who had progressive diarrhea and weight loss shortly after birth, with enterocyte auto-antibodies, as previously described.[7] His enteral inflammation was initially difficult to control and required multiple medical regimens including systemic steroids, budesonide, tacrolimus, azathioprine, cyclosporine, and infliximab. He is currently 32 years old and receives treatment with budesonide and infliximab. He has a history of autoimmune thrombocytopenia and recently developed lymphoma. The proband has one sibling who is healthy and without similar symptoms.

Results

Following receipt of informed consent from the proband's parents, exome sequencing was performed on the parent–child trio, as previously described.[10–13] Briefly, genomic DNA was prepped, enriched for the exome, and sequenced on an Illumina HiSeq 2000 instrument to a mean depth of at least 80 × (Supplement Table 1). In all samples, approximately 90% of the targeted nucleotides were covered by 20 or more reads allowing for high confidence heterozygous variant detection (Supplement Table 1). Given the family history, autosomal dominant inheritance was suspected. Initially, variants were filtered by requiring heterozygosity, shared by the proband and affected father, and not previously detected in more than 2,000 individuals sequenced at the Center for Pediatric Genomic Medicine (CPGM). A total of 61 non-synonymous, short indel, and splicing variants met these criteria (Supplement Table 2). The variants were further prioritized on the basis of occurrence in genes involved in the immune response and/or previously recognized to cause immunologic disorders (Supplement Table 3). Expert review and analysis of all 61 variants revealed a non-synonymous variant in *STAT3* (chromosome 17:40468917-40468917 G>A; c.2147C>T; p.Thr716Met) as the most probable disease-causing variant in the patient and his father. PolyPhen2 predicts the variant to be probably damaging (0.999).[14] This variant was not seen in the mother. Sanger sequencing confirmed the heterozygous

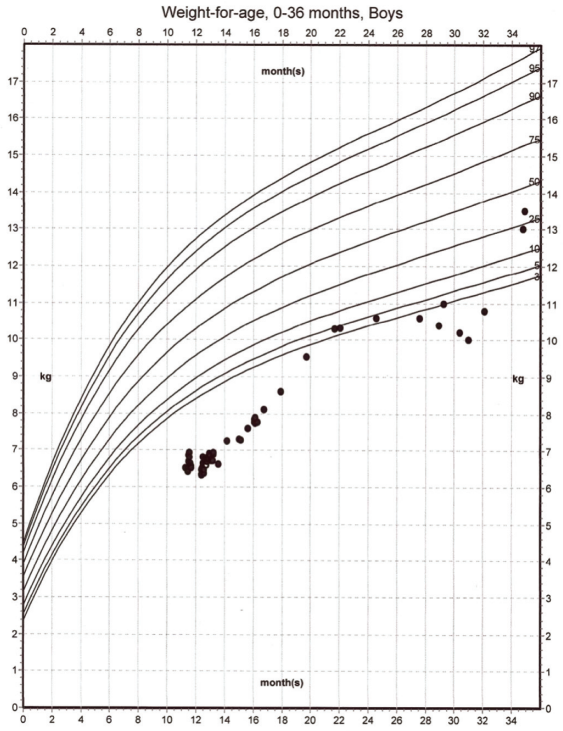

Figure 2. Centers for disease control and prevention growth chart from the time of presentation to the most recent follow-up clinic visit.

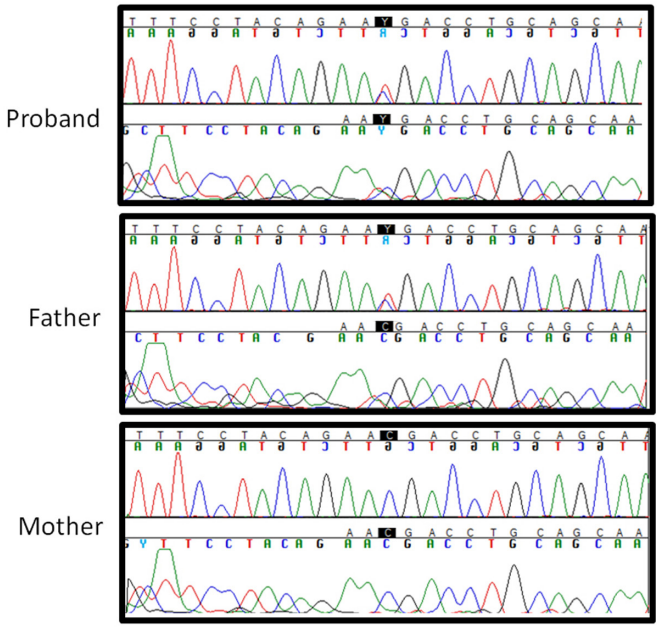

Figure 3. Sanger confirmation of proband, father, and mother.

variant in both the proband and father, but not in the mother (Supplement Fig. 1) (Fig. 3).

The *STAT3* (c.2147C>T) variant is novel, as it is not reported in dbSNP build 139, 1000 Genomes Project,[15] NHLBI Exome Sequencing Project,[16] or Complete Genomics databases.[17] *STAT3* is crucial for IL-17 producing T-helper cells. Without it, they are not able to proliferate normally. Likely, causative mutations in genes known to cause AIE, *FOXP3* and *STAT5*, were not identified.

Discussion

Previously patients with AIE often died at a young age.[9] However, with gains in therapy, patients are living longer and starting families.[7,18] Our case provided a unique opportunity to compare a father–son duo with AIE.

STAT3 is an important immune regulatory gene, and mutations have been associated with autoimmune phenomena[19,20] and Hyper IgE syndrome.[21] Neither the proband nor his father had symptoms of Hyper IgE syndrome, and the proband had normal IgE levels. While *STAT3* has previously shown association with T-cell-mediated autoimmune disease of the central nervous system,[22,23] there are no prior reports of *STAT3*-associated enteropathy. In support of AIE as a novel phenotype associated with *STAT3* variants, the threonine amino acid at position 716 is highly conserved in vertebrates; in 46 vertebrate genomes, only the tree shrew and tarsier have different reported amino acids at this position in *STAT3*. Indeed, Thr716 is found in disparate organisms such as *Xenopus tropicalis* and the Japanese rice fish indicating that this position is important for proper function, otherwise it would not be so highly conserved (Fig. 4). Studies are currently ongoing and are needed to further assess the pathogenicity of this variant.

Prior reports have characterized AIE-associated endocrinopathies as autoimmune phenomena.[1,24] While the proband had hypothyroidism, this appeared to be central in origin based on lab work and MRI, and not autoimmune. His father did not have a concomitant endocrine disorder, but did develop autoimmune thrombocytopenia. These data highlight the potential for significant clinical heterogeneity associated with AIE, even within the same family, as is frequently the case in autosomal dominant disorders. Indeed, although the proband and his father both had poor weight gain, their disease courses were quite different, with the father exhibiting profuse diarrhea while the proband had normal stools and his main symptom was poor weight gain. The father required multiple medications to control inflammation,[7] but the proband's symptoms were well controlled on a maintenance dose of budesonide. However, given the age disparity of the proband and his father, the clinic course of the proband may yet resemble that of his father. It is hoped that the lower exogenous immunosuppression in the proband will allow avoidance of side effects such as secondary infection or malignancy.

Conclusion

AIE is a complex diagnosis with several genetic causes. It has a variable clinical course even with possibly the same causative genetic variant and the spectrum of disease associated with AIE may show incomplete or variable penetrance. Genomic sequencing offers an opportunity both to achieve

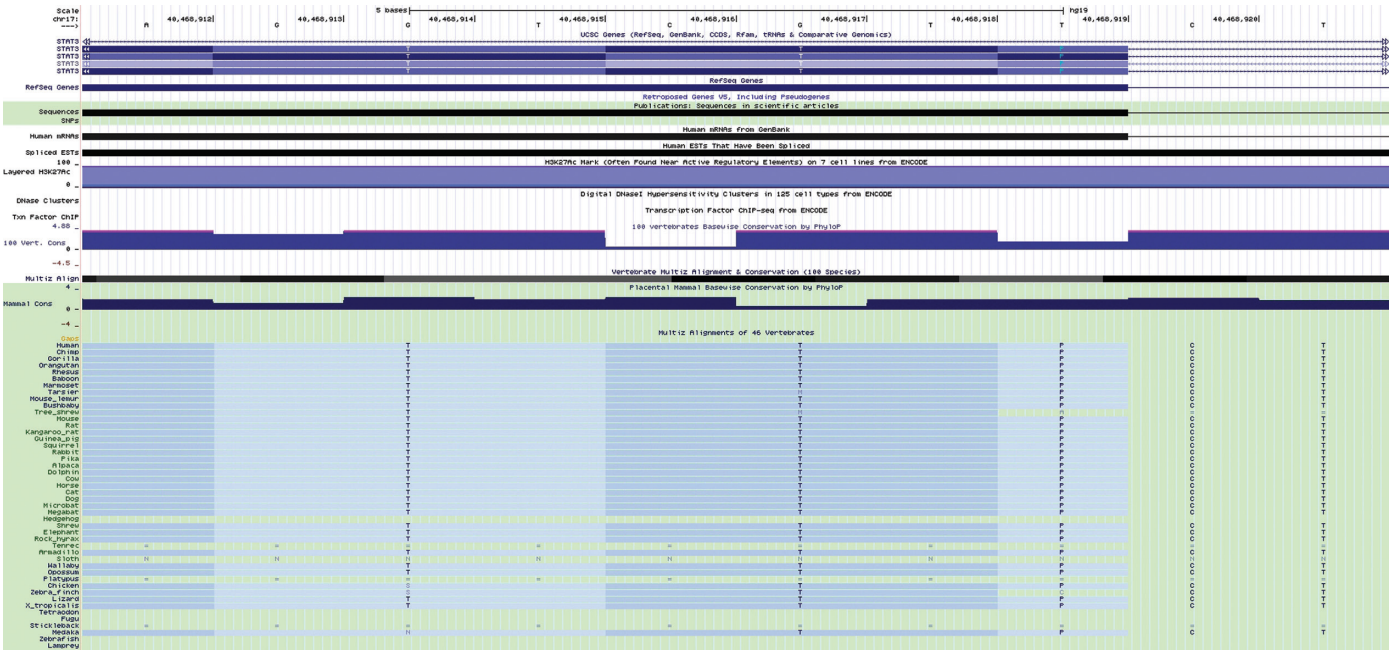

Figure 4. *STAT3* conservation across diverse organisms.

a molecular diagnosis in such conditions and also to suggest novel genotype–phenotype relationships that have the potential to increase our understanding of immune system coordination and regulation.

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Author Contributions

SK and DD conceived and designed the experiments. DD analyzed the data. VS and SS wrote the first draft of the manuscript. VS, DD, CC, LS, CS, JV, SK, and SS contributed to the writing of the manuscript. VS, DD, CC, LS, CS, JV, SK, and SS agreed with manuscript results and conclusions. VS, DD, CC, LS, CS, JV, SK, and SS jointly developed the structure and arguments for the paper. VS, DD, CC, LS, CS, JV, SK, and SS made critical revisions and approved the final version. All authors reviewed and approved the final manuscript.

DISCLOSURES AND ETHICS

As a requirement of publication the authors have provided signed confirmation of their compliance with ethical and legal obligations including but not limited to compliance with ICMJE authorship and competing interests guidelines, that the article is neither under consideration for publication nor published elsewhere, of their compliance with legal and ethical guidelines concerning human and animal research participants (if applicable), and that permission has been obtained for reproduction of any copyrighted material. This article was subject to blind, independent, expert peer review. The reviewers reported no competing interests.

Supplemental Data

Supplement table 1. Exome sequencing summary.

Supplement table 2. Heterozygous variants shared by proband and father and not previously detected in 2,072 samples sequenced at CPGM at Children's Mercy Hospital.

Supplement table 3. Shared variants in OMIM genes.

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