Rapid whole-genome sequencing identifies a novel *AIRE* variant associated with autoimmune polyendocrine syndrome type 1

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Abstract Autoimmune polyendocrine syndrome type 1 (APS-1; OMIM #240300), also referred to as autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED), is a rare monogenic autoimmune disorder caused by mutations in the autoimmune regulator (*AIRE)* gene. APS-1 is classically characterized by a triad of chronic mucocutaneous candidiasis, autoimmune hypoparathyroidism, and autoimmune adrenocortical insufficiency. We report a 5-yr-old female who presented with symptoms of tetany due to hypocalcemia and was subsequently found to be secondary to hypoparathyroidism. Rapid trio whole-genome sequencing revealed compound heterozygous variants in *AIRE* in the proband, with a paternally inherited, pathogenic, frameshift variant (c.1265delC; p.Pro422LeufsTer58) and a novel, likely pathogenic, maternally inherited missense variant (c.268T>C; p.Tyr90His).

[Supplemental material is available for this article.]

CASE PRESENTATION

The patient was a 5-yr-old female with an uncomplicated birth history and normal development. For the first two years of life, she had frequent candidal diaper dermatitis that was responsive to topical antifungal therapy. Between the ages of 2 and 3 yr, she had recurrent oral thrush. That same year she was also evaluated by allergy/immunology for 4 mo of daily urticaria. There was concern at that time for an autoimmune etiology of the urticarial eruption, but it resolved without intervention shortly thereafter. At age 4, she was referred to dermatology for dystrophic nails of the right foot and right thumb. She was diagnosed with onychomycosis which was not responsive to griseofulvin or topical therapies.

Three weeks prior to admission, the patient was noted to have abnormal movements of the upper extremities at school that were concerning for seizure activity. She was evaluated in the emergency room. An electrocardiogram (ECG) showed peaked T waves and a prolonged QT interval (QTc 486 msec, and normal is <450 msec), and the patient was referred to cardiology clinic. No laboratory studies were obtained at that time. She was seen by a

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pediatric cardiologist 1 wk later and a repeat ECG confirmed a prolonged QTc interval at 499 msec. Treatment with a beta blocker was initiated. A 24-h Holter monitor did not reveal arrhythmias. Five days prior to admission, the patient was evaluated in the emergency department (ED) for possible seizure activity. Her father observed 1 min of irregular eye movements associated with generalized hypotonia, followed by 30 min of presumed post-ictal behavior (fatigue and disorientation). The patient reportedly developed a fever earlier that morning and was discharged from the ED with a presumed febrile seizure. On the day of admission, the patient had an event at school where she fell with sustained contraction of her upper extremities lasting ~20 sec. She was evaluated in the ED and laboratory studies were performed which were remarkable for calcium 4.8 mg/dl (reference 8.8–10.8), ionized calcium 0.58 mmol/l (1.10–1.35), magnesium 1.6 mg/dl (1.8–2.3), and phosphorus 12.3 mg/dl (4.1–5.4). Parathyroid hormone (PTH) was <4.0 pg/ml (8.5–72.5). Physical exam was notable for a positive Chvostek's sign. The patient was admitted to the Pediatric Intensive Care Unit (PICU) for management.

The patient was initially treated with intravenous calcium gluconate and magnesium sulfate. She was subsequently transitioned to enteral calcium carbonate and calcitriol. Hypocalcemia persisted; the doses of enteral medications were increased, and teriparatide (recombinant PTH) was added. Her calcium and ionized calcium levels subsequently improved, and intravenous replacement therapy was stopped. A morning cortisol level was within normal limits at 13.6 µg/dl (3.7–19.4). A repeat ECG demonstrated normalization of the QTc interval (433 msec) and T wave morphology following correction of hypocalcemia, therefore beta blockade was discontinued. The patient and her parents were enrolled for trio rapid whole-genome sequencing (rWGS) to determine the etiology of her hypoparathyroidism. She was discharged on enteral calcium carbonate and calcitriol. Follow-up 1 wk after discharge confirmed normal serum calcium levels on this medication regimen.

There was no family history of seizures, thyroid disease, or autoimmune disorders, and the parents denied consanguinity.

TECHNICAL ANALYSIS AND METHODS

Blood was drawn immediately following consent for trio rWGS. DNA was subsequently extracted and sequenced on a HiSeq 4000 (Illumina). Rapid alignment and nucleotide variant calling was performed using the Dragen (Edico Genome) hardware and software (Miller et al. 2015). Yield was 162.7Gb, 149.1 Gb, and 134.5 Gb for the proband, mother, and father, respectively. This resulted in 4,841,028, 4,813,792, and 4,730,860 distinct variant calls, respectively (Supplemental Data 1). Variants were annotated and analyzed in Opal Clinical (Fabric Genomics) (Coonrod et al. 2013). Initially, variants were filtered to retain those with allele frequencies of <1% in the Exome Variant Server, 1000 Genomes Samples, and Exome Aggregation Consortium database (http://evs.gs.washington.edu/EVS/ 2016; Karczewski et al. 2017). A gene panel was built in Phenolyzer (Yang et al. 2015) using Human Phenotype Ontology (HPO) (Köhler et al. 2009, 2016). This panel included 238 genes related to the following HPO terms: hypoparathyroidism (HP: 0000829), hypocalcemia (HP: 0002901), hypocalcemic seizures (HP: 0002199), and hyperphosphatemia (HP: 0002905). Variants were further filtered to retain those mapping to these 238 genes yielding 257 proband calls (130 homozygous variants, 13 heterozygous inherited variants, and 2 heterozygous de novo variants). Manual curation revealed one variant as pathogenic (one very strong, two moderate, and two supporting criteria) and one variant as likely pathogenic (no strong, four moderate, and two supporting criteria) (Supplemental Data 2, 3) by ACMG guidelines (Richards et al. 2015). The diagnosis was made by rWGS in 5 d, and was reported following Sanger confirmation in 11 d (see Table 1).

Table 1. Characteristics of the variants leading to a diagnosis of APS-1

Gene	Genomic location	HGVS cDNA	HGVS protein	Zygosity	Parent of origin	Variant interpretation
AIRE	NC_000021.9 (on Assembly GRCh38): g.44293161delC	NM_000383.3: c.1265delC	NP_000374.1 p.Pro422LeufsTer58	Heterozygous	Father	Pathogenic
AIRE	NC_000021.9 (on Assembly GRCh38): g.44286693A>G	NM_000383.3: c.269A>G	NP_000374.1 p.Tyr90His	Heterozygous	Mother	Likely pathogenic

VARIANT INTERPRETATION

This patient was found to be compound heterozygous for a paternally inherited, known pathogenic frameshift variant (c.1265delC; p.Pro422LeufsTer58; rs764878471) and a likely pathogenic, maternally inherited, novel missense variant (c.268T>C; p.Tyr90His) in the *AIRE* (autoimmunity regulator) gene. Biallelic pathogenic variants in *AIRE* have been implicated in autoimmune polyendocrine syndrome type 1 (APS-1) also known as autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) (OMIM #240300).

The maternally inherited p.Tyr90His is a novel missense variant. It lies within the caspase activation and recruitment domain (CARD), which is essential for AIRE homo-oligomerization and nuclear localization (Abramson and Goldfarb 2016; Passos et al. 2018). The majority of reported disease-causing variants within this domain are missense changes, supporting that this region is missense intolerant. A variant at the same location with a different amino acid change, p.Tyr90Cys, has been previously reported as pathogenic (Pearce et al. 1998; Peterson et al. 2004; Oftedal et al. 2015). p.Tyr90Cys was observed in *trans* with a pathogenic *AIRE* variant. p.Tyr90Cys has been observed twice in 245,928 alleles in GNOMAD (once as a homozygote; http://gnomad.broadinstitute.org/gene/ENSG00000160224). The p.Tyr90His variant is absent from population databases, suggesting it is a rare variant. In silico analyses predict this variant to be detrimental. Based on the combined evidence, this variant is classified as likely pathogenic.

The paternally inherited p.Pro422LeufsTer58 pathogenic variant has been reported previously in a patient with autoimmune polyendocrinopathy syndrome type 1; however, no additional variant was identified in that patient (Heino et al. 1999). p.Pro422LeufsTer58 has been observed five times in 210,636 alleles in GNOMAD (none as a homozygote; http://gnomad.broadinstitute.org/gene/ENSG00000160224). The deletion causes a frameshift starting with codon Proline 422, changes this amino acid to a Leucine residue and creates a premature Stop codon at position 58 of the new reading frame. This pathogenic variant is predicted to cause loss of normal protein function either through protein truncation or nonsense-mediated mRNA decay.

DISCUSSION

The *AIRE* gene is located on Chromosome 21q22.3. It encodes a protein that has a crucial role in immunity. AIRE is a transcription factor that functions by regulating expression of tissue-restricted antigens in medullary thymic epithelial cells and, subsequently, the T cells that respond to those proteins (autoreactive) are subject to negative selection and undergo apoptosis. Proper functioning of AIRE is essential for the detection of autoreactive T cells and the prevention of autoimmune disease (Heino et al. 2001; Zaidi et al. 2017; Passos et al. 2018). Pathogenic variants in *AIRE* result in a failure to eliminate autoreactive T cells in the thymus, resulting in autoimmune manifestations seen in APS-1 (De Martino et al. 2013).

APS-1 is a rare autoimmune disease that is characterized by three major clinical features: chronic mucocutaneous candidiasis, hypoparathyroidism, and adrenal insufficiency. Development of at least two of the three major clinical features establishes a clinical diagnosis of APS-1. In addition to the major clinical features, individuals may develop many other endocrine and/or nonendocrine symptoms. Other clinical findings that have been reported in multiple affected individuals include enamel hypoplasia, diarrhea, and/or constipation resulting from malabsorption, alopecia, vitiligo, premature ovarian failure in females, autoimmune hepatitis, and type 1 diabetes mellitus (Table 2; Peterson et al. 2004; De Martino et al. 2013; Orlova et al. 2017). The main immunological finding in the endocrine disorders of APS-1 is the existence of high levels of serum antibodies reacting specifically with components of the

Table 2. Phenotypic autoimmune polyendocrine syndrome, type 1 features

Features	Prevalence of feature (%)	Mean age at onset (yr)	Proband (II-1)	Age of onset in proband
Phenotypic features				
Hypoparathyroidism	78	7	Yes	5 yr
Chronic mucocutaneous candidiasis	75	5	Yes	<2 yr
Adrenal insufficiency	67	10	No	
Primary ovarian failure (in females)	48	15	No	
Alopecia/alopecia areata	34	11	No	
Malabsorption/chronic diarrhea	25	11	No	
Dental enamel hypoplasia	28	16	No	
Hypo/hyperthyroidism	13	9	No	
Autoimmune hepatitis	11	5	No	
Insulin-dependent diabetes mellitus	9	12	No	
Vitiligo/hair depigmentation	9	12	No	
Pernicious anemia	8	11	No	
Ptosis	8	7	No	
Periodic fever with rash	5	3	Yes	
Retinopathy	4	2	No	
Chronic blepharitis/dry eyes	4	11	No	
Metaphyseal dysplasia	4	11	No	
Asplenia	2	22	No	
Ectodermal dystrophy	n.d.	n.d.	Yes	3–4 yr
Pituitary defects	n.d.	n.d.	No	
Cholelithiasis	n.d.	n.d.	No	
Lupus erythematosus	n.d.	n.d.	No	
Partial diabetes insipidus	n.d.	n.d.	No	
Novel clinical features				
Urticarial eruption			Yes	1.5–2 yr[a]
Recurrent otitis externa			Yes	
Seizures			Yes	5 yr
Long QTc interval			Yes	Secondary to hypocalcemia

The list of clinical features is based on the OMIM clinical synopsis related to AIRE (#240300; Autoimmune polyendocrine syndrome, type 1), Orlova et al. 2017, and Oftedal et al. 2015.
[a]From a single report: Ferre et al. 2016.

affected organs (Bruserud et al. 2016). A recent paper identified 23 different clinical features reported in a cohort of 112 patients, with a mean of five features presenting in each patient (Orlova et al. 2017). The initial presenting symptom(s) can vary by individual, but typically develops during childhood and teenage years. The majority of individuals first present clinically with chronic candida infections and/or hypoparathyroidism. Overall, this condition is highly variable in the number of clinical features an individual will develop and the severity of the disease.

There are approximately 400 cases of APS-1 reported worldwide (Orlova et al. 2017). It is suspected that this condition is underdiagnosed because of a lack of knowledge of the condition, the possible long intervals between the development of clinical features, the wide spectrum of clinical presentation, and the variability in disease severity. Prevalence in European countries has been estimated at ∼1/80,000–1/130,000. There is thought to be a higher prevalence in Finland (1/25,000), Sardinia (1/14,400), and for those with an Iranian Jewish (1/9,000) background because of a founder mutation effect. Inheritance of APS-1 is typically autosomal recessive. However, there have been reports of individuals presenting with a later onset, milder and more phenotypically variable form of APS-1, who have dominant-negative, missense pathogenic variants of the AIRE PHD1 domain (Cetani et al. 2001; Oftedal et al. 2015). Of note, no second variant was identified by exonic sequencing of *AIRE* in the prior patient with p.Pro422LeufsTer58-associated APS-1 (Heino et al. 1999). The current patient's parents, however, were healthy, and the variants reported herein were of types typically associated with autosomal recessive inheritance.

The diagnosis of APS-1 explained the etiology of the patient's hypoparathyroidism, which was the cause of her hypocalcemia and associated tetany and QT prolongation. Retrospectively, it also provided an explanation for the skin and nail disorders that she suffered from previously. Additionally, this diagnosis identified a profile of disorders that she is at risk of developing in the future, facilitating timely referral to appropriate subspecialty care and an opportunity for early recognition of potentially life-threatening conditions such as adrenal insufficiency. In fact, at a recent endocrinology follow-up visit she was found to have an elevated renin level, indicating that she is now in the process of developing Addison's disease.

APS-1 is a progressive syndrome, with symptoms manifesting at different time intervals. Chronic mucocutaneous candidiasis and hypoparathyroidism classically appear early in childhood, whereas adrenal insufficiency typically has an onset early in the second decade of life (Neufeld et al. 1981; Ahonen et al. 1990). The autoimmunity of APS-1 primarily affects endocrine tissues, although nonendocrine manifestations are becoming increasingly recognized (Bruserud et al. 2016; Ferre et al. 2016). Prior to the development of molecular diagnosis, the syndrome was diagnosed in patients with at least two out of three of the following: hypoparathyroidism, adrenal insufficiency, and chronic mucocutaneous candidiasis (Neufeld et al. 1981; Ahonen et al. 1990). However, given the widely variable phenotypic presentation, using the classic criteria may unnecessarily delay diagnosis of APS-1 (Ferre et al. 2016).

This case demonstrates the clear clinical utility of rWGS in assisting with timely identification of a disorder with a wide genetic differential diagnosis (hypocalcemia with hypoparathyroidism) and variably evolving phenotype. Establishing a molecular diagnosis in such disorders can allow detection of expanded phenotypic manifestations, such as urticarial, recurrent otitis externa, and long QTc interval herein. Establishing the diagnosis of APS-1-associated hypoparathyroidism revealed the etiology of QT prolongation, indicated the appropriate management to be correction of the serum calcium rather than beta blockade, and may have prevented the occurrence of additional, potentially catastrophic hypocalcemic syncope, seizures, and arrhythmias. The patient will also be closely followed by endocrinology for the development of potentially life-threatening adrenal insufficiency in the future. The presence of adrenal autoantibodies (most commonly to 21-hydroxylase) is associated with a rate of development of adrenal insufficiency of 19% per year (Ahonen et al. 1987).

Monitoring patients for development of CYP21A2 antibodies has been found to be adequately predictive for adrenal insufficiency (Soderbergh et al. 2004). Additionally, early diagnosis of APS-1, before the appearance of additional tissue-specific antibodies, provides a window of opportunity for proactive management with experimental therapies. Recently, there have been some isolated cases of success using the monoclonal antibody rituximab (Popler et al. 2012; Gouda et al. 2013).

SUMMARY

We describe a novel maternally inherited missense variant (c.268T>C, p.Tyr90His) in *AIRE*, an autoimmune regulator gene on Chromosome 21q22.3. This variant was observed in trans with a paternally inherited variant in *AIRE* that has previously been classified as pathogenic (c.1265delC, p.Pro422LeufsTer58; Heino et al. 1999). Thus, the proband was determined to be a compound heterozygote for the monogenic disorder autoimmune polyendocrine syndrome type 1 (APS-1, also referred to as APECED). A timely etiologic diagnosis was made by rWGS, which identified a syndrome that potentially affects many different organ systems and was low on the initial clinical differential diagnosis for this patient. The rapidity of WGS allowed the clinicians to avoid a prolonged stepwise search for a causative gene and facilitated timely intervention by the appropriate subspecialists.

ADDITIONAL INFORMATION

Data Deposition and Access

Ethics Statement

Informed and signed consent forms were obtained for all sequenced individuals in this study. The project is approved by the Institutional Review Board of the University of California at San Diego under protocol #160468 and has received nonsignificant risk status in a pre-Investigational Device Exemption submission to the Food and Drug Administration.

Author Contributions

E.S. contributed to manuscript preparation and phenotyping; K.W. contributed to variant interpretation and manuscript preparation; S.N. contributed to variant interpretation and analysis supervision; M.G. contributed to clinical implementation; N.G.C. contributed to clinical implementation; L.F. and D.D. contributed to manuscript preparation and supervised the study; S.F.K. contributed to variant interpretation, manuscript preparation, and supervised the study; and the RCIGM Investigators contributed to process development, infrastructure deployment, and maintenance. All authors contributed to the reviewing of the final version. The Rady Children's Institute for Genomic Medicine Investigators involved in this project were Julie Cakici, Michelle Clark, Yan Ding, Jennifer Friedman, Joseph Gleeson, Mary Gaughran, Jeffrey Gold, Amber Hildreth, Sara Martin, Julie Ryu, Lisa Salz, Nathaly Sweeney, Narayanan Veeraraghavan, and Kristin Wigby.

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