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Short Communication

# A novel heterozygous mutation of the *AIRE* gene in a patient with autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy syndrome (APECED)

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ABSTRACT

*Background:* Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy syndrome (APECED) is an autosomal recessive disease due to mutations of the autoimmune regulator (*AIRE*) gene. Typical manifestations include candidiasis, Addison's disease, and hypoparathyroidism. Type 1 diabetes, alopecia, vitiligo, ectodermal dystrophy, celiac disease and other intestinal dysfunctions, chronic atrophic gastritis, chronic active hepatitis, autoimmune thyroid disorders, pernicious anemia and premature ovarian failure are other rare associated diseases although other conditions have been associated with APECED.
*Case presentation:* What follows is the clinical, endocrinological and molecular data of a female APECED patient coming from Lithuania. The patient was affected by chronic mucocutaneous candidiasis, hypoparathyroidism and pre-clinical Addison's disease. Using direct sequencing of all the 14 exons of the *AIRE* gene in the patient's DNA, we identified in exon 6 the known mutation c.769 C>T (p.Arg257X) in compound heterozygosity with the newly discovered mutation c.1214delC (p.Pro405fs) in exon 10. The novel mutation results in a frameshift that is predicted to alter the sequence of the protein starting from amino acid 405 as well as to cause its premature truncation, therefore a non-functional Aire protein.
*Conclusions:* A novel mutation has been described in a patient with APECED with classical clinical components, found in compound heterozygosity with the c.769 C>T variation. Expanded epidemiological investigations based on *AIRE* gene sequencing are necessary to verify the relevancy of the novel mutation to APECED etiopathogenesis in the Lithuanian population and to prove its diagnostic efficacy in association with clinical and immunological findings.



## 1. Introduction

Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy syndrome (APECED) (OMIM ID: 240300) also known as Type 1 autoimmune polyglandular syndrome (APS1) is an inherited rare autosomal recessive disorder, characterized by various endocrine deficiencies [reviewed (rev) in (Fierabracci, 2011)]. While a high prevalence for the disease is reported in Finland (1:25,000) and Scandinavia (Norway) (Ahonen et al., 1990), there are also clusters of similar patients in Continental Italy (Scott et al., 1998) and Sardinia (1:14,000) (Rosatelli et al., 1998), as well as in other countries (Faiyaz-Ul-Haque et al., 2009; Orlova et al., 2010). Patients frequently develop high titers of autoantibodies against molecular targets in their affected endocrine organs. Criteria for the diagnosis of APS1 are the presence of at least two of the following disorders: chronic mucocutaneous candidiasis, hypoparathyroidism and primary adrenal insufficiency (Addison's disease). Type 1 diabetes, alopecia, vitiligo, ectodermal dystrophy, celiac disease and other intestinal dysfunctions, chronic atrophic gastritis, chronic active hepatitis, autoimmune thyroid disease, pernicious anemia and premature ovarian failure are other rare associated diseases. The locus for APS1, the *AIRE* (Autoimmune Regulator) gene has been mapped on chromosome 21q22.3 (Fierabracci, 2011). To date over 60 mutations of the *AIRE* gene were discovered. *AIRE* gene mutations may help to facilitate the genetic

*Abbreviations:* aa, amino acid(s); Abs, antibodies; ACTH, adrenocorticotropic hormone; AIRE, autoimmune regulator gene; APECED, autoimmune polyendocrinopathy–candidiasis–ectodermal dystrophy syndrome; APS1, Type 1 autoimmune polyglandular syndrome; BCT, brain computer tomography; bp, base pair(s); GADA, glutamic acid decarboxylase autoantibodies; GAD65, glutamic acid decarboxylase isoform 65; IA 2, insulinoma associated antigen 2; IFN ω, interferon ω; Ig, immunoglobulin(s); nr, normal range; PCR, polymerase chain reaction; PHD, Plant Homeo Domain (AIRE gene); PTH, parathyroid hormone; rev, reviewed; RIA, radioimmunoassay; AT, annealing temperature; ST, standard deviation; Tg, thyroglobulin; TPO, thyroperoxidase.

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diagnosis of APS1 in different groups of patients (Fierabracci, 2011). c.769 C>T (p.Arg257X) was demonstrated to be responsible for 82% of APS1 alleles in Finnish, while c.254A>G (A374G, p.Tyr85Cys) in Iranian Jewish patients (Bjőrses et al., 2000). In Sardinia c.415C>T (p.Arg139X) mutation was common in APS1 patients (Rosatelli et al., 1998). A 964del13 mutation in the *AIRE* gene encoding a p.Cys322fsX372 change was present in 71% of the British and 56% of the United States APS1 patients (rev in Bjőrses et al., 2000). All APS1 patients from 15 Irish families carried a c.967_979del (p.L323_L327>SfsX51) mutation (rev in Bjőrses et al., 2000). Despite the known monogenic etiology, there are no known genotype/phenotype correlations in different populations, except for the missense mutation in exon 6 c.682T>G (p.Gly228Trp) identified in an Italian family with APECED, closely cosegregating with hypothyroid autoimmune thyroiditis (Cetani et al., 2001).

Below is a report of a case of APECED with a newly discovered *AIRE* gene mutation.

## 2. Materials and methods

### 2.1. Molecular analysis of the AIRE gene

To confirm the diagnosis of APECED genomic leukocyte DNA was extracted from whole blood of the patient and her mother by QIAamp DNA blood mini kit (Qiagen, Hilden Germany). PCR (polymerase chain reaction) was carried out with specific primers for all 14 exons of the gene (GenBank ID: AJ009610). Primer sequences were selected as following: exon 1 forward 5′-CGTGCCAGTGTCCCGGGACCCACC-3′ and reverse 5′-GGGCGGGGTTCCTCCTGGAACTTCC-3′ [annealing temperature (AT) 55 °C] identified a product of 276 bp (base pair) (Cervato et al., 2009); exon 2 forward 5′-AGTCATGATGGAGATGGGC-3′ and reverse 5′-GAGCAGGTGACAGCAGC-3′ (AT 62.5 °C) identified a product of 330 bp; exon 3 forward 5′-GTCTGGCCAAGGTGTCC-3′ and reverse 5′-GC AGTGGGTGGGAGC-3′ (AT 58.5 °C) identified a product of 350 bp; exon 4 forward 5′-GGCACTCACCCCCACT-3′ and reverse 5′-ACACCAGGCCAGC ACG-3′ (AT 62.5 °C) identified a product of 280 bp; exon 5 forward 5′-GCATAGAGTATGTGCTTGG-3′ and reverse 5′-TCCGGTCTGCTGTGG-3′ (AT 58.5 °C) identified a product of 330 bp; exon 6/7 forward 5′-CTGG GGCCTACACGACTGC-3′ and reverse 5′-TGCCCAGGTAAAGGCAGAGG-3′ (AT 67 °C) identified a product of 650 bp; exon 8 forward 5′-AAGG AGGTGGCTCTCAGGA-3′ and reverse 5′-CTTCCCTTCAGGGTCAGTGG-3′ (AT 58.5 °C) identified a product of 310 bp (Cervato et al., 2009); exon 9 forward 5′-CGCTGTCTTGTTCTGCATGT-3′ and reverse 5′-ACAGGACTC CAGGGGACAG-3′ (AT 58.5 °C) identified a product of 251 bp (Cervato et al., 2009); exon 10 forward 5′-CCTGGGTTTCAGGGTCCC-3′ and reverse 5′-CCCCAGGCCCTGTGC-3′ (AT 65.8 °C) identified a product of 500 bp; exon 11 forward 5′-TCGGGTTGAGCTACATTTCC-3′ and reverse 5′-GTGT GGTTGTGGGCTGTATG-3′ (AT 58.5 °C) identified a product of 279 bp (Cervato et al., 2009); exon 12 forward 5′-GAGGTGGCACTCCTGCTC-3′ and reverse 5′-TCTGCCCTGAGATGTGCTC-3′ (AT 58.5 °C) identified a product of 247 bp (Cervato et al., 2009); exon 13 forward 5′-GAGC TGGGTGTAAGAATTCCC-3′ and reverse 5′-ACGGCTCAAGAGCAGTGG-3′ (AT 58.5 °C) identified a product of 270 bp. Two couple of primers were used to amplify exon 14 (Cervato et al., 2009): forward 5′-GGAG GTTCTCACCGTCACTC-3′ and reverse 5′-AGTAGGTCACCAGGCAAGGA-3′ (AT 58.5C) with a product of 344 bp; and forward 5′-AATTAAA CCCTGCCCCACTT-3′ and reverse 5′-TCCATTCAGGAAGCTGGAAC-3′ (AT 58.5 °C) with a product of 364 bp. PCR sequencing was carried out with the BigDye Terminator v.3.1 Cycle sequencing protocol (LifeTechnologies, Applied Biosystem, Paisley, Scotland, UK). Products were then purified and sequenced with the Genetic Analyzer 3500 (Applied Biosystem HITACHI system).

### 2.2. Homology modeling

The homology modeling of the second PHD finger domain of Aire protein was made with the program MODELLER (9v8) (Sali and Blundell, 1993), using as a template the first conformer of the NMR multi-model structure of the first PHD finger of Aire protein (Protein Data Bank accession code 1XWH). In particular, the model was generated for the amino acid interval 432–480 of Aire by sequence alignment (without gaps) with the amino acid interval 297–345 of the template structure (the two amino acid intervals share 35% amino acid identity). The two $Zn^{2+}$ ions bound by PHD domain have been added to the model accordingly to the positions of these metals as available in the structure of the template.

## 3. Results

### 3.1. Case presentation

A girl was born at 40 weeks gestation after an uncomplicated pregnancy and delivery. The female patient was the second daughter of non-consanguineous parents (parental age 30 years), without any family history for autoimmune diseases. The patient and her family were from Lithuania. At 7 months of age, she was referred to a local hospital in Lithuania, because of a febrile seizure. At the physical examination mucocutaneous candidiasis was revealed in an oral examination (Table 1).

**Table 1**
Clinical presentation, laboratory and instrumental parameters of the APS1 patient.

| Age | Clinical presentation/ therapy | Laboratory and instrumental parameters |
|---|---|---|
| Birth | Height +0.69SD<br>Weight −0.46SD | |
| 7 months | **Febrile seizure, oral mucocutaneous candidiasis** | |
| 6 years | **Repeated syncopes and tonic clonic seizures**<br>**Primary hypoparathyroidism**<br>**Ungual, throat, external ear canal candidiasis**<br>Poor response to topical and systemic anti-fungal agents<br>Oral 0.75 μg/day calcitriol in 3 doses and 3 g/day calcium gluconate | **Calcium 5.58 mg/dl (nr 8.5–10.5 mg/dl)**<br>**Phosphatemia 8.7 mg/dl (nr 2.5–5 mg/dl)**<br>**PTH 11.6 pg/ml (nr 10–65 pg/ml)**<br>**BCT: bilateral calcification in the basal ganglia**<br>**Present IgG Abs against *Candida***<br>**Delayed skin hypersensitivity test, lymphoblast stimulation test to Candida antigens positive**<br>Normal thyroid function; anti-Tg, anti-TPO negative<br>Anti-insulin, GADA; anti-IA2 Abs negative<br>morning fasting serum cortisol 19.76 μg/dl (nr 4–22)<br>**ACTH 58.7 pg/ml (nr 0–46)**<br>ACTH test: sufficient steroigogenic response<br>**Anti-21 hydroxylase Abs positive**<br>Normal liver function<br>Liver–kidney microsomal Abs negative |
| 9.3 years | Height −0.95SD; weight −0.57SD<br>**Transient occipital alopecia**<br>**Few episodes of tonic–clonic seizures**<br>**Preclinical Addison's disease** | Calcium 10.3 mg/dl; inorganic phosphate 5.3md/dl<br>Morning fasting serum cortisol 19.5 μg/dl<br>**Stimulated cortisol 24.93 μg/dl (nr>20)**<br>**Plasma ACTH 79.2 pg/ml**<br>Plasma renin 70 pg/ml (nr 5–143)<br>Aldosterone 361 pg/ml (nr 75–455)<br>**Anti-IFN ω Abs positive**<br>**AIRE: exon 6 c.769 C>T; exon 10 c.1214delC** |

Altered parameters are in bold.
Normal range (nr); thyroglobulin (Tg); thyroperoxidase (TPO).
Abs = antibodies; GADA = glutamic acid decarboxylase autoantibodies.
BCT = brain computer tomography.