Case Report

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Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy: report of three cases from Iran

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Abstract: Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED), also named as autoimmune polyglandular syndrome (APS) type 1, is a rare autosomal recessive disorder caused by mutations in autoimmune regulator (*AIRE*) gene. It is distinguished by an immune-mediated damage of endocrine tissues, chronic candidiasis, and ectodermal disorder. APECED has been shown to be frequent in some populations including Iranian Jews. Here we report three cases of APECED from two independent Iranian Muslim families. Addison's disease, hypoparathyroidismand mucocutaneous candidiasis were shared clinical manifestations in all patients. Mutational analyses have demonstrated a novel homozygous splice site mutation (c.1095+2T>A) in intron 9 and a previously identified homozygous nonsense mutation (c.415C>T) in exon 3 of patients respectively. Future studies are needed to evaluate the frequency of these variants in Iranian APECED patients which would facilitate genetic counseling as well as prenatal diagnosis.

Keywords: *AIRE*; autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy; mutation.

Introduction

Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED), also named as autoimmune polyglandular syndrome (APS) type 1 (OMIM 240300), is a rare autosomal recessive disorder which is distinguished by an immune-mediated damage of endocrine tissues, chronic candidiasis, and ectodermal disorder. The major clinical features are candidiasis, hypoparathyroidism, and adrenocortical failure. The presence of at least two of these major features confirms the diagnosis of APECED [1]. Although the disease usually arises in childhood, new tissue-specific symptoms may emerge during life [2]. Some other clinical manifestations such as alopecia and dental enamel hypoplasia have been detected in patients [3]. APECED has been shown to be caused by mutations of a single gene, named autoimmune regulator (*AIRE*) [4]. Its specific expression in medullary epithelial cells, thymic monocyte-derived cells, lymph nodes, the spleen, and in fetal liver implies its role in induction and maintenance of immune tolerance [1]. To date, more than 100 different mutations including nonsense and missense mutations, small insertions and deletions leading into frame shifts, and splice site mutations have been identified in different ethnic groups. APECED has been shown to be prevalent in some populations including the Finns (1:25,000), Sardinians (1:14,400), and Iranian Jews (1:9000) [5]. In Norway, its prevalence has been reported as 1/80,000 [6]. In the present report we introduce three cases of APECED from two Iranian Muslim families.

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Case presentation

Case 1

The patient was a 9-year-old girl first child of healthy third-cousin Iranian Muslim parents. Early signs of hypothyroidism were detected for the last 4 years. Afterwards, very low serum calcium and PTH levels which required supplemental calcium and vitamin D therapy led to hypoparathyroidism diagnosis. Subsequently she was demonstrated to be suffering from Addison's disease. Recently, she developed signs of mucocutaneous candidiasis that waxed and waned. Other hormone assessments as well as biochemical and hematologic tests were normal. No other autoimmune or non-autoimmune diseases were detected in the patient. She died at the age of 9 without any specific diagnosis.

Cases 2 and 3

Two siblings of healthy first-cousin Iranian Muslim parents were diagnosed as APECED. The first sibling is a 16-year-old male who suffered from Addison's disease at the age of 6, followed by a presentation of diabetes mellitus and hypothyroidism at the age of 10 and hypoparathyroidism for the previous 2 years. Additionally, he has suffered from mucocutaneous candidiasis, dry eyes and chronic diarrhea for the last 4, 5, and 6 year, respectively. His 9-year-old brother was recently diagnosed as APECED presented by Addison's disease and hypoparathyroidism. Other hormone assessments as well as biochemical and hematologic tests were normal in both siblings. No other autoimmune or non-autoimmune diseases have been detected in these siblings.

Identification of *AIRE* mutations

Blood samples were collected from the patients and their parents in EDTA tubes after informed consents were obtained according to the protocol approved by the local institutional review board. DNA was isolated using the standard salting out method. Based on the clinical features of the patients, which confirmed the diagnosis of APECED, mutational screening of the *AIRE* gene (transcript reference sequence: NM_000383) was performed. For this purpose, 100 ng of genomic DNA was amplified in a total volume of 25 μL reaction mixture by Taq DNA Polymerase Master Mix Red (Ampliqon, Denmark). The PCR conditions were as follows: denaturation at 94 °C for 4 min, then 30 cycles of denaturation at 94 °C for 30 s, annealing at a specific temperature for each primer set, and extension at 72 °C for 30 s, except for the final cycle, for which extension was for 4 min and 30 s. Primer sequences are presented in Table 1. Direct sequencing of the *AIRE* gene using the ABI Prism3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) revealed that case 1 was homozygote for a novel splice site change (variant of unknown significance) c.1095+2T>A in intron 9, which belongs to the conserved splice donor sequence. Bioinformatics analysis with Human Splicing Finder – UMD (www.umd.be/HSF3) has shown that such a nucleotide change will result in alteration of the wild type donor site, most probably affecting splicing. In addition, the Combined Annotation Dependent Depletion (CADD) tool which is a tool for scoring the deleteriousness of single nucleotide variants as well as insertion/deletions variants in the human genome indicated that this variant would be deleterious with a score of 21.0. Targeted sequencing on the parents demonstrated that both were heterozygote for the identified variant. In the second family, affected members

Table 1: Primer sequences and PCR conditions.

Exon	Forward primer (5′→3′)	Reverse primer (5′→3′)	Product size, bp	Tm, °C
1	CCTTTGCTCTTTGCGTGGTC	TAACTTGTCATCAGGGACGCC	482	60
2	CCTGGGAGCTCCACCCTCTAGT	CACCACTCCGGTTCCAGTCCA	354	64
3	TGGCCAAGGTGTCCAGTTCT	TCTAGTACCCAGAGGAGACC	388	60
4	GCAAAGGGACTACCCAGCACT	TAGGACAGGGTCTCAGAGGGCA	290	62
5	GCTGCCTGCTTCTGGCATAGA	GGCGTGGTCCTCCTTCCATCTT	264	62
6	TCTGCTAGACCCCACCCTG	GCCCCCAGCAGAGCCACT	327	58
7	GAACAGCGTTGCCTCTGG	AGTGCCCAGGTAAAGGCAG	299	59
8	GGAGTTCAGGTACCCAGAGA	TGACTCAGAACCCCTTTCCA	352	60
9	CGTGGGTTTGGGGATCTGTC	GGGACATAGTGCTATGGCTGG	310	60
10	CCACTCAGTGTGGACGCCTT	TGAATTCATCCGCCCCGTAG	417	60
11	CACACCCCATACCCCGGA	CTGGTGCAAGCCCTCGAAG	295	60
12	CCAGTGGAGCTGGGTGTAAG	GAGTTTCCACGGCTCAAGAGC	319	60
13	CCTGCGGCCTCTGTACCC	AGAGTGGGGAGCCTGGGTG	209	61
14	AGGTTCTCACCGTCACTCTGT	ACTGACAAGAGGTGGCGCTGT	219	58

were homozygous for a nonsense mutation c.415C>T (p.R139X) in exon 3 (Figure 1).

Discussion

Among autoimmune disorders, APECED has the specific feature of resulting from mutations in a single gene which facilitates genetic counseling and risk assessment. Numerous APECED causing mutations have been detected in different populations up to now. Although, APECED has been reported in Iranian Jews previously (Table 2), to our knowledge the present cases are among the first reported Iranian Muslim cases. Previous reports have identified Y85C, R139X, R257X, K50NfsX168, and L323SfsX51 mutations in Iranian patients [5, 7]. We have demonstrated a novel splice site mutation as well as a previously recognized nonsense mutation in *AIRE* gene in these Iranian families. R139X mutation has been shown to be a frequent mutation in Sardinian patients accounting for the majority of independent Sardinian APECED alleles [2]. As it had not been detected in other populations, it had been claimed to be uniquely Sardinian [2]. However, this mutation has been identified in one Egyptian patient in a different haplotype afterwards [8] and in Iranian [7] and Sicilian patients as well [9]. These studies put the previous thoughts about uniqueness of this mutation in Sardinians under question and demonstrate that it is a frequent mutation in APECED patients.

Establishment of a genotype-phenotype correlation has been demonstrated to be difficult in APECED due to the slowly developing nature of many of the symptoms of

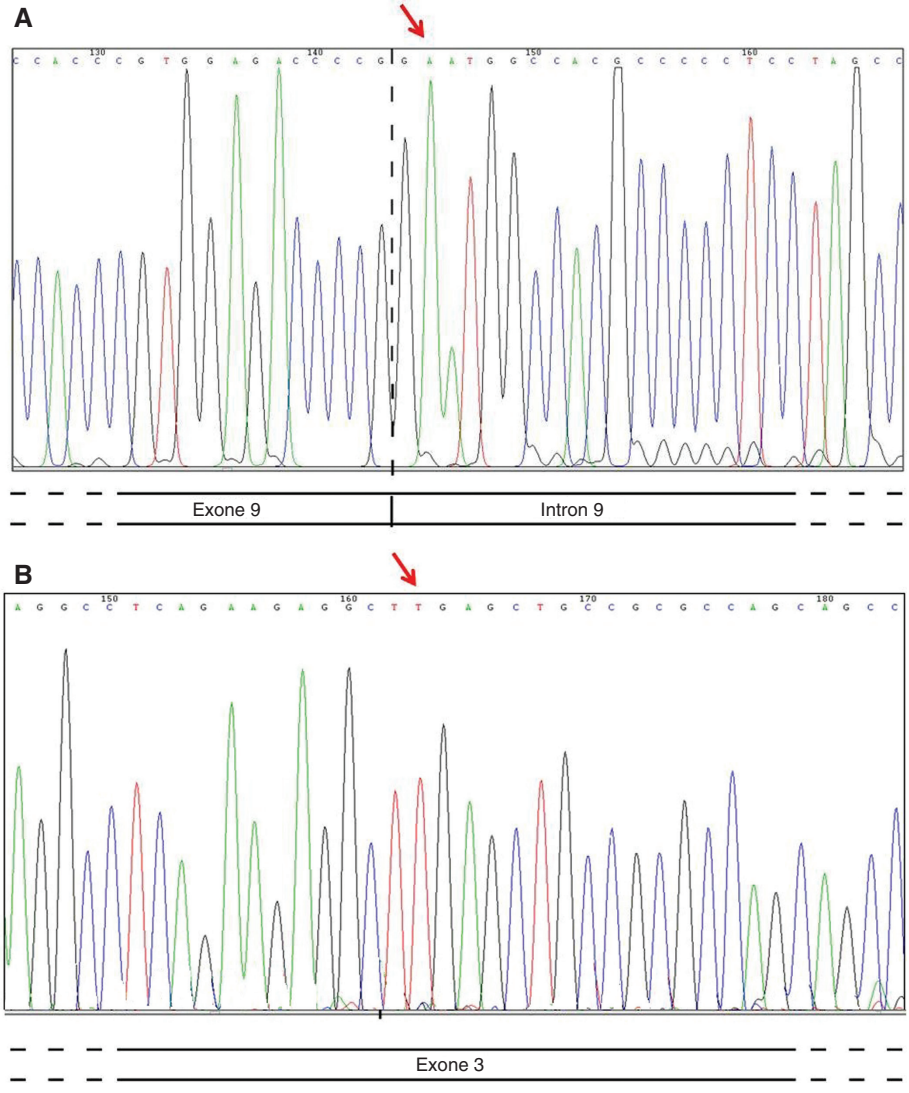

Figure 1: The results of mutation analysis.
Detected splice site variant in case 1 (A) and nonsense mutation in cases 2 and 3 (B).

Table 2: Detected mutations in *AIRE* gene in Iranian patients.

Detected mutation	Clinical presentation	Origin	References
K50NfsX168	Not mentioned	Not mentioned	[7]
R139SX	Not mentioned	Not mentioned	[7]
R257X	Not mentioned	Not mentioned	[7]
L323SfsX51	Not mentioned	Not mentioned	[7]
Y85C	Chronic mucocutaneous candidiasis is rare. Ectodermal dystrophies are not common	Iranian Jewish	[5]
c.1095+2T>A	Typical signs, mucocutaneous candidiasis	Iranian Muslim	The present study
R139X	Typical signs, mucocutaneous candidiasis	Iranian Muslim	The present study

APECED [10]. The observed phenotypes have been varied between patients homozygous for a certain mutation like R139X or even between siblings [2]. An attempt to establish genotype-phenotype correlation in a population of patients from different ethnic groups has resulted in the identification of an association between the R257X mutation and the higher prevalence of candidiasis. No other association has been found. So it has been concluded that mutation of *AIRE* by itself has little impact on the APECED phenotype, while HLA class II is a significant determinant [5]. Although previous studies have recognized candidiasis and Addison's disease as less common manifestations in the Iranian Jews patients [5], both signs have been detected in our patients. Such difference in clinical manifestations in Iranian Jewish has been connected to the prevalence of missense mutation Y85C in this population [5] which has been shown to have no effect on subcellular localization or transactivation properties of the resulted protein [11]. Besides, an extensive longitudinal study in Sardinian patients has identified R139X in 93% of the mutant *AIRE* alleles and revealed that type 1 diabetes was rare in such patients and hypothyroidism was not seen [12]. However, both clinical manifestations have been detected in our patient who has the same mutation which emphasizes the complexity in genotype-phenotype correlation in APECED. To our knowledge, the functional consequence of R139X has not been assessed yet. Future studies would help in identification of subcellular localizations and characteristics of identified mutations in our patients to establish a genotype-phenotype correlation. Besides, our study would pave the way for evaluation of mutation frequency in our population and targeted genetic counseling. The variable clinical presentation of APECED and the difficulty in initial diagnosis especially in the presence of only one of the typical manifestations necessitate increased consciousness about it which can be achieved by mutational analyses of at-risk patients following genetic counseling.

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