DOI: 10.4274/tjem.2987

Strong Similarities in Turkish and European Patients Diagnosed with APECED Syndrome

APECED Sendromu Tanısı Konulan Türk ve Avrupalı Hastalarda Güçlü Benzerlikler

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Abstract

Purpose: Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) is a rare autoimmune disease which is caused by mutations in the autoimmune regulator (*AIRE*) gene, mapping to 21q22.3. We aimed to evaluate *AIRE* gene mutations in patients with APECED syndrome and in their relatives.
Material and Method: In this study, we investigated two patients with APECED syndrome and their families in terms of the *AIRE* gene mutation. We performed mutation analysis by sequencing all the 14 exons and the intron-exon boundaries of the *AIRE* gene on the DNA extracted from the peripheral blood in 12 cases.
Results: Mutation analysis of *AIRE* gene showed that patient 1 was homozygous for the pathogenic mutation c.769C>T (p.R257X; g.8473C>T) which turns arginine coding codon 257 into a stop codon. Her father and all three sisters were heterozygous for this mutation, and no mutation was found in patient 2 and her family members.
Discussion: However phenotypic manifestations of the disease vary largely, prompting the idea of other genetic and/or environmental factors contributing to clinical presentation of the disease. The R257X mutation in exon 6 has been discovered in 89% of the alleles of the Finnish patients with APECED, but is also the most frequent one in other ethnic groups. Although this mutation has been discovered in different ethnic groups, patients with R257X mutation have similar clinical findings. The significance of our cases arises from the fact that this mutation (R257X) is demonstrated in our ethnical group and geographical area for the first time. *Turk Jem 2015; 19: 89-92*
Key words: APECED, APS I, hypoparathyroidism, candidiasis, *AIRE* gene

Özet

Amaç: Otoimmün poliendokrinopati-kandidiyazis-ektodermal displazi sendromu (APECED sendromu, OPS I) otoimmün regülatör genin (*AIRE* geni) 21q22.3 bölgesinde ortaya çıkan mutasyonun sebep olduğu nadir görülen bir otoimmün hastalıktır. Bu çalışmamızda APECED sendromlu iki hasta ile onların ailelerinde *AIRE* gen mutasyonunu araştırmayı amaçladık.
Gereç ve Yöntem: Bu çalışmada APECED sendromlu birer olgunun bulunduğu iki Türk ailede mutasyon varlığını araştırdık. Mutasyon analizini 12 olgunun periferik kanından ekstrakte edilen DNA üzerinde *AIRE* geninin 14 ekson ve intron–ekstron bağlanma bölgelerinin tamamının sekansı ile gerçekleştirdik.
Bulgular: *AIRE* geninin analizi 1. hastada arginini kodlayan 257 kodonu sonlanma kodonuna çeviren patolojik c.769>T (p.R257X; g.8473C>T) homozigot mutasyon olduğunu gösterdi. Bu hastanın babası ve 3 kız kardeşi bu mutasyon açısından heterozigot iken, 2. hastada ve ailesinde herhangi bir mutasyon saptanmadı.
Tartışma: Hastalığın fenotipik bulguları çok değişken olmakla birlikte, kliniğin ortaya çıkışında genetik ve/veya çevresel faktörlerin belirleyici olduğu düşünülmektedir. APECED sendromlu Finli olguların allellerinin %89'unda R257X mutasyonu ekson 6 üzerinde saptanmış, ayrıca diğer etnik gruplarda da en sık olarak bu mutasyon bulunmuştur. Çok farklı etnik gruplarda tanımlanmış olmasına karşın R257X mutasyon bulunan hastaların klinik bulguları benzerdir. Bizim olgularımızın önemi R257X mutasyonun bizim etnik grubumuzda ve coğrafik bölgemizde ilk kez gösterilmesinden kaynaklanmaktadır. *Turk Jem 2015; 19: 89-92*
Anahtar kelimeler: APECED, OPS I, hipoparatiroidizm, kandidiyazis, *AIRE* geni

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Introduction

Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy APECED, also called APS I, usually appears in childhood at age 3-5 years or in early adolescence. It is defined by a persistent fungal infection as chronic mucocutaneous candidiasis (CMC), the presence of hypoparathyroidism (HP), and Addison's disease (AD) (1,2,3). CMC is often the first manifestation, followed by HP and AD, which can develop at any age. Consequently, lifelong follow-up is important to allow the early detection of additional components (4,5,6,7,8). Type 1 diabetes mellitus (DM), primary hypogonadism, autoimmune thyroid disease, and lymphocytic hypophysitis may be seen as accompanying endocrinopathies. Various gastro-intestinal tract and hepato-biliary system diseases such as chronic atrophic gastritis, pernicious anemia, celiac disease, malabsorption, autoimmune hepatitis, and cholelithiasis may develop throughout life. Skin and associated tissues can be affected by some autoimmunity like in vitiligo and alopecia areata or may be involved in ectodermal dystrophy (1,2,9,10). The highest prevalence of the rare APS I has been found in populations which are characterized by a high degree of consanguinity or who are descendants of small founder populations, particularly in Iranian Jews 1: 9,000; Sardanian 1: 14500 and Finns 1: 25,000. APS I presents both in the sporadic and familial forms with monogenic autosomal recessive inheritance in a single gene (6,11,12). APECED (OMIM #240300), is an autosomal-recessive disease caused by mutations in the *AIRE* gene on chromosome 21q22.3. The *AIRE* gene consists of 14 exons and codes for a 545-amino acid protein (13,14). The phenotype of APECED is highly variable. However, conclusions regarding genotype-phenotype correlations have not been possible.

Materials and Methods

Case 1

The patient was born to Caucasian, non-consanguineous parents in 1984. At 8 years of age she developed recurrent CMC in her mouth, successfully treated with local and systemic anti fungal therapy. She developed vitiligo at 14 years of age. Total dental prosthesis was made due to severe dental enamel hypoplasia at 17 years of age. In the year 2005, she was admitted to the department of dermatology with signs and symptoms of severe CMC. She had a history of frequent upper respiratory tract infections and irregular menstrual cycles. No specific condition was found in her family history. In her physical examination there were common candidal plaques on her oral mucosa and corners of the lips. Total dental prosthesis was noticed. She had dystrophic changes on each thumb and nail. Chovostek's and Trousseau's signs were positive. In the laboratory investigation hypocalcaemia, hyperphosphatemia, undetectable parathyroid hormone (PTH), and normal vitamin-D levels were detected. 24-hour urinary excretion of calcium (Ca) and phosphorus (P) were decreased. There were no clinical and laboratory signs of hypocortisolism. Basal adrenocorticotropic hormone (ACTH), cortisol and stimulated cortisol levels with 250 µg tetracosactide (Synacthen®) were within normal ranges. Pituitary, adrenal, and sex hormone profiles were also within normal ranges. She was diagnosed with APECED syndrome with signs and symptoms of two major and three accompanying criteria. Her clinical characteristics related to APECED syndrome and time of diagnosis of these entities are shown in Table 1. Fluconazole 150 mg/day, calcium 3 gr/day, and calcitriol 1 µg/day were administered orally as therapy. Then she, her father and 3 sisters were invited to our medical genetic clinic for genetic examination. Genetic evaluation of her mother could not be performed since she died due to brain tumor 14 years ago.

Case 2

The patient was born to Caucasian, non-consanguineous parents in 1986. At 3 years of age she developed HP with signs of severe hypocalcaemia. In the laboratory investigation hypocalcaemia, hyperphosphatemia, undetectable parathyroid hormone PTH, and normal vitamin-D levels were detected. Twenty four hours urinary excretion of calcium (Ca) and phosphorus (P) were decreased. Twenty-two-year-old patient did not have any other pathological detection while he was taking Ca and calsitriol treatment meanwhile the patient applied to the dermotology clinic with total alopecia, nail dystrophy and oral candidiasis. There were no clinical and laboratory signs of hypocortisolism. Basal adrenocorticotropic hormone ACTH, cortisol and stimulated cortisol levels with 250 µg tetracosactide (Synacthen®) were within normal ranges. Pituitary, adrenal, and sex hormone profiles were also within normal ranges. No specific condition was found in her family history. She was diagnosed with APECED syndrome with signs and symptoms HP, mucocutaneous candidiasis and ectodermal displasia. Her clinical characteristics related to APECED syndrome and time of diagnosis of these entities are also shown in Table 1. Calcium 2-4 gr/day, and calcitiriol 1 µg/day and antifungal treatment were administered orally as therapy. Then she, her parents, 2 brothers and 2 sisters were invited to our medical genetic clinic for genetic examination.

Genetic Assessment

We performed mutation analysis of the *AIRE* gene on the DNA extracted from the peripheral blood of two patients and all their family members, a total of 12 persons. Mutation analysis for *AIRE* gene (GenBank Z97990.1) was performed by sequencing all the 14 exons and the intron-exon boundaries of the *AIRE* gene as described earlier (Figure 1) (15). Sequencing was done in both directions according to the Big Dye Terminator Cycle Sequencing protocol using the ABI3730x1 DNA analyzer (Applied Biosystems) in National Public Health Institute, Helsinki, Finland. While mutation analysis of the *AIRE* gene showed that index case (patient 1) was

Table 1. Clinical manifestations and time of diagnosis

Major Clinical Manifestations	Time of Diagnosis	
	Patient 1	Patient 2
Chronic mucocutaneous candidiasis	8 years	22 years
Hypoparathyroidism	24 years	3 years
Addison disease	-	-
Accompanying Clinical Manifestations		
Vitiligo	14 years	-
Enamel hypoplasia	17 years	-
Nail Dystrophy	17 years	22 years
Alopecia	-	22 years

homozygous for the pathogenic mutation c.769C>T (p.R257X; g.8473C>T), and her father and all 3 sisters were heterozygous for this mutation (Figure 1), no mutation was found in the second patient and all of her family members.

Discussion

APECED syndrome is one of the few known organ-specific autoimmune diseases caused by mutations in a single gene. The genetic penetrance of APECED is 100%, i.e. an individual inheriting two faulty alleles will inevitably develop the disease. However phenotypic manifestations of the disease vary largely, prompting the idea of other genetic and/or environmental factors contributing to clinical presentation of the disease (16,17,18,19). Actually HLA-genotypes have been shown to modify the APECED phenotype (15,20).

AIRE gene, responsible for APECED, is 13 kb in length and its 14 exons code for a protein product of 545 amino acids. The *AIRE* protein contains motifs indicative of a transcription regulator including a conserved nuclear localization signal, two PHD zinc-finger motifs, four LXXLL nuclear receptor binding motifs, and a proline rich region (Figure 2).

The nonsense mutation 769C>T/R257X, detected also in our first patient, causes an Arg codon to turn into a premature STOP codon and potentially leads to a truncated 256 residue protein lacking both PHD finger domains. Assays in transfected cells show that deletion of both PHD fingers severely reduces the homomultimerization of the protein and inhibits totally its transactivation capacity (21). However, it remains to be shown whether the truncated R257X protein is actually produced in patient cells. This mutation was initially described as Finn-major mutation and detected in 89% of Finnish APECED chromosomes examined (6,16). Later, it has also been reported in various populations (North Italian, American Caucasian, Swiss, British, New Zealander, Swedish, Dutch, German, French, Czech, Hungarian, Polish, Austrian, Slovenian, Croatian, and Russian), underlining the cross-ethnicity nature of the mutation (22,23). The mutations of the *AIRE* gene observed in APECED has been shown to be responsible for the alteration in the immunologic tolerance in humans. Subsequently, the increasing knowledge of the biological role of the *AIRE* gene has lead to new understanding on the mechanisms regulating tolerance and autoimmunity (24,25).

In a series of 68 Finnish cases reported by Ahonen and co-workers, all patients had chronic candidiasis at some time, 79% had HP, 72% had Addison's disease, and 51% had all the three of these classical components. Gonadal failure (60% in women, 14% in men) and hypoplasia of the dental enamel (77%) were also frequent findings. Other manifestations occurring less frequently included alopecia (29%), vitiligo (13%), intestinal malabsorption (18%), type 1 diabetes (18%), pernicious anemia (13%), chronic active hepatitis (12%), and hypothyroidism (4%). The major mutation in the *AIRE* gene was R257X (6). Collins et al. investigated and published same clinical findings as dermatological manifestations of APECED in 18 Irish patients (5). In another report Buzi and co workers published 3 patients with APECED syndrome who had R257X mutation of the *AIRE* gene and had similar clinical findings as in our first case and Finn cases. Like in our family, heterozygous mutations were found in the patient's family members in this report as well (4). In our series CMC, HP, ectodermal dysplasia, vitiligo, and R257X mutation were demonstrated in patient 1, HP, CMC, and ectodermal dysplasia were demonstrated in patient 2, in whom no mutation was detected. Although mutational analysis revealed R257X mutation in one of our cases and her family investigation, most of our clinical and genetic findings were in parallel with the reports in the literature. Besides a significant association between the rare allele (C961) of the *AIRE* polymorphism at position 961 and alopecia areata, the fact that women had earlier onset of HP and were younger at the time of diagnosis than men had been reported in the different reports (23,26). Walls and co-workers had pointed that enamel hypoplasia may precede the onset of HP and, despite adequate replacement therapy, may also affect teeth forming after the onset of HP (7). Gylling found a clear gender linkage with lower and later incidence in male and described

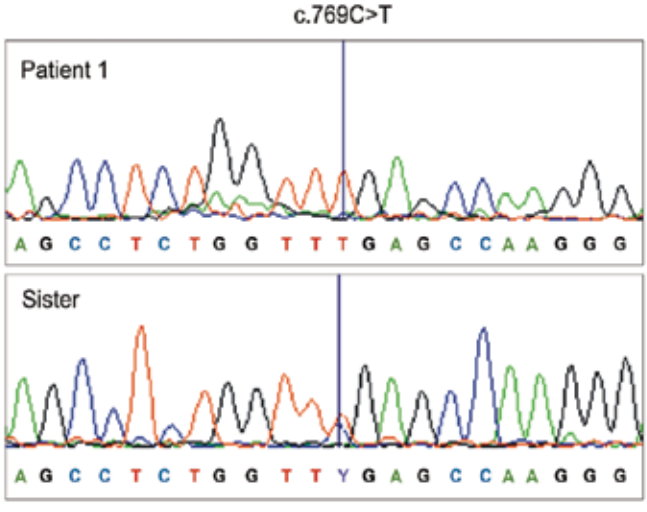

Figure 1. The electropherogram showing the mutation c.769C>T at homozygous state in patient 1, and at heterozygous state in her sister as representative of carrier family members

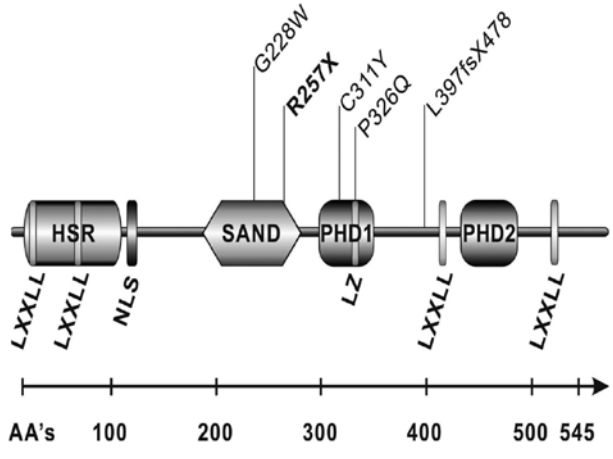

Figure 2. A schematic diagram of the functional domains of the *AIRE* protein and the location of R257X and selected other missense and nonsense mutations. NLS, nuclear localization signal; LZ, leucine zipper. The positions of the four LXXLL domains are indicated

protective effect of male sex on hypoparathyroidism in APECED (27). In our series chronologic development order of APECED signs and symptoms in patient 1 was similar to the findings of this study.

In conclusion, although our report is a single case and family investigation, most of the clinical and genetic findings of our serial were in parallel with the reports in the literature. Due to the high frequency of consanguineous marriages in Turkey, genetic counseling and detailed observations of family members are necessary in families with heterozygote carriers. Patients with APECED syndrome need life-long treatment and follow-up to detect further disease components. Increased awareness of APECED, combined with mutational analysis of the *AIRE* gene, will aid diagnosis and may prevent serious and fatal complications.

Acknowledgments:

Special thanks to Ismo Ulmanen and Tania Ilmarine (Department of Molecular Medicine, National Public Health Institute, Biomedicum, Helsinki, Finland) for their kindly cooperation and doing genetic analyses of all patients.

The skillful technical assistance of Anne Vikman in the genotyping is acknowledged.

Ethics Committee Approval: The study protocol was approved by Uludağ University Medical School Ethics Committee, **Informed Consent:** The study was conducted after obtaining informed consents from all participants, **Concept:** Metin Güçlü, Hakan Cangül, **Design:** Metin Güçlü, Hakan Cangül, **Data Collection or Processing:** Metin Güçlü, Hakan Cangül, **Analysis or Interpretation:** Hakan Cangül, **Literature Search:** Metin Güçlü, Hakan Cangül, **Writing:** Metin Güçlü, Hakan Cangül, Canan Ersoy, **Peer-review:** Externally peer-reviewed, **Conflict of Interest:** No conflict of interest was declared by the authors, **Financial Disclosure:** The study was financially supported by the 6th framework EU program EURAPS (IU, TI).

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