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Novel mutation in AIRE gene with autoimmune polyendocrine syndrome type 1

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ABSTRACT

Purpose: Autoimmune polyendocrine type 1 (APS-1) is a complex inherited autosomal recessive disorder. Classically, it appears within the first decade of life followed by adrenocortical insufficiency, mucocutaneous candidiasis, Addison's disease, and hypoparathyroidism. The clinical phenotype of APS-1 varies depending upon mutations in the autoimmune regulator gene (*AIRE*) on chromosome 21q22.3.

Methods: In this study, we performed Sanger sequencing of*AIRE* in Iranian patients to identify different variants and probable new mutations corresponding to a clinical diagnosis of APS-1.

Results: After analyzing 14*AIRE* exons, we detected a novel insertion mutation in exon 2 in a patient who presented with severe APS-1, Lys50AsnfsX168. Furthermore, the known mutations in *AIRE*, including Arg139X, Arg257X, and Leu323SerfsX51, were detected in enrolled patients.

Discussion: According to our results, sequencing analysis of*AIRE* provides a useful screening method to diagnose patients with incomplete or unusual clinical presentations of APS-1.

1. Introduction

Autoimmune polyendocrine syndrome type 1 (APS-1) is an infrequent autoimmune disease that arises from mutations in the autoimmune regulator gene, *AIRE* (OMIM 240300). Chronic mucocutaneous candidiasis (CMC), hypoparathyroidism (HPT), and primary adrenal insufficiency (AI) are the three main clinical characteristics of APS-1 (Peterson et al., 2004; Ahonen et al., 1990). The presence of two or three of those major symptoms is considered as risk factors associated with the disease (Orlova et al., 2010; Wolff et al., 2006). In addition to these three main clinical components, more than 20 other minor organ-specific conditions have been described including autoimmune hepatitis, hypothyroidism, anemia, type 1 diabetes mellitus, vitiligo, alopecia, and others (Oftedal et al., 2015). The diagnosis of APS-1 is related to both clinical features and environmental factors that can modify the phenotype, even among siblings (Meloni et al., 2012). Polyglandular Autoimmune (PGA) Syndrome is also caused by mutations in *AIRE* (Aaltonen et al., 1997). Currently, more than 100 pathogenic mutations in *AIRE* have been recognized (available at www.hgmd.org).

Although the global prevalence of PGA-1 is rare, it is observed with high frequency in some special populations such as Iranian Jews (1:9000) (Zlotogora and MSJJomg, 1992), Finns (1:25000) (Wang et al., 1998) and Sardinians (1:14400) (Rosatelli et al., 1998).

AIRE is a 13 kb gene on chromosome 21q22.3 with 545 amino acids. The AIRE protein consists of two plant homology domain (PHD) fingers in the C-terminal region, which interact with proteins involved in chromatin remodeling(Mansfield et al., 2011); a conserved nuclear localization signal for transcriptional regulation; four LXXLL nuclear receptor binding domains; a SAND domain, which is a putative DNA

Abbreviations: AIRE, autoimmune regulator gene; APECED, autoimmune polyendocrinopathy candidiasis ectodermal System; APS-1, autoimmune polyendocrine type1; HSR, homogeneously staining region; PHD, plant homology domain; PRR, proline-rich region

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binding domain consisting of amino acid 189–280; and a proline-rich motif (Nagamine et al., 1997; Mittaz et al., 1999). The homogenously staining region (HSR), contained within the first 100 amino acids of AIRE, is responsible for homodimerization. Because of the HSR's α-helical four-helix bundle structure, it is sensitive to conformational alterations. Therefore, the HSR may be considered a region with a high mutation rate, which results in APS-1 (Gibson et al., 1998; Pitkänen et al., 2000). AIRE plays a pivotal role in controlling the expression of tissue-specific antigens in thymic medullary epithelial cells, which are critical in the negative selection process of T-cells (Anderson et al., 2005). Furthermore, a parallel role for peripheral tolerance in lymph nodes has been documented (Gardner et al., 2008). AIRE facilitates the progress of early thymic regulatory T-cells by playing a controlling role in autoreactive cells (Anderson and MAJCoii, 2011).

Mutation hotspots of AIRE are exons 1, 2, 6, 8, and 10 (Cervato et al., 2009). Exons 1 and 2 encode the HSR, exon 6 is in the SAND domain, exon 8 in PHD-1 domain and exon 10 located between the two PHDs, which is a proline-rich region (PRR) (Mora et al., 2014). These findings provided a new link between histone modifications and regulation of tissue-restricted antigen expression in the thymus. Bioinformatics analyses indicated that the PHD-1 residues N295-C310 have a critical role in interactions with histone H3. Thus, mutations in the zinc-chelating cysteine disable the PHD-1 interaction with H3K4me0 (Bottomley et al., 2005). These specific interactions describe why this region is highly conserved among all species. Most of the APECED-causing (Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy) mutations result in frame-shifts that produce artificially truncated mRNA transcripts (Halonen et al., 2002). The abundance of APECED-causing mutations in AIRE PHD domains demonstrates the essential role of these domains in this disorder (Björses et al., 2000).

The most common *AIRE* mutations reported in Finnish and British populations, are R257X and 496del13, respectively (Pearce et al., 1998). Iranian Jewish patients share a common mutation, an A to G transition at residue 85, which is predicted to damage the HSR domain of AIRE protein through substitution of tyrosine 85 to cysteine (Zlotogora and MSJJomg, 1992).

In this study, we sequenced 14 exons of *AIRE* in 20 Iranian APECED patients and detected nine mutations, including a novel mutation. These findings may further facilitate the diagnosis, characterization, and understanding of APS-1.

2. Material and methods

2.1. Patients

Twenty APECED patients were enrolled in the Ethics Committee and Research Council of Mashhad University of Medical Sciences (Code; 87883). Informed consent was received from both patients and healthy volunteers. Inclusion criteria for the study were based on the development of any 2 major manifestations within the classic triad of CMC, HPT, and AI. CMC diagnostic criteria relied on the presence of frequent mucous membrane, nail, and skin infections. AI was diagnosed via the gold standard Synacthen test, demonstrating a failure to reach a serum cortisol level of 500 nmol/L. Low production of parathyroid hormone (PTH) and normal renal function are the key diagnostic factors for HPT. All patients underwent the same prospective evaluation consisting of blood testing and consultation with subspecialists in infectious disease, immunology, endocrinology, hematology, gastroenterology, pulmonology, nephrology, ophthalmology, dermatology, pediatrics, and allergy. Healthy volunteers, after obtaining informed consent, participated the same as controls. This study was performed consistent with the Helsinki Declaration.

2.2. Molecular analysis of AIRE

To confirm the APECED diagnosis, genomic DNA was extracted from the patients' peripheral whole blood according to a standard protocol (Miller et al., 1988). *AIRE*-coding exons and flanking exon-intron junctions were amplified by PCR using exon-specific oligonucleotide primers sets. The primers were identical for amplification and sequencing and are provided in Table 1. Different PCR conditions were utilized to analyze all fourteen exons, in order to optimize amplification of each exon. PCR reactions were performed in a final volume of 50 µl containing 10 pmol of each primer, 1U Taq DNA polymerase (Genet Bio, Korea), 1.5 mM MgCl2, 200 µmol of each dNTP and 50 ng genomic DNA as a template. The PCR cycling conditions were as follows: 95° for 5 min, followed by 35 cycles of 95 °C for 30 s, 55 °C for 30 s at 72 °C for 1 min followed by 4 °C hold, using TechGene thermal cycler. PCR products were purified from 1% agarose gel electrophoresis (QIAEX Gel Extraction kit, Qiagen, USA). Bi-directional sequencing was performed by Macrogen (Seoul, South Korea) using an ABI 3730XL genetic analyzer (Applied Biosystems). The sequenced fragments were analyzed with the Sequencer sequence alignment software (Gene codes) Version 4.10.1.

Table 1
Primers used for PCR-sequencing.

Primer	Forward (5'→3')	Reverse (5'→3')
Exon 1	AAGCGAGGGGCTGCCAGTGTC	GGGACTATCCCTGGCTCACAG
Exon 2	TCCACCACAAGCCGAGGAGAT	AGCTGGGCTGAGCAGGTGACA
Exon 3	CTGAGGTTGGGACCCTGCTCC	CTGGAGACCCTGGCTGGCTTC
Exon 4	GGCACTCACCCCCACTGAGAG	GCCCTGCTCTGACCCCTGAC
Exon 5	GCCCAGTGCTGCCTGCTTCTG	CCATCTTGGAGCCTGGGTCTC
Exon 6	TGCAGGCTGTGGGAACTCCAC	GGGGCATCAAGAGCCAGGCTC
Exon 7	CATGTCCACCCTCGCTGCTGA	AGAAAAAGAGCTGTACCCTGTGG
Exon 8	CACCCCAGCCCAGTCTGCATG	CTTCAGGGTCAGTGGGTGGAG
Exon 9	CTGTCACCCGCTCTGTTGTTC	GTGGCCATGTGGACAGGAGG
Exon 10	CCCAGCAGTCACTGACTCCTG	CGTAGGTCCTGGGCTCCTTGA
Exon 11	CTCGGGTTCGGGTTCAGCTAC	TGTGGGTGTGGGTTCAGGCCT
Exon 12	CATACCCCGGAGGTGGCACTC	CAGCACCGGCATGCATGGAGG
Exon 13	CTGTGGGAGTTGGGCTGACCT	AGTGGAGGAGCACCAGGAGG
Exon 14	ATGGCCATGATTGTGTGGCTG	CTCAGCACTCTCTCATCAGAG

2.3. Statistical analysis

The Statistical Package for Social Sciences SPSS 16.0 (SPSS, Chicago, IL) was employed for the examination of data. The Kolmogorov-Smirnov test was applied to evaluate the normal distribution of samples. Descriptive statistics (frequency, mean, and standard deviation) were considered for all variations. Values were reported as either mean (± SD) or median and IQR based on the requirement. A P-value < 0.05 was regarded as statistically significant.

3. Results

Twenty individuals from nineteen families (10 males and 10 females) in the 6–30 year old age group, average age of 15.3 years, fulfilled the eligibility criteria of APS-1. In all patients, minor APS-1 clinical features (e.g., autoimmune hepatitis (AH), tubulointerstitial nephritis, pigmented retinitis, and anemia) developed before the diagnosis and were retrospectively considered as disease components. The three principal components (CMC, AI, and HPT) were present in 100%, 90%, and 80% of patients, respectively.

Fifty percent of female patients were identified with primary ovarian insufficiency (POI) (mean age = 15.6 years). Enamel hypoplasia and nail dystrophy were found in more than half of our patients. Overall, 18 various clinical factors were recognized, and all of our patients were diagnosed with at least five distinctive components (range, 1–10) (Table 2). The classic dyad was diagnosed in 18 patients, and the triad was seen in 16 patients. Two patients remained oligosymptomatic, with only one major component.

Table 2
Prevalence of Disease Components in 20 Patients with APS-1. [A] Among the 10 female patients; [B] Among the 10 male patients.

	% (n)	Females/Males
APECED clinical diagnostic criteria		
Diagnostic dyad	20 (4)	1/3
Classic triad	80 (16)	9/7
Oral and infection manifestations		
Chronic mucocutaneous candidiasis	100(20)	10/10
Enamel hypoplasia	70(14)	8/6
Endocrine manifestations		
Hypoparathyroidism	80(16)	9/7
Adrenal insufficiency	90(18)	8/10
Hypothyroidism	20(4)	2/2
Growth hormone deficiency	20(4)	1/3
Ovarian failure[A]	50(5)	5/-
Testicular failure[B]	20(2)	-/2
Gastrointestinal manifestations		
Intestinal dysfunction	65(13)	6/7
Hepatitis	5(1)	1/-
Skin/nail manifestations		
Alopecia	40(8)	4/4
Vitiligo	10(2)	-/2
Nail dystrophy	55(11)	6/5
Other manifestations		
Keratoconjunctivitis	50(10)	4/6
Tubulointerstitial nephritis	15(3)	2/1
Anemia	15(3)	1/2

3.1. Chronic mucocutaneous candidiasis

All twenty patients were diagnosed with CMC (100%), with an average age of 16.7 years. The clinical course of CMC varied from affecting one or two nails in 14 patients to more severe antifungal drug-resistant cases. Candidiasis of the skin (forearm, foot) was seen in four patients. Squamous cell carcinoma or other types of cancers were not observed.

3.2. Adrenal insufficiency

In 90% of patients, AI was observed (8 females and 10 males; mean age: 14.8 years) with a wide range from 6 to 20 years old. At least one or more adrenal crises were experienced by nearly half of our patients during the follow-up period.

3.3. Hypoparathyroidism

HPT was observed in 80% of all patients (9 females and 7 males) at a mean age of 7.6 years (range, 10–30). The treatment procedure of all of them was similar, calcium, vitamin D, and calcitriol.

3.4. Enamel hypoplasia and nail dystrophy

Nearly 70% of our patients showed enamel hypoplasia (8 females and 6 males) at a mean age of 16.8 years (range, 10–30). Patients with nail dystrophy constituted about 55% of total patients (5 females and 6 males) at a mean age of 16.0 years.

3.5. Ovarian and testicular failure

Among ten females, half were diagnosed with POI at a mean age of 20.2 years. The percentage of males with testicular failure was lower; about 20% at a mean age of 15 years.

3.6. Alopecia and keratoconjunctivitis

Alopecia presented in 8 out of 20 patients, 40% (4 females and 4 males), with an average onset of 10.7 years (range, 10–20). Most patients had hair loss, four patients had one or two patches of hair loss only without changes in their eyebrows and eyelashes, and one patient developed complete loss of her eyebrows and eyelashes. Women were more likely to vocalize displeasure due to alopecia.

Keratoconjunctivitis was diagnosed in 10 patients, 50% (4 female and 6 males), depending on the presence of photophobia or blurry vision, without ocular dryness. Also in an ophthalmologic examination, there was evidence of decreased visual acuity and punctate corneal lesions.

3.7. Malabsorption

Intestinal dysfunction included chronic non-bloody diarrhea or chronic constipation, requiring chronic administration of enemas, laxatives, or both. Accompanying symptoms included abdominal pain, abdominal bloating, or foul-smelling flatulence, and the presence of greasy stools. Forty-five percent of patients suffered from severe abdominal bloating. Diarrhea and chronic constipation were diagnosed in eight patients (4 diarrhea/ 4 constipation).

3.8. Other components

Hypothyroidism was diagnosed in 20% of patients ranging from 11 to 20 years based on reduced plasma-free thyroxin and assessed plasma thyroid-stimulating hormone. Growth hormone (GH) deficiency was also observed in four patients (20%). The diagnosis of tubulointerstitial nephritis (TIN) was characterized by plasma creatinine levels and biopsy-proven inflammation in renal tubules and interstitium. TIN was diagnosed in 15% of patients (3 of 20). In 2 patients (10%), vitiligo caused depigmentation of hair, eyebrows, and eyelashes. A patient with autoimmune hepatitis (AH) was diagnosed via biopsy-proven lymphoplasmacytic hepatic inflammation.

3.9. Mortality

One patient died of unknown causes during our research project. She was 17 years, and her disease duration was seven years.

3.10. AIRE mutations

All twenty patients underwent *AIRE* PCR-sequencing, compared to the Gene Bank genomic sequence accession number ENSG00000160224. Altogether, nine different *AIRE* mutations were identified in 18 patients, 8 of which were previously known. In a 17 year-old female, we identified one novel insertion mutation in exon 2 (Lys50AsnfsX168, Gene Bank No. KT988061), which is characterized in Table 3. This frameshift mutation changed the sequence of the related polypeptide at residue 50 (lysine to asparagine), which leads to the translation of a non-functional truncated protein consisting of 168 amino acids (Fig. 1). The other eight known mutations were nonsense mutations and polymorphisms distributed throughout the coding region (Fig. 2), only two mutations were found in the first PHD domain (Wang et al., 1998; Scott et al., 1998; Saugier-Veber et al., 2001). Genetic testing was also identified a large deletion in exon 8 of the gene (c.967-979del13), which is the most frequent mutation (35%) in our patients. As expected, there were no significant differences between clinical manifestations and DNA changes ($P > 0.05$).

4. Discussion

APECED is a rare, recessive genetic disorder which is highly prevalent in certain populations such as Finns, Sardinians, and Iranian Jews (Zhang et al., 2013). Although symptoms usually appear in the first decade of life, heterogeneity of clinical features is associated with delayed diagnosis. Describing precise clinical components, the presence

Table 3
Genetic characteristics of 20 patients affected by APECED enrolled in this study.

	Mutation	RS number	Global MAF	Patients Number	Ref
Exon 2					
	c.149insTGA (p.Lys50AsnfsX168)	Novel	0.00	10	Present study
Exon 3					
	c.415C > T (p.R139X)	121,434,256*	0.00001	7	10-21
Exon 5					
	c.588C > T (p.S196S)	878081	0.1	2,3,4,12	19-21
Exon 6					
	c.769C > T (p.R257X)	121,434,254*	0.0007	19	19
	c.681C > T (p.G227 G)	1055311	0.2	8, 11, 13,17	19,20
Exon 7					
	c.834C > G (p.S278R)	1800520	0.1	1,5,14	19,20
Exon 8					
	c.967-979del (p.Leu323SerfsX51)	386,833,675*	–	2,3,4,9,12,18,20	20
Exon 10					
	c.1197 T > C (p.A399A)	1,800,521	–	1,2,3,4,5,6,9,10,12 14,18,19, 20	19-21
Exon 14					
	c.1578 T > C (p.D526D)	1133779	0.4	1,5,14,19	19,20

* pathogenic variants.

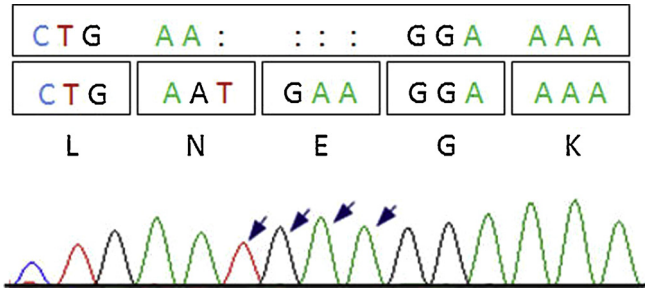

Fig. 1. Sequencing graph of the *AIRE* gene. The homozygous insertion of four nucleotides in exon 2 results in a frame shift and produces a premature stop codon at amino acid 168 (Lys50AsnfsX168).

of autoantibodies, and genetic screening are three main ways for early detection. Mazza et al. mentioned a noticeable delay between the appearance of the first symptoms and the time of APECED diagnosis (mean diagnostic delay of nearly 10 years) (29). In proteins that multimerize in order to function, single mutations can completely or partially interrupt the whole structure. As AIRE tends to form a homotetramer *in vivo* (Kumar et al., 2001), even a monoallelic variation in the *AIRE* locus can cause APS-1 or other forms of organ-specific autoimmune diseases.

Until now, more than 100 different damaging variants in *AIRE* have been reported in APECED patients (Mora et al., 2014). Like other studies, we found APECED-causing mutations spread throughout the protein, and patients showed more than one variation. 65% of our variations were identified in the PRR domain in exon 10. The SAND domain was the second most common mutation site (60%). While the third most frequently mutated domain among our patients was PHD-1 (35%). The novel mutation (c.149insTGAA, p. Lys50AsnfsX168) was located in exon 2, at the *AIRE* hotspot region. This mutation was in the homogeneously staining region (HSR) domain, which is crucial for the homo- and multimerization of the AIRE protein (Pitkänen et al., 2000) and targeting AIRE to the cell cytoskeleton (Ramsey et al., 2002). Such mutations can reduce the transcriptional activation capacity of the AIRE protein and alter the connection between the AIRE protein and the nucleolus (Meloni et al., 2005). In addition, this new frameshift mutation truncates the protein at 168 amino acids, leaving several domains such as SAND (which interacts with histone H3 in three different positions (295–298, 304–312, 331–335)), zinc finger PHD-type 1 (269–343) and PHD-type 2 (434–475) missing from the final truncation product. The pivotal role of PHD domains in the transactivation function of AIRE protein can explain how a minor variation can be sufficient for APECED pathogenesis (Fierabracci et al., 2012). In PGA syndrome, there is a difference between the mutation location and population frequency. While Y85C, in the HSR domain, is the most common mutation site in the Iranian Jewish population, R257X and R139X are common mutations in Finnish and Italian patients, both occurring in the SAND region (Bruserud et al., 2016). The 967-979del13, a deletion of 13bp in PHD-1, is often found in Norwegian and North American patients (Ferre et al., 2016). In this study, except for the Y85C mutation, we observed other common variations in our patients. R257X was found in a fourteen-year-old girl with CMC, HPT, AI as well as three other minor components, however, this mutation was not the only variation she possessed. She also harbored two synonymous mutations in *AIRE*, an A232A mutation in exon 10 and a D526D mutation in exon 14 (silent SNPs). Another stop-gain mutation (c.415 C > A, p.R139X) in exon 3 was identified in another Iranian girl with CMC, HPT, AI, and ovarian failure. Thirty-five percent of our patients carried a 13 bp *AIRE* deletion in exon 8 (c.967-979del, p. Leu323serfsx51). In addition to the variable *AIRE* mutations that are linked to APS-1, clinical manifestations of APS-1 patients with the same mutation also are varying (Zhu W, Hu Z, Liao et al., 2017), suggesting external factors that modulate disease presentation. Thus, our findings would similarly suggest that the clinical manifestations of APECED are not only as a result of mutations in its gene but also in combination with other factors like environment, lifestyle, and habits.

In addition, in some APECED patients, the lack of reliable correlation between phenotype and genotype illustrates the importance of identifying *AIRE* mutations early to establish the exact diagnosis of APECED (Mazza et al., 2011). Analysis of *AIRE* provides a practical diagnostic test in patients whose clinical features are insufficient or unconventional. Further research will continue to illuminate the role of nucleotide polymorphisms in *AIRE* and a way for genotype/phenotype

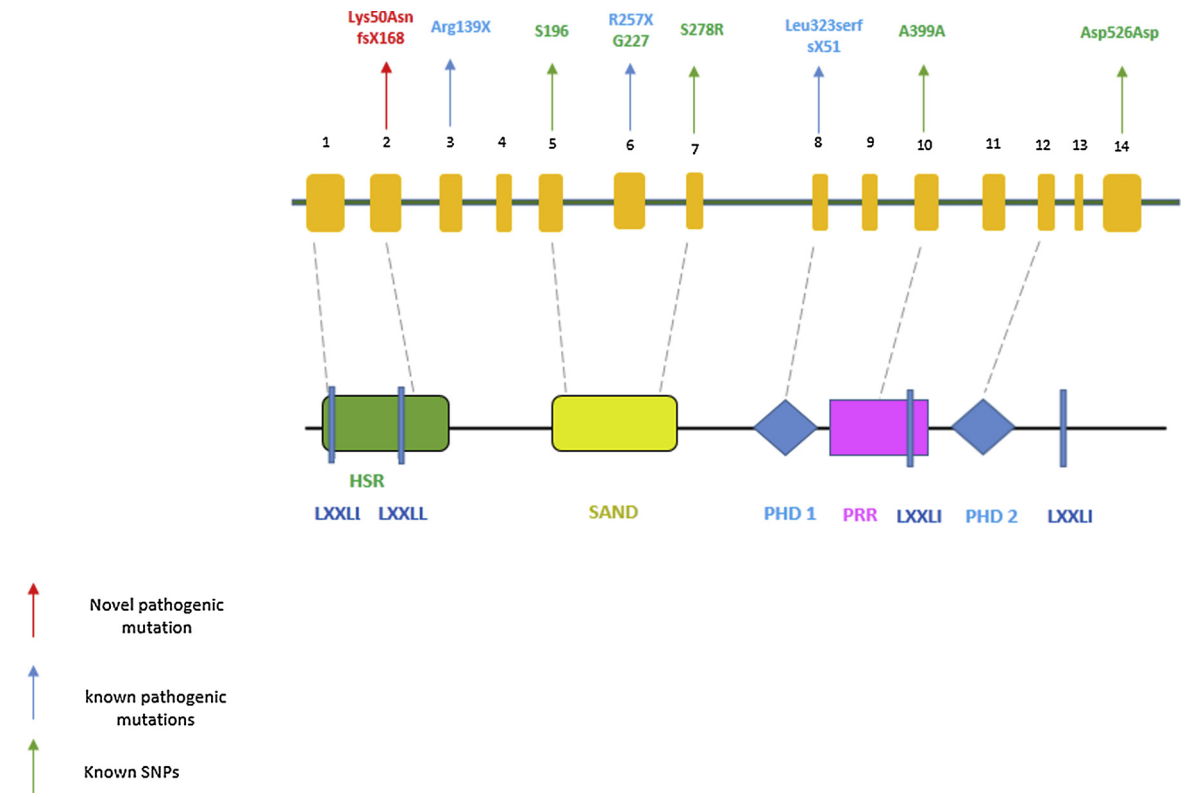

Fig. 2. AIRE protein analysis representation. Schematic representation of AIRE protein is shown along with all mutations and SNPs found in this study.

analysis that may facilitate the identification of novel therapeutic targets.

5. Conclusion

In summary, our prospective evaluation of patients with this complex disease indicates common mutations and polymorphisms in APS-1 patients, and its clinical presentation and pathogenesis.

The three most important mutation sites of APS-1 patients are R257X, A139X, and 967-979del. According to in-silico analysis, the novel mutation site (c.149insTGAA, p.Leu50AsnfsX168), reported in this paper for the first time may disturb the structure and function of the AIRE protein.

To introduce new screening diagnostic tool and reliable therapeutic strategies more research is essential in molecular mechanisms that control the disease's pathology.

Availability of data and materials

Imaging data could be provided upon request.

Authors' contributions

F.F.G; M.G.; Designed and performed experiments, analyzed data. F.F.G.; wrote the paper. N.G., R.V.; S.H.D.M.; provided the financial support and introduce patients. H.R.R; Performed bioinformatics analyses. T.E.D; revised the paper critically for important intellectual content.

M.G; Supervised the research. M.R.A; Designed experiments and co-wrote the paper.

Ethics approval and consent to participate

This study was approved by the Mashhad University of Medical Science (MUMS), and patient consent was obtained prior to the initiation of the study.

Consent for publication

Not applicable.

Competing interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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