

AIRE Gene Mutation in Chinese APS-1 Patients

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Abstract

Autoimmune polyendocrine syndrome type 1 (APS-1, OMIM 240300) is a rare autosomal recessive disease that is caused by autoimmune regulator (*AIRE*) gene. The main symptoms of APS-1 are chronic mucocutaneous candidiasis, autoimmune adrenocortical insufficiency (Addison's disease) and hypoparathyroidism. To date, more than 100 different mutations of the *AIRE* gene have been identified in APS-1 patients. These different mutations affect the structure and function of the *AIRE* protein in different ways, which eventually leads to the development of APS-1. So far, only five cases of APS-1 have been reported in the Chinese, and the main mutation sites are c.769C>T (p.R257*), c.55G>A (p.A19T), c.463G>A (p.G155fsX203), c.622G>T (p.G208W) and c.206A>C (p.Q69P).

Keywords: *AIRE* gene; Autoimmune polyglandular syndrome; Gene mutation

Introduction

Autoimmune polyendocrine syndrome type 1 (APS-1, OMIM 240300), formerly known as autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) [1], is characterized by at least two of the following major criteria: chronic mucocutaneous candidiasis, autoimmune adrenocortical insufficiency (Addison's disease) and hypoparathyroidism [2]. Patients with APS-1 also routinely exhibit additional autoimmune diseases, including vitiligo, alopecia, chronic intestinal dysfunction, type 1 diabetes mellitus (T1DM) and autoimmune hepatitis [3]. In addition, the recent literature underlines how the clinical spectrum of the syndrome is enlarging and is characterized by other autoimmune disease as lethal pulmonary involvement, chronic inflammatory demyelinating polyneuropathy (CIDP) [4,5]. APS-1 is a rare autosomal recessive disease characterized by an autoimmune response mediated by a single gene mutation of the autoimmune regulator (*AIRE*) gene [3,6], which is mainly expressed in medullary thymic epithelial cells (mTECs) [7]. *AIRE* protein activates the transcription of genes encoding for tissue-restricted antigens (TRAs) in a subset of mTECs: the presentation of TRAs to the maturing thymocytes induces the clonal deletion of self-reactive thymocytes and constitutes the main form of central tolerance. Dysregulation of thymic *AIRE* expression in genetically transmitted and acquired diseases other than APS-1 may contribute to further forms of autoimmunity [8-12].

The *AIRE* gene is located on chromosome 21q22.3, contains 14 exons spanning approximately 11.9 kb of genomic DNA, and encodes a 57-kDa transcriptional regulator of 545 amino acids. The *AIRE* protein contains several distinct domains, such as a homogeneously staining region (HSR), a conserved bipartite nuclear localization signal (NLS), a SAND domain, two plant homeodomain (PHD) zinc-finger motifs, four LXXLL nuclear receptor binding motifs (where L is leucine and X is any amino acid) and a proline-rich region (PRR) [13,14]. The HSR is responsible for the homodimerization of the *AIRE* protein and is required for *AIRE* activation. The HSR also influences many cellular

processes, including growth control, cell apoptosis and senescence [13,15-18]. The NLS directs *AIRE* to the nucleus. The SAND domain has DNA binding activity [19], which is involved in the nuclear domain compartmentalization of the *AIRE* protein. Deletion of and point mutations in the SAND domain may result in aggregation of cytoplasmic polypeptides, which interferes with normal nuclear localization [20,21]. The PHD zinc-finger motif is a cysteine-rich structure that may also act as a DNA binding domain. Mutations occurring in this region may affect protein-protein interactions [22]. The LXXLL motifs play a role in transcriptional regulation and function as co-activators of nuclear receptors and the target gene activated and transcribed by nuclear receptors. It also relates to transcriptional regulation [23].

To date, more than 100 different mutations of the *AIRE* gene have been identified in APS-1 patients, and only two mutations (c.769C>T and c.967-979del) are responsible for 95% of the mutant alleles in APS-1 patients [24-26]. So far, only five cases of APS-1 have been reported in the Chinese. Liu et al. were the first to report a Chinese APS-1 patient with mutations in the *AIRE* gene [27]. In 2015, Zhang et al. reported 2 cases of APS-1 in the Chinese population [28] and in 2016, Sun et al reported 1 case [29]. Recently, a study from our group reported 1 case [30]. The main mutation sites in these Chinese patients are c.769C>T, c.55G>A, c.463G>A, c.622G>T and c.206A>C. Many researchers have shown that different mutation sites, which are located in different domains of the *AIRE* protein, could affect the structure and function of *AIRE* in different ways. c.769C>T is a classical mutation found in the *AIRE* gene, which is responsible for more than 80% of APS-1 patients in Finnish patients [24-26]. The mutation results from a single C to T point mutation at nucleotide 769 in the *AIRE* gene. This mutation results in a truncated protein (p.R257*) lacking 288 amino acids found in the normal *AIRE* protein. In this truncated protein, at least 5 functional regions are deleted, and this seriously affects the transcriptional activation capacity of *AIRE* [31,32]. The mutation site c.463G>A located in the end of exon 3, occurred within the conservative shear sequence [33] and potentially caused a frameshift mutation in *AIRE* by shearing to skip exon 4 [34]. According to research by Zhang et al. c.463G>A mutation reduced normal splicing by generating an alternatively spliced intron 3-retaining transcript,

which resulted in a truncated protein (p.G155fsX203) containing the first 154 amino acids of *AIRE* followed by 48 aberrant amino acids [28]. Another mutation site, c.622G>T in exon 5, results in a glycine to tryptophan substitution at amino acid 208 (p.G208W) within the SAND domain of *AIRE*. The introduction of this tryptophan may impact *AIRE* function because an aromatic side chain changes the polarity of the protein. The tryptophan is a bulkier residue than glycine, so this substitution may also alter the conformation of the *AIRE* protein [29]. In our study, we found a new homozygous mutation site in the *AIRE* gene (c.206A>C) located in exon 2 that caused a glutamine to proline substitution of the amino acid 69 of the *AIRE* protein (p.Q69P) [30]. We studied the structure of the mutant *AIRE* protein and found that the new mutation was located in the HSR domain. Further research showed that many mutations located in the HSR domain can negatively influence the homodimerization of the *AIRE* protein, suppress the transcriptional activation capacity of *AIRE* and interfere with the connection between *AIRE* and the nucleolus. These negative effects on *AIRE* function can then induce a series of autoimmune diseases [35]. The yeast two-hybrid assay was used to analyse the homodimerization properties of the mutant *AIRE* protein and real time polymerase chain reaction (RT-qPCR) was used to detect the expression levels of TRAs. The above experimental results showed that the mutation site (c.206A>C) could affect the structure and function of the *AIRE* protein by influencing the homodimerization properties of *AIRE* and the expression levels of TRAs in mTECs. In the case reported by Liu et al. [27], in addition to the *AIRE* mutation site c.769C>T, Another mutation site c.55G>A, which was located in exon 1, resulted in an alanine to threonine substitution at amino acid 19 (p.A19T), a region containing a highly conserved alpha helix domain,

namely, the exon 1 N homologous chromosome end zone. p.A19T and p.Q69P, which are both located in the HSR of *AIRE*, can affect the structure and function of the *AIRE* protein by negatively influencing its dimerization characteristics.

The clinical manifestations of the five cases of APS-1 in Chinese were different. A summary of the clinical manifestations of these patients are listed in Table 1. Some of them presented with the typical APS-1 triad, but others appeared with only two of the clinical manifestations. The clinical manifestations of some patients were severe and affected multiple glands accompanied by autoimmune diseases and others were relatively mild and only involved one or two glands. In our previous study, the case we reported has not yet been infected with chronic mucocutaneous candidiasis, which is the first clinical symptom in most APS-1 patients. From the existing research reports, this clinical manifestation is not a special case, although chronic mucocutaneous *Candida* infection is the most common disease in many APS-1 patients [7,36]. This finding suggests that in certain populations, the clinical manifestations of APS-1 patients have their own characteristics. However, this cannot be statistically analysed because the number of APS-1 patients with such clinical features is small. Intriguingly, Zhu et al. reported 2 siblings with APS-1 who, although they have the same *AIRE* mutation, present different clinical manifestations [30]. In combination with other studies of APS-1 around the world, we speculate that there may be other factors in addition to the mutation of the *AIRE* gene that may be responsible for the diverse clinical symptoms and disease severity of APS-1. However, these specific factors are not clear.

MSP	Sex	YOB	Manifestations (age in years at diagnosis of component)			
			MC	HP	AD	Additional components
c.769C>T c.55G>A	Female	1994	(2)	(6)	(9)	Nyctalopia (4), Epilepsy (4), Binocular cataract (5)
c.206A>C	Male	1995	-	(2)	(19)	Enamel dystrophy (14), binocular cataract (14), chronic intestinal dysfunction (14)
c.463G>A	Male	1987	-	(18)	(18)	Epilepsy (18), Pernicious anemia (18), Chronic/tension headaches (18), Keratopathy (19), Type 1 diabetes mellitus (24)
c.463G>A	Female	1988	(1)	(15)	-	Japanese encephalitis (7), Epilepsy (15)
c.622G>T	Female	2000	(13)	(14)	-	Pernicious anemia (14), Epilepsy (14)

Table 1: The clinical manifestations of the APS-1 patient (MSP: Mutation Sites of Patients; YOB: Year of Birth; MC: Mucocutaneous Candidiasis; HP: Hypoparathyroidism; AD: Addison's Disease; numbers in parentheses represent the age in years at the diagnosis).

APS-1 is an autosomal recessive disease caused by defects of the *AIRE* gene. The disease is highly prevalent in certain genetically isolated populations, such as Finns, Sardinians and Iranian Jews [37] but is rare in the Chinese population. In recent years, there have been reports of APS-1 cases in the Chinese population, which had attracted increased attention. More and more studies show that Chinese APS-1 patients have different *AIRE* gene mutations than other populations. These studies not only improve the research about APS-1 in China but also provide data for ongoing APS-1 research around the world. At the same time, the hotspots for *AIRE* mutations in Chinese APS-1 patients are approximately consistent with mutation hotspots in the rest of the world's APS-1 patients, providing a direction for further research on APS-1.

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Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Perheentupa J (2006) Extensive clinical experience-Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy. *J Clin Endocrinol Metab* 91: 2843-2850.
2. Nagamine K, Peterson P, Scott HS, Kudoh J, Minoshima S, et al. (1997) Positional cloning of the *APECED* gene. *Nat Genet* 17: 393-398.
3. Mora M, Hanzu FA, Pradas-Juni M, Aranda GB, Halperin I, et al. (2014) New splice site acceptor mutation in *AIRE* gene in autoimmune polyendocrine syndrome type 1. *PLoS ONE* 9: e101616.
4. Valenzise M, Meloni A, Betterle C, Giometto B, Autunno M, et al. (2009) Chronic inflammatory demyelinating polyneuropathy as a possible novel component of autoimmune poly-endocrine-candidiasis-ectodermal dystrophy. *Eur J Pediatr* 168: 237-240.
5. De Luca F, Valenzise M, Alaggio R, Arrigo T, Crisafulliet G, et al. (2008) Sicilian family with autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) and lethal lung disease in one of the affected brothers. *Eur J Pediatr* 167: 1283-1288.
6. Bratanic N, Kai K, Stefanija MA, Battelino T, Podkrajsek KT (2015) Clinical, genetic and immunological characteristics of paediatric autoimmune polyglandular syndrome type 1 patients in Slovenia. *Zdr Varst* 54:112-118.
7. Podkrajsek KT, Milenkovic T, Odink R, Claahsen H, Bratanic N, et al. (2008) Detection of a complete autoimmune regulator gene deletion and two additional novel mutations in a cohort of patients with atypical phenotypic variants of autoimmune polyglandular syndrome type 1. *Eur J Endocrinol* 159: 633-639.
8. Anderson MS, Venanzi ES, Klein L, Chen Z, Berzins SP, et al. (2002) Projection of an immunological self-shadow within the thymus by the *AIRE* protein. *Science* 298: 1395-1401.
9. Perniola R (2012) Expression of the autoimmune regulator gene and its relevance to the mechanisms of central and peripheral tolerance. *Clin Dev Immunol* 207403.
10. Gardner JM, Devoss JJ, Friedman RS, Wong DJ, Tan YX, et al. (2008) Deletional tolerance mediated by extrathymic *AIRE*-expressing cells. *Science* 321: 843-847.
11. Liston A, Goodnow CC (2005) Genetic lesions in thymic T-cell clonal deletion and thresholds for autoimmunity. *Novartis Found Symp* 267: 180-192.
12. Mathis D, Benoist C (2009) *AIRE*. *Annu Rev Immunol* 27: 287-312.
13. Mittaz L, Rossier C, Heino M, Peterson P, Krohn KJ, et al. (1999) Isolation and characterization of the mouse *AIRE* gene. *Biochem Biophys Res Commun* 255: 483-490.
14. Hu Z, Zhu W, Chen X, Huang W, Zhen Z (2016) Research of the relationship between the autoimmune polyendocrine syndrome type I and *AIRE* gene. *Immunol J* 32: 548-552.
15. Borden KL (2002) Pondering the promyelocytic leukemia protein (PML) puzzle: Possible functions for PML nuclear bodies. *Mol Cell Biol* 22: 5259-5269.
16. Sternsdorf T, Grötzinger T, Jensen K, Will H (1997) Nuclear dots: actors on many stages. *Immunobiology* 198: 307-331.
17. Sternsdorf T, Jensen K, Reich B, Will H (1999) The nuclear dot protein sp100, characterization of domains necessary for dimerization, subcellular localization and modification by small ubiquitin-like modifiers. *J Biol Chem* 274: 12555-12566.
18. Guldner HH, Szostecki C, Schroder P, Matschl U, Jensen K, et al. (1999) Splice variants of the nuclear dot-associated Sp100 protein contain homologies to HMG-1 and a human nuclear phosphoprotein-box motif. *J Cell Sci* 112: 733-747.
19. Gibson TJ, Ramu C, Gemünd C, Aasland R (1998) The APECED polyglandular autoimmune syndrome protein, *AIRE*-1, contains the SAND domain and is probably a transcription factor. *Trends Biochem Sci* 23: 242-244.
20. Bottomley MJ, Collard MW, Huggenvik JI, Liu Z, Gibson TJ, et al. (2001) The SAND domain structure defines a novel DNA-binding fold in transcriptional regulation. *Nat Struct Biol* 8: 626-633.
21. Ramsey C, Bukrinsky A, Peltonen L (2002) Systematic mutagenesis of the functional domains of *AIRE* reveals their role in intracellular targeting. *Hum Mol Genet* 11: 3299-3308.
22. Peterson P, Nagamine K, Scott H, Heino M, Kudoh J, et al. (1998) APECED: A monogenic autoimmune disease providing new clues to self-tolerance. *Immunol Today* 19: 384-386.
23. Meloni A, Incani F, Corda D, Cao A, Rosatelli MC (2008) Role of PHD fingers and COOH-terminal 30 amino acids in *AIRE* transactivation activity. *Mol Immunol* 45: 805.
24. Wolff AS, Erichsen MM, Meager A, Magitta NF, Myhre AG, et al. (2007) Autoimmune polyendocrine syndrome type 1 in Norway: Phenotypic variation, autoantibodies and novel mutations in the autoimmune regulator gene. *J Clin Endocrinol Metab* 92: 595-603.
25. Björset P, Halonen M, Palvimo JJ, Kolmer M, Aaltonen J, et al. (2000) Mutations in the *AIRE* gene: Effects on Subcellular location and transactivation function of the autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy protein. *Am J Hum Genet* 66: 378-392.
26. Akirav EM, Ruddle NH, Herold KC (2011) The role of *AIRE* in human autoimmune disease. *Nat Rev Endocrinol* 7: 25-33.
27. Liu C, Shi Y, Yin H, Li H, Fan S, et al. (2010) Autoimmune regulator gene mutations in a Chinese family with autoimmune polyendocrinopathy syndrome type I. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi* 27: 18-22.
28. Zhang J, Liu H, Liu Z, Liao Y, Guo L, et al. (2013) A functional alternative splicing mutation in *AIRE* gene causes autoimmune polyendocrine syndrome type I. *PLoS ONE* 8: e53981.
29. Sun YX, Ya-Fei HE, Xia-Lian LI (2016) Clinical analysis and autoimmune regulator gene mutation of autoimmune polyendocrinopathy syndrome type I in a family: A report of one case. *Zhongguo Dang Dai Er Ke Za Zhi* 18: 147-151.
30. Zhu W, Hu Z, Liao X, Chen X, Huang W, et al. (2017) A new mutation site in the *AIRE* gene causes autoimmune polyendocrine syndrome type I. *Immunogenetics*.
31. Wang CY, Davoodi-Semiromi A, Huang W, Connor E, Shi JD, et al. (1998) Characterization of mutations in patients with autoimmune polyglandular syndrome type 1 (APS1). *Hum Genet* 103: 681-685.
32. Meloni A, Perniola R, Faà V, Corvaglia E, Cao A, et al. (2002) Delineation of the molecular defects in the *AIRE* gene in autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy patients from Southern Italy. *J Clin Endocrinol Metab* 87: 841-846.
33. Cartegni L, Chew SL, Krainer AR. (2002) Listening to silence and understanding nonsense: Exonic mutations that affect splicing. *Nat Rev Genet* 3: 285-298.
34. von Schnurbein J, Lahr G, Posovszky C, Debatin KM, Wabitsch M (2008) Novel homozygous *AIRE* mutation in a German patient with severe APECED. *J Pediatr Endocrinol Metab* 21: 1003-1009.
35. Meloni A, Fiorillo E, Corda D, Perniola R, Cao A, et al. (2005) Two novel mutations of the *AIRE* protein affecting its homodimerization properties. *Hum Mutat* 25: 319.
36. Alimohammadi M, Björklund P, Hallgren A, Pöntynen N, Szinnai G, et al. (2008) Autoimmune polyendocrine syndrome type 1 and NALP5, a parathyroid autoantigen. *N Engl J Med* 358: 1018-1028.
37. Björset P, Aaltonen J, Vikman A, Perheentupa J, Ben-Zion G, et al. (1996) Genetic homogeneity of autoimmune polyglandular disease type I. *Am J Hum Genet* 59: 879-886.