

# AIRE Gene Mutation in Chinese APS-1 Patients

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#### Abstract

Autoimmune polyendocrine syndrome type 1 (APS-1, OMIM 2403000) 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 polyendocrinopathycandidiasis-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 tissuerestricted antigens (TRAs) in a subset of mTECs: the presentation of TRAs to the maturating thymocytes induces the clonal deletion of selfreactive 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 homogenously 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<sup>\*</sup>) 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 homogzygous 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,

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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)			
			МС	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.

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