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Research article

Novel homozygous mutations in *AIRE* leading to APS-1 and potential mechanisms based on bioinformatics analysis

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A R T I C L E I N F O

Keywords:
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A B S T R A C T

Background: Autoimmune Poly-endocrine Syndrome Type 1 (APS-1), also known as autoimmune poly-endocrinopathy-candidiasis-ectodermal dystrophy (APECED), is a single-gene hereditary disorder usually characterized by chronic mucocutaneous candidiasis, hypoparathyroidism, and autoimmune adrenocortical insufficiency. This syndrome is very rare in China.

Methods: For our reported patient, we employed clinical and laboratory examinations along with genetic identification. For previously reported cases, we summarized findings based on meta-analysis principles. To investigate the *AIRE* gene's role in disease, we utilized bioinformatics analysis with existing databases and R language processing.

Results: Nucleotide sequence analysis revealed two novel homozygous missense mutations (c.74C > G; c.1612C > T) in the patient's *AIRE* gene, confirming APS-1 diagnosis. The 3D structure of these mutation sites was described for the first time, showing that altered side chains could affect *AIRE* protein function. We analyzed 16 genetically diagnosed APS-1 Chinese patients, summarized the *AIRE* genetic spectrum, and found that exons 1, 2, 3, and 5 were most commonly affected. Hypoparathyroidism and adrenal insufficiency were the most common clinical manifestations (56%–93%), followed by hypothyroidism (31.25%), hypogonadism (12.5%), type 2 diabetes (6.25%), and type 1 diabetes (6.25%). Bioinformatics analysis indicated that *AIRE* mutations cause antigen presentation abnormalities in immune cells, leading to excessive endogenous and reduced exogenous antigen presentation.

Conclusions: Our study summarized the clinical features of APS-1 caused by *AIRE* gene mutations and explored underlying mechanisms. For some patients, the prophylactic use of antimicrobial agents may be beneficial. These findings guide early genetic screening and inform potential research directions for treatment strategies.

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1. Introduction

Autoimmune polyendocrine syndrome type 1 (APS-1) is a rare genetic disorder that is characterized by autoimmune dysfunction and affects multiple organs [1]. The onset of APS-1 usually occurs during childhood or early adolescence and presents with symptoms such as chronic mucocutaneous candidiasis, hypoparathyroidism, and autoimmune adrenal insufficiency [2,3]. However, with the increasing number of reported cases, the clinical manifestations of APS-1 are expanding to include ovarian insufficiency, autoimmune thyroid disease, and type 1 diabetes, among others [4]. Interestingly, the incidence of APS-1 varies by region, with higher prevalence in certain isolated populations like Iranian Jews (1:9000), Sardinians (1:14400), and Finns (1:25000) [9–11]. However, APS-1 is an uncommon disease with few cases reported in Asia, especially in China.

The genetic basis of APS-1 is well established, with over a hundred different mutations of the *AIRE* gene identified worldwide [5,6]. The atypical expression of the *AIRE* gene in the thymus can lead to the escape of autoreactive T-cells to the periphery, resulting in impaired immune tolerance and eventually leading to autoimmune diseases [5–8].

Additionally, the *AIRE* gene and protein are also expressed in peripheral blood, lymph nodes, and the spleen, but our understanding of their functions in these locations is limited [9–11]. Encouragingly, a study provides new insights into the extrathymic immunoregulatory role of *AIRE* that the extrathymic deletion of the *AIRE* gene impairs Th17 cells, leading to excessive fungal growth [12].

In this study, we present the case of a 15-year-old Chinese girl with clinically diagnosed APS-1 with two novel homozygous missense mutations of AIRE (c.74C > G p.A25G, c.1612C > T p.R538C). Since homozygous mutations in genes may lead to a decline in normal protein function, we hold that this may be involved in the occurrence and development of the disease. Despite the heterogeneity of the clinical presentation of APS-1, we were able to retrospectively outline the clinical manifestations, onset time, and *AIRE* gene characteristics. Furthermore, using bioinformatics, we explored the potential mechanism of *AIRE* gene mutations leading to APS-1. As healthcare professionals and patients, understanding the genetic basis and clinical presentation of APS-1 is crucial for accurate diagnosis and management.

2. Method

The study was approved by the Ethics Committee of Wenzhou Yuying Children's Hospital, The Second Affiliated Hospital of Wenzhou Medical University, with approval number 2023-K-85-01.

2.1. Diagnostic criteria of APS-I and collection methods

The clinical diagnostic criteria for APS-1 require at least two of three hallmark conditions: chronic mucocutaneous candidiasis, hypoparathyroidism and Addison's disease [13]. To confirm whether the patient and his family members had *AIRE* gene mutations, we collected peripheral blood from the APS-1 patient and his family members to sequence their AIRE genes.

2.2. Patient's clinical information

Routine laboratory tests were conducted on the patient, including the most critical measurements of ACTH and cortisol levels, six sex hormone levels, and five thyroid function parameters. These measurements were obtained using direct chemiluminescence technology with a double-antibody sandwich method (instrument: Siemens CentaurXP chemiluminescence analyzer). Parathyroid hormone (PTH) levels were measured using electrochemiluminescence (instrument: Roche cobas e801 electrochemiluminescence). Blood electrolytes (Na, K, Cl, Ca, Mg, P) were analyzed using a biochemical analyzer (Siemens ADVIA2400). Imaging examinations included thyroid, parathyroid, adrenal, pituitary head MRI, liver, kidney, heart, uterus, and ovary ultrasound, chest radiography, and electrocardiography.

2.3. Genetic identification

Peripheral venous blood samples (3–4 ml) were collected from the proband and their parents after obtaining informed consent. High-throughput second-generation sequencing was employed to perform whole-exome sequencing on the proband. Rare variants were evaluated and classified according to ACMG/AMP standards and guidelines. After detecting a homozygous mutation in the *AIRE* gene in the patient, Sanger sequencing was conducted to verify the mutation site in both the proband and their parents.

2.4. Literature review data

Two researchers(HPW and YQM) independently searched the databases of PubMed, Web of Science, and China National Knowledge Infrastructure (CNKI) using the keywords "*AIRE* or APS-1″ and "case or report" until December 5th, 2022. After removing duplicate and irrelevant articles, a third researcher(CBW) verified the number, and we finally obtained 14 reported cases of *AIRE* mutations in China from 8 different publications, plus to 16 cases with 2 patients in the present hospital [20–27].

2.5. Bioinformatics analysis

Data sources: NCBI (https://www.ncbi.nlm.nih.gov/), GETx database (downloaded via UCSC - http://xena.ucsc.edu), HPA

database(https://www.proteinatlas.org/), MSigDB(http://www.gsea-msigdb.org/gsea/msigdb/),GO/KEGG(http://geneontology.org/,https://www.kegg.jp/), and DICE (databasehttps://dice-database.org/).

Analysis process: The *AIRE* gene (NM_000383) was obtained from the NCBI Gene database (https://www.ncbi.nlm.nih.gov/). The three-dimensional structures of *AIRE* wild-type and mutant were generated using the ab initio method I-TASSER automated protein tool (https://zhanglab.ccmb.med.umich.edu/I-TASSER/). *AIRE* wild-type and mutants (p.A25G, p.R538C) used 5jcss as the template, and the three-dimensional protein structures were visualized using PyMOL 2.5 software. The expression range of different tissues can be obtained by searching for the gene name on the NCBI and HPA websites. PPI and disease association analyses are based on web-based tools by searching for the gene name, These resources can provide a basic understanding of the AIRE. After calculating the correlation and significance between other genes and the AIRE gene in the expression matrix, selecting genes with high significance ($P > 0.01$) and high correlation ($R > 0.5$) to form a gene set, and then performing enrichment analysis. Enrichment analysis, including Gene Ontology and GSVA, can help us understand which pathways may be involved with the highly correlated gene groups associated with the AIRE gene, while Enrichment analysis and GSVA is performed using the R programming language, and the relevant code has already been disclosed in our previous research [14].

3. Result

3.1. Patient characteristics

A 15-year-old Chinese girl, who was the second child in a family with no known blood relation between the parents, had an older sister with a history of recurring fainting spells and seizures. Over time, the young girl experienced a range of health issues, including hypoparathyroidism, menstrual irregularities, a potential genital fungal infection, and adrenal insufficiency, leading to multiple hospitalizations due to adrenal crises.

At the age of 12, she had frequent convulsions. Blood biochemistry analysis revealed hypocalcemia (1.34 mmol/L, reference range: 2.23–2.80 mmol/L), hyperphosphatemia (3.15 mmol/L, reference range: 1.45–2.10 mmol/L), hypomagnesemia (0.62 mmol/L, reference range: 0.75–1.02 mmol/L), and low parathyroid hormone levels (5.5 pg/mL) (Table 1). Following treatment with calcium gluconate injections and oral calcium gluconate, her serum calcium levels gradually normalized, and her condition improved. She was discharged with oral alfacalcidol and calcium supplements.

A few months later, she was readmitted to the hospital with chief complaints of vomiting, fatigue, and pallor. A physical examination showed normal temperature, a pulse of 124/min, a respiratory rate of 20/min, and blood pressure of 107/60 mmHg. The patient exhibited significant lip cyanosis and pallor, while the rest of the physical examination was unremarkable. Laboratory tests confirmed hyponatremia (122 mmol/L) and hypocalcemia (2.23 mmol). These findings were indicative of Addison's disease accompanied by an adrenal crisis. Additionally, the patient experienced menstrual irregularities, amenorrhea, and a potential genital fungal infection. However, laboratory results were within normal limits (Table 2), She did not meet the diagnostic criteria for ovarian failure [15,16]. According to other ultrasound examinations, the parathyroid and adrenal glands were of normal size. Furthermore, brain MRI results showed a normal pituitary gland.

The patient gradually recovered after receiving hydrocortisone replacement therapy. She regularly took oral saline and calcitriol following hospital discharge. During the last follow-up, the levels of serum natriuretic peptide, serum calcium, serum phosphorus, parathyroid hormone, thyroid-stimulating hormone, free T4, free T3, total T3, and total T4 were 136.1 mmol/L, 1.93 mmol/L, 2.36 mmol/L, 4.4 pg/mL, 0.584 μIU/mL, 1.82 ng/dL, 2.8 pg/mL, 0.62 ng/mL, and 7.78 μg/dL, respectively (Table ss 1–3).

3.2. Genetic identification and protein expression analysis

To confirm the diagnosis of Autoimmune Polyglandular Syndrome Type 1, the patient's DNA samples were analyzed using a high-throughput sequencing platform and bioinformatics analysis, revealing two *AIRE* mutations. Sanger sequencing was subsequently conducted for the proband and family members. Whole-exome sequencing identified a homozygous C to G mutation at position 74 in exon 1 of the *AIRE* gene in the proband, resulting in a missense mutation that replaced Alanine with Glycine at amino acid position 25 (p.A25G) (Fig. 1A). This protein variant was predicted to be deleterious by both PolyPhen-2 and REVEL, while SIFT provided a benign prediction (supplement data2). A c.1612C > T mutation was observed in exon 14 of *AIRE*. At present, these two sites are based on existing screening data in the ClinVar database, but no cases resulting in ASP-1 patients have been reported to date (supplement data3).

Table 1
The electrolyte changes during follow up.

	A1	A2	B1	B2	C1	C2	Last	Normal	
Na	136	137	122	126.2	131.2	135.5	135.3	135~145	mmol/l
K	3.96	4.01	5.05	4.55	4.5	5.02	3.87	3.5–5.5	mmol/l
Cl	97	100	85	88.5	96.9	72.8	100.1	96~108	mmol/l
Ca	1.34	1.77	2.16	2.43	1.95	2.18	1.94	2.25–2.59	mmol/l
Mg	0.62	0.78	0.81	0.59	0.58	0.64	0.74	0.65–1.55	mmol/l
P	3.15	2.32	2.17	2.2	1.48	2.06	1.86	0.96–1.80	mmol/l

A1 = before the first treatment; A2 = after the first treatment; B1 = before the second treatment; B2 = after the second treatment; C1 = before the third treatment; C2 = after the third treatment.

Table 2
The change of reproductive hormone during the treatment.

Hormone	First	Second	Normal	
FSH	9.47	13.89	0.50–5.0	IU/L
LH	/	0.47	0~0.50	IU/L
PRL	9.39	11.67	2.80–29.	ng/ml
P	<0.21	<0.21	0.21–1.7	ng/ml
E2	22.85	31.14	10~30	pg/ml

FSH = follicle stimulating hormone; LH = luteinizing hormone,PRL = prolactin, P = progesterone, E2 = estradiol.

Table 3
The results of thyroid + parathyroid follow up.

hormone	First	Second	Third	Last	Normal	
PTH	5.5	7.4	4.4		15~65	pg/ml
TSH	1.397	7.311	1.808	0.584	0.55–4.78	μIU/ml
TT3	0.98	1.2	0.71	0.62	0.86–1.92	ng/ml
TT4	9.98	11.05	6.81	7.78	5.52–11.1	μg/dl
FT3	3.53	4.18	2.92	2.8	3.05–4.68	pg/ml
FT4	1.42	2.07	1.38	1.82	0.82–1.43	ug/dl

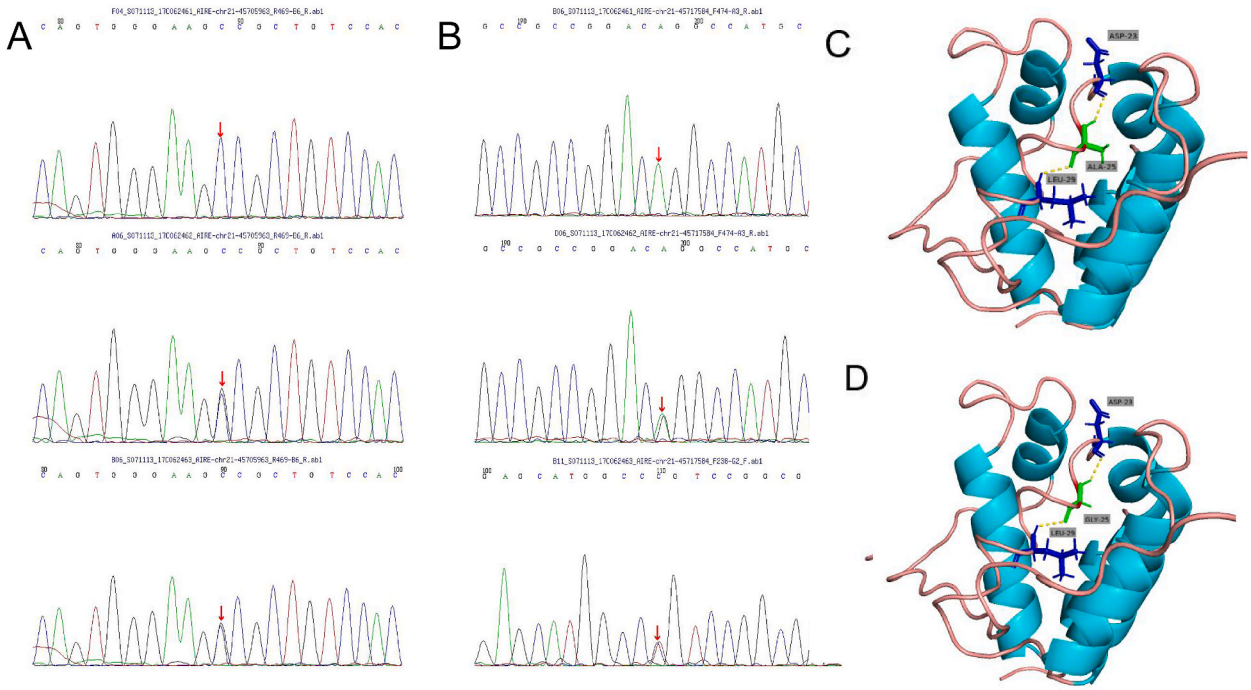

Fig. 1. Chromatogram of mutations and Three-dimensional structural model and schematic representation in *AIRE* protein. A) Whole-exome sequencing revealed a homozygous C to G mutation at position 74 in exon 1 of *AIRE* gene in the proband (Father/Mother/Patient). This leads to a missense mutation by a substitution of Alanine with Glycine at amino acid position 25 (p.A25G). The same heterozygous c.74>G mutation was identified in his parents; B) Whole-exome sequencing revealed a homozygous C to T mutation at position 1612 in exon 14 of *AIRE* gene in the proband (Father/Mother/Patient). This leads to a missense mutation by a substitution of Arginine with Cysteine at amino acid position 538 (p. R538C). The same heterozygous c.1612>T mutation was identified in his parents; C) A structural change caused by p. Ala25Gly in CARD/HSR domain. chain amino acids are altered; D) A structural change caused by p. Arg538Cy, hydrogen bonds between the side chain of Arg-538 with the side chains of Asp-502 vanished.

Additionally, whole-exome sequencing confirmed a homozygous C to T mutation at position 1612 in exon 14 of the *AIRE* gene in the proband, causing a missense mutation that replaced Arginine with Cysteine at amino acid position 538 (p.R538C) (Fig. 1B). This mutation was predicted to be pathogenic by SIFT, PolyPhen-2 protein function prediction, and REVEL. Heterozygous mutations in the same region were also detected in the patient's parents, who did not exhibit any features of APS-1. However, her parents did not manifest any symptoms of APS-1, leading us to consider these phenotypes caused by new mutations as recessive. The two novel

mutations were identified as pathogenic by REVEL (Rare Exome Variant Ensemble Learner). A three-dimensional structural model and schematic representation of the *AIRE* protein are illustrated in Fig. 1C–D. These results suggest that when homozygous mutations occur in *AIRE*, the onset of the disease may be due to the absence of normal protein expression.

3.3. Summary of clinical studies about Chinese patients

Currently, over 160 types of *AIRE* mutations have been identified in APS-1 patients, and among these, the most common types were present in exons 1, 2, 6, 8, and 10. However, exons 1, 2, 3, and 5 were the most commonly affected region in Chinese patients (Fig. 2). Sixteen APS patients from various regions in China were included in previous literature (Table 4). The results determined that the female/male ratio was 1:1 and the age of the first onset of the disease was primarily during puberty. Furthermore, all the APS-1 patients included in the study were affected by the three major diseases. Hypoparathyroidism was one of the first endocrine features of APS-1, followed by CMC and AD. Five patients (31.2%) displayed the classic triad, while ten patients (62.5%) exhibited two symptoms of the triad. Interestingly, only one patient had one manifestation of the triad. Furthermore, the patients presented with an average number of 4.3 ± 0.3 APS-1-related features. The age of onset of the first manifestation was 8.8 ± 6.2 years. The initial manifestation was hypoparathyroidism (93.7%). CMC (62.5%) included oral candidiasis, vulvodynia, and cutaneous candidiasis, with the average onset being 10 ± 8 years (range, months after birth to 27 years). Nine cases (56.3%) with AD were diagnosed at a mean age of 15 ± 4.5 years. (Fig. 3A–C). The age span of the manifestations was large. The fact that the age of diagnosis was later than reported in previous literature might be associated with the low positive rate of mycological methods and the fact that patients developing mild symptoms did not seek medical treatment during the early stage of the disease. Among all cases, hypoparathyroidism and adrenal insufficiency were the most common (56%–93%), followed by hypothyroidism (31.3%), hypogonadism (12.5%), type 2 diabetes (6.3%), and type 1 diabetes (6.3%).

Of all the mutations in Chinese patients, 14 were missense mutations, 2 were truncating ones, 3 were frameshift mutations, 2 were nonsense mutations, and 7 were de novo mutations (p.AG > TG, p.P163fsX215, p.k161fs, p.A246fs, p.G208V, p.A25G, and p.R538C). Moreover, homozygous mutations (p. P163fsX215, p. G155S, p. Y90C, p. R139X, p. Q69P, p. T16R, p. A25G, and p. R538C) were identified in nine patients (cases 4–9, 12, 14, and 16), whereas heterozygous mutations were found in seven patients (cases 1–3, 10–11, 13, and 15). The mutations mainly concentrated on CARD (p.A25G,p.Q69P, p.Y90C, p.L13P, and p.T16R), SAND(p.G208V, p.A246fs, p.G208W, p.R257X, PRR, and PHD) and some nonfunctional regions (p.R139X, p.G155S, and p.K161fs). Finally, compound heterozygous mutations were encountered in three patients (p. A19T + p.R257X, p. A246fs + p.L308F, and p. G208V + p.P124L) (Table 4).

3.4. AIRE gene analysis in relation to dual immune response in APS-1 patients

To investigate the *AIRE* gene's role in the dual immune response in APS-1 patients, we first analyzed its distribution in different tissues using the NCBI and HPA databases(Fig. 4A–B). Our analysis showed that *AIRE* was most highly expressed in thymus and lymph nodes, which is closely related to its function. Furthermore, we analyzed the PPI pathways and disease spectrum of *AIRE* (Fig. 4C-D).

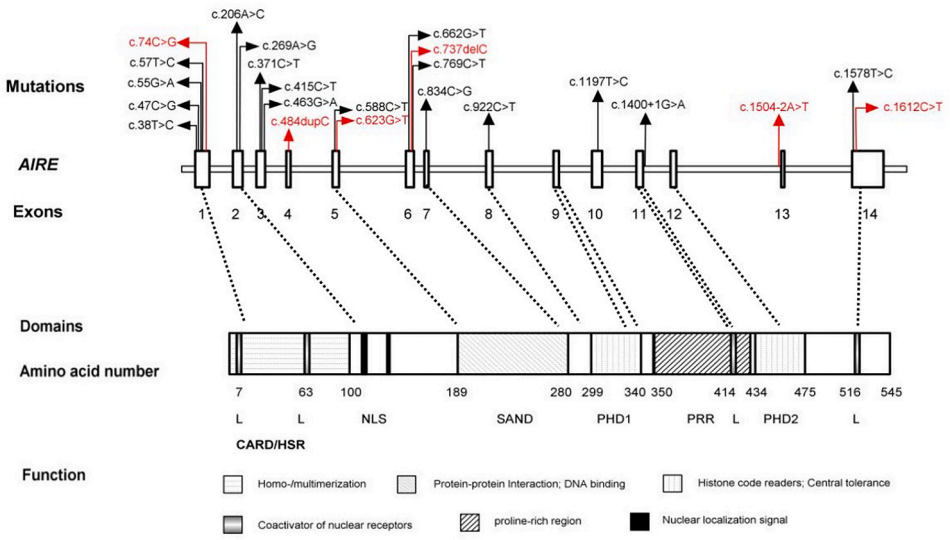

Fig. 2. Schematic diagram of the *AIRE* gene and domains of protein and the position of mutations in Chinese APS-1 patients have reported (black mutations) and novel(red mutations) in this study. HSR/CARD, homogenously staining region or caspase recruitment domain/homodimerization domain; NLS = nuclear localization signal, SAND=Sp100, *AIRE*, NucP41/75 and DEAF-1; PHD = plant homeodomain zinc finger, PRR = proline-rich region, L = the LXXLL nuclear receptor interaction motif. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 4
Characteristics of reviewed reports of APS-1 in china.

Case	Sex/Age	Clinical	Others	Sequence vacation	Amino acid change	location	Hm/Ht	Ref
1	F/15	HP(14) CMC(12)	ED(13) IM(13)	c.662 G > T/c.57T > C c.588C > T/c.834C > G c.1197T > C/c.1578T > C	p.G208W	ex5	Ht	[21]
2	M/9	HP(8.5) AD(8.9)	N/A	IVS11+1G > A	p.GT > AG	i 11	Ht	[22]
3	M/30	HP(21) AD(21) CMC(27)	T2DM(27)	c.47C > G c.1631-2A > T	p.T16R p.AG > TG	ex1 ex13	Ht	[23]
4	F/27	CMC(10) HP(18) AD(20)	HT(22)	c.483-484insC	p.P163fsX215	ex4	Hm	[24]
5	M/18	HP(18) AD(18)	EP(18) K(19) HE(18) AN(18) T1DM(24)	c.463G > A	p.G155S	ex3	Hm	[20]
6	F/21	CMC(1) HP(15)	EP(15) JE(7)	c.463G > A	p.G155S	ex3	Hm	[20]
7	M/42	HP(−) CMC(−)	K(37) A(−) HG(−)	c.415C > T	p.R139X	ex3	Hm	[25]
8	M/23	HP(15.6) AD(18)	HT(18)	c.484dupC	p.k161fs	ex4	Hm	[26]
9	F/28	HP(5) CMC(17) AD(15)	HT(15) HO(16)	C.269A > G	p.Y90C	ex2	Hm	[26]
10	F/18	HP(5) CMC(8)	ED(2) A(13) HT(9) RP(0.8)	C.38T > C	p.L13P	ex1	Ht	[26]
11	M/10	HP(5) CMC(8)	ED(−)	C.737delC + c.922C > T	p.A246fs + p.L308F	ex6	Ht	[26]
12	M/19	HP(14) AD(19)	RTA(2) ED IM	C.206A > C	p.Q69P	ex2	Hm	[26]
13	F/32	HP(7)	HO(31) HG(18) IM(31)	C.623G > T c.371C > T	p.G208V/p.P124L	ex5/ex3	Ht	[27]
14	M/15	CMC(3) HP(15) AD(15)	HT(15) S(15)	C.47C > G	p.T16R	ex1	Hm	[16]
15	F/14	AD(9) HP(6) CMC(4)	EP(4) Ca [5]	c.55G > A c769C > T	p.A19T/p.R257X	ex1	Ht	
16	F/12	AD(15) HP(12)	N/A	c.74C > G/c.1612C > T	p.A25G/p.R538C	ex1/ex14	Hm	

HP = hypoparathyroidism; CMC = chronic mucocutaneous candidiasis; AD = Addison's disease; ED = ectodermal dysplasia; A = alopecia; HT = hypothyroidism; HG = hypergonadotropic hypogonadism; K= Keratopathy; RP = retinitis pigmentosa; IM = intestinal malabsorption; HO = hematopathy; T1DM = Type 1 diabetes mellitus, JE = Japanese encephalitis, EP = Epilepsy, AN = anemia, HE= Chronic/tension headaches, S = spleen atrophy RTA = renal tubular acidosis; Ca = cataract.

To further explore the molecular mechanisms underlying the dual immune response, we focused on the genes highly associated with *AIRE* in Th17 cells and performed enrichment analysis(Fig. 4E, Supplement data1).We then analyzed the correlation between *AIRE* gene and MHC I and MHC II. We found that the down-regulation of *AIRE* may up-regulate certain genes involved in the synthesis of MHC I (such as HLA-A and HLA-B, Fig. 4F-G) while down-regulating all genes related to MHC II molecules(Fig. 4H–I). Our analysis of the relationship between *AIRE* gene and antigen presentation revealed that *AIRE* is negatively correlated with overall antibody presentation and MHCI-related antigen presentation, and positively correlated with MHCII antibody presentation (Fig. 4J).

4. Discussion

The present study showed two homozygous missense mutations, namely p. A25G and p.R538C, in the *AIRE* gene of the Chinese girl diagnosed with APS-1, which contributes to the elucidation of the genetic background of the disease. A search in the Human Gene Mutation Database and previous literature indicated That the two mutations were novel mutations, and the ClinVar database shows that the two new mutation sites are of uncertain clinical significance, which indicate that the previous phenotype performance is not obvious. However, as the number of patients with phenotypes at the same locus increases, the likelihood of coincidences occurring will gradually decrease. This is the first case of two homozygous mutations reported in the literature in China. Considering that the patient's parents were asymptomatic, this phenotype caused by novel mutation was defined as recessive.

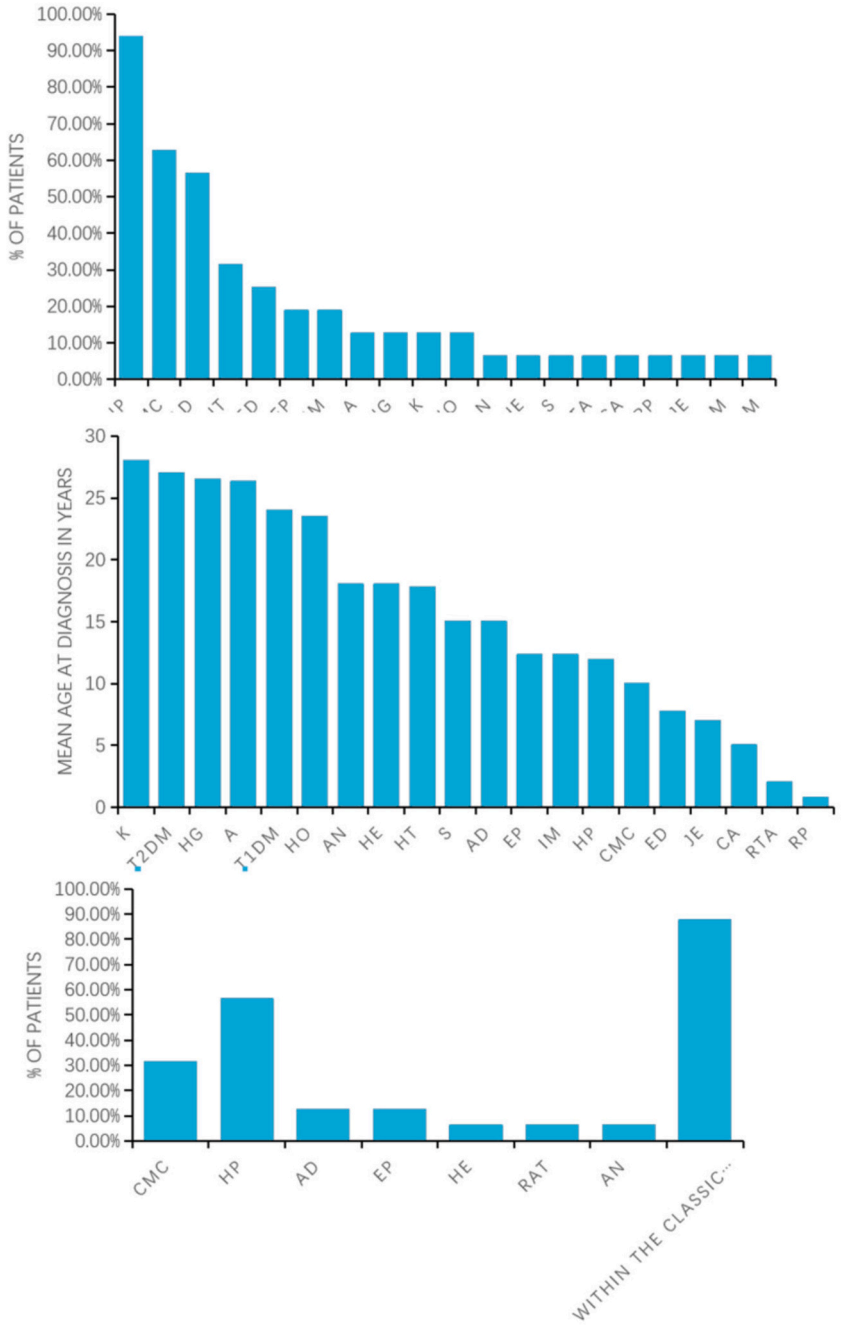

Fig. 3. The clinical feature spectrum of APS**-1 patients.** A) Prevalence of all disease manifestations in the 16 Chinese APECED patients. B) Mean age at diagnosis of all clinical manifestations among the APECED patients who developed the corresponding disease components. C) Distribution of the initial manifestation in the 16 Chinese APECED patients. Black bar denotes manifestations within the current classic diagnostic triad.

The homogenously staining region (HSR), contained within the first 100 amino acids of *AIRE*, is responsible for homodimerization [17,18]. Because of the HSR's α-helical four-helix bundle structure, it is sensitive to conformational alterations. Therefore, HSR may be considered a region with a high mutation rate, which results in APS-1 [19]. The first homozygous mutation site reported herein, p. A25G amino acid changes at codon 25, may result in conformational changes of the four-helix bundle structure in the homogenously staining region or caspase recruitment domain (HSR/CARD) of exon 1. Indeed, mutations in this domain may yield a defective functional protein that affects the homo-/heterodimerization required for transcriptional transactivation activity of *AIRE*. However, p. R538C was not within the functional threshold. Our review found that the onset of the triad and other related symptoms were relatively late in patients in the non-functional threshold. (p. R139X, p. G155S, and p. K161fs). Interestingly, although our patient was the

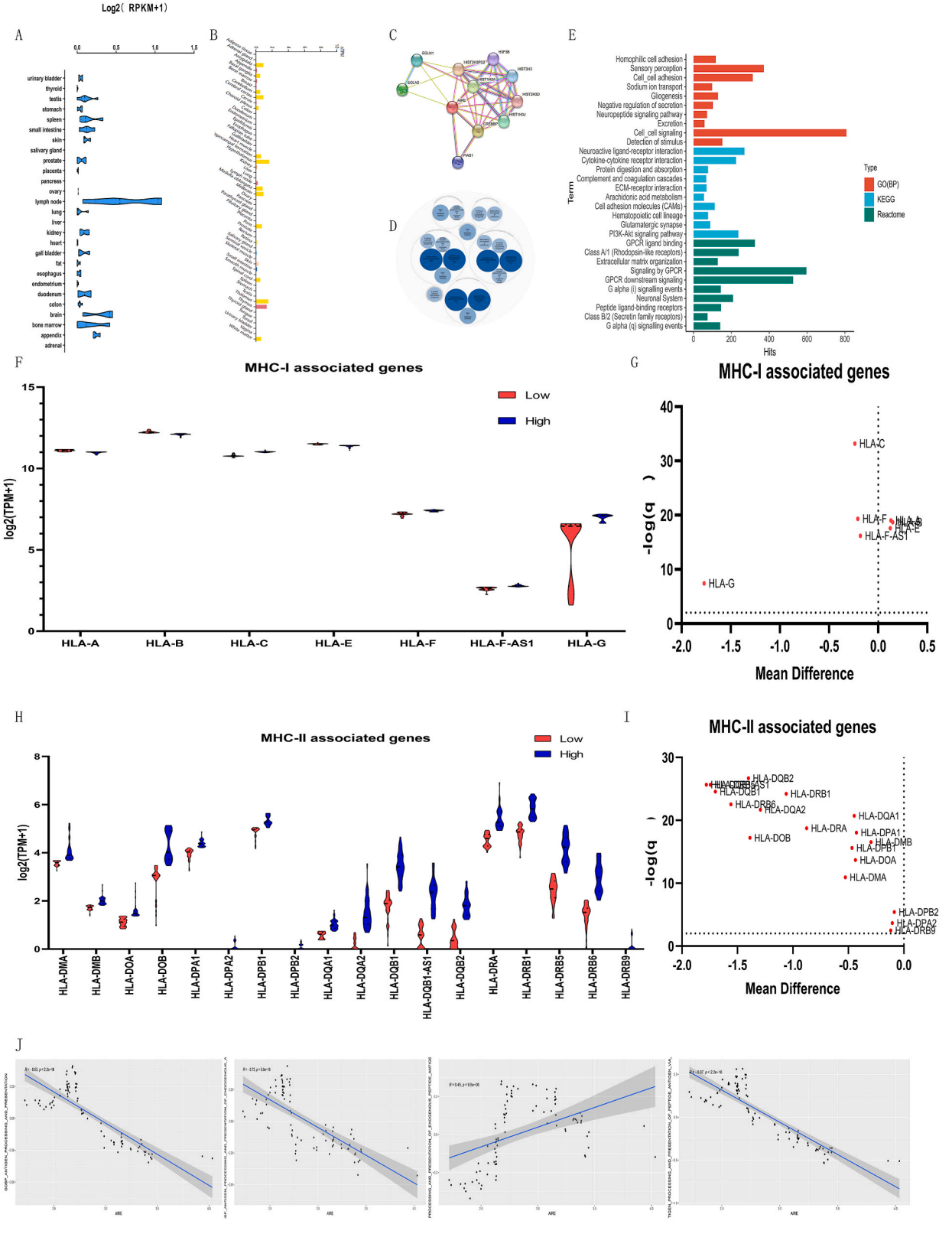

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Fig. 4. Bioinformatics analysis of the *AIRE* gene. A) Distribution levels of *AIRE* mRNA in different tissues based on the NCBI-gene database. B) Distribution levels of *AIRE* mRNA in different tissues based on the HPA database. C) PPI analysis of *AIRE* and other related proteins based on the String database. D) Analysis of the spectrum of *AIRE*-related diseases. E) Enrichment analysis of the *AIRE*-associated gene set in th17 cells. F) Expression levels of MHC I-related genes in different *AIRE* expression level groups. G) Volcano plot showing the differential expression levels of MHC I-related genes caused by high *AIRE* expression. H) Expression levels of MHC II-related genes in different *AIRE* expression level groups. I) Volcano plot showing the differential expression levels of MHC II-related genes caused by high *AIRE* expression. J) Regression analysis of *AIRE* with antigen presentation signaling pathway and MHC molecule-mediated antigen presentation.

first case of compound homozygous mutation, the clinical phenotype and other clinical manifestations of the triad were not distinct from other cases of compound heterozygous and homozygous mutations reported in China and abroad in terms of the age of onset, disease progression, disease severity or the sites of involvement.

Among all cases, the incidence of ovarian failure in China was far below that in the United States (38.1% of females), whereas hypothyroidism was significantly higher compared to the United States (22.9%) [3]. Compared with endocrine diseases, nonendocrine clinical presentations and age of onset differed significantly among the 16 individuals with mutations. The most commonly in CMC with APS-1. Except from CMC, other nonendocrine diseases included ectodermal dysplasia (25%), epilepsy (18.75%), intestinal malabsorption (18.75%), alopecia (18.75%), keratopathy (18.75%), and hematologic diseases (18.75%), while renal tubular acidosis (6.25%), anemia, splenic atrophy, cataract, and encephalitis were less commonly encountered [3].

Most APS-1 patients from China were diagnosed with the classic triad (CMC, hypoparathyroidism, and adrenal insufficiency) during their disease course. A review of the clinical record of these patients uncovered that seven patients developed a median of 3 non-triad manifestations before the diagnostic dyad, and the most common manifestations were urticarial eruption and hypothyroidism. In contrast, the most common presentations were urticarial eruption, intestinal dysfunction, and ectodermal dysplasia in the US cohort. APS-1 should be considered when patients exhibit signs of ectodermal dysplasia and present with other endocrine clinical symptoms during the disease. It was proposed that the appearance of these symptoms should be added to the clinical diagnostic criteria. Therefore, diagnosis of the disease should be enhanced to allow for early treatment.

Although the pedigrees of *AIRE* gene mutations and the clinical features of APS-1 patients are well characterized worldwide, we still know little about the underlying mechanisms. Based on our bioinformatics analysis, we have found a plausible explanation for the characteristic autoimmune activation and weakened fungal resistance observed in APS-1 patients. We suggest that the *AIRE* gene may be involved in regulating the expression levels of MHC I and II molecules and the antigen presentation pathway. As the expression of the *AIRE* gene decreases, the likelihood of presenting endogenous antigens increases while the ability to present exogenous antigens decreases. This may contribute to the immune response characteristics observed in APS-1 patients. These findings may have important implications for the development of more effective therapies for APS-1 patients.

There are several limitations to our study. Firstly, due to technical limitations, the present study did not include data on the subjects' own antibodies and in the case of APS-1 patients with no known blood relationship between parents, the probability of finding a homozygous double-site mutation is indeed very low. We did not conduct further extensive genetic testing on their family lineages. Secondly, although our bioinformatics analysis provided insights into how the *AIRE* gene contributes to the dual immune response in APS-1 patients, further laboratory research is necessary to confirm our findings. More evidence is needed to support this view. Thirdly, it is important to recognize that, unlike research on more common diseases, studies on rare diseases such as APS-1 are still in their nascent stages. Even though diagnostic criteria and associated genetic backgrounds have been established, direct evidence elucidating the causal relationship between genetic mutations and disease manifestation remains elusive. The call for increased investment and innovative research into rare diseases is urgent and holds promise not only for the treatment of these conditions but also for the potential discovery of underlying mechanisms. Such insights could profoundly deepen our understanding of genetics and immunity, possibly offering clues for other immune-related disorders. It is imperative that we value the unique perspectives that rare diseases provide as new windows into the complexities of human health.

Despite these limitations, our study results are still encouraging. Detecting APS-1 patients can be challenging due to factors such as lack of awareness, clinical heterogeneity, limited access to diagnostic tools, and cultural factors. Currently, early genetic screening for suspected patients is the primary diagnostic method. Most clinical reports in the past have been limited to clinical phenotypes, and we know little about their potential mechanisms. Our exploration of the underlying mechanisms in this study can undoubtedly help to broaden our understanding and identify suitable targets for future research.

In addition, we also need to be aware that in some cases, when APS-1 patients need to use immunosuppressants to control excessive autoimmune reactions, it may be necessary to use anti-infective drugs to prevent infections due to the possible insufficient presentation of exogenous antibodies in APS-1 patients themselves.

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Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

CRediT authorship contribution statement

Huiping Wu: Resources, Project administration, Methodology, Investigation, Data curation. **Yiqi Mo:** Writing – review & editing, Writing – original draft, Data curation. **Shiwen Yu:** Resources, Formal analysis, Data curation. **Xiaojun Ye:** Writing – review & editing, Writing – original draft, Data curation. **Yili Lu:** Writing – review & editing, Writing – original draft, Data curation. **chaoban Wang:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation. **Xiaoou Shan:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e28037.

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