

# A novel variant in *AIRE* causing a rare, non-classical autoimmune polyendocrine syndrome type 1

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**Abstract.** Autoimmune polyendocrine syndrome type 1 (APS-1) is a rare inherited autoimmune disease, characterized by a classic triad, including chronic mucocutaneous candidiasis, primary adrenocortical insufficiency and hypoparathyroidism. The present study investigated phenotypes and pathogenic variants in a Chinese woman with non-classical APS-1. Disease-associated variants in a patient with APS-1 were identified via targeted next generation sequencing and the variant was confirmed via Sanger sequencing. Serum levels of calcium, phosphorus, parathyroid hormone (PTH), follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol and urinary levels of calcium were measured. Blood count assays and bone marrow morphology were investigated. The patient was a 32-year-old woman who had suffered from typical carpopedal spasms since she was 7 years old. She developed syncope, primary amenorrhea, intermittent diarrhea and general fatigue in subsequent years. Hypocalcemia, hyperphosphatemia, low

levels of PTH and estradiol, elevated levels of FSH and LH, and absence of erythroblasts were observed, which indicated hypoparathyroidism, primary ovarian insufficiency and pure red cell aplasia. A novel heterozygous missense variant (NM\_000383.2: c.623G>T, NP\_000374.1: p.Gly208Val) in exon 5 of autoimmune regulator and a reported variant (NM\_000383.2: c.371C>T, NP\_000374.1: p.Pro124Leu) in exon 3 were detected, of which the c.623G>T variant may be a pathogenic variation that induces APS-1. Under a regular follow-up and therapeutic adjustment of calcium, calcitriol, hormone replacement therapy and methylprednisolone, the endocrine function and clinical symptoms of the patient were notably improved. The results of the present study expand the known genetic and phenotypical spectra of APS-1.

## Introduction

Autoimmune polyendocrine syndrome type 1 (APS-1; OMIM 240300), also known as autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy, is a rare disease, characterized by chronic mucocutaneous candidiasis (CMC), primary adrenocortical insufficiency (PAI) and hypoparathyroidism (HP) (1). APS-1 may present with other abnormalities, such as primary ovarian insufficiency, vitiligo, alopecia, type 1 diabetes mellitus (T1D), chronic intestinal dysfunction, enamel hypoplasia, and autoimmune hepatitis (2,3). Pure red cell aplasia (PRCA), metaphyseal dysplasia, retinitis and polyarthritis have also been reported in patients with APS-1 (4,5).

APS-1 is primarily caused by variants in the autoimmune regulator gene (*AIRE*), which is located on chromosome 21q22.3 (6). *AIRE* contains 14 exons and encodes a 57-kDa transcription regulator of 545 amino acids (7,8). *AIRE* is primarily expressed in medullary thymic epithelial cells, which serve key roles in maintaining central immunological tolerance via promoting the negative selection of autoreactive thymocytes and building the thymic microarchitecture (6). *AIRE* is also distributed in the periphery of antigen-presenting cells (9). If the functions of the *AIRE* are impaired, autoreactive T cells can escape into the periphery, resulting in a number of autoimmune abnormalities such as autoimmune attack against parathyroid and adrenal glands in APS-1 (6).

APS-1 is relatively prevalent in Iranian Jews and Sardinians, with a prevalence of 1:145,000 to 1:9,000 (10-12).

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*Abbreviations:* CMC, chronic mucocutaneous candidiasis; PAI, primary adrenocortical insufficiency; HP, hypoparathyroidism; PRCA, pure red cell aplasia; Cr, creatine; ALT, alanine aminotransferase; PTH, parathyroid hormone; FSH, follicle-stimulating hormone; LH, luteinizing hormone; FT4, free thyroxine; FT3, free triiodothyronine; TSH, thyroid-stimulating hormone; TPOAb, autoantibodies to thyroid peroxidase antigen; TGAb, autoantibodies to thyroglobulin; ACTH, adrenocorticotropic hormone; ICA, islet cell antibodies; GADA, glutamic acid decarboxylase antibodies; ANA, autoantibodies to antinuclear antibody; RO-52, 52 kDa Ro/SS-A molecule; ANCA, anti-neutrophil cytoplasmic antibodies

*Key words:* autoimmune polyendocrine syndrome type 1, heterozygous novel variant, autoimmune regulator, non-classical

Over 100 different variants in *AIRE* gene have been found in patients with APS-1, including substitutions, insertions, deletions and splice site variants [BIOBASE Human Gene Mutation Database (HGMD) professional 2019.1; hgmd.cf.ac.uk/ac/index.php]. To the best of our knowledge, however, there are very few reports of APS-1 in Chinese patients, and its pathological mechanism has not been fully elucidated.

The present study investigated the phenotype and genotype of a Chinese woman with non-classical APS-1. Characteristics of previously reported Chinese patients with APS-1 induced by *AIRE* gene variants were also summarized.

## Materials and methods

**Subjects.** A 32-year-old woman of Chinese Han origin from a non-consanguineous family was recruited following admission to the Department of Endocrinology, Peking Union Medical College Hospital (PUMCH; Beijing, China) in 2018, with suspected APS-1 based on the clinical features of syncope, tetany, primary amenorrhea, intermittent diarrhea and general fatigue.

**Phenotypic evaluation.** The functions of multiple endocrine glands and examined levels of numerous autoantibodies were evaluated. Serum levels of calcium, phosphorus, creatinine (Cr) and alanine aminotransferase (ALT), and 24-h urinary concentrations of calcium (24hUCa) and phosphorus (24hUP) were assessed via an AU5800 Beckman Automatic Biochemical Analyzer (Beckman Coulter, Inc.). Serum levels of parathyroid hormone (PTH), follicle-stimulating hormone (FSH), luteinizing hormone (LH) and estradiol, as well as serum-free thyroxine (FT4), free triiodothyronine (FT3), thyroid-stimulating hormone (TSH) and autoantibodies to thyroid peroxidase antigen (TPOAb) and thyroglobulin (TGAb) were measured using an automated electrochemiluminescence system (Roche Diagnostics). Blood samples measured for serum cortisol and plasma adrenocorticotropic hormone (ACTH) were collected at 8 AM and measured via fluorescent immunoassay (Diagnostic Products). Serum autoantibodies to islet cells antibodies (ICA) and glutamic acid decarboxylase antibodies (GADA) were measured using radioimmunoassays kits (Cisbio; Perkin Elmer, Inc.). Serum autoantibodies to anti-nuclear antibody (ANA), 52 kDa Ro/SS-A molecule (RO-52), as well as anti-neutrophil cytoplasmic antibodies (ANCA) were tested using an indirect immunofluorescence kit (ANA screen 11; cat. no. EA 1590-9601-11 G; Euroimmun AG). The blood count assays were measured using a Sysmex XE-5000 hematology analyzer (Sysmex Corporation). Serum levels of erythropoietin (EPO; cat. no. DEP00) and antibodies to IFN- $\omega$ , IFN- $\alpha$  (cat. no. 21100-1), IL-17A (cat. no. MAB317-100), IL-17F (cat. no. MAB13353-100) and IL-22 (cat. no. MAB7822-100) were detected using ELISA kits (R&D Systems Inc.).

Morphology of thyroid, abdomen, uterus and ovary were assessed via standard ultrasound scanning (Siemens Healthineers). Calcification in the central nervous system was evaluated via CT scan.

The diagnosis of hypoparathyroidism was determined by hypocalcemia, hyperphosphatemia and decreased intact PTH level. The diagnosis of primary ovarian insufficiency was based on amenorrhea, absent pubertal development and

elevated serum levels of FSH or LH. The diagnosis of PRCA was based on normal marrow in the absence of erythroblasts (<1% erythroblasts on the differential count of bone marrow cells).

**Identification of pathogenic variants.** DNA was harvested from peripheral leucocytes using the DNA Extraction kit (QIAamp DNA; Qiagen GmbH) according to the manufacturer's instructions. Targeted next-generation sequencing (NGS) was used to identify the pathogenic variants. A panel containing >700 genes of bone disease was created to capture all exons sequences of the candidate genes of APS-1 and hypoparathyroidism (such as *AIRE*, T-box transcription factor 1, tubulin-specific chaperone E and family with sequence similarity 111 member A), as previously described (13). Bioinformatic analysis was performed as previously described (13).

Fragments from the patient, her parents and 500 unrelated healthy controls covering the variant sites in *AIRE* identified by NGS were amplified via PCR. Primer sequences were as follows: Exon 3: Forward 5'-ACCCTACCCCTGGAGAAAAC-3' and reverse 5'-TGGTCCAGTGTGTGGGTCTA-3'; and exon 5: forward, 5'-GCCTGCTTCTGGCATAGAGT-3' and reverse, 5'-AACAGTCCGGTCTGCTGTG-3'.

PCR was performed using TaqMix DNA polymerase (Biomed) under the following conditions: 95°C for 3 min, followed by 35 cycles of 95°C for 30 sec, annealing at 60°C for 30 sec, and 72°C for 1 min, followed by a final extension at 72°C for 10 min. Products of PCR were purified and sequenced on the ABI377 DNA automated sequencer using Big Dye Terminators Cycle Sequencing Ready Reaction kit (Applied Biosystems; Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions.

In order to predict the potential deleterious effects of the variants, PolyPhen-2 (Polymorphism Phenotyping v2; genetics.bwh.harvard.edu/pph2/), SIFT (sift.jcvi.org/) and MutationTaster (mutationtaster.org/) were utilized. The conservation of the amino acid substitution position among species was analyzed via UniProt software (uniprot.org).

A literature review of reported Chinese patients with APS-1 exhibiting *AIRE* variants was performed and clinical and genetic characteristics were summarized (7,14-17).

**Three-dimensional modeling of AIRE.** In order to investigate the change in protein induced by the pathogenic variant in *AIRE*, the three-dimensional structure of the AIRE protein was constructed via SWISS-MODEL (swissmodel.expasy.org). Subsequently, the disease-associated p.Gly208Val substitution of AIRE protein was built using the mutagenesis module of PyMol software (v2.3.1; pymol.org/).

The present study was approved by the Scientific Ethics Committee of PUMCH (approval no. JS-2081). A total of 500 unrelated healthy individuals were recruited as controls for genetic study. Written informed consent was provided by all subjects prior to participation. The pedigree of the family of the patient is shown in Fig. 1A.

## Results

**Phenotypes of the patient.** The patient, a 32-year-old woman (height, 158.0 cm; weight, 52.5 kg; body mass index, 21.0 kg/m<sup>2</sup>),

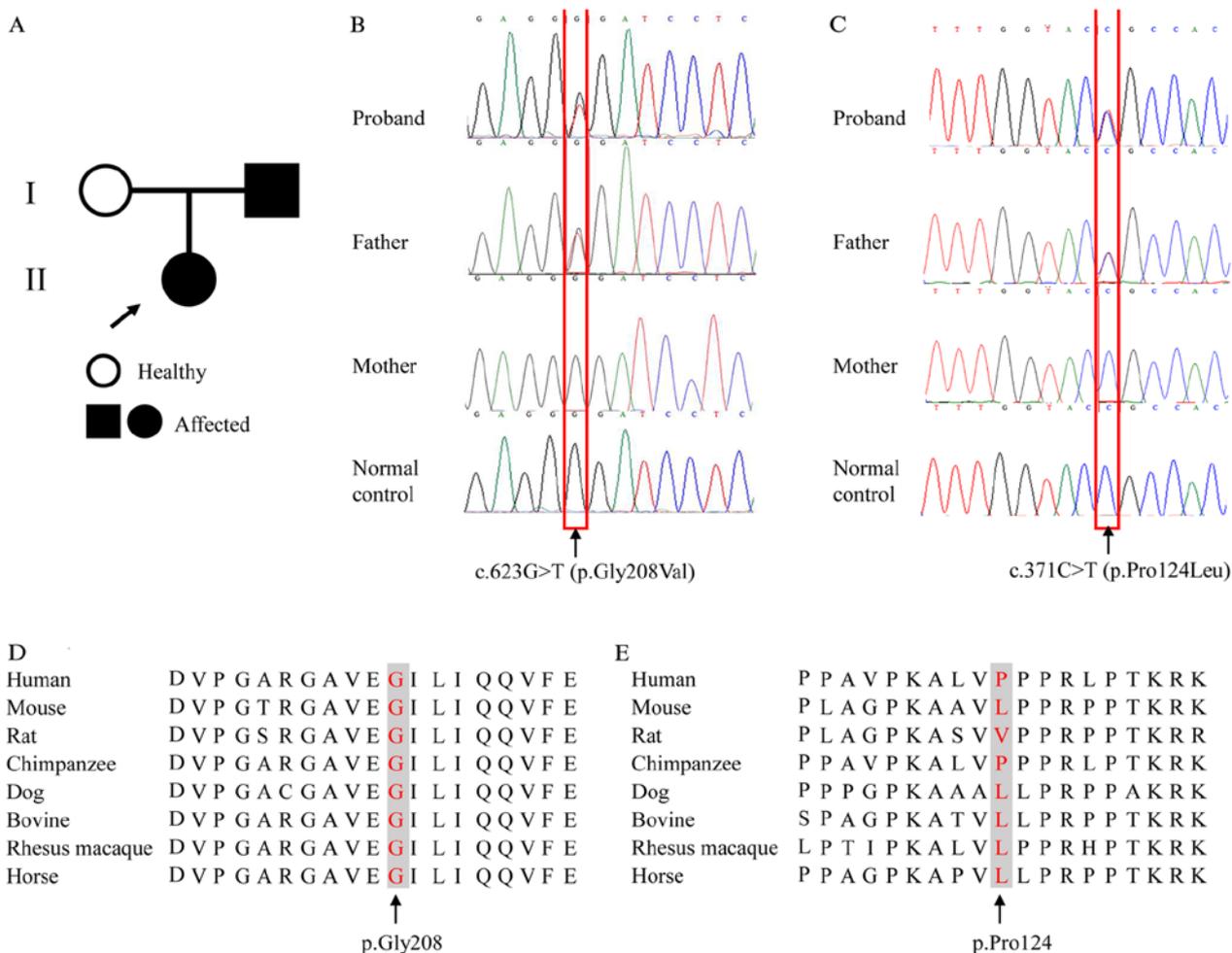


Figure 1. Novel variants in *AIRE* gene in a patient with APS-1. (A) Pedigree of the family. Black arrow indicates the patient. The sequence of *AIRE* of the family of APS-1 patient and normal controls. Two variants were identified: (B) a novel variant c.623G>T in exon 5 and (C) a reported variant c.371G>T in exon 3. (D) p.Gly208 residue in *AIRE* protein (NP\_000374.1) was highly conserved between species. (E) p.Pro124 residue in *AIRE* protein (NP\_000374.1) varied between species. *AIRE*, autoimmune regulator; APS-1, autoimmune polyendocrine syndrome type 1.

came from a Chinese non-consanguineous family with no history of autoimmune disease. At 7 years of age, she presented with tetany and hypocalcemia. She was supplemented with Calcichew D (500 mg elemental calcium, 200 IU vitamin D per tablet; GE Pharmaceutical Shanghai Co., Ltd.) three times daily and serum calcium levels were not monitored. At 17 years of age, she suffered from syncope and a generalized tonic-clonic seizure and was admitted to PUMCH for the first time. She was diagnosed with hypoparathyroidism based on PTH deficiency (PTH<1.0 pg/ml), hypocalcemia (serum total calcium, 1.35 mmol/l) and hyperphosphatemia (serum phosphorus, 1.91 mmol/l). CT scans revealed bilateral calcifications in the basal ganglia of the brain. One year later, she was admitted to the Department of Gynecology with primary amenorrhea and was diagnosed as having primary ovarian insufficiency, according to low estradiol (6.74 pg/ml), increased LH (33.7 mIU/ml) and FSH (82.6 mIU/ml) levels, as well as uterine hypoplasia in ultrasonography. At 31 years of age, she experienced intermittent diarrhea and general fatigue. Whole blood cell count revealed a notably decreased hemoglobin level (6.1 g/dl), with normal platelet count (PLT, 306,000/ $\mu$ l) and white blood cells (WBCs, 874,000/ $\mu$ l). The bone marrow aspirate showed normocellularity, a lack of erythroid precursors

(1.0% of bone marrow nucleated cells), normal granulocyte precursors and megakaryocytes with lymphocytosis (30.5%). In addition, serum level of erythropoietin was notably elevated (>776.00 mIU/ml). The patient was diagnosed with PRCA. Gastrointestinal endoscopy and histological analysis of the biopsies revealed chronic atrophic gastritis and gastric ulcers, with normal appearance of the duodenal mucosa. *Helicobacter pylori* was not present in the gastric mucosa. The abdominal ultrasound was normal. Serum cortisol, plasma ACTH, serum FT4, FT3 and TSH levels were within the normal range. For autoantibodies, RO-52, GADA and antibodies to IFN- $\omega$  and IFN- $\alpha$  were positive, whereas ANA, ANCA, TGAb, TPOAb, ICA and antibodies to IL-17A, IL-17F and IL-22 were negative.

Combined with clinical manifestations, the patient was suspected of having APS-1. The clinical manifestations of the patient are listed in Table I.

The parents did not display symptoms or signs of APS-1; therefore, they were reluctant to receive further biochemical and autoantibody profile examinations.

**Treatment and follow-up.** The patient was prescribed 3 tablets of calcium carbonate (200 mg elemental calcium per tablet) and one capsule of calcitriol (0.25  $\mu$ g per

Table I. Clinical presentation and laboratory results of a patient with APS-1.

Age, years	Clinical manifestation	Laboratory results	Reference range
7	Tetany	NA	/
17	Syncope	Serum total calcium, 1.350 mmol/l	2.13-2.70 mmol/l
	Generalized tonic-clonic seizure	Serum phosphorus, 1.910 mmol/l	0.81-1.45 mmol/l
18	Amenorrhea	Serum parathyroid hormone, <1.000 pg/ml	7.00-53.00 pg/ml
		Serum estradiol, 6.740 pg/ml	50.00-154.40 pg/ml
		Serum luteinizing hormone, 33.700 mIU/ml	4.40-7.10 mIU/ml
		Serum follicle-stimulating hormone, 82.600 mIU/ml	5.10-7.00 mIU/ml
		Ultrasonography, uterine hypoplasia	
31	Intermittent diarrhea	Hemoglobin, 6.100 g/dl	11.00-15.00 g/dl
	General fatigue	Hematocrit, 19.000%	35.00-50.00%
		Platelets, 306,000.000/ $\mu$ l	100,000.00-350,000.00/ $\mu$ l
		White blood cells, 874,000.000/ $\mu$ l	350,000.00-950,000.00/ $\mu$ l
		Plasma adrenocorticotrophic hormone, 27.000 pg/ml	0.00-46.00 pg/ml
		Serum cortisol, 19.430 $\mu$ g/dl	4.00-22.30 $\mu$ g/dl
		Serum free thyroxine, 1.100 ng/dl	0.81-1.89 ng/dl
		Serum free triiodothyronine, 3.370 pg/ml	1.80-4.10 g/ml
		Serum thyroid-stimulating hormone, 2.306 $\mu$ IU/ml	0.38-4.34 $\mu$ IU/ml
		Thyroglobulin antibodies, 12.270 IU/ml	<115.00 IU/ml
		Thyropoxidase antibodies, 10.910 IU/ml	<34.00 IU/ml
		Erythropoietin, >776.000 mIU/ml	4.5.0-31.88 mIU/ml
		Bone marrow aspirate, normocellularity, lack of erythroid precursors	
		IFN- $\omega$ Abs/IFN- $\alpha$ Abs/RO-52/GADA, Positive	GADA, <10.00 IU/ml
		IL-17A Abs/IL-17F Abs/IL-22	IFN- $\omega$ Abs/IFN- $\alpha$
		Abs/ICA/IA-2/ANA/ANCA, Negative	Abs/RO-52/IL-17A
			Abs/IL-17F Abs/IL-22
			Abs/ICA/IA-2/ANA/ANCA, Negative

NA, not available; IFN- $\omega$  Abs, IFN- $\omega$  antibodies; IFN- $\alpha$  Abs, IFN- $\alpha$  antibodies; GADA, glutamic acid decarboxylase antibodies; ANA, anti-nuclear antibodies; RO52, 52 kDa Ro/SS-A molecule; IL-17A Abs, IL-17A antibodies; IL-17F Abs, IL-17F antibodies; IL-22 Abs, IL-22 antibodies; ICA, islet cell antibodies; ANCA, anti-neutrophil cytoplasmic antibodies.

capsule) three times daily to manage hypoparathyroidism. For primary ovarian insufficiency, she received a 21-day sequential therapy with Climen (2 mg estradiol valerate, days 1-21 with 1 mg cyproterone acetate, days 12-21; Bayer, AG), followed by a 7-day treatment-free interval. The patient received a transfusion of packed red blood cells, and then 75 mg cyclosporine A was given twice daily. As the serum ALT level increased to 304 U/l one month later, she switched to methylprednisolone (4 mg per tablet). The therapeutic dosage was adjusted according to serum levels of calcium, phosphate, 24-h urinary calcium excretion and level of hemoglobin.

During the treatment, the frequency of tetany was notably decreased, and the patient experienced regular menstruation. On the final examination, serum calcium level increased to 1.84 mmol/l and inorganic phosphate decreased to 1.42 mmol/l (Fig. 2A). Urinary calcium and phosphate levels were monitored (Fig. 2B). Decreased LH and FSH were

also observed (Fig. 2C). The hemoglobin level increased to 10.2 g/dl (Fig. 2D).

**Variants in AIRE.** A heterozygous single-base-pair variation in exon 5 (NM\_000383.2: c.623G>T) of *AIRE* was identified, causing an amino acid change of glycine to valine at position 208 (NP\_000374.1: p.Gly208Val) (Fig. 1B). This variant was predicted to be pathogenic, with scores of 1.00 by PolyPhen-2 and 0.00 by SIFT. The present study also identified a sequence alteration (NM\_000383.2: c.371C>T) in exon 3 of *AIRE*, which caused substitution of proline to leucine at position 124 (NP\_000374.1: p.Pro124Leu) (Fig. 1C). This variant was predicted to be benign, with scores of 0.00 by PolyPhen-2 and 1.00 by SIFT. Moreover, the affected amino acid residue, p.Gly208, was conserved among different species, indicating the functional importance of this amino acid residue throughout evolution (Fig. 1D). The 124th amino acid of the *AIRE* protein varied between species, indicating that this

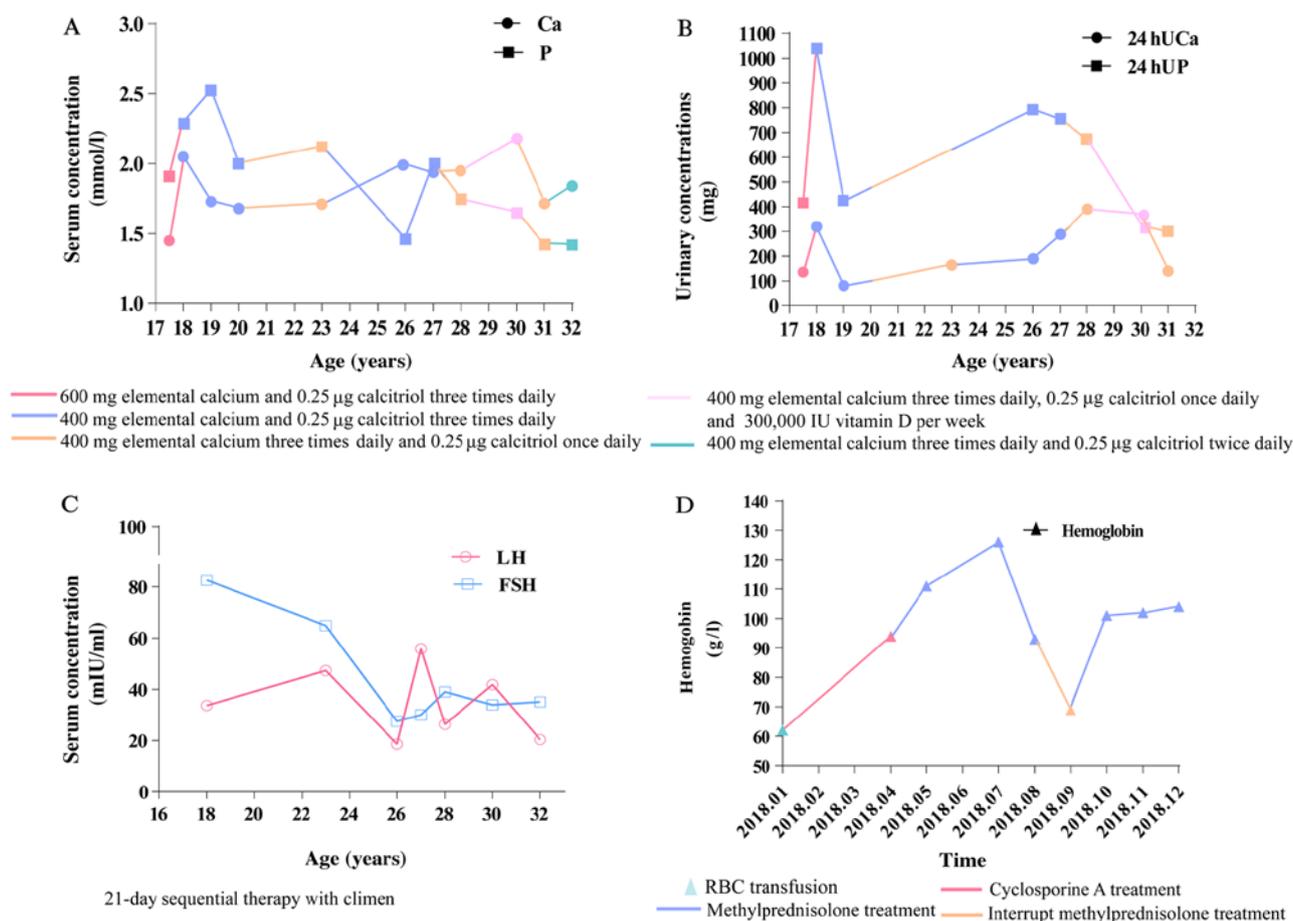


Figure 2. Effects of treatment on a patient with APS-1. (A) Changes in serum level of calcium and phosphorus following calcium and calcitriol treatment. (B) Changes in 24hUCa and 24hUP following calcium and calcitriol treatment. (C) Changes in serum levels of LH and FSH following sequential therapy with Climen. (D) Changes in hemoglobin following cyclosporine A or methylprednisolone treatment. APS-1, autoimmune polyendocrine syndrome type 1; LH, luteinizing hormone; FSH, follicle-stimulating hormone; 24hUCa, 24 h urinary concentration of calcium; 24hUP, 24 h urinary concentration of phosphorus.

residue may be less important for the function and structure of the protein (Fig. 1E).

Both sequence alterations were present in the patient's father but were absent in the mother and the 500 unaffected controls (Fig. 1B and C). Searching the HGMD ([hgmd.cf.ac.uk/ac/index.php](http://hgmd.cf.ac.uk/ac/index.php)), Leiden Open Variation Database 3.0 ([lovd.nl/3.0/home](http://lovd.nl/3.0/home)), PubMed database, the 1000 Genomes Project ([browser.1000genomes.org](http://browser.1000genomes.org)) and Exome Aggregation Consortium database ([exac.broadinstitute.org/](http://exac.broadinstitute.org/)) indicated that c.623G>T was a novel variant.

The change in the 3D structure of AIRE protein induced by the pathogenic variant present in the patient is presented in Fig. 3A. The glycine to valine substitution at position 208 changes the structure of Sp100, AIRE-1, NucP41/75, DEAF-1 (SAND).

## Discussion

The diagnosis of APS-1 is primarily based on clinical manifestations such as hypoparathyroidism, primary adrenocortical insufficiency and chronic mucocutaneous candidiasis. More recent clinical studies have demonstrated that APS-1 can be classified into classical and non-classical type (18,19). Classical APS-1 is characterized by autosomal recessive inheritance and the presence of at least two of the three main features

of hypoparathyroidism, primary adrenocortical insufficiency and chronic mucocutaneous candidiasis. Non-classical APS-1 follows an autosomal dominant inheritance pattern and presents with varying late-onset autoimmune phenotypes (18-21). To the best of our knowledge, the present study is the first to report a first Chinese patient with non-classical APS-1 caused by a novel variant in *AIRE* in an autosomal dominant fashion. At present, there are 15 known variants of *AIRE* that follow an autosomal dominant inheritance pattern (including those identified in the present study) (18,19). The majority of variants are clustered within the first plant homeodomain (PHD1) zinc finger domain (18). While two variants (p.Gly208Val and p.Gly228Trp) were in the SAND domain, one variant (p.Cys446Gly) was in the second plant homeodomain (PHD2) zinc finger domain (18,19,20). The majority of patients with a dominant inheritance often did not match the classical diagnostic criteria of APS-1. These patients exhibited more variable phenotypes, ranging from no autoimmunity to late-onset classical APS-1, and hypoparathyroidism was the most prevalent phenotype (18,19). The present patient suffered from tetany and syncope, primary amenorrhea, intermittent diarrhea and general fatigue. Upon laboratory examination, she was diagnosed as having hypoparathyroidism, primary ovarian insufficiency and PRCA. A novel heterozygous variant of c.623G>T in exon 5 was identified and regarded as

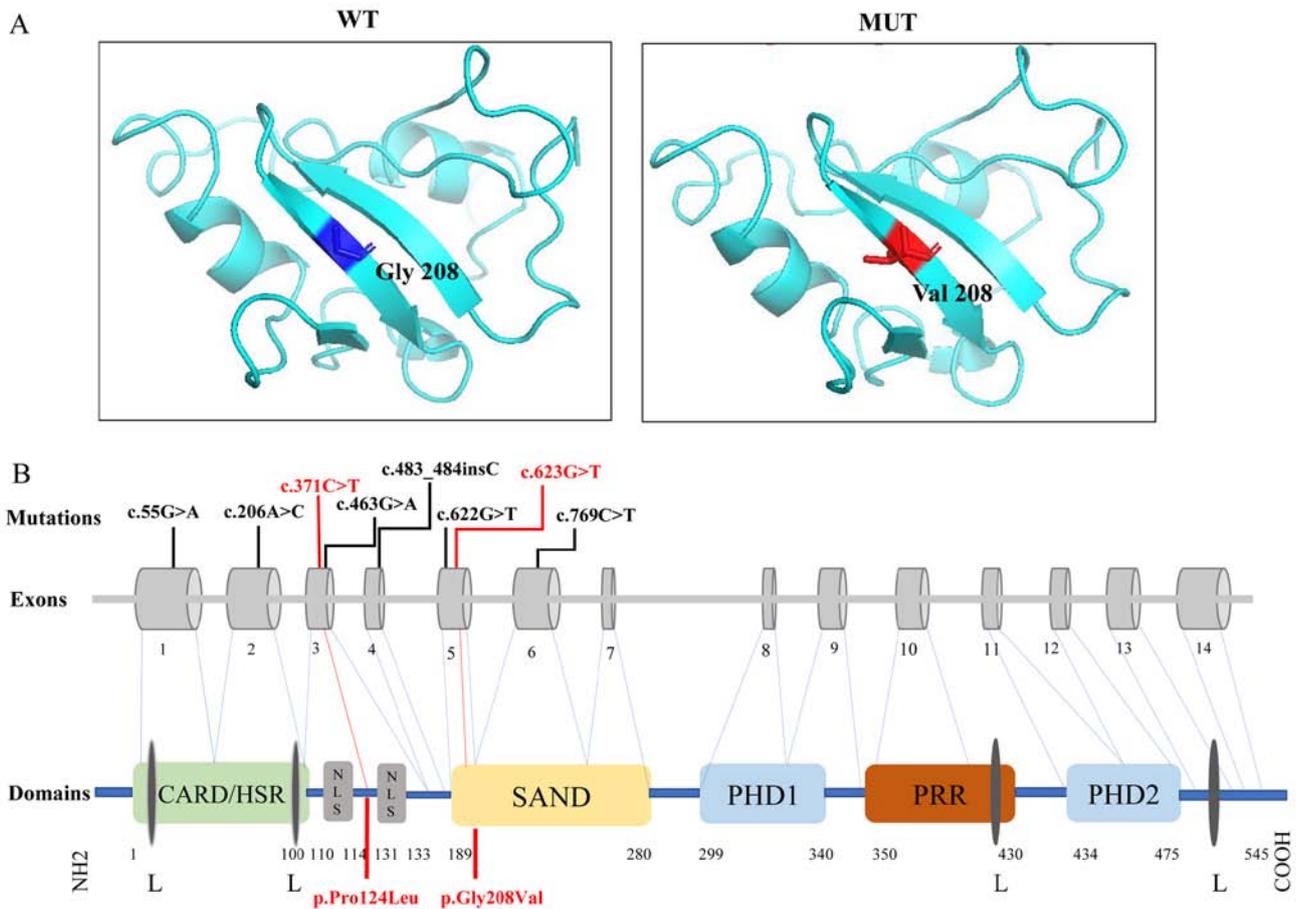


Figure 3. Three-dimensional structural model and schematic representation in AIRE protein. (A) Three-dimensional structural model of the pathogenic-mutated position in AIRE. WT: Position 208 was a glycine in normal population (blue). MUT: A structural change caused by p.Gly208Val in SAND domain (red). (B) Schematic representation of human *AIRE* variants and AIRE protein. Boxes 1-14 represent exons. AIRE protein contained CARD/HSR, NLS, SAND domain, PRR, two PHD fingers and four LXXLL motifs. Variants are highlighted in red. AIRE, autoimmune regulator; WT, wild type; MUT, mutant type; SAND, Sp100, AIRE-1, NucP41/75, DEAF-1; CARD/HSR, caspase recruitment domain/homogeneously staining region; NLS, nuclear localization signal; PRR, proline-rich region; PHD, plant homeodomain; L, leucine; X, any amino acid.

a pathogenic variant of non-classical APS-1. Another reported variant c.371C>T in exon 3 of *AIRE* was also identified.

The *AIRE* gene encodes a 57-kDa protein that promotes T-cell tolerance to self-antigens by upregulating the expression levels of tissue-specific self-antigens in medullary thymic epithelial cells (22). Impaired functional AIRE leads to the escape of self-reactive T-lymphocytes to the periphery and induces pathogenic autoimmune reactions (23). Proper subcellular location is important for the function of AIRE and its ability to maintain macromolecular interactions. Previous reports have shown that non-mutated AIRE localizes to the nucleus, whereas mutant AIRE is retained in the cytoplasm, where it is unable to regulate the transcription of a number of genes, such as major histocompatibility complex class I and class II gene products, and causes abnormal immune responses such as impaired negative selection of autoreactive thymocytes (16,23-25).

AIRE contains a number of domains, including a highly conserved caspase recruitment domain/N-terminal homogeneously staining region (CARD/HSR) domain, a conserved bipartite nuclear localization signal (NLS), a SAND domain, two plant PHD zinc-finger motifs separated by a proline-rich region, and four LXXLL (L, leucine; X, any amino acid) nuclear

receptor-binding motifs (26). The SAND domain is important for mediating the nuclear localization of AIRE (27). The variant c.623G>T in exon 5 may change the structure of the SAND domain, resulting in aggregation of AIRE in the cytoplasm and impaired DNA binding (Fig. 3A). However, p.Pro124Leu caused by c.371C>T was between bipartite of NLS, which constituted amino acids 110-114 and 131-33 (28), which may explain why this variant was not pathogenic (Fig. 3B).

A previous study demonstrated that *AIRE* variants in patients with hypoparathyroidism may induce autoantibodies to NACHT leucine-rich-repeat protein 5, which is selectively expressed in chief cells of the parathyroid glands (29). An autoimmune reaction targeting the calcium-sensing receptor was also found in parathyroid cells of patients with APS-1 (30). In addition, a loss of T-lymphocyte infiltration was observed surrounding follicles in female *AIRE*-knockout mice (22). These studies helped to elucidate the potential pathogenesis of the *AIRE* variant underlying APS-1.

APS-1 is more frequent in European countries, with a prevalence of 1:25,000 in Finland, 1: 14,000 in Sardinia, 1:43,000 in Slovenia and 1:500,000 in France (4,6,24). p.R257X, located in the SAND domain, is the most common variant in Finland, Russia and Eastern Europe (4,6,24).

Table II. Summary of clinical and genetic features of 7 Chinese patients with APS-1 and AIRE mutations.

Patient no.	Gender/onset		Manifestations			AIRE mutation/inheritance	Protein change	Domain	(Refs.)
	age, years	Major components	Other components	Major components	Other components				
1	F/4	CMC; HP; PAI	None	CMC; HP; PAI	None	Exon 1: c.55G>A/AR Exon 6: c.769C>T/AR	p.Ala19Thr p.Arg257X	CARD SAND	(14)
2	F/10	CMC; PAI	Autoimmune thyroiditis	CMC; PAI	Autoimmune thyroiditis	Exon 4: c.483_484insC/AR	Truncated protein	CARD	(16)
3	M/2	HP; PAI	Enamel dystrophy; binocular cataract; chronic intestinal dysfunction	HP; PAI	Enamel dystrophy; binocular cataract; chronic intestinal dysfunction	Exon 2: c.206A>C/AR	p.Gln69Pro	SAND	(7)
4 <sup>a</sup>	M/8	HP; PAI	Epilepsy; pernicious anemia; chronic/tension headaches; keratopathy; type 1 diabetes mellitus	HP; PAI	Epilepsy; pernicious anemia; chronic/tension headaches; keratopathy; type 1 diabetes mellitus	Exon 3: c.463G>A/AR	p.Gly155Ser	NA	(15)
5 <sup>a</sup>	F/1	CMC; HP; PAI	Epilepsy; pernicious anemia	CMC; HP; PAI	Epilepsy; pernicious anemia	Exon 3: c.463G>A/AR	p.Gly155Ser	NA	(15)
6	F/12	CMC; HP	Anemia	CMC; HP	Anemia	Exon 5: c.622G>T/NA	p.Gly208Trp	SAND	(17)
7	F/7	HP	Primary ovarian insufficiency; Pure red cell aplasia; chronic atrophic gastritis	HP	Primary ovarian insufficiency; Pure red cell aplasia; chronic atrophic gastritis	Exon 3: c.371C>T/AD Exon 5: c.623G>T/AD	p.Pro124Leu p.Gly208Val	Not domain SAND	This study

<sup>a</sup>siblings. F, female; M, male; CMC, chronic mucocutaneous candidiasis; HP, hypoparathyroidism; PAI, primary adrenocortical insufficiency; Ala, alanine; Thr, threonine; Arg, arginine; Gln, glutamine; Pro, proline; Gly, glycine; Ser, serine; Trp, tryptophan; Leu, leucine; Val, valine; AR, autosomal recessive; AD, autosomal dominant; CARD, caspase recruitment domain; SAND, Sp100, AIRE-1, NucP41/75, DEAF-1; NA, not available.

R139X is most frequent in patients from Sardinia and results in more severe phenotypes of APS-1 (10). The present study adds the clinical and genetic characteristics of the patient to those of six previously reported Chinese patients with APS-1 (7,14-17). A total of 8 variants (7 missense variants and 1 insertion variant) were detected in seven patients with APS-1 from six unrelated families (Table II). Previous research has revealed that the most common variants of the *AIRE* gene exist in exons 1, 2, 6, 8 and 10 (24,31), whereas the variants of the *AIRE* gene in Chinese patients with APS-1 occurred in exons 1-6 (c.55G>A, c.206A>C, c.371C>T, c.463G>A, c.483\_484insC, c.623G>T and c.769C>T), without obvious hotspot variants. These variants primarily affected the CARD, NLS and SAND domains (Fig. 3B). Variants in the CARD domain may influence the multimerization of the AIRE protein and suppress its transcriptional activation capacity (32). A number of gross deletions and splice site variants (such as c.483\_484insC in patient 2) have been identified as associated with APS-1 because AIRE protein translation is affected (33,34).

Regarding the clinical phenotypes of Chinese patients, the classic triad of CMC, PAI and HP were observed in two patients, with four patients exhibiting two of the three major manifestations. Generally, CMC is the most common, with an occurrence of 77-100% in European patients with APS-1 (3,35-37), but it is rare in Iranian Jews (17%) (11). CMC was only detected in four Chinese patients with APS-1 and hypoparathyroidism was the most common manifestation (six cases).

In patients with APS-1, the same pathogenic gene can lead to different clinical phenotypes. A Chinese patient with APS-1 was diagnosed as having APS-1 without CMC, but his sister exhibited this symptom (15). Cetani *et al* (19), reported a missense (p.Gly228Trp) mutation of the *AIRE* gene in a family with APS-1. The p.Gly228Trp mutation was detected in the proband as well as her mother, son and sister, and acted in a dominant manner. However, only the proband and her sister exhibited the typical components of APS-1. In another study, a proband and four of her children carried the p.Cys311Tyr variant. The proband and three of her children exhibited varying components and severity of APS-1, whereas a son was diagnosed with autoantibodies against tyrosine hydroxylase without any autoimmune manifestations (18). The patient's father exhibited the same *AIRE* variants, but he did not present with symptoms of APS-1. The mechanism governing the difference in penetration rates is not clear in patients with APS-1.

The diagnosis of APS-1 is often delayed (2). The present patient presented with major manifestations of hypoparathyroidism and other late-onset autoimmune phenotypes, which was inconsistent with the phenotypes of classic APS-1. However, APS-1 was confirmed by the detection of *AIRE* variants. Thus, *AIRE* sequencing should be performed at an early stage when patients are suspected of having APS-1.

There is currently a lack of studies concerning the effects of immunomodulatory and gene-targeted therapy on APS-1. The primary therapeutic strategy for APS-1 is hormone replacement therapy to correct endocrine function. For hypoparathyroidism, supplementation with calcium and calcitriol is given to achieve low-normal serum calcium and normal urine

calcium levels, relieve the symptoms of hypocalcemia and decrease long-term complications (38). Hormone replacement therapy is an alternative treatment used to manage primary ovarian insufficiency. Cyclosporine A, with or without concurrent corticosteroids, has been suggested to be an effective immunotherapy for pure red aplastic anemia (39). Using these approaches, the clinical symptoms of the present patient notably improved.

There were a number of limitations in the present study. First, the genotype-phenotype association was not well established due to the small sample size. Second, the lack of functional studies made it difficult to elucidate the detailed mechanism underlying how variants in *AIRE* lead to APS-1. Third, certain autoantibodies, such as 21-hydroxylase and steroid-producing cells autoantibodies, were not investigated. Last, detailed information on the patient's father was not available.

In conclusion, a novel variant of c.623G>T (p.Gly208Val) of the *AIRE* gene is associated with an APS-1 clinical syndrome of hypoparathyroidism, primary ovarian insufficiency and PRCA. The novel missense variant of c.623G>T in *AIRE* may impair its binding and regulation of the macromolecular DNA binding complex, resulting in a clinical picture of APS-1. The results of the present study expand the known genetic and phenotypic spectra of APS-1.

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#### Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

#### Authors' contributions

WBZ performed molecular genetic studies, participated in sequence alignment, analyzed data and wrote the manuscript. LJJ and DCZ collected, analyzed and interpreted the data. OW, YJ and WBX provided important medical decisions in the clinical diagnose and treatment of patient, contributed the analysis of clinical data and reviewed the manuscript. ML contributed to the conception and design of the research, acquisition and interpretation of data and revised the manuscript. All authors read and approved the final manuscript.

#### Ethics approval and consent to participate

The study was approved by the Scientific Ethics Committee of Peking Union Medical College Hospital (approval no. JS-2081).

Written informed consent was obtained from all subjects prior to participation.

### Patient consent for publication

The patient, her parents and the healthy controls provided informed consent for publication of their data.

### Competing interests

The authors declare that they have no competing interests.

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