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Clinical Research Article

Novel Gene Mutations Regulating Immune Responses in Autoimmune Polyglandular Syndrome With an Atypical Course

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Abbreviations: AAI, autoimmune adrenal insufficiency; Abs, antibodies; AIT, autoimmune thyroiditis; APS, autoimmune polyglandular syndrome; CASR, calcium-sensing receptor protein; DM1, diabetes mellitus type 1; ERC, Endocrinology Research Centre; FSH, follicle-stimulating hormone; IgG, immunoglobulin G; LH, luteinizing hormone; RR, reference range; TSH, thyrotropin.

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Abstract

Context: Autoimmune polyglandular syndrome (APS) is a cluster of endocrine disorders arising from immune dysregulation, often combined with damage to nonendocrine organs. There are 2 types of APS: type 1 and type 2 (APS-1 and APS-2, respectively). In clinical practice, an atypical course of APS is often observed.

Objective: This work aims to find a novel genetic predictor of APS.

Methods: We performed exome sequencing in 2 patients with an atypical clinical APS picture and members of their families. Patient A presented with a manifestation of APS-2 in early childhood and patient B with a late manifestation of the main components of APS-1.

Results: In patient B, we identified inherited compound mutations as a novel combination of the c.769C > T and c.821delG alleles of AIRE and genetic variation in the CIITA gene. No homozygous or compound mutations in AIRE were found in patient A, but we did reveal mutations in genes encoding regulatory proteins of innate and acquired immunity in this patient.
Conclusion: Our data revealed novel combination of mutations in the AIRE gene in atypical APS and imply that mutations in immune-related genes may modify the clinical manifestation of APS in AIRE-mutation carriers and contribute to the development of autoimmune pathology in non-AIRE carriers with atypical APS.

Key Words: exome sequencing, autoimmune polyglandular syndrome, early manifestation, atypical course, genetic predictors

Autoimmune polyglandular syndrome (APS) is a cluster of endocrine disorders arising from immune dysregulation [1], often combined with damage to nonendocrine organs. There are 2 types of APS: type 1 and type 2 (APS-1 and APS-2, respectively) [2].

APS-1 (prevalence 1:100,000) is a monogenic disease with an autosomal recessive mode of inheritance due to mutations in the autoimmune regulator gene AIRE. APS-1 is characterized by developing at least 2 of the 3 main components: chronic mucocutaneous candidiasis, hypoparathyroidism, and autoimmune adrenal insufficiency (AAI) expressed typically in childhood. The age at disease onset is variable, even among members of the same family. APS-2 (prevalence 1:20,000 [3]) is characterized by the development of 2 or more main autoimmune endocrinopathies in patients: AAI, diabetes mellitus type 1 (DM1), autoimmune thyropathies (Graves disease or autoimmune thyroiditis [AIT]) in combination with other autoimmune diseases [2, 4]. APS-2 most often manifests in adulthood [2, 5].

In clinical practice, an atypical course of APS is often observed. Thus, patients with APS-1 may have only minor components in childhood, while the main component of the disease manifests in adulthood. Some mutations in the AIRE gene may be associated with the APS-2 phenotype and even isolated organ-specific autoimmune pathologies (eg, vitiligo, pernicious anemia) with different penetrance [6]. Cases of compound-heterozygous AIRE gene mutations in APS-1 with nonclassical manifestations have been reported [7-9]. There are cases of APS manifestation in childhood in which no mutations in the AIRE gene were revealed. Therefore, the disease was classified as APS-2 [10].

Similar to other authors, we assume that autoimmune pathologies debuting at an early age may represent an independent monogenic disease unrelated to autosomal recessive mutations in the AIRE gene [2]. An example is immune dysregulation syndrome with an autosomal dominant mode of inheritance due to a mutation in the gene CTLA-4.

We hypothesized that, beyond the mutations in AIRE, atypical APS might be due to mutations in candidate genes encoding regulatory proteins of innate and acquired immunity and bearing genetic variations that have been linked to the development of autoimmune pathologies such as CTLA-4, PTPN22, CIITA, CLEC16A, MICA, STAT4, PD-L1, NALP1, FCRL3, GPR174, GATA3, NFATC1, CYP27B1, VDR, TNF, and CYP21A2 (autoimmune gene panel) [11-14] or other unknown mutant genes that have yet to be identified. To find a new genetic predictor of APS, we performed exome sequencing in 2 patients with a manifestation of the disease in childhood and an atypical clinical picture, and their family members. We paid special attention to the autoimmune gene panel when interpreting the results.

Materials and Methods

Exome Sequencing and Genetic Examination

We conducted an extended genetic examination of 2 patients with an atypical form of APS and their family members. The patients observed and examined in the Endocrinology Research Centre (ERC) demonstrated manifestation of the disease in childhood and an atypical clinical picture (anamnesis of patients is presented later). Exome sequencing was performed with primary analysis of genetic variations in the genes included in the autoimmune gene panel, followed by an analysis of all other protein-coding genes in the exome data. Genomic DNA from total peripheral human blood was extracted using a Qiagen QIAamp Midi Spin Column, following the manufacturer’s protocol. Library preparations were made using the Agilent Technologies SureSelectXT Target Enrichment System for the Illumina Platform kit, following the manufacturer’s protocol. Library validation was performed using an Agilent 2100 Bioanalyzer with a DNA High Sensitivity chip and quantified via quantitative polymerase chain reaction using a KAPA Library Quantification Illumina Kit protocol.

Paired-end libraries from patient A and her parents were sequenced using the Illumina NovaSeq 6000 S4 Reagent Kit with 2 × 151 cycles on a NovaSeq 6000 sequencer. A sample from the sister of patient A was sequenced in 2 × 101 cycles using the Illumina TruSeq SBS v3 kit on a HiSeq 2000/2500 sequencer (Illumina). Samples from patient B and her parents were sequenced in 2 × 101 cycles using the Illumina TruSeq SBS v3 kit on a HiSeq 2000/2500 sequencer (Illumina).
The resulting reads were aligned to the hg19 reference genome using a BWA-MEM algorithm (bwa v.0.7.13-r112) [15]. Residual adapters were trimmed using the Trimomatic v. 0.36 program [16]. Duplicate reads were detected using the MarkDuplicates algorithm from picard-tools v.1.133 [17] and excluded from further analysis. We used alignments with a map quality of more than 30. Genetic variants in the sequenced data were predicted using HaplotypeCaller (with default arguments) from the Genome Analysis Toolkit (GATK) package version 4.1.3.0 [18]. For all variants we performed a hard-filtering with default parameters (GATK Best Practices) for the subsequent analysis. Annotation and effect prediction of selected variants were performed in VEP [19] using the hg19 genome annotation (Ensembl v75) [20]. Additionally, variants were annotated with population allele frequencies and curated resources such as GnomAD [21, 22] and NCBI [23, 24].

In the samples from patient A and her family, we achieved the following coverage values: a mean coverage of 233.84× without duplicates, with 98.4% of the target covered at least 20× in the sample of the patient A; 133.98× without duplicates, with 78.2% of the target covered at least 20× in the sample of patient A’s mother; 66.86× without duplicates, with 64.2% of the target covered at least 20× in the sample from patient A’s father; and 66.89× without duplicates, with 55.2% of the target covered at least 20× in the sample from patient A’s healthy sister.

In the samples from patient B and her family, the resulting coverage was as follows: a mean coverage of 126.42× without duplicates, with 84.5% of the target covered at least 20× in the sample from patient B; 38.5× without duplicates, with 30% of the target covered at least 20× in the sample of patient B’s mother; and 34.54× without duplicates, with 20.1% of the target covered at least 20× in the sample from patient B’s father.

Four types of mutations were searched in the analysis: de novo, homozygous, compound, and all inherited variants. The selection of significant variants was performed according to the following algorithm:

1. Frequency of occurrence of the mutant allele less than 5% according to gnomAD allele frequency in the non-Finnish European population (gnomAD nFEAF)
2. Type of mutation: “protein_altering_variant”
3. The mutation was checked visually using the Integrative Genomics Viewer [25]

When genetic defects were detected in patients, a search for identified changes in family members was performed. This study was approved by the ethics committee of the ERC and has therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. All patients and their family members provided written informed consent prior to participation.

**Patients**

Patient A (female) was enrolled in the study at age 25 years. She had DM1 (onset at the first year of life), AAI (onset at age 22), primary hypothyroidism as a result of outcome of AIT (onset at age 22), and diffuse alopecia (onset at age 25). From age 15 years, she suffered from dermatitis, and at age 21, esophageal candidiasis was diagnosed. Both parents and sister denied any autoimmune diseases, did not have any specific complaints related to autoimmune pathology, and refused to undergo in-depth examinations. The patient received 5 mg prednisolone in the morning and 2.5 mg in the evening, 0.2 mg fludrocortisone in the morning, 75 mcg levothyroxine sodium in the morning, and insulin therapy (glargine, lispro).

Patient B (female) was enrolled in the study at age 25 years. From age 11, she noted episodes of loss of consciousness, accompanied by severe hypotension and a craving for salty food. At age 13, convulsive syndrome manifested (but the diagnosis of hypoparathyroidism was verified only 11 years later). At age 14 years, vitamin B₁₂ deficiency was diagnosed. Moreover, at the same age, the patient had alopecia areata (manifested by hair loss from the eyebrows and eyelashes). Alopecia progressed at age 23, when the patient noted hair loss at the scalp. The patient also had primary hypothyroidism as an outcome of AIT (diagnosis was made at age 22) and vulvovaginal candidiasis (diagnosis was made at age 26). Neither parent had autoimmune diseases, according to ERC examinations (specifically, antibodies [Abs] to interferons were investigated, but results were negative). The patient received levothyroxine sodium (112.5 mcg) in the morning and alfalcacidol (1.5 mcg) in the afternoon.

**Assay of Interferon Autoantibodies**

Molecular profiling of auto-Abs to interferons in serum samples was performed using a hydrogel-based, low-density microarray [26]. Antigen microarrays containing immobilized interferon omega (300 µg in 1 mL of the hydrogel, PeproTech) and interferon-α (300 µg in 1 mL of the hydrogel, PBL Assay Science) were incubated with patient serum. Antigen-auto-Ab complexes in microarray elements were detected using Cy5-labeled F(ab’)2-goat antihuman immunoglobulin G (IgG) Fc γ secondary antibody (5 µg/mL, Thermo Fisher Scientific, catalog No. 31163, RRID: AB_228214). Fluorescence signals of the microarray elements were registered using a GenePix microarray scanner.
Enzyme-Linked Immunosorbent Assay

For verification of disease autoimmune genesis and screening for new pathology biomarkers, we used the following enzyme-linked immunosorbent assay kits: for the determination of auto-Abs to P450c21 (BioVendor Laboratory Medicine, catalog No. R21E/96R, RRID:AB_2889856), tyrosine phosphatase (Medipan, catalog No. 3803, RRID:AB_2889854), islet cell antigens (Medipan, catalog No. 4129, RRID:AB_2889855), glutamic acid decarboxylase (Biomerica, catalog No. 7009, RRID:AB_2889857), insulin (Orgentec, catalog No. ORG 520, RRID:AB_2889858), zinc transporter 8 (Medipan, catalog No. ORG 531, RRID:AB_2889860), Castle intrinsic factor (Orgentec, catalog No. ORG 647, RRID:AB_2889861), and tissue transglutaminase (Orgentec, catalog No. ORG 540A, RRID:AB_2889862). Biochemical and hormonal assays were performed using kits for vitamin B12 (Beckman Coulter, catalog No. 33000, RRID:AB_2889863), cortisol (Roche, catalog No. 1187511612, RRID:AB_2889864), thyrotropin (TSH, Abbott, catalog No. 7K62, RRID:AB_2889372), renin (DiaSorin, catalog No. 310470, RRID:AB_2889866), aldosterone (DiaSorin, catalog No. 310450, RRID:AB_2889867), adrenocorticotropin (Biomerica, catalog No. 7023, RRID:AB_2889868), estradiol (Thermo Fisher Scientific, catalog No. 8552630, RRID:AB_2889869), luteinizing hormone (LH, Thermo Fisher Scientific, catalog No. 1350198, RRID:AB_2889870), and follicle-stimulating hormone (FSH, Thermo Fisher Scientific, catalog No. 1931922, RRID:AB_2889871) determination.

Results

Patient A

During the ERC examination, we diagnosed the compensation of adrenal insufficiency (sodium, 141 mmol/L, reference range [RR], 136-145 mmol/L; potassium, 4.2 mmol/L, RR, 3.5-5.1 mmol/L; and primary hypothyroidism [TSH], 2.79, RR, 0.25-3.5). However, decompensation of DM1 was diagnosed (glycated hemoglobin A1c, 10.2% [RR, 4%-6%]). Additionally, we verified the autoimmune genesis of adrenal insufficiency (antibodies to P450c21: 18.3 U/mL [RR < 0.4 U/mL]). We excluded primary hypogonadism (LH, 13.9 U/L [RR, 2.6-12.0 U/L], FSH, 2.37 U/L [RR, 1.9-11.7 U/L], estradiol 738.44 pmol/L [RR, 97.0-592.0 pmol/L]), and hypoparathyroidism (Ca total 2.49 mmol/L [RR, 2.15-2.55 mmol/L], Ca++ 1.15 mmol/L [RR, 1.03-1.29 mmol/L]). In addition, organ-specific nonendocrine diseases of autoimmune origin were screened. Celiac disease was excluded (Abs to transglutaminase 4.5 U/L [RR, 0-10 U/L]). Abs to the Castle factor were not detected; the level of vitamin B12 was within the reference values (191-663 pg/mL): 469 pg/mL; however, Abs to the parietal cells of the stomach were detected. To exclude autoimmune gastritis, esophagogastrroduodenoscopy was performed; superficial gastritis was diagnosed without signs of an exacerbation. An annual survey was recommended.

Considering the early manifestation of the disease, we assumed APS-1 in the patient. However, the Abs to α-interferon (IgG) and α2-interferon (IgG) were not detected. We also excluded mutations in the coding regions of AIRE. Among other genes included in the panel, we identified a rare (gnomAD nFEAF: 0.006392) deleterious heterozygous missense variant, c.2250G > C (rs56048322), in the PTPN22 gene. The same heterozygous variant was found in the patient’s father. This variant was not identified in the patient’s mother or sister. There were no significant variants in the coding regions of CIITA, NALP1 (NLRP1), CYP27B1, VDR, CTLA-4, CLEC16A, MICA, STAT4, PD-L1 (CD274), FCRL3, GPR174, GATA3, NAFATC1, TNF, or CYP21A2 in the patient and her relatives.

Additionally, we performed a study on clinically significant variants in all the genes of the patient and her family members using exome data. The patient was found to have rare nonsynonymous variants and compound variants in genes of regulatory proteins of the immune system (Table 1): PJA2, CASR, and DDX60L.

Patient B

During the examination at the ERC, we diagnosed AAI (adrenocorticotropin 83.09 pg/mL [RR, 7-66 pg/mL], basal cortisol 403 nmol/L [RR, 123-626 nmol/L], peak blood cortisol level during an insulin tolerance test 481.6 nmol/L [RR ≥ 500 nmol/L], aldosterone 121 pmol/L [RR, 70.9-980 pmol/L], renin > 500 IU/L [RR, 2.8-39.9 IU/L], Abs to P450c21 2.943 U/mL [RR < 0.4 U/mL]), primary hypogonadism (LH 21.7 U/L [RR, 2.6-12.0 U/L], FSH 38.2 U/L [RR, 1.9-11.7 U/L], and estradiol 78.821 pmol/L, renin > 500 IU/L [RR, 2.8-39.9 IU/L]), primary hypothyroidism (LH 21.7 U/L [RR, 2.6-12.0 U/L], FSH 38.2 U/L [RR, 1.9-11.7 U/L], and estradiol 78.821 pmol/L [RR, 97.0-592.0 pmol/L]). Therapy with hydrocortisone, fludrocortisone, estradiol, and progesterone was initiated. We diagnosed the medical compensation of hypoparathyroidism (Ca total 2.2 mmol/L [RR 2.15-2.55 mmol/L], Ca++ 1.0 mmol/L [RR, 1.03-1.29], P 1.95 mmol/L [RR, 0.74-1.52 mmol/L]) and compensation of primary hypothyroidism and vitamin B12 deficiency (TSH 1663 pg/
Table 1. Genes with rare heterozygous nonsynonymous variants and compound variants in patient A (variants shared with the mother are in bold; the remaining variants are shared with the father)

<table>
<thead>
<tr>
<th>Gene</th>
<th>Definition</th>
<th>Variant</th>
<th>SIFT/PolyPhen</th>
<th>gnomAD nFEAF</th>
</tr>
</thead>
<tbody>
<tr>
<td>PJA2</td>
<td>Praja ring finger ubiquitin ligase 2</td>
<td>c.1295G &gt; A (p.Ser432Asn), rs1428335961</td>
<td>Deleterious (0)/probably damaging (0.998)</td>
<td>0.0001297</td>
</tr>
<tr>
<td>CASR</td>
<td>Calcium-sensing receptor</td>
<td>c.2998A &gt; G (p.Arg1000Gly), rs1042636</td>
<td>Deleterious_low_confidence (0)/benign (0.051)</td>
<td>0.07433</td>
</tr>
<tr>
<td>DDX60L</td>
<td>DExD/H-Box 60 like</td>
<td>c.4496C &gt; T (p.Ala1499Val)</td>
<td>Deleterious (0.01/benign (0.124) –</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td>c.2261T &gt; C (p.Val754Ala), rs61740705</td>
<td>Tolerated (0.3)/possibly damaging (0.562)</td>
<td>0.1929</td>
</tr>
</tbody>
</table>

mL [RR, 0.25-3.5 pg/mL], vitamin B₁₂ 226 pg/mL [RR, 191-663 pg/mL]). We excluded DM1 and celiac disease (glucose 4.94 mmol/L [RR, 3.1-6.1 mmol/L], glycated hemoglobin A₁c 5.8% [RR, 4%-6%]; Abs to insulin, islet cells of the pancreas, pancreatic glutamate decarboxylase, zinc transporter 8, tyrosine phosphatase, and transglutaminase were not increased). We also verified the autoimmune genesis of the diagnosed vitamin B₁₂ deficiency (Ab to Castle in intrinsic factor was detected); esophagastroduodenoscopy revealed esophageal candidiasis and atrophic gastritis.

Considering the presence of hypoparathyroidism, AAI, and candidiasis, we assumed that the patient had APS-1. The Abs to α-interferon (IgG) and α2-interferon (IgG) tests were positive. Additionally, the patient was found to have 2 heterozygous compound variants in the coding regions of AIRE.

- **c.769C > T (p.Arg257Ter), rs121434254**: a variant with the “stop gained” annotation, leading to a nucleotide substitution with the formation of a premature stop codon, which leads to the synthesis of a truncated transcript. Prediction of pathogenicity: SIFT, deleterious; PolyPhen, probably damaging. The allele frequency in the European population is 0.0006095. This variant is the major Finnish mutation prevalent in patients with APS-1 in the Finnish population [27]. The same heterozygous variant was found in the patient’s mother.

- **c.821delG (p.Gly274AlafsTer104)**, a variant with the “frameshift variant” annotation, disrupts the translational reading frame, which leads to the termination of transcription. This variant is absent in the NCBI databases and gnomAD database but has been reported in a Russian patient with APS-1 [28]. The same heterozygous variant was found in the patient’s father.

Interestingly, the patient was found to have also rare heterozygous variants in the following autoimmune genes. CIITA: **c.4C > T (p.Arg2Cys), rs375497479**: missense variant. Prediction of pathogenicity: SIFT, deleterious low confidence; PolyPhen, possibly damaging. The allele frequency in the European population was 0.00005443.

A transcript with this variant is a target for nonsense-mediated messenger RNA degradation, which leads to the degradation of the mutant messenger RNA. The mother, who is currently asymptomatic, also has this variant.

The patient and her parents had no significant variants in the coding areas of other genes from autoimmune gene panels: PTPN22, CYP27B1, VDR, CTLA-4, CLEC16A, MICA, STAT4, PD-L1 (CD274), FCRL3, GPR174, GATA3, NFATC1, TNF, CYP21A2, and NLRP1.

**Discussion**

Thus, we present 2 clinical cases of APS with an atypical clinical picture.

**Patient A**

The family members of patient A (both parents and sister) have no clinical manifestations of the pathology. Thus, de novo mutation or autosomal recessive trait of mutation inheritance may underlie the disease. By direct comparative analysis of exomes from the family trio, father, mother, and patient A, we found no potentially deleterious de novo mutations in protein-encoding genes in patient A. Next, we searched for homozygous or compound heterozygous mutations in genes that we had previously selected for the research. We found no homozygous or compound potentially disease-causing variants in these genes, including the AIRE gene. Therefore, we searched further for rare deleterious variants in any other genes across genomes using our exome data. The genetic variant of interest was identified in the PTPN22 gene in patient A. The presence of endocrine autoimmunity in the affected child can potentially be associated with a rare (gnomAD nFEAF: 0.006392) deleterious heterozygous missense variant—c.2250G > C (rs56048322) in PTPN22. Direct inspection of the rs56048322 variant in 3609 families with DM1 revealed that this variant demonstrated a significant association with this disease [11]. However, the same heterozygous variant was found in the patient’s father, asymptomatic to the
date. Exome-sequencing analysis has revealed no mutation in any alleles of the PTPN22 gene in patient A inherited from her mother anticipated for autosomal recessive inheritance. Nonetheless, a presumable heterozygous variant in introns or regulatory regions occurring on the mother’s allele cannot yet be ruled out by analysis of the exome data. Another possible pathogenic mechanism is the dominant negative effect of the PTPN22 mutation. The mutation may have an impact on PTPN22 splicing that leads to the production of 2 alternative PTPN22 transcripts and a new isoform of the LYP protein encoded by the PTPN22 gene. The mutant nonfunctional protein isoform may compete with wild-type LYP for binding to CSK kinase. This results in decreased CD4+ T-cell reactivity in response to antigen stimulation in patients with DM1 [11]. Either a dominant-negative effect or variable penetrance of the PTPN22 mutations cannot yet be ruled out. Another putative mechanism is a synergistic effect of genetic variants in different genes in patient A. To test this hypothesis we searched for rare potentially pathogenic variants in all genes of patient A that may have a functional significance in the development of the disease. We found a rare heterozygous variant, rs1428335961, shared with the mother, in the PJA2 gene, which encodes the Praja ring finger ubiquitin ligase 2 protein. It has been shown that PJA2 participates in the SIK2-p35-CDK5-PJA2 protein complex, which is required to compensate for an insulin secretion by pancreatic β cells [29]. Impaired compensatory insulin secretion leads to prediabetic conditions and the subsequent development of diabetes. It is known that one of the first symptoms diagnosed in patient A was DM1. We found also in patient A rare heterozygous variant, rs1042636, shared with the father, in the CASR gene. This gene encodes the calcium-sensing receptor protein (CASR), which is highly expressed in the parathyroid glands as well as in the thyroid [30]. Patients with different types of APS demonstrate the presence of auto-Abs to the calcium receptor [31-33]. Presumably, defective CASR can potentially be an autoantigen, promoting the development of autoimmunity. Therefore, the combination of genetic variants in PTPN22 along with the CASR and PJA2 genes might provide a synergistic effect in patient A.

To test the autosomal recessive hypothesis further, we also searched for deleterious homozygous or compound rare variants in the exome data of patient A and her family. Compound variants were found in the DDX60L gene: a novel variant c.4496C>T, shared with her mother, and an rs61740705 variant, shared with her father. The DDX60L gene encodes a protein of the DExD/H-box helicase family, which is an interferon-dependent polypeptide with domains mediating adenosine 5′-triphosphate binding, adenosine 5′-triphosphate hydrolysis, nucleic acid binding, and RNA unwinding. It has a moderate effect on the RIG-I-dependent activation of the signaling pathway (RLR pathway) of innate immunity, performing additional functions in the binding of viral RNA [34]. Potential activation of the RLR pathway in macrophages in response to tissue colonization by Candida albicans has been shown [35]. Defective DDX60L can possibly be involved in promoting the development of the esophageal candidiasis, which was diagnosed in patient A.

In conclusion, we excluded the known genes for APS-1, including the AIRE gene, in the patient with atypical APS-2 with age of onset in early childhood. Our data suggest novel genes for APS-2 and describe the genetic variants in such genes that may contribute to an atypical clinical manifestation of the disease.

**Patient B**

The sequencing results demonstrated a new combination of nonsynonymous compound mutations in the AIRE gene in a patient with APS-1 and a rare heterozygous variant shared with her mother in the CIITA gene.

Patient B presented with the atypical course of APS-1, whereby the main component of the disease—primary adrenal insufficiency, hypoparathyroidism, and candidiasis—manifested in adulthood. Genetic testing revealed 2 heterozygous compound mutations: the first mutation (c.769C>T) is often found in the Russian population, and the second (c.821delG) was first described by Orlova and colleagues [28] in a compound heterozygous state with another mutation, p.T16M. Thus, we first described a new combination of heterozygous mutations. We compared the clinical picture of the disease in patient B and the patient described by Orlova et al [28]. Unlike our patient, Orlova’s previously described patient had a typical manifestation of the 2 main components of APS-1 (hypoparathyroidism and AAI) in childhood. A distinctive feature of Orlova’s patient was slowly progressing DM1. Although cases of APS-1 with typical manifestations have been described in patients with only one mutation (c.769C>T) in the AIRE gene [28], the patient’s mother, who is a heterozygous carrier of this variant, is healthy. Thus, the patient’s development of the disease is most likely due to the mutual influence of the variants identified in her (c.769C>T and c.821delG). However, we cannot exclude the possibility that the absence of the disease in the mother is due to some protective variant that the patient does not have.

In addition, the patient and her mother were found to have possibly pathogenic nucleotide substitutions in a heterozygous state in the CIITA gene (c.4C>T—polymorphism rs375497479), which encodes the CIITA transactivator regulating the transcription of class II human leukocyte
antigen genes [36]. There are no data on the clinical significance of this variant.

It is important to note that an immunological study confirmed the results of AIRE sequencing: Patients were tested for Abs to type I interferons ω and α2. These markers can be found in most patients with APS-1 [37-39], regardless of the presence of the main components of the disease and the position of the mutation in the AIRE gene. At the same time, patients with isolated components of APS-1 (hypoparathyroidism, mucocutaneous candidiasis, AAI), APS-2, and healthy individuals, including carriers of heterozygous mutations in the AIRE gene, have negative antibody test results. However, patients with APS-1 with a heterozygous mutation in the AIRE gene can have Abs to ω-interferon [37, 38]. Thus, the detection of Abs to ω- and α2-interferons is highly sensitive (Abs to ω-interferon, 99.4%; to α2-interferon, 95.4%) and a highly specific (Abs to ω-interferon 100%; to α2-interferon, 99.9%) method for APS-1 diagnosis [40]. The formation of these Abs is presumably due to a violation of the negative selection of T lymphocytes, specific to type I interferons, in the thymus [41]. In addition, the formation of α2-interferon Abs may be a “compensatory response” to the excessive secretion of this cytokine by dendritic cells [42].

As expected, in patient A with APS-2 Abs to interferons were not detected, and in patient B with APS-1, because of a mutation in the AIRE gene, Abs both to ω-interferon and α2-interferon were detected (thus confirming the diagnosis [43]).

Conclusion

Thus, APS, even within the same type and in affected members of the same family, is a heterogeneous disease with a variable clinical picture, and the genetic factors that determine the development of pathology are not fully understood. The obtained results do not exclude that genes PTPN22, PJA2, CASR, and DDX60L, which involved in the functioning of endocrine glands and the immune system, may be disease-causing in APS and, in particular, have a dominant-negative effect. In addition, we first described a new combination of heterozygous mutations in the AIRE gene (c.769C>T and c.821delG). Because the mutation c.821delG is absent in the NCBI databases and gnomAD database (only 2 cases were reported, including our case), and the father of the patient, who is a heterozygous carrier of this mutation, asymptomatic at the moment, clarification of its pathogenicity is required.

However, owing to the small number of patients examined, an unambiguous conclusion about the pathogenicity of these disorders cannot be made at present. A cohort of patients with APS-2, especially those with a childhood manifestation of the disease, should continue to be examined to find a new genetic marker of the disease, in addition to known disorders. In our opinion, it is also necessary to search for additional genetic predictors of the atypical course of APS-1, especially in “classic” AIRE mutations. In addition, further research is important to determine phenotype-genotypic associations in this group of diseases. Identification of new genetic predictors of APS is required for timely detection and treatment of autoimmune lesions in target organs, predicting the course of the disease, and conducting preventive measures. The data obtained showed that the AIRE mutation is not a rare predictor of APS in adults, as previously thought. All patients with adult APS should undergo AIRE sequencing after receiving a positive immunological test for Abs against type I interferons. Considering the possible development of the disease in a heterozygous variant, all patients of reproductive age should be educated about the possibilities of preimplantation diagnosis.

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**Additional Information**

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**Disclosures:** The authors have nothing to disclose.

**Data Availability:** Some or all data generated or analyzed during this study are included in this published article or the data repositories listed in “References.” The data sets generated during this study were deposited into the NCBI SRA database and accessed with the BioProject accession number PRJNA702886 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA702886).

**References**


