Search for Genetic Predictors of Adult Autoimmune Polyendocrine Syndrome in Monozygotic Twins

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ABSTRACT: Autoimmune polyendocrine syndromes (APS) are a heterogeneous group of diseases characterized by the presence of autoimmune dysfunction of 2 or more endocrine glands and other non-endocrine organs. The components of the syndrome can manifest throughout life: in childhood—APS type 1 (the juvenile type) and in adulthood—APS type 2, 3, and 4 (the adult types). Adult types of APS are more common in clinical practice. It is a polygenic disease associated with abnormalities in genes encoding key regulatory proteins of the major histocompatibility complex (MHC). The search of for candidate genes responsible for mutations in adult APS is continuing. Genetic predisposition is insufficient for the manifestation of the APS of adults, since the penetrance of the disease, even among monozygotic twins, does not approach 100% (30–70%). The article presents the case of isolated Addison's disease and APS type 2 in monozygotic twins with a revealed compound heterozygosity in the candidate gene VTCN1.

KEYWORDS: autoimmune polyendocrine syndrome, monozygotic twins, antibodies, diabetes mellitus type 1, Addison's disease, HLA

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Introduction

Autoimmune polyendocrine syndromes (APS) are a heterogeneous group of diseases characterized by autoimmune dysfunction of 2 or more endocrine glands and other non-endocrine organs. Patients with APS may develop non-endocrine autoimmune conditions such as celiac disease, alopecia, vitiligo, pernicious anemia, or autoimmune gastritis with vitamin B12 deficiency.[1] Besides, IgA deficiency and other immunodeficiency conditions are more common for patients with autoimmune diseases than in the general population.[2]

Currently, autoimmune polyendocrine syndromes are divided into 2 main groups: APS type 1 (juvenile type) and APS types 2, 3, and 4 (adult type).[3]

APS types 2, 3, and 4 are more common than the juvenile type. Prevalence among women is 3 to 4:1, and adult types are usually diagnosed at the age of 20 with a peak of manifestations in the fourth and fifth decades of life.[4]

Types 2, 3, and 4 of adult APS are multifactorial diseases with a polygenic type of inheritance, associated mainly with polymorphism of genes of the major histocompatibility complex (MHC), which encodes key regulatory proteins of the human leukocyte antigen (HLA) gene complex and leads to loss of immune tolerance.[1,4]

Addison's disease and APS type 2 are associated with the DR3-DQ2 and DR4-DQ8 haplotypes *HLA II*. The heterozygous genotype *DR3-DQ2/DR4-DQ8* considerably increases the risk of APS type 2.[5,6]

The polymorphisms of the cytotoxic T lymphocyte antigen-4 gene (*CTLA-4*), protein tyrosine phosphatase non-receptor type 22 (*PTPN22*), forkhead box P3 gene *(FOXP3)*, the transcription regulator protein *(BACH2)*, and Interleukin-2 receptor alpha *(IL2RA)*, also called CD25, were also found to be associated with APS Type 2. They determine the regulation of the immune response between antigen-presenting cells and T-cell receptors.[1] The search for the mutations of the candidate genes to APS of adults is continuing, especially in monozygotic twins' models.

Genetic predisposition is insufficient for the manifestation of the APS in adults, since the penetrance of the disease, even among monozygotic twins, does not approach 100% (30–70%). Additional environmental risk factors such as nicotine use, infections, or stress can affect the development of APS types 2–4.[7]

Case Report

Two 57-year-old male monozygotic twins (Patients 1 and 2) were observed in the Endocrinology Research Centre for 9 years. One of the patients manifested Addison's disease (Patient 1) and the other had APS type 2 (Patient 2). Patients denied having a history of severe infection, allergies, or malignancies.

Table 1. Genetic variants identified in patients and their parents.

	FCGR2B (NM_004001.4)	*VTCN1* (NM_024626.4)	*HLA-DRB1*
Patient 1	c.[817+5G>A;817+10_817+11insA]	c.[−21C>A];[−66C>T]	DRB1*03-DQA1*05:01-DQB1*02 DRB1*04-DQA1*03:01-DQB1*03:02
Patient 2	c.[817+5G>A;817+10_817+11insA]	c.[−21C>A];[−66C>T]	DRB1*03-DQA1*05:01-DQB1*02 DRB1*04-DQA1*03:01-DQB1*03:02
Father	c.[817+5G>A;817+10_817+11insA]	c.−66C>T	DRB1*04-DQA1*03:01-DQB1*03:02 DRB1*16-DQA1*01:02-DQB1*05:02
Mother	wt	c.−21C>A	DRB1*03-DQA1*05:01-DQB1*02 DRB1*03-DQA1*05:01-DQB1*02

HLA class II gene polymorphisms typing showed identical results in both patients—the heterozygous genotype *DR3 / DR4* was identified—genotype of an increased risk of developing APS type 2 (*DRB1*03-DQA1*05:01-DQB1*02/ DRB1*04-DQA1*03:01-DQB1*03:02*).

Their parents had no record of autoimmune diseases. They were tested for the *HLA II* polymorphisms. The mother was homozygous for the haplotype *DRB1*03-DQA1*0501-DQB1*02* and the father had genotype *DRB1*04-DQA1*03:01-DQB1*03:02/DRB1*16-DQA1*01:02-DQB1*05:02*. Both of them had genetic risk factors for the developing autoimmune diseases (Table 1).

Complete exome paired end (80 × 2) sequencing was performed on a NextSeq 550 System (Illumina, San Diego, CA) with a high output sequencing kit after enrichment with TruSeq Exome Enrichment Kit (Illumina), according to the manufacturer's specifications. Sequence reads were aligned to the reference human genome (hg19) using BWA-MEM algorithm, and further data analysis pipeline was performed according to Genome Analysis Toolkit (GATK) Best Practices Workflow[8] for germline SNP and Indel discovery. Variant calling was done with GATK v4.0 Haplotype Caller. The detected variants were then annotated with ANNOVAR[9] using dbSNP (build 151),[10] the Genome Aggregation Database (gnomAD),[11] the 1000 Genomes Project (August 2015),[12] ClinVar[13] and functional predictions from dbNSFP (3.0).[14] The potential variants impact on splicing was evaluated with in silico tools MMSplice[15] and SPIDEX.[16]

Complete exome sequencing revealed both affected twins to be compound heterozygotes for 2 rare variants located in the 5′ untranslated region (5′UTR) of *VTCN1* (NM_024626.4) gene: c.−21C>A (rs117000061) and c.−66C>T (rs61759536). The twins' mother and father have shown to be heterozygous carriers of rs117000061 and rs61759536 respectively.

Furthermore, in gene *FCGR2B* (NM_004001.4) twins and their father have 2 close heterozygous variants inherited in cis: c.817+5G>A (rs755556655) and c.817+10_817+11insA (rs1213913083). These variants are closely located in intron 6 near exon-intron junction. In silico analysis using MMSplise and SPIDEX tools reveals this complex variant to be likely deleterious due to possible splice donor motif disrupting.

Despite the presence of the mutation, the twins' father has no APS clinical manifestations, perhaps due to the absence of *DR3/DR4* increased risk genotype and/or his possibly protective HLA haplotype *DRB1*16-DQA1*01:02-DQB1*05:02*.

Patient 1

Patient 1 had Addison's disease manifestation when he was 25 years old. He was prescribed replacement therapy with glucocorticoids (Hydrocortisone 30 mg/day) and mineralocorticoids (Fludrocortisone 0.1 mg/day). The patient refused to take Fludrocortisone and did not see a doctor for many years.

Patient 1 was admitted to the Endocrinology Research Centre in 2011 at the age of 48. Therapy at the time of admission: Hydrocortisone 30 mg/day. Complaints at admission of Patient 1 were arterial hypotension, muscle pain and weakness, but there was no progressive weight loss or intensive skin pigmentation manifested. BMI = 25 kg/m^2. Laboratory tests revealed a high serum potassium level, low serum chlorides level, and high plasma renin activity (Table 2).

The dose of Hydrocortisone was reduced to 25 mg/day and Fludrocortisone 0.1 mg/day was prescribed. According to the results of the tests, there were no signs of impaired thyroid, kidney, or liver functions (only hepatomegaly).

Detection of circulating adrenal cortex autoantibodies (Anti 21-OH-antibodies) confirmed the diagnosis of Addison's disease. The patient had negative anti-thyroid peroxidase antibodies (anti-TPO). Islet Cell Antibodies (ICA), Protein tyrosine phosphatase Antibodies (IA-2), Glutamic Acid Decarboxylase Antibodies (GADA) levels were positive. At the same time fasting plasma glucose level and glycated hemoglobin (HbA1c) level were in the normal range. Oral glucose tolerance test did not show impairment of carbohydrate metabolism. (Table 2).

Table 2. Patient 1 and Patient 2 laboratory investigations.

PARAMETERS	PATIENT 1 VALUE (2011)	PATIENT 1 VALUE (2018)	PATIENT 2 VALUE (2011)	PATIENT 2 VALUE (2018)	REFERENCE VALUE
RBC	5.4×10^{12}/L	4.8×10^{12}/L	5.32×10^{12}/L	5.33×10^{12}/L	$4.3–5.7 \times 10^{12}$/L
Hb, g/L	**173***	159	**165**	160	130-160
Serum glucose, mmol/L	5.3	4.65	5.12	**14.92**	3.3-6.0
HbA1C, %	5.9	5.3	5.8	**8.7**	<6
S. Iron, mcmol/L	29.7	27.5	25.7	17.9	10.6-31.3
Vitamin B12, pg/mL	–	**40.8**	–	324	191-663
Vitamin D3, ng/mL	–	**9.19**	–	**15.6**	30-150
Serum creatinine, mcmol/L	88	79	83.4	98.7	62-106
AST, IU/L	37.7	26	24	25	4-32
ALT, IU/L	22.1	28	22	20	7-31
Total protein, g/L	78	74	67	68	66-83
S. Calcium, mmol/L	2.45	2.37	2.16	2.21	2.15-2.55
S. Phosphorus, mmol/L	1.58	1.52	1.2	1.07	0.78-1.45
S. Sodium, mmol/L	130	**115**	131	136	120-150
S. Potassium, mmol/L	**5.4**	**5.5**	**6.4**	4.3	3.6-5.3
S. Chlorides, mmol/L	**94**	**96**	**91**	99	97-108
Cholesterol, mmol/L	**6.2**	**6.08**	5.11	**6.28**	< 5
S.ACTH (8.00 am)	43.1	25.1	38.2	48	7.0-66
Plasma renin activity, mU/L	**106**	**98**	**500**	37.02	2.8-39.9
S. TSH, mU/L	2.4	3.0	2.89	2.78	0.25-3.5
S. FT4, pmol/L	15	16	16	14	10.8-22
Insulin, mU/mL	11.3	22.22	**96**	**102**	2.3-26.4
Anti TPO antibodies, U/mL	5.2	**31.1**	**46.2**	**43.7**	<5.6
Anti 21-OH-antibodies, U/mL	**32.1**	**36.4**	3.8	7.9	0-10
IAA, U/mL	5.1	3.7	2.36	0.29	0-10
ICA, U/mL	**3.0**	0.3	**1.1**	0.36	0-1
GADA, U/mL	**1.16**	0.25	**400**	**181.9**	0-1
IA 2, U/mL	**24**	5	–	**535**	0-10
ZnT8, U/mL	–	13.3	1.45	1.4	0-15
PCA, U/mL	3.2	1.2	1.8	1.5	0-10
IF-antibodies, U/mL	2.1	4.8	6.3	5.5	0-6
Anti-gliadin antibodies, U/mL	**78**	**168.01**	4.2	6.1	0-10
Anti-tTG antibodies, U/mL	6.7	7.7	–	16	0-15
Testosterone, nmoL/L	–	6.29	–	3.83	11-33.5
FSH, U/L	–	3.87	–	3.85	1.6-9.7
LH, U/L	–	3.63			2.5-11

"*" – bold entries is used to display parameters that are out of reference value

Abbreviations: RBC, red blood cells; Hb, Hemoglobin; HbA1C, glycated hemoglobin A1c; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ACTH, adrenocorticotropic hormone; S. FT4, serum free thyroxin; S. TSH, serum thyroid stimulating hormone; TPO, thyroid peroxidase; IAA, insulin autoantibodies; ICA, islet cell antibodies; GADA, glutamic acid decarboxylase antibodies; IA2, antibodies to protein tyrosine phosphatase; ZnT8 antibodies, Zinc transporter 8 antibodies; PCA, anti–gastric parietal cell antibodies; IF, intrinsic factor; Anti–tTG, tissue transglutaminase antibodies; FSH, follicle–stimulating hormone; LH, luteinizing hormone.

Anti-gliadin-Antibodies (AGA) level was positive, but Anti-transglutaminase Antibodies (ATA) level was normal. The patient did not take gluten free diet and did not have the symptoms of Celiac disease. Anti-parietal cell-Antibodies (APCA) and Intrinsic Factor-Antibodies (IF) were in reference range (Table 2). Patient 1 did not adhere to the treatment regimen and changed the doses of hydrocortisone and fludrocortisone on his own.

In 2018, Patient 1 was admitted to the Endocrinology Research Centre for the second time. He had the symptoms of a corticosteroid overdose (Hydrocortisone 30 mg daily), weight gain (BMI 28.7 kg/m^2). Plasma renin activity and serum potassium level remained high at a dose of Fludrocortisone 0.1 mg/day (Table 2). Based on the blood tests results the dose of Hydrocortisone was reduced (25 mg daily) and the dose of Fludrocortisone—increased (0.1 mg/day).

Screening for other autoimmune diseases in Patient 1 showed high level of anti-TPO antibodies for the first time. TSH and free T4 levels were normal. All antibodies relevant to Type 1 diabetes mellitus (DM) became negative. Fasting plasma glucose and A1c levels were normal.

AGAs were positive and their level increased compared to the previous study. ATA, APCA and IF were normal. Considering the risk of celiac disease and other gastrointestinal diseases such as gastroenteritis and prolonged overdose of corticosteroids, gastroscopy, and colonoscopy were recommended. However, the patient refused to undergo the examinations.

Thus, at the time of the last examination, Patient 1 had only 1 autoimmune endocrine disease—Addison's disease. The patient had no data for hypogonadism. Further regular screening of the patient's condition is required.

Patient 2

Patient 2 was diagnosed with Addison's disease when he was 27 years old. He received glucocorticoid replacement therapy (only Hydrocortisone 30 mg/day). He also refused to take mineralocorticoids (Fludrocortisone 0.1 mg/day).

At the age of 48 , Patient 2 was diagnosed with DM. The disease manifested with hyperglycemia up to 20 mmol/L and ketoacidosis. Insulin therapy started, however, the type of diabetes was not clearly defined. Patient 2 did not use insulin after discharge from the hospital, he decided to keep a diet. Patient 2 also reduced the dose of Hydrocortisone to 10 mg/day, as he noticed an improvement in plasma glucose levels.

For the first time Patient 2 was admitted to the Endocrinology Research Centre half a year after the onset of DM in 2011 to correct the treatment of adrenal insufficiency. He suffered from weakness, skin pigmentation and arterial hypotension. BMI was 21 kg/m^2—clinical signs of decompensated adrenal insufficiency. Laboratory tests revealed a high serum potassium level, low serum chlorides level, and high plasma renin activity (Table 2). He received parenteral Hydrocortisone 100 mg/day, later—Glucocorticoids and Mineralocorticoids in rational doses per os.

Patient 2 was examined for the serological markers of other autoimmune diseases. Anti 21-OH antibodies were positive and confirmed the diagnosis of Addison's disease. GADA and IA-2 antibodies were positive. Anti-TPO antibodies were elevated, TSH and free T4 serum levels were in the reference range (Table 2). Fasting glucose plasma level and HbA1c were in the reference range. Oral glucose tolerance test showed normal blood sugar levels. Diabetes medications were not prescribed. Apparently, Patient 2 had late autoimmune diabetes in adults (LADA) (Table 2).

A few months after hospitalization, the patient reduced the dose of Hydrocortisone again and the fasting glucose plasma level remained normal for the next 2 years.

In 2013, Patient 2 started Insulin therapy (Insulin Lispro and Insulin Glargine) due to hyperglycemia, but he had poor glycemic control and infrequent doctor visits for the next 5 years. The dose of hydrocortisone was 25 mg/day and fludrocortisone 0.1 mg/day.

Patient's 2 examination at the Endocrinology Research Centre in 2018 revealed decompensation of DM. The BMI was 28.9 kg/m^2. The level of HbA1c was 8.7% and the patient suffered from episodes of hypoglycemia at night and hyperglycemia in the morning. Insulin correcting therapy was carried out with a positive effect.

Patient 2 did not have clinical signs of decompensation of adrenal insufficiency. Laboratory tests showed a normal serum potassium, chlorides and sodium level, normal plasma renin activity level. The doses of Hydrocortisone and Fludrocortisone remained the same. The thyroid function was not impaired (Table 2).

An immunological examination was performed with the following result—Anti-TPO antibodies, IA-2 Antibodies, and ZnT8 Antibodies levels were high. AGA, ATA, APCA, and IF antibodies levels were normal (Table 2). The patient had no data for hypogonadism.

Patient 2 was diagnosed with APS type 2 (Carpenter's syndrome)—Addison's disease and late autoimmune diabetes in adults (LADA).

Conclusion

The clinical case describes monozygotic twins with autoimmune polyendocrine syndrome in adults with highly variable penetrance of the syndrome components.

With the discordant phenotype of the disease in monozygotic twins, it is necessary to take into account de novo postzygous mutations, variations in the number of copies of mutations in somatic cells, and the probable role of epigenetic changes in the pathogenesis of adult APS. The environment often influences clinical penetrance by enhancing or exacerbating the effects of inherited genetic variants.[17] In those 2 cases, we did not analyze if there were any differences in DNA methylation.

It is noteworthy that Patient 2 suffered from more autoimmune diseases than Patient 1. When analyzing other epigenetic factors, it can be noted that Patient 2 was married, had children and a permanent job, while Patient 1 was unmarried, had no children and worked sporadically. Obviously, a severe course of the disease of Patient 2 can be explained by a greater stressful environment.

The twins had great predisposition to APS type 2 and Addison's disease *HLA II* heterozygous genotype—*DR3-DQ2/DR4-DQ8*. Their mother was homozygous for the *HLA II* haplotype *DR3-DQ2* and the father had *HLA II* haplotype *DR4-DQ8*, but both of them did not develop any dependent disease. The clinical case confirms the fact that the penetrance of APS components is difficult to predict. Further studies, including genetic once, are required.

The nucleotide sequence variant in the 5'-region of the candidate gene *VTCN1*, which was found in the twins' genotype, might be the risk factor of adults APS. *VTCN1* gene encodes a protein belonging to the B7 costimulatory protein family. Proteins in this family are present on the surface of antigen-presenting cells and interact with ligand bound to receptors on the surface of T-cells.

VTCN1 gene is a potential candidate for association with autoimmune diseases. A genetic susceptibility of the *VTCN1* gene to systemic lupus erythematosus (SLE) and juvenile idiopathic arthritis was shown in a cohort of Caucasian patients, while relationship of the *VTCN1* gene with Addison's disease was not previously mentioned in studies. A significant genetic association with the *VTCN1* gene region in rheumatoid arthritis (RA) susceptibility was observed in 2 populations of northern European descent.[18-20] Radichev et al in their research examined the functionality of endogenous VTCN1 in islet cells of mice and human during T1D development, and assessed the natural VTCN1 capability to modulate autoimmune processes. They showed that defective VTCN1 presentation from pancreatic islets, due to an increased shedding, preceded T1D development. These data support the relatively distinct genetic predisposition of the *VTCN1* gene to several autoimmune diseases, including possibly Addison's disease. Further research is needed to elucidate its effects on patients with Addison's disease and adult APS.[21]

Another probable pathogenic nucleotide sequence variant in gene *FCGR2B* was found in twins. The gene encodes Fc fragment of IgG receptor IIb—a low affinity inhibitory receptor for the Fc region of immunoglobulin gamma (IgG). The receptor inhibits the functions of activating FcγRs, such as phagocytosis and pro-inflammatory cytokine release, mainly by clustering of FCGR2B with different activating receptors or with the B cell receptor by immune complexes. *FCGR2B* is one of the genes thought to influence susceptibility to several autoimmune diseases in humans. Its decreased function is associated with SLE, RA, anti-glomerular basement membrane disease, multiple sclerosis, and others.[22] Verbeek et al in their study showed that forward and reverse genetics have provided convincing evidence that *FcγRIIb* is an important autoimmune susceptibility gene, involved in the maintenance of peripheral tolerance in both humans and mice. It has been shown that in humans, an association between a SNP (*rs1050501*) in the *FCGR2B* gene, causing a missense mutation, results in impaired FCGR2B function, and susceptibility to SLE, and association with RA. A series of observations suggest that B cells impaired FcγRIIb function is affected not only by antibody titers but also by affinity maturation and memory responses of B cells and plasma cell homeostasis associated with an increase in the production of autoantibodies. To understand underlying mechanisms, and association with autoimmune endocrine diseases further studies are needed.[23]

As we mentioned earlier *FOXP3* were found to be associated with polyendocrinopathy and autoimmune diseases. Immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome is a monogenic disorder due to loss-of-function mutations in the *FOXP3*. Park et al in their research described 195 patients with IPEX-syndrome. The most frequent clinical manifestations were: enteropathy (n = 191, 97.9%), skin manifestations (n = 121, 62.1%), endocrinopathy (n = 104, 53.3%). Also, the authors identified various significant genotype-phenotype correlations that can potentially be used to predict the disease course of IPEX patients.[24] Another gene which may be present with polyendocrinopathies is mutation in the *LPS-responsive beige-like anchor* (LRBA) gene, previously considered the cause of common variable immunodeficiency with autoimmunity. LRBA deficiency has a spectrum of phenotypes, including chronic diarrhea, hypogammaglobulinemia, recurrent infections, pneumonitis, organomegaly, and autoimmune disorders such as DM1, thyroiditis, hemolytic anemia, and thrombocytopenia.[25] Charbonnier et al[26] in their work showed that LRBA deficiency can lead to IPES like syndrome and is compounded by impaired T regulatory cell phenotype and function, with decreased expression of key effector proteins involved in T regulatory cell suppression such as CD25 and CTLA-4. Sterlin D et al detected autoimmune manifestations (hepatitis, nephritis, and thyroiditis) in patients with immunodeficiency, centromeric instability, and facial dysmorphism (ICF syndrome). ICF syndrome is characterized by lack CD19+CD27+ memory B cells, which leads to recurrent infections. About 50 % of patients carry mutations of the DNA methyltransferase 3B (*DNMT3B*) gene and 30% of patients have mutation *ZBTB24* gene. The authors state that autoimmune signs are uncommon in ICF syndrome but these manifestations could reflect a breach of T-cell tolerance or defects in regulatory T-cell populations.[27] Other mutant genes associated with polyendocrinopathy are described in Table 3.

The determination of antibodies in dynamics is important for the assessment of the risk of developing new components of the syndrome, and the level of antibodies plays an important

Table 3. Mutant genes associated with polyendocrinopathy.

GENES	DISEASES	REFERENCES
PTPN22	AAI; DM1; SLE; MG; vitiligo; systemic sclerosis; JIA; Wegener's granulomatosis; psoriatic arthritis; autoimmune hepatitis type 1; GD; AIT; RA	Del Pilar Fortes et al,[28] Velaga et al,[29] and Ge et al[30]
CTLA4	DM1; GD; AIT; AAI; RA	Wolff et al[31] and Ting et al[32]
STAT4	AAI; DM1; systemic sclerosis; Sjogren's; syndrome; SLE; NUC; CD; MS; BD; GD; AIT; RA	Mitchell et al,[33] Korman et al,[34] Li et al,[35] and Yan et al[36]
CIITA	MS; AAI; BD; DM1	Ghaderi et al[37]
MICA	Celiac disease (atypical form); AAI; BD; DM1; Primary sclerosing cholangitis; AIT; GD; Psoriatic arthritis	Lopez-Vazquez et al[38] and Cho et al[39]
CLEC16A	MS; DM1 PBC; SLE; AAI; GD; AIT; RA; JIA; AA; CD	Muhali et al[40]
PD-L1	AAI; GD	Mitchell et al[41]
NALP1	AAI; DM1; Vitiligo	Magitta et al[42]
GPR174	AAI; GD	Napier et al[43]
FCRL3	AIT; GD; SLE; RA; Autoimmune pancreatitis; AAI	Chistiakov and Chistiakov[44] and Owen et al[45]
CYP27B1	AAI; AIT; GD; DM1	Lopez et al[46]
VDR	DM1; AAI; Vitiligo; AIT; GD; RA	Pani et al,[47] Pani et al,[48] and Gao and Yu[49]

Abbreviations: AAI, adrenal insufficiency; DM1, diabetes mellitus type 1; GD, Graves' disease; AIT, autoimmune thyroiditis; SLE, systemic lupus erythematosus; MG, myasthenia gravis; JIA, juvenile idiopathic arthritis; RA, rheumatoid arthritis; NUC, nonspecific ulcerative colitis; CD, Crohn's disease; MS, multiple sclerosis; PBC, primary biliary cholangitis; BD, Buerger disease; AA, alopecia areata.

role in clarifying the time of its manifestation. At the onset of DM Patient 2 had the IA-2 Antibodies level 40 times higher than the normal range. While Patient 1 had a 2.5-time increase of IA-2 Antibodies once, and then it gradually returned to normal. Patient 2 showed a slight increase in the Anti-TPO antibodies level, and the thyroid function was not impaired for 7 years.

Clinical manifestation of the new components of APS can significantly affect the compensation of already diagnosed chronic adrenal insufficiency or DM type 1/LADA and increase the risk of disease complications and life-threatening conditions such as severe hypoglycemia or Addisonian crisis.

It is also worth noting the extremely long period of lack of adequate therapy for hypocorticism in patients and diabetes mellitus in P2. Apparently, it was the excessive dose of hydrocortisone, which is known to have a mineralocorticoid effect that made it possible to remain in subcompensation for a long time. Besides, the patients during the period of the disease against the background of inadequate therapy were not subjected to significant stressful effects (operations, injuries, etc.), otherwise it could have led to an Addisonic crisis and death. More surprising is the long-term lack of treatment for diabetes mellitus against the background of extremely low doses of hydrocortisone. The tendency to hypoglycemia is due to a significant increase in the sensitivity of tissues to insulin against the background of a decrease in cortisol. Probably, the residual secretion of cortisol and insulin persisted to the period, which prevented the development of a ketoacidotic coma. It is important to add that such patients need particularly careful monitoring—if there is compensation at least once a year, otherwise, as often as necessary, not excluding hospitalization.

Therefore, all the patients with APS and those who are in the risk group of manifestation of the syndrome should undergo an annual screening (or more often) to exclude endocrine and non-endocrine autoimmune diseases. Systematic examination of clinical symptoms, laboratory tests (TSH, glucose and HbA1c, cortisol plasma levels, etc.), markers of autoimmune diseases allows predicting the risk of severe complications.

Genetic testing and search for known predisposing and protective polymorphisms of the *HLA II* and non-*HLA* genes in relation to the development of APS in adults is required in the risk groups. The risk groups include patients with 1 autoimmune disease and relatives with APS of adults; patients in nuclear APS families, including healthy first-line relatives; patients with APS type 3 in order to predict the development of APS type 2. Complete exome sequencing in the APS type 2 nuclear families facilitates the search for new rare predisposing gene mutations, including de novo mutations.

Author Contributions

Yukina MY: data analysis, writing an article, treatment of the patients, verification of the critical content of the article. Larina AA: patient recruitment, data analysis, writing an article. Vasilyev EV: performing whole exome sequencing, data analysis, checking the text of the article. Troshina EA: formulation of the purpose and objectives of the study, development of the

research concept, checking the text of the article. Dimitrova DA: treatment of the patients, writing an article.

Informed Consent

Written informed consent was obtained from both patients for the publication of this case report. The research related to human use has been conducted in compliance with all the relevant national regulations, institutional policies and in accordance with the tenets of the Declaration of Helsinki, and has been approved by the Ethics Committee of Endocrinology Research Centre with the ID №17; 28/10/2020.

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