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Uncommon constellation of multiglandular deficiency with 2 mutations in *AIRE* gene in an 18-year-old girl — 12 years of observation

Nietypowy przebieg niewydolności wielogruczołowej z współistniejącymi mutacjami genu *AIRE* u 18-letniej dziewczynki —12-letnia obserwacja

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Abstract

Autoimmune polyglandular syndromes (APS) consist of a variety of endocrine and non-endocrine disorders. The syndromes are complex and their occurrence in life does not follow any pattern. Early detection of such disorders may prevent many serious clinical consequences which are usually a result of delayed diagnosis.

We present the case of a female patient whose clinical symptoms very strongly suggested APS, however neither autoimmune background except elevated anti-thyroid peroxidase and anti-thyroglobulin antibodies of multiglandular deficiency, nor critical mutations in the AIRE gene have been confirmed or detected, yet we identified five polymorphisms and two mutations in exon1 of gene *AIRE* during 12 years of observation and treatment. **(Endokrynol Pol 2014; 65 (6): 514–518)**

Key words: *autoimmune polyglandular syndrome; hypoparathyroidism; ovarian failure; hypopituitarism; gene AIRE*

Streszczenie

Zespoły autoimmunologicznej niewydolności wielogruczołowej (APS) składają się z różnych zaburzeń, zarówno endokrynnych, jak i nieendokrynnych. Zespoły te są złożone i występują z różnymi objawami. Wczesne wykrycie zaburzeń może zapobiec wielu poważnym skutkom klinicznym, które są zazwyczaj wynikiem opóźnionego rozpoznania. W pracy przedstawiono przypadek pacjentki, której objawy kliniczne przemawiają za zespołem autoimmunologicznej niewydolności wielogruczołowej. W ciągu 12 lat obserwacji i leczenia, autoimmunologiczne tło, z wyjątkiem stwierdzenia podwyższonego miana przeciwciał przeciw peroksydazie i przeciw tyreoglobulinie, nie zostało potwierdzone, podobnie jak krytyczna mutacja w genie *AIRE*. U pacjentki zidentyfikowano 5 polimorfizmów i 2 mutacje w eksonie 1 genu *AIRE*. **(Endokrynol Pol 2014; 65 (6): 514–518)**

Słowa kluczowe: *autoimmunologiczna niewydolność wielogruczołowa; niedoczynność przytarczyc; niewydolność jajników; niedoczynność przysadki; gen AIRE*

Abbreviations:

APS — autoimmune polyglandular syndromes
APECED — autoimmune polyendocrinopathy candidiasis ectodermal dystrophy
TSH — thyrotropin
PTH — parathyroid hormone
ACTH — adrenocorticotropic hormone
aTPO — anti-thyroid peroxidase antibody
aTG — anti-thyroglobulin antibody
HbA_{1c} — glycated haemoglobin
Anti-GAD — anti-glutamic acid decarboxylase antibody
GH — growth hormone
LH — luteinising hormone
FSH — follicle stimulating hormone
ICA — islet cell antibodies
IAA — insulin autoantibodies
CT — computed tomography

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Introduction

Autoimmune polyglandular syndromes (APS) are the most common cause of multiglandular insufficiency not associated with pituitary dysfunction. A variety of genetic alterations have been observed in patients diagnosed with APS. The components of APS are various and include endocrinopathies and non-endocrine autoimmune disorders. APS can be classified into three major types (Table I) [1–4].

Autoimmune polyglandular syndromes type I is a monogenic disorder associated with mutations in AIRE gene (autoimmune regulator) which are frequently observed in autoimmune diseases [2, 5]. This gene, located on chromosome 21q22.3, covers 11.9 kb and contains 14 exons. AIRE encodes the 545 amino-acid (58-kD) nuclear protein. The presence of two zinc-finger motives (PHD-finger) suggests that AIRE protein belongs to the group of transcription factors. To date, 52 different mutations within the AIRE gene have been identified as causative alterations in several autoimmune endocrine disorders. Although these mutations are spread throughout the whole coding region of the gene, hotspot mutations have been found in exons 6 and 8.

Clinical symptoms of APS type II usually appear later in life. APS II has been proven to be associated with specific HLA antigens [2].

APS III is probably inherited in an autosomal dominant manner with incomplete penetrance. The first clinical symptoms usually develop in middle-aged patients.

Case report

The patient is an 18-year-old girl, under our care since the age of six (Table II). She was born at term with normal weight and length. No abnormalities were observed in the neonatal period and her parents are healthy. The first symptom — tetany — occurred at the age of four and was initially treated as epilepsy. However, at the time of presentation, the levels of calcium, ionised calcium and PTH were suggestive of hypoparathyroidism. The tests showed normal TSH and fT_4, fT_3 levels. The function of adrenal glands, levels of autoantibodies, glucose and HbA1c were normal

Table I. *Disorders in types I and II Autoimmune Polyglandular Syndromes compared to our patient's characteristics*
Tabela I. *Zaburzenia w APS I oraz APS II w porównaniu z zaburzeniami stwierdzanymi u opisywanej pacjentki*

	APS I	Our Patient	APS II	Our Patient
Main symptoms	Candidiasis	–	Addison's disease	–
	Hypoparathyroidism	+	Type I diabetes	–
				HbA_{1c} 5.3%, a/GAD < 5.0 IU/mL
	Addison's disease	–	Hypothyroidism	aTPO 25.5 IU/mL N
				aTG 19.8 IU/mL N
Concomitant endocrinopathies	Type I diabetes	–	Hypoparathyroidism	+
		HbA_{1c} 5.3%, anti-GAD < 5.0 IU/mL		
	Hypogonadism	+	Hypogonadism	+
			Hypergonadotropic	
	Hypopituitarism	+	Hypopituitarism	+
	Hypothyroidism	+		
		aTPO 25.5 IU/mL N		
		aTG 19.8 IU/mL N		
Concomitant nonendocrine disorders	Vitiligo	+	Vitiligo	+
	Alopecia	+	Alopecia	+
	Hepatitis	–	Coeliac disease	–
				a/endomysium (tTG) < 2 RU/mL
	Pernicious anaemia	–	Pernicious anaemia	–
	Malabsorption	–		
	Myasthenia gravis	–		
Connective tissue disease		+		

APS I — Autoimmune Polyglandular Syndrome type I; APS II — Autoimmune Polyglandular Syndrome type II

Table II. *Laboratory data during the 12 years of patient observation*
Tabela II. *Badania laboratoryjne w czasie 12-letniej obserwacji pacjentki*

	At the time of diagnosis, age 6	Before initiating GH therapy, age 11	During GH therapy, age 15	Last examination, age 18
Height [cm]	106.5	126.3	151.7	154.7
Weight [kg]	16.6	24	33.3	37
Velocity of height [cm/year]		3.8	7.1	1
Bone age (years)	4.5	9	13	15
TSH [mIU/mL]	3.53	1.88	2.58	1,61
fT_4	1.0 pg/mL	1.61 pg/mL	16.6 pmol/L	17.4 pmol/L
fT_3 [pmol/mL]	3.5	4.27	3.5	3.27
aTPO [IU/mL]	< 10	25.5	328	71
aTG [IU/mL]	< 10	19.8	> 1000	385
PTH [pg/mL]	1 [12–72]	< 3	5	6.19
LH [mIU/mL]	–	3.54	33.5	< 0.1
FSH [mIU/mL]	–	44	104	0,559
Oestradiol [pg/mL]	–	< 20	31.1	33,6
Anti-adrenal antibodies	Negative	Negative	Negative	Negative
Cortisol profile	Normal	Normal	Normal	Therapy of methyloprednisolone
ACTH [pg/mL]	35	25	20	8,09
HbA1c (range)	Normal	Normal	Normal	Normal
anti-GAD [IU/mL]	Negative	Negative	Negative	Negative
ICA	Negative	Negative	Negative	Negative
IAA	Negative	Negative	Negative	Negative
Anti-endomysial antibodies	Negative	Negative	Negative	Negative
ANA antibodies	–	–	Negative	Negative
cANCP] antibodies [RU/mL]	–	–	7.2	8
pANCP antibodies [RU/mL]	–	–	< 2.0	< 2.0

(Table I). CT of the head was normal. Consequently, no more tremors were observed. The antiepileptic treatment was discontinued and supplementary treatment was initiated. The bone age at that point was 4.5 years. An ophthalmologic abnormality — cataract — was observed in both eyes.

One year later, progressive alopecia was observed in the patient, involving the scalp, eyebrows, eyelashes and the hair on the trunk. At the same time, the tests revealed thyroid insufficiency, but the antibodies level remained normal. A supplementary therapy of L-thyroxin was initiated and the thyroid function normalised. Additionally, at the age of nine, vitiligo on the limbs and trunk was first observed.

Due to short stature and delayed bone age, confirmed by incorrect levels of GH (GH max 4.55 ng/mL) in the glucagon and L-dopa stimulation and nocturnal secretion tests, the patient was diagnosed with somatothropic hypopituitarism. MRI of the pituitary gland showed no abnormalities. Since the age of 11, the patient has been receiving 0.1 IU/kg of growth hormone daily.

Before the initiation of GH therapy, the level of LH was normal while the FSH was high and oestradiol — low. Despite the revelation of normal female karyotype (46, XX), in the examination at the age of 12, the Tanner stage was I and no hair was present on the patient's body (alopecia totalis).

The patient's height increased by 14.3 cm during the first 24 months of GH therapy, but the bone age was delayed. At the age of 13, levels of gonadotropins were higher while those of oestradiol was lower than normal for this age. No pathology was observed in the pelvis ultrasound examination. Control MRI of the pituitary gland remained normal and the Tanner stage was unchanged. Due to the short period of GH treatment, the induction of oestrogen therapy was postponed. Function of adrenal gland remained normal in repeated tests. Also, the glucose curve, HbA1c, anti-GAD, ICA and IAA levels were normal in consecutive tests repeated annually.

In the same year, conjunctivitis and keratitis were observed, while the IgE level was normal. The control levels of antibodies and liver function were still within normal limits.

At age 15, the patient presented with new symptoms: evanescent skin rash, fever and myalgia. After excluding an infectious aetiology, connective tissue disease was diagnosed in the Rheumatology Department for Children (Table II). The patient was started on methylprednisolone with good clinical response.

Currently, she receives the following medications: L-thyroxine, methylprednisolone, alfacalcidol, oestrogen and progesterone. She has so far not presented with another clinical symptom.

A genetic consultation was performed as part of the work-up (Wroclaw Medical University and Institute of Physiology and Pathology of Hearing). Cytogenetic analysis was done on GTG and CBG-banded peripheral blood lymphocyte chromosomes, following the standard protocols and revealed normal female karyotype. Molecular analysis was performed on DNA extracted from the peripheral blood lymphocytes. All exons of the *AIRE* gene were amplified by the polymerase chain reaction technique using previously described primers (Table III) [6]. Direct sequencing of all coding exons of the *AIRE* gene was performed on ABI-PRISM 377 using the dye terminator chemistry. Both strands were analysed. Precise DNA analysis of our patient allowed us to exclude the presence of two hotspot mutations (in exons 6 and 8, located between the SAND domain and the first PHD finger and in the region coding for the first PHD finger, respectively) as well as to identify five polymorphisms. Moreover, two mutations (in 1st exon 132+1_and 132+3delGTGinsCT) were detected (Fig. 1). To establish their cis or in trans configuration, direct sequencing of 1st exon was performed on DNA from both parents of our patient. We found the same two mutations in her mother (132+1_132+3delGTGinsCT), but no mutation in her father (Fig. 2). These results show that two detected mutations are located in one allele of maternal origin. The patient's mother did not present with any clinical symptoms, and in laboratory data only the level of aTPO was elevated, while the function of the thyroid was normal.

Table III. *Results of direct sequencing of the whole coding exons of the* **AIRE** *gene*

Tabela III. *Wyniki sekwencjonowania eksonów kodujących gen* **AIRE**

Exon number	Genotype	Notes
1	wt/132+1_132+3delGTGinsCT	Results showed on Fig. 1
2	Wt	
3	Wt	
4	Wt	
5	rs 41277544 A/G_rs878081 C/T	
6	Wt	
7	Wt	
8	Wt	
9	P355P (CCC > CCT)	Silent variant
10	rs60904129 T/C	
11	Wt	
12	Wt	
13	Wt	
14	rs1133779 T/C	

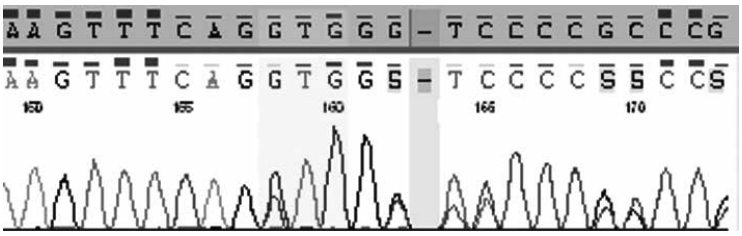

Figure 1. *Results of direct sequencing of 1 exon in the patient's DNA*

Rycina 1. *Wyniki sekwencjonowania w eksonie 1 DNA pacjentki*

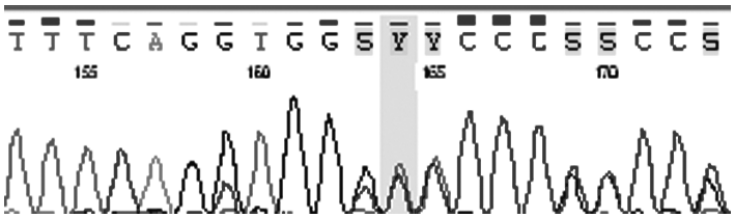

Figure 2. *Results of direct sequencing of 1 exon in the mother's DNA*

Rycina 2. *Wyniki sekwencjonowania w eksonie 1 DNA matki*

Discussion

Polyglandular deficiency in our patient affects four endocrine glands: parathyroid, thyroid, pituitary gland and ovaries, and requires supplementary treatment including L-thyroxine, methylprednisolone, oestrogen and progesterone. Additionally, non-endocrine defects specific for APS I, such as alopecia, vitiligo, keratitis and a connective tissue disorder, were diagnosed over the course of 12 years of observation. The constellation of symptoms, however, does not seem to be specific for any known type of APS. Candidiasis, typical for APS I, has never been observed and also the early onset would not be characteristic for APS II or III [3, 4, 7].

In the described case, the whole *AIRE* gene has been sequenced and heterozygous mutation in exon 1 was revealed. Since APS is inherited in a recessive manner, this change alone is not a cause of the syndrome. However, DNA sequencing does not allow for detecting large gene rearrangements in the coding areas. Thus, the possibility that these alterations are present in our patient must be taken into account.

Moreover, the function of polymorphisms found in our patient is unknown. In recent literature, increasingly frequent descriptions of rheumatic disorders in the course of APS suggest a potential role of the *AIRE* gene. Recently published data obtained by using large-scale genome-wide association (GWA) studies indicate a potential role of single nucleotide polymorphisms (SNPs) in the *AIRE* gene in the aetiology of rheumatoid arthritis or lupus erythematosus [8, 9]. The predictive value of the AIRE gene in diagnosing patterns of developing autoimmune conditions is yet to be determined [10].

Although during the 12 years of observation the consecutive components of autoimmune polyglandular syndrome developed, the level of the known antibodies, except aTPO and aTG, remained normal. Therefore, an autoimmune process affecting multiple organ systems cannot be definitely confirmed. However, a study on a group of APS patients in Norway showed that the autoimmune background of autoimmune polyglandular syndrome may not, in some cases, be confirmed by the presence of the most common autoantibodies [9]. Nonetheless, the path of the disorders observed in our patient suggests immunosurveillance disturbance as the pathogenesis of the symptoms.

It is possible that in future our patient will develop symptoms of other endocrine dysfunctions. The glucocorticoid therapy of connective tissue disease might mask, for instance, symptoms of the insufficiency of the suprarenal gland. Nevertheless, it is essential that the patient remains under strict control in order to prevent delayed diagnosis of any additional disorders that may occur.

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