

## Alkaptonuria in a boy with type 1 diabetes mellitus, vitiligo, autoimmune thyroiditis and immunoglobulin A deficiency – a case report

Alkaptonuria u chłopca z cukrzycą typu 1, bielactwem, autoimmunologicznym zapaleniem tarczycy i niedoborem immunoglobuliny A

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### Abstract

We present a 15-year-old Caucasian boy with an exceptional coincidence of a rare monogenic metabolic disease – alkaptonuria (AKU) and a cluster of autoimmune disorders: type 1 diabetes (T1DM), autoimmune thyroiditis (AIT), vitiligo, insulin infusion induced lipodystrophy and immunoglobulin A deficiency (IgAD). Alkaptonuria and type 1 diabetes in a child, especially in such an interesting coincidence with other autoimmune conditions, has not been reported so far. Our investigation, including comprehensive genetic evaluation using next generation sequencing technology, shows that alkaptonuria and T1DM were independently inherited. We also show that alkaptonuria in its pre-ochronotic phase seems to have no effect on the course of diabetes.

### Key words

type 1 diabetes, alkaptonuria, autoimmune thyroiditis, vitiligo, immunoglobulin A deficiency

### Streszczenie

W pracy przedstawiono przypadek piętnastoletniego chłopca, u którego po rozpoznaniu cukrzycy typu 1 (T1DM) zdiagnozowano alkaptonurię, autoimmunologiczne zapalenie tarczycy, bielactwo, lipoatrofię w miejscach wkłuc do pompy insulinowej oraz niedobór immunoglobuliny A (IgAD). Współwystępowanie alkaptonurii i cukrzycy typu 1 u dziecka nie zostało dotychczas opisane, zwłaszcza w skojarzeniu z wieloma innymi chorobami autoimmunologicznymi. Na podstawie badań genetycznych – sekwencjonowania nowej generacji wykazaliśmy, że alkaptonuria i T1DM wystąpiły niezależnie od siebie, a alkaptonuria w fazie przedochronotycznej wydaje się nie mieć wpływu na przebieg cukrzycy.

### Słowa kluczowe

cukrzyca typu 1, alkaptonuria, autoimmunologiczne zapalenie tarczycy, bielactwo, niedobór immunoglobuliny A

## Introduction

We present an exceptional case of a pediatric patient with familial type 1 diabetes (T1DM, vitiligo and IgA deficiency, and a rare metabolic disorder – alkaptonuria. Such a coincidence has not been reported so far.

Type 1 diabetes (T1D) is an autoimmune disease caused by immune-mediated destruction of beta cells. It can occur in association with other autoimmune and chronic inflammatory conditions. According to the results of genome-wide-association studies (GWAS), linkage analysis and association studies, to date over 60 T1D susceptibility loci have been

identified [1]. T1D coexistence with some other autoimmune disorders and/or IgA deficiency is well known and implies their common genetic background [2], however, it has yet to be identified. It was, therefore, interesting to investigate whether there is any genetic association between the presence of alkaptonuria and autoimmunity.

### Case report

A 15-year-old Caucasian boy with vitiligo was first presented to us at the age of 6 with polydipsia, polyuria of a few weeks' duration and progressive fatigue. These symptoms were preceded by upper respiratory tract infection. On admission, he was dehydrated, blood glucose was elevated - 274 mg/dl (15.2 mmol/l), but arterial pH was normal. After three days under continuous intravenous insulin infusion his condition improved and multiple insulin injection regimen therapy was introduced. Based on the presence of autoantibodies to beta cells (anti-

Zinc transporter-8 (ZnT8) (Table I) and lowered concentration of C-peptide (< 0.165 pmol/ml, normal range: 0.36-1.65ng/ml), he was diagnosed with diabetes mellitus type 1. During his stay at our Department a discoloration of his urine from yellow to dark brown (when left standing) was observed. Liquid chromatography confirmed high urinary homogentisinic acid (HGA) levels, which was consistent with the diagnosis of alkaptonuria.

The patient was referred to the Outpatient Clinic of Inborn Errors of Metabolism and treated with ascorbic acid.

His past medical history revealed that dark stains in the baby's diapers had been observed since the neonatal period. However, no inborn error in metabolism had been suspected until the diagnosis of diabetes. White patches on the skin suggesting vitiligo had been first observed early in infancy. At the same time, the boy had suffered from eczema due to cow's milk allergy and had been fed with extensively hydrolyzed formula (Nutramigen). Since infancy, he had had recurrent respiratory tract infections (including pneumonia twice a year in the first year of life).

**Table I.** Immunoglobulin and antibody profile

**Tabela I.** Profil immunoglobulin i autoprzeciwciał

Immunoglobulin class/subclass (mg/dl) Klasa/podklasa immunoglobulin (mg/dl)	Serum concentration Stężenie w surowicy	Normal range[ *] Norma[ *]
IgA (g/l)	<b>&lt;9.0</b>	62.0–230.0
IgA1 (mg/l)	<b>87.4</b>	582.0–2635.0
IgA2 (mg/l)	<b>&lt;10.7</b>	122.0–1407.0
IgM (mg/dl)	116	50.0–213.0
IgG	1110	708.0–1440.0
IgG1 (mg/dl)	825.5	400.0–1150.0
IgG2 (mg/dl)	188.0	98.0–480.0
IgG3 (mg/dl)	27.9	15.0–149.0
IgG4 (mg/dl)	4.5	3.0–210.0
IgE (IU/l)	0.3	<100
Autoantibodies / Autoprzeciwciała	Serum concentration / Stężenie w surowicy	Normal range / Norma[ *]
anti-microsomal antibody (anty-TPO) (IU/ml)	<b>7.58</b>	<5.61
anti-thyroglobulin antibody (anty-TG) (IU/ml)	3.25	<4.11
ICA 80 (JDF)	0	0
anty-GAD (U/ml)	<b>17.63</b>	<10
IA2/ICA512 (U/ml)	5.58	...
ZnT8 (IU/ml)	<b>75.28</b>	<8

\*Reference values for serum immunoglobulins G,A,M and D in children and adults, inhabitants of Mazovia district (Poland) [3]

\*Zakresy referencyjne stężeń immunoglobulin klasy G,A,M oraz D u dzieci i dorosłych z Województwa Mazowieckiego

The patient is the second child of a non-consanguineous couple of the central European descent. He was born prematurely by vaginal delivery, at 29 hbd, with the body weight of 1600g and 9 points Apgar. None of his family members is suffering from alkaptonuria. His mother, now aged 53, has been suffering from vitiligo and diabetes since she was 37 years old and was treated with insulin while being pregnant. His grandmother (mother's mother), who had suffered from ischemic heart disease and died at the age of 58, also had vitiligo and type 1 diabetes (diagnosed at the age of 26 with diabetes ketoacidosis, from the very beginning treated with insulin). However, in both women no immunologic verification of the diagnosis of type 1 diabetes has ever been performed. The boy's father is suffering from hypertension and the proband's elder sister, aged 29, is healthy.

In the further course of our medical supervision at the Diabetes Outpatient Clinic, recurrent infections causing unstable blood sugar levels were observed. Two years after the diagnosis of diabetes insulin pump therapy was instituted. The most recent insulin requirement has been 1.12j/kg/24h, which is an average insulin dose in T1D in the period of puberty at our Centre.

The metabolic control has not been satisfactory, the average annual HbA1c level being between 7.1%–8.3%. We have not observed any episodes of severe hypoglycemia or diabetic ketoacidosis. To date, he has had no physical or laboratory signs of diabetic nephropathy, retinopathy, dyslipidemia or hypertension.

Due to frequent infections (even more than ten annually), the boy underwent immunologic evaluation of immunoglobulin levels, which revealed immunoglobulin A deficiency. Further studies disclosed both IgA1 and IgA2 subclass deficiency (Table I). Despite the symptoms of allergic rhinitis, the patient has a very low total IgE and the results of skin prick tests (SPT) as well as of the specific IgE are both negative.

In the fifth year of the treatment, routine screening tests (performed every two years in every patient with autoimmune diabetes according to ISPAD guidelines) revealed an increased level of serum anti-microsomal antibodies (anti-TPO). Despite this finding, his thyroid stimulating hormone (TSH) and free thyroxin (fT4) levels are still normal. The patient's thyroid gland parenchyma shows no signs typical for Hashimoto's thyroiditis on ultrasonographic evaluation. No serological markers of celiac disease have been detected so far.

After a few years of treatment with the insulin pump, a local depression on the skin surface at the sites of insulin pump catheter insertion was observed consistent with lipoatrophy requiring a change of insulin preparation (from aspart to lispro) with no need to discontinue the insulin-pump therapy.

The patient's development has been normal. At present, his height is 173 cm (55 pc), body weight-61 kg (58 pc), BMI-20.4 kg/m<sup>2</sup> (59 percentile), IV stadium of puberty in the Tanner's scale. Apart from vitiligo and focal lipoatrophy, his physical examination is not remarkable. To date, he has no other symptoms of alkaptonuria other than urine darkening.

## Genetic evaluation

Since the patient has at least two genetically determined clinical conditions, we decided to perform his comprehensive genetic evaluation using next generation sequencing technology. DNA was extracted from the patient's blood samples using the QIAamp DNA Blood Mini Kit (Qiagen, Germany). DNA samples were normalized to a final 5 ng/ul. The Trusight One sequencing kit (Illumina, San Diego, CA), which targets 4,813 genes associated with known clinical phenotypes, was used to perform enrichment and final genetic analysis, including HGD gene. Each procedure was carried out according to the manufacturer's instructions. Compound heterozygous mutations, the c.481G>A (p.Gly161Arg) and c.184\_187dupTATA (p.Arg63IlefsTer2), affecting both alleles in HGD gene were found, being an evidence for genetic background of alkaptonuria. Moreover, several genetic variants in genes associated with the immune system were also identified, but none of them was clearly associated with the autoimmune features observed in the patient. This may suggest that both conditions, alkaptonuria and the immune related disorders are independently inherited and no shared genetic variant is responsible for their coexistence.

## Discussion

Alkaptonuria (AKU, OMIM 203500) is a rare autosomal recessive metabolic disorder caused by a deficiency of homogentisate 1,2-dioxygenase (HGD). It is mainly characterized by homogentisic aciduria (urine that turns dark brown on standing and alkalization), bluish-black pigmentation of ear cartilage and other collagenous tissues (ochronosis), physically disabling joint and spine arthritis, and destruction of the cardiac valves [4]. Those overt ochronotic manifestations of the disease are delayed, typically beginning over 30 years of age. The reason why the appearance of ochronosis is delayed, besides excessive urinary HGA excretion, remains a mystery and the natural history of the disease is not completely understood.

The disorder affects 1 in 250,000 to 1 million people worldwide. It was found to be unusually frequent in the Dominican Republic [5] and in Slovakia [6]. Until 1980, 39 cases of the disorder have been registered in Poland [7].

The defect is caused by homozygous or compound heterozygous mutations in the homogentisate 1,2-dioxygenase gene (HGD), which maps to the human chromosome 3q21–q23. The majority of these mutations, which consist mostly of missense mutations, nonsense mutations, splice sites and small insertions/deletions tend to aggregate particularly in exons 6, 8, 10 and 13 [8].

Currently, there is no effective treatment for this condition. Patients are advised dietary protein restriction in order to reduce the production of homogentisic acid. On the other hand, ascorbic acid was reported to block the bounding of the homogentisic acid to the connective

tissue [9,10]. Just recently, it has been shown that nitisinone (2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione could significantly reduce urinary homogentisic acid, an approved therapy for tyrosinemia type I, by inhibiting 4-hydroxyphenylpyruvate dioxidase, the enzyme that produces homogentisic acid [11].

Our patient is in the pre-ochronotic phase of alkaptonuria, which is treated only with low protein diet and ascorbic acid. Except for urine discoloration, he has had no other symptoms of alkaptonuria so far. On the other hand, it seems that alkaptonuria has no specific negative effect on the course of his diabetes in this phase. Searching the literature, we were able to find only one report on the coexistence of insulin dependent diabetes and alkaptonuria. It concerned a 46-year-old man suffering from diabetes for 23 years, whose ochronosis progressed much more rapidly than in his two alkaptonuric siblings, due to diabetic renal failure and excessive accumulation of homogentisic acid. Interestingly, the symptoms became less severe after the patient had received a renal transplant [12]. It is important to mention, that while having the patient with alkaptonuria and diabetes, it should be remembered that alkaptonuric urine causes false negative results while detecting glucose with paper strips [12]. It is, then, reasonable to think that, until kidney function remains normal, alkaptonuria will progress as in a non-diabetic patient. On the other hand, one may suspect that the additional metabolic error affecting the kidneys might result in earlier development of diabetic nephropathy or other chronic complications of diabetes.

The genetic evaluation of our patient, using NGS, confirmed the presence of the HGO gene mutation, indicating that the very rare metabolic disorder under discussion and type 1 diabetes occurred independently. Nevertheless, the patient remains a very interesting case, because of the coexistence of several

other autoimmune disorders (autoimmune diabetes, vitiligo, postinsulin lipoatrophy and anti-thyroid autoimmune reaction) with a humoral defect (IgA deficiency). Such a constellation of autoimmune conditions and a positive family history of type 1 diabetes and vitiligo, indicate a possibility of a shared genetic background of these disorders. Recent studies have shown the existence of a monogenic form of autoimmune diabetes and co-morbid diseases resulting from a mutation in a single gene [13,14]. This may include humoral immune defect as well, such as IgA deficiency which has been linked to a number of autoimmune diseases [15]. Individuals with IgA deficiency have type 1 diabetes, vitiligo and autoimmune thyroid disease more frequently [16]. Moreover, the risk of AIT in a person with vitiligo is around 30% [17] and autoimmune thyroid disease is the most prevalent endocrinopathy among diabetic patients [2,18]. Both focal vitiligo vulgaris and lipoatrophy were reported to be precipitated and exacerbated by the subcutaneous infusion of insulin lispro, suggesting the involvement of immunological mechanisms in their pathogenesis [19–22].

Also, familial clustering of type 1 diabetes mellitus and vitiligo seen in three generations of this family implies a common genetic background of autoimmunity. However, its genetic pathogenesis remains to be elucidated.

## Conclusion

We presented an interesting case with independently inherited alkaptonuria and a cluster of autoimmune disorders. Alkaptonuria in a pre-ochronotic phase seems to have no effect on the course of type 1 diabetes. Further studies are needed to confirm a possible common genetic background of T1D, vitiligo, AIT, IgAD and insulin infusion induced lipoatrophy.

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