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CASE REPORT

EARLY-ONSET OCULAR OCHRONOSIS IN A GIRL WITH ALKAPTONURIA (AKU) AND A NOVEL MUTATION IN HOMOGENTISATE 1,2-DIOXYGENASE (*HGD*)

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A b s t r a c t: Alkaptonuria (AKU) is a disorder of phenylalanine/tyrosine metabolism due to a defect in the enzyme homogentisate 1,2-dioxygenase *(HGD)*. This recessive disease is caused by mutations in the *HGD* gene. We report a 14-year-old girl who was referred after presenting black urine. Careful examination revealed ochronosis of the conjunctiva. There was no affection of the cardiac valves. Elevated excretion of homogentisic acid in urine was found. Sequence analysis of the *HGD* gene from genomic DNA revealed that the patient is a compound heterozygote with a previously described mutation (c.473C > T, p.Pro158Leu), and a novel one (c.821C > T, p.Pro274Leu). Her mother is heterozygous for the novel mutation, while the brother is heterozygous for the previously described mutation. In summary, we describe an alkaptonuric patient with ocular ochronosis and a novel *HGD* mutation, c.821C > T, p.Pro274Leu.

Key words: Alkaptonuria, novel mutation, ocular ochronosis.

Introduction

AKU is caused by deficiency of homogentisate 1,2-dioxygenase *(HGD)*. *HGD* converts homogentisic acid (HGA) to maleylacetoacetic acid in the tyrosine degradation pathway [1]. Darkening of the urine upon standing is a striking sign of the disease. The phenomenon is caused by the oxidation of the HGA

excreted in the urine. The bluish-black pigmentation in connective tissue (ochronosis) usually occurs after the age of 30 years [2]. Clinically important arthritis begins in the third decade and after several decades can be disabling [3]. Rare phenomena are aortic or mitral valve calcification or regurgitation [4], renal and prostate stones. AKU patients excrete a significant amount of HGA in the urine.

Ocular ochronosis is rarely seen in children [5, 6]. Inpeed, the ocular ochronosis and dark urine prompted the diagnosis of AKU in a 14year-old Macedonian girl.

Case report

The patient's parents reported staining of urine from the age of 5 months with the urine actually darkening to blackness on standing. The proband is the second child in a family of young and healty parents. Her mental and physical development is normal. She is a nice-looking, excellent stdent in high-school. No degenerative joint disease was noted until the age of 14 years. On ophthalmological examination, the patient revealed bilateral grey brown of the temporal sclera. Discoloration of the ears and an irregular thickening of auricular cartilage were not observed.

Elevated excretion of homogentisic acid in urine was found in the proband, but not in the other members of the family. *HGD* gene sequencing from genomic DNA revealed that the proband is a compound heterozygote with a previously described mutation p.Pro158Leu (c.473C > T) in exon 8), and a novel one p.Pro274Leu (c.821C > T) affecting exon 11 (Fig. 1). As can be seen in Fig. 1, her mother is heterozygous for the same novel mutation and her brother is heterozygous for the mutation in exon 8. The father's DNA was not available for analysis. Both mutations have also been submitted to the novel *HGD* mutation database under the family code AKU_DB_116.

Discussion

This patient is the first diagnosed and published AKU in Macedonia. So far, about 250 cases of alkaptonuria have been reported in whom *HGD* mutations have also been identified (*HGD* mutation database; http://hgddatabase.cvtisr.sk/, [1, 7]. Worldwide, AKU has a low prevalence of 1 : 250 000 (USA 1 : 250,000 – 1 : 1,000,000 live births). The notable exeptions are Slovakia (1 : 19 000) [8, 9, 10] and the Dominican Republic [11, 12]. It is of historical interest that AKU is the first human disease to be understood as a recessive trait [13].

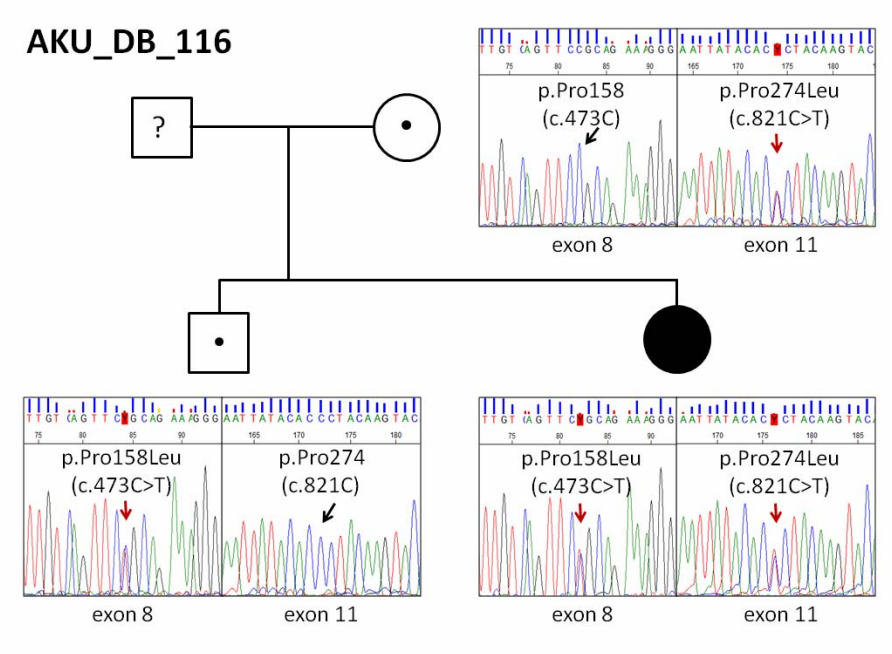

Fig. 1 – Schematic representation of the pedigree and the mutations identified. The proband is a compound heterozygote with a previously described mutation p.Pro158Leu (c.473C > T)(in exon 8) (black arrow), and a novel one p.Pro274Leu (c.821C > T) affecting exon 11 (red arrow). The mother is heterozygous for the same novel mutation in exon 11 and her brother is heterozygous for the mutation in exon 8

Dark urine or urine that turns dark on standing or exposure to an alkaline agent, ochronosis **(**bluish-black pigmentation of connective tissue: accumulation of HGA and its oxidation products), and arthritis are AKU's main characteristics.

Arthritis often begins in the spine and resembles ankylosing spondylitis in its large-joint distribution [3]. Fifty percent of individuals require at least one joint replacement by the age of 55 years [2, 14].

The ear cartilage can be slate blue or gray and thickened. Cerumen in perspiration can also be coloured and can cause discoloration of clothing.

Ocular ochronosis is a brown pigmentation of the sclera between the cornea and the outer and inner canthi. It may affect the conjunctiva and cornea, too [5]. Furthermore, ocular ochronosis usually precedes the appearance of articular symptoms [6]. It is of note that the differential diagnosis of blue black

scleral pigmentation includes malignant melanoma of the choroid and that this diagnosis can be missed [15].

Urolithiasis has been described in adults and in children with AKU e.g. [2, 16, 17]. Intellectual capacity and life expectancy are not reduced in AKU.

The diagnosis of alkaptonuria is based on the detection of a significant amount of HGA in the urine by gas chromatography-mass spectrometry analysis [1, 2]. However, biochemical testing cannot detect the heterozygotes.

The majority of the ~ 91 *HGD* mutations reported so far are missense [16]. Nonsense, frame shift, and splice-site mutations have been described. Some populations show a founder effect for the frequent mutations, such as p.Gly161Arg mutation in Slovakia [9] or p.Cys120Trp in the Dominican Republic [12]. The most prevalent mutation in Europe is p.Met368Val, while in the US, no mutational hot spot or founder effect has been identified [2]. So far no correlation has been observed between the type of *HGD* mutation and amount of HGA excreted or severity of the disease. More detailed and extended studies in larger groups of patients are necessary to follow possible genotype- phenotype correlations.

Mutation p.Pro158Leu, found in our patient, was previusly reported in a patient from the USA who carried mutation p. Ser59AlafsX52 (c174_175delA) in exon 3 on his second allele [16]. Intrestingly, mutation p.Pro158Leu affects nucleotide c.473C that has already also been found mutated into G in another case from USA, causing amminoacid change p.Pro158Arg [2].

Novel mutation p.Pro274Leu affects nucleotide c.821C that is highly conserved in *Homo sapiens, Mus musculus, Rattus norvegicus, Danio rerio, Drosophila melanogaster, Arabinopsis thaliana* and *Aspergillus nidulans* (ClustalW2, data not shown). In addition, PolyPhen-2 (Polymorphism Phenotyping v2) (21) and SNAP (Screening for non-acceptable polymorphisms) [22] programmes were used to predict an impact of this amino acid substitution on the structure and function of a human *HGD* protein (NP_000178.2). Both programmes predicted a probably damaging effect, further confirming the pathogenity of this novel *HGD* mutation.

No correlation is observed between the type of *HGD* mutation and amount of HGA excreted or severity of the disease.

So far, no effective preventive or curative treatment for AKU is available. Nitisinone is not approved in the treatment of AKU.[19] Severe restriction of phenylalanine and tyrosine may be dangerous. No studies have demonstrated the clinical efficacy of ascorbic acid [1]. A prospective study with oral bisphosphonate therapy did not demonstrate benefit [20].

This is the first Macedonian patient with AKU. A novel mutation has been described as well as an unusualy early ocular ochronosis.

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Резиме

РАН ПОЧЕТОК НА ОКУЛАРНА ОХРОНОЗА КАЈ ДЕВОЈЧЕ СО АЛКАПТОНУРИЈА (АКУ) И НОВА МУТАЦИЈА НА ХОМОГЕНТИЗАТ 1,2-ДИОКСИГЕНАЗА (ХГД)

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Алкаптонуријата е нарушување на метаболизмот на фенилаланин/тирозин како резултат на дефект во ензимот хомогентизат 1,2-диоксигеназа *(ХГД)*. Ова рецесивно нарушување е предизвикано од мутации во *ХГД* генот. Ние прикажуваме 14-годишно девојче примено заради појава на црна урина. Деталните иследувања утврдија постоење на конјуктивална охроноза, но без афекција на срцевите залистоци. Беше најдено зголемена екскреција на хомогентизинска киселина во урината. Секвенционата анализа на *ХГД* генот покажа дека пациентот е хетерозигот со претходно опишана мутација (c.473C > T, p.Pro158Leu) и една нова мутација (c.821C > T, p.Pro274Leu). Мајката и братот се исто така хетерозиготи за новата мутација. ДНК од

таткото не беше достапна за анализа. Како заклучок, опишуваме пациент со алкаптонурија со очна охроноза и нова *ХГД* мутација c.821C > T, p.Pro274Leu.

Клучни зборови: алкаптонурија, нова мутација, окуларна охроноза.

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