A rare mutation in alkaptonuria patient

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Received - 31 January 2020 Initial Review - 13 February 2020 Accepted - 21 March 2020

ABSTRACT

Alkaptonuria is a rare autosomal recessive metabolic disorder due to mutation in enzyme homogentisate 1,2-dioxygenase resulting in accumulation of homogentisic acid. The homogentisate 1,2-dioxygenase (HGD) gene has been mapped to chromosome 3q21- q23 and comprises 14 exons. A wide variety of causative mutations has been reported. Here, we are presenting a case report of a 2-year-old male child with a history of dark black-brown spots of urine on diaper with c.674G>C (p.Arg225Pro) mutation at exon 10 of HGD gene. The observed variant had a minor allele frequency of 0.0200% and 0.0004% in 1000 genomes and gnomAD database, respectively.

Key words: *Alkaptonuria, Homogentisate 1,2-dioxygenase gene, 2-dioxygenase, Homogentisic Acid*

Alkaptonuria (AKU) is a rare autosomal recessive metabolic disorder of phenylalanine-tyrosine metabolism characterized by the accumulation of homogentisic acid (HGA) in the body and extracellular fluids. The incidence ranges from 1 in 250,000 to 1,000,000 live births. Due to mutation, an affected individual lacks a functional level of enzyme homogentisate 1,2-dioxygenase (HGD). The HGD enzyme catalyzes the conversion of HGA into maleylacetoacetic acid (Fig. 1). Inability to metabolize the acid results in its increased blood levels, which is rapidly cleared by kidney. On contact with air, HGA in urine turns dark black-brown in color. Aside from dark urine, affected individuals generally remain asymptomatic during infancy or childhood and are unaware of disease until adulthood.

Mutations in the human HGD gene have been reported in a number of countries throughout the world [1,2]. Slovakia and the Dominican Republic have reported a high incidence of HGD gene [3]. However, the reports about HGD mutation in Asian population are rare. Here, we report novel mutation at exon 10 of HGD gene. The observed variant had a minor allele frequency of 0.0200% and 0.0004% in 1000 genomes and gnomAD database, respectively.

CASE REPORT

A 2-year-old male child born to non-consanguineous parents presented in our outpatient department with the chief complaint of dark black-brown spots of urine on diapers and urine that turned dark black-brown when exposed to air on standing (Fig. 2) since the age of 6 months. The patient did not present with any other complaint. The patient did not have a history of the previous hospitalization. There was no relevant family history of similar complaints. The patient did not have any dysmorphic facies, was normal in growth (weight – 11 kg, height – 86 cm, and head circumference – 48 cm), and developmental milestones were appropriate for age. Overall patient's general physical and systemic examination appeared normal.

The laboratory investigations (complete blood count, liver function tests, and RFT) were normal. Urine analysis did not show any pathology. Electrocardiogram, echocardiography, and ophthalmological examination were normal.

The diagnosis was established by molecular genetic testing, showing homozygous mutation for c.674G>C (p.Arg225Pro) at exon 10 of HGD gene that led to amino acid substitution from arginine-to-proline at codon 225. The observed variant had a minor allele frequency of 0.0200% and 0.0004% in 1000 genomes and gnomAD database, respectively. The patient was prescribed Vitamin C tablets, 1 g/day life long and is under regular monthly follow-up. No changes in clinical and laboratory features on follow-up were noticed.

DISCUSSION

AKU generally remains asymptomatic during infancy and symptoms appear in adulthood. Characteristic presentation in the pediatric age group is darkening of urine on standing. This is because the HGA polymerizes can also be observed on the addition of alkali substances [4]. Overtime, the HGA polymer is deposited within connective tissues, especially cartilages causing ochronosis [5]. Long-term ochronosis results in the development

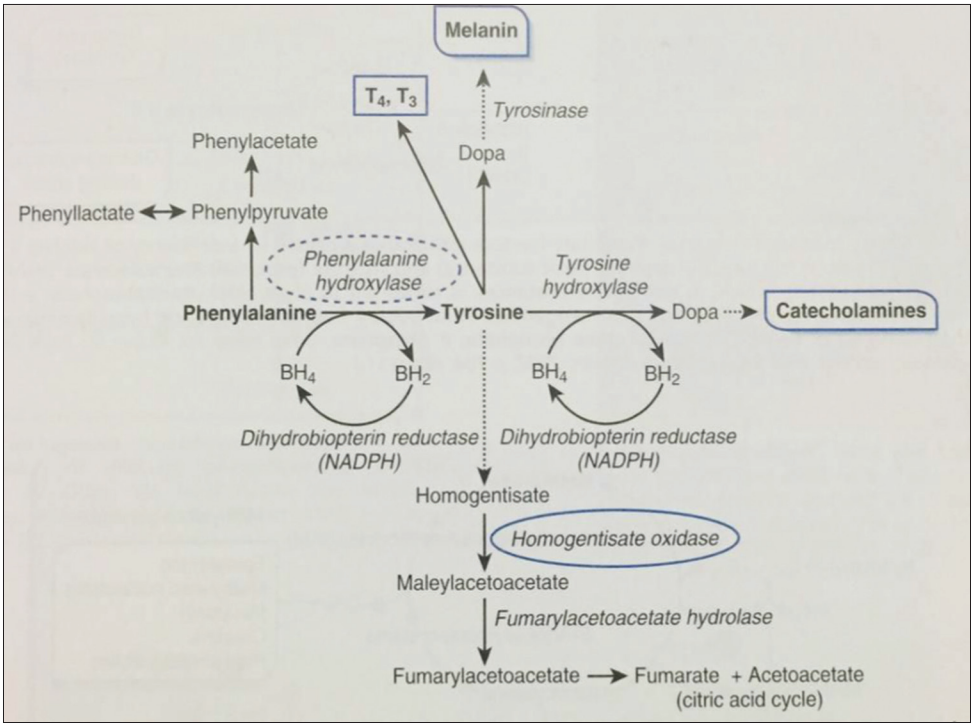

Figure 1: Alkaptonuria biochemical pathway

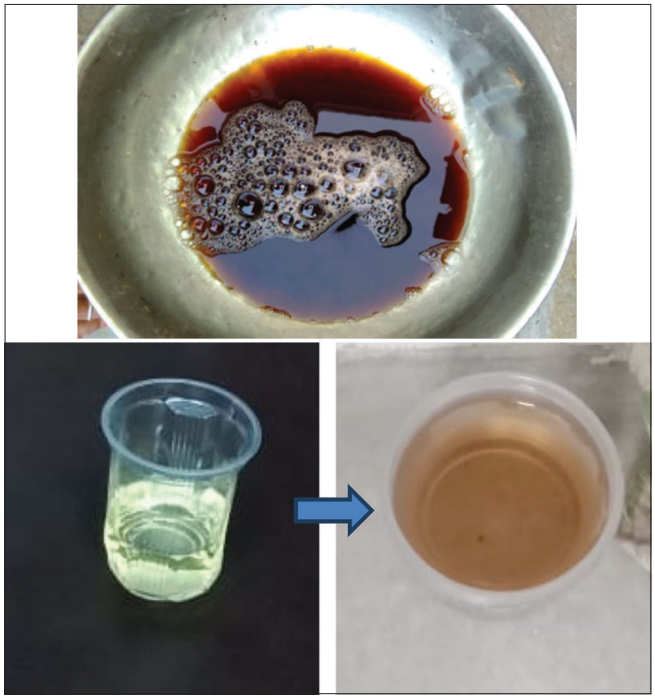

Figure 2: Urine that turned dark black-brown when exposed to air on standing

of ochronotic osteoarthropathy, especially in the spine and large joints (hip, knee, and shoulder joints) [6,7]. Arthritis can be severe and disabling with common symptoms being lower back pain, stiffness, ankylosis, kyphosis, decreased range of motion, and joint effusion.

Connective tissues, especially cartilages, turn blue-black. HGA accumulates in ligaments and tendons overtime and affected tissue becomes discolored, brittle, and weak. Some affected individuals can present with heart diseases due to the deposition of HGA on aortic and mitral valve. This accumulation can cause thickening and stenosis of valves. Aortic valve stenosis is a frequent associated finding requiring surgical replacement [8]. Although HGD expression and metabolism occur in the liver, there are no conclusive reports to demonstrate that the tissues of the liver, pancreas, gastrointestinal, lymphoreticular, or endocrine systems develop ochronosis [9].

The HGD gene has been mapped to chromosome 3q21- q23 and comprises 14 exons. The functional HGD protein is a hexamer, organized as a dimer of trimers. The HGD mutation database lists 620 variants, out of which almost 80% are missense substitutions. The remaining is deletions, duplications, insertions, or unknown. As per the HGD mutation database, the mutations are not evenly distributed, and the largest number of variants is found in exon 3, 6, 8, 10, and 13. Our patient was homozygous for mutation of HGD gene for transcript NM_000187.3 at exon 10 location with c.674G>C (p.Arg225Pro) variant due to amino acid substitution from arginine-to-proline at codon 225. The observed variant had a minor allele frequency of 0.0200% and 0.0004% in 1000 genomes and gnomAD database, respectively.

The treatment of AKU is aimed at specific symptoms arising at different stages of life in each individual. Medical therapy is used to ameliorate the rate of pigment deposition. Dietary restriction of phenylalanine and tyrosine reportedly reduces HGA excretion in the urine. Vitamin C, as much as 1 g/day, is recommended for children. The mild antioxidant nature of ascorbic acid helps to retard the process of conversion of homogentisate to the polymeric material and reduces the rate of deposition on tissues [10,11]. The limited use of nitisinone, an inhibitor of the enzyme 4-hydroxyphenylpyruvate dioxygenase, which mediates the formation of HGA, has been reported.

CONCLUSION

We report a novel mutation in AKU patient which is rare in Asian population. As the parents are carriers, prenatal testing or pre-implantation genetic diagnosis should be advised for subsequent pregnancies.

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Funding: None; Conflicts of Interest: None Stated.

How to cite this article: Agarwal A, Goyal K, Mathur P, Minocha P, Bansal K, Mathur N. A rare mutation in alkaptonuria patient. Indian J Child Health. 2020; 7(3):137-139.

Doi: 10.32677/IJCH.2020.v07.i03.011