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Aghayeva S.A.
Institute of Genetic Resources, Azerbaijan National Academy of Sciences
Huseynova L.S.
Department of Medical biology and genetics, Azerbaijan Medical University, Baku, Azerbaijan, lalahs1979@gmail.com
Kichibekov B.R.
Ministry of Health of Azerbaijan, Central Neftchilar Hospital.
Aliyeva K.A., Khalilov R.I.
Faculty of Biology, Baku State University, Baku, Azerbaijan

INHERITED METABOLIC DISEASE PHENYLKETONURIA AND DEFICIENCY OF G6PD ENZYME IN A FAMILY STUDY

Abstract. *The family with identified inherited metabolic disease of phenylketonuria lives in Masally administrative area. Masally area itself is located in South-East of Azerbaijan Republic on the slopes of Talysh mountains in subtropical zone. Members of proband's family possess deficiency of glucose-6-phosphatedehydrogenase. Phenylketonuria gene has an identified R261G (G-A) mutation. The study of erythrocyte enzyme preparation for family members have shown low electophoretic mobility for G6PD which was unknown in the world studies. Based on the Michaelis-Menten constant (K_m) applied to G6P, substrate values have manifested high biochemical polymorphism.*
Key words*: phenylketonuria, polymerase chain reaction, glucose-6-phospatedehydrogenase enzyme, biochemical polymorphism, enzyme preparation, abnormal variant, mutation.*

Introduction. Introductıon. In 1985 the gene phenylalanine-4-hydroxilase (PAH) responsible for phenylketonuria disease was identified. The gene is located on the long shoulder of chromosome 12 in q22-24.1 site. The lendth is 90 thousand nb and consists of 13 exons. Synthesized protein consists of 451 amino acid residues. Phenylalanine amino acid coming with food in oxidation process turns into different amino acide -thyrosine as a result of phenylalanine hydroxidation process. In the result of mutation in PAH gene this phenylalanine into thyrosine transformation fails. Up to 1 % cases of phenylketonuria are presented with atypical forms. The disease is inherited as to autosome-recessive type [4,10].

The prevalence rate differs in different population groups. For example, in Europeoid inhabitants in the USA it is 1 to 10000. The highest rate is in Turkey, which is 1 to 2600. In Finland and Japan the rate of phenylketonuria is extremely low: even less 1 newborn to 100000 births. In Slovakia in some gypsy populations there were found ultrahigh rates of phenylketonuria because of inbreeding: 1 case for 40 newborns [9].

According to the World Health Organisation data, there are around 100 million people suffering from glucose-6-phosphatedehydrogenase (G6PD) enzyme activity deficiency. More than 400 abnormal variants were identified, and around ¼ of them are endemically different [2,3].

One part of those abnormal G6PD variants could be characteristic for only one certain ethnic group, and another part - for several ethnic groups [5-8]. A group of people with enzyme deficiency resulted with hemolytic crisis after some specific medicines, and other people - just after eating food cooked with beans (favism) [9].

Thus, the goal of our studies was to identify gene mutations in people with PKU diagnosis and to study the physic-chemical specificities of abnormal G6PD enzyme in the proband's family members.

Materıal and methods. Venous blood samples with heparine anticoagulant were used as the study subjects. Blood was sampled from G.M. (proband) family members, who are inhabitants of Tekle village of Masally area, Azerbaijan Republic.

PKU diagnostics was carried out by means of IFA method. In identification of PKU gene mutations, complex of molecular-genetic methods were used [1].

Genomic DNA was isolated from venous blood, using readymade kits by QIAGEN (Germany) company. Intactness and quantity of isolated

genomic DNA were identified by means of electrophoresis in 1.7% agarose gel, as well as gene fragments after polymerase chain reaction (PCR). Electrophoretic apparatus and power source were BioRad (USA) manufactured. Marker for identification of synthesized DNA fragments was DNA Ladder 100 bp .

The content of PCR: 0,1-1,0 µg of genomic DNA, 0,25 µM of each dNTP, 25 µl buffer (67 mM Tris-HCL, pH 8,8: 16,6 M $(NH_4)_2SO_4$, 0,01% Twin-20, 1,5 unit DNA-polymerase. 2 µg of primers for each of exons 3,5,7,11 and 12.

Regime of PCR for PKU gene was as follows: 95°C-2 minutes, (94°C-45^{I}, 58°C-45^{I}, 72°C-45^{i} 30 cycles), 72°C-7 minutes and pause at 4°C for 10 minutes. PCR was conducted in amplifier – Professional Thermocycler, Biometra, (Germany).

Purification of DNA fragments after the first PCR stage a set of magnets was used: «AgencourtAMPure XP PCR purification» and SPRIPlate 96 Super Magnet Plate. After that purified DNA fragments were used for the further researches. The second PCR was conducted in the regime: 95°C-2 minutes, (95°C-30^{I}, 52°C-58°C - 30^{I}, 78°C-2 minutes 30 cycles), 72°C-10 minutes and pause on the amplifier at 4°C for 10 minutes. Then the standard procedure on the apparatus GENOMELabGeXP^{TM} Sequencing for the identification of nucleotide sequence of each DNA fragment was carried out [1].

G6PD enzyme activity was measured by means of modified fluorescent method [2,3].

Purification of enzyme prerarations and study of the characteristics were carried out according to the WHO standardized methods [10].

Results . Total nucleotide sequencing was done only for exon 7 as an example, where R261G mutation was identified. Being a point mutation we have found a substitution of guanine with adenine. The result of mutation was on protein level, and arginine amino acid was substituted with gluthamine amino acid.

Homozygous form was identified in 4-year-old girl (proband – III-6). Heterozygous form carriers were both parents (II-1, II-2) and one sibling (III-2). So, family members manifested one homozygous and three heterozygous forms of R261G mutation.

It's worthwhile noting, that proband's parents are children of two sisters. Marriage is identified as a 3rd cosanguineous parallel marriage type. G.M. family tree is presented in the Figure 1.

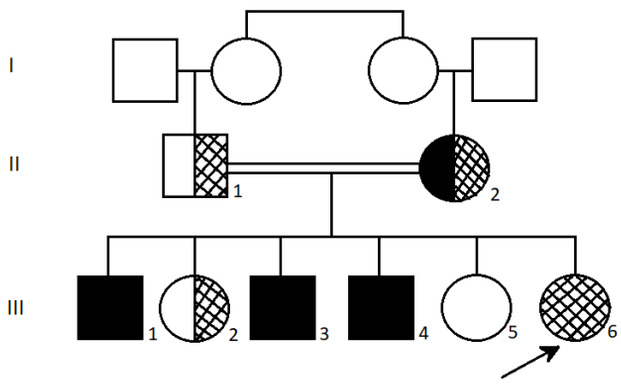

Figure 1. Family tree of G.M. family

Father (II-1) – heterozygous carrier of R261G mutation, mother (II-2) – heterozygous carrier of R261G mutation and G6PD enzyme deficiency, proband (III-6) – homozygote of R261G mutation, siblings (III-1), (III-3) and (III-4) – hemizygotes of G6PD enzyme deficiency, sibling (III-2) – heterozygous carrier of R261G mutation, sibling (III-5) – healthy.

Table 1 presents physic-chemical characteristics of G.M. family boy kids blood enzyme preparations where G6PD enzyme deficiency were identified.

G6PD enzyme deficiency was as low as 5.2-12.5% of the normal activity in three brothers in the family (Fig.1). According to the obtained activity it was relates to the II activity class. All three enzyme preparations showed low enzyme (0-10%) activity. Each of the three kids had erythrocyte hemolysis and anaemia after eating food with beans (favism).

All three enzyme preparations have the indication of pH-optimum in the normal range (pH 7,5-8,5). All enzyme preparations under research have shown low electrophoretic movbility.

Table 1

New mutation form of G6PD deficiency in Massally area.

Variant name	G6PD Activity %	EP-mobility	Km G6F µmol	2dG6P disposal	pH optimum	Thermostability	Clinical manifestation
Tekle	5,2-7,0	92-95	146,7	70,0	8,0-9,0	Extremely low	No

Based on the G6P substrate, constant of Michaelis-Menten (K_m) indication of all the enzyme preparations was high (146,7µm). For analogue of 2dG6P substrate was high disposal degree identified.

Enzyme preparations, obtained from the inhabitants of Masally area Tekle village, have manifested such physic-chemical characteristics of G6PD deficiency which was new and had no analogue in the world scientific literature.

Thus, a new biochemical variant was identified on the basis of physic-chemical indications of G6PD enzyme, and PKU inherited metabolic disease with R261G (G-A) mutation were found in the family G.M., who live in Tekle village of Masally area.

Dıscussıon. The world scientific literature researches show that European populations have mainly R408W, P281L, R261Q, R158Q, R252W, I65T, IVS10nt546, IVS12ntl. PAH gene mutations prevail over the others. These mutations are located in 3,5,7,11 and 12 exons of the gene [4,10]. With this purpose we have done amplification of PAH gene exons 3,5,7,11 and 12 genomic DNA fragments, got from lymphocytes of the G.M. family members: two parents and six their children, by means of polymerase chain reaction with 5 primer groups. Fragments of exon 3 of 112 nb, exon 5 of 162 nb, exon 7 of 218 nb, exon 11 of 222 nb and exon 12 of 177 nb were amplified.

G6PD enzyme deficiency was as low as 5.2-12.5% of the normal activity in three brothers in the family. All three enzyme preparations showed low enzyme (0-10%) activity and all three enzyme preparations have the indication of pH-optimum in the normal range (pH 7,5-8,5). All enzyme preparations under research have shown low electrophoretic movbility. According to the obtained activity it was relates to the II activity class. Each of the three kids had erythrocyte hemolysis and anaemia after eating food with beans.

Conclusıons. Phenylketonuria and G6PD enzyme deficiency were identified in one family in Tekle village of Masally area of Azerbaijan Republic.

1. Heterozygous and homozygous genetic types of phenylalanine-4-hydroxilase gene mutation R261G (G-A) were identified.
2. A new unknown to scientific literature biochemical polymorphism of G6PD enzyme was identified.

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