Khazar Journal of Science and Technology Volume 4 №1 2020, 18-24
© Khazar University Press 2020 DOI: 10.5782/2520-6133.2020.4.1.18

Molecular Analysis of Glucose-6-Phosphate Dehydrogenase Gene Mutations in Azerbaijan Republic

Aghayeva Saltanat[1*], Mammadov Ayaz[1,4], Rolfs Arndt[2], Skrahina Volha[2], Rasulov Elkhan[3], Guliyeva Elvira[1]

[1]Genetic Resources Institute of ANAS; [2]Centogene AG, Rostock, Germany;
[3]Azerbaijan State Advanced Training Institute for Doctors named after A.Aliyev;
[4]Western Caspian University
[*]Correspondin author: saltanat.genetic@gmail.com

Abstract

Genetic screening of school children in Masalli region in Azerbaijan Republic identified 23 school children with G6PD enzyme activity different deficiency (from 0 up to 60% activity). Biochemical studies were done/performed for school children with activity efficiency on enzyme preparations from erythrocytes. As to WHO Guidelines, enzymatic preparations were related to the following classes: 2nd class – 13 boys, 3rd class – 6 school boys, 4th class – 4 of them. DNA molecular analysis, isolated from blood of the index patient, was classified as the 2nd class of G6PD enzyme deficiency and has shown the substitution of Guanine nucleotide with Adenine in position 1178. As a result of the mutation in protein in the position 393, substitution of amino acids Arginine with Histidine [G6PD,1178 (G-A) Arg393His] takes place.

Keywords: G6PD, biochemical polymorphism, enzyme preparation, abnormal variant, gene, molecular-genetic analysis, mutation, nucleotide, amino acid.

Introduction

Glucose-6-phosphate dehydrogenase (G6PD: EC 1.1.1.49) is defined with gene high polymorphism. Enzyme has been identified more than 400 biochemical variants and one fourth of them differ with endemicity. One part of the G6PD enzyme abnormal variants is endemic for the certain ethnic group, and another part is specific to another ethnic group. One group of people with enzymatic deficiency gets hemolytic crisis from some medicines and another group of people gets from beans (favism) with food (Fuji *et al.,* 1984; Xu *et al.,* 1999; Zuo *et al.,* 1999).

Biochemical variants are mainly asymptomatic clinically. Big portion of biochemical variants results in hemolytic anemias under the influence of chemicals. And small portion of variants leads to severe chronic non-sphericytic anemia (Huseynova *et al.,* 2019; Aghayeva *et al.,* 2018; Beutler, 1998).

The G6PD enzyme gene is located on the X chromosome and is transferred from heterozygous mother to a son. Since one of the two X-sex chromosomes is inactivated in women, the difference is observed in the clinic of heterozygotes. In case non-affected enzyme gene becomes inactive, the affected gene mainly appears in erythrocytes and the clinic manifestations are the same as in male hemizygote patients (Aghayeva *et al.,* 2019; Boytler, 1981; Hirono *et al.,* 1995; Zuo *et al.,* 1999).

According to the data of World Health Organization (1997), around 100 million of the world population manifests G6PD enzyme deficiency (WHO, 1997; Du *et al.,* 1988).

For Azerbaijan Republic population, study of G6PD enzyme deficiency started in the 70s of the last century. Studies were satisfied with only enzymatic activity studies (Aghayeva *et al.,* 2019; Boytler, 1981).

The last century 70s' studies undertaken for Masalli area population revealed G6PD enzyme activity deficiency high frequencies (20-23%). Thus, Masalli area was not a random choice when starting our studies (Beutler, 1994; Krasnopolskaya & Shatskaya, 1987).

The goal of our studies was to research on G6PD gene molecular genetics and G6PD enzyme physic-chemical characteristics for the index patient from pupils living in Masalli area chosen from people with abnormal G6PD activities.

Material and Methods

Material was collected during screening from pupils from Arabkendi, Gullutepe, Tekle, Chakhirli, Bedalan towns of Masalli area and pupils of 7-11 grades from Masalli downtown. As a result of screening of 276 pupils, we found 23 boys with inherited hemizygous type of G6PD enzyme.

Material collected for biochemical studies was venous blood samples in tubes with EDTA anticoagulant (Huseynova *et al.,* 2019; Aghayeva *et al.,* 2018; Beutler, 1998).

G6PD enzyme activity was identified with modified fluorescence method. To make the analysis accurate and to identify the inheritance type, we got their parents and family members involved into the study. Totally, there were 302 blood samples processed (Aghayeva *et al.,* 2019; Beutler, 1998).

Purification of enzyme preparations and their classification were done according to the WHO standardized methods (WHO, 1988; Krasnopolskaya *et al.,* 1977; Krasnopolskaya *et al.,* 1985).

Results and Discussion

As a result of 23 pupils' screening on G6PD enzyme, different proportion of enzyme deficiency (activity 0-60%) was identified. According to the data of World Health Organization (WHO) in 1967, G6PD enzyme activity deficiency (based on the lack) was divided into 5 classes: the 1st class – chronic non-spherocytic anemia; the 2nd class – acute deficiency of the enzyme (lower than 10%); the 3rd class – enzyme medium deficiency (activity 10-60%); the 4th class – very mild deficiency of the enzyme (60%) and the 5th class – lower range of normal enzymatic activity.

Our studies have revealed enzyme activity which complies with the WHO 2nd, 3rd and 4th classes: 13 persons in the 2nd class, 6 boys in the 3rd and 4 pupils in the 4th classes.

Results of the genetic screening of G6PD enzyme carried in Masalli area are presented in Table 1. 276 male pupils and 24 their family members were checked up in five towns. G6PD enzyme phenotypic frequencies and gene frequencies were presented. High results were provided for Masalli town school (11.11% and 0.1111 in d.f.). The lower results were for Arabkendiand Terle town pupils (5.56% and 0.0555 in d.f.). For the total area enzymatic deficiency frequency was 8,33%, and gene frequency was equal to 0.0833.

G6PD enzyme deficit in Masalli regional center was classified as 2, 3 and 4 classes, whereas in Gullutepe and Bedalan towns, only the 2nd class was presented, in Arabkendi and Chakhirli - the 3rd class, and in Tekle both the 2nd and 3rd classes were found. In Bedalan town, hemizygote inheritance type for the enzyme was identified in F.N. index patient's 24 family members, and 6 different male persons were found with hemizygote inheritance type.

Table 1. Results of the G6PD enzyme genetic screening of school pupils in Masalli area

Place	Amount of patients	Affected patients	Phenotypic frequency (%)	Gene frequency (in decimal fraction)	Enzyme deficiency based classes
Regional center Masalli	72	8	11,11	0.1111	2 pupils – class 2 2 pupils-7 class 3 4 pupils - class 4
Gullutepe village	38	4	10.53	0.1053	3 pupils - class 2 1 pupil - class 2
Arabkendi village	42	3	5.56	0.0555	3 pupils - class 3
Tekle village	54	3	5.56	0.0555	2 pupils - class 2 1 pupil - class 3
Chakhirli village	30	2	7.14	0.0714	2 pupils - class 3
Bedalan village	40	3	7.50	0.0750	3 pupils - class 2
F.N.(index patient) for family members	24	6	25.0	0.2500	6 persons – class 2

Complying to the 2nd class requirements, 24 family members of the school pupil F.N. (index patient) from Bedalan town were screened, and 6 of them showed acute enzyme activity deficit (lower than 10%).

Table 2 presents physic-chemical characteristics of enzyme preparations made from blood samples of F.N. index patient and his six family members with the enzyme deficit in Bedalan town of Masalli area.

Table 2. Mutation variant of G6PD enzyme in school pupils from Bedalan village

Variant name	G6PD activity (%)	EP-mobility	K_m G6F mkmol	2dG6F utilization	pH optimum	Thermostability	Clinic manifestation
Bedalan	6.0-8.0	85-90	21.3-24.5	78.6-80.0	8.0-9.0	Weak low	Mild anemia

In enzyme preparations, the PH-optimum bifase variant was noted within the limits of the PH-optimum indicator norm (pH 7,5-8,5)

Electrophoretic mobility showed norm. All samples manifested lower than norm indications according to Michaelis-Menten constant (K_m) (21.3-24.5 mkm) for G6P

substrates. For 2dG6P substrate analogue, there was high utilization range (78,6-80,0).

So, Bedalan variant is classified as a variant with enzyme low activity (6.0-9.0% from norm), for G6P substrate has low range of K_m binding (24.4 mkm), for 2dG6P substrate analogue has high utilization degree (up to 80% of G6P substrate), and manifests mild anemia.

Figure 1 presents a schematic picture of X-sex chromosome structure and G6PD locus on it (q28).

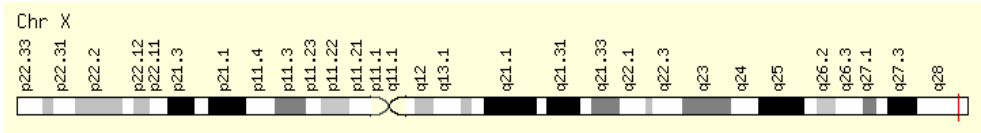

Figure 1. Scheme of X-sex chromosome structure and G6PD locus (q28).

Gene of G6Pdenzyme is located on the long shoulder of X-sex chromosome in subtelomer Xq28 site. The gene of the enzyme Q6FD is located in the subtelomer Xq28 part of the long shoulder of the X-sex chromosome

Firstly, cDNA based on mRNA of G6PD enzyme was synthesized by M. Persico in 1981. In 1986, T.Takizawa created the cDNA library using liver cells cloning. The human q6fd enzyme gene has a size of 13 kbas, consisting of 12 exons and 18 introns. The exons sizes vary from 12 bp up to 236 bp. The intron sizes vary between 97 bp and 11 kb. The Promotor part of the gene plays the role of TATA box prior to 20 nucleotide bases starting from the part of 202 position at ATTAAAT 5^1-terminal. In the promotore part, hexonucleotide sequence GGGCGGG is repeated 3 times, and complementary to that, CCCGCCC is repeated 6 times. These nucleotide sequences are usually located on 12 to 400 nucleotide bases apart from sequence ATTAAAT. GGGCGGG sequence bases are located on a distance of 70kbas from 5^1-terminal of intron 1. Transcription level is equilibrated with CAAT nucleotide sequence and is located at the distance of 220 nucleotide bases at the 5^1-terminal rich in GC nucleotide sequences. ATTG nucleotide sequence is located at the position 411.

In 1991, Chen studied 20114 bp sequences involving G6PD enzyme gene. Amino acid sequences were predicted using the G6PD enzyme gene sequence.

Fusco et al. (2012) observed Alu repeat gene at non-involved 5^1-translation part for 3 times. 12 Alu genes are located in the biggest intron 2. Capellini and Fiorelli

(2008) identified that enzyme G6PD consists of 515 amino acid bases (Aghayeva *et al.*, 2018; Du *et al.*, 1988; Hirono *et al.*, 1995; Xu *et al.*, 1999; Zuo *et al.*, 1999).

In blood of F.N. (index patient), mutation of G6PD enzyme gene was identified as a result of DNA molecular analysis. Guanine nucleotide substitution with Adenine nucleotide was identified in position 1178. Mutation resulted in substitution of Arginine amino acid with Histidine amino acid in position 393.

For the first time, Filosa et al. (1992) found that the substitution of Guanine with Adenine nucleotide happens in position 1178, and this new enzyme mutation was referred as G6PD Portici. The authors related this new G6PD enzyme mutation to the second group according to WHO classification.

Thus, as a result of genetic screening of pupils in Masalli area, 23 male pupils were identified with different G6PD enzyme deficit (in 0-60% range). Complying to WHO requirements, the identified enzyme deficiencies were shared into three classes as to their biochemical characteristics: 13 pupils –to the 2nd class, 6 pupils – to the 3rd class, and 4 pupils – to the 4th class. Complying to the 2nd class requirements, 24 family members of the school pupil F.N. (index patient) from Bedalan town were screened, and 6 of them showed acute enzyme activity deficit (lower than 10%). DNA molecular analysis of G6PD gene obtained from the blood sample of F.N. index patient identified substitution of Guanine nucleotide with Adenine nucleotide in position 1178. This mutation resulted in the protein with Arginine-to-Histidine [G6PD, 1178 (G-A) Arg393His] substitution in the position 393 [G6PD, 1178 (G-A) Arg393His].

Conclusion

So, G6PD enzyme deficiency for Masalli area has shown the following values: 8.33% for phenotypic frequency and 0.0833 (d.f.) for gene frequency. According to the WHO requirements, and according to the biochemical characteristics of the identified enzyme deficiency, findings were related to three classes: 13 pupils to the 2nd class, 6 pupils to the 3rd class, and 4 pupils to the 4th. Molecular analysis of G6PD gene identified substitution of Guanine nucleotide with Adenine nucleotide in position 1178. As a result of the mutation in protein in the position 393, there was substitution of amino acids Arginine with Histidine [G6PD, 1178 (G-A) Arg393His].

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