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MOLECULAR GENETIC ANALYSIS OF GLA GENE CAUSING FABRY INHERITED DISEASE FOR CARDIOMYOPATHY PATIENTS IN AZERBAIJAN REPUBLIC

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Abstract. In the paper for the first time in Lenkoran-Astara administrative area of Azerbaijan patients with cardiomyopathy were genetically screened for Fabry metabolic disease. Screening was carried out by means of identification of α-galactosidase enzyme activity and quantity of globotriasylsphingosine. In 12 out of 29 examined persons we got activity deficit of α-galactosidase enzyme and amount of globotriasylsphingosine was higher than the norm which were specific for Fabry disease. In 8 women manifested X-linked inheritance type as heterozygotes for Fabry disease, and 4 men were identified as hemizygotes. Molecular genetic analysis identified two different nucleotide substitutionsin GLA gene: 801+3A>G mutation in intron 5 and substitution of Adenine nucleotide with Guanine nucleotide in position 137 (137,A>G) of GLA gene. To prevent Fabry disease, it is recommended to screen affected persons family members for α-galactosidase enzyme activities.

Keywords: *Fabry, cardiomiopathy, mutation,gene, α-galactosidase, globotriasylsphingosine (lyso-Gb3), GLA.*

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1. Introduction

Fabry disease (Anderson-Fabry disease, inherited distonic lipoidosis etc.) being a lisosome metabolic disease relates to a group of orphan (rare) diseases. Anderson and Fabry in 1898 studied this disease for the first time. This disease takes the second place following to Gaucher lisosome metabolic disease in human population on frequencies. Frequency for the newborns with Fabry disease equals 1:4000-1:120000. For male population this figures are for Australia - 1:1170000, Netherlands - 1:476000 and USA - 1:40000-60000 (Volgina, 2012; Moore *et al*., 2003; Gutiérrez-Amavizca *et al*., 2017).

In men frequency for Fabry disease is 1:40000-1:117000, and in women is 1:40000. In isolates the frequency in men was even 1:3100 (Gal *et al*., 2006).

GLA gene, modifying Fabry disease clinic, locates on the X-sex chromosome long shoulder - Xq22. GLA gene encodes α-galactosidase enzyme. Up to nowadays more than 840 allelic forms were identified. Those alleles 70% are related to missense or nonsense mutations group. The most of mutations have family specificity. The most frequent pathologic alleles are R227Q and R227X.Inheritance type is dominant X-linked one. The disease in women has incomplete penetration (Ortiz *et al*., 2018; Baptista et al., 2010).

Fabry disease being one out of 60 diseases of lisosome storage diseases is a result of complete or partial α-galactosidase A enzyme activity deficiency. α-galactosidase A enzyme activity reduction causing sphyngolipidoses metabolism damage, leads to storage of globotriasylsphingosines. The disease has broad spectrum of symptoms. In the course of disease one organ is mainly damaged, problems are in heart or kidney. The disease in 95% of cases, defect gene happens to be transferred from mother to a son in men, and for women - from their father or mother. In 5% of cases *de novo* mutation occurs to cause the disease. Men have severe clinic with this disease and women also have often rather severe clinic manifestations. If parents do not have any Fabry disease pathologic gene, it does not exclude emergence of the disease (Gutiérrez-Amavizca *et al*., 2017; Shabbeer *et al*., 2010).

There identified classic and non-classic forms of Fabry disease. Basically men manifest acromegalic specific phenotype, pains in palms and feet, fast fatigue, sweating disorder and physically lack stamina. The first early diagnostic signs are presence of red-purple painful angiokeratomas. At the same time function of heart and kidney is damaged. Patients manifest psycho-emotional lesions and sometimes hearing decrease (Sigmundsdottir *et al*., 2014; El Turk & Mitchell, 2019).

This pathologic allel according to clinic testing was related to class 1 pathogenic (ClinVar ID: 197639). X-linked GLA pathologic cases is related to Fabry disease. In case α-galactosidase enzyme activity being lower than 1%, hemizygotes manifest classic clinic in childhood or in youth: periodic seizures, angiokeratomas, anhydrosis, hypohydrosis, rare cases of hyperhidrosis, specific corneal opacity and proteinuria. In men 30-50 of age, kidney function is gradually being disrupted up to its acute state and additionally heart and/or cerebral vessels are affected. Women heterozygotes differing from men hemizygotes, all those signs get in later age and in softer variant. In rare cases heterozygous women have the same severe clinic as in homozygous men (El Turk & Mitchell, 2019).

In hemizygote men, if enzyme activity deficiency is more than 1%, then patients in their 60s-80s develop atypical cardiologic phenotype alongside with Fabry disease – hypertrophy of the left ventricle, arythmia, and proteinuria are manifested (OMIM®: 301500, Mehta and Hughes, 2017 - PMID: 20301469) (Gal *et al*., 2006).

Gene GLA consists of seven exons encoding 429 amino acids of 1290 nucleotide bases. Out of 429 identified mutations 2/3 of them are located in the exon 7 (Forshaw-Hulme *et al*., 2019).

Shabbeer A. et al. (2006), carried out genetic researches of 66 non-relative families, and found in 49 families 50 new pathologic alleles of GLA gene. Out of them 29 are missense mutations: N34K, T41I, D93V, R112S, L166G, G171D, M187T, S201Y, S201F, D234E, W236R, D264Y, M267R, V269M, G271S, G271V, S276G, Q283P, A285P, A285D, M290I, P293T, Q312H, Q321R, G328V, E338K, A348P, E358A, Q386P; nine nonsense mutations: C56X, E79X, K127X, Y151X, Y173X, L177X, W262X, Q306X, E338X; in five there was a splice defectIVS4-1G>A, IVS5-2A>G, IVS5+3A>G, IVS5+4A>G, IVS6-1G>C; in four - deletions: 18delA, 457delGAC, 567delG, 1096delACCAT; in one there is a small insertion-996insC; in one 3.1 kilobase Alu-Alu deletion (in exon 2); in one case -K374R, 1124delGAG- a complex of mutations was identified (Gutiérrez-Amavizca *et al*., 2017).

In 18 families known as affected, the following 9 pathologic alleles were found: R112C, D264Y, V269M, Q312H, A143T, D264Y, Q312H, V269M, A143T (Gutiérrez-Amavizca *et al.*, 2017).

Gutiérrez-Amavizca B.E. et al (2017) have identified five mutations: P40S, IVS4+4, G328V, R363H, R404del when GLA gene screening in 65 family members.

It should be mentioned that because population of Azerbaijan Republic have never been diagnosed for Fabry disease and the disease has never been identified, there is no data on clinic, biochemistry and genetics of the disease.

The goal of the paper was to screen patients with cardiomyopathies for Fabry inherited lysosomal metabolic disease and to carry out molecular genetic analysis of GLA gene for identified patients.

2. Material and Methods

29 patient suspicious for Fabry inherited lysosomal disease venous blood samples 1ml each were taken into EDTA anticoagulant sample tube and then absorbed into special DBS (dry blood spots) cards. Cards with absorbed blood samples stay in room temperature for 1 hour and then are analyzed in the Chair of Laboratory Science (Azerbaijan State Doctors Advanced Training Institute after A.Aliyev) and CENTOGENE laboratories in Germany (Rostock city).

29 cardiologic patients from Lenkoran, Masalli and Astara Central Regional Hospitals were suspicious for Fabry disease to develop those cardiomyopathies. For that purpose α-galactosidase enzyme activities were measured and in case of enzymatic deficit amounts of globotriasylsphingosine (lyso-Gb3) were identified (Smid *et al.*, 2015).

In genetic screening fluorimetric method and liquid chromatography were used. Sanger methodique was applied for direct sequencing of GLA gene. Testing of the existing mutation in GLA gene became possible with this method. The method was developed in CENTOGENE laboratories, Rostock, Germany (Trujillano *et al.*, 2017).

3. Results and Discussion

In Lenkoran, Masalli and Astara we worked with patients who were diagnosed and treated in Central Region Hospital as having hypertrophic cardiomyopathies.

Seven patients were found in Astara, four of them – in Masalli region and one patient in Lenkoran region who had manifested deficit (deficiency) of α-galactosidase enzyme. And here we found out that all patients with deficit of α-galactosidase enzyme activities had amounts higher than norm for lyso-Gb3.

Table 1 presents results of enzyme analysis for Fabry genetically screened and diagnosis identified patients as primarily with cardiomyopathies.

In Masalli region 4 patients identified were 2 men and 2 women. In brothers T.I. and T.A. α-galactosidase enzyme activity showed lower than the norm activity as 0.8 mkmol/l/s ($n \geq 15.3$ mkmol/l/s). To verify the diagnostics, another test was carried out for lyso-Gb3 amount, and happened to be higher than the norm - 106.0 ng/ml (T.I.) and 106.0 ng/ml (T.A.) when norm was $n \leq 1.8$ ng/ml. Fabry disease having X-linked chromosome dominant inheritance type, both of brothers were hemizygous carriers of GLA gene.

α-galactosidase enzyme deficiency for sisters T.G. and A.G. being 3.2 mkmol/l/s, 1.9 mkmol/l/s, relatively, amounts of Lyso-Gb3 for them were vice versa higher – 15.6 ng/ml (T.G) and 8.3 ng/ml (A.G). For both of sisters X-linked autosomal dominant inheritance type in heterozygous state was identified (Table 1.)

Figure 1 presents family tree of the family found in Masalli having Fabry diagnosed members.

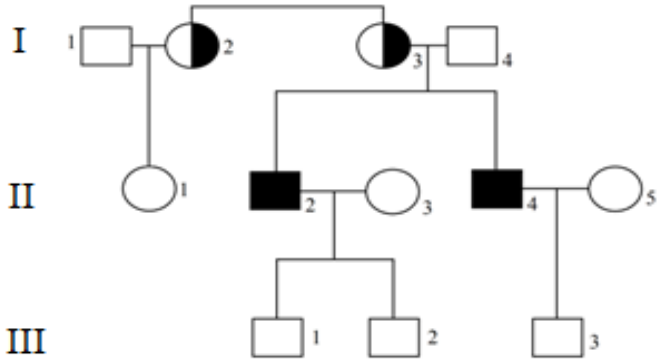

Fig.1. Family tree of index patient A.G.

I-2 – index patient (A.G), I-3 (T.G –sister of the index patient), II-2 (T.A.) və II-4 (T.I.) Fabry disease diagnosed sons of the index patient's sister.

Patient M.A. originated from Lerkoran had α-galactosidase activity lower than the norm- 2.3 mkmol/l/s, but amount Lyso-Gb3 was higher than the norm (109 ng/ml).

In Astara area in the family 6 persons were women and one man born on 12.06.1978.α-galactosidase enzyme showed "0" activity for patient K.A., and amount of Lyso-Gb3 was much higher than the norm (218.0 ng/ml).

In six women of the family, activity of α-galactosidase enzyme was in range of1.4-1.8 mkmol/l/s. The mean amount of Lyso-Gb3 was registered as 16.0 ng/ml (11.0-21.0 ng/ml).

Figure 2 presents family tree of the Fabry identified family in Astara area.

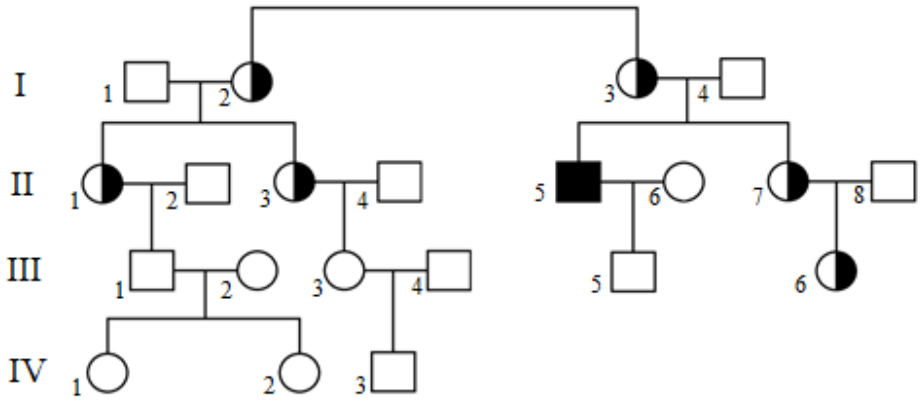

Fig.2. Family tree of the index patient K.A.

II-5 – index patient (K.A.), I-3 – mother of the index patient (K.L.), II-7 – sister of index patient (L.I.)and III-6 – daughter of his sister (A.A.), I-2 –maternal aunt of the index patient (K.E.), II-1 (K.F.) and II-3 (K.A.) – daughters of the maternal aunt.

In Masalli area all patients were members of one and the same family, thus they had the same mutation 801+3A>G of GLA gene.

801+3A>G mutation of GLA gene destroys donor splice site in exon 5. According to HGMD Professional 2019.1, this variant (PMID: 16595074) priory was presented as a cause of Fabry disease (Shabbeer *et al*., 2006).

In patient M.A. molecular genetics diagnostics of GLA gene identified substitution of Adenine nucleotide with Guanine nucleotide in position 137 (137,A>G). This mutation in position 137 of GLA gene at the time of Adenine nucleotide substitution with Guanine nucleotide causes substitution of Histidine amino acid with Arginine amino acid in position 46 in the course of protein (enzyme) biosynthesis (46 His>Arg). Identified gene mutation relates to missense (nonsense) mutations.

One and the same GLA gene polymorphism was identified in one family in Astara area. Adenine nucleotide is substituted with Guanine nucleotide position 137 (137,A>G) of GLA gene. Gene polymorphism happened in the gene caused the change of Histidine amino acid to Arginine amino acid (46 His>Arg) in position 46 while protein biosynthesising. Because of mutation relates to missense (nonsense) type, the synthesis of enzyme was completely destroyed.

Bartista M.V. et al (2010) first found the substitution of Adenine nucleotide with Guanine nucleotide in position 137 of GLA gene. That was done during genetic screening of young people in Portugal. That kind pathologic allel was related to missense mutation type.

Table 1. Results of enzyme and gene analysis of patients with Fabry metabolic disease who were genetically screened in Astara, Lenkoran and Masalli areas

Patient	Date of birth	Gender	α-galactosidase n≥15.3 mkmol/l/s	lyso-Gb3 n ≤ 1.8 nq/ml	GLA gene mutation	Genotype
T.I.	03.06.87	male	0.8 mkmol/l/s	106.0 nq/ml	801+3A>G	Hemizygote
T.A.	21.06.86	male	0.8 mkmol/l/s	117.0 nq/ml	801+3A>G	Hemizygote
T.G	19.08.62	female	3.2 mkmol/l/s	15.6 nq/ml	801+3A>G	Heterozygote
A.G.	06.09.56	female	1.9 mkmol/l/s	8.3 nq/ml	801+3A>G	Heterozygote
M.A.	20.07.12	male	2.3 mkmol/l/s	109.0 nq/ml	137, A>G	Hemizygote
K.E.	16.08.75	female	1.8 mkmol/l/s	13.0 nq/ml	137, A>G	Heterozygote
K.A.	28.07.07	female	1.6 mkmol/l/s	11.0 nq/ml	137, A>G	Heterozygote
L.I.	31.08.51	female	1.4 mkmol/l/s	19.0 nq/ml	137, A>G	Heterozygote
K.A.	12.06.78	male	0.0 mkmol/l/s	218.0 nq/ml	137, A>G	Hemizygote
K.L.	20.07.53	female	1.4 mkmol/l/s	21.0 nq/ml	137, A>G	Heterozygote
A.A.	29.05.17	female	1.3 mkmol/l/s	20.0 nq/ml	137, A>G	Heterozygote
K.F.	15.05.03	female	1.1 mkmol/l/s	18.0 nq/ml	137, A>G	Heterozygote

So, twelve patients with Fabry inherited metabolic disease were identified resulting from genetic screening in Astara, Lenkoran and Masalli areas population especially among cardiomyopathic patients for α-galactosidase enzyme and globotriasylsphingosine. Molecular genetic analysis of GLA gene identified two

different mutations: 801+3A>G and 137, A>G. Both mutations of GLA gene were found for the first time for Republic population.

To prevent Fabry disease, family members of the indexed patients are recommended to pass through screening of α-galactosidase enzyme activity.

4. Conclusions

- For the first time genetic screening was carried out for patients with cardiomyopathies in case of Fabry metabolic disease in Lenkoran-Astara area of Azerbaijan Republic. Heterozygote and hemizygote inheritance types were identified.
- Two different mutations of GLA gene were identified: 801+3A>G mutation in intron 5, and substitution of Adenine nucleotide with Guanine nucleotide in position 137 (137, A>G).
- Heterozygote and hemizygote gene GLA inheritance types were identified.

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