Molecular Genetic Studies of the Gene (GALT) of Galactose-1-Phosphaturidyltransferase Enzyme

HAJIYEVA N.M.
Baku State University, Baku, Azerbaijan

Abstract:
For the first time in Baku city, Azerbaijan Republic in maternity houses by means of immunoenzyme analysis the genetic screening for galactosemia inherited metabolism disorder was carried out, and 3 newborns with GALT gene deficiency were identified. By means of molecular genetic methods two mutations of GALT gene were identified: 1. substitution of guanine nucleotide by adenine nucleotide (G-A) in position 563 was because glycine amino acid was substituted by argenine amino acid (Gln-Arg) in 188 position; 2. substitution of guanine nucleotide by adenine nucleotide (G-A) in 184 position as a result of substitution of leucine amino acid by methionine amino acid (Leu-Met) in position 62.

Key words: galactosemiya, metabolism disorder, enzyme of galactose-1-phosphaturidyltransferase, polymerase chain reaction, mutation.

INTRODUCTION

Galactosemia is hereditary disorder of carbohydrate metabolism that affects the body's ability to convert galactose (a sugar contained in milk, including human mother's milk) to glucose (a different type of sugar). The disorder is caused by a deficiency of an enzyme galactose-1-phosphate uridyl transferase (GALT) which is vital to this process. Early diagnosis and treatment with a lactose-free diet is absolutely
Galactosemia is an autosomal recessive hereditary disorder caused by a deficiency of the enzyme galactose-1-phosphate uridyl transferase (GALT) that is needed for the breakdown of the milk sugar, galactose. Deficiency of this enzyme results in the accumulation of toxic products: galactose-1-phosphate (a derivative of galactose), and galactitol (an alcohol derivative of galactose). Galactitol accumulates in the lens of the eye where it causes lens swelling and protein precipitation and, subsequently, cataracts. Accumulation of galactose-1-phosphate is thought to cause the other signs and symptoms of disease. (1,2,3,4,5)

The deficit of galactoso-1-phosphaturidyltransferase enzyme (GALT), which plays a key role in the metabolism process, does not provide glucose degradation of galactose, resulting in excessive sugars poisoning the brain and causing galactosemic oligophrenia in the patient, cataracts in the eyes, hepatomegaly and cirrhosis of the liver, physical and mental retardation. From the first days of the disease: jaundice, neurological symptoms (cramps, nystagm, muscle hypotension), vomiting, and subsequent physical and mental retardation are observed. If the disease is discovered on time, and the galactose is excluded in the accepted food, it is possible to ensure the normal physical and mental development of the child. (6)

Since the genetics of the galactosemia gene is heterogeneous, various forms are associated with the deficiency of different enzymes. Generally, around 100 mutations of galactosemia gene were detected and authenticated. The disorder depends on the damage in three different genes found
in autosomal chromosomes 1, 9 and 17. They are: mutations occurring in the GALT gene of the galactoso-1-phosphaturidyltransferase enzyme, located in p13 of the short shoulder of autosomal chromosome 9; mutation in the GALK gene located at q23-q25 of the long shoulder of the autosome 17; and mutation in the GALE gene of the UDP-glucose-4-epimerase enzyme in the p35-p36 short shoulder of the chromosome 1. The inheritance type of all three genetic forms of galactosemia metabolic disorder is autosomal-recessive (7,8,9).

The genetics of the disease are different, as is the case in its clinics. The light clinic of galactosemia results in the absence of digestion of the milk by the body and the formation of cataract in the eye. The Dewart form of the disease passes without symptoms and there is a tendency toward liver disease in humans. Frequency of incidence is 1: 15000-20000 for homozygotes, about 1: 300 for heterozygotes. (10,11,12)

There was no screening on galactosemia inherited disorder for newborns carried out in Azerabijan Republic, and the identified affected kids haven’t been studied for its gene mutations.

Thus, the goal of our study is research of existing inherited metabolism disorder – galactosemia- in newborns of Baku city.

MATERIAL AND METHODS

Genetic screening of newborns for galactosemia inherited metabolism disorder was carried out during 2015-2018 years in Baku city maternity houses and affected kids, who appealed to Scientific-Research Institute of Pediatrics of Ministry of Healthcare. Totally 278 newborns and 38 affected kids were under study.

Samples preparation: Blood samples were taken from the heel during the first 24-72 hours of life. The newborn’s heel
was cleaned by soap and clean warm towel (40-45°C). The area where the blood will be sampled is cleaned with cotton disc with 70% alcohol (isopropanol). Then by means of a lancet (scarificator) we carefully prick the heel and absorbed blood into Wattman 903 paper (Card Gatry). It is banned to touch the absorbed into paper blood drop paper. Blood stain is dried at room temperature around 3 hours. Every sample is kept in the separate envelope. The lifetime of keeping the sample in the humidity-proof envelope is one week. The quality of blood sample, kept in the refrigerator (2-8°C), is satisfactory enough for the period of 2 months. If needed to keep the sample for a long time, then use freezer department of refrigerator. In order to keep controls and standards stable, they should be kept in the envelope of special aluminium folio and in the box. The testis carried out by means of IFA method (13.14).

Polymorphism of GALT was studied by means of molecular genetic methods based on polymerase chain reaction (PCR) (2). Genomic DNA was isolated from the venous blood by means of chemicals mixture of QIAamp genomic DNA and RNA kit (QIAGEN company), Germany. Intactness of the isolated genome DNA and amplified DNA fragments was studied in 1.7% agarose gel by means of electrophoretic method in the USA produced PowerPacBasicGelDocMEZ electrophoresis apparatus. PCR was carried out in the following temperature regime: 95°C-2 minutes (95°C-30¹, 60°C-30¹, 77°C-2 minutes. This cycle repeated 30 times), 72°C-10 minutes and 4°C break. PCR was conducted in Germany produced “Professional Thermocycler Biometra” Company apparatus. A pair of Forward and Reverse primers was used for each genome DNA fragment. Purification of each DNA fragment was done on special magnets (Agencourt AMPure XP PCR purification and SPRIPlate 96 Super Magnet Plate). Purified DNA fragments were amplified for the second time in the following regime: 95°C-2 minutes, (95°C-30¹, 55°C-30¹, 77°C-2 minutes 30 cycles and 72°C 10 minutes, break at 4°C. Then the obtained
amplificate was passed over to the “GENOMELabGeXP™ Sequencing” apparatus to sequence the nucleotide (15).

RESULTS AND DISCUSSION

GALT enzyme deficiency was identified in 3 newborns and 2 affected kids out of 276 newborns and 38 affected kids during genetic screening. In newborns there were 2 boys and 1 girl. Out of those three newborns, two had partial deficiency: 40-45% of normal activity was found. This degree of enzyme activity shows heterozygous carriage of GALT enzyme type for both newborns. In one newborn total deficiency was identified. This total deficiency shows homozygous form of GALT in newborn.

In figure 1, one of the sick children’ M.A. family tree has been presented.

![Family Tree](image)

Fig. 1. M.A.patient’s family tree.

M.A. is the third child in the family. His two brothers are healthy. The patient's parents are relatives - secondary blood relatives. Parents are children of two sisters. Since the inheritance type is autosomal-recessive, both parents are practically healthy heterozygous carriers of the GALT gene, and the risk of a childbirth in each following pregnancy is 25%. After the first stage of PCR, the DNA fragments were investigated agarose gel, and intactness and quantity were
analyzed. The DNA fragments were purified to prepare for the second stage of PCR. After stage 2 of PCR, fragments were passed on to the GENOMELab GeXPTM Sequencing apparatus where their nucleotide sequences were determined. The obtained results are shown in Table 1. (16)

Table 1. The identified GALT mutations

<table>
<thead>
<tr>
<th>Patient</th>
<th>Mutation</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Newborn Aliyev</td>
<td>563 (G-A) 188 (Gln-Arg)</td>
<td>Heterozygote</td>
</tr>
<tr>
<td>Newborn Ahmadov</td>
<td>184 (G-A) 62 (Leu-Met)</td>
<td>Heterozygote</td>
</tr>
<tr>
<td>Newborn Rasulova</td>
<td>188 (G-A)/188 (G-A)</td>
<td>Homozygote</td>
</tr>
<tr>
<td>Patient M.A.</td>
<td>563 (G-A)/563 (G-A)</td>
<td>Homozygote</td>
</tr>
<tr>
<td>Patient F.H.</td>
<td>563 (G-A)/184 (G-A)</td>
<td>Compound</td>
</tr>
</tbody>
</table>

As it is seen from the Table 1, two mutations of GALT gene were identified in the presented material. The first mutation of GALT gene is found out in the position 563 and it is a substitution of adenine by guanine (G-A). This mutation was forced by protein glycine amino acid substitution by argenine amino acid (Gln-Arg) in 188 position. The second mutation of GALT gene identified substitution of guanine nucleotide by adenine nucleotide (G-A) in 184 position. For this mutation the purpose was as substitution of leucine aminoacid by methionine amino acid (Leu-Met) in the position 62. In two of three newborns had heterozygous and one had homozygous forms of mutation.

In the affected kids the first (M.A.) had 563 (G-A) mutation as homozygous form, the second one (F.H.) had compound form – that is twice heterozygous forms of two mutations. Thus, five affected kids manifested two mutations of GALT gene: 563 (G-A)188 (Gln-Arg) and 184 (G-A) 62 (Leu-Met). Heterogote mutation were found in two kids, homozygote mutation – in two more kids, and one had twice heterozytoe mutations.

The frequencies for GALT gene mutations were counted. For 276 newborns screened the frequency for GALT
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gene was 0.0072 (in decimal quantity), for 38 affected kids it was 0.0526.

During 2006-2010 in Bashkortostan republic of Russian Federation, in maternity houses neonatal screening revealed 168 newborns with galactosemia inherited metabolism disorder. In Ufa city in Republican medical-genetic center, GALT gene mutations were identified by means of molecular methods. Among those mutations 563 (G-A)188 (Gln-Arg) and 184 (G-A) 62 (Leu-Met) mutations predominated.

One of the three affected kids with mental retardation manifested G188R and G212X mutation compound forms, and the rest two had two new unknown mutations in homozygous state. Mutation E340X was identified in the 10th, and mutation G212X was found in the 7th exons.

In the USA in white population GALT 563 (G-A) 188 (Gln-Arg) mutation was also identified.

Thus, for the first time in Baku city neonatal genetic screening of 276 newborns and 38 affected kids for galactosemia inherited metabolism disorder was carried out, which identified 2 newborns with GALT gene heterozygous, and 1 with homozygous forms. By means of molecular genetic methods two mutations of GALT gene were identified. The first mutation: substitution of guanine nucleotide by adenine nucleotide (G-A) in position 563 was because glycine amino acid was substituted by arginine amino acid (Gln-Arg) in 188 position. The second mutation: substitution of guanine nucleotide by adenine nucleotide (G-A) in 184 position as a result of substitution of leucine amino acid by methionine amino acid (Leu-Met) in position 62. Among newborns, the frequency of GALT was – 0.0072, and in 38 affected kids – 0.0526 (in decimal quantity). (17,18)
CONCLUSION

1. For the first time in Baku city, Azerbaijan Republic in maternity houses by means of immunoenzyme analysis the genetic screening for galactosemia inherited metabolism disorder was carried out, and 3 newborns with GALT gene deficiency were identified.

2. By means of molecular genetic methods two mutations of GALT gene were identified: 1. substitution of guanine nucleotide by adenine nucleotide (G-A) in position 563 was because glycine amino acid was substituted by arginine amino acid (Gln-Arg) in 188 position; 2. substitution of guanine nucleotide by adenine nucleotide (G-A) in 184 position as a result of substitution of leucine amino acid by methionine amino acid (Leu-Met) in position 62.

3. Among newborns, the frequency of GALT was – 0.0072, and in 38 affected kids – 0.0526 (in decimal quantity).

REFERENCES


