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MUTATION OF DUARTE VERSION OF GALACTOSEMIA (GALT) DISEASE FOUND IN AZERBAIJAN

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МУТАЦИОННАЯ ВЕРСИЯ ГАЛАКТОЗЕМИИ (GALT) БОЛЕЗНИ DUARTE НАЙДЕНЫ В АЗЕРБАЙДЖАНЕ

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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 for were identified.

Аннотация. Впервые в городе Баку Азербайджанской Республики в родильных домах методом иммуноферментного анализа проведен генетический скрининг на наследственное нарушение обмена веществ при галактоземии, выявлены 3 новорожденных с дефицитом гена GALT.

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

Ключевые слова: галактоземия, нарушение метаболизма, фермент галактозо-1-фосфатуридилтрансфераза, полимеразная цепная реакция, мутация.

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 essential to avoid profound intellectual disability, liver failure and death in the newborn period. Galactosemia is inherited as an autosomal recessive genetic condition. Classic galactosemia and clinical variant galactosemia can both result in life threatening health problems unless treatment is started shortly after birth. A biochemical variant form of galactosemia termed Duarte is not thought to cause clinical disease. 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: galactose1-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 [1-5].

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

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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 dipends 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-resessive [7-9].

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 incidecne is 1: 15000-20000 for homozygotes, about 1: 300 for heterozygotes [10-12].

There was no screening on galactosemia inherited disorder for newborns carried out in Azerbaijan 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 1481 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□). 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□), 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 bymeans of IFA method [13, 14].

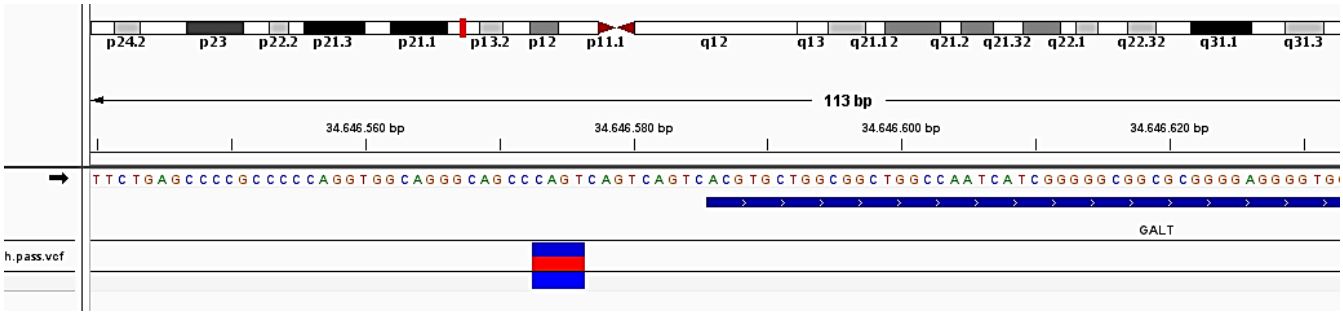
Polymorphism of GALT was studied by means of molecular genetic methods based on polymerase chain reaction (PCR) [2]. Genomic DNA was isolated from the venous blood by means of chemicals mixture of QIAampgenomic DNA and RNA kit (QIAGEN company), Germany. Intactness of the isolated genome DNA and amplified DNA fragments was studied in 1.7% agarose gel by means of electrophoretic method in the USA produced PowerPacBasicGelDocIMEZ electrophoresis apparatus. PCR was carried out in the followiing temperature regime: 95oC-2 minutes (95oC-30I , 60oC-30I , 77oC-2 minutes. This cycle repeated 30 times), 72oC-10 minutes and 4oC 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» и SPRIPlate 96 Super Magnet Plate). Purified DNA fragments were amplified for the second time in the following regime: 95oC-2 minutes, (95oC-30I , 55oC- 30I , 77oC-2 minutes 30 cycles and 72oC 10 minutes, break at 4oC. Then the obtained amplificate was passed over to the "GENOMELabGeXPTM 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 The DNA fragments were purified to prepare for the second stage of PCR.

1. GALT gene chr9 exon 1 upstream
Position 34646572-34646576
Reference: CCAGT
Patient: C
Type: INDEL
Type: Heterozygot

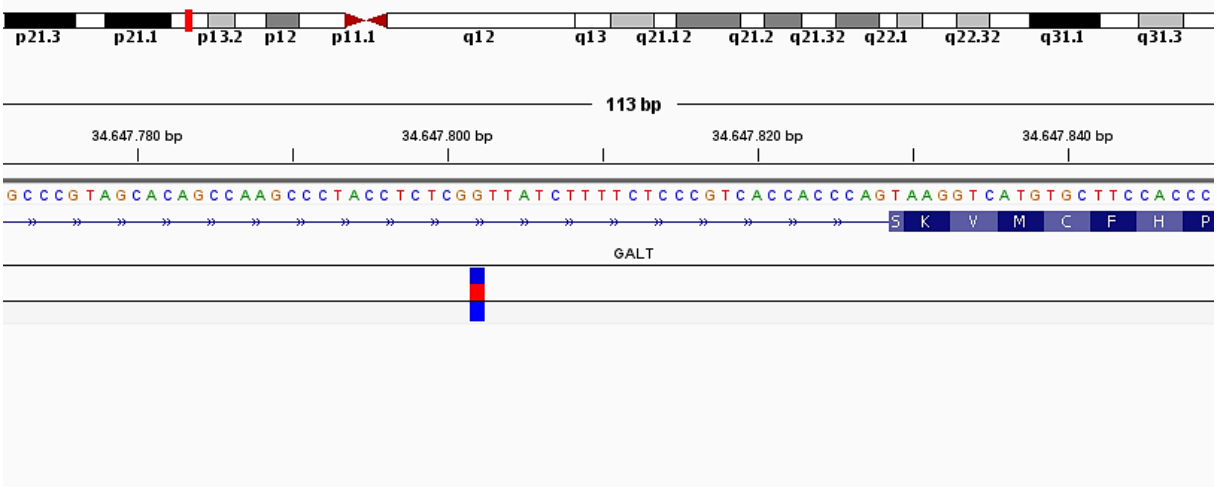


Results of molecular-genetical analysis of Galt-gene of newborn R.H.

1) To the 1st exson of Galt gene CCAGT nukleotids were added. Type of this genetical changes was heterozygote. Mutation is in 9th chromosome.

2. NM_000155.4(GALT):c.378-27G>C - intron variant
Allele ID 36500
Variant type: single nucleotide variant
Variant length: 1 bp
Cytogenetic location: 9p13.3
Genomic location 9: 34647805 (GRCh38)
Interpretation: Benign

Type: heterozygote G



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2) At 378th position of gene nitrogenic base of quanine waz replaces with cytozine nitrogenic base. İt is point mutation. Version length is 1 bp. At cytogenetic location the place of mutation is in 3rd subsegment of 13th segment of small (p)of 9th chroosome. It is a harmless variability. The type of this genetic variation was determined as heterozygous.

3. NM_000155.4(GALT):c.507+62G>A - intron variant

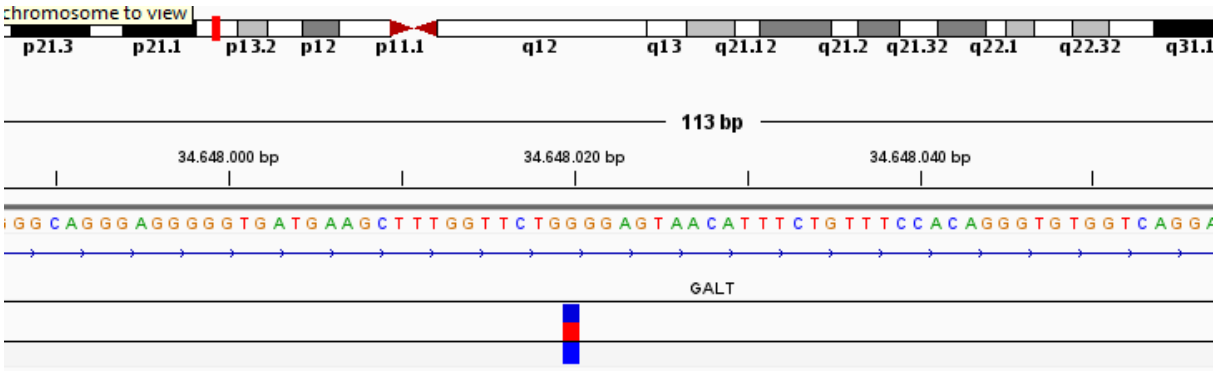
Variant type: single nucleotide variant

Cytogenetic location: 9p13.3Genomic location:

Chr9: 34648023 (on Assembly GRCh38)

Interpretation: Benign

Type: heterozygote G>A



3) A point mutation occurred in the 507th part of the gene in the intron part of the Galt gene, due to the replacement of the nitrogenous base of guanine with adenine. Its cytogenetic location is in the 3rd subsegment of the 13th segment of the small arm of the 9th chromosome. It is a harmless mutation. The type of this genetic variation is heterozygous.

4. NM_000155.4(GALT):c.508-24G>A

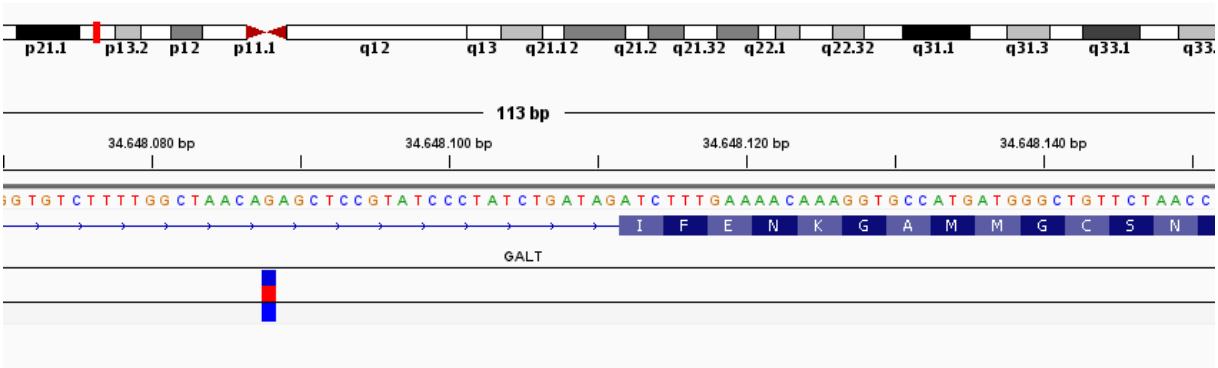
Variant type: single nucleotide variant

Cytogenetic location: 9p13.3

Genomic location: Chr9: 34648091 (on Assembly GRCh38)

Interpretation: Benign

Type: heterozygote G>A



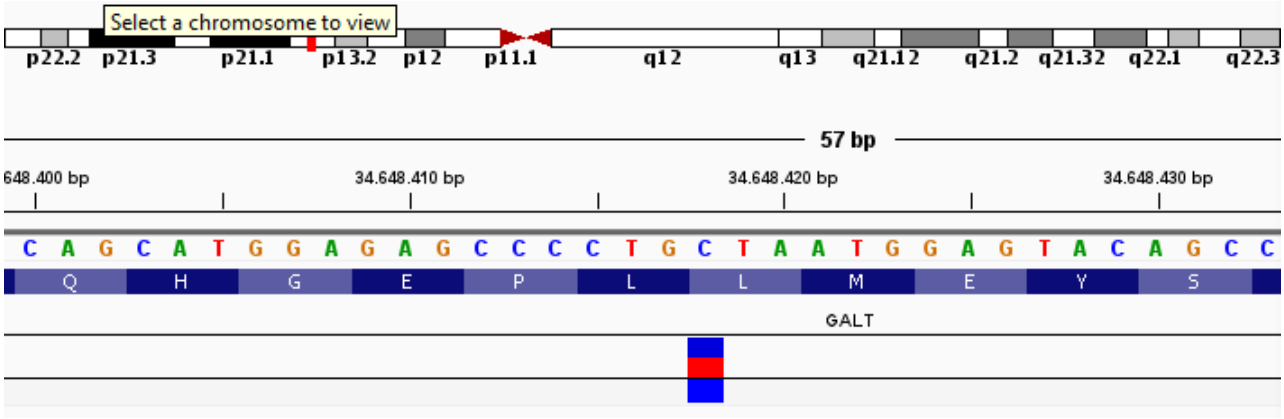
4) In the 508th part of the Galt gene, the nitrogenous base of guanine was replaced by adenine. It is a point mutation. The location of the mutation in the cytogenetic location is in the 3rd subsegment of the 13th segment of the small arm of the 9th chromosome (p). It did not cause illness.

5. GALT gene Exon number: 7, Single nucleotid variant SNV:

Clinical classification: benign, does not affect function NM_000155.3:c.652C>T (p.Leu218=)

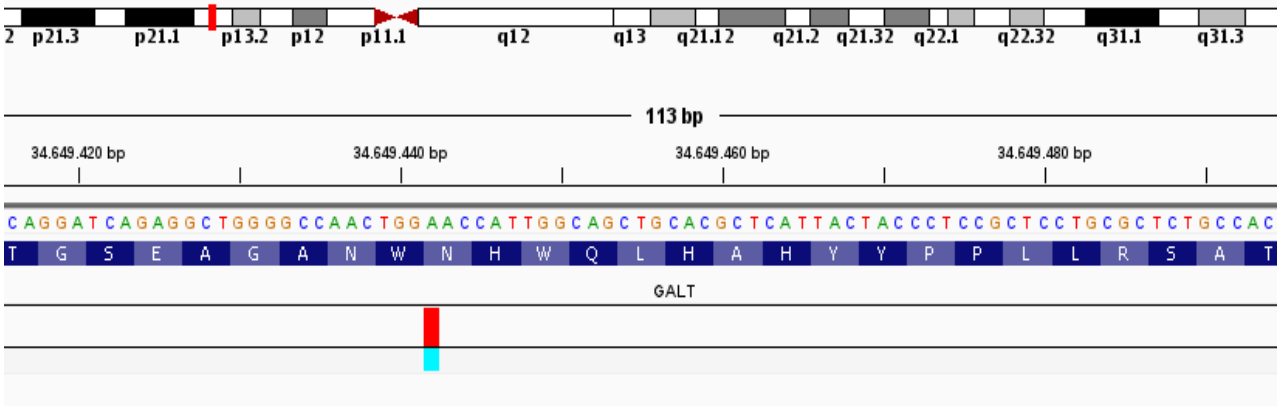
Type: heterozygote

Allele ID: 18659
Variant type: single nucleotide variant
Variant length: 1 bp
Cytogenetic location: 9p13.3
Genomic location: Chr9: 34648421 (on Assembly GRCh38)



5) In exon 7 of the Galt gene, cytosine was replaced by thymine in the 652nd part of the gene. Clinical classification: benign, does not affect function. It is polymorphism. Single nucleotide variant. Variant length 1 bp. Its cytogenetic location is in the 3rd subsegment of segment 13 of the small arm of chromosome 9 (p).

6. Gene: GALT
Variant type: single nucleotide variant
Cytogenetic location:9p13.3
Genomic location: exon 10
Chr9: 34649445 (on Assembly GRCh38)
Chr9: 34649442 (on Assembly GRCh37)
Protein change:N205D; ASN314ASP
Preferred name: NM_000155.4(GALT):c.940A>G (p.Asn314Asp)
Type: Homozygot G/G
Other names: Duarte 2; Duarte 1; Duarte1 Duarte 2; p.N314D:AAC>GAC
HGVS: NC_000009.12:g.34649445A>G
NG_009029.2:g.7857A>G
NG_028966.1:g.2261A>G



6) In exon 10 of the galt gene, the nitrogen base of adenine was replaced by the nitrogen base of guanine in the 940th part of the gene. Protein change ASN314ASPThe type of this genetic variation is homozygous G/G. Other names Duarte 2; Duarte 1.

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