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Determination of The GALT Gene in Galactosemic Cataract Patients in Azerbaijan

L. S. Huseynova[1,2], A. H. Ansarova[1]

[1]Department of Medical Biology and Genetics, Azerbaijan Medical University
[2]Ministry of Education of Azerbaijan, Institute of Genetic Resources
ORCID: 0000-0003-2563-1493[1]

**Corresponding author's E-mail: royahuseynova2006@gmail.com*

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Abstract

Galactosemia is one of the genetic diseases that can be treated thanks to early detection by genetic screening test. Galactosemia is a hereditary metabolic disease. This disease is heterogeneous and controlled by GALT, GALK, GALE and GALM genes. The presented article is dedicated to galactosemic disease among Azerbaijani patients as a result of GALT (galactose-1-phosphate uridyltransferase) gene mutations. This study reviews discusses the type of cataracts associated with galactosemia. The genetics of galactosemic patients are evaluated and details of galactose metabolism are described. This study describes the results of the GALT gene analysis of two Azerbaijanian patients with Galactosemic cataract. In one of the patients the heterzygous form of the same mutation, and the homozygous form in the other patient were found. In this study, control and experimental groups were determined, and DNA extracted from the peripheral blood of patients belonging to both groups was subjected to PCR amplification according to standard protocols and sequenced by Sanger sequence method. The symptoms of the disease were reviewed in these patients. Patients presented with jaundice, diarrhea, vomiting, malnutrition, seizures, allergies and more cataracts in the neonatal period. Commonly, Cataract disease is idiopathic origin. Also, patients with cataracts may have an underlying genetic abnormality of galactose metabolism. Elevated levels of galactose and galactose-1-phosphate and absence of GALT activity were diagnosed in the studied patients. Early biochemical and molecular-genetic diagnosis and genetic counseling of patients and their family members are extremely important in the treatment of galactosemia. We selected 25 cataract patients aged 0-45 years and 25 age and sex-matched controls for the study. Blood samples taken from the experimental and control groups, were biochemically and then molecular-genetically analyzed. Mutations Q188R, K285N and N314D were investigated in patients. Of these mutations, only the N314D mutation was detected in two patients. Both heterozygous and homozygous forms of N314D mutation were detected.

Keywords: *Cataract, disease, galactose, glucose, mutation, sequensing*

1. Introduction

Galactosemia is an inborn error of carbohydrate metabolism. The metabolic defect is a deficit in the activity of a specific enzyme, galactose-l-phosphate uridyltransferase. Infants with this condition appear normal at birth and usually develop satisfactorily for about the first two weeks. The presenting symptoms are fever, vomiting, inability to gain weight, and at times diarrhea. Jaundice, hepatomegaly, and splenomegaly are consistently found. Ascites may occur. If the disease is diagnosed at this stage and if a lactose-free diet is prescribed, the symptoms abate and the child progresses normally. If undiagnosed by four to eight weeks of age, cataractous changes usually develop. Eventually there will be mental retardation. It has been reported that after the diagnosis has been made and milk and milk-containing foods have been removed from the diet, the cataracts will regress. In our experience, some punctate opacities of the crystalline lens will remain. A comparable situationis seen in diabetes mellitus in children where the rapidity of the cataract formation may be great but in which the cataract changes may regress partially or almost completely once the disease is controlled. That a correct diet will control

galactosemia and in many cases partially reverse the lens opacities had been known for many years (Teramoto Y. et.al.2008; Haskovic M. et.al.2020; Huseynova L.S., et.al 2021).

Cataracts developed in patients from seven days to six months. If the cataracts had not appeared by the age of six months, they seemed unlikely to develop. The cataracts were irreversible even with a galactose-free diet unless the diet was started at a very early age (Ashino J., et al.1995; Timson D.J. 2019; Huseynova L.S., et.al.2023).

In reviewing the genetics of patients with galactosemia it is evident that most authors have considered the defect to be an autosomal recessive mendelian characteristic, adefect being carried by 2 heterozygous parents.

Galactosemia is a heterogeneous hereditary disease controlled by GALT, GALK 1, GALE and GALM genes. Type 1 galactosemia (Duarte galactosemia) is caused by mutations in the *GALT* gene. *GALT* gene mapped on chromosome 9p13 (Calderon F.R., et.al. 2007; Henderson H. et.al.2002).

Patients with galactosemia type 2 with galactokinase deficiency have abnormal amounts of galactose in their blood and galactitol in their urine. As a result of the accumulation of galactitol in the lens, cataracts form in them from the first periods of life. Galactokinase enzyme is encoded by GALK1 gene. The GALK1 gene is located at the 17q24 locus on autosomal chromosome 17. A mutation in the GALK 1 gene causes type 2 galactosemia. The disease is inherited in an autosomal recessive manner. Cataract development can be completely prevented if the diagnosis is made early and a galactose-restricted diet is introduced and strictly followed (Ficicioglu C., et.al.2008; Berry G.T., et al 2021; Delnoy B., et al. 2021).

Type 3 Galactosemia causes by mutations in the *GALE* gene. GALE gene located on chromosome 1p36 and encoding the UDP-galactose 4-epimerase enzyme. Galactose epimerase deficiency is caused by mutations in the *GALE* gene (Coffee B., et.al.2006; Waisbren S.E., et.al.2012).

Type 4 galactosemia is a disease manifested as a result of a malfunction of the galactose mutarotase (aldose 1-epimerase) enzyme. This enzyme is encoded by the GALM gene located at the 2p22.1 locus. Congenital galactosemia is caused by galactose mutarotase enzyme deficiency due to mutations in the GALM gene. Cataracts are mainly observed in these patients. Gastrointestinal symptoms and severe liver dysfunction are not observed. If treated early with a galactose-restricted diet, neurological and other symptoms are not observed. (Lukac-Bajalo J., et.al.2006; Kotb M.A., et. Al.2018; Pasquali M. et al. 2018; Kotb M.A., et. Al.2019).

Galactosemia is an inborn error of carbohydrate metabolism. The metabolic defect is a deficit in the activity of a specific enzyme, galactose-l-phosphate uridyl transferase. Infants with this condition appear normal at birth and usually develop satisfactorily for about the first two weeks. The presenting symptoms are fever, vomiting, inability to gain weight, and at times diarrhea. Jaundice, hepatomegaly, and splenomegaly are consistently found. Ascites may occur. If the disease is diagnosed at this stage and if a lactose-free diet is prescribed, the symptoms abate and the child progresses normally. If undiagnosed by four to eight weeks of age, cataractous changes usually develop. Eventually there will be mental retardation. It has been reported that after the diagnosis has been made and milk and milk-containing foods have been removed from the diet, the cataracts will regress (Pyhtila B.M., et al. 2015). Rogers and Berton have elaborated this point. They have shown the different amino acids in the chromatographic spectra of normal tissues such as kidney, liver, and heart and have compared them to pathological tissues. The use of paper chromatography has been one of the simplest tests but now there are more direct laboratory methods based on the demonstration of the fundamental enzyme defect. Modifications of an assay of red blood cells for transferase, first demonstrated by Anderson et.al.,25 in 1957, is the generally accepted technique. The enzyme activity is practically nil in hemolysates of galactosemic individuals and is reduced in those that are carriers (Anderson, E. P., et.al.1957; Novelli G., et.al.2000).

Chemical analysis of the plasma electrolytes is generally normal unless the infant develops severe diarrhea and dehydration. Liver function tests will vary. There may be elevation of serum bilirubin. The cephalin flocculation and the thymol turbidity may be abnormal. Bromosulfophthalein dye retention tests usually show high levels if the patient is taking a diet containing galactose but the dye retention returns to normal after the patient is on a galactose-free diet. The fasting blood glucose levels are normal and serum-alpha aminonitrogen concentrations are not elevated (Bell S.J., et al.2020).

It has been considered that a derivative of galactose rather than sugar itself was toxic to the brain and the lens of the eye. Galactose-l phosphate is known to accumulate in tissues of galactosemic patients,

and it has been postulated that this compound causes lens and brain damage. On the other hand, galactitol is the product of a possibly important alternate pathway for the disposition of galactose, and it may be toxic to tissues, at least to the lens (Katre D., et.al.2022).

2. Materials And Methods

All molecular genetic methods for detecting mutations are based on differences in the DNA sequence. An equal number of age and sex-matched healthy controls with normal vision and with no previous history of cataract surgery were recruited as controls. Material used was venous blood with anticoagulant of 50 patients in 2016–2022. 25 of them were patients with idiopathic presenile cataract, 0-45 years of age and 25 age and sex matched controls for the study. Genome DNA was obtained by automatic isolation from 200 μL of venous blood. The DNA concentration was measured by the Digital spectrometer. The venous blood for research was drawn into a tube containing EDTA or heparin. Genomic DNA and RNA kits made by Qiagen GmbH (Hilden, Germany) were used for analysis.

The integrity of the isolated genomic DNA was detected in a 2% agarose gel. Integrity and quantity of genomic DNA and polymerase chain reaction (PCR) products were identified by electrophoresis on 2% agarose gel (PowerPacBasicGelDoc™ EZ; Bio-Rad Laboratories, Hercules, CA, USA). Classical galactosemia mutations (Q188R, K285N) and Duarte galactosemia mutations (N314D) were identified by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP). The expected band pattern showed the presence or absence of mutation.

The genome DNA underwent the PCR procedure for every protein-encoding exon of the *GALT* gene. Positive PCR samples that were checked by electrophoresis in agarose gel were purified by an enzymatic method. Purified product was dyed with fluorescent dye by BiqDye Terminator V.3.1. (Applied Biosystems, Foster City, CA, USA) and processed by Cycle Sequencing PCR. Positive Cycle Sequencing PCR samples, controlled by electrophoresis in agarose gel, were extracted from the BiqDye XT (Applied Biosystems with dye-purifying agent.

Polymerase chain reaction was carried out in a following condition: denaturation at 96 °C for 30 seconds; annealing at 55 °C for 30 seconds; extension at 75 °C for 1 min. This cycle was repeated 25 times, 72 °C for 10 min. and 4 °C pause. The PCR was carried out on a Professional Thermocycler Biometra system (Biometra Biomedizinische Analytik GmbH, Göttingen, Germany). A pair of for-ward and reverse primers was used for each genomic fragment. For the purification of DNA fragments after the first stage of PCR, a set of magnets was used: Agencourt AMPure XP PCR purification and SPRIPlate 96 Super Magnet Plate (Beckman Coulter Inc., Beverly, CA, USA). The second amplification of the purified DNA fragments was carried out in the following condition: denaturation at 95 °C for 30 seconds; annealing at 55 °C for 30 seconds; extension at 77 for 2 min. This cycle was repeated 25 times, and 72 °C for 10 min. and 4 °C pause.

The polymerase chain reaction (PCR) products were sequenced with the Sanger sequencing method in an automatic sequencing system ABI 7500 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). The results were investigated using FinchTV software (version: 1.4.0; Geospiza, Seattle, WA, USA). The results of sequencing were compared to the reference sequence in the GenBank database (Figure 1).

Study has been carried out at ANAS Institute of Genetic Resources, “Laboratory of human genetics” and AFGENE laboratory in Baku, Azerbaijan.

3. Results and Discussion

For the first time in the population of the Republic of Azerbaijan, we aimed at studying the molecular-genetic characteristics of the Galactosemic cataract disease. A review and discussion of the clinical entity galactosemia has been given (Figure 1).

Biochemical and molecular-genetic analyzes were performed in both control and experimental groups. Patients were diagnosed with increased levels of galactose and galactose-1-phosphate in the blood and absence of GALT activity.

In this study we analyzed Q188R, N314D and K285N mutations among cataract disease patients. A total of 50 people, including 25 controls and 25 patients, were sampled in the study. For detect the N314D mutation we used 5′ACTGTAAAAGGGCTCTCTCTCC3′ (Forward) and 5′GCAAGCATTTCGTAGCCAA3′ (Reverse) praymers. The length of the PCR product was 171 b.p.

No mutant allele for Q188R and K285N mutations was observed in either the patients or controls. The N314D mutation was present in 2 patients. No mutant allele was observed in either the controls. In one

of the patients the heterzygous form of the same mutation, and the homozygous form in the other patient were found.

1. 2.

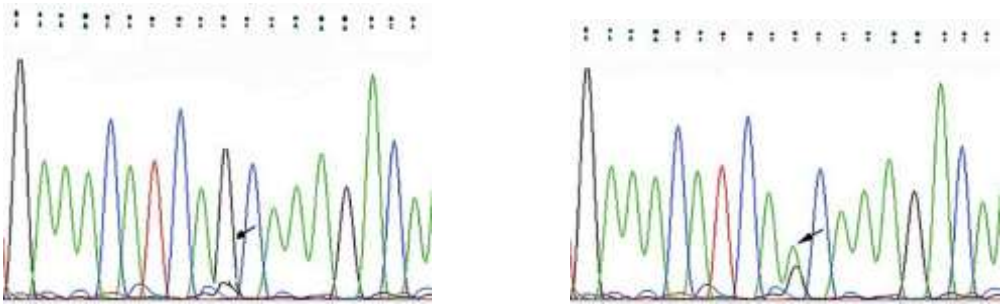

Figure 1. The results of sequencing:1-homozygous form of N314D190(A>G) mutation; 2-Heterozygous form of N314D190(A>G) mutation.

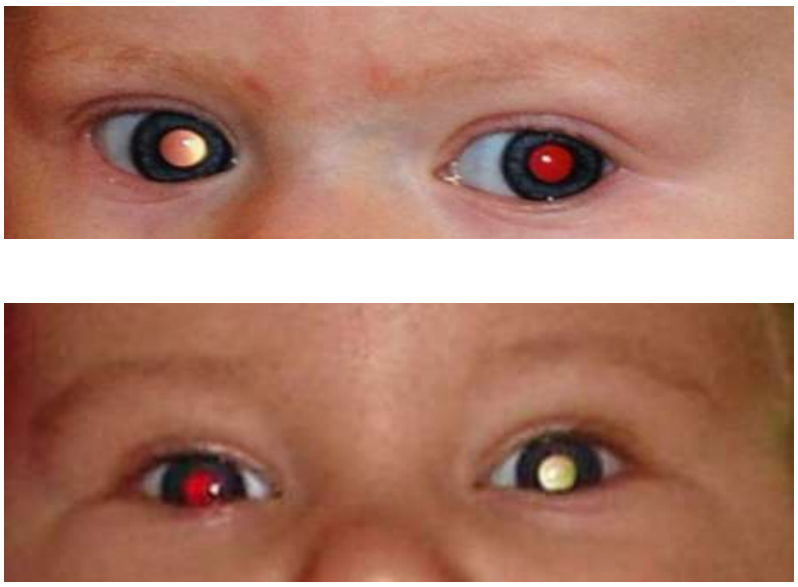

Figure 2. Galactosemic cataract patients with N314D mutation

Cataract is one of the most common symptoms of galactosemia. The genetics of galactosemic patients are evaluated and details of galactose metabolism are described. Alternate carbohydrate pathways are considered. The laboratory experiments included are concerned with a study in rats of a specific derivative of galactose, galactitol (dulcitol), which may be implicated in the development of cataracts (Lenhart P.D., et.al.2022).

In our experience, some punctate opacities of the crystalline lens will remain. A comparable situation is seen in diabetes mellitus in children where the rapidity of the cataract formation may be great but in which the cataract changes may regress partially or almost completely once the disease is controlled (Yuzyuk T. et.al.2018).

That a correct diet will control galactosemia and in many cases partially reverse the lens opacities had been known for many years, but until the true nature of the disease and its cause were ascertained many patients died undiagnosed. The presence of a reducing substance in the urine should make the physician suspect the disease. Urinalyses of infants with galactosemia also usually reveal a generalized proteinuria and amino-aciduria. The reducing substances give a positive reaction to Benedict's solution but are negative with glucose oxidase. The use of paper chromatography is now a standard means of differentiating various sugars. It has been used for the identification of other substances in many pathological conditions (Coss K.P., et.al.2013; Huseynova L.S. 2023).

Surgery has seldom been necessary for galactosemic cataracts and there is a paucity of references to it in the literature. However, any appreciable amount of lens opacity can cause visual impairment which is a handicap to the individual even if not severe enough to warrant surgical intervention (Demirbas D., et.al.2018).

Thus, an early diagnosis and the prescription of a proper diet are mandatory for the preservation of better vision. Early detection of patients and their families, prompt and correct diagnosis, and the importance of genetic counseling play an important role in the treatment of the disease.

We selected 25 cataract patients aged 0-45 years and 25 age- and sex-matched controls for the study. Mutations in the GALT gene were determined by polymerase chain reaction and sequencing methods. The most common mutations in the GALT gene, Q188R, K285N and N314D, were studied in selected patients. They were diagnosed with elevated blood levels of galactose and galactose-1-phosphate and lack of GALT activity. In the studied patients, the N314D mutation in the GALT gene was detected only in 2 patients from the experimental group. A homozygous form of N314D mutation was detected in one of these patients, and a heterozygous form in the other. This study shows that GALT mutations are ethnically specific and that galactosemia is a heterogeneous disease at the molecular level.

4. Conclusion

Galactosemia is one of the hereditary disorders of carbohydrate metabolism, accompanied by the inability to convert galactose into glucose. The present study was undertaken because of a particular interest of the author in infants and children with a clinical and laboratory diagnosis of galactosemia. Classical galactosemia mutations (Q188R, K285N) and Duarte galactosemia mutations (N314D) were identified by polymerase chain reaction and sequencing methods. The symptoms of these patients were reviewed. Patients presented with jaundice, diarrhea, vomiting, malnutrition, allergies, convulsions and mostly cataracts in the neonatal period. Galactose metabolism disorders are more likely in patients with cataracts. Biochemical and molecular-genetic analyzes were performed in both control and experimental groups. Patients were diagnosed with increased levels of galactose and galactose-1-phosphate in the blood and absence of GALT activity. In this study, we analyzed the Q188R, N314D, and K285N mutations among cataract patients. No mutations were detected in the control group. In the experimental group, the N314D mutation was detected in 2 patients. In one of the two patients, the N314D mutation was detected in a homozygous state, and in the other, in a heterozygous state. Mutations Q188R and K285N were not detected in this group. The control of the disease is affected by the elimination of lactose and galactose from the diet. Therefor early detection of patients and their families, prompt and correct diagnosis, and genetic counseling are extremely important for timely and correct treatment of the studied patients.

Conflict of Interest.

No conflict of interest.

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Data Availability

Within the article

Ethics Approval

Not ethical approval

Authorship Contributions.

H.L. collected samples, and performed the analyses. Also, H.L. and A.A. are designed the study, wrote the manuscript.

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