Molecular Characterization of Galactosemia and Identification of GALT Gene Mutations

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Abstract

Classic Galactosemia is an inherited metabolic disease with life threatening symptoms in newborn. Nevertheless, early diagnosis can be managed the symptoms and mortality. The disease caused by a severe deficiency of the enzyme galactose-1-phosphate uridyl transferase. Mutations in this gene can cause a defect in this enzyme. Identification of these mutations can play an important role in early diagnosis and disease management. It is necessary to perform galactosemia screening despite difficulties and complexities. This study identified 5 different pathogenic mutations on the GALT gene in the Azerbaijanian population with galactosemia. The identification of the mutations involved in the development of CG in the Azerbaijanian population can play an essential role in early diagnosis and intervention. The GALT gene mutations identified in this study can be used as screening markers to identify Azerbaijanian children with CG.

Keywords: Galactosemia, metabolic disorders, enzyme, metabolism, screening

Introduction

Galactosemia is one of the metabolic disorders discovered in newborn screening (1). In other words, galactosemia is an inherited disorder of galactose metabolism due to deficiency in one of the three enzymes involved in the Leloir pathway (2). Galactose-1-phosphate uridyltransferase (GALT), galactokinase, and uridine diphosphate glucose (UDP)-galactose-4-epimerase are members of the Leloir pathway of galactose metabolism (3). The deficiency of any of the three enzymes
causes galactose accumulation in plasma which causes galactosemia. Classic galactosemia (CG) is caused by mutations in the GALT gene. In CG, The GALT enzyme, which is responsible for conversion of galactose-1-phosphate (Gal-1-P) and UDP glucose into glucose-1-phosphate and UDP-galactose, loses its activity. The GALT deficiency leads to the accumulation of Gal1-P in various organs leading to the clinical presentations. The CG is an autosomal recessive disturbance of galactose metabolism (4, 5). The treatment of CG is diet limitations of lactose-containing foods (6-8). The CG occurs with a frequency of approximately 1.2 in 10,000 to 1 in 60,000 live births (9). The incidence rate of CG in southern of Iran is 1 in 28,000 live births (10). The GALT gene is located on chromosome 9p13 and contains 11 exons (11). The CG is heterogeneous at clinical and molecular levels; accordingly, 336 different mutations have been identified in recent years (12, 13). Several common mutations are detected in the GALT gene. The most prevalent observed is the Q188R mutation, which has been reported to account for 54 - 70% of CG (1315). The Q188R, L195P, and K285N mutations are common (16, 17) in Caucasian sand whites; however, the S135L mutation in African populations has been reported as the common mutation (18-20). The N314D variant is a common change in Duarte galactosemia (21). The identification of mutations involved in the development of CG in each population can play an essential role in early diagnosis and intervention (22, 23).

Materials and Methods

The genetic screening of galactosemia genetic exchange disease was carried out during 2016-2022 among children born in hospitals of Baku city and different regions, patients who applied to the Scientific Research Pediatric Institute of the Ministry of Health of the Republic of Azerbaijan. Biochemical analysis and genetic screening of a total of 494 newborns, including 80 in the control group and 414 in the experimental group, and a total of 450 patients, including 70 in the control group and 380 in the experimental group, were performed for galactosemia disease. For the investigation of the variants of the GALT gene in patients with galactosemia, all patients were subjected to exons sequencing of the GALT gene; accordingly, the blood samples were collected from each patient in tube containing ethylene di amine tetra acetic acid. Genomic deoxyribonucleic acid (DNA) was extracted from peripheral blood white cells via a genomic DNA extraction kit (Add Bio Inc., Korea) following the manufacturer’s procedure. Eight pairs of primers were designed for sequencing the GALT gene, covering all 11 exons of the gene (1,28). 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. Finally, the type and frequency of GALT gene mutations were evaluated in Azerbaijanian patients with galactosemia (Figure 1).
Table 1. Primer Sequence

<table>
<thead>
<tr>
<th>Primer Number</th>
<th>Exon Number</th>
<th>Sequence 5' - 3'</th>
<th>PCR Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>E1, E2</td>
<td>F: 5'- AAAGTGAAAGGTAGGCGACG -3'</td>
<td>750 bp</td>
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<tr>
<td></td>
<td></td>
<td>R: 5'- TGACCCAGAAGGAGTTGAC -3'</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>E3, E4</td>
<td>F: 5'- GCCTGTCCAGTCTTTGAAGC -3'</td>
<td>350 bp</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: 5'- GGTGAAAGTGATTAAGAGGGG -3'</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>E5</td>
<td>F: 5'- CACAGCGCAAGCCTACCTTC -3'</td>
<td>190 bp</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: 5'- ACCTCACAAACCTGACACCCAA -3'</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>E6</td>
<td>F: 5'- CTITTTGGCTAAGAGCTCCG -3'</td>
<td>200 bp</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: 5'- TTCCATGTCCACAGTGCTCC -3'</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>E7, E8</td>
<td>F: 5'- ACCTGCGCTTTCTCTGTC -3'</td>
<td>700 bp</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: 5'- TACTGGGAGCAACCCTCCATC -3'</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>E9</td>
<td>F: 5'- GCTGAGTAGTCCAGGCTCTGATTC -3'</td>
<td>155 bp</td>
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<td></td>
<td></td>
<td>R: 5'- CCAGAAATGTGTTGGGTGGCT -3'</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>E10</td>
<td>F: 5'- GGTTTGGAGTGGTGCTGC -3'</td>
<td>320 bp</td>
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<td></td>
<td></td>
<td>R: 5'- GGGCAACAGAAGTATCAAGG -3'</td>
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</tr>
<tr>
<td>8</td>
<td>E11</td>
<td>F: 5'- GAAATCCATGCAACCATTCT -3'</td>
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<tr>
<td></td>
<td></td>
<td>R: 5'- TTCAAGGCTTTTCTGCTTA -3'</td>
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</tr>
</tbody>
</table>

Abbreviations: PCR, polymerase chain reaction.
Research Results and Their Discussion

H132Q, M142K, R148W mutations in the 5th exon of the GALT gene, R258G, R231H mutations in the 8th exon, K285N mutation in the 9th exon, 10th Q334K, P325L and N314D mutations in the exon were studied. Four different mutations of the GALT gene (P325L, H132Q, N314D, Q334K) were detected in the samples of the experimental group in homozygous, heterozygous and compound forms. N314 mutation (4 of 9 patients), Q334K mutation (2 of 9 patients), compound form of N314D/Q334K mutations (1 of 9 patients), P325L mutation (1 of 9 patients), H132Q (1 of 9 patients also) has been detected. Thus, the gene frequency of the N314D mutation in the GALT gene was 0.0042, the gene frequency of the Q334K mutation was 0.0021, and the gene frequencies of the N314D/Q334K, P325L and H132Q mutations were recorded as 0.01.

In similar studies, Reichardt et al. reported this mutation in the United States, which results in the formation of an unstable polypeptide and an inefficient enzyme (29). This mutation is also encountered in patients of African origin (4, 19, 20). Lai et al. reported that S135L mutation in the GALT gene is a prevalent cause of galactosemia among black patients, and GALT activity varies in different tissues of patients homozygous for S135L (18).

A320T and Y209S mutations were also detected with similar frequency (3.22%), which are likely to be family- specific. The aforementioned mutations were previously reported in the Iranian and European populations (24). In the current study, the patients who carried mutations in the GALT gene showed reduced GALT enzyme activity, compared to the normal range; this finding is consistent with the results of a study conducted by Garcia et al. (6) related to GALT enzyme activity. Routine screening tests for galactosemia help diagnose the disease quickly,
manage symptoms after a positive test, carry out a careful clinical assessment quickly, and perform genotyping to determine whether the patient will be kept on a galactose restriction diet (25-27). One of the limitations of this study is the small number of the studied samples, which suggests that this test should be performed on a larger population. In addition, as previously mentioned in the background, there are other genes in the path of galactose metabolism that cause different types of galactosemia; therefore, it is recommended to check the sequence of these genes in patients.

**Conflict of Interests.** There is no conflict of interest in the submission of this manuscript.

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**References**


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