Short Communication

Detection of common mutations in the GALT gene through ARMS

Umair Mahmood a,1,2, Muhammad Imran b,2,3, Salma Iqbal Naik a, Huma Arshad Cheema c, Anjum Saeed c, Muhammad Arshad d,⁎, Saqib Mahmood d⁎

⁎ Corresponding author at: Department of Human Genetics & Molecular Biology, University of Health Sciences, Khayaban-e-Jamia Punjab, Lahore 54600, Pakistan.
⁎⁎ Present Address: Institute of Biochemistry and Biotechnology (IBB), University of Veterinary and Animal Sciences (UVAS), Lahore, 54000, Pakistan.
⁎⁎⁎ E-mail addresses: medgen@uhs.edu.pk, sqb_medgen@yahoo.com (S. Mahmood).

a Present Address: Department of Biochemistry, University of Health Sciences, Khayaban-e-Jamia Punjab, Lahore 54600, Pakistan.
b Centre for Research in Endocrinology and Reproductive Sciences, Department of Physiology and Cell Biology, UHS.
c Department of Pediatric Gastroenterology and Hepatology, The Children's Hospital & The Institute for Child Health, Lahore 54600, Pakistan.
d Department of Genetics and Molecular Biology, UHS.

A R T I C L E   I N F O

Article history:
Accepted 3 August 2012
Available online 16 August 2012

Keywords:
Galactosemia
GALT
Duarte galactosemia

A B S T R A C T

Type I galactosemia is an inborn error resulting from mutations on both alleles of the GALT gene, which leads to the absence or deficiency of galactose-1-phosphate uridylyltransferase (GALT), the second of three enzymes catalyzing the conversion of galactose into glucose. On the basis of residual GALT activity, Type I galactosemia is classified into severe “Classical” and mild “Duarte” phenotypes. Classical galactosemia is frequently associated with S135L, Q188R and K285N mutations in the GALT gene. The functionally neutral N314D variation in the GALT gene is associated with Duarte galactosemia and is widespread among various worldwide populations. The present study aimed at detecting S135L, Q188R and K285N mutations and the N314D variant in the GALT gene by PCR using amplification refractory mutation system (ARMS). ARMS assays were established using standard DNA samples and were used for 8 galactosemia patients and 190 unrelated normal subjects all of Pakistani origin. S135L and K285N mutations were present neither in galactosemia patients nor in normal subjects. Only one galactosemia patient carried Q188R mutation that was in homozygous state. However, the N314D variant was frequently found both in affected (7 out of 16 alleles) and normal subjects (55 out of 380 alleles). This finding indicates that Duarte allele D314 might be far more common in Pakistani population than in European and North American ones.

© 2012 Elsevier B.V. All rights reserved.
More than 230 mutations in the GALT gene (MIM# 606999) have been reported (Calderon et al., 2007a; Õunap et al., 2010). The most frequent of these mutations are S135L in Blacks (Lai et al., 1996) and Q188R and K285N in Caucasians (Kozak et al., 1999; Mirzajani et al., 2006; Õunap et al., 2010; Tyfield et al., 1999). A common D314 (N314D) allele of the GALT gene is widespread among worldwide populations with a pan-ethnic frequency of near 8 to 11% (Carney et al., 2009; Suzuki et al., 2001). This D314 allele is functionally neutral and is referred to as Duarte galactosemia allele (D2) when it is linked to a 4-bp deletion (c.-119→-116delGCTA) in the promoter of the GALT gene (Carney et al., 2009) and as Los Angeles galactosemia allele (D1) when it is linked to a silent substitution of thymine (CTA→TTA encoding L218L) at codon 218 of the GALT gene (Langley et al., 1997). While the 4-bp deletion is associated with impaired promoter activity and under-expression of the GALT gene (Carney et al., 2009), the CTA→TTA substitution is proposed to improve the translational efficiency of the gene transcripts (Langley et al., 1997). These molecular mechanisms may be responsible for variable GALT activity in hemolysates of Duarte (Carney et al., 2009) and Los Angeles galactosemia patients (Langley et al., 1997).

S135L, Q188R and K285N mutations and the N314D variant are commonly screened to confirm the diagnosis of galactosemia in newborn screening programs (Calderon et al., 2007b; Dobrowolski et al., 2003). In this study, we have developed ARMS-based PCR assays (Newton et al., 1989) for the screening of these common mutations. The assays were validated in Pakistani galactosemia patients and normal subjects.

### 2. Subjects and methods

A total of 8 unrelated galactosemia patients (3 males and 5 females of age range 1.6–15 months) were recruited from the Department of Pediatric Gastroenterology and Hepatology, Children’s Hospital and Institute of Child Health, Lahore. Diagnosis of galactosemia included typical clinical symptoms, elevated serum bilirubin, AST and ALT levels, normal hemoglobin level, and a positive test for urinary reducing substance with glucose level within normal range. Clinical symptoms of some of these individuals have also been used in a previous study entitled “Frequency distribution of PRNP polymorphisms in the Pakistani population” (Imran et al., 2012). Approval for the study was obtained from Ethical Review Board of University of Health Sciences, Lahore and informed signed consent was obtained from patients’ parents.

DNA was extracted from whole blood following a standard organic procedure. Allele specific primers for S135L, Q188R and K285N mutations and the N314D variant were designed using the GALT gene sequence (GeneBank accession # M96264.1). Primer sequences and annealing temperatures are provided in Table 2. PCR ingredients consisted of 50 ng DNA, 1× Taq buffer [75 mM Tris–HCl (pH 8.8 at 25 °C), 20 mM (NH4)2SO4 and 0.1% (v/v) Tween 20], 2 mM MgCl2, 200 μM of each dNTP, 5 pmol of each primer and 0.5 U of Taq DNA polymerase (Fermentas Biosciences, USA). The final reaction volume was 25 μl. Thermal PCR profile consisted of an initial denaturation at 95 °C for 5 min, 35 cycles of denaturation at 94 °C for 30 s, annealing at 54–66 °C for 30 s and extension at 72 °C for 1 min, followed by a final extension step at 72 °C for 10 min. PCR products were resolved in 2% ethidium bromide-containing agarose gels and were visualized under UV light (Fig. 1). Standard DNA samples carrying S135L, Q188R and K285N mutations were provided by Dr. Raza Mirfakhari from the National Institute of Genetic Engineering and Biotechnology, Tehran, IR, Iran. These samples served as positive controls. A DNA pool from 110 Pakistani unrelated individuals served as positive control for assaying the N314D variant. Each sample was analyzed for three times to determine specificity of the results.
**3. Results and discussion**

We have developed simple, cost-effective, and rapid ARMS-based PCR assays (Newton et al., 1989) for the screening of S135L, Q188R and K285N mutations and the N314D variant in the GALT gene. These assays are superior to widely used conventional SNP genotyping assays such as restriction fragment length polymorphism (RFLP) and allele specific oligonucleotides melting which require additional post-PCR process, time and ingredients to provide information about the genotype of a sample. In ARMS-based PCR assays, the genotype of a PCR-amplified DNA sample can directly be analyzed on agarose gel after its electrophoretic separation.

As reported by Newton et al. (1989), the PCR amplification of normal and mutant alleles was carried out in separate reactions. Both homozygous and heterozygous samples were used as positive controls. A wide range of temperature gradients (51 °C to 67 °C) was used to find stringent annealing temperature. The amplification of S135L, Q188R and K285N normal and mutant alleles was not detected when a DNA pool containing 110 unrelated DNA specimens in equivalent concentration was used as template. The N314D variant was detected in heterozygous state in this DNA pool. We considered the DNA pool to serve as positive control for subsequent PCR assays. Both homozygous and heterozygous positive controls showed specific amplification of S135L, Q188R and K285N normal and mutant alleles at annealing temperatures given in Table 2. The positive control for S135L mutation served as negative control for the screening of Q188R, K285N and N314D variations and vice versa. DNA from peripheral leukocytes of each of 8 galactosemia patients studied was isolated on 2–3 different occasions and three serial amplifications on the same day were performed for each isolated DNA specimen. The results were concordant every time. These findings indicate that the developed ARMS-based PCR assays may be useful for the diagnosis of galactosemia.

They may also be used to determine the frequency of carriers in general population.

In contrast with galactosemia patients from Western countries, our galactosemia patients presented with more severe clinical symptoms such as vomiting, diarrhea, failure to thrive, weight loss, jaundice, hepatosplenomegaly, ascites, and developmental delay. These symptoms were seen with 100% frequency in our patients (Table 1). This contrast in symptoms outcome might be due to age difference at which ours and Western galactosemia patients are hospitalized. Half of our galactosemia patients (4/8) developed bilateral cataracts. None of screened mutations was detected in two of these galactosemia patients manifesting bilateral cataracts. The remaining two galactosemia patients were homozygous for the N314D variant. Of the four galactosemia patients without cataracts, one inherited the Q188R mutation in homozgyous state and the remaining 3 the N314D variant in heterozygous state (Fig. 1). The L135, R188 and N285 alleles were not found in 190 normal subjects. The Duarte allele D314 was detected in 14.5% of normal alleles (55 out of 380 alleles). These results suggest that some other mutations in the GALT gene may be responsible for classical galactosemia in Pakistan. Therefore, sequencing of the GALT gene in our classical galactosemia patients may disclose novel pathogenic mutations. According to this study, the frequency of D314 allele is higher in Pakistan than in many Caucasian populations and in Americans (Table 3). Due to higher frequency of the D314 allele, the Duarte galactosemia (D1 or D2/G) may be more frequent in Pakistani population than classical galactosemia (G/G). However, large patients' sample size is required to confirm the results of the present study. Because the Duarte galactosemia patients usually present with milder phenotype in adulthood (Fernhoff, 2010; Ficicioglu et al., 2010), their identification during infancy is difficult to carry out. In such situations, the implementation of newborn screening programs becomes a prerequisite. It should be noticed that although the patients of Duarte galactosemia, caused by one D314 allele and the other “G” allele, usually appear to be benign, some of these patients may develop ovarian failure and presenile cataracts later in life (Bari et al., 2008). The differential production of endogenous galactose may be responsible for this difference.

**References**


---

![Fig. 1. Screening of S135L, Q188R and K285N mutations and N314D variant in eight Pakistani (1–8) galactosemia patients. PC refers to positive control and NTC to non-template control.](image-url)

**Table 3**

Allele frequencies of N314D variant in different worldwide healthy populations.

<table>
<thead>
<tr>
<th>Population</th>
<th>Number of individuals</th>
<th>Allele frequency (%)</th>
<th>Significance</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pakistan</td>
<td>190</td>
<td>14.5</td>
<td>Present study</td>
<td>Ko et al. (2010)</td>
</tr>
<tr>
<td>Korea</td>
<td>100</td>
<td>2</td>
<td>S</td>
<td>Milánkovic et al. (2009)</td>
</tr>
<tr>
<td>Hungary</td>
<td>100</td>
<td>11.5</td>
<td>NS</td>
<td>Bari et al. (2008)</td>
</tr>
<tr>
<td>Croatia</td>
<td>221</td>
<td>7.5</td>
<td>S</td>
<td>Bari et al. (2008)</td>
</tr>
<tr>
<td>Italy</td>
<td>802</td>
<td>7.7</td>
<td>S</td>
<td>Bari et al. (2008)</td>
</tr>
<tr>
<td>Ireland</td>
<td>743</td>
<td>9.9</td>
<td>S</td>
<td>Tighe et al. (2004)</td>
</tr>
<tr>
<td>Slovenia</td>
<td>174</td>
<td>8</td>
<td>S</td>
<td>Lukac-Baja et al. (2002)</td>
</tr>
<tr>
<td>USA</td>
<td>4796</td>
<td>7.8</td>
<td>S</td>
<td>Suzuki et al. (2001)</td>
</tr>
<tr>
<td>Czech Republic</td>
<td>504</td>
<td>8.2</td>
<td>S</td>
<td>Kozák et al. (1999)</td>
</tr>
<tr>
<td>Germany</td>
<td>289</td>
<td>14.9</td>
<td>NS</td>
<td>Shin et al. (1998)</td>
</tr>
<tr>
<td>UK</td>
<td>246</td>
<td>17</td>
<td>NS</td>
<td>Morland et al. (1998)</td>
</tr>
</tbody>
</table>

*P ≤ 0.05 = significant (S); P > 0.05 = non-significant (NS).


