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Background: Considering a few studies on the genetic basis of the cystinuria in the Middle East and the population-specific distribution of mutations in the SLC3A1, we tried to find genetic variants in three exons (1, 3, and 8) of SLC3A1. Materials and Methods: In this study, exons 1, 3, and 8 of SLC3A1 gene of 25 unrelated cystinuria patients searched for genetic variations by polymerase chain reaction and sequencing. Results: There were five different variations in our studied population. We found one mutation in the SLC3A1 gene including missense variant M467K and identified three polymorphisms: nonsynonymous variant G38G, c. 610 + 169C>T and c. 610 + 147C>G within the SLC3A1 gene, and one new variant. Conclusion: Our results confirm that cystinuria is a heterogeneous disorder at the molecular level and more studies are needed to identify the distribution and frequency of mutations causing cystinuria in the Iranian population.

Key words: Aminoaciduria, cystinuria, rBAT, SLC3A1, transport

INTRODUCTION

Cystinuria is an autosomal recessive disorder characterized by the abnormal urinary excretion of cysteine and the dibasic amino acids in the renal tubule and epithelial cells of small intestine.[1] Two genes including SLC3A1 and SLC7A9 are associated with cystinuria and two different types of cystinuria have been known according to genetic defects. Type I of cystinuria is caused by mutations in SLC3A1, an amino acid transporter gene located on chromosome 2 (2p16.3-21), and consists of 10 exons ranging in span from 120 to 461 bp which encodes the b0,+ transporter-related protein (rBAT). This protein creates the heavy chain of the renal cystine transport system (rBAT/b0,+ AT). Due to its biological functions, mutations in SLC3A1/rBAT probably cause protein misfolding and trafficking defects. Mutations in b0,+ AT cause loss of function of the transporter system b0,+ by defect in folding and trafficking.[2] In addition, digenic inheritance (Type AB) has been described.[3] In previous studies, about 133 mutations in SLC3A1 have been described. These mutations consist of nonsense, missense, splicing, frame shifts, and large sequence rearrangements.[2,4,5] An overview on the most frequent cystinuria mutations in SLC3A1 gene in different ethnic groups shows that the selected exons are very important.[6]

Considering a few studies on the genetic basis of the cystinuria in the Middle East and the population-specific distribution of mutations in the SLC3A1, we tried to find genetic variants in three exons (1, 3, and 8) of SLC3A1.

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MATERIALS AND METHODS

Twenty-five cystinuria unrelated patients were referred from Al-Zahra Hospital, Isfahan University of Medical Sciences, by a urologist (11 women and 14 men). All patients had a history of recurrent cysteine stones. These patients were selected according to the type of cystine stones in the patients who had been subjected to operation for removing kidney stones. The appropriate informed consent was obtained from all patients. The Ethics Committee of the Medical University of Isfahan approved this study according to the National Helsinki guidelines (Declaration of Helsinki) (Research Project Number: 294207).

About 10°C of blood was taken from each patient in tubes containing EDTA. DNA of the samples was extracted according to the standard protocol of kit (Bio Genet kit, Korea). Polymerase chain reaction was used to amplify three pairs of SLC3A1 gene primers (exons 1, 3, and 8) in chromosome 2 (2p16.3-21) [Table 1]. Primers were designed using primer blast program (htpp://www.ncbi.nlm.nih.gov/tools/primer-blast) according to the genomic sequence references available at the Genome Browser (Gene ID: 6519, updated on December 6, 2016). Finally, All samples were sequenced using Sanger sequencing method (Macro Gene Co. Korea).

RESULTS

According to direct sequencing of exons 1, 3, and 8 of SLC3A1 gene, we found five patients (C4, C6, C10, C21, C23, and C25) who were heterozygote for the polymorphism G38G in exon 1 [Figure 1a]. G38G (c. 114A>C) makes a synonymous variant of GGA (glycine). The amino acid variant M467K detected in exon 8 in one patient (C18). This mutation also was heterozygote. M467K is a missense mutation that changes methionine (ATG) to lysine (AAG) at position 179 T/A. The methionine at codon 467 of rBAT sequences was completely conserved in all species. This mutation leads to reduction in transport activity of cystine and dibasic amino acids. M467K has been described as a pathogenic mutation [Figure 1b]. Two intronic variants in intron three including c. 610+147C>T and c. 610+169C>G were also identified in one patient (C20) [Figure 1c and d].

Table 1: Sequence of primers and condition for amplification of SLC3A1 exons

<table>
<thead>
<tr>
<th>Exon</th>
<th>Primers (forward/reverse)</th>
<th>Condition of PCR</th>
<th>Size of amplified fragment (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exon 1</td>
<td>5'-TTACCCTTTCTTCCTTGCTG-3' 5'-AACCAGCTGGGTCTCGTGAG-3'</td>
<td>Initial denaturation (°C, min) 94, 4 Denaturation (°C, s) 94, 20 Annealing (°C, s) 59, 30 Extension (°C, s) 72, 50</td>
<td>758</td>
</tr>
<tr>
<td>Exon 3</td>
<td>5'-GGCAAGATGGAAGAGGTTT-3' 5'-ACTGGCCCCTCCCGATCAA-3'</td>
<td>94, 4 94, 20 62, 30 72, 50</td>
<td>742</td>
</tr>
<tr>
<td>Exon 8</td>
<td>5'-AAGGCTGGCAGTGAGTACC-3' 5'-CAGACCCCAAAGGAAGCTGA-3'</td>
<td>94, 4 94, 20 62, 30 72, 50</td>
<td>472</td>
</tr>
</tbody>
</table>

PCR = Polymerase chain reaction
African, American, European, and South Asian people, respectively (http://www.ensemble.org). All patients in our study had percutaneous nephrolithotomy. In our research, we found that 1 of the 25 (4%) patients had M467K mutation in exon 8. In addition, six of patients (24%) showed G38G polymorphism in exon 1 and 7 out of 25 (28%) had a new mutation in 5’UTR (c.‑29A>G) in the SLC3A1 gene. Both intronic mutations (c. 610+147C>T, c. 610+169C>G) found in 1 out of 25 patients.

CONCLUSION

In conclusion, our results confirm that cystinuria is a heterogeneous disorder at the molecular level. This study contributes to the understanding of the distribution and frequency of mutations causing cystinuria in the Iranian population.

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Nil.

Conflicts of interest

There are no conflicts of interest.

AUTHORS’ CONTRIBUTION

SM contributed in the acquisition, analysis of data for the work and drafting the work, and agreed for all aspects of the work. MK contributed in the conception of the work, conducting the study, revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work. AD contributed in the conception of the work, approval of the final version of the manuscript, and agreed for all aspects of the work. MM contributed in the clinical conception of the work, approval of the final version of the manuscript, and agreed for all aspects of the work.

REFERENCES


Table 2: Variants in SLC3A1 gene in cystinuria patients

<table>
<thead>
<tr>
<th>Mutation/polymorphism</th>
<th>Effect coding sequence</th>
<th>Nucleotide change</th>
<th>Exon/intron</th>
<th>Number of patients with mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>M467K</td>
<td>Met→lys</td>
<td>T→A at 1478</td>
<td>Exon 8</td>
<td>One</td>
</tr>
<tr>
<td>G38G</td>
<td>No aa change</td>
<td>C or A at 114</td>
<td>Exon 1</td>
<td>Five</td>
</tr>
<tr>
<td>c. 610+147C&gt;T</td>
<td>5’ intron 3</td>
<td>C→T at 610+147</td>
<td>Intron 3</td>
<td>One</td>
</tr>
<tr>
<td>c. 610+169C&gt;G</td>
<td>5’ intron 3</td>
<td>C→G at 610+169</td>
<td>Intron 3</td>
<td>One</td>
</tr>
<tr>
<td>c.‑29A&gt;G (new mutation)</td>
<td>5’UTR</td>
<td>G→A</td>
<td>Exon 1</td>
<td>Seven</td>
</tr>
</tbody>
</table>

M = Methionine; K = Lysine; G = Glycine; aa = Amino acid; 5’UTR = 5’ untranslated region