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Cystinuria in a Patient With a Novel Mutation in SLC7A9 Gene

Leila Koulivand, Mehrdad Mohammadi, Behrouz Ezatpour, Majid Kheirollahi

Cystinuria, one of the first inborn errors of metabolism, is characterized by hyperexcretion of cystine, arginine, lysine, and ornithine into urine. Cystinuria is genetically classified into types A and B. Mutations in the SLC3A1 gene lead to type A, and type B is caused by mutations in the SLC7A9 gene. We described a 19-year-old woman that had early onset of cystine calculus formation at the age of 3 years. After DNA extraction and polymerase chain reaction, direct sequencing was performed. By these methods, a novel nucleotide substitution c.177G>A in exon 3 of the SLC7A9 gene was found, which had not been reported elsewhere previously. This nucleotide substitution occurs in the extracellular domain of the SLC7A9 gene. In addition, a previously described intron variant c.1136+2/3delT (intron 6 of SLC3A1) in homozygosity status was detected in the patient. To our knowledge, this is the first report of novel nucleotide substitution c.177G>A in exon 3 of the SLC7A9 gene.

INTRODUCTION

Cystinuria, one of the first inborn errors of metabolism explained by Archibald Garrod (MIM# 220100), is characterized by hyperexcretion of cystine and dibasic amino acids (arginine, lysine, and ornithine) into urine. The disease is caused by the impaired transport of these compounds across the apical membrane of epithelial cells of the proximal renal tubule and gastrointestinal tract. Hyperexcretion of cystine in the urinary tract most often leads to formation of recurring cystine stones. Some of risk factors for urolithiasis include genetic, metabolic, nutritional, infectious, anatomical, and environmental disorders. Incomplete treatment or delay in the diagnosis of calculi may cause damage to the kidney and the renal parenchyma by obstruction. It has been estimated that the worldwide prevalence of the disease is 1 per 7000, although it varies considerably between specific populations, ranging from 1 per 2500 among Libyan Jewish population to 1 per 100 000 persons in Sweden.

To date, 2 genes associated with cystinuria have been identified. The SLC3A1 (2p16.3) creates the heavy subunit rBAT of the renal b0,+ transporter, and the SLC7A9 (19q13.1) encodes the light subunit b0,+AT. A novel classification for cystinuria based on genetics has been defined as types A, B, and AB. Type A cystinuria is caused by mutations in the SLC3A1 gene, type B includes mutations in the SLC7A9 gene, and type AB is caused by 1 mutation in the SLC3A1 and one mutation in the SLC7A9. Over 100 mutations in the SLC3A1 and nearly 100 mutations for SLC7A9 gene were identified. Despite the population-specific distribution of mutations in the SLC3A1 and SLC7A9 genes, there are few genetic data reported for Asian patients with cystinuria. We describe a patient with a novel mutation in the SLC7A9 gene.
CASE REPORT

A 19-year-old woman was referred to the local Alzahra Hospital, in Isfahan. She had early-onset cystine calculus formation at the age of 3 years and presented elevated urine cystine level and recurrent cystine calculi. This patient had undergone surgery for 7 times, 3 of which had been open surgery. The open surgeries had been performed on one kidney for 2 times and once on the other kidney. The other operations had been performed using the percutaneous nephrolitotomy technique. Moreover, she has had shock wave lithotripsy for 10 times. She had been treated by classic treatment including D-penicillamine, captopril, and potassium citrate. Despite treatment, many calculi were formed in her kidneys. Her 24-hour urine volume was 2.5 L to 3 L and urine pH was 6.8 to 7, which was maintained. According to the latest intravenous pyelography, both kidneys had acceptable function. Renal parenchyma was thin and multiple scars resulting from surgery were seen in the kidney tissue.

Genomic DNA was extracted from peripheral blood lymphocytes using standard procedures (Bio Genet kit, Korea). The coding sequence of the exon 3 (SLC7A9) and 6 (SLC3A1) was amplified by intron-derived primers. Primers were designed using primer blast tool (www.ncbi.nlm.nih.gov/tools/primer-blast) according to the genomic sequence references available at the Genome Browser (http://www.ensemble.org; Table 1). Polymerase chain reaction was carried out on 25 µL solution containing 150 ng of genomic DNA, 2.5 µL of 10X polymerase chain reaction buffer, 1 U of Taq-DNA-polymerase, 200 µmol/L of dNTPs, and 400 nmol/L of primer forward and reverse. The temperature profile for the 35-cycle amplification reaction is shown in Table 2. Polymerase chain reaction products were sequenced by the Applied Biosystems 3730 /Genetic Analyzer and using the BigDye terminator kit. We identified the described polymorphism c.1136+2/3delT (intron 6 of SLC3A1) in homozygosity status and a novel nucleotide substitution c.177G>A (exon 3 of SLC7A9) detected in heterozygosity status for the described patient (Table 1).

DISCUSSION

A previous study impressively reflects the population-specific distribution of mutations in cystinuric patients. Former investigations reported novel mutations for cystinuria patients in Portugal, Sweden, Turkey, Serbia, Czech, Japan, and China. We detected the previously described polymorphism c.1136+2/3delT in intron 6 SLC3A1 gene in homozygosity status and the novel nucleotide substitution c.177G>A in heterozygosity status in this case. This nucleotide substitution occurs in exon 3 and involves substitution of G by A at position c.177. It changes threonine ACA codon to ACG codon of same amino acid (Figure). Considering this

<table>
<thead>
<tr>
<th>Gene</th>
<th>Exon</th>
<th>Nucleotide Change</th>
<th>Amino Acid Change</th>
<th>Primers</th>
</tr>
</thead>
</table>
| SLC3A1 | 6 | c.1136+2/3delT | ... | 6F: 5’-TATAGAGCGAGCTGTGGGCA-3’
| | | | | 6R: 5’-TGCCTTGGCCTCCTACAGTG-3’|

| SLC7A9 | 3 | c.177G>A | T59T or activating a cryptic splice site | 3F: 5’-TACCGAGGGAGGGTGAGGGGCA-3’
| | | | | 3R: 5’-AAGAGGGGATCTGAGGGGT-3’|

<table>
<thead>
<tr>
<th>Exon</th>
<th>Initial Denaturation</th>
<th>Denaturation</th>
<th>Annealing</th>
<th>Extension</th>
<th>Final Extension</th>
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</thead>
<tbody>
<tr>
<td>3</td>
<td>94°C, 4 min</td>
<td>94°C, 20 sec</td>
<td>55.5°C, 30 sec</td>
<td>72°C, 30 sec</td>
<td>72°C, 5 min</td>
</tr>
<tr>
<td>6</td>
<td>94°C, 4 min</td>
<td>94°C, 20 sec</td>
<td>57.5°C, 30 sec</td>
<td>72°C, 40 sec</td>
<td>10 min</td>
</tr>
</tbody>
</table>
transition creates AG site, it may be important in splicing process. Some mutations which appear to be silent may not be because they affect splicing by activating a cryptic splice site or by altering an exon splice enhancer sequence. The nucleotide substitution c.177G>A occurs in the extracellular domain of the SLC7A9 gene. According to data (polyphen), this substitution is highly conserved among other species. Several studies have analyzed the relation between dietary intake of multiple nutrients and excretion of lithogenic and inhibitory substances in urine.30

ACKNOWLEDGMENTS
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CONFLICT OF INTEREST
None declared.

REFERENCES
