A Novel Mutation in *SLC7A9* Gene in Cystinuria

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Introduction. Cystinuria is an inherited disorder affecting luminal transport of cystine and dibasic amino acids. Because of the poor solubility of cystine in urine, stone formation in the kidney occurs frequently. Cystinuria is associated with mutations in the *SLC3A1* and *SLC7A9* genes. Despite the population-specific distribution of mutations in the *SLC7A9* genes, there are few genetic data reported for cystinuric patients from the Middle East.
Materials and Methods. Exon 4 of the *SLC7A9* gene was sequenced in 21 patients with cystinuria, using the polymerase chain reaction and sequencing methods.
Results. A new variation in exon 4 of the *SLC7A9* gene was identified, which was insertion of 1 adenine nucleotide between 2 cytosine nucleotides in position c.272-273 insA.
Conclusions. It seems to be important since it causes frame shift and it may be an important cause to make disease.

Keywords. Cystinuria, gene mutations; *SLC7A9* gene

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INTRODUCTION

Cystinuria is an autosomal recessive disorder with an overall prevalence of 1 per 7000 population, which varies from one area to another in the world.[1-3] The disease is caused due to defective transporter protein.[4,5] This protein has 2 subunits, the light subunit ($b^{o,+}$AT) and the heavy one (rBAT),[6] which are respectively encoded by the *SLC3A1* and *SLC7A9* genes.[7,8] *SLC3A1* is mapped on chromosome 2p16.3-21, whereas *SLC7A9* is located on 19q13.1.[9]

Classically, cystinuria is biochemically classified based on the excretion of cysteine. Dibasic aminoaciduria into type I (autosomal recessive), non-type I (autosomal dominant with incomplete penetrance), and mixed type (also known as type I/non-type I) are the three types of cystinuria disease. A new classification of cystinuria is defined according to molecular genetics data: type A (mutation in *SLC3A1*), type B (mutation in *SLC7A9*), and type AB (mutation in both genes).[8-11]

SLC7A9 gene has 13 exons, and some of previously known mutations of *SLC7A9* gene exist in exon 4 including G105R, T123M, V170M, and A126T. Among these mutations, G105R is very important and pathogenic. In addition, p.G105A accounts for approximately 20% of identified alleles and is present in nearly all ethnic groups. The second frequent mutation is p.R333W (in exon 10). Other variants are often rare.[12]

Despite the population-specific distribution of mutations in the *SLC7A9* genes, there are few genetic data reported for Asian and Middle-Eastern patients with cystinuria. The aim of the present research was to find mutations in the *SLC7A9* gene among Iranian cystinuric patients in order to find causes of the disease.

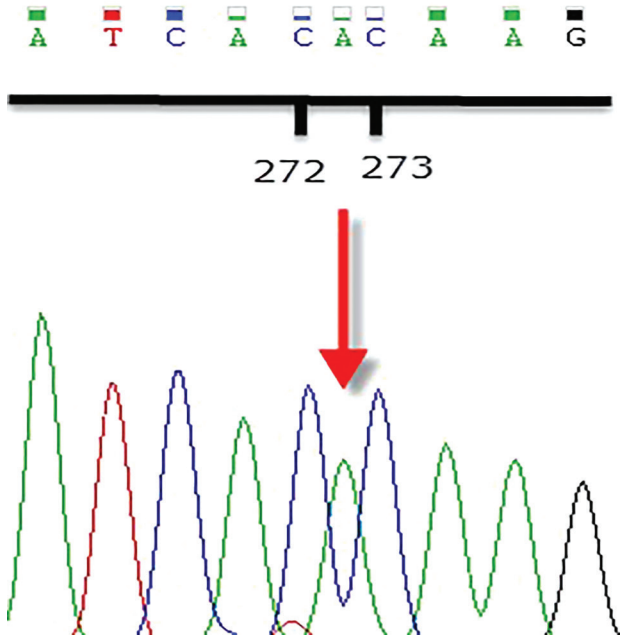
MATERIALS AND METHODS

This study was done in the genetics laboratory of Isfahan University of Medical Sciences. Blood samples were taken from patients at Alzahra Hospital. DNA was extracted from 200 µL of each blood sample using the PrimePrepTM Genomic DNA Extraction Kit (Genetbio, Korea) according to the manufacturer's instructions. Polymerase chain reaction (PCR) primers for exon 4 were designed using the PrimerIII software (Howard Hughes Medical Institute and by the National Institutes

of Health, USA). The sequences of the primers used PCR of exon 4 were as follows: Forward primer: 5′-CCA GTA TCC CTC TTG GCA CA-3′ and Reverse primer: 5′-AGA GAC TCA CTG CGG AGG AG-3′. Then, the PCR method was used to amplify exon 4 of the *SLC7A9* gene. A PCR was carried out on a 25-µL sample containing 100 ng of genomic DNA, 12.5 µL of Taq DNA Polymerase Master Mix Red (Ampliqon, Denmark), and 8.5 µL of ddH2O. The PCR program was started with an initial denaturation at 95°C for 4 minutes, followed by 30 repetitive cycles with a strand separation step at 95°C for 30 seconds, an annealing step at 56°C for 30 seconds, and an extension step at 72°C for 30 seconds that was finished with a 5-minute extension period at 72°C. The PCR product and 50-bp ladder (DNA marker) were run in 1.5% agarose gels. The PCR products were sequenced by the Applied Biosystems 3730/Genetic Analyzer and using the BigDye terminator kit.

RESULTS

Based on results of sequencing and nucleotide waves from 21 patients, a new variation in exon 4 of the *SLC7A9* gene was identified: insertion of 1 adenine nucleotide between 2 cytosine nucleotides in position c.272-273 insA. The results of electropherography and blast of nucleotides with reference sequence showed insertion of 1 adenine nucleotide in exon 4 (Figure).[13,14] The Table shows



DNA sequence analysis showing a novel mutation in the *SLC7A9* gene. Electropherography of nucleotide A insertion (c.272-273 insA). Blast of nucleotide with reference sequence shows insertion of 1 adenine nucleotide in exon 4.

Results of Screening for Mutations and c.272-273 insA Variant in Exon 4 of *SLC7A9* Gene

Patient	Age	Sex	Number of Recurrent Calculi	Age at First Diagnosis of Calculi	Homozygote Versus Heterozygote
1	55	Male	> 10	30 mo	Homozygote
2	27	Female	> 10	7 y	Homozygote
3	41	Male	3	34 y	Homozygote
4	41	Male	> 10	21 y	Homozygote
5	34	Female	> 10	23 y	Homozygote
6	57	Male	Unknown	27 y	Homozygote
7	36	Female	> 10	Unknown	Homozygote
8	42	Male	> 10	28 y	Homozygote
9	23	Female	2	Unknown	Homozygote
10	30	Female	> 10	25 y	Homozygote
11	7	Male	> 10	at first	Homozygote
12	25	Female	> 10	17 y	Homozygote
13	44	Female	1	34 y	Homozygote
14	14	Female	4	6 mo	Homozygote
15	41	Male	5	30 y	Homozygote
16	32	Female	7	12 mo	Homozygote
17	65	Male	> 10	35 y	Homozygote
18	40	Female	3	31 y	Homozygote
19	23	Male	5	18 y	Homozygote
20	47	Female	Unknown	15 y	Homozygote
21	37	Female	Unknown	Unknown	Homozygote

clinical information of patients.

DISCUSSION

Previously, studies indicated the population-specific distribution of mutations in cystinuric patients.[15] Literature showed novel mutations for cystinuric patients in Portugal, Sweden, Turkey, Serbia, Czech, Japan, and China. Approximately 95 mutations have been reported in the *SLC7A9*.[4,16-19] Despite specific demographic distribution of mutations for cystinuria disease, few studies exist on the genetic basis of this disease in the Middle East.[20-22] In this study, we observed a new insertion in position c.272-273 insA that has not been seen previously.[5,6,23] By using Ensemble program (variant effect predictor) and the UMD Software (www.umd.be/hsf), it was predicted that frameshift occurred.

CONCLUSIONS

Our newly identified insertion in position c.272-273 insA in the *SLC7A9* gene can be a key in diagnosis and treatment of cystinuric patients in Iran and potentially the Middle East. This mutation can cause serious defect in protein structure by making changes in intronic positions and create splicing sites. In addition, it can create a premature stop codon and thus produce a truncated protein. Further studies are necessary on this new mutation in other populations.

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CONFLICT OF INTEREST

None declared.

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