IJKD KIDNEY DISEASES

A Novel Mutation in *SLC3A1* Gene in Patients With Cystinuria

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Cystinuria is an inherited disease characterized by the formation of cystine calculi in the kidneys, ureters, and bladder. Cystinuria is associated with mutation in the *SLC3A1* and *SLC7A9* genes. These defects prevent appropriate reabsorption of dibasic amino acids lysine, ornithine and arginine. Cystinuria is classified as type I (silent heterozygotes) and non-type I (heterozygotes with urinary hyperexcretion of cystine). In molecular term, cystinuria is classified as type A (mutations on *SLC3A1* gene) and type B (mutations on *SLC7A9* gene). This report describes 7 patients with early onset of cystine calculus formation. We are report a new mutation in *SLC3A1* gene in exon 1. A novel nucleotide substitution c.-29A>G was found in exon 1 of the *SLC3A1* gene, which had not been reported elsewhere previously.

IJKD 2016;10:44-7
www.ijkd.org

Keywords. cystinuria, urinary calculi, gene mutations

Case Report

INTRODUCTION

Cystinuria is an autosomal recessive disorder of amino acids transport affecting the epithelial cells of the renal tubules and gastrointestinal tract.[1] It is characterized by increased urinary excretion of cystine and the dibasic amino acids (lysine, ornithine and arginine).[2,3] The average prevalence is 1 in 7000 births, but it ranges from 1 in 2500 births in Jewish Israelis of Libyan origin to 1 in 100 000 in Swedes.[4,5] Cystinuria is the only disorder which is exclusively caused by gene mutations. Two genes responsible for cystinuria have been identifying: *SLC3A1* (2p16.3-21), which encodes the heavy subunit rBAT, and *SLC7A9* (19q12-13.1), which encodes its interacting light subunit $b^{0,+}$AT. The rBAT/$b^{0,+}$AT is linked by a disulphide bridge.[6-8]

Classically, cystinuria was biochemically classified according to the excretion of cystine and 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).[6,9,10] A new classification of cystinuria has emerged based on molecular genetics data: type A (mutation in *SLC3A1)*, type B (mutation in *SLC7A9*), and type AB (mutation in both genes).[11] In this report, we identified a new mutation in exon 1 of the *SLC3A1* gene. We investigated 7 patients from Isfahan affected with cystinuria (unrelated families).

CASE REPORT

Seven unrelated patient with cystinuria were referred to the Alzahra Hospital, in Isfahan (Table 1). Family consanguinity was not reported by any of the patients. The diagnosis had been established on the basis of kidney stone formation, urinary sediment, and urinary amino acids profile. Informed consent was obtained from all of the patients. Amino acids profile in urine amino acid excretion was determined, using morning urine samples, and whenever possible, a 24-hour collection of urine. Urinary amino acid profile was determined by liquid ion-exchange chromatography.

Table 1. Characteristics of Patients With Cystinuria*

Patient	Sex	Age, y	Age at Diagnosis, y	Intervention Prior to Diagnosis	Cystine Excretion, mmol/mol	Sum of Excretions,* mmol/mol	Family History of Calculi	Calculi	Urinary Tract Infection	Medical Treatment
1	Female	30	25	Multiple ESWL	675	1032	Sister and brother	Unilateral	Yes	D-penicillamine
2	Male	7	< 1	Nephrolithotomy	367	1484	Father	Unilateral	No	No
3	Male	34	26	Multiple ESWL and PCNL	105	2287	Brother and father	Unilateral	Yes	No
4	Female	24	19	PCNL and multiple ESWL	267	1265	No	Unilateral	Yes	D-penicillamine and citrate
5	Male	41	22	Multiple PCNL and ESWL	454	1760	Sister	Unilateral	Yes	Citrate and captopril
6	Female	27	17	Multiple ESWL	472	823	No	Bilateral	Yes	D-penicillamine and citrate
7	Female	20	18	PCNL and multiple ESWL	235	1876	No	Unilateral	No	Captopril

*ESWL indicates extracorporeal shockwave lithotripsy and PCNL, percutaneous nephrolithotomy.
†Sum of arginine, lysine, ornithine, and cystine excretions in mmol/mol of creatinine

The samples were collected in tubes containing ethylenediaminetetraacetic acid.

DNA was extracted from blood sample (Bio Genet, Korea). Polymerase chain reaction (PCR) was used to amplify 1 pair primer of *SLC3A1* gene (exon 1) in chromosome 2 (2p16.3-21). 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 2). Each PCR reaction contained 30 µL of master mix, 5 µL of genomic DNA template, 1.2 µL of each primer, 0.25 µL pf Taq-DNA-polymerase, 17.8 µL of nuclease-free water, 3 µL of 10X PCR buffer, 0.9 µL of magnesium chloride, and 0.6 µL of dNTP. Polymerase chain reaction amplifications were conducted on an Eppendorf thermocycler (Hauppauge, NY, USA). The PCR conditions were denaturing at 94°C for 4 minutes, followed by 35 cycles at 94°C for 20 seconds, annealing at 59°C for 30 seconds, extension at 72°C for 50 seconds, and a final extension at 72°C for 10 seconds. For all PCR reactions, positive and negative controls were being run parallel to each test sample. For the positive control, all reaction mixtures were used except for the template DNA and other known DNA templates. For the negative control, all reaction mixtures except DNA were used, to ensure the amplification quality. The PCR product and 50-bp ladder (DNA marker) was run in 1.5% agarose gels as shown. The PCR products were sequenced by the Applied Biosystems 3730/Genetic Analyzer and using the BigDye terminator kit.

We found a novel mutation in the acceptor region in exon 1 (*SLC3A1* gene) and detected a heterozygosity status for the described patients.

DISCUSSION

Former studies indicate the population-specific distribution of mutations in cystinuric patients.[12] Literature showed novel mutations for cystinuria patients in Portugal, Sweden, Turkey, Serbia, Czech, Japan, and China. Approximately 133 mutations have been reported in *SLC3A1* and nearly 95 mutations in *SLC7A9*.[13-18] Despite the population-specific distribution of mutations for this disease, there are limited studies on the genetic bases of the cystinuria in the Middle East.[19,20] Sequencing of 7 patients with cysteine calculi represented a new mutation. We identified a novel nucleotide

Table 2. Primers for Amplifications of Exon 1 in *SLC3A1* Gene

Gene	Exon	Primer Forward	Primer Reverse	Amplified Fragment
SLC3A1	1	5'-TTA CCC TTT CTT CCT TGG CTG -3'	5'-AAC TGC TGG GTT CTG CTG AG -3'	758 bp

substitution c.-29A>G (exon 1 of *SLC3A1*) detected in heterozygosity status for the described patients (Table 1). In this study, the sequence position was 50 in 5 prime untranslated region of exon 1 and change G to A (Figure). Mutations in the 5 prime untranslated region which cause increment or decrement of translation efficiency have been recently described as a novel molecular mechanism of disease. Alterations in the consensus sequence for the translation initiation may promote context-dependent leaky scanning of ribosomes and initiation from a downstream AUG codon.

ACKNOWLEDGMENTS

We thank the Research Affairs Office of Isfahan University of Medical Sciences for financial support.

CONFLICT OF INTEREST

None declared.

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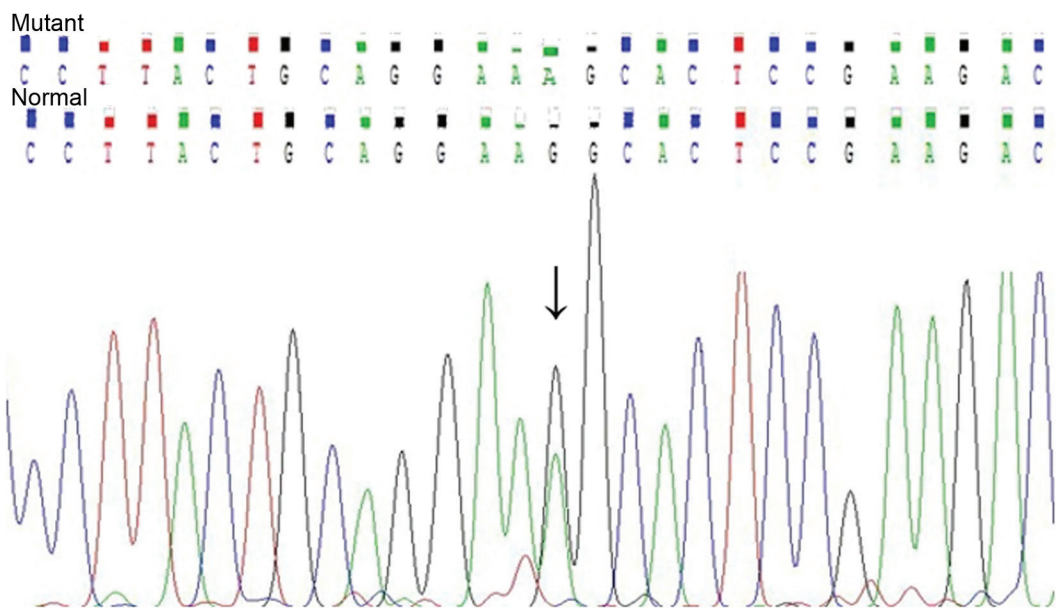

Spectrography of mutant exon 1 at coding region in *SLC3A1* gene in cystine calculi. Substitution of G to A is documented at position 50 bp of 508 bp.

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Received April 2015
Revised August 2015
Accepted September 2015