SHORT COMMUNICATION

Report of *SLC3A1*/rBAT gene mutations in Iranian cystinuria patients: A direct sequencing study

Samaneh Markazi, Majid Kheirollahi[1,2], Abbas Doosti, Mehrdad Mohammadi[3]

Department of Molecular Genetics, Faculty of Basic Sciences, Shahrekord Branch, Islamic Azad University, Sahrekord, [1]Pediatric Inherited Diseases Research Center, Research Institute for Primordial Prevention of Noncommunicable Disease, [2]Department of Genetics and Molecular Biology, School of Medicine, Isfahan University of Medical Sciences, [3]Department of Urology, Urology and Kidney Transplantation Research Center, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran

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

How to cite this article: Markazi S, Kheirollahi M, Doosti A, Mohammadi M. Report of *SLC3A1*/rBAT gene mutations in Iranian cystinuria patients: A direct sequencing study. J Res Med Sci 2017;22:33.

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 a*nd *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 $b^{0,+}$ transporter-related protein (rBAT). This protein creates the heavy chain of the renal cystine transport system (rBAT/$b^{0,+}$ AT). Due to its biological functions, mutations in *SLC3A1*/rBAT probably cause protein misfolding and trafficking defects. Mutations in $b^{0,+}$ AT cause loss of function of the transporter system $b^{0,+}$ 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*.

Access this article online

Quick Response Code:

Website: www.jmsjournal.net

DOI: 10.4103/1735-1995.202149

Address for correspondence: Dr. Majid Kheirollahi, Pediatric Inherited Diseases Research Center, Research Institute for Primordial Prevention of Noncommunicable Disease, Isfahan, Iran. Department of Genetics and Molecular Biology, School of Medicine, Isfahan University of Medical Sciences, P.O. Box: 81746-73461, Isfahan, Iran. E-mail: mkheirollahi@med.mui.ac.ir

Received: 24-10-2016; **Revised:** 10-12-2016; **Accepted:** 21-12-2016

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].

DISCUSSION

A new heterozygote mutation in exon 1 was found in seven patients. This novel variant is located in exon 1 before initiation codon in 5′ untranslated region (5′UTR) that changes G to A (c.-29A>G). Mutations in the 5′UTR cause increase or decrease of translation efficiency that recently described as a novel molecular mechanism of disease. Changes in the consensus sequence for the translation initiation may intensify context-dependent leaky scanning of ribosomes and/or initiation from a downstream AUG codon.[7] One previously identified polymorphism in codon 38 (114C-A) was also identified. G38G is synonymous variant (GGA change to GGC) because no amino acid change occurs. It only changes one codon of amino acid glycine (GGA) to another codon of glycine (GGC) at position 114 of exon 1 (c. 114A>C). In addition, the frequency of GGC codon in African, American, East Asian, European, and South Asian populations is 80%, 67%, 38%, 77%, and 86%, respectively. In our study, two known mutations (c. 610+147C>T, c. 610+169C>G) were found in intronic regions of the *SLC3A1* gene (intron 3) [Table 2]. Prevalence of c. 610+147C>T intronic mutation is 3%, 1%, 2%, and 1% in African, American, South Asian, and European people, respectively.[8-13] Intronic mutation c. 610+169C>G was found in 3%, 1%, 1% 2% of

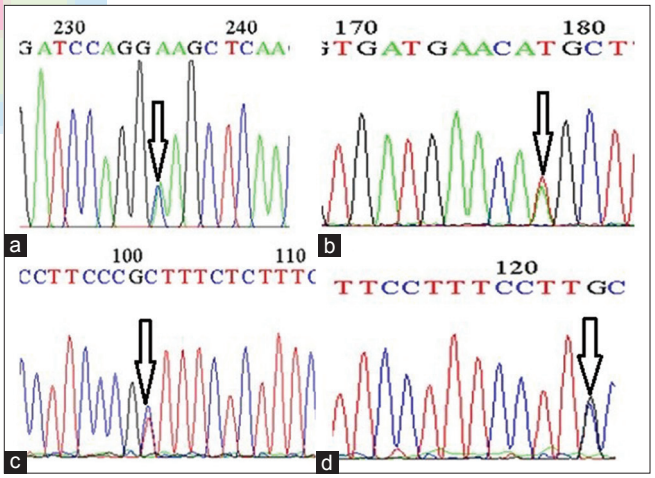

Figure 1: Electropherogram of mutations in *SLC3A1*. (a) c. 610 + 147C>T, (b) c. 610 + 169C>G, (c) M467K, (d) G38G

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

Exon	Primers (forward/reverse)	Condition of PCR				Size of amplified fragment (bp)
		Initial denaturation (°C, min)	Denaturation (°C, s)	Annealing (°C, s)	Extension (°C, s)	
Exon 1	5'-TTACCCTTTCTTCCTTGGCTG-3' 5'-AACTGCTGGGTTCTGCTGAG-3'	94, 4	94, 20	59, 30	72, 50	758
Exon 3	5'-GGCAAGATGGGGATGAGGTTT-3' 5'-ACTTGCCCCCTTCCGATAAA-3'	94, 4	94, 20	62, 30	72, 50	742
Exon 8	5'-AAGTCCAGGCTTGCTAGTACC-3' 5'-CAGACCACCAAGAAAGCTGA-3'	94, 4	94, 20	62, 30	72, 50	472

PCR = Polymerase chain reaction

Table 2: Variants in *SLC3A1* gene in cystinuria patients

Mutation/polymorphism	Effect coding sequence	Nucleotide change	Exon/intron	Number of patients with mutation
M467K	Met→lys	T→A at 1478	Exon 8	One
G38G	No aa change	C or A at 114	Exon 1	Five
c. 610+147C>T	5' intron 3	C→T at 610+147	Intron 3	One
c. 610+169C>G	5' intron 3	C→G at 610+169	Intron 3	One
c.-29A>G (new mutation)	5'UTR	G→A	Exon 1	Seven

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

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 that1of 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.

Financial support and sponsorship
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

1. Palacín M, Borsani G, Sebastio G. The molecular bases of cystinuria and lysinuric protein intolerance. Curr Opin Genet Dev 2001;11:328-35.
2. Palacín M, Nunes V, Font-Llitjós M, Jiménez-Vidal M, Fort J, Gasol E, *et al.* The genetics of heteromeric amino acid transporters. Physiology (Bethesda) 2005;20:112-24.
3. Chillarón J, Font-Llitjós M, Fort J, Zorzano A, Goldfarb DS, Nunes V, *et al.* Pathophysiology and treatment of cystinuria. Nat Rev Nephrol 2010;6:424-34.
4. Dello Strologo L, Pras E, Pontesilli C, Beccia E, Ricci-Barbini V, de Sanctis L, *et al.* Comparison between SLC3A1 and SLC7A9 cystinuria patients and carriers: A need for a new classification. J Am Soc Nephrol 2002;13:2547-53.
5. Barbosa M, Lopes A, Mota C, Martins E, Oliveira J, Alves S, *et al.* Clinical, biochemical and molecular characterization of cystinuria in a cohort of 12 patients. Clin Genet 2012;81:47-55.
6. Eggermann T, Venghaus A, Zerres K. Cystinuria: An inborn cause of urolithiasis. Orphanet J Rare Dis 2012;7:19.
7. Markazi S, Kheirollahi M, Doosti A, Mohammadi M, Koulivand L. A novel mutation in SLC3A1 gene in patients with cystinuria. Iran J Kidney Dis 2016;10:44-7.
8. Skopková Z, Hrabincová E, Stástná S, Kozák L, Adam T. Molecular genetic analysis of SLC3A1 and SLC7A9 genes in Czech and Slovak cystinuric patients. Ann Hum Genet 2005;69:501-7.
9. Tanzer F, Ozgur A, Bardakci F. Type I cystinuria and its genetic basis in a population of Turkish school children. Int J Urol 2007;14:914-7.
10. Schmidt C, Vester U, Hesse A, Lahme S, Lang F, Zerres K, *et al.* The population-specific distribution and frequencies of genomic variants in the SLC3A1 and SLC7A9 genes and their application in molecular genetic testing of cystinuria. Urol Res 2004;32:75-8.
11. Botzenhart E, Vester U, Schmidt C, Hesse A, Halber M, Wagner C, *et al.* Cystinuria in children: Distribution and frequencies of mutations in the SLC3A1 and SLC7A9 genes. Kidney Int 2002;62:1136-42.
12. Endsley JK, Phillips JA, Hruska KA, Denneberg T, Carlson J, George AL Jr. Genomic organization of a human cystine transporter gene (SLC3A1) and identification of novel mutations causing cystinuria. Kidney Int 1997;51:1893-9.
13. Eggermann T, Spengler S, Wirth J, Lahme S. Molecular genetic testing in cystinuria. Int J Hum Genet 2011;11:41-4.