Cystinuria in a Patient With a Novel Mutation in *SLC7A9* Gene

Leila Koulivand,[1] Mehrdad Mohammadi,[2] Behrouz Ezatpour,[3] Majid Kheirollahi[1]

[1]Pediatrics Inherited Diseases Research Center and department of Genetics and Molecular Biology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran
[2]Urology department, Urology and kidney transplantation research center, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran
[3]Razi Herbal Medicines Research Center, Lorestan University of Medical Sciences, Khorramabad, Iran

Keywords. cystinuria, mutation, *SLC7A9*, gene

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.

IJKD 2015;9:63-6
www.ijkd.org

INTRODUCTION

Cystinuria, one of the first inborn errors of metabolism explained by Archibald Garrod (MIM# 220100),[1] is characterized by hyperexcretion of cystine and dibasic amino acids (arginine, lysine, and ornithine) into urine.[2,3] 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.[4,5] Hyperexcretion of cystine in the urinary tract most often leads to formation of recurring cystine stones.[6,7] Some of risk factors for urolithiasis include genetic, metabolic, nutritional, infectious, anatomical, and environmental disorders.[8,9] Incomplete treatment or delay in the diagnosis of calculi may cause damage to the kidney and the renal parenchyma by obstruction.[10] 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.[7,11,12]

To date, 2 genes associated with cystinuria have been identified. The *SLC3A1* (2p16.3) creates the heavy subunit rBAT of the renal b0,+ transporter,[13,14] and the *SLC7A9* (19q13.1) encodes the light subunit b0,+AT.[15,16] 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*.[4,11,17] Over 100 mutations in the *SLC3A1* and nearly 100 mutations for *SLC7A9* gene were identified.[18-25] 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

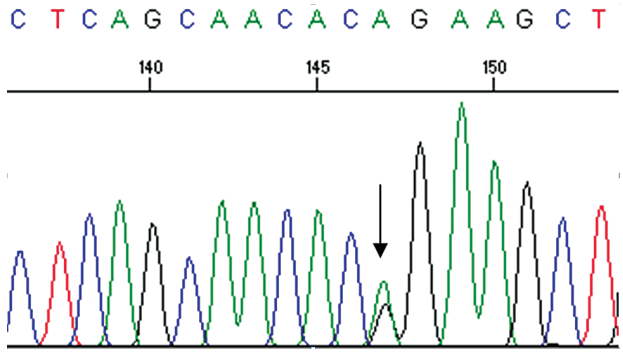
CASE REPORT

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.[26] Former investigations reported novel mutations for cystinuria patients in Portugal, Sweden, Turkey, Serbia, Czech, Japan, and China.[4,25,27-29] We detected the previously described polymorphism c.1136+2/3delT in intron 6 *SLC3A1* gene in homorozygosity status and the novel nucleotide substitution c.177G>A in heterozygosity status in this case.[27] 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



DNA sequence analysis showing a novel mutation in the *SLC7A9* genes. A nucleotide substitution c.177G>A (exon 3 of *SLC7A9*) was detected for the same patient in heterozygosity status.

Table 1. Mutations Identified in the *SLC3A1* and the *SLC7A9* Genes and Primers for Amplification

Gene	Exon	Nucleotide Change	Amino Acid Change	Primers
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'-TACCGAGGGAGGGTGGC-3' 3R: 5'-AAGAGGGATACTGGAGGGT-3'

Table 2. Temperature Profile for the 35-cycle Polymerase Chain Reaction

Exon	Initial Denaturation	Denaturation	Annealing	Extension	Final Extension
3	94°C, 4 min	94°C, 20 sec	55.5°C, 30 sec	72 °C, 30 sec	72°C, 5 min
6	94°C, 4 min	94°C, 20 sec	57.5°C, 30 sec	72 °C, 40 sec	10 min

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

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

CONFLICT OF INTEREST

None declared.

REFERENCES

1. Garrod AE. Inborn errors of metabolism (lectures I–IV). Lancet. 1908;2:1-214.
2. Palacín M, Goodyer P, Nunes V, Gasparini P. The metabolic and molecular bases of inherited diseases. New York: McGraw-Hill; 2001.
3. Palacín M, Borsani G, Sebastio G. The molecular bases of cystinuria and lysinuric protein intolerance. Curr Opin Genet Dev. 2001;11:328-35.
4. Popovska-Jankovic K, Tasic V, Bogdanovic R, et al. Five Novel Mutations in Cystinuria Genes SLC3A1 and SLC7A9. Balkan J Med Genet. 2009;12:15-20.
5. Chillarón J, Font-Llitjós M, Fort J, et al. Pathophysiology and treatment of cystinuria. Nat Rev Nephrol. 2010;6:424-34.
6. Palacín M, Nunes V, Font-Llitjós M, et al. The genetics of heteromeric amino acid transporters. Physiology. 2005;20:112-24.
7. 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.
8. Fallahzadeh MH, Zare J, Al-Hashemi GH, et al. Elevated serum levels of Vitamin D in infants with urolithiasis. Iran J Kidney Dis. 2012;6:186-91.
9. Alemzadeh-Ansari M, Valavi E, Ahmadzadeh A. Predisposing factors for infantile urinary calculus in South-west of iran. Iran J Kidney Dis. 2014;8:53-7.
10. Asl AS, Maleknejad S. Pediatric Urolithiasis An Experience of a Single Center. Iran J Kidney Dis. 2011;5:309-13.
11. Saravakos P, Kokkinou V, Giannatos E. Cystinuria: current diagnosis and management. Urology. 2014;83:693-9.
12. Knoll T, Zöllner A, Wendt-Nordahl G, Michel MS, Alken P. Cystinuria in childhood and adolescence: recommendations for diagnosis, treatment, and follow-up. Pediatr Nephrol. 2005;20:19-24.
13. Calonge MJ, Gasparini P, Chillarón J, et al. Cystinuria caused by mutations in rBAT, a gene involved in the transport of cystine. Nat Genet. 1994;6:420-5.
14. Zhang X-X, Rozen R, Hediger MA, Goodyer P, Eydoux P. Assignment of the Gene for Cystinuria (SLC3A1) to Human Chromosome 2p21 by Fluorescence< i> in Situ</ i> Hybridization. Genomics. 1994;24:413-4.
15. Feliubadaló L, Font M, Purroy J, et al. Non-type I cystinuria caused by mutations in SLC7A9, encoding a subunit (bo,+ AT) of rBAT. Nat Genet. 1999;23:52-7.
16. Škopková Z, Hrabincová E, Štástná 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.
17. Krombach P, Wendt-Nordahl G, Knoll T. Cystinuria and cystine stones: urinary tract stone disease. Springer; 2011. p. 207-15.
18. Calonge MJ, Volpini V, Bisceglia L, et al. Genetic heterogeneity in cystinuria: the SLC3A1 gene is linked to type I but not to type III cystinuria. Proc Natl Acad Sci. 1995;92:9667-71.
19. Egoshi K-I, Akakura K, Kodama T, Ito H. Identification of five novel SLC3A1 (rBAT) gene mutations in Japanese cystinuria. Kidney Int. 2000;57:25-32.
20. Font-Llitjos M, Jimenez-Vidal M, Bisceglia L, et al. New insights into cystinuria: 40 new mutations, genotype–phenotype correlation, and digenic inheritance causing partial phenotype. J Med Genet. 2005;42:58-68.
21. Guillén M, Corella D, Cabello M, et al. Identification of novel SLC3A1 gene mutations in Spanish cystinuria families and association with clinical phenotypes. Clin Genet. 2005;67:240-51.
22. Leclerc D, Boutros M, Suh D, et al. SLC7A9 mutations in all three cystinuria subtypes. Kidney Int. 2002;62:1550-9.
23. Font M, Feliubadaló L, Estivill X, et al. Functional analysis of mutations in SLC7A9, and genotype–phenotype correlation in non-Type I cystinuria. Hum Mol Genet. 2001;10:305-16.
24. Strologo LD, Pras E, Pontesilli C, 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.
25. Yuen Y, Lam C, Lai C, et al. Heterogeneous mutations in the SLC3A1 and SLC7A9 genes in Chinese patients with cystinuria. Kidney Int. 2006;69:123-8.
26. Schmidt C, Vester U, Hesse A, 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.
27. Barbosa M, Lopes A, Mota C, et al. Clinical, biochemical and molecular characterization of Cystinuria in a cohort of 12 patients. Clin Genet. 2012;81:47-55.
28. Harnevik L, Fjellstedt E, Molbæk A, Tiselius HG, Denneberg T, Söderkvist P. Identification of 12 novel mutations in the SLC3A1 gene in Swedish cystinuria patients. Hum Mutat. 2001;18:516-25.

29. Shigeta Y, Kanai Y, Chairoungdua A, et al. A novel missense mutation of SLC7A9 frequent in Japanese cystinuria cases affecting the C-terminus of the transporter. Kidney Int. 2006;69:1198-206.
30. Naseri M, Varasteh AR, Alamdaran SA. Metabolic factors associated with urinary calculi in children. Iran J Kidney Dis. 2010;4.

Correspondence to:
Majid Kheirollahi, PhD
Pediatric Inherited Diseases Research Center, Department of Genetics and Molecular Biology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran, PO Box: 81746-73461
Tel: +98 311 792 2486
Fax: +98 311 668 8597
E-mail: mkheirollahi@med.mui.ac.ir

Received March 2014
Revised July 2014
Accepted July 2014