Genotype and Phenotype Analysis in Pediatric Patients with Cystinuria

Ji Hyun Kim, Euijin Park, Hye Sun Hyun, Beom Hee Lee, Gu-Hwan Kim, Joo Hoon Lee, Young Seo Park, Hee Gyung Kang, Il-Soo Ha, and Hae Il Cheong

INTRODUCTION

Cystinuria (OMIM #220100) is an autosomal recessive genetic disorder. Cystinuria leads to defects in transepithelial transporters for dibasic amino acids, including cystine, ornithine, lysine, and arginine. Although the urine concentration of all dibasic amino acids is elevated in cystinuric patients, only cystine leads to stone disease as it is relatively insoluble at physiological pH (1–4). More than 50% of patients with cystinuria suffer from stone formation throughout their lifetime, with variable onset ages as well as a high rate of recurrence of up to 60% (5). Furthermore, since stones are likely to be formed bilaterally in more than three-quarters of cystinuric patients (4), they are at high risk of renal dysfunction and consequent poor quality of life (6).

Cystinuria types I, II, and III are traditional subgroups divided according to the urinary phenotype in heterozygotes (7). While type I heterozygotes show a normal amino aciduria, non-type I (type II and III) heterozygotes show high and moderate hyper-excretion of cystine and dibasic amino acids, respectively. Patients with type III, a mixed type, inherit type I and non-type I alleles from either parent. This classification system is still in clinical use. However, the International Cystinuria Consortium developed a new classification system to reflect the genetic and functional characteristics of the disease after the discovery of 2 genes responsible for cystinuria. Type A cystinuria is due to biallelic SLC3A1 mutations, type B cystinuria is due to dibasic SLC7A9 mutations, and type AB cystinuria is due to single heterozygous mutations in both genes (8). SLC3A1 encodes a heavy subunit of the renal cystine transport system, rBAT, and was identified as the cause of type I cystinuria. SLC7A9 encodes b+ amino acid transporter (b+AT), a light subunit of the renal cystine transport system and responsible for type II cystinuria. To date, 163 disease-causing mutations in SLC3A1 and 118 disease-causing mutations in SLC7A9 have been listed in The Human Gene Mutation Database (HGMD Professional 2016.2, https://portal.biobase-international.com/hgmd/pro/start.php).

There have been several clinical case reports and studies of Korean patients with cystinuria (6,9-14). However, only 2 case reports published include genetic studies: one was that of a 13-year-old boy with a single heterozygous SLC7A9 mutation (c.517G

Keywords: Cystinuria; Genotype; Phenotype; Gene mutation; SLC3A1 Gene; SLC7A9 Gene

Cystinuria is an inherited disorder characterized by defective renal reabsorption of cystine and dibasic amino acids leading to nephrolithiasis. This study was conducted to analyze the genotypes and phenotypes of pediatric patients with cystinuria. Eight children from Seoul National University Hospital and Asan Medical Center presenting with cystinuria from January 2003 to June 2016 were retrospectively analyzed. Mutational studies were performed by direct sequencing. Two of the 8 were male and 6 were female. The median ages at onset and diagnosis were 1.5 (range, 0.3–13.6) and 2.6 (range, 0.7–16.7) years, respectively. The median followed up was 7.7 (range, 3.4–14.0) years. Mutational analyses were performed in 7 patients and revealed biallelic SLC3A1 mutations (AA genotype) in 4 patients, a single heterozygous SLC3A1 mutation (A- genotype) in 1 patient, biallelic SLC7A9 mutations (BB genotype) in 1 patient, and a single heterozygous SLC7A9 mutation (B- genotype) in 1 patient. Two of the mutations were novel. No genotype-phenotype correlations were observed, except for earlier onset age in patients with non-AA genotypes than in patients with the AA genotype. All patients suffered from recurrent attacks of symptomatic nephrolithiasis, which lead to urologic interventions. At the last follow-up, 3 patients had a mild-to-moderate degree of renal dysfunction. This is the first study of genotypic and phenotypic analyses of patients with cystinuria in Korea.

Received: 29 August 2016
Accepted: 16 October 2016

Address for Correspondence:
Hae Il Cheong, MD
Department of Pediatrics, Seoul National University Children’s Hospital, 101 Daehak-ro, Jongno-gu, Seoul 03080, Korea
E-mail: cheonghi@snu.ac.kr

Funding: This study was supported by a grant (HI12C0014) from the Korean Health Technology R&D Project, Ministry of Health and Welfare, Republic of Korea.

© 2017 The Korean Academy of Medical Sciences.
This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/4.0) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.
Materials and Methods

Patients
Eight patients diagnosed with cystine stones during the period between January 2003 and June 2016 in 2 hospitals (Seoul National University Children’s Hospital, Seoul, Korea and Asan Medical Center Children’s Hospital, Seoul, Korea) were recruited. Their clinical presentation, clinical courses and serial laboratory findings were evaluated retrospectively.

Mutational studies
Genomic DNA was extracted from nucleated cells in peripheral blood using a commercial kit (QIAamp DNA Blood Mini Kit; Qiagen, Hilden, Germany). All coding exons and flanking introns of the SLC3A1 and the SLC7A9 genes were amplified using polymerase chain reaction followed by direct sequencing (primer sequences are available upon request).

Ethics statement
This study was approved by the Institutional Review Board at Seoul National University Hospital (IRB No. 0812-002-264). Informed consent was obtained from all individual patients included in this study or their parents.

Results
Two of the 8 patients were male and six were female. The median ages at onset and diagnosis were 1.5 years (range, 0.3–13.6 years) and 2.6 years (range, 0.7–16.7 years), respectively. They were followed up for a median period of 7.7 years (range, 3.4–14.0 years). The clinical features of the patients are summarized in Table 1. The presenting symptoms were gross hematuria (n = 3) or urinary tract infection (n = 3) in association with renal stones, while renal stones were detected incidentally in two patients. Family history of nephrolithiasis was absent in all patients, except for one (Patient 7), whose maternal uncle had a history of nephrolithiasis of unidentified composition. The initial renal function was normal (estimated glomerular filtration rate [eGFR calculated using the Schwartz formula] ≥ 90 mL/min/1.73 m²) in 2 patients, and the remaining 6 patients had various degrees of renal dysfunction. Seven patients were treated with tiopronin (thiola®) with or without potassium citrate (n = 6) and captopril (n = 1). The remaining patient (Patient 5) had a single heterozygous SLC3A1 mutation and was treated with oral sodium bicarbonate only. However, all patients suffered from recurrent attacks of symptomatic nephrolithiasis, which lead to urologic in-

Table 1. Clinical features of 8 patients with cystinuria

| Characteristics            | Patient 1 | Patient 2 | Patient 3 | Patient 4 | Patient 5 | Patient 6 | Patient 7 | Patient 8 |
|----------------------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| Gender                     | Female    | Female    | Female    | Male      | Female    | Male      | Female    | Male      |
| Age, yr                    |           |           |           |           |           |           |           |           |
| At onset                   | 12.4      | 2.7       | 13.6      | 0.4       | 1.3       | 0.4       | 0.3       | 1.6       |
| At diagnosis               | 16.7      | 2.7       | 13.6      | 0.7       | 11.4      | 0.7       | 0.8       | 2.5       |
| At last follow-up          | 23.3      | 6.9       | 17.4      | 5.5       | 19.3      | 14.2      | 8.0       | 16.3      |
| Initial presentation       | GHU       | Stone     | UTI       | UTI       | GHU       | Stone     | UTI       | GHU       |
| Family history             | –         | –         | –         | –         | –         | –         | –         | –         |
| Urolithiasis at last follow-up | +   | +         | –         | +         | +         | +         | –         | +         |
| eGFR, ml/min/1.73 m²        |           |           |           |           |           |           |           |           |
| Initial                    | 63.5      | 58.0      | 110.8     | 83.4      | 66.5      | 37.2      | 72.8      | 125.8     |
| Current                    | 74.9      | 98.6      | 98.8      | 125.0     | 83.7      | 72.9      | 135.4     | 96.4      |
| Medication                 |           |           |           |           |           |           |           |           |
| Tiopronin, mg/kg/day       | 2.9       | 2.6       | 5.8       | 7.0       | -         | 4.2       | 10.5      | 3.7       |
| Others                     | KC, Capto | KC        | KC        | -         | NaB       | KC        | KC        | KC        |
| Urologic intervention      | SWL, PNL  | PNL, UL   | SWL, UL   | SWL, UL   | SWL, UL   | SWL, UL   | SWL, UL   | SWL, PNL  |

GHU = gross hematuria, eGFR = estimated glomerular filtration rate (calculated using the Schwartz formula), KC = potassium citrate, Capto = captopril, NaB = sodium bicarbonate, SWL = shock wave lithotripsy, PNL = percutaneous nephrolithotomy, UL = ureteroscopic lithotripsy.
terventions. At the last follow-up, 3 patients had a mild-to-moderate degree of renal dysfunction.

Urinary excretion of cystine and dibasic amino acids were markedly increased in all patients, except for Patient 5 who exhibited only a mildly increased level of cystine. The cyanide nitroprusside test was performed and positive in 2 patients (Table 2). Mutational analyses revealed biallelic SLC3A1 mutations (AA genotype) in 4 patients, a single heterozygous SLC3A1 mutation (A- genotype) in one patient, biallelic SLC7A9 mutations (BB genotype) in one patient, and a single heterozygous SLC7A9 mutation (B- genotype) in one patient. In one remaining patient, mutational analysis was not performed due to unavailable samples (Table 3). The c.1820delT SLC3A1 mutation was detected in 3 of 5 patients with an AA genotype (4 of 10 alleles).

Urinary cystine excretion levels did not correlate with any clinical parameters or genotypes of the patients. We compared the clinical and laboratory findings of the 4 patients with the AA genotype to those of the others. The only difference observed was an older onset age in patients with the AA genotype. Urinary excretion levels of cystine did not correlate with any clinical parameters in the 4 patients with the AA genotype.

**DISCUSSION**

We report 8 cases of cystinuria, presenting with various symptoms. Mutational analyses revealed the AA genotype in 4 patients. Patients with the AA genotype had older onset ages than those with non-AA genotypes.

Several previous studies examining genotype-phenotype correlations in cystinuria did not show any correlation between patients with type A genotype and patients with non-A genotypes (8,15,16). In a UK study (15), patients with at least one missense mutation in SLC3A1 had significantly lower levels of lysine, arginine, and ornithine, but not cystine, than patients with all other types of SLC3A1 mutations. Another UK study (16) revealed no difference between patients with type AA and patients with type BB in a variety of clinical parameters. In addition, patients with a single mutated allele also had variable disease severity and could not be differentiated from patients with 2 mutated alleles (16). In our study, the only difference between patients with the AA genotype and those with non-AA genotypes was the later age of onset in the former group. Of the 4 patients in our study with the AA genotype, Patient 4 had 2 truncating mutations and the youngest onset age. However, urinary excretion levels of cystine were highest in Patient 2, who had 2 missense mutations. Other clinical parameters, including long-term prognosis, showed no correlation with the different genotypes.

The SLC3A1 p.T216M mutation was detected in 2 of our patients. This mutation is common in South-eastern European (17, 18), Gypsy (19), and Greek populations (20) but has not been found in Chinese (21) and Japanese (22) patients. Conversely, the SLC7A9 p.P482L mutation is common in Japanese patients (22), but has not been found in European populations. In Patient 5, we found a nucleotide variation, c.1976A>C (p.Q659P), in SLC3A1. Using Mutation Taster (http://www.mutationtaster.org/), this variation was predicted to be a polymorphism, but was not found in ExAC (http://exac.broadinstitute.org/) or 1000G (http://www.1000genomes.org/). Mild but abnormal hyperexcretion of cystine and early onset nephrolithiasis in Patient 5 suggested that the p.Q659P variation may be a hypomorphic mutation. Patient 6 had a BB genotype. However, a study of the patient’s family revealed type I cystinuria. A single heterozygous SLC7A9 mutation was detected in Patient 7. However, urinary excretion levels of branched amino acids in the patient were very high. Furthermore, her mother was heterozygous for the same mutation but was clinically silent, although urinary amino acid excretion levels were not measured in the mother. Therefore, it is quite possible that Patient 7 had another pathogenic mutation, not detected by Sanger sequencing, in SLC3A1 (AB genotype) or SLC7A9 (BB genotype). The genotype of Patient 8 was unavailable, but family study revealed type I cystinuria.

Progression to chronic kidney disease in patients with cystinuria has been reported to be between 5 and 17% (23-25), and the prevalence of end stage renal disease has been reported to be up to 5% (26). In a recent UK study (16), the level of renal impairment observed was similar across all genotypes. Recently, a recent large retrospective study conducted by the French Cystinuria Group (27) showed that 5 (1.1%) of 442 patients with cystinuria progressed to end stage renal disease at a median age of 35.0 (11.8-70.7) years. Multivariate analyses revealed that pro-

---

**Table 3. Mutations detected in the patients**

| Patients | Genes | Mutation 1 | Mutation 2 | Genotypes |
|----------|-------|------------|------------|-----------|
| Patient 1 | SLC3A1 | c.647C>T, p.T216M | c.1820delT, p.L607Hfs*4 | AA |
| Patient 2 | SLC3A1 | c.647C>T, p.T216M | c.2017T>C, p.L673R | AA |
| Patient 3 | SLC3A1 | c.46A>T, p.K16* | c.1500+1G>A, abnormal splicing | AA |
| Patient 4 | SLC3A1 | c.1820delT, p.L607Hfs*4 | c.1820delT, p.L607Hfs*4 | AA |
| Patient 5 | SLC3A1 | c.1820delT, p.L607Hfs*4 | c.1976A>C, p.Q659P | A- (or AA) |
| Patient 6 | SLC7A9 | c.1445C>T, p.P482L | c.1224+4166_1399+119del4972 | BB |
| Patient 7 | SLC7A9 | c.1224+4166_1399+119del4972 | ? | B- (?) |
| Patient 8 | Not done | - | - | Unknown |

† Novel mutations; ‡ A nucleotide variation of uncertain pathogenicity; § This large deletion causes a total deletion of exon 12.
gression to chronic kidney disease was associated with age, hypertension, severe damage of renal parenchymal defined as a history of partial or total nephrectomy, and so on (27). In our study, 3 of 8 patients had a mild degree of renal dysfunction at the last follow-up.

In conclusion, we have analyzed the genotypes and phenotypes of cystinuria in 8 pediatric patients and identified two novel mutations. We did not observe an association between clinical course and genotype, except for earlier onset age in patients with non-AA genotypes. A big limitation of this study was the small number of subjects. Therefore, a large nationwide multicenter study is recommended.

DISCLOSURE

The authors have no potential conflicts of interest to disclose.

AUTHOR CONTRIBUTION

Conceptualization: Cheong HI. Data curation: Kim JH, Park E, Hyun HS, Lee BH, Kim GH, Park YS. Investigation: Kim JH, Park E, Hyun HS, Lee BH, Kim GH, Lee JH, Park YS, Kang HG, Ha IS, Cheong HI. Writing - original draft: Kim JH, Park E, Cheong HI. Writing - review & editing: Kim JH, Park E, Kang HG, Ha IS, Cheong HI.

ORCID

Ji Hyun Kim http://orcid.org/0000-0002-3051-1691
Eujin Park http://orcid.org/0000-0002-4413-468X
Hye Sun Hyun http://orcid.org/0000-0001-8525-1471
Beom Hee Lee http://orcid.org/0000-0001-9709-2631
Gu-Hwan Kim http://orcid.org/0000-0002-7056-7446
Joo Hoon Lee http://orcid.org/0000-0001-8010-3605
Young Seo Park http://orcid.org/0000-0001-7653-2036
Hee Gyung Kang http://orcid.org/0000-0001-8323-5320
Il-Soo Ha http://orcid.org/0000-0001-5428-6209
Hae Il Cheong http://orcid.org/0000-0001-7556-1265

REFERENCES

1. Egggermann T, Venghaus A, Zerres K. Cystinuria: an inborn cause of urolithiasis. Orphanet J Rare Dis 2012; 7: 19.
2. Palacín M, Borsani G, Sebastian G. The molecular bases of cystinuria and lysinuric protein intolerance. Curr Opin Genet Dev 2001; 11: 328-35.
3. Moe OW. Kidney stones: pathophysiology and medical management. Lancet 2006; 367: 333-44.
4. Knoll T, Zühlner 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.
5. Claes DJ, Jackson E. Cystinuria: mechanisms and management. Pediatr Nephrol 2012; 27: 2031-8.
6. Shim M, Park HK. Multimodal treatments of cystine stones: an observational, retrospective single-center analysis of 14 cases. Korean J Urol 2014; 55: 515-9.
7. Rosenberg LE, Downing S, Durant JL, Segal S. Cystinuria: biochemical evidence for three genetically distinct diseases. J Clin Invest 1966; 45: 365-71.
8. Dello Strologo L, Pras E, Ponteselli C, Beccia E, Ricci-Barbini V, de Sanctis L, Ponzzone A, Gallucci M, Bisciglia L, Zelante 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.
9. Choi JE, Yun BV, Park HW, Park JH, Ha IS, Jeong HI, Choi Y, Choi H, Kim IW. Cystinuria 3 cases. J Korean Pediatr Soc 1995; 38: 245-31.
10. Kang EG, Lee JH, Lee BH, Kim GH, Park YS. A case of cystinuria with multiple renal stones in an 8-month-old girl. J Korean Soc Pediatr Nephrol 2013; 17: 122-6.
11. Lee EH, Kim YH, Hwang JS, Kim SH. Non-type I cystinuria associated with mental retardation and ataxia in a Korean boy with a new missence mutation (G173R) in the SLC7A9 gene. J Korean Med Sci 2010; 25: 172-5.
12. Lee ST, Cho H. Metabolic features and renal outcomes of urolithiasis in children. Ren Fail 2016; 38: 927-32.
13. Cho CH, Hahn KS, Park JK, Kim KH. A case of cystine stone in a child. Korean J Urol 1986; 27: 933-8.
14. Hah ST, Yoon YH, Yoon JB. Cystine stone: report of two cases. Korean J Urol 1981; 22: 451-5.
15. Wong KA, Mein R, Wiss M, Flinter E, Pardy C, Bulititude M, Thomas K. The genetic diversity of cystinuria in a UK population of patients. BJU Int 2015; 116: 109-16.
16. Rhodes HL, Yarram-Smith L, Rice SJ, Tabakser A, Coe FL. Renal function in patients with cystinuria: a population-based study. J Am Soc Nephrol 2015; 10: 1235-45.
17. Saadi I, Chen XZ, Hediger M, Ong P, Pereira P, Goodyer P, Rozen R. Molecular genetics of cystinuria: mutation analysis of SLC3A1 and evidence for another gene in type I (silent) phenotype. Kidney Int 1998; 54: 48-55.
18. Schmidt C, Vester U, Hesse A, Lahme S, Lang F, Zerres K, Egggermann T; Arbeitsgemeinschaft Pädiatrische Nephrologie. The population-specific distribution and frequencies of genotypic variants in the SLC3A1 and SLC7A9 genes and their application in molecular genetic testing of cystinuria. Urol Res 2004; 32: 75-8.
19. Popovska-Jankovic K, Tasie V, Bogdanovic R, Miljkovic P, Golubovic E, Soylu A, Saraga M, Pavicvic S, Baskin F, Akil I, et al. Molecular characterization of cystinuria in south-eastern European countries. Urolithiasis 2013; 41: 23-30.
20. Albers A, Lahme S, Wagner C, Kaiser P, Zerres K, Capasso G, Pica A, Palacin M, Lang F, Bichler KH, et al. Mutations in the SLC3A1 gene in cystinuric patients: frequencies and identification of a novel mutation. Genet Test 1999; 3: 227-31.
21. Yuen YP, Lam CW, Lai CK, Tong SE, Li PS, Tam S, Kwan EY, Chan SY, Tsang IW. Cystinuria 3 cases. J Korean Pediatr Soc 2002; 13: e806.
22. Sakamoto S, Cheong HI, Naya Y, Shigeta Y, Fujimura M, Ueda T, Mikami K, Akakura K, Masai M, Ichikawa T. Genomic characteristics of Asian cystinuria patients. Genet Test 2011; 15: 65-71.
23. Assimos DG, Leslie SW, Ng C, Streem SB, Hart LJ. The impact of cystinuria on renal function. J Urol 2002; 168: 27-30.
24. Worcester EM, Parks JH, Evan AP, Coe FL. Renal function in patients with
nephrolithiasis, J Urol 2006; 176: 600-3.
25. Worcester EM, Coe FL, Evan AP, Parks JH. Reduced renal function and benefits of treatment in cystinuria vs other forms of nephrolithiasis. BJU Int 2006; 97: 1285-90.
26. Gambaro G, Favaro S, D’Angelo A. Risk for renal failure in nephrolithiasis.
27. Prot-Bertoye C, Lebbah S, Daudon M, Tostivint I, Bataille P, Bridoux E, Brignon P, Choquenet C, Cochat P, Combe C, et al. CKD and its risk factors among patients with cystinuria. Clin J Am Soc Nephrol 2015; 10: 842-51.