Molecular genetic anaylses of cystinuria type 1 in 24 Turkish patients

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SUMMARY: Dayangaç D, Kalkanoğlu HS, Durmuş-Aydoğdu S, Erdem H, Coşkun T. Molecular genetic analysis of cystinuria type I in 24 Turkish patients. Turk J Pediatr 2001; 43: 110-113.

M467T mutation (exon 8) in rBAT gene is found to be the most common mutation in cystinuria type I patients. In our series consisting of 24 patients, the allele frequency of the M467T mutation was 8.3 percent (4/48). The second most frequent mutation at the same nucleotide position was M467K, with an allele frequency of 4.2 percent (2/48). The polymorphism which is found in linkage disequilibrium with the M467T is 231T/A (exon 1). We also found that 231T/A was associated with the M467T mutation in our series.

Key words: cystinuria type I, molecular genetics, mutation, polymorphism.

Cystinuria is an autosomal recessive disease characterized by the development of stones in the uniray tract. It is caused by the defective transport of cystine and dibasic amino acids (lysine, arginine, ornithine) through the renal tubular cells and intestinal mucosa[1]. Three different types of cystinuria (I, II and III) have been described. Type I heterozygotes excrete normal amounts of cystine in their urine, whereas types II and III heterozygotes excrete high and moderate amounts of cystine, respectively[2]. The disease has a prevalence of 1/7,000 worldwide[3]. In a screening of metabolic disorders, included 6,050 high-risk infants, the prevalence of cystinuria in Turkey was reported as 1/1,000[4]. The prevalence was also reported by Gültekin et al.[5] and Durmuş-Aydoğdu et al.[6] as 1/2,155 and 1/2,065, respectively, in a screening of normal children for cystinuria.

Cystinuria disease gene rBAT (SLC3A1), which is localized to 2p16.3-21, has been shown to be responsible for type I but not for type II and type III cystinuria[7-9]. Linkage analysis demonstrated the presence of genetic heterogeneity and mapped type III cystinuria locus to 19q13.1[10]. It was also suggested that cystinuria type II might be due to a defect at this locus[11]. Description of this new locus will allow the identification of cystinuria type II and III genes. This will be useful in understanding the biochemistry of cystine reabsorption.

The rBAT gene spans about 45 kb of genomic DNA and is composed of 10 exons[12,13]. The 2.2 kb mRNA is expressed in the kidney cortex and intestinal epithelium and encodes for a 685 amino acid, 79 kDa transport protein, for cystine and dibasic amino acids[14].

Thirty-eight mutations[15] and nine polymorphisms[14] have been described at the rBAT locus so far. M467T, which is the most common mutation, is a T→C substitution at nucleotide position 1400 (exon 8). It destroys a N1aIII restriction site in the 60 bp PCR product.

Another mutation at the same nucleotide position is M467K, which is a T→A substitution. It destroys a N1aIII restriction site and creates an AluI restriction site, yielding two fragments of 37 and 23 bp[16].

The polymorphism which is found in linkage disequilibrium with the M467T mutation is 231T/A in exon 1 in the rBAT gene. It creates a DdeI restriction site yielding two fragments of 263 and 102 bp[17].

In this study, 24 Turkish cystinuria type I patients were analyzed for the M467T and M467K mutations and 231T/A polymorphism.

Material and Methods

The cyanide-nitroprusside test was routinely applied to urine samples of all patients evaluated at the Metabolism Unit's laboratory as a component of a selective screening procedure. Patients having a positive cyanide-nitroprusside test were further analyzed by urine and blood amino acid chromatography. All patients with an increase in cystine and dibasic amino acids in their urine samples were diagnosed with cystinuria. Twenty-four patients with high urinary excretion of cystine whose parents had a normal urinary amino acid profile were diagnosed with cystinuria type I.

DNA was isolated from white blood cells according to standard protocols[18]. M467T and M467K mutations were analyzed by amplifying genomic DNA (F: 5' GCG TTT GGG GAA TCA GTA TG 3', R: 5' -GTT CCA GGG AGT GTG AAA AG 3'). For the mutations and the polymorphism, a total of 35 cycles of amplification were performed (2 min. at 94 °C, 1 min. at 55 °C and 1 min. at 72 °C). For the detection of mutations, PCR products were digested with N1aIII and AluI according to the manufacturer's instructions and were electrophoresed on a 15% polyacrylamide gel at 300V for 45 minutes[16].

231T/A polymorphism was analyzed by amplifying DNA (F: 5' AGA GAG GGC AAT GAT GGC TA 3', R: 5' GAA GGC ACT CCG AAG ACA TAA 3'). PCR products were digested with DdeI and electrophoresed on a 3% agarose gel[7]. In addition to 24 cystinuria patients, 20 healthy control individuals were analyzed for 231T/A polymorphism. Gels were stained with EtBr and viewed under UV fluorescence.

Results

Among 24 cystinuria type I patients, 75 percent had stones in their urinary tract. Consanguinity was present in 37.5 percent of the families. The characteristics of the patients and the results of DNA analysis are shown in Table I.

Table I. Characteristics of the Patients and Results of DNA Analysis

No.	Age	Sex	Consanguinity	Urinary stone	M467T	M467K	231T/A
1	3 y	M	(+)	(+)	-/-	-/-	-/-
2	8 m	M	(-)	(+)	-/-	+/-	-/-
3	12 y	M	(-)	(+)	+/-	-/-	+/-
4	3 y	M	(-)	(+)	-/-	-/-	-/-
5	6 y	M	(+)	(+)	-/-	-/-	-/-
6	2 y	M	(+)	(+)	-/-	-/-	-/-
7	7 y	M	(+)	(+)	-/-	-/-	-/-
8	2 m	F	(-)	(+)	-/-	-/-	-/-
9	11 y	M	(-)	(-)	+/-	-/-	+/-
10	9 y	F	(-)	(-)	-/-	-/-	-/-
11	6 y	F	(+)	(+)	-/-	-/-	-/-
12	5 y	M	(+)	(-)	-/-	-/-	-/-
13	25 y	M	(-)	(-)	-/-	-/-	-/-
14	6 y	M	(+)	(+)	-/-	+/-	-/-
15	8 y	M	(-)	(-)	-/-	-/-	-/-
16	?	M	(-)	(+)	-/-	-/-	-/-
17	1.5 y	M	(+)	(+)	-/-	-/-	-/-
18	2 y	M	(+)	(+)	+/+	-/-	+/+
19	3 y	M	(-)	(+)	-/-	-/-	-/-
20	?	F	(-)	(+)	-/-	-/-	-/-
21	18 y	M	(-)	(+)	-/-	-/-	-/-
22	14 y	M	(-)	(+)	-/-	-/-	-/-
23	10 y	F	(-)	(+)	-/-	-/-	-/-
24	3 y	M	(-)	(-)	-/-	-/-	-/-

Among the 24 patients, two were heterozygous and one was homozygous for the M467T mutation. The allele frequency was 8.3 percent. Another two patients were heterozygous for M467K, which is the second most frequent mutation at the same position, and the allele frequency was 4.2 percent. It has been shown previously that 231T/A polymorphism was found in linkage disequilibrium with the M467 mutation[17]. We also analyzed 231T/A in our group of patients T→A transversion was found in three patients that had the M467T mutation (8.3%). This polymorphism was not found in the rest of our patient group or in the 20 healthy control individuals.

Discussion

At present, 38 cystinuria type I mutations in the rBAT gene have been described[15] in populations in Italy, Spain, the Middle East, Eastern Europe (mainly people of Jewish origin) and Japan. Of the mutations there were missense, deletion, insertion, nonsense, splice site and frameshift mutations[19].

The most frequent mutation was M467T in Italian (8.7%) and Spanish (40%) populations[16,20]. In our patients the allele frequency of the M467T mutation (8.3%) was similar to the Italian results. M467K was analyzed in Spanish patients, and the frequency was 1.8 percent[21], which is lower than the frequency in the Turkish mutant alleles (4.2%).

In the rBAT gene, besides 38 mutations, nine polymorphisms were identified. In the Mediterranean cystinuria type I patients, the 231T/A polymorphism in exon 1 was found in linkage disequilibrium with the M467T mutation (11.8%). 231T/A polymorphism was only found in affected chromosomes, not in normal ones. This observation was confirmed by our results and the allele frequency of the 231T/A polymorphism (8.3%) was found similar to that in Mediterranean populations.

In our study, 12.5 percent of the cystinuria type I mutant alleles could be identified. This leaves a large proportion of alleles still to be analyzed. Heterogeneity of mutations in recessive disorders is observed in the Turkish population[22]. This creates a difficulty in population screening or carrier identification by DNA analysis. Further characterization of unknown mutations is underway.

REFERENCES

1. Mckusick VA. Cystinuria. In: Mendelian Inheritance in Man. Catalogs of Autosomal Dominant, Autosomal Recessive, and X-Linked Phenotypes (9th ed.). Baltimore, MD: The Johns Hopkins University Press; 1990: 1128-1129.
2. Rosenberg L, Downing S, Durant J, Segal S. Cystinuria: biochemical evidence of three genetically distinct diseases. J Clin Invest 1966; 45: 365-371.
3. Segal S, Thier SO. Cystinuria. In: Scriver CH, Beaudet AL, Sly WS, Valle D (eds). The Metabolic and Molecular Bases of Inherited Disease (7th ed). New York: McGraw-Hill; 1995: 3581-3601.
4. Özalp I, Coşkun T, Tokol S, Demircin G, Mönch E. Inherited metabolic disorders in Turkey. J Inherit Metab Dis 1990; 13: 732-738.
5. Gültekin A, Özalp İ, Tanzer F, Hasanoğlu A. Türk çocuklarında sistinüri'nin görülme sıklığı. Çocuk Sağ ve Hast Derg 1980; 23: 1-8.
6. D. Aydoğdu S, Kırel B, Coşkun T, et al. Eskişehir ilköğretim okulu çocuklarında sistinüri prevalansı. 44. Milli Pediatri Kongresi Özet Kitabı, 4-8 Eylül 2000, Bursa, P165.
7. Lee WS, Wells RG, Sabbag RV, Mohandas TK, Hediger MA. Cloning and chromosomal localization of a human kidney cDNA involved in cystine, dibasic and neutral amino acid transport. J Clin Invest 1993; 91: 1959-1963.
8. Pras E, Arber N, Aksentijevich I, et al. Localization of a gene causing cystinuria to chromosome 2p. Nature Genet 1994; 6: 415-419.
9. Calonge MJ, Nadal M, Calvano S, et al. Assignment of the gene responsible for cystinuria (rBAT) and of markers D2S119 and D2S177 to 2p16 by fluorescence in situ hybridization. Hum Genet 1995; 95: 633-636.
10. Bisceglia L, Calonge MJ, Totaro A, et al. Localization by linkage analysis of the cystinuria type III gene to chromosome 19q13.1. Am J Hum Genet 1997; 60: 611-616.
11. Wartenfeld R, Golomb E, Katz G, et al. Molecular analysis of cystinuria in Libyan Jews: Exclusion of the SLC3A1 gene and mapping of a new locus on 19q. Am J Hum Genet 1997; 60: 617-624.
12. Pras E, Sood R, Raben N, Aksentiyevich I, Chen X, Kastner DL, Genomic organization of SLC3A1, a transporter gene mutated in cystinuria. Genomics 1996; 36: 163-167.
13. Purroy J, Bisceglia L, Calonge MJ, et al. Genomic structure and organization of the human rBAT gene (SLC3A1). Genomics 1996; 37: 249-252.
14. Gitomer WL, Pak CY. Recent advances in the biochemical and molecular biological basis of cystinuria. J Urol 1996; 156: 1907-1912.
15. Egoshi KI, Akakura K, Kodama T, Ito H. Identification of five novel SLC3A1 (rBAT) gene mutations in Japanese cystinuria. Kidney Int 2000; 57: 25-32.
16. Calonge MJ, Gasparini P, Chillaron J, et al. Cystinuria caused by mutations in rBAT, a gene involved in the transport of cystine. Nature Genet 1994; 6: 420-425.

17. Gasparini P, Calonge MJ, Bisceglia L, et al. Molecular genetics of cystinuria: identification of four new mutations and seven polymorphisms and evidence for genetic heterogeneity. Am J Hum Genet 1995; 57: 781-788.
18. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucl Acids Res 1988; 16: 1215.
19. Palacin M, Chillaron J, Mora C. Role of the $b^{0,+}$ - like amino acid - transport system in the renal reabsorption of cystine and dibasic amino acids. Biochem Soc Transac 1996; 24: 856-863.
20. Sanctis L, Bruno M, Bonetti G, et al. Phenotype characterization and prevalence of rBAT M467T mutation in Italian cystinuric patients. J Inherit Metab Dis 1996; 19: 243-245.
21. Bisceglia L, Calonge MJ, Strologo LD, et al. Molecular analysis of the cystinuria disease gene: identification of four new mutations, one large deletion and one polymorphism. Hum Genet 1996; 98: 447-451.
22. Yılmaz E, Erdem H, Özgüç M, et al. Study of 12 mutations in Turkish cystic fibrosis patients. Hum Hered 1995; 45: 175-177.