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ORIGINAL PAPER

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The population-specific distribution and frequencies of genomic variants in the *SLC3A1* and *SLC7A9* genes and their application in molecular genetic testing of cystinuria

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Abstract Cystinuria is a common inherited aminoaciduria resulting in nephrolithiasis. Mutations in two genes, *SLC3A1* and *SLC7A9*, have been identified in cystinuric patients. Considering the population-specific distribution of genetic variants in the *SLC3A1* gene, we focused our study on mutations in *SLC3A1* and *SLC7A9* described more than once in the literature. We evaluated the usefulness of this restricted analysis as a diagnostic approach. Furthermore, the data obtained were used to estimate the frequency of heterozygote carriers of *SLC3A1* mutations in the general European population. A total of 22 unclassified cystinuric patients were screened for genetic variants in four exons of both *SLC3A1* and *SLC7A9* in which the most common mutations have been identified. For screening, we used single strand conformation polymorphism analysis (SSCP), restriction assays, real-time PCR and direct sequencing. In total, we identified mutations in 17 of our 22 patients, including a new mutation (R365Q) as well as a novel polymorphism (c.1035G/A) within the *SLC3A1* gene. An ethnic influence on the distribution of mutations was confirmed: T216M in *SLC3A1* is the major mutation in south-eastern Europe, whereas M467T in *SLC3A1* is mainly found in western Europe. A complex duplication in *SLC3A1* is restricted to German patients. Generally, we could show that a stepwise analysis directed to the most common mutations in the two cystinuria genes is sufficient to detect variants in more than 75% of patients of European origin. The test consists of nine different PCR-based approaches and therefore represents a low-cost, reliable and timesaving diagnostic tool.

Keywords Cystinuria · Mutations · *SLC3A1* · *SLC7A9* · Population-specific distribution

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Introduction

Cystinuria is an inherited metabolic disorder characterised by the abnormal urinary excretion of cystine and the dibasic amino acids ornithine, lysine and arginine. Formation of cystine kidney stones, recurrent infections and subsequent renal failure are the main complications of the disease. The incidence of cystinuria is estimated to be about 1:7,000 but varies considerably between specific populations [1, 2].

At the biochemical level, two subtypes of cystinuria can be distinguished: type I cystinuria is transmitted by an autosomal recessive trait, whereas non-type I cystinuria is inherited in a dominant mode with incomplete penetrance. Patients with the different types can not be distinguished on the basis of urinary cystine excretion, but their parents, as obligate heterozygotes, show different excretion patterns: type I carriers show a normal excretion of cystine, arginine, ornithine and lysine. In contrast, cystine excretion in non-type I carriers ranges from normal to severely increased.

Recently, two genes responsible for cystinuria have been identified [3, 4]. Mutations in the *SLC3A1* gene

encoding the glycoprotein rBAT cause cystinuria type I, whereas mutations in the *SLC7A9* gene have been demonstrated in non-type I cystinuria; the gene products of *SLC3A1* and *SLC7A9* form the two subunits of the apical renal heterodimeric cystine transport system rBAT/$b^{0,+}$AT. It is thought that $b^{0,+}$AT represents the catalytic subunit of the transporter complex and that rBAT is mainly involved in the trafficking and possible stabilisation of the transporter in the brush border membrane [5, 6, 7, 8]. In addition, rBAT may modify the functional transport properties of the complete transporter complex [6]. The transporter functions as a tertiary active exchanger taking up cystine, arginine, lysine, and ornithine from the urine in exchange for neutral amino acids [5, 6].

Up to now, more than 80 mutations in *SLC3A1* and 50 in *SLC7A9* have been reported in the literature. The majority of mutations in *SLC3A1* have been detected only in single patients, however, the distribution of the more frequent mutations, such as M467T and T216M in *SLC3A1* or V170M in *SLC7A9*, appears to be influenced by the ethnic background of the patients [9, 10].

Taking into account the population-specific distribution of mutations in the *SLC3A1* gene, we focused our study on genomic variants in *SLC3A1* and *SLC7A9* described more than once in the literature. The purpose of this study was to evaluate the usefulness of this restricted analysis as a diagnostic approach.

Materials and methods

In addition to our previously reported cohort of cystinurics [10, 11], we screened 22 unrelated patients referred to the contributing urological centres. The appropriate informed consent was obtained from all patients. The study was approved by the Ethics Committee of the University Hospital of Aachen. All patients had a history of recurrent cystine stones and elevated urine cystine levels. As controls, we used 50 Germans with a mean age of 25 years and without an history of renal stone formation.

The 22 newly patients were screened for genetic variants in four exons of both *SLC3A1* and *SLC7A9* (Table 1) in which the most common mutations have been identified in previous studies. In the case of exons 3, 4, 6 and 8 of *SLC3A1*, the coding sequences were amplified by PCR and subsequently digested with the appropriate restriction enzymes; exons 5, 7 and 10 of *SLC7A9* were analysed by SSCP, exon 4 was directly sequenced. Testing for the duplication spanning exons 5–9 in *SLC3A1* was performed using a PCR based approach. All protocols used were published recently [10, 11, 12].

Results

In comparison to our recent screening studies for genetic variants in all coding regions of *SLC3A1* and *SLC7A9*, we restricted our analyses in this study to those genomic regions containing the most common mutations in cystinuric patients, i.e. exons 3, 4, 6 and 8 of *SLC3A1* and exons 4, 5,7 and 10 of *SLC7A9*, as well as the duplication spanning exons 5 to 9 in *SLC3A1* (Table 1). In addition to the frequent mutations within these fragments, we also searched for further variants detectable by the respective assays.

In total, we could identify mutations in 77% of our patients (Table 2) while 59% of the cystinuria alleles could be determined. The preponderant variant was T216M in *SLC3A1*, which was detectable in 12 alleles; the majority of carriers were of south-eastern European origin. The amino acid variants M467T in *SLC3A1* and G105R in *SLC7A9* were detected in two patients, and the duplication in *SLC3A1* in three alleles. In two German patients, we detected a novel mutation in exon 6 of *SLC3A1*, a transition from G to A at nt1094 resulting in the amino acid substitution R365Q. This variant was not detected in the 50 controls.

In one patient, we found a novel but rare polymorphism in exon 6 of *SLC3A1*: by performing an *Alu*I restriction assay, which was first published to detect the mutation L346P [13], we detected a silent G to A transition at position c.1035 which destroys the same *Alu*I restriction site affected by the mutation L346P. This variant could also be detected in one out of 126 control chromosomes.

Discussion

Two recent reports impressively reflect the population-specific distribution of at least *SLC3A1* mutations in cystinuric patients: in 2001, Harnevik et al. [14] described their findings on 53 unclassified Swedish patients from 43 families. They detected 15 different *SLC3A1* mutations in 43 patients. Twelve of these mutations had never been observed in other ethnic groups. The predominant mutation was M467T, which contributed to the cystinuria phenotype in 28 Swedish patients as well as to that in an Iranian patient. In contrast, M467T has not yet been detected in the Japanese population. A study restricted to 36 Japanese patients was published by

Table 1 Methods used for the detection of the most frequent mutations in the two cystinuria genes examined in the present study. Due to the nature of the genetic tests, some rare mutations were detected by the same approach

Gene	Exon	Mutation	Method/restriction enzyme
SLC3A1	3	T216M	*Nla*III
	4	R270X/L	*Taq*I
	6	R365W/L/Q	*Msp*I
		R362C/H	*Aci*I
		L346P	*Alu*I
	8	M467T/K	*Nla*III/*Alu*I
		c.1500 + 1G > T	*Mse*I
		E483X	*Mbo*II
	5–9	Duplication of exons 5–9	PCR
SLC7A9	4	G105R, T123M, F140S, c.553delCG	Sequencing
	5	A182T, c.747delG	SSCP
	7	E244del	SSCP
	10	A331V, R333W	SSCP

Table 2 Results of screening for the most frequent mutations in the *SLC3A1* and *SLC7A9* genes in our cystinuic pateints. R365Q is a novel mutation; * the duplication spans from exon 5 to exon 9

Patient	*SLC3A1*mutations	*SLC7A9* mutations	Ethnic origin
Cys 113	M467T/R365Q	-	German
Cys 116	-	-	Turkish
Cys 117	T216M/T216M	-	Yugoslavian
Cys 123	T216M/T216M	-	Yugoslavian
Cys 126	T216M/T216M	-	Turkish
Cys 128	-	-	Turkish
Cys 129	-	E244del/-	German/Domican
Cys 130	-	G105R/A182T	German
Cys 133	R365L/R365L	-	Yugoslavian
Cys 141	Duplication*/-	-	German
Cys 142	-	c.553delCG/-	Italian
Cys 143	-	G105R/-	Yugoslavian
Cys 146	T216M/T261M	-	Yugoslavian
Cys 147	R365Q/-	-	German
Cys 148	Duplication*/Duplication*	-	German
Cys 152	-	-	German
Cys 155	T216M/T216M	-	Yugoslavian
Cys 158	-	-	German
Cys 161	-	-	Turkish
Cys 162	M467T/-	-	German
Cys 163	T216M/-	-	German
Cys 164	T216M/-	-	German

Egoshi et al. [13]: the authors did not detect any of the mutations observed in Caucasian patients but described five new variants which had never been observed in Europe.

The findings from our 22 additional unclassified patients allows us to further confirm the population-specific distribution of mutations in *SLC3A1*. In our total population of 78 cystinuria patients, M467T is the most common mutation in Germany, while T216M is mainly distributed in south-eastern Europe (Greece, the Balkans) and Turkey (Table 3). In a patient from this region, we detected the R365L mutation for a second time, possibly indicating a further population-specific mutation. Another population-specific distribution can be observed for the duplication in *SLC3A1* spanning exons 5 to 9, which seems to be restricted to German patients (Tables 2, 3) [12].

In addition to R365L, we identified a third base pair substitution affecting R365, resulting in R365Q. This variant was not detectable in the controls.

In the case of the *SLC7A9* gene, G105R is the most common mutation (Table 3). The mutation V170M was not investigated since it seems to be restricted to Libyan Jews and has never been observed in other populations [4, 9]. However, it is too early to speculate on the specific distributions and frequencies of mutations in this gene, since only few screening data are presently available.

In total, we could show that a stepwise analysis of eight fragments, as well as the search for duplications in *SLC3A1*, is sufficient for the detection of more than 55% of mutations and of at least one mutation in more than 70% of all cystinuria patients (Table 3). Thus, a diagnostic screening assay becomes more accessible. These data agree with those of a recent survey on mutations in *SLC3A1* and *SLC7A9* in 27 unclassified North American cystinurics which showed mutations in 78% of patients, resulting in a detection rate of 56% of chromosomes [9]. As in other genetic diseases, such as cystic fibrosis, this diagnostic strategy is restricted to the most common mutations, allowing the identification of

Table 3 Overview on the most frequent mutations in *SLC3A1* and *SLC7A9* detected in our total study population; the patients published recently [10, 11] are included. Numbers are percentages with the absolute number of chromosomes given in parentheses

Gene	Mutation	German ($n=90$)	Italian ($n=24$)	Greek ($n=6$)	Yugoslavian ($n=16$)	Turkish ($n=20$)	Total ($n=156$)
SLC3A1	T216M	3.3 (3)	4.2 (1)	100 (6)	62.5 (10)	30 (6)	16.7 (26)
	M467K	-	8.3 (2)	-	-	-	1.3 (2)
	M467T	15.6 (14)	4.2 (1)	-	-	5.0 (1)	10.3 (16)
	R365W/Q/L	6.7 (6)	4.2 (1)	-	25 (4)	-	7.1 (11)
	Duplication	14.4(13)	-	-	-	-	8.3 (13)
SLC7A9	G105R	6.7 (6)	8.3 (2)	-	6.3 (1)	5.0 (1)	6.4 (10)
	c.553delCG	-	4.2 (1)	-	-	-	0.6 (1)
	F140S	1.1 (1)	-	-	-	-	0.6 (1)
	A182T	2.2 (2)	-	-	-	-	1.3 (2)
	c.747delG	1.1 (1)	-	-	-	-	0.6 (1)
	E244del	1.1 (1)	4.2 (1)	-	-	-	1.3 (2)
	A331V	-	-	-	-	10 (2)	1.3 (2)
	R333W	1.1 (1)	4.2 (1)	-	-	-	1.3 (2)
Total		53.3 (48)	41.7 (10)	100 (6)	93.8 (15)	50 (10)	57.1 (89)

the majority of mutations in the patients. Of course, the ethnic origin of the patients must be considered. In the case of clinical diagnosis of cystinuria, the identification of only one mutation should be sufficient for confirmation. The detection of the novel mutation R365Q as well as of the rare polymorphism c.1035G > A in *SLC3A1* by molecular assays originally developed for the detection of other variants, shows the importance of confirming a mutation using a second technique. Finally, with this diagnostic algorithm, a low-cost, reliable and timesaving diagnostic tool for cystinuria is available.

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