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Molecular Genetic Testing in Cystinuria

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ABSTRACT Cystinuria (OMIM 220100) is caused by the defective transport of cystine and the dibasic amino acids in the proximal renal tubule and in the epithelial cells of the gastrointestinal tract. We analysed a cohort of 26 unrelated cystinuria patients diagnosed on the basis of stone formation. Direct sequencing of all coding regions and exon-intron boundaries of the *SLC3A1* and *SLC7A9* genes allowed us to identify 26 different mutations in 23 out of the 26 patients, in total they accounted for 40 affected chromosomes. Three of the 26 are novel mutations, two in *SLC3A1* and one in *SLC7A9*. Interestingly, two of our patients carried three mutations in *SLC3A1* each, one patient was mixed heterozygous for *SLC3A1* and *SLC7A9* mutations. In summary, these findings expand the spectrum of *SLC3A1* and *SLC7A9* mutations and confirm the heterogeneity and complexity of cystinuria. If we assume an autosomal recessive inheritance of the disease, our detection rate was 88.5% and thereby relatively high in comparison to other studies. Nevertheless we have to consider that at least *SLC7A9* mutations are often dominant, we therefore think that our effective detection rate is higher. Additionally, the broad pathophysiological consequences of *SLC7A9* mutations make an individual prognosis and genetic counselling difficult.

INTRODUCTION

Cystinuria (OMIM 220100) is an inherited renal disease characterised by the defective transport of cystine, lysine, ornithine and arginine in the proximal tubule and in the epithelial cells of the gastrointestinal tract. The resulting urinary hyperexcretion of cystine leads to precipitation in the distal tubule and formation of cystine stones. The prevalence ranges between 1 in 2,500 and 1 in 100,000 (for review: Palacin et al. 2001). While cystinuria is rare in adults, cystine stones represent 4-8% of renal stones in children.

So far, two genes responsible for cystinuria have been identified: *SLC3A1* (chromosome 2p21) encodes the heavy subunit rBAT of the renal $b^{0,+}$ transporter while *SLC7A9* (chromosome 19q12) encodes the light subunit $b^{0,+}$AT (Fig. 1) (Calonge et al. 1994; International Cystinuria Consortium 1999).

Historically, the classification of cystinuria was based on the urinary excretion pattern of heterozygotes. Type I heterozygotes show a normal urinary excretion of all amino acids, in type II and III heterozygotes an increased excretion of cystine and the dibasic amino acids can be observed (Rosenberg et al. 1964). After identification of the molecular basis of the disease a new molecular classification was proposed: the autosomal recessively inherited type I cystinuria which is mainly caused by *SLC3A1* mutations, and the autosomal incomplete dominant non-type I cystinuria associated with *SLC7A9* mutations (formerly types II and III). However, the identification of single *SLC3A1* mutations causing non-type I cystinuria and of recessive *SLC7A9* mutations lead to a new strictly molecular classification (Dello Strogolo et al. 2002) which does not include the biochemical phenotype: type A cystinuria represents *SLC3A1* mutations, and type B cystinuria includes *SLC7A9* mutations. Thus, three genotypes can be delineated: AA, BB, and in rare cases mixed cystinuria AB.

In addition to the classical cystinuria phenotype there are some reports on patients with the hypotonia-cystinuria syndrome. In this autosomal recessive disorder microdeletions of the two contiguous genes *SLC3A1* and *PREPL* in 2p21 are not only associated with cystinuria but also with neonatal hypotonia, failure to thrive and growth retardation (for review: Martens et al. 2007).

Here we report on our molecular findings in 26 cystinuria patients and the difficulties to interpret the genetic data in respect to prognosis and genetic counselling.

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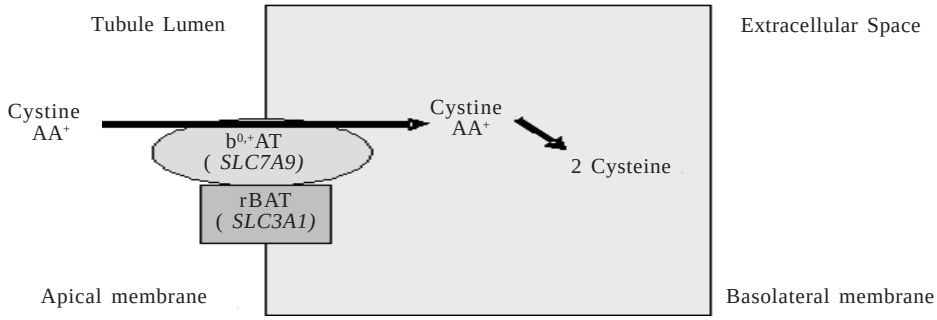

Fig. 1. Schematic drawing of the physiological function of the rBAT and b0,+AT amino acid transport system encoded by *SLC3A1* and *SLC7A9*.

Table 1: Results from molecular genetic testing in the cystinuria patient cohort

Patient	*Ethnic origin*	*SLC3A1 mutations*	*SLC7A9 mutations*	*Cystinuria type*
104	German		G105R IVS5+2T/C	BB
156	German		A382T c.799_800insA	BB
174	German		F140S F140S	BB
368	German	Q199X		A?
644	Turkish		A331V A331V	BB
780	German	T216M T216M		AA
781	German			??
916	German	R567*fs* R567*fs* Q224X		AA
1004	German		G105R R333W	BB
1166	German	T216M T216M		AA
1283	German	M467T	G105R	AB
1482	South East Europe	R365L T216M c.1136+2/3delT		AA
2487	German			??
2778	Dutch	P508R S547L		AA
3029	Turkish		N203fs	B?
3858	German	F599S F599S		AA
4057	German	Y397C R584X		AA
4289	German	M467T D179Y		AA
4611	German	M467T Q125X		AA
5335	German	G121E G121E		AA
5356				??
6100	German	R452W		A?
6245	German	M467T		A?
6291	German		S421fs	B?
6389	German	M123R M467T		AA
6670	German	M467T		A?

MATERIAL AND METHODS

The 26 patients were referred for molecular genetic testing because of (recurrent) kidney stones by external hospitals and clinicians. Patients´ genomic DNA was isolated from peripheral blood samples by a simple salting-out procedure. The whole coding region and the intron/exon boundaries of *SLC3A1* and *SLC7A9* were amplified by PCR. Primer sequences and PCR conditions have been described previously (Botzenhart et al. 2002; Font-Llitjos et al. 2005). In case of homozygosity of rare mutations large deletions were excluded by DNA microarray analysis using the 500K array system (Affymetrix, High Wycombe, UK).

RESULTS AND DISCUSSION

By screening our cohort of 26 patients with recurrent cystine kidney stones for mutations in the *SLC3A1* and the *SLC7A9* genes we detected mutations in 88.5% of cystinurics (Table 1). In 65.4%, both alleles of an individual were affected. Homozygosity or compound heterozygosity for *SLC3A1* mutations was present in 11 cystinuria patients, in another 5 cases two mutations were detectable in the *SLC7A9* gene. One patient was mixed heterozygous with mutations in both genes. In 6 probands, only one mutation in one of the two genes was detectable. In 3 patients we could not detect any mutation, neither in *SLC3A1* nor in *SLC7A9*.

On the allelic level, the detection rate was 76.9%, *SLC3A1* mutations accounted for 51.9%, *SLC7A9* mutations for 25%. The most common *SLC3A1* mutations were M467T and T216M in *SLC3A1*, G105R was the most frequent mutation in *SLC7A9* (Table 2). These data confirm observations from other studies that these three mutations are indeed the most common ones (Schmidt et al. 2004; Di Perna et al. 2008).

Interestingly, two of our patients carried three mutations in the *SLC3A1* gene (Table 1: patients 916 and 1482). The detection of three recessive mutations in one gene is a rare finding but it has also been known from other diseases like autosomal recessive polycystic kidney diseases (*PKHD1* gene). The identification of three mutations illustrates that two mutations can be localised on the same allele, this might lead to false-positive or false-negative results in carrier diagnostics.

Table 2: Localisation and frequencies of the mutations in the *SLC3A1* and *SLC7A9* genes in our study cohort (new mutations are printed in bold face)

Gene	*Exon*	*Mutation*	*Frequencies (n)*
SLC3A1	1	G121E	2
		M123R	1
		Q125X	1
		D179Y	1
		Q199X	1
	3	T216M	5
		Q224X	1
	6/Intron 6	R365L	1
		c.1136+2/3delT	1
	7	Y397C	1
	8	R452W	1
		M467T	6
	9	P508R	1
	10	S547L	1
		R567fs	2
		R584X	1
		F599S	2
SLC7A9	4	G105R	3
		F140S	2
	Intron 5	IVS5+2T/C	1
	6	N203*fs*	1
		c.799_800insA	1
	10	A331V	2
		R333W	1
	11	A382T	1
	12	S421*fs*	1

Our detection rates correspond to that published by other groups leaving 10-15% of alleles unidentified. The failure to detect mutations in single chromosomes might be explained by putative dominance of some *SLC7A9* alleles, in these cases a second mutation is not needed to affect the phenotype. Furthermore, it has been suggested that genomic variants in the SLC7A9 gene might predispose a cystinuria phenotype in case of a co-occurrence with other mutations (Schmidt et al. 2003; Chatzikyriakidou et al. 2006). However, as our results and data from other groups show a reliable diagnostic screening assay for cystinuria is accessible with a detection rate of more than 85%.

In clinical practice, the molecular genetic testing results might influence the prognosis and therapy of cystinuria. While homozygosity or compound heterozygosity for *SLC3A1* mutations are principally associated with an increased cystine excretion, the age of kidney stone formation is difficult to predict. Indeed, in sibs with the same cystinuria genotype the age of onset can differ remarkably. Nevertheless, all carriers of two *SLC3A1* or *SLC7A9* mutations (genotypes AA, BB, AB) will develop kidney stones

in their live while the majority of *SLC3A1* heterozygotes do not exhibit a biochemical phenotype. In contrast, the urinary excretion pattern in heterozygote *SLC7A9* mutation carriers is extremely variable and does therefore hardly allow a prediction of the clinical course, in particular in childhood.

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