*Klin. Biochem. Metab., 13 (34), 2005, No. 2, p. 103–106.*

# Cystinuria: Current Biochemical and Molecular Biological View

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**SUMMARY**
Cystinuria is a frequent inherited metabolic disorder in Czech population (frequency 1/5 600 of live births). It is caused by a defect in the renal transport of cystine and dibasic amino acids (arginine, lysine and ornithine). The disease is characterized by increased urinary excretion of amino acids and often leads to recurrent urolithiasis. For many years cystinuria has been classified into three subtypes based on the urinary excretion of cystine and dibasic amino acids in obligate heterozygotes and intestinal absorption in homozygotes. Cystinuria is currently classified into two subtypes (type I and type non-I) based on urinary excretion of cystine and dibasic amino acids in obligate heterozygotes. Two genes coding for proteins constituting heterodimeric transporter are currently associated with cystinuria. Cystinuria type I is caused predominantly by mutations in the SLC3A1 gene (2p16.3) encoding a heavy subunit (rBAT) of the heterodimeric transporter. Cystinuria non-I type is caused by mutations in the SLC7A9 gene (19q13.1), encoding a light subunit (b0+AT) of this transporter. Despite extensive research in the field, many questions still remain open.
*Key words:* cystinuria, SLC3A1, SLC7A9, transport.

## Introduction

Cystine stone was first described in 1810 by Wollaston. He found that a glistening yellow bladder stone was composed of an unusual substance, which he called cystic oxide because it came from the bladder. Later analysis showed it to be a sulphur-containing amino acid so that the stone ultimately gave its name not only to cystinuria but also to the amino acids cystine and cysteine (1). In 1908, Sir Archibald E. Garrod discussed cystinuria in his Croonian lectures and first described cystinuria as an inborn error of metabolism with a defect in the metabolism of cystine responsible for the disorder (2).

In 1978, Kelly concluded that the excretion rates of obligate carriers among the relatives of cystinurics are sufficient for the determination of the type of cystinuria in the proband (3).

Although cystinuria was described already in 1908, the first gene associated with this disease was not identified until 1994 (4, 5, 6).

The incidence of this disease varies considerably depending on particular population, for example: 1/100 000 in Sweden, 1/15,000 in the USA, and 1/2 500 among Libyan Jews (7). In the Czech population the incidence of cystinuria is 1/5 600, making cystinuria one of the most frequent inherited metabolic disease (8). Cystinuria is an inherited form of nephrolithiasis due to the failure of reabsorptive transport in the proximal tubule.

## Heterodimeric amino acid transporters

The heteromeric amino acid transporter (HAT) is composed of a light and a heavy subunit linked by a disulfide bridge. The heavy subunit assigned rBAT is the SLC3 member, whereas the light subunit ($b^{0,+}$ AT) is a member of the solute transporter family SLC7 of amino acids. SLC3 proteins are type II membrane glycoproteins (i. e. one single transmembrane domain and the C-terminus located outside the cell) with a bulky extracellular domain that exhibits homology with bacterial alpha-glucosidases. The rBAT consists of a single transmembrane helix and a large extracellular domain. SLC7 members are polytopic membrane transport proteins with 12–14 transmembrane helices. The light subunit $b^{0,+}$ AT consists of 12 membrane-spanning regions.

The rBAT heterodimerizes with $b^{0,+}$ AT constituting the amino acid transport $b^{0,+}$ (Fig. 1), the main system responsible for the apical reabsorption of cystine in the kidney. The heteromeric rBAT/$b^{0,+}$AT protein transports cystine as well as basic and neutral amino acids (namely lysine, arginine, ornithine, etc.) and does not accept acidic amino acids (9). The defect in this system causes cystinuria, the most common primary inherited aminoaciduria.

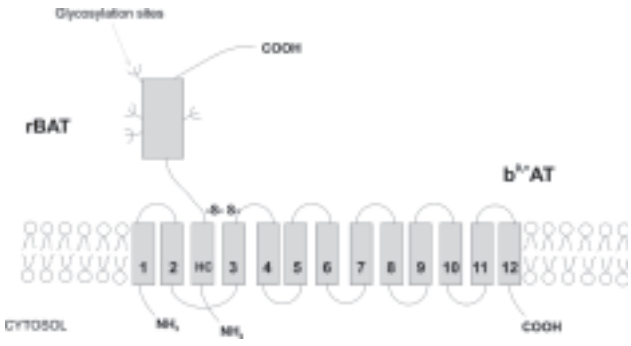


**Fig. 1.** Hypothetical heterodimeric rBAT/$b^{0,+}$ AT transporter of cystine and dibasic amino acids
Sketch shows disulfide bridge (-S–S-) between subunit, HC – heavy chain.

## Renal transport and defects

The proximal convoluted tubule starting from the glomerulus, encompasses S1 and S2 segments, and the proximal straight tubule is equivalent to the segment S3. In general, more than 90% of amino acid reabsorption take place in the proximal convoluted tubule, which applies also for cystine. The reabsorptive mechanism has a maximal capacity that is exceeded in certain disorders. In most cases aminoaciduria is caused by an extrarenal metabolic defect, which leads to the accumulation of a particular amino acid in the plasma. It is then filtered in amounts exceeding the normal capacity of the nephron for reabsorption. When excessive loss occurs in the face of normal or low plasma levels and diminished filtered loads of the amino acid, then the reabsorptive capacity of the tubule is said to be below normal and tubular dysfunction exists. The latter situation takes part in cystinuria. Excessive urinary losses of cystine and dibasic amino acids occur at normal or less than normal plasma levels of the amino acids.

Although hyperexcretion of cystine, arginine, ornithine and lysine in the urine is the hallmark of cystinuria, other amino acids have been found in higher than normal amounts in the urine of some patients (7). These include glycine, methionine, cystathionine, and homocysteine-cysteine disulfide. The amounts are related directly to the amount of cystine excreted.

Kidney stones are formed when urine becomes hypersaturated and particular substances crystallize in the urine. Excessive cystine crystals clump together to form a stone, which can block portions of the interior of the kidney or the ureter. Painful symptoms arise when the stones begin to move down the ureter.

## Biochemical classification according to Rosenberg

Three types of classic cystinuria (I, II, and III) have been described based on the urinary excretion of cystine and dibasic amino acids in obligate heterozygotes (10) and in intestinal absorption in homozygotes (7). Type I heterozygotes display normal aminoaciduria, whereas type II and III heterozygotes display a high or moderate hyperexcretion of cystine and dibasic amino acids that may eventually result in stone formation. Type I and II homozygotes show a minimal increase in blood cystine levels after oral loading, whereas type III homozygotes exhibit an almost normal response. Type II and type III heterozygotes, however, may show overlapping excretion values.

## Geneticss of cystinuria

*Cystinuria type I*

Type I cystinuria is caused by mutations in the SLC3A1 (CSNU1, MIM# 104614), an amino acid transporter gene located on chromosome 2 (2p16.3) (4, 5, 6). Analysis of the genomic structure and organization of the SLC3A1 gene was reported in 1996 (11). It was found that the gene spans 45 kb of genomic DNA and is composed of 10 exons, ranging in size from 120 till 500 to 13 000 bp. All exon/intron splice junctions conform to the eukaryotic 5-prime-donor, 3-prime-acceptor consensus splice junction GT/AG rule.

The resulting protein (rBAT) consists of 685 amino acids. It is located in the brush border plasma membrane of the proximal straight tubule (S3 segment) of the nephron and in the small intestine.

Currently, more than 80 cystinuria-specific mutations of SLC3A1 gene have been described (http://www.cysdb.mcgill.ca/). In the Czech population, other six mutations were found (12). A defective amino acid transport has been reported for seven missense mutations of SLC3A1 after expression in Xenopus laevis oocytes (13).

After discovering the first gene (SLC3A1) associated with cystinuria, three types had been thought to be due to allelism of the gene. However, the involvement of two distinct genetic loci for type I and type III cystinuria had been suggested (14). To resolve this question, a linkage study of type I and type III using the SLC3A1 gene and its nearest marker, D2S119, was performed in twenty--two cystinuria families. Homogeneity for linkage to SLC3A1 was demonstrated in type I/I families, whereas types I/III and III/III were not linked.

*Cystinuria non-type I*

Cystinuria non-type I is associated with the SLC7A9 gene (CSNU3, MIM 604144), which is located on chromosome 19 (19q13.1). SLC7A9 is organized into 13 exons with sizes ranging from 45 to 242 bp (9). The SLC7A9 cDNA is polyadenylated and contains an open reading frame. This gene encodes 487-amino acids protein $b^{0,+}$ AT and is expressed in the proximal convoluted tubules (S1 and S2 segments) of the nephron and in the small intestine. The protein belongs to the family of light subunits of amino acid transporters expressed in kidney, liver, small intestine, and placenta (9).

Subsequently, more than 50 mutations in the SLC7A9 gene in Libyan Jewish, North American, Turkish, Dutch, German, Italian and Spanish non-type I cystinuria patients have been identified (http://www.cysdb.mcgill.ca/). Other three novel mutations were described in the Czech population (12). The most frequent SLC7A9 missense mutations found were G105R, V170M, A182T, and R333W (http://www.cysdb.mcgill.ca/). Among heterozygotes carrying these mutations, A182T heterozygotes showed the lowest urinary excretion levels of cystine and dibasic amino acids, correlating with significant residual transport activity in vitro. In contrast to that, mutations G105R, V170M, and R333W were associated with a complete or nearly complete loss of transport activity, leading to a more severe urinary phenotype in heterozygotes (15). SLC7A9 mutations located in the putative transmembrane domains of $b^{0,+}$ AT and affecting the conserved amino acid residues with a small side chain were associated with a severe phenotype, while mutations in non-conserved residues gave rise to a mild phenotype (15).

Although mutations in SLC3A1 and SLC7A9 genes explained majority of reported patients in some cases genetic background was not fully explained. In our recent study on the Czech patients (12) we also observed large differences in clinical manifestation in patients with identical genotype. It raised question about other genetic or non-genetic factors contributing to pathology of cystinuria.

## New classification of cystinuria

*Biochemical classification according to Ito (type I, non--type I)*

Recent developments in the genetics and physiology do not support the traditional classification into three groups (type I, II and III). As mutations of only two genes (SLC3A1 and SLC7A9) have been found to be responsible for all three types of the disease and because the distinction between types II and III is particularly unclear, classification into two groups of patients (type I and non-type I) was proposed (16). The non-type I includes type II and III (according to Rosenberg classification). This simple classification is now commonly used.

*Genetic classification according to Dello Strologo (AB)*

The International Cystinuria Consortium set up a multinational database and collected genetic and clinical data from 224 patients affected by cystinuria (125 with full genotype definition), 189 heterozygotes and 83 healthy controls (17). Comparison of genetic data with urinary excretion patterns showed that all SLC3A1 carriers and 14% of SLC7A9 carriers had a normal amino acid urinary pattern (type I phenotype). The remaining SLC7A9 carriers had the phenotype non-I (type III, 80.5%, type II, 5.5%). On the basis of these results a new classification is suggested:

- Type A, due to two mutations of SLC3A1 (45.2% of studied patients) and heterozygotes of this type have a normal amino acid urinary pattern.
- Type B, due to two mutations of SLC7A9 (53.2% of studied patients) and in this type, heterozygotes usually have an increased cystine and dibasic amino acid urinary excretion but may also have a normal pattern.
- The possible third type AB (1.6% of studied patients) is cystinuria caused by one mutation on each of the above-mentioned genes (17).

## Conclusion

The authors review advances achieved in knowledge of biochemical and molecular genetic basis of cystinuria. Although cystine stone had been recognised almost two hundred years ago, the first gene associated with cystinuria was described in 1994. Since that time, the second disease-causing gene was found and over hundred mutations havebeen described. In a part of mutations, the defective transport activity was confirmed by expression studies. Although a lot of research was conducted in the field, many questions remain open. Are there other genes/transporters involved? Why constituents of heterodimeric transporter are not co-localized in the same segments of proximal tubule? What is the role of other nongenetic factors or genetic background in so wide differences in clinical manifestation of cystinuria? The main goal of cystinuria research is to find an effective therapy for the patients.

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The support of the grant CEZ MZ/98001/209627 of the Grant Agency of the Ministry of Health of the Czech Republic is highly acknowledged.

*Redakci předáno 24. 2. 2005.*

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