The Genetics of Heteromeric Amino Acid Transporters

Heteromeric amino acid transporters (HATs) are composed of a heavy (SLC3 family) and a light (SLC7 family) subunit. Mutations in system b0,þ (rBAT-b0,þAT) and in system y’L (4F2hc-y’LAT1) cause the primary inherited aminoacidurias (PIAs) cystinuria and lysinuric protein intolerance, respectively. Recent developments [including the identification of the first Hartnup disorder gene (B0AT1; SLC6A19)] and knockout mouse models have begun to reveal the basis of renal and intestinal reabsorption of amino acids in mammals.

Heteromeric Amino Acid Transporters

Heteromeric amino acid transporters (HATs) are composed of a heavy subunit and a light subunit (Table 1) (15, 21, 100–102). Two homologous heavy subunits (HSHATs) from the SLC3 family have been cloned and are called rBAT (i.e., related to b0,þ amino acid transport) and 4F2hc [i.e., heavy chain of the surface antigen 4F2hc, also named CD98 or fusion regulatory protein 1 (FRP1)]. Nine light subunits (LSHATs; SLC7 family members from SLC7A5 to SLC7A13) have been identified. Six of them are partners of 4F2hc (LAT1, LAT2, y’LAT1, y’LAT2, asc1, and xCT); one forms a heterodimer with rBAT (b0,þAT); and two (asc2 and AGT-1) seem to interact with as yet unknown heavy subunits (100). Members SLC7A1–SLC7A4 of family SLC7 correspond to system y’ isoforms (i.e., cationic amino acid transporters; CATs) and related proteins, which on average show <25% amino acid identity to the light subunits of HATs.

The general features of HATs are as follows (reviewed in Refs. 15, 21, 61, and 100–102; only very recent references are cited):

• The heavy subunits (molecular mass of ~90 and ~80 kDa for rBAT and 4F2hc, respectively) are type II membrane N-glycoproteins with a single transmembrane domain, an intracellular NH2 terminus, and an extracellular COOH terminus significantly homologous to insect and bacterial glucosidases (FIGURE 1). Recently, X-ray diffraction of the extracellular domain of human 4F2hc revealed a three-dimensional structure similar to that of bacterial glucosidases (a triose phosphate isomerase (TIM) barrel

Table 1. Heteromeric amino acid transporters

<table>
<thead>
<tr>
<th>Heavy Chain (HSHAT)</th>
<th>Light Chain (LSHAT)</th>
<th>Gene</th>
<th>Amino Acid Transport</th>
<th>Human Chromosome</th>
<th>Inherited Aminoaciduria</th>
</tr>
</thead>
<tbody>
<tr>
<td>4F2hc</td>
<td>y’LAT1</td>
<td>SLC7A7</td>
<td>y’L</td>
<td>14q11.2</td>
<td>LPI</td>
</tr>
<tr>
<td></td>
<td>y’LAT2</td>
<td>SLC7A6</td>
<td>y’L</td>
<td>16q22.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LAT1</td>
<td>SLC7A5</td>
<td>L</td>
<td>16q24.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LAT2</td>
<td>SLC7A8</td>
<td>L</td>
<td>14q11.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>asc1</td>
<td>SLC7A10</td>
<td>asc</td>
<td>19q12-13</td>
<td></td>
</tr>
<tr>
<td></td>
<td>xCT</td>
<td>SLC7A11</td>
<td>Xc1</td>
<td>4q28-q32</td>
<td></td>
</tr>
<tr>
<td>rBAT</td>
<td>b0,þAT</td>
<td>SLC7A1</td>
<td>b0,þ</td>
<td>2p16.3</td>
<td>cystinuria</td>
</tr>
<tr>
<td></td>
<td>AGT1</td>
<td>SLC7A9</td>
<td>b0,þ</td>
<td>19q12-13</td>
<td>cystinuria</td>
</tr>
<tr>
<td></td>
<td>asc2</td>
<td>SLC7A12</td>
<td>new</td>
<td>8q21.3</td>
<td>not present</td>
</tr>
</tbody>
</table>

Heteromeric amino acid transporters (HATs) are composed of a heavy and a light chain. Heavy subunits belong to solute carrier family SLC3, and light subunits belong to SLC7. AGT and asc2 heterodimerize with unknown heavy subunits. A functional asc2 gene is not present in the human genome. LPI, lysinuric protein intolerance.
and eight antiparallel β-strands; FIGURE 1 (unpublished observations).

- The light subunits (~50 kDa) are highly hydrophobic and not glycosylated. This results in anomalously high mobility in SDS-PAGE (35–40 kDa). Recent cysteine-scanning mutagenesis studies support a 12-transmembrane-domain topology, with the NH2 and COOH terminals located inside the cell and with a reentrant-like structure in the intracellular loop IL2-3 for xCT, as a model for the light subunits of HATs (FIGURE 1) (31, 38).

- The light and the corresponding heavy subunit are linked by a disulfide bridge (FIGURE 1). For this reason, HATs are also named glycoprotein-associated amino acid transporters (21, 102). The intervening cysteine residues are located in the putative extracellular loop EL3-4 of the light subunit and a few residues away from the transmembrane domain of the heavy subunit (FIGURE 1).

- The light subunit cannot reach the plasma membrane unless it interacts with the heavy subunit.

- The light subunit confers specific amino acid transport activity to the heteromeric complex (LAT1 and LAT2 for system L isoforms, γLAT1 and γLAT2 for system γL isoforms, asc1 and asc2 for system asc isoforms, xCT for system χL isoforms, b0,+AT for system b0,+ isoforms, and AGT-1 for a system serving aspartate and glutamate transport (Table 1). Moreover, reconstitution in liposomes shows that the light subunit b0,+AT is fully functional in the absence of the heavy subunit rBAT (71).

- HAT transport activities are, with the exception of system asc isoforms, tightly coupled amino acid antiporters (67).

**Primary Inherited Aminoacidurias**

Primary inherited aminoacidurias (PIAs) are caused by defective amino acid transport activities, which...
Cystinuria is an autosomal inherited disorder, characterized by impaired transport of cystine and dibasic amino acids in the proximal renal tubule and the gastrointestinal tract [first described by Sir Archivald Garrod in 1908 (30) and reviewed in Refs. 60 and 80]. The overall prevalence of the disease is 1/7,000 neonates, ranging from 1/2,500 neonates in Libyan Jews to 1/100,000 among Swedes (60). Patients present normal to low-normal levels in blood, hyperexcretion in urine, and intestinal malabsorption of these amino acids. High cystine concentration in the urinary tract most often causes the formation of recurring cystine stones (i.e., urolithiasis) due to the low solubility of this amino acid. This is the only symptom associated with the disease. Therefore, treatment attempts to increase cystine solubility in urine (high hydration, urine alkalinization, and formation of soluble cystine adducts with thiol drugs) (60). Cystinuria is not accompanied by malnutrition, suggesting that intestinal malabsorption is not severe. Absorption of di- and tripeptides via PepT1 may prevent malnutrition in cystinuria (17).

Traditionally, three types of cystinuria have been recognized in humans: type I, type II, and type III.

### Cystinuria

Cystinuria is an autosomal inherited disorder, characterized by impaired transport of cystine and dibasic amino acids in the proximal renal tubule and the gastrointestinal tract (first described by Sir Archivald Garrod in 1908 and reviewed in Refs. 60 and 80). The overall prevalence of the disease is 1/7,000 neonates, ranging from 1/2,500 neonates in Libyan Jews to 1/100,000 among Swedes. Patients present normal to low-normal levels in blood, hyperexcretion in urine, and intestinal malabsorption of these amino acids. High cystine concentration in the urinary tract most often causes the formation of recurring cystine stones (i.e., urolithiasis) due to the low solubility of this amino acid. This is the only symptom associated with the disease. Therefore, treatment attempts to increase cystine solubility in urine (high hydration, urine alkalinization, and formation of soluble cystine adducts with thiol drugs). Cystinuria is not accompanied by malnutrition, suggesting that intestinal malabsorption is not severe. Absorption of di- and tripeptides via PepT1 may prevent malnutrition in cystinuria.

Traditionally, three types of cystinuria have been recognized in humans: type I, type II, and type III.
This classification correlates poorly with molecular findings, and it has recently been revised to type I (MIM 220100) and non-type I (MIM 600918) cystinuria (with the latter corresponding to old types II and III). These two are distinguished on the basis of the cystine and dibasic aminoacidauria of the obligate heterozygotes (60): type I heterozygotes are silent, whereas non-type I heterozygotes present a variable degree of urinary hyperexcretion of cystine and dibasic amino acids that is higher in type II than in type III. This indicates that type I cystinuria is transmitted as an autosomal recessive trait, whereas non-type I is transmitted dominantly, with incomplete penetrance (27). Not surprisingly, urolithiasis has been described in a minority of non-type I heterozygotes (Table 3). Patients with a mixed type, inheriting type I and non-type I alleles from either parent, have also been described (33). Data on the relative proportion of the two types in specific populations are scarce. In 97 well-characterized families of the International Cystinuria Consortium (ICC) cohort of patients, mainly from Italy, Spain, and Israel, 38, 47, and 14% transmit type I, non-type I, and mixed cystinuria, respectively (Table 3) (28). This cohort is not a registry, and therefore it might not represent the whole population within those countries (20).

LPI

LPI is a PIA with an autosomal recessive mode of inheritance (reviewed in Ref. 86). LPI is predominantly reported in Finland, with a prevalence of 1/60,000. LPI also occurs in Southern Italy and Japan (prevalence of 1/50,000 in the northern part of Iwate) (see Ref. 46 and reviewed in Ref. 58).

In LPI there is massive excretion of dibasic amino acids, especially lysine, and the intestinal absorption of these amino acids is poor (reviewed in Ref. 57). As a result, plasma levels of dibasic amino acids are low. Arginine and ornithine are intermediates of the urea cycle that provide the carbon skeleton. Their reduced availability is thought to produce a functional deficiency of the urea cycle. Protein malnutrition and lysine deficiency contribute to the patient’s failure to thrive.

Several organs are affected in LPI (reviewed in Ref. 86). Patients with LPI are usually asymptomatic while being breast fed, and symptoms (e.g., vomiting, diarrhea, and hyperammonemic coma when force-fed high-protein food) appear only after weaning. After infancy, patients with LPI reject a high-protein diet and show delay in bone growth, osteoporosis, hepatosplenomegaly, muscle hypotonia, and sparse hair. Most patients show normal mental development, but some may have moderate retardation. About two-thirds of the patients have interstitial changes in chest radiographs, sometimes with acute or chronic respiratory insufficiency that can lead to fatal pulmonary alveolar proteinosis and to multiple-organ dysfunction syndrome. Further symptoms, such as glomerulonephritis and erythroblastophagia, suggest that the immune system is affected in some patients.

Low-protein diet and citrulline (i.e., a urea cycle intermediate that is not a substrate of system y+L) are used to correct the functional deficiency of the urea cycle (reviewed in Ref. 57). The final height in treated patients is slightly subnormal. This treatment neither corrects hepatosplenomegaly nor delays bone age or osteoporosis, probably due to lysine deficiency.

The transport characteristics of two of the LSHAT-associated transport systems are relevant to the inherited aminoacidurias cystinuria and LPI (Table 1): system b0,+ (due to the rBAT (SLC3A1) and b0,+AT (SLC7A9) heterodimer) is a tertiary active mechanism of renal reabsorption and intestinal absorption

### Table 3. Genotype and phenotype classification of cystinurial probands

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Phenotype</th>
<th>Total Probands</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
<td>Non-I</td>
</tr>
<tr>
<td>AA</td>
<td>29</td>
<td>2</td>
</tr>
<tr>
<td>AA(B)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>BB</td>
<td>1</td>
<td>34</td>
</tr>
<tr>
<td>B+</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>BB(A)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>78</td>
<td>100</td>
</tr>
</tbody>
</table>

%: 38.5  43.6  3.8  14.1  100

Among the 164 probands studied by the International Cystinuria Consortium, 78 are fully genotyped and phenotyped (28). Thirty-eight probands are not fully genotyped [1 mutated SLC3A1 allele (A?), 1 mutated SLC7A9 allele (B?), and no mutated allele (??)] have been identified in 11, 22, and 5 probands, respectively. Sixty-seven probands (25 AA, 23 BB, 5 A?, 11 B?, and 3 ??) have not been phenotyped due to lack or incoherent information on the amino acid excretion in urine of the obligate heterozygotes. AA, 2 mutated SLC3A1 alleles; BB, 2 mutated SLC7A9 alleles; B+, mutated SLC7A9 carrier; AA(B), 2 mutated SLC3A1 alleles and 1 mutated SLC7A9 allele; BB(A), 2 mutated SLC7A9 alleles and 1 mutated SLC3A1 allele.
of dibasic amino acid and cystine in the apical plasma membrane. It mediates the electrogenic exchange of dibasic amino acids (influx) for neutral amino acids and cystine (efflux) (10). This exchange is favored by the intracellular reduction of cystine to cysteine. System y+L, (due to a 4F2hc-y+LAT1 heterodimer) mediates the electroneutral exchange of dibasic amino acids (efflux) for neutral amino acids plus sodium (influx) (10, 13, 39). It is assumed that this transport system allows the influx of dibasic amino acids against the membrane potential at the basolateral domain of epithelial cells (FIGURE 2). The 4F2hc-y+LAT2 heterodimer mediates system y+L in many other cell types (39, 65, 96).

The Molecular Basis of Cystinuria

The above-mentioned characteristics of rBAT point-
and the other for efflux. This scenario is consistent with two additional sets of results: 1) the chicken intestinal system b\textsuperscript{0,+} has a sequential mechanism of exchange, compatible with the formation of a ternary complex (i.e., the transporter bound to its intracellular and extracellular amino acid substrates) (95); and 2) the analog aminoisobutyrate (AIB) induces an unequal exchange with other substrates through the rBAT-induced system b\textsuperscript{0,+} in oocytes (i.e., using the endogenous b\textsuperscript{0,+}-AT subunit) (16). The oligomeric structure of system b\textsuperscript{0,+} (i.e., rBAT-b\textsuperscript{0,+}-AT heteromer) is unknown. Functional coordination of two rBAT-b\textsuperscript{0,+}-AT heterodimers in a heterotetrameric structure would explain these results. If this is not the case, the transport defect associated with mutation R365W would suggest that a single b\textsuperscript{0,+}-AT subunit contains two translocation pathways.

The gene causing non-type I cystinuria was assigned to 19q12-13.1 by linkage analysis (5, 89, 103). In 1999 the non-type I cystinuria gene was identified as SLC7A9 (24). SLC7A9 was a positional candidate gene for non-type I cystinuria because it has the appropriate chromosomal location, rBAT-associated amino acid transport activity (system b\textsuperscript{0,+}), and tissue expression (mainly in kidney and small intestine). The protein product encoded by SLC7A9 was termed b\textsuperscript{0,+}-AT for b\textsuperscript{0,+} amino acid transporter. Sixty-six SLC7A9 mutations causing cystinuria have been described (see Ref. 28 and references therein). Mutation G105R is the most frequent SLC7A9 mutation in the ICC cohort of patients (27.4% of the identified alleles). In 1999 the non-type I cystinuria gene was identified as SLC7A9 (24). SLC7A9 was a positional candidate gene for non-type I cystinuria because it has the appropriate chromosomal location, rBAT-associated amino acid transport activity (system b\textsuperscript{0,+}), and tissue expression (mainly in kidney and small intestine). The protein product encoded by SLC7A9 was termed b\textsuperscript{0,+}-AT for b\textsuperscript{0,+} amino acid transporter. Sixty-six SLC7A9 mutations causing cystinuria have been described (see Ref. 28 and references therein). Mutation G105R is the most frequent SLC7A9 mutation in the ICC cohort of patients (27.4% of the identified SLC7A9 alleles). Similarly to the human disease, the Slc7a9-knockout mouse presents non-type 1 cystinuria with urolithiasis (23).

Several cystinuria-specific SLC7A9 missense mutations have been reported to lead to a transport function defect (27). Among these mutations, reconstitution in proteoliposomes showed that A182T-mutated b\textsuperscript{0,+}-AT is active but with a trafficking defect to the plasma membrane, whereas mutation A354T renders the transporter inactive (71).

### Explained alleles in cystinuria

Very recently, the ICC performed an exhaustive mutational analysis on 164 probands (28): 86.8% of the independent alleles were identified. The coverage of identified alleles was similar in all cystinuria types (type I, 90.5%; non-type I, 87.6%; mixed, 89.3%; untyped patients, 83.6%).

The unidentified alleles (13.2%) may be due to mutations in intronic or promoter regions (e.g., two cystinuria-specific sequence variants in the promoter region of SLC3A1 have been reported in Ref. 7), to SLC3A1 or SLC7A9 polymorphisms in combination with cystinuria-specific mutations in the other allele (as suggested by Schmidt et al. (76)), or to unidentified genes. These three possibilities have not been confirmed or ruled out. Functional studies of promoter sequence variants have not been reported, and cystinuria association studies and functional analysis of candidate polymorphisms are required to demonstrate their role in the disease. Of particular interest is the possibility of a third cystinuria gene. In this sense, Goodyer's group proposed SLC7A10 as a candidate (50). SLC7A10 is in the vicinity of the cystinuria gene SLC7A9 on chromosome 19q13.1 and codes for the renal light subunit asc1, which, with 4F2hc, forms the holotransporter asc with substrate specificity for cystein and other small neutral amino acids. Moreover, these authors found the missense mutation E112D associated with cystinuria. In contrast, recent studies have ruled out this hypothesis: 1) cystinuria-specific mutations are not found in patients with alleles not explained by mutations in the two cystinuria genes (67, 78), 2) the conservative mutation E112D does not affect transport of 4F2hc-asc1 (67), and 3) asc1 mRNA is expressed in the distal tubule where renal reabsorption of amino acids is not relevant but where asc1 may have a role in the regulation of osmosis (67). Another candidate is a missing light subunit of rBAT. Comimonoprecipitation studies and the SLC7A9 knockout model demonstrated that the protein products of SLC3A1 (rBAT) and SLC7A9 (b\textsuperscript{0,+}-AT) heterodimerize in mouse kidney brush border membranes, but a significant part of rBAT heterodimerizes with an unknown light subunit (X) (23, 25). Indeed, a heterodimer of rBAT is present in kidney brush-border membranes of the Slc7a9-knockout mice (23). The protein X may also be present in human kidney brush borders, but a final demonstration is needed (25). Therefore, the gene coding for X could be a candidate for cystinuria. However, the similar hyperexcretion of amino acids detected in urine of patients with mutations in SLC3A1 or in SLC7A9 does not support this hypothesis (28). If X were coded by a cystinuria gene, one would expect higher aminoaciduria in patients with mutations in SLC3A1 than in patients with mutations in SLC7A9: b\textsuperscript{0,+}-AT mutations would affect system b\textsuperscript{0,+} only, whereas rBAT mutations would affect system b\textsuperscript{0,+} and the transport activity of the rBAT/X heteromeric complex. In summary, the possibility of a third cystinuria gene cannot be discarded, but it would be relegated to a very small proportion of patients and seems improbable (Only 3% of the probands of the ICC show no mutation in either of the two cystinuria genes; Ref. 28).

### Genotype-phenotype correlations in cystinuria

Initial data suggested a one-to-one correlation between the phenotype and the mutated gene
mutations in SLC3A1 resulted in type I, and mutations in SLC7A9 resulted in non-type I) (12, 32). In contrast to this simple view, recent data show a more complex scenario. On the one hand, all SLC3A1 mutations in well-characterized families cause type I cystinuria, with the exception of mutation dupE5–E9, which shows the non-type I phenotype in four out of six heterozygotes studied (28). This mutation consists of a gene rearrangement c.(891+1524, 1618–1600)dup (initially published in Ref. 77), which results in the duplication of exons 5–9 and the corresponding in-frame duplication of amino acid residues E298–D539 of rBAT, as shown by RNA studies (28, 77). Functional studies are necessary to explain the dominant negative effect of dupE5–E9 mutation on the rBAT/b²-AT heteromeric complex. On the other hand, most of the heterozygotes carrying a SLC7A9 mutation have a non-type I urine phenotype (i.e., hyperexcretion of dibasic amino acids and cystine), but heterozygotes carrying SLC7A9 mutations may also have a type I phenotype (i.e., silent heterozygotes). Approximately 14% of the SLC7A9 heterozygotes have phenotype I in the ICC cohort of patients and their relatives (20). SLC7A9 mutations that have been found associated with phenotype I in some families are 144T, G63R, G105R, T123M, A126T, V170M (the Libyan Jewish mutation), A182T, G195R, Y232C, P261L, W69X, and c.614dupA (28, 49). There is no clear explanation of why these mutations associate with phenotype I, since some proteins show residual transport activity when expressed in heterologous expression systems but others do not (27). A182T is the most frequent SLC7A9 mutation associated with phenotype I (i.e., 6 out of 11 A182T heterozygotes in the ICC cohort), and this mutation leads to a protein with 50% residual transport activity at the plasma membrane (27, 71). Moreover, mixed cystinuria patients with two mutations in SLC7A9 presented a level of aminoaciduria in the lower range of non-type I patients with two mutations in this gene (28). This suggests that, in addition to individual and population variability, mild SLC7A9 mutations may be more prone to associate with silent phenotype in heterozygotes.

The lack of a direct relationship between the mutated cystinuria gene and the type of cystinuria led the ICC to propose two parallel classifications to describe cystinuria (20) that are 1) based on the urine phenotype of the obligate heterozygotes (type I, non-type I, and mixed, as described above) and 2) based on the genotype of the patients (type A due to mutations in SLC3A1, type B due to mutations in SLC7A9, and type AB to define a possible digenic cystinuria). Table 3 summarizes the double classification for 78 cystinuria probands by the ICC (28) as follows: 1) most type I patients have two mutations in SLC3A1 (i.e., individuals AA); 2) all non-type I patients (including type non-type I heterozygotes with urolithiasis) have mutations in SLC7A9 (i.e., individuals BB and BA); 3) patients with mixed cystinuria carry mutations in SLC3A1 (2 probands AA) or in SLC7A9 (7 probands BB); and 4) 2 out of 126 fully genotyped probands carry mutations in both genes (probands AA(B) and BB(A)), suggesting a role for digenic inheritance in cystinuria (see below).

Urolithiasis shows a clear gender and individual variability among cystinuria patients (20). In the ICC cohort, the age of onset (first stone) ranges from 2–40 yr with a median of 12 and 15 yr for males and females, respectively. The incidence of onset before the age of 3 is lower in females than males. Similarly, the number of total stone events (i.e., spontaneously emitted stones plus those surgically removed) is higher in males than females (0.42 and 0.21 events per year in males and females, respectively). Of the 224 patients studied, ten with aminoaciduria and full genetic confirmation of the disease did not develop renal stones, and two of them are over 40 years of age. In contrast, clinical symptoms (i.e., urolithiasis and its consequences) are almost identically represented in the two cystinuria types when either the clinical or the genetic classification is considered. The differences in severity between the genders and marked differences between siblings sharing the same mutations (19, 20) suggest that other lithogenic factors, genetic or environmental, contribute to the urolithiasis phenotype. Moreover, only about half of the Slc7a9-knockout mice, which have a mixed genetic background, develop urolithiasis (23). Moreover, lithiasic and nonlithiasic Slc7a9-knockout mice hyperexcrete similar levels of cystine. Studies in Slc7a9-knockout mice within different genetic backgrounds may unravel the genetic factors contributing to urolithiasis besides mutations in Slc7a9 and the cystine levels in urine.

Digenic inheritance causing partial phenotype

To our knowledge, only four patients with mutations in both cystinuria genes have been reported (28, 35). There is no report of the urine phenotype of the Swedish patient AA(B). In the ICC database, only two sisters AA(B) and one male BB(A) from 2 families out of 126 fully genotyped families have been identified. They are classified as mixed cystinuria patients (i.e., each of the two mutated alleles in the same gene is associated with phenotype I or non-phenotype I in the obligate heterozygotes). The aminoaciduria levels of these patients and their double-heterozygote (i.e., AB) relatives indicate that digenic inheritance in cystinuria has only a partial effect on the phenotype, restricted to a variable impact on the aminoaciduria. Indeed,
none of the individuals AB presented urolithiasis. Given that the frequencies of type A and B alleles are similar in this cohort, if digenic inheritance was the rule in cystinuria, we would expect a quarter of patients to be AA, a quarter of patients to be BB, and half of patients to be AB. This indicates that digenic inheritance affecting phenotype is an exception in cystinuria. However, we cannot rule out the possibility that some combinations of mutations A and B might produce enough cystine hyperexcretion to cause urolithiasis.

A working hypothesis on the biogenesis of the rBAT-b0,+AT heterodimer and the urine phenotypes in cystinuria may explain the apparent lack of full digenic inheritance in cystinuria: the rBAT protein is produced in excess in kidney, and therefore neither an rBAT mutation in heterozygosis (e.g., mouse cystinuria model with the Slc3a1 mutation D140G [64]) nor any of the human cystinuria rBAT mutations except dupE5–E9 [28] lead to hyperexcretion of amino acids (phenotype 1). Also, b0,+AT controls the expression of the functional rBAT-b0,+AT heterodimeric complex: interaction with b0,+AT stabilizes rBAT, and the excess of rBAT is degraded, as shown in transfected cells (4, 71). As a result, a half dose of b0,+AT (heterozygotes of severe human SLC7A9 mutations or of the SLC7A9 knockout mice [23, 28]) results in a significant decrease in the expression of rBAT-b0,+AT heterodimeric complex: interaction with b0,+AT stabilizes rBAT, and the excess of rBAT is degraded, as shown in transfected cells (4, 71). As a result, a half dose of b0,+AT [heterozygotes of severe human SLC7A9 mutations or of the SLC7A9 knockout mice (23, 28)] results in a significant decrease in the expression of rBAT-b0,+AT heterodimeric complex (system b0,+), which causes hyperexcretion of cystine and dibasic amino acids. In this scenario, the lack of a full phenotype because of digenic inheritance indicates that in double heterozygotes (AB), the mutated rBAT, with trafficking defects (14, 68, 74), does not interact and/or does not compromise the heterodimerization and trafficking to the plasma membrane of the half dose of wild-type b0,+AT with the half dose of wild-type rBAT. Thus individuals AB behave as heterozygotes B with a variable degree of aminoaciduria, which could be greater than that of single heterozygotes within the family, depending on the particular combination of mutations. Demonstration of this hypothesis requires a deep study of both the impact of cystinuria-specific rBAT and b0,+AT mutations (including the dominant negative rBAT mutation dupE5–E9) in the biogenesis and the functional and structural stoichiometry of subunits of the heteromeric complex rBAT/b0,+AT both in cell culture studies and in vivo with the help of Slc3a1 and Slc7a9 double-mutant mice.

The Molecular Basis of LPI

The gene responsible for LPI was localized to 14q11.2 in Finnish and non-Finnish populations (47, 48). The cloning of y+LAT1, encoded by SLC7A7 (96), revealed characteristics that made this gene an excellent candidate for LPI:

- y+LAT1 heterodimerizes with 4F2hc to express system y+L amino acid transport activity in the basolateral plasma membrane of the epithelial cells of the renal proximal tubule and the small intestine (see above).
- SLC7A7 is expressed in tissues affected in LPI (kidney, small intestine, lung, and white blood cells).
- SLC7A7 maps to the correct location for LPI.

Torrents and co-workers (97) performed mutational analysis of SLC7A7 in 1 Spanish and 31 Finnish LPI patients. A single Finnish mutant allele (1181-2A>T) was found, in which the splice site acceptor of intron 6 is inactivated and a cryptic acceptor 10 bp downstream is activated, with the result that 10 bp of the open reading frame are deleted and the reading frame is shifted. This mutation has been found in all Finnish LPI patients (i.e., “the Finnish mutation”) (54, 97). The Spanish patient with LPI was a genetic compound of two SLC7A7 mutations (a missense mutation, L334R, and a 4-bp deletion, 1291delCTCTT). Simultaneously and independently, Borsani and co-workers (6) identified the Finnish mutation in four Finnish patients and found two additional SLC7A7 mutations (1625insATAC and 242delS43) in five Italian patients with LPI. These two studies established that mutations in SLC7A7 cause LPI. Additional studies showed the nonsense mutation W242X and the insertion 1625insATAC as the most prevalent mutations in the south of Italy (88) and the nonsense mutation R410X as the most prevalent in Japan (55).

A total of 31 SLC7A7 mutations have been described in 113 patients with LPI (222 explained alleles from a total of 226 studied alleles, including consanguineous ones; reviewed in Ref. 58). Identified SLC7A7 mutations include missense, nonsense, and splicing mutations, insertions, deletions, and large genomic rearrangements. No LPI-associated mutation has been found in SLC3A2, which encodes the heavy subunit of y+LAT1 (4F2hc). This strongly suggests that SLC7A7 is the only gene involved in the primary cause of LPI. It is believed that mutations in 4F2hc would be deleterious, and indeed, the targeted disruption of Slc3a2 in mouse is lethal during embryonic life (98). 4F2hc serves as the heavy subunit of six other heteromeric amino acid transporters (see above). Therefore, a defect in 4F2hc would result in six defective amino acid transport activities expressed in many cell types and tissues. Moreover, 4F2hc is a multifunctional protein with a putative role in β1-integrin function and cellular fusion (reviewed in Ref. 15). Thus defective 4F2hc would be life threatening.
cells showed that frameshift mutations (e.g., 1291delCTTT, 1540delC, and the Finnish mutation) produce a severe defect in trafficking to the plasma membrane (54, 94). In contrast, the missense mutations G54V and L334R inactivate the transporter (e.g., the mutated proteins reach the plasma membrane when coexpressed with 4F2hc, but no transport activity is elicited) (54, 94). Recently, functional studies in oocytes showed that the LPI-specific SLC7A7 mutations M1L, M50K, T188I, W242X, S386R, Y457X, and c.1471delT/YCT lead to loss of transport function. In this last study, no attempt was made to elucidate whether these mutations affect trafficking or inactivate the transporter (Sperandeo MP and Sebastio G, personal communication).

The Renal and Intestinal Reabsorption of Amino Acids

The renal reabsorption of amino acids occurs in the proximal convoluted tube (85), and the absorption of amino acids occurs in the small intestine (52). Most of the transporters responsible for these functions are the same in kidney and intestine (FIGURE 2). The most striking exception to this rule is the proton-dependent peptide transporters. PepT1 and PepT2 are expressed in small intestine and in kidney, respectively. The physiological role of PepT2 in kidney is unknown, and the quantitative contribution of PepT1 to assimilation of amino acids has not yet been evaluated in mammals or humans (17). A deeper study of the phenotype of the PepT2-knockout mice (73) and generation and study of the PepT1 may answer these questions.

The molecular basis of cystinuria and LPI teaches us about the molecular bases of cystine and dibasic amino acid reabsorption. Mutations of the apical exchanger b0,+ (heterodimer rBAT-b0,+AT) lead to hyperexcretion of cystine and dibasic amino acids, as shown in human (11, 24), mouse (23, 64), and canine (37) cystinuria. Mutations of the basolateral exchanger y+L (heterodimer 4F2hc-y+LAT1) produce hyperexcretion of dibasic amino acids (6, 97). Similarly, there is intestinal malabsorption of cystine and dibasic amino acids in cystinuria and of dibasic amino acids in LPI (reviewed in Refs. 80 and 86). Thus the sequential transport activities of systems b0,+ (apical) and y+L (basolateral) play a major role in renal and intestinal reabsorption of amino acids. Besides these two players, not much is known about other transporters with a role in dibasic amino acid reabsorption. Cystinuria patients may show almost null cystine reabsorption in kidney, whereas dibasic reabsorption in kidney remains significant (reviewed in Ref. 25). This suggests that system b0,+ is the main (if not the only) apical reabsorption system for cystine, but other apical transport systems in addition to system b0,+ contribute to the renal reabsorption of dibasic amino acids. The study of the dibasic amino acid transport activities in the Slc7a9-knockout mice should help to identify those transporters. Similar data are not available for LPI patients, but the experiments of the Finnish group (70) suggest that system y+L is the main basolateral absorption system for dibasic amino acids in the small intestine: an oral load with the dipeptide lysyl-glycine increased glycine plasma concentrations, but plasma lysine remained almost unchanged in patients with LPI, whereas both amino acids increased in plasma of control subjects and in patients with cystinuria. This demonstrated the basolateral defect in LPI and points to a role of PepT1 in amino acid assimilation. Indeed, as a consequence of the renal and intestinal defects in LPI, plasma levels of dibasic amino acids are 1/2 to 1/3 of normal levels (reviewed in Ref. 86), whereas in human and mouse cystinuria cystine and dibasic amino acid levels in plasma are only moderately (20–30%) reduced (reviewed in Ref. 57). In all, these results suggest that system y+L has a higher impact than system b0,+ on renal and intestinal reabsorption of dibasic amino acids.

An understanding of the molecular basis of zwitterionic renal and intestinal reabsorption was helped by the identification of B0AT1 (SLC6A19) as the gene causing Hartnup disorder (45, 81). B0AT1 corresponds to system B0 (also named system neutral brush border system NBB) and catalyzes the Na+-dependent transport of most neutral amino acids (9). Patients with Hartnup disorder present hyperexcretion and malabsorption of neutral amino acids (reviewed in Ref. 51). Interestingly, they also show moderate hyperexcretion of cystine and dibasic amino acids, suggesting a metabolic link with system b0,+ (reviewed in Ref. 81) (FIGURE 2). The basolateral Na+-K+-ATPase generates the electrochemical gradient of Na+, which is used by apical (system B0; i.e., B0AT1) and basolateral (system A and ASC) Na+ cotransporters to generate a high intraepithelial concentration of zwitterionic amino acids. A reduced influx of zwitterionic amino acids when B0AT1 is defective (i.e., Hartnup disorder) would reduce the substrates for exchange with cystine and dibasic amino acids via system b0,+.

The above-mentioned transporters with a role in reabsorption of amino acids are highly expressed in the corresponding apical or basolateral plasma membrane of the epithelial cells of the proximal convoluted tubules (S1 and S2 segments) in kidney and of the enterocytes of the small intestine (18, 25, 29). The heterodimer 4F2hc-LAT2 has a similar pattern of expression in kidney and small intestine, where it is located in the basolateral plasma membrane (18, 66). 4F2hc-LAT2 is an exchanger with...
broad specificity for small and large zwitterionic amino acids with characteristics of system L (66). This suggests that 4F2hc-LAT2 may have a role in reabsorption of zwitterionic amino acids. Indeed, antisense experiments in the polarized opossum kidney cell line OK, derived from proximal convoluted epithelial cells, demonstrated a role of LAT2 (SLC7A8) in the transepithelial flux of cystine, and the basolateral efflux of cysteine and influx of alanine, serine, and threonine (intracellular concentration of cysteine increases, and that of alanine, serine, and threonine decreases in LAT2-antisense OK cells in polarized culture) (26). To our knowledge, no inherited human disease has yet been related to LAT2 mutations. A final demonstration of the role of LAT2 in reabsorption would only be possible after the generation of LAT2-knockout mouse models.

There is evidence for phenotypic variants of Hartnup disorder, suggesting that 5p15.33 (SLC6A19, and linkage to 5p15 has been excluded in one of them (45). This suggests that a defect in other amino acid transporters may result in Hartnup disorder. Several candidates are already available:

- Within the SLC6 family there are two B0AT1 sequence-related orphan transporters, XT2 (SLC6A19) and XT3 (SLC6A20). These three genes belong to a branch of amino acid transporters within the Na+-Cl–-dependent neurotransmitter family (9). SLC6A19 is located in the vicinity of SLC6A19 in 5p15, and therefore it might be excluded from linkage to Hartnup disorder (45). Functional and tissue-expression studies are needed to ascertain the role of XT2 and XT3 in reabsorption of amino acids.

- The amino acid transporter ASCT2 (also named ATB0) (SLC1A5) is expressed in the apical plasma membrane of the renal proximal convoluted and small intestine epithelial cells (1). This transporter exchanges most of the zwitterionic amino acids in a Na+-dependent manner (40, 42, 99). The role of ASCT2 in amino acid reabsorption has not been evaluated.

- The metabolic link between systems b0,+ B0, and y+L requires a basolateral transport system to mediate the efflux of neutral amino acids (FIGURE 2).

A defective amino acid transport system for basolateral efflux of zwitterionic amino acids would increase the intracellular concentration of these amino acids, resulting in their hyperexcretion in urine and intestinal malabsorption. Candidate transporters for this function may be found within families SLC16 and SLC43. Amino acid transporters in these families mediate facilitated diffusion and may therefore mediate the efflux of zwitterionic amino acids from the high intracellular concentration to the interstitial space. T-type amino acid transporter 1 (TAT1; SLC16A10) transports aromatic amino acids in a Na+- and H+ independent manner (43, 44). TAT1 is expressed in human kidney and small intestine with a basolateral location. The SLC16 family (also named MCT for monocarboxylate transporters) contains members transporting monocarboxylates and thyroid hormones as well. Eight transporters within this family are orphan transporters (MCT5–7, MCT9, and MCT11–14) (34). Knockout mouse models for TAT1, and their related orphan transporters expressed in kidney cortex and small intestine, may help to identify basolateral transporters involved in reabsorption of zwitterionic amino acids. LAT3 (2) and LAT4 (5a) within family SLC43 mediates facilitated diffusion of zwitterionic amino acids with characteristics of system L. Neither of these two transporters is expressed in epithelial cells of the renal proximal convoluted tubule or the small intestine, but the SLC43 family has a third member with no identified transport function (EEG1; Ref. 90). Functional and tissue-expression studies are needed to ascertain the role of EEG1 in reabsorption of amino acids.

The molecular basis of the renal and intestinal reabsorption of acidic amino acids is less known. The bulk (>90%) of filtered acidic amino acids is reabsorbed within segment S1 (i.e., the first part of the proximal convoluted tubule) (83, 84). Two apical acidic transport systems have been described: one of high capacity and low affinity and the other of low capacity and high affinity (reviewed in 36). The Na+-K+-dependent acidic amino acid transporter EAAT3, which localized to chromosome 9p24 (87) (also named EAAC1; SLC1A5) (system XAg) is expressed in the brush-border membranes of segments S2 and S3 of the nephron (82). The transport characteristics of SLC1A5 correspond to the high-affinity system (41). The Slatat1-knockout mice develop dicarboxylic aminoaciduria (62), demonstrating the role of this transporter in renal reabsorption of acidic amino acids. In contrast, a direct demonstration of the role of SLC1A1 in human dicarboxylic aminoaciduria is lacking. The apical low-affinity transport system for acidic amino acids in kidney has been determined in brush-border membrane preparations (104), but its molecular identity remained elusive (36). At renal basolateral plasma membranes, a high-affinity Na+-K+-dependent transport system for acidic amino acids has been reported (75), but its molec-
ular structure has not been identified. Another member of the SLC1 family, GLT1 (i.e., the glial high-affinity glutamate transporter (69)), also named EAAT2; SLC1A2, is expressed in rat kidney cortex and porcine small intestine (22, 105). To our knowledge, the expression of GLT1 protein has not been studied in kidney or intestine. SLC1a2-knockout mice show lethal spontaneous epileptic seizures (91). Reabsorption of acidic amino acids could be studied in these mice to ascertain the role of GLT1, although this is difficult because only 50% survive longer than 6 wk.

We thank Robin Rycroft for editorial help.

Studies in our laboratory referred to above were supported in part by the Spanish Ministry of Science and Technology (SAF2003-08940/01-B02), the European Union (EUGIDAT, LSHM-CT-2003-502852), the Spanish Instituto de Salud Carlos III (networks G03/054, C03/07 and C03/08), the Generalitat de Catalunya (2001 SGR00399, 2001 SGR00118), and the Comissionat per a la Universitats i Recerca (Generalitat de Catalunya). M. Font-Llitjós was a recipient of a CIRIT fellowship. M. Jiménez-Vidal was supported by BIOMED BMH4 C798-3154. L. Felipubadalo was supported by EUGIDAT.

References


4. Bauch C and Verrey F. Apical heterodimeric cystine and Vidal was supported by BIOMED BMH4 C798-3154. L. Llitjós was a recipient of a CIRIT fellowship. M. Jiménez-Vidal was supported by BIOMED BMH4 C798-3154. L. Felipubadalo was supported by EUGIDAT.

References


4. Bauch C and Verrey F. Apical heterodimeric cystine and Vidal was supported by BIOMED BMH4 C798-3154. L. Llitjós was a recipient of a CIRIT fellowship. M. Jiménez-Vidal was supported by BIOMED BMH4 C798-3154. L. Felipubadalo was supported by EUGIDAT.

References


