Lysinuric protein intolerance: one gene, many problems

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AMINO ACIDS are efficiently absorbed in intestine and kidney by epithelial cells endowed with transporters for specific groups of amino acids. Inherited renal aminocidurias arise as a result of mutations inactivating apical or basolateral transport systems. Although aminocidurias are diagnosed by urine amino acid analysis, most of the disorders affect both intestinal and renal transport. In the intestine, peptide transporters and amino acid transporters work in parallel in the apical membrane to aid in protein absorption. Mutations in apical transporters, as a result, show predominantly renal phenotypes because intestinal malabsorption is compensated by peptide transport (5). Lysinuric protein intolerance (LPI), by contrast, is an autosomal recessive disorder affecting the basolateral transporter for cationic amino acids in the kidney and intestine (12). It is generally assumed that a block of basolateral membrane transport is more severe than mutations affecting apical transport because peptides are largely hydrolyzed inside epithelial cells and, therefore, no shunt pathway exists in protein absorption across the basolateral membrane (8). The phenotype of LPI has puzzled researchers for many years. It is a multiorgan disorder with a variety of clinical symptoms. Nausea and vomiting are observed as acute symptoms after protein ingestion. Chronic symptoms include an enlarged liver and spleen, growth retardation, muscle weakness, and osteoporosis. Some patients also develop renal insufficiency and pulmonary alveolar proteinosis. The related lung disorder is characterized by lipoprotein deposits derived from surfactant. LPI is diagnosed by the presence of excessive amounts of dibasic amino acids in the urine combined with high plasma ammonia levels, particularly after protein ingestion. Lysine levels in the urine are more elevated than other cationic amino acids (6). A variety of mechanisms are discussed to explain the multiplicity of symptoms. The poor intestinal absorption of arginine, ornithine, and lysine combined with their loss in the kidney causes low levels of these amino acids in the blood of LPI patients. This, in turn, results in low activity of the urea cycle, causing hyperammonemia. The above-mentioned acute symptoms after protein ingestion, such as nausea and vomiting, are likely to be caused by this mechanism. LPI is therefore treated by strict avoidance of protein-rich food and supplementation of citrulline. Citrulline is absorbed by neutral amino acid transporters and can subsequently replenish the urea cycle (9). The reduced capacity of the urea cycle probably also causes a compensatory enlargement of the liver. Reduced growth, alveolar proteinosis, and osteoporosis are more difficult to explain. These symptoms are not alleviated by citrulline supplementation, and it is rather likely that they are related to reduced lysine availability (13). Lysine is important for the cross-linking of collagen fibrils, and low plasma lysine concentrations appear also to be correlated with reduced levels of growth hormone (4). Studies using lysine plus citrulline supplementation to treat LPI unfortunately have remained inconclusive due to the small number of LPI patients (13).

A breakthrough was achieved in 1999 with the identification of the LPI gene by two independent groups (1, 15). The transporter belongs to a larger family of heteromeric amino acid transporters, which consist of a heavy subunit (called 4F2 or CD98, SLC3A2) and a light subunit (7, 14). The light subunit forms the translocation pore, whereas the heavy subunit is essential for the trafficking of the assembled protein to the plasma membrane. The heavy subunit 4F2 can associate with a variety of light chains forming amino acid transporters of different substrate specificity (19, 20). LPI is exclusively caused by mutations in the so-called y⁺LAT1 light chain (SLC7A7). Mutations in the 4F2 heavy chain have not been observed and would affect a variety of essential amino acid transport systems. In fact, 4F2 knockout mice die early during embryogenesis (17). In addition to renal and intestinal epithelia, the y⁺LAT1 light chain is also expressed in lymphocytes, alveolar macrophages, and epithelial cells of the lung, but not in the liver (10). The related transporter y⁺LAT2 (SLC7A6) also forms a heterodimer with 4F2 and is ubiquitously expressed (2). Both transporters export cationic amino acids from the cell in exchange for neutral amino acids. Due to the low expression levels of y⁺LAT2 in the intestine and kidney, it cannot replace the function of y⁺LAT1 (11). LPI is more frequent in Finland than in other countries. All Finnish LPI patients carry a founder mutation that is persistent in this population (1, 15). Despite their genetic commonality, Finnish LPI patients show large variability of their clinical symptoms, ranging from normal growth and little protein aversion to cases with severe protein intolerance, an enlarged liver and spleen, osteoporosis, and alveolar proteinosis.

The expression pattern of y⁺LAT1 suggests explanations for some of the clinical symptoms of LPI. Reduced clearance of lung surfactant by alveolar macrophages has been implicated in the generation of alveolar proteinosis (16). If y⁺LAT1 expression is essential for alveolar macrophage function, mutations would incapacitate macrophages and allow the build up of lung surfactant proteins. The y⁺LAT1 protein is also found in blood lymphocytes. The expression in these cells could be related to the enlarged spleen observed in LPI patients, but the precise cause is unknown. Many patients with LPI show signs of disturbed immune function. Humoral immune responses are defective in some patients, and Varicella viral infections may be exceptionally severe (13).

The unresolved questions of LPI pathology, together with the possibility of controlled studies using different dietary supplements, call for a mouse model of this disorder. Sperandeo et al. (12a) reported on the generation of a slc7a7 nullizygous mouse. In contrast to humans, slc7a7⁺/⁻ mice experience intrauterine growth restriction, resulting in small pups, which were mostly cannibalized by their mothers. As a result, only two pups could be rescued and studied further. These two animals showed symptoms that closely resembled...
those of LPI. Using a protein-reduced diet and citrulline, the animals survived, and the female mouse could even produce offspring. Returning the animals to a protein-rich diet caused progressive hypotonia, tremors, and weight loss followed by death. It appears likely that the death of these animals was caused by acute hyperammonemia leading to brain edema and coma (18). In humans with LPI, intrauterine development appears to be normal. Symptoms are usually not observed while breastfeeding but appear after weaning (6). Further investigation of the intrauterine growth restriction in the mice showed that expression of IGF-1 and IGF-2 was significantly reduced in slc7a7−/− animals. Growth hormone exerts many of its effects via stimulation of IGF-1–2 released from the liver and other tissues. Interestingly, it appears that high concentrations of cationic amino acids in blood plasma increase the secretion of growth hormone. In agreement with this notion, growth hormone deficiency has been reported in a patient with LPI (3). Growth restriction in humans with LPI appears to be a postnatal event, whereas retarded growth of mice is already evident in utero.

Microarray expression analysis revealed a number of compensatory changes in slc7a7−/− mice. The cationic amino acid transporter Cat-2 (slc7a2) was upregulated in liver tissue, most likely improving capture of arginine and ornithine from the blood plasma. The related y+LAT2 (slc7a6) transporter was found to be upregulated, but this compensation did not prevent LPI. Surprisingly, it was found that the intestinal Na+/P+ cotransporter (slc34a2) was strongly downregulated, which may contribute to osteoporosis and reduced growth. However, the relevance of this finding needs further investigation in view of normal plasma and urine phosphate levels in LPI patients (12). The slc7a7 nullizygous mouse provides a promising start to the investigation of LPI pathology and will now allow many of these questions to be addressed experimentally. In the longer term, this mouse model may allow improved treatment of patients with LPI, but progress will be accelerated if the severity of the mouse phenotype can be attenuated by breeding onto a different genetic background or by generating a conditionally nullizygous mouse.

REFERENCES