Neutral amino acid transporters of the kidney and intestine

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The epithelial cells of the renal proximal tubule and of the intestine are important sites of amino acid transport. A defect in this amino acid transport leads to compromised amino acid absorption, which manifests as an excess of amino acids in the urine and/or faeces. Several such disorders have been identified, including Hartnup disorder and iminoglycinuria.

Hartnup disorder is an autosomal recessive inherited disorder, which is characterised by the excretion of large amounts of neutral amino acids in the urine. Clinical symptoms are variable, and can include a photosensitive skin rash, episodes of cerebellar ataxia and other neurological symptoms. Recently, we cloned and characterised a novel sodium-dependent neutral amino acid transporter called B^0AT1. We and others have since identified mutations in B^0AT1 that cause Hartnup disorder by inactivating the transporter (Seow *et al.*, 2004; Kleta *et al.*, 2004). However, causative mutations in B^0AT1 were not found in all affected individuals. Together with the variability of clinical symptoms, this suggests that Hartnup disorder may be genetically heterogeneous.

To investigate this further, we studied several newly acquired families and reevaluated those families where causative mutations were not previously detected. Genomic sequencing of B^0AT1 revealed disease-associated mutations in each of these Hartnup disorder families, including six novel missense mutations and one nonsense mutation. Functional analysis of these mutations by expression of B^0AT1 cRNA in *Xenopus laevis* oocytes*, demonstrated that the transporter is inactivated by all novel mutations detected in these families. This suggests that B0AT1 is the major gene involved in Hartnup disorder.

Iminoglycinuria is an autosomal recessive disorder characterized by increased excretion of imino acids (proline and hydroxyproline) and glycine in the urine. While the renal transport of imino acids and glycine is always defective in iminoglycinuria, intestinal transport is not always affected. Moreover, some obligate heterozygotes show hyperglycinuria, while others do not. This variability suggests the involvement of multiple genes and/or alleles (Chesney, 2001).

Among the known renal and intestinal amino acid transporters, several candidates exist that are potentially involved in iminoglycinuria – these include IMINO, PAT1, PAT2 and XT2. IMINO is a sodium-dependent proline transporter that we have cloned and characterised. PAT1 and PAT2 are both proton-dependent proline and glycine transporters, while XT2 is a putative glycine transporter. We are currently investigating the role of these candidates in iminoglycinuria.

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**Xenopus laevis* oocytes were harvested by surgery of anaesthesized frogs (MS-222, 1.5g/l). The procedure was approved by the Animal Experimentation Ethics Committee of the Australian National University.