

The molecular basis of neutral amino acidurias

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Abstract. Hartnup disorder is an autosomal recessive disorder caused by mutations in the neutral amino acid transporter B⁰AT1. Iminoglycinuria, by contrast is likely to be a multigene disorder. The proton amino acid transporters PAT1 and PAT2, the IMINO transporter and the glycine transporter XT2 appear to play key roles in the resorption of glycine and proline. (www.actabiomedica.it)

Key words: Hartnup disorder, Iminoglycinuria, solute carrier family 6

Introduction

Several transporters contribute to the resorption of neutral amino acids in both kidney and intestine (Fig. 1). Functional studies indicate the presence of dominant transport activity for almost all neutral amino acids in the apical membrane of enterocytes and kidney epithelial cells, called system B⁰ or NBB. Ad-

ditional transporters are involved in the transport of proline and glycine. A common high-affinity transporter is expressed in kidney, additionally a glycine-specific and a proline-specific transporter have been reported (1).

Hartnup disorder is characterised by an elevated secretion of most neutral amino acid in the urine. Intestinal transport is affected as well. Mutations in the neutral amino acid transporter SLC6A19 have been identified as the cause underlying most cases (2, 3). However, failure to identify SLC6A19 mutations in some families was considered evidence for a second Hartnup gene. Iminoglycinuria appears to be multigene disorder, perhaps involving several transporters for proline and glycine in kidney and intestine (1).

Materials and Methods

Messenger RNA encoding SLC6 amino acid transporters was reverse transcribed and the coding sequence amplified by PCR using specific primers. The coding region of the transporter cDNA's was excised and subcloned into the oocyte expression vector pGem-He-Juel. Oocytes were injected with 30 ng of

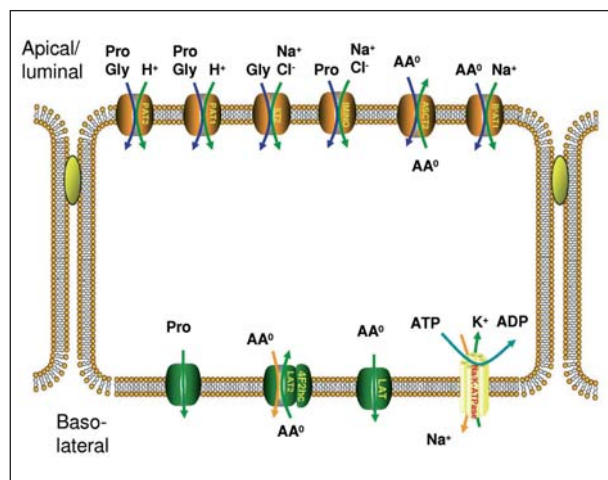


Figure 1. Neutral amino acid transport systems in epithelial cells

in vitro transcribed cRNA encoding transporters. Transport measurements were carried out after 3-10 days of expression. Mutations identified in individuals with Hartnup disorder were replicated by a PCR-based site-directed mutagenesis procedure. Flux experiments in oocytes were performed as described recently (4). Amino acid induced currents were analysed by two-electrode voltage clamp recording. In situ hybridization was carried out as described previously (5).

Outcomes

Following the identification of SLC6A19 as the system B⁰ transporter, we analysed several members of the SLC6 amino acid transporter branch (II). Humans have only one SLC6A20 gene, whereas mouse and rat have two homologues at the syntenic chromosomal location. In mouse they are referred to as XT3 and XT3s1, in rat the corresponding genes are XT3s1 and rB21a, respectively. Mouse XT3s1 (corresponding to rat rB21a) can be functionally expressed in *Xenopus laevis* oocytes, whereas XT3 (corresponding to rat XT3s1) does not elicit any activity. Both genes are expressed in the kidney, but only the functional isoform XT3s1 is found in the brain and in the intestine. To unify the nomenclature between the gene products in rat, mouse and humans we suggest calling the functional variant IMINO^B (expressed in the brain in all species) and the non-functional variant IMINO^K because it is expressed in kidney only. IMINO^B transports amino acids with secondary and tertiary amino-groups, such as proline, N-methyl-aminoisobutyric acid, betaine and nipecotic acid (5). Due to its expression in kidney and intestine, mutations of IMINO^B could be involved in iminoglycinuria.

Similar to IMINO, two homologous transporters are found next to each other at the locus of Hartnup disorder on chromosome 5p15, namely B⁰AT1 (SLC6A19) and XT2 (SLC6A18). Sequencing of SLC6A18 in a cohort of Australian Hartnup patients revealed the absence of any mutations in this gene. The expression of SLC6A18 increases from the kidney cortex to the medulla (Fig. 2), suggesting that it encodes a high-affinity, low-capacity transporter. In

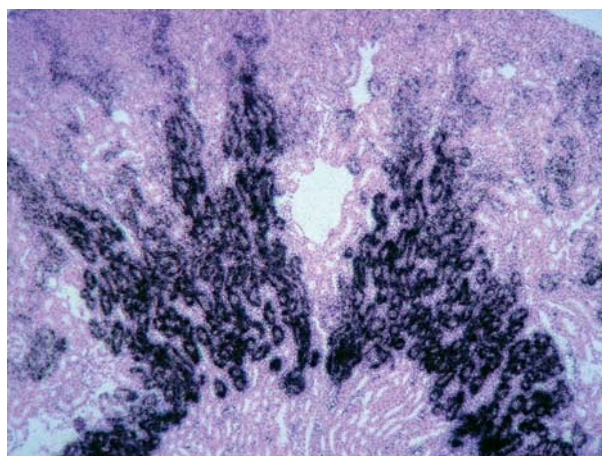


Figure 2. In situ hybridization of mouse XT2 (*slc6a18*) in kidney cortex. Expression is particularly strong close to the cortex-outer medulla border

agreement with this notion, XT2 ko-mice lack a high-affinity glycine transport activity (6).

Characterization of B⁰AT1 by two-electrode voltage clamp recording indicates that it transports all neutral amino acids including proline and glycine. The transporter is expressed in kidney and intestine, where it carries the bulk load of neutral amino acid transport. However, proline and glycine do not exceed normal levels in the urine of Hartnup disorder patients, demonstrating the presence of additional transporters for these amino acids such as PAT1 (SLC36A1), PAT2 (SLC36A2), XT2 (SLC6A18) and IMINO (SLC6A20).

Conclusion

Recent progress in the cloning and identification of epithelial amino acid transporters has provided the basis for the identification of genes involved in Hartnup disorder and Iminoglycinuria. Sequence analysis of these genes is currently in progress.

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