# The transporters SLC22A7 (OAT2) and SLC22A13: substrate elucidation, localization, discovery of an orotic acid transporter

The solute carriers SLC22A7 (OAT2, organic anion transporter 2) and SLC22A13 are conserved across several mammalian species. Human OAT2 is predominantly expressed in the liver, whereas SLC22A13 is specifically found in the kidney. For both the physiological significances are unknown. Basically transporters are defined through their substrate spectrum. For this reason substrates were elucidated via LC-MS- (Liquid Chromatography-Mass Spectrometry) difference shading. For SLC22A13h (human) guanidiniumsuccinate (GSA) was found and for OAT2h trigonelline (Trig). In addition, other related compounds or substrates from the other organic anion transporters were accepted by SLC22A13: the organic anions α-ketoglutarate, glutarate, glycocholate, guanidiniumglutarate (GGA), nicotinate, para-aminohippurate, pantothenate (PA), orotate, and urate as well as the zwitterions glycylproline (GP), prolylglycine (PG) and creatine. The highest transport efficiency (TE) was measured for nicotinate (15 µl $min^{-1}$ mg $protein^{-1}$). Due to the fact that intracellular concentrations of GSA, GGA, GP, PG and PA were decreased in SLC22A13 expressing cells it could be hypothesized, that this transporter acts as an exchanger. This interpretation was confirmed by trans-stimulation experiments. Furthermore, the exact localization of SLC22A13 in the rat kidney was determined by immunohistochemical staining with a self designed antibody. It was indicated for the first time, that the transporter is located in the basolateral membrane of intercalated cells in the collection duct. Based on these results, the hypothesis has been developed that SLC22A13 is involved in the renal excretion and/or reabsorption of organic anions in cooperation with the transporter SLC22A9 (located in the apical membrane of intercalated cells).

As mentioned above, Trig is a specific substrate for OAT2 from human and rat (TE = 4 µl $min^{-1}$ mg $protein^{-1}$, $K_m$ = 409 µM). Further experiments have shown that the glutamic acid at position 441 in the human protein is relevant for Trig uptake. Afterwards, several other related compounds were tested and nicotinate riboside was found to be another substrate (TE = 7 µl $min^{-1}$ mg $protein^{-1}$). But the highest TE was measured for the pyrimidine nucleotide precursor orotic acid (TE = 74-99 µl $min^{-1}$ mg $protein^{-1}$, $K_m$ = 234 µM). This uptake is exclusively carried out by the rat transporter. Because of this experimental result, the model is proposed that OAT2 is involved in the development of the fatty liver of rats (hepatic steatosis) derived due to the increased uptake of orotic acid with the nutriment. Thus, for the first time an orotic acid transporter was discovered in eukaryotes.