doi:10.2533/chimia.2022.1011 Chimia 76 (2022) 1011–1018

Structure, Function and Pharmacology of SLC7 Family Members and Homologues

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Abstract: Amino acids are essential components of all living cells serving as building blocks of proteins, as energy source, and as precursors of metabolites and signaling molecules. Amino acid transporters are membrane proteins that mediate the transfer of amino acids across the plasma membrane, and between compartments in cells, different cells and organs. The absence, overexpression or malfunction of specific amino acid transporters have been associated with human disease. One of the projects within the Swiss National Centre of Competence in Research (NCCR) TransCure was directed at SLC7 family amino acid transporters, with a particular focus on the heteromeric amino acid transporters 4F2hc-LAT1 (SLC3A2-SLC7A5) and 4F2hc-LAT2 (SLC3A2-SLC7A8), and the bacterial homologue AdiC. The project addressed questions of basic research (function and structure), pharmacology (identification of potent inhibitors and activators), and pre-clinical medicine (*e.g.*, physiological role in the placenta) and disease models (*e.g.*, tumor progression) of specific SLC7 family amino acid transporters. This review presents, summarizes and discusses selected main results obtained in this NCCR TransCure project.

Keywords: Amino acid transporter · Inhibitor · LAT1 · LAT2 · SLC7

Introduction

Amino acids (AAs) are composed of an aminoethanoic acid backbone to which different side chains are attached. Those are structurally diverse and range from polar to charged and small to bulky hydrophobic side chains. Proteinogenic amino acids are used as building blocks for proteins and play critical roles as metabolites, nutrients, signaling molecules and precursors of hormones.[1] As a consequence, it is of fundamental physiological importance to enable and control transport of AAs through biological membranes, which is mediated by membrane-embedded amino acid transporters (AATs).[2,3] Moreover, dysfunction, overexpression or absence of AATs may lead to human diseases.[4] Currently, the table of solute carriers (SLCs) lists eleven families containing AATs,[5] which are further classified as different systems based on their substrate specificity and mechanism of transport.[2,3] Among AATs, heteromeric amino acid transporters (HATs) are structurally unique since they are comprised of a light and a heavy subunit, which are covalently linked *via* a disulfide bridge.[6–8] Based on substrate selectivity and ion coupling preference, HATs are grouped into five distinct systems, *i.e.*, system L, system y^+L, system x_c^-, system asc and system $b^{0,+}$.[8] From a medical perspective, HATs are associated with viral infections (*e.g.*, as receptor for viral cell entry),[9,10] and HAT mutations with inherited metabolic diseases such as aminoacidurias, *e.g.*, cystinuria and lysinuric protein intolerance,[6–8] and tumor growth.[8,11–14] Whereas the glycoprotein-associated L-type amino acid transporters (LATs) from the SLC7 family are the light and catalytic subunits of HATs,[15–17] the type II membrane N-glycoproteins are the heavy subunits and belong to the SLC3 family.[7,8,18] Examples of the latter ancillary proteins are 4F2hc (SLC3A2, CD98) and rBAT (SLC3A1). They are responsible for the correct trafficking of LATs to the plasma membrane in mammalian cells.[7,8,18]

Our project team within the Swiss National Centre of Competence in Research (NCCR) TransCure focused on the research of the two system L human HATs, *i.e.*, 4F2hc-LAT1 (SLC3A2-SLC7A5) and 4F2hc-LAT2 (SLC3A2-SLC7A8). In addition to act as a LAT-specific chaperone in mammalian cells,[8] the heavy chain subunit 4F2hc is also involved in cell adhesion and cell fusion, regulation of macrophage activation *via* galectin-3 and integrin signaling.[19–22] Only recently, we described 4F2hc to be a modulator for substrate affinity and specificity of LAT1 and LAT2.[23] Structurally, 4F2hc is composed of a cytoplasmic N-terminal domain, followed by a single transmembrane α-helix (TM), and a large extracellular C-terminal domain. The latter has a fold similar to bacterial glucosidases but is enzymatically inactive.[24,25] The light chains LAT1 and LAT2 are isoforms and share about 48% amino acid sequence identity[26] and are composed of twelve TMs. In the human body, they are differentially expressed. Whereas LAT1 is mainly found in tissues such as brain, spleen, testis, and placenta,[27,28] LAT2 is ubiquitously expressed.[8] In addition, LAT1 is expressed in high amounts at the blood-brain barrier[8,29] and in many cancer types, *e.g.*, brain,[30] lung,[31] pancreatic,[32] gastric,[33] breast,[34] prostate,[35] urologic,[36] and renal cell cancer,[37] and also other tumors.[11,38] Due to the expression level and tissue distribution, LAT1 is a putative marker for an unfavorable prognosis in cancer patients[14,39] and at the same time it could be a possible drug-delivery vehicle for brain and tumor targeting.[11,14,38,40,41] Dysfunction and expression levels of LAT2 also impact human health, *e.g.*, age-related hearing loss and cataract formation were linked to a damaged LAT2-dependent substrate transport function,[8,42,43] and basal cell carcinoma and pancreatic cancer were attributed to elevated expression levels of LAT2.[44,45] In addition, the development of autism spectrum disorder was associated to LAT2 coding variants[46] or LAT1 impaired amino acid

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transport at the blood–brain barrier.[46,47] Both HAT light chain transporters, *i.e.*, LAT1 and LAT2, function as Na^+-independent obligatory exchangers of an amino acid or amino acid derivate with another amino acid or amino acid derivate in a 1:1 stoichiometry.[7,8] The substrate preferences differ to some extent. While the thyroid hormones T3 and T4 are transported by both transporters,[48–50] large neutral amino acids with branched or aromatic side chains are preferably transported by LAT1[51–54] of which L-leucine and L-histidine show highest affinities.[17,23,50,52,53] Neutral as well as small amino acids are the preferred substrates of LAT2.[23,54–56] 2-Aminobicyclo-[2.2.1]heptane-2-carboxylic acid (BCH) is an unspecific inhibitor of system L transporters as LAT1 and LAT2.[51] Potent and specific inhibition of the LAT1 transporter is achieved by the tyrosine-analog JPH203 (KYT-0353), a compound that has shown growth inhibitory effects on different cancer cells[57,58] and also impeded *in vivo* tumor growth in HT-29 tumor-carrying nude mice[57] as well as in a fully immunocompetent mouse model of thyroid cancer.[59]

The medically relevant heteromeric amino acid transporters 4F2hc-LAT1 and 4F2hc-LAT2 were studied in an interdisciplinary manner within the SLC7 group of the NCCR TransCure. The SLC7 project team was composed of four subgroups, each led by the principal investigators: Dimitrios Fotiadis (structural and functional characterization of membrane proteins), Jürg Gertsch (compound screening and molecular pharmacology), Karl-Heinz Altmann (organic/medicinal chemistry) and Christiane Albrecht (human placenta). Small molecule synthesis was combined with ligand transport studies using various cell types and tissues, and the macromolecular structures of HATs and a bacterial SLC7 homologue were determined by cryogenic-electron microscopy (cryo-EM) or X-ray crystallography. Selected main research achievements obtained during the NCCR TransCure period are presented, summarized and discussed in the following sections.

Structure and Function of HATs, and SLC7 Family Members and Homologues

The main mission of this NCCR TransCure subproject was to unveil new mechanistic insights into ligand binding and transport from selected HATs and SLC7 amino acid transporters using functional and structural approaches. Specific target proteins were the human HATs 4F2hc-LAT1 and 4F2hc-LAT2, because of their physiological and pathophysiological importance in humans.[8] For certain biochemical and biophysical assays as well as for structure determination, milligram amounts of pure and functional protein are a prerequisite. Thus, the first challenge of this project represented the heterologous overexpression of functional human 4F2hc-LAT1 and 4F2hc-LAT2 in a selected host cell. Overexpression of functional human 4F2hc-LAT1 and 4F2hc-LAT2, but also of LAT1 and LAT2 alone was successfully achieved using the methylotrophic yeast *Pichia pastoris*.[23,50,60,61] This microorganism was shown to be excellent for the overexpression of functional membrane proteins and the subsequent isolation of milligram amounts of protein for structural studies.[62] Importantly, yeast allows the use of bioreactors to obtain very high biomass[63] when compared to other eukaryotic protein expression systems. Finally, and in contrast to mammalian cell lines, *P. pastoris* is able to traffic LAT1 and LAT2 to the plasma membrane in the absence of the ancillary protein 4F2hc: another attractive feature of this cell system.[16] Having selected *P. pastoris* clones overexpressing human 4F2hc-LAT1, 4F2hc-LAT2, LAT1 or LAT2, uptake assays for functional characterization were successfully established. This opened the unique opportunity to unveil new possible functional differences in transport by the HATs (4F2hc-LAT1 and 4F2hc-LAT2), and the corresponding LATs (LAT1 and LAT2). A comparative study performed within the NCCR TransCure described a new function of 4F2hc, namely the modulation of substrate affinity and specificity. For example, the K_m value of LAT2 for L-leucine determined in the complex with 4F2hc (4F2hc-LAT2) differs significantly when compared to the K_m measured for LAT2 alone, *i.e.*, in the absence of 4F2hc (Fig. 1a).[23] In addition, the established uptake assays using *P. pastoris* allowed the determination of kinetic parameters, *e.g.*, K_m and IC_{50} of substrates and inhibitors, respectively, for the HATs 4F2hc-LAT1 and 4F2hc-LAT2, and the LATs LAT1 and LAT2:[50,61] for a representative example, see IC_{50} determination of the potent and specific human 4F2hc-LAT1 inhibitor JPH203 in Fig. 1b determined using our yeast cell uptake assay. This IC_{50} value of about 200 nM nicely compares to results obtained in different laboratories using different experimental set-ups.[57,59] From a structural point of view, we provided the first 3D view onto the supramolecular organization of a HAT using purified human 4F2hc-LAT2 overexpressed in *P. pastoris*, and single-particle negative stain transmission electron microscopy and 3D reconstruction.[16,64] At that time, *i.e.*, in the years 2013–2014, cryo-EM was not yet developed to today's level, and X-ray crystallography was the method of choice for structure determination at high-resolution. Because growth of 3D crystals of HATs for X-ray crystallographic analysis was an extremely challenging and time-consuming endeavor, we determined and studied the structure of a prokaryotic homologue of LAT1 and LAT2, *i.e.*, of the L-arginine/agmatine AdiC transporter from *E. coli*. AdiC represents a valuable model transporter with amino acid sequence identities to LAT1 and LAT2 of 21.7% and 23.1%, respectively.[65] In this effort, three high-resolution AdiC structures (one at the impressive resolution of 1.7 Å) were determined in the presence and absence of ligand, unravelling important mechanistic insights into transport and ligand binding, and the role of structural water.[66,67] Additionally, mechanistic insights on LAT1 ligand binding were acquired using a LAT1 homology model based on the crystal structure of the prokaryotic SLC7 homologue AdiC.[65] The milligram amounts of detergent-solubilized, purified AdiC protein produced for 3D crystallization allowed us further to establish within the NCCR TransCure the scintillation proximity assay (SPA) for transport proteins, a radioligand- and bead-based binding assay.[68] SPA is an excellent tool for target-ligand validation, because it represents a cell-free system devoid of endogenous transporters and other potentially cross-reacting proteins. This SPA was used for the characterization of AdiC variants,[67,69] and was further developed and applied to the human HATs 4F2hc-LAT1[61] and 4F2hc-LAT2.[64] The advent of cryo-EM began shortly after our 3D reconstruction of a human HAT from single negatively stained 4F2hc-LAT2 particles.[16] Using the new direct electron detectors, state-of-the-art cryo-electron microscopes and newly developed powerful image processing, single-particle analysis and 3D reconstruction software, we progressively managed to improve our structural view onto the human HAT 4F2hc-LAT2 over the years, finally obtaining an atomic model (Fig. 1d).[70–72]

In summary, important functional and structural insights as well as valuable cell- and protein-based transport and binding assays, *e.g.*, for the characterization of new HAT inhibitors, could successfully be obtained and contributed to the scientific community thanks to the longstanding support of the NCCR TransCure from the Swiss National Science Foundation (SNSF).

Development of LAT1 Inhibitors

The overexpression of LAT1 in many types of tumors makes it an interesting and potentially very specific target for anticancer drug discovery.[14] However, at this point, only few potent inhibitors of LAT1 have been reported and almost all of them are derived from the substrate amino acids phenylalanine or tyrosine.[14] The most advanced of these compounds is the tyrosine derivative JPH203 (**1**) (Fig. 2), which is currently undergoing Phase II clinical studies.[73] JPH203 (**1**) inhibits LAT1-mediated [^{3}H]-L-leucine uptake in HT-29 human colon carcinoma cells with an IC_{50} value

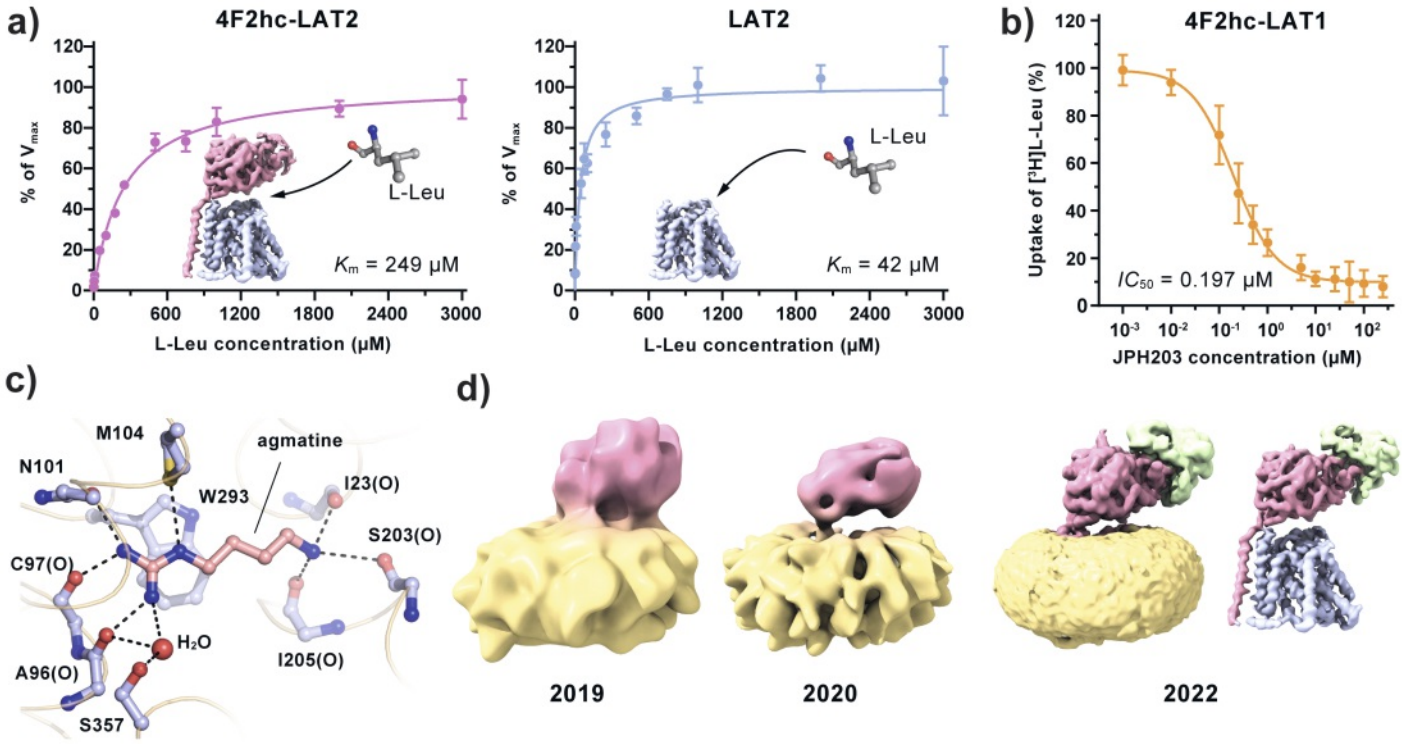

Fig. 1. Overview of functional and structural results obtained during the Swiss NCCR TransCure. a) Plots of L-leucine (L-Leu) K_m determination of 4F2hc-LAT2 (*left*) and LAT2 alone (*right*). b) Plot of IC_{50} determination of JPH203 against L-Leu (heterologous competition). c) View into the substrate binding pocket of the agmatine-bound AdiC structure (PDB code 5J4N). The main chain of the protein (yellow ribbon), residues (light blue sticks) and a water molecule (red sphere) in close contact to the agmatine (pink sticks) are discerned, interactions are highlighted with dotted lines, and respective residues are labeled. Additionally, main chain interactions with the carbonyl oxygen or nitrogen atoms are labeled with (O) or (N). d) Progress in structural elucidation of human 4F2hc-LAT2 in terms of resolution (year 2019, ~13 Å resolution;[70] year 2020, 7.5 Å resolution[71] and year 2022, 3.2 Å resolution[72]). Shown are the cryo-EM densities of the detergent micelle (yellow), 4F2hc (pink), LAT2 (light blue) and the 4F2hc-binding protein (green). Data points shown in a) and b) represent the mean with SD and if not visible, error bars are smaller than symbols.

of around 150 nM;[57,59] the compound inhibits the proliferation of HT-29 cells with µM potency and it has been shown to retard the growth of HT-29 tumors in a mouse xenograft model[57] and to induce tumor growth arrest in an immunocompetent mouse model of thyroid cancer.[59]

Our own work has focused on the discovery of new LAT1 inhibitors based on the substrate amino acids L-tryptophan and L-tyrosine as starting points for design and optimization. The former had not been explored as a template for LAT1 inhibitors prior to the initiation of our work. In a collaboration with the Novartis Institute for Biomedical Research (NIBR) in Basel (led by Jürg Gertsch), we have also attempted to identify non-amino acid LAT1 inhibitors. However, the screening of >20k compounds from the NIBR public collection delivered very few viable hits, two of which are being further studied as possible LAT1/LAT2 modulators.

L-Tryptophan-based Inhibitors[74]

Our strategy towards the identification of L-tryptophan-based LAT1 inhibitors was based on the premise that the addition of steric bulk to the indole side chain would result in the conversion of a substrate amino acid into a potent inhibitor. This would be in broad analogy to the relationship between L-tyrosine and JPH203 (**1**).[14] The implementation of this concept in a first phase involved the preparation of the four isomeric benzyloxy-tryptophans **2**, **3**, **4** and **5** (Fig. 2). Although a benzyloxy group is clearly less bulky than the (2-phenyl-benzo[d]oxazol-7-yl)methoxy substituent in **1**, we hoped that it would at least produce a weak inhibitory effect that could then be enhanced by further modification. Out of the four benzyloxy-L-tryptophans **2**–**5**, **3** was able to inhibit [^{3}H]-L-leucine uptake into HT-29 human colon carcinoma cells, which are known to express high levels of LAT1, with an IC_{50} of 19 µM: **2**, **4**, and **5** were not active up to concentrations of 100 µM. Our data are in line with independent findings by Ecker and co-workers for **3**[40] and, more recently, by Hutchinson *et al*. for **2**–**5**.[75] Modification of 5-benzyloxy-tryptophan **3** then included the attachment of different (hetero)aromatic moieties to the 5-benzyloxy substituent and the replacement of the benzyl group by the 2-(phenylbenzo[d]oxazol-7-yl)methoxy substituent from **1**. However, increasing the steric bulk of the plain benzyloxy group did not result in substantial changes in potency (IC_{50} values between 5.5 and 40 µM). Additional structure–activity relationship (SAR) work will be required to fully explore the potential of 5-substituted L-tryptophans as LAT1 inhibitors. At the same time, our data clearly suggest that the attachment of a bulky group to the 5-position of the indole ring system in L-tryptophan leads to a loss of substrate properties. This makes this position unsuitable as an attachment point for drug moieties in LAT1-directed prodrugs.

L-Tyrosine-based Inhibitors

Our work on tyrosine-based LAT1 inhibitors built on earlier literature findings on the LAT1 binding affinity of bicyclic analogs of the cancer drug melphalan.[76,77] Replacement of the nitrogen-mustard moiety by a benzyloxy group and the assessment of the most favorable site for the attachment of this group on the aromatic part of the scaffold eventually led to the discovery of a series of bicyclic *meta*-tyrosine derivatives, such as **6**, **7** or **8** that are at least equipotent inhibitors of [^{3}H]-L-leucine uptake into HT-29 cells as **1** (IC_{50} values: **6**, 165 nM; **7**, 121 nM; **8**, 234 nM). While the initial SAR studies on bicyclic *meta*-tyrosines were performed with racemic mixtures, we have recently shown for two examples that the activity of these compounds is highly linked to an (*S*)-configuration of the α-carbon atom. Finally, in collaboration with the group of Prof. Qiang Zhou at Westlake University, Hangzhou, China, cryo-EM structures were obtained

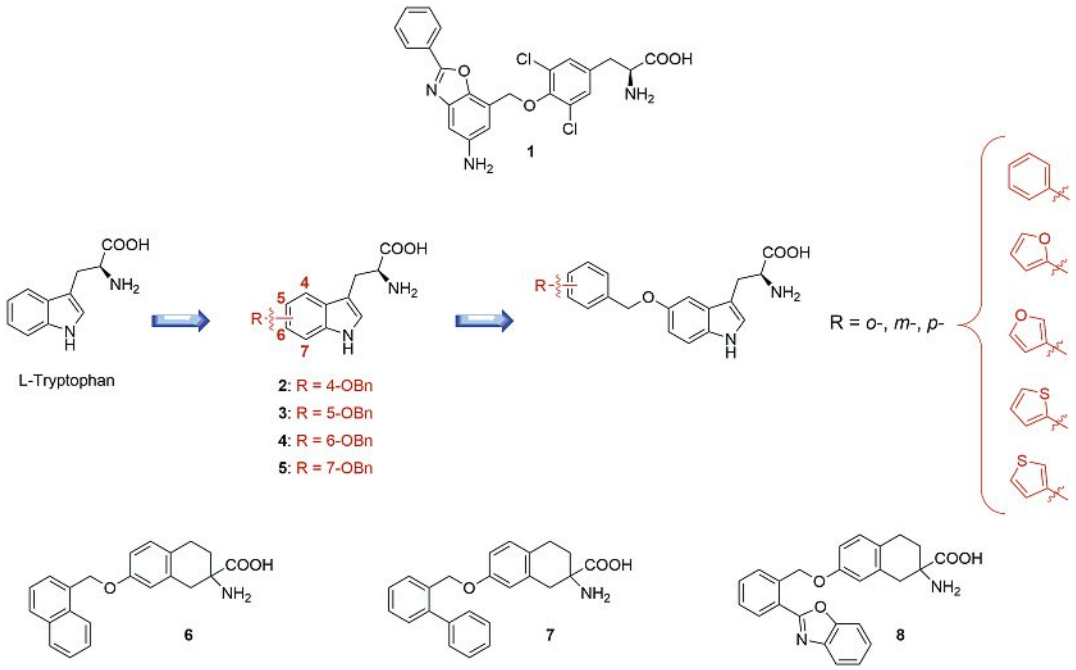

Fig. 2. Structures of the Phase II compound JPH203 (**1**) and of L-tryptophan- and *meta*-L-tyrosine-derived LAT1 inhibitors.

for complexes of **6**, **7** and **8** with LAT1.[78] Intriguingly, the transporter was found to adopt an outward-open conformation in all three structures, in contrast to the inward-open conformation that had been seen in previous structures of the apo-protein[79,80] and of a complex with the non-specific transport inhibitor 2-aminobicyclo[2.2.1]heptane-2-carboxylic acid (BCH).[79] We consider the outward-open conformation to be more relevant for inhibitor design, a notion that has recently been re-confirmed by modeling studies by Hutchinson *et al*.[75]

Pharmacology of LAT1 Inhibitors

An increasing number of experimental studies and reviews advocate the amino acid transporter LAT1 (SLC7A5) as a validated drug target for the treatment of different kinds of cancer.[11,14,57,73] Capitalizing on the observation that LAT1 preferentially nourishes fast growing tumor cells with large, neutral and essential L-amino acids (Phe, Trp) and drives mTOR signaling, the development of LAT1-selective inhibitors is a possible anticancer strategy. To date, proof of concept for LAT1 as anticancer target has been obtained in different pre-clinical rodent experiments, mainly employing xenograft models.[57] In the NCCR TransCure, we aimed to better understand the 'druggability' of LAT1 and its role in physiology, focusing on cancer and the placenta (see below). Since LAT1 is essential for rather diverse biological processes, like transport of T3/T4 thyroid hormones, and the passage of amino acids and other metabolites across the blood brain barrier (BBB), the question regarding the potential toxicity of very potent LAT1 inhibitors is an important aspect of drug discovery. Systemic homozygous LAT1 gene deletion causes embryonic lethality[81] and LAT1 appears to be essential for the development of a properly functional central nervous system (CNS). For instance, mutations in LAT1 have been shown to potentially be associated to autism,[46,47] and Phe excess in the brain leads to mental retardation in untreated patients with phenylketonuria.[82] Yet, mutations in the LAT1 gene, which lead to its loss of function, may be responsible for milder clinical manifestations such as lower brain toxicity of hyperphenylalaninaemia observed in certain patients.[83] Employing nontoxic reversible LAT1 inhibitors to target diseases in which this transporter causes a pathophysiology may be an ultimately successful therapeutic strategy. To our knowledge, only one Phase I clinical trial has been accomplished with the LAT1 inhibitor JPH203, showing its lack of toxicity and good tolerability in humans. A limited Phase I clinical trial was performed on five different solid tumors and showed that JPH203 is well-tolerated and seems promising for the treatment of bile duct cancer.[84] According to a recent press release by J-Pharma Co., Ltd., JPH203 is now in Phase II to treat bile duct cancer.[85] In mice, the half-life of JPH203 is relatively short given its limited metabolic stability (unpublished observation), thus the toxicology data from JPH203 cannot be generalized and will have to be determined for each individual LAT1 inhibitor. LAT1 has also been proposed as a novel pharmacological target for the treatment of glioblastoma.[86] Recently, Quadriga BioSciences initiated Phase I clinical trials with a LAT1 substrate QBS10072S, which specifically accumulates in LAT1 expressing brain cancers.[87] Preclinical studies have demonstrated that the amino acid-based small-molecule nitrogen mustard compound QBS10072S significantly suppressed tumor growth and improved survival in different glioblastoma mouse models.[88]

In the 2nd phase of the NCCR TransCure, different mouse models bearing the BRAF mutations were established in the laboratory of Roch-Philippe Charles to test the involvement of LAT1 and the efficacy of LAT1 inhibitors. The role of LAT1 in anaplastic thyroid carcinoma (ATC), a highly aggressive malignancy for which no effective therapy exists, could be studied. JPH203 administered chronically at high doses (50 mg/kg) showed significant tumor growth arrest in ACT.[59] However, it appears that the emerging concept of LAT1 as a pan-cancer drug target[89] is too confident as LAT1 does not seem to be important in all cancer types. Mutations in the B-Raf proto-oncogene serine/threonine kinase (BRAF) have been observed to be a major cause of tumor development and growth. In our studies, we expressed the well-studied driver V600E mutation in the BRAF gene in which valine (V) is substituted by glutamic acid (E) at amino acid position 600.[90] We recently characterized BRAF-driven melanoma in mice, in which BRAF(V600E) cooperates with Pten loss to induce metastatic melanoma, in which we could not observe a role for LAT1 (unpublished data).

To develop LAT1 inhibitors is a pharmacological challenge in which not only potency but also the physicochemical properties of the compounds, their tissue distribution and metabolic stability play crucial roles for *in vivo* efficacy. Within the NCCR TransCure Screening Profiling and Analytical Facility (SPAF),

we established methods to measure the pharmacodynamics of LAT1 inhibitors in different tissues by LC-MS/MS, generating quantitative amino acid signatures *ex vivo*. Moreover, we established LAT1 overexpressing mammalian cell lines, in addition to heterologous expression systems (see above), to tackle the rather complex amino acid flux *via* LAT1, and network effects on metabolism *in vitro*. Another open question relates to the mode of action of the LAT1 inhibitors. While it remains unclear how JPH203 interacts with LAT1, the inhibitors developed in the consortium appear to target the LAT1 outward-open conformation.[78] Although none of these potent (IC_{50} = 80–200 nM) substrate-competitive (*i.e.*, orthosteric) LAT1 inhibitors behave as substrates of LAT1, we cannot exclude the possibility that a fraction is transported. An interesting question related to the antitumor mode of action of LAT1 inhibitors is related to the fact that their cytotoxicity (antiproliferative, pro-apoptotic effects, *etc.*) is relatively inefficient *in vitro*, in contrast to the observed efficient antitumor effects *in vivo*. We therefore tried to mimic the low amino acid concentrations found in tumors.[91] This unmasked the antiproliferative effects of the LAT1 inhibitors, though at about 5–10 times higher concentrations than their respective IC_{50} values for LAT1 inhibition. Cancer cells transport essential amino acids *via* different transport systems, and it may not be enough to just target one transporter. For instance, human epithelial and mesenchymal HCC cell lines adapt to ASCT2 or LAT1 knockout.[92] Thus, amino acid exchangers like ASCT2 and LAT1 may act as 'harmonizers', not drivers, of amino acid accumulation and signaling in cancer cells, despite their long-established dominant role in initial-rate amino acid transport.[92] Based on the available data, it cannot be excluded that the antitumor effects observed *in vivo* are mediated, at least in parts, *via* the immune system and not only through the deprivation of cancer cells from essential amino acids. We believe that the advancement of LAT1 inhibitors to treat cancer requires a better understanding of the involvement of this transporter in immune and neurophysiological processes. Moreover, the chronic effects of LAT1 inhibitors on liver and brain need to be established. As a result of the NCCR TransCure research on LAT1, studies will be ongoing on improving the pharmacokinetics of a novel class of LAT1 inhibitors developed within the consortium, and to assess their chronic toxicology, focusing on the brain. In addition to cancer, LAT1 may be a drug target for other diseases[93] and it is thus important to have drug leads that show tissue-relevant pharmacodynamic efficacy.

Amino Acid Transporters in the Human Placenta

A successful pregnancy and the birth of a healthy baby depends to a great extent on the controlled supply of essential nutrients *via* the placenta. Inspired by clinical observations and in close collaboration with clinical obstetricians, the Albrecht group focused on the implementation of biochemical and cell biological *in vitro* models to mimic and investigate physiological relationships between the human placenta and gestational diseases.

The placenta maintains the balance between nutrition and growth control of the fetus through selective and regulated supply of macronutrients such as amino acids and carbohydrates, and critical micronutrients like minerals and vitamins.[94] Placental nutrient transport does not just play a role in fetal development, but also affects the newborn's life through metabolic programming until adulthood.[95] Appropriate fetal growth is largely dictated by the availability of amino acids in the maternal circulation, which is ensured during pregnancy by the capacity of the placenta to transfer amino acids from the maternal into the fetal circulation (Fig. 3a).[96,97] For most amino acids, the concentrations in the umbilical vein are two- to threefold higher than in the uterine vein, demonstrating that the transfer of amino acids is an active energy-dependent process against counter-directed feto-maternal gradients (Fig. 3a and 3c).[98–100]

Amino acid transporters expressed in the human placenta are categorized into accumulative transporters, exchangers and facilitators and at least 20 different amino acid transporters have been identified in trophoblast cells.[101] Within the clinically oriented NCCR TransCure studies, the Albrecht group predominantly investigated the contribution of SLC7 family amino acid transporters in the transplacental transfer of essential amino acids. Na^+-independent placental leucine transport is mainly attributed to the system L transporters LAT1/SLC7A5 and LAT2/SLC7A8 (Fig. 3c).[101,102] We determined physiological amino acid gradients of paired maternal and fetal serum samples by ion exchange chromatography and correlated them with the anthropometric parameters. We found significant associations between reduced materno-fetal amino acid gradients, maternal gestational weight gain and blood pressure, suggesting a potential role of amino acid gradients in reduced intrauterine growth or even in hypertensive diseases like preeclampsia.[103]

In the next step, the functional effects of a physiological gradient versus equimolar leucine concentrations were tested using *in vitro* uptake and Transwell®-based transfer assays. Interestingly, materno-fetal leucine transfer was significantly stimulated against a counter-directed gradient.[103] These results suggest a currently underestimated effect of transplacental amino acid gradients on leucine transfer efficiency and underline their potential impact on pregnancy diseases associated with impaired fetal growth. In the same context, the Albrecht group also investigated the relevance of SLC7 transporters in placental leucine transfer by applying the LAT1-specific inhibitor JPH203 and the system L-specific inhibitor JX009 synthesized by the collaborators in the NCCR TransCure network. These studies identified LAT1 as the major accumulative transporter in the placenta, but other system L transporters like LAT2 are rate-limiting for leucine efflux to the fetus.[104]

Finally, previous and currently ongoing studies performed in the Albrecht group revealed that placental LAT1 plays an important role in fetal programming of metabolic disease.[105,106] Placental LAT1 is significantly up-regulated by several forms of prenatal stress, both in humans and in mice and it plays a central role in the transport of methionine from the mother to the fetus increasing methionine uptake under stress. Changes in methionine availability subsequently affect placental and fetal hypothalamic global DNA methylation, with long-lasting effects on hypothalamic gene regulation in the adult offspring. Our results suggest a newfound role of placental LAT1 in the life-long programming of the offspring's metabolic homeostasis, with intriguing sex-specific effects.

Conclusion

This NCCR TransCure project mainly addressed questions of basic research, pharmacology and pre-clinical medicine and disease models related to the human heteromeric amino acid transporter 4F2hc-LAT1 and 4F2hc-LAT2 from the SLC3 and SLC7 families. The project was highly interdisciplinary and depended on close interactions between the different research groups in the project. Important mechanistic insights on ligand binding, transport mechanism and mode of action of inhibitors were gained. Novel potent LAT1 inhibitors were successfully obtained and characterized *in vitro*, *ex vivo* and *in vivo* in accordance with the translational NCCR Transcure vision "from gene to drug". The project will continue beyond the NCCR TransCure at the Institute of Biochemistry and Molecular Medicine of the University of Bern.

Received: October 2, 2022

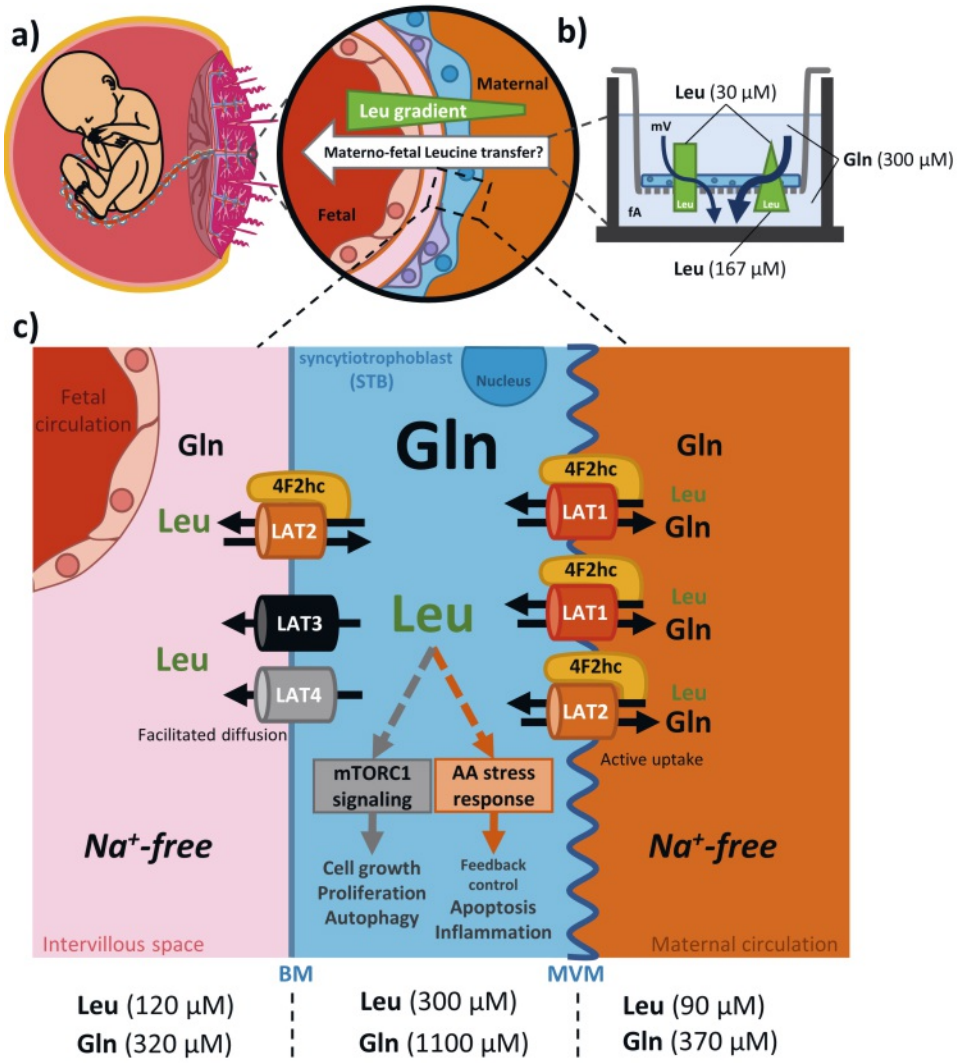

Fig. 3. Leucine transport across the placental barrier induced through a counter-directed gradient and maintained by asymmetric LAT1/LAT2 expression. a) The placental barrier is formed mainly by the syncytiotrophoblast (STB), which is in direct contact with the maternal circulation. Since leucine (Leu) concentrations are higher in the fetal as compared to the maternal blood, the placenta needs to overcome a counter-directed Leu gradient by active transport mechanisms (panel a, *right*). b) The comparison of Na^+-independent Leu transfer across the placental barrier *in vitro* using the Transwell® technique demonstrated an increased materno-fetal Leu transfer when a counter-directed Leu gradient was applied. c) The Na^+-independent uptake of Leu from the maternal circulation (orange) across the microvillous membrane (MVM) into the STB (blue) represents the active step of placental Leu transport and is mediated by an intracellular co-substrate (*e.g.*, glutamine, Gln) in the interplay with the amino acid exchangers LAT1/SLC7A5 and LAT2/SLC7A8. LAT1 and LAT2, both expressed at the MVM, exchange one branched-chain amino acid amino acid (BCAA, *e.g.*, Leu) for another amino acid (*e.g.* Gln). LAT1 has been shown to be the major Leu uptake transporter at the maternal facing MVM. Under Na^+-free conditions, there is no possibility for the STB to build up a Na^+-gradient driving system A amino acid uptake, hence only Na^+-independent system L transport is active. In addition, Leu is transferred across the basal membrane (BM) by facilitators such as LAT3- and LAT4-mediated diffusion driven by the extracellularly directed concentration gradient on the fetal side. The relevance of LAT1 compared to the other system L transporters was revealed by SLC7-specific inhibition studies performed within the TransCure network.[104] The font size of the amino acid in the 3-letter code represents an estimation of their relative concentration in the fetal (*left*, pink and red), intracellular (*middle*, blue) and maternal (*right*, orange) compartment. Intracellular amino acid concentrations were taken from Philipps and co-workers.[107]

[1] G. Wu, *Amino Acids* **2009**, *37*, 1, https://doi.org/10.1007/s00726-009-0269-0.
[2] H. N. Christensen, *Physiol. Rev.* **1990**, *70*, 43, https://doi.org/10.1152/physrev.1990.70.1.43.
[3] J. D. McGivan, M. Pastor-Anglada, *Biochem. J.* **1994**, *299 (Pt 2)*, 321, https://doi.org/10.1042/bj2990321.
[4] S. Bröer, M. Palacin, *Biochem. J.* **2011**, *436*, 193, https://doi.org/10.1042/BJ20101912.
[5] P. Kandasamy, G. Gyimesi, Y. Kanai, M. A. Hediger, *Trends Biochem. Sci.* **2018**, *43*, 752, https://doi.org/10.1016/j.tibs.2018.05.003.
[6] J. Chillaron, R. Roca, A. Valencia, A. Zorzano, M. Palacin, *Am. J. Physiol. Renal Physiol.* **2001**, *281*, F995, https://doi.org/10.1152/ajprenal.2001.281.6.F995.
[7] F. Verrey, E. I. Closs, C. A. Wagner, M. Palacin, H. Endou, Y. Kanai, *Pflugers Arch.* **2004**, *447*, 532, https://doi.org/10.1007/s00424-003-1086-z.
[8] D. Fotiadis, Y. Kanai, M. Palacin, *Mol. Aspects. Med.* **2013**, *34*, 139, https://doi.org/10.1016/j.mam.2012.10.007.
[9] J. A. Kaleeba, E. A. Berger, *Science* **2006**, *311*, 1921, https://doi.org/10.1126/science.1120878
[10] J. Rabinowitz, H. J. Sharifi, H. Martin, A. Marchese, M. Robek, B. Shi, A. A. Mongin, C. M. C. de Noronha, *Virology* **2021**, *556*, 149, https://doi.org/10.1016/j.virol.2021.01.008.
[11] P. Häfliger, R.-P. Charles, *Int. J. Mol. Sci.* **2019**, *20*, E2428, https://doi.org/10.3390/ijms20102428.
[12] X. Lu, *Curr. Cancer Drug Targets* **2019**, *19*, 863, https://doi.org/10.2174/1568009619666190802135714.
[13] Y. Saito, T. Soga, *Cancer Sci.* **2021**, *112*, 2958, https://doi.org/10.1111/cas.15006.
[14] Y. Kanai, *Pharmacol. Ther.* **2022**, *230*, 107964, https://doi.org/10.1016/j.pharmthera.2021.107964.
[15] N. Reig, J. Chillaron, P. Bartoccioni, E. Fernandez, A. Bendahan, A. Zorzano, B. Kanner, M. Palacin, J. Bertran, *EMBO J.* **2002**, *21*, 4906, https://doi.org/10.1093/emboj/cdf500.

[16] A. Rosell, M. Meury, E. Alvarez-Marimon, M. Costa, L. Perez-Cano, A. Zorzano, J. Fernandez-Recio, M. Palacin, D. Fotiadis, *Proc. Natl. Acad. Sci. USA* **2014**, *111*, 2966, https://doi.org/10.1073/pnas.1323779111.

[17] L. Napolitano, M. Scalise, M. Galluccio, L. Pochini, L. M. Albanese, C. Indiveri, *Int. J. Biochem. Cell Biol.* **2015**, *67*, 25, https://doi.org/10.1016/j.biocel.2015.08.004.

[18] M. Palacin, Y. Kanai, *Pflugers Arch*. **2004**, *447*, 490, https://doi.org/10.1007/s00424-003-1062-7.

[19] M. Tsurudome, Y. Ito, *Crit. Rev. Immunol.* **2000**, *20*, 167.

[20] C. A. Fenczik, T. Sethi, J. W. Ramos, P. E. Hughes, M. H. Ginsberg, *Nature* **1997**, *390*, 81, https://doi.org/10.1038/36349.

[21] A. C. MacKinnon, S. L. Farnworth, P. S. Hodkinson, N. C. Henderson, K. M. Atkinson, H. Leffler, U. J. Nilsson, C. Haslett, S. J. Forbes, T. Sethi, *J. Immuno*. **2008**, *180*, 2650, https://doi.org/10.4049/jimmunol.180.4.2650.

[22] C. C. Feral, N. Nishiya, C. A. Fenczik, H. Stuhlmann, M. Slepak, M. H. Ginsberg, *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 355, https://doi.org/10.1073/pnas.0404852102.

[23] S. Kantipudi, J.-M. Jeckelmann, Z. Ucurum, P. D. Bosshart, D. Fotiadis, *Int. J. Mol. Sci*. **2020**, *21*, https://doi.org/10.3390/ijms21207573.

[24] J. Fort, L. R. de la Ballina, H. E. Burghardt, C. Ferrer-Costa, J. Turnay, C. Ferrer-Orta, I. Uson, A. Zorzano, J. Fernandez-Recio, M. Orozco, M. A. Lizarbe, I. Fita, M. Palacin, *J. Biol. Chem*. **2007**, *282*, 31444, https://doi.org/10.1074/jbc.M704524200.

[25] J. Fort, A. Nicolas-Arago, M. Palacin, *Molecules* **2021**, *26*, https://doi.org/10.3390/molecules26206231.

[26] J.-M. Jeckelmann, D. Fotiadis, *Int. J. Mol. Sci.* **2020**, *21*, https://doi.org/10.3390/ijms21197094.

[27] E. Nakamura, M. Sato, H. Yang, F. Miyagawa, M. Harasaki, K. Tomita, S. Matsuoka, A. Noma, K. Iwai, N. Minato, *J. Biol. Chem*. **1999**, *274*, 3009, https://doi.org/10.1074/jbc.274.5.3009.

[28] P. D. Prasad, H. Wang, W. Huang, R. Kekuda, D. P. Rajan, F. H. Leibach, V. Ganapathy, *Biochem. Biophys. Res. Commun.* **1999**, *255*, 283, https://doi.org/10.1006/bbrc.1999.0206.

[29] M. Scalise, M. Galluccio, L. Console, L. Pochini, C. Indiveri, *Front. Chem*. **2018**, *6*, 243, https://doi.org/10.3389/fchem.2018.00243.

[30] H. Nawashiro, N. Otani, N. Shinomiya, S. Fukui, H. Ooigawa, K. Shima, H. Matsuo, Y. Kanai, H. Endou, *Int. J. Cancer* **2006**, *119*, 484, https://doi.org/10.1002/ijc.21866.

[31] K. Kaira, N. Oriuchi, H. Imai, K. Shimizu, N. Yanagitani, N. Sunaga, T. Hisada, S. Tanaka, T. Ishizuka, Y. Kanai, H. Endou, T. Nakajima, M. Mori, *Br. J. Cancer* **2008**, *98*, 742, https://doi.org/10.1038/sj.bjc.6604235.

[32] K. Kaira, Y. Sunose, K. Arakawa, T. Ogawa, N. Sunaga, K. Shimizu, H. Tominaga, N. Oriuchi, H. Itoh, S. Nagamori, Y. Kanai, A. Segawa, M. Furuya, M. Mori, T. Oyama, I. Takeyoshi, *Br. J. Cancer* **2012**, *107*, 632, https://doi.org/10.1038/bjc.2012.310.

[33] M. Ichinoe, T. Mikami, T. Yoshida, I. Igawa, T. Tsuruta, N. Nakada, N. Anzai, Y. Suzuki, H. Endou, I. Okayasu, *Pathol. Int*. **2011**, *61*, 281, https://doi.org/10.1111/j.1440-1827.2011.02650.x.

[34] S. Kurozumi, K. Kaira, H. Matsumoto, M. Kurosumi, T. Yokobori, Y. Kanai, C. Sekine, C. Honda, A. Katayama, M. Furuya, S. Shiino, T. Makiguchi, N. P. Mongan, E. A. Rakha, T. Oyama, T. Fujii, K. Shirabe, J. Horiguchi, *Sci. Rep*. **2022**, *12*, 2742, https://doi.org/10.1038/s41598-022-06615-8.

[35] T. Sakata, G. Ferdous, T. Tsuruta, T. Satoh, S. Baba, T. Muto, A. Ueno, Y. Kanai, H. Endou, I. Okayasu, *Pathol. Int*. **2009**, *59*, 7, https://doi.org/10.1111/j.1440-1827.2008.02319.x.

[36] K. Nakanishi, S. Ogata, H. Matsuo, Y. Kanai, H. Endou, S. Hiroi, S. Tominaga, S. Aida, H. Kasamatsu, T. Kawai, *Virchows Arch*. **2007**, *451*, 681, https://doi.org/10.1007/s00428-007-0457-9.

[37] H. Betsunoh, T. Fukuda, N. Anzai, D. Nishihara, T. Mizuno, H. Yuki, A. Masuda, Y. Yamaguchi, H. Abe, M. Yashi, Y. Fukabori, K. Yoshida, T. Kamai, *BMC Cancer* **2013**, *13*, 509, https://doi.org/10.1186/1471-2407-13-509.

[38] Q. Wang, J. Holst, *Am. J. Cancer Res*. **2015**, 5, 1281.

[39] J. J. Lu, P. Li, Y. Yang, L. Wang, Y. Zhang, J. Y. Zhu, X. R. Zhu, M. B. Chen, *PLoS One* **2020**, *15*, e0233629, https://doi.org/10.1371/journal.pone.0233629.

[40] N. Singh, M. Scalise, M. Galluccio, M. Wieder, T. Seidel, T. Langer, C. Indiveri, G. F. Ecker, *Int. J. Mol. Sci.* **2018**, *20*, https://doi.org/10.3390/ijms20010027.

[41] M. Scalise, R. Scanga, L. Console, M. Galluccio, L. Pochini, C. Indiveri, *Molecules* **2021**, *26*, https://doi.org/10.3390/molecules26216562.

[42] E. B. Knöpfel, C. Vilches, S. M. R. Camargo, E. Errasti-Murugarren, A. Staubli, C. Mayayo, F. L. Munier, N. Miroshnikova, N. Poncet, A. Junza, S. S. Bhattacharya, E. Prat, V. Berry, W. Berger, E. Heon, A. T. Moore, O. Yanes, V. Nunes, M. Palacin, F. Verrey, B. Kloeckener-Gruissem, *Front. Physiol*. **2019**, *10*, 688, https://doi.org/10.3389/fphys.2019.00688.

[43] M. Espino Guarch, M. Font-Llitjos, S. Murillo-Cuesta, E. Errasti-Murugarren, A. M. Celaya, G. Girotto, D. Vuckovic, M. Mezzavilla, C. Vilches, S. Bodoy, I. Sahun, L. Gonzalez, E. Prat, A. Zorzano, M. Dierssen, I. Varela-Nieto, P. Gasparini, M. Palacin, V. Nunes, *eLife* **2018**, *7*, https://doi.org/10.7554/eLife.31511.

[44] E. Tina, S. Prosen, S. Lennholm, G. Gasparyan, M. Lindberg, A. Gothlin Eremo, *Br. J. Dermatol*. **2019**, *180*, 130, https://doi.org/10.1111/bjd.16905.

[45] M. Feng, G. Xiong, Z. Cao, G. Yang, S. Zheng, J. Qiu, L. You, L. Zheng, T. Zhang, Y. Zhao, *J. Exp. Clin. Cancer Res.* **2018**, *37*, 274, https://doi.org/10.1186/s13046-018-0947-4.

[46] L. Cascio, C. F. Chen, R. Pauly, S. Srikanth, K. Jones, C. D. Skinner, R. E. Stevenson, C. E. Schwartz, L. Boccuto, *Mol. Genet. Genomic Med*. **2020**, *8*, e1036, https://doi.org/10.1002/mgg3.1036.

[47] D. C. Tarlungeanu, E. Deliu, C. P. Dotter, M. Kara, P. C. Janiesch, M. Scalise, M. Galluccio, M. Tesulov, E. Morelli, F. M. Sonmez, K. Bilguvar, R. Ohgaki, Y. Kanai, A. Johansen, S. Esharif, T. Ben-Omran, M. Topcu, A. Schlessinger, C. Indiveri, K. E. Duncan, A. O. Caglayan, M. Gunel, J. G. Gleeson, G. Novarino, *Cell* **2016**, *167*, 1481, https://doi.org/10.1016/j.cell.2016.11.013.

[48] E. C. Friesema, R. Docter, E. P. Moerings, F. Verrey, E. P. Krenning, G. Hennemann, T. J. Visser, *Endocrinology* **2001**, *142*, 4339, https://doi.org/10.1210/endo.142.10.8418.

[49] C. Zevenbergen, M. E. Meima, E. C. Lima de Souza, R. P. Peeters, A. Kinne, G. Krause, W. E. Visser, T. J. Visser, *Endocrinology* **2015**, *156*, 4345, https://doi.org/10.1210/en.2015-1140.

[50] S. Kantipudi, D. Fotiadis, *Front. Mol. Biosci*. **2021**, *8*, 676854, https://doi.org/10.3389/fmolb.2021.676854.

[51] Y. Kanai, H. Segawa, K. Miyamoto, H. Uchino, E. Takeda, H. Endou, *J. Biol. Chem*. **1998**, *273*, 23629, https://doi.org/10.1074/jbc.273.37.23629.

[52] L. Mastroberardino, B. Spindler, R. Pfeiffer, P. J. Skelly, J. Loffing, C. B. Shoemaker, F. Verrey, *Nature* **1998**, *395*, 288, https://doi.org/10.1038/26246.

[53] O. Yanagida, Y. Kanai, A. Chairoungdua, D. K. Kim, H. Segawa, T. Nii, S. H. Cha, H. Matsuo, J. Fukushima, Y. Fukasawa, Y. Tani, Y. Taketani, H. Uchino, J. Y. Kim, J. Inatomi, I. Okayasu, K. Miyamoto, E. Takeda, T. Goya, H. Endou, *Biochim. Biophys. Acta - Biomembranes* **2001**, *1514*, 291, https://doi.org/10.1016/s0005-2736(01)00384-4.

[54] C. Meier, Z. Ristic, S. Klauser, F. Verrey, *EMBO J.* **2002**, *21*, 580, https://doi.org/10.1093/emboj/21.4.580.

[55] M. Pineda, E. Fernandez, D. Torrents, R. Estevez, C. Lopez, M. Camps, J. Lloberas, A. Zorzano, M. Palacin, *J. Biol. Chem*. **1999**, *274*, 19738, https://doi.org/10.1074/jbc.274.28.19738.

[56] G. Rossier, C. Meier, C. Bauch, V. Summa, B. Sordat, F. Verrey, L. C. Kuhn, *J. Biol. Chem*. **1999**, *274*, 34948, https://doi.org/10.1074/jbc.274.49.34948.

[57] K. Oda, N. Hosoda, H. Endo, K. Saito, K. Tsujihara, M. Yamamura, T. Sakata, N. Anzai, M. F. Wempe, Y. Kanai, H. Endou, *Cancer Sci*. **2010**, *101*, 173, https://doi.org/10.1111/j.1349-7006.2009.01386.x.

[58] D. W. Yun, S. A. Lee, M. G. Park, J. S. Kim, S. K. Yu, M. R. Park, S. G. Kim, J. S. Oh, C. S. Kim, H. J. Kim, J. S. Kim, H. S. Chun, Y. Kanai, H. Endou, M. F. Wempe, D. K. Kim, *J. Pharmacol. Sci*. **2014**, *124*, 208, https://doi.org/10.1254/jphs.13154fp.

[59] P. Häfliger, J. Graff, M. Rubin, A. Stooss, M. S. Dettmer, K. H. Altmann, J. Gertsch, R.-P. Charles, *J. Exp. Clin. Cancer Res*. **2018**, *37*, 234, https://doi.org/10.1186/s13046-018-0907-z.

[60] M. Costa, A. Rosell, E. Alvarez-Marimon, A. Zorzano, D. Fotiadis, M. Palacin, *Protein Expr. Purif.* **2013**, *87*, 35, https://doi.org/10.1016/j.pep.2012.10.003.

[61] S. Kantipudi, D. Harder, S. Bonetti, D. Fotiadis, J.-M. Jeckelmann, *Methods Protoc*. **2021**, *4*, https://doi.org/10.3390/mps4030051.

[62] B. Byrne, *Curr. Opin. Struct. Biol*. **2015**, *32C*, 9, https://doi.org/10.1016/j.sbi.2015.01.005.

[63] V. Looser, B. Bruhlmann, F. Bumbak, C. Stenger, M. Costa, A. Camattari, D. Fotiadis, K. Kovar, *Biotechnol. Adv.* **2015**, *33*, 1177, https://doi.org/10.1016/j.biotechadv.2015.05.008.

[64] M. Meury, M. Costa, D. Harder, M. Stauffer, J.-M. Jeckelmann, B. Bruhlmann, A. Rosell, H. Ilgü, K. Kovar, M. Palacin, D. Fotiadis, *PLoS One* **2014**, *9*, e109882, https://doi.org/10.1371/journal.pone.0109882.

[65] H. Ilgü, J.-M. Jeckelmann, C. Colas, Z. Ucurum, A. Schlessinger, D. Fotiadis, *Int. J. Mol. Sci*. **2018**, *19*, https://doi.org/10.3390/ijms19030918.

[66] H. Ilgü, J.-M. Jeckelmann, D. Kalbermatter, Z. Ucurum, T. Lemmin, D. Fotiadis, *BMC Biol*. **2021**, *19*, 179, https://doi.org/10.1186/s12915-021-01102-4.

[67] H. Ilgü, J.-M. Jeckelmann, V. Gapsys, Z. Ucurum, B. L. de Groot, D. Fotiadis, *Proc. Natl. Acad. Sci. USA* **2016**, *113*, 10358, https://doi.org/10.1073/pnas.1605442113.

[68] D. Harder, D. Fotiadis, *Nature Protoc*. **2012**, *7*, 1569, https://doi.org/10.1038/nprot.2012.090

[69] M. Meury, D. Harder, Z. Ucurum, R. Boggavarapu, J.-M. Jeckelmann, D. Fotiadis, *Biol. Chem*. **2011**, *392*, 143, https://doi.org/10.1515/BC.2011.004.

[70] J.-M. Jeckelmann, D. Fotiadis, *Int. J. Mol. Sci*. **2019**, *20*, https://doi.org/10.3390/ijms20040931.

[71] J.-M. Jeckelmann, D. Fotiadis, *Int. J. Mol. Sci*. **2020**, *21*, https://doi.org/10.3390/ijms21197094.

[72] J.-M. Jeckelmann, T. Lemmin, M. Schlapschy, A. Skerra, D. Fotiadis, *Sci. Rep*. **2022**, 12, 18269, https://doi.org/10.1038/s41598-022-23270-1.

[73] Webpage, UMIN Clinical Trials Registry, UMIN000034080-Randomized Controlled Phase 2 Clinical Trial of JPH203 in Patients

With Advanced Biliary Tract Cancers (Accessed on June 1, 2022; website last modified on Sept. 21, 2021) - https://upload.umin.ac.jp/cgi-open-bin/ctr_e/ctr_view.cgi?recptno=R000038852
[74] J. Graff, J. Muller, A. Sadurni, M. Rubin, I. A. Canivete Cuissa, C. Keller, M. Hartmann, S. Singer, J. Gertsch, K. H. Altmann, *ChemMedChem*. **2022**, *17*, e202200308, https://doi.org/10.1002/cmdc.202200308.
[75] K. Hutchinson, D. B. Silva, J. Bohlke, C. Clausen, A. A. Thomas, M. Bonomi, A. Schlessinger, *bioRxiv* **2022**, 2022.05.03.490502, https://doi.org/10.1101/2022.05.03.490502.
[76] Y. Takada, D. T. Vistica, N. H. Greig, D. Purdon, S. I. Rapoport, Q. R. Smith, *Cancer Res*. **1992**, *52*, 2191.
[77] J. Matharu, J. Oki, D. R. Worthen, Q. R. Smith, P. A. Crooks, *Bioorg. Med. Chem. Lett*. **2010**, *20*, 3688, https://doi.org/10.1016/j.bmcl.2010.04.086.
[78] R. Yan, Y. Li, J. Muller, Y. Zhang, S. Singer, L. Xia, X. Zhong, J. Gertsch, K. H. Altmann, Q. Zhou, *Cell Discov*. **2021**, *7*, 16, https://doi.org/10.1038/s41421-021-00247-4.
[79] R. Yan, X. Zhao, J. Lei, Q. Zhou, *Nature* **2019**, *568*, 127, https://doi.org/10.1038/s41586-019-1011-z.
[80] Y. Lee, P. Wiriyasermkul, C. Jin, L. Quan, R. Ohgaki, S. Okuda, T. Kusakizako, T. Nishizawa, K. Oda, R. Ishitani, T. Yokoyama, T. Nakane, M. Shirouzu, H. Endou, S. Nagamori, Y. Kanai, O. Nureki, *Nat. Struct. Mol. Biol*. **2019**, *26*, 510, https://doi.org/10.1038/s41594-019-0237-7.
[81] R. Ohgaki, T. Ohmori, S. Hara, S. Nakagomi, M. Kanai-Azuma, K. Kaneda-Nakashima, S. Okuda, S. Nagamori, Y. Kanai, *Mol. Cell. Biol*. **2017**, *37*, https://doi.org/10.1128/MCB.00427-16.
[82] K. Ashe, W. Kelso, S. Farrand, J. Panetta, T. Fazio, G. De Jong, M. Walterfang, *Front. Psychiatry* **2019**, *10*, 561, https://doi.org/10.3389/fpsyt.2019.00561.
[83] M. Bik-Multanowski, J. J. Pietrzyk, *J. Inherit. Metab. Dis*. **2006**, *29*, 684, https://doi.org/10.1007/s10545-006-0285-0.
[84] N. Okano, D. Naruge, K. Kawai, T. Kobayashi, F. Nagashima, H. Endou, J. Furuse, *Invest. New Drugs* **2020**, *38*, 1495, https://doi.org/10.1007/s10637-020-00924-3.
[85] Webpage, as a PDF link - https://www.j-pharma.com/en/wp-content/uploads/2022/04/ca78dc166426a36846c58dc0608d8c19.pdf.
[86] N. Cappoli, M. D. Jenkinson, C. Dello Russo, D. Dickens, *Biochem. Pharmacol*. **2022**, *201*, 115103, https://doi.org/10.1016/j.bcp.2022.115103.
[87] Webpage, ClinicalTrials.gov Identifier: NCT04430842 (Accessed on June 1, 2022; website last modified on April 19, 2022) - https://clinicaltrials.gov/ct2/show/NCT04430842.
[88] T. Ozawa, M. Rodriguez, G. Zhao, T. W. Yao, W. N. Fischer, B. Jandeleit, K. Koller, T. Nicolaides, *Cureus* **2021**, *13*, e17595, https://doi.org/10.7759/cureus.17595.
[89] A. Achmad, S. Lestari, H. A. Holik, D. Rahayu, M. H. Bashari, A. Faried, A. H. S. Kartamihardja, *Processes* **2021**, *9*, 1170, https://doi.org/10.3390/pr9071170.
[90] O. ElMokh, D. Ruffieux-Daidie, M. A. Roelli, A. Stooss, W. A. Phillips, J. Gertsch, M. S. Dettmer, R. P. Charles, *Oncotarget* **2017**, *8*, 24604, https://doi.org/10.18632/oncotarget.15599.
[91] Y. Miyagi, M. Higashiyama, A. Gochi, M. Akaike, T. Ishikawa, T. Miura, N. Saruki, E. Bando, H. Kimura, F. Imamura, M. Moriyama, I. Ikeda, A. Chiba, F. Oshita, A. Imaizumi, H. Yamamoto, H. Miyano, K. Horimoto, O. Tochikubo, T. Mitsushima, M. Yamakado, N. Okamoto, *PLoS One* **2011**, *6*, e24143, https://doi.org/10.1371/journal.pone.0024143.
[92] P. J. Bothwell, C. D. Kron, E. F. Wittke, B. N. Czerniak, B. P. Bode, *Int. J. Mol. Sci*. **2018**, *19*, https://doi.org/10.3390/ijms19072093.
[93] J. Zhang, Y. Xu, D. Li, L. Fu, X. Zhang, Y. Bao, L. Zheng, *Front. Chem*. **2020**, *8*, 564809, https://doi.org/10.3389/fchem.2020.564809.
[94] S. Roos, T. L. Powell, T. Jansson, *Biochem. Soc. Trans*. **2009**, *37*, 295, https://doi.org/10.1042/BST0370295.
[95] G. J. Burton, A. L. Fowden, K. L. Thornburg, *Physiol. Rev*. **2016**, *96*, 1509, https://doi.org/10.1152/physrev.00029.2015.
[96] P. W. Aldoretta, W. W. Hay, *Nut. Res*. **1994**, *14*, 929, https://doi.org/10.1016/S0271-5317(05)80493-2.
[97] O. R. Vaughan, F. J. Rosario, T. L. Powell, T. Jansson, *Prog. Mol. Biol. Transl. Sci*. **2017**, *145*, 217, https://doi.org/10.1016/bs.pmbts.2016.12.008.
[98] J. K. Cleal, R. M. Lewis, *J. Neuroendocrinol*. **2008**, *20*, 419, https://doi.org/10.1111/j.1365-2826.2008.01662.x.
[99] T. Jansson, *Pediatr. Res*. **2001**, *49*, 141, https://doi.org/10.1203/00006450-200102000-00003.
[100] I. Cetin, M. S. de Santis, E. Taricco, T. Radaelli, C. Teng, S. Ronzoni, E. Spada, S. Milani, G. Pardi, *Am. J. Obstet. Gynecol*. **2005**, *192*, 610, https://doi.org/10.1016/j.ajog.2004.08.011.
[101] Y. Sadovsky, T. Jansson, in 'Knobil and Neill's Physiology of Reproduction (Fourth Edition)', Eds. T. M. Plant, A. J. Zeleznik, Academic Press, San Diego, **2015**, p. 1741, https://doi.org/10.1016/B978-0-12-397175-3.00039-9.
[102] F. Gaccioli, I. L. Aye, S. Roos, S. Lager, V. I. Ramirez, Y. Kanai, T. L. Powell, T. Jansson, *Reprod. Biol. Endocrinol.* **2015**, *13*, 57, https://doi.org/10.1186/s12958-015-0054-8.
[103] J. Zaugg, F. Ziegler, J. M. Nuoffer, R. Moser-Hassig, C. Albrecht, *J. Nutr. Biochem*. **2021**, *96*, 108760, https://doi.org/10.1016/j.jnutbio.2021.108760.
[104] J. Zaugg, X. Huang, F. Ziegler, M. Rubin, J. Graff, J. Muller, R. Moser-Hassig, T. Powell, J. Gertsch, K. H. Altmann, C. Albrecht, *J. Cell. Mol. Med*. **2020**, *24*, 12681, https://doi.org/10.1111/jcmm.15840.
[105] M. Schroeder, M. Jakovcevski, T. Polacheck, Y. Drori, A. Luoni, S. Roh, J. Zaugg, S. Ben-Dor, C. Albrecht, A. Chen, *Nat. Commun*. **2018**, *9*, 1596, https://doi.org/10.1038/s41467-018-03836-2.
[106] M. Schroeder, M. Jakovcevski, T. Polacheck, Y. Drori, S. Ben-Dor, S. Roh, A. Chen, *Mol. Metab*. **2018**, *17*, 1, https://doi.org/10.1016/j.molmet.2018.08.005.
[107] A. F. Philipps, I. R. Holzman, C. Teng, F. C. Battaglia, *Am. J. Obstet. Gynecol*. **1978**, *131*, 881, https://doi.org/10.1016/s0002-9378(16)33136-2.