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Novel (sulfated) thyroid hormone transporters in the solute carrier 22 family

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Abstract

Objective: Thyroid hormone (TH) transport represents a critical first step in governing intracellular TH regulation. It is still unknown whether the full repertoire of TH transporters has been identified. Members of the solute carrier (SLC) 22 family have substrates in common with the known TH transporters of the organic anion-transporting peptide family. Therefore, we screened the SLC22 family for TH transporters
Methods: Uptake of 1 nM of iodothyronines or sulfated iodothyronines in COS1 cells expressing SLC22 proteins was performed.
Results: We first tested 25 mouse (m) SLC22 proteins for TH uptake and found that the majority of the organic anion transporter (OAT) clade were capable of 3,3',5-triiodothyronine and/or thyroxine (T4) transport. Based on phylogenetic tree analysis of the mouse and human (h) SLC22 family, we selected eight hSLC22s that grouped with the newly identified mouse TH transporters. Of these, four tested positive for uptake of one or more substrates, particularly hSLC22A11 showed robust (3-fold over control) uptake of T4. Uptake of sulfated iodothyronines was strongly (up to 17-fold) induced by some SLC22s, most notably SLC22A8, hSLC22A9, mSLC22A27 and mSLC22A29. Finally, the zebrafish orthologues of SLC22A6/8 drOatx and drSlc22a6l also transported almost all (sulfated) iodothyronines tested. The OAT inhibitors lesinurad and probenecid inhibited most SLC22 proteins.
Conclusions: Our results demonstrated that members of the OAT clade of the SLC22 family constitute a novel, evolutionary conserved group of transporters for (sulfated) iodothyronines. Future studies should reveal the relevance of these transporters in TH homeostasis and physiology.

Key Words
- thyroid hormone
- transporter
- solute carrier 22 family
- thyroid hormone sulfate
- organic anion transporter
- OAT3
- OAT4
- OAT7
- probenecid
- lesinurad

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Introduction

Thyroid hormone (TH) is important for development, metabolism and tissue homeostasis. The intracellular bioavailability of TH is dependent on plasma membrane transporters that mediate TH influx and efflux (1). The relevance of TH transporters is clearly demonstrated by the severe disease features in patients with defective monocarboxylate transporter 8 (MCT8) (2). At present, approximately 15 transporters that are able to transport TH have been identified. They comprise MCT8 and MCT10, solute carrier (SLC)17A4 and members of the large amino acid transporters (LATs) and the organic anion-transporting polypeptides (OATPs), as well as the ATP-binding cassette (ABC) family transporter ABCB1 (reviewed in (3)).

It is unknown whether the full repertoire of TH transporters has been identified so far. In this context, the SLC22 family is of potential interest. The SLC22 family is categorized into two major clades based on their substrate preferences: the organic anion transporter (OAT) clade and organic cation transporter (OCT) clade (4). Some members in the OAT major clade share common substrates with the known TH transporters in the OATP family, such as estrone 3 sulfate, prostaglandin E2, estradiol glucuronide and taurocholic acid (5, 6, 7, 8, 9, 10, 11, 12). In addition, the recently identified TH transporter SLC17A4 transports urate (13, 14), which is also a physiologically relevant substrate for some members of the SLC22 family (15, 16, 17, 18, 19).

Apart from iodothyronines, some TH transporters, notably OATP1B1 (20), can also transport iodothyronines that are conjugated at the phenolic group with sulfate. Sulfation of iodothyronines contributes to TH clearance as it greatly enhances inner ring deiodination by the type I deiodinase (DIO1) in the liver and kidney (21). In addition, sulfated TH may be a local source in critical tissues, such as the brain and liver, during fetal development (22). As (sulfated) iodothyronines are organic anions, we hypothesized that some members in the SLC22 family could transport TH and/or its metabolites.

To test our hypothesis, we screened all mouse SLC22 family members and several human SLC22 family members from the OAT clade for the uptake of iodothyronines as well as iodothyronine sulfates. We here report the identification of ten novel iodothyronine (sulfate) transporters, thereby greatly expanding the repertoire of potentially relevant TH transporters.

Materials and methods

Reagents

Nonradioactive 3,3'-diiodothyronine (3,3'-T2), 3,3',5'-triiodothyronine (rT3), 3,3',5-triiodothyronine (T3) and thyroxine (T4) were purchased from Sigma-Aldrich (Zwijndrecht, the Netherlands). [^{125}I]-3,3'-T2, [^{125}I]-rT3, [^{125}I]-T3, [^{125}I]-T4, non-radioactive and [^{125}I]-labeled T3 sulfate (T3S) and T4 sulfate (T4S) were prepared as previously described (23). Probenecid and lesinurad (Sigma-Aldrich) were dissolved in 0.15N NaOH and DMSO, respectively.

Constructs, cell culture, transfection and uptake assays

All constructs were commercially purchased or generated from previous studies except for human SLC22A11 (24, 20, 25, 26) (Supplementary Tables 1, 2, 3 and 4 in Supplementary Material 1, see section on supplementary materials given at the end of this article). Generation of the expression construct of human SLC22A11 is described in Supplementary Material 1. Cell culture, transfection and uptake assays were essentially performed as described previously (26, 27). COS1 cells were used for transfection as they have little deiodinase activity that would compromise the transport of the iodothyronines (24). For the uptake assays, we co-transfected the iodothyronine-binding protein CRYM (μ-crystallin) to minimize the efflux of the (sulfated) iodothyronines (26, 28). Full details are provided in Supplementary Material 1.

Phylogenetic tree of mouse and human SLC22 family

The protein sequences of human and mouse SLC22s (accession numbers in Supplementary Table 5 in Supplementary Material 1) were collected from the National Center for Biotechnology Information (NCBI) protein database (https://www.ncbi.nlm.nih.gov/). The phylogenetic tree was generated through http://www.phylogeny.fr/alacarte.cgi using T-coffee multiple alignments of the protein sequences without any curation and constructed with ProtDist/FastDist+BioNJ distances.

Analysis of common genetic variation in newly identified SLC22 TH transporters

We analyzed whether common genetic variation in the *SLC22* genes (h*SLC22A8*, h*SLC22A9*, h*SLC22A11*

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and h*SLC22A24*) was associated with serum circulating TH concentrations (free T4 (FT4), and T3/T4 ratios). FT4 analyses were performed using data from 72,167 individuals of the ThyroidOmics Consortium (13,14). For the FT3/FT4 ratio, we conducted a separate analysis within the two cohorts (SHIP-START-0 and SHIP-TREND-0) of the German population-based Study of Health in Pomerania (29). For the total T3 (TT3)/total T4 (TT4) ratio, a separate analysis was conducted in the Dutch population-based study NIMA (non-invasive measurements of atherosclerosis) (30). Detailed information on the analysis is provided in Supplementary Material 1. All subjects from the above cohorts gave informed consent. The Medical Ethics Committee of the Radboud University Medical Centre approved the study protocol of the NBS/NIMA study and the Medical Ethics Committee of the University of Greifswald approved the study protocol of the SHIP study. Both are in accordance with the Declaration of Helsinki.

Statistical analysis

The statistical analysis was performed using GraphPad Prism version 8.4.0. The sample size and the methods of statistical analysis used were indicated in the figure legends. $P < 0.05$ was considered significant.

Results

Uptake of iodothyronines by mouse SLC22s

We initially screened all members of the mouse (m) SLC22 family for iodothyronine transport. We first verified whether the mSLC22 proteins were properly expressed at

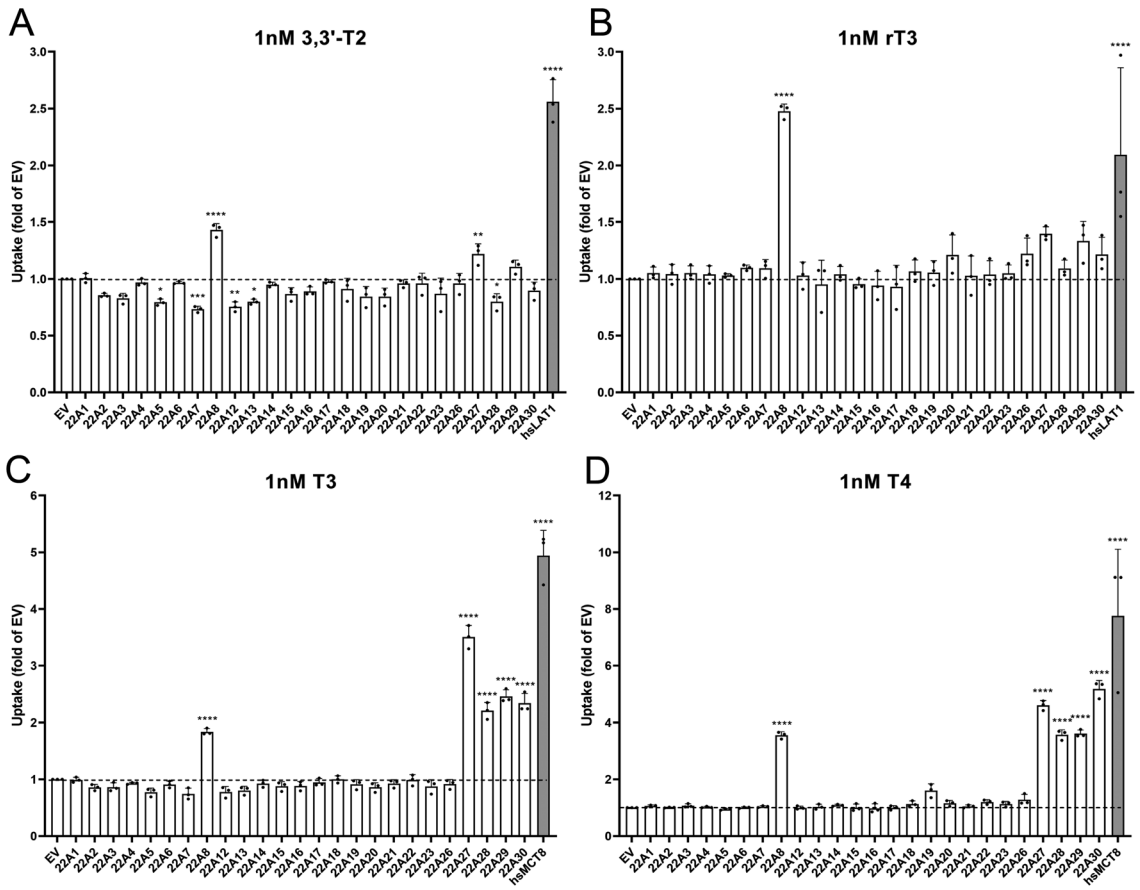

Figure 1
Uptake of 1 nM 3,3′-T2, rT3, T3 and T4 in COS1 cells transfected with mouse *Slc22s* and h*CRYM* (μ-crystallin). COS1 cells were incubated with the substrates for 10 min (3,3′-T2 and rT3) or 30 min (T3 and T4) in DPBS/0.1% glucose/0.1% BSA. The uptake of mouse *Slc22s* transfected cells is expressed as fold induction of that in the cells transfected with empty vector (EV). Human LAT1 (for 3,3′-T2 and rT3 uptake) and MCT8 (for T3 and T4 uptake) were used as positive controls (gray bars). Data are represented as mean ± S.D. ($n = 3$). One-way ANOVA plus Dunnett's *post hoc* test (compared to the EV group) was used for statistical analysis. *$0.01 < P < 0.05$; **$0.001 < P \leq 0.01$; ***$0.0001 < P \leq 0.001$; ****$P \leq 0.0001$.

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the plasma membrane by immunoblotting biotinylated cell surface proteins with anti-FLAG antibodies. Except for mSLC22A22, whose expression was hardly detectable, all mSLC22s were expressed on the cell membrane (Supplementary Fig. 1 in Supplementary Material 1). The majority of the mSLC22 proteins displayed more than one band suggesting post-translational modifications, di- or oligomerization or protein aggregation, or some protein degradation.

Next, we tested 3,3'-T2, rT3, T3 and T4 uptake in COS1 cells transfected with m*Slc22* expression constructs together with the intracellular (sulfated) iodothyronine binding protein CRYM (28, 31). Uptake assays were performed in DPBS/0.1% glucose in the absence (representing a non-stringent screening strategy) or presence (representing a more physiological condition) of 0.1% bovine serum albumin (BSA).

None of the mSLC22 transporters that belong to the OCT major clade (mSLC22A1-5, mSLC22A15, mSLC22A16 and mSLC22A21) (4) facilitated uptake of any of the substrates (Fig. 1 and Supplementary Fig. 2).

In contrast, several members of the OAT major clade induced the uptake of one or more of the substrates tested. Mouse SLC22A8 induced uptake of all tested substrates, especially 1 nM rT3 and T4 (>2 fold compared to empty vector (EV) transfected cells) (Fig. 1). The closely related OAT members mSLC22A27-30 increased uptake of 1 nM T3 and T4 to a similar extent (Fig. 1). In addition, mSLC22A19 and mSLC22A26 induced T4 uptake but to a lesser extent and only in the absence of BSA (Supplementary Fig. 2). Uptake of 3,3'-T2 by mSLC22A8 was only induced in the presence of BSA. For some transporters, intracellular accumulation of 3,3'-T2 was reduced (Fig. 1 and Supplementary Fig. 2). This may indicate increased efflux of substrates, however, this was not further tested.

Taken together, these results suggest that mSLC22A8 and mSLC22A27-30 transport several iodothyronines, whereas under less stringent conditions, mSLC22A19 and mSLC22A26 transport T4.

Phylogenetic tree of mouse and human SLC22s

Next, we generated a phylogenetic tree to identify the human orthologues of the mSLC22 members that were tested positive for TH uptake. The phylogenetic tree shows that six subclades (OAT, OAT-like, OAT-related, OCT, OCTN and OCT/OCTN related) are separately clustered, in agreement with previous findings (4) and all members that transport iodothyronines reside within the OAT subclade

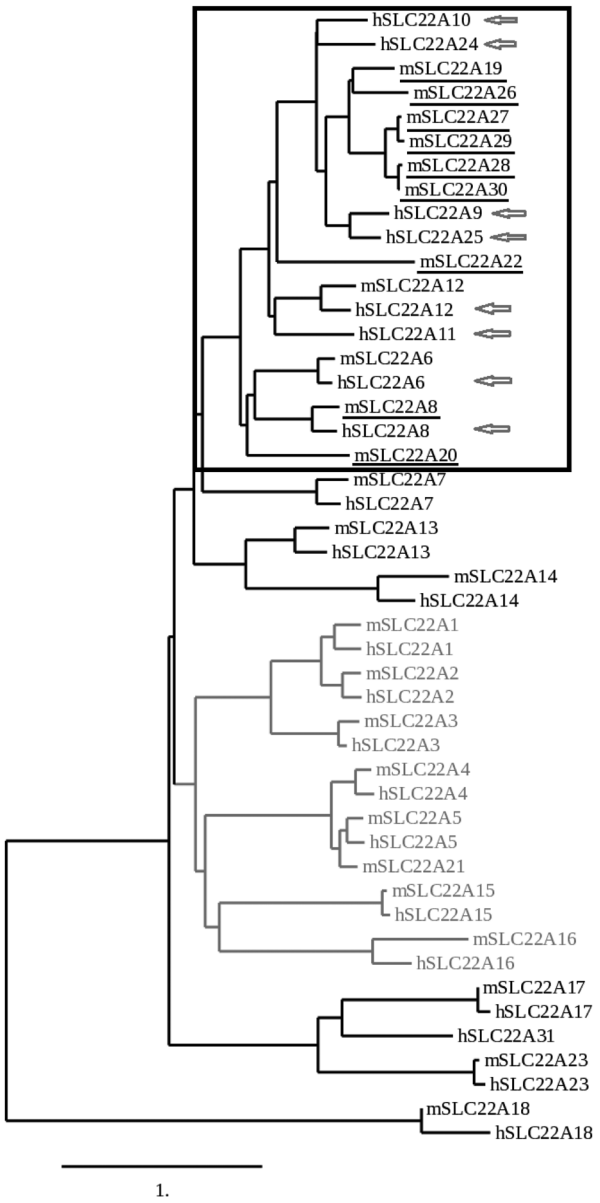

Figure 2
The phylogenetic tree of mouse and human SLC22s. According to literature (reviewed in 'The SLC22 Transporter Family: A Paradigm for the Impact of Drug Transporters on Metabolic Pathways, Signaling, and Disease' by Nigam *et al.* in 2018), the mouse and human SLC22s in gray belong to the organic cation (OCT) major clade and the ones in black belong to the organic anion (OAT) major clade. The OCT major clade consists of three subclades (OCT (SLC22A1-3), OCTN (SLC22A4-5 and 22A21 and OCT/OCTN-related (SLC22A15-16)). The OAT major clade consists of three subclades (OAT-like (SLC22A13-14), OAT-related (SLC22A17-18, 22A23 and 22A31) and OAT (the others)). The mouse SLC22s that are able to transport iodothyronines are underlined in black. The clusters that contain mouse SLC22s transporting iodothyronines were grouped in a black box and the human SLC22 orthologues in the box with arrowheads were selected for further test of uptake of iodothyronines. The number of amino acid substitutions per site was depicted as the length of branches. The line and the number '1' underneath the tree indicate the scale bar of branch length which is one amino acid substitution per site.

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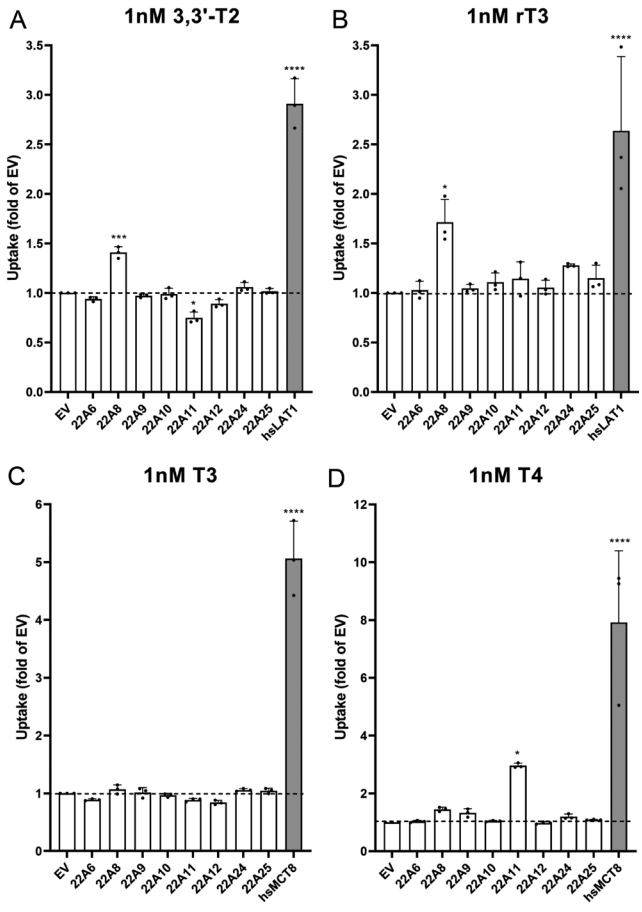

Figure 3
Uptake of 1 nM 3,3'-T2, rT3, T3 and T4 in COS1 cells transfected with human *SLC22s* and h*CRYM*. COS1 cells were incubated with the substrates for 10 min (3,3'-T2 and rT3) or 30 min (T3 and T4) in DPBS/0.1% glucose/0.1% BSA. The uptake of human *SLC22s* transfected cells is expressed as fold induction of that in the cells transfected with EV. Human LAT1 (for 3, 3'-T2 and rT3 uptake) and MCT8 (for T3 and T4 uptake) were used as positive controls (gray bars). Data are represented as mean ± S.D. ($n = 3$). One-way ANOVA plus Dunnett's *post hoc* test (compared to the EV group) was used for statistical analysis. *0.01 < P < 0.05; ***0.0001 < $P \leq 0.001$; ****$P \leq 0.0001$.

(Fig. 2). Within the OAT clade, six closely related mouse iodothyronine transporters namely mSLC22A27-30, and mSLC22A19 and mSLC22A26, have no human orthologues, but group closely to human (h)SLC22A9, hSLC22A25, hSLC22A10 and hSLC22A24. In addition to these four human SLC22s, we also selected hSLC22A6, hSLC22A8 and hSLC22A11-12 that are grouped in the same cluster to test iodothyronine transport (Fig. 2).

Uptake of iodothyronines by human SLC22s

The selected hSLC22s showed membrane expression, although expression of hSLC22A25 was relatively low (Supplementary Fig. 3). Like the mouse orthologue,

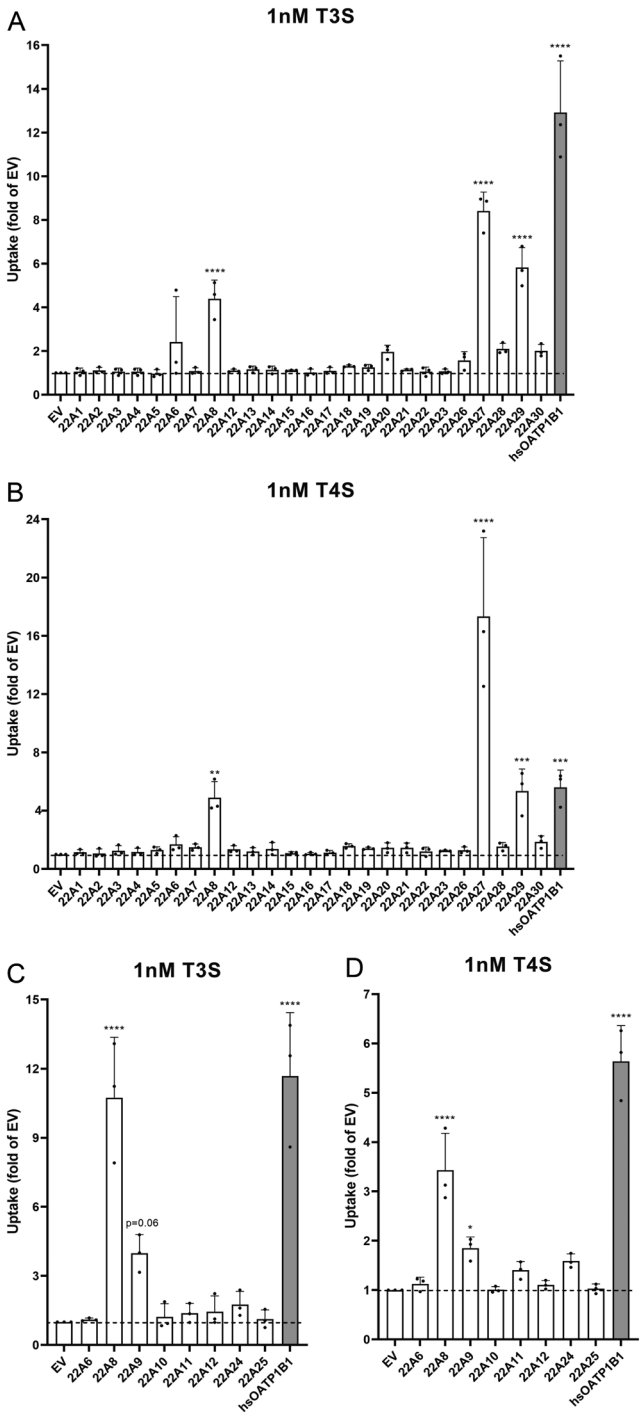

Figure 4
Uptake of 1 nM T3S and T4S in COS1 cells transfected with mouse (A and B) and human (C and D) *SLC22s* in combination with h*CRYM*. COS1 cells were incubated with 1 nM T3S (A and C) or T4S (B and D) for 30 min in DPBS/0.1% glucose/0.1% BSA. The uptake of *SLC22s* transfected cells is expressed as fold induction of that in the cells transfected with EV. Human OATP1B1 was used as a positive control (gray bars). Data are represented as mean ± S.D. ($n = 3$). One-way ANOVA plus Dunnett's *post hoc* test (compared to the EV group) was used for statistical analysis. *0.01< P < 0.05; **0.001 < $P \leq 0.01$; ***0.0001 < $P \leq 0.001$; ****$P \leq 0.0001$.

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hSLC22A8 induced uptake of all iodothyronines tested (Fig. 3 and Supplementary Fig. 4). Human SLC22A11 induced robust uptake (2.5-fold) of 1 nM T4 but not of any of the other iodothyronines (Fig. 3 and Supplementary Fig. 4). In addition, hSLC22A9 induced weak uptake of T3 and T4 (Fig. 3 and Supplementary Fig. 4). In contrast, hSLC22A6, hSLC22A10, hSLC22A12 and hSLC22A25 did not induce uptake of any of the iodothyronines tested. These results indicate that several of the hSLC22 members of the OAT clade can transport T3 and/or T4.

Uptake of sulfated TH by mouse and human SLC22s

Because some OATPs can transport iodothyronine sulfates, we also tested uptake of the sulfated iodothyronines T3S and T4S by mouse and human SLC22 proteins (Fig. 4 and Supplementary Fig. 5). Mouse SLC22A8, mSLC22A27 and mSLC22A29 induced robust uptake (>4-fold for T3S and >3-fold for T4S compared to the EV), with mSLC22A27 showing ~3-fold higher uptake of T4S than the positive control hOATP1B1 (20) (Fig. 4). Of the human SLC22s, hSLC22A8 potently transported T3S and T4S, as evidenced by the ~10-fold increased uptake of T3S and 3-fold increased uptake of T4S, comparable to hOATP1B1 (Fig. 4). In addition, hSLC22A9 efficiently transported T3S (~4-fold) and to a lesser extent T4S (1.8-fold) (Fig. 4). In summary, our results show that some SLC22 members efficiently transport sulfated iodothyronines.

The specificity and efficacy of lesinurad and probenecid for the mouse and human SLC22s

SLC22A8 (also known as OAT3) and SLC22A11 (OAT4), as well as SLC22A12 (URAT1) are important for urate resorption in the kidney (7, 18, 32). Excess serum urate (hyperuricaemia) can cause gout, a form of inflammatory arthritis. Lesinurad and probenecid are FDA-approved drugs for the treatment of gout that act by reducing urate resorption. To determine whether these inhibitors can inhibit (sulfated) iodothyronine uptake by the newly identified TH transporters in the SLC22 family, we tested uptake of the preferred (sulfated) iodothyronines by these transporters in the presence or absence of lesinurad or probenecid (Table 1).

Lesinurad reduced substrate uptake by all the SLC22 proteins tested in a concentration-dependent manner, except for mSLC22A27, mSLC22A29 and mSLC22A30 (Supplementary Fig. 6). Probenecid inhibited mSLC22A8, hSLC22A8, hSLC22A11 and hSLC22A24 in a concentration-dependent manner but not mSLC22A26-27 and mSLC22A29-30 and only at the highest concentration inhibited mSLC22A19, mSLC22A28 and hSLC22A9 (Supplementary Fig. 7). For mouse and human SLC22A8, the IC50s of lesinurad and probenecid were in the low micromolar range (Table 1). Taken together, lesinurad and probenecid inhibit all the hSLC22s tested and mSLC22A8 (Table 1).

Uptake of (sulfated) iodothyronines for zebrafish SLC22A6 and SLC22A8 orthologues

Among the human (sulfated) iodothyronine transporters, SLC22A9, SLC22A11 and SLC22A24 have only orthologues in mammals but not in other vertebrates. According to the conserved synteny analysis of human and zebrafish (Danio rerio, dr) *SLC22/slc22* genes, dr*oatx* and dr*slc22a6l* are orthologues of SLC22A6/8 (33). To determine whether (sulfated) iodothyronine transport by OATs is a conserved trait, we tested drOatx and drSlc22a6l for uptake.

Our results showed that both drOatx and drSlc22a6l transported all the tested substrates, except for T3S and T4S transport by drSlc22a6l (Fig. 5). In particular, drOatx is a very potent transporter of 3,3'-T2, rT3, T3S and T4S, comparable with the positive controls hLAT1 and hOATP1B1 (Fig. 5).

Common genetic variation in newly identified SLC22 TH transporters

As a first step to explore the possible relevance of these newly identified SLC22 transporters for human TH homeostasis, we analyzed whether genetic variations in these SLC22 genes (h*SLC22A8*, h*SLC22A9*, h*SLC22A11* and h*SLC22A24*) were associated with serum circulating TH concentrations (FT4, ratios of free T3 (FT3)/FT4 and TT3/TT4. However, none of the single nucleotide polymorphisms (SNPs) that are located in the vicinity of the *SLC22* genes are significantly associated after correction for multiple testing (false discovery rate, FDR < 0.05) with the serum FT4 levels, FT3/FT4 or TT3/TT4 ratios (Supplementary Material 2).

Discussion

In the present study, we identified ten novel (sulfated) iodothyronine transporters in the SLC22 family including six mouse-specific and three human-specific members, thereby greatly expanding the repertoire of TH transporters. Among them, seven transporters transport

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Table 1 Overview of the newly identified transporters of iodothyronines and/or iodothyronine sulfates in mouse and human SLC22 family. For the specificity and efficacy of lesinurad and probenecid, IC50 values were listed. SLC22A9, SLC22A11 and SLC22A24 are human specific, whereas SLC22A19, SLC22A26-30 are mouse specific.

	Mouse					Human				
Transporter	Substrates	Expression in tissues[a]	Substrate tested for inhibition	Lesinurad IC50	Probenecid IC50	Substrates	Expression in tissues[b]	Substrate tested for inhibition	Lesinurad IC50	Probenecid IC50
SLC22A8	3,3'-T2, rT3, T3, T4, T3S, T4S	Kidney, frontal lobe, brain (endothelial cells)[c]	T4	1.3 μM	6.0 μM	3,3'-T2, rT3, T3, T4, T3S, T4S	Kidney, choroid plexus	T3S	0.6 μM	3.5 μM
SLC22A9	Not in mouse					T3, T4, T3S, T4S	Liver	T3S	50 μM	413 μM
SLC22A11	Not in mouse					T4, T3S	Placenta, kidney, epididymis	T4	8.8 μM	57 μM
SLC22A19	T4	Kidney	T4	5.4 μM	773 μM	Not in human				
SLC22A24	Not in mouse					T4	Kidney	T3S[d]	11 μM	24 μM
SLC22A26	rT3, T4, T4S	Liver	T4	17 μM	x	Not in human				
SLC22A27	3, 3'-T2, rT3, T3, T4, T3S, T4S	Liver	T4	x	x	Not in human				
SLC22A28	T3, T4	Liver, kidney	T4	26 μM	>>1000 μM	Not in human				
SLC22A29	rT3, T3, T4, T3S, T4S	Kidney	T4	x	x	Not in human				
SLC22A30	T3, T4	Liver	T4	x	x	Not in human				

[a]The tissue expression in mouse was adopted from the mouse ENCODE transcriptome data project ('A comparative encyclopedia of DNA elements in the mouse genome' by Yue *et al.* in 2014) (34). Expression less than five reads per kilo base per million mapped reads (RPKM) are neglected; [b]The tissue expression in human was adopted from the HPA datasets from https://www.proteinatlas.org/. Expression less than three transcripts per million (TPM) are neglected; [c]Cell-specific expression in brain was adopted from http://www.brainrnaseq.org/; [d]T3S was used as the substrate to test for inhibition effect because hSLC22A24 induced T3S uptake more robustly though the induction did not reach statistical significance. >>1000 μM means within the tested doses, IC50 was not reached; N.T., not tested; X, no inhibition effect.

(sulfated) iodothyronines in the more physiological condition of added TH-binding proteins. In addition, we demonstrated that zebrafish orthologues of SLC22A6 and SLC22A8 are capable of transporting almost all the (sulfated) iodothyronines tested, indicating an evolutionary conserved function for (sulfated) iodothyronine transport within the OAT clade. These findings may contribute to the understanding of TH homeostasis in tissues in humans. Furthermore, the identification of (sulfated) iodothyronine transport of mouse and zebrafish homologues paves the way to study the physiological relevance of these transporters in animal models.

Our phylogenetic analysis showed that all newly identified transporters are grouped within the OAT major clade (mainly in the OAT subclade) but not in the OCT major clade (4). In the human genome, most *SLC22* genes encoding OATs are located in clusters on chromosome 11, with the exception of *SLC22A7*. Of these, *SLC22A6* and *SLC22A8* have phylogenetic and syntenic relationships with the dr*oatx* and dr*Slc22a6l* genes, which are clustered on chromosome 21 in the zebrafish genome (33). The transporters encoded by the zebrafish genes both tested positive for nearly all substrates tested, hinting at an evolutionary conserved capacity for (sulfated) iodothyronine transport. This root function is further substantiated by the fact that similar to the zebrafish orthologues, both mouse and human SLC22 had the broadest range of substrates. Gene duplication events resulted in clusters of *SLC22* genes some of which specific for either mouse (*mSlc22a19* and *mSlc22a26-30*) or human (*SLC22A9*, *SLC22A11* and *SLC22A24*). Most of the transporters encoded by these genes retained the capacity for (sulfated) iodothyronine transport, although with some changes in substrate preference. A notable exception is the cluster formed by *SLC22A11* and *SLC22A12* in the human genome. We found that the human-specific SLC22A11 is a potent T4 transporter, whereas neither hSLC22A12 or mSLC22A12 transported any of the substrates tested.

The physiological function of the newly identified TH transporters for TH homeostasis is as yet unclear. As SLC22 proteins transport a broad range of substrates and have a redundant expression in multiple tissues (Table 1), it is uncertain whether all contribute to TH homeostasis *in vivo*, particularly those transporters that only showed modest uptake in our *in vitro* assays. Of interest, many SLC22 proteins are expressed in the liver and kidney (Table 1). Both tissues are important for TH clearance via TH sulfation by sulfotransferases

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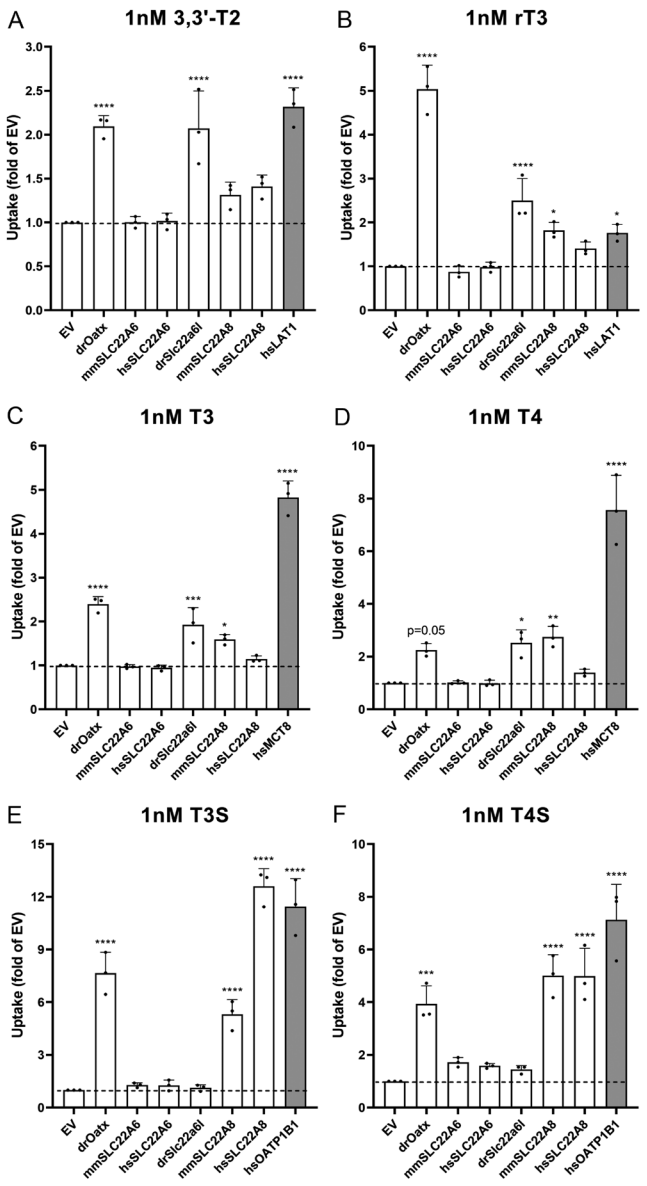

Figure 5

Uptake of 1 nM 3,3′-T2, rT3, T3, T4, T3S and T4S in COS1 cells transfected with zebrafish (dr), mouse (mm) and human (hs) SLC22A6 and 22A8 orthologues in combination with h*CRYM*. COS1 cells were incubated with the substrates for 30 min (10 min for 3,3′-T2 and rT3) in DPBS/0.1% glucose/0.1% BSA. The uptake of SLC22s transfected cells is expressed as fold induction of that in the cells transfected with EV. Human LAT1 (for 3,3′-T2 and rT3), MCT8 (for T3 and T4) and OATP1B1(for T3S and T4S) were used as positive controls (gray bars). Data are represented as mean ± S.D. ($n = 3$). One-way ANOVA plus Dunnett's *post hoc* test (compared to the EV group) was used for statistical analysis. *$0.01 < P < 0.05$; **$0.001 < P \leq 0.01$; ***$0.0001 < P \leq 0.001$; ****$P \leq 0.0001$.

and subsequent deiodination by DIO1. The inner ring deiodinating activity of DIO1 is markedly increased when the substrates are sulfated (21). The balance of influx and efflux of sulfated TH by transporter proteins is therefore an important determinant of the rate of TH clearance and thus circulating TH concentrations. We found several SLC22 members with robust uptake of sulfated iodothyronines, comparable to (hSLC22A8 for T3S and mSLC22A29 for T4S) or even exceeding (mSLC22A27 for T4S) the uptake by hOATP1B1, which is an efficient hepatic transporter for sulfated iodothyronines (20). Human SLC22A8 and SLC22A9 are strongly expressed in human kidney and liver, respectively, whereas the mouse mSlc22A27 and mSlc22A29 are expressed in both tissues (Table 1) (https://www.proteinatlas.org/) (34). We speculate that these transporters may play a role in renal and hepatic clearance of TH via the transport of sulfated iodothyronines. A recent GWAS study showed that the SNPs rs12282281 in the *SLC22A9* gene and rs11822642 in the intergenic region between *SLC22A9* and *HRASLS5* are associated with total serum T4 concentrations and the T3/T4 ratio respectively in a Croatian cohort (35). However, we found no association of *SLC22A9* or any other selected *SLC22* gene with either FT4 or free or total T3/T4 ratios in the look-up GWAS analysis in the ThyroidOmics Consortium which has a much larger sample size. Of course, our finding does not rule out the population-specific relevance of SLC22A9 and TH levels in the Croatian population. On the other hand, no association of a TH transporter with circulating TH levels does not necessarily mean it has no physiological relevance. For example, common genetic variation in *MCT8*, mutations which cause neurocognitive phenotypes, has no association with thyroid stimulating hormone (TSH) or FT4 levels (14).

A second process for which TH transport by SLC22 proteins is potentially relevant is the trans-placental transport of TH. Maternal-to-fetal transfer of TH is important for fetal development, particularly during the first trimester when the fetal thyroid gland has not developed yet (36). Although several transporters have been identified, they do not account for all transport, particularly T4 (27). SLC22A11 is strongly expressed in placenta, with its protein present at the basolateral membrane of the syncytiotrophoblasts (37) in the third trimester and its RNA in both the cytotrophoblasts and the syncytiotrophoblasts in the first trimester of pregnancy (38). With its expression in the placenta being higher than MCT8, MCT10, LAT2 and OATPs (39), it may play a role in trans-placental T4 transport to or from the fetal circulation, which is faced by the basolateral surface of the placental syncytium, is a likely possibility. Interestingly, SLC22A11 is specific for humans and to our knowledge no functional homologues from the SLC22 family are expressed in the murine placenta, indicating that putative

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placental transport of T4 by an SLC22 transporter may be human specific.

Apart from placenta, SLC22A11 is also highly expressed in the kidney. Both SLC22A11 and SLC22A12 are located on the apical membrane of the renal proximal tubule cells, facilitating the resorption of urate (40, 41, 7). Reduced urate secretion from the kidney can cause gout, a chronic disease of monosodium urate crystal deposition in soft tissues due to hyperuricaemia (42). Lesinurad and probenecid are registered drugs against gout that act by blocking urate transport by SLC22A11 and SLC22A12 (43). We found that lesinurad and probenecid also inhibited T4 transport by SLC22A11 with IC50 values in a comparable range as reported for urate (40, 43). In fact, lesinurad and probenecid inhibited (sulfated) iodothyronines by most SLC22 transporters, in particular, hSLC22A8 when tested for T3S transport. Considering the potential role of SLC22 transporters in hepatic and renal TH uptake and metabolism, these drugs could potentially interfere with TH homeostasis in these tissues.

We acknowledge some limitations in our study. First, the transport assays were performed in the presence of CRYM, an intracellular (sulfated) iodothyronine binding protein to minimize the efflux of substrates. This allows us to measure the full extent of substrate uptake, but not efflux capacity. Second, there were differences in the amount of cell surface expression for the different SLC22 proteins. For example, the abundance of mSLC22A22 and hSLC22A25 was particularly low compared to the other transporters and we therefore may have missed the capacity of these proteins to transport (sulfated) iodothyronines. In addition, the differences in expression warrant caution when comparing the capacity to transport (sulfated) iodothyronines between transporters.

In summary, we identified an evolutionary conserved function for several members within the OAT clade of the SLC22 family as (sulfated) iodothyronine transporters. These findings have substantially expanded the repertoire of TH transporters and may contribute to understanding the complexity of TH availability and homeostasis *in vivo*.

Supplementary materials

This is linked to the online version of the paper at https://doi.org/10.1530/ETJ-23-0023.

Declaration of interest

The authors declare that they have no conflicts of interest with the contents of this article.

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Author contribution statement

Z.C., M.E.M, W.E.V and R.P.P designed the study. Z.C., W.F., L.J.d.R. and S.Y performed the experiments and processed the experimental data. Z.C. analyzed the data. A.T. and R.B.T.M.S. performed genetic analyses. Z.C., M.E.M, W.E.V, A.T., R.B.T.M.S. and R.P.P wrote the manuscript. All authors critically reviewed, revised and approved the manuscript.

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