REVIEW

Molecular aspects of thyroid hormone transporters, including MCT8, MCT10, and OATPs, and the effects of genetic variation in these transporters

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Abstract

Thyroid hormone is a pleiotropic hormone with widespread biological actions. For instance, adequate levels of thyroid hormone are critical for the development of different tissues such as the central nervous system, but are also essential for the regulation of metabolic processes throughout life. The biological activity of thyroid hormone depends not only on serum thyroid hormone levels, but is also regulated at the tissue level by the expression and activity of deiodinases, which activate thyroid hormone or mediate its degradation. In addition, thyroid hormone transporters are necessary for the uptake of thyroid hormone into target tissues. With the discovery of monocarboxylate transporter 8 (MCT8) as a specific thyroid hormone transporter and the finding that mutations in this transporter lead to a syndrome of severe psychomotor retardation and elevated serum 3,3',5-tri-iodothyronine levels known as the Allan–Herndon–Dudley syndrome, the interest in this area of research has greatly increased. In this review, we will focus on the molecular aspects of thyroid hormone transporters, including MCT8, MCT10, organic anion transporting polypeptides, and the effects of genetic variation in these transporters.

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Introduction

Thyroid hormone is a pleiotropic hormone with widespread biological actions. The follicular cells of the thyroid gland produce predominantly thyroxine (T_4), but it is mainly 3,3',5-tri-iodothyronine (T_3) that binds to the nuclear thyroid hormone receptor (Yen 2001). The biological activity of T_3 is therefore largely determined by the intracellular T_3 concentration which is dependent on a) the circulating T_3 concentration; b) the transport of thyroid hormone across the cell membrane; and c) the presence of iodothyronine deiodinases, which activate or inactivate thyroid hormone. To date, three deiodinases have been characterized as homologous selenoproteins (Bianco *et al.* 2002). Both D1 and D2 converts T_4 to T_3 , whereas D3 catalyzes the degradation of T_4 to reverse T_3 (rT_3) and of T_3 to 3,3'- T_2 .

The deiodinases are membrane proteins with their active sites located in the cytoplasm (Bianco *et al.* 2002). Therefore, transport across the cell membrane is essential for thyroid hormone action and metabolism. Based on the lipophilic structure of thyroid hormones, it is long thought that thyroid hormone

enters the cell through passive diffusion. However, it has become increasingly clear that there are specific thyroid hormone transporters, and that the activity of these transporters in part determines the intracellular thyroid hormone concentration (Jansen *et al.* 2005).

To date, several transporters with high affinity for thyroid hormone, but with different tissue distributions and ligand affinities have been identified. This review will focus on the molecular aspects of the monocarboxylate transporter 8 (MCT8) and MCT10, and several members of the organic anion transporting polypeptide (OATP) family. Other transporters, such as the Na^+ -taurocholate co-transporting polypeptide, the L-type amino acid transporters (LAT1 and LAT2), and fatty acid translocase (CD36) will not be discussed as little is known about the effects of genetic variation in these transporters on cellular thyroid hormone transport. A review including these transporters has been published recently (van der Deure *et al.* 2007).

Besides molecular aspects, this review will focus on the effect of genetic variation in these transporters on cellular thyroid hormone transport. Polymorphisms are variations in the nucleotide sequence of the human genome that occurs in at least 1% of the

general population. These polymorphisms determine differences between individuals, such as eye or hair color, but they also play a role in the variation of serum thyroid hormone levels that exists between individuals. Moreover, recent papers have indicated that polymorphism studies provide new insights into the role and activity of different proteins involved in thyroid hormone synthesis, metabolism, and transport (Peeters *et al.* 2006).

MCT8 and MCT10

Monocarboxylates, such as lactate, pyruvate, and ketone bodies, play an important role in energy metabolism in different tissues, in particular brain (Nehlig & Pereira de Vasconcelos 1993). The transport of these substances across the cell membrane is carried out by the proton-linked MCT1–4 (Halestrap & Meredith 2004). They belong to a larger family, consisting of 14 homologous proteins, but the function of most other members remains to be elucidated (Friesema *et al.* 2003, 2008). However, MCT10 has been characterized by Kim *et al.* (2002) as a T-type amino acid transporter, facilitating the cellular uptake and efflux of aromatic amino acids. Although this was not immediately clear, we have later shown that MCT10 is an active iodothyronine transporter (Friesema *et al.* 2008). This is reminiscent of the work of the group of Zhou *et al.* (1990, 1992), showing that thyroid hormone uptake into erythrocytes and other cell types is mediated by a T-type amino acid transporter.

The human *MCT10* gene is located on chromosome 6q21–q22 and consists of six exons and five introns, of which the first intron is particularly large, i.e. ~100 kb. This gene structure is identical to that of the human *MCT8* gene, which was already identified in 1994 by Lafrenière *et al.* who showed that it is located on chromosome Xq13.2. On the basis of the presence of 12 putative transmembrane domains (TMDs), they hypothesized that the predicted protein represents a transporter (Lafreniere *et al.* 1994). The MCT8 and MCT10 proteins are highly homologous (Fig. 1), with an amino acid identity of 49% (Friesema *et al.* 2008). This homology is highest in the TMDs and lowest in the N- and C-terminal domains that are both located intracellularly.

In humans, *MCT8* may be expressed as a long 613-amino acid protein or a short 539-amino acid protein, depending on which of the two translation start sites (TLSs) is used. The *MCT8* gene in most of the other species including rats and mice, only have the downstream TLS coding for the short MCT8 protein. The possible function of the N-terminal extension in the long human MCT8 protein as well as the possible differential regulation of the expression of the long versus short MCT8 proteins remains to be elucidated. In all species, MCT10 has only one TLS, giving rise to the production of the 'short' protein.

Friesema *et al.* (2003) demonstrated that rat and human MCT8 are active and specific iodothyronine transporters. Despite its high homology with MCT10

Figure 1 Comparison of the protein structures of human MCT8 and MCT10. The yellow part of the MCT8 structure denotes the N-terminal domain that is present in the long MCT8 protein generated by use of the upstream translation start site. Indicated in green are the identical amino acid residues that occupy corresponding positions in MCT8 and MCT10. The blue lines represent the plasma membrane. The top of the figure is extracellular and the bottom is intracellular.

and MCT8 it does not transport (aromatic) amino acids. MCT8 also does not transport sulfonated iodothyronines or monocarboxylates such as lactate. The mechanisms by which MCT8 and MCT10 facilitate thyroid hormone uptake are unknown, but it has been demonstrated that iodothyronine transport is Na-independent. In view of the proton coupling of monocarboxylate transport by MCT1–4, the possible pH dependence of iodothyronine uptake by MCT8 and MCT10 needs to be investigated.

In transfected cells, both MCT8 and MCT10 increase the intracellular availability of iodothyronines, as evidenced by the marked increase in their intracellular deiodination by co-transfected deiodinases. However, both MCT8 and MCT10 facilitate not only the cellular uptake but also the efflux of iodothyronines. Since MCT10 appears more important for the export than for the import of aromatic amino acids (Ramadan *et al.* 2006, 2007), it is quite possible that cellular uptake of thyroid hormone is driven by the cellular efflux of aromatic amino acids through the same transporter. The trans-stimulation of T₃ and T₄ uptake by intracellular Trp has indeed been described in the studies of Francon and Blondeau (Zhou *et al.* 1990, 1992).

MCT8 is expressed in many tissues, including liver, kidney, heart, skeletal muscle, brain, and, strangely enough, thyroid. Mutations in the *MCT8* gene cause a syndrome of severe psychomotor retardation and high serum T₃ levels in affected male patients, known as the Allan–Herndon–Dudley syndrome. The neurological deficits are probably explained by an impeded uptake of T₃ in MCT8-expressing central neurons and, hence, an impaired brain development. This has been reviewed in detail elsewhere (Dumitrescu *et al.* 2004, Friesema *et al.* 2004). Since mutations in the *MCT8* gene have such profound effects, the question arises whether small changes in the *MCT8* gene may affect transport activity as well.

Only two studies exist on the relationship between MCT8 polymorphic variants and serum thyroid hormone levels (van der Deure *et al.* 2007, Lago-Leston *et al.* 2008; Table 1). Lago-Leston *et al.* (2008) studied

the serine-to-proline change at position 107 (Ser107Pro; rs6647476), which is the only established nonsynonymous polymorphism in MCT8. In their study, 276 healthy Spanish men were genotyped for this polymorphism. They found no association with serum thyroid hormone levels or with mRNA levels coding for MCT8 or thyroid hormone-responsive genes in white blood cells or in T₃-stimulated fibroblasts.

We also genotyped this polymorphism in a population of 156 healthy men and women, and found no association between this variant and serum thyroid parameters either. Hemizygous carriers of a different polymorphism, i.e. rs5937843, located in intron 5 of the *MCT8* gene, had lower free T₄ (FT₄) levels compared with wild-type male subjects. However, we failed to replicate these findings in the homozygous female carriers in the same population (van der Deure *et al.* 2007).

To date, only one study has been published regarding the possible association of genetic variation in the *MCT10* gene with serum thyroid parameters. We showed that a common polymorphism (rs14399) in the 3'-UTR region of the *MCT10* gene is not associated with serum thyroid hormone levels (van der Deure *et al.* 2007). The only established nonsynonymous polymorphism identified in human MCT10 is a lysine to glutamine change at position 508 (Lys508Gln; rs17072442), with a minor allele frequency of ~2%. Considering the type of amino acid change, it would be interesting to investigate the association of this polymorphism with serum thyroid parameters and other thyroid-related endpoints.

So far, patients with mutations in MCT10 have not been identified. Considering the wide tissue distribution of MCT10 expression and its swift T₃ transport, it is quite likely that MCT10 mutations are associated with significant alterations in tissue and/or serum thyroid hormone concentration. As is the case with MCT8, mutations in MCT10 may well result in a significant impairment of tissue T₃ uptake and, thus, in manifestations of thyroid hormone resistance. At present, it is unclear, however, if MCT10 is significantly expressed in the pituitary and/or

Table 1 Effect of common variation in monocarboxylate transporter-8 (MCT8) and MCT10

	rs Number polymorphism	Location	Change	Effect on serum thyroid hormone levels	In vitro effect	References
Gene						
MCT8	rs6647476	Exon	Ser107Pro	No effect observed	No effect observed	van der Deure <i>et al.</i> (2007) and Lago-Leston <i>et al.</i> (2008)
	rs5937843	Intron	G > T	Not consistent	Not determined	van der Deure <i>et al.</i> (2007)
MCT10	rs14399	3'-UTR	C > A	No effect observed	Not determined	van der Deure <i>et al.</i> (2007)

hypothalamus. Therefore, it remains to be seen if mutations in MCT10 result in a defect in the negative feedback control of TSH secretion like that seen in patients with thyroid hormone resistance because of mutations in the TR β receptor.

To predict the phenotype of patients with MCT10 mutations, it would be highly interesting to study MCT10 knockout mice, although it should be realized that MCT8 knockout mice do not show any neurological abnormality in sharp contrast with the clinical condition of patients with MCT8 mutations (Dumitrescu *et al.* 2006, Trajkovic *et al.* 2007, Heuer & Visser 2009). However, MCT8 knockout mice replicate the endocrinological changes found in patients with the Allan–Herndon–Dudley syndrome: a marked increase in serum T₃ and a decrease in serum T₄ and rT₃ (Dumitrescu *et al.* 2006, Trajkovic *et al.* 2007). The lack of any overt neurological abnormality may be explained by differences in expression patterns and specificities of thyroid hormone transporters between humans and rodents.

In the mouse brain, MCT8 is expressed not only in neurons but also in capillaries and the choroid plexus (Heuer *et al.* 2005, Roberts *et al.* 2008). The importance of MCT8 in the blood–brain barrier and in the blood–cerebral spinal fluid (CSF) barrier is strongly suggested by the almost complete block in brain T₃ uptake in MCT8 knockout mice (Trajkovic *et al.* 2007). The lack of a neurological phenotype in these animals is explained by a compensatory increase in local T₄ to T₃ conversion by D2 expressed in astrocytes and the expression of alternative T₃ transporters in neurons in rodents. Brain T₄ uptake is not affected in MCT8 knockout mice, which is explained by the expression of the T₄ transporter OATP1C1 in brain capillaries. Recently, Roberts *et al.* (2008) have shown that OATP1C1 is strongly expressed in mouse and rat cerebral microvessels, but not in human microvessels. This high expression of OATP1C1 in microvessels in rodents compared with human brain may contribute to the relatively mild phenotype observed in MCT8-null mice, in contrast to humans lacking functional MCT8.

OATPs

The OATPs are a large family of transporters responsible for Na⁺-independent transmembrane transport of amphipathic organic compounds, including bile salts, bromosulphophthalein (BSP), steroid hormones, and numerous drugs (Konig *et al.* 2006). So far, ~40 OATPs have been identified in humans, rats, and mice. All OATPs are large proteins of 652–848 amino acids in length with 12 TMDs. Most OATP proteins are expressed in multiple tissues, including liver, kidney, brain (blood–brain barrier, choroid plexus), lung, heart,

placenta, testis, eye, and small intestine (Hagenbuch & Meier 2003). However, some members show a tissue-specific distribution; OATP1B1 and OATP1B3 are exclusively expressed in liver (Hsiang *et al.* 1999, König *et al.* 2000), whereas OATP1C1 is only present in the brain and in the Leydig cells of the testis (Pizzagalli *et al.* 2002). Furthermore, most OATP family members are expressed at the basolateral membrane of polarized cells (Hsiang *et al.* 1999, König *et al.* 2000, Lee *et al.* 2005).

OATPs are generally involved in transport of both endo- and xenobiotics. Among the many ligands transported by OATPs, several members of this large family also facilitate uptake of thyroid hormone. These include members of the OATP1 subfamily: OATP1A2 (Fujiwara *et al.* 2001, Kullak-Ublick *et al.* 2001), OATP1B1 (Abe *et al.* 1999, Kullak-Ublick *et al.* 2001), OATP1B3 (Kullak-Ublick *et al.* 2001), and OATP1C1 (Pizzagalli *et al.* 2002); members of the OATP4 subfamily: OATP4A1 (Fujiwara *et al.* 2001) and OATP4C1 (Mikkaichi *et al.* 2004); and a member of the OATP6 subfamily: OATP6C1 (Suzuki *et al.* 2003). Interestingly, all human members of the OATP1 subfamily that transport thyroid hormone form a gene cluster together with a related pseudogene on chromosome 12p. In addition, they share on average almost 50% amino acid identity. The focus in this review will be on these four human members of the OATP1 subfamily, i.e. OATP1A2, OATP1B1, OATP1B3, and OATP1C1.

OATP1A2

OATP1A2, first cloned and characterized in 1995 as a transporter for bile salts and BSP in human liver by Kullak-Ublick *et al.* (1995), has been shown to transport T₃ and T₄ with Km values of 7 and 8 μ M respectively (Fujiwara *et al.* 2001). In addition, we recently demonstrated that OATP1A2 facilitates not only transport of T₄, T₃, and rT₃ but also of their sulfates T₄S, T₃S, and rT₃S in transfected cells (unpublished data). OATP1A2 is expressed in multiple tissues, among which are liver, brain, and kidney (Kullak-Ublick *et al.* 1995, Gao *et al.* 2000). Based on its expression pattern, OATP1A2 could play a role in the delivery of thyroid hormone across the blood–brain barrier.

The high apparent Km values of OATP1A2-mediated T₄ and T₃ transport may not preclude a physiological role of OATP1A2 as a thyroid hormone transporter; they are in the same range as those determined for T₄ and T₃ transport by the physiologically important transporter MCT8. However, the low specificity of OATP1A2 suggests that thyroid hormone bioavailability for instance in the brain is not importantly regulated by this transporter. It may serve as a back-up transport system in case other transporters malfunction or are saturated, or when thyroid hormone concentrations are higher than normal. Alternatively, OATP1A2 could play

a role in the clearance of thyroid hormone and its metabolites, for instance through elimination via bile or urine. Since iodothyronine sulfates, such as T₄S, T₃S, and rT₃S, are regarded as waste products of thyroid hormone metabolism (Wu *et al.* 1993), this hypothesis seems to be plausible.

Like many OATPs, OATP1A2 plays a role in drug transport (Konig *et al.* 2006). Therefore, several papers have been published on the effect of OATP1A2 polymorphisms on drug pharmacokinetics. Badagnani *et al.* (2006) showed that genetic variation in the *OATP1A2* gene might contribute to variation in methotrexate disposition and response. Two common (Ile13Thr and Glu172Asp) and two rare (Arg168Cys and Asn278X) protein-altering variants of OATP1A2 showed altered transport function. In a different study, Glu172Asp and Asn135Ile variants showed markedly reduced transport of the OATP1A2 substrates estrone sulfate (E1S) and deltorphin II (Lee *et al.* 2005). Other variants (Ala187Thr and Thr668Ser) appeared to have substrate-dependent changes in transport activity (Lee *et al.* 2005).

Since OATP1A2 transports thyroid hormone, we tested whether polymorphisms in the *OATP1A2* gene have an effect on thyroid hormone transport *in vitro*. In addition, the variants were analyzed for association with serum thyroid parameters in two populations, i.e. a population of Caucasian blood donors (Peeters *et al.* 2003) and a large population of elderly Caucasian men and women of the Rotterdam Scan Study (Breteler 2000). We only studied the Ile13Thr and

Glu172Asp polymorphisms, as other polymorphisms have an allele frequency <1% in Caucasians (Lee *et al.* 2005, Badagnani *et al.* 2006). The OATP1A2-Ile13Thr polymorphism was associated with higher T₃ levels (Table 2). The OATP1A2-Glu172Asp was not consistently associated with serum thyroid hormone levels (Table 2).

Except for transport of T₄S, no differences in thyroid hormone transport were observed between OATP1A2-Ile13Thr and wild-type OATP1A2 *in vitro*. However, cells transfected with the OATP1A2-Glu172Asp variant showed decreased transport compared with cells transfected with wild-type OATP1A2 (Fig. 2). Metabolism of (sulfated) iodothyronines co-transfected deiodinases was decreased in a similar manner in cells transfected with OATP1A2-Glu172Asp compared with cells transfected with wild-type OATP1A2 (data not shown). Our *in vitro* data are in line with previous data from Badagnani *et al.* (2006). Lee *et al.* (2005) however, also showed decreased transport by the OATP1A2-Ile13Thr variant. The discrepancy between their and our findings might be explained by substrate-dependent changes in transport activity.

Although the OATP1A2-Glu172Asp showed decreased transport activity *in vitro*, this variant was not associated with serum thyroid parameters in two populations of Caucasians. Therefore, OATP1A2 may not play an important role in thyroid hormone transport in a physiological situation. Alternatively, the polymorphism may affect tissue thyroid hormone concentrations independent of serum levels.

Table 2 Serum thyroid hormone levels by OATP1A2 genotypes in a population of Caucasian blood donors and in Caucasian men and women of the Rotterdam Scan Study

	Blood donors			Rotterdam Scan Study		
	OATP1A2-Ile13Thr			OATP1A2-Ile13Thr		
	Wild-type (116)	Carriers (33+1)	P			
TSH ^a	1.31±0.07 ^b	1.40±0.13	0.26	ND		ND
FT ₄	15.2±0.2	14.5±0.4	0.14	ND		ND
T ₃	1.94±0.02	2.06±0.04	0.007	ND		ND
rT ₃	0.32±0.01	0.30±0.01	0.15	ND		ND
T ₄ S ^a	17.53±0.55 ^c	17.55±1.03	0.82	ND		ND
	OATP1A2-Glu172Asp			OATP1A2-Glu172Asp		
	Wild-type (136)	Carriers (14)	P	Wild-type (839)	Carriers (100+6)	P
TSH ^a	1.34±0.07 ^d	1.24±0.20	0.90	1.41±0.04	1.35±0.10	0.16
FT ₄	15.1±0.2	15.0±0.7	0.84	17.7±0.1	17.7±0.3	0.96
T ₃	1.97±0.02	1.91±0.06	0.39	2.01±0.01	1.99±0.02	0.52
rT ₃	0.31±0.01	0.36±0.02	0.02	0.34±0.01	0.35±0.01	0.21
T ₄ S ^a	17.50±0.51 ^e	17.22±1.61	0.95	ND	ND	

Data are corrected for age and gender and shown as mean ± s.e.m. ND, not determined.

^aDue to a nonnormal distribution, TSH and T₄S were logarithmically transformed in the analysis.

^bN=115.

^cN=117.

^dN=135.

^eN=137.

Figure 2 [^{125}I]T₃, [^{125}I]T₄, [^{125}I]T₃S, and [^{125}I]T₄S uptake by COS1 cells transfected with empty vector, wild-type OATP1A2, OATP1A2-Ile13Thr, or OATP1A2-Glu172Asp. Cells were co-transfected with μ -crystallin (Vie *et al.* 1997). Cells were incubated for 30 min at 37 °C with 1 nM [^{125}I]T₃, T₄, T₃S, or T₄S. Data are expressed as percentage uptake of added radioactivity. Results are the means $\pm$ S.E.M. of three experiments. * $P < 0.05$ for OATP1A2-Ile13Thr or OATP1A2-Glu172Asp versus wild-type OATP1A2.

OATP1B1 and OATP1B3

OATP1B1 and OATP1B3 are exclusively expressed in liver and share ~80% amino acid identity with each other (Abe *et al.* 1999, 2001). Both transporters have broad substrate specificities as they transport numerous compounds such as bilirubin, bile salts, drugs such as pravastatin and digoxin, but also steroid hormone conjugates (Hagenbuch & Meier 2003). Many studies have been published on the role of these two proteins in drug absorption, distribution, and excretion (Konig *et al.* 2006, Niemi 2007). They are involved in the cellular uptake of drugs into the liver which, besides the intestine and the kidneys, is an important tissue determining the pharmacokinetics of drugs.

Recent studies have shown that OATP1B1 markedly stimulates uptake of the iodothyronine sulfates T₄S, T₃S, and rT₃S but has little activity towards nonsulfated T₄, T₃, and rT₃ (van der Deure *et al.* 2008b). Like OATP1B1, OATP1B3 preferentially transports the sulfated iodothyronines as well as rT₃ (unpublished data, WM van der Deure, ECH Friesema, RP Peeters & TJ Vissers). Under normal conditions, D1 in liver rapidly degrades rT₃ and sulfated iodothyronines, keeping the serum concentrations of these metabolites low (Chopra *et al.* 1992, 1993, Wu *et al.* 1992, 1993, 2005,

Figure 3 [^{125}I]T₄S and [^3H]E1S uptake by COS1 cells transfected with empty vector, OATP1B1-Val¹⁷⁴, or OATP1B1-Ala¹⁷⁴. Cells were incubated at 37 °C with 1 nM [^{125}I]T₄S or 100 nM [^3H]E1S for different time periods. Data are expressed as percentage uptake of added radioactivity. Results are the means $\pm$ S.E.M. of three experiments. (a) $P < 0.05$ for OATP1B1-Val¹⁷⁴ versus empty vector and (b) $P < 0.05$ for OATP1B1-Ala¹⁷⁴ versus empty vector.

Bianco *et al.* 2002). However, serum iodothyronine sulfates and rT₃ levels are high in preterm infants and during critical illness, possibly due to decreased degradation (Chopra *et al.* 1992, 1993, Peeters *et al.* 2005). It is therefore of interest that both OATP1B1 and OATP1B3 only facilitate transport of the iodothyronine sulfates and rT₃. This suggests that OATP1B1 and OATP1B3 play an important role in hepatic transport and metabolism of iodothyronine sulfates, reflecting their role in the transport of endo- and xenobiotics that are metabolized in the liver and excreted in the bile.

Polymorphisms in the OATP1B1 and OATP1B3 genes have been extensively studied as they impact on the interindividual variability of drug disposition and drug response (Smith *et al.* 2005). To date, only one study has

focused on associations between a polymorphism in the *OATP1B1* gene, OATP1B1-Val174Ala, and serum thyroid hormone levels. Niemi *et al.* (2005) have previously shown that the OATP1B1-Val174Ala polymorphism leads to decreased function of OATP1B1 and thereby increases the systemic bioavailability of lipid-lowering drugs.

As we showed that OATP1B1 preferentially transports sulfated hormones, i.e. T₄S, T₃S, rT₃S, and E1S (van der Deure *et al.* 2008b), we expected that the OATP1B1-Val174Ala polymorphism would be associated with serum levels of iodothyronine sulfates and E1S. Indeed, this polymorphism was associated with higher serum T₄S levels in 155 blood donors, while in a larger cohort of elderly Caucasians this same polymorphism was associated with 40% higher serum E1S levels. Unfortunately, we were not able to replicate these findings as no serum was left to determine E1S and T₄S levels in the healthy blood donors and elderly Caucasians respectively. However, *in vitro* OATP1B1-Ala¹⁷⁴ showed a 40% lower induction of transport and metabolism of these substrates than OATP1B1-Val¹⁷⁴ in transfected COS1 cells (van der Deure *et al.* 2008b; Fig. 3). Decreased hepatic uptake of T₄S and E1S by OATP1B1-Ala¹⁷⁴ compared with OATP-Val¹⁷⁴ *in vivo* thus gives rise to higher serum T₄S and E1S levels.

Concerning the association of the OATP1B1-Val174Ala polymorphism and E1S levels, it would be interesting to study OATP1B1 as a risk factor in the development of breast cancer. In contrast to the role of sulfation in thyroid hormone metabolism, the sulfation of estrogens is readily reversible by estrogen sulfatase (Reed *et al.* 2005). E1S is thought to serve as a reservoir for active estrogens, for instance in breast tissue, since serum concentrations of E1S are 10–20 times higher than those of the unconjugated estrogens (Ruder *et al.* 1972).

Estrogens play an important role in the initiation and growth of breast cancer. Therefore, therapies aim at blocking their interaction with the estrogen receptor by use of an anti-estrogen, or by inhibiting the conversion of androstenedione to estrone with an aromatase inhibitor (Cole *et al.* 1971, Smith & Dowsett 2003). Local formation of estrogens in breast tumors might be more important than circulating estrogens for growth and survival of estrogen-dependent breast cancer in postmenopausal women (Nakata *et al.* 2003). Serum E1S is a major source of estrogens for breast tissue through local sulfatase activity (Gamage *et al.* 2006). The role of OATP1B1 in the risk of developing breast cancer should therefore be investigated, since carriers of the OATP1B1-Val174Ala polymorphism have life-long higher serum E1S levels (van der Deure *et al.* 2008b).

To date, no studies have been published on associations between genetic variation in the *OATP1B3* gene and serum thyroid hormone levels. A recent paper

by Smith *et al.* demonstrated that genetic variation in OATP1B3 played a limited role in the disposition of the anti-cancer agent paclitaxel. They studied three non-synonymous polymorphisms, i.e. Ser112Ala, Met233Ile, and Gly522Cys. We genotyped the Ser112Ala and Met233Ile polymorphisms in a population of 156 healthy men and women. These polymorphisms are in complete linkage disequilibrium with each other (www.ncbi.nlm.nih.gov) and show no association with serum thyroid parameters (Table 3).

OATP1C1

Virtually all OATPs transport numerous substrates. A notable exception to this multi-specific transport capacity is OATP1C1. Pizzagalli *et al.* (2002) demonstrated that it shows a high preference for T₄ and rT₃. We recently extended these findings by showing that T₄S uptake is also facilitated by OATP1C1 although less effectively than T₄ (van der Deure *et al.* 2008c). Together, with the almost exclusive expression at the blood–brain barrier, this suggests that OATP1C1 is critical for T₄ uptake into the brain. This important role is substantiated by Sugiyama *et al.* (2003) who showed that expression levels of OATP1C1 in isolated rat brain capillaries are regulated by thyroid hormone concentrations. Oatp1c1 is up-regulated in hypothyroid rats and down-regulated in hyperthyroid rats (Sugiyama *et al.* 2003). Together with changes in D2 expression (Forrest *et al.* 2002), OATP1C1 counteracts the effect of alterations in serum T₄ to ensure stable thyroid hormone concentrations in the brain.

Table 3 Serum thyroid hormone levels by OATP1B3 genotypes in a population of Caucasian blood donors

	Blood donors		
	Wild-type (102)	Carriers (45 + 6)	P
OATP1B3-Ser112Ala			
TSH ^a	1.28 ± 0.08	1.38 ± 0.11 ^b	0.13
FT ₄	14.9 ± 0.2	15.5 ± 0.3	0.13
T ₃	1.97 ± 0.02	1.96 ± 0.03	0.80
rT ₃	0.31 ± 0.01	0.32 ± 0.01	0.20
T ₄ S ^a	17.61 ± 0.59 ^c	17.54 ± 0.84	0.82
OATP1B3-Met233Ile			
TSH ^a	1.28 ± 0.08	1.38 ± 0.11 ^b	0.13
FT ₄	14.9 ± 0.2	15.5 ± 0.3	0.13
T ₃	1.97 ± 0.02	1.96 ± 0.03	0.80
rT ₃	0.31 ± 0.01	0.32 ± 0.01	0.20
T ₄ S ^a	17.61 ± 0.59 ^c	17.54 ± 0.84	0.82

Data are corrected for age and gender and shown as mean ± s.e.m.

^aOwing to a non-normal distribution, TSH and T₄S were logarithmically transformed in the analysis.

^bN = 50.

^cN = 103.

Table 4 Effect of common variation in OATP1A2, OATP1B1, OATP1B3 and OATP1C1

	Polymorphism	Location	Change	Effect on serum thyroid hormone levels	In vitro effect	References
Gene						
OATP1A2	rs57921276	Exon	Ile13Thr	No effect observed	No effect observed	Table 2 and Fig. 2
	rs57550534	Exon	Glu172Asp	No effect observed	Decreased transport capacity	
OATP1B1	rs4149056		Val174Ala	Higher bilirubin, T ₄ S, E1S levels, lower T ₃ /rT ₃ ratio	Decreased transport capacity	van der Deure <i>et al.</i> (2008b)
OATP1B3	rs4149117	Exon	Ser112Ala	No effect observed	Not determined	Table 3
OATP1C1	rs7311358	Exon	Met233Ile	No effect observed	Not determined	
	rs10770704	Intron	C/T	No effect observed	Not determined	van der Deure <i>et al.</i> (2008c)
	rs36010656	Exon	Pro143Thr	Higher rT ₃ levels, however, effect not consistent	No effect observed	
	rs10444412	3'-UTR	C3035T	Higher FT ₄ and rT ₃ levels, however, effect not consistent	No effect observed	

Recently, OATP1C1 homologs have been identified in chicken and mice (Tohyama *et al.* 2004, Nakao *et al.* 2006). Similar to its human ortholog, mouse Oatp1c1 transports T₄ and rT₃ with high affinity and is widely expressed in brain, suggesting an important role in T₄ transport across the blood–brain barrier (Tohyama *et al.* 2004). Nakao *et al.* demonstrated that also in chicken Oatp1c1 transports T₄.

Whether findings regarding the role of OATP1C1 in mice, rats, and chicken can be extrapolated to humans, remains to be investigated. For instance, in a recent report Roberts *et al.* (2008) showed the expression of OATP1C1 is higher in rodents compared with human brain. However, due to the high level of homology in structure and substrate specificity between rodent and human OATP1C1, it seems plausible that OATP1C1 is also important for T₄ uptake in the human brain. This is supported by our data showing that polymorphisms in the OATP1C1 gene are associated with fatigue and depression in a cohort of hypothyroid patients (van der Deure *et al.* 2008a). Considering the presumed function of T₄ transport across the blood–brain barrier, mutations in OATP1C1 are expected to have a significant impact on brain development and function. Loss of OATP1C1 function may well lead to neuronal deficits similar to those seen in subjects with untreated congenital hypothyroidism or in patients with MCT8 mutations (Sugiyama *et al.* 2003). It seems worthwhile to study this in OATP1C1 knockout mice.

OATP1C1 is capable of T₄, T₄S, and rT₃ transport, but polymorphisms in the OATP1C1 gene are not consistently associated with serum thyroid hormone levels (van der Deure *et al.* 2008c). Although, the OATP1C1-Pro143Thr and OATP1C1-C3035T polymorphisms

were associated with serum thyroid parameters in 156 blood donors, we could not replicate these findings in a much larger cohort of Danish twins. Nor did we observe any differences in uptake and metabolism of T₄ and rT₃ between these variants and wild-type OATP1C1. In addition, no associations were found between the OATP1C1-intron3C>T polymorphism and serum thyroid hormone levels (van der Deure *et al.* 2008c).

However, both OATP1C1-intron3C>T and OATP1C1-C3035T polymorphisms, but not the OATP1C1-Pro143Thr polymorphism, were associated with symptoms of fatigue and depression in a population of adequately treated hypothyroid patients (van der Deure *et al.* 2008a). This is of interest as recently a number of papers have reported on effects of polymorphisms in thyroid hormone pathway genes, independent of an effect on serum thyroid hormone levels (Mentuccia *et al.* 2002, Canani *et al.* 2005). In a recent review, Panicker has shown that most of the associations between polymorphisms in thyroid hormone pathway genes and thyroid hormone-related endpoints were independent of serum thyroid hormone levels, which highlight the importance of local regulation of thyroid hormones in tissues (Dayan & Panicker 2009). In addition, it demonstrates that polymorphism studies can provide new insights into the role of thyroid hormone in the human body. It should be stressed, however, that functional consequences of polymorphisms in OATP1C1 have not been demonstrated *in vitro*.

Table 4 presents a summary of the studies discussed above regarding the possible effects of polymorphisms in the different OATP1 transporters on serum thyroid hormone levels *in vivo* and on the rate of iodothyronine transport *in vitro*.

Concluding remarks and future studies

Until only a few years ago, transport of thyroid hormone was thought to be a passive diffusion process. However, it has become clear that thyroid hormone requires active transport across cell membrane to carry out its biological functions. The importance of transport for thyroid hormone action was highlighted by the discovery of MCT8 as an active thyroid hormone transporter and by the notion that mutations in the *MCT8* gene lead to the Allan–Herndon–Dudley syndrome. In this review, we have discussed the molecular aspects of MCT8, MCT10 and the human members of the OATP1 family. In addition, we have discussed several studies dealing with polymorphisms in different thyroid hormone transporters.

It is surprising that few studies have been published investigating the association of polymorphisms in these transporters with serum thyroid parameters or thyroid hormone-related endpoints, especially since polymorphism studies have yielded new insights into the role of thyroid hormone in several processes in the human body. For instance, a genome-wide linkage scan identified the type 2 deiodinase as a susceptibility locus for osteoarthritis (Meulenbelt *et al.* 2008). In addition, genetic variation seems to play a role in psychological well-being (van der Deure *et al.* 2008a). However, there is also conflicting data about the role of D2 in diabetes (Mentuccia *et al.* 2002, Maia *et al.* 2007, Peeters *et al.* 2007). In addition, some associations are preliminary and need to be replicated in larger cohorts. Nevertheless, these studies provide a powerful tool to study thyroid hormone action in humans.

Many genes are involved in thyroid hormone action and metabolism. So far, studies have been published focusing on polymorphisms in the TSH receptor, in the deiodinases (for a recent review see Peeters *et al.* (2006)) and to a lesser extent also in thyroid hormone transporters and thyroid hormone receptors using a candidate gene approach. Owing to disappointing results of studies that employ a candidate gene approach, more and more studies use a genome-wide association (GWA) strategy, which is made possible through rapid technical progress over the recent years. In such a GWA study, the genome of each individual in the population is genotyped for more than 500 000 polymorphisms to search for variants that are associated with the phenotype of interest.

Although a GWA study is complicated, as it requires large samples sizes, replication and reliable geno- and phenotyping, it will unravel previously unknown pathways involved in thyroid hormone metabolism. Panicker *et al.* (2008) identified several loci associated with serum FT₄ and TSH by a genome-wide linkage scan with 737 microsatellite markers, but as expected from an underpowered linkage scan in related subjects,

they did not identify the actual genes explaining the variation in serum thyroid hormone levels. Arnaud-Lopez *et al.* (2008) have recently demonstrated that polymorphisms in the phosphodiesterase 8B gene are associated with serum TSH levels and thyroid function. It is, therefore, likely that more new insights will be obtained in the near future.

Declaration of interest

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References

- Abe T, Kakyō M, Tokui T, Nakagomi R, Nishio T, Nakai D, Nomura H, Unno M, Suzuki M, Naitoh T *et al.* 1999 Identification of a novel gene family encoding human liver-specific organic anion transporter LST-1. *Journal of Biological Chemistry* **274** 17159–17163.
- Abe T, Unno M, Onogawa T, Tokui T, Kondo TN, Nakagomi R, Adachi H, Fujiwara K, Okabe M, Suzuki T *et al.* 2001 LST-2, a human liver-specific organic anion transporter, determines methotrexate sensitivity in gastrointestinal cancers. *Gastroenterology* **120** 1689–1699.
- Arnaud-Lopez L, Usala G, Ceresini G, Mitchell BD, Pilia MG, Piras MG, Sestu N, Maschio A, Busonero F, Albai G *et al.* 2008 Phosphodiesterase 8B gene variants are associated with serum TSH levels and thyroid function. *American Journal of Human Genetics* **82** 1270–1280.
- Badagnani I, Castro RA, Taylor TR, Brett CM, Huang CC, Stryke D, Kawamoto M, Johns SJ, Ferrin TE, Carlson EJ *et al.* 2006 Interaction of methotrexate with organic-anion transporting polypeptide 1A2 and its genetic variants. *Journal of Pharmacology and Experimental Therapeutics* **318** 521–529.
- Bianco AC, Salvatore D, Gereben B, Berry MJ & Larsen PR 2002 Biochemistry, cellular and molecular biology, and physiological roles of the iodothyronine selenodeiodinases. *Endocrine Reviews* **23** 38–89.
- Breteler MM 2000 Vascular involvement in cognitive decline and dementia. Epidemiologic evidence from the Rotterdam Study and the Rotterdam Scan Study. *Annals of the New York Academy of Sciences* **903** 457–465.
- Canani LH, Capp C, Dora JM, Meyer EL, Wagner MS, Harney JW, Larsen PR, Gross JL, Bianco AC & Maia AL 2005 The type 2 deiodinase A/G (Thr92Ala) polymorphism is associated with decreased enzyme velocity and increased insulin resistance in patients with type 2 diabetes mellitus. *Journal of Clinical Endocrinology and Metabolism* **90** 3472–3478.
- Chopra IJ, Wu SY, Teco GN & Santini F 1992 A radioimmunoassay for measurement of 3,5,3'-triiodothyronine sulfate: studies in thyroidal and nonthyroidal diseases, pregnancy, and neonatal life. *Journal of Clinical Endocrinology and Metabolism* **75** 189–194.
- Chopra IJ, Santini F, Hurd RE & Chua Teco GN 1993 A radioimmunoassay for measurement of thyroxine sulfate. *Journal of Clinical Endocrinology and Metabolism* **76** 145–150.
- Cole MP, Jones CT & Todd ID 1971 A new anti-oestrogenic agent in late breast cancer. An early clinical appraisal of ICI46474. *British Journal of Cancer* **25** 270–275.

- Dayan CM & Panicker V 2009 Novel insights into thyroid hormones from the study of common genetic variation. *Nature Reviews. Endocrinology* **5** 211–218.
- van der Deure WM, Peeters RP & Visser TJ 2007 Genetic variation in thyroid hormone transporters. *Best Practice and Research. Clinical Endocrinology and Metabolism* **21** 339–350.
- van der Deure WM, Appelhof BC, Peeters RP, Wiersinga WM, Wekking EM, Huyser J, Schene AH, Tijssen JG, Hoogendijk WJ, Visser TJ *et al.* 2008a Polymorphisms in the brain-specific thyroid hormone transporter OATP1C1 are associated with fatigue and depression in hypothyroid patients. *Clinical Endocrinology* **69** 804–811.
- van der Deure WM, Friesema EC, de Jong FJ, de Rijke YB, de Jong FH, Uitterlinden AG, Breteler MM, Peeters RP & Visser TJ 2008b Organic anion transporter 1B1: an important factor in hepatic thyroid hormone and estrogen transport and metabolism. *Endocrinology* **149** 4695–4701.
- van der Deure WM, Hansen PS, Peeters RP, Kyvik KO, Friesema EC, Hegedus L & Visser TJ 2008c Thyroid hormone transport and metabolism by organic anion transporter 1C1 and consequences of genetic variation. *Endocrinology* **149** 5307–5314.
- Dumitrescu AM, Liao XH, Best TB, Brockmann K & Refetoff S 2004 A novel syndrome combining thyroid and neurological abnormalities is associated with mutations in a monocarboxylate transporter gene. *American Journal of Human Genetics* **74** 168–175.
- Dumitrescu AM, Liao XH, Weiss RE, Millen K & Refetoff S 2006 Tissue-specific thyroid hormone deprivation and excess in monocarboxylate transporter (MCT) 8-deficient mice. *Endocrinology* **147** 4036–4043.
- Forrest D, Reh TA & Rusch A 2002 Neurodevelopmental control by thyroid hormone receptors. *Current Opinion in Neurobiology* **12** 49–56.
- Friesema EC, Ganguly S, Abdalla A, Manning Fox JE, Halestrap AP & Visser TJ 2003 Identification of monocarboxylate transporter 8 as a specific thyroid hormone transporter. *Journal of Biological Chemistry* **278** 40128–40135.
- Friesema EC, Grueters A, Biebermann H, Krude H, von Moers A, Reeser M, Barrett TG, Mancilla EE, Svensson J, Kester MH *et al.* 2004 Association between mutations in a thyroid hormone transporter and severe X-linked psychomotor retardation. *Lancet* **364** 1435–1437.
- Friesema EC, Jansen J, Jachtenberg JW, Visser WE, Kester MH & Visser TJ 2008 Effective cellular uptake and efflux of thyroid hormone by human monocarboxylate transporter 10. *Molecular Endocrinology* **22** 1357–1369.
- Fujiwara K, Adachi H, Nishio T, Unno M, Tokui T, Okabe M, Onogawa T, Suzuki T, Asano N, Tanemoto M *et al.* 2001 Identification of thyroid hormone transporters in humans: different molecules are involved in a tissue-specific manner. *Endocrinology* **142** 2005–2012.
- Gamage N, Barnett A, Hempel N, Duggleby RG, Windmill KF, Martin JL & McManus ME 2006 Human sulfotransferases and their role in chemical metabolism. *Toxicological Sciences* **90** 5–22.
- Gao B, Hagenbuch B, Kullak-Ublick GA, Benke D, Aguzzi A & Meier PJ 2000 Organic anion-transporting polypeptides mediate transport of opioid peptides across blood–brain barrier. *Journal of Pharmacology and Experimental Therapeutics* **294** 73–79.
- Hagenbuch B & Meier PJ 2003 The superfamily of organic anion transporting polypeptides. *Biochimica et Biophysica Acta* **1609** 1–18.
- Halestrap AP & Meredith D 2004 The SLC16 gene family—from monocarboxylate transporters (MCTs) to aromatic amino acid transporters and beyond. *Pflügers Archiv* **447** 619–628.
- Heuer H & Visser TJ 2009 Minireview: pathophysiological importance of thyroid hormone transporters. *Endocrinology* **150** 1078–1083.
- Heuer H, Maier MK, Iden S, Mittag J, Friesema EC, Visser TJ & Bauer K 2005 The monocarboxylate transporter 8 linked to human psychomotor retardation is highly expressed in thyroid hormone-sensitive neuron populations. *Endocrinology* **146** 1701–1706.
- Hsiang B, Zhu Y, Wang Z, Wu Y, Sasseville V, Yang WP & Kirchgesner TG 1999 A novel human hepatic organic anion transporting polypeptide (OATP2). Identification of a liver-specific human organic anion transporting polypeptide and identification of rat and human hydroxymethylglutaryl-CoA reductase inhibitor transporters. *Journal of Biological Chemistry* **274** 37161–37168.
- Jansen J, Friesema EC, Milici C & Visser TJ 2005 Thyroid hormone transporters in health and disease. *Thyroid* **15** 757–768.
- Kim DK, Kanai Y, Matsuo H, Kim JY, Chairoungdua A, Kobayashi Y, Enomoto A, Cha SH, Goya T & Endou H 2002 The human T-type amino acid transporter-1: characterization, gene organization, and chromosomal location. *Genomics* **79** 95–103.
- König J, Cui Y, Nies AT & Keppler D 2000 A novel human organic anion transporting polypeptide localized to the basolateral hepatocyte membrane. *American Journal of Physiology. Gastrointestinal and Liver Physiology* **278** G156–G164.
- König J, Seithel A, Gradhand U & Fromm MF 2006 Pharmacogenomics of human OATP transporters. *Naunyn-Schmiedeberg's Archives of Pharmacology* **372** 432–443.
- Kullak-Ublick GA, Hagenbuch B, Stieger B, Schteingart CD, Hofmann AF, Wolkoff AW & Meier PJ 1995 Molecular and functional characterization of an organic anion transporting polypeptide cloned from human liver. *Gastroenterology* **109** 1274–1282.
- Kullak-Ublick GA, Ismail MG, Stieger B, Landmann L, Huber R, Pizzagalli F, Fattinger K, Meier PJ & Hagenbuch B 2001 Organic anion-transporting polypeptide B (OATP-B) and its functional comparison with three other OATPs of human liver. *Gastroenterology* **120** 525–533.
- Lafreniere RG, Carrel L & Willard HF 1994 A novel transmembrane transporter encoded by the XPC1 gene in Xq13.2. *Human Molecular Genetics* **3** 1133–1139.
- Lago-Leston R, Iglesias MJ, San-Jose E, Areal C, Eiras A, Araujo-Vilar D, Lado-Abeal J & Dominguez-Gerpe L 2008 Prevalence and functional analysis of the S107P polymorphism (rs6647476) of the monocarboxylate transporter 8 (SLC16A2) gene in the male population of Northwest Spain (Galicia). *Clinical Endocrinology*.
- Lee W, Glaeser H, Smith LH, Roberts RL, Moeckel GW, Gervasini G, Leake BF & Kim RB 2005 Polymorphisms in human organic anion-transporting polypeptide 1A2 (OATP1A2): implications for altered drug disposition and central nervous system drug entry. *Journal of Biological Chemistry* **280** 9610–9617.
- Maia AL, Dupuis J, Manning A, Liu C, Meigs JB, Cupples LA, Larsen PR & Fox CS 2007 The type 2 deiodinase (DIO2) A/G polymorphism is not associated with glycemic traits: the Framingham Heart Study. *Thyroid* **17** 199–202.
- Mentuccia D, Proietti-Pannunzi L, Tanner K, Bacci V, Pollin TI, Poehlman ET, Shuldiner AR & Celi FS 2002 Association between a novel variant of the human type 2 deiodinase gene Thr92Ala and insulin resistance: evidence of interaction with the Trp64Arg variant of the beta-3-adrenergic receptor. *Diabetes* **51** 880–883.
- Meulenbelt I, Min JL, Bos S, Riyazi N, Houwing-Duistermaat JJ, van der Wijk HJ, Kroon HM, Nakajima M, Ikegawa S, Uitterlinden AG *et al.* 2008 Identification of DIO2 as a new susceptibility locus for symptomatic osteoarthritis. *Human Molecular Genetics* **17** 1867–1875.
- Mikkaichi T, Suzuki T, Onogawa T, Tanemoto M, Mizutamari H, Okada M, Chaki T, Masuda S, Tokui T, Eto N *et al.* 2004 Isolation and characterization of a digoxin transporter and its rat homologue expressed in the kidney. *PNAS* **101** 3569–3574.
- Nakao N, Takagi T, Iigo M, Tsukamoto T, Yasuo S, Masuda T, Yanagisawa T, Ebihara S & Yoshimura T 2006 Possible involvement of organic anion transporting polypeptide 1c1 in the photoperiodic response of gonads in birds. *Endocrinology* **147** 1067–1073.
- Nakata T, Takashima S, Shiotsu Y, Murakata C, Ishida H, Akinaga S, Li PK, Sasano H, Suzuki T & Saeiki T 2003 Role of steroid sulfatase in local formation of estrogen in post-menopausal breast cancer patients. *Journal of Steroid Biochemistry and Molecular Biology* **86** 455–460.

- Nehlig A & Pereira de Vasconcelos A 1993 Glucose and ketone body utilization by the brain of neonatal rats. *Progress in Neurobiology* **40** 163–221.
- Niemi M 2007 Role of OATP transporters in the disposition of drugs. *Pharmacogenomics* **8** 787–802.
- Niemi M, Backman JT, Kajosaari LI, Leathart JB, Neuvonen M, Daly AK, Eichelbaum M, Kivisto KT & Neuvonen PJ 2005 Polymorphic organic anion transporting polypeptide 1B1 is a major determinant of repaglinide pharmacokinetics. *Clinical Pharmacology and Therapeutics* **77** 468–478.
- Panicker V, Wilson SG, Spector TD, Brown SJ, Kato BS, Reed PW, Falchi M, Richards JB, Surdulescu GL, Lim EM *et al.* 2008 Genetic loci linked to pituitary–thyroid axis setpoints: a genome-wide scan of a large twin cohort. *Journal of Clinical Endocrinology and Metabolism* **93** 3519–3523.
- Peeters RP, van Toor H, Klootwijk W, de Rijke YB, Kuiper GG, Uitterlinden AG & Visser TJ 2003 Polymorphisms in thyroid hormone pathway genes are associated with plasma TSH and iodothyronine levels in healthy subjects. *Journal of Clinical Endocrinology and Metabolism* **88** 2880–2888.
- Peeters RP, Kester MH, Wouters PJ, Kaptein E, van Toor H, Visser TJ & Van den Berghe G 2005 Increased thyroxine sulfate levels in critically ill patients as a result of a decreased hepatic type I deiodinase activity. *Journal of Clinical Endocrinology and Metabolism* **90** 6460–6465.
- Peeters RP, van der Deure WM & Visser TJ 2006 Genetic variation in thyroid hormone pathway genes; polymorphisms in the TSH receptor and the iodothyronine deiodinases. *European Journal of Endocrinology* **155** 655–662.
- Peeters RP, van der Deure WM, van den Beld AW, van Toor H, Lamberts SW, Janssen JA, Uitterlinden AG & Visser TJ 2007 The Asp727Glu polymorphism in the TSH receptor is associated with insulin resistance in healthy elderly men. *Clinical Endocrinology* **66** 808–815.
- Pizzagalli F, Hagenbuch B, Stieger B, Klenk U, Folkers G & Meier PJ 2002 Identification of a novel human organic anion transporting polypeptide as a high affinity thyroxine transporter. *Molecular Endocrinology* **16** 2283–2296.
- Ramadan T, Camargo SM, Summa V, Hunziker P, Chesnov S, Pos KM & Verrey F 2006 Basolateral aromatic amino acid transporter TAT1 (Slc16a10) functions as an efflux pathway. *Journal of Cell Physiology* **206** 771–779.
- Ramadan T, Camargo SM, Herzog B, Bordin M, Pos KM & Verrey F 2007 Recycling of aromatic amino acids via TAT1 allows efflux of neutral amino acids via LAT2-4F2hc exchanger. *Pflugers Archiv* **454** 507–516.
- Reed MJ, Purohit A, Woo LW, Newman SP & Potter BV 2005 Steroid sulfatase: molecular biology, regulation, and inhibition. *Endocrine Reviews* **26** 171–202.
- Roberts LM, Woodford K, Zhou M, Black DS, Haggerty JE, Tate EH, Grindstaff KK, Mengesha W, Raman C & Zerangue N 2008 Expression of the thyroid hormone transporters monocarboxylate transporter-8 (SLC16A2) and organic ion transporter-14 (SLCO1C1) at the blood–brain barrier. *Endocrinology* **149** 6251–6261.
- Ruder HJ, Loriaux L & Lipssett MB 1972 Estrone sulfate: production rate and metabolism in man. *Journal of Clinical Investigation* **51** 1020–1033.
- Smith IE & Dowsett M 2003 Aromatase inhibitors in breast cancer. *New England Journal of Medicine* **348** 2431–2442.
- Smith NF, Figg WD & Sparreboom A 2005 Role of the liver-specific transporters OATP1B1 and OATP1B3 in governing drug elimination. *Expert Opinion on Drug Metabolism and Toxicology* **1** 429–445.
- Sugiyama D, Kusuhara H, Taniguchi H, Ishikawa S, Nozaki Y, Aburatani H & Sugiyama Y 2003 Functional characterization of rat brain-specific organic anion transporter (Oatp14) at the blood–brain barrier: high affinity transporter for thyroxine. *Journal of Biological Chemistry* **278** 43489–43495.
- Suzuki T, Onogawa T, Asano N, Mizutamari H, Mikkaichi T, Tanemoto M, Abe M, Satoh F, Unno M, Nunoki K *et al.* 2003 Identification and characterization of novel rat and human gonad-specific organic anion transporters. *Molecular Endocrinology* **17** 1203–1215.
- Tohyama K, Kusuhara H & Sugiyama Y 2004 Involvement of multispecific organic anion transporter, Oatp14 (Slc21a14), in the transport of thyroxine across the blood–brain barrier. *Endocrinology* **145** 4384–4391.
- Trajkovic M, Visser TJ, Mittag J, Horn S, Lukas J, Darras VM, Raivich G, Bauer K & Heuer H 2007 Abnormal thyroid hormone metabolism in mice lacking the monocarboxylate transporter 8. *Journal of Clinical Investigation* **117** 627–635.
- Vie MP, Evrard C, Osty J, Breton-Gilet A, Blanchet P, Pomerance M, Rouget P, Francon J & Blondeau JP 1997 Purification, molecular cloning, and functional expression of the human nicotinamide-adenine dinucleotide phosphate-regulated thyroid hormone-binding protein. *Molecular Endocrinology* **11** 1728–1736.
- Wu SY, Huang WS, Polk D, Florsheim WH, Green WL & Fisher DA 1992 Identification of thyroxine-sulfate (T₄S) in human serum and amniotic fluid by a novel T₄S radioimmunoassay. *Thyroid* **2** 101–105.
- Wu SY, Huang WS, Polk D, Chen WL, Reviczky A, Williams J III, Chopra IJ & Fisher DA 1993 The development of a radioimmunoassay for reverse triiodothyronine sulfate in human serum and amniotic fluid. *Journal of Clinical Endocrinology and Metabolism* **76** 1625–1630.
- Wu SY, Green WL, Huang WS, Hays MT & Chopra IJ 2005 Alternate pathways of thyroid hormone metabolism. *Thyroid* **15** 943–958.
- Yen PM 2001 Physiological and molecular basis of thyroid hormone action. *Physiological Reviews* **81** 1097–1142.
- Zhou Y, Samson M, Osty J, Francon J & Blondeau JP 1990 Evidence for a close link between the thyroid hormone transport system and the aromatic amino acid transport system T in erythrocytes. *Journal of Biological Chemistry* **265** 17000–17004.
- Zhou Y, Samson M, Francon J & Blondeau JP 1992 Thyroid hormone concentrative uptake in rat erythrocytes. Involvement of the tryptophan transport system T in countertransport of tri-iodothyronine and aromatic amino acids. *Biochemical Journal* **281** 81–86.

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