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 (T₄), but it is mainly 3,3',5-tri-iodothyronine (T₃) that binds to the nuclear thyroid hormone receptor (Yen 2001). The biological activity of T₃ is therefore largely determined by the intracellular T₃ concentration which is dependent on a) the circulating T₃ 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 T₄ to T₃, whereas D3 catalyzes the degradation of T₄ to reverse T₃ (rT₃) and of T₃ to 3,3'-T₂.

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 (OATP) family, 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...
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 intra-aromatic amino acids through the same transporter.

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 et al. (2007) |
|                  |                     |          |                |                               |                | and Lago-Leston et al. (2008) |
| MCT10            | rs5937843           | Intron   | G>T            | Not consistent                | Not determined | van der Deure et al. (2007) |
|                  | rs14399            | 3′-UTR   | C>A            | No effect observed            | Not determined | van der Deure et al. (2007) |

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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 TRβ 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, bromosulfophthalaein (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, Konig 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, Konig 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 μM 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 T₃S, T₄S, 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 compared to 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 (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 (Table 2). The OATP1A2-Glu172Asp was associated with serum thyroid hormone levels (Table 2). The OATP1A2-Glu172Asp was not consistently associated with serum thyroid hormone levels (Table 2).

### 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

<table>
<thead>
<tr>
<th>Blood donors</th>
<th>OATP1A2-Ile13Thr</th>
<th>Rotterdam Scan Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSH³</td>
<td>Wild-type (116)</td>
<td>Carriers (33 + 1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P</td>
</tr>
<tr>
<td>TSH³</td>
<td>1:31 ± 0.07b</td>
<td>1:40 ± 0.13</td>
</tr>
<tr>
<td>FT₄</td>
<td>15:2 ± 0:2</td>
<td>14:5 ± 0:4</td>
</tr>
<tr>
<td>T₃</td>
<td>1:94 ± 0:02</td>
<td>2:06 ± 0:04</td>
</tr>
<tr>
<td>rT₃</td>
<td>0:32 ± 0:01</td>
<td>0:30 ± 0:01</td>
</tr>
<tr>
<td>T₄S²</td>
<td>17:53 ± 0:55</td>
<td>17:55 ± 1:03</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>OATP1A2-Glu172Asp</th>
<th>Wild-type (136)</th>
<th>Carriers (14)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSH³</td>
<td>1:34 ± 0:07d</td>
<td>1:24 ± 0:20</td>
<td>0.90</td>
</tr>
<tr>
<td>FT₄</td>
<td>15:1 ± 0:2</td>
<td>15:0 ± 0:7</td>
<td>0.84</td>
</tr>
<tr>
<td>T₃</td>
<td>1:97 ± 0:02</td>
<td>1:91 ± 0:06</td>
<td>0.39</td>
</tr>
<tr>
<td>rT₃</td>
<td>0:31 ± 0:01</td>
<td>0:36 ± 0:02</td>
<td>0.02</td>
</tr>
<tr>
<td>T₄S²</td>
<td>17:50 ± 0:51</td>
<td>17:22 ± 1:61</td>
<td>0.95</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>OATP1A2-Glu172Asp</th>
<th>Wild-type (839)</th>
<th>Carriers (100 + 6)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSH³</td>
<td>1:41 ± 0:04</td>
<td>1:35 ± 0:10</td>
<td>0.16</td>
</tr>
<tr>
<td>FT₄</td>
<td>17:7 ± 0:1</td>
<td>17:7 ± 0:3</td>
<td>0.96</td>
</tr>
<tr>
<td>T₃</td>
<td>2:01 ± 0:01</td>
<td>1:99 ± 0:02</td>
<td>0:52</td>
</tr>
<tr>
<td>rT₃</td>
<td>0:34 ± 0:01</td>
<td>0:35 ± 0:01</td>
<td>0:21</td>
</tr>
<tr>
<td>T₄S²</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
</tbody>
</table>

Data are corrected for age and gender and shown as mean ± S.E.M. ND, not determined.

³N=115.
⁴N=117.
⁵N=135.
⁶N=137.
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, 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 T1S 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 T1S levels in the healthy blood donors and elderly Caucasians respectively. However, in vitro OATP1B1-Ala\textsuperscript{174} showed a 40% lower induction of transport and metabolism of these substrates than OATP1B1-Val\textsuperscript{174} in transfected COS1 cells (van der Deure et al. 2008b; Fig. 3). Decreased hepatic uptake of T1S and E1S by OATP1B1-Ala\textsuperscript{174} compared with OATP-Val\textsuperscript{174} in vivo thus gives rise to higher serum T1S 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. 2006). 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

<table>
<thead>
<tr>
<th>Blood donors</th>
<th>Wild-type (102)</th>
<th>Carriers (45 + 6)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>OATP1B3-Ser112Ala</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TSH\textsuperscript{a}</td>
<td>1.28 ± 0.08</td>
<td>1.38 ± 0.11\textsuperscript{b}</td>
<td>0.13</td>
</tr>
<tr>
<td>FT\textsubscript{4}</td>
<td>14.9 ± 0.2</td>
<td>15.5 ± 0.3</td>
<td>0.13</td>
</tr>
<tr>
<td>T\textsubscript{3}</td>
<td>1.97 ± 0.02</td>
<td>1.96 ± 0.03</td>
<td>0.80</td>
</tr>
<tr>
<td>rT\textsubscript{3}</td>
<td>0.31 ± 0.01</td>
<td>0.32 ± 0.01</td>
<td>0.20</td>
</tr>
<tr>
<td>T\textsubscript{1S}\textsuperscript{c}</td>
<td>17.61 ± 0.59</td>
<td>17.54 ± 0.84</td>
<td>0.82</td>
</tr>
<tr>
<td>OATP1B3-Met233Ile</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TSH\textsuperscript{a}</td>
<td>1.28 ± 0.08</td>
<td>1.38 ± 0.11\textsuperscript{b}</td>
<td>0.13</td>
</tr>
<tr>
<td>FT\textsubscript{4}</td>
<td>14.9 ± 0.2</td>
<td>15.5 ± 0.3</td>
<td>0.13</td>
</tr>
<tr>
<td>T\textsubscript{3}</td>
<td>1.97 ± 0.02</td>
<td>1.96 ± 0.03</td>
<td>0.80</td>
</tr>
<tr>
<td>rT\textsubscript{3}</td>
<td>0.31 ± 0.01</td>
<td>0.32 ± 0.01</td>
<td>0.20</td>
</tr>
<tr>
<td>T\textsubscript{1S}\textsuperscript{c}</td>
<td>17.61 ± 0.59</td>
<td>17.54 ± 0.84</td>
<td>0.82</td>
</tr>
</tbody>
</table>

Data are corrected for age and gender and shown as mean ± S.E.M.

\textsuperscript{a}Owing to a non-normal distribution, TSH and T\textsubscript{1S} were logarithmically transformed in the analysis.

\textsuperscript{b}N=50.

\textsuperscript{c}N=103.
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 population of adequately treated 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, T3, and rT3 transport, but polymorphisms in the OATP1C1 gene are not consistently associated with serum thyroid hormone levels (van der Deure et al. 2008a). 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 vivo.

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.

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

<table>
<thead>
<tr>
<th>Gene</th>
<th>Polymorphism</th>
<th>Location</th>
<th>Change</th>
<th>Effect on serum thyroid hormone levels</th>
<th>In vitro effect</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>OATP1A2</td>
<td>rs57921276</td>
<td>Exon</td>
<td>Ile13Thr, Glu172Asp</td>
<td>No effect observed</td>
<td>No effect observed</td>
<td>Table 2 and Fig. 2</td>
</tr>
<tr>
<td></td>
<td>rs57550534</td>
<td>Exon</td>
<td></td>
<td>No effect observed</td>
<td>Decreased transport capacity</td>
<td>van der Deure et al. (2008b)</td>
</tr>
<tr>
<td>OATP1B1</td>
<td>rs4149056</td>
<td>Intron</td>
<td></td>
<td>Higher bilirubin, T4, T3S, rT3 levels, lower T4/T3 ratio</td>
<td>Decreased transport capacity</td>
<td>van der Deure et al. (2008b)</td>
</tr>
<tr>
<td>OATP1B3</td>
<td>rs4149117</td>
<td>Exon</td>
<td>Ser112Ala, Met233Ile</td>
<td>No effect observed</td>
<td>Not determined</td>
<td>Table 3</td>
</tr>
<tr>
<td></td>
<td>rs7311358</td>
<td>Exon</td>
<td></td>
<td>No effect observed</td>
<td>Not determined</td>
<td>van der Deure et al. (2008c)</td>
</tr>
<tr>
<td>OATP1C1</td>
<td>rs36010656</td>
<td>Exon</td>
<td>Pro143Thr</td>
<td>Higher rT3 levels, however, effect not consistent</td>
<td>No effect observed</td>
<td>van der Deure et al. (2008c)</td>
</tr>
<tr>
<td></td>
<td>rs10770704</td>
<td>Intron</td>
<td></td>
<td>No effect observed</td>
<td>Not determined</td>
<td>van der Deure et al. (2008c)</td>
</tr>
<tr>
<td></td>
<td>rs36010656</td>
<td>Exon</td>
<td></td>
<td>No effect observed</td>
<td>Not determined</td>
<td>van der Deure et al. (2008c)</td>
</tr>
<tr>
<td></td>
<td>rs10444412</td>
<td>3′-UTR</td>
<td>C3035T</td>
<td>Higher FT4 and rT3 levels, however, effect not consistent</td>
<td>No effect observed</td>
<td>van der Deure et al. (2008c)</td>
</tr>
</tbody>
</table>
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

The authors have nothing to disclose.

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