Thyroid hormone transporters

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Abstract
Thyroid hormone is important for development of various tissues, in particular brain, and for regulation of metabolic processes throughout life. The follicular cells of the thyroid gland produce predominantly T4 (thyroxine), but the biological activity of thyroid hormone is largely exerted by T3 (3,3′,5′-tri-iodothyronine). The deiodinases involved in T4-to-T3 conversion or T4 and T3 degradation, as well as the T3 receptors, are located intracellularly. Therefore the action and metabolism of thyroid hormone require transport of iodothyronines across the cell membrane via specific transporters. Recently, a number of transporters capable of cellular uptake of iodothyronines have been identified. The most specific transporters identified so far are OATP1C1 and MCT8, which appear to be involved in T4 transport across the blood–brain barrier, and in T3 transport into brain neurons, respectively. The MCT8 gene is located on human chromosome Xq13, and mutations in MCT8 are associated with X-linked severe psychomotor retardation and elevated serum T3 levels.

Introduction
Most biological actions of thyroid hormone are initiated by binding of T3 (3,3′,5′-tri-iodothyronine) to its nuclear receptor [1]. This receptor is associated with regulatory elements of T3-responsive genes, and forms a complex with other proteins. Binding of T3 to the receptor induces the release of co-repressor proteins and the recruitment of co-activator proteins, resulting in the activation of the basic transcription machinery and, thus, increased gene expression. This is the case for positively regulated genes; the exact mechanism for negative regulation of gene transcription by T3 is less clear.

The biological activity of thyroid hormone is determined by the intracellular T3 concentration, which is dependent, among other things, on: (a) the circulating concentrations of T3 and its precursor T4 (thyroxine); (b) the activity of transporters mediating the cellular uptake and/or efflux of T4 and T3 [2]; and (c) the activity of the deiodinases catalysing the ORD (outer-ring deiodination) of T4 to T3 or the IRD (inner-ring deiodination) of T4 and T3 to receptor-inactive metabolites [3] (Figure 1).

Three iodothyronine deiodinases (D1–D3) have been identified as homologous selenoproteins [3]. D1 has both ORD and IRD activities, and is expressed in liver, kidney and the thyroid. Its ORD activity is responsible for the production of the major part of circulating T3. D2 has only ORD activity, and is expressed in the brain, pituitary, human thyroid and skeletal muscle. Although the enzyme in skeletal muscle may contribute to circulating T3, the major function of D2 lies in the local generation of T3 in tissues such as the brain and the pituitary. D3 has only IRD activity; it is expressed in adult brain, and at higher levels in fetal brain as well as in other fetal tissues, placenta and pregnant uterus. D3 converts T4 into reverse (r)T3, (3,3′,5′-tri-iodothyronine) and T3 into 3,3′-T2 (3,3′-di-iodothyronine) [3].

Transports
Although conflicting data have been reported for D3, the deiodinase proteins are integrated in the plasma membrane or the endoplasmic reticulum with their active centres located in the cytoplasm, which contains the necessary cofactors for reductive deiodination [3,4]. Therefore both action and metabolism of thyroid hormone are intracellular events, requiring uptake of iodothyronines through the plasma membrane by specific transporters. Evidence has been accumulated over the past three decades for the existence of multiple thyroid hormone transporters in different tissues [2]. However, only recently some of these transporters have been characterized at the molecular level (Table 1). The transporters known to facilitate cellular thyroid hormone uptake may be categorized as organic anion transporters and amino acid transporters.

Organic anion transporters
Cellular thyroid hormone uptake by organic anion transporters was considered when it was found that transporters expressed in rat liver mediated uptake not only of different iodothyronines, but also of their sulphonated derivatives [5]. This was confirmed by expression studies in Xenopus laevis oocytes, demonstrating that the multispecific NTCP (sodium taurocholate co-transporting polypeptide) and OATP1 (Na-independent organic anion co-transporting polypeptide 1)
Figure 1 | Role of transporters and deiodinases in the regulation of intracellular thyroid hormone levels and, thus, in thyroid hormone metabolism and action
RXR, retinoid-X receptor; TR, thyroid hormone receptor; TRE, thyroid hormone response element.

Table 1 | Characteristics of thyroid hormone transporters
CP, choroid plexus; NT, not tested.

<table>
<thead>
<tr>
<th>Gene (new)</th>
<th>Gene (new)</th>
<th>Accession code</th>
<th>Chromosome distribution*</th>
<th>Tissue transport</th>
<th>Reference</th>
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<tr>
<td>SLC10A1</td>
<td>NTCP</td>
<td>NP_003040</td>
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<td>NP_0058807</td>
<td>4q14</td>
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<td>T4, T3, T3, T2 [31]</td>
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<td>OATP2</td>
<td>NP_571981</td>
<td>4</td>
<td>Liver, brain, retina</td>
<td>T4, T2, T3, T3 [31,34]</td>
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<tr>
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<td>OATP3</td>
<td>NP_110465</td>
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<td>T3, T4 [32,34]</td>
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<td>9</td>
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*The tissue distribution information shown for genes SLC10A1, SLC21A20, SLC7A5 and SLC7A8 applies to the rat as well as humans.
facilitated uptake of the different iodothyronines, as well as their sulphate and sulphamate derivatives [6]. NTCP is expressed exclusively in the liver, and is a member of the SLC10 (solute carrier 10) family [7]. The other members of the SLC10 family have not been tested for thyroid hormone transport, except for P3 (or SLC10A3), which was found to be inactive (E.C.H. Friesma, T. Abe and T.J. Visser, unpublished work).

OATPs represent a large family of homologous proteins, many of which have been shown to transport different iodothyronines (Table 1) [8]. The genes were initially assigned to the SLC21 family, but according to the new nomenclature based on the homology between the different members, they are now referred as the SLCO family. The proteins have corresponding new OATP codes. The OATPs accept a wide range of ligands, not only anionic but also neutral and sometimes even cationic compounds. Some members are expressed in a single tissue, whereas others have a wider tissue distribution. Interestingly, the SLCO1A2, 1B1, 1B3 and 1C1 genes are clustered together with a related pseudogene on human chromosome 12p12. The encoded OATPs, formerly known as OATP-A, OATP-C, OATP8 and OATP-F, have all been shown to transport iodothyronines (Table 1). Of these, OATP1B1 and 1B3 are expressed liver-specifically, OATP1C1 is expressed only in the brain and testis, while OATP1A2 is expressed in the brain, liver and kidney. In terms of thyroid hormone transport, OATP1C1 is the most intriguing, since it shows a high specificity and affinity towards iodothyronines, in particular T4 and rT3. In brain, it is localized preferentially in capillaries, suggesting that OATP1C1 is particularly important for transport of T4 across the blood–brain barrier [9–11].

Amino acid transporters
Iodothyronines are a particular class of amino acids built from two tyrosine residues. Therefore, it is no surprise that amino acid transporters, in particular the L- and T-type amino acid transporters, have been shown to be involved in thyroid hormone uptake into several tissues [12–16]. L-type transporters mediate uptake of large neutral, branched-chain and aromatic amino acids, whereas T-type transporters are specific for the aromatic amino acids phenylalanine, tyrosine and tryptophan.

Recently, two LATs (L-type amino acid transporters; LAT1 and LAT2) have been identified among the members of the HAT (heterodimeric amino acid transporter) family. HATs consist of a heavy chain and a light chain, linked through a disulphide bond [17]. There are two possible heavy chains [4F2hc and rBAT (related to basic amino acid transport)], belonging to the SLC3 gene family, and seven possible light chains, belonging to the SLC7 gene family. The 4F2 or CD98 cell-surface antigen is expressed in many tissues, especially on activated lymphocytes and tumour cells. The 4F2hc and rBAT heavy chains are glycosylated proteins with a single transmembrane domain, whereas the light chains are not glycosylated and have 12 transmembrane domains. The 4F2hc heavy chain forms functional HATs with six light chains (LAT1, LAT2, y+LAT1, y+LAT2, Asc1 and XCT), whereas rBAT dimerizes only with one light chain (b5+AT) [17].

We have demonstrated significant Na-independent transport of the iodothyronines T4, T3, rT3 and 3,3’-T2 by heterodimeric transporters consisting of human 4F2hc and either human LAT1 or mouse LAT2 in Xenopus oocytes [18]. No iodothyronine uptake was observed in oocytes expressing 4F2hc alone or LAT1 or LAT2 alone, or in oocytes expressing 4F2hc together with either y+LAT1 or y+LAT2. At sub-saturating ligand concentrations, the rate of iodothyronine uptake by the 4F2hc/LAT1 transporter decreased in the order 3,3’-T2 > T3 ≈ rT3 > T4. Apparent $K_m$ values were found to be in the micromolar range, being lowest for T3 (1.5 µM), which is the lowest value reported for any ligand of the 4F2hc/LAT1 transporter [17].

Ritchie et al. [19] have reported on the stimulation of T3 transport in oocytes injected with mRNA for 4F2hc and for the IU12 Xenopus LAT1 homologue. These workers have also demonstrated that overexpression of the heterodimeric L-type transporter in cells results in increased intracellular T3 availability and, thus, augmented T3 action [20]. Furthermore, they demonstrated T3 uptake via the 4F2hc/LAT1 transporter into the human BeWo placental choriocarcinoma cell line, suggesting that this transporter plays an important role in supplying the placenta and developing fetus with thyroid hormone [21].

TAT1, a T-type amino acid transporter, has recently been cloned from rats and humans, and shown to transport phenylalanine, tyrosine and tryptophan, but not iodothyronines [22,23]. This protein is a member of the MCT (monocarboxylate transporter) family, and is also called MCT10. Since, among the 14 known MCT family members, MCT8 shows the highest homology with MCT10, we hypothesized that MCT8 is a TAT that also transports iodothyronines. This proved to be partially true: MCT8 was indeed found to transport iodothyronines, but it was inactive towards amino acids such as leucine, phenylalanine, tyrosine and tryptophan, as well as towards monocarboxylates such as lactate and pyruvate. The latter are ligands for MCT1–4, but the compounds transported by most other MCTs, in spite of their name, have not been identified. According to the SLC nomenclature, MCT8 and MCT10 are now also referred to as SLC16A2 and SLC16A10 respectively.

MCT8
The human MCT8 gene is located on chromosome Xq13.2, consists of six exons and codes for a protein of 539 or 613 amino acids, depending on which of the two alternative TLSs (translation start sites) is used (Figure 2) [24]. Both forms of the protein contain 12 predicted transmembrane domains, characteristic of a transporter protein. The N-terminal end of the protein contains a so-called PEST domain, rich in proline, glutamate, serine and threonine residues. PEST domains serve as proteolytic signals, targeting the protein for rapid degradation [25]. This is why MCT8 was initially called X-linked.
PEST-containing transporter (XPCT; [24]). MCT8 is expressed in many tissues, including human liver, kidney, heart, brain, placenta, lung and skeletal muscle.

After the cloning of MCT8 in 1994, no reports on the biological function or the transported ligands had been published, until Friesema et al. [26] identified rat MCT8 as a specific thyroid hormone transporter. Expression of MCT8 in *Xenopus* oocytes was shown to induce an approx. 10-fold increase in uptake of T4, T3, rT3 and 3,3′-T2, which is much greater than the activity of any other transporter tested in this system, including rat NTCP, rat OATP1 and human LAT1 [26]. Although rat MCT8 does not discriminate between the different iodothyronines, it does not transport iodothyronine sulphates and sulphamates, the amino acids phenylalanine, tyrosine, tryptophan and leucine, or the monocarboxylates lactate and pyruvate. Apparent \( K_m \) values were found to be in the range of 2–5 \( \mu \)M for the different iodothyronines in the absence of protein in the medium. T4 transport is modestly inhibited in the absence of Na\(^{+}\), whereas transport of T3 is completely Na-independent [26].

To date it is unknown whether either the long (613 amino acids) or the short (539 amino acids) isoform of human MCT8, or both, are expressed in *vivo*. It is also unknown if there are any functional differences between them. In rats and mice, only the short form of MCT8 exists due to the lack of the first TLS. We have characterized the short form of human MCT8 also as an active and specific thyroid hormone transporter, showing a slight preference for T3 as the ligand. In this review, the numbering of amino acids starts at the first TLS; in contrast with our recent publication [27], where positions of mutations were related to the second TLS.

We have obtained dramatic evidence for the pathophysiological importance of thyroid hormone transport by MCT8 in studies of five young boys with a novel syndrome of severe psychomotor retardation and strongly elevated serum T3 levels [27]. The boys vary in age from 1.5 and 6 years, and in none of them is there any development of speech. Communication skills are limited to smiling and crying. None of the boys can sit or stand independently. Poor head control due to truncal hypotonia is observed in all subjects. All patients were found to have a mutation in MCT8 (Figure 2). One patient showed a deletion of 24.5 kb in size, encompassing exon 1, and another patient had a deletion of 2.4 kb in size, including part of exon 3 and the entire exon 4. The other patients show single nucleotide mutations, which is associated with the introduction of a premature stop in one boy, and with amino acid substitutions in two boys, i.e. an Ala-to-Val substitution in the second transmembrane domain, and a Leu-to-Pro substitution in the ninth transmembrane domain. The mothers of all patients proved to be carriers of the mutations. None of the mothers showed psychomotor retardation, and their serum thyroid hormone levels are mostly within the normal range.

Mutations in MCT8 have also been reported by Dumitrescu et al. [28], who reported on two unrelated patients with similar severe neurological abnormalities and increased serum T3 levels. In one patient, a point mutation in exon 5 was detected, resulting in a Leu-to-Pro substitution in the fifth intracellular loop. In the other patient, a single nucleotide deletion in exon 3 results in a frameshift and the generation of a premature stop in the seventh transmembrane domain.

From the observations in these patients, we hypothesize that MCT8 plays an essential role in T3 supply to neurons in the central nervous system, which are the primary targets of T3 action, in particular during brain development. These neurons also express D3, involved in the termination of T3 action in these cells. However, neurons do not express D2, which is required for local production of T3 from T4. For this, neurons depend on the neighbouring astrocytes. Indeed, in the rodent brain expression of D2 has been demonstrated in astrocytes, whereas MCT8 and D3 are almost exclusively

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expressed in neurons [29,30]. Inactivating mutations in MCT8 will result in an impaired supply of T3 to the neuron, which will have detrimental effects on neuronal migration, differentiation and myelination, causing the psychomotor phenotype in our patients. In addition, inactivation of MCT8 will block the access of T3 to neuronal D3, resulting in a decreased T3 clearance and, thus, in the increased serum T3 levels in our patients. However, the possibility that MCT8 is involved in the transport of other, still unidentified ligands, whose reduced bioavailability may contribute to the phenotype is not excluded.

Mutations in MCT8 appear to cause tissue-specific hypothyroidism in the brain. Since the gene is located on the X chromosome, mutations in MCT8 represent a novel cause of X-linked psychomotor retardation. These findings demonstrate the dramatic consequences of a mutation in a thyroid hormone transporter, resulting in an impaired tissue thyroid hormone supply and representing a novel mechanism for thyroid hormone resistance.

References

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