Scope and limitations of iodothyronine deiodinases in hypothyroidism

Balázs Gereben, Elizabeth A. McAninch, Miriam O. Ribeiro and Antonio C. Bianco

Abstract | The coordinated expression and activity of the iodothyronine deiodinases regulate thyroid hormone levels in hypothyroidism. Once heralded as the pathway underpinning adequate thyroid-hormone replacement therapy with levothyroxine, the role of these enzymes has come into question as they have been implicated in both an inability to normalize serum levels of tri-iodothyronine (T_3) and the incomplete resolution of hypothyroid symptoms. These observations, some of which were validated in animal models of levothyroxine monotherapy, challenge the paradigm that tissue levels of T_3 and thyroid-hormone signalling can be fully restored by administration of levothyroxine alone. The low serum levels of T_3 observed among patients receiving levothyroxine monotherapy occur as a consequence of type 2 iodothyronine deiodinase (DIO2) in the hypothalamus being fairly insensitive to ubiquitination. In addition, residual symptoms of hypothyroidism have been linked to a prevalent polymorphism in the *DIO2* gene that might be a risk factor for neurodegenerative disease. Here, we discuss how these novel findings underscore the clinical importance of iodothyronine deiodinases in hypothyroidism and how an improved understanding of these enzymes might translate to therapeutic advances in the care of millions of patients with this condition.

Gereben, B. *et al. Nat. Rev. Endocrinol.* **11**, 642–652 (2015); published online 29 September 2015; doi:10.1038/nrendo.2015.155

Introduction

Thyroid hormones are iodinated molecules produced by the thyroid gland that regulate development, growth, energy homeostasis, cardiovascular systems, musculoskeletal systems and cognitive function. Insufficient levels of the thyroid hormones tetraiodothyronine (T_4) and tri-iodothyronine (T_3) result in hypothyroidism, a prevalent condition that affects more than 8 million patients in the USA alone[1] and 1–2% of individuals living in iodine-replete communities.[2]

Over the past 150 years, treatment modalities for hypothyroidism have been developed around thyroid hormone 'replacement' through administration of thyroid gland extracts,[3] which remained the mainstay of therapy for nearly a century. However, with the discovery in 1970 that, in humans, iodothyronine deiodinases produce most of the circulating T_3,[4] clinical standards abruptly shifted to align with the assumption that levothyroxine monotherapy would maintain the pool of T_4 and that a group of enzymes known as the iodothyronine deiodinases would provide physiologic regulation of the T_3 availability to tissues.[5]

For the past few decades, clinicians have displayed an almost dogmatic reliance upon the ability of the iodothyronine deiodinases to mediate conversion of T_4 to T_3 and thereby regulate the availability of serum levels of T_3 among patients with hypothyroidism who are treated with levothyroxine.[6] However, we now understand that ~12% of all patients treated with levothyroxine are biochemically euthyroid (that is, they have normal serum levels of TSH) but continue to experience residual symptoms of hypothyroidism, including psychological[7–9] and metabolic effects.[10,11] This finding represents a major public-health concern given the high prevalence of hypothyroidism and the fact that not all parameters are restored by levothyroxine monotherapy. With such strong reliance on the iodothyronine deiodinases and, therefore, the efficacy of levothyroxine monotherapy, clinicians were left with few options to explain or treat these residual symptoms.

As awareness of this subpopulation of patients with hypothyroidism has improved, investigators have made advances in understanding the aetiology of this phenomenon. Serum levels of T_3 might not be fully normalized among such patients owing to insufficient T_4-to-T_3 conversion,[12] which could explain why a minority remain symptomatic despite treatment with levothyroxine. Some patients demonstrate improved well-being and a treatment preference when co-administered levothyroxine and liothyronine;[13,14] however, this issue has remained contentious as the majority of clinical trials have failed to demonstrate an objective benefit of combination therapy.[15,16]

Differences within subgroups of patients with hypothyroidism could make them more or less responsive to levothyroxine monotherapy versus combination therapy.[13] Therefore, the iodothyronine deiodinases that were once almost universally accepted as the key clinical strategy to thyroid-hormone regulation in hypothyroidism might actually cause continued symptoms among a substantial proportion of patients.

Department of Endocrine Neurobiology, Institute of Experimental Medicine, Hungarian Academy of Sciences, Szigony Street 43, Budapest H-1083, Hungary (**B.G.**). Division of Endocrinology and Metabolism, Rush University Medical Center, 212 Cohn Building, 1735 West Harrison Street, Chicago, IL 60612, USA (**E.A.M.**, **A.C.B.**). Developmental Disorders Program, Center for Biological and Health Science, Mackenzie Presbyterian University, Rua da Consolação 930, Building 16, São Paulo, SP 01302, Brazil (**M.O.R.**).

Correspondence to: A.C.B. abianco@deiodinase.org

Competing interests
The authors declare no competing interests.

Key points

- Levothyroxine monotherapy at doses that normalize serum levels of TSH does not universally restore parameters of thyroid hormone levels for patients with hypothyroidism
- The iodothyronine deiodinases provide a cell-specific, prereceptor mechanism that controls thyroid-hormone signalling
- Localized thyroid-hormone signalling has a critical role in different areas of the brain, as mediated by thyroid hormone transporters and the iodothyronine deiodinases
- DIO2 ubiquitination induced by tetraiodothyronine (T_4) normally decreases tri-iodothyronine (T_3) production, but not in the hypothalamus
- The levothyroxine dose that normalizes serum levels of TSH in an animal model is lower than the dose that normalizes serum levels of T_3, which explains the increased serum T_4 to T_3 ratio observed in patients treated with levothyroxine
- If patients carrying the Thr92AlaD2 polymorphism derive benefit from combination therapy with levothyroxine and liothyronine, then genotyping for this single nucleotide polymorphism might become a component of the management of hypothyroidism

Here, we review the physiologic role of the iodothyronine deiodinases, as well as the changes that occur in hypothyroidism, with particular focus on their role in preserving thyroid hormone levels in the brain. The latest studies indicate that unique biochemical aspects of the iodothyronine deiodinases in the hypothalamus prevent normalization of serum levels of T_3 in patients treated with levothyroxine monotherapy. In combination with a prevalent *DIO2* polymorphism, this finding could also explain the insufficient symptomatic response experienced by an appreciable proportion of patients with hypothyroidism.

Animal models of thyroid replacement

Further insight into the regulation of iodothyronine deiodinases and tissue-specific thyroid-hormone signalling is required to develop new strategies to treat hypothyroidism. However, studies in humans are limited as they require tissue biopsy samples. Thus, animal models have been developed that provide the basis for how unique aspects of iodothyronine deiodinase regulation might affect different thyroid-hormone replacement therapies.[17–20]

The applicability of such models reflects the extensive similarities in thyroid physiology between humans and small rodents.[21] Interspecies differences exist but their interference can be minimized by correct experimental planning. For example, the half-lives of T_4 and T_3 are longer in humans (~1 week and 1 day, respectively) than in rodents (~8 h and 2 h, respectively) and so blood and/or tissue sampling should be planned accordingly.[21] Iodothyronine deiodinases are conserved across species at the amino acid level but they have species-specific differences in tissue distribution. Type 2 iodothyronine deiodinase (DIO2) is expressed in the human thyroid gland and heart but not in these tissues in rodents.[22,23] Furthermore, DIO2 is the only activating iodothyronine deiodinase in the human brain, whereas both Dio2 and type 1 iodothyronine deiodinase (Dio1) are expressed in the rat brain.[24,25]

With these differences in mind, studies in thyroidectomized rats have conclusively shown that only therapy with a combination of levothyroxine and liothyronine could normalize serum and tissue levels of T_3.[18,19] Similar conclusions were obtained for normalization of T_3-dependent biological parameters, such as lipid profile, mitochondrial content and the expression of T_3-target genes, in the brains of thyroidectomized rats.[17] Mouse models were also instrumental in the discovery that preserving the stability of serum concentrations of T_3 is a biological priority, which is enforced even when Dio1 and/or Dio2 are genetically inactivated in all tissues[26] or in specific tissues.[27,28] Therefore, animal models have provided substantial advances in our understanding of thyroid-hormone replacement therapy.

Thyroid-hormone signalling

In humans, thyroid hormones are secreted into the circulation predominantly as a prohormone (T_4), and only ~20% is secreted as the biologically active T_3 form. T_4 and T_3 enter almost all cells through transporters in the plasma membrane and remain in equilibrium between plasma and cells (Figure 1). Once inside a cell, T_3 diffuses into the nucleus and binds to a thyroid-hormone receptor (either TRα or TRβ) to modulate gene expression. The T_3–TR complex controls expression of specific sets of genes that are responsive to T_3, thus promoting T_3-dependent biological effects. Tissues contain different combinations of TRα and/or TRβ as well as other transcriptional coregulators of the T_3–TR complex. The net effect of T_3, therefore, depends on these combinations and is highly cell and/or tissue-specific.[29,30]

Studies in patients, animal models and *in vitro* cell models with mutations in the genes encoding TRα or TRβ have highlighted the mechanisms involved in the positive stimulation of gene transcription by T_3.[29] Given the free T_3 concentration in the plasma (about 5×10^{-12} M) and the affinity of TRs for T_3 (about 1×10^9 l/mol), the ratio of occupied to unoccupied TRs is 1:1 in most tissues. Even when unoccupied, TRs are predominantly bound to specific *cis*-acting elements in T_3-responsive genes, such as ectonucleotide pyrophosphatase/phosphodiesterase 2 (*ENPP2*) and myelin basic protein (*MBP*). Unoccupied TRs have a high affinity for negative coregulators of transcription (also known as corepressors), which actively inhibits gene expression. Upon binding to T_3, TRs lose their affinity for corepressors and gain affinity for coactivators, which triggers transcriptional activation of T_3-dependent genes. Therefore, the clinical syndrome of an individual with hypothyroidism is largely the result of transcriptional repression of T_3-responsive genes mediated by unoccupied TRs. The goal of thyroid-hormone replacement therapy is to provide sufficient T_3 to relieve TR-mediated gene repression and to promote T_3-dependent transactivation of target genes.[29] The unique role of the unoccupied TRs is further illustrated by the fact that mice with a knockout for the genes encoding TRα and TRβ exhibit only a mild phenotype, mainly because no transcriptional repression occurs.[29] Therefore, the intensity of thyroid-hormone signalling depends on the ratio between occupied and unoccupied TRs, which is a function of the plasma T_3 concentration, presence of plasma membrane transporters, iodothyronine deiodinase activity and (ultimately) the nuclear concentration of T_3.

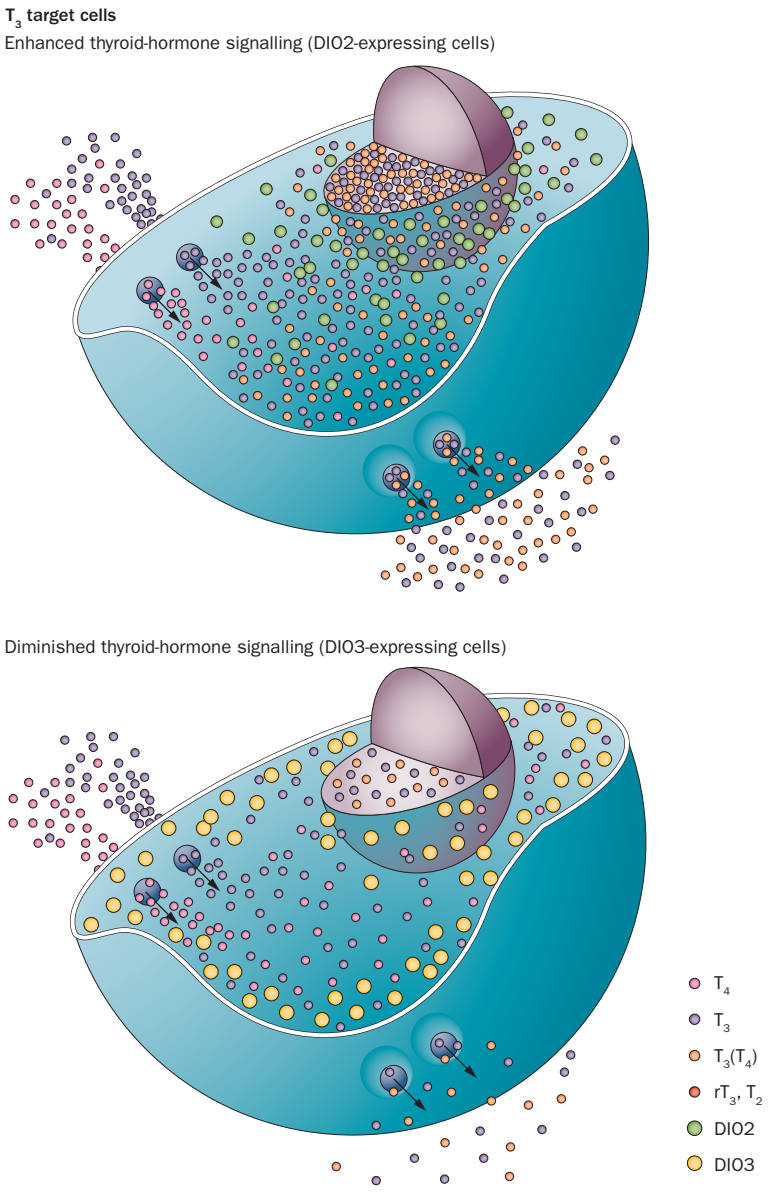

Figure 1 | Iodothyronine deiodinases modulate thyroid-hormone signalling in T_3 target cells. T_4 and T_3 enter almost all cells via membrane transporters and are then modified in a cell-specific manner by DIOs to either enhance (DIO2) or diminish (DIO3) thyroid-hormone signalling. Consequently, the flow of T_3 diffusing from the cell membrane to the nucleus is increased by $T_3(T_4)$, which represents T_3 generated locally from T_4 via DIO2. By contrast, the DIO3 pathway decreases the flow of T_3 to the nucleus by terminally inactivating T_3 to T_2 and T_4 to rT_3 (rT_3,T_2). DIO2 generates T_3 in a cell compartment adjacent to the nucleus. By contrast, DIO3 largely resides in the periphery of the cell in the plasma membrane and early endosomes. Once inside cells, T_3 can diffuse to the nucleus to modulate gene expression. Abbreviations: DIO, iodothyronine deiodinase; rT_3, reverse tri-iodothyronine; T_2, di-iodothyronine; T_3, tri-iodothyronine; T_4, tetra-iodothyronine. Courtesy of BiancoLab.org.

Iodothyronine deiodinases

Iodothyronine deiodinases are small, highly homologous, integral membrane enzymes that modify thyroid-hormone signalling. They comprise a single N-terminal, transmembrane segment connected to a larger globular cytosolic domain with a selenocysteine-containing active centre embedded in a thioredoxin-like fold. The molecular structure has been modelled using hydrophobic cluster analysis[31] and confirmed with supportive experimental data in the case of type 3 iodothyronine deiodinase (DIO3).[32] The selenium in the active centre provides enhanced substrate affinity and a fast turnover rate for the deiodination reaction.

Both T_4 and T_3 can be deiodinated, a process that results in either activation of T_4 (by DIO1 and DIO2) or inactivation of T_4 and T_3 (by DIO3). Consequently, the intracellular environment can be enriched with additional T_3 supplied by DIO2 (enhancing thyroid-hormone action) or depletion of thyroid hormones by DIO3 (dampening thyroid-hormone action; Figure 1). A key property of iodothyronine deiodinases in thyroid-hormone signalling is their unique subcellular localization. DIO2 is usually retained in the endoplasmic reticulum;[33,34] however, it is also closely associated with the cell nucleus,[35] but not with the Golgi apparatus.[33] As a result, the nuclear environment can be greatly affected by DIO2-generated T_3 owing to this physical proximity. By contrast, DIO1 is located in the plasma membrane; it has low affinity for T_4 and so DIO1-generated T_3 rapidly diffuses from the cells and reaches the plasma without appreciably affecting nuclear concentrations of T_3.[34,36]

DIO3 is generally anchored in the plasma membrane, where it is internalized to become part of vesicles known as early endosomes that can be recycled back to the plasma membrane.[34,37] In the rat central nervous system, Dio3 is observed in dense-core vesicles of hypothalamic neurosecretory axon varicosities with the active centre containing the C-terminus of Dio3 at the outer surface of these organelles.[38] However, this peripheral distribution of Dio3 can change depending on oxygen availability. For example, after unilateral induction of ischaemia and hypoxia in the rat brain, Dio3 is found predominantly in the nucleus of the neurons in the pyramidal and granular ipsilateral layers, as well as in the hilus of the dentate gyrus of the hippocampal formation.[39] In isolated mouse hippocampal neurons grown in culture and in a human neuroblastoma cell line, hypoxia redirects active DIO3 to the nucleus via the HSP40 pathway, a shuttle mechanism known to direct proteins to the nucleus. In the human neuroblastoma cell line, preventing nuclear DIO3 import by *HSP40* knockdown almost doubles the thyroid-hormone-dependent glycolytic rate and quadruples the transcription of thyroid-hormone target gene *ENPP2*. By contrast, overexpression of HSP40 increases nuclear import of DIO3 and minimizes the effects of thyroid hormones in cell metabolism.[39] Rerouting DIO3 to the nucleus decreases thyroid-hormone signalling and might function to reduce ischaemia-induced hypoxic brain damage.

The corollary to these findings is that the activity of DIO2 and DIO3 are viewed as a cell-specific, prereceptor mechanism that controls thyroid-hormone signalling, the intensity of which cannot be predicted on the basis of circulating levels of T_3.[40] For example, cold and/or sympathetic nervous system stimulation of Dio2 expression in rat brown adipose tissue accelerates transcription of T_3-responsive genes, such as *Ucp1* and *Pgc1α*.[41] Furthermore, ectopic expression of DIO3 in the human

heart and brain during ischaemia or hypoxia[42] decreases the amount of T_3-dependent transcription in these organs, curbing thyroid-hormone signalling.[43,44] Although no humans have been identified with loss-of-function mutations in the iodothyronine deiodinases,[45] a few individuals have been identified with defects in selenoprotein synthesis that results in a reduction in DIO2 activity and possible reduction in the activities of DIO1 and DIO3. These patients exhibit increased serum levels of TSH, increased serum levels of T_4 and low serum levels of T_3, but otherwise have a mild phenotype.[46]

Effects on brain function

The fundamental impact of thyroid hormones on brain function was established by the striking link between untreated congenital hypothyroidism and developmental retardation of cognitive function.[47–49] Myelination, neuronal and/or glial proliferation, differentiation and neuronal migration represent crucial targets of thyroid-hormone-mediated events in the brain.[48,50,51] Access of thyroid hormones to the brain is selective, with T_4 in plasma having greater ease of access than T_3 in plasma because of the types of transporters expressed in the blood–brain barrier.[52,53]

Uptake of thyroid hormones via the blood–brain barrier and into cells inside the brain parenchyma is mediated by transporters, including monocarboxylate transporter 8 (MCT8) and solute carrier organic anion transporter family member 1C1 (SLCO1C1, also known as OATP1C1).[54–56] These transporters are plasma membrane proteins with multiple membrane-spanning domains that have a half-life of several days. Although levels of *Mct8* and *Slco1c1* mRNA are decreased in a rodent model of nonthyroidal illness,[57] currently no direct evidence exists that quick, adaptive changes in the transport of thyroid hormones regulate thyroid hormone availability in the brain. However, the observation that patients with Allan–Herndon–Dudley syndrome express a mutant MCT8 protein explained the molecular background of this rare X-linked disorder, which is characterized by neurological abnormalities (central hypotonia, hearing problems, rotatory nystagmus and spasticity) and developmental delay.[58,59]

The link between mutations in the genes that encode thyroid hormone transporters and neurological symptoms highlights the critical importance of localized thyroid-hormone signalling in different areas of the brain. In contrast to the majority of tissues, a large portion of T_3 in the rodent brain is produced locally via activation of T_4. A dual-labelling approach was used to assess this process directly[60] and the findings were later confirmed in *Dio2* knockout mice that exhibited ~50% reductions in levels of T_3 in the brain.[61] Thus, local metabolism of thyroid hormones (that is, via the DIO2 and DIO3 pathways) is currently viewed as the major factor that regulates the intensity of thyroid-hormone signalling in the brain. Both enzymes exhibit homeostatic regulation in response to changes in serum levels of thyroid hormones. The activity of DIO2 increases but that of DIO3 decreases in the presence of low concentrations of thyroid hormones (for example, in conditions of hypothyroidism or iodine deficiency), a finding that has been interpreted largely as a mechanism that delays adverse effects of hypothyroidism in the brain.[62,63]

Neurons are critically important targets of T_3; however, these cells lack DIO2 and are unable to generate T_3 via this pathway.[64–66] Indeed, enrichment of *Dio2* expression is detected in glial cells when examining the transcriptome of astrocytes, neurons and oligodendrocytes.[67] Furthermore, a >95% drop in *Dio2* mRNA and activity occurred in the brain of a mouse model with a transgene conferring glia-specific inactivation of this enzyme.[28] Thus, the current paradigm predicts that T_3 generated by DIO2 located in astrocytes acts in a paracrine fashion to stimulate TRs in neurons.[68–70] This hypothesis has been modelled and validated *in vitro* by co-culturing human DIO2-expressing glioma cells with human neuronal cells that express DIO3.[70] In such a system, T_3 generated by glial cells (via DIO2 activity) has a paracrine effect on the co-cultured neurons and activates T_3-regulated neuronal genes. Such pathways might also affect thyroid-hormone signalling *in vivo*. For example, DIO3 is activated by hypoxia inducible factor 1-α under conditions of ischaemia and/or hypoxia.[43] Furthermore, DIO2 activity is downregulated and *DIO3* expression is activated by the morphogenic hedgehog protein family.[71,72]

Logically, in responding to ischaemia or hypoxia and other cues known to regulate these enzymes, iodothyronine deiodinases enhance or dampen thyroid-hormone signalling in discrete groups of cells or areas of the brain with clear consequences. For example, in rodent models, Dio2 is involved in the generation of Tsh-releasing hormone (Trh) and/or Tsh feedback (Figure 2). DIO2 is also implicated in the hypothalamic regulation of nonthyroidal illness, seasonal breeding in birds and the onset of hearing in rodents.[73–76] Direct evidence suggests that iodothyronine deiodinases have similar roles in humans. For example, in the human fetal hypothalamus, a developmental dependency and coordinated expression of DIO2 and DIO3 occurs in combination with the various thyroid-hormone transporters.[77]

In the rodent and avian retina, Dio3 dampens the actions of thyroid hormones, limiting T_3 exposure to the cone cells and preserving survival and/or patterning of opsins, which is required for cone function.[78] Some evidence supports a role for iodothyronine deiodinase-mediated thyroid-hormone signalling in the human brain. For example, a correlation exists between the levels of thyroid hormones and enzyme activity in different areas of the developing human brain.[79] During recovery from intraventricular haemorrhage in preterm infants, which depends on maturation of oligodendrocytes and myelination, *DIO2* expression is decreased and *DIO3* expression increased, which is indicative of localized hypothyroidism.[80] Reversing this effect with levothyroxine treatment promotes neurological recovery in infants and in a model of intraventricular haemorrhage in rabbits and, if confirmed, this approach could improve the neurodevelopmental outcome of preterm infants with intraventricular haemorrhage.[80]

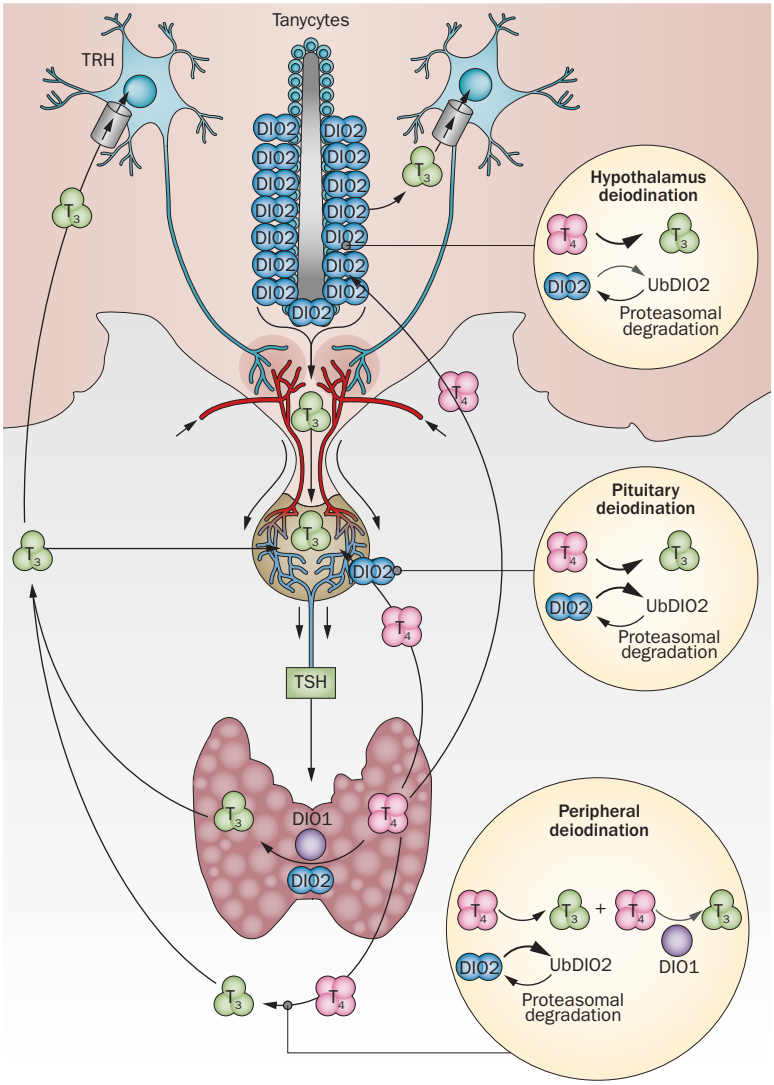

Figure 2 | T_4-induced DIO2 ubiquitination in thyroid hormone homeostasis. In response to thyroid hormone signals from the periphery and DIO2-expressing tanycytes, hypophysiotropic TRH-expressing neurons release TRH into the portal blood. TRH is transported to the anterior pituitary gland where TSH is secreted and stimulates the thyroid gland to produce and secrete T_4 and T_3. Hypothalamic T_3 is generated locally by tanycytes and enters the systemic circulation. T_3 can also be generated in the periphery via DIO1. In most peripheral tissues, exposure to T_4 accelerates inactivation of DIO2 (UbDIO2) and UbDIO2 targeting to the proteasomal system; however, UbDIO2 can be reactivated and rescued fromproteasomal destruction by deubiquitination. Peripheral deiodination is very sensitive to T_4-induced DIO2 ubiquitination: a mild increase in the serum T_4:T_3 ratio favours DIO2 inactivation and decreases fractional T_4-to-T_3 conversion and peripheral T_3 production. However in the hypothalamus, DIO2 is less susceptible to T_4-induced ubiquitination than in other tissues. Thus, T_4 signalling via DIO2-mediated T_3 production is very effective in the hypothalamus, whereas T_3 production via DIO2 is easily inhibited in the periphery. Abbreviations: DIO, iodothyronine deiodinase; T_2, di-iodothyronine; T_3, tri-iodothyronine; T_4, tetra-iodothyronine; TRH, TSH-releasing hormone; UbDIO2, ubiquitinated DIO2. Permission obtained from American Society for Clinical Investigation © Werneck de Castro, J. P. *et al. J. Clin. Invest.* **125**, 769–781 (2015).[17]

What changes in hypothyroidism?

The amount of thyroid hormone entering a cell is equivalent to the amount exiting that cell, characterizing a state of equilibrium. Studies in animal models and human cells grown in culture suggest that the net flow of T_4 and T_3 depends on the type of iodothyronine deiodinase expressed in each given cell type (Figure 1). The expression of *DIO2* creates an inward net flow of T_4 and an outward net flow of T_3.[40] Expression of *DIO3* creates inward net flows of both T_4 and T_3, whereas the net flow is neutral if no iodothyronine deiodinase is expressed.[40]

Iodothyronine deiodinase activity also affects circulating levels of T_4 and T_3. In humans, ~70% of the circulating T_3 is produced via the extrathyroidal DIO2 pathway, whereas ~15% originates from the DIO1 pathway. Given that DIO1 is positively regulated by T_3,[81] whereas the opposite is seen for DIO2, the contribution of DIO1 to the circulating T_3 pool is increased in patients with hyperthyroidism. Notably, DIO1 activity is inhibited by propylthiouracil, glucocorticoids and β blockers, which explains at least part of the clinical efficacy of these drugs.[40]

Most circulating T_3 is metabolized via the DIO3 pathway.[82] This pathway acquires particular clinical relevance during pregnancy because *DIO3* is highly expressed in the human placenta,[83] and, therefore, increases the daily requirements for thyroid hormone for patients on thyroid hormone replacement therapy.[84] Increased expression of *DIO3* is also observed in some disease states, including in the liver, lungs, heart and brain of patients experiencing ischaemia or hypoxia.[42] In rare cases, *DIO3* is expressed in infantile haemangioma to such an extent that it inactivates T_3 at a faster rate than the hormone can be produced (consumptive hypothyroidism).[85] Similarly, treatment of patients who have cancer with the tyrosine kinase inhibitors imatinib and sunitinib is associated with the development of hypothyroidism, which seems to be the result of marked overexpression of *DIO3* within the tumour cells.[86]

Hypothyroidism is an important example of the mass effect of the iodothyronine deiodinases changing their levels of activity in a coordinated fashion. As a result of low thyroid hormone levels, the activity of DIO2 is accelerated in almost all tissues, increasing the whole-body fractional conversion of T_4 to T_3 that helps preserve serum levels of T_3 (the opposite is observed during hyperthyroidism).[87] In addition, *DIO3* is a T_3-responsive gene and thus T_3 clearance is reduced in hypothyroidism. These coordinated and reciprocal enzyme responses explain why measuring serum concentrations of T_3 is of little diagnostic value in patients with hypothyroidism.

The DIO2-adaptive response to hypothyroidism is possible because of the unique sensitivity of this enzyme to T_4, its natural substrate. DIO2 has a short half-life (~60 min) that becomes even shorter (20 min) as a result of interacting with T_4 and/or its catalytic activity.[88,89] In other words, by converting T_4 to T_3, DIO2 is inactivated and degraded. This mechanism is explained by an 18-amino acid instability loop unique to the DIO2 protein that is located adjacent to its globular catalytic domain.[71] This loop mediates binding to the hedgehog-inducible protein WD repeat and SOCS box-containing protein 1 (WSB-1), a ubiquitin ligase adaptor that mediates DIO2 ubiquitination and targeting for proteasomal degradation.[90] Truncation analyses identified a core of six amino acids within the loop as the minimal requirements critical for recognition of DIO2 by

WSB-1.[91] The loop explains the short half-life of DIO2, a characteristic that can be transferred between proteins if the loop is fused to an otherwise stable protein.[33]

As with the other iodothyronine deiodinases, DIO2 exists as a dimer that is maintained by interacting surfaces within its transmembrane and globular cytosolic domains.[92] Upon binding T_4, DIO2 is ubiquitinated with ubiquitin chains formed at the Lys48 position,[93] which in turn inactivates the enzyme by interfering with globular interacting surfaces critical for dimerization and catalytic activity.[90] This inactive state can be transient or permanent, depending on whether ubiquitinated DIO2 is reactivated by DIO2-interacting deubiquitinases (such as ubiquitin specific peptidase 33),[94] or retrotranslocated to the cytoplasm via the p97–ATPase complex and delivered to the proteasomes.[93] The continuous association of DIO2 with this regulatory protein complex supports rapid cycles of deiodination, conjugation to ubiquitin and enzyme reactivation by deubiquitination, which enables tight control of thyroid-hormone action (Figure 2).

Treatment of hypothyroidism

Limitations of levothyroxine monotherapy

Unique aspects of hypothalamic DIO2 define limitations of levothyroxine replacement therapy and the use of TSH as a therapeutic goal. Circulating T_4 and T_3 exert negative feedback on the secretion of TRH and TSH.[76] In rodents, Tsh-secreting cells co-express *Dio2*;[95] in the rodent hypothalamus, *Dio2* is expressed in specialized glial cells known as tanycytes (Figure 2). These cells are located in the mediobasal hypothalamus, as well as on the floor and infralateral wall of the third ventricle. Tanycytes are distributed from the end of the optic chiasm, along the mammillary recess, and their processes also reach the median eminence outside the blood–brain barrier.[64,65,76] The presence of DIO2 in both locations is critical for T_4-mediated negative feedback.[28,96] In the pituitary gland, T_4 must be converted to T_3 to suppress secretion of TSH. In the hypothalamus, T_3 generation via DIO2 in tanycytes is likely to negatively affect TRH expression in the paraventricular nucleus.[97] However, the full extent of the function of DIO2 at both sites is only just starting to be appreciated.[17] Given that DIO2 activity is increased in most tissues during hypothyroidism and decreased in hyperthyroidism (via ubiquitination), its presence in the mediobasal hypothalamus and thyrotropic cells would seem to be counterproductive to the feedback mechanism. Thus, for many years it remained unclear how fluctuations in plasma levels of T_4 could be faithfully transduced in the nuclei of thyrotropic cells and hypophysiotropic TRH neurons in the hypothalamic paraventricular nucleus to ultimately regulate serum concentrations of TSH.[95]

The key new element in this feedback mechanism is the observation that rat hypothalamic Dio2 is stable and largely refractory to the levels of thyroid hormones.[17] In contrast to the rest of the body, DIO2-mediated T_4-to-T_3 conversion in the hypothalamus is not accelerated during hypothyroidism nor is it diminished by hyperthyroidism or administration of thyroid hormones. Indeed, a sensitivity gradient exists in the DIO2 response to hypothyroidism and thyroid hormones between the hypothalamus and the rest of the brain and body,[17] which is similar to previous observations in rodent thyrotropic cells.[95] The corollary of these experiments is that the secretion of Trh and Tsh in rodents is controlled by a steady process of Dio2-mediated T_4-to-T_3 conversion, thus transducing minor changes in serum levels of T_4 (Figure 2). By contrast, the rate of Dio2-mediated T_4-to-T_3 conversion in the rest of the body progressively decreases with administration of thyroid hormones because of Dio2 ubiquitination, so that peripheral T_3 production in levothyroxine-treated hypothyroid rats is progressively decreased and self-limiting. As a result, the dose of levothyroxine required to normalize serum concentrations of TSH is lower than the dose that normalizes serum levels of T_3. Given that the rat model reproduces the findings observed among patients treated with levothyroxine, similar pathways in humans are expected to provide the mechanistic basis for the observation of an increased ratio of serum T_4 to T_3 in the setting of normalized serum levels of TSH that is frequently observed among patients treated with levothyroxine.[12]

Differences in hypothalamic DIO2 susceptibility to ubiquitination explain localized sensitivity to levothyroxine.[17] Both *in vivo* studies in mice harbouring astrocyte-specific inactivation of *Wsb1* and *in vitro* analysis of DIO2 ubiquitination induced by different tissue extracts indicated that DIO2 ubiquitination in the hypothalamus is lower or that deubiquitination is faster than in other tissues.[17] As a result, in contrast to other DIO2-expressing tissues, the hypothalamus is wired to have increased sensitivity to T_4.

Hypothalamic DIO2 is also sensitive to other stimuli that are involved in the hypothalamic–pituitary–thyroid axis, including nutritional and inflammatory signals.[76] The regulation of DIO2 activity in tanycytes, therefore, also integrates other signals that override the input provided by circulating levels of thyroid hormone. For example, in a rodent model of lipopolysaccharide-induced nonthyroidal illness, Dio2 is upregulated in tanycytes, which results in increased local thyroid-hormone signalling that in turn suppresses TRH expression despite falling serum levels of thyroid hormone.[98,99] Notably, lipopolysaccharide-mediated downregulation of Trh does not occur in mice with global inactivation of *Dio2*, which indicates that this pathway is required for this process.[70] Studies in transgenic mice suggest that mice without appreciable *Dio2* expression in astrocytes, but with an intact tanycyte Dio2 pathway, can efficiently regulate their hypothalamic–pituitary–thyroid axis.[28]

Normalization of T_3 levels in hypothyroidism

Many patients receiving levothyroxine do not achieve serum levels of T_3 within the normal range and have raised serum levels of T_4, which results in a high serum T_4:T_3 ratio.[12,100,101] Although this phenomenon has been recognized for many decades, clinicians generally adopted the hypothesis that iodothyronine deiodinases would appropriately regulate the pool of available T_3 at the cellular and tissue level, thus leaving patients treated with levothyroxine euthyroid. However, some

investigators have questioned whether a low serum level of T_3 and/or a high T_4:T_3 ratio could have clinical implications. Specifically, whether this situation causes the residual symptoms experienced by a minority of patients treated with levothyroxine.[9,102]

The clinical data remain contentious.[15,18,19] However, studies in rats indicate that levothyroxine administration alone does not normalize serum Tsh, serum T_3 and tissue T_3 levels at the same time,[18] and that a serum Tsh level within the normal range coexists with reduced serum and tissue T_3 levels. These issues have been confirmed in an animal study in which thyroidectomized rats receiving levothyroxine monotherapy exhibited low levels of serum T_3 and a high T_4:T_3 ratio.[17] The novelty of this study was not only that these abnormalities in systemic parameters were confirmed but also that tissue-specific markers of hypothyroidism were evaluated. Mitochondrial content and α-glycerophosphate dehydrogenase activity—both known markers of T_3-responsiveness in the liver and skeletal muscle—were normalized in rats receiving levothyroxine plus liothyronine combination therapy but not in those treated with levothyroxine alone.[17] In addition, serum levels of cholesterol were normalized in rats receiving combination therapy but not in those receiving levothyroxine monotherapy.

Therefore, in the rodent model, levothyroxine replacement therapy that results in a high ratio of T_4 to T_3 and low serum levels of T_3 seem to exert consequences on markers of hypothyroidism at the tissue level (liver and skeletal muscle). The application of these findings to human studies will be important to determine whether patients treated with levothyroxine experience tissue-specific hypothyroidism and, if so, whether this effect can be reversed with combination therapy.

Thyroid-hormone homeostasis in the brain

The plasma contributes approximately half of the T_3 present in the rodent brain, with the remainder produced locally via deiodination of T_4;[61] therefore, a drop in serum levels of T_4 or T_3 could negatively affect thyroid-hormone signalling in the brain. However, this effect is largely minimized by the homeostatic actions of the iodothyronine deiodinases.[63] Accordingly, normalization of T_3 levels in the cerebral cortex, but not in the cerebellum, in levothyroxine-treated thyroidectomized rats is achieved when circulating levels of T_4 and T_3 are half that of euthyroid control animals that did not undergo thyroidectomy.[18] In addition, levels of T_3 in the cerebral cortex remain within the normal range, despite an up to 20-fold increase in the levothyroxine infusion doses.

These landmark studies provide the rationale for very tight control of thyroid hormone actions in the brain. These findings suggest that thyroid-hormone signalling is preserved in the brains of patients with mild hypothyroidism. However, the different behaviour of rat cerebral cortex and cerebellum confirm that thyroid-hormone signalling in the brain is highly compartmentalized (that is, not all brain areas behave in the same way). To address this issue, the action of thyroid hormone was studied using 16 genetic markers (such as *ENPP2*) of T_3-responsiveness in the cerebral cortex, cerebellum and hippocampus of thyroidectomized rats treated with either levothyroxine monotherapy or levothyroxine plus liothyronine combination therapy at doses that normalize serum levels of Tsh.[17] Expression of all genetic markers returned to normal levels in thyroidectomized rats receiving combination therapy, whereas 10 markers failed to be normalized in those receiving monotherapy, which is indicative of cerebral hypothyroidism.[17] The animals receiving monotherapy had reduced serum levels of T_3, which might explain some of these observations. An additional explanation is that the reduction of Dio2 activity in the brain observed in levothyroxine-treated animals (caused by the raised serum T_4:T_3 ratio) could lead to decreased T_3 production via Dio2 and add to the localized hypothyroidism.[17]

These findings challenge the idea that the impact of reduced DIO2 activity on T_3 production following exposure to T_4 is compensated for by increased substrate availability, thus preserving or even increasing T_3 production. Although logical, this rationale might not apply to all DIO2-expressing tissue or cell types given that in a cell system in which conversion of T_4 to T_3 via DIO2 was monitored while cells were exposed to progressively higher T_4 levels, T_3 production was lower than the T_4 concentration in the medium only to a limited extent.[95] This change was followed by an inflection point at which further increases in T_4 concentration reduced DIO2 activity, and T_3 production fell abruptly; the level of T_4 at the inflection point depends on the cell type, presumably because of differences in DIO2 ubiquitination.[95] This finding is supported by the observations that Dio2 activity in mice with brain-specific inactivation of *Wsb-1* respond differently to T_4 administration across different brain areas.[17] For example, Wsb-1 mediates Dio2 inactivation in response to levothyroxine administration in brain areas such as the cerebral cortex and hippocampus, whereas in the cerebellum, Wsb-1 does not mediate loss of Dio2 activity. Furthermore, levothyroxine-induced Dio2 inactivation is minimal (almost nonexistent), in the hypothalamus.[17]

Mood disorders and cognition

To what extent do the mechanisms mediated by iodothyronine deiodinase that regulate thyroid-hormone signalling underpin clinical phenotypes observed in patients with hypothyroidism? These patients have variable degrees of cognitive dysfunction, lethargy, poor motor coordination, memory impairment, depression and mood disorders. In addition, the efficacy of antidepressant agents among euthyroid individuals is potentiated when associated with the administration of thyroid hormone, either levothyroxine or liothyronine.[103,104] In some clinical studies, but not all,[105] a residual cognitive dysfunction exists in patients with hypothyroidism who are treated with levothyroxine.[9,53,106,107] Whether these residual symptoms are a consequence of low serum levels of T_3, or a high T_4 to T_3 ratio,[108,109] remains to be determined. In addition, whether combination therapy with levothyroxine and liothyronine can restore cognitive function is an interesting hypothesis that needs to be tested.

Some evidence suggests that thyroid hormones act in the brain via serotonergic pathways, a system involved in the pathogenesis of affective disorders and response to psychotropic agents.[110] Arising from discrete brainstem nuclei, serotonin circuits project to cortical and subcortical brain areas, including the prefrontal cortex, hippocampus and amygdala, which explains their influence on behaviours such as mood, sleep and appetite.[111] The use of MRI spectroscopy and PET has confirmed that thyroid hormones act in the human limbic system, with substantial differences observed in the posterior cingulate cortex and the inferior parietal lobe of hypothyroid versus hyperthyroid individuals.[112,113]

Considering that iodothyronine deiodinases modify thyroid-hormone signalling in the rodent brain,[114] it seems logical to assume that the activity of these enzymes might affect mood and/or have a role in the variability of the response of patients with depression to T_3. In animal models, Dio2 activity in the brain is increased by antidepressants of various classes, including selective serotonin reuptake inhibitors.[115–117] In addition, hippocampal expression of Dio2 and Dio3 is increased in a mouse model that is resistant to stress-induced depression and after antidepressant treatment in mice.[108] However, a mouse with global inactivation of *Dio2* exhibits only a mild motor phenotype and no behavioural phenotype.[61,118] By contrast, a mouse model with global inactivation of *Dio3* exhibits brain hyperthyroidism, aggressive behaviour and indifference towards pups.[119] Furthermore, a negative correlation exists between *Dio3* expression and T_3 levels in the frontal cortex and hippocampus of a mouse model of depressive behaviour and cognitive deficits, which is partially restored by administration of levothyroxine.[120–122]

The available clinical data are inconsistent for DIO2 and DIO3 but strong for DIO1, which is expressed at low levels in the human brain.[24,79] A study of 1,447 individuals enrolled from the general population indicated that a polymorphism in *DIO1*, called C785T, is associated with the lifetime risk of depression in white female individuals in high-risk cohorts[123] and with the antidepressant efficacy of T_3 in a trial of the selective serotonin reuptake inhibitor sertraline.[124]

Genetic risk and brain disease

A prevalent single nucleotide polymorphism in the *DIO2* gene (which results in a single amino acid substitution of Thr for Ala at position 92 in DIO2, thus called Thr92AlaD2) has been identified,[125] with estimates suggesting that ~12–36% of individuals in the general population are homozygous. This variant has been associated with metabolic derangements, including insulin resistance[125,126] and type 2 diabetes mellitus.[127] Furthermore, this polymorphism has been implicated in mental and psychological disorders,[45] such as mental retardation,[128] bipolar disorder[129] and low IQ.[130] Conversely, Thr92AlaD2 might confer protection from recurrent depression.[131] The clinical relevance of Thr92AlaD2 was strengthened in a large clinical trial, in which the substitution was correlated with reduced well-being and a preference for levothyroxine plus liothyronine combination therapy versus levothyroxine monotherapy among carriers with hypothyroidism.[13]

These findings have stimulated much intrigue in the field with most initial hypotheses focusing on a possible defect in thyroid-hormone signalling. However, the single amino acid substitution associated with Thr92AlaD2 does not alter the enzyme kinetics of DIO2 when transiently expressed in cells[132,133] and only indirect evidence suggests that the conversion of T_4 to T_3 via Thr92AlaD2 could be reduced in patients.[134,135] Perhaps this observation is not unexpected given that the mutation lies not within the catalytic site but rather within the 18-amino acid loop that controls susceptibility to T_4-induced ubiquitination of DIO2.[31] The putative functional abnormality associated with expression of the variant protein was defined in studies performed in a human embryonic kidney cell line that had been engineered to stably express this mutation. The mutated protein has a longer half-life than the native form and is ectopically located in the Golgi apparatus.[35] This aberrant subcellular localization was associated with abnormal Golgi structure, such that circular (rather than linear or ribbon-like) stacks of Golgi membrane were observed. These cellular abnormalities resulted in alterations at the transcriptional level that were independent of DIO2-mediated T_3 production given that the cell line studied does not express TRs[136] and that the observed gene expression pattern lacked typical indicators of T_3-responsiveness.[114] Remarkably, when human cerebral cortex samples from patients harbouring the polymorphism were studied, overlap in the expression of 81 genes was found, which defines a molecular fingerprint associated with the Thr92AlaD2 variant across these human cell and brain models.[35]

In addition, robust analysis of microarray data derived from human brain samples demonstrated a gene expression profile reminiscent of that of neurological diseases, particularly the gene expression profile associated with Huntington disease.[35] This finding implies that the Thr92AlaD2 polymorphism is a novel risk factor for neurodegenerative disease or impaired cognition, which might explain the neuropsychological impairment of some patients; however, further studies are needed before definite conclusions can be drawn.[35] In particular, elucidating whether the high T_4:T_3 ratio observed in patients with hypothyroidism who are treated with levothyroxine[12,100] might perturb the cellular abnormalities associated with expression of the variant protein will be important. This relationship could explain the improved well-being and preference for combination therapy recorded in a subgroup of patients, as no evidence of reduced thyroid-hormone signalling in the samples was found (T_3-independent).[13]

These findings represent a step towards determining the relationship between the cellular abnormalities associated with variant protein expression and the associated clinical phenotype. Future studies are needed to fully characterize the molecular basis for the clinical observations. Nevertheless, clinical trials among this subgroup of patients could be warranted to determine whether patients

with hypothyroidism who carry the polymorphism would benefit from alternative therapeutic strategies. If it can be rigorously demonstrated that such patients derive benefit from combination therapy, then genotyping for Thr92AlaD2 could become a routine component of hypothyroidism management, thus bringing the concept of personalized medicine to the field.

Conclusions

Major advances have been made in the treatment of hypothyroidism over the past century; however, refined therapeutic regimens might still be needed to ensure that all patients can become asymptomatic and clinically euthyroid. Once heralded as the ultimate regulators of thyroid-hormone availability and the key to levothyroxine treatment efficacy, iodothyronine deiodinases might actually underpin the inability to normalize serum levels of TSH and T_3 in patients receiving levothyroxine monotherapy and the insufficient symptomatic response experienced by an appreciable proportion of patients with hypothyroidism. Future studies exploring the role of the iodothyronine deiodinases in hypothyroidism hold real promise for determining therapeutic regimens that can normalize systemic and tissue-level parameters for all patients with hypothyroidism, including new approaches that might even be genotype-directed.

1. Aoki, Y. *et al.* Serum TSH and total T_4 in the United States population and their association with participant characteristics: National Health and Nutrition Examination Survey (NHANES 1999–2002). *Thyroid* **17**, 1211–1223 (2007).
2. Vanderpump, M. P. The epidemiology of thyroid disease. *Br. Med. Bull.* **99**, 39–51 (2011).
3. Lindholm, J. & Laurberg, P. Hypothyroidism and thyroid substitution: historical aspects. *J. Thyroid Res.* **2011**, 809341 (2011).
4. Braverman, L. E., Ingbar, S. H. & Sterling, K. Conversion of thyroxine (T_4) to triiodothyronine (T_3) in athyreotic subjects. *J. Clin. Invest.* **49**, 855–864 (1970).
5. Larsen, P. R. & Ingbar, S. in *Textbook of Endocrinology* (eds Wilson, J. D. *et al.*) 357–487 (W. B. Saunders, Co., 1992).
6. Garber, J. R. *et al.* Clinical practice guidelines for hypothyroidism in adults: cosponsored by the American Association of Clinical Endocrinologists and the American Thyroid Association. *Endocr. Pract.* **18**, 988–1028 (2012).
7. Taylor, S., Kapur, M. & Adie, R. Combined thyroxine and triiodothyronine for thyroid replacement therapy. *Br. Med. J.* **2**, 270–271 (1970).
8. Roberts, N. D. Psychological problems in thyroid disease. *British Thyroid Foundation Newsletter* **18**, 3 (1996).
9. Saravanan, P. *et al.* Psychological well-being in patients on 'adequate' doses of l-thyroxine: results of a large, controlled community-based questionnaire study. *Clin. Endocrinol.* **57**, 577–585 (2002).
10. Gorman, C. A., Jiang, N. S., Ellefson, R. D. & Elveback, L. R. Comparative effectiveness of dextrothyroxine and levothyroxine in correcting hypothyroidism and lowering blood lipid levels in hypothyroid patients. *J. Clin. Endocrinol. Metab.* **49**, 1–7 (1979).
11. Franklyn, J. A. *et al.* Thyroxine replacement therapy and circulating lipid concentrations. *Clin. Endocrinol.* **38**, 453–459 (1993).
12. Gullo, D. *et al.* Levothyroxine monotherapy cannot guarantee euthyroidism in all athyreotic patients. *PLoS ONE* **6**, e22552 (2011).
13. Panicker, V. *et al.* Common variation in the *DIO2* gene predicts baseline psychological well-being and response to combination thyroxine plus triiodothyronine therapy in hypothyroid patients. *J. Clin. Endocrinol. Metab.* **94**, 1623–1629 (2009).
14. Bunevicius, R., Kazanavicius, G., Zalinkevicius, R. & Prange, A. J. Jr. Effects of thyroxine as compared with thyroxine plus triiodothyronine in patients with hypothyroidism. *N. Engl. J. Med.* **340**, 424–429 (1999).
15. Escobar-Morreale, H. F. *et al.* Thyroid hormone replacement therapy in primary hypothyroidism: a randomized trial comparing L-thyroxine plus liothyronine with L-thyroxine alone. *Ann. Intern. Med.* **142**, 412–424 (2005).
16. Jonklaas, J. *et al.* Guidelines for the treatment of hypothyroidism: prepared by the American Thyroid Association task force on thyroid hormone replacement. *Thyroid* **24**, 1670–1751 (2014).
17. Werneck de Castro, J. P. *et al.* Differences in hypothalamic type 2 deiodinase ubiquitination explain localized sensitivity to thyroxine. *J. Clin. Invest.* **125**, 769–781 (2015).
18. Escobar-Morreale, H. F., Obregon, M. J., Escobar del Rey, F. & Morreale de Escobar, G. Replacement therapy for hypothyroidism with thyroxine alone does not ensure euthyroidism in all tissues, as studied in thyroidectomized rats. *J. Clin. Invest.* **96**, 2828–2838 (1995).
19. Escobar-Morreale, H. F., Rey, F., Obregon, M. J. & Escobar, G. M. Only the combined treatment with thyroxine and triiodothyronine ensures euthyroidism in all tissues of the thyroidectomized rat. *Endocrinology* **137**, 2490–2502 (1996).
20. Escobar-Morreale, H. F., Obregon, M. J., Hernandez, A., Escobar del Rey, F. & Morreale de Escobar, G. Regulation of iodothyronine deiodinase activity as studied in thyroidectomized rats infused with thyroxine or triiodothyronine. *Endocrinology* **138**, 2559–2568 (1997).
21. Bianco, A. C. *et al.* American Thyroid Association guide to investigating thyroid hormone economy and action in rodent and cell models. *Thyroid* **24**, 88–168 (2014).
22. Gereben, B., Salvatore, D., Harney, J. W., Tu, H. M. & Larsen, P. R. The human, but not rat, *Dio2* gene is stimulated by thyroid transcription factor-1 (TTF-1). *Mol. Endocrinol.* **15**, 112–124 (2001).
23. Salvatore, D., Tu, H., Harney, J. W. & Larsen, P. R. Type 2 iodothyronine deiodinase is highly expressed in human thyroid. *J. Clin. Invest.* **98**, 962–968 (1996).
24. Campos-Barros, A. *et al.* Phenolic and tyrosyl ring iodothyronine deiodination and thyroid hormone concentrations in the human central nervous system. *J. Clin. Endocrinol. Metab.* **81**, 2179–2185 (1996).
25. Visser, T. J., Leonard, J. L., Kaplan, M. M. & Larsen, P. R. Kinetic evidence suggesting two mechanisms for iodothyronine 5'-deiodination in rat cerebral cortex. *Proc. Natl Acad. Sci. USA* **79**, 5080–5084 (1982).
26. Christoffolete, M. A. *et al.* Mice with impaired extrathyroidal thyroxine to 3,5,3'-triiodothyronine conversion maintain normal serum 3,5,3'-triiodothyronine concentrations. *Endocrinology* **148**, 954–960 (2007).
27. Fonseca, T. L. *et al.* Tissue-specific inactivation of type 2 deiodinase reveals multilevel control of fatty acid oxidation by thyroid hormone in the mouse. *Diabetes* **63**, 1594–1604 (2014).
28. Fonseca, T. L. *et al.* Coordination of hypothalamic and pituitary T_3 production regulates TSH expression. *J. Clin. Invest.* **123**, 1492–1500 (2013).
29. Brent, G. A. Mechanisms of thyroid hormone action. *J. Clin. Invest.* **122**, 3035–3043 (2012).
30. Heuer, H. & Visser, T. J. The pathophysiological consequences of thyroid hormone transporter deficiencies: Insights from mouse models. *Biochim. Biophys. Acta* **1830**, 3974–3978 (2013).
31. Callebaut, I. *et al.* The iodothyronine selenodeiodinases are thioredoxin-fold family proteins containing a glycoside hydrolase clan GH-A-like structure. *J. Biol. Chem.* **278**, 36887–36896 (2003).
32. Schweizer, U., Schlicker, C., Braun, D., Kohrle, J. & Steegborn, C. Crystal structure of mammalian selenocysteine-dependent iodothyronine deiodinase suggests a peroxiredoxin-like catalytic mechanism. *Proc. Natl Acad. Sci. USA* **111**, 10526–10531 (2014).
33. Zeold, A. *et al.* Metabolic instability of type 2 deiodinase is transferable to stable proteins independently of subcellular localization. *J. Biol. Chem.* **281**, 31538–31543 (2006).
34. Baqui, M. M., Gereben, B., Harney, J. W., Larsen, P. R. & Bianco, A. C. Distinct subcellular localization of transiently expressed types 1 and 2 iodothyronine deiodinases as determined by immunofluorescence confocal microscopy. *Endocrinology* **141**, 4309–4312 (2000).
35. McAninch, E. A. *et al.* Prevalent polymorphism in thyroid hormone-activating enzyme leaves a genetic fingerprint that underlies associated clinical syndromes. *J. Clin. Endocrinol. Metab.* **100**, 920–933 (2015).
36. Schneider, M. J. *et al.* Targeted disruption of the type 1 selenodeiodinase gene (*Dio1*) results in marked changes in thyroid hormone economy in mice. *Endocrinology* **147**, 580–589 (2006).
37. Baqui, M. *et al.* Human type 3 iodothyronine selenodeiodinase is located in the plasma membrane and undergoes rapid internalization to endosomes. *J. Biol. Chem.* **278**, 1206–1211 (2003).
38. Kallo, I. *et al.* A novel pathway regulates thyroid hormone availability in rat and human hypothalamic neurosecretory neurons. *PLoS ONE* **7**, e37860 (2012).
39. Jo, S. *et al.* Neuronal hypoxia induces hsp40-mediated nuclear import of type 3 deiodinase as an adaptive mechanism to reduce cellular metabolism. *J. Neurosci.* **32**, 8491–8500 (2012).

40. Gereben, B. *et al.* Cellular and molecular basis of deiodinase-regulated thyroid hormone signaling. *Endocr. Rev.* **29**, 898–938 (2008).
41. Bianco, A. C. & McAninch, E. A. The role of thyroid hormone and brown adipose tissue in energy homoeostasis. *Lancet Diabetes Endocrinol.* **1**, 250–258 (2013).
42. Peeters, R. P. *et al.* Reduced activation and increased inactivation of thyroid hormone in tissues of critically ill patients. *J. Clin. Endocrinol. Metab.* **88**, 3202–3211 (2003).
43. Simonides, W. S. *et al.* Hypoxia-inducible factor induces local thyroid hormone inactivation during hypoxic-ischemic disease in rats. *J. Clin. Invest.* **118**, 975–983 (2008).
44. Olivares, E. L. *et al.* Thyroid function disturbance and type 3 iodothyronine deiodinase induction after myocardial infarction in rats a time course study. *Endocrinology* **148**, 4786–4792 (2007).
45. Bianco, A. C. & Casula, S. Thyroid hormone replacement therapy: three 'simple' questions, complex answers. *Eur. Thyroid J.* **1**, 88–98 (2012).
46. Dumitrescu, A. M. *et al.* Mutations in *SECISBP2* result in abnormal thyroid hormone metabolism. *Nat. Genet.* **37**, 1247–1252 (2005).
47. Ord, W. M. Report of the committee of the Clinical Society of London nominated December 14, 1883, to investigate the subject of myxoedema. *Trans. Clin. Soc. Lond.* **21** (Suppl.), 1–215 (1888).
48. Oppenheimer, J. H. & Schwartz, H. L. Molecular basis of thyroid hormone-dependent brain development. *Endocr. Rev.* **18**, 462–475 (1997).
49. Obregon, M. J., Escobar del Rey, F. & Morreale de Escobar, G. The effects of iodine deficiency on thyroid hormone deiodination. *Thyroid* **15**, 917–929 (2005).
50. Desouza, L. A. *et al.* Thyroid hormone regulates hippocampal neurogenesis in the adult rat brain. *Mol. Cell. Neurosci.* **29**, 414–426 (2005).
51. Bernal, J. Thyroid hormone receptors in brain development and function. *Nat. Clin. Pract. Endocrinol. Metab.* **3**, 249–259 (2007).
52. Morte, B. & Bernal, J. Thyroid hormone action: astrocyte-neuron communication. *Front. Endocrinol.* **5**, 82 (2014).
53. Greenberg, J. H. *et al.* Imaging triiodothyronine binding kinetics in rat brain: a model for studies in human subjects. *Synapse* **60**, 212–222 (2006).
54. Visser, T. J. Thyroid hormone transporters. *Horm. Res.* **68** (Suppl. 5), 28–30 (2007).
55. Ceballos, A. *et al.* Importance of monocarboxylate transporter 8 for the blood-brain barrier-dependent availability of 3,5,3'-triiodo-L-thyronine. *Endocrinology* **150**, 2491–2496 (2009).
56. Liao, X. H. *et al.* Distinct roles of deiodinases on the phenotype of Mct8 defect: a comparison of eight different mouse genotypes. *Endocrinology* **152**, 1180–1191 (2011).
57. Wittmann, G. *et al.* Parallel regulation of thyroid hormone transporters OATP1c1 and MCT8 during and after endotoxemia at the blood-brain barrier of male rodents. *Endocrinology* **156**, 1552–1564 (2015).
58. Friesema, E. C. *et al.* Association between mutations in a thyroid hormone transporter and severe X-linked psychomotor retardation. *Lancet* **364**, 1435–1437 (2004).
59. Dumitrescu, A. M., Liao, X. H., Best, T. B., Brockmann, K. & Refetoff, S. A novel syndrome combining thyroid and neurological abnormalities is associated with mutations in a monocarboxylate transporter gene. *Am. J. Hum. Genet.* **74**, 168–175 (2004).
60. Crantz, F. R., Silva, J. E. & Larsen, P. R. Analysis of the sources and quantity of 3,5,3'-triiodothyronine specifically bound to nuclear receptors in rat cerebral cortex and cerebellum. *Endocrinology* **110**, 367–375 (1982).
61. Galton, V. A. *et al.* Thyroid hormone homeostasis and action in the type 2 deiodinase-deficient rodent brain during development. *Endocrinology* **148**, 3080–3088 (2007).
62. Silva, J. E. & Matthews, P. S. Production rates and turnover of triiodothyronine in rat-developing cerebral cortex and cerebellum: responses to hypothyroidism. *J. Clin. Invest.* **74**, 1035–1049 (1984).
63. Peeters, R. *et al.* Regional physiological adaptation of the central nervous system deiodinases to iodine deficiency. *Am. J. Physiol. Endocrinol. Metab.* **281**, E54–E61 (2001).
64. Guadano-Ferraz, A., Obregon, M. J., St Germain, D. L. & Bernal, J. The type 2 iodothyronine deiodinase is expressed primarily in glial cells in the neonatal rat brain. *Proc. Natl Acad. Sci. USA* **94**, 10391–10396 (1997).
65. Tu, H. M. *et al.* Regional distribution of type 2 thyroxine deiodinase messenger ribonucleic acid in rat hypothalamus and pituitary and its regulation by thyroid hormone. *Endocrinology* **138**, 3359–3368 (1997).
66. Mohacsik, P., Zeold, A., Bianco, A. C. & Gereben, B. Thyroid hormone and the neuroglia: both source and target. *J. Thyroid Res.* **2011**, 215718 (2011).
67. Cahoy, J. D. *et al.* A transcriptome database for astrocytes, neurons, and oligodendrocytes: a new resource for understanding brain development and function. *J. Neurosci.* **28**, 264–278 (2008).
68. Bernal, J., Guadano-Ferraz, A. & Morte, B. Perspectives in the study of thyroid hormone action on brain development and function. *Thyroid* **13**, 1005–1012 (2003).
69. Visser, W. E., Friesema, E. C., Jansen, J. & Visser, T. J. Thyroid hormone transport by monocarboxylate transporters. *Best Pract. Res. Clin. Endocrinol. Metab.* **21**, 223–236 (2007).
70. Freitas, B. C. *et al.* Paracrine signaling by glial cell-derived triiodothyronine activates neuronal gene expression in the rodent brain and human cells. *J. Clin. Invest.* **120**, 2206–2217 (2010).
71. Dentice, M. *et al.* The Hedgehog-inducible ubiquitin ligase subunit WSB-1 modulates thyroid hormone activation and PTHrP secretion in the developing growth plate. *Nat. Cell Biol.* **7**, 698–705 (2005).
72. Dentice, M. *et al.* Sonic hedgehog-induced type 3 deiodinase blocks thyroid hormone action enhancing proliferation of normal and malignant keratinocytes. *Proc. Natl Acad. Sci. USA* **104**, 14466–14471 (2007).
73. Fliers, E., Alkemade, A., Wiersinga, W. M. & Swaab, D. F. Hypothalamic thyroid hormone feedback in health and disease. *Prog. Brain Res.* **153**, 189–207 (2006).
74. Yoshimura, T. *et al.* Light-induced hormone conversion of T_4 to T_3 regulates photoperiodic response of gonads in birds. *Nature* **426**, 178–181 (2003).
75. Campos-Barros, A. *et al.* Type 2 iodothyronine deiodinase expression in the cochlea before the onset of hearing. *Proc. Natl Acad. Sci. USA* **97**, 1287–1292 (2000).
76. Fekete, C. & Lechan, R. M. Central regulation of hypothalamic–pituitary–thyroid axis under physiological and pathophysiological conditions. *Endocr. Rev.* **35**, 159–194 (2014).
77. Friesema, E. C. *et al.* Thyroid hormone transporters and deiodinases in the developing human hypothalamus. *Eur. J. Endocrinol.* **167**, 379–386 (2012).
78. Ng, L. *et al.* Type 3 deiodinase, a thyroid-hormone-inactivating enzyme, controls survival and maturation of cone photoreceptors. *J. Neurosci.* **30**, 3347–3357 (2010).
79. Kester, M. H. *et al.* Iodothyronine levels in the human developing brain: major regulatory roles of iodothyronine deiodinases in different areas. *J. Clin. Endocrinol. Metab.* **89**, 3117–3128 (2004).
80. Vose, L. R. *et al.* Treatment with thyroxine restores myelination and clinical recovery after intraventricular hemorrhage. *J. Neurosci.* **33**, 17232–17246 (2013).
81. Zavacki, A. M. *et al.* Type 1 iodothyronine deiodinase is a sensitive marker of peripheral thyroid status in the mouse. *Endocrinology* **146**, 1568–1575 (2005).
82. Bianco, A. C., Salvatore, D., Gereben, B., Berry, M. J. & Larsen, P. R. Biochemistry, cellular and molecular biology, and physiological roles of the iodothyronine selenodeiodinases. *Endocr. Rev.* **23**, 38–89 (2002).
83. Roti, E., Fang, S. L., Green, K., Emerson, C. H. & Braverman, L. E. Human placenta is an active site of thyroxine and 3,3',5-triiodothyronine tyrosyl ring deiodination. *J. Clin. Endocrinol. Metab.* **53**, 498–501 (1981).
84. Alexander, E. K. *et al.* Timing and magnitude of increases in levothyroxine requirements during pregnancy in women with hypothyroidism. *N. Engl. J. Med.* **351**, 241–249 (2004).
85. Huang, S. A. *et al.* Severe hypothyroidism caused by type 3 iodothyronine deiodinase in infantile hemangiomas. *N. Engl. J. Med.* **343**, 185–189 (2000).
86. Abdalla, S. M. & Bianco, A. C. Defending plasma T_3 is a biological priority. *Clin. Endocrinol.* **81**, 633–641 (2014).
87. Bianco, A. C. & Kim, B. W. Deiodinases: implications of the local control of thyroid hormone action. *J. Clin. Invest.* **116**, 2571–2579 (2006).
88. Steinsapir, J., Harney, J. & Larsen, P. R. Type 2 iodothyronine deiodinase in rat pituitary tumor cells is inactivated in proteasomes. *J. Clin. Invest.* **102**, 1895–1899 (1998).
89. Gereben, B., Goncalves, C., Harney, J. W., Larsen, P. R. & Bianco, A. C. Selective proteolysis of human type 2 deiodinase: a novel ubiquitin-proteasomal mediated mechanism for regulation of hormone activation. *Mol. Endocrinol.* **14**, 1697–1708 (2000).
90. Sagar, G. D. *et al.* Ubiquitination-induced conformational change within the deiodinase dimer is a switch regulating enzyme activity. *Mol. Cell. Biol.* **27**, 4774–4783 (2007).
91. Egri, P. & Gereben, B. Minimal requirements for ubiquitination-mediated regulation of thyroid hormone activation. *J. Mol. Endocrinol.* **53**, 217–226 (2014).
92. Curcio-Morelli, C. *et al. In vivo* dimerization of types 1, 2, and 3 iodothyronine selenodeiodinases. *Endocrinology* **144**, 937–946 (2003).
93. Arrojo, E. D. R., Egri, P., Jo, S., Gereben, B. & Bianco, A. C. The type II deiodinase is retrotranslocated to the cytoplasm and proteasomes via p97/Atx3 complex. *Mol. Endocrinol.* **27**, 2105–2115 (2013).
94. Curcio-Morelli, C. *et al.* Deubiquitination of type 2 iodothyronine deiodinase by von Hippel-Lindau protein-interacting deubiquitinating enzymes regulates thyroid hormone activation. *J. Clin. Invest.* **112**, 189–196 (2003).

95. Christoffolete, M. A. *et al.* Atypical expression of type 2 iodothyronine deiodinase in thyrotrophs explains the thyroxine-mediated pituitary thyrotropin feedback mechanism. *Endocrinology* **147**, 1735–1743 (2006).
96. Schneider, M. J. *et al.* Targeted disruption of the type 2 selenodeiodinase gene (*DIO2*) results in a phenotype of pituitary resistance to T_4. *Mol. Endocrinol.* **15**, 2137–2148 (2001).
97. Fekete, C. & Lechan, R. M. Negative feedback regulation of hypophysiotropic thyrotropin-releasing hormone (TRH) synthesizing neurons: role of neuronal afferents and type 2 deiodinase. *Front. Neuroendocrinol.* **28**, 97–114 (2007).
98. Boelen, A. *et al.* Simultaneous changes in central and peripheral components of the hypothalamus–pituitary–thyroid axis in lipopolysaccharide-induced acute illness in mice. *J. Endocrinol.* **182**, 315–323 (2004).
99. Fekete, C. *et al.* Lipopolysaccharide induces type 2 iodothyronine deiodinase in the mediobasal hypothalamus: implications for the nonthyroidal illness syndrome. *Endocrinology* **145**, 1649–1655 (2004).
100. Sawin, C. T., Surks, M. I., London, M., Ranganathan, C. & Larsen, P. R. Oral thyroxine: variation in biologic action and tablet content. *Ann. Intern. Med.* **100**, 641–645 (1984).
101. Ito, M. *et al.* TSH-suppressive doses of levothyroxine are required to achieve preoperative native serum triiodothyronine levels in patients who have undergone total thyroidectomy. *Eur. J. Endocrinol.* **167**, 373–378 (2012).
102. Wiersinga, W. M., Duntas, L., Fadeyev, V., Nygaard, B. & Vanderpump, M. P. 2012 ETA Guidelines: The use of L-T_4 + L-T_3 in the treatment of hypothyroidism. *Eur. Thyroid J.* **1**, 55–71 (2012).
103. Williams, G. R. Neurodevelopmental and neurophysiological actions of thyroid hormone. *J. Neuroendocrinol.* **20**, 784–794 (2008).
104. Ritchie, M. & Yeap, B. B. Thyroid hormone: influences on mood and cognition in adults. *Maturitas* **81**, 266–275 (2015).
105. Parsaik, A. K. *et al.* Hypothyroidism and risk of mild cognitive impairment in elderly persons: a population-based study. *JAMA Neurol.* **71**, 201–207 (2014).
106. Wekking, E. M. *et al.* Cognitive functioning and well-being in euthyroid patients on thyroxine replacement therapy for primary hypothyroidism. *Eur. J. Endocrinol.* **153**, 747–753 (2005).
107. Nasr, A., Lauterio, T. J. & Davis, M. W. Unapproved drugs in the United States and the Food and Drug Administration. *Adv. Ther.* **28**, 842–856 (2011).
108. Markova, N. *et al.* Hippocampal gene expression of deiodinases 2 and 3 and effects of 3,5-diiodo-L-thyronine T_2 in mouse depression paradigms. *Biomed. Res. Int.* **2013**, 565218 (2013).
109. de Jong, F. J. *et al.* Thyroid function, the risk of dementia and neuropathologic changes: the Honolulu-Asia aging study. *Neurobiol. Aging* **30**, 600–606 (2009).
110. Bauer, M., Heinz, A. & Whybrow, P. C. Thyroid hormones, serotonin and mood: of synergy and significance in the adult brain. *Mol. Psychiatry* **7**, 140–156 (2002).
111. Morrissette, D. A. & Stahl, S. M. Modulating the serotonin system in the treatment of major depressive disorder. *CNS Spectr.* **19** (Suppl. 1), 57–67 (2014).
112. Zhang, Q. *et al.* Monitoring glutamate levels in the posterior cingulate cortex of thyroid dysfunction patients with TE-averaged PRESS at 3T. *Magn. Reson. Imaging* **33**, 774–778 (2015).
113. Schreckenberger, M. F. *et al.* Positron emission tomography reveals correlations between brain metabolism and mood changes in hyperthyroidism. *J. Clin. Endocrinol. Metab.* **91**, 4786–4791 (2006).
114. Hernandez, A., Morte, B., Belinchon, M. M., Ceballos, A. & Bernal, J. Critical role of types 2 and 3 deiodinases in the negative regulation of gene expression by T_3 in the mouse cerebral cortex. *Endocrinology* **153**, 2919–2928 (2012).
115. Eravci, M., Pinna, G., Meinhold, H. & Baumgartner, A. Effects of pharmacological and nonpharmacological treatments on thyroid hormone metabolism and concentrations in rat brain. *Endocrinology* **141**, 1027–1040 (2000).
116. Campos-Barros, A. *et al.* The influence of desipramine on thyroid hormone metabolism in rat brain. *J Pharmacol. Exp. Ther.* **268**, 1143–1152 (1994).
117. Baumgartner, A., Dubeyko, M., Campos-Barros, A., Eravci, M. & Meinhold, H. Subchronic administration of fluoxetine to rats affects triiodothyronine production and deiodination in regions of the cortex and in the limbic forebrain. *Brain Res.* **635**, 68–74 (1994).
118. Barez-Lopez, S. *et al.* Abnormal motor phenotype at adult stages in mice lacking type 2 deiodinase. *PLoS ONE* **9**, e103857 (2014).
119. Hernandez, A., Martinez, M. E., Fiering, S., Galton, V. A. & St Germain, D. Type 3 deiodinase is critical for the maturation and function of the thyroid axis. *J. Clin. Invest.* **116**, 476–484 (2006).
120. Sittig, L. J., Shukla, P. K., Herzing, L. B. & Redei, E. E. Strain-specific vulnerability to alcohol exposure *in utero* via hippocampal parent-of-origin expression of deiodinase-III. *FASEB J.* **25**, 2313–2324 (2011).
121. Wilcoxon, J. S., Kuo, A. G., Disterhoft, J. F. & Redei, E. E. Behavioral deficits associated with fetal alcohol exposure are reversed by prenatal thyroid hormone treatment: a role for maternal thyroid hormone deficiency in FAE. *Mol. Psychiatry* **10**, 961–971 (2005).
122. Wilcoxon, J. S. & Redei, E. E. Prenatal programming of adult thyroid function by alcohol and thyroid hormones. *Am. J. Physiol. Endocrinol. Metab.* **287**, E318–E326 (2004).
123. Philibert, R. A. *et al.* The relationship of deiodinase 1 genotype and thyroid function to lifetime history of major depression in three independent populations. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* **156B**, 593–599 (2011).
124. Cooper-Kazaz, R. *et al.* Preliminary evidence that a functional polymorphism in type 1 deiodinase is associated with enhanced potentiation of the antidepressant effect of sertraline by triiodothyronine. *J. Affect. Disord.* **116**, 113–116 (2009).
125. Mentuccia, D. *et al.* 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 β-3-adrenergic receptor. *Diabetes* **51**, 880–883 (2002).
126. Estivalet, A. A. *et al.* D2 Thr92Ala and PPARγ2 Pro12Ala polymorphisms interact in the modulation of insulin resistance in type 2 diabetic patients. *Obesity (Silver Spring)* **19**, 825–832 (2010).
127. Dora, J. M., Machado, W. E., Rheinheimer, J., Crispim, D. & Maia, A. L. Association of the type 2 deiodinase Thr92Ala polymorphism with type 2 diabetes: case-control study and meta-analysis. *Eur. J. Endocrinol.* **163**, 427–434 (2010).
128. Guo, T. W. *et al.* Positive association of the DIO2 (deiodinase type 2) gene with mental retardation in the iodine-deficient areas of China. *J. Med. Genet.* **41**, 585–590 (2004).
129. He, B. *et al.* Association of genetic polymorphisms in the type II deiodinase gene with bipolar disorder in a subset of Chinese population. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **33**, 986–990 (2009).
130. Taylor, P. *et al.* Effect of low thyroid hormone bioavailability on childhood cognitive development: data from the Avon Longitudinal Study of Parents and Children birth cohort. *Lancet* **383**, S100 (2014).
131. Galecka, E. *et al.* Association of the *DIO2* gene single nucleotide polymorphisms with recurrent depressive disorder. *Acta Biochim. Pol.* **62**, 297–302 (2015).
132. Canani, L. H. *et al.* 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. *J. Clin. Endocrinol. Metab.* **90**, 3472–3478 (2005).
133. Peeters, R. P. *et al.* Polymorphisms in thyroid hormone pathway genes are associated with plasma TSH and iodothyronine levels in healthy subjects. *J. Clin. Endocrinol. Metab.* **88**, 2880–2888 (2003).
134. Torlontano, M. *et al.* Type 2 deiodinase polymorphism (threonine 92 alanine) predicts L-thyroxine dose to achieve target thyrotropin levels in thyroidectomized patients. *J. Clin. Endocrinol. Metab.* **93**, 910–913(2008).
135. Butler, P. W. *et al.* The Thr92Ala 5′ type 2 deiodinase gene polymorphism is associated with a delayed triiodothyronine secretion in response to the thyrotropin-releasing hormone-stimulation test: a pharmacogenomic study. *Thyroid* **20**, 1407–1412 (2011).
136. Zhang, C., Kim, S., Harney, J. W. & Larsen, P. R. Further characterization of thyroid hormone response elements in the human type 1 iodothyronine deiodinase gene. *Endocrinology* **139**, 1156–1163 (1998).

Acknowledgements

B.G. is supported by the Hungarian Brain Research Program and Hungarian Scientific Research Fund (OTKA 109415). M.O.R. is supported by CAPES and FAPESP. A.C.B. is supported by the NIDDK.

Author contributions

All authors contributed to researching the data for the article and the writing the article. B.G., E.A.M. and A.C.B. provided a substantial contribution to discussions of the content and reviewed and/or edited the manuscript before submission. B.G. and E.A.M. contributed equally to this Review.