

## Iodothyronine deiodinases and reduced sensitivity to thyroid hormones

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### TABLE OF CONTENTS

1. Abstract
2. Introduction
3. Iodothyronine deiodinases
  - 3.1. Iodothyronine deiodinases: general overview
  - 3.2. Iodothyronine deiodinase activity and the regulation of negative feedback loop
4. Impaired iodothyronine deiodinase activity: a possible model of “syndrome of reduced sensitivity to thyroid hormone”
  - 4.1. Experimental models of impaired iodothyronine deiodinase activity: targeted disruption of DIO genes
  - 4.2. Iodothyronine deiodinase defects in the syndromes of reduced sensitivity to THs
  - 4.3. LT4 replacement therapy and iodothyronine deiodinase polymorphisms
5. Conclusions
6. Acknowledgment
7. References

### 1. ABSTRACT

Iodothyronine deiodinases are selenoproteins that regulate thyroid hormone metabolism. Of the three types of deiodinases, type 2 is the major regulator of intracellular triiodothyronine concentration in both the hypothalamus and pituitary, and therefore the major regulator of thyrotropin secretion. A defect in iodothyronine deiodinase activity can lead to a reduced sensitivity to thyroid hormones action and the most recent literature includes these defects in the so-called “syndromes of reduced sensitivity to thyroid hormones”. To date, the pathogenic variants of the selenocysteine insertion sequence-binding protein 2 (*SECISBP2*) gene are the first and only inherited disorder of iodothyronine metabolism described. Moreover, there is a growing interest in understanding the possible role of polymorphisms of *DIO1* and *DIO2* genes in some pathological conditions and in determining the requirement of

levothyroxine replacement and the role of combined levothyroxine-liothyronine therapy in carrying subjects affected by hypothyroidism and who need replacement therapy. Results on this topic are still conflicting and more studies are needed to assess the efficacy of combined levothyroxine-liothyronine replacement therapy in this subset of patients.

### 2. INTRODUCTION

Thyroid hormones (TH) activity is essential for many diverse processes in nearly all vertebrate tissues. The thyroid gland, in response to a cascade of signals including thyrotropin-releasing hormone (TRH) release from the hypothalamus and thyrotropin (TSH) release from the anterior pituitary (1), is responsible for the synthesis and release of triiodothyronine (T3) and thyroxine (T4), which exert their biological effects by binding to nuclear receptors (TH receptors, TRs).

## Iodothyronine deiodinases and reduced sensitivity to thyroid hormones

**Table 1.** TR isoform expression in human tissues

Isoform	Predominantly expression	Lower expression
TRbeta1	kidneys, liver, brain, heart, thyroid	skeletal muscle, lungs, spleen
TRbeta2	brain, hypothalamus, pituitary, retina, inner ears	lungs, heart
TRbeta3	kidneys, liver, lungs	skeletal muscle, spleen, brain, heart
TRalfa1, TRalfa2	brain	kidneys, skeletal muscle, lungs, heart, testes, liver

TRs belong to the superfamily of nuclear receptors which includes the retinoid X receptor, vitamin D receptor, and oestrogen receptor (2). These receptors are ligand-dependent transcription factors that consist of a single polypeptide chain with three modular functional domains: (1) an amino-terminal domain that varies between the different isoforms and (2) a central DNA-binding domain and (3) a carboxy-terminal ligand-binding domain that are conserved between the different isoforms (3).

TRs are derived from two structurally similar human genes, *thyroid hormone receptor alpha (THRA)* and *thyroid hormone receptor beta (THRB)*, located on chromosome 17 and chromosome 3, respectively. The alternately splicing of these two genes results in several homologous nuclear receptor isoforms that mainly differ in their amino-terminal domain and tissue distribution (4, 5). In humans, the alternative splicing of the TRbeta gene (*THRB*) gives rise to two T3-binding nuclear receptor isoforms (TRbeta1, TRbeta2), and to one truncated variant (TRbeta4) (6, 7). Similarly, the TRalfa gene (*THRA*) encodes one T3-binding variant (TRalfa1) and three splicing variants (TR alfa2, TR alfa3 and TR alfaDeltaE6), which have no T3-binding activity (8, 9).

The expression of TR isoforms is tissue-dependent: TRalfa1 is constitutively expressed since embryonic development, while TRbeta is expressed toward the later stages of development (10). Their distribution in human tissues is reported in Table 1.

Even though both T3 and T4 can exert biological effects, T3 is considered to be the

biologically active thyroid hormone that binds to TRs, while T4 is considered a “prohormone” that must be converted to T3 in order to gain biological activity.

THs metabolism, including T4-to-T3 conversion, depends on iodothyronine deiodinase expression and activity, which can vary among different tissues and according to the age of development. In fact, the thyroid produces only about 20% of the active hormone T3, while 80% of the circulating levels of T3 in healthy human subjects is derived from the activity of iodothyronine deiodinase (11).

However, several studies have demonstrated that T4 has non-genomic roles that do not require conversion to T3 (12). Indeed, some observations indicate that TR alfa1 in brain can be activated by T4 also in absence of T3 (13). This phenomenon is crucial in pregnancy, considering the direct evidences of the role of maternal T4 in neurogenesis (14).

There are three kinds of iodothyronine deiodinase but type 2 iodothyronine deiodinase (D2), expressed in the pituitary, brain, brown adipose tissue, placenta, intestinal wall and reproductive tract, is the major T4-activating iodothyronine deiodinase, producing T3 by the removal of an iodine residue from the outer phenolic ring of T4 (15-17). Furthermore, deiodinases are involved in the enterohepatic recycling of iodothyronines and a possible interference of microbiota on the peripheral TH metabolism has also been proposed (18).

D2 generates intracellular T3 from T4 in several human tissues, including the hypothalamus and pituitary. Importantly enough, the intracellular T3 concentration at both hypothalamic and pituitary levels is the major determinant of the control of TSH secretion. Therefore, D2 activity plays a crucial role in the regulation of the hypothalamus–pituitary–thyroid (HPT) axis, both in physiological and in pathological conditions. For example, the pharmacological effects of several drugs (e.g. amiodarone, propylthiouracil (PTU)) are secondary to their effects on iodothyronine deiodinase activities (19, 20), and the reduced TRH synthesis observed during the so-called “non-thyroidal illness syndrome”

(NTIS) seems to be related to a marked increase in D2 mRNA expression both in tanycytes and in the hypothalamus (21). Furthermore, an impaired iodothyronine deiodinase activity can also result in different degrees of impaired sensitivity to TH action. To the best of our knowledge, the only known inherited defect of thyroid hormone metabolism involving iodothyronine deiodinase activity is the mutation of selenocysteine insertion sequence-binding protein 2 (SECISBP2, also known as SBP2), that is involved in iodothyronine deiodinase synthesis and degradation. This mutation interferes with the conversion of T4 to T3, resulting in a low T3 and a high T4 and reverse T3 (rT3). However, different polymorphism of *DIO2* gene have been described in association with several diseases.

In this review, the role of iodothyronine deiodinases activity in the regulation of the HPT axis and the possible implications in conditions of altered sensitivity to TH action will be discussed.

### 3. IODOTHYRONINE DEIODINASES

#### 3.1. Iodothyronine deiodinases: general overview

T4 is the main form of TH produced by the thyroid gland, but it is only minimally active. T3 is the fully active TH responsible for biological actions, its affinity for TRs being about 10-fold higher than that of T4. In humans, approximately 80% of serum T3 is derived from T4. T3 has a shorter half-life than T4 (~12 h vs. 8 days, respectively) (22), and it is mostly produced outside of the thyroid gland through the removal of the outer ring (5') iodine from T4 (23). Conversely, the removal of the inner T4 or T3 ring iodine produces reverse T3 and T2, respectively, which are biologically inactive molecules. These crucial steps are mediated by the iodothyronine deiodinases.

The deiodination cascade is also involved in the synthesis of functional molecules, similar to TH. Iodothyronamine (3-T1AM) and thyronamine (T0AM) are endogenous signaling molecules with similar structure to TH. Their physiological mode of action is still controversial and sites of their biosynthesis are not fully elucidated. The biosynthesis of these

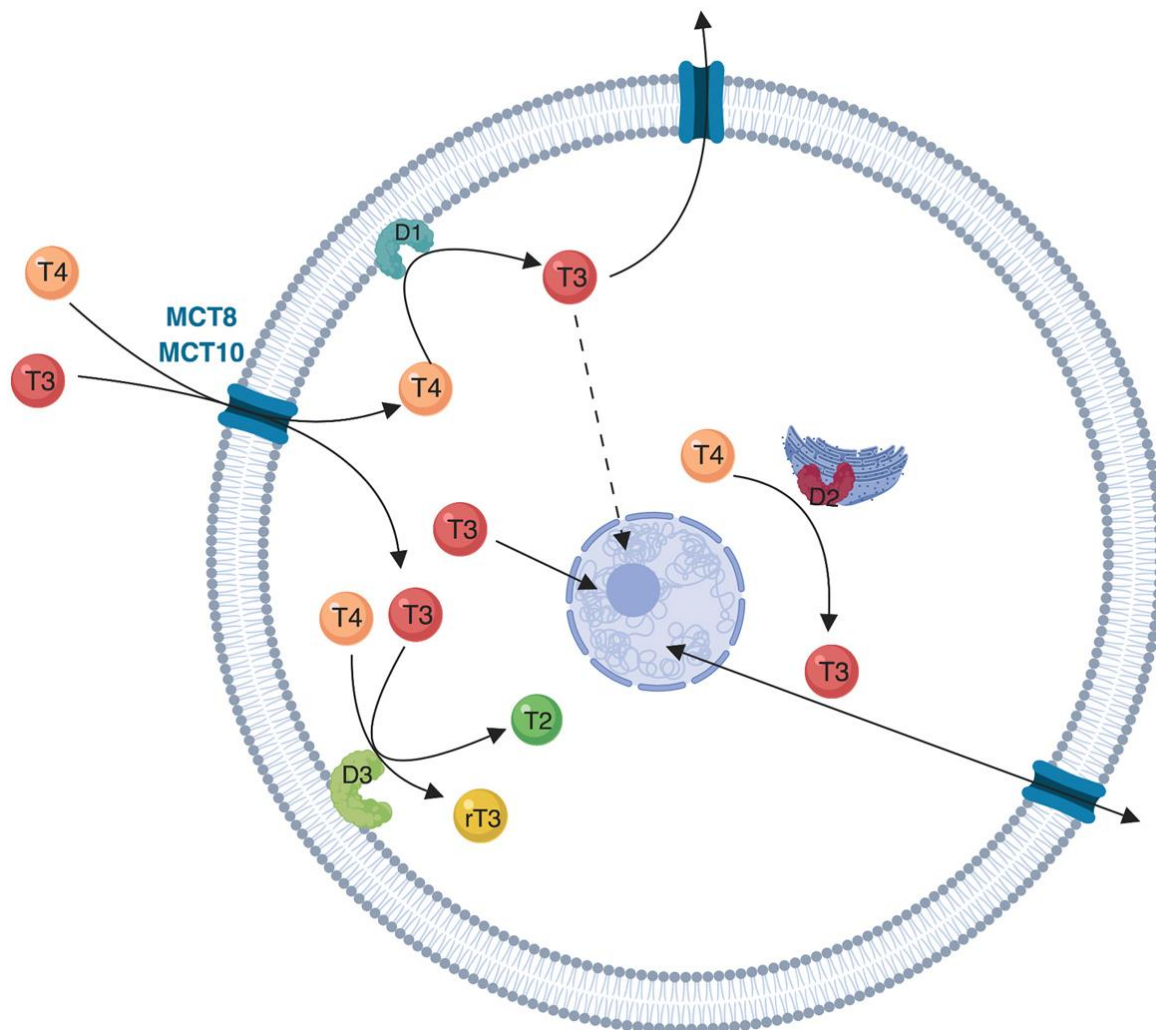
compounds involves decarboxylation and more or less extensive deiodination (24). Monoiodothyronine (T1) and diiodothyronine (T2), the precursor of TH which undergo a coupling reaction to produce T3 and T4, seem to have a biological active role. In particular, T2 is considered a “biologically active iodothyronine”, which targets mostly mitochondria and is involved in bioenergetic metabolism regulation (25).

Iodothyronine deiodinases (encoded by *DIO* genes *DIO1*, *DIO2*, and *DIO3*) are selenoproteins that contribute to the intracellular regulation of TH action (Figure 1). They are homodimers anchored in cell membranes via a single transmembrane domain, while the catalytic globular domain faces the cytosol, and dimerization is required for their catalytic activity (26). Recently, some authors showed that the iodothyronine deiodinases share an evolutionary conserved TH-binding domain, which is similar to that of TH nuclear receptors and TH cell membrane transporters and which could represent the product of a common ancestor gene (27).

*DIO1* gene, maps on the short (p) arm of chromosome 1 at position 32.3 (1p32.3) (GRCh38/hg38: chr1:53,891,239-53,911,086; NC\_000001.11) and encodes a protein (D1) of 294 amino acids. *DIO2* and *DIO3* genes, mapping on the long arm (q) of chromosome 14 in positions 31.1 (14q31.1) (GRCh38/hg38: chr14:80,197,525-80,387,757; NC\_000014.9) and 32.31 (14q32.31) (GRCh38/hg38: chr14:101,561,351-101,563,452; NC\_000014.9) respectively, encode proteins of 273 (D2) and 304 (D3) amino acids, respectively. These selenoproteins contain the rare amino acid selenocysteine (Sec) at their active site. Sec is encoded by the UGA codon, which normally signals translation termination. The 3' UTRs of selenoprotein mRNAs contain a conserved stem-loop structure, designated the Sec insertion sequence (SECIS) element, that is necessary for the recognition of UGA as a Sec codon, rather than as a stop signal.

D1 and D2 activate TH, whereas D3, and to a lesser degree D1, inactivate both T4 and T3. The activity of iodothyronine deiodinase is related to the type of iodothyronine deiodinase expressed and to

## Iodothyronine deiodinases and reduced sensitivity to thyroid hormones



**Figure 1.** Schematic representation of deiodinases localization and function. The cellular influx and efflux of thyroid hormones is facilitated by protein transporters (mainly MCT8 and MCT10). The intracellular metabolism of thyroid hormones depends on the activity of three iodothyronine deiodinases (D1, D2 and D3). D1 is a plasma membrane protein and is the only deiodinase that can function both as an outer ring deiodination, resulting in T4 to T3 conversion, and as an inner ring deiodination resulting in T4 to rT3 conversion (not shown). D2 is an endoplasmic reticulum protein that functions as an outer ring deiodinase converting T4 to T3. D2 is closer to the nucleus when compared to D1, and D2-originated T3 diffuses to the nucleus (solid line arrow) and remains in the target cell for a longer period of time when compared to D1-originated T3 (dotted line arrow). D3 is an inner ring deiodinase located in the plasma membrane that inactivates T4 and T3, reducing TH

the degree of the enzyme activity in each tissue (23).

As mentioned previously, T3 binds to nuclear TRs to exert its biological effects. Since TR saturation depends on the intracellular concentration of T3, D2 and D3 play an opposite role in TH action by inversely affecting intracellular T3 levels. In fact, D2 produces T3 and thus activates TH signaling, whereas D3 inactivates T4 and T3, reducing TH

signaling. Both pathways are relatively independent of circulating T4 or T3 levels (28).

D1 is predominantly expressed in the pituitary, thyroid, liver, and kidney. D1 is the only iodothyronine deiodinase that can function as either an outer- or an inner -ring iodothyronine deiodinase, and was the first to be cloned (29). D1 is located in the plasma membrane,

## Daily T3 production

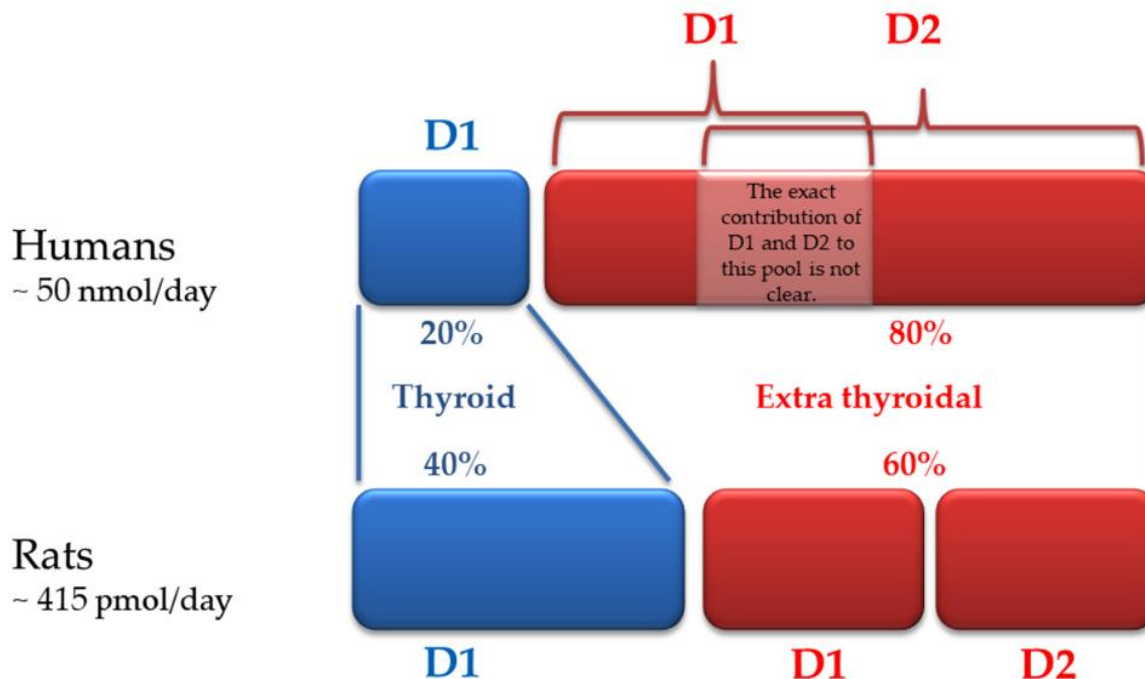


Figure 2. Daily T3 production in humans and rodents: contribution of each deiodinase (modified from reference 31).

and the amount of T3 produced by this pathway is thought to equilibrate with the plasma pool of T3. If in rodents D1 is responsible for about 50% of the daily extrathyroidal production of T3, in humans D1 plays a lesser role in maintaining circulating T3 (Figure 2) (30, 31).

D2 is an endoplasmic reticulum resident protein. Its expression is low in most tissues during development, but it increases in the perinatal period (32). D2-mediated activity is an intracellular event: D2-originated T3, before exiting the cell and contributing to plasma T3 levels, diffuses to the nucleus and remains in the target cell for a longer period of time when compared to D1-originated T3. This is probably explained by the fact that D2-originated T3 exerts intracellular biological effects through the interaction with the local TRs (30). Tissues in which D2 plays a role are the cochlea, bone, brown adipose tissue, liver, and perhaps the muscle (22). D2 contributes to adaptive

thermogenesis and metabolic control under norepinephrine control (33), and its expression is in turn regulated by food availability, which stimulates D2-mediated T3 production in mouse skeletal muscle (34). D2 also has a role in behavior and mood regulation, and animal models based on D2KO mouse exhibit a neurological phenotype characterized by diminished agility, altered global gait pattern, impairment of coordination and prehensile reflex, and reduced muscle strength (35). The human myocardium expresses D2 that is potentially capable of generating local T3. This is also the physiological basis contributing to the antiarrhythmic efficacy of amiodarone, which inhibits the deiodination of T4 to T3 (19). In fact, myocardial D2 increases fractional shortening, the velocity of circumferential fiber shortening, peak aortic outflow velocity, and aortic velocity acceleration. The noncompetitive D2 inhibitor amiodarone can potentially decrease thyroid hormone signaling in the heart. Furthermore, D2 expression has been



## Iodothyronine deiodinases and reduced sensitivity to thyroid hormones

detected in minimal amounts in both humans and murine muscle tissue, playing a role in muscle regeneration and exercise (36).

D3 is an inner-ring iodothyronine deiodinase with a half-life of approximately 12 h (22) that is expressed in numerous tissues in fetal life, but only in the central nervous system and placenta in adults. This enzyme is located in the plasma membrane and it is inserted into the membrane to easily inactivate TH (37). D3 activity predominates in developing tissues, preventing the exposure of cells to TH. D3-mediated T3 inactivation occurs in the brain, skin, and placenta: the latter could explain the need to increase TH replacement therapy during pregnancy (30, 38). On the other hand, in mature embryos, D3 expression is minimized and D2 increases, enhancing thyroid hormone signaling. However, D3 expression can be reactivated in multiple tissues in disease states, contributing to the low circulating levels of T3 observed in these conditions (39). D3 is expressed in both embryonic and adult pancreatic beta-cells in humans and mice, and D3 knock-out (KO) animals are glucose-intolerant, without changes in peripheral sensitivity to insulin (40). Severe heart diseases associated with ischemia or hypoxia increase the cardiac expression of D3 determining a local hypothyroidism (41). Increased D3 expression has been observed in animal models of heart remodeling due to myocardial infarction or chronic pulmonary hypertension with right ventricular hypertrophy (42). D3 overexpression has been found to be a common paraneoplastic syndrome in gastrointestinal stromal tumors (43), causing the so-called “consumptive hypothyroidism” in affected patients. Consumptive hypothyroidism is a systemic condition characterized by an accelerated degradation of circulating TH, at rates exceeding the synthetic capacity of the normal thyroid, due to an aberrant uncontrolled expression of D3 (44). This condition has previously been described in hemangiomas (45) and hemangioendotheliomas (46) and it is probably due to the great expression of D3 in these tumors (30). The diagnosis of consumptive hypothyroidism can be performed by the evidence of increased TH inactivation (elevated levels of serum reverse triiodothyronine or abnormal TH replacement therapy requirement). Similarly, the antineoplastic use of tyrosine kinase inhibitors imatinib and sunitinib

is associated with hypothyroidism, which seems to result from the paradoxical increase in D3 observed in some tumors after exposure to these drugs (43). However, the cause of hypothyroidism in these situations is not fully understood, and it could be multifactorial, including enhanced D3 expression in other tissues (47).

A debate regarding the most important source of plasma T3 in humans has been going on for many years. In fact, although it was initially thought that D1 provided the majority of plasma T3 in humans, further investigations suggest that D2 have a major contribution (48). In line with this, treatment with PTU (which inhibits D1 activity) only causes an approximately 30% decrease in serum T3 in patients with primary hypothyroidism receiving fixed doses of exogenous T4, suggesting an important potential role of D2 in the generation of plasma T3 (49). The most supported hypothesis is that the human D1- or D2-derived T3 production varies significantly according to the thyroid status. As *DIO1* expression is positively regulated by T3, D1-catalyzed T4 conversion to T3 is decreased in the hypothyroid state (50). In contrast, D2 half-life and *DIO2* transcription was increased by low TH levels. In the opposite scenario, D1-catalyzed T3 production, in particular from the thyroid gland, predominates in the hyperthyroid state (51). In this condition, the thyroidal contribution to serum T3 levels increases in parallel with the disease severity, reaching nearly two-thirds of the total T3 production in severe hyperthyroidism (52). A decreased D1 activity has been reported in the NTIS and in several human neoplasia of epithelial origin, except for the follicular thyroid tumors and breast cancer, in which D1 expression is increased (29).

In conclusion, the ratio of D2 and D3 expression is important both in physiological conditions and in thyroid disease states. In fact, D2 expression and activity are both increased in TH deficiency, whereas they are reduced in thyrotoxicosis. In contrast, D3 expression is regulated reciprocally. Thus, this balance determines the homeostatic control of T3 availability to the nuclear receptor and it is particularly important in specific tissues, such as the brain, with the aim of “protecting” from possible deleterious changes in TH levels.

### 3.2. Iodothyronine deiodinase activity and the regulation of negative feedback loop

Normal thyroid function results from the complex and well-regulated activity of the HPT axis, which in conjunction with external factors maintains normal concentrations of TH. Pituitary TSH secretion represents the main regulator of thyroid development, growth, and function. The set point of the normal concentration of TSH is determined by the balance between the stimulatory action of TRH and the negative feedback of THs, as well as by other regulators originating from the central nervous system, peripheral tissues, and local pituitary mechanisms. Both circulating T4 and T3 play a role in the negative feedback loop that inhibits both TRH and TSH secretion: as mentioned previously, T4 is only minimally active, while T3 represents the “active” hormone which negatively regulates *TSH* and *TRH* genes.

The negative feedback loop is regulated by the so-called “hypophysiotropic TRH-synthesizing neurons”, located in the paraventricular nucleus (PVN) (53). Interestingly, low levels of THs increase TRH expression only in the hypophysiotropic neurons (54), while TRH gene expression is not regulated by T3 in non-hypophysiotropic TRH neurons (54). In addition to the negative feedback exerted by TH on TRH, other factors also contribute to the modulation of TRH neurons (e.g., neuropeptide Y (NPY), leptin, alpha-melanocortin-stimulating hormone (alpha-MSH), and agouti-related peptide (AgRP)) (23).

The TR-beta2 isoform is the dominant isoform mediating both the negative regulation of the *TSH-beta* gene expression and the negative regulation of the *TRH* gene expression in the hypothalamus (55). Interestingly, mice lacking the beta isoforms of TR (TRbetaKO) have significantly higher TH and TSH levels compared with mice KO for both TR and TRH (double KO), which have reduced TH and TSH levels. In fact, hypothyroid double KO mice failed to mount a significant increase in serum TSH levels. This confirms that TRH is absolutely required for both TSH and TH synthesis, even if it is not necessary for thyrotroph cell development (56).

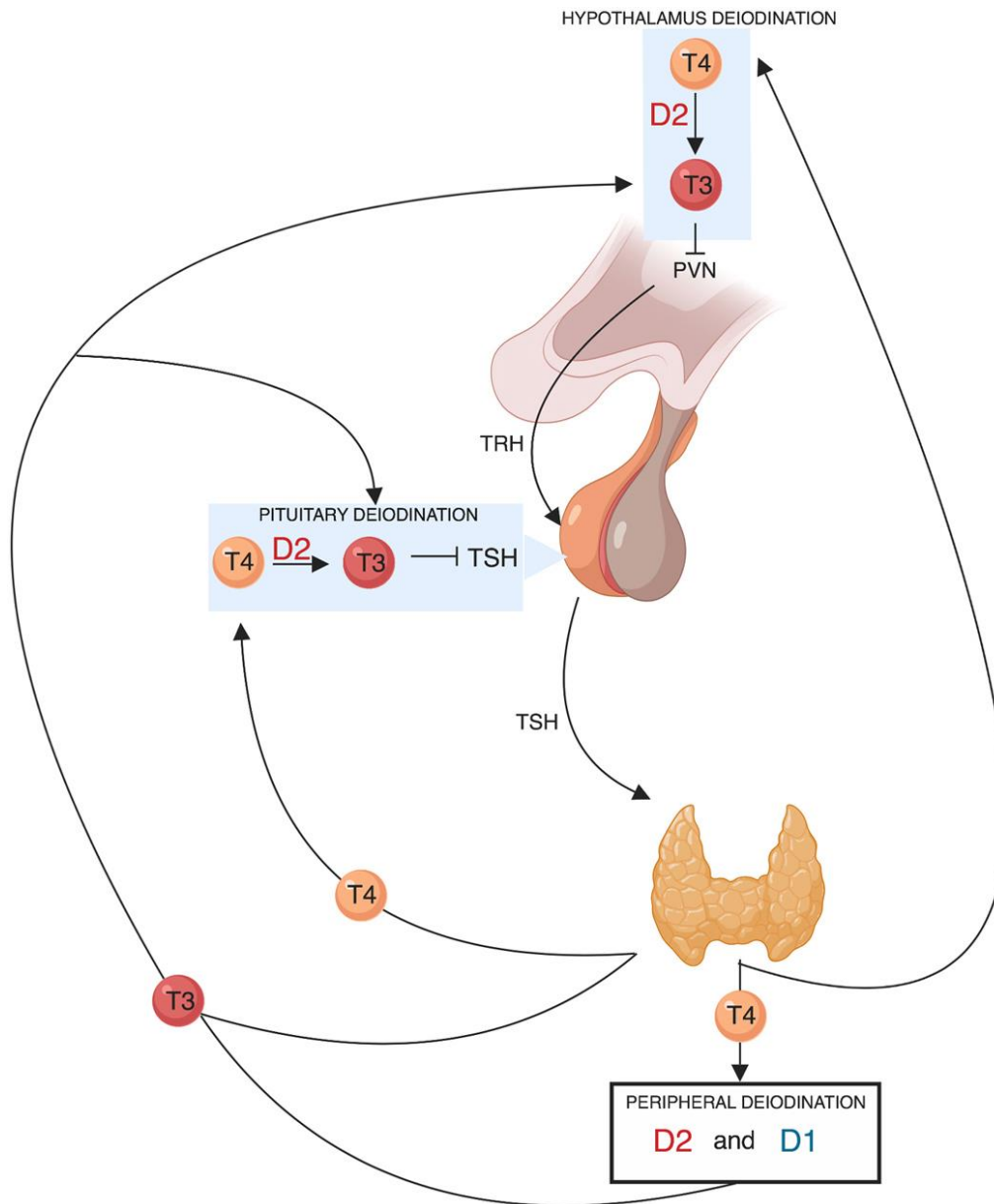
An increase in circulating T4 is expected to increase T3 concentration and to reduce TSH gene expression, while a drop in serum T4 has the opposite effects. Importantly enough, variations of circulating T3 concentration have a direct effect on the hypothalamic and pituitary expression of TRH and TSH genes, while variations of T4 concentration have effect on HPT axis only thanks to D2 activity. However, the vast majority of T3 present in the brain is generated locally by D2-mediated deiodination of T4 (57). Therefore, D2 can be considered a key regulator of the negative feedback loop mediated by local TH (Figure 3).

D2 has a relatively short half-life (approximately 40 minutes) related to its ubiquitination and destruction in the proteasomes. This process is accelerated by the interaction with T4, which is its natural substrate (51). This mechanism is at the basis of the rapid control of intracellular T3 production and of its interaction with TRs. Furthermore, D2 activity in HPT regulation is modulated by the pituitary adenylate cyclase-activating polypeptide (PACAP), which increases intracellular cAMP levels—a second messenger known to stimulate the *DIO2* gene (58). D2 is co-expressed with TSH in pituitary thyrotrophs (59).

Tanycytes are specialized glial cells located in the ventrolateral walls and floor of the third ventricle, that seems to be crucial in regulating the negative feedback exerted by TH. Tanycytes are essential in guaranteeing strict TH levels in the hypothalamus. Tanycytes express TH transporters (particularly MCT8 and OATP1C1) and both D2 and D3. Therefore, while D2 is usually expressed by astrocytes in other brain regions, in the hypothalamus D2 is expressed in the tanycytes. D2 activity in tanycytes is crucial to permit the TH control of TRH expression. Tanycyte D2 has access to plasma or cerebral-spinal fluid-born T4, which is converted to T3. T3 in turn reaches the PVN or the pituitary gland via portal blood flow (60) and it enters TRH neurons via the MCT8 transporter where it regulates prepro-TRH expression and the processing of pro-TRH into TRH peptide (23). This process is rapid, occurring in about 5 hours (61).

Iodothyronine deiodinase activity has been

### Iodothyronine deiodinases and reduced sensitivity to thyroid hormones



**Figure 3.** Schematic representation of the role of deiodinases in the hypothalamic-pituitary-thyroid axis feedback regulation TRH derived from the parvocellular neurons of the hypothalamic paraventricular nucleus (PVN), stimulates TSH synthesis and secretion from thyrotroph cells located in the anterior pituitary gland. In turn, TSH regulates thyroid hormone production and release via binding to the TSH-receptor in thyroid follicular cells. The thyroid gland secretes T4 and about 20% of circulating T3. About 80% of circulating T3 is derived from the peripheral deiodination of T4 that is mediated by D2 (mainly) and D1. The circulating T3 signals a negative feedback mechanism both at the hypothalamic level by suppressing TRH secretion and at the pituitary level by suppressing TSH secretion. D2 is also expressed in the hypothalamus and in the anterior pituitary gland. The pituitary and hypothalamic deiodination of T4 to T3 and the local action exerted by the latter before exiting the cell explains how circulating T4 plays a key and probably even predominant role in regulating the negative feedback mechanism.

considered to regulate the response of the HPT axis in different pathological conditions, including critical illness. However, data on this matter are still

conflicting. The most accepted theory is that the extra-thyroidal conversion of T4 to T3 is reduced during critical illness due to the reduction in both



hepatic/renal D1 activity and in skeletal muscle D2 activity (49). Furthermore, an increase in hepatic and skeletal muscle D3 activity may also be involved (62). Interestingly, the systemic administration of bacterial lipopolysaccharide (LPS) in normal mice increases D2 mRNA and its activity and reduces TRH expression. The T3 generated by the D2 pathway also directly affects the thyrotrophs, inhibiting TSH secretion (63). Nevertheless, recent evidences criticize these conclusions, arguing that the modifications in iodothyronine deiodinase expression in NTIS may be a consequence of changes occurring in T3 and T4, rather than the cause of these hormonal changes (64). In line with this, studies on both D3KO mice and D1/D2 KO mice subjected to treatment with LPS showed that the changes in T3 and T4 that occurred in response to LPS were essentially no different from those seen in wild-type (WT) animals (49). In addition, in WT mice treated with LPS, the decrease in plasma total T3 and total T4 has been found to precede the fall in hepatic D1 (65).

### **4. IMPAIRED IODOTHYRONINE DEIODINASE ACTIVITY: A POSSIBLE MODEL OF “SYNDROME OF REDUCED SENSITIVITY TO THYROID HORMONE”**

Iodothyronine deiodinase activity is the most important regulator of TH availability both in brain and in peripheral tissue. As a consequence, impaired D1 and D2 activity will virtually result in a reduced sensitivity to TH action. Until a few years ago, the mechanism of “resistance” to TH was considered as related almost exclusively to mutations in TRs, such that “Refetoff’s syndrome” was synonymous with “resistance to TH syndrome”. The recent literature has revised this concept, coining the so-called “syndrome of reduced sensitivity to thyroid hormone”, which includes other conditions that reduce the action or the availability of THs on target tissues. Defects that affect the synthesis of selenoproteins have also been included in this group. Furthermore, there is growing interest in the possible role of *DIO1* and *DIO2* polymorphisms in some pathological conditions and in determining the requirement of LT4 replacement therapy in carrying subjects.

In this section, the role of an impaired

iodothyronine deiodinase activity as a model of reduced sensitivity to TH will be discussed, starting from the animal model of targeted disruption of *DIO* genes up to the possible consequences in clinical practice.

### **4.1. Experimental models of impaired iodothyronine deiodinase activity: targeted disruption of DIO genes**

Studies aimed to evaluate the activity of iodothyronine deiodinase can be based on several approaches. One of these would be to specifically inhibit their activity by drugs. Unfortunately, there is no known pharmacological agent that specifically and completely inhibits the activity of each iodothyronine deiodinase individually. Furthermore, some of these compounds (e.g., PTU) also inhibit TH biosynthesis, and thus they are not ideal for *in vivo* studies where intact thyroid function is required. An alternative approach is to create animal models that lack the active form of an iodothyronine deiodinase enzyme. These elegant models might contribute to understanding the role of iodothyronine deiodinases not only in physiological, but also in pathological conditions. In particular, it is interesting to evaluate if animal models of impaired iodothyronine deiodinase activity can represent a model of reduced sensitivity to TH.

Schneider *et al.* produced a mouse strain lacking D2 activity using homologous recombination (66). Mice homologous for the targeted deletion (D2KO), in which no D2 activity was observed in any tissue (including astrocytes) in basal conditions, did not show abnormalities in their phenotype, and their development appeared normal except for a mild growth retardation in males. In the liver, thyroid, and pituitary, D1 levels were the same in WT and D2KO mice, while cerebral D3 activity in D2KO animals was twice that in WT. Interestingly, there were no differences in serum T3 levels between WT and D2KO animals, while serum T4 and TSH levels were significantly higher in the KO model. These findings suggest a pituitary resistance to the negative feedback exerted by plasma T4 in D2KO mice. Furthermore, the administration of both T4 and T3 was effective in hypothyroid WT mice in terms of TSH reduction, while only T3 was effective in D2KO

## Iodothyronine deiodinases and reduced sensitivity to thyroid hormones

models (66). In fact, in the presence of a targeted inactivation of the *DIO2* gene, the exogenous administration of T3 overrode the absence of T4 activation in T3, restoring a “normal” feedback.

A more recent study performed in mice with inactivated pituitary D2, showed that basal TSH levels in neonatal D2KO mice were two-fold higher when compared with controls, but the TSH increase in response to hypothyroidism was reduced compared to controls, despite an identical level of pituitary TSH $\alpha$  and beta-subunit mRNAs. Therefore, a congenital severe reduction of thyrotroph D2 activity seems to cause a major impairment of the TSH response to hypothyroidism (67). Surprisingly, even if D2KO mice have elevated TSH levels, their TRH mRNA levels in the PVN were not increased, suggesting that the resistance to T4 suppression results mainly from *DIO2* deficiency in the pituitary rather than in the hypothalamus (19).

It is well-known that analysis of the HPT axis in D2KO mice has demonstrated normal T3 levels, probably related to notably elevated T4 levels which are converted to T3 by the residual D1 activity (68). In contrast, other studies seem to demonstrate that iodothyronine deiodinase action is not critical for the maintenance of plasma T3 concentrations. In fact, D1 and D2 KO mice maintain normal plasma T3 concentrations (49). However, D2 KO animals have alterations in TSH regulations and thermogenesis, and the brain T3 concentration is reduced despite increased brain T4. Combined D1/D2 KO mice have plasma T4 levels almost twice those found in WT animals, and a plasma TSH 2.6 times the level found in WT or D1 KO (69). The most likely explanation for the maintenance of plasma T3 in D1/D2 KO animals is that the increased TSH concentration promotes an enhanced thyroidal production of T3. This also confirms that, despite the normal T3 plasma concentration, hypothalamic and pituitary T3 levels are decreased, causing an impaired negative feedback activity and an increase of TSH (60).

The role of D2 in regulating the sensitivity to THs is also related to its action in guaranteeing the normal development of TRs. Studies performed in zebrafish embryos and targeting D2 showed that, besides delays in phenotypic development, there is a

reduction in THR-beta expression. Due to depletion of intracellular T3 levels, these effects were reversible by T3 administration (70).

In D1-deficient mice (D1KO), the general health and the reproductive capacity were unimpaired. The serum levels of T4 and rT3 were elevated, whereas those of TSH and T3, as mentioned previously, were unchanged. However, D1 deficiency caused marked changes in the metabolism and excretion of iodothyronines, including a significant increase in the fecal excretion of endogenous iodothyronines (71). This finding confirms the scavenging function for D1 that might be particularly important in the setting of iodine deficiency (29).

### 4.2. Iodothyronine deiodinase defects in the syndromes of reduced sensitivity to THs

The intracellular levels of T3 define how much T3 is bound to TRs and the intensity of TH signaling, the so-called “thyroid status”. The “thyroid status” is the result of all T3-dependent signaling events and depends not only by the circulating T3 levels but also by the factors influencing the intracellular concentration of T3. In fact, the peripheral tissues can feel the “thyrotoxic” or “hypothyroid” status independently of serum TH thanks to cell specific factors which can alter the intracellular THs concentration and/or their activities. An example of a condition characterized by a mismatch between THs and TSH levels and tissue-specific “thyroid status”, is represented by the so-called “syndromes of reduced sensitivity to THs”, that include inherited disorders of the transport, metabolism, and action of THs, as recently stated by a new reclassification (72).

The first condition was described by Refetoff in 1967 (“resistance to thyroid hormone syndrome or “Refetoff’s syndrome”) (73). The most important clinical features of this condition are represented by elevated serum THs levels and non-suppressed TSH (“inappropriate TSH secretion”), often associated with goiter and no clear symptoms and signs of thyrotoxicosis, due to the possible coexistence of decreased and increased THs action in different tissues (74). This condition is

## Iodothyronine deiodinases and reduced sensitivity to thyroid hormones

predominantly due to a mutation in the *THRB* gene (75), but mutations in the *THRA* gene, which are related to a distinct phenotype, have recently been detected (76). Furthermore, a syndrome with reduced cellular access of T3 due to defects of THs cell membrane transport was identified (77, 78). Monocarboxylate transporter 8 (MCT8) and 10 (MCT10) are the most important TH transporters, which facilitate both TH transport into the cell and TH efflux from cells (79). Inactivating mutations in MCT8 cause a syndrome characterized by alterations in serum TH levels and neurological abnormalities (77, 78). Interestingly enough, some authors showed that over-expression of these transporter induces a transient elevation in intracellular T3 concentration but only a modest increase in nuclear T3 activity (79). Of note, mouse models with combined MCT8 and deiodinases deficiency have been generated to investigate the interplay of thyroid hormone metabolism and transport both in physiological and pathological conditions (80-83).

In this field, iodothyronine deiodinase activity plays a crucial role in assuring a normal intracellular “thyroid status”. In fact, D2 catalyzes the conversion of T4 to T3, increasing intracellular T3 levels and potentially leading to local thyrotoxicosis. The opposite is observed for D3 activity, which depletes the cell of T3 by deiodination to T2, causing local hypothyroidism. Therefore, due to the crucial role exerted in the regulation of TH metabolism, there is growing interest in how genetic variation and abnormalities in the iodothyronine deiodinases may influence thyroid function, not only by altering TH levels in blood, but also by altering their intracellular activity. Until just over 10 years ago, all of the known defects of TH metabolism observed in humans were acquired. For example, the most frequent defect is observed in “non-thyroidal illness syndrome” which produces the so-called “low T3 syndrome” (84).

In 2005, Dumitrescu *et al.* reported the first inherited disorder of iodothyronine metabolism, caused by a reduction in the synthesis of iodothyronine deiodinases (85). The involved gene, the selenocysteine insertion sequence-binding protein2 (*SECISBP2*), maps on the long (q) arm of chromosome 9 at position 22.2 (9q22.2) and spans a region of 46.644 bp (GRCh38/hg38:

chr9:89,318,473-89,367,117; NC\_000009.12) (86). The protein, composed of 854 amino acids, is one of the essential components of the machinery involved in co-translational insertion of selenocysteine (Sec) into selenoproteins. Sec is encoded by the UGA codon, which normally signals translation termination. The recoding of UGA as Sec codon requires a Sec insertion sequence (SECIS) element; present in the 3' untranslated regions of eukaryotic selenoprotein mRNAs. This protein specifically binds to the SECIS element, which is stimulated by a Sec-specific translation elongation factor.

Pathogenic variants in *SECISBP2* gene have been associated with reduction in enzymatic activity of D2 and abnormal thyroid hormone metabolism. To date, 13 *SECISBP2* variants have been reported as pathogenic. The most important clinical characteristics have been reported in Table 2. In particular, Dumitrescu described two unrelated families where affected children were either homozygous (p.Arg540Gln) or compound heterozygous (p.Lys438Ter/NM\_024077.4:c.1212+29G>A) for *SECISBP2* pathogenic variants (87). Significant clinical features were short stature, delayed bone age and delayed puberty. Accordingly with the impaired conversion of T4 to T3, laboratory findings are represented by high T4, low T3, high rT3, and slightly elevated serum TSH. When compared with their normal siblings, the affected children required higher doses and serum concentrations of T4 to reduce their TSH. In contrast, similar doses of T3 used in the affected and unaffected siblings had equal suppressive effects on TSH, suggesting a defect in T3 conversion.

Studies performed on affected patients' skin fibroblasts showed reduced baseline and cAMP-stimulated D2 enzymatic activity when compared to fibroblasts from unaffected individuals. However, the phenotype described by Dumitrescu is mild, because the deficiency is not complete, while a more severe deficiency might have more severe consequences (88).

There is no agreement about the best treatment in these patients. Supplementation with organic and inorganic selenium has been

## Iodothyronine deiodinases and reduced sensitivity to thyroid hormones

**Table 2.** Pathogenic variants identified in *SECISBP2* gene

Family	<i>SECISBP2</i> gene	Protein	N. affected	Defect	Main clinical characteristics	TFTs abnormalities	Overall phenotype	Ref.
1	c.1619 G>A	p.Arg540Gln	3	homozygous	short stature, delayed bone age,	high T4, low T3, elevated TSH	mild	85
2	c.1312 A>T	p.Lys438Ter	1	compound heterozygous	transient growth retardation,	high T4, low T3, elevated TSH	mild	85
	IVS8ds+29 G>A	fs						
3	c.382 C>T	p.Arg128Ter	1	homozygous	growth retardation,	high T4, low T3, elevated TSH	mild	91
4	c.358 C>T	p.Arg120Ter	1	compound heterozygous	delayed bone maturation, impairment of mental and motor coordination,	high T4, low T3, elevated TSH	severe	92
	c.2308 C>T	p.Arg770Ter						
5	c.668delT	fs255Ter	1	compound heterozygous	delayed motor and speech milestones, hearing problems, infertility (azoospermia), axial muscular dystrophy, cutaneous photosensitivity	high T4, normal T3 and TSH	mild	94
	intron 6-155 delC	fs						
6	c.2071 T>C	p.Cys691Arg	1	compound heterozygous	global developmental delay, short stature, hearing problems, axial muscular dystrophy	high T4, low T3, normal TSH	mild	94
	intronic SNP	fs						
7	c.1529_1541dup CCAGCGCCCACT	p.Met515 fs563Ter	1	compound heterozygous	short stature, delayed bone maturation	high T4, low T3, elevated TSH	mild	95
	c.235 C>T	p.Gln79Ter						
8	c.800_801insA	p.Lys267LysfsTer2	1	homozygous	attention deficit disorder, muscle weakness	high T4, low T3, normal TSH	mild	96

<sup>a</sup>Defects are reported as described by authors

administered, but even if total serum selenium concentrations in *SECISBP2*-deficient subjects increased after selenomethionine supplementation, the effect was not indicative of improved selenoprotein synthesis (89). Treatment with LT3 showed an increase in height velocity and advancement in bone age with a narrowing of the gap between chronological and bone age (90).

Few years later, Di Cosmo reported a novel homozygous *SECISBP2* variant, the p.Arg128Ter, producing an early arrest in the synthesis of the protein (91) while Azevedo (92) described a compound heterozygous 12-year-old girl carrying two new truncated variants (the p.Arg120Ter and the p.Arg770Ter). She was affected by a more severe phenotype due to impairment of the selenoprotein N-1 (SEPN-1)-related myopathy gene (93, 94).

Schoenmakers *et al.* detected compound heterozygous defects in two unrelated patients: proband 1 was heterozygous for a paternally

inherited frameshift variant causing a premature stop codon at aminoacid 255 and a splicing defect causing misincorporation of additional intronic sequence between exon 6 and 7 on the other allele. Patient 2 was heterozygous for the maternally inherited missense variant p.Cys691Arg together with a paternally derived defect generating aberrantly spliced transcripts (Delta2-3-4 and Delta3-4) (94).

Finally, a pathogenic *SECISBP2* genotype (p.M515SerfsTer48/p.Q79X) has been identified in Japanese boy treated with LT3 and recombinant growth hormone therapy to normalize thyroid function tests and improve bone maturation (95). The last reported case is a patient carrying the homozygous p.K267KfsTer2 variant (96).

The most impressive way to describe the impact of *SECISBP2* pathogenic variants in clinical practice and the complexity of the underlying mechanisms which are mostly still unknown is represented by the refined words of Duntas who,

referring to Greek mythology, compares the complexity of the SECISBP2 syndrome to Daedalean labyrinth: *“the way out must be sought among the many mutations of SECISBP2—several now known and others that remain to be identified—which express various defective selenoproteins that cause disease. In this crucial quest, better understanding of the thyroid hormone phenotype as well as of the Se concentrations could well prove to be Ariadne’s invaluable clue”* (97).

### 4.3. LT4 replacement therapy and iodothyronine deiodinase polymorphisms

Even though Gabriela Morreale de Escobar published two studies in 1995 and 1996 demonstrating that only the combined T4 + T3 treatment ensured euthyroidism in all tissues of thyroidectomized rats (98, 99), LT4 replacement therapy is considered the current standard of care for patients affected by hypothyroidism, and no consistently strong evidence supports the superiority of alternative therapies. The regulated peripheral conversion of LT4 to T3 in humans has previously been demonstrated (100). Such conversion, dependent upon the activity of iodothyronine deiodinases, allows normal T3 levels to be achieved with traditional LT4 therapy alone in patients who underwent near-total or total thyroidectomy, suggesting that T3 administration is not necessary to maintain serum T3 values at their endogenous prethyroidectomy levels (11). The goal of LT4 treatment is to normalize serum TSH levels and improve the biological markers of hypothyroidism (101).

However, it is mandatory to consider all the situations that could make TSH evaluation not reliable. Different drugs (glucocorticoids, dopamine agonists, somatostatin analogues, rexinoids, anti-epileptic drugs) can affect thyroid function and reduce TSH, as well as secondary hypothyroidism related to a concomitant pituitary disease (102, 103).

On the other hand, in conditions of elevated TSH, the impact of gastrointestinal disorders which can affect LT4 absorption must be considered (104).

The adequacy of this strategy to replace

physiological requirements and reverse patients’ symptoms remains controversial. Therefore, for the subset of patients treated with LT4 and complaining of persistent hypothyroid symptoms (i.e., memory loss, weight gain, fatigue, depression, and reduced quality of life) despite normal TSH levels, studies comparing clinical improvement between LT4 monotherapy and combination therapy with LT4 and LT3 have been performed. Some authors reported the superiority of combination therapy for mood, anxiety, and depression (105), whereas others observed no significant superiority or only partial improvement (106). However, in consideration of the insufficient evidence of substantial benefit and the lack of long-term LT3 safety, combination therapy is not currently recommended for the routine treatment of hypothyroidism.

Aside from the lack of a feeling of well-being, which is a subjective parameter, another problem is represented by the difficulty in reaching adequate TSH levels in some subjects on TH replacement. In these cases, a possible degree of reduced sensitivity to THs could be suspected. Nevertheless, twin and family studies are able to estimate that the heritability of TSH levels can be 65% and of FT4 up to 39%, suggesting the strong role of genetic predisposition (107). Furthermore, in approximately 20% of patients, LT4 did not ensure physiological T3 levels (108), even if results on this matter are controversial (11). In this setting, a different genetic background, which causes a reduced bioavailability of both endogenous and synthetic LT4, could be helpful in explaining these biochemical discrepancies. Single nucleotide polymorphisms (SNPs) in *DIO* genes represent a widely studied field in this context.

Polymorphisms in *DIO* genes are associated with variations in serum TSH and iodothyronine concentrations in healthy subjects (109). The C-allele of *DIO1* SNP (rs2235544) is associated with increased D1 activity, which results in slightly higher FT3 and lower FT4 and, in LT4-treated hypothyroid patients, in higher FT3/FT4 as compared to WT (110). Minor genotypes of others *DIO1* variants (rs11206244, rs2294512, and rs4926616) have been associated with reduced psychological well-being (111).



## Iodothyronine deiodinases and reduced sensitivity to thyroid hormones

The C/C genotype of the rs225014T>C (NP\_001311391.1:p.Thr92Ala) SNP was reported in 10.7% of the general population and in 11.3% of LT4 users (112). However, the effects induced by this polymorphism on D2 enzymatic activity and on thyroid function tests are unclear.

Torlontano (113) reported that athyreotic differentiated thyroid cancer (DTC) patients homozygous for rs225014C allele needed a higher LT4 dose compared with carriers of the rs225014T allele. However, this observation could be applied only to patients with serum TSH concentrations in the range of semi-suppression (0.1–0.5 mU/L), but not in the “suppressed group” (TSH <0.1 mU/L).

Castagna found an association between low FT3 values and the rs225014 polymorphism in thyroidectomized patients. In particular, the mean post-surgery FT3 levels were significantly lower in patients carrying the rs225014C allele than in patients with the rs225014T allele, in whom FT3 postsurgical levels were similar to pre-surgery levels. On the basis of these findings, thyroidectomized patients carrying the p.Thr92Ala variant are at increased risk of reduced intracellular and serum T3 concentrations, and could be inadequately compensated for by LT4 (114). The most recent study found that the homozygous genotype *DIO2* rs225014 (T/T) and the homozygous genotype rs225015 (G/G) were associated with higher levels of TSH compared with their other types. Therefore, patients carrying these genotypes present higher LT4 requirement (115).

In contrast, other studies excluded the effects of the rs225014 polymorphism on serum TSH, FT4, or T3 (116, 117). A recent paper evaluating LT4 therapy in athyreotic patients with differentiated thyroid cancer showed no significant association with *DIO2* rs225014 and rs12885300 polymorphisms in predicting the dose requirement for TSH suppression. In this study, neither *DIO1* SNP seemed to influence HPT axis regulation (118).

Interestingly, the p.Thr92Ala variant has been associated with diabetes, psychological well-being, hypertension, and the risk of osteoarthritis (116, 119, 120). In African Americans, the presence

of the p.Thr92Ala is associated with a higher risk, compared to European Americans, of developing Alzheimer’s disease, supporting the hypothesis that p.Thr92Ala might represent one factor contributing to racial discrepancies in incident AD.

Furthermore, some studies have reported the association between polymorphism in *DIO1* and *DIO2* and autoimmune thyroid disease, reporting a low frequency of the T/T genotype for D2 rs225014 polymorphism (which is associated with higher D2 activity) in autoimmune thyroid diseases, and especially in Hashimoto’s thyroiditis (121).

## 5. CONCLUSIONS

Since their first discovery, iodothyronine deiodinases have represented a very challenging topic in TH biology, both in physiological and pathological conditions. The superfine regulation of the HPT axis by iodothyronine deiodinases is the result of a harmonious concert of the three enzymes, which guarantees a physiological steady-state in thyroid function. In pathological conditions, iodothyronine deiodinase activity is regulated to “protect” each tissue from both over- and under-exposition of THs, and several drugs (e.g., amiodarone) exert their pharmacological activity via their effects on iodothyronine deiodinases.

Animal models created by the targeted disruption of *DIO* genes have unveiled some of the mechanisms at the basis of these complex events. However, it is difficult to fully apply certain paradigms deriving from these experiments due to the different contribution of each iodothyronine deiodinase to the circulating and local pools of T3 that exists between animals (in particular rodents) and humans.

The biggest challenge is to better characterize the genetic background and the clinical features of the syndromes related to defects of the *SBP2* gene, which is the only known genetic defect related to iodothyronine deiodinase activity. Unfortunately, probably due to the relatively recent discovery of this syndrome, few cases have been reported in the literature. Nevertheless, this condition should be considered and eventually properly evaluated in the differential diagnosis of the reduced

sensitivity to TH syndromes.

A more difficult trail to run across is represented by the role of iodothyronine deiodinase polymorphism both in the presence of persistent symptoms of hypothyroidism during “biochemically adequate” LT4 replacement therapy and in the presence of abnormal increases of LT4 replacement therapy to normalize TSH levels. Data are conflicting, on the one hand authors considers the genetic background an important element to guarantee a “personalized medicine” instead of empirical treatment, on the other hand different studies exclude any significant association between patients carrying the polymorphism and controls. In conclusion, further studies are needed to evaluate the efficacy of a combined LT4–LT3 replacement therapy in this subset of patients.

## 6. ACKNOWLEDGMENT

The authors contributed equally to this work.

## 7. REFERENCES

1. R. Mullur, Y. Y. Liu and G. A. Brent: Thyroid hormone regulation of metabolism. *Physiol Rev*, 94(2), 355-82 (2014)  
DOI: 10.1152/physrev.00030.2013  
PMid:24692351 PMCID:PMC4044302
2. T. M. Ortiga-Carvalho, A. R. Sidhaye and F. E. Wondisford: Thyroid hormone receptors and resistance to thyroid hormone disorders. *Nat Rev Endocrinol*, 10(10), 582-91 (2014)  
DOI: 10.1038/nrendo.2014.143  
PMid:25135573 PMCID:PMC4578869
3. S. Y. Cheng, J. L. Leonard and P. J. Davis: Molecular aspects of thyroid hormone actions. *Endocr Rev*, 31(2), 139-70 (2010)  
DOI: 10.1210/er.2009-0007  
PMid:20051527 PMCID:PMC2852208
4. M. A. Lazar: Thyroid hormone receptors: multiple forms, multiple possibilities. *Endocr Rev*, 14(2), 184-93 (1993)  
DOI: 10.1210/edrv-14-2-184  
DOI: 10.1210/er.14.2.184  
PMid:8325251
5. R. C. Ribeiro, P. J. Kushner and J. D. Baxter: The nuclear hormone receptor gene superfamily. *Annu Rev Med*, 46, 443-53 (1995)  
DOI: 10.1146/annurev.med.46.1.443  
PMid:7598477
6. G. R. Williams: Cloning and characterization of two novel thyroid hormone receptor beta isoforms. *Mol Cell Biol*, 20(22), 8329-42 (2000)  
DOI: 10.1128/MCB.20.22.8329-8342.2000  
PMid:11046130 PMCID:PMC102140
7. K. Moriyama, H. Yamamoto, K. Futawaka, A. Atake, M. Kasahara and T. Tagami: Molecular characterization of human thyroid hormone receptor beta isoform 4. *Endocr Res*, 41(1), 34-42 (2016)  
DOI: 10.3109/07435800.2015.1066801  
PMid:26513165
8. T. Mitsushashi, G. E. Tennyson and V. M. Nikodem: Alternative splicing generates messages encoding rat c-erbA proteins that do not bind thyroid hormone. *Proc Natl Acad Sci U S A.*, 85(16), 5804-8 (1988)  
DOI: 10.1073/pnas.85.16.5804  
PMid:2901090 PMCID:PMC281853
9. F. Casas, M. Busson, S. Grandemange, P. Seyer, A. Carazo, L. Pessemesse, C. Wrutniak-Cabello and G. Cabello: Characterization of a novel thyroid hormone receptor alpha variant involved in the regulation of myoblast differentiation. *Mol Endocrinol*, 20(4), 749-63 (2006)

- DOI: 10.1210/me.2005-0074  
PMid:16322094
10. D. J. Bradley, H. C. Towle and W. S. Young, 3rd: Spatial and temporal expression of alpha- and beta-thyroid hormone receptor mRNAs, including the beta 2-subtype, in the developing mammalian nervous system. *J Neurosci*, 12(6), 2288-302 (1992)  
DOI: 10.1523/JNEUROSCI.12-06-02288.1992  
PMid:1607941
  11. J. Jonklaas, B. Davidson, S. Bhagat and S. J. Soldin: Triiodothyronine levels in athyreotic individuals during levothyroxine therapy. *JAMA*, 299(7), 769-77 (2008)  
DOI: 10.1001/jama.299.7.769  
PMid:18285588
  12. P. J. Davis, F. B. Davis, H. Y. Lin, S. A. Mousa, M. Zhou and M. K. Luidens: Translational implications of nongenomic actions of thyroid hormone initiated at its integrin receptor. *Am J Physiol Endocrinol Metab*, 297(6), E1238-46 (2009)  
DOI: 10.1152/ajpendo.00480.2009  
PMid:19755667
  13. A. C. Schroeder and M. L. Privalsky: Thyroid hormones, t3 and t4, in the brain. *Front Endocrinol (Lausanne)*, 5, 40 (2014)  
DOI: 10.3389/fendo.2014.00040  
PMid:24744751 PMCid:PMC3978256
  14. G. Morreale de Escobar, M. J. Obregon and F. Escobar del Rey: Role of thyroid hormone during early brain development. *Eur J Endocrinol*, 151 Suppl 3, U25-37 (2004)  
DOI: 10.1530/eje.0.151u025
  15. G. Canettieri, A. Franchi, M. D. Guardia, I. Morante, M. G. Santaguida, J. W. Harney, P. R. Larsen and M. Centanni: Activation of thyroid hormone is transcriptionally regulated by epidermal growth factor in human placenta-derived JEG3 cells. *Endocrinology*, 149(2), 695-702 (2008)  
DOI: 10.1210/en.2007-0779  
PMid:17991726 PMCid:PMC2219305
  16. G. Canettieri, A. Franchi, R. Sibilla, E. Guzman and M. Centanni: Functional characterisation of the CRE/TATA box unit of type 2 deiodinase gene promoter in a human choriocarcinoma cell line. *J Mol Endocrinol*, 33(1), 51-8 (2004)  
DOI: 10.1677/jme.0.0330051  
PMid:15291742
  17. L. Sabatino, G. Iervasi, P. Ferrazzi, D. Francesconi and I. J. Chopra: A study of iodothyronine 59-monodeiodinase activities in normal and pathological tissues in man and their comparison with activities in rat tissues. *Life Sci.*, 68(2), 191-202 (2000)  
DOI: 10.1016/S0024-3205(00)00929-2
  18. C. Virili and M. Centanni: "With a little help from my friends" - The role of microbiota in thyroid hormone metabolism and enterohepatic recycling. *Mol Cell Endocrinol*, 458, 39-43 (2017)  
DOI: 10.1016/j.mce.2017.01.053  
PMid:28167127
  19. M. L. Rosene, G. Wittmann, R. Arrojo e Drigo, P. S. Singru, R. M. Lechan and A. C. Bianco: Inhibition of the type 2 iodothyronine deiodinase underlies the elevated plasma TSH associated with amiodarone treatment. *Endocrinology*, 151(12), 5961-70 (2010)  
DOI: 10.1210/en.2010-0553

- PMid:20926587 PMCID:PMC2999495
20. D. Villar, S. M. Rhind, P. Dicks, S. R. McMillen, F. Nicol and J. R. Arthur: Effect of propylthiouracil-induced hypothyroidism on thyroid hormone profiles and tissue deiodinase activity in cashmere goats. *Small Ruminant Research*, 29(3), 317-324 (1998)  
DOI: 10.1016/S0921-4488(97)00130-2
21. E. M. de Vries, E. Fliers and A. Boelen: The molecular basis of the non-thyroidal illness syndrome. *J Endocrinol*, 225(3), R67-81 (2015)  
DOI: 10.1530/JOE-15-0133  
PMid:25972358
22. A. C. Bianco and R. R. da Conceicao: The Deiodinase Trio and Thyroid Hormone Signaling. *Methods Mol Biol*, 1801, 67-83 (2018)  
DOI: 10.1007/978-1-4939-7902-8\_8  
PMid:29892818
23. T. M. Ortiga-Carvalho, M. I. Chiamolera, C. C. Pazos-Moura and F. E. Wondisford: Hypothalamus-Pituitary-Thyroid Axis. *Compr Physiol*, 6(3), 1387-428 (2016)  
DOI: 10.1002/cphy.c150027  
PMid:27347897
24. J. Kohrle and H. Biebermann: 3-Iodothyronamine-A Thyroid Hormone Metabolite With Distinct Target Profiles and Mode of Action. *Endocr Rev*, 40(2), 602-630 (2019)  
DOI: 10.1210/er.2018-00182  
PMid:30649231
25. R. Senese, P. de Lange, G. Petito, M. Moreno, F. Goglia and A. Lanni: 3,5-Diiodothyronine: A Novel Thyroid Hormone Metabolite and Potent Modulator of Energy Metabolism. *Front Endocrinol (Lausanne)*, 9, 427 (2018)
- DOI: 10.3389/fendo.2018.00427  
PMid:30090086 PMCID:PMC6068267
26. G. D. Sagar, B. Gereben, I. Callebaut, J. P. Mornon, A. Zeold, C. Curcio-Morelli, J. W. Harney, C. Luongo, M. A. Mulcahey, P. R. Larsen, S. A. Huang and A. C. Bianco: The thyroid hormone-inactivating deiodinase functions as a homodimer. *Mol Endocrinol*, 22(6), 1382-93 (2008)  
DOI: 10.1210/me.2007-0490  
PMid:18356288 PMCID:PMC2422829
27. S. Benvenga and F. Guarneri: Deiodinases share an evolutionarily conserved thyroid hormone-binding motif. *Front Biosci (Landmark Ed)*, 23, 2195-2203 (2018)  
DOI: 10.2741/4699
28. B. Gereben, A. M. Zavacki, S. Ribich, B. W. Kim, S. A. Huang, W. S. Simonides, A. Zeold and A. C. Bianco: Cellular and molecular basis of deiodinase-regulated thyroid hormone signaling. *Endocr Rev*, 29(7), 898-938 (2008)  
DOI: 10.1210/er.2008-0019  
PMid:18815314 PMCID:PMC2647704
29. A. L. Maia, I. M. Goemann, E. L. Meyer and S. M. Wajner: Deiodinases: the balance of thyroid hormone: type 1 iodothyronine deiodinase in human physiology and disease. *J Endocrinol*, 209(3), 283-97 (2011)  
DOI: 10.1530/JOE-10-0481  
PMid:21415143
30. S. M. Abdalla and A. C. Bianco: Defending plasma T3 is a biological priority. *Clin Endocrinol (Oxf)*, 81(5), 633-41 (2014)  
DOI: 10.1111/cen.12538  
PMid:25040645 PMCID:PMC4699302
31. A. C. Bianco, D. Salvatore, B. Gereben,

- M. J. Berry and P. R. Larsen: Biochemistry, cellular and molecular biology, and physiological roles of the iodothyronine selenodeiodinases. *Endocr Rev*, 23(1), 38-89 (2002)  
DOI: 10.1210/edrv.23.1.0455  
PMid:11844744
32. J. M. Bates, D. L. St Germain and V. A. Galton: Expression profiles of the three iodothyronine deiodinases, D1, D2, and D3, in the developing rat. *Endocrinology*, 140(2), 844-51 (1999)  
DOI: 10.1210/endo.140.2.6537  
PMid:9927314
33. E. A. McAninch and A. C. Bianco: Thyroid hormone signaling in energy homeostasis and energy metabolism. *Ann N Y Acad Sci*, 1311, 77-87 (2014)  
DOI: 10.1111/nyas.12374  
PMid:24697152 PMCID:PMC4451242
34. L. J. Lartey, J. P. Werneck-de-Castro, O. S. I, T. G. Unterman and A. C. Bianco: Coupling between Nutrient Availability and Thyroid Hormone Activation. *J Biol Chem*, 290(51), 30551-61 (2015)  
DOI: 10.1074/jbc.M115.665505  
PMid:26499800 PMCID:PMC4683275
35. S. Barez-Lopez, D. Bosch-Garcia, D. Gomez-Andres, I. Pulido-Valdeolivas, A. Montero-Pedrazuela, M. J. Obregon and A. Guadano-Ferraz: Abnormal motor phenotype at adult stages in mice lacking type 2 deiodinase. *PLoS One*, 9(8), e103857 (2014)  
DOI: 10.1371/journal.pone.0103857  
PMid:25083788 PMCID:PMC4118963
36. M. Dentice, A. Marsili, R. Ambrosio, O. Guardiola, A. Sibilio, J. H. Paik, G. Minchiotti, R. A. DePinho, G. Fenzi, P. R. Larsen and D. Salvatore: The FoxO3/type 2 deiodinase pathway is required for normal mouse myogenesis and muscle regeneration. *J Clin Invest*, 120(11), 4021-30 (2010)  
DOI: 10.1172/JCI43670  
PMid:20978344 PMCID:PMC2964991
37. S. Jo, I. Kallo, Z. Bardoczi, R. Arrojo e Drigo, A. Zeold, Z. Liposits, A. Oliva, V. P. Lemmon, J. L. Bixby, B. Gereben and A. C. Bianco: Neuronal hypoxia induces Hsp40-mediated nuclear import of type 3 deiodinase as an adaptive mechanism to reduce cellular metabolism. *J Neurosci*, 32(25), 8491-500 (2012)  
DOI: 10.1523/JNEUROSCI.6514-11.2012  
PMid:22723689 PMCID:PMC3752066
38. E. K. Alexander, E. Marqusee, J. Lawrence, P. Jarolim, G. A. Fischer and P. R. Larsen: Timing and magnitude of increases in levothyroxine requirements during pregnancy in women with hypothyroidism. *N Engl J Med*, 351(3), 241-9 (2004)  
DOI: 10.1056/NEJMoa040079  
PMid:15254282
39. S. A. Huang and A. C. Bianco: Reawakened interest in type III iodothyronine deiodinase in critical illness and injury. *Nat Clin Pract Endocrinol Metab*, 4(3), 148-55 (2008)  
DOI: 10.1038/ncpendmet0727  
PMid:18212764 PMCID:PMC3133953
40. M. C. Medina, J. Molina, Y. Gadea, A. Fachado, M. Murillo, G. Simovic, A. Pileggi, A. Hernandez, H. Edlund and A. C. Bianco: The thyroid hormone-inactivating type III deiodinase is expressed in mouse and human beta-cells and its targeted inactivation impairs insulin secretion. *Endocrinology*, 152(10), 3717-27 (2011)



- DOI: 10.1210/en.2011-1210  
PMid:21828183 PMCID:PMC3176649
41. W. S. Simonides, M. A. Mulcahey, E. M. Redout, A. Muller, M. J. Zuidwijk, T. J. Visser, F. W. Wassen, A. Crescenzi, W. S. da-Silva, J. Harney, F. B. Engel, M. J. Obregon, P. R. Larsen, A. C. Bianco and S. A. Huang: Hypoxia-inducible factor induces local thyroid hormone inactivation during hypoxic-ischemic disease in rats. *J Clin Invest*, 118(3), 975-83 (2008)  
DOI: 10.1172/JCI32824  
PMid:18259611 PMCID:PMC2230657
42. E. L. Olivares, M. P. Marassi, R. S. Fortunato, A. C. da Silva, R. H. Costa-e-Sousa, I. G. Araujo, E. C. Mattos, M. O. Masuda, M. A. Mulcahey, S. A. Huang, A. C. Bianco and D. P. Carvalho: Thyroid function disturbance and type 3 iodothyronine deiodinase induction after myocardial infarction in rats a time course study. *Endocrinology*, 148(10), 4786-92 (2007)  
DOI: 10.1210/en.2007-0043  
PMid:17628010
43. M. A. Maynard, A. Marino-Enriquez, J. A. Fletcher, D. M. Dorfman, C. P. Raut, L. Yassa, C. Guo, Y. Wang, C. Dorfman, H. A. Feldman, M. C. Frates, H. Song, R. H. Jugo, T. Taguchi, J. M. Hershman, P. R. Larsen and S. A. Huang: Thyroid hormone inactivation in gastrointestinal stromal tumors. *N Engl J Med*, 370(14), 1327-34 (2014)  
DOI: 10.1056/NEJMoa1308893  
PMid:24693892 PMCID:PMC4186889
44. C. Luongo, L. Trivisano, F. Alfano and D. Salvatore: Type 3 deiodinase and consumptive hypothyroidism: a common mechanism for a rare disease. *Front Endocrinol (Lausanne)*, 4, 115 (2013)
- DOI: 10.3389/fendo.2013.00115  
PMid:24027558 PMCID:PMC3761349
45. K. Bessho, Y. Etani, H. Ichimori, Y. Miyoshi, N. Namba, A. Yoneda, T. Ooue, T. Chihara, E. Morii, T. Aoki, M. Murakami, S. Mushiake and K. Ozono: Increased type 3 iodothyronine deiodinase activity in a regrown hepatic hemangioma with consumptive hypothyroidism. *Eur J Pediatr*, 169(2), 215-21 (2010)  
DOI: 10.1007/s00431-009-1009-x  
PMid:19548001
46. F. Mouat, H. M. Evans, W. S. Cutfield, P. L. Hofman and C. Jefferies: Massive hepatic hemangioendothelioma and consumptive hypothyroidism. *J Pediatr Endocrinol Metab*, 21(7), 701-3 (2008)  
DOI: 10.1515/JPEM.2008.21.7.701  
PMid:18780606
47. F. Torino, A. Barnabei, R. Paragliola, R. Baldelli, M. Appetecchia and S. M. Corsello: Thyroid dysfunction as an unintended side effect of anticancer drugs. *Thyroid*, 23(11), 1345-66 (2013)  
DOI: 10.1089/thy.2013.0241  
PMid:23750887
48. A. L. Maia, B. W. Kim, S. A. Huang, J. W. Harney and P. R. Larsen: Type 2 iodothyronine deiodinase is the major source of plasma T3 in euthyroid humans. *J Clin Invest*, 115(9), 2524-33 (2005)  
DOI: 10.1172/JCI25083  
PMid:16127464 PMCID:PMC1190373
49. M. H. Warner and G. J. Beckett: Mechanisms behind the non-thyroidal illness syndrome: an update. *J Endocrinol*, 205(1), 1-13 (2010)  
DOI: 10.1677/JOE-09-0412  
DOI: 10.1677/joe.0.0770001

- PMid:20016054
50. A. L. Maia: Effect of 3,5,3'-Triiodothyronine (T3) administration on *dio1* gene expression and T3 metabolism in normal and type 1 deiodinase-deficient mice. *Endocrinology*, 136(11), 4842-4849 (1995)  
DOI: 10.1210/endo.136.11.7588215  
PMid:7588215
51. B. Gereben, C. Goncalves, J. W. Harney, P. R. Larsen and A. C. Bianco: Selective proteolysis of human type 2 deiodinase: a novel ubiquitin-proteasomal mediated mechanism for regulation of hormone activation. *Mol Endocrinol*, 14(11), 1697-708 (2000)  
DOI: 10.1210/mend.14.11.0558  
PMid:11075806
52. P. Laurberg, H. Vestergaard, S. Nielsen, S. E. Christensen, T. Seefeldt, K. Helleberg and K. M. Pedersen: Sources of circulating 3,5,3'-triiodothyronine in hyperthyroidism estimated after blocking of type 1 and type 2 iodothyronine deiodinases. *J Clin Endocrinol Metab*, 92(6), 2149-56 (2007)  
DOI: 10.1210/jc.2007-0178  
PMid:17389703
53. A. Kadar, E. Sanchez, G. Wittmann, P. S. Singru, T. Fuzesi, A. Marsili, P. R. Larsen, Z. Liposits, R. M. Lechan and C. Fekete: Distribution of hypophysiotropic thyrotropin-releasing hormone (TRH)-synthesizing neurons in the hypothalamic paraventricular nucleus of the mouse. *J Comp Neurol*, 518(19), 3948-61 (2010)  
DOI: 10.1002/cne.22432  
PMid:20737594 PMCID:PMC2932658
54. T. P. Segerson, J. Kauer, H. C. Wolfe, H. Mobtaker, P. Wu, I. M. Jackson and R. M. Lechan: Thyroid hormone regulates TRH biosynthesis in the paraventricular nucleus of the rat hypothalamus. *Science*, 238(4823), 78-80 (1987)  
DOI: 10.1126/science.3116669  
PMid:3116669
55. E. D. Abel, R. S. Ahima, M. E. Boers, J. K. Elmquist and F. E. Wondisford: Critical role for thyroid hormone receptor beta2 in the regulation of paraventricular thyrotropin-releasing hormone neurons. *J Clin Invest*, 107(8), 1017-23 (2001)  
DOI: 10.1172/JCI10858  
PMid:11306605 PMCID:PMC199552
56. A. A. Nikrodhanond, T. M. Ortiga-Carvalho, N. Shibusawa, K. Hashimoto, X. H. Liao, S. Refetoff, M. Yamada, M. Mori and F. E. Wondisford: Dominant role of thyrotropin-releasing hormone in the hypothalamic-pituitary-thyroid axis. *J Biol Chem*, 281(8), 5000-7 (2006)  
DOI: 10.1074/jbc.M511530200  
PMid:16339138
57. F. R. Crantz and P. R. Larsen: Rapid thyroxine to 3,5,3'-triiodothyronine conversion and nuclear 3,5,3'-triiodothyronine binding in rat cerebral cortex and cerebellum. *J Clin Invest*, 65(4), 935-8 (1980)  
DOI: 10.1172/JCI109749  
PMid:7358853 PMCID:PMC434484
58. P. Egri, C. Fekete, A. Denes, D. Reglodi, H. Hashimoto, B. D. Fulop and B. Gereben: Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP) Regulates the Hypothalamo-Pituitary-Thyroid (HPT) Axis via Type 2 Deiodinase in Male Mice. *Endocrinology*, 157(6), 2356-66 (2016)  
DOI: 10.1210/en.2016-1043  
PMid:27046436
59. M. A. Christoffolete, R. Ribeiro, P. Singru,

- C. Fekete, W. S. da Silva, D. F. Gordon, S. A. Huang, A. Crescenzi, J. W. Harney, E. C. Ridgway, P. R. Larsen, R. M. Lechan and A. C. Bianco: Atypical expression of type 2 iodothyronine deiodinase in thyrotrophs explains the thyroxine-mediated pituitary thyrotropin feedback mechanism. *Endocrinology*, 147(4), 1735-43 (2006)  
DOI: 10.1210/en.2005-1300  
PMid:16396983
60. E. D. R. Arrojo, T. L. Fonseca, J. P. Werneck-de-Castro and A. C. Bianco: Role of the type 2 iodothyronine deiodinase (D2) in the control of thyroid hormone signaling. *Biochim Biophys Acta*, 1830(7), 3956-64 (2013)  
DOI: 10.1016/j.bbagen.2012.08.019  
PMid:22967761 PMCID:PMC4979226
61. M. L. Sugrue, K. R. Vella, C. Morales, M. E. Lopez and A. N. Hollenberg: The thyrotropin-releasing hormone gene is regulated by thyroid hormone at the level of transcription *in vivo*. *Endocrinology*, 151(2), 793-801 (2010)  
DOI: 10.1210/en.2009-0976  
PMid:20032051 PMCID:PMC2817611
62. L. J. De Groot: Non-thyroidal illness syndrome is a manifestation of hypothalamic-pituitary dysfunction, and in view of current evidence, should be treated with appropriate replacement therapies. *Crit Care Clin*, 22(1), 57-86, vi (2006)  
DOI: 10.1016/j.ccc.2005.10.001  
PMid:16399020
63. C. Fekete, B. Gereben, M. Doleschall, J. W. Harney, J. M. Dora, A. C. Bianco, S. Sarkar, Z. Liposits, W. Rand, C. Emerson, I. Kacs Kovics, P. R. Larsen and R. M. Lechan: Lipopolysaccharide induces type 2 iodothyronine deiodinase in the mediobasal hypothalamus: implications for the nonthyroidal illness syndrome. *Endocrinology*, 145(4), 1649-55 (2004)  
DOI: 10.1210/en.2003-1439  
PMid:14684601
64. Y. Debaveye, B. Ellger, L. Mebis, V. M. Darras and G. Van den Berghe: Regulation of tissue iodothyronine deiodinase activity in a model of prolonged critical illness. *Thyroid*, 18(5), 551-60 (2008)  
DOI: 10.1089/thy.2007.0287  
PMid:18466079
65. J. Kwakkel, O. Chassande, H. C. van Beeren, W. M. Wiersinga and A. Boelen: Lacking thyroid hormone receptor beta gene does not influence alterations in peripheral thyroid hormone metabolism during acute illness. *J Endocrinol*, 197(1), 151-8 (2008)  
DOI: 10.1677/JOE-07-0601  
PMid:18372241
66. M. J. Schneider, S. N. Fiering, S. E. Pallud, A. F. Parlow, D. L. St Germain and V. A. Galton: Targeted disruption of the type 2 selenodeiodinase gene (DIO2) results in a phenotype of pituitary resistance to T4. *Mol Endocrinol*, 15(12), 2137-48 (2001)  
DOI: 10.1210/mend.15.12.0740  
PMid:11731615
67. C. Luongo, C. Martin, K. Vella, A. Marsili, R. Ambrosio, M. Dentice, J. W. Harney, D. Salvatore, A. M. Zavacki and P. R. Larsen: The selective loss of the type 2 iodothyronine deiodinase in mouse thyrotrophs increases basal TSH but blunts the thyrotropin response to hypothyroidism. *Endocrinology*, 156(2),

- 745-54 (2015)  
DOI: 10.1210/en.2014-1698  
PMid:25456070 PMCID:PMC4298316
68. M. A. Christoffolete, R. Arrojo e Drigo, F. Gazoni, S. M. Tente, V. Goncalves, B. S. Amorim, P. R. Larsen, A. C. Bianco and A. M. Zavacki: Mice with impaired extrathyroidal thyroxine to 3,5,3'-triiodothyronine conversion maintain normal serum 3,5,3'-triiodothyronine concentrations. *Endocrinology*, 148(3), 954-60 (2007)  
DOI: 10.1210/en.2006-1042  
PMid:17138654
69. V. A. Galton, M. J. Schneider, A. S. Clark and D. L. St Germain: Life without thyroxine to 3,5,3'-triiodothyronine conversion: studies in mice devoid of the 5'-deiodinases. *Endocrinology*, 150(6), 2957-63 (2009)  
DOI: 10.1210/en.2008-1572  
PMid:19196796 PMCID:PMC2689801
70. C. N. Walpita, A. D. Crawford, E. D. Janssens, S. Van der Geyten and V. M. Darras: Type 2 iodothyronine deiodinase is essential for thyroid hormone-dependent embryonic development and pigmentation in zebrafish. *Endocrinology*, 150(1), 530-9 (2009)  
DOI: 10.1210/en.2008-0457  
PMid:18801906
71. M. J. Schneider, S. N. Fiering, B. Thai, S. Y. Wu, E. St Germain, A. F. Parlow, D. L. St Germain and V. A. Galton: Targeted disruption of the type 1 selenodeiodinase gene (*Dio1*) results in marked changes in thyroid hormone economy in mice. *Endocrinology*, 147(1), 580-9 (2006)  
DOI: 10.1210/en.2005-0739  
PMid:16223863
72. S. Refetoff, J. H. Bassett, P. Beck-Peccoz, J. Bernal, G. Brent, K. Chatterjee, L. J. De Groot, A. M. Dumitrescu, J. L. Jameson, P. A. Kopp, Y. Murata, L. Persani, J. Samarut, R. E. Weiss, G. R. Williams and P. M. Yen: Classification and proposed nomenclature for inherited defects of thyroid hormone action, cell transport, and metabolism. *Thyroid*, 24(3), 407-9 (2014)  
DOI: 10.1089/thy.2013.3393.nomen  
PMid:24588711 PMCID:PMC3950730
73. S. Refetoff, L. T. DeWind and L. J. DeGroot: Familial syndrome combining deaf-mutism, stippled epiphyses, goiter and abnormally high PBI: possible target organ refractoriness to thyroid hormone. *J Clin Endocrinol Metab*, 27(2), 279-94 (1967)  
DOI: 10.1210/jcem-27-2-279  
PMid:4163616
74. S. Refetoff, R. E. Weiss and S. J. Usala: The syndromes of resistance to thyroid hormone. *Endocr Rev*, 14(3), 348-99 (1993)  
DOI: 10.1210/edrv-14-3-348  
DOI: 10.1210/er.14.3.348  
PMid:8319599
75. S. J. Usala, G. E. Tennyson, A. E. Bale, R. W. Lash, N. Gesundheit, F. E. Wondisford, D. Accili, P. Hauser and B. D. Weintraub: A base mutation of the *C-erbA* beta thyroid hormone receptor in a kindred with generalized thyroid hormone resistance. Molecular heterogeneity in two other kindreds. *J Clin Invest*, 85(1), 93-100 (1990)  
DOI: 10.1172/JCI114438  
PMid:2153155 PMCID:PMC296391
76. E. Bochukova, N. Schoenmakers, M. Agostini, E. Schoenmakers, O. Rajanayagam, J. M. Keogh, E. Henning,

- J. Reinemund, E. Gevers, M. Sarri, K. Downes, A. Offiah, A. Albanese, D. Halsall, J. W. Schwabe, M. Bain, K. Lindley, F. Muntoni, F. Vargha-Khadem, M. Dattani, I. S. Farooqi, M. Gurnell and K. Chatterjee: A mutation in the thyroid hormone receptor alpha gene. *N Engl J Med*, 366(3), 243-9 (2012)  
DOI: 10.1056/NEJMoa1110296  
PMid:22168587
77. A. M. Dumitrescu, X. H. Liao, T. B. Best, K. Brockmann and S. Refetoff: A novel syndrome combining thyroid and neurological abnormalities is associated with mutations in a monocarboxylate transporter gene. *Am J Hum Genet*, 74(1), 168-75 (2004)  
DOI: 10.1086/380999  
PMid:14661163 PMCID:PMC1181904
78. E. C. H. Friesema, A. Grueters, H. Biebermann, H. Krude, A. von Moers, M. Reeser, T. G. Barrett, E. E. Mancilla, J. Svensson, M. H. A. Kester, G. G. J. M. Kuiper, S. Balkassmi, A. G. Uitterlinden, J. Koehrle, P. Rodien, A. P. Halestrap and T. J. Visser: Association between mutations in a thyroid hormone transporter and severe X-linked psychomotor retardation. *The Lancet*, 364(9443), 1435-1437 (2004)  
DOI: 10.1016/S0140-6736(04)17226-7
79. A. A. van Mullem, A. L. M. van Gucht, W. E. Visser, M. E. Meima, R. P. Peeters and T. J. Visser: Effects of thyroid hormone transporters MCT8 and MCT10 on nuclear activity of T3. *Mol Cell Endocrinol*, 437, 252-260 (2016)  
DOI: 10.1016/j.mce.2016.07.037  
PMid:27492966
80. J. P. Stohn, M. E. Martinez, K. Matoin, B. Morte, J. Bernal, V. A. Galton, D. St Germain and A. Hernandez: MCT8 Deficiency in Male Mice Mitigates the Phenotypic Abnormalities Associated With the Absence of a Functional Type 3 Deiodinase. *Endocrinology*, 157(8), 3266-77 (2016)  
DOI: 10.1210/en.2016-1162  
PMid:27254003 PMCID:PMC4967121
81. X. H. Liao, C. Di Cosmo, A. M. Dumitrescu, A. Hernandez, J. Van Sande, D. L. St Germain, R. E. Weiss, V. A. Galton and S. Refetoff: Distinct roles of deiodinases on the phenotype of Mct8 defect: a comparison of eight different mouse genotypes. *Endocrinology*, 152(3), 1180-91 (2011)  
DOI: 10.1210/en.2010-0900  
PMid:21285310 PMCID:PMC3040057
82. J. Muller and H. Heuer: Understanding the hypothalamus-pituitary-thyroid axis in mct8 deficiency. *Eur Thyroid J*, 1(2), 72-9 (2012)  
DOI: 10.1159/000339474  
PMid:24783000 PMCID:PMC3821472
83. E. K. Wirth, E. Rijntjes, F. Meyer, J. Kohrle and U. Schweizer: High T3, Low T4 Serum Levels in Mct8 Deficiency Are Not Caused by Increased Hepatic Conversion through Type I Deiodinase. *Eur Thyroid J*, 4(Suppl 1), 87-91 (2015)  
DOI: 10.1159/000381021  
PMid:26601078 PMCID:PMC4640264
84. R. J. Koenig: Regulation of type 1 iodothyronine deiodinase in health and disease. *Thyroid*, 15(8), 835-40 (2005)  
DOI: 10.1089/thy.2005.15.835  
PMid:16131326
85. A. M. Dumitrescu, X. H. Liao, M. S. Abdullah, J. Lado-Abeal, F. A. Majed, L. C. Moeller, G. Boran, L. Schomburg, R. E. Weiss and S. Refetoff: Mutations in SECISBP2 result in abnormal thyroid



- hormone metabolism. *Nat Genet*, 37(11), 1247-52 (2005)  
DOI: 10.1038/ng1654  
PMid:16228000
86. A. Lescure, C. Allmang, K. Yamada, P. Carbon and A. Krol: cDNA cloning, expression pattern and RNA binding analysis of human selenocysteine insertion sequence (SECIS) binding protein 2. *Gene*, 291(1-2), 279-85 (2002)  
DOI: 10.1016/S0378-1119(02)00629-7
87. A. M. Dumitrescu and S. Refetoff: Novel biological and clinical aspects of thyroid hormone metabolism. *Endocr Dev*, 10, 127-39 (2007)  
DOI: 10.1159/000106824  
PMid:17684394
88. S. Refetoff and A. M. Dumitrescu: Syndromes of reduced sensitivity to thyroid hormone: genetic defects in hormone receptors, cell transporters and deiodination. *Best Pract Res Clin Endocrinol Metab*, 21(2), 277-305 (2007)  
DOI: 10.1016/j.beem.2007.03.005  
PMid:17574009
89. L. Schomburg, A. M. Dumitrescu, X. H. Liao, B. Bin-Abbas, J. Hoeflich, J. Kohrle and S. Refetoff: Selenium supplementation fails to correct the selenoprotein synthesis defect in subjects with SBP2 gene mutations. *Thyroid*, 19(3), 277-81 (2009)  
DOI: 10.1089/thy.2008.0397  
PMid:19265499 PMCid:PMC2858371
90. A. M. Dumitrescu, C. Di Cosmo, X. H. Liao, R. E. Weiss and S. Refetoff: The syndrome of inherited partial SBP2 deficiency in humans. *Antioxid Redox Signal*, 12(7), 905-20 (2010)  
DOI: 10.1089/ars.2009.2892  
PMid:19769464 PMCid:PMC2864657
91. C. Di Cosmo, N. McLellan, X. H. Liao, K. K. Khanna, R. E. Weiss, L. Papp and S. Refetoff: Clinical and molecular characterization of a novel selenocysteine insertion sequence-binding protein 2 (SBP2) gene mutation (R128X). *J Clin Endocrinol Metab*, 94(10), 4003-9 (2009)  
DOI: 10.1210/jc.2009-0686  
PMid:19602558 PMCid:PMC2758735
92. M. F. Azevedo, G. B. Barra, L. A. Naves, L. F. Ribeiro Velasco, P. Godoy Garcia Castro, L. C. de Castro, A. A. Amato, A. Miniard, D. Driscoll, L. Schomburg and F. de Assis Rocha Neves: Selenoprotein-related disease in a young girl caused by nonsense mutations in the SBP2 gene. *J Clin Endocrinol Metab*, 95(8), 4066-71 (2010)  
DOI: 10.1210/jc.2009-2611  
PMid:20501692
93. B. Moghadaszadeh, N. Petit, C. Jaillard, M. Brockington, S. Quijano Roy, L. Merlini, N. Romero, B. Estournet, I. Desguerre, D. Chaigne, F. Muntoni, H. Topaloglu and P. Guicheney: Mutations in SEP1N1 cause congenital muscular dystrophy with spinal rigidity and restrictive respiratory syndrome. *Nat Genet*, 29(1), 17-8 (2001)  
DOI: 10.1038/ng713  
PMid:11528383
94. E. Schoenmakers, M. Agostini, C. Mitchell, N. Schoenmakers, L. Papp, O. Rajanayagam, R. Padidela, L. Ceron-Gutierrez, R. Doffinger, C. Prevosto, J. Luan, S. Montano, J. Lu, M. Castanet, N. Clemons, M. Groeneveld, P. Castets, M. Karbaschi, S. Aitken, A. Dixon, J. Williams, I. Campi, M. Blount, H. Burton, F. Muntoni, D. O'Donovan, A. Dean, A. Warren, C. Brierley, D. Baguley, P. Guicheney, R. Fitzgerald, A. Coles, H.

- Gaston, P. Todd, A. Holmgren, K. K. Khanna, M. Cooke, R. Semple, D. Halsall, N. Wareham, J. Schwabe, L. Grasso, P. Beck-Peccoz, A. Ogunko, M. Dattani, M. Gurnell and K. Chatterjee: Mutations in the selenocysteine insertion sequence-binding protein 2 gene lead to a multisystem selenoprotein deficiency disorder in humans. *J Clin Invest*, 120(12), 4220-35 (2010)  
DOI: 10.1172/JCI43653  
PMid:21084748 PMCID:PMC2993594
95. T. Hamajima, Y. Mushimoto, H. Kobayashi, Y. Saito and K. Onigata: Novel compound heterozygous mutations in the SBP2 gene: characteristic clinical manifestations and the implications of GH and triiodothyronine in longitudinal bone growth and maturation. *Eur J Endocrinol*, 166(4), 757-64 (2012)  
DOI: 10.1530/EJE-11-0812  
PMid:22247018
96. G. Catli, H. Fujisawa, O. Kirbiyik, M. S. Mimoto, P. Gencpinar, T. R. Ozdemir, B. N. Dundar and A. M. Dumitrescu: A Novel Homozygous Selenocysteine Insertion Sequence Binding Protein 2 (SECISBP2, SBP2) Gene Mutation in a Turkish Boy. *Thyroid* (2018)  
DOI: 10.1089/thy.2018.0015  
PMid:29882503
97. L. H. Duntas: On the trail of the SBP2-syndrome: clues in a Daedalean maze. *J Clin Endocrinol Metab*, 95(8), 3618-21 (2010)  
DOI: 10.1210/jc.2010-1325  
PMid:20685891
98. H. F. Escobar-Morreale, M. J. Obregon, F. Escobar del Rey and G. Morreale de Escobar: Replacement therapy for hypothyroidism with thyroxine alone does not ensure euthyroidism in all tissues, as studied in thyroidectomized rats. *J Clin Invest*, 96(6), 2828-38 (1995)  
DOI: 10.1172/JCI118353  
PMid:8675653 PMCID:PMC185993
99. H. F. Escobar-Morreale, F. E. del Rey, M. J. Obregon and G. M. de Escobar: Only the combined treatment with thyroxine and triiodothyronine ensures euthyroidism in all tissues of the thyroidectomized rat. *Endocrinology*, 137(6), 2490-502 (1996)  
DOI: 10.1210/endo.137.6.8641203  
PMid:8641203
100. L. E. Braverman, S. H. Ingbar and K. Sterling: Conversion of thyroxine (T4) to triiodothyronine (T3) in athyreotic human subjects. *J Clin Invest*, 49(5), 855-64 (1970)  
DOI: 10.1172/JCI106304  
PMid:4986007 PMCID:PMC535757
101. J. Jonklaas, A. C. Bianco, A. J. Bauer, K. D. Burman, A. R. Cappola, F. S. Celi, D. S. Cooper, B. W. Kim, R. P. Peeters, M. S. Rosenthal, A. M. Sawka and R. American Thyroid Association Task Force on Thyroid Hormone: Guidelines for the treatment of hypothyroidism: prepared by the american thyroid association task force on thyroid hormone replacement. *Thyroid*, 24(12), 1670-751 (2014)  
DOI: 10.1089/thy.2014.0028  
PMid:25266247 PMCID:PMC4267409
102. B. R. Haugen: Drugs that suppress TSH or cause central hypothyroidism. *Best Pract Res Clin Endocrinol Metab*, 23(6), 793-800 (2009)  
DOI: 10.1016/j.beem.2009.08.003  
PMid:19942154 PMCID:PMC2784889
103. R. M. Paragliola, A. Prete, P. W. Kaplan,

- S. M. Corsello and R. Salvatori: Treatment of hypopituitarism in patients receiving antiepileptic drugs. *The Lancet Diabetes & Endocrinology*, 3(2), 132-140 (2015)  
DOI: 10.1016/S2213-8587(14)70081-6
104. C. Virili, A. Antonelli, M. G. Santaguida, S. Benvenga and M. Centanni: Gastrointestinal Malabsorption of Thyroxine. *Endocr Rev*, 40(1), 118-136 (2019)  
DOI: 10.1210/er.2018-00168  
PMid:30476027
105. R. Bunevicius, G. Kazanavicius, R. Zalinkevicius and A. J. Prange, Jr.: Effects of thyroxine as compared with thyroxine plus triiodothyronine in patients with hypothyroidism. *N Engl J Med*, 340(6), 424-9 (1999)  
DOI: 10.1056/NEJM199902113400603  
PMid:9971866
106. P. Saravanan, D. J. Simmons, R. Greenwood, T. J. Peters and C. M. Dayan: Partial substitution of thyroxine (T4) with tri-iodothyronine in patients on T4 replacement therapy: results of a large community-based randomized controlled trial. *J Clin Endocrinol Metab*, 90(2), 805-12 (2005)  
DOI: 10.1210/jc.2004-1672  
PMid:15585551
107. V. Panicker, S. G. Wilson, T. D. Spector, S. J. Brown, M. Falchi, J. B. Richards, G. L. Surdulescu, E. M. Lim, S. J. Fletcher and J. P. Walsh: Heritability of serum TSH, free T4 and free T3 concentrations: a study of a large UK twin cohort. *Clin Endocrinol (Oxf)*, 68(4), 652-9 (2008)  
DOI: 10.1111/j.1365-2265.2007.03079.x  
PMid:17970774
108. D. Gullo, A. Latina, F. Frasca, R. Le Moli, G. Pellegriti and R. Vigneri: Levothyroxine monotherapy cannot guarantee euthyroidism in all athyreotic patients. *PLoS One*, 6(8), e22552 (2011)  
DOI: 10.1371/journal.pone.0022552  
PMid:21829633 PMCID:PMC3148220
109. R. P. Peeters, H. van Toor, W. Klootwijk, Y. B. de Rijke, G. G. Kuiper, A. G. Uitterlinden and T. J. Visser: Polymorphisms in thyroid hormone pathway genes are associated with plasma TSH and iodothyronine levels in healthy subjects. *J Clin Endocrinol Metab*, 88(6), 2880-8 (2003)  
DOI: 10.1210/jc.2002-021592  
PMid:12788902
110. V. Panicker, C. Cluett, B. Shields, A. Murray, K. S. Parnell, J. R. Perry, M. N. Weedon, A. Singleton, D. Hernandez, J. Evans, C. Durant, L. Ferrucci, D. Melzer, P. Saravanan, T. J. Visser, G. Ceresini, A. T. Hattersley, B. Vaidya, C. M. Dayan and T. M. Frayling: A common variation in deiodinase 1 gene DIO1 is associated with the relative levels of free thyroxine and triiodothyronine. *J Clin Endocrinol Metab*, 93(8), 3075-81 (2008)  
DOI: 10.1210/jc.2008-0397  
PMid:18492748 PMCID:PMC2515080
111. Y. Young Cho, H. Jeong Kim, H. Won Jang, T. Hyuk Kim, C. S. Ki, S. Wook Kim and J. Hoon Chung: The relationship of 19 functional polymorphisms in iodothyronine deiodinase and psychological well-being in hypothyroid patients. *Endocrine*, 57(1), 115-124 (2017)  
DOI: 10.1007/s12020-017-1307-4  
PMid:28466400
112. H. J. Wouters, H. C. van Loon, M. M. van der Klauw, M. F. Elderson, S. N. Slagter, A. M. Kobold, I. P. Kema, T. P. Links, J. V. van Vliet-Ostaptchouk and B. H.

- WolffenbutTel: No Effect of the Thr92Ala Polymorphism of Deiodinase-2 on Thyroid Hormone Parameters, Health-Related Quality of Life, and Cognitive Functioning in a Large Population-Based Cohort Study. *Thyroid*, 27(2), 147-155 (2017)  
DOI: 10.1089/thy.2016.0199  
PMid:27786042
113. M. Torlontano, C. Durante, I. Torrente, U. Crocetti, G. Augello, G. Ronga, T. Montesano, L. Travascio, A. Verrienti, R. Bruno, S. Santini, P. D'Arcangelo, B. Dallapiccola, S. Filetti and V. Trischitta: Type 2 deiodinase polymorphism (threonine 92 alanine) predicts L-thyroxine dose to achieve target thyrotropin levels in thyroidectomized patients. *J Clin Endocrinol Metab*, 93(3), 910-3 (2008)  
DOI: 10.1210/jc.2007-1067  
PMid:18073314
114. M. G. Castagna, M. Dentice, S. Cantara, R. Ambrosio, F. Maino, T. Porcelli, C. Marzocchi, C. Garbi, F. Pacini and D. Salvatore: DIO2 Thr92Ala Reduces Deiodinase-2 Activity and Serum-T3 Levels in Thyroid-Deficient Patients. *J Clin Endocrinol Metab*, 102(5), 1623-1630 (2017)  
DOI: 10.1210/jc.2016-2587  
PMid:28324063
115. M. Arici, E. Oztas, F. Yanar, N. Aksakal, B. Ozcinar and G. Ozhan: Association between genetic polymorphism and levothyroxine bioavailability in hypothyroid patients. *Endocr J*, 65(3), 317-323 (2018)  
DOI: 10.1507/endocrj.EJ17-0162  
PMid:29321381
116. V. Panicker, P. Saravanan, B. Vaidya, J. Evans, A. T. Hattersley, T. M. Frayling and C. M. Dayan: 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(5), 1623-9 (2009)  
DOI: 10.1210/jc.2008-1301  
PMid:19190113
117. B. C. Appelhof, R. P. Peeters, W. M. Wiersinga, T. J. Visser, E. M. Wekking, J. Huyser, A. H. Schene, J. G. Tijssen, W. J. Hoogendijk and E. Fliers: Polymorphisms in type 2 deiodinase are not associated with well-being, neurocognitive functioning, and preference for combined thyroxine/3,5,3'-triiodothyronine therapy. *J Clin Endocrinol Metab*, 90(11), 6296-9 (2005)  
DOI: 10.1210/jc.2005-0451  
PMid:16144953
118. A. B. Santoro, D. D. Vargens, C. Barros Filho Mde, D. A. Bulzico, L. P. Kowalski, R. M. Meirelles, D. P. Paula, R. R. Neves, C. N. Pessoa, C. J. Struchine and G. Suarez-Kurtz: Effect of UGT1A1, UGT1A3, DIO1 and DIO2 polymorphisms on L-thyroxine doses required for TSH suppression in patients with differentiated thyroid cancer. *Br J Clin Pharmacol*, 78(5), 1067-75 (2014)  
DOI: 10.1111/bcp.12437  
PMid:24910925 PMCID:PMC4243881
119. M. Medici, L. Chaker and R. P. Peeters: A Step Forward in Understanding the Relevance of Genetic Variation in Type 2 Deiodinase. *J Clin Endocrinol Metab*, 102(5), 1775-1778 (2017)  
DOI: 10.1210/jc.2017-00585  
PMid:28482082

120. W. M. Wiersinga: THERAPY OF ENDOCRINE DISEASE: T4 + T3 combination therapy: is there a true effect? *Eur J Endocrinol*, 177(6), R287-R296 (2017)

DOI: 10.1530/EJE-17-0645

PMid:28855267

121. N. Inoue, M. Watanabe, Y. Katsumata, N. Ishido, Y. Hidaka and Y. Iwatani: Functional Polymorphisms of the Type 1 and Type 2 Iodothyronine Deiodinase Genes in Autoimmune Thyroid Diseases. *Immunol Invest*, 47(5), 534-542 (2018)

DOI: 10.1080/08820139.2018.1458861

PMid:29648895

OATPs, Triiodothyronine: T3, Type 1 iodothyronine deiodinase: D1, Type 2 iodothyronine deiodinase: D2, Type 3 iodothyronine deiodinase: D3, Wild-type: WT

**Key Words:** Iodothyronine Deiodinases, Thyroid Hormone, Hypothalamus–Pituitary–Thyroid Axis, SECISBP2, DIO Polymorphism, Reduced Sensitivity To Thyroid Hormone, Review

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**Abbreviations:** alfa-melanocortin-stimulating hormone: alfa-MSH, Agouti-related peptide: AgRP, Differentiated thyroid cancer: DTC, Hypothalamus-pituitary-thyroid: HPT, L-amino acid transporters: LATS, Lipopolysaccharide: LPS, Loss of heterozygosity: LOH, Monocarboxylate transporters: MCT, Neuropeptide Y: NPY, Non-thyroidal illness syndrome: NTIS, Paraventricular nucleus: PVN, Peroxisome proliferator-activated receptor: PPAR, Phosphatidylinositol 3-OH kinase: PI3K, Pituitary adenylate cyclase-activating polypeptide: PACAP, Pituitary transforming gene: PTTG, Propylthiouracil: PTU, Resistance to thyroid hormone: RTH, Retinoid X receptors: RXR, Reverse T3: rT3, Selenocysteine insertion sequence-binding protein 2: SECISBP2 or SBP2, Selenocysteine-specific elongation factor tRNA: tRNA<sup>Sec</sup>, Selenocysteine-specific elongation factor: EF<sup>Sec</sup>, Single nucleotide polymorphisms: SNPs, Thyroid hormone receptor alfa gene: THRA, Thyroid hormone receptor beta gene: THRB, Thyroid hormone receptors: TRs, Thyroid hormones: THs, Thyroid response elements: TREs, Thyrotropin: TSH, Thyrotropin-releasing hormone: TRH, Thyroxine: T4, Transporters of organic anions: