
IMPAIRED SENSITIVITY TO THYROID HORMONE: DEFECTS OF TRANSPORT, METABOLISM AND ACTION

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ABSTRACT

Resistance to thyroid hormone (RTH), a syndrome of reduced responsiveness of target tissues to thyroid hormone (TH) was identified in 1967 (1). An early report proposed various mechanisms including defects in TH transport, metabolism and action (2). However, with the identification of *TH receptor beta* (*THRB*) gene mutations in 1989 (3, 4), the term RTH became synonymous with defects of this specific gene (5). Subsequent discoveries of genetic defects that reduce the effectiveness of TH through altered cell membrane transport (6, 7) and metabolism (8) have broadened the definition of TH hyposensitivity to encompass all defects that can interfere with the biological activity of a chemically intact hormone secreted in normal or even excessive amounts. In this chapter, we have retained the acronym RTH to denote the syndrome produced by reduced intracellular action of the active TH, triiodothyronine (T_3). However, with the identification of mutations in the *TH receptor alpha* (*THRA*) gene (9), RTH syndromes are designated as RTH α and RTH β . The term of impaired sensitivity to TH (ISTH) has been therefore proposed (10-12) to denote altered effectiveness of TH in a broader sense.

TH SECRETION, CELL-MEMBRANE TRANSPORT, METABOLISM AND ACTION

Normal TH action requires 1) adequate synthesis and secretion of TH, 2) its transport across cell membranes, 3) hormone activation through intracellular metabolism, 4) cytosolic processing and nuclear translocation, 5) binding to receptors, and 6) interaction of the receptors with co-regulators or other post receptor effects mediating the TH effect. In addition to nuclear actions of TH, non-genomic actions are also of physiological relevance (13, 14).

Maintenance of TH supply is insured by a feedback control mechanism involving the hypothalamus, pituitary, and thyroid gland (Figure 1A). A decrease in the circulating TH concentration induces a hypothalamus-mediated stimulation of thyroid stimulating hormone (TSH) secretion mediated by TSH-releasing hormone (TRH) from the pituitary thyrotrophs, which stimulates the thyroid follicular cells to synthesize and secrete more hormone. In contrast, TH excess attenuates or suppresses the system through the same pathway, in order to maintain homeostasis. This centrally regulated system, does not respond to changing requirements for TH in a particular organ or cell.

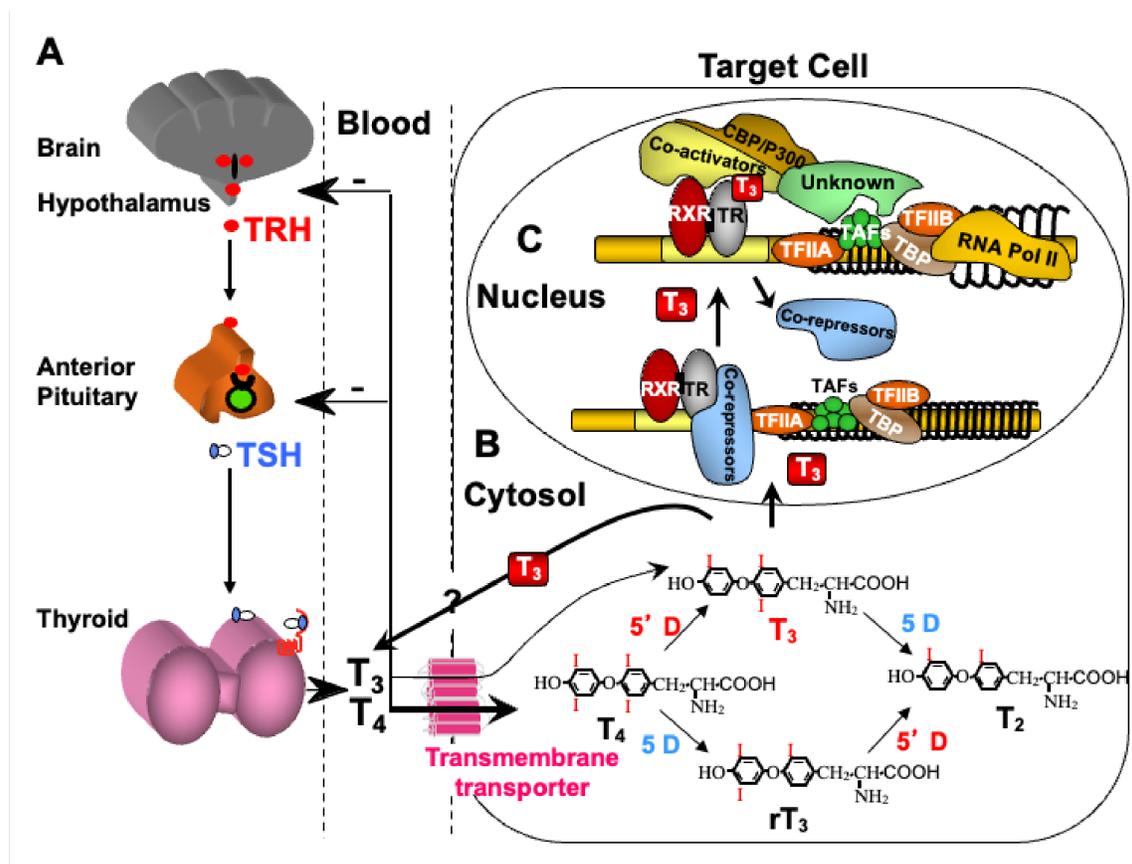


Figure 1. Regulation of TH supply, metabolism and genomic action. (A) Feedback control that regulates the amount of TH in blood. (B) Intracellular metabolism of TH, regulating TH bioactivity. (C) Genomic action of TH. For details see text. CBP/P300, cAMP-binding protein/general transcription adaptor; TFIIA and TFIIB, transcription intermediary factor II, A and B; TBP, TATA-binding protein; TAF, TBP-associated factor.

Additional systems operate to accommodate for local TH requirements. One such system is the control of TH entry into the cell through active transmembrane transporters (15). Another is the activation of the hormone precursor thyroxine (T₄) by removal of the outer ring iodine (5'-deiodination) to form T₃ or, inactivation of T₄ and T₃ by removal of the inner ring iodine (5-deiodination) to form reverse T₃ (rT₃) and T₂, respectively (Chapter 6) (Figure 1B). Cell specific adjustment in deiodinase activity allows for additional local regulation of hormone supply (16).

Finally, the types and abundance of TH receptors (TRs), through which TH action is mediated, determine the nature and degree of the response. TH action takes place in the cytosol as well as in the nucleus (13). The latter, known as genomic or type 1 effect, has been more extensively studied (14, 17, 18) (Figure 1C). TRs are ligand-regulated transcription factors that bind to DNA of genes whose expression they regulate either positively or negatively.

THE PARADOX OF COEXISTING MANIFESTATIONS OF THYROID HORMONE DEFICIENCY AND EXCESS

TH deficiency and excess are associated with typical symptoms and signs reflecting the global effects of lack and excess of the hormone, respectively, on all organs. A departure from this became apparent with the identification of the RTH β syndrome. Subjects with RTH β have high serum TH levels without TSH suppression. This paradox encompasses biochemical and clinical observations suggesting TH deficiency, sufficiency, and excess, depending on the degree and nature of the TR protein abnormality in affected individuals (5). The syndrome of TH cell membrane transport defect (THCMTD) presents a similar paradox, as subjects have high serum T₃ concentration but the cellular uptake of TH is not uniform in all tissues and cell types (19).

THYROID HORMONE ACTION DEFECTS KNOWN AS RESISTANCE TO THYROID HORMONE (RTH)

The first syndrome recognized to impair the sensitivity to TH was that of reduced TH action at the cellular level (1), and it was described as Resistance to Thyroid Hormone (RTH) (2). After the clinical recognition of the syndrome, it took 22 years until the molecular defect could be unraveled by demonstrating mutations in the *THRB* gene in 1989 (3). Twenty-three years after this discovery, mutations in the *THRA* gene led to the recognition of a distinct syndrome, RTH α , in 2012 (9, 20). In addition to these two syndromes, RTH β and RTH α , other causes that impair the sensitivity to TH have been identified during the last two decades.

RESISTANCE TO THYROID HORMONE-BETA (RTH β)

Patients with RTH β are identified by their persistent elevation of circulating free TH associated with non-

suppressed serum TSH levels, in the absence of intercurrent illnesses, drugs, or alterations of TH transport serum proteins. In addition, laboratory testing reveals that unusually high doses of exogenous TH are required to produce the expected suppressive effect on the secretion of pituitary TSH and the expected metabolic responses in peripheral tissues.

Although the apparent resistance to TH may vary in severity, it is always partial. The variability in clinical manifestations may be due to the severity of the hormonal resistance, the effectiveness of compensatory mechanisms, the presence of modulating genetic factors, and the effects of prior therapy. The magnitude of the hormonal resistance is, in turn, dependent on the nature of the underlying genetic defect, most commonly, a mutation in the *THRB* gene (5, 21)

Despite a variable clinical presentation, the common features characteristic of the RTH β syndrome are: 1) elevated serum levels of free T₄ and to a lesser degree T₃, particularly in older individuals, 2) normal or slightly increased serum TSH levels that respond to thyrotropin releasing hormone (TRH), 3) an absence of the usual symptoms and metabolic consequences of TH excess, and 4) goiter.

Clinical Classification

The diagnosis is based on the clinical findings and standard laboratory tests and confirmed by genetic studies. Before *THRB* gene defects were recognized, the proposed sub-classification of RTH β was based on symptoms, signs and laboratory parameters of tissue responses to TH (22). Notwithstanding the assessment of TSH feedback regulation by TH, the measurements of most other responses to TH have low sensitivity and are relatively nonspecific. For this reason, all tissues other than the pituitary have been grouped together under the term *peripheral tissues*, on which the impact of TH was roughly assessed by a

combination of clinical observation and laboratory tests.

The majority of patients who appear to be eumetabolic and maintain a near normal serum TSH concentration have been classified as having **Generalized Resistance to TH (GRTH)**. In such individuals, the defect seems to be compensated by the high levels of TH. In contrast, patients with equally high serum levels of TH and non-suppressed TSH levels, who appear to be hypermetabolic, because they have signs such as sinus tachycardia, are classified as having selective **pituitary resistance to TH (PRTH)**. TSH-producing pituitary adenomas caused by somatic mutations or isoform specific TR β s mutants also fall into this category of RTH (23, 24). Finally, the occurrence of isolated **peripheral tissue resistance to TH (PTRTH)** was reported in a single patient (25). No mutation in the *THRB* gene of this patient could be identified (26), and no similar cases have been reported. Thus, it is uncertain whether PTRTH exists as a true entity. The earliest suggestion that PRTH may not constitute an entity distinct from GRTH was reported by Beck-Peccoz et al. (27). A comprehensive study involving 312 patients with GRTH and 72 patients with PRTH, has conclusively shown a significant overlap in all parameters examined including tachycardia, emotional disturbance and hyperactivity in the two categories (28). More importantly, identical mutations were found in individuals classified as having GRTH and PRTH, many of whom belonged to the same family (29). This led to the conclusion that these two seemingly different forms of RTH are in fact related to a spectrum of subjective symptoms, as well as the individual's target organ susceptibility to changes of TH, a phenomenon also observed in subjects with

thyroid dysfunction in the absence of RTH (See section on the Molecular Basis of the Defect).

Incidence and Inheritance

The precise incidence of RTH is unknown. Because most routine neonatal screening programs are based on the determination of TSH, RTH β is rarely identified by this means (30). A limited neonatal survey by measuring blood T₄ concentrations suggested the occurrence of one case per 40,000 live births (31, 32). Known cases with *THRB* gene mutations surpass 4,000 affected individuals.

Although most thyroid diseases occur more commonly in women, RTH β has been found with equal frequency in females and males. The condition appears to have wide geographic distribution and has been reported in Caucasians, Africans, Asians and Amerindians. The prevalence may vary among different ethnic groups.

Familial occurrence of RTH β has been documented in approximately 75% of cases. Taking into account only those families in whom both parents of the affected subjects have been studied, the true incidence of sporadic cases or *de novo* mutations is 19% (Table 1). Not uncommonly, hypothyroid adults given supraphysiological amounts of TH on subjective basis are labeled as having acquired RTH. Such individuals have no mutations in the *THRB* gene but a compensatory upregulation of the TH degrading enzyme deiodinase 3. This can be demonstrated by the very high concentration of reverse T3 but normal T3 (33).

Table 1. Types of TR β Gene Mutations					
Type		Number of occurrences	Number of families		Effect on TR β
		qt different sites	(total)	(authors)'	
Substitution	Single nucleotide	190	610	286	Single a.a. substitution;
		5	15	9	Premature stop: C434*, K443*, E445* C446*, E449*
	Dinucleotide	3	3	1	Single a.a. substitution: P453N, P453Y; Premature stop: F451*
Deletion	Single nucleotide	2	2	2	FrSh at codon 438 and 440; stop at codon 442*
	Trinucleotide	6	10	4	Single a.a. deletion: E248 Δ , I276 Δ , T337 Δ , M430 Δ , G432 Δ , P452 Δ
	Eight nucleotides	1	1	0	FrSh at codon 443 normal stop at codon 462
	Eleven nucleotides	1	2	1	FrSh at codon 449 stop at codon 459
	All coding sequence	1	1	1	Complete deletion
Insertion	Single nucleotide	8	20	12	FrSh at codons: 436, 443, 448, 451, 454, 456 stop at 464)
	Trinucleotide	1	0	1	Single a.a. insertion (328S)
Duplication	Seven nucleotides	1	1	0	At codon 452 FrSh and a.a. 464 (extended with 2 a.a.)
TOTAL		219	665	317	
Mutations at CpG dinucleotides		20	212 ^a	111 ^a	35% of families with single nucleotide substitution and 39% of similar families studied in the authors' laboratory
De novo mutations			^b	60 ^c	19% of families studied in the authors' laboratory

a.a., amino acid. FrSh, frame shift

^a Not included are 10 families in which the mutation did not follow the rule of G to A or C to T transition.

^b Not counted as publications do not always include parental genotype

^c Families with THRB gene mutations excluding those with a single affected individual when both parents were not tested.

The inheritance of RTH β is typically autosomal dominant. Transmission was only recessive in a single family (1, 34). The biallelic expression of the mutant

TR β due to consanguinity in three families with dominant inheritance of RTH β , as well as the possible deletion of the paternal allele in another family, has led

to very severe clinical manifestations in the affected children (35, 36).

Etiology and Genetics

Using the technique of restriction fragment length polymorphism, Usala et al. (37) were first to demonstrate linkage between the *THRB* locus on chromosome 3 and the RTH β phenotype. Subsequent studies at the University of Chicago and at the National Institutes of Health have identified distinct point mutations in the *THRB* gene of two unrelated families with RTH β (3, 4). In both families, only one of the two *THRB* alleles was mutated, which was consistent with the dominant mode of inheritance.

Mutations in the *THRB* gene have now been identified in subjects with RTH β of 665 families (Table 1). They comprise 219 different mutations including the initially reported index family, which was found to have complete deletion of the *THRB* gene (34), a finding that contrasts with the usually observed point mutations. Forty-eight of the known mutations have not been published (33). The majority of the families, 625, have single nucleotide substitutions resulting in single amino acid replacements: in 15 families, mutations leading to premature stop codons result in truncated TR β proteins. In the remaining 40 families, the sequence alterations consisted of dinucleotide substitutions, insertions, deletions of nucleotides

ranging from a single base pair to 11 nucleotides, and a duplication of 7 bases (for details see Table 1).

Given that there are 446 more families than the 219 different mutations, 64 of the mutations are shared by more than one family. Haplotyping of intragenic polymorphic markers showed that, in most instances, identical mutations have developed independently in different families (38). These occur more often, though not exclusively, in CpG dinucleotide hot spots. In fact, *de novo* mutations are twice as frequent in CpG dinucleotides with the largest number of families, namely 41 harboring the same mutation, R338W. In addition, different mutations resulting in more than one amino acid substitution at the same codon have been found at 44 different sites. Mutations in codons 345 and 451 result both in 5 different amino acid replacements (G345R,S,A,V,D; F451I,L,S,C,*), and those in codon 453 include 8 different substitutions (P453T,S,A,N,Y,H,L,R), as well as an insertion and a deletion, and a total of 74 families harbor mutations of this particular codon.

The detected mutations are located in the last four exons of the gene and include 8, 27, 89 and 90 mutations in exons 7, 8, 9 and 10, respectively. These involve 56, 40, 292 and 278 families (Figure 2). The following mutations have been identified in more than 20 families: R243Q, A317T, R320C, R338W, R438H and P453T. Of note the first five are in CpG dinucleotides and the last in a stretch of six cytidines.

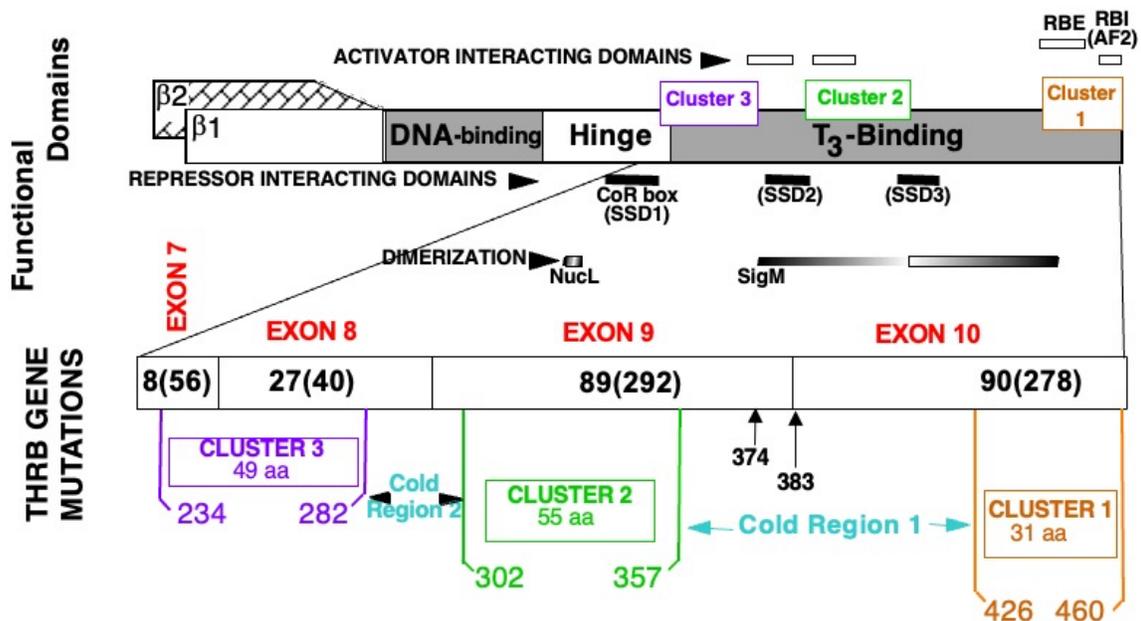


Figure 2. Location of mutations in the TR β protein in subjects with RTH β .

TOP PORTION: Schematic representation of TR β and its functional domains for interaction with TREs (DNA-binding), with hormone (T₃-binding), with activating (298), repressing (299-301) cofactors and with nuclear receptor partners (dimerization) (74, 302, 303). Note their relationship to the three clusters of natural mutations.

BOTTOM PORTION: The T₃-binding domain and distal end of the hinge region, which contain the three mutation clusters, are expanded. The four terminal exons containing all so far identified mutation are shown with the number different mutation and number of families in parenthesis (published and our unpublished data). Amino acids are numbered consecutively starting at the amino terminus of the TR β 1 molecule according to the consensus statement of the First International Workshop on RTH (304). TR β 2 has 15 additional residues at the N-terminus. Mutations occur in three clusters as indicated. A silent region between cluster 1 and 2, located in the dimerization domain contains two mutations (Q374K and R383H), indicated with arrows.

AF2, Hormone-dependent activation function (12th amphipathic helix) (305, 306); RBE, corepressor-binding enhancer; RBI, corepressor-binding inhibitor (306); SSD, silencing subdomain (301); Nucl, nuclear localization (307); SigM, signature motif (308). aa, amino acid.

All *THRB* gene mutations are located in the functionally relevant T₃-binding domain and its adjacent hinge region. Three mutational clusters have been identified with intervening cold regions (Figure 2). No mutations have been identified in the DNA binding domain or in the amino termini characterizing TR β 1 and TR β 2. A report of a putative mutation, C36Y, in the amino terminus (39) represents a

polymorphism that does not alter the biological properties of the TR β 1 molecule (40). With the exception of the family with *THRB* gene deletion, the inheritance of all others is autosomal dominant.

Somatic mutations in the *THRB* gene have been identified in some TSH-secreting pituitary tumors (23, 41). These mutations can be identical to those

occurring in the germline. However, which affects the negative regulation of TSH by TH, is responsible for the development of the pituitary tumor.

In 14% of families, RTH β occurs in the absence of mutations in the *THRB* genes (nonTR β -RTH) (42). Such individuals may have a defect in one of the cofactors involved in the mediation of TH action (see Animal Models of RTH, below).

Molecular Mechanisms of TR Action

The two TH receptor genes located on chromosome 17 and 3 encode TR α and TR β , which have substantial structural and sequence similarities. Both genes produce two isoforms, α 1 and α 2 by alternative splicing, and β 1 and β 2 by different transcription start points. TR α 2 binds to TH response elements (TREs) but due to a sequence difference in the ligand-binding domain (LBD), it does not bind TH (43) and appears to have a weak antagonistic effect (44). Additional TR isoforms, including a TR β with a shorter amino terminus (TR β 3), a truncated TR β 3, TR α 1 and TR α 2 lacking the DNA-binding domain (DBD) have been identified in rodents (45, 46), and TR β 4 that lacks the LBD has been reported in selected human tissues (47). The significance of these variants in humans remains unknown (48). Finally, a p43 protein, translated from a downstream AUG of TR α 1, is believed to mediate the TH effect in mitochondria (49).

The relative expression of the two TR genes and the distribution of the encoded proteins vary among tissues and during different stages of development (50-52). The abundance of several splice variants involving the 5'-untranslated region of the human TR β 1 (53, 54) is regulated developmentally and varies among tissues. Although TR β and TR α are interchangeable (55, 56) to a certain degree, the absence of one or the other receptor does not produce equivalent phenotypes. Some TH effects are entirely TR isoform specific (see Animal Models of RTH, below).

TREs, located in TH regulated genes, consist of half-sites that contain the consensus sequence AGGTCA, and vary in number, spacing and orientation (57, 58). Each half-site usually binds a single TR molecule (monomer), two half-sites bind two TRs (dimer) or one TR and a heterologous partner (heterodimer), the most prominent being the retinoid X receptor γ (RXR). Dimer formation is facilitated by the presence of an intact "leucine zipper" motif located in the middle of the LBD of TRs. Occupation of TREs by unliganded TRs, also known as aporeceptors, inhibits the constitutive expression of genes that are positively regulated by TH (59) through association with corepressors such as the nuclear corepressor (NCoR) or the silencing mediator of retinoic acid and TH receptors (SMRT) (60). Transcriptional repression is mediated through the recruitment of the mammalian homologue of the *Saccharomyces* transcriptional corepressor (mSin3A) and histone deacetylases (HDAC) (61). This latter activity compacts nucleosomes into a tight and inaccessible structure, effectively shutting down gene expression (Figure 1C). This effect is relieved by the addition of TH, which releases the corepressor, reduces the binding of TR dimers to TRE, enhances the occupation of TREs by TR/RXR heterodimers (62) and recruits coactivators (CoA) such as p/CAF (CREB binding protein-associated factor) and the nuclear coactivator (NCoA) (63) with histone acetylation (HAT) activity (60, 64). This results in the loosening of the nucleosome structure making the DNA more accessible to transcription factors (Figure 1C). The ligand-dependent association with TR associated proteins, in conjunction with the general coactivators PC2 and PC4, act to mediate transcription by RNA polymerase II and general initiation factors (65). Furthermore, it is believed that T₃ exerts its effect by inducing conformational changes of the TR molecule and that TR associated proteins (TRAP) stabilize the association of TRs with TREs.

In addition to these genomic effects, TH can also act at the cell membrane and in the cytosol through non-genomic actions (13, 66). These non-genomic type 2 effects (14) include oxidative phosphorylation and

mitochondrial gene transcription and involve the generation of intracellular secondary messengers with induction of $[Ca^{2+}]$ (I), cyclic adenosine monophosphate (cAMP) AMP or protein kinase signaling cascades.

Properties of Mutant TR β Receptors and Associated Dominant Negative Effect

THRB gene mutations produce two forms of RTH. The less common, described in only one family (1), is caused by deletion of all coding sequences of the *THRB* gene and is inherited as an autosomal recessive trait (34). The complete lack of TR β in these individuals results in severe deafness and is associated with mutism (1), as well as monochromatic vision (67) because TR β is required for the cochlear maturation and the development of cone photoreceptors that mediate color vision (68) (see Animal Models of RTH, below). Heterozygous individuals that express a single *THRB* gene have no clinical or laboratory abnormalities. This is not due to compensatory overexpression of the single normal allele of the *THRB* gene nor that of the *THRA* gene (69). However, because subjects with complete TR β gene deletion preserve some TH responsiveness, it is logical to conclude that TR α 1 is capable of partially substituting for the function of TR β (see Animal Models of RTH, below).

The more common form of RTH β is inherited in an autosomal dominant fashion and is characterized by defects in one allele of the *THRB* gene, principally missense mutations. This contrasts with the lack of a phenotype in individuals that express a single *THRB* allele. The RTH β phenotype does not result from a lack of a functional allele (haploinsufficiency) caused by the mutant TR β s (mutTRs) but by interfering with the function of the wild-type (WT) TR (dominant negative effect, DNE). This has been clearly demonstrated in experiments in which mutTRs are coexpressed with WT TRs (70, 71).

Studies have established two basic requirements for mutTRs to exert a DNE: 1) preservation of binding to TREs on DNA and 2) the ability to dimerize with a homologous (72, 73) or heterologous partner (74, 75). These criteria apply to mutTRs with predominantly impaired T₃-binding activity (Figure 3). In addition, a DNE can be exerted through impaired association with a cofactor even in the absence of important impairment of T₃-binding. Increased affinity of a mutTR for a corepressor (CoR) (76, 77), or reduced association with a coactivator (CoA) (78-80), have been found to play a role in the dominant expression of RTH. The introduction in a mutTR of an additional artificial mutation that abolishes either DNA binding, dimerization or the association with a CoR results in the abrogation of its DNE (75, 81, 82).

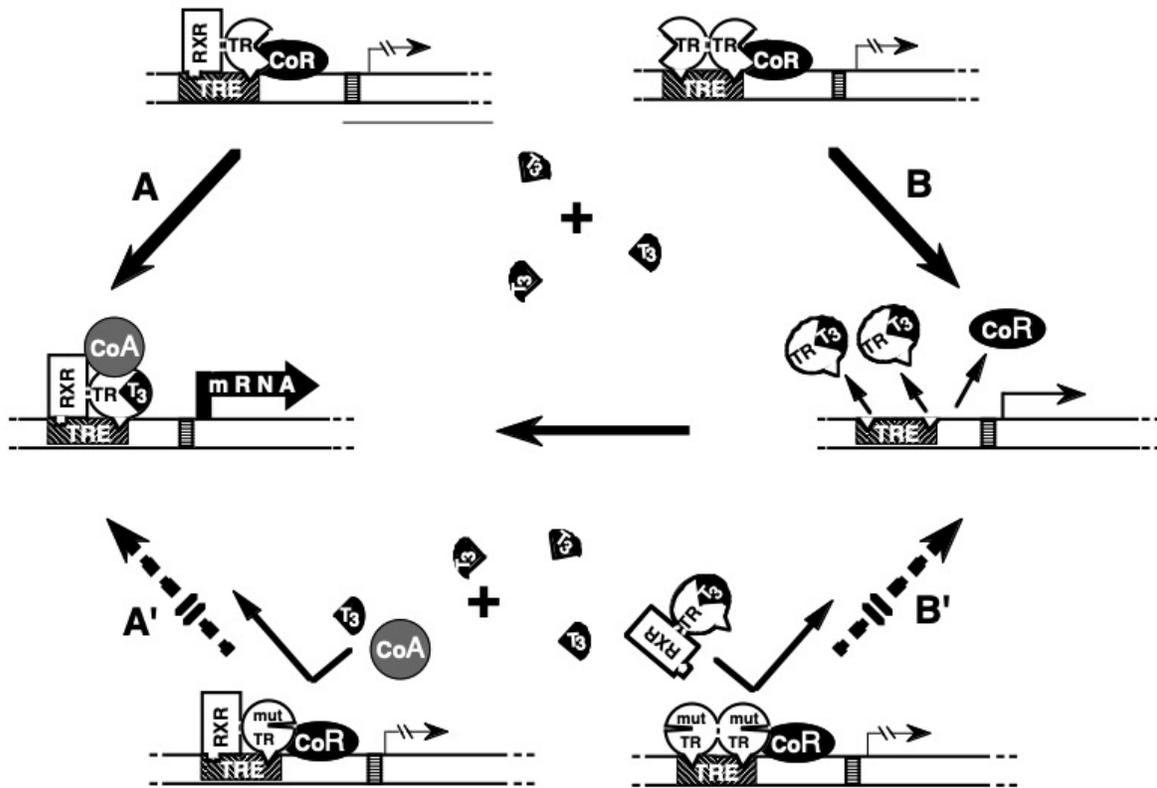


Figure 3. Mechanism of the dominant expression of RTH: In the absence of T₃, occupancy of TRE by TR heterodimers (TR-TRAP) or dimers (TR-TR) suppresses transactivation through association with a corepressor (CoR). (A) T₃-activated transcription mediated by TR-TRAP heterodimers involves the release of the CoR and association with coactivators (CoA) as well as (B) the removal of TR dimers from TRE releases their silencing effect and liberates TREs for the binding of active TR-TRAP heterodimers. The dominant negative effect of a mutant TR (mutTR), that does not bind T₃, can be explained by the inhibitory effect of mutTR-containing-dimers and heterodimers that occupy TRE. Thus, T₃ is unable to activate the mutTR-TRAP heterodimer (A') or release TREs from the inactive mutTR homodimers (B'). [Modified from Refetoff et al. (5)].

The distribution of *THRB* gene mutations associated with RTH β reveals a conspicuous absence of mutations in regions of the molecule that are important for dimerization, for the binding to DNA, and for the interaction with CoRs (Figure 2). These "cold regions" contain CpG hot spots, suggesting that they may not be devoid of naturally occurring mutations. Rather, mutations would escape detection owing to their failure to produce clinically significant RTH β in heterozygotes, as tested *in vitro* (83). This was

recently confirmed in a study of a family in which one member has been fortuitously identified to have a mutation in the cold region (84). Nevertheless, mutation in other regions of the TR β could also be phenotypically silent, particularly if not occurring near the T₃ binding pocket (85). Structural studies of the DBD and LBD have provided further understanding about the clustered distribution of mutTR β s associated RTH and defects in the association with cofactors (86-89).

Based on the early finding that RTH β is associated with mutations confined to the LBD of the TR β , it was anticipated that the clinical severity of RTH β would correlate with the degree of T₃-binding impairment. While this was true in 12 different natural mutTR β s, in 5 others, the severity of the resistance was less pronounced despite virtually complete absent T₃-binding. This is explained by the reduced dominant negative potency due to diminished ability to form homodimers (for example R316H and R338W) (90). Weakened association of TR β with DNA or CoR can produce the same effect.

Less evident was the observation of relatively severe interference with the function of the WT TR β , despite very mild impairment or no T₃-binding defect at all. This was the case when hormone-binding was tested in two mutTR β s, located in the hinge region of the receptor (R243Q and R243W) (91). However, reduced T₃-binding could be demonstrated after binding of the mutTR β to TRE, indicating a change in the mutTR β configuration when bound to genomic DNA (91, 92). Other mechanisms and examples of DNE in the presence of normal or slightly attenuated T₃-binding include a decreased interaction of L454V with CoA (78), and a delay of R383H to release CoR (93).

In general, the relative degree of impaired function among various mutTR β s is similar whether tested using TRE-containing reporter genes that are negatively or positively regulated by T₃. Exceptions to this rule are the mutTR β s R383H and R429Q that show greater impairment of transactivation on negatively rather than positively regulated promoters (90, 93, 94). In this respect, these two mutTR β s are candidates for a predominant PRTH phenotype, even though they have been clinically described as producing GRTH (95), as well as PRTH (96, 97). Substitution of these charged amino acids (in this case arginine) disrupts the unique property of TR β 2 to bind certain coactivators through multiple contact surfaces (98). The result is a decrease in the normal T₃-mediated feedback suppression mediated by TR β 2

through the conversion of TR β 2 to a TR β 1-like molecule with altered CoA binding. As a consequence, the mutation affects predominantly TR β 2 mediated action. *In vivo* support for a TR β 2 predominant impairment of the mutTR β R429Q was also obtained in mice (99). Another putative mechanism for isolated PRTH was illustrated by the occurrence of a double-hit combining in *cis* the *THRB* mutation R338W and a single nucleotide polymorphism (SNP) located in an intronic enhancer shown to play a critical role in the pituitary expression of the TR β 2 isoform (100). The presence of a thymidine in this SNP, leads to over-expression of the mutant allele in GH3 pituitary-derived cells, thus having the potential to generate a tissue-specific dominant-negative condition. However, the T/C nucleotides of this SNP have not been correlated with the clinical presentation in individuals with this most common TR β R338W mutation. No mutations specific to the TR β 2 involved in the hypothalamic-pituitary feedback regulation have been identified.

Molecular Basis of the Variable RTH β Phenotype

The extremes of the RTH β phenotype have a readily apparent molecular basis. Subjects heterozygous for a *THRB* gene deletion are normal because the expression of a single *THRB* allele is sufficient for normal function. RTH β manifests in homozygotes completely lacking the *THRB* gene and in heterozygotes that express a mutTR β with DNE. The most severe form of RTH β , with extremely high TH levels and signs of both hypothyroidism and thyrotoxicosis, occur in homozygous individuals expressing only mutTR β s (35, 36). The severe hypothyroidism manifesting in bone and brain of such subjects can be explained by the silencing effect of a double dose of mutTR and its interference with the function of TR α (72), a situation which does not occur in homozygous subjects who lack TR β . In contrast, the manifestation of thyrotoxicosis in other tissues, such as the heart, may be explained by the effect of high TH levels on tissues that normally express predominantly TR α 1 (101, 102) (see Animal Models of RTH, below).

For this same reason, tachycardia is a relatively common finding in RTH β (103).

Various mechanisms can be postulated to explain the tissue differences in TH resistance within the same subject and among individuals. The distribution of receptor isoforms varies from tissue to tissue (50, 104, 105). This likely accounts for greater hormonal resistance of the liver as compared to the heart. Differences in the degree of resistance among individuals harboring the same mutTR β could be explained by the relative level of mutant and WT TR expression. Such differences have been found in one study using cultured fibroblasts (106) but not in another (69). Various reasons for a predominant TR β 2 dysfunction have been presented in the preceding section.

Although in a subset of mutTR β s a correlation was found between their functional impairment and the degree of thyrotroph hyposensitivity to TH, this correlation is not maintained in terms of hormonal resistance in peripheral tissues (90). Subjects with the same mutations, even belonging to the same family, show different degrees of hormone resistance. A most striking example is that of family G.H. in which the mutTR β R316H did not co-segregate with the RTH β phenotype in all family members (107). This variability of clinical and laboratory findings was not observed in affected members of two other families with the same mutation (29, 108). A study in a large family with the mutTR β R320H, suggests that genetic variability of factors other than TR may modulate the phenotype of RTH β (109).

RTH β Without *THRB* Gene Mutations (nonTR β -RTH)

The molecular basis of nonTR β -RTH remains unknown. Since the first demonstration of nonTR β -RTH (21), more than 75 families with the phenotype of RTH β have been identified, in which affected individuals did not harbor germline mutations in the

THRB, 39 of which in the authors' laboratory (42, 110-113). The phenotype is indistinguishable from that in subjects with *THRB* gene mutations. Distinct features are an increased female to male ratio and a high prevalence of sporadic cases. While it has been postulated that nonTR β -RTH is likely caused by a defect in one of the cofactors involved in the mediation of TH action, proof supporting this contention is lacking (114). Recently, in-depth targeted new generation sequencing revealed mosaicism of previously reported *THRB* gene mutations in 19% of families (33) as previously identified in one family (115). Two families with more than one affected individual were found to harbor a *THRB* gene mutation that had been missed when early sequencing required cloning of amplified fragments into plasmids (33).

Animal Models of RTH β

Understanding the action of TH *in vivo*, and the mechanisms underlying the abnormalities observed in patients with RTH β , has been bolstered by observations made in genetically manipulated mice. Three types of genetic manipulations have been applied: (a) transgenic mice that overexpress a receptor; (b) deletion of the receptor (knockout or KO); and (c) introduction of mutations in the receptor (knockin or KI). The latter two types of gene manipulation, species differences notwithstanding, have yielded true models of the recessively and dominantly inherited forms of RTH β (116).

The features of RTH β found in patients homozygous for TR β deletion also manifest in the TR β deficient mouse (117-119). Special features, such as sensorineural deafness and monochromatic vision are characteristic and shared by mouse (120) (121) and man (1, 122). The mouse model allowed for investigations in greater depth into the mechanisms responsible for the development of these abnormalities. Thus, TR β deficiency retards the expression of fast-activating potassium conductance in inner hair cells of the cochlea that transforms the

immature cells from spiking pacemakers to high-frequency signal transmitters (123). TR β 2 interacts with transcription factors providing timed and spatial order for cone differentiation. Its absence results in the selective loss of M-opsin (121). The down regulation of hypothalamic TRH is also TR β 2 specific (124). Mice deficient in TR β have increased heart rate that can be decreased to the level of the WT mouse by reduction on the TH level (119). This finding, together with the lower heart rate in mice selectively deficient in TR α 1 (101), indicates that TH dependent changes in heart rate are mediated through TR α , and explains the tachycardia observed in some patients with RTH β .

The combined deletion of TR α 1 and α 2, produces no important alterations in TH or TSH concentrations in serum (55). The complete lack of TRs, both α and β , is compatible with life (55, 56). This contrasts with the complete lack of TH which, in the athyreotic Pax8 deficient mouse, results in death prior to weaning, unless rescued by TH treatment (125). The survival of mice deficient in both TR α and β is not due to expression of a yet unidentified TR but to the absence of the noxious silencing effects of aporeceptors. Indeed, removal of the *Thra* gene rescues the Pax8 KO mice from death (126). The combined *Thr β* and *Thra* deficient mice have serum TSH levels that are 500-fold higher than those of the WT mice, and T₄ concentrations 12-fold above the average normal mean (55). Thus, the presence of an aporeceptor does not seem to be required for the upregulation of TSH but no amount of TH causes its downregulation.

The first animal model of the dominantly inherited organ-limited RTH β utilized somatic transfer of a *mutTR β 1 G345R* mutation by means of a recombinant adenovirus (127). The liver of these mice was resistant to TH, and overexpression of the WT TR β increased the severity of hypothyroidism, confirming that the unliganded TR has a constitutive effect both *in vivo* as *in vitro*. True mouse models of dominantly inherited RTH β have been generated by targeted mutations in the *Thr β* gene (128, 129). The mutations were modeled on those identified in humans with RTH

[frame-shift resulting in 16 carboxylterminal nonsense amino acids (PV mouse) and T337 Δ]. As in humans, the phenotype seen in the heterozygous KI animals was more severe in mice lacking both *Thr β* alleles.

NcoA (SRC-1) deficient mice have RTH β with typical increase in T₄, T₃ and TSH concentrations (130). A milder form of RTH β was identified in mice deficient in RXR γ (131). These animals show reduced sensitivity to L-T₃ in terms of TSH downregulation but not in metabolic rate. These data indicate that abnormalities in cofactors can produce RTH β .

Pathogenesis

The reduced sensitivity to TH in subjects with RTH β is shared to a variable extent by most tissues. The hyposensitivity of the pituitary thyrotrophs results in a non-suppressed serum TSH, which in turn, increases the synthesis and secretion of TH. The persistence of TSH secretion in the face of high levels of serum free TH contrasts with the low TSH levels in the more common forms of TH hypersecretion that are TSH-independent. This apparent paradoxical dissociation between TH and TSH is responsible for the wide use of the term "inappropriate secretion of TSH" to designate the syndrome. However, TSH hypersecretion is not at all inappropriate, given the fact that the response to TH is reduced. It is compensatory and appropriate for the level of TH action mediated through a defective TR β . As a consequence, most patients are eumetabolic, though the compensation is variable among affected individuals and among tissues in the same individual. However, the level of tissue responses does not correlate with the level of TH, probably owing to discordance between the hormonal effect on the pituitary and other body tissues. Thyroid gland enlargement occurs with chronic, though minimal TSH hypersecretion due to increased biological potency of this glycoprotein through increased sialylation (132). Administration of supraphysiological doses of TH is required to

suppress TSH secretion without induction of global thyrotoxic changes in peripheral tissues.

Thyroid-stimulating antibodies, which are responsible for the hyperactivity of the thyroid gland in Graves' disease, have been conspicuously absent in patients with RTH β . Another potential thyroid stimulator, human chorionic gonadotropin, has not been found in serum of subjects with RTH β (133, 134).

The selectivity of the resistance to TH has been convincingly demonstrated. When tested at the pituitary level, both thyrotrophs and lactotrophs were less sensitive only to TH. Thyrotrophs responded normally to the suppressive effects of the dopaminergic drugs L-dopa and bromocriptine (135, 136), as well as to glucocorticoids (136-138). Studies carried out in cultured fibroblasts confirm the *in vivo* findings of selective resistance to TH. The responsiveness to dexamethasone, measured in terms of glycosaminoglycan (139) and fibronectin synthesis (140), was preserved in the presence of T₃ insensitivity.

Several of the clinical features encountered in some patients with RTH β may be the manifestation of selective tissue deprivation of TH during early stages of development. These clinical features include retarded bone age, stunted growth, mental retardation or learning disability, emotional disturbances, attention deficit/hyperactivity disorder (ADHD), hearing defects, and nystagmus (5). A variety of associated somatic abnormalities appear to be unrelated pathogenically and may be the result of involvement of other genes such as in major deletions of DNA sequences (34). However, no gross chromosomal abnormalities have been detected on karyotyping (1, 141).

Pathology

Little can be said about the pathologic findings in tissues other than the thyroid. Electron microscopic examination of striated muscle obtained by biopsy from one patient revealed mitochondrial swelling, also known to be encountered in thyrotoxicosis (1). This is compatible with the predominant expression of TR α in muscle, responding to the excessive amount of circulating TH (142). Light microscopy of skin fibroblasts stained with toluidine blue showed moderate to intense metachromasia (2) as described in myxedema. However, in contrast to patients with TH deficiency, treatment with the hormone failed to induce the disappearance of the metachromasia in fibroblasts from patients with RTH β .

Thyroid tissue, obtained by biopsy or at surgery, revealed various degrees of hyperplasia of the follicular epithelium (136, 143-145). Specimens have been described as "adenomatous goiters", "colloid goiters", and normal thyroid tissue. When present, lymphocytic infiltration is due to the coexistence of thyroiditis (146).

Clinical Features

Characteristic of the RTH β syndrome is the paucity of specific clinical manifestations. When present, manifestations are variable from one patient to another. Investigations leading to the diagnosis of RTH β have been undertaken because of the presence of goiter, hyperactive behavior or learning disabilities, developmental delay and sinus tachycardia (Figure 4). Fortuitous detection of RTH β on laboratory testing can become more common with the increased frequency of routine thyroid testing. The finding of elevated serum TH levels in association with a non-suppressed TSH is usually responsible for the pursuit of further studies leading to the diagnosis.

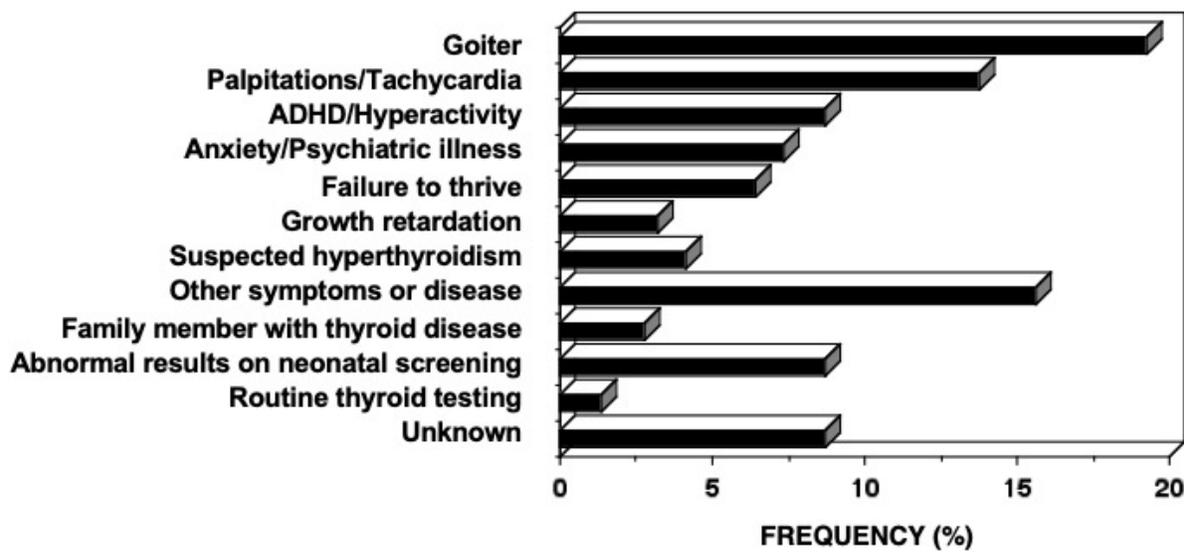


Figure 4. The reasons prompting further investigation of the index member of each family with RTHβ.

The degree of compensation for tissue hyposensitivity by the increased levels of TH is variable among individuals, as well as in different tissues. As a consequence, clinical and laboratory evidence of TH deficiency and excess often coexist. For example, RTHβ can present with a mild to moderate growth retardation, delayed bone maturation and learning

disabilities suggestive of hypothyroidism, alongside with hyperactivity and tachycardia compatible with thyrotoxicosis. The common clinical features and their frequency are given in Table 2. Frank symptoms of hypothyroidism are more common in those individuals who, because of erroneous diagnosis, have received treatment to normalize their circulating TH levels.

Table 2. Clinical Features of RTHβ	
FINDINGS	FREQUENCY (%)
Thyroid gland	
Goiter	66-95
Nervous System	
Hyperkinetic behavior	33-68
Attention deficit hyperactivity disorder	40-60
Learning disability	30
Mental retardation (IQ <70)	4-16
Hearing loss (sensorineural)	10-22
Growth and Development	
Short stature (<5 th percentile)	18-25
Delayed bone age >2 SD	29-47
Low body mass index (in children)	33
Recurrent Ear and Throat Infections	
	55

Goiter is the most common abnormality. It has been reported in 66-95% of cases and is almost always detected by ultrasonography. The enlargement of the gland is usually diffuse; nodular changes and gross asymmetry are found in recurrent goiters after surgery. Goiter is more often present in children with RTH β born to normal than to affected mothers (96).

Sinus tachycardia is also very common, with some studies reporting a frequency as high as 80% (28). Palpitations often bring the patient to medical attention, and the finding of tachycardia is the most common reason for the erroneous diagnosis of autoimmune thyrotoxicosis or the suspicion of PRTH.

About one-half of subjects with RTH β have some degree of learning disability with or without ADHD (5, 147). One-quarter have intellectual quotients (IQ) less than 85, but frank mental retardation (IQ <60) has been found only in 3% of cases. Impaired mental function was found to be associated with impaired or delayed growth (<5th percentile) in 20% of subjects, although isolated growth retardation is rare (4%) (5). Despite the high prevalence of ADHD in patients with RTH, the occurrence of RTH β in children with ADHD must be very rare, none having been detected in 330 such children studied (148, 149). The higher prevalence of low IQ scores appear to confer a higher likelihood for subjects with RTH β to exhibit ADHD symptoms (108). A retrospective survey has shown an increased miscarriage rate and low birth weight of normal infants born to mothers with RTH β (150). These same individuals, exposed to high TH levels during embryonic life, develop reduced sensitivity to TH as adults despite the absence of *THRB* gene mutations. This epigenetic effect is transmitted along the male line for at least three generations (151).

A variety of physical defects that cannot be explained on the basis of TH deprivation or excess have been recorded. These include major or minor somatic defects, such as winged scapulae, vertebral anomalies, pigeon breast, prominent pectoralis,

birdlike facies, scaphocephaly, short 4th metacarpals, as well as Besnier's prurigo, congenital ichthyosis, and bull's eye type macular atrophy (5). Some may be related to the severity of the hormonal resistance as they manifest in homozygotes (36). An infant compound heterozygous for a *THRB* gene mutation (R338W and R429W) presented with a cone photoreceptor disorder associated with severe thyroid hormone resistance (152).

Course of Disease

The course of the disease is as variable as is its presentation. Most subjects have normal growth and development, and lead a normal life at the expense of high TH levels and a small goiter. Others present variable degrees of mental and growth retardation. Symptoms of hyperactivity tend to improve with age as it does in subjects with ADHD only.

Goiter has recurred in every patient who underwent incomplete thyroid surgery. As a consequence, some subjects have been submitted to several consecutive thyroidectomies or treatments with radioiodine (145, 153-155). Thyroid cancer has been rarely reported in individuals with RTH β and, when occurring, the outcome has not been unfavorable despite incomplete TSH suppression (156).

Laboratory Findings

TH AND ITS METABOLITES

In the untreated patient, elevation in the concentration of serum free T₄ is a *sine qua non* requirement for the diagnosis of RTH β . It is often accompanied by high serum levels of T₃, but less so with advancing age. Serum thyroid binding globulin (TBG) and transthyretin (TTR) concentrations are normal. The

resin T₃ uptake is usually high as is the case in patients with thyrotoxicosis.

Serum T₄ and T₃ values range from just above to several fold the upper limit of normal. Although the levels may vary in the course of time in the same patient (28), the T₃:T₄ ratio remains normal (5). This contrasts with the disproportionate increase in serum T₃ concentration characteristic of autoimmune thyrotoxicosis (157).

Reverse T₃ concentration is also high in patients with RTH β as is that of another product of T₄ degradation, 3,3'-T₂ (144). Serum thyroglobulin level tends also to be high and the degree of its elevation reflects the level of TSH induced thyroid gland hyperactivity.

In vivo turnover kinetics of T₄ showed a normal or slightly increased volume of distribution and fractional disappearance rate of the hormone. However, because of the large extrathyroidal pool, the absolute daily production of T₄ and T₃ are increased by about two- to four-fold (2, 153, 158, 159), but the extrathyroidal conversion of T₄ to T₃ remains normal (159).

THYROTROPIN AND OTHER THYROID STIMULATORS

A characteristic feature of the syndrome is the preservation of the TSH response to TRH despite the elevated TH levels (160). In most cases, the basal serum TSH concentration is normal and the circadian rhythm is unaltered (161, 162). TSH values above 6 mIU/L indicate a decrease in thyroidal reserve due to treatment directed to the thyroid or associated thyroid disease. The severity of the central RTH β can be quantitated, even in the presence of reduced thyroidal reserve, using the thyrotroph T₄ resistance index (TT₄RI); the product of serum FT₄, expressed as

percent of the upper limit of normal, and the TSH level (91).

TSH has increased biological activity (132, 163) and the free alpha subunit (α -SU) is not disproportionately high. Antibodies against thyroglobulin and thyroid peroxidase indicating the presence of autoimmune thyroid disease, have a higher prevalence in RTH β (164).

THYROID GLAND ACTIVITY AND INTEGRITY OF HORMONE SYNTHESIS

The fractional uptake of radioiodine by the thyroid gland is high as is the absolute amount of accumulated iodide. The latter is normally organified as demonstrated by the retention of radioiodine following the administration of perchlorate (1, 153, 165).

IN VIVO EFFECTS OF TH

The impact of TH on peripheral tissues, assessed *in vivo* by a variety of tests, suggests a reduced biologic response to the hormone in some tissues but not in others. Early studies measuring the metabolic rate (BMR) evaluated by measurement of oxygen consumption showed normal results (2). However resting energy expenditure, measured subsequently by indirect calorimetry was increased, but not the rate of ATP synthesis, measured by magnetic resonance spectroscopy (166). This indicates that in subjects with RTH β , the basal mitochondrial substrate oxidation is increased and energy production in the form of ATP synthesis is decreased. Yet, the metabolic response to the administration of TH is reduced compared to normal individuals (5). With the exception of increased resting pulse rate in about one half of the patients with RTH β , the cardiac function is only minimally altered. Two-dimensional and Doppler echocardiography showed findings consistent with a mild excess of TH

on cardiac systolic and diastolic function whereas other parameters, such as ejection and shortening fractions of the left ventricle, systolic diameter, and left ventricle wall thickness, were normal (103). Findings suggestive of hypothyroidism have also been reported (167). The Achilles tendon reflex relaxation time has been normal or slightly prolonged.

In contrast to overt thyrotoxicosis, serum parameters of TH action on peripheral tissues are usually in the reference range. These include, serum cholesterol, carotene, triglycerides, creatine kinase, alkaline phosphatase, angiotensin-converting enzyme, sex hormone-binding globulin (SHBG), ferritin and osteocalcin. Urinary excretion of magnesium, hydroxyproline, creatine, creatinine, carnitine, and cyclic adenosine monophosphate (cAMP), all found to be elevated in thyrotoxicosis, have been normal or low, suggesting normal or slightly reduced TH effect. The prolactin hyper-responsiveness in some patients with RTH β may be due to the functional TH deprivation at the level of the lactotrophs (160).

Radiological evidence of delayed bone maturation has been observed in one-half of patients with RTH β diagnosed during infancy or childhood (5). However, the majority achieve normal adult stature.

Evaluation of endocrine function by a variety of tests has failed to reveal significant defects other than those related to the thyroid (5).

***In Vitro* Tests of Thyroid Hormone Action**

Cultured skin fibroblasts from patients with RTH β showed reduced responses to L-T₃ added to the medium in terms of degradation rate of lipoproteins (155), synthesis of glycosaminoglycans (139) and fibronectin (140). This was also true for L-T₃-induced changes on specific messenger ribonucleic acid (mRNA) (168). The normal responses of

dexamethasone were preserved indicating that the activity of the glucocorticoid receptor was preserved.

Responses to the Administration of Thyroid Hormone

Because reduced responsiveness to TH is central in the pathogenesis of the syndrome, patients have been given TH in order to observe their responses and thereby establish the presence of hyposensitivity to the hormone. Unfortunately, the data have been discrepant, not only because of differences in the relative degree of resistance to TH among patients, but also because of differences in the manner in which tests have been carried out.

The dose of TH that suppresses the TSH secretion, and eventually abolishes the TSH response to TRH, is greater than that required for unaffected individuals. The decreased TSH secretion during the administration of supraphysiological doses of TH is accompanied by a reduction in the thyroidal radioiodine uptake and, when exogenous L-T₃ is given, a reduction in the pretreatment level of serum T₄ (133, 134, 145, 153, 155).

Various responses of peripheral tissues to the administration of TH have been quantitated. Most notable are measurements of the BMR, pulse rate, reflex relaxation time, serum cholesterol, lipids, enzymes, osteocalcin and SHBG, and urinary excretion of hydroxyproline, creatine, and carnitine. Either no significant changes were observed, or they were much reduced relative to the amount of TH given (5).

Of great importance are observations on the catabolic effect of exogenous TH. In some subjects with RTH β , L-T₄ given in doses of up to 1000 μ g/day, and L-T₃ up to 400 μ g/day, failed to produce weight loss without a change in caloric intake, nor did they induce a negative nitrogen balance (2, 133, 136). In contrast,

administration of these large doses of TH over a prolonged period of time was apparently anabolic as evidenced by a dramatic increase in growth rate and accelerated bone maturation (30, 136).

Effects of Other Drugs

As expected, administration of the TH analogue, 3,5,3'-triiodo-L-thyroacetic acid (TRIAc) to patients with RTH β produced attenuated responses (2, 162, 169).

Administration of glucocorticoids promptly reduced the TSH response to TRH and the serum T₄ concentration (133, 136, 137, 143, 158).

Administration of L-dopa and bromocriptine produced a prompt suppression of TSH secretion, as well as a diminution of the thyroidal radioiodine uptake and serum T₃ level (135, 136, 143). Domperidone, a dopamine antagonist, caused a rise in the serum TSH level when given to patients with RTH β (162). These observations indicate that, in this syndrome, the normal inhibitory effect of dopamine on TSH is intact.

The response to antithyroid drugs has shown some variability. Methimazole and propylthiouracil, in doses usually effective in reducing the high serum TH level of autoimmune hyperthyroidism, had no effect in two patients (2). However, in other cases of RTH β , antithyroid drugs induced some decrease in the circulating level of TH, producing a reciprocal change in the TSH concentration (3, 141, 165, 170). Administration of 100 mg of iodine daily had a similar effect in one patient (134), but 4 mg potassium iodide per day produced no changes in another (2).

The β adrenergic blockers, propranolol and atenolol, produce a significant reduction in heart rate.

Differential Diagnosis

Because the clinical presentation of RTH β is variable, detection requires a high degree of suspicion. The differential diagnosis includes all possible causes of hyperthyroxinemia (Table 3). The sequence of diagnostic procedures listed in Table 4 is suggested.

Table 3. Serum Thyroid Function Tests in the Differential Diagnosis of Impaired Sensitivity to Thyroid Hormone

Defect	T4	T3	rT3	T3/rT3 ratio	TSH	FT4 Dialysis	Other common manifestations
RTH β	↑	↑ or N	↑	N	N or ↑	↑	tachycardia, goiter, ADHD
RTH α	N or sl↓	N or sl↑	N sl↓	↑	N or sl↑	N or sl↓	growth and mental delay, constipation
TSHoma	↑	↑	↑	N	sl↑ or N	↑	thyrotoxicosis
<i>MCT8</i> mut	N or ↓	↑↑	↓↓	↑↑	N or sl↑	↓	neuropsychomotor delay
<i>SBP2</i> mut ^a	↑	↓	↑	↓↓	N or sl↑	↑	growth delay
FDH (<i>ALB</i> mut)	↑	N or sl↑ ^b	↑	↓	N	N or ↑	none
TBG excess	↑	↑	↑	N	N	N	none
Acute NTI	↑	↓↓	↑	↓	N	N or ↑	variable depending on illness

sl: slightly; N: normal; ↑: increased; ↓: decreased; mut: mutation

ADHD: attention deficit hyperactivity disorder; NTI: non-thyroidal illness

FDH: familial dysalbuminemic hyperthyroxinemia

^a Low serum selenium

^b High in ALB L66P

Table 4. Suggested Sequence of Diagnostic Procedures in Suspected RTH β

1. Usual presentation: high serum levels of free T ₄ with non-suppressed TSH.
2. Confirm the elevated serum level of free T ₄ and exclude interfering substance, such as antibodies to T ₄ , and other serum TH transport defects, especially if T ₃ is normal and obtain free T ₄ measurement by equilibrium dialysis
3. Obtain tests of thyroid function in first-degree relatives; parents, sibs and children
4. Sequence the <i>THRB</i> gene and if a mutation is detected shown to have an impaired function, the diagnosis of RTH β is secured
5. In the absence of <i>THRB</i> gene mutation and lack of abnormal thyroid function tests in other family members, the presence of a TSHoma should be excluded by measurement of the α -SU in serum and other appropriate tests (T ₃ -suppression, TRH stimulation and MRI).
6. Demonstrate a blunted TSH-suppression and metabolic response to the administration of praphysiological doses of TH (see response to L-T ₃ protocol, Figure 5).
7. Blunted TSH response to L-T ₃ with absence of <i>THRB</i> gene mutation in indicates nonTR β -RTH, which includes possible <i>THRB</i> mosaicism.

The presence of an elevated serum T₄ concentration with a non-suppressed TSH needs to be confirmed by repeated testing. The possibility of an inherited or acquired increase in T₄-binding to serum TH transport proteins must be excluded by direct measurement and by estimation of the circulating free T₄ level. The presence of a high serum T₃ is helpful, though normal levels do not exclude RTH β . Examples of instances when serum T₃ is not high are: transiently during the administration of some drugs, or with concomitant nonthyroidal illnesses (see other Endotext chapters), and permanently with advanced age, familial dysalbuminemic hyperthyroxinemia (FDH) and inherited defects of iodothyronine metabolism (see the THMD Section in this Chapter). In FDH, free T₄ measured by automated direct methods but not by equilibrium dialysis may be falsely elevated. A rare cause of elevated serum T₄ and T₃ level is the endogenous production of antibodies directed against these iodothyronines, which can be excluded by direct testing.

Measurement of the serum TSH is an absolute requirement. Under most circumstances, patients with high concentrations of circulating free TH have virtually undetectable serum TSH levels, which fail to respond to TRH. This is true even when the magnitude of TH excess is minimal and therefore subclinical, either on physical examination or by other laboratory tests. The combination of elevated serum levels of free TH and non-suppressed TSH, narrows the differential diagnosis to one of the syndromes of reduced sensitivity to TH and autonomous hypersecretion of TSH associated with pituitary tumors (TSHomas). The clinical and laboratory findings of the latter mimic those of RTH β with a few exceptions. TSHomas have: 1) a disproportionate abundance in serum free α -SU relative to whole TSH (171); 2) lack similar thyroid test abnormalities in parents and first degree relatives; 3) with rare exceptions (172), their serum TSH fails to respond to TRH or suppress with supra-physiologic doses of TH; 4) often have concomitant

hypersecretion of growth hormone and or prolactin; and 5) in the majority of cases, tumors can be demonstrated by computerized tomography (CT) or by magnetic resonance imaging (MRI) of the pituitary.

Rarely, subjects with autoimmune thyrotoxicosis may have endogenous antibodies to TSH or some of the test components, that can give rise to false increase in serum TSH values (173). Ectopic production of TSH and endogenous TRH hypersecretion could theoretically result in TSH-induced hyperthyroidism. The presence of high serum free T₃ or free T₄ only, in the presence of a non-suppressed TSH, is characteristic of the syndromic abnormalities of TH cell transport and metabolism, respectively (see the THCMTD and THMD Sections in this Chapter).

Proving the existence of isolated peripheral tissue resistance to TH is not simple. Lack of clinical symptoms and signs of hypermetabolism are insufficient to establish the diagnosis of RTH β and symptoms suggestive of thyrotoxicosis are not uncommon in RTH β . Because resistance to the hormone is variable in different tissues, no single test measuring a particular response to TH is diagnostic. Furthermore, results of most tests that measure the effect of TH on peripheral tissues show considerable overlap among thyrotoxic, euthyroid and hypothyroid subjects. The value of these tests is enhanced if measurements are obtained before and following the administration of supraphysiological doses of TH.

While the demonstration of a *THRB* gene mutation is sufficient to establish the diagnosis of RTH β , lack of cosegregation of the *THRB* haplotype with the phenotype has been used to exclude the involvement of TR β in the individuals suspected of having RTH β (174). This does not exclude mosaicism (115) when a single member of the family is affected (see nonTR β -RTH Section in this Chapter). In such cases, *in vivo* demonstration of tissue resistance to TH is required. A standardized diagnostic protocol, using short-term

administration of incremental doses of L-T₃, and outlined in Figure 5, is recommended. It is designed to assess several parameters of central and peripheral tissue effects of TH in the basal state and in comparison to those determined following the administration of L-T₃. The three doses, given to adults in sequence, are a replacement dose of 50 µg/day and two supraphysiological doses of 100 and 200 µg/day. The hormone is administered in a split dose every 12 hours and each incremental dose is given for the period of 3 days. Doses are adjusted in children and in adults of unusual size to achieve the same level of serum T₃ (for details see reference (5)).

L-T₃, rather than L-T₄, is used because of its direct effect on tissues, bypassing potential defects of T₄ transport and metabolism, which may also produce attenuated responses. In addition, the more rapid onset and shorter duration of T₃ action reduces the period required to complete the evaluation and shortens the duration of symptoms that may arise in individuals with normal responses to the hormone. Responses to each incremental dose of L-T₃ are expressed as increments and decrements or as a percent of the value measured at baseline. The results of such a study are shown in Figure 6.

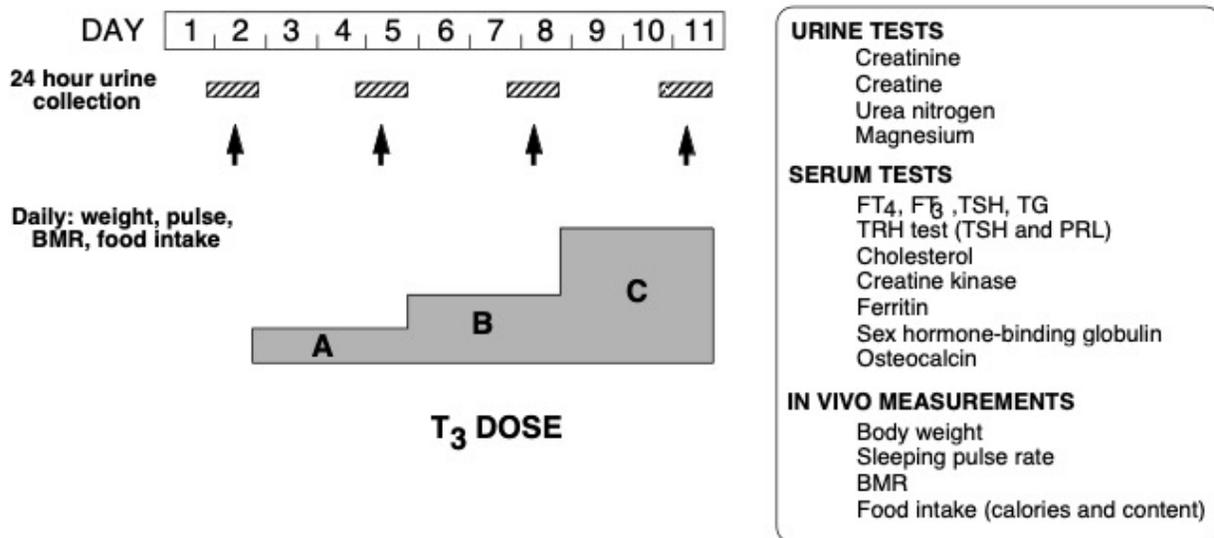


Figure 5. Schematic representation of a protocol for the assessment of the sensitivity to TH using incremental doses of L-T₃. For details see text.

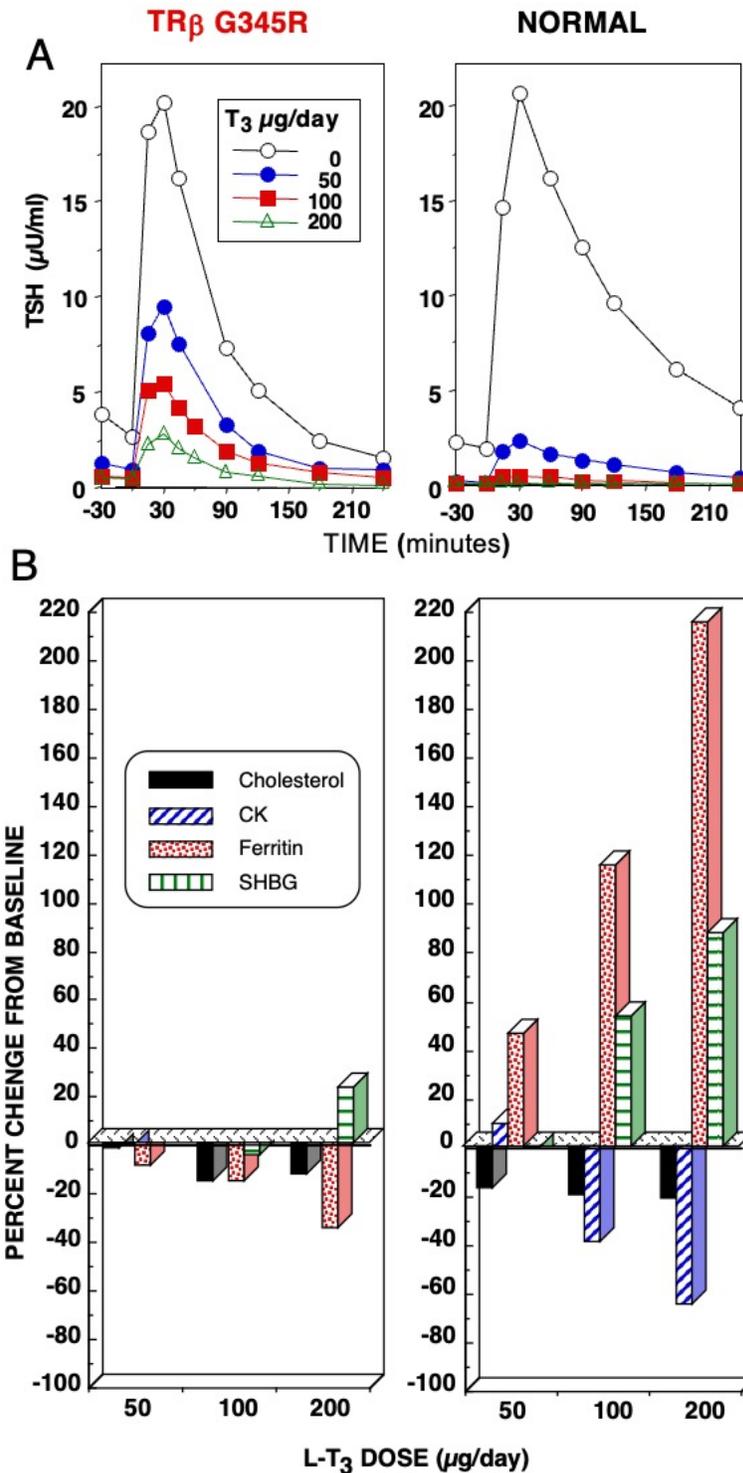


Figure 6. Responses to the administration of L-T₃ in a subject with RTH β harboring TR β G345R mutant and an unaffected individual. The hormone was given in three incremental doses, each for 3 days as illustrated in Figure 5. Results are shown at baseline and after each dose of L-T₃. (A) TSH responses to TRH stimulation. Note the reduced suppression of the TSH response by L-T₃ in the individual with

RTH β . (B) Responses of peripheral tissues. Note the stimulation of ferritin and sex hormone binding globulin (SHBG) and the suppression of cholesterol and creatine kinase (CK) in the normal subject. Responses in the affected subject were blunted or paradoxical.

The diagnosis of RTH β is particularly challenging when the latter is associated with other thyroid diseases, such as autoimmune thyrotoxicosis that suppresses the TSH level (175) or with congenital (176, 177) or acquired (178) hypothyroidism. Failure to differentiate RTH β from ordinary thyrotoxicosis continues to result in inappropriate treatments. The diagnosis requires awareness of the possible presence of RTH β , usually suspected when high levels of circulating TH are not accompanied by a suppressed TSH.

Treatment

No specific treatment is available to fully and specifically correct the defect. Theoretically, an ideal treatment for RTH β caused by mutant TR β s with altered TH binding would be to design mutation-specific TH analogues that overcome the binding defect (179). The ability to identify specific mutations in the *THRB* gene provides a means for prenatal diagnosis and appropriate family counseling. This is particularly important for families whose affected members show evidence of growth or mental retardation. Fortunately, in most cases of RTH β , the partial tissue resistance to TH appears to be adequately compensated for by an increase in the endogenous supply of TH. Thus, treatment need not be given to such patients. This is not the case in patients who have undergone ablative therapy or have a concomitant condition limiting their thyroidal reserve. In these patients, the serum TSH level can be used as a guideline for hormone dosage.

Not infrequently, some peripheral tissues in patients with RTH β appear to be relatively more resistant than the pituitary. Thus, compensation for the defect at the level of peripheral tissues is incomplete. In such instances, judicious administration of

supraphysiological doses of the hormone is indicated. Since the dose varies greatly among cases, it should be individually determined by assessing tissue responses. In childhood, particular attention must be paid to growth, bone maturation and mental development. It is suggested that TH be given in incremental doses and that the BMR, nitrogen balance, serum SHBG and osteocalcin be monitored at each dose, and bone age and growth on a longer term. Development of a catabolic state is an indication of overtreatment.

The exact criteria for treatment of RTH β in infancy have not been established. This is often an issue when the diagnosis is made at birth or in early infancy. In infants with elevated serum TSH levels, subclinical hypothyroidism may be more harmful than treatment with TH. Indications for treatment may include a TSH level above the upper limit of the reference range, retarded bone development, and failure to thrive. This may not apply to children homozygous for a mutant TR β . The outcome of affected older members of the family who did not receive treatment may serve as a guideline. Longer follow-up and psychological testing of infants who have been given treatment will determine the efficacy of early intervention.

It is unclear at this time whether intervention during early gestation is appropriate. However, limited experience suggests that the T₄ of mothers with RTH β carrying a normal embryo should not be allowed to be higher than 50% the upper limit of normal in order to prevent low birth weight (180). The concept of *in utero* treatment is questionable (181, 182).

Patients with more severe thyrotroph resistance and symptoms of thyrotoxicosis may require therapy. Usually, symptomatic treatment with an adrenergic β blocking agent, preferably atenolol, would suffice.

Treatments with antithyroid drugs or thyroid gland ablation increase TSH secretion and may result in thyrotroph hyperplasia. Development of true pituitary tumors, even after long periods of thyrotroph overactivity, is extremely rare (183).

Treatment with supraphysiological doses of L-T₃, given as a single dose every other day, is successful in reducing goiter size without causing side effects (184). Such treatment is preferable considering that postoperative recurrence of goiter is the rule. The L-T₃ dose must be adjusted until TSH and TG are suppressed and reduction of goiter size is observed. L-T₃ has been also used with some success in the treatment of ADHD in an individual with RTH β resistant to conventional treatments with stimulants (185).

Among the TH analogues used to alleviate symptoms of apparent TH excess (186), TRIAC has had the widest use (187, 188). It has a relatively greater affinity than T₃ for some mutant TR β s (189). In general, TRIAC's short half-life produces greater effect centrally than on peripheral tissues. This, in turns, reduces TSH and TH secretion with apparent amelioration of hypermetabolism. The value of treatment with D-T₄ is questionable. Theoretically, the ideal treatment is development of mutant-specific TH analogues that would rescue the dominant negative effect of the mutant TR β (179).

Patients with presumed isolated peripheral tissue resistance to TH present a most difficult therapeutic dilemma. The problem is, in reality, diagnostic rather than therapeutic. Many, if not most patients falling into this category, are habitual users of TH preparations. Gradual reduction of the TH dose and psychotherapy are recommended.

RESISTANCE TO THYROID HORMONE-ALPHA (RTH α)

Background

Following the identification of a mutation in the *THRB* gene in 1989, finding one in the *THRA* gene was implicit. However, this did not come to pass for the next 23 years. With the development of mice deficient in Tr α (knockout) (55) and further, mice harboring *Thra* gene mutations (knockin), modeled after human mutations known to occur in the *THRB* gene (190, 191), a phenotype was defined to aid in the identification of similar *THRA* gene defects in humans. Yet, laboratories searching for mutations in the *THRA* gene in individuals with low IQ and short without growth hormone deficiency did not succeed. It is through whole exome sequencing that in 2012 the first few families with *THRA* gene mutations were identified (9, 20, 192, 193). In retrospect, the failure to identify *THRA* gene mutation using the candidate gene approach was the lack of signature serum thyroid test abnormalities characteristic to individuals with *THRB* gene mutations.

Incidence and Inheritance

The precise incidence of RTH α is unknown. Because most routine neonatal screening programs are based on the determination of TSH, as is the case of RTH β , subjects with RTH α cannot be detected owing to their normal blood TSH. The RTH α in key cases of half of the reported families has been caused by *de novo* mutations (194). While the ethnic origin in most reported cases is not specified, white European is presumed based on pictures.

Etiology and Genetics

Mutations in the *THRA* gene have now been identified in 32 subjects with RTH α belonging to 19 families. They comprise 18 different mutations, 15 of which have been published (9, 195-203) and reviewed (194, 204). E403*, located in a CpG hot-spot was found in two unrelated families (9, 200). All are located in the ligand-binding domain of TR α and 6 of the 18 mutations involve both TR α 1 and TR α 2, but none

affect the *REV-RRB α* gene transcription from the opposite strand of the *THRA* locus. Given the 85% amino acid homology between the hinge region and ligand binding domains of TR α 1 and TR β with the exception of *THRA* N359Y, all have been found to have corresponding mutations in the homologous regions of the *THRB* and five are located in CpG hot spots. As expected, in three of them (A263V/S, R384C/H and E403K/*) mutations have produced more than one codon alteration (Figure 7).

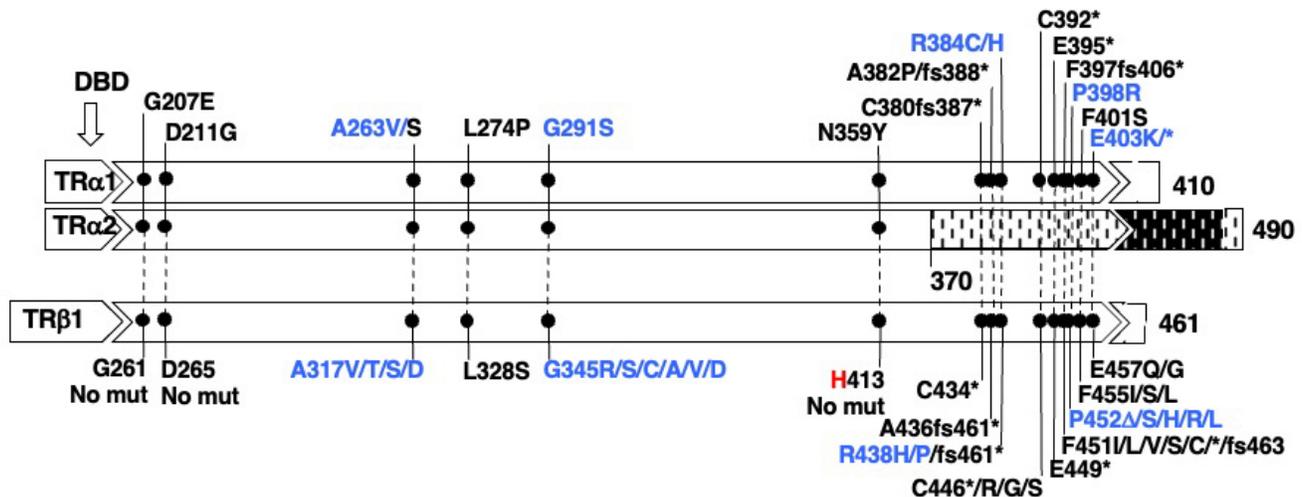


Figure 7. Mutations in TR α 1 and TR α 2 and in the corresponding amino acid mutations in TR β 1 are aligned according to amino acid sequence homology. The single difference is indicated in red. In blue are mutations occurring in hot spots (CpG or CG-rich regions). The ligand binding domain (LBD) containing the mutations is expanded and the locations of mutation is in scale. The DNA binding domain (DBD) is upstream of the mutations. Sequences from amino acid 370 to 490 of the TR α 2 diverge from those of TR α 1 due to alternative splicing. Data on *THRA* gene mutations courtesy of Carla Moran, University of Cambridge, United Kingdom

Molecular Basis of the Defect and Properties of the Mutant TR α

The *THRA* gene is located on chromosome 17. It generates two protein isoforms, TR α 1 and TR α 2 by alternative splicing. TR α 2 is devoid of a ligand binding and its physiological function through binding on DNA remains unclear. TR α 1 functions in the same manner as TR β but there are some differences in the

interaction with cofactors and in tissue distribution. It is the latter that is responsible for the differences in the phenotype in individuals harboring mutations in these two transcription factors. By virtue of TR α 1 expression predominantly in the central nervous system, bone, intestine and heart, manifestations in these organs dominate. Differences in the severity among mutations can be explained by the degree of loss of function and

by the amount of L-T₃ required to demonstrate reversibility *in vitro* (197).

The mechanism causing the defect were convincingly demonstrated in the first reported patient with RTH α harboring a nonsense mutation, produces a truncated TR α 1 (E403*) that lacks the C-terminal alpha-helix of the molecule (9). As a consequence, in addition to negligible T₃-binding, the mutation promoted corepressor binding while abolishing binding of the coactivator, both contributing to a strong DNE as demonstrated *ex vivo*. The 6-year-old girl, harboring this mutation, presented with chronic constipation noted upon weaning at 7 months of age, and growth and developmental delay. Hypothyroidism manifested in organs expressing predominantly TR α , including bone, gastrointestinal tract, heart, striated muscle and central nervous system. More specifically X-rays showed patent cranial sutures with Wormian bones, delayed dentition, femoral epiphyseal dysgenesis and retarded bone age. In addition, diminished colonic motility with megacolon, slow heart rate, reduced muscle strength were suggestive of hypothyroidism, as was her placid affect, slow monotonous speech and cognitive impairment. Thyroid function tests, were of subtle nature somewhat reminiscent of MCT8 defects, presumably due to alterations in iodothyronine metabolism (Table 3) (see the THCMTD Section in this Chapter).

Animal Models of RTH α (See also animal models under RTH β , above)

The question of why mutations in the *THRA* gene have not been identified earlier in man was partially answered by the study of mice with targeted gene

manipulations. As stated in an earlier section, *THRA* gene deletions, total or only α 1, failed to produce a RTH β -type serum phenotype. Several human mutations known to occur in the *THRB* gene were targeted in homologous regions of the *Thra* gene of the mouse. These are, the PV frame-shift mutation, Tr α 1 R384C (equivalent to Tr β R438C), Tr α P398H (equivalent to Tr β P452H) and Tr α L400R (corresponding to Tr β 454) (205). While the resulting phenotypes were somewhat variable, none exhibited thyroid test abnormalities characteristic of RTH β . Common features in heterozygous mice were retarded post-natal development and growth, decreased heart rate, and difficulty in reproducing. Also, all were lethal in the homozygous state, in accordance with the noxious effect of unliganded Tr α 1.

Clinical Features

The earliest clinical observations are poor feeding, coarse cry and macroglossia. Growth retardation with shorter lower limbs was also noted in infancy (9, 20). Other somatic abnormalities and clinical findings are listed in Table 5. Unusual somatic defects including clavicle agenesis, marked abnormalities of fingers, toes and elbow joints were observed in a single patient with a *THRA* N359Y mutation (195). It is unlikely that these findings are related to the *THRA* gene defect. Constipation is a common finding that results in fecal impaction. Bowel dilatation is seen on X-rays. Decreased peristalsis and delayed intestinal transit have been documented. In general, symptoms and signs are compatible with hypothyroidism. These include delayed fontanel closure, slow mentation and motor activity, reduced global IQ, and bradycardia.

Table 5. List of Clinical Features of RTH α		
System	Infant and Child	Adult
Early features	poor feeding; coarse cry; umbilical hernia	
Developmental	delayed milestones; growth retardation	short stature (short limbs)
Somatic defects (Dysmorphism)	macroglossia, broad and coarse face, broad face, thick lips, flat nasal bridge	
		skin tags
Skeletal	delayed fontanel closure epiphyseal dysgenesis	serpiginous cranial sutures cranial and cortical hyperostosis
Gastrointestinal	constipation; bowel dilatation	constipation
Cardiovascular	bradycardia	bradycardia, low blood pressure
Neurological	delayed speech; dyspraxia	dysarthria, slow motor initiation ataxia, dysdiadochokinesis, low IQ
Metabolic	Low metabolic rate, reduced resting energy expenditure, peripheral markers of TH action compatible with hormone deprivation	
Hematological	mild anemia	mild anemia

Data derived from references (193, 298)

Laboratory Findings

Thyroid test abnormalities are not as prominent as in other syndromes of impaired sensitivity to TH and explain the failure to readily recognize the defect. There is a trend for serum T₄ and rT₃ to be low and T₃ to be high. However, in most instances the concentrations of these iodothyronines are not outside of the reference range. Yet, the T₃/rT₃ ratio is consistently high and a good laboratory biomarker (Table 3). TSH is usually within the upper part of the reference range. A number of test results are compatible with reduced TH action in peripheral thyroid tissues but not centrally. There is a trend for insulin like growth factor I (IGF-1) to be low and for low density lipoprotein (LDL) cholesterol and creatine kinase to be elevated. Bone mineral density is increased. Anemia is a common finding and has been observed as early as 7 months of age (33). Anemia is normocytic with normal reticulocyte count, hemolytic indices, vitamin B12 and folate.

Differential Diagnosis

Suspicion for the presence of RTH α is based on clinical rather than laboratory findings. The serum thyroid test abnormalities are subtle and their relative pattern somewhat reminiscent of those found in MCT8 deficiency. However, individuals with *THRA* gene mutations do not manifest the severe neuropsychomotor defect characteristic of MCT8 deficiency. Symptoms of hypothyroidism are disproportionate to the thyroid function test abnormalities and TSH is not elevated as in primary hypothyroidism. The fact that in most cases diagnosis was made by whole exome sequencing attests to the difficulty in identifying patients based on clinical and standard laboratory evaluation. Clinical findings suggestive of congenital hypothyroidism without elevated TSH and a high T₃/rT₃ ratio should rise a high degree of suspicion.

Treatment

The majority of individuals with *THRA* gene mutations have received L-T₄ treatment. The treatment has shown beneficial effect on symptoms and signs caused by functional thyroid hormone deprivation in peripheral tissues. These include, constipation and bradycardia but not anemia. In children, L-T₄ treatment resulted in catch-up growth, motor development and in one case reduced hypotonia (201). The effectiveness of L-T₄ treatment in children appears to depend on the severity of the defect [frame shift mutations (203)] and time of treatment initiation. There is no experience with treatment beginning in early infancy.

L-T₄ treatment resulted in normalization of low serum T₄ and rT₃ levels but T₃ concentration remained elevated. Serum TSH, on the other hand was suppressed, even with physiological L-T₄ doses and FT₄ levels within the reference range. This confirms an intact hypothalamic-pituitary axis, which may even be hypersensitive to TH. Peripheral tissue markers of TH action, such as serum SHBG, LDL cholesterol and creatine kinase also responded appropriately to L-T₄ treatment.

No treatments with TH analogues have been reported. The use of TR α -specific agonist may be helpful but, being directed to the WT-receptor it will not abrogate the DNE of the mutant TR α . The development of mutant specific analogues, as suggested for RTH β , may be an option.

THYROID HORMONE CELL MEMBRANE TRANSPORTER DEFECT (THCMTD)

Patients with THCMTD caused by X-linked MCT8 deficiency are usually boys identified in infancy or in early childhood with feeding difficulties, severe cognitive deficiency, infantile hypotonia and poor head control. They develop progressive spastic

quadriplegia, diminished muscle mass with weakness, joint contractures, and dystonia. Early and characteristic thyroid abnormalities are high serum T₃, low T₄, and a slightly elevated TSH.

The neurological phenotype is severe and incapacitating in affected individuals, with minimal variability across families. Most importantly, this phenotype is not consistent with classical generalized hyperthyroidism or hypothyroidism. Depending on the type of TH transporters expressed, different tissues manifest the consequences of TH excess or deprivation. Tissues expressing other transporters than MCT8 respond to the high circulating T₃ level, resulting in a hyperthyroid state, while tissues dependent on MCT8 for TH transport, are hypothyroid. This complicates treatment as standard TH replacement fails to reach some tissues, while it worsens the hyperthyroidism in others.

The complex and severe neurodevelopmental phenotype together with the pathognomonic thyroid tests including high serum T₃, low rT₃, low or low normal serum T₄ concentrations and normal or slightly elevated serum TSH levels represent the characteristic presentation of the MCT8 deficiency syndrome.

Cell Membrane Transporters of TH

The identification and characterization of several classes of molecules that transport TH across membranes (206), has changed the previously held paradigm of passive TH diffusion into cells (207). These proteins belong to different families of solute carriers: 1) Na⁺/taurocholate cotransporting polypeptide (NTCP) (208); 2) fatty acid translocase (209); 3) multidrug resistance-associated proteins (210); 4) L-amino acid transporters (LAT) (211), among which LAT1 and LAT2 have been shown to transport TH; 5) members of the organic anion-transporting polypeptide (OATP) family (212), of which OATP1B1 and OATP1B3 are exclusively expressed in liver and transport the sulfated iodothyronines, T₄S,

T₃S, and rT₃S and less efficiently the corresponding non-sulfated analogues, whereas OATP1C1 is localized preferentially in brain capillaries and shows a high specificity and affinity towards T₄. The latter suggests that OATP1C1 may be important for transport of T₄ across the blood-brain barrier (213); and 6) within the monocarboxylate transporter (MCT)

family (214), MCT8 and MCT10 are specific TH transporters (215, 216). Differences in tissue distribution and transport kinetics of TH and of other ligands, impart their distinctive roles in the cell-specific delivery of TH.

Early studies using the expression of rat Mct8 in an heterologous system, showed that it potentiated the uptake of T₄, T₃, rT₄, and 3,3'-T₂ by 10-fold, but it had no effect on the uptake of sulfated T₄, the aromatic amino acids phenylalanine, tyrosine, and tryptophan, or lactate (216). Furthermore, transfection of human MCT8 in mammalian cells enhanced the metabolism of iodothyronines by endogenous deiodinases (217). These studies demonstrated the potent and iodothyronine-specific cell membrane transport function of MCT8.

The importance of MCT8 was most convincingly demonstrated by the identification of the first inherited THCMTD caused by mutations in the *MCT8* gene (6, 7). Although presence of the defect is suspected on the basis of clinical findings and standard laboratory tests, genetic confirmation is mandatory. Recently a novel neurodegenerative disease associated with a homozygous missense mutation in the T₄ transporter *OATP1C1* was reported (218). The patient was a 15.5-year-old girl with normal development during the first year of life, who gradually developed dementia with spasticity and intolerance to cold. Brain imaging demonstrated gray and white matter degeneration and severe glucose hypometabolism. When studied *in vitro*, decreased uptake of T₄ was shown, however, serum thyroid function tests were normal (218). At this point it cannot be excluded that the deficit in another

substrate is responsible for or contributes to the phenotype.

Inheritance and Incidence

MCT8 deficiency is a recessive X-linked defect that affects males, while females harboring a mutation are carriers. There is 100% penetrance in males that inherit a deleterious mutation. They manifest neuro-psychomotor and characteristic thyroid test abnormalities, whereas carrier females may show only mild thyroid test abnormalities (6, 219, 220). A female with typical features of *MCT8*-specific THCMTD had a *de novo* translocation disrupting the *MCT8* gene and unfavorable nonrandom X-inactivation (221). No affected male has reproduced. The defect has been reported in individuals of all races and diverse ethnic origins.

The exact incidence of this defect is not known. As most routine neonatal screening programs are based on the determination of TSH, MCT8 deficiency is rarely identified at birth by this mean. In neonatal screening programs based on T₄ measurements, a low concentration could potentially identify new cases. The yield is expected to be low given the high frequency of low T₄ levels in newborns.

The identification of more than 200 families with *MCT8* gene defects during the last 16 years indicates that this syndrome is more common than initially suspected. *MCT8* gene mutations can be maintained in the population because carrier females are asymptomatic and fertile, which precludes negative selection. Familial occurrence of MCT8 defects has been documented in many instances, however, genetic information on mothers of affected males is not always available.

Etiology

The clinical condition was first recognized in 1944, in a large family with X chromosomal mental retardation

presenting with motor abnormalities (222), a form of syndromic X-linked mental retardation, subsequently named the Allan-Herndon-Dudley syndrome (AHDS). In 1990, the syndrome was mapped to a locus on chromosome Xq21 (223). Following the identification of *MCT8*, gene mutations in subjects with thyroid abnormalities and neuropsychomotor manifestations were identified (6, 7), and subsequently *MCT8* mutations were found in other affected males, including the original family described in 1944 (224). The affected subjects have the characteristic thyroid test abnormalities, which went unnoticed in the past.

A large-scale screening of 401 males with X-linked mental retardation has identified *MCT8* gene mutations in only 3 subjects, and two of them had the characteristic thyroid phenotype. The other one had a normal serum T₃, but the mutation was also found in an unaffected relative (221). This underscores the importance of performing thyroid tests prior to undertaking gene sequencing, in individuals suspected of having a *MCT8* defect on the basis of the neurological phenotype.

Given the existence of other types of TH transporters and their different tissue distributions, it is anticipated that defects in such transport molecules would result in distinct phenotypes, the nature of which is difficult to predict. However, as mice deficient in specific TH transporters become available, some predictions about the nature of such diseases may be deduced despite species constraints. In this regard, mice with targeted inactivation of the *Lat2* (*Slc7a8*), *Mct10* (*Slc16a10*) and *Oatp1c1* (*Slco1c1* or *Oatp14*) TH transporters showed normal development, growth and normal thyroid function tests (225-227). The distribution of *Oatp1c1* differs in the brain of mice compared to humans, with mouse *Oatp1c1* being predominantly localized in capillary endothelial and only weak OATP1C1 staining being detected by immunohistochemistry in capillary endothelial cells in human brain sections (228). No mutations have been reported in humans in the *LAT2* and *MCT10*

transporters, however a human case of *OATP1C1* defect has been recently reported (218).

The *MCT8* Gene and Mutations

The *MCT8* gene was first cloned during the physical characterization of the Xq13.2 region known to contain the X-inactivation center (229). It has 6 exons and a large first intron that encompasses >100 kb. It belongs to a family of genes, named *SLC16*, the products of which catalyze proton-linked transport of monocarboxylates, such as lactate, pyruvate and ketone bodies. The deduced products of the *MCT8* (*SLC16A2*) gene are proteins of 613 and 539 amino acids (translated from two in-frame start sites) containing 12 transmembrane domains (TMD) with both amino- and carboxyl- ends located within the cell (230). The furthest upstream translation start site is absent in most species, including mouse and rat. Thus, the importance of the additional N-terminal sequence of the longer human *MCT8* protein is unknown. The demonstration that the rat homologue is a specific transporter of TH into cells in 2003 opened the field to clinical and genetic investigation (215).

Over 150 different *MCT8* gene mutations are known (33, 231) without counting the larger deletions that are not uncommon in *MCT8* due to the large intron 1. Mutations are distributed throughout the coding region of the gene. At the protein level, amino acids in the extracellular and intracellular loops are relatively underrepresented. One could speculate that missense mutations in these domains could putatively result in a milder phenotype, escaping detection, as sequences in these regions are less conserved across species compared to the TMD regions (232).

The types of *MCT8* gene mutations other than the large deletions involving one or more exons are shown in Figure 8. Coding single nucleotide substitutions are most common at 66%, while insertions/deletions (in/dels) causing frameshift or in frame insertions or deletions represent 30% and only 4% of defects are

located at splice sites. At the protein level, missense mutations are most common at 46%, followed by in/del frameshifts with premature termination at 26%, and nonsense mutations at 20%. A similar small proportion of 4% are in frame in/dels and defects with abnormal splicing. Some mutations have occurred in more than one family, for example, three different single amino acid in frame deletions (F229 Δ , F501 Δ and F554 Δ) have occurred in more than 5 families. Different

mutations in the same codon have been found to produce 2 or 3 mutant amino acids for example codon 224 (GCC) was mutated as A224T, V and E. In the families studied in our laboratory, 10% of the mutations occurred *de novo*, while 52% were present in at least one female carrier and one third occurred in mutation hotspots such as CpG dinucleotides C repeats or A repeats (33).

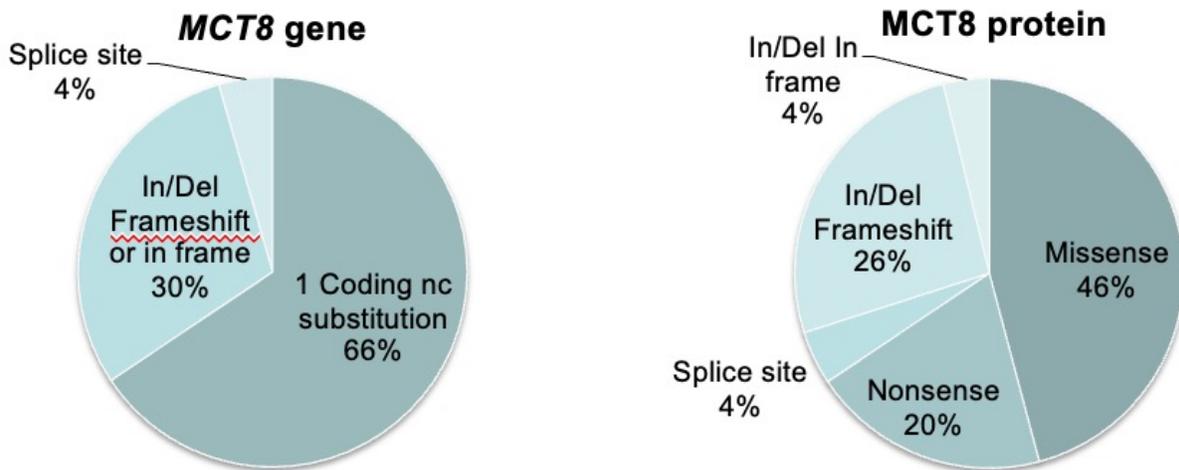


Figure 8. Representation of A. *MCT8* gene mutations and B. resulting mutant *MCT8* proteins associated with THCMTD, using both data reported by other groups and our published and unpublished cases, for a total of 157 different mutations.

More recently, variants of unknown significance (VUS) in the *MCT8* gene have been identified by whole exome sequencing performed in individuals with less typical neurodevelopmental abnormalities. The clinical relevance of these variants has been questioned, especially if the thyroid function tests are not characteristic of *MCT8* deficiency. This remains an area of investigation that could further expand the knowledge on genotype-phenotype correlations of VUS in the *MCT8* gene.

Clinical Features and Course of the Disease

Male subjects that are later found to have *MCT8* gene mutations, are referred for medical investigation during infancy or early childhood because of neurodevelopmental abnormalities. The clinical presentation of affected males with *MCT8* gene mutations is very similar, with characteristic thyroid test abnormalities and severe psychomotor retardation.

Newborns have normal Apgar scores and in most cases there is a history of normal gestation. However, polyhydramnios and reduced fetal movements have been reported (33, 224, 233). It is unclear whether this is an intrauterine manifestation of the syndrome. At birth there were no typical signs of hypothyroidism.

Truncal hypotonia and feeding problems are the most common early signs of the defect, appearing in the first 6 months of life. Only in a few cases they manifested within the first few days of life. Characteristically the neurological manifestations progress from flaccidity to limb rigidity and impairment of psychomotor development, leading to spastic quadriplegia with advancing age. With the exception of a few, subjects are unable to walk, stand or sit independently and they do not develop speech. To date, the ability to walk or talk has been reported only in the members of three families (224, 234). These are patients harboring L568P, L434W and F501del mutations who walked with ataxic gait or support, and had a limited and dysarthric speech. A possible explanation for the milder neurological phenotype in these patients is a residual 15-37% TH-binding activity of their mutant MCT8 molecules (235).

Dystonia and purposeless movements are common and characteristic paroxysms of kinesigenic dyskinesias have been reported in several patients, particularly severe in one boy, who presented up to 150 dyskinetic episodes per day (236). These are usually triggered by somatosensory stimuli, such as changing clothes or lifting the child. The attacks consist of extension of the body, opening of the mouth, and stretching or flexing of the limbs lasting for 2 or less than a minute (237). In addition to these non-epileptic events, true seizures can also occur. An altered sleep pattern with difficulty falling asleep and frequent awakening, can represent an important clinical issue for caregivers (236). Reflexes are usually brisk, clonus is often present, but nystagmus and extension plantar responses are less common.

With advancing age, weight gain lags and microcephaly becomes apparent, while linear growth proceeds normally (238). Muscle mass is diminished and there is generalized muscle weakness with typical poor head control, originally described as “limber neck” (222). A common and pronounced feature in MCT8 deficient patients is the failure to thrive, which can be severe, requiring the placement of gastric feeding tubes in some cases. Possible causes for low weight and muscle wasting are the neurologic dysphagia, and increased metabolism due to the thyrotoxic state of peripheral tissues as indicated by reduced cholesterol, and increased transaminases, SHBG, and lactate levels found in some patients with MCT8 mutations (236, 239-241).

Common facial findings that may be attributed to the prenatal and infantile hypotonia include ptosis, open mouth, and a tented upper lip. Ear length is above the 97th centile in about half of adults. Cup-shaped ears, thickening of the nose and ears, upturned earlobes, and a decrease in facial creases have been also reported. Pectus excavatum and scoliosis are common, most likely the result of hypotonia and reduced muscle mass.

While the cognitive impairment is severe, MCT8 deficient patients tend to present a non-aggressive behavior. Generally, affected individuals are attentive, friendly, and docile. Death during childhood or teens is not uncommon, usually caused by recurrent infections and/or aspiration pneumonia. However in a few instances of more mild neurologic involvement, survival beyond age 70 years has been observed (224). Data accumulated by a parent support group between 2009 and 2018 on the causes of death in 24 children and adolescents with MCT8 deficiency with age ranging between 1 day and 23 years, shows respiratory causes with aspiration, pneumonia, sometimes leading to septic shock or death during sleep as being common causes of demise (Figure 9).

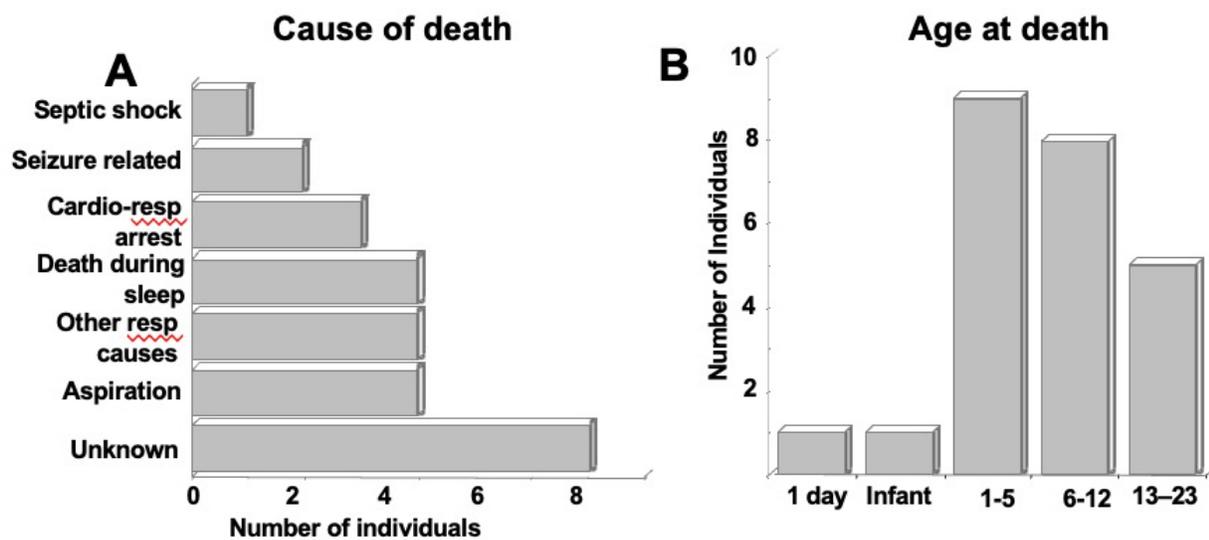


Figure 9. Death in 24 children and adolescents with MCT8 deficiency, between 2009-2018, age range 1 day to 23 years causes (A) and age (B).

Female carriers do not manifest any of the psychomotor abnormalities described above. However, intellectual delay and frank mental retardation have been reported in six carrier females (6, 221, 224, 240). Although an unfavorable nonrandom X-inactivation could alter the phenotype in these females (224), cognitive impairment can be due to a variety of causes. Thus, the causative link of *MCT8* mutations in heterozygous females with cognitive impairment remains to be proven (220).

Laboratory Findings

SERUM TESTS OF THYROID FUNCTION

Most characteristic, if not pathognomonic, are the high serum total and free T₃ and low rT₃ concentrations. T₄ is reduced in most cases and TSH levels can be slightly elevated but rarely above 6 mU/L (Figure 10).

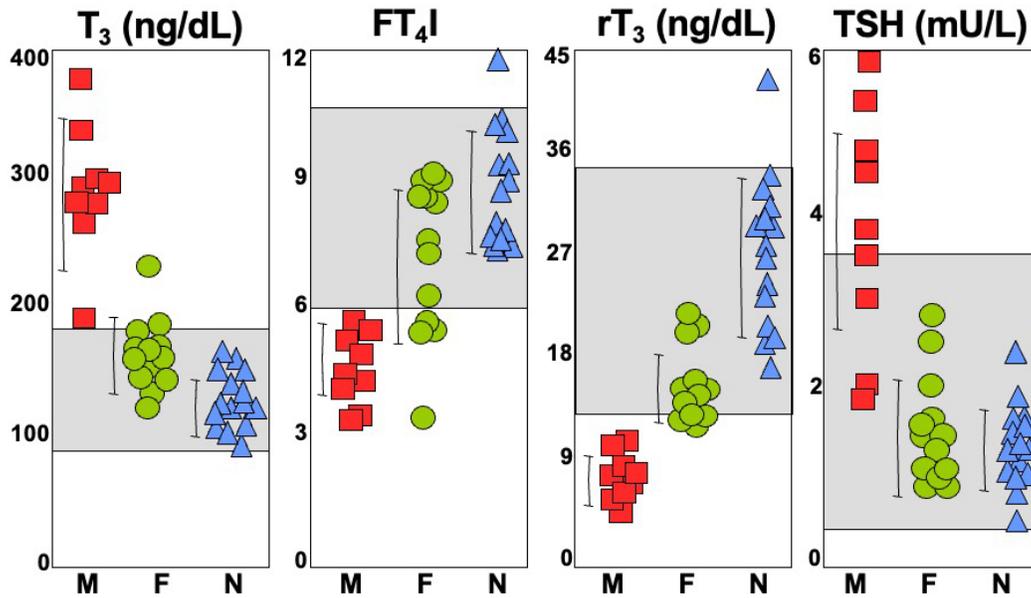


Figure 10. Thyroid function tests in several families with MCT8 deficiency studied in the authors' laboratory. Grey regions indicate the normal range for the respective test. Hemizygous males (M) are represented as red squares, heterozygous carrier females (F), as green circles and unaffected members of the families, as blue triangles (N). With the exception of TSH, mean values of iodothyronines in carrier females are significantly different than those in affected males and normal relatives.

TSH was normal at neonatal screening in most cases. Information about neonatal T₄ levels available in 8 cases revealed low values in 6 and normal levels in 2 subjects (33, 224, 236). However, low T₄ concentrations at birth are not uncommon, and are more often associated with low levels of T₄-binding protein and prematurity. Information regarding the T₃ and rT₃ concentration in the first days of life is not available. However, within one month the typical thyroid test abnormalities of MCT8 deficiency become apparent. In infants and children, tests results should be interpreted using age-specific reference ranges (see Chapter 62). This is particularly important for T₃ and rT₃, which are higher than those in adults. The ratio of T₃ to rT₃ is characteristically high in MCT8 deficiency while, with the exception of RTH α , it is low in other more common causes of abnormal T₃ and rT₃

levels, such as binding defects, iodine deficiency and non-thyroidal illness (Table 3).

Heterozygous female carriers can have iodothyronine concentrations that are on average intermediate between affected males and unaffected family members (6, 224, 240). While they are significantly different compared to both affected and unaffected individuals, overlapping values are observed in both groups. Serum TSH concentrations are, however, normal (Figure 10).

OTHER SERUM TESTS

Some patients have undergone extensive testing prior to the diagnosis of MCT8 deficiency. Results are summarized here and in the subsequent sections. Urinary organic acids, serum amino acids and fatty

acids, CSF neurotransmitters, glucose and lactate were normal. Other test results were abnormal only in some patients. These included, elevated serum SHBG, transaminases, ammonia, lactate and pyruvate, mildly elevated medium chain products in plasma acylcarnitine profile, elevated hydroxybutyric acid in urine (33, 233, 240) and reduced serum cholesterol. While the relation of some test abnormalities with MCT8 deficiency is unclear, others can be ascribed to the effect of the high serum T₃ levels on peripheral tissues. These are reduced cholesterol, and increased SHBG, and lactate.

Other endocrine tests, including pituitary function were normal when tested in a few individuals. However, administration of incremental doses of L-T₃, using the protocol devised for the study of patients with RTH β , showed reduced pituitary sensitivity to the hormone (33). This is probably due the reduced feedback effect of T₃ on the hypothalamo-pituitary axis, as well as the reduced incremental effect of the hormone on peripheral tissues already exposed to high levels of T₃.

X-RAYS AND IMAGING

Bone age has been inconsistently reported, and was found to be delayed in four cases and was slightly advanced in one (33, 240, 242, 243). The consequences of the MCT8 defects on bone are not clear at this time.

Mild to severe delayed myelination or dysmyelination (33, 244, 245) is a common finding when brain MRI is performed in early life. However, this can be missed as the delay in myelination is usually less apparent by approximately 4 years of age, and an adequate MRI technique is required for optimal interpretation. This distinguishes MCT8 deficiency from other leukodystrophies in which the myelination defect is persistent. Other reported MRI abnormalities in single cases might be non-specific and include subtle cortical

and subcortical atrophy (239), mild cerebellar atrophy (240), putaminal lesions (246), and a small corpus callosum (33). Increased choline and myoinositol levels and decreased N-acetyl aspartate were detected by MR-spectroscopy, and these abnormalities in brain metabolism were associated with the degree of dysmyelination according to MRI findings (247).

A recent study aimed to better define the spectrum of motor disability in MCT8 deficiency and to elucidate the neuroanatomic basis of the motor impairments using clinical observation and brain MRI in a cohort of 6 affected pediatric patients (248). T1- and T2-weighted brain MRI sequences revealed hypomyelination involving the subcortical U-fibers and periventricular white matter tracts that became more conspicuous over time in all 6 patients. In contrast, the callosal and cortical spinal tracts showed near normal myelination. Diffusion tensor imaging, performed in 3 of the patients, showed poor definition of the white matter association tracts relative to normal controls, suggesting the presence of subtle microstructural abnormalities. The same 3 subjects had a normal-appearing corpus callosum. These findings are consistent with the presence of dystonia in the MCT8 deficient patients studied. These imaging biomarkers suggest that rehabilitation efforts targeting dystonia may be more beneficial than those targeting spasticity in the prepubertal pediatric MCT8 deficiency population as the combination of hypotonia and dystonia presents a neurorehabilitation challenge for these patients and therapies directed only toward spasticity have commonly produced suboptimal responses (248).

TESTS IN TISSUES

Altered activity of mitochondrial complexes II and IV was identified in muscle biopsies from two cases (33, 249). It is unclear if this is due to the abnormal TH status of the muscle or to a yet unidentified effect of MCT8 on the mitochondria.

Cultured skin fibroblasts from males with MCT8 deficiency showed a significant reduction of T₄ and T₃ uptake while D2 enzymatic activity was higher, compared to fibroblasts from normal individuals (33, 234). Fibroblasts from carrier females gave results intermediate to those of affected males and normal individuals. Cellular T₃-uptake of cell lines transfected with different mutant MCT8 molecules (235), demonstrated or predicted complete inactivation in about 2/3 of mutations, while in the remaining 1/3, T₃-uptake ranged from 8.6 to 33% that of the WT MCT8. In particular, three missense mutations, S194F, L434W, and L598P showed significant residual transport capacity of more than 15% of normal MCT8, which may underlie the relatively milder phenotype observed in patients with these mutations (see section on Clinical Features and Course of the Disease, above).

Histological analysis of brain sections from a 30th gestational week male fetus and an 11-year-old boy with MCT8 deficiency was performed using as controls, brain tissue from a 30th and 28th gestational week male and female fetuses, respectively, and a 10-year-old girl and a 12-year-old boy (250). The MCT8-deficient fetus showed a delay in cortical and cerebellar development and myelination (Figure 11), loss of parvalbumin expression, abnormal calbindin-D28k content, impaired axonal maturation, and diminished biochemical differentiation of Purkinje cells. The 11-year-old boy showed altered cerebellar structure with deficient myelination, deficient synaptophysin and parvalbumin expression, and abnormal calbindin-D28k expression. This study showed that brain damage in MCT8 deficiency is diffuse, without evidence of focal lesions, that was present from fetal stages despite apparent normality at birth, and the deficient hypomyelination was found to persist at 11 years of age (250).

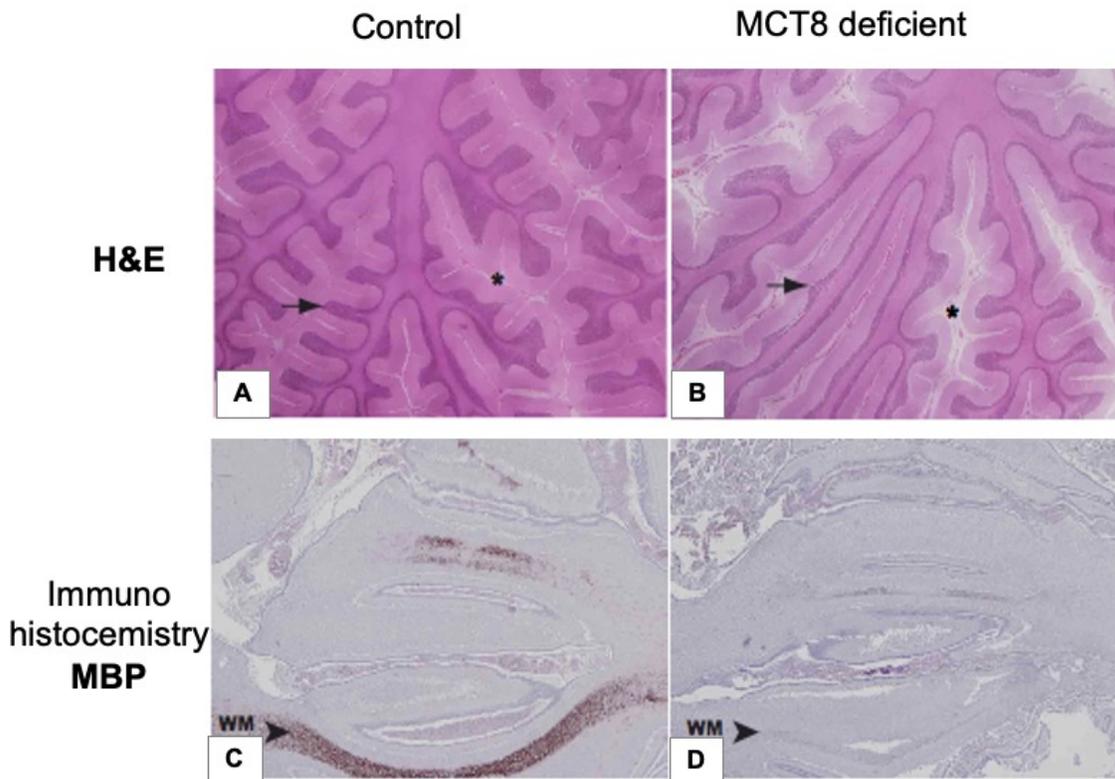


Figure 11. Structure and myelination of the fetal and juvenile cerebellum. Representative images showing hematoxylin-eosin (H&E) staining (A, B) and myelin basic protein (MBP) immunostaining (C, D) of tissue sections from the cerebellar vermis from 10-year-old control child (A), and 11-year-old MCT8 deficient child (B), control fetus and (C) MCT8-deficient fetus (D), both 30 weeks gestational age. In panels A and B, asterisks indicate the subarachnoid space in the cerebellum (wider in the MCT8-deficient subject) and arrows point to cerebellar folia (thinner size in the cerebellum of the MCT8-deficient boy). In panels C and D, arrowheads indicate immunopositive axons (lower proportion of immunopositive axons in the vermis from the MCT8-deficient fetus) WM, white matter. [Reproduced with permission from reference (250)].

GENETIC TESTING

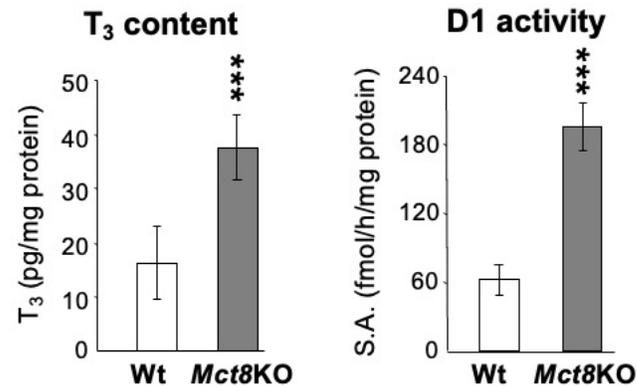
By definition, a defect in the *MCT8* gene is present in all patients. Genetic testing by sequencing is available in commercial laboratories and can detect nucleotide substitutions and small deletions and insertions. However, larger deletions and splice defects may require application of more in-depth genetic investigations, such as Southern blotting and haplotyping, available in research laboratories. Carrier testing for relatives at-risk and prenatal testing of pregnant carriers should be offered to families (251).

Animal Models of MCT8 Deficiency

Mct8-deficient recombinant (*Mct8KO*) mice (19, 252) replicate the characteristic thyroid test abnormalities found in humans and, thus, helped in understanding the mechanisms responsible for the thyroid phenotype (253). Measurements of tissue T₃ content showed the variable availability of the circulating hormone to

tissues, depending on the redundant presence of TH cell membrane transporters. In *Mct8KO* mice, tissues such as the liver, that express other transporters than Mct8 (15), have high T₃ concentrations reflecting the high levels in serum and are, therefore, “thyrotoxic” as demonstrated by an increase in the D1 enzymatic activity (Figure 12A). In accordance with a thyrotoxic state, serum cholesterol concentration is decreased and serum alkaline phosphatase is increased. In contrast, tissues with limited redundancy in cell membrane TH transporters, such as the brain (15), have decreased T₃ content in *Mct8KO* mice, which together with the increase in D2, indicate “hypothyroidism” in this tissue (Figure 12B). The role of D2 is to maintain local levels of T₃ in the context of TH deficiency and its activity is inversely regulated by TH availability (16). These findings of coexistent T₃ excess and deficiency in the *Mct8KO* mouse tissues explain, in part, the mechanisms responsible for the tissue specific manifestation of TH deficiency and excess in humans with MCT8 deficiency.

A LIVER



B BRAIN

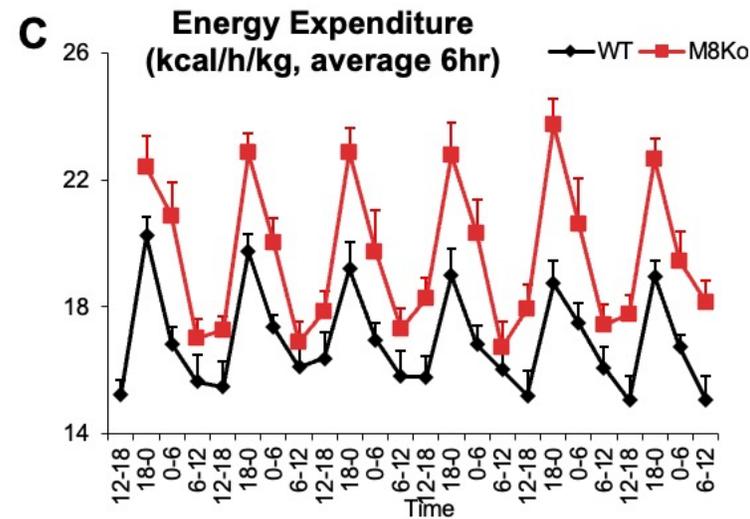
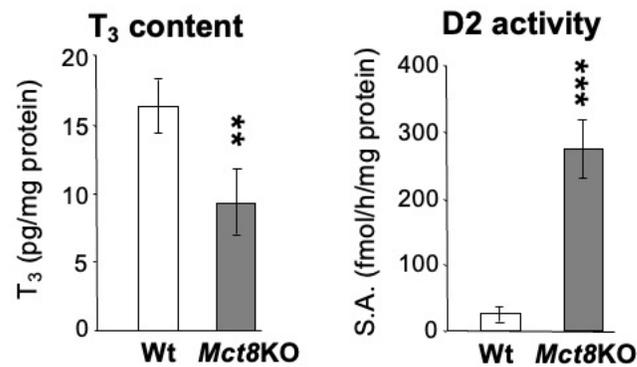


Figure 12. Data from *Mct8KO* vs Wt mice. A. T₃ content and D1 enzymatic activity in liver. B. T₃ content and D2 enzymatic activity in brain. Data from *Mct8KO* mice are represented as grey bars and those from Wt littermates are in open bars. ** p-value <0.01, *** p-value <0.001. C. Total energy expenditure (TEE) flow chart represented as 6h average over 6 days, *Mct8KO* mice are shown in red.

Metabolic and body composition studies showed that *Mct8KO* mice were leaner and had increased total energy expenditure (TEE) with increased food and water intake, while the activity level was normal (254). T_3 -treated *Wt* mice also showed increased food intake and TEE, and increased T_3 content, TH action and increased glucose metabolism in skeletal muscle similar to *Mct8KO* mice. Thus, the hypermetabolic state in MCT8 deficiency is due to the high serum T_3 and is responsible for the failure to maintain weight despite adequate caloric intake. Normalizing serum T_3 level by deleting deiodinase 1 in the combined *Mct8Dio1KO* mice was able to improve body composition and the metabolic alterations caused by the *Mct8* deficiency (254). Treatment of adult mice with the TH analog diiodothyropropionic acid (DITPA), which enters cells independently of *Mct8* transport, revealed amelioration of the thyrotoxic state in liver and improving the hypermetabolism of the *Mct8KO* mice (255), making this thyromimetic chemical suitable for the treatment of the hypermetabolism in patients with MCT8 deficiency.

Mct8 also has a role in TH efflux in the kidney and secretion from the thyroid gland (256, 257). The content of T_4 and T_3 in kidney is increased and their local actions increase D1 activity which enhances the local generation of T_3 . In the thyroid, *Mct8* is localized at the basolateral membrane of thyrocytes. Thyroidal T_4 and T_3 content is increased in *Mct8KO* mice as is the rate of their secretion and appearance in serum is reduced (257).

These observations from the *Mct8* deficient mice have helped understanding the mechanisms involved in producing the thyroid abnormalities in mice and humans. The increased D1 and D2 activities, stimulated by opposite states of intracellular TH availability, have an additive consumptive effect on T_4 levels and result in increased T_3 generation. The important contribution of D1 in maintaining a high serum T_3 level is supported by the observation in mice

deficient in both *Mct8* and D1. These mice have a normal serum T_3 and rT_3 (258). The low serum T_4 in *Mct8* deficiency is not only the result of attrition through deiodination but also due to reduced secretion from the thyroid gland and possibly increased renal loss.

In MCT8 deficient subjects serum TSH is usually modestly increased, a finding that may be compatible with the decreased serum T_4 concentration but not with the elevated serum T_3 level. However, MCT8 is expressed in the hypothalamus and pituitary, and its inactivation likely interferes with the negative feedback of TH at both sites (259). In *Mct8KO* mice, hypothalamic TRH expression is markedly increased and high T_3 doses are needed to suppress it, indicating T_3 resistance particularly at the hypothalamic level.

Mct8KO mice have been valuable in testing thyromimetic compounds for their potential to bypass the *Mct8* defect in tissues. One such TH analogue, DITPA has been tested. It was found to be effective in equal doses in the *Mct8KO* and *Wt* animal to replace centrally (pituitary and brain) and peripherally (liver) the TH requirements in animals rendered hypothyroid (260). In contrast, 2.5 and 8-fold higher doses of L- T_4 and L- T_3 , respectively, were required to produce a central effect in the *Mct8KO* compared to that in *Wt* animal. These high doses of TH produced “hyperthyroidism” in peripheral tissues of the *Mct8KO* mice. Importantly, DITPA given to pregnant mice carrying *Mct8*-deficient embryos was able to cross the placenta and to have similar thyromimetic action on the expression of TH-dependent genes in brain of *Mct8KO* and *Wt* pups at similar DITPA serum levels (261). The similar serum TSH in *Mct8KO* vs *Wt* pups prenatally treated with DITPA, demonstrated better accessibility of DITPA at the pituitary compared to L- T_4 thus making DITPA a candidate for the prenatal treatment of MCT8 deficiency (261).

Mct8KO mice were also used to test the possibility of gene therapy in MCT8 deficiency. Normal MCT8 was delivered using an AAV9 vector, injected either intravenously (IV) and/or intracerebroventricularly (ICV) into postnatal day 1 *Mct8KO* and *Wt* mice (262). Analysis of brains at 28 postnatal days, after L-T₃ injection for four days showed that MCT8 delivery to the blood brain barriers by IV but not ICV injection is necessary for its proper function and resulted in increased T₃ in the brain tissue triggering expression of known TH-regulated genes (262). These studies have introduced the consideration for gene therapy in the patients with MCT8 deficiency.

The lack of a neurological phenotype in *Mct8KO* mice limits their use as a model for understanding the mechanisms of the neurological manifestations in patients with MCT8 deficiency. If combined with deficiencies of other TH transporters in brain, *Mct8* has the potential of producing an obvious neurological phenotype. Thus, mice deficient in both *Mct8* and another TH transporter such as *Lat2*, *Mct10* and *Oatp1c1* were generated (225, 226, 228). From them, the *Mct8/Oatp1c1* double KO (DKO) mice showed alterations in peripheral TH homeostasis that were similar to those in *Mct8KO* mice; while, uptake of both T₃ and T₄ into the brain of *Mct8/Oatp1c1* DKO mice was strongly reduced (228). TH deprivation in the CNS of *Mct8/Oatp1c1* DKO mice with marked decrease in brain TH content and in TH target gene expression manifested with delayed cerebellar development, reduced myelination and compromised differentiation of GABAergic interneurons in the cerebral cortex (228). *Mct8/Oatp1c1* DKO mice displayed pronounced locomotor abnormalities and currently are being used to assess the pathogenic mechanisms underlying the neurologic phenotype in *Mct8* deficiency and as models to test the effect treatment modalities on neurodevelopmental defects observed in humans.

Molecular Basis of the Disorder

In vitro studies using mutant MCT8 alleles as well as observations from animals deficient in *Mct8*, serve to explain the mechanism leading to the defect. All mutant MCT8 alleles tested by transfection or in fibroblast derived from affected individuals show absent or greatly reduced ability to transport iodothyronines, primarily T₃ (235). Although MCT8 mRNA is widely expressed in human and rat tissues, including brain, heart, liver, kidney, adrenal gland, and thyroid (263, 264), repercussions due to its absence manifest primarily in tissues and cells in which MCT8 is the principal, if not unique TH transporter.

Analysis of the MCT8 mRNA expression pattern in the mouse brain by *in situ* hybridization revealed a distinct localization of this transporter in specific neuronal populations known to be highly dependent on proper TH supply, indicating that a defective MCT8 will perturb T₃-dependent neuronal function. Moreover, high transcript levels for MCT8 were observed in choroid plexus structures and in capillary endothelial cells, suggesting that MCT8 also contributes to the passage of THs via the blood-brain barrier and/or via the blood-cerebrospinal fluid barrier (265, 266). In the thyroid, it has been demonstrated that MCT8 is involved in the secretion of TH into the bloodstream (257, 267).

The magnitude of serum T₃ elevation does not correlate with the degree of T₃ transport defect produced by a particular *MCT8* mutation. This is probably due to the important contribution of the concomitant perturbation in iodothyronine metabolism on the production of T₃, as demonstrated in the *Mct8KO* mice (see the section above). Similarly, there is no correlation between the magnitude of serum T₃ elevation or rT₃ reduction in affected males compared to their carrier mothers (33). Some imperfect correlation does appear to exist between the degree of the MCT8 defect and clinical consequences. Patients that are least severely affected and capable of some locomotion have mutations with partial preservation of T₃ transport function (see Clinical

Features and Course of the Disease, above). In contrast, early death is more common in patients with mutations that completely disrupt the MCT8 molecule. However, it should be kept in mind that genetic factors, variability in tissue expression of MCT8, and other iodothyronine cell membrane transporters could be responsible for the lack of a stronger phenotype/genotype correlation. The possibility that MCT8 is involved in the transport of other ligands, or has functions other than TH transport, cannot be excluded.

There has been a great deal of effort in trying to understand how MCT8 transports TH into cells, and how MCT8 distinguishes TH substrates from structurally closely related compounds is not known. One starting point used was the fact that T₃ is bound between a His-Arg clamp in the crystal structure of the T₃ receptor/T₃ complex. To investigate whether such a motif might potentially be relevant for T₃ recognition in MCT8, several mutations occurring in patients, or generated *in vitro* have been tested in combination with amino acid specific chemical modification. Molecular modeling has demonstrated a perfect fit of T₃ poised into the substrate channel between His415 and Arg301 and observing the same geometry as in the T₃ receptor (268). Similarly, cysteine residues Cys481 and Cys497 were found to probably be located at or near the substrate-recognition site in MCT8 (269). The question whether MCT8 functions as a monomer or as an oligomer has also been investigated. Although several mutations have been shown to affect oligomerization *in vitro*, currently, it is not known whether there is an obligatory functional need for dimerization of MCT8, or whether there is substrate-induced or constitutive oligomerization versus monomerization (270). However, different from the tight interaction of TH with receptors as ligand and with the deiodinases as substrate, the transporters have to achieve specific interactions with TH, while avoiding tight binding, as this would stall the transport process. Characterizing the mechanism is of

fundamental interest and will be key for the design of specific MCT8 modulators.

Differential Diagnosis

MCT8-dependent THCMTD is syndromic, presenting a thyroid and a neuropsychomotor component. However, the majority of patients come to medical attention because of retarded development, and neurological deficits. Although the thyroid abnormalities are most characteristic, they escape detection by neonatal screening. TSH concentration is not elevated above the diagnostic cut off level and although T₄ is commonly low, it more often accompanies premature births and low levels of TH-binding serum proteins. Studies in *Mct8KO* mice suggest that rT₃ could turn out to be a good marker for the early detection of *MCT8* defects in humans.

Hypotonia is an early manifestation, but is not specific. Reduced myelin, documented by brain MRI, places MCT8 in the category of other diseases showing reduced myelination, among them Pelizaeus–Merzbacher disease (PMD; OMIM# 312080). The latter is also X-linked, and is a leukodystrophy caused by an inborn error of myelin formation due to defects in the *PLP1* gene located on Xq22. In fact, a survey of 53 families affected by hypomyelinating leukodystrophies of unknown etiology, classified as PMD, resulted in the identification of *MCT8* gene mutations in 11% (244), and the affected subjects were subsequently found to have the typical thyroid test abnormalities. Patients with PMD do not exhibit the thyroid phenotype of MCT8 deficiency and their myelination defect is persistent, rather than transient.

All children above the age of 1 month found to have *MCT8* gene mutations show the characteristic thyroid test abnormalities. This underscores the importance of performing thyroid tests in patients diagnosed with mental retardation or syndromic X-linked phenotypes suggestive of a MCT8 defect, prior to sequencing the *MCT8* locus. Most useful is the finding of a high serum T₃ and low rT₃. A reduced (at the low limit or below

normal) serum total or free T₄, and a normal or slightly elevated TSH are also present. In cases with increased T₃ due to other causes, calculating the ratio of T₃/rT₃ is helpful in differentiating them from cases of MCT8 defects, in whom the ratio will be above 10.

Treatment

Treatment options for patients with *MCT8* gene mutations are currently limited (251). Supportive measures include the use of braces to prevent malpositioned contractures that may ultimately require orthopedic surgery. Intensive physical, occupational, and speech therapies have subjective but limited objective beneficial effects. Diet should be adjusted to prevent aspiration and a permanent gastric feeding tube may be placed to avert malnutrition. Dystonia could be ameliorated with medications such as anticholinergics, L-DOPA carbamazepine and lioresol. Drooling might be improved with glycopyrolate or scopolamine. Seizures should be treated with standard anticonvulsants. When refractory to the latter, a ketogenic diet, as well as administration of supraphysiological doses of L-T₄, has been successful. Experience with such treatments is, however, limited to only a few cases.

Detection of low T₄ levels by neonatal screening has led to treatment with L-T₄ in several infants. As expected, no improvement has been noted when used in physiological doses, because of the impaired uptake of the hormone by MCT8-dependent tissues. Under these circumstances it would be logical to treat with supraphysiological doses of L-T₄, thereby increasing the availability of TH to the brain. However, the presence of an already increased D1, as observed in *Mct8* deficient mice (see Animal Models in a preceding section of this Chapter), is likely to aggravate the hypermetabolic state of the patient by generating more T₃ from the exogenous L-T₄. Therefore, high L-T₄ dose treatment has been used in combination with propylthiouracil (PTU), which is a specific inhibitor of D1. This results in reduction of the

conversion of T₄ to T₃ in peripheral tissues by D1, while it allows the local generation of T₃ by D2 in tissues. Although this treatment allowed an increase in serum L-T₄ level without increasing the hypermetabolism and weight loss, it did not improve the neuropsychomotor deficit (33, 241).

Other possible treatments that have been tested include the administration of the thyromimetic drug DITPA, that seems to be effectively transported into mouse brain in the absence of *Mct8* (260) (see Animal Models in a preceding section of this Chapter). DITPA given to children with MCT8 deficiency in doses of 1–2 mg/kg/d fully normalizes the serum thyroid tests, and reduces the hypermetabolism with improvement in the nutritional status with no objective change in the neuropsychiatric deficit (271).

In vitro treatment with DITPA of oligodendrocytes derived from a human embryonic stem cell reporter line expressing MCT8 (272) was found to up-regulate oligodendrocyte differentiation transcription factors and myelin gene expression and to promote myelination of retinal ganglion axons in co-culture. Pharmacological and genetic blockade of MCT8 induced significant oligodendrocyte apoptosis, impairing myelination, and DITPA treatment was able to limit this effect (272). As MCT8 seems to be essential for TH transport in human oligodendrocytes development, DITPA has the potential to be a promising treatment for developmentally regulated myelination in AHDS (272).

Other TH metabolites, such as TRIAC and its precursor tetraiodothyroacetic acid (TETRAC) have been tested. Recently, a multicenter, open-label, single-arm, phase 2, clinical trial investigated the effectiveness and safety of oral TRIAC in male pediatric and adult patients with MCT8 deficiency assessed at baseline and after 12 months of TRIAC administered in a predefined escalating dose schedule at a dose ranging from 23–48 µg/kg per day (273). All serum thyroid tests decreased, including T₃; TSH,

total free T₄, and reverse T₃ further decreased from their already low baseline levels in patients with MCT8 deficiency. While the reduction in serum T₃ concentrations was associated with improvements in body weight, cardio-vascular status, and markers of TH action in different tissues, causality cannot be proven directly because the open-label study design did not include a control group. Moreover, there are concerns that the further reduction in circulating T₃ concentrations under TRIAC treatment aggravates the hypothyroid state in the brain in individuals with MCT8 deficiency (273).

Another treatment option tested *in vitro* consists of the use of chemical and pharmacological chaperones on the expression and transport activity of several MCT8 mutant proteins. The chemical chaperone sodium phenylbutyrate (NaPB), has been used to treat patients with cystic fibrosis and urea cycle defects for extended periods of time. *In vitro* testing showed that NaPB could rescue the expression and activities of MCT8 mutations that retain some residual activity (274). Testing of another chemical chaperone, 4-phenylbutyric acid (PBA), demonstrated that it was effective in potentiating the T₃ uptake in transiently transfected COS-1 cells with WT MCT8 and the F501Δ mutant, but only minor effects were observed in F501Δ fibroblasts (275). Thus, the applicability of chemical and pharmacological chaperones may be limited to only a small number of patients with certain mutations. In addition, because the magnitude of the effect of chaperone therapy strongly depends on the disease model, more extensive preclinical studies are warranted before clinically available chaperones should be considered as a treatment option in patients with MCT8 deficiency.

Treatments that reduce the high serum T₃ level, including combining PTU with L-T₄, DITPA or TRIAC, have all beneficial effect on the hypermetabolism and reduce weight loss. This often obviates the need for feeding through a G-tube. However, no demonstrable effect on the neuropsychomotor abnormalities has

been observed even when treatment was initiated during infancy. It is noteworthy that none of the treatments discussed above have been initiated before the age of 6 months. It is possible that for any TH mediated treatment to be effective on brain development, it will have to be initiated before birth. However, intra-amniotic treatment with L-T₄ of an embryo beginning at 17 weeks of gestation failed to prevent the neurological complications, even though myelination proceeded normally (33). This suggests that earlier brain damage, unrelated to myelin formation, is involved and that treatment should begin at 10 weeks of gestation, when brain TH receptors becomes fully active (276).

Use of thyromimetic drugs is supported by the defect in the transport of authentic THs. However, it is possible that a deficiency in a different substrate or that the loss of a putative constitutive effect harbored by the intact MCT8, play a role in the observed brain morbidity.

THYROID HORMONE METABOLISM DEFECT

Recessive mutation in the *SBP2* (*SECISBP2*) gene encoding selenocysteine insertion sequence-binding protein 2 is an inherited condition causing a TH metabolism defect (THMD). The mutations affect selenoprotein synthesis including the deiodinases, which are selenoenzymes. Only twelve families with this defect have been identified so far. Affected individuals present with short stature and characteristic thyroid test abnormalities with high serum T₄, low T₃, high rT₃ and normal or slightly elevated serum TSH. In addition, they also have decreased serum selenium (Se) and decreased selenoprotein levels and activities in serum and tissues. The overall clinical phenotype is complex. Affected individuals may have delayed growth and puberty, and in severe cases failure to thrive, developmental delay, infertility, myopathy, hearing impairment, photosensitivity, and immune deficits.

Another inherited condition also reported to cause THMD is the mutation in the *TRU-TCA1-1* gene encoding the selenocysteine transfer RNA (tRNA^{Sec}), which has a role in selenoprotein synthesis. The affected individual had high serum T₄, high rT₃, normal T₃, normal TSH with low Se level (277)

Mutations in the in selenocysteine synthase (SEPSECS) have also been identified, however affected individuals do not manifest abnormal thyroid function tests (278). Affected children manifest a complex neurodevelopmental and neurodegenerative disorder involving, among other features, microcephaly, delayed motor and intellectual development, spasticity, and seizures.

Intracellular Metabolism of TH

The requirement for TH varies among tissues, cell types, and the timing in development. In order to provide the proper intracellular hormone supply, TH entry into cells is controlled by membrane transporters, and further fine-tuned by its intracellular metabolism, regulated by three selenoprotein iodothyronine deiodinases (D1, D2, D3). D1 and D2 are 5'-iodothyronine deiodinases that catalyze TH activation by converting T₄ to T₃. D3, a 5-deiodinase is the main TH inactivator through conversion of T₄ to rT₃

and T₃ to T₂ (Figure 1B) (see other Endotext chapters for details)

Deiodinases are selenoproteins containing the rare amino acid selenocysteine (Sec), present in the active center of the molecule and required for their enzymatic activity. They are differentially expressed in tissues and in response to alterations in the intracellular environment, further regulated at the level of transcription, translation and metabolism (16). D2 activity can change very rapidly as its half-life is more than 15-fold shorter than that of D1 and D3. T₄ accelerates D2 inactivation through ubiquitination, a reversible process that can regenerate active D2 enzyme through de-ubiquitination.

Deiodinases share with other selenoproteins the synthesis through a unique mode of translation. The codon used for insertion of Sec is UGA, which under most circumstances serves as a signal to stop synthesis. This recoding of UGA is directed by the presence of a selenocysteine insertion sequence (SECIS) in the 3'-untranslated region of the selenoprotein messenger RNA. It is the SECIS-binding protein 2 (in short SBP2) that recognizes the SECIS and recruits an elongation factor (EFSec) and the specific selenocysteine transfer RNA (tRNA^{Sec}) for addition of Sec at this particular UGA codon (Figure 13) (279).

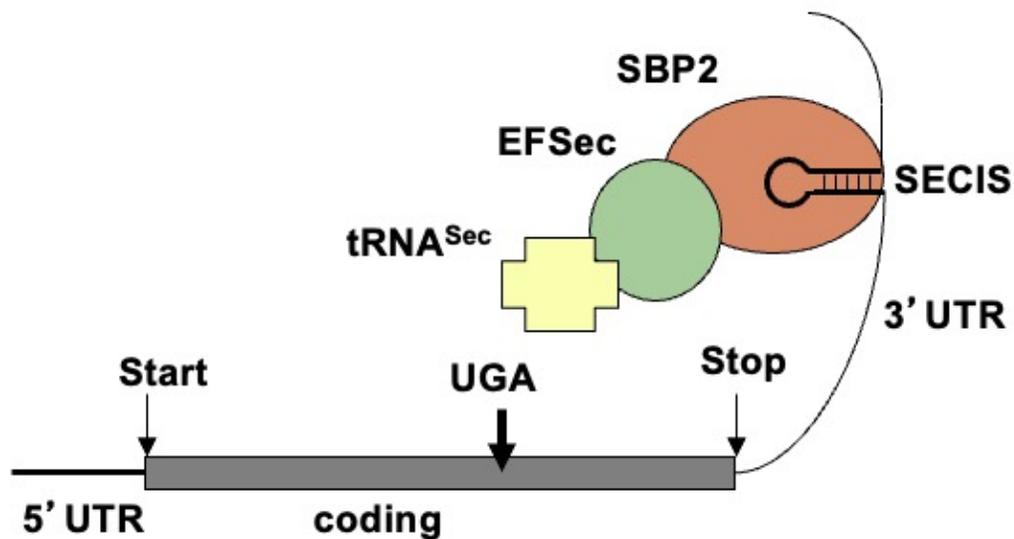


Figure 13. Components involved in Sec incorporation central in the synthesis of selenoproteins. Elements present in the mRNA of selenoproteins: an in frame UGA codon and Sec incorporation sequence (SECIS) element, a stem loop structure located in the 3'UTR (untranslated region). SBP2 binds SECIS and recruits the Sec-specific elongation factor (EFSec) and Sec-specific tRNA (tRNA^{Sec}) thus resulting in the recoding of the UGA codon and Sec incorporation.

Etiology and Genetics

Abnormalities in TH metabolism have first been observed in humans as acquired condition, the “low T3 syndrome”, which occurs during non-thyroidal illness or starvation (see Chapter 14) (280). The first inherited disorder of TH metabolism in humans was reported in 2005 (8), and was found to be caused by a mutation in the *SBP2* gene. Later, mutations in *TRU-TCA1-1* gene were also reported to cause THMD in a single individual (277). Both genes are involved in selenoprotein synthesis, thus the mutations result in the defect of selenoproteins including the deiodinases. It is anticipated that mutations in other genes causing defective TH metabolism may have different phenotypes. So far, no humans have been reported with mutations in the deiodinase genes or in other proteins involved in selenoprotein synthesis.

Incidence and Inheritance

The incidence of THMD caused by *SBP2* deficiency is unknown. Ten additional families have been identified since the description of the initial two families (33, 281-285). The inheritance is autosomal recessive and males and females are equally affected. For this reason, the likelihood of being affected is less than that for autosomal dominant or X-linked conditions. The ethnic origins of the reported patients include Bedouin from Saudi Arabia, African, Irish, Brazilian, English, Japanese, Turkish and Argentinian individuals.

THE *SBP2* GENE AND MUTATIONS

The human *SBP2* gene, cloned in 2002, is located on chromosome 9 and encodes a protein of 854 amino acids widely expressed in most tissues (286). The C-terminal domain encompassing codons 399-774 of the protein is the minimal functional protein domain

required for SECIS binding, ribosome binding and Sec incorporation (287), which is mandatory for SBP2 function. The role of the N-terminal region is not fully understood. *In vitro* studies have characterized a nuclear localization signal located in the N-terminal part and nuclear export signal in the C-terminal part. These domains enable SBP2 to shuttle between the nucleus and the cytoplasm (288), and they play a role in the function of SBP2 in the nucleus *in vivo*.

The finding of *SBP2* defects was made possible by extensive genetic studies of a large family with three affected and four unaffected children (8). Although affected individuals had clinical evidence of abnormal TH metabolism, the sequences of the deiodinase genes, as well as those of genes encoding proteins involved in the ubiquitination and de-ubiquitination of the deiodinase 2 were normal. Subsequently, mutational analysis of the *SBP2* gene revealed that the affected individuals were homozygous for a R540Q mutation, while both parents were heterozygous carriers. It is likely that the parents, which were not directly related but both members of the same Bedouin tribe, had a common ancestor. The affected child of the 2nd family, of mixed

African/European background, was compound heterozygous for a paternal nonsense mutation (K438*), and a maternal mutation located in intron 8 (+29bp G->A), causing alternative splicing, but allowing 24% expression of a normal transcript. Since this initial report, at least ten more families with mutations in *SBP2* have been identified (33, 281-285). Affected individuals harbor homozygous or compound heterozygous mutations. The mutations are summarized in Table 6 and are schematically represented in Figure 14. Among the 20 mutations identified, four are missense mutations located within the functional domain (codon 399-774) causing deleterious effects on protein function, while the other six result in a prematurely truncated protein disrupting the functional domain. Nine mutations cause either transcripts with abnormal splicing or truncations, affecting the N-terminal part of the protein. The generation of shorter isoforms translated from downstream ATGs at codons 139, 233, and 300, which all contain the intact C-terminal functional domain results in preservation of partial *SBP2* function (281). One remaining mutation, Q782*, results in a truncated protein downstream of the functional domain 399-774 and is likely subject to nonsense-mediated decay.

Table 6. Mutations in *SBP2* Gene

Family (# affected)	Mutations	Protein change	Comments on putative defect	Status	Ref.
1 (3)	c.1619G>A	R540Q	Predicted damaging (PolyPhen-2 score 1)	Homozygous	(8)
2 (1)	c.1312A>T	K438*	Truncated functional domain	Compound heterozygous	(8)
	c.1283+29G>A Abnormal splicing	Frameshift	Truncated functional domain		
3 (1)	c.382C>T	R128*	Shorter isoforms ^a	Homozygous	(280)
4 (1)	c.358C>T	R120*	Shorter isoforms ^a	Compound heterozygous	(281)
	c.2308C>T	R770*	Truncated functional domain		

5 (1)	c.668delT	F223Ffs*32	Shorter isoform ^a	Compound heterozygous	(282)
	c.881-155T>A, abnormal splicing	Frameshift	Shorter isoform ^a		
6 (1)	c.2071T>C	C691R	Predicted damaging (PolyPhen-2 score 1)	Compound heterozygous	(282)
	Intronic SNP, abnormal splicing	Frameshift	Shorter isoforms ^a		
7 (1)	c.1529_1541dup CCAGCGCCCCACT	M515Qfs*48	Truncated functional domain	Compound heterozygous	(283)
	c.235C>T	Q79*	Shorter isoforms ^a		
8 (1)	c.800_801insA	K267Kfs*2	Shorter isoform ^a	Homozygous	(284)
9 (1)	c.589C>T	R197*	Shorter isoforms ^a	Compound heterozygous	(37)
	c.2037G>T	E679D	Predicted damaging (PolyPhen-2 score 1)		
10 (1)	c.2344C>T	Q782*	Truncated after functional domain, NMD	Compound heterozygous	(37)
	c.2045_2048delAACA	K682Tfs*2	Truncated functional domain		
11 (2)	c.1588A>G	T530A	Predicted damaging (PolyPhen-2 score 1)	Compound heterozygous	(37)
	c.1711C>T	Q571*	Truncated functional domain		
12 (1)	c.283delT	Y95lfs*31	Shorter isoforms ^a	Compound heterozygous	(37)
	c.589C>T	R197*	Shorter isoforms ^a		

The nomenclature as transcript ID ENST00000375807.7 (854-amino acid long)

^a Shorter isoform(s) generated from downstream ATG(s) (M139, M233, M300) containing C-terminal functional domain NMD, nonsense mediated decay

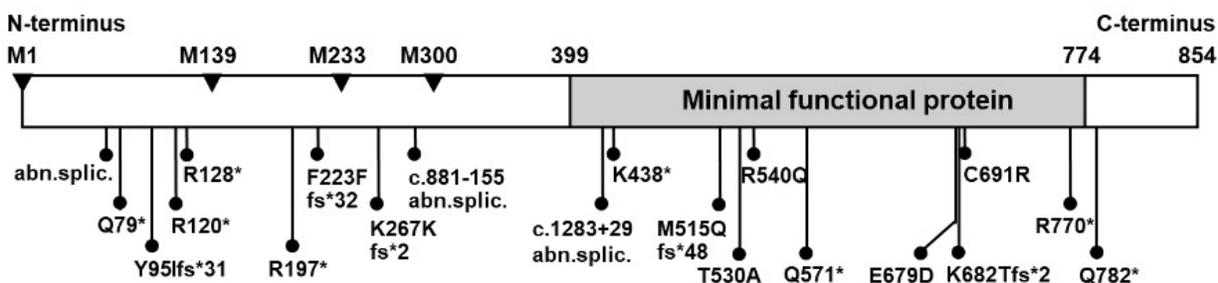


Figure 14. Schematic representation of human SBP2 showing the location of the mutations. Region of minimal functional protein encompassing codon 399-774 is showed in gray. The positions of methionine used as alternative translational initiation sites (M1, M139, M233, M300) are indicated as arrowheads.

Clinical Features and Course of the Disease

Age at presentation ranged from 4 months to 14 years, with the exception of one adult patient who had been identified at the age of 35 years (283). Delayed growth and bone maturation are the main manifestations that brought the affected individuals to medical attention. The severity of growth delay varies among patients. Some patients developed failure to thrive during infancy, while some presented with short stature during mid-childhood. Delayed motor and intellectual milestones were also recognized in seven cases (33, 282-284). Affected individuals started walking and talking around the age of 1.5 to 3 years. More severe developmental delay was found in one patient who started to walk, but still was unable to talk, at the age of 4.5 years (33).

Congenital myopathy with characteristic MRI findings was reported in five cases (282-285). A patient developed hypotonia and muscle weakness early in life and still had hip girdle weakness, impaired motor coordination, waddling gait, and positive Gower's sign when she was 11 years old (282). The adult patient was reported to have delayed motor milestones during childhood and still had difficulty walking and running during adolescence, with genua valga and external rotation of the hip requiring orthotic footwear (283). The other two patients were evaluated for muscle weakness during mid-childhood (284, 285) while

another came to medical attention at the age of 2 years (283).

Sensorineural hearing loss was reported in three cases (282, 283) and conductive hearing loss following recurrent exudative otitis media was reported in one another case (284). Rotatory vertigo was also found (283, 284). Obesity was documented in two cases (282, 285). In addition, another two cases had an increased fat mass index (283), paradoxically associated with low fasting insulin with enhanced insulin sensitivity, elevated adiponectin levels, and a favorable lipid profile. One of them, a 2-year-old boy, had recurrent fasting nonketotic hypoglycemia with low insulin levels requiring supplemental enteral nutrition.

Pubertal development was documented only in 4 cases, while the information is not available in the remaining cases. In females, an affected girl had Tanner stage II breasts when she was 11 years old (33) and she had her menarche at the age of 13 years. In males, one affected boy was prepubertal at the age of 14 years but had a pubertal growth spurt within the following 2 years (8), another one was in Tanner stage III with a testicular volume of 10 mL at the age of 11.5 years (285). The only adult affected individual was reported to have normal pubertal development, despite unilateral orchidectomy at age 15 years following an episode of testicular torsion. He had infertility in adulthood and the investigations revealed

azoospermia despite the presence of a sonographically normal remaining testis (283). Other manifestations found in the adult patient were severe Raynaud disease, skin photosensitivity with evidence of enhanced UV-mediated oxidative DNA damage and mild reduction in red blood cell and total lymphocyte counts with impaired T cell proliferation and abnormal cytokine production (283). This, together with the variety of the aforementioned manifestations, suggests that the defects in the *SBP2* gene result in a multi-organ involvement. The consequences of *SBP2* deficiency could still be underestimated since the features associated with oxidative damage such as neoplasia, neurodegeneration and premature aging may develop later in life. A detailed longitudinal evaluation of affected individuals is needed to further understand and characterize the spectrum of the clinical manifestations in *SBP2* deficiency.

Laboratory Findings

The characteristic thyroid test abnormalities in subjects with *SBP2* gene mutations are high total and free T₄, low T₃, high rT₃, and a normal or slightly elevated serum TSH (8) (Figure 15A). *In vivo* studies assessing the hypothalamo-pituitary-thyroid axis show that compared to normal siblings, affected children require higher doses of L-T₄ and higher serum concentrations of T₃, but not T₃, to reduce their TSH levels compared to unaffected siblings, suggesting impaired conversion of T₄ to T₃ (Figure 15B). None of the affected individuals was reported to have an enlarged thyroid gland on physical examination. Thyroid ultrasonography showed a normal thyroid gland in one patient (282) and a hypoplastic thyroid gland in another patient (284). Delay in bone age was reported in the majority of the patients investigated, except one patient who presented with obesity and a bone age of 11 years at the chronological age of 10 years (285).

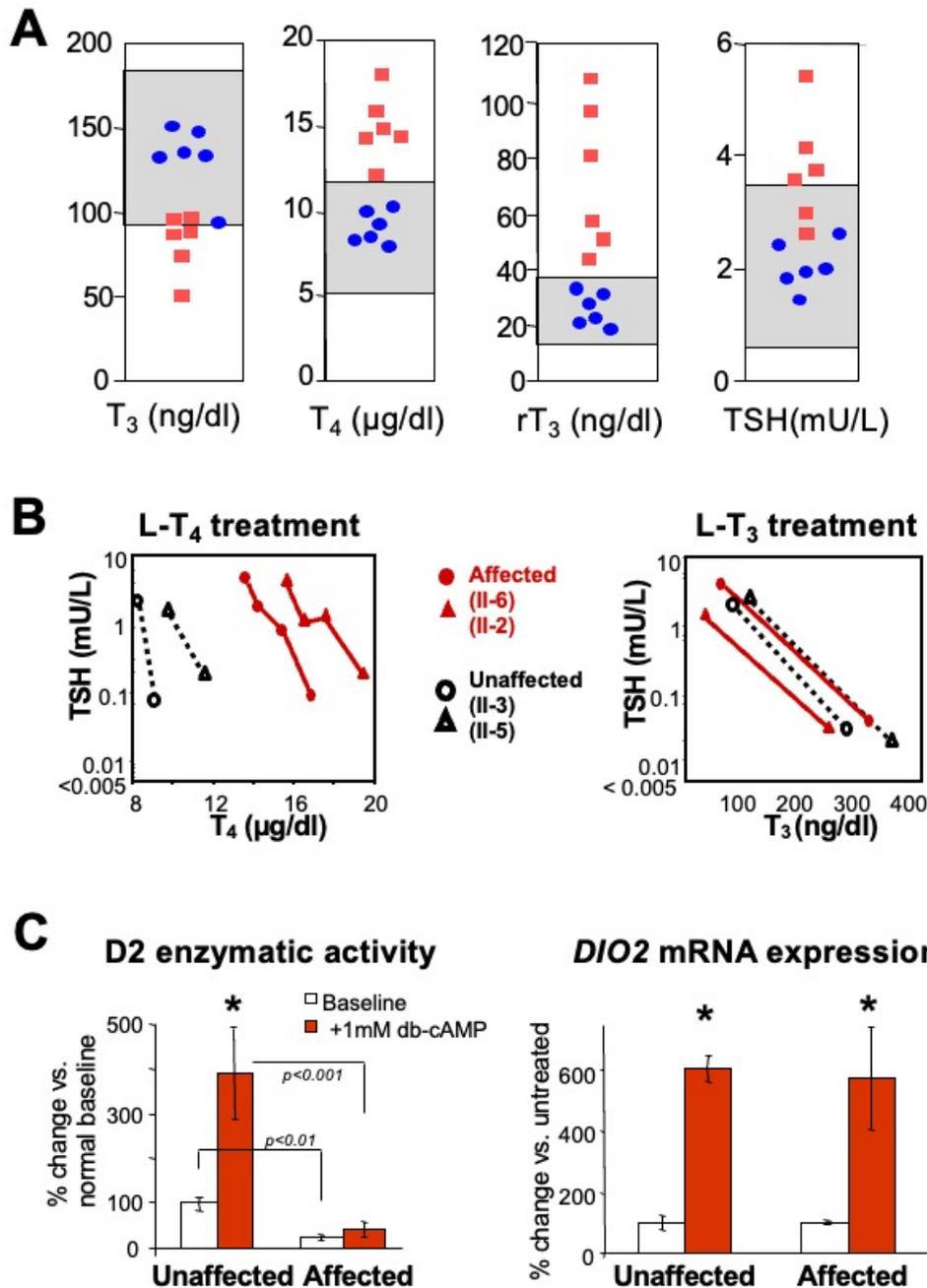


Figure 15. Thyroid function tests in several families with SBP2 deficiency studied in the authors' laboratory. Grey regions indicate the normal range for the respective test. Affected individuals are represented as red squares and unaffected members of the families, as blue circles. B. *In vivo* studies: Serum TSH and corresponding serum T₄ and T₃ levels, before and during the oral administration of incremental doses of L-T₄ and L-T₃. Note the higher concentrations of T₄ required to reduce serum TSH in the affected subjects; C. *In vitro* studies: Deiodinase 2 enzymatic activity and mRNA expression in cultured fibroblasts. Baseline and stimulated D2 activity is significantly lower in affected. There is a significant increase of *DIO2* mRNA with dibutyryl cyclic adenosine monophosphate [(db)-cAMP)], in both

unaffected and affected ($*p < 0.001$) while there are no significant differences in baseline (db)-cAMP stimulated *DIO2* mRNA in affected versus the unaffected.

Skin fibroblasts obtained from affected individuals and propagated in cell culture, showed reduced baseline and cAMP-stimulated D2 enzymatic activity, compared to fibroblasts from unaffected individuals. However, baseline and cAMP-stimulated D2 mRNA levels were not different compared to those in fibroblast from normal individuals (Figure 15C).

As SBP2 is epistatic to selenoprotein synthesis, SBP2 deficiency is expected to affect multiple selenoproteins. Indeed, serum concentrations of selenium, selenoprotein P and other selenoproteins are reduced, and skin fibroblasts have decreased D2 and glutathione peroxidase (Gpx) activities in affected individuals (8).

Thigh MRI of affected patients with muscle weakness showed muscle hypotrophy and increased signal in T1-weighted images suggesting of connective tissue / fatty infiltration in the thigh muscles, predominantly in the adductor magnus, biceps femoris and sartorius, with relative sparing of other muscle groups (282, 283, 285). This pattern is similar to that of individuals with myopathies caused by selenoprotein N1 (SEPN1) deficiency, suggesting that SEPN1 is also affected in the individuals with SBP2 deficiency. Detailed evaluation in the adult patient with multi-systemic involvement also demonstrated deficiencies in multiple selenoproteins: lack of testis-enriched selenoproteins resulting in failure of the latter stages of spermatogenesis and azoospermia, cutaneous deficiencies of antioxidant selenoenzymes causing increased cellular reactive oxygen species (ROS), and reduced selenoproteins in peripheral blood cells resulting in immune deficits (283).

Deficiencies of other selenoproteins of unknown function, such as SELH, SELT, SELW, SELI, were found and their consequences are as yet unknown (283). In some of these patients, multiple tissues and

organs show damage mediated by reactive oxygen species, and it is conceivable that other pathologies linked to oxidative damage such as neoplasia, neurodegeneration, premature ageing, may manifest with time.

Molecular Basis of the Disorder

Clinical and laboratory investigations have established that the mutations in the *SBP2* gene fully explain the observed abnormalities, as SBP2 is a major determinant in the incorporation of Sec during selenoprotein synthesis. Complete lack of SBP2 function is predicted to be lethal, as its immunodepletion eliminates Sec incorporation. The absence of lethality in the reported patients with SBP2 deficiency is attributed to the preservation of partial SBP2 activity and the hierarchy in the synthesis of selenoproteins.

The thyroid test abnormalities in subjects with SBP2 deficiency are consistent with a defect in TH metabolism due to the deficiency in deiodinases and have been found in all cases, even those with a relative mild phenotype. The mutant R540Q SBP2 behaves as a hypomorphic allele in *in vitro* studies using the corresponding R531Q mutation of the rat *Sbp2* (289). The mutant molecule showed no binding to some, but not all SECIS elements, resulting in selective loss in the expression of a subset of selenoproteins. The affected child of another family was compound heterozygous and expressed ~24% of a normal transcript. In the case of the homozygous R128* mutation, smaller SBP2 isoforms translated from downstream ATGs were preserved which contained the intact C-terminus functional domains.

As the human selenoproteome comprises at least 25 selenoproteins (290, 291) it is not surprising that the

phenotype of SBP2 deficiency is complex and goes beyond the thyroid test abnormalities that dominate the mild cases. The more severe phenotype reported in three families is due to a more extensive impairment in SBP2 function (292). In the patient with two nonsense mutations (282), the R770* mutation truncates the C-terminal functional domain in all the isoforms and likely abolishes SBP2 function. However, the R120* allele likely generates smaller functionally active SBP2 isoforms, but the overall amount would be less than that of the homozygous R128* patient (281), thus explaining the more severe phenotype. Low expression of functional SBP2 also explains the phenotype of the two patients from the United Kingdom. Increased proteasomal degradation was demonstrated for the C691R mutation, and Western blotting of skin fibroblasts from both probands showed lack of full length SBP2 protein expression (283)

THMD Caused by Mutation in the *TRU-TCA1-1* Gene

Another inherited condition also reported to cause THMD is the mutation in the *TRU-TCA1-1* gene encoding selenocysteine transfer RNA (tRNA^{Sec}) (277), an essential molecule in the Sec-incorporation pathway. The first and only patient reported to date was found to have elevated serum T₄ and rT₃ levels, together with a normal serum T₃, suggestive of impaired deiodinase activity, during the investigations for his abdominal pain, fatigue and muscle weakness. A low plasma selenium level was also found, together with undetectable red cell and plasma GPX, low plasma selenoprotein P (SEPP1), mild signaling intensity change in muscle imaging, and negligible SEPN1 expression in fibroblasts. However, SBP2 protein expression was normal and no mutation in the

SBP2 gene was identified, leading to the suspicion of a defect in another gene involved in the selenoprotein synthesis pathway. A homozygous missense mutation in the *TRU-TCA1-1* gene, resulting in the amino acid substitution C65G, was then identified in the proband and segregated with the phenotype in the family. Primary cells from the proband showed marked reduction of the mcm⁵Um isoform of tRNA^{Sec}, needed mainly for the synthesis of stress-related selenoproteins, whereas the mcm⁵U isoform of tRNA^{Sec}, mainly responsible for the synthesis of housekeeping selenoproteins, was relatively preserved.

Animal Models of THMD

To bypass the embryonic lethality of lacking *Sbp2* (293), a global partial *Sbp2* deficiency mouse model, *Sbp2* conditional knockout (*Sbp2* iCKO) was generated using tamoxifen-inducible Cre-ER induced at P35 (294). The *Sbp2* iCKO mice replicate most of the characteristic thyroid function tests in patients, with high serum T₄, rT₃ and TSH, whereas serum T₃ is normal (Figure 16A). The enzymatic activity of D1 in the liver, and the D2 enzymatic activity and Dio3 mRNA expression in the cerebrum were decreased in *Sbp2* iCKO compared to Wt littermates (Figure 16B) (294). In addition to the affected selenoenzymes deiodinases, *Sbp2* iCKO mice also had decreased expression and/or activity of other selenoproteins in the liver, cerebrum and serum (294). Decreased body weight was observed in *Sbp2* iCKO mice by 2 weeks after tamoxifen injection, similar to the failure-to-thrive phenotype in patients. Other phenotypes are being further investigated in *Sbp2* iCKO mice in order to understand the mechanisms of SBP2 deficiency as a multi-organ syndrome.

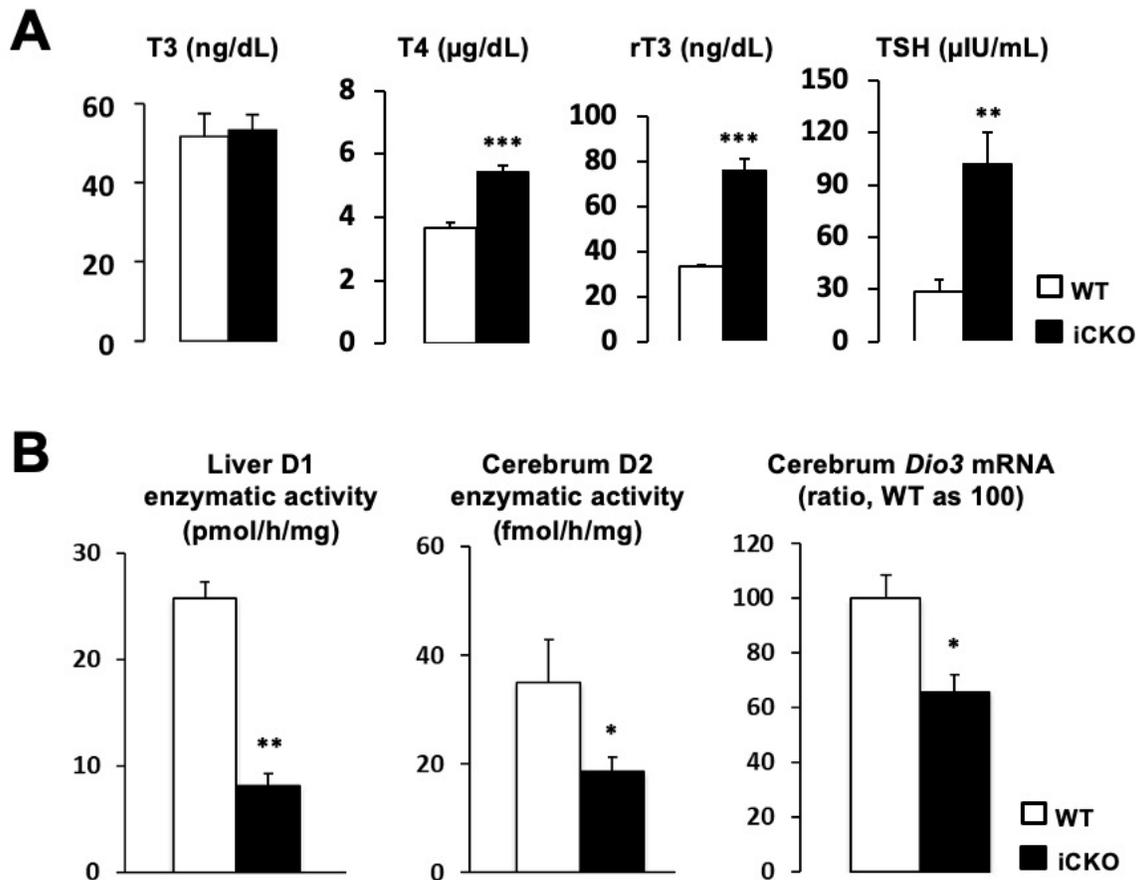


Figure 16. Data from *Sbp2* iCKO vs *Wt* male mice. **A.** Serum TFTs in *Sbp2* iCKO vs *Wt* male mice. **B.** Enzymatic activity of the D1 in liver, D2 in cerebrum and mRNA expression of *Dio3* in cerebrum. *Sbp2* iCKO mice represented as black bars and *Wt* littermates in open bars *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Differential Diagnosis

From the point of view of the thyroid phenotype, the combination of non-suppressed (normal or slightly elevated) serum TSH with increased concentrations of T_4 and decreased T_3 levels, is characteristic for the TH metabolism defects due to SBP2 deficiency. An elevated TSH and a general medical evaluation would help distinguishing the thyroid test abnormalities from those encountered in acute non-thyroidal illness, which in terms of iodothyronines could be similar. It is important to confirm the abnormalities by repeat

testing several weeks or months apart because the consequence of a variety of non-thyroidal illnesses and some drugs are often transient. For a comprehensive thyroid evaluation, it is recommended to perform the entire panel of thyroid tests, including the free TH levels by dialysis, to exclude abnormalities in serum TH-binding proteins.

Because the clinical presentations of a THMD can be variable, broad and non-specific, including short stature and growth delay, the differential diagnosis can be extensive. Obtaining thyroid tests in first-degree relatives is important in determining the inheritance

pattern of the phenotype, and identification of other affected individuals can help in categorizing the symptoms and signs. Given the recessive mode of inheritance, investigation of relatives is helpful in large families and when the patient has multiple siblings. For SBP2 deficiency in particular, measuring serum Se and SePP levels as well as GPX activity can avoid more invasive tests such as skin or muscle biopsies.

Finding a mutation in the *SBP2* gene can be sufficient to provide a diagnosis if the mutation is predicted and/or demonstrated to result in a functionally defective protein or results in failure to synthesize the protein. Linkage analysis in smaller families is particularly helpful in excluding the involvement of SBP2. Failure to detect a *SBP2* mutation by sequencing only coding regions of the gene is not sufficient, as putative mutations can exist in introns and regulatory elements. In this case, measuring the TSH responses to incremental doses of L-T₄ and/or L-T₃ could be used to confirm the biochemical diagnosis of TH metabolism defect, as described in the section on Laboratory Tests.

Treatment

Identification of the metabolic pathway responsible for the phenotype in these patients and the demonstration of defects in the *SBP2* gene provided further insight into targeted treatment possibilities. Three such options, namely, administration of Se, TH and vitamin E were tested (270, 281, 295).

Administration of up to 400 mcg of selenium (295), in the form of selenomethionine but not selenite, normalized the serum selenium concentration but not selenoprotein P levels and did not restore the TH metabolism dysfunction. Se supplementation in form of selenomethionine contained in Se-rich yeast seems to be more effective as it can be incorporated nonspecifically into all circulating serum proteins (296), whereas selenite is metabolized and inserted as

selenocysteine into the growing peptide chain of selenoproteins (297), therefore resulting in different Se bioavailability.

The effect of L-T₃ administration was tested in three patients as it was demonstrated to equally suppress serum TSH concentration in affected and unaffected subjects, bypassing the defect (8). Delayed linear growth can be improved with L-T₃ supplementation (281), but experience with TH administration in these patients is limited.

As increased oxidative stress state was documented in SBP2 deficiency, treatment with vitamin E was evaluated in a patient. The level of 7 β -hydroxycholesterol, a free radical-mediated lipid peroxidation product, was found to be elevated in the patient at baseline, and was reduced to control levels after 2 weeks of α -tocopherol acetate treatment. The effect persisted during 2 years of treatment and at least 7 months after withdrawal (270). Other clinical features of SBP2 defects are treated symptomatically. Physical, occupational and speech therapy was required in some of these patients with developmental delay.

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