IMPAIRED SENSITIVITY TO THYROID HORMONE: Defects of Transport, Metabolism and Action

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ABSTRACT

Defects along the pathways leading to TH action can manifest as impaired sensitivity to TH. Six steps are presumed to be required for the circulating thyroid hormone (TH) to exert its action on target tissues. For three of these steps four distinct phenotypes have been identified in humans. The clinical, laboratory, genetic and molecular characteristics of these defects are the subject of this chapter.

The first defect, recognized almost 50 years ago, produces reduced sensitivity to TH and was given the acronym RTH, for resistance to thyroid hormone. Its major cause, found in more than 3,000 individuals, is mutations in the *TH receptor ß (THRB*) gene. More recently mutations in the *THRA* gene were found to produce a different phenotype owing to the distinct tissue distribution of this TH receptor. Two other gene mutations, affecting TH action, but acting at different sites were identified in the last 10 years. One of them, caused by mutations in the TH cell-membrane transporter *MCT8*, produces severe psychomotor defects. It has been identified in more than 320 males. A defect of the intracellular metabolism of TH, identified in 11 members from 9 families, is caused by mutations in the *SECISBP2* gene required for the synthesis of selenoproteins, including TH deiodinases.

Knowledge of the molecular mechanisms involved in mediation of TH action allows the recognition of the phenotypes caused by genetic defects in the involved pathways. While these defects have opened the avenue for novel insights into thyroid physiology, they continue to pose therapeutic challenges. For complete coverage of this and related areas in Endocrinology, visit the free online web-textbook, www.endotext.org.

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 22 years later (3,4), the term RTH become synonymous with defects of this 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 could interfere with the biological activity of a chemically intact hormone secreted in normal or even excess amounts. In this revised chapter, we

cover all syndromes resulting from impaired sensitivity to TH, using the recently proposed nomenclature (9) (see Table 1).

Table 1.Inheritable Forms of Impaired Sensitivity to Thyroid Hormone

LEVEL OF THE DEFECT

Commonly used name (References Are for first Reported Cases)	**Synonyms**	**Gene Involved & Inheritance (OMIM)**	**Phenotype**	
			Consistent (Pathognomonic)	**Common**
THYROID HORMONE CELL MEMBRANE TRANSPORT DEFECTS (THCMTD)				
Monocarboxylate transporter 8 (MCT8) defect	Allan-Herndon-Dudley syndrome	*MCT8 (SLC16A2*) gene (300095) X-chromosome linked	High T_3, low rT_3 and T_4, normal or slightly elevated TSH; low BMI; hypotonia, spastic quadriplegia; not walking or rarely ataxic gait; no speech or dysarthria, mental retardation	Hypermetabolism, paroxysmal dyskinesia, reduced muscle mass, seizures, poor head control, difficulty sitting independently.
Idiopathic & other THCMTDs		To be determined	Unknown	
THYROID HORMONE METABOLISM DEFECTS (THMD)				
Selenocysteine insertion sequence binding protein 2 (SBP2) defect		*SBP2* (*SECISBP2*) gene (607693) recessive	High T_4 and rT_3, low T_3, normal or slightly elevated TSH; growth retardation	Azoospermia, immunodeficiency, photosensitivity, delayed bone maturation, myopathy, hearing impairment, delayed developmental milestones
Idiopathic & other THMDs		To be determined	Unknown	
THYROID HORMONE ACTION DEFECTS (THAD): nuclear receptor and other				

Resistance to thyroid hormone (RTH)[a]	Thyroid hormone unresponsiveness, Generalized RTH, RTH beta;	*THRB* gene (190160) dominant negative (rarely recessive)	High serum FT_4 and non suppressed TSH.	High serum FT_3 and rT_3, high thyroglobulin, goiter, attention deficit hyperactivity disorder (ADHD), tachycardia
nonTR-RTH[b]		Unknown	Same as above	Same as above
RTH alpha1[c]	Congenital nongoitrous hypothyroidism 6	*THRA* gene (190120) dominant negative	Low serum T_4/T_3 ratio; cognitive impairment, short lower limbs, delayed closure of skull sutures, delayed bone and dental development, skeletal dysplasia, macrocephaly; constipation; anemia	Low rT_3, seizures, placid behavior.
Hypersensitivity to thyroid hormone (HTH)		Unknown	Low FT_4 and FT_3 with normal TSH, euthyroid and no serum transport defects	Normal thyroid gland
Idiopathic & other THADs		To be determined	Unknown	

Abbreviations: FT_3, free T_3; FT_4, free T_4; BMI, body mass index; nonTR-RTH, RTH without mutations in the *THRB* or *THRA* genes.

[a] Proposed future terminology: RTH beta.

[b] RTH without mutations in the THRB gene.

[c] A single case with a mutation involving both TR alpha1 and TR allpha2 presented a more complex phenotype, including severe bone malformations, hyper-calcaemia with hyperparathyroidism, and diarrhea rather than constipation. It is unclear if all observed abnormalities are due to the *THRA* gene mutation alone

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TH SECRETION, CELL-MEMBRANE TRANSPORT, METABOLISM AND ACTION

Proper TH action requires 1) an intact TH, 2) its transport across cell membrane, 3) hormone activation through intracellular metabolism, 4) cytosolic processing and nuclear translocation, 5) binding to the TH receptors (TRs) and 6) interaction with co-regulators or other post receptor effects mediating the TH effect.

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

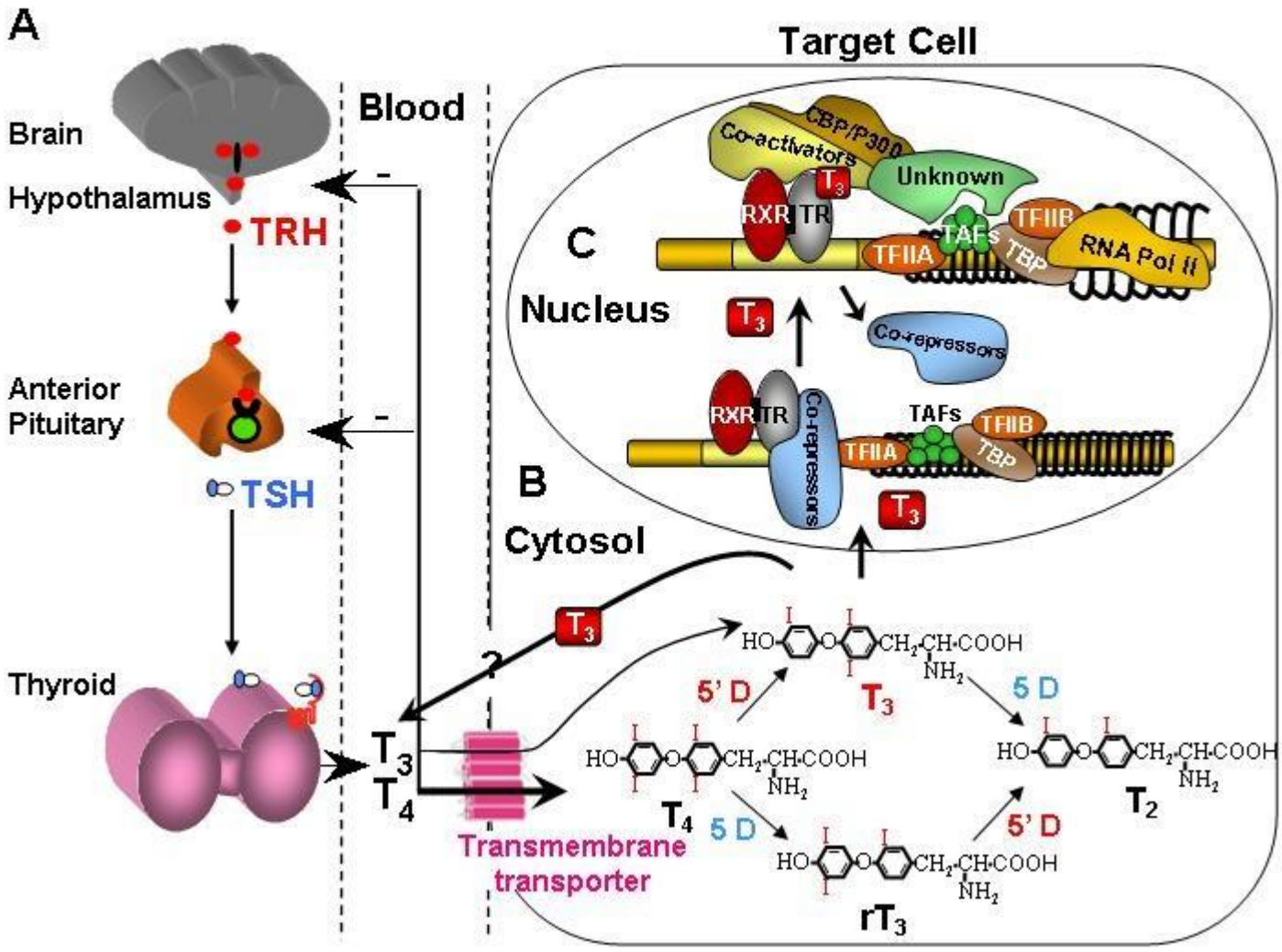

FIG. 1. Regulation of TH supply, metabolism and genomic action. (A) Feedback control that regulates the amount of TH in blood. (B) Intracellular metabolism of TH, regulating TH bioactivity. (C) Genomic action of TH. For details see text.
CBP/P300, cAMP-binding protein/general transcription adaptor ; 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 (10). Another is the activation of the hormone precursor thyroxine (T_4) by removal of the outer ring iodine (5'-deiodination) to form triiodothyronine (T_3) or, inactivate T_4 and T_3 by removal of the inner ring iodine (5-deiodination) to form reverse T_3 (rT_3) and T_2, respectively (See Fig.1B). Cell specific adjustment in deiodinase activity allows for additional local regulation of hormone supply (11).

Finally, the types and abundance of 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 (12). The latter, known as genomic effect, has been more extensively studied (13,14) (See Fig.1C). TRs are transcription factors that bind to DNA of genes whose expression they regulate.

HOW THYROID HORMONE DEFICIENCY AND EXCESS COEXIST

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 body tissues. A departure from this became apparent with the identification of the RTHß syndrome. Subjects with RTHß have high TH levels without TSH suppression. This paradox encompasses other biochemical and clinical observations suggesting, TH deficiency, sufficiency, and excess, depending on the degree and nature of the TR abnormality (5). The syndrome of TH cell membrane transport defect (THCMTD) presents a similar paradox, as subjects have high serum T_3 concentration but the uptake of TH is not uniform in all tissues and cell types (15).

RESISTANCE TO THYROID HORMONE (RTH)

Until recently the term RTH has been applied to the phenotype characteristic for mutations in the *THRB* gene. With the identifications of mutations in the *TH receptor alpha* (*THRA*) gene (16), which presents a different phenotype, the syndromes are now identified as RTH-beta (RRTß) and RTH-alpha (RTHα). A syndrome clinically and biochemically indistinguishable from RTHß but without *THRB* gene mutations has been named nonTR-RTH (Table 1)

RECEPTOR MEDIATED TH ACTION

TH receptor genes located on chromosome 17 and 3, generate a TRα and a TRß molecules, respectively, with substantial structural and sequence similarities. Both genes produce two isoforms; α1 and α2 by alternative splicing and ß1 and ß2 by different transcription start points. TRα2 binds to TH response elements (TREs) but, due to a sequence difference at the ligand-binding domain (LBD) site, it does not bind TH (17) and appears to have a weak antagonistic effect (18). Additional TR isoforms, including a TRß with shorter amino terminus (TRß3), truncated TRß3, TRα1 and TRα2, lacking the DNA-binding domain (DBD) have been identified in rodents (19,20) and TRß4 that lacks the LBD in selected human tissues (21). Their significance in humans remains unknown (22). Finally, a p43 protein, translated from a downstream AUG of TRα1, is believed to mediate the TH effect in mitochondria (23).

The relative expression of the two *THR* genes and the distribution of their products vary among tissues and during different stages of development (24-26). The abundance of several splice variants involving the 5'-untranslated region of the human TRß1 (27,28) is developmentally and tissue regulated. Although TRß and TR□ are interchangeable (29,30) to a certain degree, the absence of one or the other receptor do not produce equivalent phenotypes. Some TH effects are absolutely TR isoform specific (see Animal Models of RTH, below).

TREs, located in TH regulated genes, consist of half-sites having the consensus sequence of AGGTCA and vary in number, spacing and orientation (31,32). Each half-site usually binds a single TR molecule (monomer) and 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 (without hormone) TRs, also known as aporeceptors, inhibits the constitutive expression of genes that are positively regulated by TH (33) through association with corepressors such as the nuclear corepressor (NCoR) or the silencing mediator of retinoic acid and TH receptors (SMRT) (34). Transcriptional repression is mediated through the recruitment of the mammalian homologue of the Saccaromyces transcriptional corepressor (mSin3A) and histone deacetylases (HDAC) (35). This latter activity compacts nucleosomes into a tight and inaccessible structure, effectively shutting down gene expression (See Fig. 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 (36) and recruits coactivators (CoA) such as p/CAF (CREB binding protein-associated factor) and nuclear coactivators (NCoA) (37) with HAT (histone acetylation) activity (34,38). This results in the loosening of the nucleosome structure making the DNA more accessible to transcription factors (See Fig.1C). Actually, 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 (39). Furthermore, it is believed that T_3 exerts its effect by inducing conformational changes of the TR molecule and that TR associated proteins (TRAP) stabilizes the association of TR with TRE.

In addition to the genomic effect described above, TH acts at the cell membrane and cytosol (12). These non-genomic effects include oxidative phosphorylation and mitochondrial gene transcription and involve the generation of intracellular secondary messengers with induction of [Ca(2+)](I), cyclic adinosine monophosphate (cAMP) AMP or protein kinase signaling cascades.

RTHß MUTATIONS CAUSING TH INSENSITIVITY

In practice, patients with RTHß are identified by their persistent elevation of circulating free TH levels association with non-suppressed serum TSH, and in the absence of intercurrent illness, drugs, or alterations of TH transport serum proteins. More importantly, higher 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. With the exception of nnTR-RTH, the defect involes a mutation in the *THRB* gene (5,40)

Despite a variable clinical presentation, the common features characteristic of the RTHß syndrome are: 1) elevated serum levels of free T_4 and to a lesser degree T_3, particularly in older individuals, 2) normal or slightly increased TSH level that responds to TRH, 3) 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 was based on symptoms, signs and laboratory parameters of tissue responses to TH (41). Not withstanding the assessment of TSH feedback regulation by TH, the measurements of most other responses to the hormone are insensitive and 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 appeared to be eumetabolic and maintained a near normal serum TSH concentration. They were classified as having **generalized resistance to TH (GRTH).** In such individuals, the defect seemed to be compensated by the high levels of TH. In contrast, patients with equally high levels of TH and nonsuppressed TSH that appeared to be hypermetabolic, because they were restless or had sinus tachycardia, were classified as having selective **pituitary resistance to TH (PRTH).** Finally, the occurrence of isolated **peripheral tissue resistance to TH (PTRTH)** was reported in a single patient (42). No mutation in the *THRB* gene of this patient was found (43) and no similar cases have been reported. More common in clinical practice is the apparent tolerance of some individuals to the ingestion of supraphysiological doses of TH.

The earliest suggestion that PRTH may not constitute an entity distinct from GRTH can be found in a study by Beck-Peccoz et al (44). A comprehensive study involving 312 patients with GRTH and 72 patients with PRTH, has conclusively shown that the response of sex hormone-binding globulin (SHBG) and other peripheral tissue markers of TH action, were equally attenuated in GRTH and PRTH (45). More importantly, identical mutations were found in individuals classified as having GRTH and PRTH, many of whom belonged to the same family (46). It was, therefore, concluded that these two forms of RTH are the product of the subjective nature of symptoms as well as the individual's target organ susceptibility to changes of TH also observed in subjects with thyroid dysfunction in the absence of RTH (See section on the Molecular Basis of the Defect). True thyrotroph specific TH has been identified in association with TSH-producing pituitary adenomas caused by expression of somatic mutations or isoform specific TRßs (47,48).

Incidence And Inheritance

The precise incidence of RTHß is unknown. Because routine neonatal screening programs are based on the determination of TSH, RTHß is rarely identified by this means (49). A limited

neonatal survey by measuring blood T_4 concentration, suggested the occurrence of one case per 40,000 live births (50,51). Known cases surpass 3,000.

Although most thyroid diseases occur more commonly in women, RTHß has been found with equal frequency in both genders. 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 RTHß 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, is 21.0%. This is in agreement with current estimate of the frequency of de novo mutations of 20.8% (See Table 2). The reports of acquired RTH are seriously questioned.

Inheritance is autosomal dominant. Transmission was clearly recessive in only one family (1,52). Consanguinity in three families with dominant inheritance of RTHß has produced homozygous children with very severe clinical manifestations (53,54).

Table 2. Types of *TRß* Gene Mutations

Type		***Number of Occurrences at different sites***	***Number of families***		***Effect on TRß***
			total	***authors'***	
Substitution	Single nucleotide	148	430	191	Single a.a. substitution; Premature stop (C434X, K443X, E445X, C446X, E449X)
	Dinucleotide	3	3	1	Single a.a. substitution (P453Y, P453Y); Premature stop (F451X)
Deletion	Single nucleotide	4	0	4	FrSh and stop (441X) of two a.a. extension
	Trinucleotide	5	6	2	Single a.a. deletion (T276Δ, T337Δ, M430Δ, G432Δ,
	Eight nucleotides	1	1	0	FrSh normal stop at a.a. 461
	All coding sequences	1	1	1	Complete deletion

Insertion	Single nucleotide	7	14	10	FrSh and two a.a. extension
	Trinucleotide	1	1	0	Single a.a. insertion (328S)
Duplication	Seven nucleotides	1	1	0	FrSh and two a.a. extension
Mutations at CpG dinucleotides		10	184 **a**	88**a**	42.8% of 430 families with single nucleotide substitutions and 46.1% of 191 similar families studied in the authors' laboratory
De novo mutations	Total		**b**	43**c**	20.6% of 209 families studied in the authors' laboratory
	in CpGs	6	**b**	21	48.8% of the de novo mutations
No TRß gene mutations		**d**	40**e**	34	14.0% of 243 families studied in the authors' and in whom the *THRB* gene was sequenced

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

a Not included are 7 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 TRß gene mutations excluding those with a single affected individual when both parents were not tested.

d Non applicable.

e Total number of families is grossly underestimated because usually they are not reported

Etiology And Genetics

Using the technique of restriction fragment length polymorphism, Usala et al (55) were first to demonstrate linkage between a *THRB* locus on chromosome 3 and the RTHß phenotype. Subsequent studies at the University of Chicago and at the National Institutes of Health identified distinct point mutations in the *THRB* gene of two unrelated families with RTHß (3,4). In both families only one of the two *THRB* alleles was involved, compatible with the apparent dominant mode of inheritance.

Mutations in the *THRB* gene have now been identified in subjects with RTHß belonging to 457 families (See Table 2 and Fig. 2). They comprise 170 different mutations. With the exception of the index family, found to have complete deletion of the *THRB* gene (52), the majority (430 families) have single nucleotide substitutions resulting in single amino acid replacements in 419

instances and stop codons in 11 others, producing truncated molecules. In addition, deletions, insertions and a duplication were identified in 20 families (for details see Table 2).

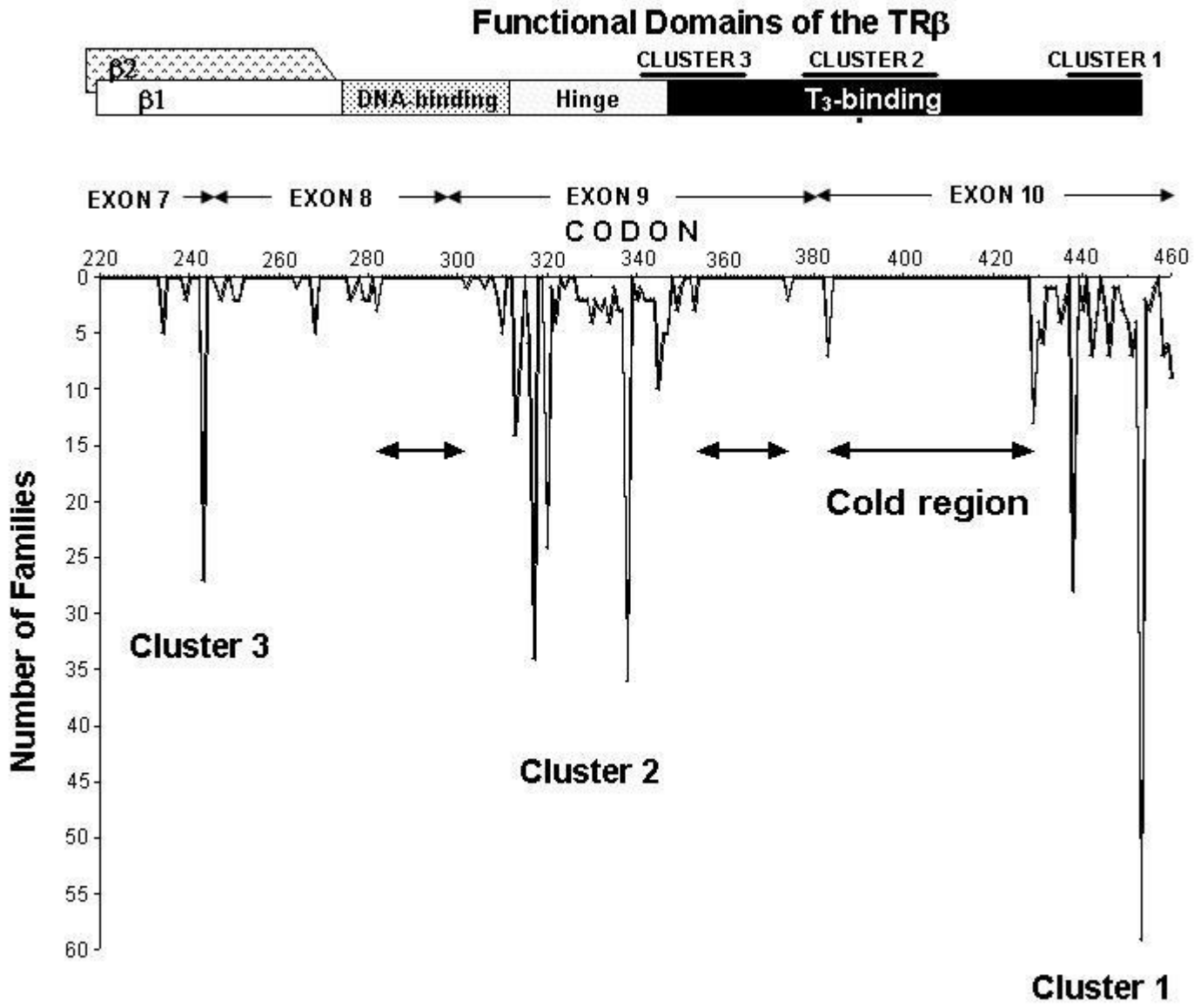

FIG. 2. Location of natural mutations in the TRß molecule associated with RTHß.

TOP PORTION: Schematic representation of the TRß and its functional domains for interaction with TREs (DNA-binding) and with hormone (T_3-binding). Their relationship to the three clusters of natural mutations is also indicated. TRß2 has 15 more residues than TRß1 at the aminoterminus.

BOTTOM PORTION: The location of the 170 different mutations detected and their frequencies in the total of 457 unrelated families (published and our unpublished data). Amino acids are

numbered consecutively starting at the amino terminus of the TRß1 molecule according to the consensus statement of the First International Workshop on RTH (258). "Cold regions" are areas devoid of mutations associated with RTHß.

Given that there are 287 more families than the 170 different mutations, 78 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 (56). These occur more often, though not exclusively, in CpG dinucleotide hot spots. In fact, *de-novo* mutations are twice as frequent in CpG dinucleotides. In addition, different mutations producing more than one amino acid substitution at the same codon have been found at 44 different sites. Mutations in codons 345 and 451 produced each 5 different amino acid replacements (G345R,S,A,V,D; F451I,L,S,C,X) while those in codon 453, seven (P453T,S,A,N,Y,H,L) not counting an insertion and a deletion. A total of 59 families harbor mutations at codon 453. Mutations are located in the last four exons of the gene: 6, 17, 73 and 73 mutations in exons 7, 8, 9 and 10, respectively. These involve 35, 23, 202 and 196 families (See Fig. 2). The following mutations have been identified in more than 15 families: R243Q, A317T, R338W, R423H and P453T. Of note the first three are in CpG dinucleotides and the last in a stretch of six cytidines. Thirty-three unrelated families share the R338W mutation.

All *THRB* gene mutations are located in the functionally relevant domain of T_3-binding and its adjacent hinge region. Three mutational clusters have been identified with intervening cold regions (See Fig. 2). With the exception of the family with *THRB* gene deletion, in all others inheritance is autosomal dominant.

Somatic mutations in the *THRB* gene have been identified in some TSH-secreting pituitary tumors (47,57). These mutations can be identical to those occurring in the germline. However, because their expression is limited to the thyrotrophs, the phenotype, as in other TSHomas, is that of TSH induced thyrotoxicosis. It is postulated that defective TR interfering with the negative regulation of TSH by TH is responsible for the development of the pituitary tumor.

In 14% of families, RTHß occurs in the absence of mutations in the *TR* genes (nonTR-RTH) (58) (see below). 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 Basis Of The Defect

Properties of Mutant TRßs and Dominant Negative Effect

THRB gene mutations produce two forms of RTHß. 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 (52). The complete lack of TRß in these individuals produces severe deafness, resulting in mutism (1), as well as monochromatic vision (59is) as TRß is required for the cochlear maturation and the development of cone photoreceptors that mediate color vision (60) (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 (61). However, because subjects with complete *THRB* gene deletion preserve some TH responsiveness, it is logical to

conclude that TRα1 is capable of partially substituting for the function of TRß (see Animal Models of RTH, below).

The more common form of RTHß is inherited in a dominant fashion and is characterized by defects in one allele of the *THRB* gene, principally missense mutations. This contrasts with the lack of phenotype in individuals that express a single *THRB* allele. These mutant TRßs (mTRs) do not act by reducing the amount of a functional TR (haploinsufficiency) 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 mTRs are coexpressed with WT TRs (62,63).

Studies have established two basic requirements for mTRs to exert a DNE: 1) preservation of binding to TREs on DNA and 2) the ability to dimerize with a homologous (64,65) or a heterologous (66,67) partner. These criteria apply to mTRs with predominantly impaired T_3-binding activity (See Fig. 3). In addition, a DNE can be exerted through impaired association with a cofactor even in the absence of important impairment of T_3-binding. Increased affinity of a mTR for a corepressor (CoR) (68,69), or reduced association with a coactivator (CoA) (70-72), have been found to play a role in the dominant expression of RTHß. The introduction in a mTR of an additional artificial mutation that abolishes either DNA binding, dimerization or the association with a CoR results in the abrogation of its DNE (67,73,74).

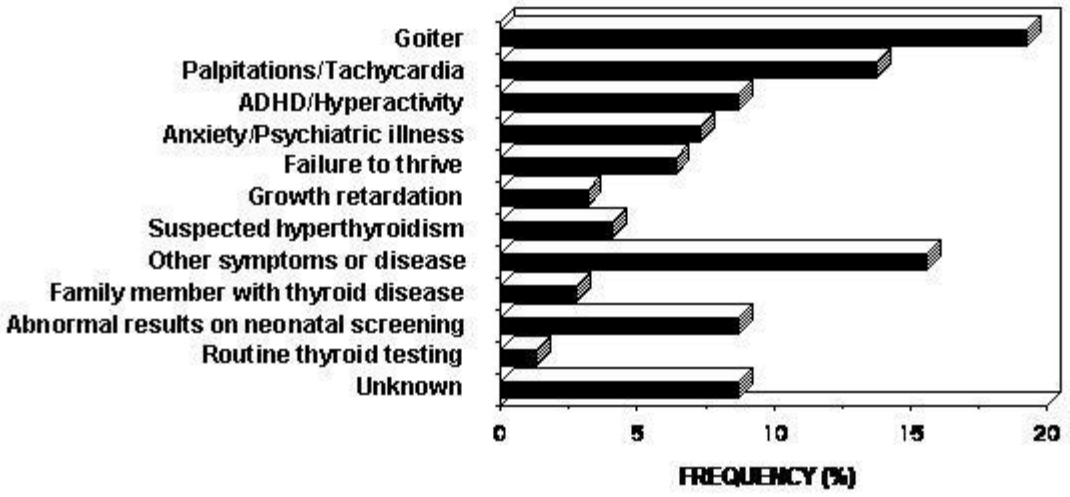

FIG. 3. Mechanism of the dominant expression of RTHß: In the absence of T_3, occupancy of TRE by TR heterodimers (TR-TRAP) or dimers (TR-TR) suppresses transactivation through association with a corepressor (CoR). (A) T_3-activated transcription mediated by TR-TRAP heterodimers involves the release of 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 (mTR), that does not bind T_3, can be explained by the inhibitory effect of mTR-containing-dimers and

heterodimers that occupy TRE. Thus, T_3 is unable to activate the mTR-TRAP heterodimer (A') or release TREs from the inactive mTR homodimers (B'). [Modified from Refetoff et al (5)].

The distribution of *THRB* gene mutations associated with RTHß reveals conspicuous absence of mutations in regions of the molecule that are important for dimerization, for the binding to DNA and for the interaction with CoR (See Fig. 2). These "cold regions" contain CpG hot spots, suggesting that they may not be devoid of natural mutations. Rather, mutations would escape detection owing to their failure to produce clinically significant RTHß in heterozygotes, as tested *in vitro* (75). Structural studies of the DBD and LBD have provided further understanding about the clustered distribution of mTRßs associated RTHß and defects in the association with cofactors (76-79).

Based on the early finding that RTHß is associated with mutations confined to the LBD of the TRß, it was anticipated that the clinical severity of RTHß would correlate with the degree of T_3-binding impairment. While this was true in 12 different natural mTRßs, in 5 others, the severity of RTHß was lesser despite virtually complete absence T_3-binding. This was explained by the reduced dominant negative potency due to diminished ability to form homodimers (for example R316H and E338W) (80). Weakened association of TRß with DNA or CoR can produce the same effect.

Less evident was the observation of relatively severe interference with the function of the WT TRß, despite very mild impairment or no T_3-binding defect at all. This was the case when hormone-binding was tested in two mTRßs, located in the hinge region of the receptor (R243Q and R243W) (81). However, reduced T_3-binding could be demonstrated after complexing to TRE, indicating a change in the mTRß configuration when bound to T_3 (81,82). Other mechanisms and examples of DNE in the presence of normal or slightly attenuated T_3-binding are: decreased interaction of L454Vwith the CoA (70) and delay of R383H to release the CoR (83).

In general the relative degree of impaired function among various mTRßs is similar whether tested using TREs controlled reporter genes that are negatively or positively regulated by T_3. Exceptions to this rule are the mTRßs, R383H and R429Q that show greater impairment of transactivation on negatively rather than positively regulated promoters (80,83,84). In this respect these two mTRßs are candidates for predominantly PRTH, even though they have been clinically described as producing GRTH (85) as well as PRTH (86,87). Later work suggests that the substitution of these charged aminoacids (here arginines) disrupts the unique property of TRß2 to bind certain coactivators through multiple contact surfaces (88). The result is a decrease in the normal T_3-mediated feedback suppression by converting the TRß2 to a TRß1-like single mode of coactivator binding. As a consequence, the mutation affects predominantly TRß2 mediated action. *In vivo* support for a TRß2 predominant impairment of the mTRß R429Q was obtained in mice (89). Another possible mechanism for PRTH is a double-hit combining a single nucleotide polymorphism (SNP) and the mTRß R338W (90). The presence of a thymidine in a SNP, located in the enhancer region of the *THRB* gene, leads to over-expression of the mutant allele in GH3 pituitary-derived cells. However, the T/C nucleotides of this SNP have not been correlated with the clinical presentation in individuals with this most common *TRß* R338W mutation.

Moleular Basis of the Variable Phenotype of RTHß

The extremes of the RTHß phenotype have a clear molecular basis. Subjects heterozygous for a *THRB* gene deletion are normal because the expression of a single TRß allele is sufficient for normal function. RTHß manifests in homozygotes completely lacking the *THRB* gene and in heterozygotes that express a mTRß with DNE. The most severe form of RTHß, with extremely high TH levels and signs of both hypothyroidism and thyrotoxicosis, occur in homozygous individuals expressing only mTRßs (53,54). The severe hypothyroidism manifesting in bone and brain of such subjects can be explained by the silencing effect of a double dose of mTR and its interference with the function of TRα (64); a situation which does not occur in homozygous subjects with TRß deletion. In contrast, the manifestation of thyrotoxicosis in other tissues, such as the heart, may be explained by the effect high TH levels have on tissues that normally express predominantly TRα1 (91,92) (see Animal Models of RTH, below). It is for this same reason that tachycardia is a relatively common finding in RTHß (93).

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 (24,94,95). 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 mTRß could be explained by the relative level of mutant and WT TR expression. Such differences have been found in one study using cultured fibroblast (96) but not in another (61). Various reasons for a predominant TRß2 dysfunction have been presented in the section on “Receptor mediated TH action” (see above).

Although in a subset of mTRßs a correlation was found between their functional impairment and the degree of thyrotroph hyposensitivity to TH, this correlation was not maintained with regards to the hormonal resistance of peripheral tissues (80). Subjects with the same mutations, even belonging to the same family, show different degrees of RTH. A most striking example is that of family G.H. in which the mTRß R316H did not cosegregate with the RTH phenotype in all family members (97). This variability of clinical and laboratory findings was not observed in affected members of two other families with the same mutation (46,98). A study in a large family with the mTRß R320H, suggests that genetic variability of factors other than TR may modulate the phenotype of RTH (99).

Pathogenesis

The reduced sensitivity to TH in subjects with RTH is shared to a variable extent by all tissues. The hyposensitivity of the pituitary thyrotrophs results in nonsuppressed serum TSH, which in turn, increases the synthesis and secretion of TH. The persistence of TSH secretion in the face of high levels of 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. 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 do not correlate with the level of TH, probably owing to a 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 (100). Administration of supraphysiological doses of TH is required to suppress TSH secretion without induction of thyrotoxic changes in peripheral tissues.

Thyroid-stimulating antibodies, which are responsible for the thyroid gland hyperactivity in Graves' disease, have been conspicuously absent in patients with RTH. Another potential thyroid stimulator, human chorionic gonadotropin, has not been found in serum of subjects with RTH (101,102).

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 (103,104) as well as to glucocorticoids (104-106). 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 (107) and fibronectin synthesis (108), was preserved in the presence of T_3 insensitivity.

Several of the clinical features encountered in some patients with RTH 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 (52). However, no gross chromosomal abnormalities have been detected on karyotyping (1,109).

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□ in muscle, responding to the excessive amount of circulating TH (110). 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.

Thyroid tissue, obtained by biopsy or at surgery, revealed various degrees of hyperplasia of the follicular epithelium (104,111-113). Specimens have been described as "adenomatous goiters", "colloid goiters" and normal thyroid tissue. When present, lymphocytic infiltration is due to the coexistence of thyroiditis (114).

Clinical Features

Characteristic of the RTHß syndrome is the paucity of specific clinical manifestations. When present, manifestations are variable from one patient to another. Investigations leading to the diagnosis of RTHß have been undertaken because of the presence of goiter, hyperactive behavior or learning disabilities, developmental delay and sinus tachycardia (See Fig. 4). The finding of elevated serum TH levels in association with nonsuppressed TSH is usually responsible for the pursuit of further studies leading to the diagnosis.

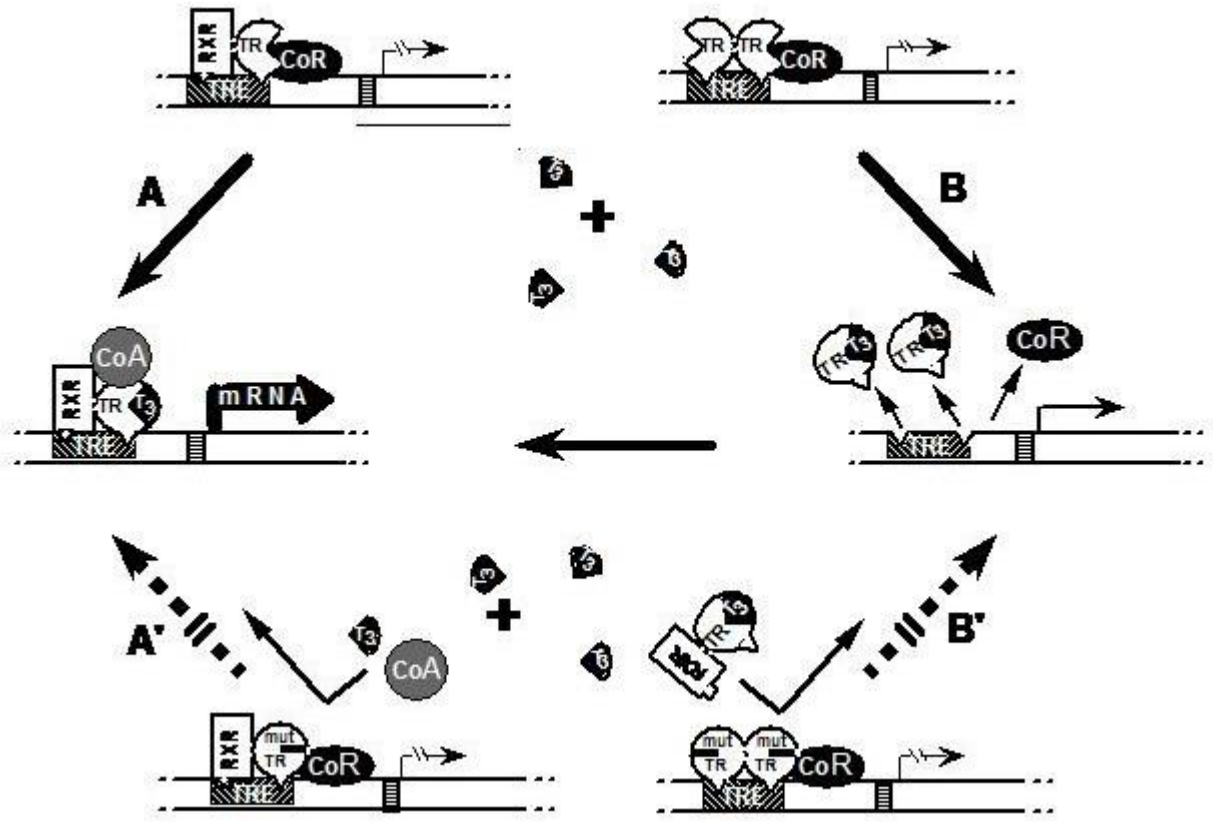

FIG. 4 The reasons prompting further investigation of the index member of each family with RTHß.

The degree of compensation to tissues hyposensitivity by the high 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, RTH 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 more common clinical features and their frequency are given in Table 3. Frank symptoms of hypothyroidism are more common in those individuals who, because of erroneous diagnosis, have received treatment to normalize their circulating TH levels.

Goiter is by far the most common abnormality. It has been reported in 66-95% of cases and is almost always detected by ultrasonography. Gland enlargement is usually diffuse; nodular changes and gross asymmetry are found in recurrent goiters after surgery.

Sinus tachycardia is also very common, with some studies reporting frequency as high as 80% (45). Palpitations often bring the patient to the physician 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 RTHß have some degree of learning disability with or without ADHD (5,115). One-quarter have intellectual quotients (IQ) lesser 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 though growth retardation alone is rare (4%) (5). Despite the high prevalence of ADHD in patients with RTHß, the occurrence of RTHß in children with ADHD must be very rare, none having been detected in 330 such children studied (116,117). The higher prevalence of low IQ scores appears to confer a higher likelihood for subjects with RTH to exhibit ADHD symptoms (98). A retrospective survey has shown an increased miscarriage rate and low birth weight of normal infants born to mothers with RTHß (118).

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, craniosynostosis, 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 (53).

Table 3. CLINICAL FEATURES

FINDINGS	**FREQUENCY (%)**
Thyroid gland	
Goiter	66-95
Heart	
Tachycardia	33-75
Nervous System	

Emotional disturbances	60
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%)	18-25
Delayed bone age >2 SD	29-47
Low Body mass index (in children)	33
Recurrent Ear and Throat Infections	55

Data derived from references (5,45,86)

Course Of The 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 thyroid surgery. As a consequence, some subjects have been submitted to several consecutive thyroidectomies or treatments with radioiodide (113,119-121).

Laboratory Findings

TH and its metabolites

In the untreated patient, elevation in the concentration of serum free T_4 is a *sine qua non* requirement for the diagnosis of RTHß. It is often accompanied by high serum levels of T_3, but less so with advancing age. Serum TBG and TTR concentrations are normal. The resin T_3 uptake is usually high as is the case in patients with thyrotoxicosis.

Serum T_4 and T_3 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 (45), the T_3 to T_4 ratio remains normal (5). This contrasts with the disproportionate increase in serum T_3 concentration characteristic of autoimmune thyrotoxicosis (122).

Reverse T_3 concentration is also high in patients with RTHß as is that of another product of T_4 degradation, 3,3'-T2 (112). 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 T_4 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 T_4 and T_3 are increased by about two- to four-fold (2,119,123,124), but the extrathyroidal conversion of T_4 to T_3 remains normal (124).

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 (125). In most cases, the basal serum TSH concentration is normal and the circadian rhythm is unaltered (126,127). TSH values above 6 mU/L indicate a decrease in thyroidal reserve due to treatment or associated thyroid disease. The severity of the central RTHß can be quantitated, even in the presence of reduced thyroidal reserve, using the thyrotroph T_4 resistance index (TT_4RI); the product of serum FT_4, expressed as percent the upper limit of normal, and the TSH (81).

Thyrotropin has increased biological activity (100,128) and the free α subunit (α -SU) is not disproportionately high. Antibodies against thyroglobulin and thyroid peroxidase indicate the presence of autoimmune thyroid disease, having a higher prevalence in RTHß (129).

Thyroid Gland Activity and Integrity of Hormone Synthesis

The fractional uptake of radioiodide 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 radioiodide following the administration of perchlorate (1,119,130).

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 (131). This indicates that in subjects with RTHß, 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 relative to normal individuals (5). With the exception of increased resting pulse rate in about one half of the patients with RTHß, the cardiac

function is only minimally altered. Two-dimensional and Doppler echocardiography showed mild hyperthyroid effect on cardiac systolic and diastolic function of the myocardium whereas other parameters, such as ejection and shortening fractions of the left ventricle, systolic diameter, and left ventricle wall thickness, were normal (93). Findings suggestive of hypothyroidism have been also reported (132). The Achilles tendon reflex relaxation time has been normal or slightly prolonged.

Serum parameters of TH action on peripheral tissues are usually in the normal range. These include, serum cholesterol, carotene, triglycerides, creatine kinase, alkaline phosphatase, angiotensin-converting enzyme, 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 PRL hyper-responsiveness in some patients with RTHß may be due to the functional TH deprivation at the level of the lactotrophs (125).

Radiological evidence of delayed bone maturation has been observed in one-half of patients with RTH 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 showed reduced responses to L-T_3 added to the medium in terms of degradation rate of lipoproteins (121), synthesis of glycosaminoglycans (107) and fibronectin (108). This was also true for L-T_3-induced changes on specific messenger ribonucleic acid (mRNA) (133). Fibroblasts preserved normal responses to dexamethasone.

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, data generated 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 radioiodide uptake and, when exogenous T_3 is given, a reduction in the pretreatment level of serum T_4 (101,102,113,119,121).

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 RTHß, L-T_4 given in doses of up to 1000 µg/day, and L-T_3 up to 400 µg/day, failed to produce weight loss without a change in calorie intake, nor did they induce a negative nitrogen balance (2,101,104). 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 (49,104).

Effects Of Other Drugs

As expected, administration of the TH analogue, 3,5,3'-triiodo-L-thyroacetic acid (TRIAC) to patients with RTHß produced attenuated responses (2,127,134).

Administration of glucocorticoids promptly reduced the TSH response to TRH and the serum T_4 concentration (101,104,105,111,123).

Administration of L-dopa and bromocriptine produced a prompt suppression of TSH secretion, as well as a diminution of the thyroidal radioiodide uptake and serum T_3 level (103,104,111). Domperidone, a dopamine antagonist, caused a rise in the serum TSH level when given to patients with RTHß (127). 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 RTHß, antithyroid drugs induced some decrease in the circulating level of TH, producing a reciprocal change in the TSH concentration (3,109,130,135). Administration of 100 mg of iodine daily had a similar effect in one patient (102), but 4 mg potassium iodide per day produced no changes in another (2).

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

Differential Diagnosis

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

The presence of elevated serum T_4 concentration with nonsuppressed TSH needs to be confirmed by repeated testing. The possibility of an inherited or acquired increase in serum TBG must be excluded by direct measurement and by estimation of the circulating free T_4 level. The presence of a high serum T_3 is helpful, though normal levels do not exclude RTHß. This may occur transiently with concomitant nonthyroidal illnesses or during the administration of some drugs (see **The Non-Thyroidal Illness Syndrome** and **Effects of the Environment, Chemicals and Drugs on Thyroid Function**), and permanently with advanced age, familial dysalbuminemic

hyperthyroxinemia (FDH) (see Abnormal Thyroid Hormone Transport) and inherited defects of iodothyronine metabolism (see the THMD Section in this Chapter). In FDH free T_4 measured by automated direct methods but not by equilibrium dialysis may be falsely elevated. A rare cause of elevated serum T_4 and T_3 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, both on physical examination or by other laboratory tests (see **Assay of Thyroid Hormones and Related Substances**). The combination of elevated serum levels of free TH and non suppressed TSH, narrows the differential diagnosis to one of the syndromes of impaired sensitivity to TH and autonomous hypersecretion of TSH associated with pituitary tumors (TSHomas). The clinical and laboratory findings of the latter mimic those of RTHß with a few exceptions. TSHomas have: 1) disproportionate abundance in serum free α -SU relative to whole TSH (136); 2) lack similar thyroid tests abnormalities in either parents of the patient; 3) with rare exceptions (137), their serum TSH fails to respond to TRH or suppress with large doses of TH; 4) often have concomitant hypersecretion of growth hormone and or prolactin; 5) in the majority of cases, tumors can be demonstrated by computerized tomography 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. Ectopic production of TSH and endogenous TRH hypersecretion could theoretically result in TSH-induced hyperthyroidism. The presence of high serum free T_3 or free T_4 only, in the presence of nonsuppressed 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 RTHß and symptoms suggestive of thyrotoxicosis are not uncommon in RTHß. 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.

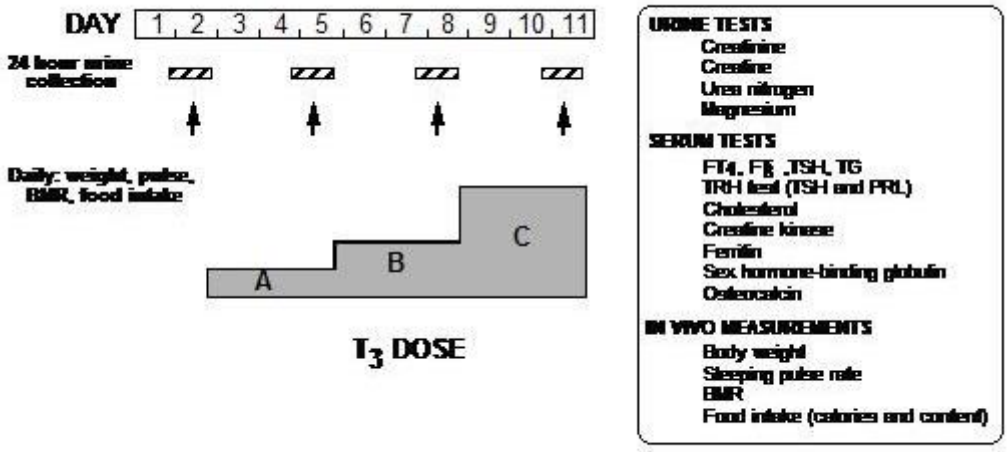

FIG. 5. Schematic representation of a protocol for the assessment of the sensitivity to TH using incremental doses of L-T_3. For details see text.

While the demonstration of *THRB* gene mutation is sufficient to establish the diagnosis of RTHß, a firm exclusion of TRß involvement includes lack of cosegregation of the *THRB* haplotype with the phenotype of RTHß (138), the exclusion of mosaicism (139), and sequencing of TRß cDNA. 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-T_3, and outlined in Fig. 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-T_3. 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 T_3 (for details see reference (5)). L-T_3, rather than L-T_4, is used because of its direct effect on tissues, bypassing potential defects of T_4 transport and metabolism, which may also produce attenuated responses. In addition, the more rapid onset and shorter duration of T_3 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-T_3 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 Fig. 6.

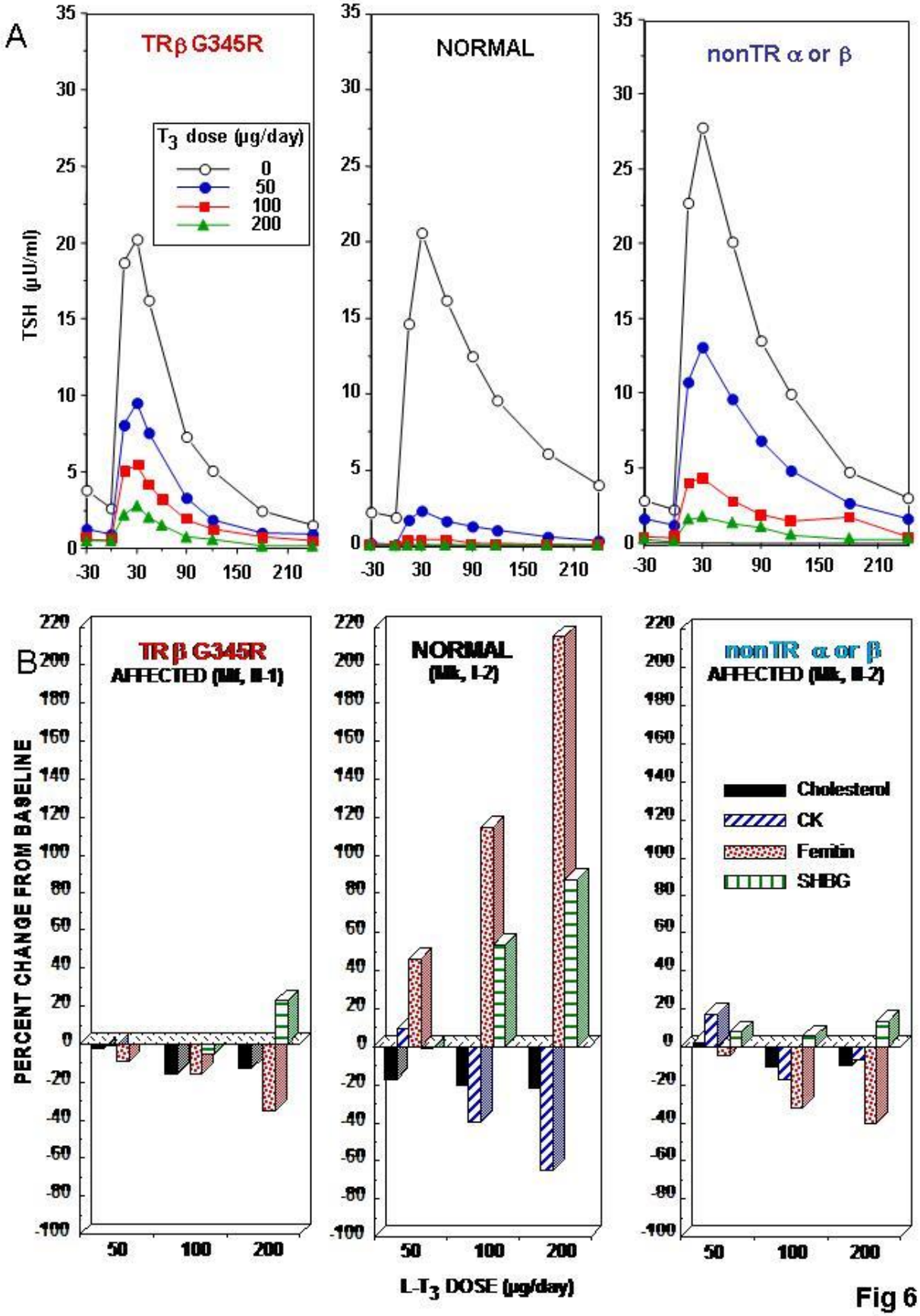
A
TRβ G345R
NORMAL
nonTR α or β
T3 dose (μg/day)
0
50
100
200
TSH (μU/ml)
B
TRβ G345R
AFFECTED (Mk, II-1)
NORMAL
(Mk, I-2)
nonTR α or β
AFFECTED (Mk, II-2)
Cholesterol
CK
Ferritin
SHBG
PERCENT CHANGE FROM BASELINE
L-T3 DOSE (μg/day)

Fig 6

FIG. 6. Responses to the administration of L-T_3 in subjects with RTHß, with and without mutations in the *THRB* gene and in a normal individual. The hormone was given in three incremental doses, each for 3 days as illustrated in Fig. 5. Results are shown at baseline and after each dose of L-T_3 in patients with RTHß in the presence (left) or absence (right) of a *THRB* gene mutation, and the unaffected mother of the patient with nonTR-RTH (center). (A) TSH responses to TRH stimulation. (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 affected subjects, with or without a *THRB* gene mutation, were blunted or paradoxical.

The diagnosis of RTHß is particularly challenging when the latter is associated with other thyroid diseases, such as autoimmune thyrotoxicosis that suppresses the TSH level (140) or with congenital (141,142) or acquired (143) hypothyroidism. Failure to differentiate RTHß from ordinary thyrotoxicosis continues to result in inappropriate treatments. The diagnosis requires awareness of the possible presence of RTHß, usually suspected when high levels of circulating TH are not accompanied by a suppressed TSH.

TABLE 4. Suggested Sequence of Diagnostic Procedures in Suspected RTH

1. Usual presentation: high serum levels of free T_4 with nonsuppressed TSH.
2. Confirm the elevated serum level of free T_4 and exclude 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 TRß gene which, when present and shown to have an impaired function, secures the diagnosis of RTH.
5. In the absence of TRß gene mutation and abnormal thyroid function tests in other family members, the presence of a TSHoma should be excluded by measurement of the α-SU in serum.
6. Demonstrate a blunted TSH-suppression and metabolic response to the administration of supraphysiological doses of TH (see response to L-T_3 protocol, Fig. 6).
7. Blunted TSH response to L-T_3 with absence of TRß gene mutation indicates nonTR-RTH.

Treatment

No specific treatment is available to fully and specifically correct the defect. Theoretically, such ideal treatment for RTHß caused by mutant TRßs with altered TH-binding would be to design mutation-specific TH analogues that would overcome the binding defect (144). However, 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 RTHß, 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 RTHß 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 RTHß 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 normal, retarded bone development and failure to thrive. This may not apply to children homozygous for a *THRB* gene mutation. 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 T_4 of mothers with RTHß carrying a normal embryo should not be allowed to be higher than 20% above the upper limit of normal in order to prevent low birth weight. The wisdom of in utero treatment is questionable (145,146).

Patients with more severe thyrotroph resistance and symptoms of thyrotoxicosis may require therapy. Usually symptomatic treatment with an adrenergic ß 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 (147).

Treatment with supraphysiological doses of L-T_3, given as a single dose every other day, is successful in reducing goiter size without causing side effects (148). Such treatment is preferable considering that postoperative recurrence of goiter is the rule. The L-T_3 dose must be adjusted until TSH and TG are suppressed and reduction of goiter size is observed.

Among the TH analogues used to alleviate symptoms of apparent TH excess (149), TRIAC has had the widest use (150,151). It has a relatively greater affinity than T_3 for some mutant TRßs (152). 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-T_4 is questionable.

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.

Non-TR-RTH

NonTR-RTH refers to the occurrence of the RTHß in the absence of a *THRB* gene mutation. The molecular basis of nonTR-RTH remains unknown. Since the first demonstration of nonTR-RTH (40), 49 subjects belonging to 35 different families have been identified (58,153,154). The phenotype is indistinguishable from that in subjects harboring *TRß* gene mutations (see differential diagnosis, below). Distinct features are an increased female to male ratio of 3.5:1 and the high prevalence of sporadic cases. As a matter of fact, of the 35 families in which both parents, all sibling and progeny were examined, the occurrence of RTHß in another family member was documented in only 6. In several of these families, inheritance is autosomal dominant and mutations in *THRB* gene have been excluded by the absence of genetic co-segregation and by sequencing, thus ruling out mosaicism. Based on observations in mice (155,156) and studies in humans (40) mutations of one of the cofactors that interact with the receptors may be responsible for the resistance in these families (40,58).

THRA MUTATIONS CAUSING TH INSENSITIVITY

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. *TRα* gene deletions, total or only α1 and mice harboring mutation in the *Thra* gene, modeled after those in the *Thrb* gene, failed to produce serum thyroid tests abnormalities. Further, there was no evidence for central hypothyroidism and perturbations in metabolic regulations (157);. The first individual found to harbor a *THRA* gene defect was identified in 2012 by Bochukova et al. in a 6 year old girl by whole-exome sequencing (16).

Incidence, Prevalence

As of this writing, 13 subjects belonging to 9 families have been identified with *THRA* gene mutations (16,158-162) (Table 5). The proposita of the first case was, a white female of European descent. She was heterozygous for a de novo mutation in the *THRA* gene. A second family, of Greek ancestry, had an affected father and daughter. A fourth case was diagnosed at age 42 years in a woman with epilepsy, growth retardation, constipation and macrocephaly. Six additional families with *THRA* gene mutations were recently presented. The prevalence remains unknown (16,158-162).

Table 5. THRA gene mutations in humans

THRA gene **Nucleotide** #	**THRA protein**	**FT4 % lower limit of normal**	**FT3 % upper limit of normal**	**TrT3 % lower limit of normal**	**TSH mU/L**	**Known THRB gene mutations in correspond-ding codons**	**Refetere nce**

806 C>T	A263 V	99*	90*	<63*	4.2*	A317V/T/S/D	(161)
1075 A>T	N359 Y	114	100	121	0.3		(160)
1144 delG	A382f sM38 8X	100	140	91	5.8	A436T/fs M442V/T	(159)
1176 C>A	C392 X	107	148		2.8	C446X/G/R	(162)
1193 C>G	P398 R	72	70		0.5	P452H/L/□	(162)
1207 G>T	E403 X	63	80		1.0	E457G	(16)
1207 G>T	E403 X	NA	NA		NA	E457G	(162)
1207 G>A	E403 K	106	90	33	1.9	E457G	(162)
1190 insT	F397f s E406 X	Low Nl	High Nl	l	Nl	E460K	(158)

del, deletion; ins, insertion; Nl, normal; NA, data not available prior to treatment with L-T4.

* average for 3 affected adult members of the family.

The alternative splicing giving rise to TRa2 isoforms occurs at amino acid 372.

Clinical Features

In general RTHα presents with a range of features that are characterized by growth retardation with skeletal dysplasia resulting in short lower limbs and large head, mental retardation constipation, bradycardia and reduced muscle strength. Other associated problems are seizures and red cell macrocytosis. These clinical findings are compatible with the known tissue distribution of the TR α in bone, brain, intestine, heart and muscle.

Laboratory Findings And Differential Diagnosis

Thyroid function tests in RTHα have consistently demonstrated markedly low serum rT_3, slightly low or low normal T_4, relatively high normal T_3 and normal TSH. This constellation of thyroid tests is not explained by a defective feedback regulation at the hypothalamus and pituitary level, as these

are mainly the functions of RTHβ. It has been demonstrated that TH regulation of deiodinase 3 (DIO3) is dependent on TRα (163). Therefore, subjects with RTHα may have a reduced DIO3 resulting in a low rT_3. The differential diagnosis would include TH cell transport defects, such as MCT8 deficiency, although the lack of severe psychomotor retardation in RTHα and the lack of bone and growth defects in the MCT8 deficient patients are distinguishing features.

Genetic Pathophysiology

The inheritance is autosomal dominant. As is the case of TRß defects, the mutant TRα exerts dominant negative effect on the wild-type TR□1 that binds T_3. The first three families reported had mutations that truncated the TRα; these were M338X, E403X and E406X. Functionally, they corresponded to the following mutations in the TRß molecule: M442X, E457X and E460X, one of which, C446X has been reported (see table 5). The latter produce a very severe form of RTHß phenotype (164). More recently missense mutation that involve both the TRα1 and TRα2 isoforms have been reported, A263V and N359Y (160,161). The mutation affecting the TRα2 isoform does not seam to contribute to the phenotype (161). While A382PfsX7 and N359Y both have dominant negative effects on TRα1 and TRα2, the A382PfsX7 mutant retains constitutive corepressor binding and there is an absence of coactivator recruitment. The reason for the unusual manifestations and somatic defects present in the subject with the THRA N359Y (160) remain unexplained.

Treatment

Given the limited experience with RTHα, there is no established therapy. Affected subjects have received trials of $L\text{-}T_4$ therapy that alleviated the constipation. Unless treatment is instituted in early life, it is unclear whether there will be improvement on mental function.

ANIMAL MODELS OF RTH

Understanding the action of TH *in vivo*, and the mechanisms underlying the abnormalities observed in patients with RTHß, has been bolstered by observations made in genetically manipulated mice. Three types of genetic manipulations have been applied: (a) transgenic mice that over express 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 not withstanding, have yielded true models of the recessively and dominantly inherited forms of RTHß, respectively (165).

The features of RTHß found in patients homozygous for TRß deletion also manifest in the Trß deficient mouse (166-168). Special features, such as sensorineural deafness and monochromatic vision are characteristic and shared by mouse (169) (170) and man (1,59). The mouse model allowed for investigations in greater depth into the mechanisms responsible for the development of these abnormalities. Thus, TRß 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 (171). TRß2 interacts with transcription factors providing timed and spatial order for cone differentiation. Its absence results in the selective loss of

M-opsin (170). The down regulation of hypothalamic TRH is also TRß2 specific (172). Mice deficient in TRß have increased heart rate that can be decreased to the level of the WT mouse by reduction on the TH level (168). This finding, together with the lower heart rate in mice selectively deficient in TR□1 (91), indicates that TH dependent changes in heart rate are mediated through TR□, and explains the tachycardia observed in some patients with RTHß.

The combined deletion of TRα1 and α2, produces no important alterations in TH or TSH concentrations in serum (29). The complete lack of TRs, both α and ß, is compatible with life (29,30). 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 (173). The survival of mice deficient in both TRα1and ß is not due to expression of a yet unidentified TR but to the absence of the noxious effect of unligaded recetors, known as aporeceptors. Indeed, removal of the *Thra* gene rescues the Pax8 KO mice from death (174). The combined TRß and TRα deficient mice have serum TSH levels that are 500-fold higher than those of the WT mice, and T_4 concentrations 12-fold above the average normal mean (29). Thus, the presence of an aporeceptor does not seem to be required for the upregulation of TSH but no amount of TH can cause its downregulation in the absence THR.

The first animal model of a dominantly inherited organ-limited RTHß utilized somatic transfer of a *mTRß1 G345R* cDNA by means of a recombinant adenovirus (175). The liver of these mice was resistant to TH, and overexpression of the WT TRß increased the severity of hypothyroidism, confirming that the unliganded TR has a constitutive effect *in-vivo* as *in-vitro*. True mice models of dominantly inherited RTHß have been generated by targeted mutations in the *Thrb* gene (176,177). Mutations were modeled on those identified in humans with RTHß [frame-shift resulting in 16 carboxylterminal nonsense amino acids (PV mouse) and T337□]. As in humans, the phenotype manifested in the heterozygous KI animals and manifestations were more severe in the homozygotes.

NcoA (SRC-1) deficient mice have RTH with typical increase in T_4, T_3 and TSH concentrations (156). A more mild form of RTH was identified in mice deficient in RXRγ (155). Animals show reduced sensitivity to L-T_3 in terms of TSH downregulation but not in metabolic rate. These data indicate that abnormalities in cofactors can produce RTH. The significance and mechanism of the hypotalamo-pituitary-thyroid activation in the Jun N-terminal kinase 1 (Jnk1) KO mouse has not been yet determined (178).

TRα gene deletions, total or only α1, failed to produce a RTHß phenotype. Similarly, mice with targeted *Thra* gene mutations failed to manifest the phenotype of RTHß. 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□1 R384C* (equivalent to *TRß R438C*) in the and *TR□□P398H* (equivalent to *TRß P452H*) and *TRa L400R* (corresponding to TRß454) (179). While the resulting phenotypes were somewhat variable, none exhibited thyroid tests abnormalities characteristic of RTHß. A common feature in heterozygotes was 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.

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 T_3 low T_4, and slightly elevated TSH.

The neurological phenotype is severe and incapacitating in all patients, 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 T_3 level, resulting in a hyperthyroid state, while tissues dependent predominantly 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.

All affected subjects tested to date have 1) a complex and severe neurodevelopmental phenotype and 2) pathognomonic thyroid tests including high serum T_3 and low rT_3. Serum T_4 concentrations are often reduced, but may be within the low normal range, while serum TSH levels are normal or slightly elevated.

Cell Membrane Transporters Of Th

The identification and characterization of several classes of molecules that transport TH across membranes (180), has changed the previously accepted paradigm of passive TH diffusion into cells (181). These proteins belong to different families of solute carriers: 1) Na^+/taurocholate cotransporting polypeptide (NTCP) (182); 2) fatty acid translocase (183); 3) multidrug resistance-associated proteins (184); 4) L-amino acid transporters (185), among which LAT1 and LAT2 have been shown to also transport TH; 5) members of the organic anion-transporting polypeptide (OATP) family (186), of which OATP1B1 and OATP1B3 are exclusively expressed in liver and transport the sulfated iodothyronines, T_4S, T_3S, and rT_3S and less the corresponding non-sulfated analogues. OATP1C1 is localized preferentially in brain capillaries and shows a high specificity and affinity towards T_4. The latter suggests that OATP1C1 may be important for transport of T_4 across the blood-brain barrier (187); 6) From the monocarboxylate transporter (MCT) family (188), MCT8 and MCT10 are specific TH transporters (189,190). 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 by 10-fold the uptake of T_4, T_3, rT_3, and 3,3′-T_2, but it had no effect on the uptake of sulfated T_4, the aromatic amino acids Phe, Tyr, and Trp, and lactate (190). Furthermore, transfection of human MCT8 in mammalian cells enhanced the metabolism of iodothyronines by endogenous deiodinases (191). 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 in two different laboratories of the first inherited THCMTD caused by mutations in the *MCT8* gene (6,7). Although

presence of the defect is suspected on the based of clinical findings and standard laboratory tests, genetic confirmation is mandatory.

Inheritance And Incidence

MCT8 deficiency is a recessive X-linked defect that affects males, while females are carriers. The mutation has 100% penetrance in males that inherit the mutation. They manifest the neuropsychomotor and characteristic thyroid tests abnormalities, whereas carrier females may show only mild thyroid test abnormalities (6,192,193). A single female with typical features of *MCT8*-specific THCMTD had a *de-novo* translocation disrupting the *MCT8* gene and unfavorable nonrandom X-inactivation (194). No affected male has reproduced. The defect has been reported in individuals of all races and diverse ethnic origins. *De-novo* mutations have been identified in 21% of families.

The incidence of this recently recognized defect is not known. As most routine neonatal screening programs are based on the determination of TSH, MCT8 defects are rarely identified at birth by this mean. In neonatal screening programs based on T_4 measurements, a low concentration could potentially identify new cases. The yield is expected to be low given the high frequency of low T_4 in newborns.

The identification in 11 years of more than 250 individuals with MCT8 defects, belonging to more than 130 families, indicates that this syndrome is more common than initially suspected. Individuals of all races and diverse ethnic origin harbor more than 80 different mutations. *MCT8* gene mutations can be maintained in the population because carrier females are asymptomatic and fertile, which precludes negative selection to take place. Familial occurrence of MCT8 defects has been documented in more than half of the cases. However, genetic information on all mothers of affected males is not available.

Etiology

The clinical condition was first recognized in 1944, in a large family with X chromosome-linked mental retardation presenting with motor abnormalities (195), a form of syndromic X-linked mental retardation, subsequently named the Allan Herndon Dudley syndrome. In 1990, the syndrome was mapped to a locus on chromosome Xq21 (196). Following the identification of *MCT8* gene mutations in subjects with TH abnormalities and psychomotor manifestations (6,7), mutations in the same gene were found in other males, including the original family described in 1944 (197). The affected subjects presented the characteristic thyroid tests abnormalities, not previously suspected.

A large-scale screening of 401 males with X-linked mental retardation has identified *MCT8* gene mutations in only 3, two of whom had the characteristic thyroid phenotype. The other one had normal serum T_3 but the mutation was also found in an unaffected relative (194). 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 idea about the nature of such diseases may be deduced despite species

constraints. In this regard, mice with targeted inactivation of the Lat2 (Slc7a8), which also transports TH, showed normal development, growth, circulating TH levels and TSH (198). Presumably, alternative transporters compensated for the absence of Lat2. No *LAT2* mutations have been reported in humans.

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 (199). It has 6 exons and a large, >100 kb first intron. 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 (200). 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 in 2003 that the rat homologue was a specific transporter of TH into cells (189) opened the field to clinical and genetic investigation.

We now know of 132 families with *MCT8* gene mutations (201,202). Mutations are distributed throughout the coding region of the gene with apparent increased distribution in the TMDs (See Fig. 7). Except for TMD 4, mutations have been reported in all remaining 11 TMDs. Mutations are relatively underrepresented in the extracellular and intracellular loops. One could speculate that missense mutations in these domains could putatively result in milder phenotype, escaping detection, as sequences in these regions are less conserved across species compared to the TMD regions (203).

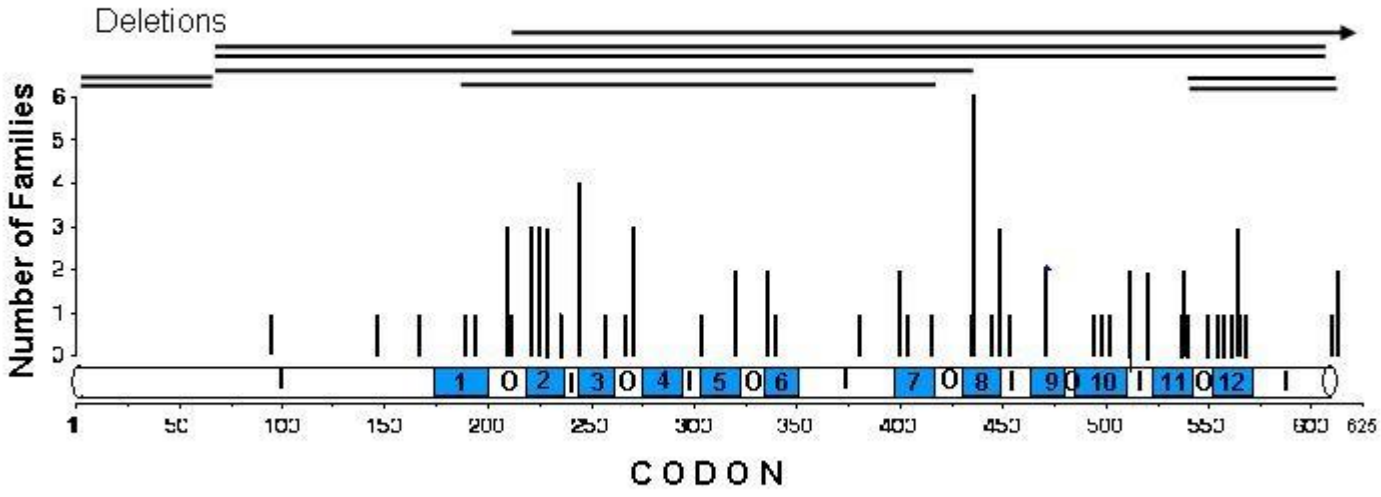

FIG. 7. Location of mutations in the MCT8 molecule associated with THCMTD

Location of the 58 known MCT8 mutations and their frequency in 80 families (published and our unpublished data) are shown by the vertical lines. Horizontal lines indicate the mutations with deletions of large regions. Numbering is consecutive, starting at the amino terminus of the 613 amino acid human molecule. TMDs are indicated in blue and numbered. Loops predicted to be outside the cell are indicated by an O and those inside the cell, by an I.

The types of *MCT8* gene mutations are listed in Table 6. Single amino acid substitutions causing missense mutations were found in 61 families and in 24 they resulted in nonsense mutations. One to 4 nucleotide deletions were observed in 17 families and insertions in 11. Three different single amino acid in frame deletions (F229Δ, F501Δ and F554Δ) occurred in 5 families and single amino acid insertions (189I and 236V) in 2 families. Large deletions involving one or more exons were observed in 9 families. Different mutations in codon 224 (GCG) produced 3 mutant amino acids A224T, V and E. Fifteen different mutations occurred at least in 2 families, the most frequent being R245X which occurred in 5 unrelated families. Of note is the observation that only 6 of the 15 mutations found in multiple families did not occur in mutation hotspots, the remaining 9 occurring either in CpG dinucleotides (G221R, R271H, R245X, G401R and Q564R), C repeats (c.962C->T and c.1614insC) or A repeats (c.629insA). As is the case with *THRB* gene mutations, of the 61 families with single nucleotide substitutions, mutations in 42.6% occurred in CpG dinucleotides, and represented 25% of the de-novo mutations.

Table 6. Types of *MCT8* Gene Mutations reported

Type		**Number of different mutations**	**Number of families**	**Effect on MCT8 protein (number or name of different mutations)**
Substitution	Single nucleotide	37	61	Single a.a. substitution (27 mutations, 45 families). Premature stop (10 mutations, 16 families)
Deletion	Single nucleotide	7	8	FrSh with premature termination (6) and extension with 64 aa (1)
	Trinucleotide	3	7	Single a.a. deletion (F229Δ, F501Δ, F554Δ)
	Eight nucleotides	1	1	FrSh with premature termination
	Fourteen nucleotides	1	1	FrSh with premature termination
	Large	11	11	Lacking part of the gene
Insertion	Single nucleotide	6	9	FrSh with premature termination

	Four nucleotides	1	1	FrSh with premature termination
	InDel	1	1	FrSh with premature termination (c.1678 insA delCC
Duplication	Three nucleotides	2	2	Single a.a. insertion (189I, 236V)
	Four nucleotides	2	2	FrSh with premature termination)
	Six nucleotides	1	1	Two a.a. insertion (c.127 insGGCAGC -> p.43insGS)
	Eight nucleotides	1	1	FrSh with premature termination
Splice site mutation		1	1	IVS3as -1 G->C, alternative splicing and in frame deletion of 94 aa
Chr translocation involving MCT8		1	1	Balanced translocation 46,X,t(X;9)(q13.2;p24)
Mutations at CpG dinucleotides	at CpG dinucleotides	8	26	42.6% of 61 families with single nucleotide substitution
	in C repeats	5	8	Missense (2), 1nc insertion (1), 1nc deletion (1), InDel (
	in A repeats	1	2	FrSh with premature termination (c.629insA)
De novo mutations	Total	12[a]	13[a]	Mutation F229Δ occured de novo in two different families
	In CpGs	3	3	25% of the de novo mutation

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

[a]Might be underestimated, as publications do not always include parental genotype.

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 the 320 known males with *MCT8* gene mutations is very similar, with characteristic thyroid tests abnormalities and severe psychomotor retardation.

Newborns have normal Apgar scores and in most cases there is history of normal gestation. However, polyhydramnios and reduced fetal movements have been reported (197,202,204). 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 with advancing age to spastic quadriplegia. 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 (197,205). These are patients harboring L568P, L434W and F501del mutations that walked with ataxic gait or support and had a limited and dysarthric speech. A possible explanation for milder neurological phenotype in these patients is a residual 15-37% TH-binding activity of their mutant MCT8 molecules (201).

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 (206). 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 (207). In addition to these non-epileptic events, true seizures can also occur. An altered sleep pattern with difficulty falling asleep and frequent awakenings, can represent an important clinical issue for caregivers (206). 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 (208). Muscle mass is diminished and there is generalized muscle weakness with typical poor head control, originally described as “limber neck” (195). A common and pronounced feature in MCT8 deficient patients is the failure to thrive, which can be severe, requiring the placement of a gastric feeding tube in some cases. Possible causes for low weight and muscle wasting are difficulty swallowing, on neurological basis, 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 (206,209-211).

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 (197).

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 (6,194,197,210). Although an unfavorable nonrandom X-inactivation could alter the phenotype in these females (197), cognitive impairment can be due to a variety of causes. Thus, the causative link of *MCT8* mutations in heterozygotes and cognitive impairments remains to be proven (193).

Laboratory Findings

Serum Tests of Thyroid Function

Most characteristic, if not pathognomonic, are the high serum total and free T_3 and low rT_3 concentrations. T_4 is reduced in most cases and TSH levels can be slightly elevated but rarely above 6 mU/L (See Fig. 8).

TSH was normal at neonatal screening in most cases. Information about neonatal T_4 levels available in 8 cases revealed low values in 6 and normal in 2 (197,202,206). However, low T_4 concentrations at birth are not uncommon, and are more often associated with low levels of T_4-binding protein and prematurity. Information regarding the T_3 and rT_3 concentration in the first days of life is not available. However, within one month the typical thyroid tests abnormalities of MCT8 deficiency become apparent. In infants and children, tests results should be interpreted using age-specific reference range (see Assay of Thyroid Hormones and Related Substances). This is particularly important for T_3 and rT_3, which are higher than those in adults. The ratio of T_3 to rT_3 is characteristically high in MCT8 deficiency while it is low in other causes of abnormal T_3 and rT_3 levels, such as binding defects, iodine deficiency and non-thyroidal illness (see corresponding chapters).

Heterozygous female carriers can have all three serum iodothyronine concentrations intermediate between affected males and unaffected family members (6,197,210). While on average they are significantly different than both affected and unaffected individuals, overlapping values are observed with both groups. Serum TSH concentrations are, however, normal (See Fig. 8).

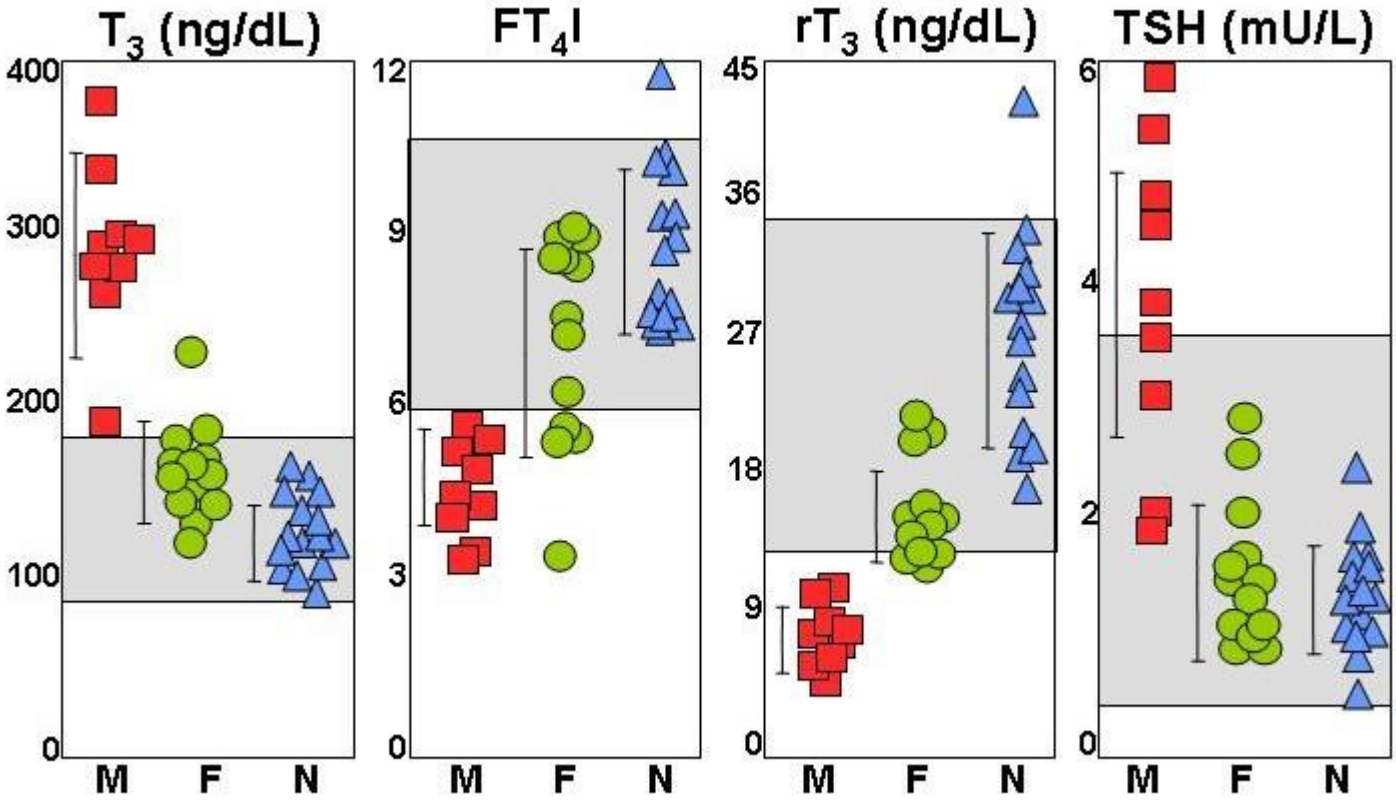

FIG. 8. Thyroid function tests in several families with MCT8 deficiency studied in the authors' laboratory. Grey regions indicate the normal range for the respective test. Hemizygous males (M) are represented as red squares, heterozygous carrier females (F), as green circles and unaffected members of 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.

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 (202,204,210) 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 T_3 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-T_3, using the protocol devised for the study of

patients with RTH, showed reduced pituitary sensitivity to the hormone (202). This is probably due the reduced feedback effect of T_3 on the hypothalamo-pituitary axis, as well as the reduced incremental effect of the hormone on peripheral tissues already exposed to high levels of T_3.

X-rays and Imaging

Bone age has been inconsistently reported, and was found delayed in four cases and was slightly advanced in one (202,210,212,213). The consequences of the MCT8 defects on bone are not clear at this time.

Mild to severe delayed myelination or dysmyelination (202,214,215) is a common finding when brain MRI is performed in early life. However this can be missed as the delay in myelination usually is less apparent by approximately 4 years of age, and an adequate MRI technique, with T1 inverted images and comparison with age and gender matched standards, 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 (209), mild cerebellar atrophy (210), putaminal lesions (216) and a small corpus callosum (202). 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 dysmyelinization according to MRI findings (217).

Tests in Tissues

Altered activity of mitochondrial complexes II and IV was identified in muscle biopsies from two cases (202,218). 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 T_4 and T_3 uptake while D2 enzymatic activity was higher, compared to fibroblasts from normal individuals (202,205). Fibroblasts from carrier females gave results intermediate to those of affected males and normal individuals. Cellular T_3-uptake of cell lines transfected with different mutant MCT8 molecules (201), demonstrated or predicted complete inactivation in about 2/3 of mutations, while in the remaining 1/3, T_3-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).

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 (219).

Animal Models Of Mct8 Deficiency

Mct8-deficient recombinant (*Mct8*KO) mice (15,220) replicate the characteristic thyroid tests abnormalities found in humans and, thus, helped in understanding the mechanisms responsible for the thyroid phenotype (221). Measurements of tissue T_3 content showed the variable availability of the circulating hormone to tissues, depending on the redundant presence of TH cell membrane transporters. In *Mct8*KO mice, tissues such as the liver, that express other transporters than Mct8 (10), have high T_3 concentrations reflecting the high levels in serum and are, therefore, "thyrotoxic" as demonstrated by an increase in the D1 enzymatic activity (See Fig. 9A). 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 (10), have decreased T_3 content in *Mct8*KO mice, which together with the increase in D2, indicate "hypothyroidism" in this tissue (See Fig. 9B). The role of D2 is to maintain local levels of T_3 in the context of TH deficiency and its activity is inversely regulated by TH availability (11). These findings of coexistent T_3 excess and deficiency in the *Mct8*KO mouse tissues explain, in part, the mechanisms responsible for the tissue specific manifestation of TH deficiency and excess in humans with MCT8 deficiency.

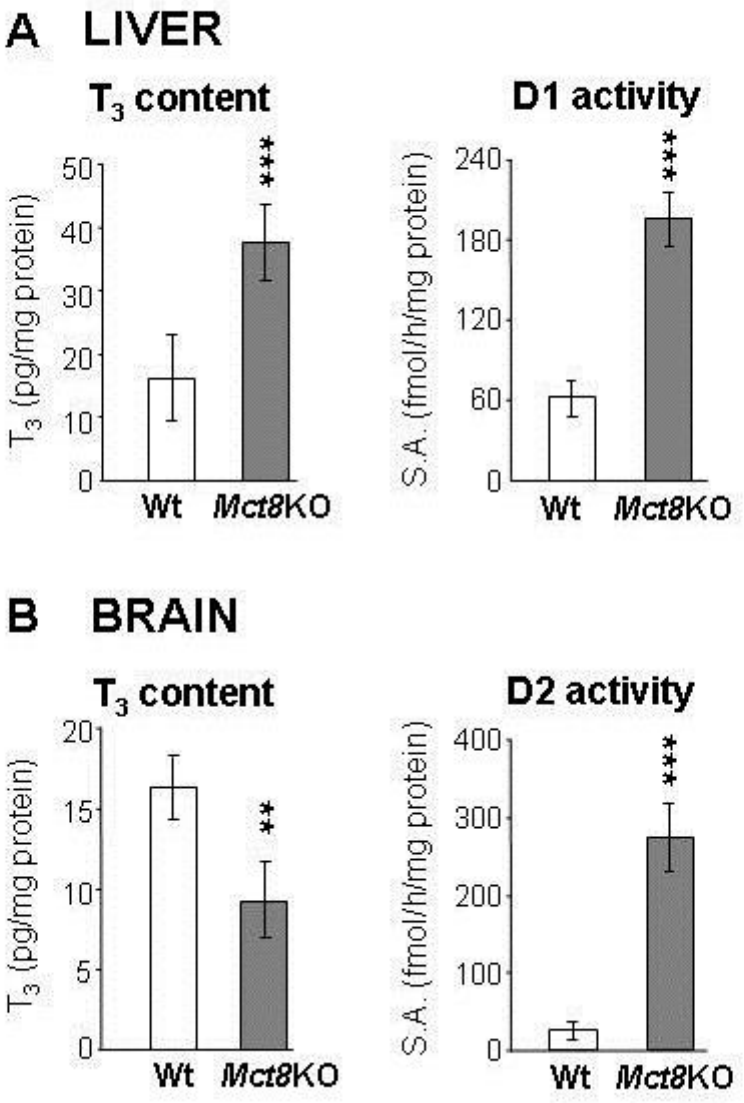

FIG. 9. T3 content and its effect in two tissues of *Mct8*KO and *Wt* mice. **A**. T_3 content and D1 enzymatic activity in liver. **B**. T_3 content and D2 enzymatic activity in brain. Data from *Mct8*KO mice are represented as grey bars and those from *Wt* littermates are in open bars. ** p-value <0.01, *** p-value <0.001.

Mct8 also has a role in TH efflux in the kidney and secretion from the thyroid gland (222,223). 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 *Mct8*KO mice as is the rate of their secretion and appearance in serum is reduced (223).

These observations from the Mct8 deficient mice have helped understand 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 (224). 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 (225). In *Mct8*KO 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.

*Mct8*KO mice have been valuable in testing thyromimetic compound for their potential to bypass the Mct8 defect in tissues. One such TH analogue, diiodothyropropionic acid (DITPA) has been tested. It was found to be effective in equal doses in the *Mct8*KO and Wt animal to replace centrally (pituitary and brain) and peripherally (liver) the TH requirements in animals rendered hypothyroid (226). 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 *Mct8*KO compared to that in Wt animal. These high doses of TH produced “hyperthyroidism” in peripheral tissues of the Mct8KO mice.

The lack of a neurological phenotype in *Mct8*KO mice limits their use as a model for understanding the mechanisms of the neurological manifestations in patients with MCT8 deficiency. Recently a mouse deficient I both Mct8 and Oatp1c1 has been generated that manifests some of the neurological abnormalities observed in humans, supporting the notion that the latter are caused by the deficiency of TH in brain (227)..

Molecular Basis Of The Disorder

In vitro studies using mutant MCT8 molecules as well as observations from animals deficient in Mct8, serve to explain the mechanism leading to the defect. All mutant MCT8 molecules tested by transfection or in fibroblast derived from affected individuals show absent or greatly reduced ability to transport iodothyronines, primarily T_3 (201). Although MCT8 mRNA is widely expressed in human and rat tissues, including brain, heart, liver, kidney, adrenal gland, and thyroid (228,229), 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 T_3-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 (230,231). In thyroid it has been recently demonstrated that MCT8 is involved in the secretion of TH into the bloodstream (223,232).

The magnitude of serum T_3 elevation does not correlate with the degree of T_3 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 T_3, as demonstrated in the *Mct8*KO mice (see the section above). Similarly, there is no correlation between the magnitude of serum T_3 elevation or rT_3 reduction in affected males compared to their carrier mothers (202). Some imperfect correlation does appear to exist between the degree of the MCT8 defect and clinical consequences. Patient that are least severely affected and capable of some locomotion have mutations with partial preservation of T_3 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.

Diferential 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 T_4 is commonly low, it more often accompanies premature births and low levels of TH-binding serum protein. Studies in *Mct8KO* mice suggest that rT_3 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 which Pelizaeus–Merzbacher disease (PMD; MIM 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 (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% (214) and were subsequently found to have the typical thyroid tests 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 thyroid tests abnormalities typical of the defects. This underscores the importance of performing thyroid tests in patients diagnosed with syndromic X-linked phenotypes suggestive of MCT8 defect, prior to sequencing the *MCT8* locus. Most useful is the finding of high serum T_3 and low rT_3. A reduced (at the low limit or below normal) serum total or free T_4 and a normal or slightly above normal TSH are also present. In cases with increase of T_3 due to other causes, calculating the ratio of T_3/rT_3 is helpful in differentiating them from cases of MCT8 defects, in which the ratio will be above 10.

Treatment

Treatment options for patients with *MCT8* gene mutations are currently limited (219). Supportive measures include the use of braces to prevent mal-positioned 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 anticonvulsivants. When refractory to the latter, a ketogenic diet has been successful as well as administration of supraphysiological doses of L-T_4. Experience with such treatments is, however, limited to only a few cases.

Detection of low T_4 by neonatal screening has led to treatment with L-T_4 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-T_4 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 T_3, from the exogenous L-T_4. Therefore, high L-T_4 dose treatment has been used in combination with propylthiouracil (PTU), which is a specific inhibitor of D1. This combination treatment results in reduction of the conversion of T_4 to T_3 by D1 in peripheral tissues while it allows the local generation of T_3 by D2 in tissues. Although this treatment allowed an increase in serum L-T_4 level without increasing the hypermetabolism and weight loss, it did not improve the neuropsychomotor deficit (202,211).

Other possible treatments currently being tested include, administration of the thyromimetic drug DITPA, that seems to be effectively transported into mouse brain in the absence of Mct8 (226) (see Animal Models in a preceding section of this Chapter). Preliminary results from compassionate use in humans (233) show normalization of the thyroid tests and possible improvement in the nutritional status but no objective change in the neuropsychiatric deficit. Other TH metabolites, such as TRIAC is being tested. It is of note that the earliest treatment by any of the above mentioned means has not 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 at, or before birth.

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 (THMD)

The only known inherited TH metabolism defect (THMD), is that caused by recessive mutations in the *selenocysteine insertion sequence-binding protein 2* (*SECISBP2,* in short *SBP2*) gene affecting selenoprotein synthesis, among which are the selenoenzymes deiodinases. Nine families with this defect have been so far identified. Affected individuals present with short stature and characteristic thyroid tests abnormalities, high serum T_4, low T_3, high rT_3 and normal or slightly elevated serum TSH. In addition they also have decreased serum selenium (Se) and decreased selenoprotein levels and activity 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, mental retardation, infertility, myopathy, hearing impairment, photosensitivity, and immune deficits.

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 (Ds). D1 and D2 are 5'-iodothyronine deiodinases that catalyze TH activation by converting T_4 to T_3. D3, a 5-deiodinase is the main TH inactivator through conversion of T_4 to rT_3 and T_3 to T_2 (See Fig. 1B)

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 (11). D2 activity can change very rapidly as its half-life is more than 15-fold shorter that that of D1 and D3. T_4 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 and the specific selenocysteine transfer RNA ($tRNA^{Sec}$) for addition of Sec at this particular UGA codon (See Fig. 10) (234).

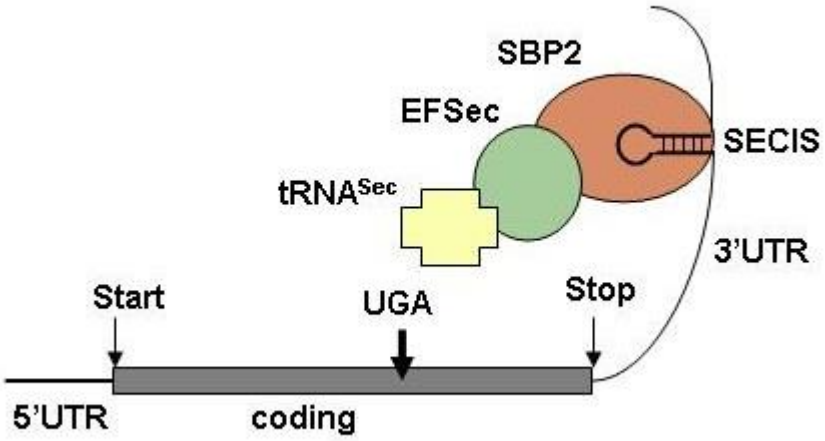

FIG. 10. Components involved in Sec incorporation central in the synthesis of selenoproteins. Elements present in the mRNA of selenoproteins: an in frame UGA codon and Sec incorporation sequence (SECIS) element, a stem loop structure located in the 3'UTR (untranslated region). SBP2 binds SECIS and recruits 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

Until recently, known defects of TH metabolism observed in man were acquired. The most frequent alteration produces the "low T_3 syndrome" of non-thyroidal illness (235) (see The Non-Thyroidal Illness Syndrome). The first inherited disorder of iodothyronine metabolism in a human, was reported in 2005 by Dumitrescu et al. (8). The mutant gene, *SBP2* affects the synthesis 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 genes of other proteins involved in selenoprotein synthesis.

Incudence And Inheritance

The incidence of THMD caused by SBP2 deficiency is unknown. Six additional families have been identified since the description of the initial two families (236-241). 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 are Bedouin from Saudi Arabia, African, Irish, Brazilian, English, Turkish, Japanese and Argentinian.

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 (242). The C-terminal domain of the protein is required for SECIS binding, ribosome binding and Sec incorporation (243) which is mandatory for SBP2 function. The role of the N-terminal region is not fully understood. Recent *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 (244) and 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. The affected were found to be homozygous for R540Q mutation while both parents, members of the same Bedouin tribe, were heterozygous carriers. It is likely that the parents, even though not directly related, had a common ancestor. The affected child of the 2nd family, of mixed African/European background, was compound heterozygous for a paternal nonsense mutation (K438X), and a maternal mutation located in intron 8 (+29bp G->A), causing alternative splicing, but allowing 24% expression of a normal transcript. The 3rd family is originally from Ghana and the affected child was found to harbor a homozygous early termination R128X. The carrier parents were not directly related but belonged to the same tribe.

A Brazilian patient was reported to be compound heterozygous for two nonsense mutations R120X/R770X (237) while the parents were carriers. Two patients were reported from the UK. One was the only adult subject with SBP2 defect reported to date and was heterozygous for a paternally inherited frameshift/premature stop mutation in exon 5 c.668delT fs223 225X, and a splicing defect

causing misincorporation of an additional intronic sequence, believed to be due to a de novo single nucleotide change at –155 bp in intron 6. The second subject from the UK was heterozygous for a maternally inherited missense mutation (C691R), together with a paternally derived defect generating aberrantly spliced SBP2 transcripts lacking exonic sequences (238). The affected subject of a Turkish family was compound heterozygous for two nonsense mutations (240). That of an Argentinian family was compound heterozygous for an early nonsense and a missense mutation in the carboxylterminus. (241) (Table 7).

Table 7. Mutations in the *SBP2* gene

Family	SBP2 gene	Protein	Comments on putative defect	No of affected	Defect	References
1	c.1619 G>A	R540Q	hypomorphic allele	3	homozygous	(8)
2	c.1312 A>T	K438X	missing C terminus	1	compound heterozygous	(8)
	IVS8ds+29 G>A	fs	abnormal splicing			
3	c.382 C>T	R128X	smaller isoforms*	1	homozygous	(236)
4	c.358 C>T	R120X	smaller isoforms*	1	compound heterozygous	(237)
	c.2308 C>T	R770X	disrupted C-terminus			
5	c.668delT	F223fs 255X	truncation and smaller isorforms*	1	compound heterozygous	(238)
	intron 6 -155 delC	fs	abnormal splicing, missing C-terminus			
6	c.2071 T>C	C691R	increased proteasomal degradation	1	compound heterozygous	(238)
	intronic SNP	fs	transcripts lacking exons 2-4, or 3-4			

7	c.1529_1541dup CCAGCGCCCCACT	M515 fs 563X	missing C terminus	1	compound heterozygous	(239)
	c.235 C>T	Q79X	smaller isoforms*			
8	c.2344 C>T	Q782X	missing C terminus	1	compound heterozygous	(240).
	c.2045-2048 delAACA	K682 fs 683X	missing C terminus			
9	c.589 C>T	R197X	smaller isoforms*	1	compound heterozygous	(241)
	c.2037 G>T	E679D	disrupted SECIS binding			

* generated from downstream ATGs; fs – frame shift.

Clinical Features And Course Of The Disease

The probands of the initial three families were brought to clinical attention because of growth delay (8,236). All three were boys ranging in age from 6 to 14.5 years. The proband of a fourth family was a 12-yr-old girl who presented with delayed bone maturation, congenital myopathy, impaired mental and motor coordination development, and bilateral sensorineural loss (237). In a 5th family, a male child, presented at age 2 years with progressive failure to thrive in infancy, followed by global developmental delay and short stature that prompted further investigation. Other features in this patient are an early diagnosis of eosinophilic colitis, fasting nonketotic hypoglycemia with low insulin levels requiring supplemental parenteral nutrition, muscle weakness and mild bilateral high-frequency hearing loss (238). Affected individuals of the 8th and 9th had, in addition to short stature, mild mental retardation and developmental delay, respectively.

The only adult with SBP2 deficiency presented at age 35 years with primary infertility, skin photosensitivity, fatigue, muscle weakness, and severe Raynaud disease (digital vasospasm), impaired hearing, and rotatory vertigo (238). In childhood, both motor and speech developmental milestones were delayed, requiring speech therapy. Hearing problems persisted despite myringotomies for secretory otitis media at 6 years of age. Additional features became obvious with advancing age. He had difficulty walking and running in adolescence, with genu valgus and external rotation of the hip requiring orthotic footwear. At the age of 13 years, marked sun photosensitivity was noted with abnormal UV responses on phototesting. Pubertal development was normal but, at the age of 15 years, he developed unilateral testicular torsion requiring orchiectomy and fixation of the remaining testis. His final stature of 1.67 m, was compatible with the mean parental height of 1.69 m.

Some of the clinical features, in particular delayed growth and bone age, prompted thyroid testing in these patients. All affected subjects were found to have characteristic serum thyroid test abnormalities (detailed in the Laboratory Findings). None of the subjects had an enlarged thyroid gland confirmed by ultrasound examinations.

SBP2 defects could have as yet undetermined consequences and the identification of additional patients, and their long term follow up, will help to further characterize this recently described defect.

Laboratory Findings

The characteristic thyroid tests abnormalities in subjects with *SBP2* gene mutations are high total and free T_4, low T_3, high rT3 and slightly elevated serum TSH (8) (See Fig. 11A). *In vivo* studies assessing the hypothalamo-pituitary-thyroid axis show that compared to normal siblings, affected children required higher doses and serum concentrations of T_4, but not T_3, to reduce their TSH levels (See Fig. 11B).

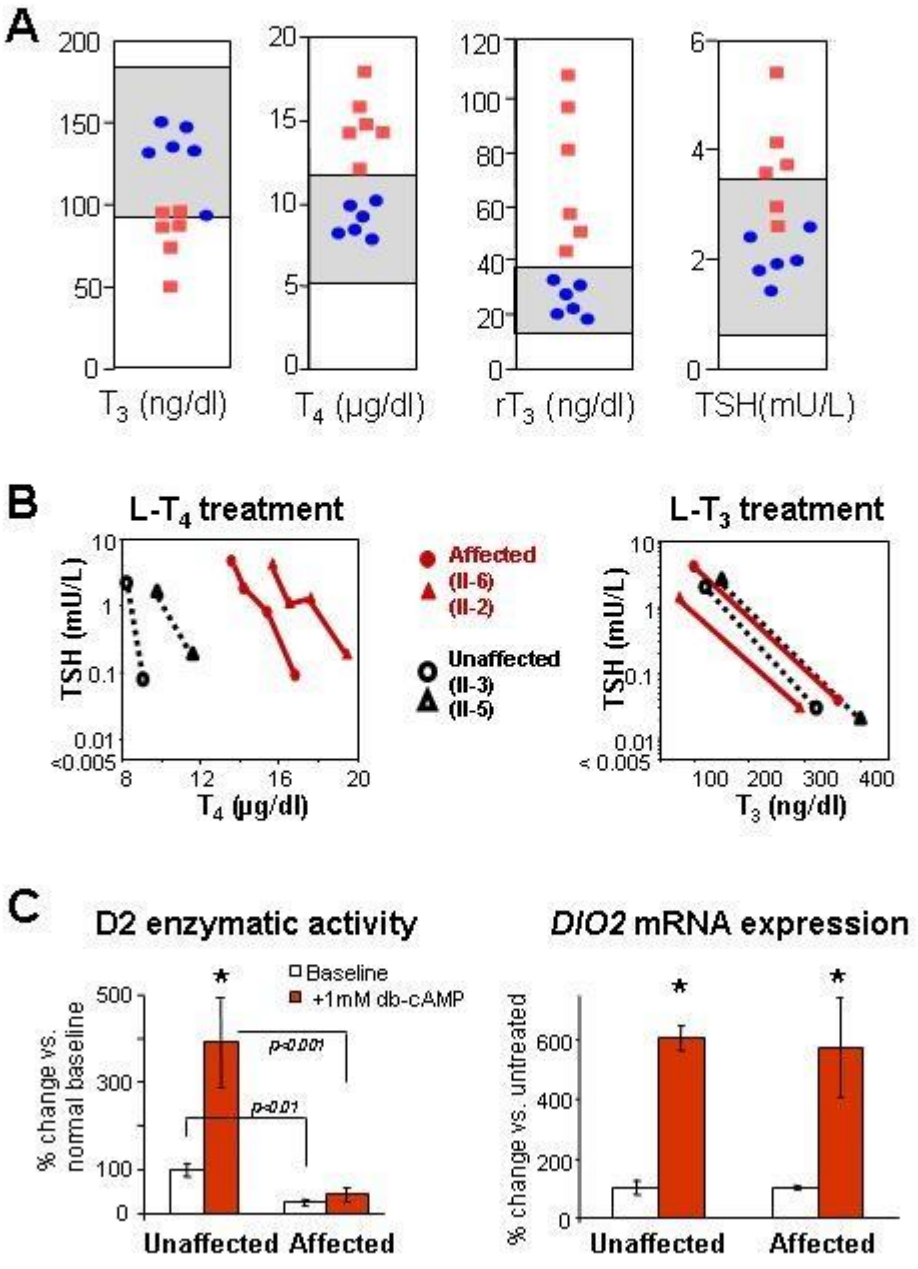

FIG. 11. **A**. Thyroid function tests in several families with SBP2 deficiency studied in the authors' laboratory. Grey regions indicate the normal range for the respective test. Affected individuals are represented as red squares and unaffected members of the families, as blue circles. **B**. *In-vivo* studies**:** Serum TSH and corresponding serum T_4 and T_3 levels, before and during the oral administration of incremental doses of L-T_4 and L-T_3. Note the higher concentrations of T_4 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 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 the 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 than those in fibroblast from normal individuals (See Fig. 11C).

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 (8) in affected individuals.

Detailed evaluation of three recent cases with severe SBP2 deficiency (237,238) demonstrated deficiencies in multiple selenoproteins: lack of testis-enriched selenoproteins resulting in failure of the latter stages of spermatogenesis and azoospermia; selenoprotein N (SEPN) like myopathy resulting in axial muscular dystrophy; cutaneous deficiencies of antioxidant selenoenzymes causing increased cellular reactive oxygen species (ROS) and reduced selenoproteins in peripheral blood cells resulting in immune deficits (238).

Deficiencies of other selenoproteins of unknown function, such as SELH, SELT, SELW, SELI, were found and their consequences are as yet unknown (238). 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 tests abnormalities in subjects with SBP2 deficiency are consistent with a defect in TH metabolism due to the deficiency in deiodinases 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 (245). 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 transcripts. In the case of the homozygous R128X 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 (246,247) it is not surprising that the phenotype of SBP2 deficiency is complex and goes beyond the thyroid tests abnormalities that dominate the mild cases. The more severe phenotype, recently reported in three families, is due to a more extensive impairment in SBP2 function (248). In the patient with two nonsense mutations (237), the R770X mutation truncates the C-terminal functional domain in all the isoforms and likely abolishes SBP2 function. However, the R120X allele likely generates smaller functionally active SBP2 isoforms, but the overall amount would be less than that of the homozygous R128X patient (236), thus explaining the more severe phenotype. Low expression of functional SBP2 also explains the phenotype of the two patients from the UK. 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 (238).

Animal Models

There is no mouse model of a SBP2 defect or components of the Sec incorporation machinery other than $tRNA^{Sec}$ (249). However, a partial synthesis defect results in uneven deficiency in the different types of selenoproteins, reflecting the hierarchy in selenoprotein expression known to occur under conditions of selenium deprivation.

Mice deficient in each of the three deiodinases have been created by homologous recombination (250-252). *Dio1*KO mice have elevated levels of T_4 and rT_3 while the concentrations of T_3 and TSH are unimpaired. *Dio2*KO mice have significantly elevated serum T_4 and normal T_3 levels but contrary to *Dio1*KO mice, TSH concentration is elevated. In addition, *Dio2*KO mice show some growth retardation and defective auditory function (253). Finally, lack of D3 is most deleterious. Total deficiency is associated with partial embryonic and neonatal lethality. Surviving mice exhibit severe growth retardation, impaired reproductive function and central hypothyroidism (252). Mice with combined *Dio1* and *Dio2* targeted disruptions have also been reported and have high serum T_4, and rT_3, reminiscent of the phenotype in SBP2 deficient patients. However, different from the patients, their T_3 is normal while TSH is markedly elevated (224). The putative, partial and uneven involvement of all three deiodinases in the thyroid phenotype of SBP2 defect, including that of D3, might explain the noted difference in the thyroid tests abnormalities. Deletion of the Sbp2 in the mous is incompatible with life (254). Generation of mouse models of partial and conditional Sbp2 deficiency will be crucial for the understanding of the pathophysiology of the complex phenotype of patients with SBP2 defects in humans.

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 distinguish the thyroid tests abnormalities from those encountered in acute non-thyroidal illness, which in terms of iodothyronines could be similar (see chapter The Non-Thyroidal Illness Syndrome). It is important to confirm the abnormalities by repeat testing several weeks or months apart, 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 are 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-T_3 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. Two such options, namely, administration of Se and TH were tested (236,255).

Administration of up to 400 mcg of selenium (255), 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 (256), whereas selenite is metabolized and inserted as selenocysteine into the growing peptide chain of selenoproteins (257), therefore resulting in different Se bioavailability.

The effect of L-T_3 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-T_3 supplementation (236), but experience with TH administration in these patients is limited. Other clinical features of SBP2 defects are treated symptomatically.

Acknowledgments

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REFERENCES

1. Refetoff S, DeWind LT, DeGroot LJ. Familial syndrome combining deaf-mutism, stippled epiphyses, goiter, and abnormally high PBI: possible target organ refractoriness to thyroid hormone. J Clin Endocrinol Metab 1967; 27:279-294

2. Refetoff S, DeGroot LJ, Benard B, DeWind LT. Studies of a sibship with apparent hereditary resistance to the intracellular action of thyroid hormone. Metabolism 1972; 21:723-756

3. Sakurai A, Takeda K, Ain K, Ceccarelli P, Nakai A, Seino S, Bell GI, Refetoff S, DeGroot LJ. Generalized resistance to thyroid hormone associated with a mutation in the ligand-binding domain of the human thyroid hormone receptor b. Proc Natl Acad Sci U S A 1989; 86:8977-8981

4. Usala SJ, Tennyson GE, Bale AE, Lash RW, Gesundheit N, Wondisford FE, Accili D, Hauser P, Weintraub BD. A base mutation of the c-erbAb thyroid hormone receptor in a kindred with generalized thyroid hormone resistance. Molecular heterogeneity in two other kindreds. J Clin Invest 1990; 85:93-100

5. Refetoff S, Weiss RE, Usala SJ. The syndromes of resistance to thyroid hormone. Endocr Rev 1993; 14:348-399

6. Dumitrescu AM, Liao XH, Best TB, Brockmann K, Refetoff S. A Novel Syndrome Combining Thyroid and Neurological Abnormalities Is Associated with Mutations in a Monocarboxylate Transporter Gene. Am J Hum Genet 2004; 74:168-175

7. Friesema EC, Grueters A, Biebermann H, Krude H, von Moers A, Reeser M, Barrett TG, Mancilla EE, Svensson J, Kester MH, Kuiper GG, Balkassmi S, Uitterlinden AG, Koehrle J, Rodien P, Halestrap AP, Visser TJ. Association between mutations in a thyroid hormone transporter and severe X-linked psychomotor retardation. Lancet 2004; 364:1435-1437

8. Dumitrescu AM, Liao X-H, Abdullah SYM, Lado-Abeal J, Abdul-Majed F, Moeller LC, Boran G, Schomburg L, Weiss RE, Refetoff S. Mutations in SECISBP2 result in abnormal thyroid hormone metabolism. Nat Genet 2005; 37:1247-1252

9. Refetoff S, Bassett JH, Beck-Peccoz P, Bernal J, Brent G, Chatterjee K, De Groot LJ, Dumitrescu AM, Jameson JL, Kopp PA, Murata Y, Persani L, Samarut J, Weiss RE, Williams GR, Yen PM. Classification and proposed nomenclature for inherited defects of thyroid hormone action, cell transport, and metabolism. Thyroid 2014; 24:407-409

10. Friesema EC, Jansen J, Milici C, Visser TJ. Thyroid hormone transporters. Vitam Horm 2005; 70:137-167

11. Bianco AC, Salvatore D, Gereben B, Berry MJ, Larsen PR. Biochemistry, cellular and molecular biology, and physiological roles of the iodothyronine selenodeiodinases. Endocr Rev 2002; 23:38-89

12. Bassett JH, Harvey CB, Williams GR. Mechanisms of thyroid hormone receptor-specific nuclear and extra nuclear actions. Mol Cell Endocrinol 2003; 213:1-11

13. Zhang J, Lazar MA. The mechanism of action of thyroid hormones. Annu Rev Physiol 2000; 62:439-466

14. Yen PM, Ando S, Feng X, Liu Y, Maruvada P, Xia X. Thyroid hormone action at the cellular, genomic and target gene levels. Mol Cell Endocrinol 2006; 246:121-127

15. Dumitrescu AM, Liao XH, Weiss RE, Millen K, Refetoff S. Tissue specific thyroid hormone deprivation and excess in Mct8 deficient mice. Endocrinology 2006:4036-4043

16. Bochukova E, Schoenmakers N, Agostini M, Schoenmakers E, Rajanayagam O, Keogh JM, Henning E, Reinemund J, Gevers E, Sarri M, Downes K, Offiah A, Albanese A, Halsall D, Schwabe JWR, Bain M, Lindley K, Muntoni F, Vargha Khadem F, Dattani M, Farooqi IS, Gurnell M, Chatterjee K. A Mutation in the Thyroid Hormone Receptor Alpha Gene. N Engl J Med 2012; 366:243-249

17. Mitsuhashi T, Tennyson GE, Nikodem VM. Alternative splicing generates messages encoding rat c-erbA proteins that do not bind thyroid hormones. Proc Natl Acad Sci USA 1988; 85:5804-5805

18. Macchia PE, Takeuchi Y, Kawai T, Cua K, Gauthier K, Chassande O, Seo H, Hayashi Y, Samarut J, Murata Y, Weiss RE, Refetoff S. Increased sensitivity to thyroid hormone in mice with complete deficiency of thyroid hormone receptor alpha. Proc Natl Acad Sci U S A 2001; 98:349-354

19. Chassande O, Fraichard A, Gauthier K, Flamant F, Legrand C, Savatier P, Laudet V, Samarut J. Identification of transcripts initiated from an internal promoter in the c-erb-Aa locus that encode inhibitors of retinoic acid receptor-a and triiodothyronine receptor activities. Mol Endocrinol 1997; 11:1278-1290

20. Williams GR. Cloning and Characterization of Two Novel Thyroid Hormone Receptor beta Isoforms. Mol Cell Biol 2000; 20:8329-8342

21. Tagami T, Yamamoto H, Moriyama K, Sawai K, Usui T, Shimatsu A, Naruse M. Identification of a novel human thyroid hormone receptor beta isoform as a transcriptional modulator. Biochem Biophys Res Commun 2010; 396:983-988

22. Gauthier K, Plateroti M, Harvey CB, Williams GR, Weiss RE, Refetoff S, Willott JF, Sundin V, Roux J-P, Malaval L, Hara M, Samarut J, Chassande O. Genetic analysis reveals different functions for the products of the thyroid hormone receptor alpha locus. Mol Cell Biol 2001; 21:4748-4760

23. Casas F, Rochard P, Rodier A, Cassar-Malek I, Marchal-Victorion S, Wiesner RJ, Cabello G, Wrutniak C. A variant form of the nuclear triiodothyronine receptor c-ErbAalpha1 plays a direct role in regulation of mitochondrial RNA synthesis. Mol Cell Biol 1999; 19:7913-7924

24. Hodin RA, Lazar MA, Chin WW. Differential and tissue-specific regulation of the multiple rat c-erbA messenger RNA species by thyroid hormone. J Clin Invest 1990; 85:101-105

25. Macchia E, Nakai A, Janiga A, Sakurai A, Fisfalen ME, Gardner P, Soltani K, DeGroot LJ. Characterization of site-specific polyclonal antibodies to c-erbA peptides recognizing human thyroid hormone receptors a_1, a_2, and ß and native 3,5,3'-triiodothyronine receptor, and study of tissue distribution of the antigen. Endocrinology 1990; 126:3232-3239

26. Strait KA, Schwartz HL, Perez-Castillo A, Oppenheimer JH. Relationship of c-erbA mRNA content to tissue triiodothyronine nuclear binding capacity and function in developing and adult rats. J Biol Chem 1990; 265:10514-10521

27. Mannavola D, Moeller LC, Beck-Peccoz P, Persani L, Weiss RE, Refetoff S. A novel splice variant involving the 5' untranslated region of the thyroid hormone receptor ß1 (TRß1). J Endocrinol Invest 2004; 27:318-322

28. Frankton S, Harvey CB, Gleason LM, Fadel A, Williams GR. Multiple mRNA variants regulate cell-specific expression of human thyroid hormone receptor ß1. Mol Endocrinol 2004; 18:1931-1642

29. Gauthier K, Chassande O, Platerotti M, Roux J-P, Legrand C, Rousset B, Weiss R, Trouillas J, Samarut J. Different functions for the thyroid hormone receptors TRa and TRß in the control of thyroid hormone production and post-natal development. EMBO J 1999; 18:623-631

30. Göthe S, Wang Z, Ng L, Kindblom JM, Campos barros A, Ohlsson C, Vennström B, Forrest D. Mice devoid of all known thyroid hormone receptors are viable but exhibit disorders of the pituitary-thyroid axis, growth, and bone maturation. Genes & Dev 1999; 13:1329-1341

31. Forman BM, Casanova J, Raaka BM, Ghysdael J, Samuels HH. Half-site spacing and orientation determines whether thyroid hormone and retinoic acid receptors and related factors bind to DNA response elements as monomers, homodimers, or heterodimers. Mol Endocrinol 1992; 6:429-442

32. Glass CK. Differential recognition of target genes by nuclear receptor monomers, dimers and heterodimers. Endocr Rev 1994; 15:391-407

33. Brent GA, Dunn MK, Harney JW, Gulick T, Larsen PR, Moore DD. Thyroid hormone aporeceptor represses T_3-inducible promoters and blocks activity of the retinoic acid receptor. New Biol 1989; 1:329-336

34. Koenig RJ. Thyroid hormone receptor coactivators and corepressors. Thyroid 1998; 8:703-713

35. Pazin MJ, Kadonaga JT. What's up and down with histone deacetylation and transcription? Cell 1997; 89:325-328

36. Yen PM, Darling DS, Carter RL, Forgione M, Umeda PK, Chin WW. Triiodothyronine (T3) decreases binding to DNA by T3-receptor homodimers but not receptor-auxiliary protein heterodimers. J Biol Chem 1992; 267:3565-3568

37. Yu J, Koenig RJ. Regulation of Hepatocyte Thyroxine 5'-Deiodinase by T3 and Nuclear Receptor Coactivators as a Model of the Sick Euthyroid Syndrome. J Biol Chem 2000; 275:38296-38301

38. Glass CK, Rose DW, Rosenfeld MG. Nuclear receptor coactivators. Current Opinion Cell Biol 1997; 9:222-232

39. Fondell JD, Guermah M, Malik S, Roeder RG. Thyroid hormone receptor-associated proteins and general positive cofactors mediate thyroid hormone receptor function in the absence of the TATA box-binding protein-associated factors of TFIID. Proc Natl Acad Sci USA 1999; 96:1959-1964

40. Weiss RE, Hayashi Y, Nagaya T, Petty KJ, Murata Y, Tunka H, Seo H, Refetoff S. Dominant inheritance of resistance to thyroid hormone not linked to defects in the thyroid hormone receptors a or ß genes may be due to a defective co-factor. J Clin Endocrinol Metab 1996; 81:4196-4203

41. Weintraub BD, Gershengorn MC, Kourides IA, Fein H. Inappropriate secretion of thyroid stimulating hormone. Ann Intern Med 1981; 95:339-351

42. Kaplan MM, Swartz SL, Larsen PR. Partial peripheral resistance to thyroid hormone. Am J Med 1981; 70:1115-1121

43. Usala SJ. Molecular diagnosis and characterization of thyroid hormone resistance syndromes. Thyroid 1991; 1:361-367

44. Beck-Peccoz P, Roncoroni R, Mariotti S, Medri G, Marcocci C, Brabant G, Forloni F, Pinchera A, Faglia G. Sex hormone-binding globulin measurement in patients with inappropriate secretion of thyrotropin (IST): Evidence against selective pituitary thyroid hormone resistance in nonneoplastic IST. J Clin Endocrinol Metab 1990; 71:19-25

45. Beck-Peccoz P, Chatterjee VKK. The variable clinical phenotype in thyroid hormone resistance syndrome. Thyroid 1994; 4:225-232

46. Adams M, Matthews C, Collingwood TN, Tone Y, Beck-Peccoz P, Chatterjee KK. Genetic analysis of 29 kindreds with generalized and pituitary resistance to thyroid hormone: identification of thirteen novel mutations in the thyroid hormone receptor ß gene. J Clin Invest 1994; 94:506-515

47. Ando S, Sarlis NJ, Krishan J, Feng X, Refetoff S, Zhang MQ, Olfield EH, Yen PM. Aberrant alternative splicing of thyroid hormone receptor in a TSH-secreting pituitary tumor is a mechanism for hormone resistance. Mol Endocrinol 2001; 15:1529-1538

48. Tagami T, Usui T, Shimatsu A, Beniko M, Yamamoto H, Moriyama K, Naruse M. Aberrant expression of thyroid hormone receptor beta isoform may cause inappropriate secretion of TSH in a TSH-secreting pituitary adenoma. J Clin Endocrinol Metab 2011; 96:E948-952

49. Weiss RE, Balzano S, Scherberg NH, Refetoff S. Neonatal detection of generalized resistance to thyroid hormone. JAMA 1990; 264:2245-2250

50. LaFranchi SH, Snyder DB, Sesser DE, Skeels MR, Singh N, Brent GA, Nelson JC. Follow-up of newborns with elevated screening T4 concentrations. J Pediatr 2003; 143:296-301

51. Tajima T, Jo W, Fujikura K, Fukushi M, Fujieda K. Elevated free thyroxine levels detected by a neonatal screening system. Pediatr Res 2009; 66:312-316

52. Takeda K, Sakurai A, DeGroot LJ, Refetoff S. Recessive inheritance of thyroid hormone resistance caused by complete deletion of the protein-coding region of the thyroid hormone receptor-ß gene. J Clin Endocrinol Metab 1992; 74:49-55

53. Ferrara AM, Onigata K, Ercan O, Woodhead H, Weiss RE, Refetoff S. Homozygous thyroid hormone receptor beta gene mutations in resistance to thyroid hormone: Three new cases and review of the literature. J Clin Endocrinol Matab 2012; 97:1328-1336

54. Ono S, Schwartz ID, Mueller OT, Root AW, Usala SJ, Bercu BB. Homozygosity for a "dominant negative" thyroid hormone receptor gene responsible for generalized resistance to thyroid hormone. J Clin Endocrinol Metab 1991; 73:990-994

55. Usala SJ, Bale AE, Gesundheit N, Weinberger C, Lash RW, Wondisford FE, McBride OW, Weintraub BD. Tight linkage between the syndrome of generalized thyroid hormone resistance and the human c-erbAb gene. Mol Endocrinol 1988; 2:1217-1220

56. Weiss RE, Weinberg M, Refetoff S. Identical mutations in unrelated families with generalized resistance to thyroid hormone occur in cytosine-guanine-rich areas of the thyroid hormone receptor beta gene: Analysis of 15 families. J Clin Invest 1993; 91:2408-2415

57. Ando S, Sarlis NJ, Oldfield EH, Yen PM. Somatic mutation of TRbeta can cause a defect in negative regulation of TSH in a TSH-secreting pituitary tumor. J Clin Endocrinol Metab 2001; 86:5572-5576

58. Sadow P, Reutrakul S, Weiss RE, Refetoff S. Resistnace to thyroid hormone in the absence of mutations in the thyroid hormone receptor genes. Curr Opin Endocrinol Diabetes 2000; 7:253-259

59. Newell FW, Diddie KR. Typische Monochromasie, angeborene Taubheit und Resistenz gegenüber der intrazellulären Wikung des Thyroideahormons. Klin Mbl Augenheilk 1977; 171:731-734

60. Jones I, Srinivas M, Ng L, Forrest D. The thyroid hormone receptor beta gene: structure and functions in the brain and sensory systems. Thyroid 2003; 13:1057-1068

61. Hayashi Y, Janssen OE, Weiss RE, Murata Y, Seo H, Refetoff S. The relative expression of mutant and normal thyroid hormone receptor genes in patients with generalized resistance to thyroid hormone determined by estimation of their specific messenger ribonucleic acid products. J Clin Endocrinol Metab 1993; 76:64-69

62. Sakurai A, Miyamoto T, Refetoff S, DeGroot LJ. Dominant negative transcriptional regulation by a mutant thyroid hormone receptor ß in a family with generalized resistance to thyroid hormone. Mol Endocrinol 1990; 4:1988-1994

63. Chatterjee VKK, Nagaya T, Madison LD, Datta S, Rantoumis A, Jameson JL. Thyroid hormone resistance syndrome. Inhibition of normal receptor function by mutant thyroid hormone receptors. J Clin Invest 1991; 87:1977-1984

64. Yen PM, Sugawara A, Refetoff S, Chin WW. New insights on the mechanism(s) of the dominant negative effect of mutant thyroid hormone receptor in generalized resistance to thyroid hormone. J Clin Invest 1992; 90:1825-1831

65. Piedrafita FJ, Ortiz MA, Pfahl M. Thyroid hormone receptor-ß mutants, associated with generalized resistance to thyroid hormone show defects in their ligand-sensitive repression function. Mol Endocrinol 1995; 9:1533-1548

66. Au-Fliegner M, Helmer E, Casanova J, Raaka BM, Samuels HT. The conserved ninth C-terminal heptad in thyroid hormone and retinoic acid receptors mediates diverse responses by affecting heterodimer but not homodimer formation. Mol Cel Endocrinol 1993; 13:5725-5737

67. Nagaya T, Jameson JL. Thyroid hormone receptor dimerization is required for the dominant negative inhibition by mutations that cause thyroid hormone resistance. J Biol Chem 1993; 268:15766-15771

68. Yoh SM, Chatterjee VKK, Privalsky ML. Thyroid hormone resistance syndrome manifests as an aberrant interaction between mutant T_3 receptor and transcriptional corepressor. Mol Endocrinol 1997; 11:470-480

69. Tagami T, Gu W-X, Peairs PT, West BL, Jameson JL. A novel natural mutation in the thyroid hormone receptor defines a dual functional domain that exchanges nuclear receptor corepressors and coactivators. Mol Endocrinol 1998; 12:1888-1902

70. Collingwood TN, Rajanayagam O, Adams M, Wagner R, Cavaillès V, Kalkhoven E, Matthews C, Nystrom E, Stenlof K, Lindstedt G, Tisell L, Fletterick RJ, Parker MG, Chatterjee VKK. A natural transactivation mutation in the thyroid hormone ß receptor: Impaired interaction with putative transcriptional mediators. Proc Natl Acad Sci USA 1997; 94:248-253

71. Liu Y, Takeshita A, Misiti S, Chin WW, Yen PM. Lack of coactivator interaction can be a mechanism for dominant negative activity by mutant thyroid hormone receptors. Endocrinology 1998; 139:4197-4204

72. Collingwood TN, Wagner R, Matthews CH, Clifton-Bligh RJ, Mark G, Rajanayagam O, Agostini M, Fletterick RJ, Beck-Peccoz P, Reinhardt W, Binder G, Ranke MB, Hermus A, Hesch RD, Lazarus J, Paul N, Parfitt V, Ragatt P, de Zegher F, Chatterjee VKK. A role for helix 3 of the TRß ligand-binding domain in coactivator recruitment identified by characterization of a third cluster of mutations in resistance to thyroid hormone. EMBO J 1998; 17:4760-4770

73. Nagaya T, Madison LD, Jameson JL. Thyroid hormone receptor mutants that cause resistance to thyroid hormone. Evidence for receptor competition for DNA sequences in target genes. J Biol Chem 1992; 267:13014-13019

74. Nagaya T, Fujieda N, Seo H. Requirement of corepressor binding of thyroid hormone receptor mutants for dominant negative inhibition. Biochem Biophys Res Commun 1998; 247:620-623

75. Hayashi Y, Sunthornthepvarakul T, Refetoff S. Mutations of CpG dinucleotides located in the triiodothyronine (T_3)-binding domain of the thyroid hormone receptor (TR) ß gene that appears to be devoid of natural mutations may not be detected because they are unlikely to produce the clinical phenotype of resistance to thyroid hormone. J Clin Invest 1994; 94:607-615

76. Rastinejad F, Perlmann T, Evans F, Sigler P. Structural determinants of nuclear receptor assembly on DNA direct repeats. Nature 1995; 375:203-211

77. Wagner RL, Apriletti JW, McGrath ME, West BL, Baxter JD, Fletterick RJ. A structural role for hormone in the thyroid hormone receptor. Nature 1995; 138:690-697

78. Marimuthu A, Feng W, Tagami T, Nguyen H, Jameson JL, Fletterick RJ, Baxter JD, West BL. TR surfaces and conformations required to bind nuclear receptor corepressor. Mol Endocrinol 2002; 16:271-286

79. Huber BR, Desclozeaux M, West BL, Cunha-Lima ST, Nguyen HT, Baxter JD, Ingraham HA, Fletterick RJ. Thyroid hormone receptor-beta mutations conferring hormone resistance and reduced corepressor release exhibit decreased stability in the N-terminal ligand-binding domain. Mol Endocrinol 2003; 17:107-116

80. Hayashi Y, Weiss RE, Sarne DH, Yen PM, Sunthornthepvarakul T, Marcocci C, Chin WW, Refetoff S. Do clinical manifestations of resistance to thyroid hormone correlate with the functional alteration of the corresponding mutant thyroid hormone-ß receptors? J Clin Endocrinol Metab 1995; 80:3246-3256

81. Yagi H, Pohlenz J, Hayashi Y, Sakurai A, Refetoff S. Resistance to thyroid hormone caused by two mutant thyroid hormone receptor ß, R243Q and R243W, with marked impairment of function that cannot be explained by altered in-vitro 3,5,3'-triiodothyronine binding affinity. J Clin Endocrinol Metab 1997; 82:1608-1614

82. Safer JD, Cohen RN, Hollenberg AN, Wondisford FE. Defective release of corepressor by hinge mutants of the thyroid hormone receptor found in patients with resistance to thyroid hormone. J Biol Chem 1998; 273:30175-30182

83. Clifton-Bligh RJ, de Zegher F, Wagner RL, Collingwood TN, François I, Van Helvoirt M, Fletterick RJ, Chatterjee VKK. A novel mutation (R383H) in resistance to thyroid hormone syndrome predominantly impairs corepressor release and negative transcriptional regulation. Mol Endocrinol 1998; 12:609-621

84. Flynn TR, Hollenberg AN, Cohen O, Menke JB, Usala SJ, Tollin S, Hegarty MK, Wondisford FE. A novel C-terminal domain in the thyroid hormone receptor selectively mediates thyroid hormone inhibition. J Biol Chem 1994; 629:32713-32716

85. Taniyama M, Ishikawa N, Momotani N, Ito K, Ban Y. Toxic multinodular goitre in a patient with generalized resistance to thyroid hormone who harbours the R429Q mutation in the thyroid hormone receptor beta gene. Clin Endocrinol (Oxf) 2001; 54:121-124

86. Brucker-Davis F, Skarulis MC, Grace MB, Benichou J, Hauser P, Wiggs E, Weintraub BD. Genetic and clinical features of 42 kindreds with resistance to thyroid hormone. The National Institutes of Health prospective study. Ann Intern Med 1995; 123:573-583

87. Safer JD, O'Connor MG, Colan SD, Srinivasan S, Tollin SP, Wondisford FE. The thyroid hormone receptor-ß gene mutation R383H is associated with isolated central resistance to thyroid hormone. J Clin Endocrinol Metab 1999; 84:3099-3109

88. Lee S, Young BM, Wan W, Chan IH, Privalsky ML. A Mechanism for Pituitary-Resistance to Thyroid Hormone (PRTH) Syndrome: a Loss in Cooperative Coactivator Contacts by Thyroid Hormone Receptor (TR){beta}2. Mol Endocrinol 2011; 25:1111-1125

89. Machado DS, Sabet A, Santiago LA, Sidhaye AR, Chiamolera MI, Ortiga-Carvalho TM, Wondisford FE. A thyroid hormone receptor mutation that dissociates thyroid hormone regulation of gene expression in vivo. Proc Natl Acad Sci U S A 2009; 106:9441-9446

90. Alberobello AT, Congedo V, Liu H, Cochran C, Skarulis MC, Forrest D, Celi FS. An intronic SNP in the thyroid hormone receptor beta gene is associated with pituitary cell-specific over-expression of a mutant thyroid hormone receptor beta2 (R338W) in the index case of pituitary-selective resistance to thyroid hormone. J Transl Med 2011; 9:144

91. Johansson C, Göthe S, Forrest D, Vennsrtöm B, Thorén P. Cardiovascular phenotype and temperature controlin mice lacking tyroid hormone receptor ß or both a1 and ß. Am J Physiol 1999; 276:H2006-H2012

92. Gloss B, Trost SU, Bluhm WF, Swanson EA, Clark R, Winkfein R, Janzen KM, Giles W, Chassande O, Samarut J, Dillmann WH. Cardiac Ion Channel Expression and Contractile Function in Mice with Deletion of Thyroid Hormone Receptor alpha or beta. Endocrinology 2001; 142:544-550.

93. Kahaly GJ, Matthews CH, Mohr-Kahaly S, Richards CA, Chatterjee VK. Cardiac involvement in thyroid hormone resistance. J Clin Endocrinol Metab 2002; 87:204-122

94. Lazar MA. Thyroid hormone receptors: multiple forms, multiple possibilities. Endocr Rev 1993; 14:184-193

95. Falcone M, Miyamoto T, Fierro-Renoy F, Nacchia E, DeGroot LJ. Antipeptide polyclonal antibodies specifically recognize each human thyroid hormone receptor isoform. Endocrinology 1992; 131:2419-2429

96. Mixson AJ, Hauser P, Tennyson G, Renault JC, Bodenner DL, Weintraub BD. Differential expression of mutant and normal beta T3 receptor alleles in kindreds with generalized resistance to thyroid hormone. J Clin Invest 1993; 91:2296-2300

97. Geffner ME, Su F, Ross NS, Hershman JM, Van Dop C, Menke JB, Hao E, Stanzak RK, Eaton T, Samuels HH, Usala SJ. An arginine to histidine mutation in codon 311 of the C-erbAß gene results in a mutant thyroid hormone receptor that does not mediate a dominant negative phenotype. J Clin Invest 1993; 91:538-546

98. Weiss RE, Stein MA, Duck SC, Chyna B, Phillips W, O'Brien T, Gutermuth L, Refetoff S. Low intelligence but not attention deficit hyperactivity disorder is associated with resistance to thyroid hormone caused by mutation R316H in the thyroid hormone receptor ß gene. J Clin Endocrinol Metab 1994; 78:1525-1528

99. Weiss RE, Marcocci C, Bruno-Bossio G, Refetoff S. Multiple genetic factors in the heterogeneity of thyroid hormone resistance. J Clin Endocrinol Metab 1993; 76:257-259

100. Persani L, Borgato S, Romoli R, Asteria C, Pizzocardo A, Beck-Peccoz P. Changes in the degree of sialylation of carbohydrate chain modify the biological properties of circulating thyrotropin isoforms in various physiological and pathological states. J Clin Endocrinol Metab 1998; 83:2486-2492

101. Bode HH, Danon M, Weintraub BD, Maloof F, Crawford JD. Partial target organ resistance to thyroid hormone. J Clin Invest 1973; 52:776-782

102. Tamagna EI, Carlson HE, Hershman JM, Reed AW. Pituitary and peripheral resistance to thyroid hormone. Clin Endocrinol (Oxf) 1979; 10:431-441

103. Bajorunas DR, Rosner W, Kourides IA. Use of bromocriptine in a patient with generalized resistance to thyroid hormone. J Clin Endocrinol Metab 1984; 58:731-735

104. Refetoff S, Salazar A, Smith TJ, Scherberg NH. The consequences of inappropriate treatment because of failure to recognize the syndrome of pituitary and peripheral tissue resistance to thyroid hormone. Metabolism 1983; 32:822-834

105. Refetoff S, DeGroot LJ, Barsano CP. Defective thyroid hormone feedback regulation in the syndrome of peripheral resistance to thyroid hormone. J Clin Endocrinol Metab 1980; 51:41-45

106. Kaplowitz PB, D'Ercole AJ, Utiger RD. Peripheral resistance to thyroid hormone in an infant. J Clin Endocrinol Metab 1981; 53:958-963

107. Murata Y, Refetoff S, Horwitz AL, Smith TJ. Hormonal regulation of glycosaminoglycan accumulation in fibroblasts from patients with resistance to thyroid hormone. J Clin Endocrinol Metab 1983; 57:1233-1239

108. Ceccarelli P, Refetoff S, Murata Y. Resistance to thyroid hormone diagnosed by the reduced response of fibroblasts to the triiodothyronine induced suppression of fibronectin synthesis. J Clin Endocrinol Metab 1987; 65:242-246

109. Mäenpää J, Liewendahl K. Peripheral insensitivity to thyroid hormones in a euthyroid girl with goitre. Arch Dis Child 1980; 55:207-212

110. White P, Burton KA, Fowden AL, Dauncey MJ. Developmental expression analysis of thyroid hormone receptor isoforms reveals new insights into their essential functions in cardiac and skeletal muscles. FASEB J 2001; 15:1367-1376

111. Cooper DS, Ladenson PW, Nisula BC, Dunn JF, Chapman EM, Ridgway EC. Familial thyroid hormone resistance. Metabolism 1982; 31:504-509

112. Lamberg B-A, Liewendahl K. Thyroid hormone resistance. Ann Clin Res 1980; 12:243-253

113. Vandalem JL, Pirens G, Hennen G. Familial inappropriate TSH secretion: evidence suggesting a dissociated pituitary resistance to T3 and T4. J Endocrinol Invest 1981; 4:413-422

114. Lamberg BA, Sandström R, Rosengård S, Säarinen P, Evered DC. Sporadic and familial partial peripheral resistance to thyroid hormone. In: Harland WA, Orr JS, eds. Thyroid Hormone Metabolism. London: Academic Press; 1975:139-161.

115. Hauser P, Zametkin AJ, Martinez P, Vitiello B, Matochik JA, Mixson AJ, Weintraub BD. Attention deficit-hyperactivity disorder in people with generalized resistance to thyroid hormone. N Engl J Med 1993; 328:997-1001

116. Weiss RE, Stein MA, Trommer B, Refetoff S. Attention-deficit hyperactivity disorder and thyroid function. J Pediatr 1993; 123:539-545

117. Elia J, Gulotta C, Rose SR, Martin G, Rapoport J. Thyroid function and attention-deficit hyperactivity disorder. J Am Acad Child Adolesc Psychiatry 1994; 33:169-172

118. Anselmo J, Cao D, Karrison T, Weiss RE, Refetoff S. Fetal loss associated with excess thyroid hormone exposure. JAMA 2004; 292:691-695

119. Lamberg BA. Congenital euthyroid goitre and partial peripheral resistance to thyroid hormones. Lancet 1973; 1:854-857

120. Bantle JP, Seeling S, Mariash CN, Ulstrom RA, Oppenheimer JH. Resistance to thyroid hormones: A disorder frequently confused with Graves' disease. Arch Intern Med 1982; 142:1867-1871

121. Chait A, Kanter R, Green W, Kenny M. Defective thyroid hormone action in fibroblasts cultured from subjects with the syndrome of resistance to thyroid hormones. J Clin Endocrinol Metab 1982; 54:767-772

122. Schimmel M, Utiger R. Thyroidal and peripheral production of thyroid hormones: Review of recent findings and their clinical implications. Ann Intern Med 1977; 87:760-768

123. Gómez-Sáez JM, Fernández Castañer M, Navarro MA, Bonnin MR, Soler Ramón J, Roca A. Resistencia parcial a las hormonas tiroideas con bocio y eutiroidismo. Med Clin 1981; 76:412-416

124. Gheri RG, Bianchi R, Mariani G, Toccafondi R, Cappelli G, Brat A, Borghi A, Giusti G, Forti G. A new case of familial partial generalized resistance to thyroid hormone: Study of 3,5,3'-triiodothyronine (T3) binding to lymphocyte and skin fibroblast nuclei and *in vivo* conversion of thyroxine to T3. J Clin Endocrinol Metab 1984; 58:563-569

125. Sarne DH, Sobieszczyk S, Ain KB, Refetoff S. Serum thyrotropin and prolactin in the syndrome of generalized resistance to thyroid hormone: Responses to thyrotropin-releasing hormone stimulation and triiodothyronine suppression. J Clin Endocrinol Metab 1990; 70:1305-1311

126. Kasai Y, Aritaki S, Utsunomiya M, Matsuno T. Twin sisters with Refetoff's Syndrome. J Jap Pediat Assoc 1983; 87:1203-1212

127. Hughes IA, Ichikawa K, DeGroot LJ, John R, Jones MK, Hall R, Scanlon MF. Non-adenomatous inappropriate TSH hypersecretion and euthyroidism requires no treatment. Clin Endocrinol 1987; 27:475-483

128. Persani L, Asteria C, Tonacchera M, Vitti P, Chatterjee VKK, Beck-Peccoz P. Evidence for secretion of thyrotropin with enhanced bioactivity in syndromes of thyroid hormone resistance. J Clin Endocrinol Metab 1994; 78:1034-1039

129. Barkoff MS, Kocherginsky M, Anselmo J, Weiss RE, Refetoff S. Autoimmunity in Patients with Resistance to Thyroid Hormone. J Clin Endocrinol Metab 2010; 95:3189-3193

130. David L, Blanc JF, Chatelain P, Rouchon A, François R. Goitre congéntal avec résistance péripherique partielle aux hormones thyroïdiennes. Ou syndrome de pseudo-hyperthyroïdie. Pediatrie 1979; 34:443-449

131. Mitchell CS, Savage DB, Dufour S, Schoenmakers N, Murgatroyd P, Befroy D, Halsall D, Northcott S, Raymond-Barker P, Curran S, Henning E, Keogh J, Owen P, Lazarus J, Rothman DL, Farooqi IS, Shulman GI, Chatterjee K, Petersen KF. Resistance to thyroid hormone is associated with raised energy expenditure, muscle mitochondrial uncoupling, and hyperphagia. J Clin Invest 2010; 120:1345-1354

132. Pulcrano M, Palmieri EA, Ciulla DM, Campi I, Covelli D, Lombardi G, Biondi B, Beck-Peccoz P. Impact of resistance to thyroid hormone on the cardiovascular system in adults. J Clin Endocrinol Metab 2009; 94:2812-2816

133. Moeller LC, Dumitrescu AM, Walker RL, Meltzer PS, Refetoff S. Thyroid hormone responsive genes in cultured human fibroblasts. J Clin Endocrinol Metab 2005; 90:936-943

134. Kunitake JM, Hartman N, Henson LC, Lieberman J, Williams DE, Wong M, Hershman JM. 3,5,3'-triiodothyroacetic acid therapy for thyroid hormone resistance. J Clin Endocrinol Metab 1989; 69:461-466

135. Magner JA, Petrick P, Menezes-Ferreira MM, Stelling M, Weintraub BD. Familial generalized resistance to thyroid hormones: report of three kindreds and correlation of patterns of affected tissues with the binding of [^{125}I] triiodothyronine to fibroblast nuclei. J Endocrinol Invest 1986; 9:459-470

136. Beck-Peccoz P, Persani L, Faglia G. Glycoprotein hormone a-subunit in pituitary adenomas. Trends Endocrinol Metab 1992; 3:41-45

137. Mornex R, Tommasi M, Cure M, Farcot J, Orgiazzi J, Rousset B. Hyperthyroidie associee a un hypopituitarisme au cours de l'evolution d'une tumeur hypophysaire secretant T.S.H. Ann d'Endocrinol (Paris) 1972; 33:390-396

138. Pohlenz J, Weiss RE, Macchia P, E., Pannain S, Lau IT, Ho H, Refetoff S. Five new families with resistance to thyroid hormone not caused by mutations in the thyroid hormone receptor ß gene. J Clin Endocrinol Metab 1999; 84:3919-3928

139. Mamanasiri S, Yesil S, Dumitrescu AM, Liao XH, Demir T, Weiss RE, Refetoff S. Mosaicism of a Thyroid Hormone Receptor (TR) Beta Gene Mutation in Resistance to Thyroid Hormone (RTH). J Clin Endocrinol Metab 2006; 91:3471-3477

140. Shiwa T, Oki K, Awaya T, Nakanishi S, Yamane K. Resistance to thyroid hormone accompanied by graves' disease. Intern Med 2011; 50:1977-1980

141. Grasberger H, Ringkananont U, Croxson M, Refetoff S. Resistance to thyroid hormone in a patient with thyroid dysgenesis. Thyroid 2005; 15:730-733

142. Borck G, Seewi O, Jung A, Schonau E, Kubisch C. Genetic causes of goiter and deafness: Pendred syndrome in a girl and cooccurrence of Pendred syndrome and resistance to thyroid hormone in her sister. J Clin Endocrinol Metab 2009; 94:2106-2109

143. Sabet A, Pallotta JA. Dichotomous responses to thyroid hormone treatment in a patient with primary hypothyroidism and thyroid hormone resistance. Thyroid 2011; 21:559-561

144. Hassan AQ, Koh JT. Selective chemical rescue of a thyroid-hormone-receptor mutant, TRbeta (H435Y), identified in pituitary carcinoma and resistance to thyroid hormone. Angew Chem Int Ed Engl 2008; 47:7280-7283

145. Asteria C, Rajanayagam O, Collingwood TN, Persani L, Romoli R, Mannavola D, Zamperini P, Buzi F, Ciralli F, Chatterjee VKK, Beck-Peccoz P. Prenatal diagnosis of thyroid hormone resistance. J Clin Endocrinol Metab 1999; 84:405-410

146. Weiss RE, Refetoff S. Treatment of resistance to thyroid hormone--primum non nocere. J Clin Endocrinol Metab 1999; 84:401-404

147. Safer JD, Colan SD, Fraser LM, Wondisford FE. A pituitary tumor in a patient with thyroid hormone resistance: a diagnostic dilemma. Thyroid 2001; 11:281-291.

148. Anselmo J, Refetoff S. Regression of a large goiter in a patient with resistance to thyroid hormone by every other day treatment with triiodothyronine. Thyroid 2004; 14:71-74

149. Iglesias P, Diez JJ. [Therapeutic possibilities in patients with selective pituitary resistance to thyroid hormones.]. Med Clin (Barc) 2008; 130:345-350

150. Beck-Peccoz P, Piscitelli G, Cattaneo MG, Faglia G. Successful treatment of hyperthyroidism due to nonneoplastic pituitary TSH hypersecretion with 3,5,3'-triiodothyroacetic acid (TRIAC). J Endocrinol Invest 1983; 6:217-223

151. Radetti G, Persani L, Molinaro G, Mannavola D, Cortelazzi D, Chatterjee VKK, Beck-Peccoz P. Clinical and hormonal outcome after two years of triiodothyroacetic acid treatment in a child with thyroid hormone resistance. Thyroid 1997; 7:775-778

152. Takeda T, Suzuki S, Liu R-T, DeGroot LJ. Triiodothyroacetic acid has unique potential for therapy of resistance to thyroid hormone. J Clin Endocrinol Metab 1995; 80:2033-2040

153. Vlaeminck-Guillem V, Margotat A, Torresani J, D'Herbomez M, Decoulx M, Wemeau JL. Resistance to thyroid hormone in a family with no TRß gene anomaly: pathogenic hypotheses. Ann Endocrinol (Paris) 2000; 61:194-199

154. Parikh S, Ando S, Schneider A, Skarulis MC, Sarlis NJ, Yen PM. Resistance to thyroid hormone in a patient without thyroid hormone receptor mutations. Thyroid 2002; 12:81-86

155. Brown NS, Smart A, Sharma V, Brinkmeier ML, Greenlee L, Camper SA, Jensen DR, Eckel RoH, Krezel W, Chambon P, Haugen BR. Thyroid hormone resistance and increased metabolic rate in the RXR-g-deficient mouse. J Clin Invest 2000; 106:73-79

156. Weiss RE, Xu J, Ning G, Pohlenz J, O'Malley BW, Refetoff S. Mice deficient in the steroid receptor coactivator-1 (SRC-1) are resistant to thyroid hormone. EMBO J 1999; 18:1900-1904

157. Liu YY, Schultz JJ, Brent GA. A thyroid hormone receptor alpha gene mutation (P398H) Is associated with visceral adiposity and impaired catecholamine-stimulated lipolysis in mice. J Biol Chem 2003; 278:38913-38920

158. van Mullem A, van Heerebeek R, Chrysis D, Visser E, Medici M, Andrikoula M, Tsatsoulis A, Peeters R, Visser TJ. Clinical phenotype and mutant TRalpha1. N Engl J Med 2012; 366:1451-1453

159. Moran C, Schoenmakers N, Agostini M, Schoenmakers E, Offiah A, Kydd A, Kahaly G, Mohr-Kahaly S, Rajanayagam O, Lyons G, Wareham N, Halsall D, Dattani M, Hughes S, Gurnell M, Park SM, Chatterjee K. An adult female with resistance to thyroid hormone mediated by defective thyroid hormone receptor alpha. J Clin Endocrinol Metab 2013; 98:4254-4261

160. Espiard S, Savagner F, Flamant F, Vlaeminck-Guillem V, Guyot R, Munier M, d'Herbomez M, Bourguet W, Pinto G, Rose C, Rodien P, Wemeau JL. A novel mutation in THRA gene associated with an atypical phenotype of resistance to thyroid hormone. J Clin Endocrinol Metab 2015:jc20151120

161. Moran C, Agostini M, Visser WE, Schoenmakers E, Schoenmakers N, Offiah AC, Poole K, Rajanayagam O, Lyons G, Halsall D, Gurnell M, Chrysis D, Efthymiadou A, Buchanan C, Aylwin S, Chatterjee KK. Resistance to thyroid hormone caused by a mutation in thyroid hormone receptor (TR)alpha1 and TRalpha2: clinical, biochemical, and genetic analyses of three related patients. Lancet Diabetes Endocrinol 2014;

162. Tylki-Szymanska A, Acuna-Hidalgo R, Krajewska-Walasek M, Lecka-Ambroziak A, Steehouwer M, Gilissen C, Brunner HG, Jurecka A, Rozdzynska-Swiatkowska A, Hoischen A, Chrzanowska KH. Thyroid hormone resistance syndrome due to mutations in the thyroid hormone receptor alpha gene (THRA). J Med Genet 2015; 52:312-316

163. Barca-Mayo O, Liao XH, Alonso M, Di Cosmo C, Hernandez A, Refetoff S, Weiss RE. Thyroid hormone receptor alpha and regulation of type 3 deiodinase. Mol Endocrinol 2011; 25:575-583

164. Groenhout EG, Dorin RI. Severe generalized thyroid hormone resistance due to a sporadic mutation in the c-erbAß gene producing a turncated T3 receptor protein. Paper presented at: Annual Meeting of The Endocrine Society, San Antonio, TX1992

165. Flamant F, Samarut J. Thyroid hormone receptors: lessons from knockout and knock-in mutant mice. Trends Endocrinol Metab 2003; 14:85-90

166. Forrest D, Hanebuth E, Smeyne RJ, Evereds N, Stewart CL, Wehner JM, Curran T. Recessive resistance to thyroid hormone in mice lacking thyroid hormone receptor ß: evidence for tissue-specific modulation of receptor function. EMBO J 1996; 15:3006-3015

167. Weiss RE, Forrest D, Pohlenz J, Cua K, Curran T, Refetoff S. Thyrotropin regulation by thyroid hormone in thyroid hormone receptor ß-deficient mice. Endocrinology 1997; 138:3624-3629

168. Weiss RE, Murata Y, Cua K, Hayashi Y, Seo H, Refetoff S. Thyroid hormone action on liver, heart and energy expenditure in thyroid hormone receptor ß deficient mice. (Erratum, 141:4767, 2000). Endocrinology 1998; 139:4945-4952

169. Forrest D, Erway LC, Ng L, Altschuler R, Curran T. Thyroid hormone receptor ß is essential for development of auditory function. Nature Genet 1996; 13:354-357

170. Ng L, Hurley JB, Dierks B, Srinivas M, Salto C, Vennstrom B, Reh TA, Forrest D. A thyroid hormone receptor that is required for the development of green cone photoreceptors. Nat Genet 2001; 27:94-98

171. Rüsch A, Erway LC, Oliver D, Vennström B, Forrest D. Thyroid hormone receptor ß-dependent expression of a potassium conductance in inner hair cells at the onset of hearing. Proc Natl Acad Sci U S A 1998; 95:15758-15762

172. Abel ED, Boers ME, Pazos-Moura C, Moura E, Kaulbach H, Zakaria M, Lowell B, Radovick S, Liberman MC, Wondisford F. Divergent roles for thyroid hormone receptor beta isoforms in the endocrine axis and auditory system. J Clin Invest 1999; 104:291-300

173. Mansouri A, Chawdhury K, Gruss P. Follicular cells of the thyroid gland require Pax8 gene function. Nature Genet 1998; 19:87-90

174. Flamant F, Poguet AL, Plateroti M, Chassande O, Gauthier K, Streichenberger N, Mansouri A, Samarut J. Congenital Hypothyroid Pax8(-/-) Mutant Mice Can Be Rescued by Inactivating the TRalpha Gene. Mol Endocrinol 2002; 16:24-32

175. Hayashi Y, Mangoura D, Refetoff S. A mouse model of resistance to thyroid hormone produced by somatic gene transfer of a mutant thyroid hormone receptor. Mol Endocrinol 1996; 10:100-106

176. Kaneshige M, Kaneshige K, Zhu X-g, Dace A, Garrett L, Carter TA, Kazlauskaite R, Pankratz DG, Wynshaw-Boris A, Refetoff S, Weintraube B, Willingham MC, Barlow C, Cheng S-y. Mice with a targeted mutation in the thyroid hormone ß receptor gene exhibit impaired growth and resistanceto thyroid hormone. Proc Natl Acad Sci U S A 2000; 97:13209-13214

177. Hashimoto K, Curty FH, Borges PP, Lee CE, Abel ED, Elmquist JK, Cohen RN, Wondisford FE. An unliganded thyroid hormone receptor causes severe neurological dysfunction. Proc Natl Acad Sci U S A 2001; 98:3998-4003

178. Sabio G, Cavanagh-Kyros J, Barrett T, Jung DY, Ko HJ, Ong H, Morel C, Mora A, Reilly J, Kim JK, Davis RJ. Role of the hypothalamic-pituitary-thyroid axis in metabolic regulation by JNK1. Genes Dev 2010; 24:256-264

179. Mittag J, Wallis K, Vennstrom B. Physiological consequences of the TRalpha1 aporeceptor state. Heart Fail Rev 2010; 15:111-115

180. Hennemann G, Docter R, Friesema EC, de Jong M, Krenning EP, Visser TJ. Plasma membrane transport of thyroid hormones and its role in thyroid hormone metabolism and bioavailability. Endocr Rev 2001; 22:451-476

181. Ekins R. The free hormone hypothesis and measurement of free hormones. Clin Chem 1992; 38:1289-1293

182. Friesema EC, Docter R, Moerings EP, Stieger B, Hagenbuch B, Meier PJ, Krenning EP, Henneman G, Visser TJ. Identification of thyroid hormone transporters. Biochem Biophys Res Commun 1999; 254:479-501

183. van der Putten HH, Friesema EC, Abumrad NA, Everts ME, Visser TJ. Thyroid hormone transport by the rat fatty acid translocase. Endocrinology 2003; 144:1315-1323

184. Mitchell AM, Tom M, Mortimer RH. Thyroid hormone export from cells: contribution of P-glycoprotein. J Endocrinol 2005; 185:93-98

185. Taylor PM, Ritchie JW. Tissue uptake of thyroid hormone by amino acid transporters. Best Pract Res Clin Endocrinol Metab 2007; 21:237-251

186. Hagenbuch B. Cellular entry of thyroid hormones by organic anion transporting polypeptides. Best Pract Res Clin Endocrinol Metab 2007; 21:209-221

187. Pizzagalli F, Hagenbuch B, Stieger B, Klenk U, Folkers G, Meier PJ. Identification of a novel human organic anion transporting polypeptide as a high affinity thyroxine transporter. Mol Endocrinol 2002; 16:2283-2296

188. Visser WE, Friesema EC, Jansen J, Visser TJ. Thyroid hormone transport by monocarboxylate transporters. Best Pract Res Clin Endocrinol Metab 2007; 21:223-236

189. Friesema EC, Ganguly S, Abdalla A, Manning Fox JE, Halestrap AP, Visser TJ. Identification of monocarboxylate transporter 8 as a specific thyroid hormone transporter. J Biol Chem 2003; 278:40128-40135

190. Friesema EC, Jansen J, Jachtenberg JW, Visser WE, Kester MH, Visser TJ. Effective cellular uptake and efflux of thyroid hormone by human monocarboxylate transporter 10. Mol Endocrinol 2008; 22:1357-1369

191. Friesema EC, Kuiper GG, Jansen J, Visser TJ, Kester MH. Thyroid hormone transport by the human monocarboxylate transporter 8 and its rate-limiting role in intracellular metabolism. Mol Endocrinol 2006; 20:2761-2772

192. Papadimitriou A, Dumitrescu AM, Papavasiliou A, Fretzayas A, Nicolaidou P, Refetoff S. A novel monocarboxylate transporter 8 gene mutation as a cause of severe neonatal hypotonia and developmental delay. Pediatrics 2008; 121:e199-202

193. Schwartz CE, Stevenson RE. The MCT8 thyroid hormone transporter and Allan-Herndon-Dudley syndrome. Best Pract Res Clin Endocrinol Metab 2007; 21:307-321

194. Frints SG, Lenzner S, Bauters M, Jensen LR, Van Esch H, des Portes V, Moog U, Macville MV, van Roozendaal K, Schrander-Stumpel CT, Tzschach A, Marynen P, Fryns JP, Hamel B, van Bokhoven H, Chelly J, Beldjord C, Turner G, Gecz J, Moraine C, Raynaud M, Ropers HH, Froyen G, Kuss AW. MCT8 mutation analysis and identification of the first female with Allan-Herndon-Dudley syndrome due to loss of MCT8 expression. Eur J Hum Genet 2008; 16:1029-1037

195. Allan W, Herndon CN, Dudley FC. Some examples of the inheritance of mental deficiency: apparently sex-linked idiocy and microcephaly. Am J Ment Defic 1944; 48:325-334

196. Schwartz CE, Ulmer J, Brown A, Pancoast I, Goodman HO, Stevenson RE. Allan-Herndon syndrome. II. Linkage to DNA markers in Xq21. Am J Hum Genet 1990; 47:454-458

197. Schwartz CE, May MM, Carpenter NJ, Rogers RC, Martin J, Bialer MG, Ward J, Sanabria J, Marsa S, Lewis JA, Echeverri R, Lubs HA, Voeller K, Simensen RJ, Stevenson RE. Allan-Herndon-Dudley Syndrome and the Monocarboxylate Transporter 8 (MCT8) Gene. Am J Hum Genet 2005; 77:41-53

198. Braun D, Wirth EK, Wohlgemuth F, Reix N, Klein MO, Gruters A, Kohrle J, Schweizer U. Aminoaciduria, but normal thyroid hormone levels and signalling, in mice lacking the amino acid and thyroid hormone transporter Slc7a8. Biochem J 2011; 439:249-255

199. Lafreniere RG, Carrel L, Willard HF. A novel transmembrane transporter encoded by the XPCT gene in Xq13.2. Hum Mol Genet 1994; 3:1133-1139

200. Halestrap AP, Meredith D. The SLC16 gene family-from monocarboxylate transporters (MCTs) to aromatic amino acid transporters and beyond. Pflugers Arch 2004; 447:619-628

201. Friesema EC, Visser WE, Visser TJ. Genetics and phenomics of thyroid hormone transport by MCT8. Mol Cell Endocrinol 2010;

202. Personal-Observation.

203. Kleinau G, Schweizer U, Kinne A, Kohrle J, Gruters A, Krude H, Biebermann H. Insights into molecular properties of the human monocarboxylate transporter 8 by combining functional with structural information. Thyroid Res 2011; 4 Suppl 1:S4

204. Holden KR, Zuniga OF, May MM, Su H, Molinero MR, Rogers RC, Schwartz CE. X-linked MCT8 gene mutations: characterization of the pediatric neurologic phenotype. J Child Neurol 2005; 20:852-857

205. Visser WE, Jansen J, Friesema EC, Kester MH, Mancilla E, Lundgren J, van der Knaap MS, Lunsing RJ, Brouwer OF, Visser TJ. Novel pathogenic mechanism suggested by ex vivo analysis of MCT8 (SLC16A2) mutations. Hum Mutat 2008; 30:29-38

206. Boccone L, Mariotti S, Dessi V, Pruna D, Meloni A, Loudianos G. Allan-Herndon-Dudley syndrome (AHDS) caused by a novel SLC16A2 gene mutation showing severe neurologic features and unexpectedly low TRH-stimulated serum TSH. Eur J Med Genet 2010; 53:392-395

207. Brockmann K, Dumitrescu AM, Best TT, Hanefeld F, Refetoff S. X-linked paroxysmal dyskinesia and severe global retardation caused by defective MCT8 gene. J Neurol 2005; 252:663-666

208. Visser WE, Friesema EC, Visser TJ. Minireview: thyroid hormone transporters: the knowns and the unknowns. Mol Endocrinol 2011; 25:1-14

209. Biebermann H, Ambrugger P, Tarnow P, von Moers A, Schweizer U, Grueters A. Extended clinical phenotype, endocrine investigations and functional studies of a loss-of-function mutation A150V in the thyroid hormone specific transporter MCT8. Eur J Endocrinol 2005; 153:359-366

210. Herzovich V, Vaiani E, Marino R, Dratler G, Lazzati JM, Tilitzky S, Ramirez P, Iorcansky S, Rivarola MA, Belgorosky A. Unexpected Peripheral Markers of Thyroid Function in a Patient with a Novel Mutation of the MCT8 Thyroid Hormone Transporter Gene. Horm Res 2007; 67:1-6

211. Wemeau JL, Pigeyre M, Proust-Lemoine E, d'Herbomez M, Gottrand F, Jansen J, Visser TJ, Ladsous M. Beneficial effects of propylthiouracil plus L-thyroxine treatment in a patient with a mutation in MCT8. J Clin Endocrinol Metab 2008; 93:2084-2088

212. Fuchs O, Pfarr N, Pohlenz J, Schmidt H. Elevated serum triiodothyronine and intellectual and motor disability with paroxysmal dyskinesia caused by a monocarboxylate transporter 8 gene mutation. Dev Med Child Neurol 2009; 51:240-244

213. Namba N, Etani Y, Kitaoka T, Nakamoto Y, Nakacho M, Bessho K, Miyoshi Y, Mushiake S, Mohri I, Arai H, Taniike M, Ozono K. Clinical phenotype and endocrinological investigations in a patient with a mutation in the MCT8 thyroid hormone transporter. Eur J Pediatr 2008; 167:785-791

214. Vaurs-Barriere C, Deville M, Sarret C, Giraud G, Des Portes V, Prats-Vinas JM, De Michele G, Dan B, Brady AF, Boespflug-Tanguy O, Touraine R. Pelizaeus-Merzbacher-Like disease presentation of MCT8 mutated male subjects. Ann Neurol 2009; 65:114-118

215. Gika AD, Siddiqui A, Hulse AJ, Edwards S, Fallon P, McEntagart ME, Jan W, Josifova D, Lerman-Sagie T, Drummond J, Thompson E, Refetoff S, Bönnemann CG, Jungbluth H. White matter abnormalities and dystonic motor disorder associated with mutations in the SLC16A2 gene. Dev Med & Child Neurol 2010; 52:475-482

216. Kakinuma H, Itoh M, Takahashi H. A novel mutation in the monocarboxylate transporter 8 gene in a boy with putamen lesions and low free T4 levels in cerebrospinal fluid. J Pediatr 2005; 147:552-554

217. Sijens PE, Rodiger LA, Meiners LC, Lunsing RJ. 1H magnetic resonance spectroscopy in monocarboxylate transporter 8 gene deficiency. J Clin Endocrinol Metab 2008; 93:1854-1859

218. Crushell E, Reardon W. Elevated TSH levels in a mentally retarded boy. Eur J Pediatr 2009; 169:573-575

219. Dempsey MA, Fu J, Dumitrescu AM, Refetoff S. MCT8 (SLS16A2)-Specific thyroid hormone cell transporter deficiency. GeneReviews 2013.
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=20301789.

220. Trajkovic M, Visser TJ, Mittag J, Horn S, Lukas J, Darras VM, Raivich G, Bauer K, Heuer H. Abnormal thyroid hormone metabolism in mice lacking the monocarboxylate transporter 8. J Clin Invest 2007; 117:627-635

221. Bernal J. Role of Monocarboxylate Anion Transporter 8 (MCT8) in Thyroid Hormone Transport: Answers from Mice. Endocrinology 2006; 147:4034-4035

222. Trajkovic-Arsic M, Visser TJ, Darras VM, Friesema EC, Schlott B, Mittag J, Bauer K, Heuer H. Consequences of Monocarboxylate Transporter 8 Deficiency for Renal Transport and Metabolism of Thyroid Hormones in Mice. Endocrinology 2010; 151:802-809

223. Di Cosmo C, Liao XH, Dumitrescu AM, Philp NJ, Weiss RE, Refetoff S. Mice deficient in MCT8 reveal a mechanism regulating thyroid hormone secretion. J Clin Invest 2010; 120:3377-3388

224. Liao XH, Di Cosmo C, Dumitrescu AM, Hernandez A, Van Sande J, St Germain DL, Weiss RE, Galton VA, Refetoff S. Distinct Roles of Deiodinases on the Phenotype of Mct8 Defect: A Comparison of Eight Different Mouse Genotypes. Endocrinology 2011; 152:1180-1191

225. Fliers E, Unmehopa UA, Alkemade A. Functional neuroanatomy of thyroid hormone feedback in the human hypothalamus and pituitary gland. Mol Cell Endocrinol 2006; 251:1-8

226. Di Cosmo C, Liao XH, Dumitrescu AM, Weiss RE, Refetoff S. A thyroid hormone analogue with reduced dependence on the monocarboxylate transporter 8 (MCT8) for tissue transport. Endocrinology 2009; 150:4450-4458

227. Mayerl S, Muller J, Bauer R, Richert S, Kassmann CM, Darras VM, Buder K, Boelen A, Visser TJ, Heuer H. Transporters MCT8 and OATP1C1 maintain murine brain thyroid hormone homeostasis. J Clin Invest 2014; 124:1987-1999

228. Nishimura M, Naito S. Tissue-specific mRNA expression profiles of human solute carrier transporter superfamilies. Drug Metab Pharmacokinet 2008; 23:22-44

229. Price NT, Jackson VN, Halestrap AP. Cloning and sequencing of four new mammalian monocarboxylate transporter (MCT) homologues confirms the existence of a transporter family with an ancient past. Biochem J 1998; 329 (Pt 2):321-328

230. Heuer H, Maier MK, Iden S, Mittag J, Friesema EC, Visser TJ, Bauer K. The monocarboxylate transporter 8 linked to human psychomotor retardation is highly expressed in thyroid hormone sensitive neuron populations. Endocrinology 2005;

231. Roberts LM, Woodford K, Zhou M, Black DS, Haggerty JE, Tate EH, Grindstaff KK, Mengesha W, Raman C, Zerangue N. Expression of the thyroid hormone transporters MCT8 (SLC16A2) and OATP14 (SLCO1C1) at the blood-brain barrier. Endocrinology 2008; 149:6251-6261

232. Trajkovic-Arsic M, Muller J, Darras VM, Groba C, Lee S, Weih D, Bauer K, Visser TJ, Heuer H. Impact of Monocarboxylate Transporter-8 Deficiency on the Hypothalamus-Pituitary-Thyroid Axis in Mice. Endocrinology 2010; 151:5053-5062

233. Verge CF, Konrad D, Cohen M, Di Cosmo C, Dumitrescu AM, Marcinkowski T, Hameed S, Hamilton J, Weiss RE, Refetoff S. Diiodothyropropionic Acid (DITPA) in the Treatment of MCT8 Deficiency. J Clin Endocrinol Metab 2012; 97:4515-4523

234. Driscoll DM, Copeland PR. Mechanism and regulation of selenoprotein synthesis. Annu Rev Nutr 2003; 23:17-40

235. Koenig RJ. Regulation of type 1 iodothyronine deiodinase in health and disease. Thyroid 2005; 15:835-840

236. Di Cosmo C, McLellan N, Liao XH, Khanna KK, Weiss RE, Papp L, Refetoff S. Clinical and molecular characterization of a novel selenocysteine insertion sequence-binding protein 2 (SBP2) gene mutation (R128X). J Clin Endocrinol Metab 2009; 94:4003-4009

237. Azevedo MF, Barra GB, Naves LA, Ribeiro Velasco LF, Godoy Garcia Castro P, de Castro LC, Amato AA, Miniard A, Driscoll D, Schomburg L, de Assis Rocha Neves F. Selenoprotein-related disease in a young girl caused by nonsense mutations in the SBP2 gene. J Clin Endocrinol Metab 2010; 95:4066-4071

238. Schoenmakers E, Agostini M, Mitchell C, Schoenmakers N, Papp L, Rajanayagam O, Padidela R, Ceron-Gutierrez L, Doffinger R, Prevosto C, Luan J, Montano S, Lu J, Castanet M, Clemons N, Groeneveld M, Castets P, Karbaschi M, Aitken S, Dixon A, Williams J, Campi I, Blount M, Burton H, Muntoni F, O'Donovan D, Dean A, Warren A, Brierley C, Baguley D, Guicheney P, Fitzgerald R, Coles A, Gaston H, Todd P, Holmgren A, Khanna KK, Cooke M, Semple R, Halsall D, Wareham N, Schwabe J, Grasso L, Beck-Peccoz P, Ogunko A, Dattani M, Gurnell M, Chatterjee K. Mutations in the selenocysteine insertion sequence-binding protein 2 gene lead to a multisystem selenoprotein deficiency disorder in humans. J Clin Invest 2010; 120:4220-4235

239. Hamajima T, Mushimoto Y, Kobayashi H, Saito Y, Onigata K. Novel compound heterozygous mutations in the SBP2 gene: characteristic clinical manifestations and the implications of GH and triiodothyronine in longitudinal bone growth and maturation. Eur J Endocrinol 2012; 166:757-764

240. Sillers L, Gönç N, Kandemir N, Alikasifoglu A, Dumitrescu AM, Weiss RE, Refetoff S. 2012 Selenoprotein deficiency syndrome caused by novel compound heterozygous mutations in the SBP2 gene. 94th Annual Meeting of the Endocrine Societ; 2012; Houston, TX.

241. Fu J, Liao X-H, Menucci MB, Dumitrescu AM, Weiss RE. 2014 Thyroid hormone metabolism defect caused by novel compound heterozygous mutations in the SBP2 Gene. 96th Annual Meeting of the Endocrine Society; 2014; Chicago, IL.

242. Lescure A, Allmang C, Yamada K, Carbon P, Krol A. cDNA cloning, expression pattern and RNA binding analysis of human selenocysteine insertion sequence (SECIS) binding protein 2. Gene 2002; 291:279-285

243. Copeland PR. Regulation of gene expression by stop codon recoding: selenocysteine. Gene 2003; 312:17-25

244. Papp LV, Lu J, Striebel F, Kennedy D, Holmgren A, Khanna KK. The redox state of SECIS binding protein 2 controls its localization and selenocysteine incorporation function. Mol Cell Biol 2006; 26:4895-4910

245. Bubenik JL, Driscoll DM. Altered RNA-binding activity underlies abnormal thyroid hormone metabolism linked to a mutation in Sec insertion sequence binding protein 2. J Biol Chem 2007; 282:34653-34662

246. Kryukov GV, Castellano S, Novoselov SV, Lobanov AV, Zehtab O, Guigo R, Gladyshev VN. Characterization of mammalian selenoproteomes. Science 2003; 300:1439-1443

247. Papp LV, Lu J, Holmgren A, Khanna KK. From selenium to selenoproteins: synthesis, identity, and their role in human health. Antioxid Redox Signal 2007; 9:775-806

248. Dumitrescu A, Di Cosmo C, Liao XH, Weiss R, Refetoff S. The syndrome of inherited partial SBP2 deficiency in humans. Antioxid Redox Signal 2010; 12:905-920

249. Bosl MR, Takaku K, Oshima M, Nishimura S, Taketo MM. Early embryonic lethality caused by targeted disruption of the mouse selenocysteine tRNA gene (Trsp). Proc Natl Acad Sci U S A 1997; 94:5531-5534

250. Schneider MJ, Fiering SN, Thai B, Wu SY, St Germain E, Parlow AF, St Germain DL, Galton VA. Targeted disruption of the type 1 selenodeiodinase gene (dio1) results in marked changes in thyroid hormone economy in mice. Endocrinology 2006; 147:580-589

251. Schneider MJ, Fiering SN, Pallud SE, Parlow AF, St Germain DL, Galton VA. Targeted Disruption of the Type 2 Selenodeiodinase Gene (DIO2) Results in a Phenotype of Pituitary Resistance to T(4). Mol Endocrinol 2001; 15:2137-2148.

252. Hernandez A, Martinez ME, Fiering S, Galton VA, St Germain D. Type 3 deiodinase is critical for the maturation and function of the thyroid axis. J Clin Invest 2006; 116:476-484

253. Ng L, Goodyear RJ, Woods CA, Schneider MJ, Diamond E, Richardson GP, Kelley MW, Germain DL, Galton VA, Forrest D. Hearing loss and retarded cochlear development in mice lacking type 2 iodothyronine deiodinase. Proc Natl Acad Sci USA 2004; 101:3474-3479

254. Seeher S, Atassi T, Mahdi Y, Carlson BA, Braun D, Wirth EK, Klein MO, Reix N, Miniard AC, Schomburg L, Hatfield DL, Driscoll DM, Schweizer U. Secisbp2 is essential for embryonic development and enhances selenoprotein expression. Antioxid Redox Signal 2014; 21:835-849

255. Schomburg L, Dumitrescu AM, Liao XH, Bin-Abbas B, Hoeflich J, Kohrle J, Refetoff S. Selenium supplementation fails to correct the selenoprotein synthesis defect in subjects with SBP2 gene mutations. Thyroid 2009; 19:277-281

256. Burk RF, Norsworthy BK, Hill KE, Motley AK, Byrne DW. Effects of chemical form of selenium on plasma biomarkers in a high-dose human supplementation trial. Cancer Epidemiol Biomarkers Prev 2006; 15:804-810

257. Xu J, Yang F, An X, Hu Q. Anticarcinogenic activity of selenium-enriched green tea extracts in vivo. J Agric Food Chem 2007; 55:5349-5353

258. Beck-Peccoz P, Chatterjee VKK, Chin WW, DeGroot LJ, Jameson JL, Nakamura H, Refetoff S, Usala SJ, Weintraub BD. Nomenclature of thyroid hormone receptor ß-gene mutations in resistance to thyroid hormone: Consensus statement from the first workshop on thyroid hormone resistance, July 10-11, 1993, Cambridge, United Kingdom. J Clin Endocrinol Metab 1994; 78:990-993