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 $\beta$ (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**

<table>
<thead>
<tr>
<th>Commonly used name (References Are for first Reported Cases)</th>
<th>Synonyms</th>
<th>Gene Involved &amp; Inheritance (OMIM)</th>
<th>Phenotype</th>
<th>Consistent (Pathognomonic)</th>
<th>Common</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>THYROID HORMONE CELL MEMBRANE TRANSPORT DEFECTS (THCMTD)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Monocarboxylate transporter 8 (MCT8) defect</td>
<td>Allan-Herndon-Dudley syndrome</td>
<td>MCT8 (SLC16A2) gene (300095) X-chromosome linked</td>
<td>High T3, low rT3 and T4, normal or slightly elevated TSH; low BMI; hypotonia, spastic quadriplegia; not walking or rarely ataxic gait; no speech or dysarthria, mental retardation</td>
<td>Hypermetabolism, paroxysmal dyskinesia, reduced muscle mass, seizures, poor head control, difficulty sitting independently.</td>
<td></td>
</tr>
<tr>
<td>Idiopathic &amp; other THCMTDs</td>
<td>To be determined</td>
<td>Unknown</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>THYROID HORMONE METABOLISM DEFECTS (THMD)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Selenocysteine insertion sequence binding protein 2 (SBP2) defect</td>
<td>SBP2 (SECISBP2) gene (607693) recessive</td>
<td>High T4 and rT3, low T3, normal or slightly elevated TSH; growth retardation</td>
<td>Azoospermia, immunodeficiency, photosensitivity, delayed bone maturation, myopathy, hearing impairment, delayed developmental milestones</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Idiopathic &amp; other THMDs</td>
<td>To be determined</td>
<td>Unknown</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>THYROID HORMONE ACTION DEFECTS (THAD): nuclear receptor and other</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resistance to thyroid hormone (RTH)a</td>
<td>Thyroid hormone unresponsiveness , Generalized RTH, RTH beta; THR gene (190160) dominant negative (rarely recessive)</td>
<td>High serum FT₄ and rT₃, high thyroglobulin, goiter, attention deficit hyperactivity disorder (ADHD), tachycardia</td>
<td>High serum FT₃ and rT₃</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-------------------------------------</td>
<td>----------------------------------------------------------------------------------------------------------------------------------</td>
<td>--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
<td>--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>nonTR-RTHb</td>
<td>Unknown</td>
<td>Same as above</td>
<td>Same as above</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RTH alpha¹c</td>
<td>Congenital nongoitrous hypothyroidism 6</td>
<td>THRA gene (190120) dominant negative</td>
<td>Low rT₃, seizures, placid behavior.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Low serum T₄/T₃ ratio; cognitive impairment, short lower limbs, delayed closure of skull sutures, delayed bone and dental development, skeletal dysplasia, macrocephaly; constipation; anemia</td>
<td>Low serum T₄/T₃ ratio; cognitive impairment, short lower limbs, delayed closure of skull sutures, delayed bone and dental development, skeletal dysplasia, macrocephaly; constipation; anemia</td>
<td>Low serum T₄/T₃ ratio; cognitive impairment, short lower limbs, delayed closure of skull sutures, delayed bone and dental development, skeletal dysplasia, macrocephaly; constipation; anemia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypersensitivity to thyroid hormone (HTH)</td>
<td>Unknown</td>
<td>Low FT₄ and FT₃ with normal TSH, euthyroid and no serum transport defects</td>
<td>Normal thyroid gland</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Idiopathic &amp; other THADs</td>
<td>To be determined</td>
<td>Unknown</td>
<td>Unknown</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: FT₃, free T₃; FT₄, free T₄; BMI, body mass index; nonTR-RTH, RTH without mutations in the THR or THRA genes.

a Proposed future terminology: RTH beta.

b RTH without mutations in the THR gene.

c A single case with a mutation involving both TR alpha1 and TR alpha2 presented a more complex phenotype, including severe bone malformations, hyper-calcemia 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 (T4) by removal of the outer ring iodine (5'-deiodination) to form triiodothyronine (T3) or, inactivate T4 and T3 by removal of the inner ring iodine (5-deiodination) to form reverse T3 (rT3) and T2, 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 RTHβ syndrome. Subjects with RTHβ 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 T3 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 THRβ 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 (RRTB) and RTH-alpha (RTHα). A syndrome clinically and biochemically indistinguishable from RTHβ but without THRβ 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 TRβ molecules, respectively, with substantial structural and sequence similarities. Both genes produce two isoforms; α1 and α2 by alternative splicing and β1 and β2 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 TRβ with shorter amino terminus (TRβ3), truncated TRβ3, TRα1 and TRα2, lacking the DNA-binding domain (DBD) have been identified in rodents (19,20) and TRβ4 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 TRα 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 T3 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 involves 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 T4 and to a lesser degree T3, 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). Notwithstanding 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 euthyroid 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 T4 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, RTHβ 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 RTHβ 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 RTHβ has produced homozygous children with very severe clinical manifestations (53,54).

Table 2. Types of TRβ Gene Mutations

<table>
<thead>
<tr>
<th>Type</th>
<th>Number of Occurrences at different sites</th>
<th>Number of families</th>
<th>Effect on TRβ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Substitution</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Single nucleotide</td>
<td>148</td>
<td>430</td>
<td>191</td>
</tr>
<tr>
<td>Dinucleotide</td>
<td>3</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Deletion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single nucleotide</td>
<td>4</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Trinucleotide</td>
<td>5</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>Eight nucleotides</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>All coding sequences</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Insertion</td>
<td>Single nucleotide</td>
<td>7</td>
<td>14</td>
</tr>
<tr>
<td>-----------</td>
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<td>---</td>
<td>----</td>
</tr>
<tr>
<td></td>
<td>Trinucleotide</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Duplicati</td>
<td>Seven nucleotides</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

| Mutations at CpG dinucleotides | 10 | 184 | 88a | 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 | 43c | 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 | 40e | 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 TRβ molecule associated with RTHβ.

TOP PORTION: Schematic representation of the TRβ and its functional domains for interaction with TREs (DNA-binding) and with hormone (T₃-binding). Their relationship to the three clusters of natural mutations is also indicated. TRβ2 has 15 more residues than TRβ1 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 TRβ1 molecule according to the consensus statement of the First International Workshop on RTH (258). “Cold regions” are areas devoid of mutations associated with RTHβ.

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; F451L,S,C,X) while those in codon 453, seven (P453T,S,A,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 THRβ gene mutations are located in the functionally relevant domain of T3-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 THRβ gene deletion, in all others inheritance is autosomal dominant.

Somatic mutations in the THRβ 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, RTHβ 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 TRβs and Dominant Negative Effect**

THRβ gene mutations produce two forms of RTHβ. The less common, described in only one family (1), is caused by deletion of all coding sequences of the THRβ gene and is inherited as an autosomal recessive trait (52). The complete lack of TRβ in these individuals produces severe deafness, resulting in mutism (1), as well as monochromatic vision (59is ) as TRβ 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 THRβ gene have no clinical or laboratory abnormalities. This is not due to compensatory overexpression of the single normal allele of the THRβ gene nor that of the THRα gene (61). However, because subjects with complete THRβ gene deletion preserve some TH responsiveness, it is logical to
conclude that TRα1 is capable of partially substituting for the function of TRβ (see Animal Models of RTH, below).

The more common form of RTHβ 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 TRβs (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 T₃-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 T₃-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 RTHβ. 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 RTHβ: In the absence of T₃, occupancy of TRE by TR heterodimers (TR-TRAP) or dimers (TR-TR) suppresses transactivation through association with a corepressor (CoR). (A) T₃-activated transcription mediated by TR-TRAP heterodimers involves the release of the CoR and association with coactivators (CoA) as well as (B) the removal of TR dimers from TRE releases their silencing effect and liberates TREs for the binding of active TR-TRAP heterodimers. The dominant negative effect of a mutant TR (mTR), that does not bind T₃, can be explained by the inhibitory effect of mTR-containing-dimers and
heterodimers that occupy TRE. Thus, T3 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 RTHβ 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 RTHβ in heterozygotes, as tested *in vitro* (75). Structural studies of the DBD and LBD have provided further understanding about the clustered distribution of mTRβs associated RTHβ and defects in the association with cofactors (76-79).

Based on the early finding that RTHβ is associated with mutations confined to the LBD of the TRβ, it was anticipated that the clinical severity of RTHβ would correlate with the degree of T3-binding impairment. While this was true in 12 different natural mTRβs, in 5 others, the severity of RTHβ was lesser despite virtually complete absence T3-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 TRβ with DNA or CoR can produce the same effect.

Less evident was the observation of relatively severe interference with the function of the WT TRβ, despite very mild impairment or no T3-binding defect at all. This was the case when hormone-binding was tested in two mTRβs, located in the hinge region of the receptor (R243Q and R243W) (81). However, reduced T3-binding could be demonstrated after complexing to TRE, indicating a change in the mTRβ configuration when bound to T3 (81,82). Other mechanisms and examples of DNE in the presence of normal or slightly attenuated T3-binding are: decreased interaction of L454V with the CoA (70) and delay of R383H to release the CoR (83). In general the relative degree of impaired function among various mTRβs is similar whether tested using TREs controlled reporter genes that are negatively or positively regulated by T3. Exceptions to this rule are the mTRβs, R383H and R429Q that show greater impairment of transactivation on negatively rather than positively regulated promoters (80,83,84). In this respect these two mTRβs 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 TRβ2 to bind certain coactivators through multiple contact surfaces (88). The result is a decrease in the normal T3-mediated feedback suppression by converting the TRβ2 to a TRβ1-like single mode of coactivator binding. As a consequence, the mutation affects predominantly TRβ2 mediated action. *In vivo* support for a TRβ2 predominant impairment of the mTRβ R429Q was obtained in mice (89). Another possible mechanism for PRTH is a double-hit combining a single nucleotide polymorphism (SNP) and the mTRβ 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 TRβ R338W mutation.
Molecular Basis of the Variable Phenotype of RTHβ

The extremes of the RTHβ phenotype have a clear molecular basis. Subjects heterozygous for a TRβ gene deletion are normal because the expression of a single TRβ allele is sufficient for normal function. RTHβ manifests in homozygotes completely lacking the TRβ gene and in heterozygotes that express a mTRβ with DNE. The most severe form of RTHβ, with extremely high TH levels and signs of both hypothyroidism and thyrotoxicosis, occur in homozygous individuals expressing only mTRβs (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 TRβ 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 RTHβ (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 mTRβ 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 TRβ2 dysfunction have been presented in the section on “Receptor mediated TH action” (see above).

Although in a subset of mTRβs 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 mTRβ 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 mTRβ 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 T₃ 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 TRα 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 RTHβ syndrome is the paucity of specific clinical manifestations. When present, manifestations are variable from one patient to another. Investigations leading to the diagnosis of RTHβ 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 RTHβ.

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 RTHβ 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 RTHβ, the occurrence of RTHβ 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 RTHβ (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

<table>
<thead>
<tr>
<th>FINDINGS</th>
<th>FREQUENCY (%)</th>
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<tr>
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<tr>
<td>Goiter</td>
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<td></td>
</tr>
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<td>33-75</td>
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<td>-------</td>
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<tr>
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<tr>
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<tr>
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<td>Short stature (&lt;5%)</td>
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<tr>
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<td>Low Body mass index (in children)</td>
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</tr>
<tr>
<td>Recurrent Ear and Throat Infections</td>
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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 T₄ and T₃ values range from just above to several fold the upper limit of normal. Although the levels may vary in the course of time in the same patient (45), the T₃ to T₄ ratio remains normal (5). This contrasts with the disproportionate increase in serum T₃ concentration characteristic of autoimmune thyrotoxicosis (122).

Reverse T₃ concentration is also high in patients with RTHβ as is that of another product of T₄ 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 T₄ showed a normal or slightly increased volume of distribution and fractional disappearance rate of the hormone. However, because of the large extrathyroidal pool, the absolute daily production of T₄ and T₃ are increased by about two- to four-fold (2,119,123,124), but the extrathyroidal conversion of T₄ to T₃ 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 RTHβ can be quantitated, even in the presence of reduced thyroidal reserve, using the thyrotroph T₄ resistance index (TT₄RI); the product of serum FT₄, expressed as percent 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 RTHβ (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 RTHβ, 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 RTHβ, 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 RTHβ 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-T4 given in doses of up to 1000 µg/day, and L-T3 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-thyraocetic 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 T4 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 T3 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 T4 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 T4 level. The presence of a high serum T3 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 T₄ measured by automated direct methods but not by equilibrium dialysis may be falsely elevated. A rare cause of elevated serum T₄ and T₃ level is the endogenous production of antibodies directed against these iodothyronines, which can be excluded by direct testing.

Measurement of the serum TSH is an absolute requirement. Under most circumstances, patients with high concentrations of circulating free TH have virtually undetectable serum TSH levels, which fail to respond to TRH. This is true even when the magnitude of TH excess is minimal and therefore subclinical, 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 RTHβ 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 T₃ or free T₄ 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 RTHβ and symptoms suggestive of thyrotoxicosis are not uncommon in RTHβ. 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-T3. For details see text.

While the demonstration of THRB gene mutation is sufficient to establish the diagnosis of RTHβ, a firm exclusion of TRβ involvement includes lack of cosegregation of the THRB haplotype with the phenotype of RTHβ (138), the exclusion of mosaicism (139), and sequencing of TRβ 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-T3, 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-T3. The three doses, given to adults in sequence, are a replacement dose of 50 µg/day and two supraphysiological doses of 100 and 200 µg/day. The hormone is administered in a split dose every 12 hours and each incremental dose is given for the period of 3 days. Doses are adjusted in children and in adults of unusual size to achieve the same level of serum T3 (for details see reference (5)). L-T3, rather than L-T4, is used because of its direct effect on tissues, bypassing potential defects of T4 transport and metabolism, which may also produce attenuated responses. In addition, the more rapid onset and shorter duration of T3 action reduces the period required to complete the evaluation and shortens the duration of symptoms that may arise in individuals with normal responses to the hormone. Responses to each incremental dose of L-T3 are expressed as increments and decrements or as a percent of the value measured at baseline. The results of such a study are shown in Fig. 6.
FIG. 6. Responses to the administration of L-T₃ in subjects with RTHβ, with and without mutations in the THRβ 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-T₃ in patients with RTHβ in the presence (left) or absence (right) of a THRβ 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 THRβ gene mutation, were blunted or paradoxical.

The diagnosis of RTHβ 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 RTHβ from ordinary thyrotoxicosis continues to result in inappropriate treatments. The diagnosis requires awareness of the possible presence of RTHβ, 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 T₄ with nonsuppressed TSH. |
| 2. Confirm the elevated serum level of free T₄ and exclude TH transport defects, especially if T₃ is normal and obtain free T₄ measurement by equilibrium dialysis |
| 3. Obtain tests of thyroid function in first-degree relatives; parents, sibs and children. |
| 4. Sequence the TRβ gene which, when present and shown to have an impaired function, secures the diagnosis of RTH. |
| 5. In the absence of TRβ 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-T₃ protocol, Fig. 6). |
| 7. Blunted TSH response to L-T₃ with absence of TRβ gene mutation indicates nonTR-RTH. |

Treatment

No specific treatment is available to fully and specifically correct the defect. Theoretically, such ideal treatment for RTHβ caused by mutant TRβs 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 THRβ 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 RTHβ, 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 RTHβ 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 RTHβ 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 T4 of mothers with RTHβ 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 β blocking agent, preferably atenolol, would suffice. Treatments with antithyroid drugs or thyroid gland ablation increase TSH secretion and may result in thyrotroph hyperplasia. Development of true pituitary tumors, even after long periods of thyrotroph overactivity, is extremely rare (147).

Treatment with supraphysiological doses of L-T3, given as a single dose every other day, is successful in reducing goiter size without causing side effects (148). Such treatment is preferable considering that postoperative recurrence of goiter is the rule. The L-T3 dose must be adjusted until TSH and TG are suppressed and reduction of goiter size is observed.

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 T3 for some mutant TRβs (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-T4 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 RTHβ 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 $TR\beta$ 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 RTHβ 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**

<table>
<thead>
<tr>
<th>THRA gene Nucleotide #</th>
<th>THR A protein</th>
<th>FT4 % lower limit of normal</th>
<th>FT3 % upper limit of normal</th>
<th>TrT3 % lower limit of normal</th>
<th>TSH mU/L</th>
<th>Known THRB gene mutations in corresponding codons</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td>-----</td>
<td>-----</td>
<td></td>
<td></td>
</tr>
<tr>
<td>806 C&gt;T</td>
<td>A263 V</td>
<td>99*</td>
<td>90*</td>
<td>&lt;63*</td>
<td>4.2*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1075 A&gt;T</td>
<td>N359 Y</td>
<td>114</td>
<td>100</td>
<td>121</td>
<td>0.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1144 delG</td>
<td>A382f sM38 8X</td>
<td>100</td>
<td>140</td>
<td>91</td>
<td>5.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1176 C&gt;A</td>
<td>C392 X</td>
<td>107</td>
<td>148</td>
<td>2.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1193 C&gt;G</td>
<td>P398 R</td>
<td>72</td>
<td>70</td>
<td>0.5</td>
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<td></td>
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</tr>
<tr>
<td>1207 G&gt;T</td>
<td>E403 X</td>
<td>63</td>
<td>80</td>
<td>1.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1207 G&gt;T</td>
<td>E403 X</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1207 G&gt;A</td>
<td>E403 K</td>
<td>106</td>
<td>90</td>
<td>33</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1190 insT</td>
<td>F397fs E406 X</td>
<td>Low Ni</td>
<td>High Ni</td>
<td>I</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

del, deletion; ins, insertion; Ni, 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 TRα2 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 rT3, slightly low or low normal T4, relatively high normal T3 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 \( \text{RTH}\beta \). It has been demonstrated that TH regulation of deiodinase 3 (DIO3) is dependent on TR\( \alpha \) (163). Therefore, subjects with \( \text{RTH}\alpha \) may have a reduced DIO3 resulting in a low \( \text{rT}3 \). The differential diagnosis would include TH cell transport defects, such as MCT8 deficiency, although the lack of severe psychomotor retardation in \( \text{RTH}\alpha \) 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 TR\( \beta \) defects, the mutant TR\( \alpha \) exerts dominant negative effect on the wild-type TR\( \beta 1 \) that binds \( \text{T}_3 \). The first three families reported had mutations that truncated the TR\( \alpha \); these were M338X, E403X and E406X. Functionally, they corresponded to the following mutations in the TR\( \beta \) molecule: M442X, E457X and E460X, one of which, C446X has been reported (see table 5). The latter produce a very severe form of RTH\( \beta \) phenotype (164). More recently missense mutation that involve both the TR\( \alpha 1 \) and TR\( \alpha 2 \) isoforms have been reported, A263V and N359Y (160,161). The mutation affecting the TR\( \alpha 2 \) isoform does not seam to contribute to the phenotype (161). While A382PfsX7 and N359Y both have dominant negative effects on TR\( \alpha 1 \) and TR\( \alpha 2 \), 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 THR A N359Y (160) remain unexplained.

**Treatment**

Given the limited experience with \( \text{RTH}\alpha \), 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 RTH\( \beta \), 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 notwithstanding, have yielded true models of the recessively and dominantly inherited forms of RTH\( \beta \), respectively (165).

The features of RTH\( \beta \) found in patients homozygous for TR\( \beta \) deletion also manifest in the Tr\( \beta \) 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, TR\( \beta \) 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). TR\( \beta 2 \) 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 TRβ2 specific (172). Mice deficient in TRβ 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 TRα1 (91), indicates that TH dependent changes in heart rate are mediated through TRα, and explains the tachycardia observed in some patients with RTHβ.

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 β, 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 TRα1 and β is not due to expression of a yet unidentified TR but to the absence of the noxious effect of unliganded receptors, known as aporeceptors. Indeed, removal of the Thra gene rescues the Pax8 KO mice from death (174). The combined TRβ and TRα deficient mice have serum TSH levels that are 500-fold higher than those of the WT mice, and T4 concentrations 12-fold above the average normal mean (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 RTHβ utilized somatic transfer of a mTRβ1 G345R cDNA by means of a recombinant adenovirus (175). The liver of these mice was resistant to TH, and overexpression of the WT TRβ 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 RTHβ have been generated by targeted mutations in the Thrb gene (176,177). Mutations were modeled on those identified in humans with RTHβ [frame-shift resulting in 16 carboxyterminal 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 T4, T3 and TSH concentrations (156). A more mild form of RTH was identified in mice deficient in RXRγ (155). Animals show reduced sensitivity to L-T3 in terms of TSH downregulation but not in metabolic rate. These data indicate that abnormalities in cofactors can produce RTH. 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 RTHβ phenotype. Similarly, mice with targeted Thra gene mutations failed to manifest the phenotype of RTHβ. Several human mutations known to occur in the THRβ gene were targeted in homologous regions of the Thra gene of the mouse. These are, the PV frame-shift mutation, TRβ1 R384C (equivalent to TRβ R438C) in the and TRβ P398H (equivalent to TRβ P452H) and TRα L400R (corresponding to TRβ454 ) (179). While the resulting phenotypes were somewhat variable, none exhibited thyroid tests abnormalities characteristic of RTHβ. 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.
Patients with THCMTD caused by X-linked MCT8 deficiency are usually boys identified in infancy or in early childhood with feeding difficulties, severe cognitive deficiency, infantile hypotonia and poor head control. They develop progressive spastic quadriplegia, diminished muscle mass with weakness, joint contractures, and dystonia. Early and characteristic thyroid abnormalities are high serum T₃, low T₄, and 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 T₃ 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 T₃ and low rT₃. Serum T₄ 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, T₄S, T₃S, and rT₃S and less the corresponding non-sulfated analogues. OATP1C1 is localized preferentially in brain capillaries and shows a high specificity and affinity towards T₄. The latter suggests that OATP1C1 may be important for transport of T₄ across the blood-brain barrier (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 T₄, T₃, rT₃, and 3,3′-T₂, but it had no effect on the uptake of sulfated T₄, 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 basis 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 T4 measurements, a low concentration could potentially identify new cases. The yield is expected to be low given the high frequency of low T4 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 T3 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](image-url)
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 THRBL 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

<table>
<thead>
<tr>
<th>Type</th>
<th>Numbr of different mutations</th>
<th>Number of families</th>
<th>Effect on MCT8 protein (number or name of different mutations)</th>
</tr>
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<tbody>
<tr>
<td>Substitution</td>
<td>Single nucleotide</td>
<td>37</td>
<td>61 Single a.a. substitution (27 mutations, 45 families). Premature stop (10 mutations, 16 families)</td>
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<tr>
<td>Deletion</td>
<td>Single nucleotide</td>
<td>7</td>
<td>8 FrSh with premature termination (6) and extension with 64 aa (1)</td>
</tr>
<tr>
<td>Trinucleotide</td>
<td></td>
<td>3</td>
<td>7 Single a.a. deletion (F229Δ, F501Δ, F554Δ)</td>
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<tr>
<td>Eight</td>
<td>nucleotides</td>
<td>1</td>
<td>1 FrSh with premature termination</td>
</tr>
<tr>
<td>Fourteen</td>
<td>nucleotides</td>
<td>1</td>
<td>1 FrSh with premature termination</td>
</tr>
<tr>
<td>Large</td>
<td></td>
<td>11</td>
<td>11 Lacking part of the gene</td>
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<tr>
<td>Insertion</td>
<td>Single nucleotide</td>
<td>6</td>
<td>9 FrSh with premature termination</td>
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<tr>
<td>Nucleotides</td>
<td>Count</td>
<td>Type</td>
<td>Description</td>
</tr>
<tr>
<td>-------------</td>
<td>-------</td>
<td>------</td>
<td>-------------</td>
</tr>
<tr>
<td>Four</td>
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<td>1</td>
<td>FrSh with premature termination</td>
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<td>InDel</td>
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<td>1</td>
<td>FrSh with premature termination (c.1678 insA delCC)</td>
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<td>Duplication</td>
<td>Three nucleotides</td>
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<td>Single a.a. insertion (189I, 236V)</td>
</tr>
<tr>
<td>Duplication</td>
<td>Four nucleotides</td>
<td>2</td>
<td>FrSh with premature termination</td>
</tr>
<tr>
<td>Duplication</td>
<td>Six nucleotides</td>
<td>1</td>
<td>Two a.a. insertion (c.127 insGGCAGC -&gt; p.43insGS)</td>
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<td>Duplication</td>
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<td>1</td>
<td>FrSh with premature termination</td>
</tr>
<tr>
<td>Splice site mutation</td>
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<td>1</td>
<td>IVS3as -1 G-&gt;C, alternative splicing and in frame deletion of 94 aa</td>
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<td>Chr translocation involving MCT8</td>
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<td>1</td>
<td>Balanced translocation 46,X,t(X;9)(q13.2;p24)</td>
</tr>
<tr>
<td>Mutations at CpG dinucleotides</td>
<td>8</td>
<td>26</td>
<td>42.6% of 61 families with single nucleotide substitution</td>
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<tr>
<td>in C repeats</td>
<td>5</td>
<td>8</td>
<td>Missense (2), 1nc insertion (1), 1nc deletion (1), InDel (1)</td>
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<tr>
<td>in A repeats</td>
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<td>2</td>
<td>FrSh with premature termination (c.629insA)</td>
</tr>
<tr>
<td>De novo mutations</td>
<td>Total</td>
<td>12a</td>
<td>13a</td>
</tr>
<tr>
<td>In CpGs</td>
<td>3</td>
<td>3</td>
<td>25% of the de novo mutation</td>
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</table>

*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 \textit{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 a 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 \textit{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 97\% 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\textsubscript{3} and low rT\textsubscript{3} concentrations. T\textsubscript{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\textsubscript{4} levels available in 8 cases revealed low values in 6 and normal in 2 (197,202,206). However, low T\textsubscript{4} concentrations at birth are not uncommon, and are more often associated with low levels of T\textsubscript{4}-binding protein and prematurity. Information regarding the T\textsubscript{3} and rT\textsubscript{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\textsubscript{3} and rT\textsubscript{3}, which are higher than those in adults. The ratio of T\textsubscript{3} to rT\textsubscript{3} is characteristically high in MCT8 deficiency while it is low in other causes of abnormal T\textsubscript{3} and rT\textsubscript{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 T₃ levels on peripheral tissues. These are reduced cholesterol, and increased SHBG, and lactate.

Other endocrine tests, including pituitary function were normal when tested in a few individuals. However, administration of incremental doses of L-T₃, using the protocol devised for the study of
patients with RTH, showed reduced pituitary sensitivity to the hormone (202). This is probably due the reduced feedback effect of T₃ on the hypothalamo-pituitary axis, as well as the reduced incremental effect of the hormone on peripheral tissues already exposed to high levels of T₃.

**X-rays and Imaging**

Bone age has been inconsistently reported, and was found 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 T₄ and T₃ 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 T₃-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, T₃-uptake ranged from 8.6 to 33% that of the WT MCT8. In particular, three missense mutations, S194F, L434W, and L598P showed significant residual transport capacity of more than 15% of normal MCT8, which may underlie the relatively milder phenotype observed in patients with these mutations (see section on Clinical Features and Course of the Disease, above).

**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).
Mct8-deficient recombinant (*Mct8KO*) 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 T₃ content showed the variable availability of the circulating hormone to tissues, depending on the redundant presence of TH cell membrane transporters. In *Mct8KO* mice, tissues such as the liver, that express other transporters than Mct8 (10), have high T₃ 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 T₃ content in *Mct8KO* 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 T₃ in the context of TH deficiency and its activity is inversely regulated by TH availability (11). These findings of coexistent T₃ excess and deficiency in the *Mct8KO* mouse tissues explain, in part, the mechanisms responsible for the tissue specific manifestation of TH deficiency and excess in humans with MCT8 deficiency.
FIG. 9. T3 content and its effect in two tissues of Mct8KO and Wt mice. A. T3 content and D1 enzymatic activity in liver. B. T3 content and D2 enzymatic activity in brain. Data from Mct8KO mice are represented as grey bars and those from Wt littermates are in open bars. ** p-value <0.01, *** p-value <0.001.

Mct8 also has a role in TH efflux in the kidney and secretion from the thyroid gland (222,223). The content of T4 and T3 in kidney is increased and their local actions increase D1 activity which enhances the local generation of T3. In the thyroid, Mct8 is localized at the basolateral membrane of thyrocytes. Thyroidal T4 and T3 content is increased in Mct8KO 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 T4 levels and result in increased T3 generation. The important contribution of D1 in maintaining a
high serum T₃ level is supported by the observation in mice deficient in both Mct8 and D1. These mice have a normal serum T₃ and rT₃ (224). The low serum T₄ 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 T₄ concentration but not with the elevated serum T₃ 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 Mct8KO mice, hypothalamic TRH expression is markedly increased and high T₃ doses are needed to suppress it, indicating T₃ resistance particularly at the hypothalamic level.

Mct8KO 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 Mct8KO 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-T₄ and L-T₃, respectively, were required to produce a central effect in the Mct8KO compared to that in Wt animal. These high doses of TH produced “hyperthyroidism” in peripheral tissues of the Mct8KO mice.

The lack of a neurological phenotype in Mct8KO mice limits their use as a model for understanding the mechanisms of the neurological manifestations in patients with MCT8 deficiency. Recently a mouse deficient in 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 T₃ (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 T₃-dependent neuronal function. Moreover, high transcript levels for MCT8 were observed in choroid plexus structures and in capillary endothelial cells, suggesting that MCT8 also contributes to the passage of THs via the blood-brain barrier and/or via the blood-cerebrospinal fluid barrier (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 T₃ elevation does not correlate with the degree of T₃ transport defect produced by a particular MCT8 mutation. This is probably due to the important contribution of the
concomitant perturbation in iodothyronine metabolism on the production of T₃, as demonstrated in the Mct8KO mice (see the section above). Similarly, there is no correlation between the magnitude of serum T₃ elevation or rT₃ reduction in affected males compared to their carrier mothers (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 T₃ transport function (see Clinical Features and Course of the Disease, above). In contrast, early death is more common in patients with mutations that completely disrupt the MCT8 molecule. However, it should be kept in mind that genetic factors, variability in tissue expression of MCT8, and other iodothyronine cell membrane transporters could be responsible for the lack of a stronger phenotype/genotype correlation. The possibility that MCT8 is involved in the transport of other ligands, or has functions other than TH transport, cannot be excluded.

Differential Diagnosis

MCT8-dependent THCMTD is syndromic, presenting a thyroid and a neuropsychomotor component. However, the majority of patients come to medical attention because of retarded development, and neurological deficits. Although the thyroid abnormalities are most characteristic, they escape detection by neonatal screening. TSH concentration is not elevated above the diagnostic cut off level and although T₄ is commonly low, it more often accompanies premature births and low levels of TH-binding serum protein. Studies in Mct8KO mice suggest that rT₃ could turn out to be a good marker for the early detection of MCT8 defects in humans. Hypotonia is an early manifestation, but is not specific. Reduced myelin, documented by brain MRI, places MCT8 in the category of other diseases showing reduced myelination, among 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 T₃ and low rT₃. A reduced (at the low limit or below normal) serum total or free T₄ and a normal or slightly above normal TSH are also present. In cases with increase of T₃ due to other causes, calculating the ratio of T₃/rT₃ 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 loresol. 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-T4. Experience with such treatments is, however, limited to only a few cases.

Detection of low T4 by neonatal screening has led to treatment with L-T4 in several infants. As expected, no improvement has been noted when used in physiological doses, because of the impaired uptake of the hormone by MCT8-dependent tissues. Under these circumstances it would be logical to treat with supraphysiological doses of L-T4 increasing the availability of TH to the brain. However, the presence of an already increased D1, as observed in Mct8 deficient mice (see Animal Models in a preceding section of this Chapter), is likely to aggravate the hypermetabolic state of the patient by generating more T3, from the exogenous L-T4. Therefore, high L-T4 dose treatment has been used in combination with propylthiouracil (PTU), which is a specific inhibitor of D1. This combination treatment results in reduction of the conversion of T4 to T3 by D1 in peripheral tissues while it allows the local generation of T3 by D2 in tissues. Although this treatment allowed an increase in serum L-T4 level without increasing the hypermetabolism and weight loss, it did not improve the neuropsychomotor deficit (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 T4, low T3, high rT3 and normal or slightly elevated serum TSH. In addition they also have decreased serum selenium (Se) and decreased selenoprotein levels and 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 T₄ to T₃. D3, a 5-deiodinase is the main TH inactivator through conversion of T₄ to rT₃ and T₃ to T₂ (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 than that of D1 and D3. T₄ accelerates D2 inactivation through ubiquitination, a reversible process that can regenerate active D2 enzyme through de-ubiquitination.

Deiodinases share with other selenoproteins the synthesis through a unique mode of translation. The codon used for insertion of Sec is UGA, which under most circumstances serves as a signal to stop synthesis. This recoding of UGA is directed by the presence of a selenocysteine insertion sequence (SECIS) in the 3'-untranslated region of the selenoprotein messenger RNA. It is the SECIS-binding protein 2 (in short SBP2) that recognizes the SECIS and recruits an elongation factor and the specific selenocysteine transfer RNA (tRNA^Sec) for addition of Sec at this particular UGA codon (See Fig. 10) (234).

![Diagram](Image)

**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 T₃ 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.

Incidence 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 carboxyterminus. (241) (Table 7).

Table 7. Mutations in the SBP2 gene

<table>
<thead>
<tr>
<th>Family</th>
<th>SBP2 gene</th>
<th>Protein</th>
<th>Comments on putative defect</th>
<th>No of affected</th>
<th>Defect</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>c.1619 G&gt;A</td>
<td>R540 Q</td>
<td>hypomorphic allele</td>
<td>3</td>
<td>homozygous</td>
<td>(8)</td>
</tr>
<tr>
<td>2</td>
<td>c.1312 A&gt;T</td>
<td>K438 X</td>
<td>missing C terminus</td>
<td>1</td>
<td>compound heterozygous</td>
<td>(8)</td>
</tr>
<tr>
<td></td>
<td>IVS8ds+29 G&gt;A</td>
<td>fs</td>
<td>abnormal splicing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>c.382 C&gt;T</td>
<td>R128 X</td>
<td>smaller isoforms*</td>
<td>1</td>
<td>homozygous</td>
<td>(236)</td>
</tr>
<tr>
<td>4</td>
<td>c.358 C&gt;T</td>
<td>R120 X</td>
<td>smaller isoforms*</td>
<td>1</td>
<td>compound heterozygous</td>
<td>(237)</td>
</tr>
<tr>
<td></td>
<td>c.2308 C&gt;T</td>
<td>R770 X</td>
<td>disrupted C-terminus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>c.668delT</td>
<td>F223 fs 255X</td>
<td>truncation and smaller isoforms*</td>
<td>1</td>
<td>compound heterozygous</td>
<td>(238)</td>
</tr>
<tr>
<td></td>
<td>intron 6 -155 delC</td>
<td>fs</td>
<td>abnormal splicing, missing C-terminus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>c.2071 T&gt;C</td>
<td>C691 R</td>
<td>increased proteasomal degradation</td>
<td>1</td>
<td>compound heterozygous</td>
<td>(238)</td>
</tr>
<tr>
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<td>fs</td>
<td>transcripts lacking exons 2-4, or 3-4</td>
<td></td>
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</table>
### 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 T₄, low T₃, 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 T₄, but not T₃, 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 T₄ and T₃ levels, before and during the oral administration of incremental doses of L-T₄ and L-T₃. Note the higher concentrations of T₄ required to reduce serum TSH in the affected subjects; C. In-vitro studies: Deiodinase 2 enzymatic activity and DIO2 mRNA expression.
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 azoosperma; 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\textsuperscript{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). Dio1KO mice have elevated levels of T\textsubscript{4} and rT\textsubscript{3} while the concentrations of T\textsubscript{3} and TSH are unimpaired. Dio2KO mice have significantly elevated serum T\textsubscript{4} and normal T\textsubscript{3} levels but contrary to Dio1KO mice, TSH concentration is elevated. In addition, Dio2KO 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\textsubscript{4}, and rT\textsubscript{3}, reminiscent of the phenotype in SBP2 deficient patients. However, different from the patients, their T\textsubscript{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\textsubscript{4} and decreased T\textsubscript{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 as skin or muscle biopsies. Finding a mutation in the SBP2 gene can be sufficient to provide a diagnosis if the mutation is predicted and/or demonstrated to result in a functionally defective protein or results in failure to synthesize the protein. Linkage analysis in smaller families is particularly helpful in excluding the involvement of SBP2. Failure to detect a SBP2 mutation by sequencing only coding regions of the gene is not sufficient, as putative mutations can exist in introns and regulatory elements. In this case, measuring the TSH responses to incremental doses of L-T4 and/or L-T3 could be used to confirm the biochemical diagnosis of TH metabolism defect, as described in the section on Laboratory Tests.

Treatment

Identification of the metabolic pathway responsible for the phenotype in these patients and the demonstration of defects in the SBP2 gene provided further insight into targeted treatment possibilities. 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-T3 administration was tested in three patients as it was demonstrated to equally suppress serum TSH concentration in affected and unaffected subjects, bypassing the defect (8). Delayed linear growth can be improved with L-T3 supplementation (236), but experience with TH administration in these patients is limited. Other clinical features of SBP2 defects are treated symptomatically.

Acknowledgments

REFERENCES


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


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


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 α or β genes may be due to a defective co-factor. J Clin Endocrinol Metab 1996; 81:4196-4203


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


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

47. Ando S, Sarlis NJ, Krishan J, Feng X, Refetoff S, Zhang MQ, Oldfield 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


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


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


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


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


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 α3 in generalized resistance to thyroid hormone. J Clin Invest 1992; 90:1825-1831

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


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 T3 receptor and transcriptional corepressor. Mol Endocrinol 1997; 11:470-480


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


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

75. Hayashi Y, Sunthornthepvarakul T, Refetoff S. Mutations of CpG dinucleotides located in the triiodothyronine (T3)-binding domain of the thyroid hormone receptor (TR) β 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


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


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-β 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 β, 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


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

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


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 LI. Antipeptide polyclonal antibodies specifically recognize each human thyroid hormone receptor isoform. Endocrinology 1992; 131:2419-2429


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 β gene. J Clin Endocrinol Metab 1994; 78:1525-1528


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

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


110. White P, Burton KA, Fowden AL, Dauncey M. 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


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


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


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


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


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


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


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

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


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


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


164. Groenhout EG, Dorin RI. Severe generalized thyroid hormone resistance due to a sporadic mutation in the c-erbAß gene producing a truncated T3 receptor protein. Paper presented at: Annual Meeting of The Endocrine Society, San Antonio, TX1992
Flamant F, Samarut J. Thyroid hormone receptors: lessons from knockout and knock-in mutant mice. Trends Endocrinol Metab 2003; 14:85-90


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

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 β deficient mice. (Erratum, 141:4767, 2000). Endocrinology 1998; 139:4945-4952

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


Rüssch A, Erway LC, Oliver D, Vennström B, Forrest D. Thyroid hormone receptor β-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


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


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


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


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;


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


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


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


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


