MINIREVIEW

Syndrome of Resistance to Thyroid Hormone: Insights into Thyroid Hormone Action (43951)

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Abstract. **Thyroid hormones (T3, T4) exert multiple cellular effects through nuclear thyroid hormone receptors (TRα, TRβ). Thyroid hormone receptors are transcription factors that act by altering patterns of gene expression. Resistance to thyroid hormone (RTH) is a rare disorder caused by mutations in the TRβ gene. Biochemically, the syndrome is defined by elevated circulating levels of free thyroid hormones due to reduced target tissue responsiveness and normal, or elevated, levels of thyroid-stimulating hormone (TSH). This "inappropriate" TSH elevation contrasts with the situation in hyperthyroidism, where the pituitary secretion of TSH is suppressed. Patients with RTH usually present with goiter and an euthyroid or mildly hypothyroid metabolic state. Thus, pituitary resistance results in hypersecretion of TSH, which compensates, at least in part, for hormone resistance in peripheral tissues. Despite this compensation, clinical effects of RTH can include short stature, delayed bone maturation, hyperactivity, learning disabilities, and hearing defects, as well as variable features of hyper- and hypothyroidism.**

With the exception of a single sibship, which harbored a deletion of the entire coding sequence of the TRβ gene and a recessive pattern of inheritance, all other cases of RTH have been inherited in an autosomal dominant manner or have been *de novo* heterozygous mutations of the TRβ gene. The dominant pattern of inheritance is explained by the functional properties of the mutant receptors which act in a dominant negative manner to block the activity of normal TRα and TRβ receptors. Now that a large number of different RTH mutations have been identified, it is striking that the mutations are clustered within restricted domains in the carboxyterminal region of the receptor. Mutations in these regions have been shown to preserve critical receptor functions such as dimerization and DNA binding, while inactivating other activities such as T3 binding and transcriptional activation. The examination of patients with RTH and their mutated receptors has provided important insights into the mechanisms of thyroid hormone action, the structure-function relationship of the receptors, and the molecular mechanisms of dominant negative activity. [P.S.E.B.M. 1996, Vol 211]

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Molecular and Cellular Basis of Thyroid Hormone Receptor Action

Physiologic Effects of Thyroid Hormones.

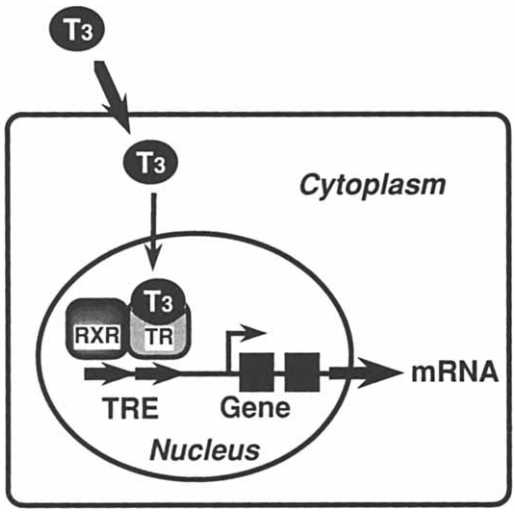
There are two major forms of circulating thyroid hormones, triiodothyronine (T3) and thyroxine (T4). In addition to its synthesis in the thyroid gland, T3

is in major part derived from T4 in peripheral tissues by enzymes referred to as 5′-deiodinases. Although circulating T4 is more abundant, T3 is more potent, because it has a higher affinity for thyroid hormone receptors. Aside from their affinities for the receptors, the hormones appear to have indistinguishable biological actions. Thyroid hormones have myriad physiologic effects, causing alterations in essentially all metabolic pathways and organs as well as effects on cellular differentiation and development (reviewed in Ref. 1).

Some of the most prominent effects of thyroid hormone occur during fetal development and early childhood. In humans, the requirement for thyroid hormone during development is manifest dramatically in the syndrome of cretinism in which fetal hypothyroidism, often combined with maternal hypothyroidism, causes irreversible mental retardation and growth impairment if not treated early. Similarly, childhood hypothyroidism is characterized by striking impairment of linear growth. In adults, the primary effects of thyroid hormone are apparent by alterations in metabolism. Clinical features of hypothyroidism such as slowed mentation and speech, depression, hypothermia, skin changes, bradycardia, constipation, and reproductive dysfunction serve as emphatic reminders that thyroid hormone causes pleiotropic effects on many different organ systems (1).

Thyroid Hormone Acts via Nuclear Receptors to Modulate Gene Expression. Based upon an elegant series of experiments in which injected T3 was shown to induce gene transcription, followed by incorporation of amino acids into protein, Tata suggested in 1966 that alterations in gene expression might be the most proximal step in thyroid hormone action (2). In 1972, nuclear binding sites for thyroid hormone were demonstrated (3), and subsequent experiments showed that the affinity of various thyroid hormones for the receptor paralleled their biologic potencies (4).

In contrast to some of the steroid hormone receptors, thyroid hormone binding does not seem to be required for receptor transport into the nucleus. The receptors are tightly associated with chromatin (5), consistent with their proposed role as DNA binding proteins that act to regulate gene expression. The current model for thyroid hormone action is shown in Figure 1. According to this view, thyroid hormone binds to nuclear receptors which in turn act upon thyroid hormone response elements (TREs) in specific target genes. At least in part, thyroid hormone receptors act in conjunction with thyroid receptor accessory proteins (TRAPs) that dimerize with the TR and enhance their binding to DNA. After activation by thyroid hormone, the receptor causes alterations in gene expression, either stimulating or repressing the transcriptional activity of the target gene. Changes in gene



Thyroid Hormone Action

Figure 1. Model for thyroid hormone action. Thyroid hormones (T4 and T3) enter the cell and bind to nuclear receptors. Thyroid hormone receptors (TRs) interact with thyroid hormone response elements (TREs) in the promoter regions of target genes. TRs typically bind to DNA as homodimers or as heterodimers with partners such as retinoid X receptors (RXRs). Thyroid hormone receptors alter patterns of gene transcription either by stimulating or repressing transcription from specific target genes.

transcription are reflected in alterations in mRNA levels and subsequently, changes in protein biosynthesis. A number of genes that respond to thyroid hormone, either by being activated or repressed, have been identified (1).

Cloning, Structure, and Expression of Thyroid Hormone Receptors. Thyroid hormone receptors were cloned based upon their relationship to other members of the steroid receptor superfamily of nuclear receptors. Sap *et al.* (6) isolated a cDNA encoding a 46-kDa protein now referred to as the thyroid receptor α (TRα) isoform. Weinberger *et al.* (7) isolated a β form of the TR from a human placental library. The TRα and β isoforms are encoded by separate genes that are located on Chromosomes 17 and 3, respectively. Both receptors bind thyroid hormones with high affinity, but they differ in some important properties including their developmental patterns of expression, tissue distributions, and patterns of splicing to create additional isoforms (see below).

Functional Domains of Nuclear Receptors

The overall domain structure of the thyroid hormone receptors is depicted in Figure 2. Major functional domains include the central DNA binding domain and a carboxyterminal ligand binding domain. These domains are remarkably modular as indicated by domain swapping experiments in which the functional features of a specific domain are preserved in

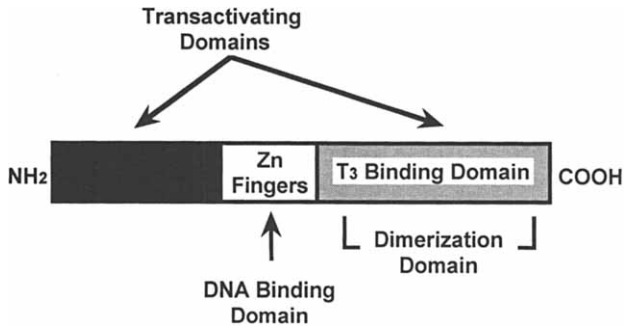


Figure 2. Domain structure of thyroid hormone receptors (TRs). The locations of different TR domains are depicted. The functional properties of these domains are described in the text.

chimeric receptors (8). The carboxyterminus of the receptor also contains nuclear localization signals, dimerization domains, and transactivation functions. The functional properties of the aminoterminal region of the receptor have not been determined, although a role in transactivation is postulated based upon analogy to other nuclear receptors and interactions with transcription factor TFIIB (9).

DNA Binding Domain. The DNA binding domains of nuclear receptors, including the thyroid hormone receptors, are comprised of two distinct zinc fingers in which a single zinc atom is coordinated tetrahedrally with four cysteines. Elegant mutational analyses, in combination with X-ray crystallography and NMR, have defined the key structural determinants for binding to specific DNA sequences. These experiments show that a small stretch of amino acids at the base of the first finger (referred to as the P-box) define the specificity for binding to DNA (10). A second region of the DNA binding domain (referred to as the D-box) is located between the first two cysteines of the second zinc finger, and is thought to be involved in spacing between receptor homodimers. The thyroid hormone receptors can also bind as heterodimers with related proteins such as RXR (11). In the case of heterodimers, distinct dimerization surfaces in the DNA binding domain are used to specify protein-protein interactions. DNA binding domain swapping experiments and x-ray crystallographic studies demonstrate that the D domain of the second zinc finger in RXR still makes contacts with its heterodimeric partner, but the orientation of the TR is rotated such that the RXR D domain contacts the first zinc finger of the TR (12, 13). As discussed below, the arrangement of TRE half-sites specifies how the DNA binding domains will be oriented, which in turn dictates interactions between dimerization surfaces.

Dimerization Domains. In addition to dimerization contacts in the DNA binding domain, a strong dimerization interface also exists in the carboxyterminal region of the receptors. Although there are probably a number of dimerization contacts within this region of the receptor, a series of repeated hydrophobic sequences have been most clearly implicated (14). Mutations in the so-called ninth heptad repeat near the end of the receptor disrupt heterodimerization and adjacent mutations prevent homodimerization (15, 16). Receptor dimerization is important for high-affinity binding of most receptors. Thus, the dimerization domains encode specificity determinants for receptor pairs as well as modulating DNA binding.

Transactivation Domains. The transactivation domains (TADs) are receptor regions that are thought to make contacts with other transcription factors to induce or repress transcription. These domains have been mapped either by showing that the transactivation property can be transferred to another protein and/or by demonstrating that a mutation in the receptor alters transcription without modifying other known properties such as hormone binding, nuclear localization, dimerization, or DNA binding. Although the TADs have not been mapped extensively in the TR, there are domains in the carboxyterminus that are involved in basal repression and in hormone-induced transactivation (17). Recently, there have been some insights into possible molecular mechanisms for TAD action. TRs have been shown to interact with transcription factor TFIIB, which is a key component of the basal transcription machinery (9). The thyroid hormone receptor also interacts with Trip (thyroid-hormone-receptor interacting protein), the homolog of Sug1, which is a co-activator in yeast (18).

Ligand-Binding Domain. The ligand binding pocket of the thyroid hormone receptors has not been clearly defined. Although a 250 amino acid carboxy-terminal fragment is sufficient for high-affinity T3 binding, deletions from either end of this fragment cause loss of hormone binding (19). In addition, a variety of natural and artificial mutations scattered throughout the carboxyterminus reduce or eliminate hormone binding (20, 21). Taken together, these data suggest that extensive tertiary structure is required to form the T3 binding pocket. Of note, T3 binding induces marked conformational changes as reflected by circular dichroism, mobility on nondenaturing gels, or susceptibility to proteases (22). Although the ligand binding domain overlaps the dimerization domains, there is no evidence that dimerization is required for binding or that hormone binding induces dimerization. On certain binding sites (e.g., lap, see below), hormone binding causes homodimers to dissociate from one another and from DNA, presumably because of conformational changes in the protein (23).

Structural Variants of the Thyroid Hormone Receptors. The TRα gene is alternately spliced to result in a number of distinct carboxyterminal protein prod-

ucts (24). One variant, referred to as α2, is identical to TRα1 through the first 370 amino acids, but then the sequences diverge completely reflecting the splicing of alternative exons (Fig. 3). The functional consequences of substituting the carboxyterminal sequences of α1 with those of α2 are profound. First, α2 no longer binds thyroid hormone because of substitution of critical amino acids at the extreme carboxyterminal end of the protein. The α2 isoform has been proposed to be an endogenous inhibitor of thyroid hormone receptor function (25), in part because thyroid hormone action is thought to be blunted in some of the tissues in which α2 is highly expressed. In contrast to the TRα gene, the splicing variants of the TRβ gene involve the aminoterminus of the receptor. One variant, referred to as TRβ2, is expressed predominantly in the pituitary and the hypothalamus (24). In TRβ2, the aminoterminal region of the receptor is distinct, reflecting the use of a tissue-specific promoter as well as alternate splicing. The functional consequences of alterations in the aminoterminus of the thyroid hormone receptor are not known but may involve modulation of translation efficiency, interactions with other cellular proteins (including transcriptional co-factors), tissue-specific expression from different promoters, or control of receptor turnover.

In general, the α and β receptor isoforms are distributed widely and exhibit overlapping patterns of expression. Spleen and testis are notable for their relative deficiencies of α1 and β1 receptors, a feature that is consistent with data indicating that these tissues have minimal metabolic responses to thyroid hormone. The α2 isoform is highly expressed in many tissues, particularly brain, kidney, and testis.

Specific DNA Recognition Sequences for TRs in Target Genes

Characterization of Thyroid Hormone Response Elements (TREs). The thyroid hormone response elements, or TREs, have been delineated by performing detailed mutagenesis of the promoters of thyroid hormone responsive genes (26). Although there is considerable heterogeneity in the TREs of different target genes, certain common features emerge when a large number of such sequences are compared. Most TREs contain two or more "half sites" which correspond to the minimal recognition motif for a receptor monomer. Although thyroid hormone receptors can bind to selected DNA sequences as monomers, they generally bind with greater affinity as homodimers or as heterodimers with structurally related nuclear receptors (see below). The consensus TRE half-site consists of the DNA sequence, AGGTCA. Dimers bind to repeated copies of this minimal element. Remarkably, receptor dimers can bind TRE half sites in a variety of different orientations. For example, half-sites can be arranged as a palindrome, a direct repeat, or as an inverted repeat, also called lap (an eponym created by inversion of *pal*indrome). The ability of TR dimers to bind to TREs in different orientations raises the possibility of flexibility in the determinants of the protein-protein interface. Receptor binding to these repeats is greatly influenced by neighboring nucleotides as well as by the spacing between the elements. In fact, the spacing between elements provides one of the primary determinants of receptor specificity, allowing a half-site sequence that can bind to a large repertoire of nuclear receptors (e.g., thyroid hormone receptors, retinoid receptors, vitamin D receptors) to selectively bind some classes of receptors and not others (27).

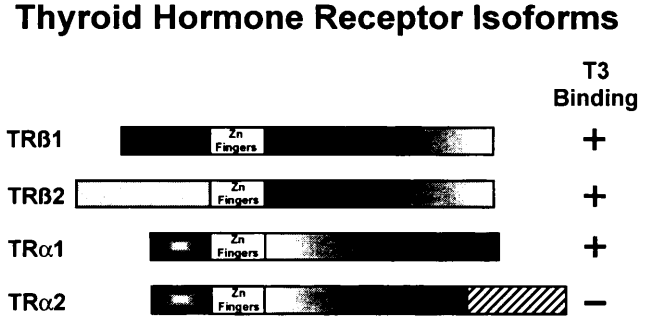


Figure 3. Splicing variants of the TRs give rise to different receptor isoforms. As described in the text, both the TRα and TRβ genes give rise to different receptor isoforms. TRβ1 and TRβ2 differ at the aminoterminus whereas TRα1 and TRα2 differ at the extreme carboxyterminus. T3 binding is absent for the TRα2 isoform.

Interactions of Thyroid Hormone Receptors with Other Nuclear Proteins. As noted above, TRs contain a series of hydrophobic sequences that overlap with the ligand binding domain to comprise dimerization surfaces. In addition, to the thyroid hormone receptor itself, a variety of dimerization partners have been identified, and all are members of the nuclear receptor superfamily. Three such proteins are referred to as RXR α, β, and γ, reflecting their previous identification as retinoic acid receptor related proteins (28). Transcription factor COUP, the peroxisome proliferator activating protein (PPAR), and retinoic acid receptors (RARs) also dimerize with TR, although probably less well than RXRs. The most striking feature of dimerization partners such as RXR proteins is their ability to enhance receptor binding to DNA. The receptor heterodimers are known to form in solution, but are probably stabilized by binding to DNA.

The observation that the thyroid hormone receptors bind to target sequences as heterodimers has major implications for physiologic responses to thyroid hormone action. The tissue distributions of the various RXR isoforms are different and it remains to be seen whether there are differences in transcriptional enhancement of thyroid hormone receptor function by

different partners. Although the RXRs were initially classified as "orphan receptors" because they had no apparent ligand, it is now known that these receptors bind the stereoisomer, 9-*cis* retinoic acid, with high affinity (29). Therefore, ligands that bind to TR dimerization partners have the potential of modulating transcriptional responses to TRs.

Transcriptional Effects of TRs. The steps that occur after TRs bind to TREs are being actively investigated. In the absence of T3, TRs generally have an inhibitory effect on basal gene transcription (17, 26). When T3 binds to its receptor, it induces conformational changes in the receptor which relieve basal repression and cause additional transcriptional enhancement. Thus, the TR acts as a very potent transcriptional switch which can exert both repressive and stimulatory effects on transcription depending upon occupancy by T3. The mechanism of transcriptional induction is postulated to involve transcriptional activation domains (TADs) in the TR which contact transcriptional adaptor proteins or components of the basal transcription apparatus. By interacting with proteins such as TFIIB, the activated TR is thought to recruit rate-limiting proteins to the transcriptional initiation complex, thereby increasing the rate of transcription.

Resistance to Thyroid Hormone (RTH)

History. The concept of resistance to hormones was introduced in 1937 by Albright in his description of pseudohypoparathyroidism (30, 31). Since then, clinical, biochemical and genetic studies of hormone resistance syndromes have flourished. There have been descriptions of resistance to insulin, growth hormone, vasopressin, luteinizing hormone, thyroid stimulating hormone, androgen, estrogen, glucocorticoid, and thyroid hormones (32). These disorders share in common that they are caused by mutations in the hormone receptors.

Resistance to thyroid hormone (RTH) was first reported in 1967 by Refetoff, DeWind, and DeGroot (33). An historical perspective of the discovery of RTH and its further molecular characterization has been published recently (34). In the initial report, Refetoff described two sibs of a consanguineous marriage presenting with goiter, high levels of protein-bound iodine, deaf-mutism, delayed bone age, and stippled epiphyses, without signs of hyperthyroidism (33). Abnormalities in thyroid hormone itself, or its transport into tissues were excluded, and it was hypothesized that target organ resistance was the explanation for the absence of signs and symptoms of thyrotoxicosis. Thyroid hormone receptors had not been characterized at the time of these initial studies. Subsequent studies of thyroid hormone receptors in these and other patients with RTH failed to show consistent abnormalities in receptor binding or numbers. This apparent paradox is now accounted for by the fact that there is more than one type of hormone receptor. Thus, hormone binding to the α receptor is preserved even when mutations involve one, or both, β receptor genes.

The cloning of the two genes encoding thyroid hormone receptors (c-*erb*Aα for TRα on Chromosome 17; c-*erb*Aβ for TRβ on Chromosome 3) in 1986 was fundamental for the further molecular elucidation of the syndrome (6, 7). Subsequently, linkage analysis demonstrated that c-*erb*Aβ is tightly linked to RTH, whereas no association could be demonstrated to the c-*erb*Aα gene (35, 36). The ultimate proof that a defect in the receptor is the cause of RTH was provided in 1989 by the demonstration of mutations in the TRβ gene in affected, but not in unaffected individuals with the disorder (37, 38). This observation has now been confirmed in multiple reports (reviewed in Refs. 20 and 21), and a database has been established (39). Although the true incidence of RTH remains unknown, it appears to be a relatively rare disorder. About 100 families have been reported, but it is likely that the recognition of the disorder will increase with the availability of genetic testing.

Classification. The key finding in RTH is the elevation of thyroid hormones with concomitantly unsuppressed TSH (Fig. 4). Based on clinical features, two forms of RTH are discriminated: general and pituitary resistance to thyroid hormone (GRTH, PRTH) (20). The eponym PRTH was coined in 1975, when

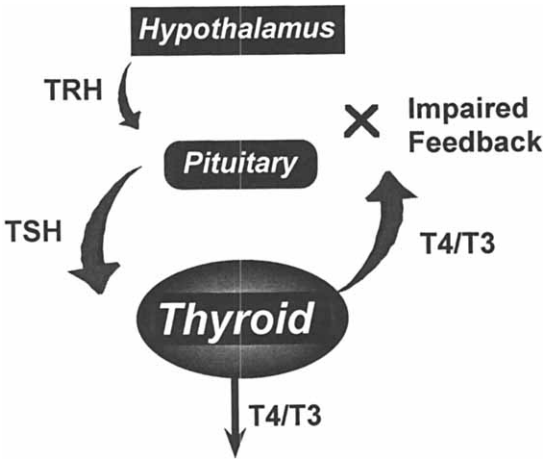


Figure 4. Feedback inhibition of TSH by thyroid hormone is impaired in RTH. Inhibition by thyroid occurs at the hypothalamic (TRH) and pituitary (TSH) levels. In RTH, inhibition is impaired resulting in inappropriately normal TSH levels in the setting of increased circulating T4 and T3. Variable degrees of thyroid hormone resistance also occur in peripheral tissues giving rise to a range of phenotypic variability in the disorder.

Gershengorn and Weintraub reported a patient with the same biochemical characteristics as seen in GRTH, but with associated signs of hyperthyroidism in peripheral tissues, suggesting selective resistance at the level of the pituitary thyrotroph cells (40).

Most patients present with GRTH in which the resistance includes peripheral tissues as well as the thyrotroph cells of the pituitary gland (20, 21). The hyposensitivity of the pituitary results in unsuppressed TSH levels; this TSH elevation in turn leads to an increase of thyroid hormone. The raised thyroid hormone levels overcome, at least in part, the hormone resistant state. Most of the patients with GRTH are therefore in a nearly eumetabolic state. This compensation may, however, be variable among affected individuals, even within the same kindred (41). In addition, signs of hormone excess and deficiency frequently coexist in the same individual. Administration of pharmacological doses of thyroid hormones are required to suppress the basal TSH secretion and they do not necessarily induce the signs of thyrotoxicosis at the level of peripheral tissues (20). Almost all patients with GRTH exhibit goiter, due to chronic stimulation of the thyroid by elevated TSH levels. In some patients with GRTH, signs reminiscent of those encountered in thyroid hormone deprivation during early life are encountered. These can include stunted growth, delayed bone development, learning disabilities, mental retardation, sensorineural deafness, and nystagmus.

Although the distinction between these two forms of RTH seems clearcut, there is considerable overlap between these two phenotypes and spontaneous temporal variations can even be observed in an individual patient (41).

Peripheral tissue resistance to thyroid hormone (PTRTH) has also been reported in a single patient requiring high doses of T3 to maintain an eumetabolic state (42). In this situation, the thyrotrophs of the pituitary gland are spared and there is no compensatory increase in TSH. While there are many anecdotal clinical observations of patients tolerating supraphysiological doses of T3 to achieve a clinically eumetabolic condition, this condition has not been shown to be caused by true hormone resistance.

Inheritance and Incidence. The analysis of the first RTH family suggested an autosomal recessive pattern of inheritance (33). In this family, the disorder has been shown to be caused by a deletion of the entire coding sequence of the TRβ gene. Only the homozygous patients were affected, and the heterozygous relatives had no evidence of RTH (43). All other reported families with RTH exhibit autosomal dominant transmission of the disorder. A single subject who inherited two doses of dominantly acting TRβ alleles from consanguinous parents showed the most severe form of RTH and died at the age of seven (44). This is the only known death that is directly related to RTH. A minority of the patients present with sporadic *de novo* mutations.

Table I. Clinical Features of RTH

Clinical features
Common signs
Elevated free T4 and T3 combined with inappropriately normal or elevated TSH.
Goiter in 95% of patients. Frequent recurrences of goiter and hyperthyroxinemia after ablative therapy.
Variable signs
Phenotypic variability with features compatible with hyper and/or hypothyroidism.
Tachycardia.
Attention deficit hyperactivity disorder (ADHD).
Short stature.
Delayed bone age and dentition.
Deafness.

The true incidence of this rare disease is unknown, as is its prevalence among different ethnic groups. In contrast to many other thyroid diseases which are more frequent in women, RTH occurs with equal frequency in males and females. In a comprehensive review of the syndrome by Refetoff *et al.*, published in 1993, 296 patients of 98 families were listed (20) and the worldwide registry for subjects with RTH, established at the University of Chicago, currently contains data on more than 404 patients.[1]

The clinical features of RTH are outlined in Table I. Goiter and abnormalities in thyroid function tests usually prompt further investigation (20, 41). The most striking feature of the thyroid function tests is the nonsuppressed TSH in the setting of elevated thyroid hormone levels. Since TSH is chronically elevated, an enlargement of the thyroid gland occurs because TSH stimulates cell proliferation and growth as well as thyroid hormone biosynthesis (45). Goiter is present in the vast majority (92%–95%) of patients with RTH and varies from minimal enlargement to the formation of huge goiters with recalcitrant regrowth (46). In the few cases in which no goiter was found, this likely reflects the difficulty of detecting a small enlargement of the thyroid. Because patients with RTH have historically been misdiagnosed with Graves' hyperthyroidism, many have been subjected to thyroid surgery or radioiodine therapy. Recurrence of goiter after thyroid surgery occurs in about 80% of patients (20). As in other instances of long-standing goiter, nodular transformation of the initially diffuse gland can also occur (47).

Roughly one-third of the patients present with

[1] This registry is accessible on the Internet: gopher.uchicago.edu → Subfolder Medicine → RTH registry. (J Clin Endocrinol Metab 79:1560, 1994.

tachycardia. In older patients, atrial fibrillation has been reported in a few patients, and this constellation leads to suspicion of hyperthyroidism, particularly in combination with goiter.

Many of the classical signs and symptoms compatible with hyper- or hypothyroidism can be associated with RTH. In children, short stature, hyperactivity, learning disability, and goiter suggest the possibility of RTH. Although many reports make an association between RTH, growth retardation, and short stature, this classic constellation is relatively uncommon. Of 72 patients with known height, six (8%) were found to be below the third percentile utilizing North American growth standards. Not infrequently, bone age is significantly retarded (<2 SD) in patients with GRTH, consistent with hypothyroidism at the level of the bone (20). In patients displaying signs of toxic effects in peripheral tissues, bone age is rarely accelerated (20).

In a study of 49 affected and 55 unaffected members of 18 families with RTH, a strong association of attention deficit-hyperactivity disorder (ADHD) and RTH was found (50% vs 7% in adults, 70% vs 20% in children) (48). The overall likelihood of having ADHD was 15 times higher in adults and 10 times higher in children with RTH than in the unaffected family members with a similar genetic and environmental background. Consistent with the observation that ADHD is more prevalent in male patients in the general population, the risk of ADHD was 3.2 times higher in males with RTH. Although an ADHD-like syndrome is strongly associated with RTH, RTH is a rare cause of the relatively common problem of ADHD. Other studies have noted a slight reduction in intelligent quotient (IQ) tests in some families with RTH, and it has been suggested that this may confer a higher likelihood of exhibiting ADHD symptoms (49). It is noteworthy that mild mental retardation has also been reported in children with congenital hyperthyroidism, suggesting that high intrauterine levels of thyroid hormones might impair brain development (50, 51). In contrast to patients with RTH, the most prevalent psychiatric diagnoses in patients with hyperthyroidism are anxiety and mood disorders (48).

One of the most striking and puzzling features of RTH is its marked intra- and interfamilial heterogeneity in clinical phenotypes, a phenomenon that has been addressed in several studies (41, 52). Some patients seem to compensate for resistance through elevation of thyroid hormone levels such that their growth and mental development is relatively normal. Other patients with thyroid hormone levels in the same range may present with signs of hormone deficiency and/or excess (20). The degree of impairment of T3 binding of the mutant TR does not correlate well with the severity of organ hyposensitivity. Initially, it was anticipated that differences in the functional impairment due to different mutations would explain the high degree of variation of RTH among affected kindreds (53). However, as an example of these types of analyses, a mutation reducing T3 binding affinity 5-fold caused more severe resistance than a mutation with 100-fold reduced T3 binding affinity (37, 38, 53–55).

Family members harboring an identical mutant TRβ may also present with very variable severity in the clinical presentation, presumably because of genetic variability of other factors contributing to thyroid hormone action (52). Some mutants may produce clinical symptoms in some members of a family while sparing others (56). Noticeable temporal variations have even been reported within individual patients (41). Variable tissue resistance with signs of hyperthyroidism with tachycardia and signs of hypothyroidism with delayed bone age may coexist in the same patient (20, 53). The phenotypic variability within an individual might be due to differing tissue distributions of TRα and TRβ receptors. According to one report the relative proportion of wild type to mutant receptors in a given tissue may vary among individuals and during development (57); others, however, did not find significant differences in the expression of wild-type and mutant receptors (58, 59). The understanding of the clinical variability of RTH at the molecular level is complicated by the fact that various receptor isoforms with variable tissue distribution are present and other nuclear factors are involved in the mediation of thyroid hormone action. The phenotypic variability may also depend on the location of the mutation as well as on compensatory mechanisms and effects of prior therapy.

Because of the presentation of some patients with signs of hyperthyroidism and elevated thyroid hormone levels, the most common misdiagnosis is hyperthyroidism (20). In primary hyperthyroidism due to Graves' disease, or an autonomous uni- or multinodular goiter, the TSH levels are suppressed and are now easily distinguishable from normal levels using ultrasensitive assays. Measurement of thyroid stimulating antibodies or anti-thyroglobulin and anti-peroxidase antibodies, which are often elevated in autoimmune hyperthyroidism, can also be helpful in the discrimination of the two entities. The same biochemical constellation as in RTH can be found with a TSH-secreting pituitary adenoma (60). To confirm or exclude the presence of the latter, CT scan or MRI of the pituitary and measurement of the α-glycoprotein subunit,[2] which is usually increased in a TSH-secreting pituitary adenoma. Abnormalities of thyroid hormone

[2] TSH is formed of an α and β subunit; the α subunit is common to the glycoproteins TSH, follicle stimulating hormone (FSH), and LH (luteinizing hormone).

Table II. Differential Diagnosis of RTH

Disorder	Clinical status	Inheritance	Diagnostic test
Generalized RTH	Euthyroid	Autosomal dominant	Thyroid receptor β mutation
Pituitary RTH	Hyperthyroid	Autosomal dominant	Thyroid receptor β mutation
TSH-secreting Tumor	Hyperthyroid	Sporadic	Increased α-subunit; MRI
FDH	Euthyroid	Autosomal dominant	Serum electrophoresis
TBPA Variant	Euthyroid	Autosomal dominant	Serum electrophoresis
TBG Excess	Euthyroid	X-Linked	↑ TBG
Anti-thyronine Ab	Euthyroid/Hypo	Sporadic	Precipitation of T4 Tracer
Acute systemic illness	Euthyroid	Sporadic	Spontaneous Resolution

Note. FDH, Familial dysalbuminemic hyperthyroxinemia; TBPA, Thyroxine binding prealbumin; TBG, Thyroxine binding globulin.

transport proteins such as an increase of thyroxine binding globulin (TBG), thyroxine binding prealbumin (TBPA), or familial dysalbuminemic hyperthyroxinemia (FDH) are other conditions which have to be differentiated from RTH (Table II) (20).

The variability in clinical presentation and the lack of pathognomonic signs requires a high degree of suspicion for the detection of RTH. Although the diagnosis is usually suspected after documenting elevated thyroid hormone levels together with a "inappropriate," nonsuppressed TSH in association with a goiter (Table III), the formal proof of the diagnosis requires demonstration of hyposensitivity to thyroid hormone action at the level of the pituitary gland as well as in peripheral tissues. This is performed by administering a stepwise increase in T3 over several days (20). An array of parameters of thyroid hormone action can be assessed including basal metabolic rate, systolic time intervals, nitrogen balance, sex hormone–binding globulin, cholesterol, ferritin, bone Gla protein, angiotensin converting enzyme, sodium content of erythrocytes and serum-soluble interleukin-2 receptor (sIL-2R). The effect of T3 on the pituitary TSH secretion is assessed by measuring TSH before and after administration of TRH.

Lastly, the possibility of identifying mutations in the TRβ gene provides a definitive means for making the diagnosis.

Therapy. The diagnosis of RTH and an attempt to distinguish GRTH and PRTH is crucial for defining the therapeutic approach. In patients with GRTH who are in an eumetabolic, compensated state, no treatment is required. In those with signs of hypothyroidism (e.g., children with growth retardation) or in whom misdiagnosis leads to inappropriate ablation of the thyroid gland and thus hypothyroidism, supplementation with levothyroxine is indicated. In these instances, levothyroxine should be administered in supraphysiologic doses to allow for compensation of the resistance. The administration of levothyroxine should aim at normalizing TSH levels, which should be monitored carefully together with other parameters of thyroid hormone action. Normalization of TSH in these patients will also prevent recurrent goiter growth.

In PRTH, reduction of thyroid hormone levels because of toxic signs may be required. Antithyroid drugs are not ideal because their administration results in further increase of the goitrous gland. Compounds suited for this purpose should inhibit TSH secretion together with diminishing thyromimetic actions in peripheral tissues. Bromocriptine or octreotide are of limited value because an escape of their inhibitory effect occurs in the long term (20, 41). Triac (61, 62) and D-T4 (63) have been administered successfully. Triac, as well as 3,5,3′-triiodothyroproprinonic acid (Triprop) have a higher affinity than T3 for the TRβ receptor, but their affinity for TRα receptor is the same (64, 65). In transient transfection assays with several TRβ mutants, these compounds resulted in 50% induction at lower levels compared with T3 and higher maximal transactivation (65). These *in vitro* results suggest that Triac may be able to partially restore the function of a subset of mutant TRβ receptors that retain partial hormone binding. Triprop has analogous properties, but does not seem to be suited for this purpose because it also alters the transactivation of TRα. In view of the spontaneous variations in the dis-

Table III. Diagnosis of RTH

Diagnosis of RTH
Important Diagnostic Features
Elevated free thyroxine with inappropriately normal or elevated TSH
Goiter
Familial pattern of paradoxical nonsuppressed TSH (Except sporadic cases)
Absence of TSH secreting pituitary adenoma
Incompletely suppressed basal and TRH-stimulated TSH after administration of T3
Mutations in the TRβ gene
Features of Altered T3 Action in Peripheral Tissues
Basal metabolic rate
Nitrogen balance
Delayed bone age
Sex hormone binding globulin (SHBG)
Cholesterol
Hydroxyproline excretion
Creatine phosphokinase (CPK)

order, periodic cessation of therapy and reevaluation of the clinical status is indicated and measures aimed at thyroid ablation should only be considered as last resort or in cases with huge goiters (41).

Molecular Pathophysiology of RTH

Localization of TRβ Mutations That Cause RTH. Genetic linkage studies firmly established a relationship between the TRβ gene locus and RTH (35, 36). Subsequently, specific mutations in the TRβ gene confirmed the prediction of the linkage studies (37, 38). Consistent with the autosomal dominant inheritance pattern in all but one kindreds, mutations in the TRβ gene are heterozygous. A large number of different mutations have now been identified (20, 21, 66, 67). When their locations in the TRβ protein are shown in aggregate, it is apparent that the mutations are clustered within restricted domains in the carboxyterminal region of the protein (Fig. 5). In most cases, the mutations are nucleotide substitutions that result in single amino acid replacements. In a few cases, the mutations cause frameshifts, either altering the reading frame, or causing premature termination of translation.

The locations of the reported mutations provide important clues for the molecular pathophysiology of RTH. For example, mutations are not found randomly throughout the receptor. No mutations have been identified within the DNA binding domain or in the amino terminus, with the exception of a large deletion of the entire coding region in a recessive example of RTH (43). The dominant mutations are largely clustered between amino acids 310–347 (domain I) and amino acids 438–461 (domain II) (20, 21). A more limited number of mutations are also found in the so-called hinge region that separates the DNA binding and ligand binding domains (20, 67, 68). A high prevalence of mutations occur in CpG dinucleotide sequences, especially at sites where recurring mutations have been reported (69). Of note, no mutations have been found in the region between the two hot spots, despite the presence of CG-rich sequences within this area. As described below, it is likely that these clusters of mutations represent a requirement to spare the dimerization domain which resides between domains I and II (14).

How Do Mutant Receptors Cause RTH? In most cases, RTH mutations reduce T3 binding to its receptor (20). However, there is a wide spectrum of altered T3 binding, ranging from no detectable binding to little effect on T3 affinity. The simplest explanation for the molecular pathogenesis of RTH could be that absent or reduced hormone response of mutant receptors leads to a relative deficiency of functional receptors, resulting in partial resistance to hormone action. However, in the original kindred with a complete deletion of the coding region of the TRβ gene, the clinical manifestations were only seen in homozygotes with no expression of this gene. In contrast, heterozygotes with a single allele of the wild-type TRβ were clinically and biochemically normal (33, 43). This important piece of information implies that in the presence of TRα, a single copy of the TRβ gene is sufficient for mediating normal thyroid hormone action. In other words, defective function of one TRβ allele is not sufficient to cause thyroid hormone resistance. This observation raises the possibility that RTH is caused by interference of normal receptor function by the mutant receptors, a mechanism referred to as dominant negative inhibition (70). To test this hypothesis, wild-type thyroid hormone receptors were introduced into receptor-deficient cells either in the absence or presence of RTH mutants. As predicted, the mutant TRβ inhibited the ability of wild-type receptors to activate thyroid hormone responsive reporter genes (54, 71). This dominant negative activity of the RTH mutants has been observed using a broad array of positively regu-

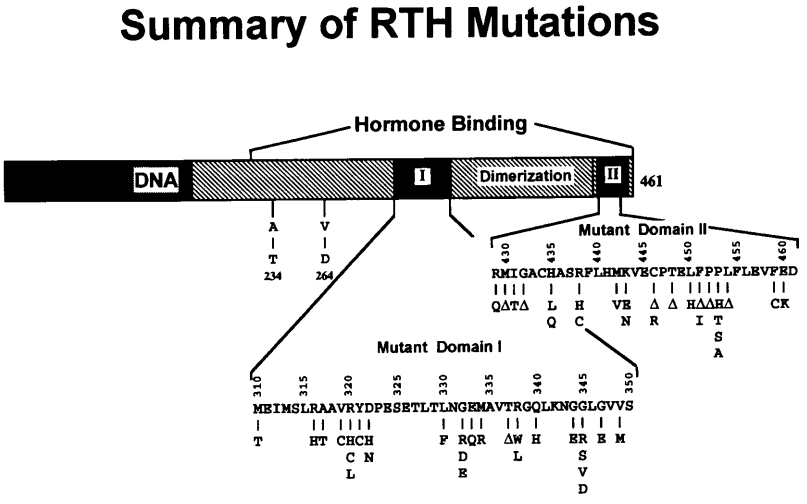


Figure 5. Locations of mutations in TRβ that cause RTH. The structure of the TRβ protein is illustrated schematically. Two regions on either side of the dimerization domain (mutant domains I and II) have been expanded to illustrate the locations of specific amino acid substitutions. In some instances, there have been several independent occurrences of the same amino acid change in different families, but these are not shown.

lated genes as well as genes that are repressed by T3 (54, 55, 66).

Molecular Basis of the Dominant Negative Inhibition by Mutant TRs. Three molecular mechanisms were initially postulated to explain how mutant TRs might inhibit the function of normal receptors (72): (i) mutant receptors might bind to DNA as an inactive complex and compete with normal TRs at the level of the thyroid hormone response elements (TREs) in target genes; (ii) because TRs are known to form homo- and heterodimers, they might be able to dimerize with normal receptors, or other cellular partners, and thereby titrate out these normal proteins by forming inactive dimers; or (iii) mutant TRs might be able to interact with critical downstream targets in the transcriptional machinery and impair access (sometimes called squelching) of normal receptors to these proteins. It should be noted that these mechanisms are not mutually exclusive as it is possible that each of these pathways could contribute to a variable degree to hormone resistance.

Mechanism (i) was tested first as competition for TRE occupancy seemed likely as no mutations were identified within the DNA binding domain. This observation suggested that if mutations occurred in this region, they might preclude dominant negative activity even though they create an inactive receptor that was still able to form dimers with itself and other cellular proteins. To test the requirement for DNA binding in dominant negative activity, mutations were introduced into the first or second zinc finger of the DNA binding domain of the wild type receptor as well as the receptors containing an RTH mutation (73). The DNA binding domain mutations had no effect on the thyroid hormone binding, but did eliminate DNA binding from both the wild-type and RTH mutant receptors when tested in gel mobility shift assays. When co-transfected with wild-type receptors, the DNA binding domain mutation alone did not act in a dominant negative manner, indicating that dimerization alone (Mechanism [ii]) is insufficient to account for dominant negative activity. However, the introduction of the DNA binding mutations into the RTH mutants (double mutants) eliminated the dominant negative potential of the RTH mutant. This finding strongly supports the idea that the dominant negative activity of mutant TRs is due to competition at the level of DNA binding. In this mechanism, the possible competitors may include inactive monomers, complexes of mutant-RXR heterodimers, mutant-mutant homodimers, and also wild-type mutant dimers.

In view of the fact that naturally occurring mutations are confined to the ligand binding domain, but spare the dimerization domain, it still seemed reasonable to test whether dimerization was important for dominant negative potential. To assess the role of dimerization, a novel mutation (L428R) was introduced into the highly conserved ninth heptad repeat of the dimerization domain (15). Unexpectedly, this mutant selectively impaired heterodimerization, without having a detectable effect on receptor homodimerization. This heterodimerization mutant was functionally inactive by itself (loss of T3 binding), but it failed to exert dominant negative activity when co-transfected with wild-type TR. Moreover, when it was inserted into an RTH mutant (i.e., G345R/L428R), the dimerization mutant abrogated the dominant negative activity of the RTH mutant (supporting Mechanism [ii]). Although these findings initially seem to contradict the results found with the DNA binding mutations, a parsimonious explanation is that dimerization is important for high-affinity binding to DNA (Fig. 6). Because this particular dimerization mutant selectively impairs heterodimerization while sparing homodimerization, these results also implicate the heterodimer as a quantitatively important component of the inhibitory complex at the level of DNA binding. The possibility that mutant receptors could also compete for limiting amount of transcriptional co-factors remains to be rigorously tested and could comprise part of the way in which DNA-bound mutants function.

Future Perspectives

It is apparent from the amount of new information that has been accumulated over the last 5 years that there is strong interest in RTH both from the basic science as well as the clinical communities. RTH has been a topic for plenary discussions at most recent endocrinology meetings and two international workshops have been held recently in an effort to speed progress even further. Despite the advances in molecular pathophysiology and the intense interest in this syndrome, a number of important issues remain unresolved. Para-

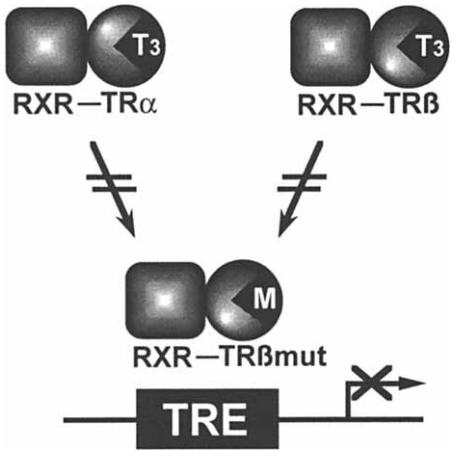


Figure 6. Molecular pathophysiology of RTH. Mutant TRβ retain the ability to dimerize and to bind to TREs. Access of wild-type receptors to thyroid hormone responsive genes is impaired by the inactive mutant receptors.

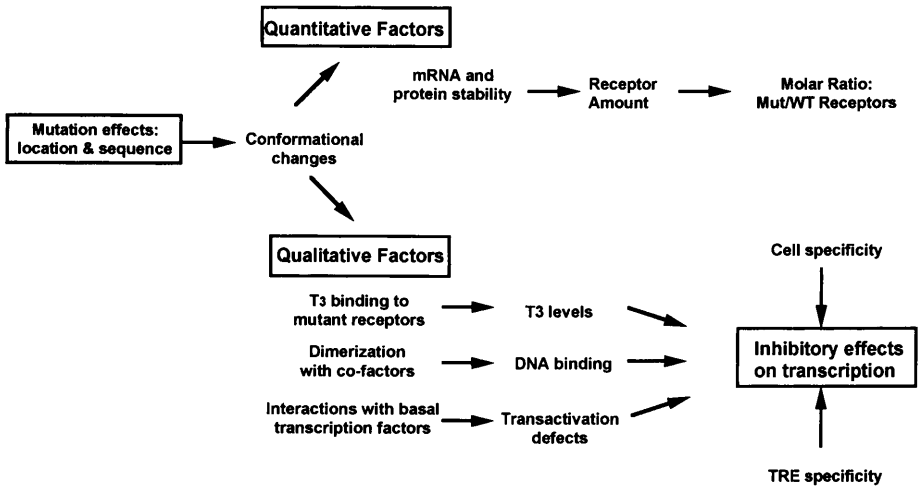


Figure 7. Factors that contribute to dominant negative activity in RTH. Some of the different quantitative and qualitative factors that contribute to phenotypic variability in RTH are illustrated.

mount among these is our inability to fully explain the phenotypic variability that occurs in RTH. As depicted in Figure 7, an array of quantitative factors (such as levels of receptor expression in different tissues) and qualitative factors (such as specific effects that mutations have on mutant receptor properties) may contribute to variations in hormone resistance. An important challenge in the future is to determine whether there is any correlation between genotype and phenotype. Although such an association has not yet emerged, this may be because we still do not have highly quantitative means of classifying RTH aside from thyroid function tests (which mainly assess hypothalamic/pituitary resistance).

It is also apparent that the syndrome of RTH is intensifying the debate about how normal TRs function. Are there specific functions for different receptor isoforms? Do receptor monomers, homodimers, and heterodimers each play a role in thyroid hormone action? If so, do these various species of receptor account in part for variable degrees of hormone resistance for different target genes? The list of thyroid hormone responsive target genes continues to accumulate. However, we are still lacking effective peripheral tissue markers of thyroid hormone action that can be used clinically. Finally, a long-term goal is to use these new molecular insights to intervene therapeutically when necessary. In this regard, it is important to understand how much of the RTH phenotype is established during early development versus postnatally when it is more practical to intervene. Transgenic models of RTH may help to address this issue. Given that ADHD is more prevalent in RTH, we need to understand its cause: Is it because regions of the central nervous system are too resistant (and relatively hypothyroid), or because that are not resistant enough for the circulating levels of thyroid hormone (and relatively hyperthyroid)? Clearly, there are many exciting prospects and goals for future work.

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1. Jameson J, DeGroot L. Mechanisms of thyroid hormone action. In: DeGroot LJ, Ed. Endocrinology (3rd ed). Philadelphia: W. B. Saunders, pp583–601, 1994.
2. Tata JR, Widnell CC. Ribonucleic acid synthesis during the early action of thyroid hormone. Biochem J **98**:604–620, 1966.
3. Shadlow AR, Surks MI, Schwartz HL, Oppenheimer JH. Specific triiodothyronine binding sites in the anterior pituitary of the rat. Science **176**:1252–1254, 1972.
4. Koerner D, Schwartz HL, Surks MI, Oppenheimer JH. Binding of selected iodothyronine analogues to receptor sites of isolated rat hepatic nuclei. High correlation between structural requirements for nuclear binding and biological activity. J Biol Chem **250**(16):6417–6423, 1975.
5. MacLeod KM, Baxter JD. Chromatin receptors for thyroid hormones: Interactions of the solubilized proteins with DNA. J Biol Chem **251**:7380–7387, 1976.
6. Sap J, Munoz A, Damm K, Goldberg Y, Ghysdael J, Leutz A, Beug H, Vennstrom B. The c-*erb*-A protein is a high-affinity receptor for thyroid hormone. Nature **324**(6098):635–640, 1986.
7. Weinberger C, Thompson CC, Ong ES, Lebo R, Gruol DJ, Evans RM. The c-*erb*-A gene encodes a thyroid hormone receptor. Nature **324**(6098):641–646, 1986.
8. Thompson CC, Evans RM. Trans-activation by thyroid hormone receptors: Functional parallels with steroid hormone receptors. Proc Natl Acad Sci USA **86**(10):3494–3498, 1989.
9. Baniahmad A, Ha I, Reinberg D, Tsai S, Tsai MJ, O'Malley BW. Interaction of human thyroid hormone receptor beta with transcription factor TFIIB may mediate target gene derepression and activation by thyroid hormone. Proc Natl Acad Sci USA **90**(19):8832–8836, 1993.

10. Umesono K, Evans RM. Determinants of target gene specificity for steroid/thyroid hormone receptors. Cell **57**(7):1139–1146, 1989.
11. Yu VC, Delsert C, Andersen B, Holloway JM, Devary OV, Naar AM, Kim SY, Boutin JM, Glass CK, Rosenfeld MG. RXR beta: A coregulator that enhances binding of retinoic acid, thyroid hormone, and vitamin D receptors to their cognate response elements. Cell **67**(6):1251–1266, 1991.
12. Zechel C, Shen XQ, Chambon P, Gronemeyer H. Dimerization interfaces formed between the DNA binding domains determine the cooperative binding of RXR/RAR and RXR/TR heterodimers to DR5 and DR4 elements. Embo J **13**(6):1414–1424, 1994.
13. Rastinejad F, Perlmann T, Evans RM, Sigler PB. Structural determinants of nuclear receptor assembly on DNA direct repeats. (see comments) Nature **375**(6528):203–211, 1995.
14. Forman BM, Samuels HH. Interactions among a subfamily of nuclear hormone receptors: The regulatory zipper model. Mol Endocrinol **4**:1293–1301, 1990.
15. Nagaya T, Jameson JL. Thyroid hormone receptor dimerization is required for dominant negative inhibition by mutations that cause thyroid hormone resistance. J Biol Chem **268**(21):15766–15771, 1993.
16. Au-Fliegner M, Helmer E, Casanova J, Raaka B, Samuels H. The conserved ninth C-terminal heptad in thyroid hormone and retinoic acid receptors mediates diverse responses by affecting heterodimer but not homodimer formation. Mol Cell Biol **13**:5725–5737, 1993.
17. Baniahmad A, Kohne AC, Renkawitz R. A transferable silencing domain is present in the thyroid hormone receptor, in the v-*erb*A oncogene product and in the retinoic acid receptor. Embo J **11**(3):1015–1023, 1992.
18. Lee J, Ryan F, Swaffield J, Johnston S, Moore D. Interaction of thyroid hormone receptor with a conserved transcriptional mediator. Nature **374**:91–94, 1995.
19. Lin KH, Parkison C, McPhie P, Cheng SY. An essential role of domain D in the hormone-binding activity of human beta 1 thyroid hormone nuclear receptor. Mol Endocrinol **5**(4):485–492, 1991.
20. Refetoff S, Weiss RE, Usala SJ. The syndromes of resistance to thyroid hormone. Endocr Rev **14**(3):348–399, 1993.
21. Adams M, Matthews C, Collingwood TN, Tone Y, Beck-Peccoz P, Chatterjee KK. Genetic analysis of 29 kindreds with generalized and pituitary resistance to thyroid hormone. Identification of thirteen novel mutations in the thyroid hormone receptor beta gene. J Clin Invest **94**(2):506–515, 1994.
22. Toney JH, Wu L, Summerfield AE, Sanyal G, Forman BM, Zhu J, Samuels HH. Conformational changes in chicken thyroid hormone receptor alpha 1 induced by binding to ligand or to DNA. Biochemistry **32**(1):2–6, 1993.
23. Yen PM, Darling DS, Carter RL, Forgione M, Umeda PK, Chin WW. Triiodothyronine (T3) decreases binding to DNA by T3-receptor homodimers but not receptor-auxiliary protein heterodimers. J Biol Chem **267**(6):3565–3568, 1992.
24. Lazar MA. Thyroid hormone receptors: Multiple forms, multiple possibilities. Endocr Rev **14**:184–193, 1993.
25. Koenig RJ, Lazar MA, Hodin RA, Brent GA, Larsen PR, Chin WW, Moore DD. Inhibition of thyroid hormone action by a non-hormone binding c-*erb*A protein generated by alternative mRNA splicing. Nature **337**:659–661, 1989.
26. Brent GA, Moore DD, Larsen PR. Thyroid hormone regulation of gene expression. Annu Rev Physiol **53**(17):17–35, 1991.
27. Glass C. Differential recognition of target genes by nuclear receptor monomers, dimers, and heterodimers. Endocr Rev **15**:391–407, 1994.
28. Lazar MA. Thyroid hormone receptors: Update 1994. Endocr Rev **3**:280–283, 1994.
29. Heyman RA, Mangelsdorf DJ, Dyck JA, Stein RB, Eichele G, Evans RM, Thaller C. 9-*cis* retinoic acid is a high affinity ligand for the retinoid X receptor. Cell **68**:397–406, 1992.
30. Albright F, Butler AM, Bloomber E. Rickets resistant to vitamin D therapy. Am J Dis Child **54**:531–547, 1937.
31. Albright F, Burnett C, Smith P, Parson W. Pseudohypoparathyroidism—An example of "Seabright-Bantam syndrome." Endocrinology **30**:922–932, 1942.
32. Jameson J. Applications of molecular biology in endocrinology. In: DeGroot LJ, Ed. Endocrinology (3rd ed). Philadelphia: W. B. Saunders, pp119–147, 1994.
33. Refetoff S, DeWind L, DeGroot L. Familial syndrome combining deaf-mutism, stippled epiphyses, goiter, and abnormally high PBI; Possible target organ refractoriness to thyroid hormone. J Clin Endocrinol Metab **27**:279–294, 1967.
34. Refetoff S. Resistance to thyroid hormone: An historical overview. Thyroid **4**:345–349, 1994.
35. Usala SJ, Tennyson GE, Bale AE, Lash RW, Wondisford FE, McBride OW, Weintraub BD. Tight linkage between the syndrome of generalized thyroid hormone resistance and the human c-*erb*Aβ gene. Mol Endocrinol **2**:1217–1220, 1988.
36. Fein HG, Burman KD, Djuh YY, Usala SJ, Bale AE, Weintraub BD, Smallridge RC. Tight linkage of the human c-*erb*A beta gene with the syndrome of generalized thyroid hormone resistance is present in multiple kindreds. J Endocrinol Invest **14**(3):219–223, 1991.
37. Sakurai A, Takeda K, Ain K, Ceccarelli P, Nakai A, Seino S, Bell GI, Refetoff S, DeGroot LJ. Generalized resistance to thyroid hormone associated with a mutation in the ligand-binding domain of the human thyroid hormone receptor β. Proc Natl Acad Sci USA **86**:8977–8981, 1989.
38. Usala SJ, Tennyson GE, Bale AE, Lash RW, Gesundheit N, Wondisford FE, Accili D, Hauser P, Weintraub BD. A base mutation of the C-*erb*A beta thyroid hormone receptor in a kindred with generalized thyroid hormone resistance. Molecular heterogeneity in two other kindreds. J Clin Invest **85**(1):93–100, 1990.
39. Beck-Peccoz P, Chatterjee VKK, Chin WW, DeGroot LJ, Jameson JL, Nakamura H, Refetoff S, Usala SJ, Weintraub BD. Nomenclature of thyroid hormone receptor β-gene mutations in resistance to thyroid hormone: Consensus statement from the first workshop on thyroid hormone resistance, July 10–11, 1993, Cambridge, UK. J Clin Endocrinol Metab **78**:990–993, 1994.
40. Gershengorn MC, Weintraub BD. Thyrotropin-induced hyperthyroidism caused by selective pituitary resistance to thyroid hormone. A new syndrome of "inappropriate secretion of TSH." J Clin Invest **56**(3):633–642, 1975.
41. Beck-Peccoz P, Chatterjee VKK. The variable clinical phenotype in thyroid hormone resistance syndrome. Thyroid **4**:225–231, 1994.
42. Kaplan MM, Swartz SL, Larsen PR. Partial peripheral resistance to thyroid hormone. Am J Med **70**:1115–1121, 1981.
43. Takeda K, Sakurai A, DeGroot LJ, Refetoff S. Recessive inheritance of thyroid hormone resistance caused by complete deletion of the protein-coding region of the thyroid hormone receptor–beta gene. J Clin Endocrinol Metab **74**(1):49–55, 1992.
44. Ono S, Schwartz ID, Mueller OT, Root AW, Usala SJ, Bercu BB. Homozygosity for a "dominant negative" thyroid hormone receptor gene responsible for generalized resistance to thyroid hormone. J Clin Endocrinol Metab **73**:990–994, 1991.
45. Dumont JE, Maenhaut C, Pirson I, Baptist M, Roger PP. Growth factors controlling the thyroid gland. Baillère's Clin Endocrinol Metab **5**:727–754, 1991.
46. Agerbaek H. Congenital goiter presumably resulting from tissue resistance to thyroid hormones. Israel J Med **8**:1859–1860, 1973.
47. Kopp P, Kimura ET, Aeschimann S, Oestreicher M, Tobler A, Fey MF, Studer H. Polyclonal and monoclonal thyroid nodules

coexist within human multinodular goiters. J Clin Endocrinol Metab **79**:134–139, 1994.
48. Hauser P, Zametkin AJ, Martinez P, et al. Attention deficit-hyperactivity disorder in people with generalized resistance to thyroid hormone. N Engl J Med **328**(14):997–1001, 1993.
49. Weiss RE, Stein MA, Duck SC, et al. Low intelligence but not attention deficit hyperactivity disorder is associated with resistance to thyroid hormone caused by mutation R316H in the thyroid hormone receptor beta gene. J Clin Endocrinol Metab **78**(6):1525–1528, 1994.
50. Hollingsworth DR. Neonatal hyperthyroidism. In: Delange F, Fisher DA, Malvaux P, Ed. Pediatric Thyroidology. Basel: Karger, Vol **14**:pp210–222, 1985.
51. Kopp P, van Sande J, Parma J, Duprez L, Gerber H, Joss E, Jameson JL, Dumont JE, Vassart G. Congenital hyperthyroidism caused by a mutation in the thyrotropin-receptor gene. N Engl J Med **332**:150–154, 1995.
52. Weiss RE, Marcocci C, Bruno-Bossio G, Refetoff S. Multiple genetic factors in the heterogeneity of thyroid hormone resistance. J Clin Endocrinol Metab **76**:257–259, 1993.
53. Magner JA, Petrick P, Menezes-Ferreira MM, Stelling M, Weintraub BD. Familial generalized resistance to thyroid hormones: Report of three kindreds and correlation of patterns of affected tissues with the binding of [^{125}I] triiodothyronine to fibroblast nuclei. J Endocrinol Invest **9**(6):459–470, 1986.
54. Chatterjee VKK, Nagaya T, Madison LD, Datta S, Rentoumis A, Jameson JL. Thyroid hormone resistance syndrome. Inhibition of normal receptor function by mutant thyroid hormone receptors. J Clin Invest **87**:1977–1984, 1991.
55. Meier CA, Dickstein BM, Ashizawa K, McClaskey JH, Muchmore P, Ransom SC, Menke JB, Hao EH, Usala SJ, Bercu BB, Cheng SY, Weintraub BD. Variable transcriptional activity and ligand binding of mutant β1 3,5,5′-triiodothyronine receptors from four families with generalized resistance to thyroid hormone. Mol Endocrinol **6**:248–258, 1992.
56. Geffner M, Su F, Ross N, Hershman JM, van Dop C, Menke JB, Hao E, Stanzak RK, Eaton T, Samuels HH. An arginine to histidine mutation in codon 311 of the C-*erb*Aβ gene results in a mutant thyroid hormone receptor that does not mediate a dominant negative phenotype. J Clin Invest **91**:538–546, 1993.
57. Mixson AJ, Hauser P, Tennyson G, Renault JC, Bodenner DL, Weintraub BD. Differential expression of mutant and normal beta T3 receptor alleles in kindreds with generalized resistance to thyroid hormone. J Clin Invest **91**(5):2296–2300, 1993.
58. Hayashi Y, Janssen OE, Weiss RE, Murata Y, Seo H, Refetoff S. The relative expression of mutant and normal thyroid hormone receptor genes in patients with generalized resistance to thyroid hormone determined by estimation of their specific messenger ribonucleic acid products. J Clin Endocrinol Metab **76**:64–69, 1993.
59. Klann RC, Torres B, Menke JB, Holbrook CT, Bercu BB, Usala SJ. Competitive polymerase chain reaction quantitation of c-*erb*A beta 1, c-*erb*A alpha 1, and c-*erb*A alpha 2 messenger ribonucleic acid levels in normal, heterozygous fibroblasts of kindred S with thyroid hormone resistance. J Clin Endocrinol Metab **77**:969–975, 1993.
60. Jameson JL, Kay TWH. Glycoprotein hormone pituitary tumors. In: Mazzaferri E, Ed. Advances in Endocrinology and Metabolism. New York: Mosby-Year Book, Vol **2**:pp125–147, 1991.
61. Beck-Peccoz P, Piscitelli G, Cattaneo MG, Fagila G. Successful treatment of hyperthyroidism due to nonneoplastic pituitary TSH hypersecretion with 3,5,3′-triiodothyroacetic acid (TRIAC). J Endocrinol Invest **6**:217–223, 1983.
62. Salmela PI, Wide L, Juustila H, Ruokonen A. Effects of thyroid hormones (T4, T3), bromocriptine and TRIAC on inappropriate TSH hypersecretion. Clin Endocrinol (Oxford) **28**:497–507, 1988.
63. Dorey F, Strauch G, Gayno J. Thyrotoxicosis due to pituitary resistance to thyroid hormones. Successful control with D-thyroxine: A study in three patients. Clin Endocrinol (Oxford) **32**:221–227, 1990.
64. Schueler PA, Schwartz HL, Strait KA, Mariash CN, Oppenheimer JH. Binding of 3,5,3′-triiodothyronine (T3) and its analogs to the in vitro translational products of c-*erb*A protooncogenes: differences in the affinity of the α- and β-forms for the acetic acid analog and failure of the human testis and kidney α-2 products to bind T3. Mol Endocrinol **4**:227–234, 1990.
65. Takeda T, Suzuki S, Liu RT, DeGroot LJ. Triiodothyroacetic acid (Triac) has unique potential for therapy of resistance to thyroid hormone. J Clin Endocrinol Metab **80(7)**:2033–2040, 1995.
66. Collingwood TN, Adams M, Tone Y, Chatterjee VK. Spectrum of transcriptional, dimerization, and dominant negative properties of twenty different mutant thyroid hormone beta-receptors in thyroid hormone resistance syndrome. Mol Endocrinol **8**(9): 1262–1277, 1994.
67. Matthews C, Collingwood TN, Adams M, Rajanayagam O, Chatterjee VKK. Novel mutations in the hinge domain of the thyroid hormone β receptor and a transactivation mutant in thyroid hormone resistance syndrome. 77th annual meeting of the Endocrine Society, Washington, DC. Abstract P3–437:578, 1995.
68. Behr M, Loos U. A point mutation (Ala229 to Thr) in the hinge domain of the c-*erb*A beta thyroid hormone receptor gene in a family with generalized thyroid hormone resistance. Mol Endocrinol **6**(7):1119–1126, 1992.
69. 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 **91**(6): 2408–2415, 1993.
70. Herskowitz I. Functional inactivation of genes by dominant negative mutations. Nature **329**:219–222, 1987.
71. Sakurai A, Miyamoto T, Regetoff S, DeGroot LJ. Dominant negative transcriptional regulation by a mutant thyroid hormone receptor–beta in a family with generalized resistance to thyroid hormone. Mol Endocrinol **4**(12):1988–1994, 1990.
72. Jameson JL. Thyroid hormone resistance: Pathophysiology at the molecular level. J Clin Endocrinol Metab **74**(4):708–711, 1992.
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 **267**(18):13014–13019, 1992.