Homozygous Thyroid Hormone Receptor β-Gene Mutations in Resistance to Thyroid Hormone: Three New Cases and Review of the Literature

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Context: The most common cause of resistance to thyroid hormone (RTH) is heterozygous thyroid hormone receptor β (THRB) gene mutations. Homozygous mutations in the THRB gene are a rare event.

Objective: In this study, the clinical findings of three new patients (belonging to two families) homozygous for mutations in the THRB gene are compared to three other families in which affected individuals lack a normal TRβ.

Methods: We conducted clinical studies and genetic analyses.

Results: The clinical presentation in all three homozygous subjects was unusually severe; their phenotype was characterized by compromised intellectual development, tachycardia, goiter, growth retardation, and hearing loss. This was comparable with one other reported patient homozygous for mutant TRβ, but not in RTH due to THRB gene deletions.

Conclusion: We report three new subjects, from two families, in whom RTH was associated with homozygous mutations in the THRB gene. They represent an important addition to the single known patient homozygous for a mutant TRβ. The clinical and laboratory abnormalities indicate a strong dominant-negative effect and are in agreement with data obtained from mice expressing a mutant Thrb in both alleles. This report strengthens the concept that the mutated TRβ interferes with the function of the TRα1 in humans. (J Clin Endocrinol Metab 97: 1328–1336, 2012)
We now report two families with RTH in which three subjects are homozygous for two different point mutations in the \textit{THRB} gene. The clinical manifestations of these three individuals are more severe not only than all heterozygotes reported but also those homozygotes for gene deletion, indicating a deleterious effect of occupancy of TH response elements (TRE) by unliganded receptor and possibly the interference with TRα function.

\section*{Patients and Methods}

\subsection*{Cases Reports}

\subsection*{Family Mbyd}

The proposita (subject IV-I; Fig. 1A), with XX karyotype, was born to a 17-yr-old single primigravida of mixed Australian Aborigine and White European ancestry. The mother of the proposita (III-1) revealed that her maternal uncle (II-1) was the father of the proposita. The weight at birth was 2470 g. The

\begin{figure}
\centering
\includegraphics[width=\textwidth]{image1.png}
\caption{Pedigrees showing the genotype and T3 results. Results are aligned with each symbol. Values represent the mean of two determinations on separate samples collected several months apart. In two instances (A, II-1 and IV-1), when samples were obtained almost 4 yr apart, both values are given. Ages represent those at the time of blood sampling. Tx, Thyroidectomy.}
\end{figure}
neonate was not frankly dysmorphic but had a prominent nasal bridge, backward-sloping forehead, and overriding fourth toes and was later noted to have an intermittent squint. No goiter was appreciated on physical examination. Because of very high serum TSH levels, a 99mTc-percotechnetate scan was obtained, which showed a thyroid gland of normal size, contour, and position with increased uptake. Treatment with a β-blocker for tachycardia was started at 1 wk of age.

At 1 month of age, brainstem-evoked response audiometry showed a moderate mixed hearing loss bilaterally: air and bone conduction at 2000 Hz was 50 and 35, respectively, for both ears [sensorineural component considered to be mild to moderate at 2000 Hz]. The proposita was fitted with hearing aids at 3 yr and 2 months of age, the proband underwent an auditory brainstem investigation. At 3 yr and 3 months of age, she was started on propranolol (20 mg/d) because of sinus tachycardia. She had decreased visual attention while performing motor tasks and tended not to look at the task she was performing. She required help with expressive speech and chewing. She produced long utterances using appropriate sentence-like intonation with unintelligible words. Her receptive language abilities were appropriate when compared with her peers with hearing impairment, but the rate of progress indicated that she was at risk of developing receptive language delay. Overall, her speech skills were delayed, and she was not participating in conversations with others. IQ score [Wechsler Preschool and Primary Scale of Intelligence (WPPSI) III test] was 72. Bone age at 3 yr was 9 months.

No facial dysmorphism was present (Fig. 2A), but a diffuse visible goiter was now evident (Fig. 2B). TFT at that time are also shown in Fig. 1A.

At 4 yr and 8 months, her IQ score (WPPSI III test) was 61. Her height was 92.2 cm (<3rd centile), weight was 13 kg (<3rd centile), and the head circumference is 47.2 cm (3rd centile). Bone age at 4 yr remained at 9 months. Testing with Interacoustics Diagnostic Audiometer (AD28) indicated a hearing loss of 45 dB at 500 Hz, sloping to 55 dB at 4000 Hz on the right and left ears.

**Family members.** The mother (III-1) had a normal serum TSH but high T₄ and T₃ levels (Fig. 1A). Her height, weight, and body mass index were 159.6 cm, 80.2 kg, and 31.5 kg/m², respectively. She did not have tachycardia, but she had a small goiter and a mild hearing deficit. She completed secondary school but for 3 yr was placed in a support class.

The proposita’s designated father had a hearing deficit of unknown etiology and was on levothyroxine (L-T₄) treatment for an undetermined thyroid problem. He declined any form of investigation.

Family members I-1, II-2, and III-2 had elevated serum iodothyronine concentrations and normal TSH as shown in Fig. 1A, all had hearing impairment.

**Family Mozv**

The proposita (IV-2; Fig. 1B), with XX karyotype, was the second child born to consanguineous Turkish parents. She had dysmorphic features consisting of birdlike facies with prominent nasal bridge (Fig. 2, C and D) and hearing impairment (evoked responses, 60 dB normalized hearing level right ear, and at 65 dB left ear) requiring hearing aids.

At 3 yr and 3 months of age, she was started on propranolol (20 mg/d) because of sinus tachycardia. She had TFT compatible with RTH, and she was noted to have a goiter.

At 7 yr of age, serum concentration of free T₄ (FT₄) was greater than 6.0 ng/dl (normal range, 0.7–1.9 mU/liter), free T₃ (FT₃) was greater than 30 pg/ml (normal range, 1.8–4.2...
mU/liter), and TSH was 14.4 mU/liter. To lower the TSH, 0.26 mg triiodothyroacetic acid (TRIAC) was given in three divided doses, and 1 yr later, the dose was doubled.

At 8.75 yr of age, because of a further increase in goiter size and the presence of macronodules, she underwent thyroidectomy and was given l-T₄ 75 µg/d. Pathological diagnosis was diffuse adenomatous hyperplasia. Her growth was delayed, with height and weight below the 3rd centile. Bone age was also greatly delayed with a catch-up at 11 yr (Fig. 3). She entered puberty at 9 yr but has no menarche at 11.6 yr. TFT results are shown in Fig. 1B.

At 11 yr and 7 months of age, her verbal, performance, and total IQ scores (Wechsler Intelligence Scale for Children-Revised intelligence test) were 49, 55, and 50, respectively, categorized as being mildly mentally retarded, with significant speech defect. She was also hyperactive, with improvement in the last 3–4 yr. Frequent upper respiratory tract infections occurred throughout childhood.

The brother of the proposita (IV-3; Fig. 1B), with XY karyotype, had similar dysmorphic features as his sister, IV-2 (Fig. 2, E and F). He had a mild hearing impairment (evoked responses 60 dB left and 80 dB normalized hearing level right ear) requiring hearing aid. There was no tachycardia.

At the age of 5 yr, because of a large goiter, he was started on TRIAC (0.26 mg/d) given in three divided doses, with an increase to 0.525 mg/d at age 8 yr. He grew poorly, height being at the 10th centile throughout age 6, then at the 3rd centile at 8 yr. Weight has followed the 3rd centile throughout. Bone age was delayed to a similar degree as his sister (Fig. 3).

At the age of 9 yr and 6 months, his verbal, performance, and total IQ scores were 49, 50, and 46, respectively, categorized as having a moderate mental retardation, and he was unable to speak. He was also hyperactive from age 5 yr, with improvement after age 8.5 yr. His TFT are shown in Fig. 1B. He had fewer episodes of upper respiratory tract infections than his sister.

Family members. Subject IV-1, the eldest sister (Fig. 1B), was 150 cm in height and weighed 48 kg at age 15 yr. She had no facial dysmorphism, no goiter (Fig. 2, G and H), and no tachycardia. Her serum TSH level was mildly elevated, accompanied by positive thyroid peroxidase (TPO) antibodies, consistent with subclinical hypothyroidism due to autoimmune thyroid disease (Fig. 1B).

The mother of the proposita (III-3; Fig. 1B) had neither goiter nor tachycardia. She had high serum total T₄ and FT₄ concentrations but also had positive TPO antibodies.

The father of the proposita (III-2; Fig. 1B) underwent thyroidectomy for goiter at the age of 28 yr. On 100 µg l-T₄/d, his serum TSH level was elevated at 34 mU/liter despite a FT₄I index (FT₄I) above the upper limit of normal. He did not have tachycardia.

The paternal grandfather (II-1; Fig. 1B) and the maternal uncle (III-4; Fig. 1B) had elevated serum FT₄I with a normal TSH concentration. They had no hearing impairment, goiter, or tachycardia.

Studies were approved by the review boards of the University of Chicago, and written consents were obtained from all subjects studied and guardians of minors.

Thyroid function tests

Blood was collected locally and shipped at room temperature for analyses in Chicago, IL. Total T₄, total T₃, and TSH were measured by chemiluminescence immunoassays using the Elecsys Automated System (Roche Molecular Biochemicals GmbH and Hitachi, Ltd., Indianapolis, IN). Total rT₃ was measured by RIA (Adalitis Italia S.p.A., Bologna, Italy), and thyroglobulin was measured by an in-house RIA. Serum FT₄I and FT₃ index were calculated as the product of their total serum concentrations of each iodothyronine and the normalized resin T₄ uptake ratio. Antibodies to thyroglobulin and TPO were measured by passive hemagglutination (Fujirebio, Inc., Tokyo, Japan).

DNA isolation and THRB sequencing

DNA was isolated from peripheral blood leukocytes using QIAamp DNA Mini Kit (QIAGEN, Valencia, CA), following the manufacturer’s protocol. Exons and adjacent intronic THRB gene sequences were amplified by the PCR and sequenced using automated fluorescence-based sequencing (3730XL 96-capillary; Applied Biosystems, Carlsbad, CA). The primers used for amplification and sequencing are available upon request. Nucleotide and amino acid numbering is according to established consensus (9).

Results

Thyroid function tests

In family Mbyd, the proposita’s mother (III-1), grandmother (II-1), great-grandmother (I-1), and uncle (III-2) had TFT consistent with RTH (Fig. 1A). In family Mozv, the proposita’s parents (III-2 and III-3) had TFT suggestive of RTH, taking into consideration that the father underwent thyroid surgery and was inadequately replaced with l-T₄. The eldest sister and mother had autoimmune thyroid disease likely responsible for the sister’s subclinical hypothyroidism. Patients II-1 and III-4, who are heterozygous carriers of the THRBI gene mutation, have a slightly elevated FT₄I but the remaining TFT were in the normal range. For unknown reasons, subject II-2, who does not carry the mutant allele, also has an elevated value of FT₄I.
Mutation identification and genotyping of family members

**Family Mbyd**

The proposita (IV-1) was found to have a single nucleotide substitution in the TRβ gene. The normal guanosine 1325 was replaced with an adenosine, resulting in the substitution of the normal amino acid glycine 347 (GGG) with a glutamic acid (GAG) (G347E). The proposita (IV-1) has only the mutant allele, whereas other affected members of the family (III-1, III-2, II-2, I-1) are heterozygous.

**Family Mozv**

The proposita (IV-2) and her brother (IV-3) were found to have the normal cytosine 1231 of the TRβ gene replaced with a thymidine. This nucleotide substitution is responsible for the replacement of the normal arginine 316 (CGC) with a cysteine (TGC) (R316C). Both are homozygous for the mutation, whereas their parents (III-2 and III-3) and the paternal grandfather (II-1) and maternal uncle (III-4) are heterozygous for the mutation.

The electropherograms of heterozygous and mutant homozygous sequences are showed in Supplemental Fig. 1 (published on The Endocrine Society’s Journals Online web site at http://jcem.endojournals.org).

**Discussion**

In this study, we described three new patients with RTH carrying two different homozygous mutations in the TRβ gene. The mutation G347E, found in family Mbyd, was previously reported in heterozygotes of one family (10), but it has not undergone *in vitro* characterization. The mutation R316C, found in patients from family Mozv, has been recently described in a heterozygous subject with RTH associated to lingual thyroid (11). Functional studies showed that R316C TRβ had a decreased T3-binding and caused impaired transcription of genes both positively and negatively regulated by T3.

So far, individuals from only three families have been shown to have only a mutant allele. In the first family reported to have RTH (family G) (12), the three affected children were homozygous for TRβ gene deletion (4). They had stippled epiphyses, dysmorphic features (bird-like facies, pigeon breast, winged scapulae), deaf-mutism, and color blindness (12, 13). Although growth was delayed, they reached an adult height above the parental mean. Furthermore, their IQ were normal compared with the average for hearing-impaired individuals (Table 1). All heterozygous family members, including both consanguineous parents and some of the siblings, were phenotypically normal (12, 14).

### TABLE 1. Clinical features of subjects devoid of TRβ or expressing only mutant TRβ

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<th>F</th>
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<th>Mozv</th>
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<td>R316C</td>
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<td>Severe myopia</td>
<td>Squint, decreased acuity</td>
<td>Significant defect</td>
<td>Significant defect</td>
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</table>

NI, Normal; URTI, upper respiratory tract infection; ?, unknown; +, mild; ++, moderate; ++++, severe; ++++, very severe.

<sup>a</sup> Patent ductus arteriosus, tricuspid insufficiency, inguinal hernia, noncommunicating, hydrocephalus requiring ventriculoperitoneal shunt, seizures, hydrocoele, recurrent diahrhea.

<sup>b</sup> Patent ductus arteriosus, patent foramen ovale.

<sup>c</sup> Enlarged tonsils (adenoidectomy at age 4 yr), chronic otitis media.

<sup>d</sup> Graded according to the degree in achieving milestones, such as sitting, standing, feeding, walking, talking.

<sup>e</sup> Catching up in adolescence.

<sup>f</sup> Compared to deaf mute children of variable etiology.

<sup>g</sup> B. B. Bercu, personal communication.
The proband of family S, a boy born to consanguineous parents, had threonine-337 deleted from both alleles (15). This mutant TRβ/H9252 has virtually no ability to bind T3 (7). At birth he presented with respiratory distress, hyperbilirubinemia, hypofibrinogenemia, polycythemia, and very high T4 and TSH (Table 2). He was later found to have a goiter and heart malformation (Table 1). His linear growth and bone age were severely delayed (at 2 1/4 yr, bone age was 4 months), and he had profound mental retardation, complicated by seizures, and a noncommunicating hydrocephalus. The patient had bilateral sensorineural hearing impairment and myopia (B. B. Bercu, personal communication). Treatments with TRβ-blockers, propylthiouracil, and D-T4 caused little or no improvement. This patient remained quite tachycardic and had great difficulty gaining weight, even with rigorous attempts at alimentation. The child died at 8 yr of age from cardiogenic shock complicating staphylococcal pneumonia (B. B. Bercu, personal communication). The heterozygous family members had goiter, tachycardia, and variable elevation of the FT4 with nonsuppressed TSH, typical for RTH (7, 15, 16).

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The proband of family F was born to nonconsanguineous parents but was found to harbor only the mutant I280S THRB allele inherited from the mother, who was heterozygous for this mutation. It is unclear whether the father, who was not available, had a deletion that included the THRB gene (8). In contrast to all other patients with absent wild-type (WT) THRB gene, the patient had normal serum T4 and TSH levels at birth. However, within 3 months, goiter; greatly increased T4, T3, and TSH levels; sweating; and tachycardia were noted (Table 2). She had myopia, a mixed sensorineural and conductive hearing defect. Development was severely retarded; she was unable to walk at age 3 yr; and at 25 yr of age, intellectual development was severely impaired. Until 3 yr of age, her height and weight were in the 3rd centile; at the age of 7 yr, in the 10th centile; and at the age of 10 and beyond, in the 25th centile. Because of a large goiter, she underwent three thyroidectomies (Table 2). Her mother had thyroidectomy because of goiter with tracheal compression but maintained a normal FT4 and TSH with 100 μg/d L-T4 replacement (8).

The cases reported herein presented with the classic findings of RTH, including abnormal TFT, tachycardia, and goiter. However, as with the previously reported case homozygous for THRB gene mutations, the severity of symptoms and magnitude of TFT abnormalities are greater than those found in subjects with heterozygous mutations. This is attributed to the DNE of the mutant receptor combined with the absence of a WT TRβ/H9252. In fact, with the exception of deaf-mutism, the manifestations of RTH and the magnitude of TFT abnormalities of homozygotes in family G were the least severe (Tables 1 and 3) and comparable to many subjects carrying heterozygous mutations.

We used the thyrotroph T4 resistance index (TT4RI), the product of FT4I and TSH (17), to estimate the relative resistance of the hypothalamo-pituitary axis to TH (calculated using data from Table 3). This allows the inclusion of individuals on L-T4 replacement. In increasing order of resistance, subjects without WT TRβ from the five fam-

<table>
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<th>Table 2. Other characteristics of the probands</th>
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<tr>
<td>G</td>
</tr>
<tr>
<td>F</td>
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<tr>
<td>Mbyd</td>
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<td>Mozv</td>
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NI, Normal; Tx, thyroidectomy.
a Premature birth at 35 wk.
b Third born tested because of known thyroid defect in sibling.
TABLE 3. Mean values of FT₄, FT₃, and TSH in homozygous (Homo) and heterozygous (Hetero) individuals belonging to five families with different THRβ gene mutations

<table>
<thead>
<tr>
<th>Mutation (family ID)</th>
<th>FT₄ (% ULN)</th>
<th>FT₃ (% ULN)</th>
<th>TSH (mU/liter)</th>
<th>No. of subjects</th>
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<td>Hetero</td>
<td>Homo</td>
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<td>THRβdel (G)</td>
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<td>G347E (Mbyd)</td>
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<td>R316C (Mozv)</td>
<td>299</td>
<td>113 ± 9</td>
<td>&gt;714</td>
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Values for individual homozygotes were derived from an average of two to more than 10 determinations off treatment, except for members of family G for whom data before thyroidectomy were not available. When more than one subject, values are expressed as mean ± s. ULN, Upper limit of normal; PDAS, personal data from the senior author.

ᵃ Originally reported as T33, prior to the identification of an error in the initial report of the human TRβ sequence (33) which added five amino acids to the amino terminus of the molecule (9).

ᵇ Values ranged from 440 to 680% ULN for FT₄ and from 155 to 395 mU/liter for TSH.

ᶜ Thyroidectomy, both on 100 μg l-T4/d.

ildes ranked as follows: G<F<Mozv<S<Mbyd, with scores of 920, 4,920, 10,456, 140,290, and 239,120 (normal, 136 ± 73), respectively. It is of interest that heterozygous relatives ranked in almost similar order F<G<Mozv<S<Mbyd, with scores of 129, 200, 293, 330, and 830, respectively. Note that the TT₄RI of heterozygotes of families G and F are well within the normal range.

Ranking the effect of the various mutations on the global manifestations is more difficult. However, taking into account the severity of manifestations as listed in Table 1, ranking is similar to that of the TT₄RI, with the exception of the proband of family F, who had more severe mental and physical incapacity. This may be due to the effect of a major gene deletion on one of the two chromosomes 3. Furthermore, in the homozygous member of family S, the additional hydrocephalus and cardiac abnormalities, possibly not directly related to the THRβ gene defect, may have contributed to the severity of the phenotype and undoubtedly to his early demise.

It is noteworthy that findings less commonly seen in RTH with heterozygous mutations, such as effects on growth, bone, cognitive development, and hearing loss, were found in all subjects of the four families expressing only mutant THRβ molecules.

The severity of RTH is dependent on the magnitude of impaired ligand-binding of the mutant THRβ and, in its heterozygous form, on the degree of DNE. The mutant THRβ may interfere with the WT THRβ function in several ways: by occupying TRE on target genes or by engaging the WT TRβ in homodimerization or heterodimerization with retinoid X receptor and possibly with TRα (18). Some mutant THRβ have increased affinity to corepressors (19) or reduced association with coactivators (20). Thus, mutant THRβ with complete inability to bind T₃ can produce minimal dysfunction if their association to corepressors is also reduced (21). Therefore, several mechanisms may account for the variability of clinical manifestation in individuals carrying a mutant THRβ gene in one or both alleles.

Some clinical and laboratory findings in patients with RTH can be explained in light of the information derived from animal models for THRβ gene mutations (22–28). Deletion of the THRβ gene in the mouse (Thrβ⁻/⁻) reproduces a phenotype similar to that observed in subjects of family G. Heterozygous mice are normal, and homozygotes have abnormal TFTs, goiter, sensorineural deafness, and monochromatic vision but normal growth and normal learning abilities (22, 23, 27). However, as in humans, the magnitude of TFT abnormalities in the absence of THRβ is not as pronounced as in mice homozygous for point mutations in the THRβ gene (Thrβmut/mut) (25–27). Such mice, as do the subjects of families S, Mbyd, and Mozv that express only a mutant THRβ in both alleles, show goiter, growth delay, and learning disabilities. An accurate mouse model for family S, with deletion of threonine-337, has been generated in Wondisford’s laboratory, and the effect of this deletion on the heart has been reported in the heterozygous animals (29). The homozygotes were described as small, frail, and weak, similar to the homozygote of family S known to the same investigator (F. E. Wondisford, personal communication).

We can speculate that the severe phenotype observed in the Thrβmut/mut mouse compared with the Thrβ⁻/⁻ mouse and, similarly, that of patients S, Mbyd, and Mozv compared with those of G, are the result of several effects of the mutant THRβ in addition to the complete loss of THRβ function. First was the maintenance of aporeceptor-mediated
repression or stimulation of genes regulated positively or negatively, respectively, by TH. This is achieved by the occupation of TRE by the mutant TRβ. Second was the interference with TRα1 function through formation of inactive TRα1/TRβmut heterodimers and/or direct binding of the mutant TRβ to TREs normally occupied by TRα1 molecules (30).

The amount of the mutant TRβ, acting as aporeceptor, undoubtedly has an effect on the severity of the phenotype, even in the absence of a WT TRβ. Thus, the proband of family F, expressing a single mutant TRβ allele, manifests a pituitary resistance intermediate to that of homozygotes of the G family with no TRβ and all other families with two copies of a mutant TRβ, the TT4RI values being 920, 4,920, and at least 10,456 for G, F, and all others.

TRα1 is essential for heart function, including heart rate up-regulation, for bone maturation, and for the function of specific regions of the brain (cerebellum and hippocampus) and intestine. All patients expressing only the mutant TRβ had marked growth retardation with different degrees of neurological impairment. Using the above reasoning, it is expected that interference of the mutant TRβ with the function of TRα1 should, as in the Tbrh⁻/⁻/Thra1°° mouse (31), reduce the tachycardia by blocking TRα1-mediated TH action. This is not the case, probably because of the effect of the astronomically high TH levels of these animals on the WT TRα1, overriding the effect of the mutant TRβ expressed at a low level in the heart (32, 33).

In conclusion, we report two new families of RTH associated to homozygous mutations in the THRB gene. They represent an important addition to the single patient homozygous for a mutant TRβ with DNE. The clinical and laboratory abnormalities are in agreement with data obtained from mouse models and strengthen the concept of the interference of the mutated TRβ on the function of the TRα1.

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References

5. Jameson JL 1994 Mechanisms by which thyroid hormone receptor mutations cause clinical syndromes of resistance to thyroid hormone. Thyroid 4:485–492
11. Nakajima Y, Yamada M, Horiguchi K, Satoh T, Hashimoto K, Tokuhiro E, Onigata K, Mori M 2010 Resistance to thyroid hormone due to a novel thyroid hormone receptor mutant in a patient with hypothyroidism secondary to lingual thyroid and functional characterization of the mutant receptor. Thyroid 20:917–926
sibship with apparent hereditary resistance to the intracellular action of thyroid hormone. Metabolism 21:723–726
17. Yagi H, Pohlenz J, Hayashi Y, Sakurai A, Refetoff S 1997 Resistance to thyroid hormone caused by two mutant thyroid hormone receptors β, R243Q and R243W, with marked impairment of function that cannot be explained by altered in vitro 3,3,5-triiodothyronine binding affinity. J Clin Endocrinol Metab 82:1608–1614
19. Yoh SM, Chatterjee VK, Privalsky ML 1997 Thyroid hormone resistance syndrome manifests as an aberrant interaction between mutant T3 receptors and transcriptional corepressors. Mol Endocrinol 11:470–480
21. Weiss RE, Hayashi Y, Nagaya T, Petty KJ, Murata Y, Tunca H, Seo H, Refetoff S 1996 Dominant inheritance of resistance to thyroid hormone not linked to defects in the thyroid hormone receptor α or β genes may be due to a defective cofactor. J Clin Endocrinol Metab 81:4196–4203
26. Suzuki H, Cheng SY 2003 Compensatory role of thyroid hormone receptor (TR) α1 in resistance to thyroid hormone: study in mice with a targeted mutation in the TR β gene and deficient in TR α1. Mol Endocrinol 17:1647–1655
27. Chassande O, Flamant F, Samarut J 1999 Thyroid hormone receptor knockouts: their contribution to our understanding of thyroid hormone resistance. Curr Opin Endocrinol Diabet 6:293–300
28. Weiss RE, Murata Y, Cua K, Hayashi Y, Seo H, Refetoff S 1998 Thyroid hormone action on liver, heart, and energy expenditure in thyroid hormone receptor β-deficient mice. Endocrinology 139:4945–4952
32. Dillmann WH 2002 Cellular action of thyroid hormone on the heart. Thyroid 12:447–452
33. Swanson EA, Gloss B, Beltce DD, Kaneshige M, Cheng SY, Dillmann WH 2003 Cardiac expression and function of thyroid hormone receptor β and its PV mutant. Endocrinology 144:4820–4825