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Genotyping of resistance to thyroid hormone in South American population. Identification of seven novel missense mutations in the human thyroid hormone receptor β gene

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ABSTRACT

Thyroid Hormone Receptor β (THRB) defects, typically transmitted as autosomal dominant traits, cause Resistance to Thyroid Hormone (RTH). We analyzed the THRB gene in thirteen South American patients with clinical evidence RTH from eleven unrelated families. Sequence analysis revealed seven novel missense mutations. Four novel mutations were identified in exon 9. The first, a c.991A>G transition which originates a substitution of asparagine by aspartic acid (p.N331D). The second nucleotide alteration consists of a guanine to cytosine transversion at position 1003 (c.1003G>C) and results in substitution of the alanine at codon 335 by proline (p.A335P). The third mutation, a c.1022T>C transition produces a change of leucine by proline (p.L341P). The fourth mutation detected in exon 9 was a c.1036C>T transition which replaces the leucine at codon 346 by phenylalanine (p.L346F). The sequencing of the exon 10 detected three novel missense mutations. The first, a c.1293A>G transition changing isoleucine 431 for methionine (p.I431M). The second, the cytosine at position 1339 was replaced by adenine (c.1339C>A) resulting in the replacement of proline by threonine (p.P447T). The third mutation detected in exon 10 was a c.1358C>T transition resulting in the substitution of proline at codon 453 by leucine (p.P453L). Finally, sequencing analysis of the THRB gene revealed three substitutions previously described (p.A268G, p.P453T and p.F459C). The p.P453T was found in two patients.
In conclusion, we report thirteen patients with RTH caused by heterozygous mutations of the THRB gene. Seven of the identified mutations correspond to novel substitutions.
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1. Introduction

The syndrome of resistance to thyroid hormone (RTH) is an inherited disorder characterized by an variable tissue hyposensitivity to 3,5,3′-L-triiodothyronine (T_3), with persistent elevation of circulating free T_3 and free thyroxine (T_4) levels in association with nonsuppressed serum thyrotropin (TSH) [1–3]. The clinical presentation exhibits marked phenotypic variability and ranges from isolated abnormal findings on routine thyroid testing to a combination of features that includes goiter, variable symptoms of hyper- and hypothyroidism, delayed bone maturation, short stature and attention deficit-hyperactivity disorder [2]. The incidence of RTH is 1 in 40.000 life births [3]. RTH is linked to the thyroid hormone receptor β (THRB) gene located on chromosome 17 and covers 377 Kb of genomic DNA (GenBank Accession Number: NC_000003) [4]. Human THRB gene encodes two N-terminal variants, THRB1 and THRB2, which are transcribed from separate promoters [5,6]. The THRB1N terminus is encoded by two exons (3 and 4) that are replaced by a single exon in THRB2 [7]. These exons are alternatively spliced to six common exons (5–10)

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that encode the DNA binding and T_3 binding domains and dimerization domains of the receptor. The open reading frame from THRB1 mRNA consists of 1386 nucleotides and encodes a protein of 461 amino acids (GenBank Accession Number: NM_000461) [8]. The general organization of the THRB1 5′-untranslated region (UTR) showed eight alternately spliced exons preceding the first 44 bp of exon 3, suggesting that THRB1 mRNAs may originate from multiple transcription initiation sites to form at least seven 5′-UTRs [7]. The relative expression and product distribution of the THRB gene vary among tissues and during different stages of development [9–11].

THRs are members of the nuclear receptor superfamily which includes receptors for steroid hormones, vitamins, retinoids, prostaglandins, fatty acids, and orphan receptors for which no ligands are known. THRs regulate transcription of target genes by binding to thyroid hormone-response elements (TREs) in their promotor regions [12]. In the absence of T_3, THRs interact and associate with other molecules, the coregulator retinoid X receptor and corepressors [12]. These complexes have a silencing effect on genes positively regulated by T_3. THRs have 2 functional domains, the ligand binding domain (LBD) which recognizes T_3 at the carboxyterminus and the DNA binding domain (DBD) near the middle of the molecule [2].

The mutations are located in the last 4 exons, which code for the hinge region and the LBD of the receptor. The 85% of cases are heterozygous for the mutant THRB allele, consistent with the autosomal dominant pattern of inheritance [13]. Therefore, mutant THRBs interfere with the function of the wild-type receptor by a dominant negative mechanism [14]. Transmission was clearly recessive in only one family [15,16]. In this case, a complete deletion of the protein-coding region of the THRB gene was identified. In 15% of the patients with the phenotype of RTH no mutations in the THRB can be found [17].

In the present study screening by direct sequencing analysis revealed seven novel mutations and three previously reported mutations in the exons 8–10 of the THRB gene, in South American patients with RTH.

2. Materials and methods

2.1. Patients

The study was carried out on thirteen Caucasian individuals diagnosed with RTH from eleven unrelated families. All patients had elevated concentrations of free T_4 (FT_4) and normal or high TSH values. Written informed consent was obtained from the parents of the children or adult individuals involved in this study and the research project was approved by the institutional review board.

2.2. Genomic DNA isolation

Genomic DNA was isolated from peripheral blood leucocytes by the standard cetyltrimetilammonium bromide (CTAB) method [18].

2.3. PCR THRB gene amplification

The exons 8–10 of the human THRB gene, including splicing signals and the flanking intronic regions were amplified from the affected patients. Polymerase chain reaction (PCR) were performed in 100 μl, using a standard 5X Colorless Go Taq Buffer (Promega, Madison, WI, USA), containing 200 ng of genomic DNA, 1.5 mM $MgCl_2$, 200 μM of each dNTP, 4% dimethylsulfoxide, 2 U Taq polymerase (Promega) and 50 pmol of each forward and reverse intronic primers. The oligonucleotide sequences and positions of their 5′coding sequence ends are shown in Table 1. Samples were denatured at 95 °C for 3 min followed by 40 cycles of amplification. Each cycle consisted of denaturation at 95 °C for 30 s, primer annealing at 57 °C for 30 s, and primer extension at 72 °C for 1 min. After the last cycle, the samples were incubated for additional 10 min at 72 °C.

2.4. DNA sequencing

The THRB PCR products were purified by GFX PCR DNA and Gel Band Purification Kit (GE Healthcare, Little Chalfont, Buckinghamshire, UK) and then sequenced by utilizing an automated sequencing system (ABI Prism 3100 DNA sequencer, Applied Biosystems, Weiterstadt, Germany). Both the sense and antisense strands were sequenced using the same THRB-specific primers used in the amplification.

2.5. Single-strand conformation polymorphism (SSCP) analysis

The gel matrix for SSCP analysis contained 10% polyacrylamide (29:1) without glycerol. Samples were electrophoresed during 26 h at a constant temperature (4 °C). DNA was visualized by silver-staining.

2.6. Protein homology analysis

Amino acid sequence homology between several species of THRB was compared using the MegAlign software programs (DNASTAR, Hauser University of California-SF).

2.7. Protein secondary structure prediction analysis

The deduced amino acids of the wild-type and mutated human THRB proteins were submitted for computer analysis of the protein secondary structure prediction to the nnPredict-UCSF internet site (http://www.cmpharm.ucsf.edu/nomi/nnpredict.html) and SSpro internet site (http://scratch.proteomics.ics.uci.edu).

2.8. Nucleotide and amino acid nomenclatures

Position numbering is according to THRB1 mRNA reference sequences (GenBank Accession Number: NM_000461). The A of the ATG of the initiator methionine codon is denoted by nucleotide +1. The codon for the initiator methionine is codon 1.

3. Results

3.1. Genotype analysis

The exons 8–10 of the THRB gene, including the flanking intronic sequences were screened by direct sequencing from thirteen Caucasian patients with RTH from eleven unrelated families. Sequence analysis revealed seven novel missense mutations and three previously reported substitutions [19,20]. The eleven mutations identified were all heterozygous. The novel mutations were identified in exons 9 (c.991A>G [p.N331D], c.1003G>C [p.A335P], c.1022T>C [p.L341P], c.1036C>T [p.L346F]) and 10 (c.1293A>G [p.I431M], c.1339C>A [p.P447T], c.1358C>T [p.P453L]) (Fig. 1). The substitution p.P447T was present in the patient FA and her sister FT, whereas the p.P453L in CE and her brother CL.

We ruled out the possibility that the all new missense mutations could be polymorphisms since they were not detected in 100 chromosomes from the general population by SSCP analysis.

The three previously reported substitutions observed were: p.A268G in exon 8 [19] and, p.P453T [20] and p.F459C [20] in exon

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Table 1
Summary of THRB primers used for PCR amplification and sequencing

Exon	Forward primers		Fragment size (bp)	Reverse primers	
	Position of 5′ end	Nucleotide sequence (5′ → 3′)		Position of 5′ end	Nucleotide sequence (5′ → 3′)
8	−124	tgtaaaacgacggccagtgatgtgaaaactggaaactgtacagg	431	+124	caggaaacagctatgaccgacgttgctttcatatgattaagtccc
9	−40	ctggcattttgaattcgttctttgctg	344	+42	aaagctctttggatccccactaacgag
10	−44	ccttccatctctgcagcaatgtccatc	312	+26	gaattatgagaatgaattcagtcagt

The intronic nucleotide position is numbered from the exon end: negative numbers start from the g of the ag splice acceptor site, positive numbers start from the g of the gt splice donor site. The M13 sequences included in the respective primers are underlined.

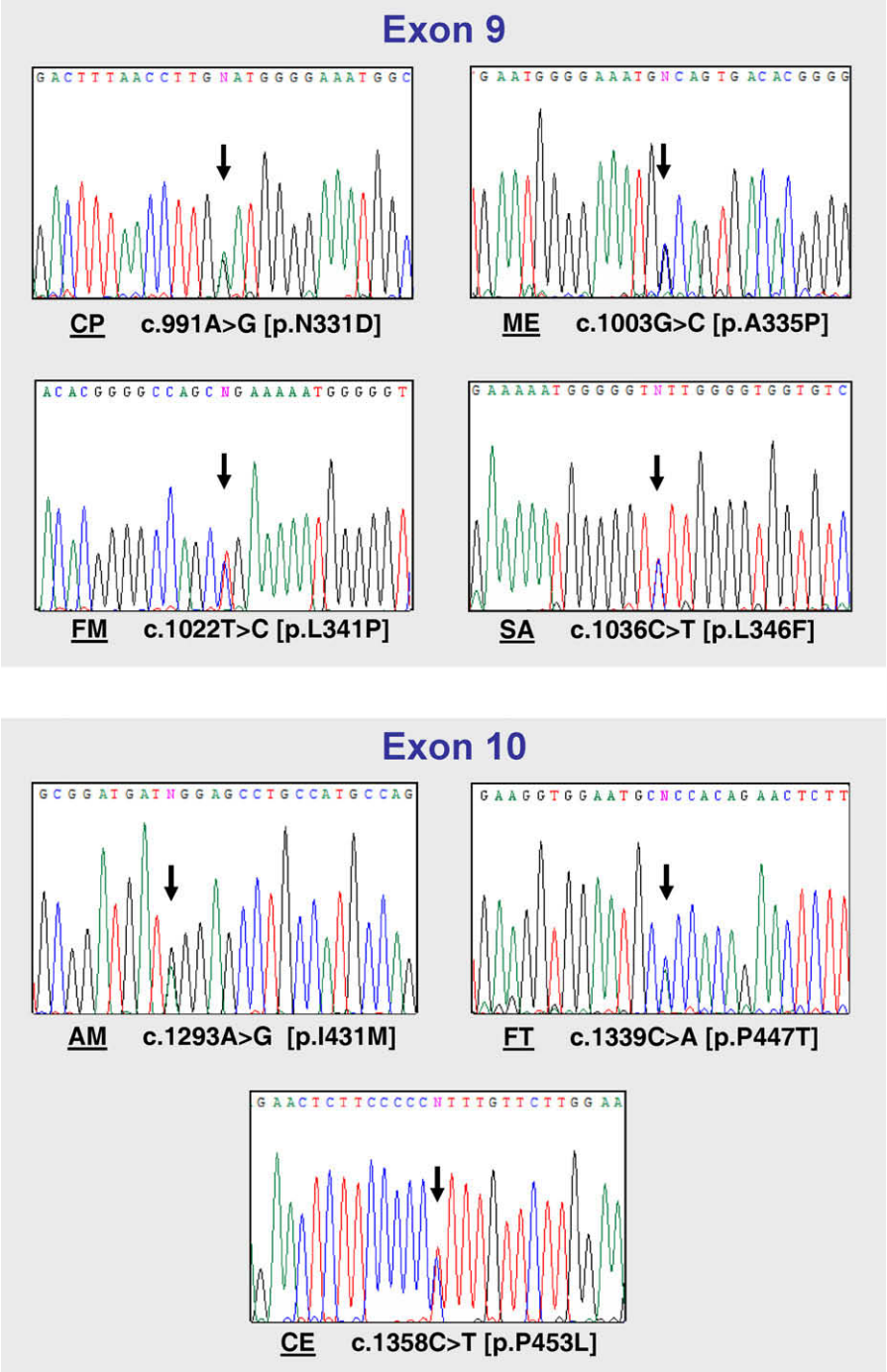

Fig. 1. Sequencing analysis of the THRB gene in patients with RTH. Partial DNA sequence (coding strands) of exons 9 and 10 making use of forward primers. The arrows point to the mutation in the index patients. Wild-type allele coexists in each case with the mutated allele.

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10 (data not shown). The p.P453T was found in two unrelated patients.

In addition, sequencing analysis revealed a recently reported g.IVS9+9G>A polymorphism [21]. From 50 controls analyzed by SSCP, the homozygous variants G/G and A/A were represented by the 71.7% and 3.8%, respectively whereas the heterozygous form appeared in a frequency of 24.5%.

3.2. Clinical findings and segregation analysis

The main clinical characteristics of the index cases with the identified novel THRB mutations, their hormone concentrations and thyroid function tests are shown in Table 2. The nine patients have elevated concentrations of FT_4 with normal level of TSH, except for the patient CP who presented elevated TSH level. Six patients present increased thyroid volume (patients FM, SA, CP, AM, FA and FT) and two of them (patients FM and CP), anti-thyroid antibodies. Administration of exogenous L-T_3 (50, 100, 200 μg/1.70 m^2 of body surface, each given for 3 days) in patients FM, SA, CP, CE and CP did not result in the usual TSH suppression (Table 2), prompting the clinical diagnosis of RTH. Patients FM, SA, CP, CE, CL, AM and FA were submitted to an intravenous TRH test at base line condition (7 μg/kg of body weight for children or 200 μg for adults). All patients studied showed a normal TSH response to the thyrotropin test (Table 2). The patient FA was diagnosed with RTH at the age of 62 years. She underwent subtotal thyroidectomy at the age of 22 years due to goiter.

Segregation studies were performed by SSCP analysis. CP, FM and SA patient's mother evaluation revealed that they were heterozygous carriers of the p.N331D, p.L341P and p.L346F mutations, respectively, and have biochemical data of RTH. Whereas all unaffected fathers were homozygous for the wild-type THRB. In contrast, the genetic study in family of patient AM allowed us to demonstrate a *de novo* mutation in the index case. Analysis of members of the family of patients CE and CL showed that p.P453L was also present in the father who presented diagnosis of RTH. This variation was not detected in the mother and sister. The mutation p.A335P has not been found in the mother of patient ME. Unfortunately, the father was not available for segregation analysis. Finally, p.P447T mutation that was identified in FA and FT sisters could not be determined in their parents.

3.3. Protein homology analysis

The deleterious effect of the all new missense mutations was therefore evaluated by assessing the degree of evolutionary conservation of the respective amino acids, among several human and other animal wild-type THRB. Multiple sequence alignment of the human THRB (*Homo sapiens* [NP_000452]) with sequences found in the GenBank database (*Macaca mulatta* [XP_001090554], *Macaca fascicularis* [BAE89698], *Mus musculus* [BAE34683], *Rattus norvegicus* [P18113], *Equus caballus* [XP_001494341], *Canis familiaris* [XP_862690], *Conger myriaster* [BAD27471], *Danio rerio* [CAM56478], *Gallus gallus* [NP_990778], *Xenopus laevis* [NP_001090182]), using Clustal method, revealed that wild-type asparagine^{331}, alanine^{335}, leucine^{341}, leucine^{346}, isoleucine^{431}, proline^{447} and proline^{453} are strictly conserved in all THRB species.

3.4. Protein secondary structure prediction analysis

We submitted the human THRB amino acid sequence to the nnPredict-UCSF internet site for protein secondary structure prediction to explore putative effects of the p.N331D, p.A335P, p.L341P, p.L346F, p.I431M, p.P447T and p.P453L mutations on the protein structure. The presence of aspartic acid^{331} caused a reduced stretch of the β-sheet structure. The presence of proline^{335} and proline^{341} caused a reduced stretch of helix structure. On the contrary, the presence of methionine^{431}, threonine^{447} and leucine^{453} produce an extended stretch of the helix structure. In addition, the presence of the proline^{341} introduces a β-sheet. Although no changes in the protein secondary structure were observed with p.L346F mutation using nnpredict analysis, in contrast, structural variations were detected by means of SSpro program.

4. Discussion and conclusions

In the present report we have described the genotypic analysis of thirteen South American patients with RTH. We found, seven different novel missense mutations and three previously reported substitutions, all located in the LBD of the THRB. More than 2000 affected individuals belonging to about 500 families have been identified. 85% of patients harbor mutations in the THRB [13]. Up to now, approximately 124 different mutations have been discovered [3]. Mutations described have been classified as missense and

Table 2
Clinical and laboratory data in patients with resistance to thyroid hormone and novel missense mutations in the THRB gene, identified in this study.

Patients	Gender	Age at diagnosis (years)	Thyroid size	TT_4 (μg/dl)	FT_4 (ng/dl)	TT_3 (ng/dl)	Basal TSH (mU/L)	TSH post T_3[a] (mU/L)	TSH post TRH[b] (mU/L)	Anti-TPO antibodies	Anti-TG antibodies
FM	F	9.1	Increased	19.9	3.2	324	2.9	0.22	28	Positive	ND
SA	F	10	Increased	24.4	5.2	425	3.6	0.38	17	Negative	Negative
CP	M	12.9	Increased	12.9	2.4	222	15.2	0.70	100	Positive	Positive
CE	M	12.7	Increased	17.1	3.4	238	2.7	0.21	17.3	Negative	Negative
CL	M	0.52	Normal	20.6	3.07	314	2.3	0.70	7.6	Negative	Negative
Normal range (ECLIA-Elecsys system, Roche)				6–14	0.8–2.2	80–220	0.5–5				
AM	M	9.8	Increased	22.1	3.74	225	1.58	ND	11.9	Negative	ND
Normal range (MEIA-Axsym system, Abbot)				6.5–12.3	0.86–1.82	101–214	0.45–6.5				
ME	F	1	Normal	ND	4.1	224	2.7	ND	ND	Negative	Negative
Normal range (Immulite system, DPC)					0.8–2.4	105–269	0.7–6.4				
FA	F	62	Increased	16.4	2.7	207	1.8	ND	11.9	Negative	ND
FT	F	50	Increased	15.3	ND	195	0.7	ND	ND	Negative	ND
Normal range (Immulite system, DPC)				5–11.5	0.9–2	85–175	0.6–4				

F, Female; M, Male; TSH, thyroid stimulating hormone; TRH, thyrotropin releasing hormone; TT_4, total thyroxine; FT_4, free thyroxine; TT_3, total 3,3′,5-triiodo-L-thyronine; ND, not determined.

CE and CL are brothers. FA and FT are sisters.

[a] TSH responses to oral administration of supraphysiological doses of L-T_3 (50, 100, 200 μg/1.70 m^2 of body surface, each given for 3 days).

[b] TSH responses to intravenous administration of TRH (7 μg/kg of body weight for children or 200 μg for adults).

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nonsense mutations, frameshift mutations by single nucleotide deletion or insertion and duplication of seven nucleotides producing frameshifts and proteins containing two extra amino acids [2,3]. The majority has single nucleotide substitution resulting in single amino acid replacement. One of the mutations described was a deletion of the entire coding region [15,16]. Somatic THRB gene mutations have been identified in two TSH-producing pituitary adenomas [22]. Mutations were found in 3 regions of the LBD of the gene, named as clusters 1, 2 and 3, at codons 429–461, 310–353 and 234–282, respectively [3]. In our patients, five mutations are located in cluster 1 (p.I431M, p.P447T, p.P453L, p.P453T and p.F459C), four in cluster 2 (p.N331D, p.A335P, p.L341P and p.L346F) and the remaining one in cluster 3 (p.A268G). However, there was no correlation between the phenotype and location of mutations in the receptor gene [23]. Within each cluster, mutations occur at higher frequency in some of the codons (e.g. 243, 313, 317, 320, 338, 438, 453) located in CpG dinucleotides or in guanine and/or cytosine rich areas, conforming hot spot regions [2,24]. CpG dinucleotide motifs are frequent sites of point mutation in mammalian DNA and could be caused by deamination of a methylated cytosine resulting in a thymine. In the present study the novel mutations c.1358C>T (p.P453L) and c.1339C>A (p.P447T), and the recurrent mutation c.1357C>A (p.P453T) are located in a cytosine tract of the exon 10.

In the last two decades only nine different THRB mutations have been reported in South American subjects displaying clinical manifestations of RTH. We have described two new mutations: p.M313T [25], 1297-1304delGCCTGCCA [26] and two previously reported substitutions: p.R338W (1012C>T) [27] and p.P453T (1357C>A) [28] in Argentinian patients. The 1297-1304delGCCTGCCA produces a frameshift at amino acid 433 and introduces a stop codon TGA at position 461, 85 nt downstream from deletion. Patient's parents had only the wild-type sequences, indicating a *de novo* mutational event [26]. Other report showed a new p.M310L substitution in a Brazilian patient who presented diffuse goiter, short stature and learning difficulties [29]. Also direct sequencing analysis of the THRB gene revealed four documented mutations: p.A317T (c.949G>A), p.R438H (c.1313G>A), p.P453T (c.1357C>A) and c.1358dupC (stop codon at 463) in four unrelated Brazilian families with diagnosis of RTH [30].

RTH with elevated thyroid hormones, nonsuppressed thyrotropin levels and relatively eumetabolic with variable manifestations of hyper- and hypothyroidism suggests a generalized disorder, whereas RTH associated with hyperthyroidism symptoms indicates a possible selective pituitary resistance [2]. However, this subclassification does not have a logical basis because the second form is encountered in individuals with the same mutation [3]. It is interesting to observe that within the same family, the same mutation may be associated with thyrotoxic characteristics in some individuals or clinically euthyroid in others with comparably elevated thyroid hormone [23]. Another report shows that the same mutation has been associated with biochemical resistance in one patient or normal thyroid function tests in others members of the same family [31]. These different clinical findings suggest marked phenotypic variability of this disorder. Our observation shows that in all patients analyzed, the mutations were associated with clinical and biochemical manifestations of RTH. An autosomal dominant mode of inheritance was demonstrated in six patients and a sporadic manner was observated in only two cases. It is important to point out that the majority of our patients had other clinical manifestations apart from goiter (Table 2). Some of patients showed sinus tachycardia (FM, CE, CP and SA), attention deficit-hyperactivity disorder (SA), learning disability (FM and CE) and emotional disturbances (SA). Goiter is by far the most common finding, reported in 66–95% of cases [3]. Enlargement is usually diffuse. As the patients FM and CP were positive for anti-thyroid antibodies, we diagnosed them as having RTH with coincidental autoimmune thyroiditis. It appeared that in patient CP decreased thyroid function caused by chronic thyroiditis resulted in an increased TSH level. Several cases of RTH associated with autoimmune thyroid disease (AITD) have been reported in the literature [32–41]. Traditionally, the coexistence of RTH and AITD was considered fortuitous. In contrast, a recent study hypothesizes that the chronic TSH elevation in two patients with RTH stimulates TNF-α secretion [41]. TNF-α, in turn mediates thyroid cell destruction by binding to its receptors on thyrocytes, or indirectly by potentiating antibody formation or cytotoxic T lymphocyte production [41]. However, sequential laboratory analysis of further cases and experimental studies are required to define if this hypothesis is true.

In conclusion, the present report represents the first comprehensive genotyping analysis in South American patients with RTH. Seven novel THRB gene missense mutations were identified. Mutational analysis of the THRB gene allows definitive diagnosis of RTH and may help to avoid potential missdiagnosis and inappropriate treatment. Also the identification of new mutations in THRB system might be helpful for understanding the pathophysiology of this disease. Additionally, we demonstrated that the direct sequencing of exons 8–10 of the THRB gene is well suited for routine use in laboratories engaged in screening programs of hereditary thyroid diseases, facilitating the rapid detection of new patients with RTH.

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References

[1] Refetoff S, Weiss RE, Usala SJ. The syndromes of resistance to thyroid hormone. Endocr Rev 1993;14:348–99.
[2] Weiss RE, Refetoff S. Resistance to thyroid hormone. Rev Endocr Metab Disord 2000;1:97–108.
[3] Refetoff S, Dumitrescu AM. Syndromes of reduced sensitivity to thyroid hormone: genetic defects in hormone receptors, cell transporters and deiodination. Best Pract Res Clin Endocrinol Metab 2007;21:277–305.
[4] Sakurai A, Nakai A, DeGroot LJ. Structural analysis of human thyroid receptor β gene. Mol Cell Endocrinol 1990;71:83–91.
[5] Williams GR. Cloning and characterization of two novel thyroid hormone receptor β isoforms. Mol Cell Biol 2000;20:8329–42.
[6] Cheng S-Y. Thyroid hormone receptor mutations and disease: beyond thyroid hormone resistance. Trends Endocrinol Metab 2005;16:176–82.
[7] Frankton S, Harvey CB, Gleason LM, Fadel A, Williams GR. Multiple messenger ribonucleic acid variants regulate cell-specific expression of human thyroid hormone receptor β1. Mol Endocrinol 2004;18:1631–42.
[8] Weinberger C, Thompson CC, Ong ES, Lebo R, Gruol DJ, Evans RM. The c-erb-A gene encodes a thyroid hormone receptor. Nature 1986;324:641–6.
[9] Iskaros J, Pickard M, Evans I, Sinha A, Hardiman P, Ekins R. Thyroid hormone receptor gene expression in first trimester human fetal brain. J Clin Endocrinol Metab 2000;85:2620–3.
[10] Zhang XY, Kaneshige M, Kamiya Y, Kaneshige K, McPhie P, Cheng S-Y. Differential expression of thyroid hormone receptor isoforms dictates the dominant negative activity of mutant β receptor. Mol Endocrinol 2002;6:2077–92.
[11] O'Shea PJ, Harvey CB, Suzuki H, Kaneshige M, Kaneshige K, Cheng S-Y, et al. A thyrotoxic skeletal phenotype of advanced bone formation in mice with resistance to thyroid hormone. Mol Endocrinol 2003;17:1410–24.

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[12] Harvey CB, Williams GR. Mechanism of thyroid hormone action. Thyroid 2002;12:441–6.
[13] Refetoff S. Resistance to thyroid hormone: one of several defects causing reduced sensitivity to thyroid hormone. Nat Clin Pract Endocrinol Metab 2008;4:1.
[14] Sakurai A, Miyamoto T, Refetoff S, DeGroot LJ. Dominant negative transcriptional regulation by a mutant thyroid hormone receptor-β in a family with generalized thyroid hormone resistance. Mol Endocrinol 1990;4:1988–94.
[15] Refetoff S, DeWind LT, DeGroot LJ. Familial syndrome combining deaf-mutism, stippled epiphyses, goiter, and abnormally high PBI: possible target organ refractoriness to thyroid hormone. J Clin Endocrinol Metab 1967;27:279–94.
[16] Takeda K, Sakurai A, DeGroot LJ, Refetoff S. Recessive inheritance of thyroid hormone resistance caused by complete deletion of the protein-coding region of the thyroid hormone receptor-ß gene. J Clin Endocrinol Metab 1992;74:49–55.
[17] Weiss RE, Hayashi Y, Nagaya T, Petty KJ, Murata Y, Tunca H, et al. Dominant inheritance of resistance to thyroid hormone not linked to defects in the thyroid hormone receptors α or ß genes may be due to a defective co-factor. J Clin Endocrinol Metab 1996;81:4196–203.
[18] Murray MG, Thompson WF. Rapid isolation of high molecular-weight plant DNA. Nucleic Acids Res 1980;8:4321–5.
[19] Jézéquel P, Guilhem I, Hespel JP, Le Treut A, Le Gall JY, Allannic H, et al. Identification of a novel mutation (A268G) in exon 8 of the TRß gene in a large family with thyroid hormone resistance. Hum Mutat 1996;8:396.
[20] Beck-Peccoz P, Chatterjee VK, Chin WW, DeGroot LJ, Jameson JL, Nakamura H, et al. 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, United Kingdom. J Clin Endocrinol Metab 1994;78:990–3.
[21] Sørensen HG, van der Deure WM, Hansen PS, Peeters RP, Breteler MMB, Kyvik KO, et al. Identification and consequences of polymorphisms in the thyroid hormone receptor alpha and beta genes. Thyroid 2008;18:1087–94.
[22] Ando S, Sarlis NJ, Oldfield EH, Yen PM. Somatic mutation of TRβ can cause a defect in negative regulation of TSH in a TSH-secreting pituitary tumor. J Clin Endocrinol Metab 2001;86:5572–6.
[23] 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 β gene. J Clin Invest 1994;94:506–15.
[24] Weiss RE, Weinberg M, Refetoff S. Identical mutations in unrelated families with generalized resistance to thyroid hormone occur in cytosine-guanine-rich areas of the thyroid hormone receptor beta gene: analysis of 15 families. J Clin Invest 1993;91:2408–15.
[25] Di Fulvio M, Chiesa AE, Baranzini SE, Gruñeiro-Papendieck L, Masini-Repiso AM, Targovnik HM. A new point mutation (M313T) in the thyroid hormone receptor β gene in a patient with resistance to thyroid hormone. Thyroid 1997;7:43–4.
[26] Rivolta CM, Mallea Gill MS, Ballarino C, Ridruejo MC, Miguel C, Gimenez S, et al. A novel 1297-1304delGCCTGCCA mutation in the exon 10 of the thyroid hormone receptor β gene causes resistance to thyroid hormone with severe cardiological dysfunction. Mol Diagn 2004;8:163–9.
[27] Rivolta CM, Herzovich V, Esperante SA, Lazzati JM, Iorcansky S, Targovnik HM. Identificación de una transición heterocigota 1012C>T (R338W) en el exón 9 del gen del receptor β de hormonas tiroideas en una familia con resistencia generalizada a hormonas tiroideas. Rev Argent Endocrinol Metab 2003;40:165–71.
[28] Rivolta CM, Feijoo MC, Targovnik HM, Funes A. Identificación de una transversión heterocigota 1357C>A (P453T) en el exón 10 del gen del receptor de hormonas tiroideas β en una familia con resistencia a hormonas tiroideas. Rev Argent Endocrinol Metab 2003;40:13–22.
[29] Furlanetto TW, Kopp P, Peccin S, Gu WX, Jameson JL. A novel mutation (M310L) in the thyroid hormone receptor β causing resistance to thyroid hormone in a Brazilian kindred and a neonate. Mol Genet Metab 2000;71:520–6.
[30] Künzle Ribeiro Magalhães P, Rodriguez Dare GL, Rodriguez dos Santos S, Nogueira CR, de Castro M, Zanini Maciel LM. Clinical features and genetic analysis of four Brazilian kindreds with resistance to thyroid hormone. Clin Endocrinol (Oxf) 2007;67:748–53.
[31] Geffner ME, Su F, Ross NS, Hershman JM, Van Dop C, Menke JB, et al. An arginine to histidine mutation in codon 311 of the C-erbA β gene results in a mutant thyroid hormone receptor that does not mediate a dominant negative phenotype. J Clin Invest 1993;91:538–46.
[32] Robinson DB, Michaelis RD, Shakir KM. Autoimmune hypothyroidism in a patient with generalized resistance to thyroid hormone. South Med J 1993;86:1395–7.
[33] Weiss RE, Chyna B, Duell PB, Hayashi Y, Sunthornthepvarakul T, Refetoff S. A new point mutation (C446R) in the thyroid hormone receptor-β gene of a family with resistance to thyroid hormone. J Clin Endocrinol Metab 1994;78:1253–6.
[34] Refetoff S, Tunca H, Wilansky DL, Mussey VC, Weiss RE. A mutation in the thyroid hormone receptor (TR) β gene (M313T) not previously reported in two unrelated families with resistance to thyroid hormone (RTH). Thyroid 1996;6:571–3.
[35] Reinhardt W, Jockenhovel F, Deuble J, Chatterjee VK, Reinwein D, Mann K. Thyroid hormone resistance: variable clinical manifestations in five patients. Nuklearmedizin 1997;36:250–5.
[36] Pohlenz J, Weiss RE, Macchia PE, Pannain S, Lau IT, Ho H, et al. Five new families with resistance to thyroid hormone not caused by mutations in the thyroid hormone receptor β gene. J Clin Endocrinol Metab 1999;84:3919–28.
[37] Fukata S, Brent GA, Sugawara M. Resistance to thyroid hormone in Hashimoto's thyroiditis. N Engl J Med 2005;352:517–8.
[38] Aksoy DY, Gurlek A, Ringkananont U, Weiss RE, Refetoff S. Resistance to thyroid hormone associated with autoimmune thyroid disease in a Turkish family. J Endocrinol Invest 2005;28:379–83.
[39] Sato H, Sakai H. A family showing resistance to thyroid hormone associated with chronic thyroiditis and its clinical features: a case report. Endocr J 2006;53:421–5.
[40] Tran HA. Difficulties in diagnosing and managing coexisting primary hypothyroidism and resistance to thyroid hormone. Endocr Pract 2006;12:288–93.
[41] Gavin C, Meggison H, Ooi TC. Proposing a causal link between thyroid hormone resistance and primary autoimmune hypothyroidism. Med Hypotheses 2008;70:1024–8.

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