

# Resistance to Thyroid Hormone: Probable De Novo Mutation (P453S) in the Receptor Beta Gene

## *Tiroid Hormon Rezistansı: Reseptör Beta Geninde Muhtemel Yeni Mutasyon (P453S)*

Banu Kale Köroğlu, Sunee Mamanasiry\*, Adem Küçük\*\*, Mehmet Numan Tamer, Ramazan Yılmaz\*\*\*, Samuel Refetoff\*

Süleyman Demirel University, Department of Endocrinology and Metabolism, Isparta, Turkey

\*University of Chicago, Department of Medicine, Chicago, USA

\*\*Süleyman Demirel University, Department of Internal Medicine, Isparta, Turkey

\*\*\*Süleyman Demirel University, Department of Medical Biology, Isparta, Turkey

*B.K.K. and S.M. should be considered co-first authors.*

### Abstract

Resistance to thyroid hormone (RTH) is an inherited syndrome of reduced sensitivity to thyroid hormone (TH), usually due to mutations in the thyroid hormone receptor (TR)  $\beta$ , gene. We studied a Turkish family whose proband, a 19-year-old male, presented with diffuse goiter, nervousness, and palpitation. Thyroid function tests revealed elevated levels of TH and nonsuppressed thyrotropin (TSH). Gene sequencing revealed a mutation in one allele of the TR $\beta$  gene in the proband, his two brothers, and father. It involved the substitution of the normal cytosine 1642 with a thymidine, resulting in the replacement of the normal proline 453 with a serine (P453S) in the T3-binding domain of the TR $\beta$  which is known to have one quarter to one third the T3-binding affinity of the normal TR $\beta$ . Genetic study of the family suggests that the mutation may have occurred de-novo in the father of the proband. *Türk Jem 2009; 13: 43-6*

**Key words:** Resistance to thyroid hormone, thyroid hormone receptor beta, mutation

### Özet

Tiroid hormon rezistansı (THR), genellikle tiroid hormon reseptör (TR)  $\beta$  genindeki mutasyon nedeniyle tiroid hormonuna (TH) duyarlılığın azaldığı kalıtsal bir sendromdur. Probandı difüz guatr, sinirlilik ve çarpıntı ile başvuran 19 yaşında bir genç erkek olan bir Türk ailesini çalıştık. Tiroid fonksiyon testleri, artmış TH seviyesi ve baskılanmamış tirotropin (TSH) gösteriyordu. Gen dizimi, proband, iki erkek kardeşi ve babasında TR $\beta$  geni bir alelinde bir mutasyon olduğunu gösterdi. Normal sitozin 1642 yerine timidinin yer değiştirmesini kapsar ki bu da normal TR $\beta$ 'ya T3 bağlanma afinitesinin dörtte birden üçte bire kadar azalmasına neden olan TR $\beta$ 'nin T3 bağlanma zincirinde normal prolin 453 ile serinin (P453S) yer değiştirmesi ile sonuçlanır. Ailenin genetik analizi, mutasyonun, probandın babasında de-novo oluştuğunu telkin etmektedir. *Türk Jem 2009; 13: 43-6*

**Anahtar kelimeler:** Tiroid hormon rezistansı, tiroid hormon reseptör beta, mutasyon

### Introduction

Resistance to thyroid hormone (RTH) is a syndrome characterized by decreased responsiveness of target tissues to the action of thyroid hormone (TH) (1). The common features of RTH include elevated serum TH and normal or slightly increased thyrotropin (TSH) concentrations. The clinical presentation of RTH is variable. The majority of individuals are completely asymptomatic. Some may manifest symptoms suggestive of TH deprivation such as growth retardation, impaired cognitive ability, and learning disabilities, while

others show signs of TH excess such as advanced bone age or hyperactivity and tachycardia (1,2). The first case of RTH was reported by Refetoff in 1967 (3). Mosaicism of the TR $\beta$  gene was described for the first time in a Turkish patient by Mamanasiri as a cause of variable sensitivity to TH in different tissues (4).

RTH is generally transmitted in an autosomal dominant manner, but sporadic, de novo cases are also common (5). RTH is mostly caused by mutations in the thyroid hormone receptor (TR)  $\beta$  gene (6). The mutant TR molecules have either reduced affinity for triiodothyronine (T3) or impaired interaction with one of the

**Address for Correspondence:** Banu Kale Köroğlu, MD, Süleyman Demirel University, Department of Endocrinology and Metabolism, Isparta, Turkey  
Phone: +90 246 211 26 00 Fax: +90 246 237 02 40 E-mail: banukale@yahoo.com **Received:** 04.10.2009 **Accepted:** 04.10.2009

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cofactors involved in the mediation of TH action (1,7). The linkage between RTH and the TRβ gene was elucidated in 1988 (8) and the first demonstration of a TRβ mutation was in 1989 (9). Thereafter, by using sequencing techniques, approximately 122 different mutations in TRβ gene have been identified in subjects with RTH belonging to 300 families (10).

In this study, we described a Turkish family with RTH caused by a single nucleotide change in exon 10 of the TRβ gene resulting in the substitution of serine for proline at codon 453 (P453S). This mutation has been identified in 11 other families, 9 of which have been published (11-16).

**Materials and Methods**

**Patients**

The proband was a 19-year-old male who presented with diffuse goiter, nervousness, and palpitations that have persisted for approximately 5 years. Treatment with propylthiouracil (PTU) was initiated 3 years earlier, assuming that he had a toxic diffuse goiter. He was admitted to the Endocrinology Unit of Suleyman Demirel University with the above mentioned complaints after having ceased the PTU treatment. He weighed 50 kg, his height was 172 cm, blood pressure was 120/80 mmHg, and pulse rate was 96 beats/min. He had a moderate, diffuse goiter and anxious mood. Physical examination was otherwise normal. Laboratory evaluation revealed normal blood count, renal and hepatic function tests and urinalysis.

Thyroid function tests showed a TSH of 1.79 mU/L (normal range 0.4-4.0), a free thyroxine (T4) of 4.4 ng/dl (normal range 0.8-1.8), and a free T3 of 5.6 pg/ml (normal range 1.6-4.6). Magnetic resonance imaging revealed a normal pituitary gland. Administration of L-T3 did not result in the usual TSH suppression. Thyroid function tests of first-degree relatives demonstrated that his father, sister and two brothers had elevated serum TH levels with normal TSH concentrations. The patient and 21 family members underwent laboratory and genetic analysis.

**Laboratory analyses**

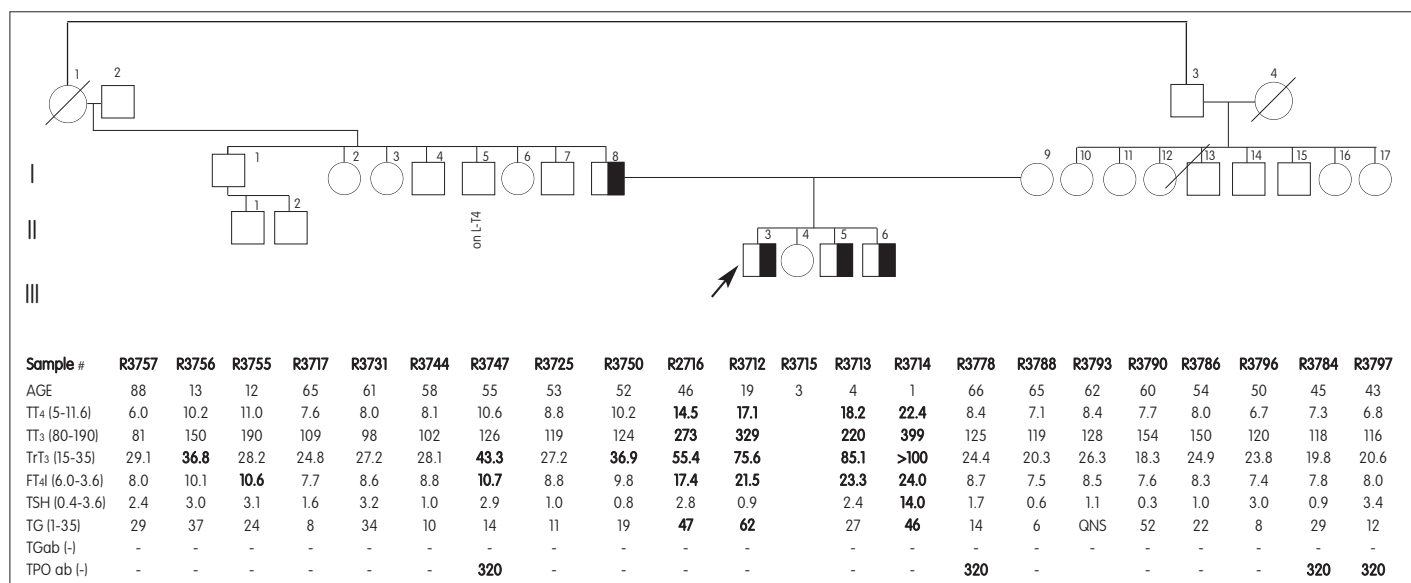
Total T4, total T3, and TSH were measured by chemiluminescence using Elecsys 2010 technology (Roche Molecular Biochemicals GmbH and Hitachi, Ltd., both located in Indianapolis, IN). Total reverse T3 and thyroglobulin (TG) were measured by RIAs. FT4 index was estimated by calculating the ratio of T3 resin uptake and the total T4 concentration. Thyroid peroxidase and TG antibodies were measured by an agglutination method.

Genomic DNA was extracted from peripheral blood leukocytes of the proband and 21 family members. Genomic DNA was amplified by the polymerase chain reaction and all coding exons and intron junctions of the TRβ1 gene were sequenced using oligonucleotide primers, described previously (12).

A written informed consent was obtained from all subjects for genetic analysis and the study was approved by the local ethics committee. The study was also approved by the Institutional Review Board of the University of Chicago.

**Results**

We identified the same mutation in one allele of the TRβ gene in the proband (subject III-3 in Figure 1), his two brothers (III-5 and III-6) and father (II-8). It involves the substitution of the normal cytosine 1642 with a thymidine. This results in the replacement of the normal proline 453 with a serine (P453S) in the T3-binding domain of the TRβ. All four subjects harbouring the mutation exhibited the typical phenotype of RTH including increase in the concentration of all iodothyronines (T4, T3 and rT3), normal or slightly elevated TSH and, with the exception of subject III-5, high serum TG concentration. The mild increase in FT4I and rT3 concentrations of subject II-5 is compatible with the use of L-T4. We have no explanation for the slight increase of rT3 in subjects II-7 and III-1, and for the slight reduction of TSH in subject II-13. Four individuals had autoimmune thyroid disease based on the detection of thyroperoxidase antibodies (see Fig. 1). None of the 6 sibling of the affected father (II-1-7), nor the paternal grandfather (I-1), or 8 nephews and nieces of the deceased paternal grandmother of the proband (II-10-17) expressed the RTH phenotype. Thus, it is likely that the father has a de novo TRβ gene mutation.



**Figure 1.** Pedigree of family Mdn and results of thyroid function tests and genotyping. Circles are females and squares indicate males. A line through the symbol indicates that the subject is deceased. The proband is indicated by an arrow. Generations are indicated by Roman numerals and subjects by Arabic numbers to the right of each symbol. Results of thyroid function tests are aligned below each symbol. Abnormal values are in bold numbers. Half shaded symbols indicate the presence of the P453S mutations in one allele of the TR gene. Note that the sister of the proband was not tested due to loss of her blood sample during shipping.

## Discussion

RTH is characterized by elevated serum levels of TH and normal or slightly increased serum TSH concentration that responds to TRH (17). Although the precise incidence of RTH is unknown, a limited neonatal survey estimated an incidence of 1 case per 40.000-50.000 live births (18). While most thyroid diseases show a female preponderance, RTH occurs in males and females with equal frequency (17). Familial occurrence of RTH has been documented in approximately 75% of cases. Inheritance is autosomal dominant, with the exception of the RTH caused by complete deletion of the protein-coding of the TR $\beta$  gene, which had autosomal recessive inheritance (19).

Before TR gene defects were recognized, patients with RTH were clinically classified as having generalized resistance to TH (GRTH) and selective pituitary resistance to TH (PRTH), based principally on symptoms and signs. Those with GRTH appeared to be eumetabolic and those with PRTH, hypermetabolic, based on restlessness and tachycardia. However, it has been suggested that PRTH may not constitute an entity distinct from GRTH (20). In fact, the clinical manifestations are variable among the families with RTH and also among the affected family members. Furthermore, one and the same subject with RTH may exhibit symptoms and signs of hypothyroidism in one tissue, while in another tissue, findings may be suggestive of thyrotoxicosis. This situation results from the dissimilar distribution of TR isoforms among tissues. For example, TR $\alpha$  is expressed predominantly in the heart, bone and brain, whereas TR $\beta$  is more abundant in the liver and kidney (22).

At least three different molecular alterations may cause reduced sensitivity to TH in humans: (a) mutations in the gene encoding TR $\beta$  isoform causing RTH, (b) mutations of the specific TH transporter, monocarboxylate transporter 8 (MCT8), and (c) mutations in selenocysteine insertion sequence binding protein 2 (SECISBP2) which reduces the synthesis of selenoproteins, including the TH deiodinases (22).

Approximately 85% of subjects with RTH, studied at the gene level, have mutations in the TR $\beta$  gene, located on chromosome 3. The TR $\beta$  molecule contains a DNA-binding domain, a hinge region, and a ligand (T3)-binding domain. Mutations are located in the T3-binding domain and its adjacent hinge region. Most are single amino acid substitutions with fewer single amino acid insertions or deletions and even less large deletions. Almost all mutations cluster around the ligand-binding pocket observed in the ligand-binding domain of the TR $\beta$  crystal structure (1,23). 122 different mutations have been so far identified in approximately 300 families (10).

We identified a mutation in one allele of the TR $\beta$  gene of the proband, his two brothers and father. The single nucleotide substitution resulted in the replacement of the normal proline 453 with a serine (P453S) in the T3-binding domain of the TR $\beta$ . This identical mutation was reported in 9 other families (11-16 and personal observations) and shown to have a reduced affinity to T3, being 25 to 36% of the normal one (12,14). In three families the mutations had occurred de novo. It should be noted that the mutation occurs in a cytosine-rich region (5 consecutive cytosines) which has been shown to be prone to mutations (24). Although the paternal grandmother could not be tested, because she was deceased, based on the fact that 6 of 7 siblings of the father and 8 nephews of the paternal grandmother had no TR $\beta$  gene mutation, it is likely that the mutation also occurred de novo in the

father of the proband. Familial occurrence of RTH has been documented in approximately 75% of cases. Taking into account only those families in whom both parents of the affected subjects have been studied, the true incidence of sporadic cases is 21.3%. This is in agreement with current estimate of the frequency of de novo mutations of 22.5% (10).

In addition, the same codon has been found to harbour five different mutations other than P453S: in 7 families-P453A (12,16,25-28 and personal observations), 16 families-P453T (12,16, 29-36 and personal observations), 1 family-P453N (personal observation), 1 family-P453Y (16), and 3 families-P453H (11,31,37).

RTH is rarely diagnosed at birth as neonatal screening programs are most often based on the determination of TSH concentration in dried blood. Screening programs that measure both TSH and T4, and using an assay reliable in the high T4 range, would rarely identify a case at birth. However, children of women with known TR $\beta$  gene mutations should be tested either prenatally or at birth.

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