An Identical Neo-mutation in the Thyroid Hormone Receptor ß Gene (A317T) in 2 Unrelated Thai Families with Resistance to Thyroid Hormone

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Abstract

We reported two unrelated Thai girls with resistance to thyroid hormone. The affected patients presented with goiter and no other stigmata of hyperthyroidism. Their serum T_4, T_3, free T_4 and free T_3 concentrations were high and they had normal levels of TSH. The affected girl in family 1 was treated with an antithyroid drug for 1-9/12 years. The affected girl in family 2 was only observed her thyroid function tests. TRH test showed normal TSH response in both girls. Analysis of the thyroid hormone receptor ß gene of both affected girls revealed the same missense mutation, changing the guanine in nucleotide 1234 to an adenine which results in the replacement of the normal alanine (GCT) with a threonine (ACT) at codon 317. Two proposita were heterozygous, and this mutation was not present in their parents compatible with a neo-mutation.

Key word : Resistance to Thyroid Hormone, Two Unrelated Thai Families

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Resistance to thyroid hormone (RTH) was first described in 1967 by Refetoff et al[1]. RTH is a syndrome of reduced responsiveness of the target tissues to thyroid hormone[2]. The clinical manifestations of RTH patients present with highly variable clinical features ranging from goiter, mental retardation and delayed bone maturation (generalized resistance to thyroid hormone, GRTH) to signs and symptoms of thyrotoxicosis (pituitary resistance to thyroid hormone, PRTH). The thyroid function test showed high concentrations of serum thyroxine (T_4), triiodothyronine (T_3) and free T_4 with non-suppressed thyrotropin (TSH) levels.

Between the first description of this order in 1967 and the exciting discovery of the genes encoding for thyroid hormone receptors in 1986, many cases of RTH have been reported in the literature[3-5]. So far, mutations in the thyroid hor-

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mone receptor (TR) ß gene have been identified in subjects with RTH belonging to 115 families[6]. Most are located in two hot spot areas in the T_3-binding domain of the TRß protein. Since only 65 of the mutations found in 115 families are unique, 46 occur in more than one family. We herein report two unrelated Thai patients manifesting the RTH phenotype, the first one was reported as the first case of RTH in Thailand in 1997[7]. We report the two cases together since they had the same TRß gene neo-mutation at codon 317 which resulted in the replacement of the normal alanine (GCT) with a threonine (ACT) and we could compare the clinical presentation and thyroid function test of this mutation.

PATIENTS AND METHOD

Family 1

The proposita was 6-10/12 years old in June 1995 when she came to medical attention because of goiter. Except for tachycardia, she had no other stigmata of thyrotoxicosis despite a serum T_4 of 17.2 ug/dl (normal range: 4.5-12.5), T_3 of 280 ng/dl (normal range: 50-200), and free T_4 of 5.0 ng/dl (normal range: 1.0-2.8). TSH was not measured and she was treated with propylthiouracil based on the diagnosis of thyrotoxicosis. Three months later, her T_4 was 14.5 ug/dl, T_3 of 315 ng/dl, free T_4 of 4.4 ng/dl, and TSH of 14.3 mU/L (normal range: 0.3-5.0). L-thyroxine 50 ug/day was added to the antithyroid drug, but her serum T_4, T_3, free T_4 remained high with no suppression of serum TSH. Her height and weight were 90^{th} and 50^{th} percentile, respectively. Her bone age was compatible with her chronological age. She had no attention deficit disorder and no hearing loss, but her intellectual quotient was 83 per cent. At age 8-7/12 years old, after stopping all medication for 5 months, her T_4 was 16.7 ug/dl, T_3 of 272 ng/dl, free T_4 of 4.68 ng/dl, free T_3 8.72 pg/ml (normal range: 1.6-4.5) and basal TSH was normal 1.0 mU/L increasing to 10.9 mU/L, 20 min after the administration of 200 ug of TRH. Antimicrosomal thyroid antibodies were negative. Her parents and her sister were clinically and biochemically normal (Fig. 1, Family 1).

Family 2

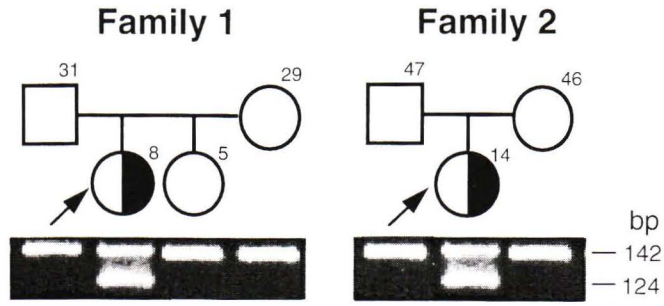
The proposita was 14 years old in October 1994 when she came to medical attention because of goiter. She had no tachycardia, weight loss or other stigmata of thyrotoxicosis. Her serum free T_4 was 4.98 ng/dl (normal range: 0.7-1.74), T_3 of 164 ng/dl (normal range: 68-145), and TSH of 4.53 mU/L (normal range: 0.36-3.77). Serum T_4 was not measured at that time. Antimicrosomal antibodies and antithyroglobulin were negative. 24 hour radioiodine (RAI) uptake was 48 per cent which was in the normal range. Her height and weight were 90^{th} and 97^{th} percentile, respectively. Since she had clinically euthyroid and normal serum TSH with normal RAI uptake, she was not treated with any medication. She had yearly follow-up and had clinical euthyroid but she still had high free T_4 (5.41 ng/dl), high serum T_3 (184 ng/dl) and normal TSH (2.38 mU/L). She had no attention deficit disorder and no hearing loss. She was referred to Rajavithi Hospital for further investigations in January 1997. At 17 years old, her T_4 was 17.0 ug/dl (normal range:4.5-11.5), T_3 of 181 ng/dl (normal range: 65-170), free T_4 of 3.12 ng/dl (normal range: 0.73-2.01), free T_3 5.73 pg/ml (normal range: 1.6-4.5) and TSH of 1.97 mU/L (normal range: 0.35-5.2). TRH test was done and showed normal basal TSH, 3.14 mU/L, and increasing to 31.98 mU/L 30 min after the administration of 200 ug of TRH. Her parents were clinically and biochemically normal (Fig. 1, Family 2).

Tests of Thyroid Function

Serum T_4, T_3 and free T_4 concentrations were measured by double-antibody technique radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA, USA). Serum concentration of TSH was measured by GammaCoat (Clinical Assays, Incstar Corporation, Stillwater, MN, USA). Antibodies directed against thyroglobulin (TGHA) and microsomal antigen (MCHA) were measured using hemagglutination technique (Murex, Dartford, UK).

Genomic DNA Extraction and DNA Sequencing

Genomic DNAs were extracted from circulating white blood cells of all members of the 2 families and used for TRß gene sequencing and genotyping. The coding exons 9 and 10 and their flanking intronic sequences were amplified by PCR using conditions described previously[8]. The oligonucleotide primers used for PCR and DNA sequencing are shown in Table 1. The amplified DNA fragments were sequenced in the sense and antisense directions using 373 DNA Sequencer, (Applied



									Normal Range
T4	ug/dl	5.5	**16.7**	7.2	8.7	3.9	**17.0**	6.5	4.5-11.5
T3	ng/dl	85	**272**	88	108	71	**181**	67	65-170
FT4	ng/dl	1.35	**4.69**	1.28	1.10	0.96	**3.12**	1.16	0.73-2.01
TSH	mU/L	0.54	0.96	1.62	1.16	1.49	1.97	1.16	0.35-5.2

Fig. 1. Pedigree, genotyping for the TRß gene mutation (see text), and results of thyroid function tests. Age to the right of symbols. The proposita is indicated by an arrow. Abnormal values are in bold numbers.

Table 1. Sequences of oligonucleotide primers and their application.

Oligonucleotide primer sequences[a]	Region amplified[b]
For TR-ß Gene Sequencing	
tatgttgttcctgactggca	Exon 9 (s)
gattggaattagcgctagac	Exon 9 (a)
cctggaattggacaaagcaa	Exon 10 (s)
agagctaggcaatggaatga	Exon 10 (a)
For Confirmation of Mutation A317T	
TGGAGATCATGTCCCTGCGC	Exon 9 (s)
CCCAGGTCAAAGATGGCGTC	Exon 9 (a)

[a] Degenerated nucleotides are underlined and intronic sequences are in lower case letter.
[b] (a), antisense primer; (s), sense primer.

Biosystems, Perkin-Elmer Corporation, Foster City, CA, USA).

Confirmation of the Mutation

The presence of mutation, G to A transition of nucleotide 1234 in codon 317 of the TRß gene, was determined in genomic DNA. A degenerate oligonucleotide sense primer was synthesized that is complementary to sequences near but not overlapping the mutant nucleotide (Table 1). It was designed so that their product of amplification would create a unique restriction site (Fsp I) only if the template contained the mutant nucleotide (endonuclease digestion-allele specific primer method). The antisense primer is shown in Table 1.

Following amplification of the subjects' genomic DNAs by PCR, the products were digested with Fsp I restriction enzyme and then submitted to electrophoresis in 3 per cent Nuseive/1 per cent agarose. Partial cleavage of the DNA fragment with Fsp I indicated that the mutant nucleotide was present in one of the two alleles.

RESULTS

DNA sequencing showed that both patients had a G to A transition of nucleotide 1234 in codon 317 located in exon 9 of TRß gene (Fig. 2). The mutation causes in the replacement of the normal Alanine (GCT) with a Threonine (ACT) at codon 317.

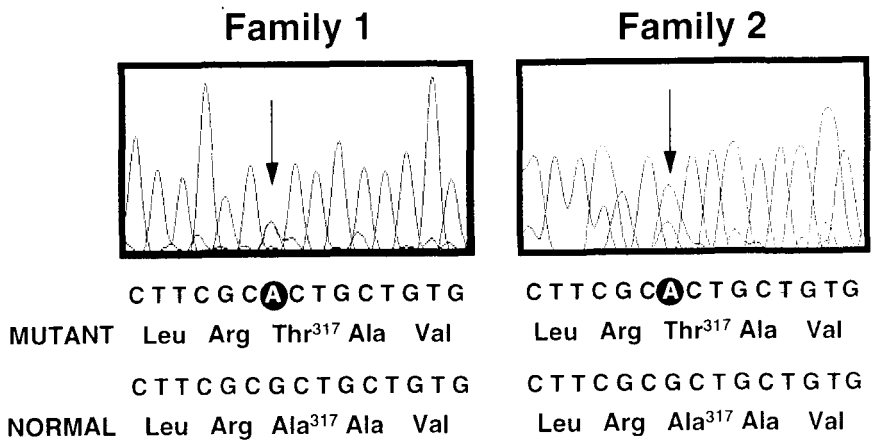


Fig. 2. Segment of DNA sequence showing the mutation in codon 317 of the TRß gene. The first nucleotide of normal codon 317 (GCT), a guanine, was substituted by an adenine (ACT), resulting in the replacement of the normal alanine (Ala) by threonine (Thr).

Confirmation of the mutation showed partial digestion of amplified DNA fragments from genomic DNA of the two proposita, but not other members of the family, confirmed that the mutation in the proposita occurred de novo (Fig. 1).

DISCUSSION

These two girls presented with goiter and clinical euthyroidism. Patient 1 had goiter and tachycardia with high serum T_4, T_3, and free T_4 levels. Despite TSH level at the beginning, her doctor would easily misinterpret and manage as hyperthyroidism. After this patient took antithyroid drug for three months, TSH concentration was inappropriately high compared to thyroid hormone levels. The patient could be RTH and TSH producing pituitary tumor. Although clinical features of RTH patients are euthyroidism, tachycardia can be found in common[5,9]. TRH test showed good response of TSH levels which confirmed the diagnosis of RTH. Patient 2 had goiter with high levels of free T_4, T_3 but she had clinical euthyroidism and no tachycardia compatible with normal TSH level. So her doctor only observed her thyroid function test for three years without any medication. Baseline TSH level is necessary and very important for management. TRH test was done and showed normal response of TSH which suggested the patient could be RTH.

We decided to sequence exon 9 and exon 10 of TRß gene in both affected patients since both exons contained almost all mutations reported in RTH[5]. The mutation reported herein, replacing Ala (GCT) with Thr (ACT) at codon 317, has been observed in 8 other families[10-16] (Table 2). The phenotype of this mutation is GRTH, and most of these RTH patients had high serum T_4, T_3, free T_4, free T_3 concentrations and normal serum TSH level. Two kindreds of these, kindred [F89] and [100], were treated with thyroid gland ablation and the patients had permanent hypothyroidism and needed thyroid hormone to maintain normal physiological requirement. In 4 of these[12-15], as in our patient, the mutation occurred de novo. This is compatible with its location in a CpG dinucleotide (CGCT -> CACT) hot spot. The change of CG to CA (CG to TG antisense) is the consequence of methylation of cytosine to 5-methylcytosine and the subsequent spontaneous deamination of 5-methylcytosine to thymine, which escapes the surveillance mechanism[17]. Our report lends further support to the high frequency of neo-mutations resulting in RTH. The affinity of the mutant TRß for T_3 is 13-22 per cent the normal TRß as previously reported [10,13,14] (Table 2).

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Table 2. Thyroid function tests, mode of inheritance and binding affinity of kindreds with mutation in the hTRß gene (A317T).

Kindred	Sex	Age at Presentation	T4 (ug/dl)	T3 (ng/dl)	FT4 (ng/dl)	FT3 (pg/ml)	TSH (mIU/L)	Phenotype	Inheritance*	Ka	Ka M/ Ka WT†	Members Analyzed‡	Reference
(XVIII) [F89]#	F	31	27.9 (5.0-12.0)§	205 (90-184)§	-	-	4.2 (0.5-4.0)§	GRTH	NS (Adopted)		0.16	1A	11,12
(XIII) [F52]	M	8.7	22.2 (5.0-12.0)§	326 (90-184)§	-	-	4.4 (0.5-4.0)§	GRTH	Sporadic (Affected identical twins, both parents unaffected)		0.16	1A, 2U	11,12
E.D. [F100]#	M	11	1.2 (4.5-12.5)§	643 (86-158)§	1.20 (1.0-1.9)§	-	4.7 (0.5-4.0)§	GRTH	Sporadic (both parents unaffected)	0.70	0.22	1A, 2U	10,12
C.M.	F	22	-	-	4.12 (0.70-1.55)§	10.39 (1.95-4.87)§	1.5 (0.4-4.0)§	GRTH	Familial (AD)	0.28	0.13	2A, 1U	14
P.C.	F	12	-	-	1.79 (0.70-1.55)§	10.39 (1.95-4.87)§	2.3 (0.4-4.0)§	GRTH	Sporadic	0.28	0.13	2A, 6U	14
T.P. (Patient no 1 this report)	F	6.8	16.7 (4.5-11.5)§	272 (65-170)§	4.69 (0.73-2.01)§	-	0.96 (0.35-5.2)§	GRTH	Sporadic (both parents unaffected)	-	-	1A, 3U	7
P.C. (Patient no 2 this report)	F	14	17.0 (4.5-11.5)§	181 (65-170)§	3.12 (0.73-2.01)§	5.73 (1.6-4.5)§	1.97 (0.35-5.2)§	GRTH	Sporadic (both parents unaffected)	-	-	1A, 2U	-

* NS, family not studied; SP, sporadic; F, familial; AD, autosomal dominant.
† Ka, affinity constants for T3 binding by receptors; M, mutant; WT, wild type.
‡ A, mutation with abnormal thyroid function; U, no mutation with normal thyroid function.
\# Treated with T4 [F89] and T3 [F100] during testing because of previous thyroid gland ablation.
§ Normal range

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Neo–mutation ใน thyroid hormone receptor ß gene (A317T) ในผู้ป่วยคนไทยที่มีภาวะดื้อต่อธัยรอยด์ฮอร์โมนสองครอบครัว

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คณะผู้วิจัยได้รายงานผู้ป่วยเด็กหญิงไทย 2 คนจากคนละครอบครัวที่มีภาวะดื้อต่อธัยรอยด์ฮอร์โมน ผู้ป่วยทั้ง 2 รายมีต่อมธัยรอยด์โตแต่ไม่มีลักษณะทางคลินิกของต่อมธัยรอยด์เป็นพิษ ระดับ T4, T3, free T4 และ free T3 อยู่ในเกณฑ์สูงกว่าปกติ แต่ระดับ TSH อยู่ในเกณฑ์ปกติ ผู้ป่วยเด็กหญิงในครอบครัวที่ 1 ได้รับการรักษาด้วย antithyroid drug อยู่นานถึง 1 ปี 9 เดือนเนื่องจากได้รับการวินิจฉัยว่าเป็นต่อมธัยรอยด์เป็นพิษ ส่วนผู้ป่วยเด็กหญิงในครอบครัวที่ 2 ได้รับการติดตามหน้าที่ต่อมธัยรอยด์เท่านั้น TRH สามารถกระตุ้น TSH ให้สูงขึ้นได้ในเกณฑ์ปกติ ในผู้ป่วยทั้ง 2 คน การศึกษา thyroid hormone receptor ß gene ของผู้ป่วยทั้ง 2 รายพบว่ามี mutation ของนิวคลีโอไทด์ที่ตำแหน่ง 1234 โดยเปลี่ยนจากกัวนีนเป็นอะดีนีน ทำให้เปลี่ยนกรดอะมิโนที่ตำแหน่ง 317 จากอะลานีนเป็นทรีโอนีน ผู้ป่วยทั้ง 2 รายเป็น hetorozygous ของ mutation นี้ ซึ่งไม่พบ mutation ในบิดาและมารดาของผู้ป่วยทั้ง 2 แสดงว่าเป็น neo–mutation.

คำสำคัญ : ภาวะดื้อต่อธัยรอยด์ฮอร์โมน, สองครอบครัวที่ไม่เกี่ยวพันธ์กัน

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