Lack of Association Between a Polymorphism of Human Thyrotropin Receptor Gene and Autoimmune Thyroid Disease

THONGKUM SUNTHORNTHEPVARAKUL, M.D.*,
SUPUNNEE NGOWNGARMRATANA, M.D.*,
CHAICHAN DEEROCHANAWONG, M.D.*,
SUGUNYA PHONGVIRATCHAI, B.Sc.*
SUREERAT KITVITAYASAK, B.Sc.*,
PATTRAPORN KONTHONG, B.N.*,
VEERASAK SARINNAPAKORN, M.D.*,

Abstract

A polymorphism in codon 52 of the human thyrotropin receptor results in a proline to threonine substitution in the extracellular domain of the receptor, but the association with autoimmune thyroid disease has been uncertain and there is no report the prevalence of this polymorphism in Orientals. To investigate this polymorphism and the association with autoimmune thyroid disease, we studied 113 normal unrelated individuals, 142 autoimmune thyroid disease patients including 112 Graves' disease and 30 Hashimoto's thyroiditis in the Thai population. We screened genomic DNAs of these subjects for the presence of A253 by PCR amplification using a degenerate oligonucleotide primer which produces a Tth111 I restriction site only in the presence of A253. The variant allele was present in 5.3 per cent of normal and 3.5 per cent of autoimmune thyroid disease, 2.7 per cent of Graves' disease and 6.7 per cent of Hashimoto's thyroiditis. The allele distribution in autoimmune thyroid disease patients did not differ significantly from that observed in controls. No association was found between this TSH-R polymorphism and the occurrence of autoimmune thyroid disease.

Key word : TSH-R Gene, Polymorphism, Autoimmune Thyroid Disease

Thyrotropin receptor (TSH-R) is a member of the family of G-protein-coupled receptors and is located on chromosome 14q31[1,2]. The cloning and sequencing of the chromosomal DNA (cDNA) sequence of TSH-R has been reported[3,4]. The TSH-R gene is composed of 10 exons and the sequences at the exon-intron junctions have been published[5]. Recently, the authors and others have reported the existence of a polymorphism at the nucleotide position 253 which is the first position of codon 52 of TSH-R[6,7]. The substitution of the wild-type cytosine with an adenine results in the replacement of proline (CCC) with threonine (ACC).

Two recent studies suggested that allelic variation in the TSH-R gene is associated with Graves' disease or its complications[6,8]. Three

* Department of Medicine, Rajavithi Hospital, Department of Medical Services, Ministry of Public Health, Bangkok 10400, Thailand.

more studies involving 246, 336 and 425 subjects showed no association of this polymorphism and Graves' disease[9-11]. Although Cuddihy et al concluded that the polymorphism was associated with autoimmune thyroid diseases in the female population[9], Kotsa et al found contradictory results[11]. So, the association of this polymorphism of TSH-R and Graves' disease remains unclear.

The prevalence of this polymorphism in the normal Caucasians is about 6.6-11.7 per cent[6-11]. There is no study on the prevalence of this polymorphism in the Orientals. The objective of this paper was to study the prevalence of this polymorphism in the Thai population and the association of this polymorphism with autoimmune thyroid disease.

MATERIAL AND METHOD

Subjects

We studied 113 normal unrelated individuals, 142 autoimmune thyroid disease patients including 112 Graves' disease and 30 Hashimoto's thyroiditis. All subjects included in this study were Thai. All normal subjects had no goiter, no clinical manifestations of thyroid disorders and normal thyroid function tests including negative for thyroid antibodies. Graves' disease was defined by the presence of hyperthyroidism and a diffuse goiter supported by the presence of antimicrosomal antibody (MCHA) or antithyroglobulin antibody (TGHA). Hashimoto's thyroiditis was defined by the presence of goiter with positive thyroid antibodies; MCHA or TGHA titers $\geq$ 1:100 and their thyroid function tests may be euthyroid or hypothyroid range. All subjects consented to participation in this study.

Serum thyroxine (T_4), triiodothyronine (T_3) concentrations and T_3-resin uptake values were measured by double-antibody technique radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA, USA). The serum free T_4 index (FT_4I) was calculated as the product of the serum T_4 and T_3-resin uptake values. Serum concentration of thyrotropin (TSH) was measured by immunoradiometric assay (Incstar Corporation, Stillwater, MN, USA). MCHA and TGHA were measured using hemagglutination technique (Murex, Dartford, UK).

DNA Preparation and Genotyping of the Population

Genomic DNA was isolated from peripheral blood leukocytes using Wizard™ Genomic DNA Purification Kit (Promega, Madison, WI). For detection of the polymorphic nucleotide A253 in Exon 1 of the TSH-R gene, we used a degenerate oligonucleotide primer that produces a restriction site for Tth111 I in the presence of A253 but not C253 in a strategy previously described[12]. Primer sequences were: 5'-GCGATTTCGGAGGATGGAGAA-3' (sense primer) and 5'-ccgggtactcac AGAGTCTGCG ACCTG-3' (antisense primer; degenerated nucleotides are underlined and intronic sequence is in lower case). The conditions of amplification by PCR were as follows: A volume of 100 µl containing 0.2 µg genomic DNA, 100 pmol of each primer, 200 µM each dNTP, 2.5 µM $MgCl_2$, 5 mM Tris-HCl pH 8.0, 10 mM NaCl, 10 mM EDTA, 0.5 mM DTT, 5 per cent glycerol, 0.1 per cent Triton-X100 and 0.8 units of Taq DNA polymerase (Promega, Madison, WI). Initial denaturation was at 94°C for 5 minutes, followed by 35 cycles of 94°C for 30 seconds, 53°C for 30 seconds, 72°C for 50 seconds and a final extension at 72°C for 15 minutes.

Amplification using the primers, whose sequences are listed above, generates a 227 bp fragment spanning 43 bp upstream from the translation start site to a 12 bp intronic sequence downstream from exon 1 of the TSH-R[5]. Overnight digestion of the PCR product with Tth111 I (New England BioLabs, Inc., Beverly, MA) generates two fragments of 201 and 26 bp only in the presence of A253 (Fig. 1) which was detected by electrophoresis on a 3 per cent Nuseive/1 per cent agarose gel.

Statistical Analysis

The statistical significance of differences between individuals with autoimmune thyroid disease and normal controls with respect to the presence or absence of the codon 52 TSH-R polymorphism was determined using 2 x 2 contingency tables and Chi-square analysis with Yates correction for continuity. Corrected p-value less than 0.05 were considered to indicate statistical significance. Similar analyses, using 2 x 2 contingency tables and either Chi-square analysis or the Fisher exac test, were used for the disease specific Graves' disease, Hashimoto's thyroiditis. Data were analyzed using EPISTAT Program.

RESULTS

Clinical and biochemical characteristics of normal controls and autoimmune thyroid disease patients are shown in Table 1. Thyroid antibodies

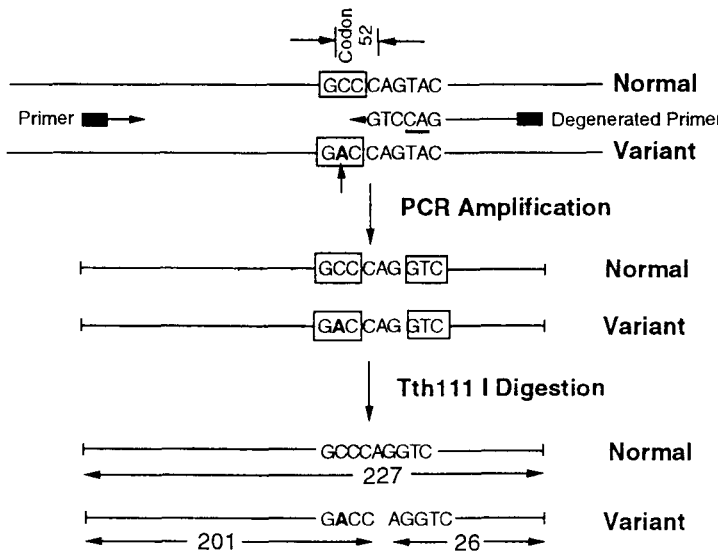

Fig. 1. Method for detection of the polymorphic allele A253-^{52}Thr.

Table 1. Clinical and biochemical characteristic of normal controls and autoimmune thyroid disease patients.

	Normal Range	Normal	Graves' disease	Hashimoto's thyroiditis
Total Number	-	113	112	30
Female:Male	-	72:41	94:18	30:0
Age (yr)	-	30.3±8.0*	35.4±11.3*	36.8±12.8*
T_4 (μg/dl)	4.5-11.5	7.9±1.8*	17.2±5.9*	7.1±3.0*
T_3 (ng/dl)	65-170	105±26*	402±167*	94±53*
FT_4I	1.33-3.67	2.13.±0.43*	5.61±3.16*	2.08±0.79*
TSH (mlU/L)	0.35-5.2	1.19±0.51*	0.11±0.17*	10.4±22.8*

* mean ± SD

were negative in all normal subjects and were equal or higher than 1:100 in Hashimoto's thyroiditis patients.

The distribution of genotypes in the difference patient groups in this paper compared to the others are shown in Table 2. The prevalence of the TSH-R codon 52 polymorphism in normal, Graves' disease and Hashimoto's thyroiditis were 5.3 per cent, 2.7 per cent and 6.7 per cent, respectively. There was no significant difference in the distribution of genotypes among autoimmune thyroid disease group and normal subjects. All subjects who had variant nucleotide 253A are heterozygous for this polymorphism.

DISCUSSION

A proline to threonine substitution at codon 52 of the TSH-R gene was originally described as a point mutation in a patient with Graves' disease and its complications[6,8]. Computer analysis predicted that the proline to threonine substitution would disrupt a potential loop in the extracellular domain of TSH-R[6], a region shown to be important for the binding of immunoglobulins[13,14]. However, this allelic variant was also identified in normal subjects and subsequent studies correctly identified the variation as a polymorphism[7,10].

The frequency of polymorphism in codon 52 of TSH-R gene in the normal Thai population is

Table 2. Prevalence and statistical significance of the human TSH receptor codon 52 polymorphism among the various categories of patients and individuals with normal thyroid function.

	Group	Total number	Genotypes			Statistical significance of association (p)
			+/-[a]		+/+[b]	
			number	%	number	
Bohr et al 1993 (6)	Normal	50	4	8	46	-
Bahn et al 1994 (8)	Graves'	22	2	9.1	20	NS (0.31)†
	Normal	17	0	0	17	-
	Total	39	2	5.1	37	-
Sunthornthepvarakul et al 1994 (7)	Normal	60	7	11.7	53	-
Cuddihy et al 1995 (9)	AITD	125	20	16	105	0.03‡
	Graves'	91	15	16.5	76	0.04‡
	Hashimoto's	34	5	14.7	29	NS (0.13)†
	Normal	121	8	6.6	113	-
	Total	246	28	11.4	218	-
Watson et al 1995 (10)	AITD	180	14	7.8	166	NS (0.86)‡
	Graves'	141	11	7.8	166	NS (0.86)‡
	Hashimoto's	39	3	7.7	36	NS (0.65)†
	Normal	156	12	7.7	144	-
	Total	336	26	7.7	310	-
Kotsa et al 1997 (11)	Graves'	180	15	8.3	165	NS (0.85)‡
	Normal	245	18	7.3	227	-
	Total	425	33	7.8	392	-
The present report	AITD	142	5	3.5	137	NS (0.35)†
	Graves'	112	3	2.7	109	NS (0.25)†
	Hashimoto's	30	2	6.7	28	NS (0.53)†
	Normal	113	6	5.3	107	-
	Total	255	11	4.3	244	-

a +/-, heterozygous for CCC (Pro) and ACC (Thr) at codon 52.
b +/+, homozygous for CCC (Pro) at codon 52.
† 2-Tailed Fisher Exact Test, ‡ Chi-square with Yates Corrected.

5.3 per cent that is lower than other reports in Caucasians (6.7-11.7%) (Table 2). The prevalence of this polymorphism in autoimmune thyroid disease group was not significantly different from normal subjects. This result is compatible with that of Watson and Kotsa et al(10,11). Recently, there was a study of analysis of thyrotropin receptor gene in familial Graves' disease using microsatellite markers within the TSH-R introns(15). The linkage analysis strongly rejected the hypothesis that TSH-R is linked to Graves' disease(15). These results and our result argue against the idea that this polymorphism is important in the susceptibility to Graves' disease. Because of the rarity of this polymorphism in Thailand, the study would be confirmed by the analysis of greater patient numbers to reveal any association.

The present study showed no significant difference in the frequency of this polymorphism in patients with autoimmune thyroid disease compared to that in normal subjects.

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การขาดความสัมพันธ์ระหว่าง Polymorphism ของ Human Thyrotropin Receptor Gene กับ Autoimmune Thyroid Disease

ทองคำ สุนทรเทพวรากุล, พ.บ., สุรีรัตน์ กิจวิทยาศักดิ์, วท.บ. (รังสีเทคนิค)*,*
สุพรรณี เงางามรัตน์, พ.บ., ภัทราพร ก้อนทอง, วท.บ. (พยาบาล)*,*
ชัยชาญ ดีโรจนวงศ์, พ.บ., วีระศักดิ์ ศรินนภากร, พ.บ.*, สุกัญญา พงษ์วิรัชไชย, วท.บ.**

มีการตรวจพบ Polymorphism ใน codon 52 ของ human thyrotropin receptor ทำให้มีการเปลี่ยนแปลงในกรดอะมิโน โปรลีน เป็น ทรีโอนีน จากการศึกษาหาความสัมพันธ์ของ Polymorphism ตำแหน่งนี้กับ autoimmune thyroid disease แต่ผลการศึกษายังไม่แน่นอนว่ามีความสัมพันธ์หรือไม่ ประกอบกับยังไม่มีการศึกษาหาอุบัติการณ์ของ Polymorphism นี้ในชาวตะวันออก. คณะผู้วิจัยได้ทำการศึกษาในคนไทย คนปกติจำนวน 113 คน, ผู้ป่วย autoimmune thyroid disease 142 คน เป็น Graves' disease 112 คน และ Hashimoto's thyroiditis 30 คน ได้ตรวจสอบหาว่ามีนิวคลีโอไทด์อะดีนีน ที่ตำแหน่ง 253 ของ thyrotropin receptor gene โดยวิธี PCR และตัดด้วยเอ็นไซม์ Tth 111 I. พบว่ามี Polymorphism ในคนปกติ 5.3%, ในผู้ป่วย Graves' disease 2.7% และในผู้ป่วย Hashimoto's thyroiditis 6.7% ซึ่งไม่มีความแตกต่างกันอย่างมีนัยสำคัญทางสถิติ จากการศึกษานี้สนับสนุนว่า Polymorphism ที่ตำแหน่ง 52 ของ thyrotropin receptor gene ไม่มีความสัมพันธ์กับ autoimmune thyroid disease

คำสำคัญ : TSH-R Gene, Polymorphism, Autoimmune Thyroid Disease

* กลุ่มงานอายุรกรรม, โรงพยาบาลราชวิถี, กรุงเทพ ฯ 10400