Int J Endocrinol Metab. 2010;8(2):64-67
International Journal of
Endocrinology & Metabolism
Journal home page: www.IJEM.org

Association between serum level of anti-TPO titer and polymorphisms G1193/C Exon 8 and C2145/T Exon 12 of thyroid peroxidase gene in an Iranian population

Mehdi Hedayati [1*], Marzieh Salehi Jahromi [1], Marjan Zarif Yeganeh [1], Maryam Sadat Daneshpour [1], Laleh Hoghooghi Rad [1], Fereidoun Azizi [2]

[1] Obesity Research Center, Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, IR Iran
[2] Endocrine Research Center, Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, IR Iran

ARTICLE INFO

Article Type:
Original Article

Article history:
Received: 13 Jun 2010
Revised: 10 Jul 2010
Accepted: 1 Aug 2010

Keywords:
Anti-thyroid peroxidase
TPO gene
G1201/T and A2257/C polymorphisms
Autoimmune thyroid diseases

ABSTRACT

Background: Autoimmune thyroid diseases (AITD) are common, with important epidemiological data, including family and twin studies, supporting a strong genetic background on the etiology of AITD.
Objectives: The aim of this study was to assess the relationship between two polymorphisms of Thyroid Peroxidase gene (TPO) and serum level of Anti-TPO titer in an Iranian population.
Patients and Methods: A sample of 184 participants from the Tehran Lipid and Glucose Study was selected as the case (No.=112) and control (No.=72) groups. Inclusion criteria for cases were Anti-TPO and Anti-Tg>100U/L with a history of hypothyroidism. Anti-TPO level in subjects was measured by the ELISA kit. Genomic DNA was extracted using Salting-out/Proteinase K method. Polymorphism detection of Exon 8 and 12 was done using the PCR-RFLP method. The PCR products were incubated with restriction enzymes SacII and BsrI, respectively.
Results: The C allele frequency of C2145/T polymorphism Exon 12 (rs732608) was observed in 71.2% of patients and 28.8% of normal individuals. This allele was significantly associated with increased levels of anti-TPO [(TT, 140±330 pmol/L; vs. CC, 436±380 pmol/L; $P < 0.001$), (OR: 9.2)]. The G1193/C was not polymorphic and no association between this SNP and the level of serum anti-TPO was found in this study.
Conclusions: We found that the C allele polymorphism in C2145/T exon 12 is associated with the high level of serum anti-TPO and that carriers of this allele are predisposed to disease 9.2 times more than those, who have no C allele. The selected polymorphism of exon 8 has no effect on increased levels of anti-TPO.

Please cite this paper as:
Hedayati M, Salehi Jahromi M, Zarif Yeganeh M, Daneshpour MS, Hoghooghi Rad L, Azizi F. Association between serum Level of anti-TPO titer and polymorphisms G1193/C Exon 8 and C2145/T Exon 12 of thyroid peroxidase gene in an Iranian population. *Int J Endocrinol Metab*. 2010;**8**(2):64-67.

Background

Over the last 20 years, different loci, such as MHC, CTLA-4, PTPN22, and TSHR genes have been linked to autoimmune thyroid disease (AITD). Moreover, several single nucleotide polymorphisms (SNPs) have been identified in AITD (1). Thyroid peroxidase (TPO) is the major thyroid autoantigen recognized by serum autoantibodies in patients with Graves' disease or Hashimoto's thyroiditis (2-4). This enzyme catalyses the oxidation of iodine to an iodinating species that forms iodotyrosines in a thyroglobulin (Tg) molecule and subsequently iodotyronines, which ultimately result in thyroid hormone synthesis such as T3 and T4 (5-10). In AITD, the immune system

* *Corresponding author at:* Mehdi Hedayati, Obesity Research Center, Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, PO Box: 19395-4763, Tehran, IR Iran. Tel: +98-2122432500, Fax: +98-2122416264.
E-mail: Hedayati@endocrine.ac.ir

produces specific antib-odies against thyroid tissue and causes tissue destruction and hypothyroidism, and thus diminishes the production of thyroid hormones (11). The humoral response in Hashimoto's thyroiditis is manifest by heterog-eneous IgG class autoantibodies to TPO, of which and around 180 human TPO antibodies have been identified (12, 13). Several studies have shown that Anti-TPO level is increased in Hashimoto's disease. Anti-TPO antibodies indicate an autoimmune reaction in which immune cells target the TPO proteins and thyroid gland, leading to thyroid diseases such as Hashimoto's thyroiditis, Grave's disease or postpartum thyroiditis (14).

In a twin study in the UK, the concordance rates for Tg antibodies were 59% and 23% in MZ and DZ twins, respectively. The concordance rates for TPO-autoantibodies were also 47% and 29% in MZ and DZ twins, respectively (15). These data suggest that Hashimoto's thyroiditis has a substantial inherited susceptibility, and seems to be a polygenic disease with a complex mode of inheritance. Immunomodulatory genes are expected to play an important role in predisposing and modulating the pathogenesis of Hashimoto's thyroiditis (16). Many patients with defects in mechanisms involved in the thyroid hormone biosynthesis have a mutation in the TPO gene (4, 5).

Objectives

This study hypothesizes that immune dysfunction is not the only major factor in autoimmune thyroid disorders and that that most likely TPO molecule alterations contr-ibute to affect the immune responses, indicating that thyroid tissue, should also be involved in this disorder. Based on this hypothesis, two common polymorphisms G1193/C in exon 8 and C2145/T in exon 12 of TPO gene, were selected for investigation.

Patients and Methods

The Tehran Lipid and Glucose Study (TLGS) was designed to determine the risk factors for major non-communicable disorders, including atherosclerosis and to promote healthy lifestyles in district 13 of Tehran, Iran. In this cross sectional study, 184 TLGS participants were selected as the control (No. = 72) and case (No. = 112) groups. Inclusion criteria for cases were anti-TPO and anti-Tg > 100U/L with a history of hypothyroidism, while those with anti-TPO and anti-Tg < 100U/L and without hypothyroidism were included in the control group (17). Written informed consent was obtained from each subject. Two polymorphisms of the TPO gene, G1193/C in exon 8 (rs2175977) and C2145/T in exon 12 (rs732608) were investigated in these two groups.

The amounts of anti-TPO and anti-Tg antibodies were measured by the ELISA method (Labodia Company, Geneva, Switzerland). For analysis of the TPO polymorphism, buffy coats were separated from non coagulated blood samples and stored at -70°C until processing when genomic DNA was extracted by the standard Salting out/ Proteinase K method (18). The polymerase chain reaction (PCR) was used to amplify a 698 bp fragment in exon 8 of the TPO gene using the oligonucleotide primers F: 5'- GTC TGC CCT TCT ACC GCT CTT C -3' and R: 5'- CAC GAT GAC CCT CCA CAC G TC -3' and a 177 bp fragment in the exon 12 of TPO gene using the oligonucleotide primers F: 5'- CTG TCT CGG GTC ATC TGT G -3' and R: 5'- GTA ACG TGG TGT GAG AGG AGA C -3'. Each amplification was performed using

Table 1. Genotype and allele frequencies of G1193/C and C2145/Tt polymorphisms in male and female participants (Total: 73 Male, 111 Female)

G polymorphism, G1201/T, Exon 8			A polymorphism, A2257/C, 12 Exon		
Genotype/Allele	**Male** [No. (%)]	**Female** [No. (%)]	**Genotype/Allele**	**Male** [No. (%)]	**Female** [No. (%)]
GG	73 (100)	111 (100)	TT	45 (62)	73 (66)
GC	0	0	CT	23 (31)	28 (25)
CC	0	0	CC	5 (6)	10 (9)
G	1	1	T	0.774	0.783
C	0	0	C	0.226	0.216

Table 2. Clinical variables, anthropometric measurements, and serum laboratory values in three different genotypes of C2145/T polymorphism

	Genotypes			p-value
	TT (118)	**CT** (51)	**CC** (15)	
Age (year)	43 ± 13	44 ± 14	43 ± 14	0.86
Weight (kg)	72 ± 12	74 ± 12	70 ± 8	0.53
Anti- TPO (U/L)	140 ± 330	500 ± 824	436 ± 380	p < 0.001
Anti-Tg (U/L)	270 ± 941	250 ± 370	450 ± 516	0.68
Log anti- TPO	2.96 ± 1.9	4.7 ± 2	5.8 ± 0.7	p < 0.001
Log anti-Tg	3.3 ± 2.2	4.5 ± 1.7	5.5 ± 1.1	p < 0.001

(Data are presented as Mean ± SD)

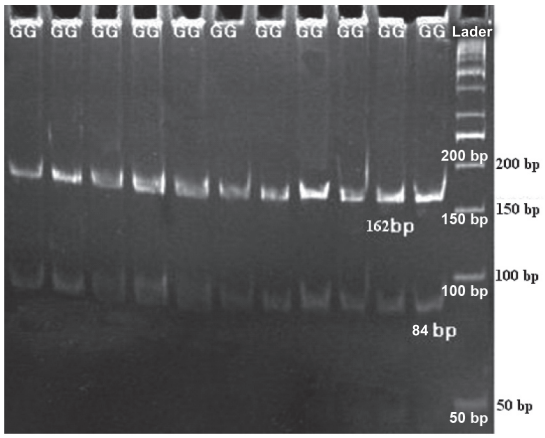

Figure 1. PCR-RFLP pattern produced by Sac II restriction enzyme on 12% polyacrylamide gel (Exon 8)

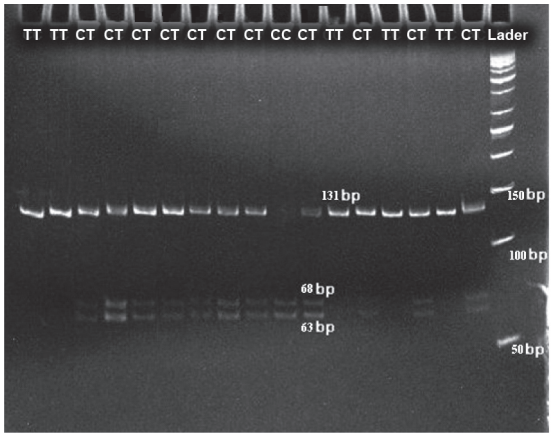

Figure 2. PCR-RFLP pattern produced by BsrIrestriction enzyme on 12% polyacrylamide gel (Exon 12)

100 ng of total genomic DNA in a final volume of 25 μL containing 40 pmol of each oligonucleotide, 0.2 mmol L-1 of each dNTP, 1.5 mmol L-1 MgCl2, 10 mmol L-1 Tris (pH 8.4) and 0.25 units of Taq polymerase (Fermentase Co. Canada). Hybridization was carried out in a DNA thermal cycler (Corbett co. Australia) in which DNA templates were denatured at 95°C for 5 minutes, amplification, consisting of 35 cycles at 95°C for 45 seconds, 60°C for 1 minute and 72°C for 1 minute, with a final extension at 72°C for 5 minutes. The PCR products were subjected to restriction enzyme analysis by digestion at 37°C for 2 hours with 10 U/μL of SacI restriction endonuclease in each 10 μL of PCR sample in the buffer recommended by the manufacturer of the endonuclease (Roche Co. City, Germany). The fragments were separated by electrophoresis on a 12% acrylamid gel,which after electrophoresis, was treated with 1μg/dl ethidium bromide solution for 10 minutes, and DNA fragments were visualized by gel documentation (Optigo Co. City, Holland).

Statistical analysis was performed by the SPSS program. For quantitative variables Mean ± SD was used and qualitative variables were expressed as percentage. In addition, to compare the findings, two-tailed ANOVA followed by the post-hoc Tukey multiple tests were used. Significance level was set at $p < 0.001$.

Table 3. Serum anti-TPO in C2145/T polymorphism

Allele	Anti-TPO (+)	Anti-TPO (-)
T (118)	25 (21.2%) [a]	93 (78.8%)
C (66)	47 (71.2%) [a]	19 (28.8)

[a] $P < 0.001$

Table 4. The association between the C and T alleles of the C2145/T polymorphism and serum anti-Tg levels

Allele	Anti-Tg (+) (100pm/L)	Anti-Tg (-) (100pm/L)	p-value
T (118)	33 (28.0%)	85 (72.0%)	$p < 0.001$
C (66)	38 (57.6%)	28 (42.4%)	$p < 0.001$

Results

The results of genotype and allele frequency determination of G1193/C polymorphism in both genders are shown in *Table 1.* The allele frequency for G allele was 100%, whereas the C allele was not observed in this population. Digestion with SacII and BsrI restriction enzymes revealed the polymorphic sites, which were produced by a single nucleotide substitution in positions of G1193/C exon 8 and C2145/T exon 12 of TPO gene, respectively *(Figures 1 and 2, Table 1).*

The association between three different genotypes of C2145/T polymorphism, clinical variables, anthropometric measurements, and serum laboratory values are given in *Table 2.* Due to population dispersion and wide ranges of anti-TPO and anti-Tg, the logarithm of these values was used in the calculations. Significant differences were found among anti-TPO levels and three genotypes, namely TT, CT, and CC ($p < 0.001$), but there was no significant correlation between mean age and weight in these three genotypes. The C and T allele associations between C2145/T polymorphism and anti-TPO levels in this study population is given in *Table 3.*

In the TPO + group, the frequencies of the T and CA alleles were 21.2% and 71.2%, respectively. On the other hand, in the TPO- group, 78.8% and 28.8% of individuals carried the T and C alleles, respectively, with a significant correlations found in both groups ($p < 0.001$). The effect of C allele on autoimmune hypothyroidism was evaluated by odds ratio (OR = 9.2). A significant correlation was also seen between increased amounts of anti-Tg and C alleles. The association between C and T alleles of C2145/T polymorphism and anti-Tg levels is given in *Table 4*, which demonstrates that individuals with C allele have significantly higher levels of anti-Tg ($p < 0.001$) than T allele carriers.

Discussion

In this study the effect of two common polymorphisms of TPO gene i.e. G1193/T in exon 8 and C2145/T in exon 12

and their correlation with anti-TPO serum levels were investigated and the results showed that G1193/T was not polymorphic and no association of this SNP with serum anti-TPO level of was found, whereas the presence of C allele in the C2145/T polymorphism was found to have a correlation with increased levels of serum anti-TPO, and it was also significantly associated with anti-Tg serum levels. Hashimoto's disease, a thyroid autoimmune disease in which the thyroid gland is gradually destroyed by a variety of cells and antibody mediated immune processes, is a common cause of hypothyroidism. Specific anti-TPO production by the immune system results in thyroid tissue destruction, as a result of which, the amount of the thyroid peroxidase enzyme is reduced, thereby also decreasing or causing cessation of thyroid hormone production. Since in AITD the process of TPO enzyme production is defective, for the first time this hypothesis is being proposed that mutation in the TPO gene may contribute to the occurrence of these diseases (19-21). Autoimmune hypothyroidism can lead to numerous complications such as metabolic disorders, mental retardation in children, obesity, nervous system disorders, insomnia (sleeplessness), and infertility, all problems that obviously strongly affect the patient's quality of life and society (22, 23). Infertility treatment programs for women impose enormous financial burdens and waste of time on both the individual and society, indicating the criticality of the early diagnosis of this complication; after puberty, its diagnosis is possible through antibody measurement, facilitating possibly disease progression; however early diagnosis is not possible with low antibody levels before puberty (24). Germline polymorphisms in this disease can be detected at birth using genetic tests, thereby saving much time and cost. To conclude, the results of this study reflect one of the genetic causes of the Hashimoto's disease in an Iranian population, findings which may be used to facilitate identification of genetic susceptibility and timely diagnosis.

Financial support

None declared.

Conflict of interest

None declared.

References

1. Zeitlin AA, Simmonds MJ, Gough SC. Genetic developments in autoimmune thyroid disease: an evolutionary process. *Clin Endocrinol (Oxf)*. 2008;**68**(5):671-82.
2. Ruf J, Toubert ME, Czarnocka B, Durand-Gorde JM, Ferrand M, Carayon P. Relationship between immunological structure and biochemical properties of human thyroid peroxidase. *Endocrinology*. 1989;**125**(3):1211-8.
3. Ewins DL, Barnett PS, Tomlinson RW, McGregor AM, Banga JP. Mapping epitope specificities of monoclonal antibodies to thyroid peroxidase using recombinant antigen preparations. *Autoimmunity*. 1992;**11**(3):141-9.
4. Jaume JC, Burek CL, Hoffman WH, Rose NR, McLachlan SM, Rapoport B. Thyroid peroxidase autoantibody epitopic 'fingerprints' in juvenile Hashimoto's thyroiditis: evidence for conservation over time and in families. *Clin Exp Immunol*. 1996;**104**(1):115-23.
5. Medeiros-Neto GA, Billerbeck AE, Wajchenberg BL, Targovnik HM. Defective organification of iodide causing hereditary goitrous hypothyroidism. *Thyroid*. 1993;**3**(2):143-59.
6. Bakker B, Bikker H, Vulsma T, de Randamie JS, Wiedijk BM, De Vijlder JJ. Two decades of screening for congenital hypothyroidism in The Netherlands: TPO gene mutations in total iodide organification defects (an update). *J Clin Endocrinol Metab*. 2000;**85**(10):3708-12.
7. Furtmuller PG, Zederbauer M, Jantschko W, Helm J, Bogner M, Jakopitsch C, et al. Active site structure and catalytic mechanisms of human peroxidases. *Arch Biochem Biophys*. 2006;**445**(2):199-213.
8. Taurog A, Dorris ML, Doerge DR. Mechanism of simultaneous iodination and coupling catalyzed by thyroid peroxidase. *Arch Biochem Biophys*. 1996;**330**(1):24-32.
9. Dunn JT, Dunn AD. Update on intrathyroidal iodine metabolism. *Thyroid*. 2001;**11**(5):407-14.
10. Phillips JA. Thyroid peroxidase (TPO). [cited 2011 May 8]; Available from: http://www.ncbi.nlm.nih.gov/omim/606765.
11. Zois C, Stavrou I, Kalogera C, Svarna E, Dimoliatis I, Seferiadis K, et al. High prevalence of autoimmune thyroiditis in schoolchildren after elimination of iodine deficiency in northwestern Greece. *Thyroid*. 2003;**13**(5):485-9.
12. McLachlan SM, Rapoport B. Genetic and epitopic analysis of thyroid peroxidase (TPO) autoantibodies: markers of the human thyroid autoimmune response. *Clin Exp Immunol*. 1995;**101**(2):200-6.
13. Chardes T, Chapal N, Bresson D, Bes C, Giudicelli V, Lefranc MP, et al. The human anti-thyroid peroxidase autoantibody repertoire in Graves' and Hashimoto's autoimmune thyroid diseases. *Immunogenetics*. 2002;**54**(3):141-57.
14. Swain M, Swain T, Mohanty BK. Autoimmune thyroid disorders—An update. *Indian J Clin Biochem*. 2005;**20**(1):9-17.
15. Phillips DI, Osmond C, Baird J, Huckle A, Rees-Smith B. Is birthweight associated with thyroid autoimmunity? A study in twins. *Thyroid*. 2002;**12**(5):377-80.
16. Chistiakov DA. Immunogenetics of Hashimoto's thyroiditis. *J Autoimmune Dis*. 2005;**2**(1):1.
17. Holl RW, Bohm B, Loos U, Grabert M, Heinze E, Homoki J. Thyroid autoimmunity in children and adolescents with type 1 diabetes mellitus. Effect of age, gender and HLA type. *Horm Res*. 1999;**52**(3):113-8.
18. Truett GE, Heeger P, Mynatt RL, Truett AA, Walker JA, Warman ML. Preparation of PCR-quality mouse genomic DNA with hot sodium hydroxide and tris (HotSHOT). *Biotechniques*. 2000;**29**(1):52-4.
19. Czarnocka B, Ruf J, Ferrand M, Carayon P, Lissitzky S. Purification of the human thyroid peroxidase and its identification as the microsomal antigen involved in autoimmune thyroid diseases. *FEBS Lett*. 1985;**190**(1):147-52.
20. Portmann L, Hamada N, Heinrich G, DeGroot LJ. Anti-thyroid peroxidase antibody in patients with autoimmune thyroid disease: possible identity with anti-microsomal antibody. *J Clin Endocrinol Metab*. 1985;**61**(5):1001-3.
21. Kotani T, Umeki K, Matsunaga S, Kato E, Ohtaki S. Detection of autoantibodies to thyroid peroxidase in autoimmune thyroid diseases by micro-ELISA and immunoblotting. *J Clin Endocrinol Metab*. 1986;**62**(5):928-33.
22. DeBoer MD, LaFranchi S. Differential presentation for children with autoimmune thyroiditis discovered because of symptom development or screening. *J Pediatr Endocrinol Metab*. 2008;**21**(8):753-61.
23. Aszalos Z. [Some neurologic and psychiatric complications in endocrine disorders: the thyroid gland]. *Orv Hetil*. 2007;**148**(7):303-10.
24. Acharya SV, Mathew BK, Menon PS, Bandgar TR, Shah NS. Rare Cases of Precocious Puberty With Hypothyroidism: A Case Series With Review of Literature. *Endocrinologist*. 2010;**20**(2):78.