



The association of FokI and ApaI polymorphisms in vitamin D receptor gene with autoimmune thyroid diseases in the northwest of Iran

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Abstract

Background: Some genetic factors are involved in the etiology of Hashimoto thyroiditis and Graves' disease as autoimmune thyroid diseases (AITDs). Effects of vitamin D receptor gene polymorphisms in AITDs development have already been investigated in some previous studies. However, no study has been done on the association between VDR FokI and ApaI polymorphisms and AITDs in an Iranian population. In this study, the possible effects of FokI and ApaI polymorphisms on AITDs were investigated in the population of northwest of Iran.

Methods: A total of 121 AITDs adult patients and 117 healthy controls matched by age and sex in the same population were included in this study. FokI and ApaI polymorphisms were genotyped by polymerase chain reaction- restriction fragment length polymorphism (PCR-RFLP). Chi-square test and odds ratio (OR) with 95% CI were used to analyze the data.

Results: FokI and ApaI genotypes frequencies were not significantly different between the 2 groups ($p=0.06$, $p=0.73$, respectively). However, FokI "CC" and "CT" genotypes were related to AITDs risk ($p=0.03$; OR= 3.75; 95% CI, 1.16-12.17 and $p=0.04$; OR= 3.41; 95% CI, 1.03-11.28, respectively).

Conclusion: These data suggest that FokI polymorphisms are involved in AITDs susceptibility in the population of northwest of Iran.

Keywords: Vitamin D receptor, Genetic polymorphisms, Hashimoto disease, Graves' disease

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Introduction

Autoimmune thyroid diseases (AITD) are common autoimmune disorders that result from T-cell mediated autoimmunity (1). Graves' disease (GD) and Hashimoto thyroiditis (HT) are 2 major categories of AITDs (2). AITDs have multifactorial etiology, and some environmental and genetic factors are involved in the pathogenesis of these disorders. Moreover, women are more susceptible to such diseases. Lymphocytes diffused infiltration, subsequent thyroid tissue atrophy, and hypothyroidism are characteristics of HT. Anti-thyroid peroxidase (TPO) and anti-thyroglobulin are 2 major auto-antibodies in HT. Hyperthyroidism of GD is caused by auto-antibody production against thyroid-stimulating hormone (TSH) receptor (3).

It has been recently shown that patients with AITD had lower levels of 25-hydroxy vitamin D than healthy individuals (4). Vitamin D acts by binding the active form of vitamin D (1,25dihydroxyvitamin D) to its nuclear receptor (vitamin D receptor). Vitamin D receptor (VDR) is expressed in monocytes, macrophages, dendritic cells, and T and B lymphocytes. Vitamin D can prevent the immune system from stimulation of thyroid gland autoimmunity by binding to its receptor in mentioned immune cells (5).

VDR polymorphisms are defined as genetic variants in regulatory parts of the gene (6). The VDR gene is mapped on chromosome 12q13.11; and the BsmI (rs1544410), ApaI (rs7975232), and TaqI (rs731236) polymorphisms

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↑What is "already known" in this topic:

Vitamin D receptor (VDR) gene polymorphisms are related to the susceptibility of autoimmune thyroid diseases (AITDs) in different populations.

→What this article adds:

In the northwest of Iran population, VDR FokI polymorphisms affect AITDs risk. VDR ApaI polymorphisms do not have any association with AITDs.

Table 1. Primers sequences and PCR thermic conditions

	Primer sequences	PCR conditions
FokI rs2228570	F: 5'-AGCTGGCCCTGGCACTGACTCTGCTCT-3' R: 5'-ATGGAAACACCTTGCTTCTTCTCCCTC-3'	95° 5 min, 35X(93°C 45 s,66°C30 s, 72°C 45 s), 72° 10 min
ApaI rs7975232	F:5'-CAGAGCATGGACAGGGAGCAAG-3' R: 5'-GCAACTCCTCATGGCTGAGGTCTCA-3'	95° 5 min, 35X(93°C 45 s,66°C30 s, 72°C 45 s), 72° 10 min

are located in Intron 8, Intron 8, and Exon 9, respectively. The FokI (rs2228570) and the EcoRV (rs4516035) polymorphisms are located in the start codon and the promoter region of the VDR gene, respectively (7).

Two different length proteins can exist corresponding to the 2 FokI polymorphism, and the shorter form of the VDR protein (F allele) is more active than the long form (f allele) (8). Differences in protein size corresponding to FokI polymorphism may lead to changes in vitamin D status with feedback mechanisms. Furthermore, FokI polymorphism can alter transcription capacity of some immune-related proteins, for instance, NF-κB, and so they may be correlated with a different immune response and autoimmunity. ApaI polymorphisms may also alter 25(OH)D serum levels and affect vitamin D function (9, 10). Thus, FokI and ApaI polymorphisms may be associated with some autoimmune diseases, such as vitiligo, multiple-sclerosis, inflammatory bowel disease, and AITDs (11-14).

There are some studies about the association of FokI and ApaI polymorphisms and AITD, but the results have been inconsistent (15). Furthermore, in different populations, the same polymorphisms may have different effects on the susceptibility to autoimmune diseases (16). No study has been conducted in Iran on the relationship between FokI and ApaI polymorphisms and AITD. Thus, this study aimed at evaluating FokI and ApaI gene polymorphisms in AITDs patients and healthy controls as potential genetic risk factors for AITDs in the population of northwest of Iran.

Methods

Participants

A total of 121 adult patients (19 males, 102 females; 16 with GD, 105 with HT) aged 18 and 65 years (mean: 38.7±11.8 years), who referred to the endocrinology clinic of Urmia Imam Khomeini Teaching hospital, were selected to participate in this case-control study. HT diagnosis criteria included diffuse goiter, appearance on thyroid sonogram, positive serum antibodies against thyroid antigens, low serum free-T₄, and high serum TSH levels; and GD diagnosis criteria included diffuse goiter, ophthalmopathy, hyperthyroidism clinical symptoms (for instance, heat intolerance, weight loss, weakness, and fatigue), high serum free-T₄, low serum TSH levels, and elevated radioactive iodine uptake (17, 18). All diagnoses criteria in these patients were approved by an endocrinologist.

For control group, we carefully selected 117 adults (24 males and 93 females) aged 18 and 65 years (mean: 39.5±10.5 years), with no personal and family history of HT and GD. The 2 groups were matched by age and sex and were selected from the same population.

In this case-control study, those with Type 1 diabetes,

Addison's disease, primary biliary cirrhosis, autoimmune hepatitis, multiple sclerosis, primary hyperparathyroidism, osteoporosis, cancers, and other autoimmune diseases were excluded from the study due to the relationship between these diseases and VDR gene polymorphism (19). All participants signed an informed consent.

Genotyping: Genomic DNA was extracted from EDTA whole blood by Cinnagen DNP™ kit (Cinnagen, Iran). ApaI (rs7975232) Intron 8 (A/C) and FokI (rs2228570) Exon 2 (C/T) polymorphisms in VDR gene were amplified with polymerase chain reactions (PCR). For these reactions, 12.5 uL of Master Mix 2X (including 3Mm/mL MgCl₂, 0.08 units/uL Taq DNA polymerase and 0.4 mM/mL of each dNTPs) (Cinnagen, Iran), 0.5 uL of forward primer, 0.5 uL of reverse primer, 1 uL of extracted genomic DNA, and 10.5 uL of sterile deionized water were used. PCR thermic conditions and forward and reverse primer sequences are displayed in Table 1. PCR products obtained were 265 bp and 740 bp for FokI and ApaI on 2.5% agarose gel, respectively.

After the production of PCR products, FokI and ApaI restriction enzymes (Thermo Fisher Scientific, USA) and enzyme buffer were used for digestion of PCR products (RFLP or Restriction fragment length polymorphism). FokI PCR product was digested for 2 hours in 55°C, ApaI PCR product was digested for 2 hours in 37°C, and electrophoresis of digestion products on 2.5% agarose gel was used to determine FokI and ApaI polymorphisms. For FokI site, 265 bp and 169+96 bp represented alleles C and T, respectively (Fig. 1). For ApaI, 740 bp and 530+210 bp represented alleles A and C, respectively (Fig. 2).

Statistical analysis

Data were analyzed using SPSS 24 software (SPSS, Chicago, IL). Chi-square test was used to compare the

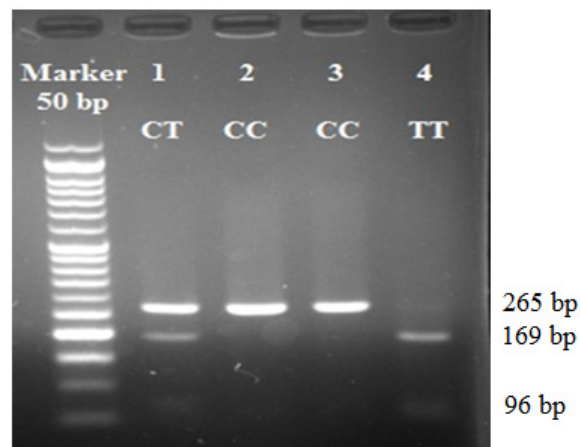


Fig. 1. Analyzing FokI genotypes (C/T) via PCR-RFLP in 4 samples

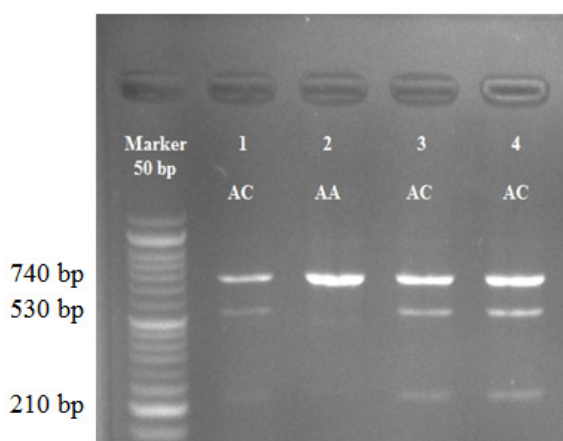


Fig. 2. Analyzing ApaI genotypes (A/C) via PCR-RFLP in 4 samples

differences of genotypes and alleles between AITDs and control groups. To evaluate the association between FokI and ApaI polymorphisms and AITDs risk, odds ratios (ORs) with 95% confidence interval (CI) were calculated using main effects model by multinomial logistic regression for genotypes. Age and sex were entered as covariates in the model. Univariate logistic regression with Enter method was used to calculate ORs in Alleles. In all tests, probability value less than 0.05 was considered statistically significant.

Results

To test genotypes frequencies, Hardy-Weinberg equilibrium (HWE) test was performed and results showed that both FokI and ApaI polymorphisms were in HWE in both case and control groups ($p > 0.05$). According to our analysis, FokI "CC" and ApaI "AC" genotypes were higher in patients with AITDs, but the differences were not statistically significant. However, when we considered FokI "TT" as the reference genotype adjusted for age and sex effects (adjusted OR= 1; CI 95%), we found that FokI "CC" and "CT" genotypes were related to higher risk of AITDs ($p = 0.03$; adjusted OR= 3.75; 95% CI, 1.16-12.16, and $p = 0.04$; adjusted OR= 3.41; 95% CI, 1.03-11.28, respectively). Also, FokI "CC" genotype was associated with higher risk of Hashimoto ($p = 0.04$; adjusted OR=

3.38; 95% CI, 1.04-11), while ApaI genotypes and alleles were not associated with risk of AITDs ($p > 0.05$) (Table 2).

Discussion

Some genetic factors including vitamin D receptor gene polymorphisms are involved in etiology of AITDs (20). Hashimoto thyroiditis and Graves' disease are 2 main manifestations of AITDs and occur by autoantibodies production against thyroid gland (21). A previously conducted study suggested that VDR polymorphisms may affect vitamin D metabolism, and thereby are involved in AITDs etiology (22).

VDR is the target nuclear receptor of vitamin D and has an important role in vitamin D function (23). VDR gene on Chromosome 12 has some single nucleotide polymorphisms (SNP), for instance, FokI and ApaI and both mentioned polymorphisms are involved in vitamin D status (9).

This study was the first investigation of the VDR FokI and ApaI possible effects on genetic susceptibility to AITDs in Iranian Azeri population. Our results revealed that FokI "CC" and "CT" genotypes have a significant effect on susceptibility to AITDs. Moreover, FokI "CC" genotype was considered as a risk factor for Hashimoto thyroiditis.

In a previous study on Chinese population in Taiwan, FokI genotypes and alleles differences between Hashimoto and control groups were statistically significant, but in other studies conducted by Inoue et al. and Meng et al. on Japanese and Chinese populations, respectively, it was demonstrated that FokI genotypes and alleles distribution among patients with AITDs and control groups were not statistically different (22, 24, 25). Our results are not consistent with those of a previous study reported by Djurovic et al., which demonstrated that individuals with FokI "CC" genotype have higher risk of Hashimoto disease (26).

In our study, ApaI genotypes and alleles did not have any association with AITDs. With the same results, a meta-analyze study performed by Feng et al. showed that ApaI polymorphisms were not related to AITDs pathogenesis (15). However, Inoue et al. revealed that Japanese

Table 2. Genotype and allelic frequencies of FokI and ApaI polymorphisms among patients and controls

	AITDs patients (n=121)	Control subjects (n=117)	p^{\ddagger}	Adjusted OR* (95% CI)	p
SNP FokI					
CC	70 (57.9)	60 (51.3)		3.75 (1.16-12.16)	0.03
CT	47 (38.8)	44 (37.6)	0.06	3.41 (1.03-11.28)	0.04
TT	4 (3.3)	13 (11.1)		1.00 (reference)	
Allele FokI					
C	187 (77.3)	164 (70.9)	0.08	1.46 (0.96-2.21)	0.07
T	55 (22.7)	70 (29.1)		1.00 (reference)	
SNP ApaI					
AA	39 (32.2)	42 (35.9)		1.00 (reference)	
AC	57 (47.1)	49 (41.9)	0.73	1.2 (0.66-2.15)	0.55
CC	25 (20.7)	26 (22.2)		1.04 (0.52-2.1)	0.91
Allele ApaI					
A	135 (55.8)	130 (55.6)	1	1.00 (reference)	0.95
C	107 (44.2)	104 (44.4)		1.00 (0.69-1.42)	

CI: confidence interval; OR: odds ratio; SNP: single-nucleotide polymorphism

\ddagger Two-sided χ^2 analysis

*Adjusted for age and sex

patients with AITDs had higher proportion of "C" allele compared to the control group (22).

Our results are helpful for broad understanding of underlying causes of AITDs. More investigations of VDR polymorphisms are needed to understand the effects of genetic factors in AITDs, especially in Iranian population. A study of interaction between FokI and ApaI polymorphisms with Vitamin D status is necessary to realize the complex effects of genetic and environmental factors related to AITDs etiology and describe the possible molecular mechanisms of VDR polymorphisms involvement in AITDs pathogenesis. The limitation of our study was low number of Graves's patients and low quality of registration system. Thus, more studies with large sample sizes, especially on Graves' disease, should be conducted on Iranian population in the future.

Conclusion

We found that VDR FokI and ApaI genotypes and allele frequencies did not differ among AITDs and healthy groups. However, FokI "CC" and "CT" genotypes were associated with higher risk of AITDs.

Acknowledgments

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Ethical approval

The ethic committee of Urmia University of Medical sciences (code: Ir.umsu.rec.1394.412) approved the research protocol.

Informed consent

Informed consent was obtained from all individual participants in the study.

Conflict of Interests

The authors declare that they have no competing interests.

References

- Antonelli A, Ferrari SM, Corrado A, Di Domenicantonio A, Fallahi P. Autoimmune thyroid disorders. *Autoimmun Rev.* 2015;14(2):174-80.
- DeGroot LJ, Quintans J. The causes of autoimmune thyroid disease. *Endocr Rev.* 1989;10(4):537-62.
- Dong Y, Fu D. Autoimmune thyroid disease: mechanism, genetics and current knowledge. *Eur Rev Med Pharmacol Sci.* 2014;18(23):3611-8.
- Wang J, Lv S, Chen G, Gao C, He J, Zhong H, et al. Meta-analysis of the association between vitamin D and autoimmune thyroid disease. *Nutrients.* 2015;7(4):2485-98.
- Bizzaro G, Shoenfeld Y. Vitamin D and autoimmune thyroid diseases: facts and unresolved questions. *Immunol Res.* 2015;61(1-2):46-52.
- Valdivielso JM, Fernandez E. Vitamin D receptor polymorphisms and diseases. *Clin Chim Acta.* 2006;371(1):1-12.
- Lee YH, Gyu Song G. Vitamin D receptor FokI, BsmI, TaqI, ApaI, and EcoRV polymorphisms and susceptibility to melanoma: a meta-analysis. *J BUON.* 2015;20:235-43.
- Uitterlinden AG, Fang Y, van Meurs JB, Pols HA, van Leeuwen JP. Genetics and biology of vitamin D receptor polymorphisms. *Gene.* 2004;338(2):143-56.
- Li K, Shi Q, Yang L, Li X, Liu L, Wang L, et al. The association of vitamin D receptor gene polymorphisms and serum 25-hydroxyvitamin D levels with generalized vitiligo. *Br J Dermatol.* 2012;167(4):815-21.
- van Etten E, Verlinden L, Giuliotti A, Ramos-Lopez E, Branisteanu DD, Ferreira GB, et al. The vitamin D receptor gene FokI polymorphism: functional impact on the immune system. *Eur J Immunol.* 2007;37(2):395-405.
- Birlea S, Birlea M, Cimponeriu D, Apostol P, Cosgarea R, Gavrilă L, et al. Autoimmune diseases and vitamin D receptor Apa-I polymorphism are associated with vitiligo in a small inbred Romanian community. *Acta Derm Venereol.* 2006;86(3):209-14.
- Huang J, Xie ZF. Polymorphisms in the vitamin D receptor gene and multiple sclerosis risk: a meta-analysis of case-control studies. *J Neurol Sci.* 2012; 313(1):79-85.
- Wang L, Wang Z, Hu J, Fan R, Zhou J, Zhong J. Polymorphisms of the vitamin D receptor gene and the risk of inflammatory bowel disease: a meta-analysis. *Genet Mol Res.* 2014;13(2):2598-610.
- Ramos-Lopez E, Kurylowicz A, Bednarczuk T, Paunkovic J, Seidl C, Badenhoop K. Vitamin D receptor polymorphisms are associated with Graves' disease in German and Polish but not in Serbian patients. *Thyroid.* 2005;15(10):1125-30.
- Feng M, Li H, Chen SF, Li WF, Zhang FB. Polymorphisms in the vitamin D receptor gene and risk of autoimmune thyroid diseases: a meta-analysis. *Endocrine.* 2013;43(2):318-26.
- Škrabić V, Zemunik T, Šitum M, Terzić J. Vitamin D receptor polymorphism and susceptibility to type 1 diabetes in the Dalmatian population. *Diabetes Res Clin Pract.* 2003;59(1):31-5.
- Caturegli P, De Remigis A, Rose NR. Hashimoto thyroiditis: clinical and diagnostic criteria. *Autoimmun Rev.* 2014;13(4-5):391-7.
- Ginsberg J. Diagnosis and management of Graves' disease. *CMAJ.* 2003;168(5):575-85.
- Valdivielso JM, Fernandez E. Vitamin D receptor polymorphisms and diseases. *Clin Chim Acta.* 2006;371(1):1-12.
- Guarneri F, Benvenga S. Environmental factors and genetic background that interact to cause autoimmune thyroid disease. *Curr Opin Endocrinol Diabetes Obes.* 2007;14(5):398-409.
- Trbojevic B, Djurica S. Diagnosis of autoimmune thyroid disease. *Srp Arh Celok Lek.* 2005;133 Suppl 1:25-33.
- Inoue N, Watanabe M, Ishido N, Katsumata Y, Kagawa T, Hidaka Y, et al. The functional polymorphisms of VDR, GC and CYP2R1 are involved in the pathogenesis of autoimmune thyroid diseases. *Clin Exp Immunol.* 2014;178(2):262-9.
- Kato S. The function of vitamin D receptor in vitamin D action. *J Biochem.* 2000; 127(5):717-22.
- Lin WY, Wan L, Tsai CH, Chen RH, Lee CC, Tsai FJ. Vitamin D receptor gene polymorphisms are associated with risk of Hashimoto's thyroiditis in Chinese patients in Taiwan. *J Clin Lab Anal.* 2006; 20(3):109-12.
- Meng S, He ST, Jiang WJ, Xiao L, Li DF, Xu J, et al. Genetic susceptibility to autoimmune thyroid diseases in a Chinese Han population: Role of vitamin D receptor gene polymorphisms. *Ann Endocrinol (Paris).* 2015;76(6):684-9.
- Djurovic J, Stojkovic O, Ozdemir O, Silan F, Akurut C, Todorovic J, et al. Association between FokI, ApaI and TaqI RFLP polymorphisms in VDR gene and Hashimoto's thyroiditis: preliminary data from female patients in Serbia. *Int J Immunogenet.* 2015;42(3):190-4.