Research article

TPO gene rs2071400 polymorphisms as an independent risk factor for hypothyroidism in Iraqi patients

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ABSTRACT

Introduction and Aim: Thyroid peroxidase (TPO) is a thyroid-specific antigen, and the presence of TPO antibodies is known to be associated with developing hypothyroidism. In this study we aimed to investigate the genotypes prevalent for the SNP rs2071400 of the TPO gene and its association to the development of autoimmune thyroid disease.

Materials and Methods: This study involved 43 patients with hypothyroidism and 44 healthy controls. Genotyping of the TPO SNP rs2071400 (C/T) was undertaken using Quantitative Real-Time Polymerase Chain Reaction using the TaqMan® Assay. Data obtained was subjected to statistical analysis using the SPSS 25 software

Results: TPO rs2071400 T carriers (CT + TT genotypes) were more frequent in patients with hypothyroidism compared with healthy control (P=0.0006), with an adjusted odds ratio of 8.0. Serum levels of Anti-TPOAb were also significantly higher in hypothyroid patients. There was no significant correlation between rs2071400 T mutation and the presence of high Anti-TPOAb.

Conclusion: Study revealed TPO rs2071400 polymorphism to be an independent risk factor for developing hypothyroidism in those with or without high serum levels of TPOAb.

Keywords: Thyroid peroxidase gene; Hypothyroidism; Anti-Thyroid Peroxidase antibody; rs2071400.

INTRODUCTION

The term "hypothyroidism" refers to a thyroid gland that is underactive, releasing inadequate thyroid hormone for proper body function maintenance. Hypothyroidism occurs due to insufficient thyroid hormone in the blood. Common causes include Hashimoto's thyroiditis, a lack of iodine in the diet, thyroid surgery, and radiation treatments (1). Hypothyroidism may not cause noticeable symptoms in the early stage but over time, untreated hypothyroidism can cause a number of health issues such as weight gain, joint pain, infertility, enlarged goiter and heart disease (1, 2). Overt or clinical primary hypothyroidism is diagnosed by measuring the thyroid-stimulating hormone (TSH) concentrations in blood (2). Numerous studies have indicated the pathogenesis of autoimmune thyroid disease to have a genetic component. Hypothyroid patients have a background inherited tendency for autoimmunity, as well as triggering environmental and hormonal factors that contribute to the disease's development (3). Genetic predisposition to autoimmune thyroid disease has been linked to a variety of immune-related genes. Hashimoto's disease (HD) and other autoimmune thyroid disorders (AITDs) are prototypical organ-specific autoimmune illnesses (4). Some HD patients develop hypothyroidism early in childhood, while others remain euthyroid throughout their lives. AITDs are caused by a breakdown in immunological tolerance to thyroid-specific antigens such as thyroid peroxidase (TPO) and thyroglobulin (TG) (5). High concentrations of TPOAb and anti-TPOAb present in the serum of most autoimmune thyroid disease patients, is considered a useful biomarker of autoimmune thyroiditis (6, 7).

The TPO gene, located on the short arm of chromosome 2 consists of 17 exons and is approximately 150 kb in length (8, 9). TPO gene polymorphisms are linked to the development of autoimmune thyroid illness and serum levels of anti-thyroid peroxidase antibodies (10, 11). In this investigation, we undertook to genotype the single nucleotide polymorphism (SNP) rs2071400 of the TPO gene to determine its relationship to AITD development and diagnosis.

MATERIALS AND METHODS

This cross-sectional case/control study carried out between December 2021 and February 2022, included 87 individuals comprising 43 hypothyroid patients and 44 seemingly healthy volunteers (control) aged between 25-60 years. Pregnant women, biotin users, patients who underwent thyroidectomy, and patients with renal or liver disease were excluded from the study. Data pertaining to age, gender, height, weight and body mass index (BMI) of each participant was recorded. Thyroid-stimulating hormone (TSH),

thyroxine (T4), and immunological parameters including anti-TPO antibody and anti-Tg antibody were measured. Additionally, Real-Time PCR was used to genotype participants for SNP (rs2071400). DNA was extracted from fresh or frozen blood samples that were collected in EDTA tubes and then treated with DNA purification kits. The DNA was extracted using the AddPrep Genomic DNA Extraction Kit (Add bio Solutions Company) following the manufacturer's instructions. Genotyping for the TPO gene SNPs (rs2071400) was carried out with TaqMan real-time PCR Master (PROBE) FAM and VIC dye assay using a real-time thermocycler (Ro-tor-Gene Q Qiagen/Germany). The probes used in Genotyping studies are given in Table 1. Biochemical tests were performed by Electrochemiluminescence (ECL) technology by Cobas E411 System (Roche, Germany).

Statistical analysis

All statistical analyses were performed using the SPSS 25 software (SPSS Inc., Chicago, IL, USA). Numeric variables were expressed with mean and standard deviation (SD). Categorical variables were expressed with numbers and percentages. Pearson's correlation was used for bivariate correlations, and multiple linear regression was used to adjust variables and to identify independence. A statistically significant level of the P-value was considered to be less than 0.05. Chi-square test was used to significantly compare between percentage (0.05 and 0.01 probability) and to estimate the Odds ratio and CI.

RESULTS

Among the total of 87 participants in the present study, 71 (81.6%) were females and 16 (18.39%) were males. Forty-three individuals were already diagnosed with hypothyroidism, while the other 44 participants were apparently healthy. 34 (79.1%) of the patients with hypothyroidism exhibited elevated serum levels of anti-thyroid antibodies. Only 9 (20.9%) of the control group had high anti-TPOAb as shown in Table 2.

There was a statistically significant positive correlation between hypothyroidism and each of the aforementioned auto-antibodies (Table 2). The odds ratio of having high anti-TPOAb was 14.69, and the odds ratio of anti-TGAb was 18.66 (Table 3). Results of genetic analysis of SNP (rs2071400) revealed that 30.3% (n=13) of the hypothyroid patients to be homozygous (TT), 37.2% (n=16) to be heterozygous (CT), and 32.5% (n=14) to be of wild (CC) genotype. Similarly, in the control group, 79.5% (n=35) were of wild-type CC, 15.9% (n=7) heterozygous CT, and 4.6% (n=2) homozygous TT genotypes.

Table 1: Primers used in RT-PCR in this study

Probe	**Primer**	**Sequence From 5-3**	**Product size**	**Company origin**
TPO gene SNP rs2071400 **[C/T]**	Forward	GTCATCTCCCAGTGTTCGGA	114 bp	Alpha DNA, Canada
	Revers	AGCATGCATTCTCCCCTCAA		
Wild probe	GTGACCTTCCTACAGAACATCTC This oligo was 5-end 6-FAM labeled This oligo was 3-end BHQ-1 modification			
Mutant probe	GTGACTTTCCTACAGAACATCTCT This oligo was 5-end VIC- replacement dye This oligo was 3-end BHQ-1 modification			

Table 2: Anti-TPO Ab categorical group

Count		**Group**		**Total**
		Patients(43)	**Control(44)**	
Anti-TPOAb category	Positive	34 (79.1%)	9 (20.9%)	43
	Negative	9 (20.5%)	35 79.5%)	44
Total		43	44	**87**
Odd ratio (95% CI)		14.69 (5.20-41.46)		

Table 3: Anti-Tg Ab categorical group

Count		**Group**		**Total**
		Patients(43)	**Control(44)**	
Anti-TgAb category	Positive	28 (87.5%)	4(12.5%)	32
	Negative	15 (27.3%)	40 (72.7%)	55
Total		43	44	**87**
Odd ratio (95% CI)		18.66 (5.60-62.22)		

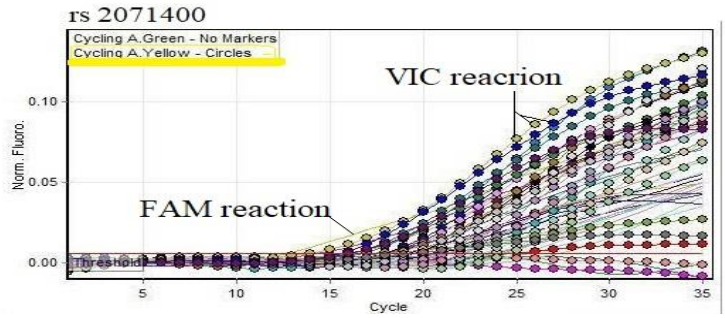


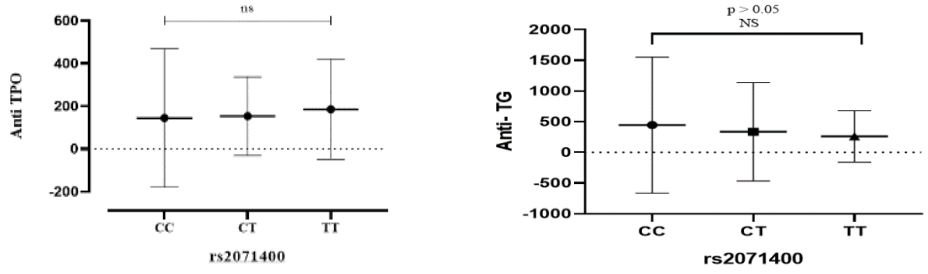
Fig. 1: Quantitative Real-Time Polymerase Chain Reaction - TaqMan® VIC (Cycling A. Yellow) channel in hypothyroidism patients for TPO gene of SNPrs 2071400

The TT genotype frequency was significantly higher in hypothyroid patients than in apparently healthy control (p=0.0007) indicating that homo-mutant genotype rs2071400 TT was at a higher risk of hypothyroidism than the wild type rs2071400 CC and was shown to be a risk factor for hypothyroidism (Odds ratio =16.2) compared with those carrying the wild-type CC. Those with heterozygous mutation (rs2071400 CT genotype) had a 5.7 fold higher risk than the wild-type CC (p=0.001). Our study found that the frequency of the TPO SNP (rs2071400) T carriers (CT + TT genotypes) was higher in hypothyroid patients compared with control group, with an odds ratio of carrying any mutant allele (both homozygous and heterozygous) = 8 (odds ratio= 8).

In our study was the association between different genotypes of rs2071400 (CC, CT, and TT) polymorphism and serum laboratory values measurements for Anti-TPO ab and, Anti-Tg ab.No Significant differences were found among anti-TPO levels and three genotypes, namely TT, CT, and CC (p=0.883). The mean (±SD) in CC, CT, and TT genotypes was (144.5816 ± 324.37469), (153.4265 ± 184.31108), and (185.6900± 234.27096) respectively (Fig.2). Also, no significant differences were found between anti-Tg antibody levels and the three genotypes. Similarly no significant correlation was observed between anti-TgAb and the three genotypes (P=0.71). The mean (±SD) values for CC, CT, and TT genotypes was (461.3576 ± 1085.71233), (302.4526 ± 841.36597), and (283.1267± 417.40294) respectively (Fig.3).

Table 4: The genotypes and allele frequency distributions for SNP rs2071400 of the TPO gene

***TPO* polymorphism rs2071400 C >T**	**Frequencies (%)**		**P value**	**Odd ratio (95% CI)**
	Control group n=44)	**Patients Group (n=43)**		
Codominant				
CC	79.5% (n=35)	32.5% (n=14)	-----	1.00 (Reference)
CT	15.9% (n=7)	37.2% (n=16)	0.001	5.7
TT	4.6% (n=2)	30.3% (n=13)	0.0007	16.2
Dominant				
CC	79.5% (n=35)	32.5% (n=14)	----	1.00 (Reference)
CT+ TT	20.5% (n=9)	67.5% (n=29)	0.0006	8.0
Recessive				
CC+CT	95.4% (n=42)	69.7% (n=30)	-----	1.00 (Reference)
TT	4.6% (n=2)	30.3% (n=13)	0.005	9.1
Allele Frequency				
C	87.5% (n=77)	51.1% (n=44)	-----	1.00 (Reference)
T	12.5% (n=11)	48.8% (n=42)	0.0001	6.6



a **b**

Fig. 2. The association between the CC, CT, and TT genotype of the rs2071400 polymorphism and serum anti-TPO antibody levels (**b**) The association between the CC, CT, and TT genotype of the rs2071400 polymorphism and serum anti-Tg antibody levels.

DISCUSSION

In this study, among the hypothyroid patients 79.0% tested positive for anti-TPOAb, while 20.9% tested negative. Similarly, among healthy volunteers 81.8% were found negative for the anti-TPOAb while 18.1% were positive.

Our study also showed the frequency of CT + TT genotypes in the TPO SNP rs2071400 to be increased in hypothyroidism patients. Since, this SNP rs2071400 (is located in the promoter region, this SNP may affect the expression of TPO. These results agree with Tomari *et al.*, (10) who found a statistically significantly higher interaction of hypothyroidism with rs2071400 polymorphisms frequency of T carriers (CT + TT genotypes) patients than in control subjects (p-value= 0.0488). The C (wild-type) allele frequencies were 87.5% in apparently healthy control but in hypothyroidism patients 51.1%. The T allele (variant) frequencies were 12.5% in apparently healthy control, and in hypothyroidism patients 48.8%, was significantly higher (p=0.0001) in hypothyroidism patients than in apparently healthy control. This result was in agreement with the results of a study conducted by Ahmed *et al.*, where it was found the TT genotype of rs2071400 C/T and the T allele were significantly more frequent in patients with hypothyroidism than in the control group (11). Results in this study are in agreement to these earlier studies showing that allele T of TPO (rs2071400 C>T) is a risk factor for the disease while allele C is a protective factor against the hypothyroidism disease. Furthermore, association of C1858T polymorphism has been made to increase AITD susceptibility (11). The C1858T variant of rs2071400 has been shown to optimize the activity of the SNP *PTPN*22 620W allele, which has been shown to be associated with multiple autoimmune phenotypes including increased susceptibility to AITD (12).

CONCLUSION

The present case-control study found a correlation between the TPO gene SNP rs2071400 (C>T) genotypes to the risk of developing hypothyroidism among the Iraqi population. In contrast to the C allele, which was considered to be protective against developing hypothyroidism, the T allele of TPO (rs2071400 C>T) was deemed a risk factor.

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CONFLICTS OF INTEREST

Authors declare no conflicts of interest.

REFERENCES

1. Chaker, L., Antonio, C. B., Jonklaas, J., Peters, R. P. Hypothyroidism. The Lancet. 2017;390(10101): 1550-1562.
2. Koehler, V. F., Reincke, M., Spitzweg, C. Hypothyroidism-when and how to treat? Der Internist, 2018; 59(7): 644-653.
3. Dvornikova, K. A., Bystrova, E. Y., Platonova, O. N., Churilov, L. P. (2020). Polymorphism of toll-like receptor genes and autoimmune endocrine diseases. Autoimmunity reviews. 2020; 19(4): 102496.
4. Casto, C., Pepe, G., Li, P.A., Corica, D., Aversa, T., Wasniewska, M. Hashimoto's thyroiditis and Graves' disease in genetic syndromes in pediatric age. Genes, 2021;12(2): 222.
5. Mizuma, T., Watanabe, M., Inoue, N., Arakawa, Y., Tomari, S., Hidaka, Y., Iwatani, Y. Association of the polymorphisms in the gene encoding thyroglobulin with the development and prognosis of autoimmune thyroid disease. Autoimmunity. 2017; 50(6): 386-392.
6. McLachlan, S.M., Rapoport, B. Autoimmune response to the thyroid in humans: thyroid peroxidase-the common auto antigenic denominator. Int Rev Immunol 2000; 19: 587-618.
7. Watanabe, M., Yamamoto, N., Maruoka, H., Tamai, H., Matsuzuka, F, *et al*., Independent involvement of CD8+ CD25+ cells and thyroid autoantibodies in disease severity of Hashimoto's disease. Thyroid. 2002; 12: 801-808.
8. Endo, Y., Onogi, S., Umeki, K., Yamamoto, I., Kotani, T. *et al.*, Regional localization of the gene for thyroid peroxidase to human chromosome 2p25 and mouse chromosome 12C. Genomics 1995; 25: 760-761.
9. Kimura, S., Hong, Y. S., Kotani, T., Ohtaki, S., Kikkawa, F. Structure of the human thyroid peroxidase gene: comparison and relationship to the human myeloperoxidase gene. Biochemistry. 1989; 28: 4481-4489.
10. Tomari, S., Watanabe, M., Inoue, N., Mizuma, T., Yamanaka, C.,Hidaka, Y. *et al*., The polymorphisms in the thyroid peroxidase gene were associated with the development of autoimmune thyroid disease and the serum levels of anti-thyroid peroxidase antibody, Endocrine Journal, 2017; 64(10):1025-1032.
11. Ahmed, H., Nsrallah, A., Abdel-Fatah, A., Mahmoud, A. and Fikry, A. Association of thyroid peroxidase gene polymorphisms and serum Anti-TPO levels in Egyptian patients with autoimmune hypothyroidism, Endocr Metab Immune Disord Drug Targets. 2021; 21(4):734-742.
12. Criswell, L.A., Pfeiffer, K.A., Lum, R.F., Gonzales, B., Novitzke, J., Kern, M., *et al*., Analysis of families in the multiple autoimmune disease genetics consortium (MADGC) collection: The PTPN22 620W allele associates with multiple autoimmune phenotypes. Am. J. Hum. Genet. 2005;76:561-571.