Al-Rafidain J Med Sci. 2025;8(1):208-214.
DOI: https://doi.org/10.54133/ajms.v8i1.1684

AJMS
Online ISSN (2789-3219)

Research Article

Role of FOXP3 (rs3761548) Polymorphism in Modulating FOXP3 Protein Level in Iraqi Patients with Thyroid Disorder

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Received: 6 January 2025; Revised: 7 March 2025; Accepted: 10 March 2025

Abstract

Background: Thyroid disorders are a major clinical concern that negatively impacts the thyroid gland. Thyroid issues have been connected to the FOXP3 gene, which is an important immune system regulator. FOXP3 protein levels can be affected by genetic differences, which may impair regulatory T-cell activity and exacerbate immunological abnormalities. ***Objectives***: To investigate the influence of FOXP3 gene polymorphisms on circulating FOXP3 protein levels and their association with thyroid dysfunction. ***Methods***: This study included 100 patients with thyroid disorders and 50 healthy controls. Thyroid function was assessed by measuring serum T3, T4, and TSH levels using a Cobas analyzer. Serum FOXP3 protein levels were quantified by ELISA. Genomic DNA was extracted and analyzed for FOXP3 gene variations using PCR and sequencing. ***Results***: No significant differences were observed in serum T3 and T4 levels between patients and controls. However, TSH levels were significantly elevated in the patient group. Serum FOXP3 levels were significantly lower in patients compared to controls. The distribution of the three FOXP3 gene genotypes (AA, AC, and CC) did not differ significantly between patients and controls. Individuals with the heterozygous (AC) genotype exhibited significantly higher serum FOXP3 levels compared to the other two genotypes. ***Conclusions***: Individuals with thyroid disorders had reduced levels of FOXP3, which may indicate an association between thyroid disease and impaired immunity. The study emphasizes the complicated interaction of genetic and environmental factors, even if it could not identify an obvious connection with the rs3761548 gene variation.

Keywords: Forkhead box p3 (FOXP3), Polymorphism, rs3761548, Thyroid disorder.

دراسة دور تعدد الأشكال الجيني (rs3761548) FOXP3 في تعديل مستوى بروتين FOXP3 لدى مرضى اضطرابات الغدة الدرقية العراقيين

الخلاصة

الخلفية: تُعد اضطرابات الغدة الدرقية مشكلة سريرية رئيسية نظرًا لتأثيرها السلبي على وظيفتها. وقد تم ربط مشاكل الغدة الدرقية بجينFOXP ، وهو منظم مهم للجهاز المناعي. يمكن أن تتأثر مستويات بروتين FOXP3 بالاختلافات الجينية، مما قد يؤدي إلى ضعف نشاط الخلايا التائية التنظيمية وتفاقم الشذوذات المناعية. **الأهداف**: التحقيق في تأثير تعدد أشكال جين FOXP3 على مستويات FOXP3المتداولة وارتباطها بضعف وظيفة الغدة الدرقية. **الطرائق**: شارك في هذه الدراسة 100 مريض مصاب باضطرابات الغدة الدرقية و50 فردًا سليمًا. تم تقييم وظيفة الغدة الدرقية عن طريق قياس مستويات هرمونات الثيروكسين الحر، ثلاثي يودوثيرونين وهرمون منبه الغدة الدرقية في الدم وتم تحديد كمية بروتين FOXP3 في المصل باستخدام تقنية ELISA تم استخراج الحمض النووي الجينومي وتحليله للكشف عن تغيرات جين FOXP3 باستخدام تقنيتي تفاعل البوليميراز المتسلسل (PCR) والتسلسل الجيني. **النتائج**: لم تسجل فروقًا معنوية في مستويات T3 و T4في الدم بين المرضى والأصحاء. كانت مستويات TSHمرتفعة بشكل ملحوظ في المرضى. أن مستويات FOXP3في المصل كانت أقل بشكل معنوي لدى المرضى مقارنة بالأفراد الأصحاء. ولم يختلف توزيع الأنماط الجينية الثلاثة لجين FOXP3 بشكل معنوي بين المرضى والأفراد الأصحاء. ومن المثير للاهتمام أن الأفراد الذين يحملون النمط الجيني المتغاير (AC) أظهروا مستويات FOXP3في المصل أعلى بشكل معنوي مقارنة مع النمطين الجينيين الآخرين. **الاستنتاجات**: إن الأفراد الذين يعانون من مشاكل في الغدة الدرقية لديهم انخفاض FOXP3 وهو بروتين أساسي للوظيفة المناعية، مما قد يشير إلى وجود ارتباط بين أمراض الغدة الدرقية وضعف المناعة. تؤكد الدراسة على التفاعل المعقد للعوامل الوراثية والبيئية، حتى لو لم تتمكن من تحديد ارتباط واضح مع تعدد الأشكال الجيني .rs3761548 من أجل الحصول على فهم أفضل لدور FOXP3 وتعدد أشكاله الجينية في أمراض الغدة الدرقية، هناك حاجة إلى مزيد من الدراسات مع مجموعات مرضى أكبر ومعايير أكثر دقة للاختيار.

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Article citation: Muayad BA, Jassim HA. Role of FOXP3 (rs3761548) Polymorphism in Modulating FOXP3 Protein Level in Iraqi Patients with Thyroid Disorder. *Al-Rafidain J Med Sci*. 2025;8(1):208-214. doi: https://doi.org/10.54133/ajms.v8i1.1684

INTRODUCTION

The endocrine system is a network of glands that secrete hormones into the bloodstream. These hormones act as chemical messengers, traveling throughout the body to regulate various bodily functions, including growth, development, metabolism, and reproduction [1]. Thyroid disorders are prevalent in women more than in males and can range from inflammatory lesions to neoplastic tumors [2,3]. About 40% of people worldwide have a thyroid problem [4]. While 2-5% of people have autoimmune thyroid disease (AID) [5]. The risk of developing these conditions is increased by various variables. Iodine consumption, pollution from the environment, smoking, some medicines, and genetics are a few of them [4]. Hashimoto's thyroiditis (HT) and Graves' disease (GD) are prevalent autoimmune thyroid gland illnesses that cause hypothyroidism and hyperthyroidism, respectively [6,7]. Hypothyroidism refers to an underactive thyroid gland. As a result, the thyroid gland's ability to function is reduced. Under secretion of thyroid hormone, primarily thyroxine (T4) and

triiodothyronine (T3), is the result of this condition. Although it can affect anyone at any age, it is more likely in women and elderly individuals [8]. A prominent cause of hypothyroidism in iodine-rich locations is chronic autoimmune thyroiditis, often known as Hashimoto's thyroiditis. Genetic factors, infections, stress, radiation exposure, environmental triggers, and interactions between genetic and environmental factors may all be associated with the beginning of disease [9]. The term "overt hyperthyroidism" refers to elevated levels of triiodothyronine (T3) and free thyroxine (FT4) as well as suppressed thyrotropin (formerly known as thyroid-stimulating hormone). About 0.2% to 1.4% of people worldwide suffer from overt hyperthyroidism, while about 0.7% to 1.4% of persons worldwide suffer from subclinical hyperthyroidism, which is characterized by low thyrotropin concentrations and normal T3 and FT4 values. The clinical manifestation of disorders of the thyroid is identified by changes in blood levels of thyroid-stimulating hormone, L-3,5,3-triiodothyronine (T3), and L-3,5,3,5-tetraiodothyronine (T4) [10]. Among thyroid problems, hyperthyroidism and hypothyroidism as well as hypothyroxinemia are the most common. Cell differentiation, metabolism, and function are all impacted by these conditions [11]. Thyroid hormones (T3 and T4) are produced with the help of iodine. As a result, a lack of iodine causes a decrease in T3 and T4 synthesis, resulting in hypothyroidism. High iodine intakes cause an increase in the rate of T3 and T4 production, leading to hyperthyroidism [12]. The forkhead box p3 (*FOXP3)* is an effective marker for the stimulation and growth of regulatory T cells [13,14]. The transcription factor *FOXP3* plays a crucial role in avoiding autoimmune disorders by controlling the growth and activity of Treg cells. Variations in FOXP3 can impair Treg cell's ability to suppress, resulting in autoimmune disorders [15,16]. The *FOXP3* gene plays a crucial role in immune tolerance as a transcription factor, and its absence results in immunological dysregulation [17,18]. Numerous autoimmune illnesses, including Graves disease (GD), have been linked to the forkhead box p3 (FOXP3) gene polymorphisms [19]. The *FOXP3* gene is located on the X chromosome and controls both the development and functions of Tregs. Functional mutations in the *FOXP3* gene have been associated with susceptibility to various autoimmune diseases. Moreover, mutation of the *FOXP3* gene is a risk in unexplained recurrent spontaneous abortions, Grave's disease, and allergic rhinitis [20]. The *FOXP3* gene contains 11 coding exons and 3 non-coding exons in the 5' upstream region in humans; the FOXP3 protein contains multiple structural domains [21]. This study aims to investigate the potential association between serum FOXP3 levels and the Foxp3 gene polymorphism (rs3761548) in Iraqi patients with thyroid disorders. By examining this genetic variation and its influence on FOXP3 serum level, we expect to gain insights into the immunological mechanisms underlying thyroid disease and potentially identify novel biomarkers for disease diagnosis and prognosis.

METHODS

Study design and setting

Two distinct groups were included in this study: the Control Group, which comprised 50 healthy individuals randomly selected from a pool of hospital visitors and relatives, and the Patient Group, which encompassed 100 individuals diagnosed with thyroid disorders. These patients were recruited from the National Diabetes Center, Mustansiriyah University, and Al-Yarmouk Teaching Hospital in Baghdad.

Inclusion criteria

Patients were included in the study if they were diagnosed with either hypothyroidism or hyperthyroidism, as determined by standard laboratory tests measuring thyroid-stimulating hormone (TSH), triiodothyronine (T3), and thyroxine (T4) levels.

Exclusion criteria

The following individuals were excluded from the study. Pregnant women, Women using hormonal contraceptives or menstrual cycle regulators, individuals with a history of thyroidectomy Patients with underlying liver or kidney disease and individuals currently using biotin.

Ethical Considerations

The study protocol was approved by the Council of the Institute of Genetic Engineering and Biotechnology at the University of Baghdad. Before enrollment, all participants provided written informed consent. Additionally, necessary approvals were obtained from the collaborating centers, hospitals, and laboratories.

Biochemical tests

The Cobas E411 system is a highly accurate and efficient automated platform used for biochemical diagnostic testing, including thyroid hormone assessments (TSH, T3, and T4). Frozen serum samples are thawed and loaded into the system using labeled sample cups. The TSH is dependent on the Sandwich Principle, with a biotinylated antibody that binds to the TSH antigen in the sample, and a ruthenium-labeled antibody that binds to a different epitope on the TSH antigen. Streptavidin-coated paramagnetic microparticles capture the biotinylated antibody-antigen complex. Furthermore, upon application of electrical potential, the ruthenium label emits light, and the intensity of the light is directly proportional to the amount of TSH in the sample. The competitive principles of T3 and T4 include a ruthenium-labeled antibody that competes with the

antigen (T3 or T4) in the sample for binding to a specific binding site. Streptavidin-coated paramagnetic microparticles capture the remaining unbound ruthenium-labeled antibody. Upon application of electrical potential, the ruthenium label emits light, and the intensity of the light is inversely proportional to the amount of antigen in the sample. The FOXP3 serum level is evaluated using an Enzyme-Linked Immunosorbent Assay (ELISA) kit from Innova Biotech CO.LTD Ltd., China, with cat. no. In.hu2287. This technique involves coating a microplate with antibodies specific to FOXP3. The serum sample or a standard containing a known amount of FOXP3 is then added to the wells. A second antibody, conjugated to horseradish peroxidase (HRP), binds to the captured FOXP3. After washing away unbound components, a substrate (TMB) is added, which is catalyzed by HRP to produce a blue color. The intensity of this color, measured at 450 nm, is directly proportional to the amount of FOXP3 in the sample. By comparing the sample's absorbance to a standard curve, the concentration of FOXP3 can be accurately determined [22].

Genomic DNA Extraction

Genomic DNA was extracted from both fresh and frozen blood samples collected in EDTA tubes. Frozen samples were stored for a maximum of seven days before extraction. DNA isolation was performed using the Easy Pure Blood Genomic DNA Extraction Kit from Trans Gen Biotech, China, with cat. no. (EE121), a protocol designed for efficient and rapid extraction of genomic DNA from up to 200 µL of whole blood. DNA concentration and purity were assessed using a Nanodrop spectrophotometer. TE buffer was used as a blank, and 2 microliters of extracted DNA were measured at 260 nm and 280 nm. Concentrations ranged from 32 to 57 ng/µl, and A260/A280 ratios were between 1.7 and 1.9, indicating pure DNA.

Polymerase chain reaction

A specific primer was designed according to the current study to detect FOXP3 SNP (rs3761548) with forward sequence (TCTTGCTCGCTCTTTGTGTG) and reverse sequence (GCCCCACAATCAAGGTTTT) with product size (582bp). To assess the FOXP3 gene's association with thyroid disorder, the PCR reaction was optimized using a partial gene sequence. An annealing temperature of 56°C was found to be optimal for producing clear bands on an agarose gel. The 25 µl PCR reaction was prepared using 2xEasyTaq® PCR SuperMix, forward and reverse primers, DNA template, and nuclease-free water. The PCR program involved an initial denaturation step at 94°C, followed by 35 cycles of denaturation at 94°C, annealing at 56°C, and extension at 72°C. A final extension step at 72°C for 5 minutes was included to ensure complete DNA synthesis.

Gel Electrophoresis

DNA samples, including extracted genomic DNA and PCR products, were separated using agarose gel electrophoresis. Agarose gels of 1% and 2% concentration were prepared with TBE buffer and ethidium bromide for DNA extraction and PCR product detection, respectively. DNA samples were mixed with loading dye and loaded into the wells of the gel. Electrophoresis was performed at 100 volts for 60 minutes, causing DNA fragments to migrate towards the positive electrode. The gel was then stained with ethidium bromide and visualized under UV light. The DNA bands were captured using a gel documentation system and analyzed using specialized software.

DNA Sequencing

The amplified PCR fragments were sequenced using Sanger sequencing on an ABI3730XL automated DNA sequencer. The obtained sequences were aligned with a reference sequence using Blast to determine the genotypes. Geneious Prime software was used for further analysis, including sequence alignment and other molecular biology tasks.

Statistical analysis

Statistical analysis was conducted using SPSS 26. Data was presented as mean and standard deviation. Normality was assessed using the Shapiro-Wilk test. Independent t-tests were used to compare group means. Hardy-Weinberg equilibrium was evaluated using a web-based tool. Chi-square tests were used to compare genotype and allele frequencies between groups. Odds ratios with 95% confidence intervals were calculated to assess the association between FOXP3 gene SNPs and thyroid disorders. Statistical significance was determined at a *p*-value of less than 0.05 [23,24].

RESULTS

The current study revealed that the mean age of patients in the intervention group (43.54 ± 1.94 years) was slightly higher than that of the control group (39.03 ± 1.54 years). However, this difference in age between the two groups did not reach statistical significance (p= 0.07) (Table 1). The gender distribution between the study groups was not statistically different (p= 0.8). Both groups exhibited a female predominance, with females comprising 78% and 80% of the disease and healthy groups, respectively, and males comprising the remaining 22% and 20%, respectively (Table 1). Biochemical analysis of thyroid hormones revealed no significant differences between patients and healthy individuals for T3 (p= 0.1) and T4 (p= 0.3). However, a significant increase in TSH levels was observed in patients compared to healthy controls (p= 0.001).

Table 1: Comparison between patients and control regarding age and sex.

Parameters		Groups		*p*-value
		Patients (n=100)	Control (n=50)	
Sex	Female	78(87)	40(80)	0.8
	Male	22(22)	10(20)	
Age (year)		43.54±1.94	39.02±1.54	0.07

Values are expressed as frequency, percentage, and mean±SE

The mean TSH level in patients was (5.66±0.66), while in healthy individuals it was (2.08±0.17). Serum FOXP3 levels were significantly lower in patients compared to healthy controls (p= 0.02), with mean values of 631.1±29.92 and 714.92±20.66, respectively (Table 2).

Table 2: Comparison between patients and control groups in T4, T3, TSH, and FOXP

Groups	Results		*p*-value
	Patients	Control	
T3 (µIU/mL)	73.55±5.82	61.63±5.36	0.1
T4 (µIU/mL)	1.85±0.20	1.68±0.05	0.3
TSH (µIU/mL)	5.66±0.66	2.08±0.17	0.001
FOXP3 (pg/ml)	631.1±29.92	714.92±20.66	0.02

Values are expressed as mean±SD.

Analysis of the rs3761548 region using the sequence technique revealed no statistically significant differences in genotype frequencies between patients and healthy controls. Specifically, no significant associations were observed for the AA, AC, and CC genotypes (p= 0.7, 0.5, and 0.6, respectively, for the A and C alleles (p= 0.8) (Table 3).

Table 3: The genotypes and allele frequency of rs3761548 in patients and control

SNP rs3761548	Frequencies n(%)		*p*-value	Odd ratio (95% CI)
	Patients	Control		
AA	30(30)	16(32)	0.7	0.91 (0.44 to 1.93)
AC	36(36)	15(30)	0.5	1.31(0.63 to 2.77)
CC	34(34)	19(38)	0.6	0.84 (0.41 to 1.72)
A	96(48)	47(47)	0.8	1.04 (0.64 to 1.69)
C	104(52)	53(53)	0.8	0.96 (0.59 to 1.56)

Table 4 elucidates Hardy-Weinberg equilibrium results among study groups. Deviation from Hardy-Weinberg equilibrium was noted in both patient and control groups (p= 0.02 for both). Within the patient group, the AC genotype exhibited the highest frequency (36), followed by CC of 34 and AA of 30. In contrast, the CC genotype was most prevalent in the control group, followed by AA and AC (19, 16%, and 15%, respectively).

Table 4: Hardy-Weinberg equilibrium result among study groups

Groups rs3761548		AA	AC	CC	*P*
Patients	Observed No.	30	36	34	0.02
	Expected No.	23.04	49.92	27.04	
Control	Observed No.	16	15	19	0.02
	Expected No.	11.045	24.91	14.045	

Subsequent ROC curve analysis yielded an AUC of 0.62 with p= 0.03, indicating moderate discriminative ability. While sensitivity was high at 92%, specificity was relatively low at 32%, suggesting potential utility as a screening tool but necessitating further investigation with a larger sample size to enhance diagnostic accuracy [22] (Figure 1).

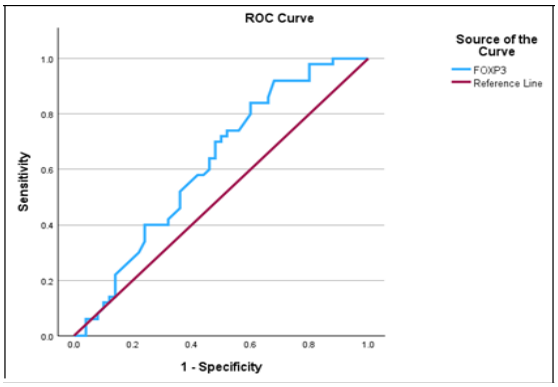

Figure 1: The ROC Curve of FOXP3 serum level (AUC= 0.621, p= 0.031, the best cut-off value = 532.50, Sensitivity (92%), and Specificity (32%).

The effect of a specific genetic polymorphism, rs3761548 on the severity of thyroid disorder patients among individuals in the study group is shown in Table 5. Analysis of serum FOXP3 levels revealed a significant decrease in patients carrying the AC genotype compared to healthy controls. The mean FOXP3 level in patients with the AC genotype was 585.56, while it was 795.73 in healthy controls with the same genotype. Within the healthy group, a significant decrease in mean FOXP3 levels was observed across all three genotypes (CC, AA, and AC). The mean levels for these genotypes were 659.89, 704.50, and 795.73, respectively.

Table 5: The effect of rs3761548 genotypes on FOXP3 level

Genotypes			Groups		
			Patients	Control	*p*-value
rs3761548	AA	FOXP3	615.67±62.31	704.50±42.83	0.2
	AC	FOXP3	585.56±43.96	795.73±31.34	0.001
	CC	FOXP3	692.94±50.04	659.89±26.63	0.5
	p-value		0.3	0.02	

Values are expressed as mean±SE.

DISCUSSION

Autoimmune thyroid disease (AITD) is a common organ-specific autoimmune disorder, primarily affecting middle-aged women [25]. AITD occurs in 2–4% of women and up to 1% of men, with increasing prevalence with age and influenced by genetic, immune, and environmental factors [26,27].The AITDs arise from a breakdown in the body's immune tolerance, leading to the production of antibodies against thyroid antigens [28]. This often involves dysfunction of T lymphocytes, which

play a crucial role in regulating the immune response. The development of AITDs is likely influenced by a complex interplay between environmental triggers and genetic predisposition [29,30]. T regulatory (Treg) cells, particularly the CD4+CD25+ subset, are essential for maintainin immune tolerance by suppressing self-reactive immune cells [31]. Dysfunction or depletion of these cells has been implicated in the pathogenesis of autoimmune disease. Evidence suggests that patients with these diseases may have reduced numbers of CD4+CD25 high Treg cells. Furthermore, studies have shown that enhancing Treg cell function can suppress experimental autoimmune thyroiditis [32]. FOXP3 is a master regulator of Treg cell differentiation, development, and function. Its deficiency impairs Treg cell suppressive activity, leading to unchecked activation of self-reactive T cells and the development of autoimmunity. Notably, Foxp3 is located on chromosome Xp11.23, a region previously linked to an autoimmune disorder [16, 33, 34]. The study observed a consistent increase in TSH levels, T3, and T4 levels in patients in comparison with control, aligning with previous research [35] in T3 and T4 while different in TSH levels. This study found significantly lower levels of serum FOXP3 in individuals with thyroid disorders compared to healthy controls. FOXP3 is a crucial protein that regulates the activity of regulatory T cells (Tregs), which are essential for maintaining a balanced immune system. Previous research on the association between serum FOXP3 levels and autoimmune diseases has been limited. A study by Paradowska *et al*. in a Polish population about rheumatoid arthritis (RA) suggested a potential link between elevated Foxp3 protein levels and RA susceptibility. Their findings revealed significantly higher serum Foxp3 levels in RA patients compared to healthy controls ($p< 0.0001$). Specifically, 138 patients exhibited positive Foxp3 levels, while 134 were negative. In the control group, 54 individuals were positive, and 241 were negative [36]. Another study by Ikram [37], revealed that serum Foxp3 levels had a statistically significant increase in RA patients; this result was assessed in 68 RA patients and 68 healthy controls using ELISA. Furthermore, a positive correlation was found between serum Foxp3 levels and both disease activity score and disease grade. However, these findings suggest that serum Foxp3 levels may not accurately reflect Treg-mediated immune regulation in the context of RA. Patients with thyroid conditions had substantially reduced serum FOXP3 levels, according to this study. For T-reg cells, which typically maintain immunological homeostasis, to grow and operate, FOXP3 is a critical transcription factor. The immune system is greatly impacted by thyroid disease, which includes both hyperthyroidism and hypothyroidism. The observed decline in FOXP3 levels suggests the possibility of a correlation between the emergence of thyroid diseases and compromised Treg function. The observed drop in FOXP3 levels may be caused by Treg activity being disrupted by chronic inflammation within the thyroid gland. Thyroid dysfunction, including both overactive and underactive thyroid function, can disrupt the immune system. The observed decrease in FOXP3 suggests that Tregs may not be functioning properly in individuals with thyroid disorders. The previous study conducted by Sedyabane *et al*. [38] investigated the association between serum FOXP3 levels and cervical lesions in a cohort of 90 cervical cancer (CC) cases, 90 cervical intraepithelial neoplasia (CIN) cases, and 90 healthy controls, using a quantitative ELISA. The results demonstrated significantly higher mean FOXP3 levels in serum samples from CC cases compared to both CIN cases and healthy controls ($p< 0.001$). Notably, over half (58%) of CC cases exhibited serum FOXP3 concentrations exceeding 0.0545 ng/ml ($P < 0.001$). While elevated serum FOXP3 levels were observed in CC cases, no significant association was found between increased FOXP3 expression and CIN. Furthermore, multivariate analysis revealed that increased serum FOXP3 concentrations were associated with a two-fold increased risk of developing CC (Odds Ratio: 2.094, $p= 0.038$, 95% CI: 1.042-4.209). The study observed a decline in FOXP3 levels in patients after Percutaneous Coronary Intervention (PCI), indicating a disruption in immune balance. However, this reduction was not specific to patients who developed contrast-induced nephropathy (CIN). This suggests that PCI itself induces inflammation, and further investigation is warranted to understand the interplay between inflammation markers and FOXP3 in the development of complications like CIN [39]. The study also analyzed a specific gene (rs3761548) but found no significant differences in its genetic variations between patients and healthy controls. This lack of association may be due to factors such as a limited number of participants, potential biases in how the study group was selected, and the complex nature of autoimmune diseases. The results of the current investigation differed from those of the previous study regarding the finding that the AC and AA genotypes of rs3761548 were significantly more frequent in patients with Graves' disease (GD) compared to healthy controls in the Turkish population. This suggests that these genotypes may be associated with an increased risk of developing GD. However, the study did not find any association between rs3761548 and the development of Graves' ophthalmopathy (GO) within the GD patient group [19]. The previous systematic review and meta-analysis carried out by Li and their team demonstrated a significant association between the variant allele of rs3761548 and an increased risk of Graves' disease (GD), specifically in Asian populations (OR: 1.31, 95% CI 1.04, 1.64; $p= 0.02$). This suggests that this genetic variation may play a role in the development of GD in individuals of Asian descent. However, no such association was observed among Caucasian populations [40]. The current study did not identify any significant associations between the analyzed SNPs (rs3761548) and the presence of this SNP in study participants. This finding is consistent with the results of a

previous study conducted by Bossowski in 2014, which also failed to demonstrate any significant differences in these SNPs between females with and without rs3761548 [41].

Study limitations

The limitations of the current investigation include a relatively small sample size, a focused analysis of a limited set of genetic polymorphisms (rs3761548), and a lack of control for potential confounding environmental factors. These limitations may restrict the generalizability of the findings and hinder the establishment of a definitive causal association. Furthermore, the diverse range of thyroid disorders present in the sample population suggests that future research should be more specific, with dedicated studies on Graves' disease and Hashimoto's thyroiditis.

Conclusion

This study found significantly lower levels of FOXP3 in patients with thyroid disorders. This suggests a possible link between the impaired function of regulatory T cells (Tregs) and the development of thyroid disease. While the rs3761548 gene variation showed no significant association with thyroid disease. The findings highlight the complex interplay of genetic and environmental factors in autoimmune diseases.

Conflict of interests

No conflict of interest was declared by the author.

Funding source

The author did not receive any source of funds.

Data sharing statement

Supplementary data can be shared with the corresponding author upon reasonable request.

REFERENCES

1. Hiller-Sturmhöfel S, Bartke A. The endocrine system: an overview. *Alcohol Health Res World.* 1998;22(3):153–164. PMID: 15706790.
2. Mammen JSR, Cappola AR. Autoimmune thyroid disease in women. *JAMA.* 2021;325(23):2392–3. doi: 10.1001/jama.2020.22196.
3. Abd Aon MAA-K. Using HPRT gene mutation assay for detection of reasons of thyroid disorders in patients at Al-Zuaaffaranya city. *Iraqi J Biotechnol*. 2017;16(3).
4. Mendoza-León MJ, Mangalam AK, Regaldiz A, González-Madrid E, Rangel-Ramírez MA, Álvarez-Mardonez O, et al. Gut microbiota short-chain fatty acids and their impact on the host thyroid function and diseases. *Front Endocrinol (Lausanne).* 2023;14:1192216. doi: 10.3389/fendo.2023.1192216.
5. Ludgate ME, Masetti G, Soares P. The relationship between the gut microbiota and thyroid disorders. *Nat Rev Endocrinol*. 2024 Sep;20(9):511–525. doi: 10.1038/s41574-024-01003-w.
6. Milo T, Korem Kohanim Y, Toledano Y, Alon U. Autoimmune thyroid diseases as a cost of physiological autoimmune surveillance. *Trends Immunol*. 2023;44(5):365–371. doi: 10.1016/j.it.2023.03.007.
7. Mohamad Fawzi S, Abdulhassan I, Mahdi B. Correlation between thyroid hormones and anti-TSHR Ab in graves' disease Introduction. *Iraqi J Biotechnol.* 2018;17(1):60–66.
8. Zamwar UM, Muneshwar KN. Epidemiology, types, causes, clinical presentation, diagnosis, and treatment of hypothyroidism. *Cureus.* 2023;15 (9):e46241. doi: 10.7759/cureus.46241
9. Mohammed S, Al-Jumaily E, Al-Hilal M. Evolution of Thyroid Autoantibodies and Thyroid Parameters in Iraqi Hypothyroidism Patients. *Iraqi J Biotechnol.* 2022;172–177.
10. Van Uytfanghe K, Ehrenkranz J, Halsall D, Hoff K, Loh TP, Spencer CA, et al. Thyroid stimulating hormone and thyroid hormones (triiodothyronine and thyroxine): An American Thyroid Association-Commissioned Review of Current Clinical and Laboratory Status. *Thyroid*. 2023;33(9):1013–1028. doi: 10.1089/thy.2023.0604.
11. Shulhai A-M, Rotondo R, Petraroli M, Patianna V, Predieri B, Iughetti L, et al. The role of nutrition on thyroid function. *Nutrients*. 2024;16(15):2496. doi: 10.3390/nu16152496.
12. Lisco G, De Tullio A, Triggiani D, Zupo R, Giagulli VA, De Pergola G, et al. Iodine deficiency and iodine prophylaxis: An overview and update. *Nutrients*. 2023;15(4):1004. doi: 10.3390/nu15041004.
13. Hasan Al-Saadi B, Jasim S. FOXO3 gene expression associated with oxidative stress in β-thalassemia Iraqi patients. *Iraqi J Biotechnol.* 2024;19(11). doi: 10.25303/1911rjbt1010107.
14. Tan G, Zheng G, Li J, Zhu Y, Liang Z, Li H, et al. Association of genetic variations in FoxP3 gene with Graves' disease in a Southwest Chinese Han population. *Immun, Inflamm Dis*. 2023;11(10):e1046. doi: 10.1002/iid3.1046.
15. Hasan Al-Saadi B. Polymorphism of FOXO3a gene and its association with incidence of asthma in Iraqi patients. *Iraqi J Biotechnol*. 2019;17(3):67–77.
16. Golzari-Sorkheh M, Zúñiga-Pflücker JC. Development and function of FOXP3+ regulators of immune responses. *Clin Exp Immunol*. 2023;213(1):13–22. doi: 10.1093/cei/uxad048.
17. Goodi GAK, AL-Saadi BQ. Polymorphism of FOXO3a Gene and its association with incidence of asthma in Iraqi Patients. *Iraqi J Biotechnol*. 2018;17(3).
18. Borna S, Meffre E, Bacchetta R. FOXP3 deficiency, from the mechanisms of the disease to curative strategies. *Immunol Rev*. 2024;322(1):244–258. doi: 10.1111/imr.13289.
19. Yaylacıoğlu Tuncay F, Serbest Ceylanoğlu K, Güntekin Ergün S, Ergün G, Konuk O. The role of FOXP3 polymorphisms in Graves' disease with or without ophthalmopathy in a Turkish population. *Turkish J Ophthalmol*. 2024;54(2):69–75. doi: 10.4274/tjo.galenos.2024.37897.
20. Shehjar F, Afroze D, Misgar RA, Malik SA, Laway BA. Association of FoxP3 promoter polymorphisms with the risk of Graves' disease in ethnic Kashmiri population. *Gene*. 2018;672:88–92. doi: 10.1016/j.gene.2018.06.023.
21. Zhang Y, Zhang J, Liu H, He F, Chen A, Yang H, et al. Meta-analysis of FOXP3 gene rs3761548 and rs2232365 polymorphism and multiple sclerosis susceptibility. Medicine (Baltimore). 2019;98(38):e17224. doi: 10.1097/md.0000000000017224.
22. Mohammed MQ, Alwan AH, Almukhtar AA. Estimation of serum TLR-9, TNF-α, and IL-6 levels in the Iraqi patients diagnosed as acute myelogenous leukemia. *Baghdad Sci J*. 2023;21(7):2182–2190. doi: 10.21123/bsj.2023.9056.
23. SPSS. Statistical Package for the Social Sciences. 2019. p. Version 26.
24. Qasim Mohammed M, Hussein Alwan A, Amer Almukhtar A, Kareem Aneed Al-Saedi M. Revealing of TLR-9 gene polymorphisms by qPCR HRM technique and their influence on TLR-9 serum level in acute myeloid leukemia patients: Case-control study. *Cytokine*. 2024;182:156730. doi: 10.1016/j.cyto.2024.156730.
25. Fadhil M, Razaq S, Al-Kareem A, Al-Kazaz A. Study the association between IL-17 level and autoimmune antibodies in hypo and hyperthyroidism patients. *Iraqi J Sci*. 2019:60(9):1967-1976. doi: 10.24996/ijs.2019.60.9.9.
26. Abed RM, Abdulmalek HW, Yaaqoob LA, Altaee MF,

Kamona ZK. Serum level and genetic polymorphism of IL-38 and IL-40 in autoimmune thyroid disease. *Iraqi J Sci*. 2023:64(6):2786-2797. doi: 10.24996/ijs.2023.64.6.12.
27. Yassin AH, Al-Kazaz AK., Rahmah AM, Ibrahim TY. Association of CTLA-4 single nucleotide polymorphisms with autoimmune hypothyroidism in Iraqi patients. *Iraqi J Sci*. 2022:63(7):2891-2899. doi: 10.24996/ijs.2022.63.7.13.
28. Fröhlich E, Wahl R. Thyroid autoimmunity: Role of anti-thyroid antibodies in thyroid and extra-thyroidal diseases. *Front Immunol*. 2017;8:521. doi: 10.3389/fimmu.2017.00521.
29. Rydzewska M, Jaromin M, Pasierowska IE, Stożek K, Bossowski A. Role of the T and B lymphocytes in pathogenesis of autoimmune thyroid diseases. *Thyroid Res*. 2018;11:2. doi: 10.1186/s13044-018-0046-9.
30. Bogusławska J, Godlewska M, Gajda E, Piekiełko-Witkowska A. Cellular and molecular basis of thyroid autoimmunity. *Eur Thyroid J*. 2022;11(1). doi: 10.1530/etj-21-0024.
31. Oparaugo NC, Ouyang K, Nguyen NPN, Nelson AM, Agak GW. Human regulatory T cells: Understanding the role of Tregs in select autoimmune skin diseases and post-transplant nonmelanoma skin cancers. *Int J Mol Sci*. 2023;24(2). doi: 10.3390/ijms24021527.
32. Goswami TK, Singh M, Dhawan M, Mitra S, Bin ET, Rabaan AA, et al. Regulatory T cells (Tregs) and their therapeutic potential against autoimmune disorders-Advances and challenges. *Hum Vaccin Immunother*. 2022;18(1):2035117. doi: 10.1080/21645515.2022.2035117.
33. Rudensky AY. Regulatory T cells and Foxp3. *Immunol Rev*. 2011;241(1):260–268. doi: 10.1111/j.1600-065x.2011.01018.x.
34. Fakour P, Shirizadeh A, Tahamoli-Roudsari A, Solgi G. Epigenetic regulation of FOXP3 gene expression in relation to impaired function of regulatory T cells in systemic lupus erythematosus. *Explor Immunol*. 2024;4(5):640–657. doi: 10.37349/ei.2024.00164.
35. Akber NT, Yenzeel JH. Evaluation of some biochemical parameters in Iraqi patients with hyperthyroidism. *Iraqi J Biotechnol*. 2023;22(1).
36. Paradowska-Gorycka A, Jurkowska M, Felis-Giemza A, Romanowska-Próchnicka K, Manczak M, Maslinski S, et al. Genetic polymorphisms of Foxp3 in patients with rheumatoid arthritis. *J Rheumatol*. 2015;42(2):170–180. doi: 10.3899/jrheum.131381.
37. Ikram EM, Allam AA, Meawed TE, Abd El-Wahab SM, Ramadan RA. Forkhead Box P3 (Foxp3) serum level and Foxp3 Gene-Promoter polymorphisms in Egyptian rheumatoid arthritis patients: A case-control study. *Egypt J Immunol*. 2021;28(2):53–64.
38. Ssedyabane F, Niyonzima N, Ngonzi J, Nambi Najjuma J, Mudondo H, Okeny C, et al. FOXP3 serum concentration; a likely predictor of CIN and cervical cancer: Secondary analysis from a case-control study at a clinic in Southwestern Uganda. *Gynecol Oncol Rep*. 2024;55:101466. doi: 10.1016/j.gore.2024.101466
39. Widaningsih Y, Natsir R, Arif M, Bahrun U, Kasim H, Kadir NA, et al. Foxp3 serum levels in contrast-induced nephropathy patients after the administration of contrast media. *Int J Biomed Mater Res*. 2017;5(2):25–28. doi: 10.11648/j.ijbmr.20170502.12.
40. Li HN, Li XR, Du YY, Yang ZF, Lv ZT. The association between Foxp3 polymorphisms and risk of Graves' disease: A systematic review and meta-analysis of observational studies. *Front Endocrinol* (Lausanne). 2020;11:392. doi: 10.3389/fendo.2020.00392
41. Bossowski A, Borysewicz-Sańczyk H, Wawrusiewicz-Kurylonek N, Zasim A, Szalecki M, Wikiera B, et al. Analysis of chosen polymorphisms in FoxP3 gene in children and adolescents with autoimmune thyroid diseases. *Autoimmunity*. 2014;47(6):395–400. doi: 10.3109/08916934.2014.910767.