Research Article

Association of CTLA-4 Gene Polymorphisms (CT60 and +49 A/G) with Hypothyroidism: A Case-Control Study in a Tertiary Care Hospital

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Abstract

Background: Hypothyroidism is a major endocrine burden in India. While iodine deficiency is common, autoimmune thyroiditis is an increasingly recognized etiology. CTLA-4 gene polymorphisms are known susceptibility loci for autoimmune disorders, but data in the Indian population remain limited.

Objective: To evaluate the association of CTLA-4 exon 1 (+49 A/G) and 3' UTR (CT60) polymorphisms with hypothyroidism and autoimmune status in a Gujarati cohort.

Methods: This case-control study included 156 hypothyroid patients and 156 controls. Genotyping was performed using PCR-RFLP. Biochemical markers, including TSH and anti-TPO antibodies, were measured. Statistical analysis utilized Chi-square tests and Odds Ratios (OR) to assess genetic associations.

Results: No significant difference in age or gender was observed between groups. The CTLA-4 CT60 polymorphism showed a significant association with hypothyroidism ($\chi 2=12.491$, $p=0.002$), with the GG genotype conferring a 3.07-fold increased risk (95% CI: 1.62–5.84). Stratification by anti-TPO status revealed that the AA genotype for both CT60 and +49 A/G polymorphisms exerted a protective effect against autoimmunity (OR=0.39, p=0.032 and OR=0.22, p=0.001, respectively). Patients with the CT60 GG genotype who were anti-TPO positive exhibited significantly higher TSH levels (p=0.009).

Conclusion: CTLA-4 gene polymorphisms are significant genetic determinants of autoimmune hypothyroidism in the Gujarati population. The G allele and GG genotype are associated with increased susceptibility, while the AA genotype confers relative protection. Genotyping CTLA-4 may serve as a valuable diagnostic adjunct for risk stratification and personalized management of autoimmune thyroid disease.

Introduction

Hypothyroidism is one of the most prevalent endocrine disorders globally, characterized by the thyroid gland's inability to produce sufficient hormones to meet metabolic demands [1]. In India, the clinical burden is significant, with recent estimates suggesting an 11% prevalence rate, far exceeding figures reported in the United Kingdom or the United States [2]. While iodine deficiency historically played a primary role, Hashimoto's thyroiditis—an organ-specific autoimmune disorder—is now the leading cause of thyroid failure in iodine-sufficient regions [3].

Despite its high prevalence and the availability of standard diagnostic markers such as serum thyrotropin (TSH) and free thyroxine (T4), the pathogenesis of autoimmune hypothyroidism remains complex. It involves a sophisticated interplay of environmental triggers and genetic susceptibility [4]. Among the various candidate genes implicated in the development of autoimmune thyroid disorders, the Cytotoxic T-lymphocyte antigen-4 (CTLA-4) gene has emerged as a major susceptibility locus [5]. Located on chromosome 2q33, CTLA-4 encodes a critical negative regulator of T-cell activation, playing an essential role in maintaining peripheral self-tolerance [6].

Various polymorphisms within CTLA-4, most notably the promoter -318 C/T, exon 1 +49 A/G, and the 3' untranslated region (3' UTR) CT60, have been identified as risk factors for autoimmune endocrinopathies [7]. While several studies have linked these variants to Hashimoto's thyroiditis and Graves' disease in diverse ethnic populations, data specific to the Indian population remain limited [8]. Understanding the genetic architecture of hypothyroidism in this demographic is essential, as it may improve early risk stratification and facilitate a move toward precision medicine in clinical practice [9].

Given the current lack of localized genetic data, this study aims to evaluate the distribution of CTLA-4 (CT60 and +49 A/G) gene polymorphisms in patients with hypothyroidism attending a tertiary care hospital in Bhavnagar. By correlating these genotypes with clinical variables, this research seeks to establish a foundational genetic database to aid in the identification of individuals with high susceptibility to thyroid dysfunction.

Methodology

Patient Recruitment

The study was conducted in the Department of Biochemistry, Government Medical College, Bhavnagar. Informed consent was taken from all the patients who participated in this study. A total of 156 Group I: Hypothyroidism (Case) and 156 Group II: Non-Hypothyroidism (Control) were included in the present study. The study protocol was approved by the Institutional Ethics Committee of the Government Medical College, Bhavnagar (Approval No: 1201/2022). This was a case control type and was conducted from the period of October 2022 to September 2023.

Inclusion and exclusion criteria

Patients were eligible for inclusion if they were between 25 and 75 years of age and had a confirmed clinical diagnosis of primary hypothyroidism. To ensure the study focused specifically on primary hypothyroidism, several exclusion criteria were applied. Subjects were excluded if they presented with secondary or tertiary hypothyroidism resulting from pituitary or hypothalamic dysfunction. Furthermore, patients with a history of thyroid surgery, pregnancy, or those requiring the continuous or periodic use of corticosteroids were excluded from the study. We also excluded individuals with severe comorbidities, such as active malignancies or severe systemic infections, which could influence TSH levels or immunological status. Rather than excluding autoimmune disease, our cohort intentionally included patients with Hashimoto's thyroiditis—identified by elevated anti-TPO antibody titers - to allow for a comprehensive evaluation of the association between CTLA-4 gene polymorphisms and autoimmune-mediated thyroid dysfunction.

Diagnostic Criteria for Hypothyroidism

The diagnosis of primary hypothyroidism is typically based on the demonstration of an elevated Serum Thyroid-Stimulating Hormone (TSH) level in conjunction with a low Serum Free Thyroxine (FT4) level. Subclinical Hypothyroidism: Characterized by an elevated TSH with a normal FT4 level. Overt Hypothyroidism: Characterized by an elevated TSH with a low FT4 level. The most widely accepted guidelines used in clinical research are those published by the American Thyroid Association (ATA) and the European Thyroid Association (ETA) [10,11].

Participants' Examination and Measurements

Patients were interviewed for medical history. The present and past history of each case were recorded in detail regarding their general information i.e. name, age, sex, address, occupation, economic status, personal habits especially smoking, medication, and history suggestive of any systemic illness especially hypothyroidism. The history of Hypothyroidism was based on the patient's self-report of a prior physician diagnosis and was under treatment with any drug.

Sampling and Biochemical Analysis

After completing the initial history taking, 5ml of peripheral venous blood was collected by vein puncture from each of the subjects. In Plain & EDTA vacutainer for TSH, Free T3 , Free T4, Anti TPO test were done by chemiluminescence based assay kit on fully automated analyser and EDTA used for isolation of DNA.

DNA Extraction

Genomic DNA was extracted from peripheral blood leukocytes collected in EDTA-anticoagulated tubes using the HiPurA™

Blood Genomic DNA Miniprep Purification Kit (HiMedia Laboratories, India), based on the selective binding of DNA to a silica-based membrane in the presence of chaotropic salts. The procedure was performed in accordance with the manufacturer's protocol. Briefly, 200 µL of whole blood was transferred to a collection tube and treated with 20 µL of Proteinase K (20 mg/mL) and 20 µL of RNase A solution (20 mg/mL), followed by brief vortexing. The sample was incubated at room temperature for 2 minutes to ensure enzymatic digestion. Subsequently, 200 µL of lysis buffer was added, the mixture was vortexed to achieve homogeneity, and incubated at 55°C for 10 minutes. Following the lysis reaction, 200 µL of absolute ethanol (96–100%) was added to the lysate to facilitate binding conditions.

The resulting mixture was loaded onto a HiElute™ Miniprep Spin Column and centrifuged at 10,000 rpm for 1 minute; the flow-through was discarded. The column underwent a two-stage washing process: first, with 500 µL of pre-wash solution (centrifuged at 10,000 rpm for 1 minute), and second, with 500 µL of wash solution (centrifuged at 13,000 rpm for 3 minutes). To ensure the complete removal of residual ethanol, an additional centrifugation step was performed for 1 minute at the same speed. Finally, the genomic DNA was eluted in two sequential 100 µL aliquots of Elution Buffer (10 mM Tris-Cl, pH 8.5) by incubating for 1 minute at room temperature followed by centrifugation at 6,500 x g. The extracted DNA was assessed for quality and subsequently stored at -20°C until further amplification, which was completed within 72 hours of extraction.

Concentration, yield and purity of DNA

Concentration and the purity of the genomic DNA in each sample were accessed in IMPLEN nanophotometer p300. Elution Buffer (ET) to dilute samples and to calibrate the nanophotometer, absorbance was measured at 260 nm, 280 nm, and 320 nm. The 320 nm absorbance was used to correct for background absorbance. An absorbance of 1.0 at 260 nm corresponds to approximately 50 µg/ml of DNA. The A260 - A320 /A280 -A320 ratio should be 1.6-1.9. Purity was determined by calculating the ratio of absorbance at 260 nm to absorbance at 280 nm.

Genetic Polymorphism of CTLA4 gene

The CTLA-4 gene polymorphisms (+49 A/G and 3' UTR CT60) were genotyped using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique. Amplification was performed in a Bio-Rad T100™ Thermal Cycler using the HiMedia 2X PCR Taq Master Mix. The final 25 µL reaction volume consisted of 12.5 µL of 2X Taq Master Mix, 0.4 µL of each forward and reverse primer (100 ng/µL), 1 µL of genomic DNA template (50 ng/µL), and 10.7 µL of nuclease-free water. The primer sequences and conditions were selected based on validated protocols for human genomic DNA analysis. SNP/Gene CTLA4 +49A/G primers, forward: 5' AAGGCTCAGCTGAACCTGGT 3' and reverse: 5' CTTTGCCTTATTTGCTGCCGC 3' sequences and SNP/Gene CTLA4 CT60 A/G primers, forward: 5' CACCACTATTTGGGATATACC 3' and reverse: 5' AGCTCTATATTTCAGGAAGGC 3' sequences were employed. The thermal cycling profile included an initial denaturation at 95°C for 10 minutes, followed by 30 cycles of denaturation (95°C for 30 seconds), annealing (60°C for 30 seconds), and extension (72°C for 30 seconds), with a final extension at 72°C for 5 minutes. Following amplification, 15 µL of the PCR products were digested for 16 hours at 37°C using 5 U of the specific restriction endonucleases BstEII for the +49 A/G polymorphism and NcoI for the CT60 polymorphism. For the +49 A/G polymorphism, digestion of the 271 bp PCR product with BstEII yielded distinct banding patterns: the A allele was characterized by cleavage into 249 bp and 22 bp fragments, whereas the G allele remained undigested at 271 bp. Similarly, for the 3' UTR CT60 A/G polymorphism, digestion of the 216 bp PCR product with NcoI allowed for genotype discrimination: the A allele was identified by fragments of 174 bp and 42 bp, while the G allele remained as an undigested 216 bp band. Based on these banding patterns, three genotypes were identified for both polymorphisms via 2% agarose gel electrophoresis: homozygous wild-type (AA), heterozygous (AG), and homozygous variant (GG). All samples were scored independently to ensure accuracy, and a subset of samples was re-analyzed to confirm the reproducibility of the genotyping results (Figure 1 and 2).

Figure 1: 49AG 271,249,22 bp fragment (lane no. 3,6) suggestive of AG genotype and 271 bp fragment (lane No.1,2,4) suggestive of GG genotype and 249,22 bp fragment (lane No.5,7) suggestive of AA genotype, distinguished using ethidium bromide under UV (ultra-violet) light in Gel Document system.

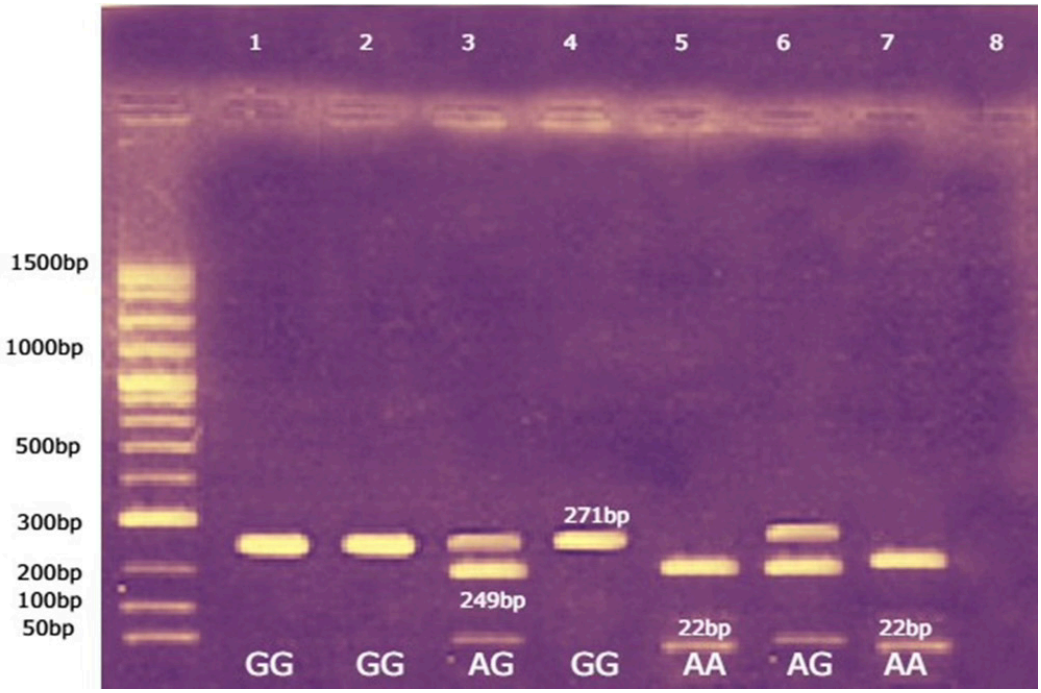
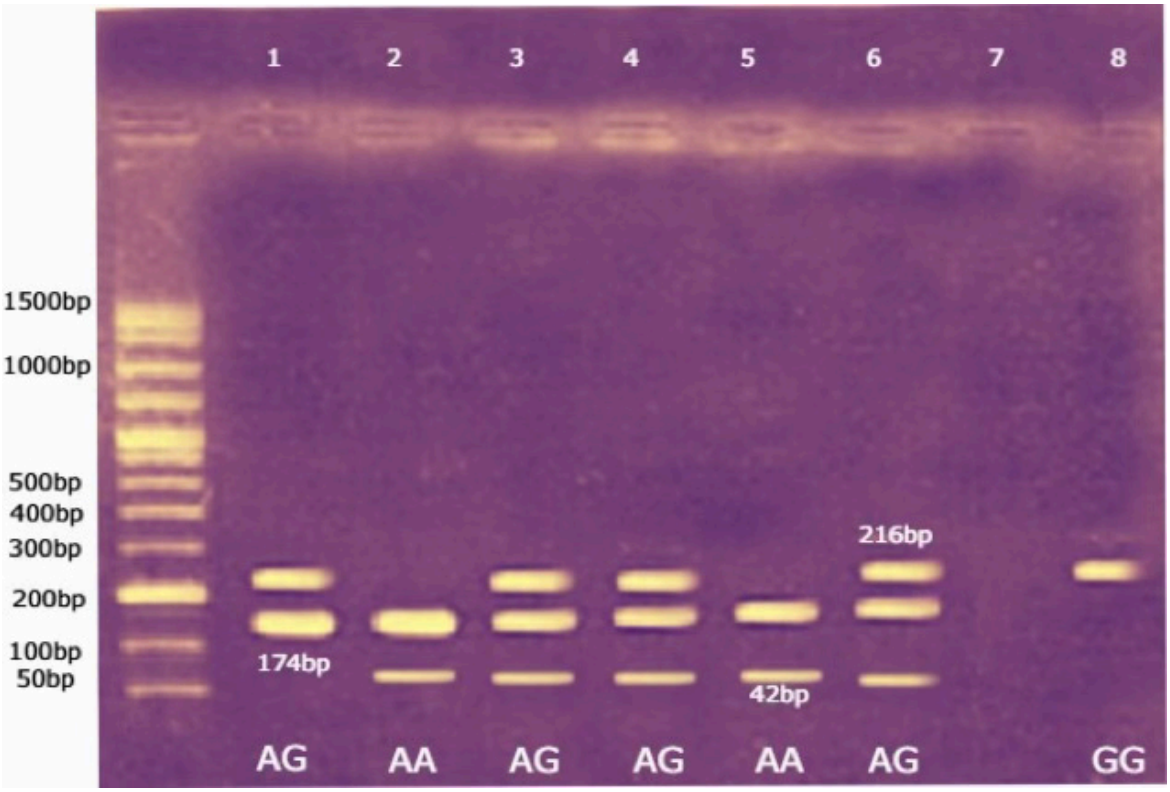


Figure 2: CT60 216,174,42 bp fragment (lane No.1,3,4,6) suggestive of AG genotype 216, bp fragment (lane no. 8) suggestive of GG genotype and 174,42 bp fragment (lane No.2,5) suggestive of AA genotype, distinguished using ethidium bromide under UV (ultra-violet) light in Gel Document system.



Data analysis

All data were analyzed using web-based statistical platforms (SPSS 25 version). Continuous variables were expressed as mean ± standard deviation (SD) and compared between groups using the student t-test. Categorical variables were evaluated using the Chi-square test. To determine whether the allele distribution within the study groups adhered to the Hardy-Weinberg equilibrium, the chi2 goodness-of-fit test was applied. The association between CTLA-4 gene polymorphisms and hypothyroidism was assessed by calculating the Odds Ratio (OR) with a 95% confidence interval (CI). All probability values were two-sided, with $p<0.05$ considered statistically significant, $p<0.001$ as highly significant, and $p>0.05$ as not significant.

Result

Demographic and biochemical status

The baseline characteristics of the study population are summarized in Table 1. There was no statistically significant difference in mean age between the hypothyroid group (39.89±12.38 years) and the control group (41.19±14.89 years; p=0.402). The gender distribution was comparable across both groups, with a higher prevalence of females (70.5% in Group I vs. 72.4% in Group II). As expected, biochemical analysis revealed a highly significant difference ($p<0.001$) in thyroid profile markers, with elevated TSH and anti-TPO levels, and lower FT3 and FT4 levels observed in the hypothyroid cohort compared to the control group.

Table 1: Demographic and Clinical Characteristics of the Study Population.

Parameter	Group I: Hypothyroidism (n=156)	Group II: Controls (n=156)	pvalue
Age (years), Mean ± SD	39.89 ± 12.38	41.19 ± 14.89	0.402
Gender, n (%)			
Male	46 (29.49)	43 (27.56)	0.692
Female	110 (70.51)	113 (72.44)	
Biochemical Markers			
TSH (µIU/mL)	19.03 ± 25.78	1.97 ± 0.90	<0.001*
FT3 (pg/mL)	1.90 ± 0.84	2.88 ± 0.30	<0.001*
FT4 (ng/dL)	0.95 ± 0.73	1.62 ± 0.38	<0.001*
AntiTPO (IU/mL)	31.12 ± 16.44	17.70 ± 5.11	<0.001*

*The Chi-square test was used to calculated the categorial data (gender) and the student t-test was used to calculate the p-value for continuous data. *p<0.05 was considered as statistically significant.*

CTLA-4 Genotypes on Thyroid Hormone Profiles in Hypothyroid Patients (Group I)

We analyzed the correlation between CTLA-4 genotypes and thyroid hormone profiles stratified by anti-TPO status. Notably, in patients carrying the CT60 GG genotype, there was a statistically significant difference in mean TSH levels between anti-TPO positive and negative cohorts (p=0.009), with anti-TPO positive individuals exhibiting markedly higher TSH concentrations. No significant associations were observed between the +49 A/G genotypes and thyroid hormone levels, nor were there significant correlations identified for FT3 or FT4 levels across any of the studied genotypes (Table 2).

Table 2: Influence of CTLA-4 Genotypes on Thyroid Hormone Profiles in Hypothyroid Patients (Group I).

Polymorphism	Genotype	TSH (µIU/mL) (n=105) (TPOve)	TSH (µIU/mL) (n=51) (TPO+ve)	pvalue	FT3 (pg/mL) (n=105) (TPOve)	FT3 (pg/mL) (n=51) (TPO+ve)	pvalue
+49 A/G	AG	16.33	25.64	0.350	1.54	1.74	0.659
	GG	23.47	24.85	0.199	1.87	2.12	0.480
	AA	15.69	8.45	0.086	2.02	1.89	0.480
CT60	AG	20.35	27.49	0.568	1.75	1.90	0.741
	GG	15.98	27.59	**0.009***	1.93	1.90	0.659
	AA	16.34	7.15	0.062	2.01	1.72	0.659

*The student t-test was used to calculate the p-value for continuous data. *p<0.05 was considered as statistically significant.*

Genotype and Allele Distribution of CTLA-4 Polymorphisms

The genotype distribution and allele frequencies of the CTLA-4 +49 A/G and CT60 polymorphisms are summarized in Table 3. The genotype frequencies for the +49 A/G polymorphism did not show a statistically significant difference between the hypothyroid group and the control group ($\chi2=3.510$, p=0.173). The A allele frequency was 57% in the hypothyroid group compared to 68% in the control group, while the G allele frequency was 42% and 31%, respectively.

In contrast, the CT60 polymorphism revealed a significant association with hypothyroidism ($\chi2=12.491$, p=0.002). While the distribution of the AA genotype was lower in the hypothyroid group (63 vs. 83), the homozygous variant GG genotype was markedly more prevalent in the hypothyroid group (42 vs. 18). Consequently, individuals carrying the CT60 GG genotype exhibited a significantly increased risk of hypothyroidism (OR = 3.07; 95% CI: 1.62–5.84). The allele frequencies for CT60 further supported this finding, with the G allele frequency being 44% in the hypothyroid group versus 29% in the control group.

Table 3: Genotype and Allele Frequencies of CTLA-4 Polymorphisms in Hypothyroid.

Polymorphism	Genotype	Group I (Hypothyroidism)		Group II (Non-Hypothyroidism)		χ2 d.f.2	P value d.f.2	OR	OR(95% CI)	
									Lower Bound	Upper Bound
49A/G	AA	67		78		3.510	0.1729	0.87	0.53	1.43
	AG	56		57				1.14	0.7	1.87
	GG	33		21				1.83	0.97	3.46
	Allele	A	G	A	G					
	Frequency	0.57%	0.42%	0.68%	0.31%					
CT60	AA	63		83 55 18		12.491	0.0019*	0.82	0.5	1.35
	AG	51						1.22	0.74	2.02
	GG	42						3.07	1.62	5.84
	Allele	A	G	A	G					
	Frequency	0.55%	0.44%	0.70%	0.29%					

Chi-square test; df: degrees of freedom; OR: Odds Ratio; CI: Confidence Interval. p<0.05 indicates statistical significance. The risk (OR) for genotypes AG and GG was calculated relative to the homozygous wild-type (AA) genotype.

Association of CTLA-4 Polymorphisms with Autoimmune Status

The association between CTLA-4 genotype distributions and autoimmune status (anti-TPO positive vs. negative) in patients with hypothyroidism is presented in Table 4. Using the GG genotype as the reference, we evaluated the risk profile for variant genotypes. For the CT60 polymorphism, the AA genotype was significantly underrepresented in the autoimmune (TPO positive) group compared to the TPO-negative group (OR = 0.40; 95% CI: 0.16–0.98, p<0.05). For the +49 A/G polymorphism, the AA genotype demonstrated a highly significant protective effect against autoimmune thyroiditis compared to the GG reference group (OR = 0.22; 95% CI: 0.09–0.57, p<0.001). These findings indicate that while the GG genotype serves as a risk marker for autoimmunity in this population, the AA genotype potentially confers a degree of genetic resistance to the development of autoimmune thyroid antibodies (Table 4).

Table 4: Association of CTLA-4 Polymorphisms with Autoimmune Status (Anti-TPO Positive) in Hypothyroid Patients.

Polymorphism	Genotype	(TPO+; n=51)	(TPO; n=105)	OR (95% CI)	pvalue
CT60	GG (Ref)	18 (35.29%)	26 (24.76%)	1.00	
	AG	20 (39.22%)	31 (29.52%)	0.93 (0.41–2.12)	0.866
	AA	13 (25.49%)	48 (45.71%)	0.39 (0.17–0.92)	**0.032***
+49 A/G	GG (Ref)	17 (33.33%)	21 (20.00%)	1.00	
	AG	25 (49.02%)	31 (29.52%)	0.99 (0.43–2.28)	0.992
	AA	9 (17.65%)	53 (50.48%)	0.22 (0.08–0.54)	**0.001***

*Data are presented as n (%). TPO+: Anti-TPO positive (Autoimmune); TPO-: Anti-TPO negative. OR: Odds Ratio calculated using the GG genotype as the reference group. CI: 95% Confidence Interval. *Significant at p<0.05; Highly significant at p<0.001.*

Discussion

The present study showed that low iodine intake is not the sole etiological candidate for hypothyroidism in India, as approximately one-third of the patients included in this study tested positive for anti-TPO antibodies. This trend aligns with recent large-scale epidemiological studies across India, which identified anti-TPO positivity as a primary indicator for thyroid disease, though those studies did not specifically isolate the prevalence of autoimmune hypothyroidism [12]. No relationship between the age of the patient and the presence of anti-TPO antibodies was found, a finding consistent with previous longitudinal research [13].

Our study provides evidence that CTLA-4 gene polymorphisms—specifically exon 1 (+49 A/G) and the 3' UTR (CT60)—are significant susceptibility loci for autoimmune hypothyroidism in the Gujarati population. We observed that the G allele at both loci correlates with increased disease risk, whereas the AA genotype consistently appears to confer a degree of genetic protection.

The association between CTLA-4 variants and hypothyroidism is multifaceted. We found that the CT60 GG genotype is a robust marker of susceptibility, demonstrating a 3.07-fold increased risk of disease (p=0.0019, OR=3.07). This finding is consistent with literature from Caucasian and Japanese populations, suggesting that the CT60 locus serves as a conserved marker for autoimmune thyroid disease across diverse ethnic groups [14, 15]. Furthermore, our analysis of thyroid profiles revealed that among hypothyroid patients, those carrying the CT60 GG genotype who are anti-TPO positive exhibit significantly higher TSH concentrations (p=0.009) compared to their anti-TPO negative counterparts. This suggests that these genetic variants not only predispose individuals to the disease but may also exacerbate the severity of the hypothyroid phenotype in the presence of an active autoimmune response [16].

A critical observation in our study is that while iodine deficiency is a known etiological factor in India, it is not the sole driver of hypothyroidism. Approximately one-third of our hypothyroid patients were anti-TPO positive, highlighting the significant burden of autoimmune-mediated thyroid failure. In our cohort, we found no significant relationship between patient age, gender, and the presence of anti-TPO antibodies (p=0.299), suggesting that genetic predisposition—rather than demographic factors—is a primary driver of the autoimmune component [17].

Our data further elucidates the role of CTLA-4 haplotypes. We identified that the AG and GG haplotypes for the +49 A/G and CT60 sites were more frequently observed in anti-TPO positive patients. Specifically, the G allele at both sites was associated with disease susceptibility, corroborating findings in Japanese

and other global populations [18, 19]. However, the presence of autoimmune hypothyroidism in some patients with "protective" AA genotypes, and the absence of disease in some individuals with "risk-associated" GG genotypes, reinforces the conclusion that autoimmune hypothyroidism is a complex, multifactorial disorder [20]. These discrepancies likely reflect the interplay between genetic susceptibility, epigenetic modifications, and environmental triggers, such as dietary iodine intake or other lifestyle factors.

By closely matching our study and control groups for age and sex, we were able to minimize confounding variables, allowing for a clearer association between CTLA-4 genotypes and thyroid dysfunction. While our findings support the status of CTLA-4 on chromosome 2q33 as a critical susceptibility locus [21, 22], they also emphasize the need to look beyond single-gene models to fully understand the pathogenesis of autoimmune thyroid disease.

Strengths and Limitations

The primary strength of this study lies in the rigorous matching of the hypothyroid group and controls for age, sex, and clinical parameters, which minimizes confounding variables. Furthermore, by stratifying patients by anti-TPO status, we were able to specifically isolate the genetic contribution to the autoimmune component of hypothyroidism.

The study has some limitations. First, the sample size, while adequate for a preliminary regional study, may not be sufficient to detect the influence of rare variants or low-frequency haplotypes. Second, as a hospital-based case-control study in a tertiary care center, there is a potential for selection bias. Finally, while we have established a genetic association, functional analysis to determine how these polymorphisms alter CTLA-4 protein expression or T-cell regulatory capacity in this specific population was outside the scope of this study and warrants future investigation.

Conclusion

This study concludes that CTLA-4 gene polymorphisms are significant genetic determinants of autoimmune hypothyroidism in the studied population. Specifically, the G allele and the GG genotype at the +49 A/G and CT60 loci are associated with increased susceptibility to the disease and the development of anti-TPO antibodies. These findings support the role of CTLA-4 as a critical susceptibility locus for autoimmune thyroid pathogenesis. We propose that CTLA-4 genotyping could serve as a valuable diagnostic adjunct for risk stratification and the early identification of individuals predisposed to autoimmune thyroid dysfunction, potentially facilitating personalized management approaches.

Statements and Disclosures

CRediT Author Statement

KR Gopala Krishnan: Conceptualization, Methodology, Writing – Original Draft.
Hariom Sharma: Formal analysis.
Piyush Tailor: Resources, Project administration.
Shailesh Patel: Formal analysis
D Shreshtta: Software, Formal analysis.
Hina Baraiya: Writing – Review & Editing.
As the corresponding author, Dr. KR Gopala Krishnan ensures that these descriptions are accurate and have been agreed upon by all authors.

Declaration of Conflicting Interests

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

Ethical Approval and Compliance

This study protocol was approved by the Institutional Ethics Committee of the Government Medical College, Bhavnagar (Approval No: 1201/2022). All procedures involving human subjects were conducted in strict compliance with the ethical principles outlined in the 1964 Declaration of Helsinki and its later amendments. Written informed consent was explicitly obtained from all individual patients prior to their enrollment in the study.

Artificial Intelligence (AI) Statement

The authors declare that an AI tool was utilized only for refine technical language formatting. The human authors reviewed, edited, and take full responsibility for the final text and intellectual content of this manuscript.

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Data Availability Statement

The genetic and biochemical datasets analyzed during the current study are available from the corresponding author upon reasonable request.

References

1. Chiovato L, Magri F, Carlé A. Hypothyroidism in context: where we've been and where we're going. Adv Ther. 2019;36(Suppl 2):47-58. Available from: https://pubmed.ncbi.nlm.nih.gov/31485975/ (accessed: 05/06/2026).
2. Unnikrishnan AG, Menon UV. Thyroid disorders in India: An epidemiological perspective. Indian J Endocrinol Metab. 2011;15(Suppl 2):S78-81. Available from: https://pubmed.ncbi.nlm.nih.gov/21966515/ (accessed: 08/06/2026).
3. Caturegli P, De Remigis A, Rose NR. Hashimoto thyroiditis: clinical and diagnostic criteria. Autoimmun Rev. 2014;13(4-5):391-397. Available from: https://pubmed.ncbi.nlm.nih.gov/24434360/ (accessed: 05/06/2026).
4. Tomer Y, Huber A. The etiology of autoimmune thyroid

disease: a story of genes and environment. J Autoimmun. 2009;32(3-4):231-239. Available from: https://pubmed.ncbi.nlm.nih.gov/19307093/ (accessed: 12/06/2026).
5. Tomer Y. The genetics of autoimmune thyroid disease. Endocrinol Metab Clin North Am. 2009;38(2):331-348. Available from: https://pubmed.ncbi.nlm.nih.gov/19446217/ (accessed: 12/06/2026).
6. van Kooten C, Boon L, Paanen-Visser J, et al. CTLA-4: A checkpoint in the immune system. Immunol Lett. 2017;186:13-20. Available from: https://pubmed.ncbi.nlm.nih.gov/28325732/ (accessed: 12/06/2026).
7. Ueda H, Howson JM, Esposito L, et al. Association of the CTLA-4 gene with autoimmune disease. Nature. 2003;423(6939):506-511. Available from: https://pubmed.ncbi.nlm.nih.gov/12717450/ (accessed: 15/06/2026).
8. Banerjee A, Saxena A, Sinha N, et al. Association of CTLA-4 gene polymorphisms with autoimmune thyroid disease in an Indian population. J Clin Endocrinol Metab. 2008;93(5):1848-1852. Available from: https://pubmed.ncbi.nlm.nih.gov/18319323/ (accessed: 15/06/2026).
9. Vanderpump MP. The epidemiology of thyroid disease. Br Med Bull. 2011;99:39-51. Available from: https://pubmed.ncbi.nlm.nih.gov/21831967/ (accessed: 15/06/2026).
10. Garber JR, Cobin RH, Gharib H, et al. Clinical practice guidelines for hypothyroidism in adults: cosponsored by the American Association of Clinical Endocrinologists and the American Thyroid Association. Endocr Pract. 2012;18(6):988-1028. Available from: https://pubmed.ncbi.nlm.nih.gov/23246686/ (accessed: 15/06/2026).
11. Pearce SH, Brabant G, Duntas LH, et al. 2013 ETA Guideline: Management of Subclinical Hypothyroidism. Eur Thyroid J. 2013;2(4):215-228. Available from: https://pubmed.ncbi.nlm.nih.gov/24455467/ (accessed: 15/06/2026).
12. Unnikrishnan AG, Kalra S, Sahay RK, et al. Thyroid epidemiology in India: A multi-city study. Indian J Endocrinol Metab. 2013;17(3):473-475. Available from: https://pubmed.ncbi.nlm.nih.gov/23869304/ (accessed: 15/06/2026).
13. Vanderpump MP, Tunbridge WM, French JM, et al. The Whickham survey: 20-year follow-up. Clin Endocrinol (Oxf). 1995;43(1):55-68. Available from: https://pubmed.ncbi.nlm.nih.gov/7641412/ (accessed: 15/06/2026).
14. Tomer Y. Genetics of autoimmune thyroid disease: a comprehensive review. J Autoimmun. 2014;53:1-12. Available from: https://pubmed.ncbi.nlm.nih.gov/24856545/ (accessed: 15/06/2026).
15. Kotsa K, Polyzos SA, Gazouli M, et al. CTLA-4 gene polymorphisms in autoimmune thyroid disease. Thyroid. 2007;17(5):455-461. Available from: https://pubmed.ncbi.nlm.nih.gov/17542699/ (accessed: 15/06/2026).
16. Chistiakov DA. CTLA-4 and autoimmune diseases. Immunol Lett. 2008;117(1):1-10. Available from: https://pubmed.ncbi.nlm.nih.gov/18243354/ (accessed: 15/06/2026).
17. Zaletel K, Gaberšček S, Pirnat E, et al. The influence of age and gender on the prevalence of thyroid autoimmunity. Thyroid. 2011;21(3):289-294. Available from: https://pubmed.ncbi.nlm.nih.gov/21190448/ (accessed: 15/06/2026).
18. Akbarzadeh S, Miresmaeili SM, Gholamrezaei MS, et al. CTLA-4 gene polymorphisms in Iranian patients with autoimmune thyroid disease. Endocr J. 2012;59(1):1-7. Available from: https://pubmed.ncbi.nlm.nih.gov/22033560/ (accessed: 15/06/2026).
19. Jin Y, Wang L, Zhang S, et al. Association of CTLA-4 gene polymorphisms with AITD: a meta-analysis. Endocrine. 2013;43(3):570-580. Available from: https://pubmed.ncbi.nlm.nih.gov/22996160/ (accessed: 15/06/2026).
20. Tomer Y, Huber A. The etiology of autoimmune thyroid disease. J Autoimmun. 2009;32(3-4):231-239. Available from: https://pubmed.ncbi.nlm.nih.gov/19307093/ (accessed: 15/06/2026).
21. Banerjee A, Saxena A, Sinha N, et al. CTLA-4 and thyroid autoimmunity. J Clin Endocrinol Metab. 2008;93(5):1848-1852. Available from: https://pubmed.ncbi.nlm.nih.gov/18319323/ (accessed: 15/06/2026).
22. Noh JY, Sato A, Suzuki K, et al. CTLA-4 as a susceptibility gene for autoimmune thyroid disease. Endocr J. 2010;57(8):723-729. Available from: https://pubmed.ncbi.nlm.nih.gov/20595785/ (accessed: 15/06/2026).

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