ORIGINAL ARTICLE

HLA CLASS I AND II ALLELE DISTRIBUTION IN ALBANIAN PATIENTS WITH AUTOIMMUNE THYROID DISEASE

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ABSTRACT

Background. Autoimmune thyroid diseases (ATD), including Hashimoto's thyroiditis and Graves' disease, are among the most common autoimmune disorders and arise from complex interactions between genetic susceptibility and environmental factors. The human leukocyte antigen (HLA) region on chromosome 6p21 plays a major role in immune regulation and disease susceptibility. However, the distribution of HLA alleles varies across populations, and data from Balkan populations remain limited. This study aimed to investigate the distribution of HLA class I and class II alleles in Albanian patients with autoimmune thyroid disease.

Methods. This study included 68 patients diagnosed with autoimmune thyroid disease at the Endocrinology Unit of the University Hospital Center "Mother Teresa", Tirana, between 2023 and 2025. The cohort comprised 51 patients with Hashimoto's thyroiditis and 17 with Graves' disease. Genomic DNA was extracted from peripheral blood samples, and allele-level HLA genotyping for class I (HLA-A, HLA-B, HLA-C) and class II (HLA-DRB1, HLA-DQB1) loci was performed using PCR with sequence-specific primers (PCR-SSP). Allele frequencies were calculated by direct gene counting and expressed as percentages with 95% confidence intervals.

Results. The mean age of participants was 42.2 ± 11 years, with a strong female predominance (91%). Among HLA class I alleles, A02:01 was the most frequent allele (38.0%), followed by A01:01 and A24:02 (9.9% each). At the HLA-B locus, B51:01 (16.2%), B18:01 (14.6%), and B35:01 (13.8%) were the most common alleles. For the HLA-C locus, C07:01 was predominant (22.3%), followed by C12:03 (17.4%) and C02:02 (10.7%). Among class II alleles, DRB111:01 showed the highest frequency (26.6%), followed by DRB116:01 (18.8%) and DRB104:01 (12.5%). At the HLA-DQB1 locus, DQB103:01 was the most frequent allele (35.5%), followed by DQB105:02 (19.4%) and DQB102:01 (12.1%). Compared with previously published European data, the Albanian cohort demonstrated a relatively higher frequency of DRB111 and lower frequency of DRB1*03.

Conclusion. This study provides the first characterization of HLA class I and class II allele frequencies in Albanian patients with autoimmune thyroid disease. The predominance of DRB111 and DQB103 suggests a distinctive immunogenetic profile that partially overlaps with other Mediterranean populations. These findings contribute to understanding the

population-specific genetic background of autoimmune thyroid disease and highlight the need for larger studies in Balkan populations.

Keywords. Autoimmune thyroid disease, HLA polymorphism, Albanian population

SHPËRNDARJA E ALELEVE HLA TË KLASËS I DHE II TE PACIENTËT SHQIPTARË ME SËMUNDJE AUTOIMUNE TË GJËNDRËS TIROIDE

ABSTRAKT

Hyrja. Sëmundjet autoimune të tiroides (SAT), përfshirë tiroiditin e Hashimotos dhe sëmundjen e Graves, janë ndër çrregullimet autoimune më të shpeshta dhe lindin nga ndërveprimi kompleks midis predispozitës gjenetike dhe faktorëve mjedisorë. Rajoni i antigjenit leukocitar human (HLA) në kromozomin 6p21 luan një rol të rëndësishëm në rregullimin e përgjigjes imune dhe në ndjeshmërinë ndaj sëmundjes. Megjithatë, shpërndarja e aleleve HLA ndryshon ndërmjet popullatave dhe të dhënat për popullatat e Ballkanit janë ende të limituara. Qëllimi i këtij studimi ishte të imvestigoje shpërndarjen e aleleve të klasës I dhe klasës II të HLA tek pacientët shqiptarë me sëmundje autoimune të tiroides.

Metodat. Studimi përfshiu 68 pacientë të diagnostikuar me sëmundje autoimune të tiroides në Shërbimin e Endokrinologjisë të Qendrës Spitalore Universitare "Nënë Tereza", Tiranë, gjatë periudhës 2023–2025. Grupi përbëhej nga 51 pacientë me tiroidit kronik Hashimoto dhe 17 pacientë me sëmundje të Graves. ADN-ja gjenomike u ekstraktua nga mostrat e gjakut periferik dhe gjenotipizimi i HLA në nivel aleli për lokuset e klasës I (HLA-A, HLA-B, HLA-C) dhe klasës II (HLA-DRB1, HLA-DQB1) u krye duke përdorur metodën PCR me primerë specifikë për sekuencën (PCR-SSP). Frekuencat alelike u llogaritën me metodën e numërimit direkt të gjeneve dhe u shprehën në përqindje me intervale besueshmërie 95%.

Rezultatet. Mosha mesatare e pjesëmarrësve ishte 42.2 ± 11 vjeç, me predominancë të theksuar të femrave (91%). Ndër alelet e klasës I të HLA, A02:01 ishte aleli më i shpeshtë (38.0%), i ndjekur nga A01:01 dhe A24:02 (secila 9.9%). Në lokusin HLA-B, alelet më të shpeshta ishin B51:01 (16.2%), B18:01 (14.6%) dhe B35:01 (13.8%). Për lokusin HLA-C, C07:01 ishte aleli predominues (22.3%), i ndjekur nga C12:03 (17.4%) dhe C02:02 (10.7%). Ndër alelet e klasës II, DRB111:01 kishte frekuencën më të lartë (26.6%), i ndjekur nga DRB116:01 (18.8%) dhe DRB104:01 (12.5%). Në lokusin HLA-DQB1, DQB103:01 ishte aleli më i shpeshtë (35.5%), i ndjekur nga DQB105:02 (19.4%) dhe DQB102:01 (12.1%). Krahasuar me të dhënat e publikuara më parë për popullatat evropiane, kohorta shqiptare

tregoi një frekuencë relativisht më të lartë të DRB111 dhe një frekuencë më të ulët të DRB1*03.

Përfundimi. Ky studim paraqet karakterizimin e parë të frekuencave të aleleve të klasës I dhe klasës II të HLA tek pacientët shqiptarë me sëmundje autoimune të tiroides. Predominanca e aleleve DRB1*11 dhe DQB1*03 sugjeron një profil imunogjenetik të veçantë që mbivendoset pjesërisht me popullatat e tjera mesdhetare. Këto rezultate hedhin bazat për kuptimin e bazës gjenetike specifike për popullatën të sëmundjeve autoimune të tiroides dhe theksojnë nevojën për studime të tjera në popullatat e Ballkanit.

Fjalë kyçe: sëmundjet autoimune të gjëndrës tiroide, polimorfizmi i HLA, popullata shqiptare

INTRODUCTION

Autoimmune thyroid diseases (ATD), primarily Hashimoto's thyroiditis and Graves' disease, are among the most common autoimmune disorders and affect approximately 5–10% of the population worldwide (1). These diseases arise from a complex interplay between genetic susceptibility and environmental triggers that ultimately leads to immune-mediated damage or stimulation of thyroid tissue. Among the genetic determinants implicated in ATD, the human leukocyte antigen (HLA) region located on chromosome 6p21 has been consistently identified as a major susceptibility locus (2). HLA molecules are responsible for antigen presentation to T lymphocytes and therefore play a central role in adaptive immune responses. Numerous studies have demonstrated associations between autoimmune thyroid diseases and specific HLA class II alleles**,** particularly HLA-DR and HLA-DQ variants**,** which influence CD4+ T-cell activation and immune tolerance mechanisms (3). However, the distribution of HLA alleles varies considerably across ethnic groups and geographic regions.

Previous research has identified associations between autoimmune thyroid diseases and several HLA alleles in European, Asian, and Middle Eastern populations (4–6). Nevertheless, data from Balkan populations remain limited**,** and the immunogenetic background of autoimmune thyroid disease in this region is poorly characterized.

The aim of the present study was therefore to investigate the distribution of HLA class I and class II alleles in Albanian patients with autoimmune thyroid disease**.**

MATERIAL AND METHODS

Study design and populations

Study Population

This study involved 68 consecutive individuals diagnosed with Autoimmune thyroid disease (ATD) at the Endocrinology Unit of the University Hospital Center "Mother Teresa" between 2023 and 2025. Diagnosis of ATD disease was established based on clinical hyperthyroidism or hypothyroidism, altered serum thyroid-stimulating hormone (TSH), and positivity for TSH receptor antibodies (TRAb), thyroid peroxidase antibody AntiTPO and/or antithyroglobulin antibodies (AntiTg) 7,8. When feasible, thyroid ultrasound and scintigraphy findings were used to support the diagnosis.

Ethical approval

The study protocol received approval from the Ethics Committee of the University of Medicine of Tirana. Written informed consent was obtained from all participants before enrollment.

HLA genotyping

Genomic DNA was extracted from buffy coat samples using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany). Allele-level HLA genotyping of class I (HLA-A*, -B*, -C*) and class II (HLA-DRB1*, -DQB1*) loci was performed using PCR with sequence-specific primers (PCR-SSP) and Micro SSP™ DNA Typing Trays (One Lambda, Thermo Fisher Scientific, USA).

Data analysis

Allele frequencies were calculated through direct gene counting and reported as proportions. Quantitative independent variables with normal distribution across groups were tested using a Student t-test, with the null hypothesis that the variance is equal across groups being checked using Levene's test for equality of variance. All statistical analyses were performed using R software (R Foundation for Statistical Computing, version 4.3.1, R Core Team, 2023, Vienna, Austria).

RESULTS

Characteristics of the study sample populations

This study included 68 consecutive individuals diagnosed with ATD at the Endocrinology Unit at the University Hospital Center "Mother Teresa" between 2023 and 2025, comprising 51 patients with Hashimoto's thyroiditis (75.0%) and 17 patients with Graves' disease

(25.0%). Mean age was 42.2 ± 11years while age distribution was normal across the entire patient cohort. Females predominated over males, 91% and 9% respectively.

HLA genotyping results

HLA allele distribution in autoimmune thyroid disease

HLA Class I

At the HLA-A locus, the most frequent allele was A*02:01, with an allele frequency of 38.0% (95% CI 29.4–47.4). Other common alleles included A*01:01 and A*24:02, each with a frequency of 9.9% (95% CI 5.7–16.5), followed by A*68:01 with 8.3% (95% CI 4.5–14.6) and A*03:01 with 7.4% (95% CI 3.8–13.7).

At the HLA-B locus, B*51:01 was the most frequent allele (16.2%, 95% CI 10.9–23.4), followed by B*18:01 (14.6%, 95% CI 9.6–21.6) and B*35:01 (13.8%, 95% CI 9.0–20.7).

At the HLA-C locus, C*07:01 was the predominant allele (22.3%, 95% CI 15.7–30.6), followed by C*12:03 (17.4%, 95% CI 11.8–24.8) and C*02:02 (10.7%, 95% CI 6.4–17.2),

Table 1. HLA allele frequencies in AITD patients

Locus	Allele	Frequency (%)	95% CI
HLA-A	A*02:01	38.0	29.4–47.4
	A*01:01	9.9	5.7–16.5
	A*24:02	9.9	5.7–16.5
	A*68:01	8.3	4.5–14.6
	A*03:01	7.4	3.8–13.7
HLA-B	B*51:01	16.2	10.9–23.4
	B*18:01	14.6	9.6–21.6
	B*35:01	13.8	9.0–20.7
HLA-C	C*07:01	22.3	15.7–30.6
	C*12:03	17.4	11.8–24.8
	C*02:02	10.7	6.4–17.2
HLA-DRB1	DRB1*11:01	26.6	19.7–34.9
	DRB1*16:01	18.8	12.9–26.7
	DRB1*04:01	12.5	7.8–19.4
HLA-DQB1	DQB1*03:01	35.5	27.5–44.4
	DQB1*05:02	19.4	13.6–26.8
	DQB1*02:01	12.1	7.7–18.5

HLA Class II

At the HLA-DRB1 locus, the most common allele was DRB1*11:01 with a frequency of 26.6% (95% CI 19.7–34.9), followed by DRB1*16:01 (18.8%, 95% CI 12.9–26.7) and DRB1*04:01 (12.5%, 95% CI 7.8–19.4).

At the HLA-DQB1 locus, DQB1*03:01 represented the most frequent allele (35.5%, 95% CI 27.5–44.4). This was followed by DQB1*05:02 (19.4%, 95% CI 13.6–26.8) and DQB1*02:01 (12.1%, 95% CI 7.7–18.5) (Table 1).

The most frequent alleles observed were HLA-A02:01, HLA-B51:01, HLA-C07:01, HLA-DRB111:01, and HLA-DQB1*03:01 (Fig. 1).

Comparison of HLA allele frequencies in Albanian ATD patients and other populations

When comparing the allele frequencies observed in the Albanian ATD cohort with previously published data from other populations (Table 2), several differences become apparent. The DRB1*11 allele showed a higher frequency in our cohort (26.6%) compared with most European populations, where frequencies typically range between 10–18% (9,10). Conversely, DRB1*03, a classical susceptibility allele for autoimmune thyroid disease in Caucasian populations, appeared less frequent in the Albanian cohort (Table 2). These findings suggest that the immunogenetic background of autoimmune thyroid disease in Albania may differ from that observed in Northern European populations, possibly reflecting regional genetic variation within the Mediterranean and Balkan populations (Table 2) (3,4, 6).

Table 2. Comparison of HLA allele frequencies in Albanian ATD patients and other populations

Locus	**Allele**	**Albania ATD**	**Europe**	**Asia**
HLA-A	A*02	38%	25–35%	20–30%
HLA-B	B*51	16.2%	10–15%	<10%
HLA-C	C*07	22.3%	15–25%	10–15%
HLA-DRB1	DRB1*11	26.6%	10–18%	<10%
HLA-DRB1	DRB1*03	8.3%	20–30%	10–15%
HLA-DQB1	DQB1*03	35.5%	20–30%	15–25%
HLA-DQB1	DQB1*02	12.1%	20–30%	10–15%

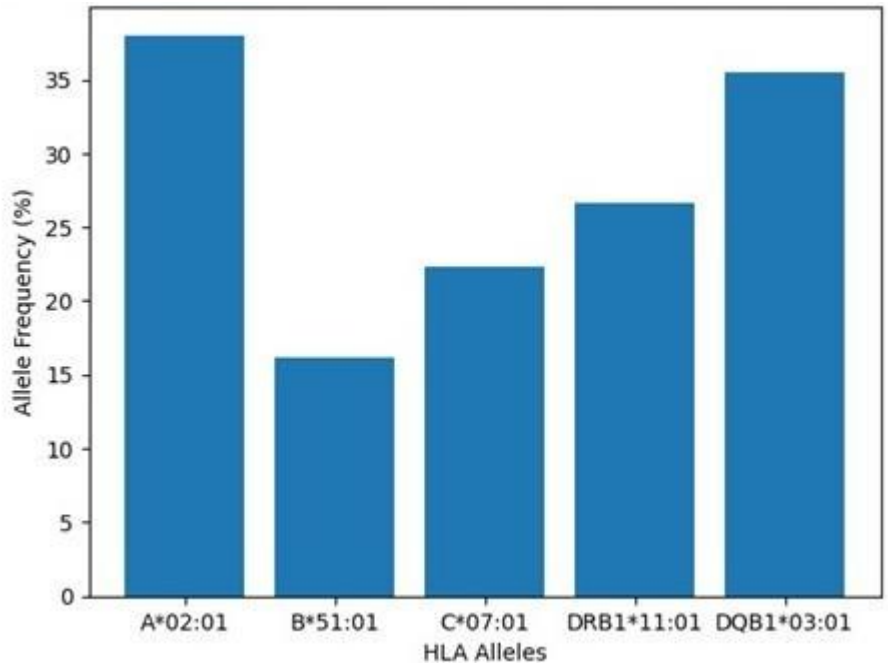

Figure 1. Most frequent HLA alleles in Albanian patients with Autoimmune Thyroid Disease.

DISCUSSION

Autoimmune thyroid diseases, including Hashimoto's thyroiditis and Graves' disease, are among the most common autoimmune disorders worldwide and represent the leading cause of thyroid dysfunction in iodine-sufficient regions (1). These diseases arise from a complex interaction between genetic predisposition and environmental triggers, ultimately leading to immune-mediated destruction or stimulation of thyroid tissue. Among the genetic factors involved, the human leukocyte antigen (HLA) region on chromosome 6p21 plays a critical role in determining individual susceptibility to autoimmune thyroid disease (2,3).

In the present study, we analyzed the distribution of HLA class I and class II alleles in Albanian patients with autoimmune thyroid disease. The most frequent alleles observed were HLA-A02:01, HLA-B51:01, HLA-C07:01, HLA-DRB111:01, and HLA-DQB1*03:01. These findings highlight the extensive polymorphism of the HLA region and support its involvement in autoimmune thyroid disease susceptibility.

Previous studies in European populations have frequently reported associations between autoimmune thyroid diseases and HLA class II alleles, particularly HLA-DRB103 and HLA-

DQB102, which form the well-known DR3-DQ2 haplotype linked to Graves' disease (4). In contrast, the results of the present study showed a predominance of DRB111 and DQB103 alleles, suggesting a somewhat different immunogenetic profile in the Albanian population.

Interestingly, studies conducted in Mediterranean populations have reported similar findings. Research in Turkish cohorts demonstrated associations between autoimmune thyroid diseases and DRB1*11 as well as DQB1*03 alleles, indicating that these variants may contribute to disease susceptibility in this geographic region (5). The relatively high frequency of DRB1*11 and DQB1*03 observed in our cohort may therefore reflect shared genetic backgrounds within populations of the Eastern Mediterranean and Balkan regions.

At the HLA-DQB1 locus, DQB1*03:01 represented the most frequent allele in the present study. This finding is consistent with previous studies reporting increased frequencies of DQB1*03 alleles in autoimmune endocrine disorders, including autoimmune thyroid disease and type 1 diabetes (6). Because HLA-DQ molecules participate in antigen presentation to CD4+ T cells, structural differences in these molecules may influence the presentation of thyroid autoantigens and thereby contribute to disease development.

The present study also demonstrated relatively high frequencies of class I alleles, particularly A*02:01 and B*51:01. Although the role of class I HLA alleles in autoimmune thyroid diseases is less well established than that of class II alleles, increasing evidence suggests that CD8+ cytotoxic T cells may participate in thyroid tissue damage, especially in Hashimoto's thyroiditis (8). Class I molecules may therefore contribute to autoimmune processes through cytotoxic immune mechanisms directed against thyroid follicular cells.

Comparison of our findings with studies from other populations highlights the substantial geographic variability in HLA allele distribution. Studies from East Asian populations, for example, have reported associations between autoimmune thyroid disease and different HLA alleles, including specific DRB1 variants and certain class I alleles (6). Such differences underscore the importance of investigating the genetic architecture of autoimmune diseases in specific populations, as susceptibility patterns may vary considerably according to ethnic background.

The present study provides the first characterization of HLA allele frequencies in Albanian patients with autoimmune thyroid disease. The allele distribution observed suggests that the

Albanian population shares certain immunogenetic characteristics with other Mediterranean populations while also demonstrating distinctive features, particularly the relatively high frequency of DRB1*11 and DQB1*03 alleles.

Several limitations should be considered when interpreting these findings. First, the sample size was relatively modest, which may limit the ability to detect associations involving less frequent alleles. Second, the present study focused on allele frequency analysis rather than haplotype structures, and further studies examining linkage disequilibrium and haplotype patterns may provide additional insight into the genetic susceptibility to autoimmune thyroid disease. Despite these limitations, the results contribute to the growing body of evidence supporting the role of HLA polymorphisms in autoimmune thyroid disease susceptibility. Future studies involving larger cohorts and multicenter collaborations within the Balkan region may help clarify the immunogenetic mechanisms underlying autoimmune thyroid diseases and improve our understanding of population-specific genetic risk factors.

CONCLUSION

This study represents the first analysis of HLA class I and class II allele frequencies in Albanian patients with autoimmune thyroid disease**.** The results demonstrate a predominance of HLA-A*02:01, HLA-B*51:01, HLA-C***07**:01, *HLA-DRB1*11:01, and HLA-DQB1*03:01, highlighting the considerable polymorphism of the HLA region within this population. The allele distribution observed in the present cohort shows partial similarities with other Mediterranean populations but also suggests distinctive immunogenetic characteristics in the Albanian population. Further studies involving larger cohorts and comparative analyses across different populations are needed to confirm these observations and to clarify the contribution of HLA variation to the pathogenesis of autoimmune thyroid diseases.

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Conflict of interest: Nothing to declare

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