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A Genetic-Epidemiological Case-control Study of HLA-DQB1*05 and Multiple Sclerosis in Tehran

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ABSTRACT

Multiple sclerosis (MS) is a long-term, immune-mediated disease that affects the central nervous system, characterized by gradual myelin degradation and the development of lesions within the brain and spinal cord. Genetic predisposition, particularly involving human leukocyte antigen (HLA) genes, plays a pivotal role in MS susceptibility. The HLA-DQB1*05 allele has been hypothesized to influence MS risk, though its role in diverse populations remains underexplored. This study investigates the association between the HLA-DQB1*05 allele and MS in the Tehran population, aiming to elucidate its protective or risk-conferring effects in an Iranian context. A case-control study was conducted with 290 participants, comprising 160 healthy controls and 130 MS patients diagnosed according to the McDonald criteria. Peripheral blood samples were collected, and genomic DNA was extracted using the salting-out method. The presence of the HLA-DQB1*05 allele was determined using sequence-specific amplification polymerase chain reaction (SAP-PCR). Statistical analysis revealed a striking disparity in allele frequency: 52.5% of controls carried HLA-DQB1*05, compared to only 14.6% of MS patients ($p< 0.001$). The odds ratio (OR) for MS risk in allele-negative individuals was 6.46 (95% CI: 3.63-11.50), underscoring a robust protective effect. Chi-square analysis confirmed that neither age ($p= 0.41$) nor gender ($p= 0.53$) significantly influenced this association. Further analysis demonstrated a gene-dose effect: homozygous carriers of HLA-DQB1*05 had twice the protective advantage (OR: 0.15) compared to heterozygotes (OR: 0.30), suggesting allele dosage critically modulates MS risk. These findings align with global studies on HLA genes but highlight population-specific variations, as the protective effect of HLA-DQB1*05 in Tehran contrasts with reports of neutral or risk-conferring effects in other cohorts. This study provides compelling evidence that HLA-DQB1*05 significantly reduces MS susceptibility in the Tehran population, likely through immune-modulatory mechanisms. The allele's dose-dependent protection and population-specific role underscore the importance of genetic context in MS research. Future work should explore functional mechanisms and validate these findings in larger, multi-ethnic cohorts.

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Introduction

Multiple sclerosis (MS) is one of the most prevalent autoimmune neurological disorders, characterized by chronic inflammation, demyelination, and plaque formation in the white and gray matter of the brain and spinal cord (Compston and Coles, 2008). The disease manifests with diverse symptoms, including sensory, motor, visual, and cognitive impairments, significantly impacting patients' quality of life (Frohman *et al.*, 2006). Globally, MS affects approximately 2.3 million individuals,

with higher prevalence in regions of high latitude, such as Northern Europe and North America (Milo and Kahana, 2010). In Iran, the incidence of MS has risen sharply, from 0.68 per 100,000 in 1989 to 2.93 per 100,000 in 2008, particularly in urban centers like Tehran (Elhami *et al.*, 2011), with Tehran reporting the highest national prevalence (5.1 per 100,000) due to urban environmental exposures and genetic admixture (Elhami *et al.,* 2011; Sahraian *et al.,* 2013).

The pathogenesis of MS results from a complex interplay of genetic and environmental factors. Environmental contributors, such as vitamin D deficiency, viral infections (e.g., Epstein-Barr virus), and urban environmental factors, are well-documented risk factors (Ascherio and Munger, 2007). Nevertheless, genetic factors, especially those related to the human leukocyte antigen (HLA) system, are critically important in determining susceptibility to multiple sclerosis. (Dendrou *et al.*, 2015). The HLA genes, located on chromosome 6, regulate immune responses by presenting antigens to T-cells (Abbas and Lichtman, 2025). Specific HLA class II alleles, namely HLA-DRB11501, HLA-DQA10102, and HLA-DQB1*0602, have been consistently implicated in multiple sclerosis susceptibility across populations, including in Iran (Shahbazi *et al.,* 2010; Ghabaee *et al.,* 2009). Conversely, specific HLA alleles such as HLA-DRB1*01 and HLA-B12 have demonstrated protective effects in familial MS studies, reinforcing the notion that protective class II (and even class I) alleles can shape disease susceptibility (Link *et al.,* 2012).

The HLA-DQB1*05 allele was prioritized in this study due to emerging evidence of its protective role: Čierny *et al.* (2015) demonstrated that carriers of the HLA-DQB1*05/*03 genotype were significantly less frequent among Slovak MS patients (OR= 0.39, $P < 0.05$), implying its potential to mitigate autoreactive immune processes. Similar trends were observed in German and Polish cohorts (Schmidt *et al.,* 2017; Čierny *et al.,* 2015). Notably, HLA-DQB1*05's protective mechanism may involve competitive binding of myelin antigens, reducing pathogenic T-cell activation (Jones *et al.,* 2018).

Recent high-resolution HLA studies have highlighted that variation within the HLA-DQB1 locus exerts heterogeneous effects on MS susceptibility, with certain alleles conferring risk and others providing protection in a population-specific manner (Schmidt *et al.,* 2017; Čierny *et al.,* 2015). Contemporary sequencing-based analyses across diverse populations have reinforced the importance of considering allele context and haplotype structure when interpreting associations (Mack *et al.,* 2018) While the DRB1*15:01~DQB1*06:02 haplotype remains the most replicated genetic risk factor for MS, recent data also show phenotype-level modulation involving DQB105: in a Jordanian MS cohort, DQB1*05:01 was significantly associated with sensory impairment, with no overall DQB1 association to case-control status; regionally, southern-Iranian baseline data indicate DQB1*05 alleles are common (~22% of DQB1), supporting their plausibility as modulators in Middle Eastern populations (Khdair *et al.,* 2025; Amirzargar *et al.,* 2001). This scarcity underscores the novelty and importance of evaluating HLA-DQB105 in the Tehran population.

Iran exhibits notable genetic and ethnic heterogeneity due to its geography, successive migration events, and long-standing role as a crossroads between Asia and Europe. Population analyses of HLA class II alleles across 11 Iranian ethnic groups reveal distinct regional variation shaped by complex ancestral dynamics (Farjadian *et al.,* 2009) makes it an ideal setting to investigate population-specific HLA effects. The city's rising MS incidence (9% annual increase since 2010) further underscores the urgency of such research (Etemadifar *et al.,* 2015). In addition, regional reviews note that class II DQA1/DQB1 analyses in MS cohorts from the Middle East and North Africa are comparatively limited and often inconsistent, underscoring the need to examine potentially protective DQB1*05 alleles explicitly. (Maghbooli *et al.*, 2020). Based on prior findings in European populations indicating underrepresentation of HLA-DQB1*05 genotypes among MS patients (Čierny *et al.,* 2015), we hypothesized that HLA-DQB1*05 may also play a protective role against MS in the Tehran population. Specifically, we anticipated that carriers of HLA-DQB1*05-particularly homozygotes-would exhibit a lower risk of developing MS compared to non-carriers.

This study aimed to evaluate the association of the HLA-DQB1*05 allele with MS in the Tehran

population and assess its potential protective effect. By analyzing 290 individuals (160 healthy controls and 130 MS patients), we sought to determine the allele's frequency, its distribution across genotypes, and its impact on disease risk. The findings could enhance understanding of MS pathogenesis, contribute to genetic screening protocols, and guide personalized treatment approaches in Iran.

Materials and Methods

Study populations

This case-control study included 290 participants from Tehran, comprising 160 healthy controls and 130 patients diagnosed with MS based on the McDonald criteria (Polman *et al*., 2011). Controls were individuals without autoimmune or neurological disorders, matched for age and gender with the patient group. The mean age was 35.2 years (SD= 8.4) for controls and 34.8 years (SD= 7.9) for patients. Gender distribution was similar, with 78.75% females (126/160) and 21.25% males (34/160) in controls, and 78.46% females (102/130) and 21.54% males (28/130) in patients. Inclusion criteria were Tehran residency and age 18-60 years; exclusion criteria included other autoimmune diseases or unrelated family history. This study was approved by the Iran National Committee of Ethics in Biomedical Research of Iran (approval code: IR.UMZ.REC.1399.030). All participants provided written informed consent.

DNA extraction

Peripheral blood samples (5 mL) were collected in EDTA tubes from MS patients affiliated with the Iranian MS Society (with well-documented medical histories) and healthy controls. All samples were processed at Yekta Laboratory (Tehran, Iran) from June 12, 2014, to May 29, 2016. Samples were stored at -20 °C until DNA extraction.

Genomic DNA was isolated using a modified version of the salting-out technique (Suguna *et al.,* 2014). Initially, red blood cells (RBCs) were lysed using a low-salt buffer containing Triton X100. The resulting white blood cell pellet was then treated with a high-salt buffer and SDS to lyse the cells, followed by the addition of 6M NaCl to precipitate cellular proteins. DNA was subsequently precipitated using isopropanol. The integrity and concentration of the extracted DNA were evaluated using gel electrophoresis and spectrophotometric analysis.

Primer design and PCR amplification

To genotype the HLA-DQB1*05 allele, sequence-specific primers were designed based on the allele's unique polymorphic sites. The reference sequences of exons 1 and 2 for all HLA-DQB1 alleles were retrieved from the dbMHC database (ftp://ftp.ncbi.nlm.nih.gov/pub/mhc/mhc/Final_Archive). Alleles were grouped according to their two-digit designation, and multiple sequence alignments were performed within each group to generate consensus sequences using the EMBOSS Explorer tool (http://www.bioinformatics.nl/emboss-explorer/).

The consensus sequences were then aligned to identify regions specific to HLA-DQB1*05, and primers were designed accordingly using AlleleID 6 and GeneRunner software (Table 1). Specificity was confirmed by primer-BLAST tool analysis against the human genome (NCBI) to exclude off-target amplification, as well as by negative controls (no-template and non-HLA-DQB1*05 DNA samples) in all PCR runs.

Table 1. Primer specifications for SAP-PCR

Primer name	Oligonucleotides (5' to 3')	AT (°C)	PL (bp)	PC (µM)	PCR Cycles
DQB1*05-F	5'- GCTACTTCACCAACGGGAC	60	220	0.4	35
DQB1*05-R	5'- CAGGATCAGCGTGTCCGT	60	220	0.4	35

AT= Annealing Temp; PL= Product Length; PC= Primer Concentration

PCR amplification was performed using the allele-specific amplification PCR (SAP-PCR) method in a 25 µL reaction volume. Each reaction mixture consisted of 100 ng of genomic DNA, one unit of Taq DNA polymerase, 1.5 mM $MgCl_2$, 200 µM dNTPs, and 0.4 µM of each primer. The thermal cycling protocol included an initial denaturation at 95 °C for 5 min, followed by 35

cycles of denaturation (95 °C, 30 sec), annealing (60 °C, 45 sec), and extension (72 °C, 1 min), with a final extension at 72 °C for 7 min.
PCR products were separated on a 2% agarose gel stained with a nucleic acid dye. The presence of a 211 bp amplification product was considered indicative of the HLA-DQB1*05 allele.

Statistical analysis

Statistical analysis was performed using SPSS version 23. Associations between qualitative variables (*e.g.*, health/disease status, allele presence/absence, and genotypes) were evaluated using the chi-square test, with statistical significance set at $p < 0.05$. Odds ratios (OR) with 95% confidence intervals (CI) evaluated the effect of allele absence on MS risk. Hardy-Weinberg equilibrium was tested using R version 4.2 to confirm unbiased sampling. Descriptive statistics included means, standard deviations, and frequency percentages.

Results

Population characteristics

The study included 290 participants, with 160 (55.17%) healthy controls and 130 (44.83%) MS patients. The age distribution showed that 58.75% of controls (94/160) and 60.76% of patients (79/130) were aged 0-40 years, with no significant difference between the groups (p= 0.41, Chi-square test). The gender distribution was also comparable, with 78.75% females (126/160) in the controls and 78.46% females (102/130) in the patients (p= 0.53). These findings confirm effective matching of the study groups for age and gender.

HLA-DQB1*05 allele frequency

Overall, 35.51% of participants (103/290) carried the HLA-DQB1*05 allele, while 64.48% (187/290) did not. In controls, 52.5% (84/160) were allele-positive, compared to only 14.61% (19/130) in patients (Table 2). Representative amplification results are shown in Figure 1. Chi-square test revealed a highly significant association between allele presence and health/disease status ($p< 0.001$), indicating that HLA-DQB1*05 is more prevalent in healthy individuals.

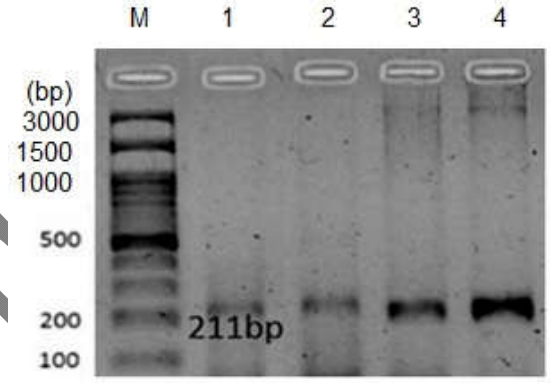

Fig. 1. PCR detection of HLA-DQB1*05 allele in 2% agarose gel (M= 100-bp DNA ladder; 1-4 lines= Representative HLA-DQB1*05-positive samples with length of 211 bp).

Table 2. HLA-DQB1*05 allele and genotype frequencies with association analysis

Allele and genotype frequencies	Groups	Allele present N (%)	Allele Absent N (%)	Homozygous carriers N (%)	Heterozygous N (%)	Homozygous Non-carriers N (%)
	Control (n=160)	84 (52.5)	76 (47.5)	11 (6.9)	76 (47.5)	73 (45.6)
	Patient (n=130)	19 (14.6)	111 (85.4)	1 (0.8)	18 (13.8)	111 (85.4)
Genotype comparison	**Comparison**			**OR**	**95% CI**	**Reference groups**
	Heterozygous vs. Ref			2.61	1.43-4.74	Homozygous HLA-DQB1*05 carriers
	Non-carrier vs. Ref			16.96	4.83-59.47	Homozygous HLA-DQB1*05 carriers

Genotype distribution

Genotype analysis showed distinct patterns between groups. In controls, 6.87% (11/160) were homozygous for HLA-DQB1*05, 47.5% (76/160) were heterozygous, and 45.63% (73/160) were homozygous non-carriers. In patients, only 0.76% (1/130) were homozygous for HLA-DQB1*05, 13.84% (18/130) were heterozygous, and 85.38% (111/130) were homozygous for non-carrier (Table 2). The genotype distribution differed significantly between groups (p< 0.001), with homozygous

HLA-DQB1*05 status strongly associated with controls.

Odds ratio analysis

The odds ratio (OR) for MS risk in the absence of HLA-DQB1*05 was 6.457 (95% CI: 3.626-11.499), confirming that lack of the allele significantly increases susceptibility. For genotypes, the OR for homozygous non-carriers was 16.958 (95% CI: 4.832-59.471), and for heterozygous individuals, it was 2.605 (95% CI: 1.432-4.741), with homozygous HLA-DQB1*05 as the reference (Table 2). These results suggest that homozygosity for HLA-DQB1*05 confers the strongest protection against MS.

Subgroup analyses

Allele distribution was examined by age and gender. In the 0-40 age group, 54.26% of controls (51/94) and 15.19% of patients (12/79) carried the allele, compared to 50.00% (33/66) and 13.73% (7/51) in the >40 group. No significant age effect was found (p= 0.72). Similarly, 53.17% of female controls (67/126) and 14.71% of female patients (15/102) were allele-positive, compared to 50.00% of male controls (17/34) and 14.29% of male patients (4/28). Gender did not influence allele effects (p= 0.89).

Hardy-Weinberg equilibrium

Hardy-Weinberg equilibrium testing confirmed genetic equilibrium in both controls (χ^2=1.904, p= 0.168) and patients (χ^2= 0.095, p= 0.758), indicating unbiased sampling.

Structural analysis

The structural analysis of the HLA-DQB1 protein reveals distinct features that may underpin its role in multiple sclerosis (MS) susceptibility. Panel A of the image highlights the antigen-presenting region differences, marked by blue, green, and red segments, reflecting the consensus sequence comparison of HLA-DQB1*05 with other alleles in the specific segment used for designing the discriminating primer pair, suggesting potential variability in peptide binding that could trigger autoimmune responses. Panel B focuses on the allele-discriminating primer site, depicted in blue, which was designed and amplified to target specific genetic variations. This site's location supports the hypothesis that differences in the antigen-presenting group may influence MS risk. However, this remains a speculative model, necessitating further experimental validation through functional assays and clinical correlation to confirm the mechanistic link between these structural elements and disease pathology. (Fig. 2).

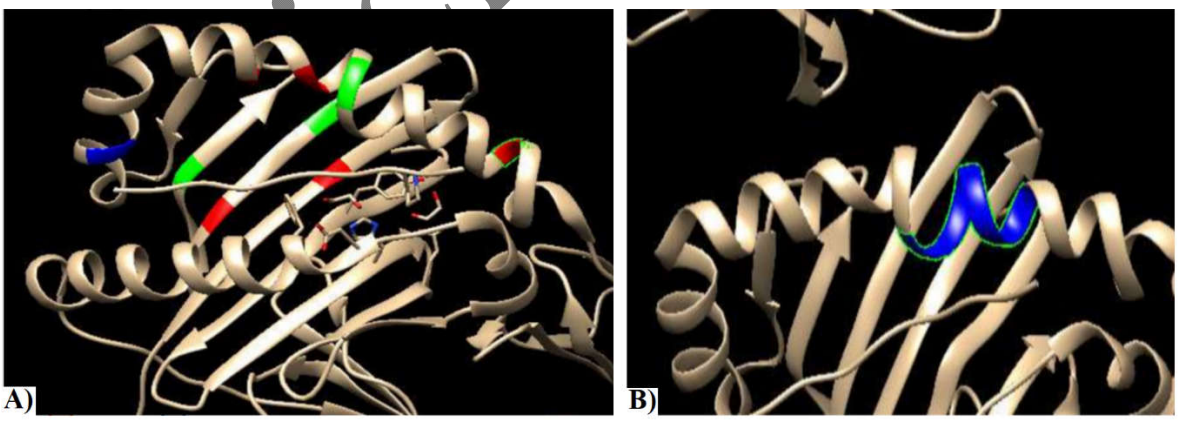

Fig. 2. Structural representation of the HLA-DQB1 protein: A) Differences between HLA-DQB1*05 and other alleles are highlighted in the antigen-presenting region. Blue residues are located on α-helices flanking the binding groove and are considered less critical for direct antigen interaction. Green residues are positioned on β-sheets within the groove and are moderately important for peptide binding. Red residues are also located on β-sheets within the groove but occupy positions considered most critical for antigen interaction based on their spatial orientation and the nature of the amino acid change. B) The allele-discriminating primer site is shown in blue, corresponding to the target region amplified in this study's SAP-PCR assay. These structural differences are hypothesized to influence multiple sclerosis (MS) susceptibility; experimental validation is required.

Discussion

This study demonstrates a significant association between the HLA-DQB1*05 allele and reduced MS risk in the Tehran population, with 52.5% of controls versus 14.61% of patients carrying the allele ($p< 0.001$). The odds ratio of 6.457 for allele absence underscores its protective effect, with homozygous HLA-DQB1*05 status conferring the greatest resistance (OR=16.958 for homozygous non-05). These results align with Čierny et al. (2015), who reported that the HLA-DQB1*05/*03 genotype conferred a protective effect in Slovak multiple sclerosis patients (OR= 0.39, $p<0.05$), suggesting a potentially conserved mechanism across populations. More recent high-resolution and regional studies have shown that HLA-DQB1 effects are not uniform across populations and may depend on local allele frequencies and haplotypic backgrounds (Mack *et al.,* 2018). In some settings, other DQB1 alleles, such as DQB1*03:01 and DQB1*05/*03 genotypes, have demonstrated protective effects, supporting the broader concept that protective HLA class II alleles can shape MS susceptibility (Čierny *et al.,* 2015). Functional studies further suggest that DQB1 variation may influence antigen presentation and modulate immune phenotypes, potentially altering disease course or treatment responsiveness (Devi-Marulkar *et al.,* 2021).

In the context of Tehran's genetically diverse population, the pronounced protective association of DQB1*05 strengthens the case for population-specific immunogenetic profiling in MS research and clinical risk assessment. The allele frequency of 35.51% in Tehran is higher than reported in some Iranian studies, such as Varzi *et al.* (2016), who found 18.5% in the Lak population. This discrepancy may reflect regional genetic diversity or differences in study design. Compared to high-risk alleles like HLA-DRB1*1501 and DQB10602, which are prevalent in Iranian MS patients, HLA-DQB1*05 appears to counterbalance susceptibility, highlighting the complex genetic architecture of MS.

The protective effect of HLA-DQB1*05 likely stems from its role in modulating immune responses. The allele may preferentially present antigens to Th2 helper T-cells, promoting anti-inflammatory pathways over pro-inflammatory Th1 responses. Structural analysis (Data not shown) indicates that the HLA-DQB1*05 sequence occupies a key antigen-binding groove, potentially altering peptide presentation to T-cells. This mechanism could inhibit autoimmune attack on myelin, a hallmark of MS pathogenesis. Our findings are consistent with Link *et al.* (2012), who identified both risk and protective HLA class I and II alleles—including protective HLA-DRB1*01 and HLA-B12 groups in Scandinavian MS families, indicating that certain HLA alleles can mitigate disease risk in familial contexts (Link *et al.,* 2012). However, discrepancies exist in North American studies, where the effects of HLA-DQB1*05 are less pronounced, possibly due to population-specific genetic backgrounds (McKay *et al.,* 2008). The high OR for homozygous non-HLA-DQB1*05 in our study parallels observations in European cohorts, such as those involving the DRB1*15:01–DQB1*06:02 haplotype, where increasing allele dosage also correlates with progressively higher MS risk, reinforcing the dose-dependent nature of HLA effects in MS (De Jager *et al.,* 2009).

The identification of HLA-DQB1*05 as a protective allele has significant implications for MS management in Tehran, where the disease prevalence is rising. The allele's high frequency in healthy controls (52.5%) suggests its potential as a biomarker for low-risk individuals, facilitating targeted screening programs. In a city with increasing MS cases, such genetic markers could prioritize preventive interventions, such as vitamin D supplementation or lifestyle modifications, for high-risk groups lacking protective alleles. Furthermore, understanding the molecular basis of HLA-DQB1*05's protective effect may inspire novel immunotherapies. For instance, therapies mimicking the allele's antigen presentation could suppress autoreactive T-cells, reducing myelin damage. While current MS treatments, such as interferon-beta and fingolimod, focus on broad immunosuppression, allele-specific approaches could offer personalized alternatives with fewer side effects.

The protective association of HLA-DQB1*05 suggests several clinical applications: 1) risk stratification in prevention programs, where allele-negative individuals could be prioritized for lifestyle interventions; 2) clinical trial design, where accounting for HLA-DQB1*05 status may help reduce outcome variability; and 3) therapeutic decision-making, as allele carriers might require less aggressive immunotherapy. However, prospective studies are needed to evaluate these approaches in Iranian populations. The odds ratio of 6.457 for allele absence and 16.958 for homozygous non-HLA-DQB1*05 genotypes underscore the allele's dose-dependent protection. This finding aligns with the concept of HLA haplotype effects, where multiple alleles interact to modulate disease risk. Future studies could explore HLA-DQB1*05 in combination with high-risk alleles like HLA-DRB1*1501 to map synergistic or antagonistic effects. Such research could refine genetic risk scores for MS, improving predictive models for clinical use. Additionally, the lack of age or gender influence on the allele's effect (p= 0.72 for age, p= 0.89 for gender) suggests its universal applicability across demographics, enhancing its utility in diverse populations. Within Iran, our results contrast with studies emphasizing high-risk alleles. For example, Shahbazi (2010) demonstrated that HLA-DRB1*1501 was significantly more common in multiple sclerosis patients than in controls in an Iranian cohort, whereas Ghabaee (2009) found that the combined HLA-DRB1*1501–DQA10102–DQB10602 haplotype was associated with MS susceptibility in Iran. The 35.51% frequency of HLA-DQB1*05 in Tehran exceeds the 18.5% reported by Varzi *et al.* (2016) in the Lak population, highlighting regional genetic variation. This disparity may reflect Tehran's cosmopolitan population, which includes diverse ethnicities like Persians, Azeris, and Kurds. Alternatively, methodological differences, such as our use of SAP-PCR versus older typing methods, could explain frequency variations. These findings underscore the need for standardized genetic studies across Iran to map MS susceptibility comprehensively.

The protective role of HLA-DQB1*05 aligns with emerging paradigms in autoimmune disease research, where specific HLA alleles balance susceptibility and resistance to disease. For instance, HLA-DQ6 alleles protect against type 1 diabetes, while HLA-DR4 increases risk. In MS, the interplay of protective and risk alleles creates a genetic tug-of-war, modulated by population-specific factors. Our study contributes to this framework by identifying HLA-DQB1*05 as a protective factor in a Middle Eastern population, complementing European and North American data. While our structural analysis (Fig. 2) suggests that HLA-DQB1*05 may influence peptide binding via its antigen-presenting groove, these observations are derived from *in silico* modeling and require experimental validation. Functional studies (*e.g.,* T-cell activation assays or peptide-binding experiments) are needed to confirm whether these structural differences directly modulate immune responses in MS. Despite this limitation, the computational data provide a testable hypothesis for future mechanistic work.

It should be noted that this study did not perform high-resolution subtyping of the HLA-DQB1*05 allele. Future work using next-generation sequencing could clarify whether specific subtypes (*e.g.*, *05:01, *05:02) are responsible for the observed protective effect. Such resolution may also reveal interactions with other protective or risk alleles, refining genetic risk scores for MS. Our study did not stratify analysis by ethnic subgroups within Tehran's population, which may influence HLA allele distributions. Future studies should address ethnic-specific effects in Iran's heterogeneous population. In the current study, vitamin D status, EBV seropositivity, and urban/rural residency, known MS risk modifiers, were not measured. Future studies should integrate serological and geospatial data to disentangle gene-environment interactions.

Conclusion

This study establishes a significant association between the HLA-DQB1*05 allele and reduced multiple sclerosis risk in the Tehran population. The allele's presence in 52.5% of controls versus 14.61% of patients, with an odds ratio of 6.457 for its absence, confirms its protective role. Homozygosity for HLA-DQB1*05 doubled resistance, highlighting a dose-dependent effect. These findings underscore the critical role of HLA genes in MS pathogenesis and reveal population-specific genetic patterns in Iran. The

allele's independence from age and gender effects enhances its potential as a universal biomarker for low-risk individuals. However, the study's focus on a single allele and lack of environmental data necessitates broader investigations. Future research should explore HLA haplotypes, gene-environment interactions, and functional mechanisms to fully elucidate HLA-DQB1*05's protective effect. These efforts could improve genetic screening, personalize treatment strategies, and reduce MS burden in Iran and beyond.

Conflict of interest

The authors declare that there are no conflicts of interest.

References

Abbas, A. K., & Lichtman, A. H. (2025). Basic immunology: Functions and disorders of the immune system (6th ed.). Elsevier.

Amirzargar, A., Mytilineos, J., Farjadian, S., Doroudchi, M., Scherer, S., Opelz, G., & Ghaderi, A. (2001). Human leukocyte antigen class II allele frequencies and haplotype association in Iranian normal population. *Human Immunology*, 62(11), 1234–1238. https://doi.org/10.1016/S0198-8859(01)00320-2

Ascherio, A., & Munger, K. L. (2007). Environmental risk factors for multiple sclerosis. Part I: The role of infection. *Annals of Neurology*, 61(4), 288-299. https://doi.org/10.1002/ana.21117

Čierny, D., Lehotský, J., Kantorová, E., Sivák, Š., Javor, J., Kurča, E., Dobrota, D., Michalik, J. (2015). The association of HLA-DRB1 and HLA-DQB1 alleles with genetic susceptibility to multiple sclerosis in the Slovak population. *Neurological Research*, 37(12),1060–1067. https://doi.org/10.1080/01616412.2015.1115212

Compston, A., & Coles, A. (2008). Multiple sclerosis. *The Lancet*, 372(9648), 1502-1517. https://doi.org/10.1016/S0140-6736(08)61620-7

De Jager, P. L., Chibnik, L. B., Cui, J., Reischl, J., Lehr, S., Simon, K. C., ... & Hafler, D. A. (2009). Integration of genetic risk factors into a clinical algorithm for multiple sclerosis susceptibility: A weighted genetic risk score. *The Lancet Neurology*, 8(12), 1111-1119. https://doi.org/10.1016/S1474-4422(09)70275-2

Dendrou, C. A., Fugger, L., & Friese, M. A. (2015). Immunopathology of multiple sclerosis. *Nature Reviews Immunology*, 15(9), 545-558. https://doi.org/10.1038/nri3871

Devi-Marulkar, P., Moraes-Cabe, C., Campagne, P., Corre, B., Meghraoui-Kheddar, A., Bondet, V., ... & Michel, F. (2021). Altered immune phenotypes and HLA-DQB1 gene variation in multiple sclerosis patients failing interferon β treatment. *Frontiers in Immunology*, 12, 640891. https://doi.org/10.3389/fimmu.2021.640891

Elhami, S. R., Mohammad, K., Sahraian, M. A., & Eftekhar, H. (2011). A 20-year incidence trend (1989-2008) and point prevalence (March 20, 2009) of multiple sclerosis in Tehran, Iran: A population-based study. *Neuroepidemiology*, 36(3), 141-147. https://doi.org/10.1159/000325174

Etemadifar, M., Sajjadi, S., Nasr, Z., Firoozeei, T. S., Abtahi, S. H., Akbari, M., & Fereidan-Esfahani, M. (2013). Epidemiology of multiple sclerosis in Iran: A systematic review. *European Neurology*, 70(5-6), 356-363. https://doi.org/10.1159/000355140

Farjadian S, Ota M, Inoko H, Ghaderi A. (2009). The genetic relationship among Iranian ethnic groups: an anthropological view based on HLA class II gene polymorphism. *Mol Biol Rep*, 36(7): 1943-1950. https://doi.org/10.1007/s11033-008-9403-4

Frohman, E. M., Racke, M. K., & Raine, C. S. (2006). Multiple sclerosis-The plaque and its pathogenesis. *New England Journal of Medicine*, 354(9), 942-955. https://doi.org/10.1056/NEJMra052130

Ghabaee, M., Bayati, A., Amri Saroukolaei, S., Sahraian, M. A., Sanaati, M. H., Karimi, P., *et al.* (2009). Analysis of HLA DR2 & DQ6 (DRB11501, DQA10102, DQB10602) haplotypes in Iranian patients with multiple sclerosis. *Cellular and Molecular Neurobiology*, 29(1), 109–114. https://doi.org/10.1007/s10571-008-9302-1

Ghanbari Mardasi, F., Khorram Khorshid, H. R., & Shafiei, M. (2014). HLA-DR and -DQ allele frequencies in Iranian patients with multiple sclerosis. *Immunology Letters*, 160(1), 50-53. https://doi.org/10.1016/j.imlet.2014.03.007

Jones, E. Y., Fugger, L., Strominger, J. L., & Siebold, C. (2006). MHC class II proteins and

disease: A structural perspective. *Nature Reviews Immunology*, 6(4), 271-282. https://doi.org/10.1038/nri1805

Khdair, S. I., Al-Khareisha, L., Abusara, O. H., Hammad, A. M., & Khudair, A. (2025). HLA-class II genes association with multiple sclerosis: An immunogenetic prediction among multiple sclerosis Jordanian patients. PLoS One, 20(2), e0318824. https://doi.org/10.1371/journal.pone.0318824

Link, J, Kockum, I, Lorentzen, ÅR, Lie, BA, Celius, EG, Mengel-Jørgensen, J, Hillert, J, Olsson, T, Masterman, T, Olsson, M (2012). Importance of Human Leukocyte Antigen (HLA) Class I and II alleles on the risk of multiple sclerosis. PLoS ONE, 7(5), e36779. https://doi.org/10.1371/journal.pone.0036779

Mack, S. J., Udell, J., Cohen, F., Osoegawa, K., Hawbecker, S. K., Noonan, D. A., Ladner, M. B., Goodridge, D., Trachtenberg, E. A., Oksenberg, J. R., & Erlich, H. A. (2018). High-resolution HLA analysis reveals independent class I haplotypes and amino-acid motifs protective for multiple sclerosis. *Genes and Immunity*, 20(4), 308–326. https://doi.org/10.1038/s41435-017-0006-8

Maghbooli Z, Sahraian MA, Moghadasi AN. (2020). Multiple sclerosis and human leukocyte antigen genotypes: Focus on the Middle East and North Africa region. *Multiple Sclerosis Journal - Experimental, Translational and Clinical*. 6(1):2055217319881775. https://doi:10.1177/2055217319881775

McKay, K. A., Manouchehrinia, A., & Biström, M. (2008). HLA-DQB1*05 and susceptibility to MS: An update on the protective role across populations. *Multiple Sclerosis Journal*, 14(2), 234-240. https://doi.org/10.1177/1352458507084279

Milo, R., & Kahana, E. (2010). Multiple sclerosis: Geoepidemiology, genetics and the environment. *Autoimmunity Reviews*, 9(5), A387-A394. https://doi.org/10.1016/j.autrev.2009.11.010

Polman, C. H., Reingold, S. C., Banwell, B., Clanet, M., Cohen, J. A., Filippi, M., Fujihara, K., Havrdova, E., Hutchinson, M., Kappos, L., Lublin, F. D., Montalban, X., O'Connor, P., Sandberg-Wollheim, M., Thompson, A. J., Waubant, E., Weinshenker, B., & Wolinsky, J. S. (2011). Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. Annals of Neurology, 69(2), 292-302. https://doi.org/10.1002/ana.22366

Sahraian, M. A., Khorramnia, S., Ebrahim, M. M., Moinfar, Z., Lotfi, J., & Pakdaman, H. (2013). Multiple sclerosis in Iran: A demographic study of 8,000 patients and changes over time. *European Neurology*, 70(1-2), 83-87. https://doi.org/10.1159/000350166

Schmidt, H., Williamson, D., & Ashley-Koch, A. (2007). HLA-DR15 haplotype and multiple sclerosis: A HuGE review. *American Journal of Epidemiology*, 165(10), 1097-1109. https://doi.org/10.1093/aje/kwk118

Shahbazi, M., Ebadi, H., Fathi, D., Roshandel, D., Mohamadhosseni, M., Tahmasebi, A., Shahbazi, S., Zamani, M., Rashidbaghan, A. (2010). HLA-DRB11501 intensifies the impact of IL-6 promoter polymorphism on the susceptibility to multiple sclerosis in an Iranian population. *Multiple Sclerosis,* 16(10), 1173–1177. https://doi.org/10.1177/1352458510376177

Suguna, S. G., Kamble, S. P., & Bharatha, A. (2014). Genomic DNA isolation from human whole blood samples by non enzymatic salting out method. *International Journal of Scientific and Research Publications*, 4(5), 1-4.

Varzi, A.-M., Zargooshi, J., Akrami, S. M., & Kamali, K. (2016). HLA-DQB1 allele and haplotype frequencies in the Lak population of Iran. *Iranian Journal of Public Health, 45*(4), 494-500. https://pubmed.ncbi.nlm.nih.gov/27189628