Journal of
Kerman University of Medical Sciences

https://jkmu.kmu.ac.ir
10.34172/jkmu.3317
JKMU. 2025;32:3317

Original Article

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HLA-DQB1 Polymorphisms and Juvenile Idiopathic Arthritis: A Case-Control Association Study Among Iranian Children

Mahsa Kazerani[1], Reza Shiari[2], Shervin Afzali[1], Reza Sinaei[3,4], Shirin Farivar[1*]

[1]Department of Cell and Molecular Biology, Faculty of Life Sciences and Biotechnology, Shahid Beheshti University, Tehran, Iran
[2]Department of Pediatrics, Mofid Children's Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[3]Department of Pediatrics, School of Medicine, Kerman University of Medical Sciences, Kerman, Iran
[4]Clinical Research Development Unit, Afzalipour Hospital, Kerman University of Medical Sciences, Kerman, Iran

*Corresponding Author: Shirin Farivar, Email: S_farivar@sbu.ac.ir

Abstract

Background: Juvenile idiopathic arthritis (JIA) is an important cause of inflammatory arthritis during childhood. HLA class II genes have been recognized as susceptibility factors for JIA development. This study aimed to investigate the association between HLA-DQB1 allele polymorphisms and JIA and its subtypes in a population of Iranian children.

Methods: In this case-control study, HLA typing was carried out on 60 children with systemic-onset JIA (28 patients) and oligoarticular JIA (32 patients) and 50 controls with the same age range for HLA-DQB1 alleles. Genotyping was performed by polymerase chain reaction using sequence-specific primers (PCR-SSP) and touchdown PCR methods. Finally, the sequencing of HLA polymorphisms was done.

Results: There was a significant association among DQ2 (DQB1*02:01) ($P=0.028$), DQ7 (DQB1*03:01, *03:04) ($P=0.046$) alleles, and JIA. None of the tested alleles demonstrated protective effects. The frequencies of DQ7 (DQB1*03:01, *03:04) ($P<0.001$) and DQ5 (DQB1*05:01- *05:04) ($P=0.03$) alleles were significantly higher in patients with oligoarticular JIA compared to the control group. In patients with systemic-onset JIA, DQ2 (DQB1*02:01) subtype also showed a significant association with this disorder's subtype ($P<0.001$).

Conclusion: It was found that distinct polymorphisms in the HLA-DQB1 locus could confer susceptibility to JIA in Iranian children. In this regard, the observed associations between HLA-DQB1 and JIA would be applicable for developing diagnostic, preventive, and therapeutic approaches.

Keywords: Juvenile idiopathic arthritis, Genetic association study, Case-control study, HLA-DQB1 locus, HLA typing

Citation: Kazerani M, Shiari R, Afzali S, Sinaei R, Farivar S. HLA-DQB1 polymorphisms and juvenile idiopathic arthritis: a case-control association study among Iranian children. *Journal of Kerman University of Medical Sciences*. 2025;32:3317. doi:10.34172/jkmu.3317

Received: April 19, 2022, **Accepted**: August 9, 2023, **ePublished**: October 6, 2025

Introduction

The term "Juvenile Idiopathic Arthritis" (JIA) commonly refers to a collection of childhood-onset inflammatory rheumatic diseases characterized by the involvement of the joints as well as varying degrees of systemic symptoms (1). In countries around the Persian Gulf, the incidence rate of JIA has been mentioned to be in the lower range compared to that of the global estimate (less than 22 compared to up to 400 cases per 100000 worldwide) (2). JIA can be subcategorized into seven distinct subtypes with differences in their clinical patterns, diagnosis, and immunological aspects (3). Based on epidemiological studies, the largest share of the population of JIA patients is categorized into the oligoarticular subtype of JIA in Western countries (4).

Similar to other autoimmune disorders, evidence indicates that JIA is a complex polygenic disease with distinct genetic markers varying among its diverse subsets (5). Several studies have reported the role of specific *human leukocyte antigen* (HLA) alleles in developing various subtypes of JIA (4).

The major histocompatibility complex (MHC) is an indispensable feature of the immune system, encompassing more than 200 genes on chromosome 6. Accordingly, this chromosomal region includes genes encoding HLA, an extremely polymorphic locus (6, 7). However, the distribution of HLA alleles may vary among different races and ethnic groups, so the genetic effect of HLA on JIA development would also differ among diverse populations (8-10).

The significant loci within the HLA class II region (HLA-DR, -DQ, and -DP) encode α and β chains of the multiple HLA class II molecules and have been shown to confer a predisposition to many autoimmune diseases

(10-12). The HLA-DQB1 gene, which encodes the β chain of the HLA-DQB1 heterodimer, is highly polymorphic. Since antibodies primarily bind to the β-chain of the DQ molecule, they can be considered the main determinant of the DQ antigen (12, 13).

While well-documented associations have been discovered between HLA-DQB1 and some autoimmune diseases, several studies have proposed possible associations between HLA-DQB1 polymorphisms and JIA. Accordingly, in a previous study, HLA-DQB1* 04 was shown to be a susceptibility locus for oligoarticular JIA (14). In addition, systemic JIA was found to be linked with HLA-DQB1* 04:01 (15). In a study performed on patients with JIA in the UK, the DQB1*04 allele was reported to confer an increased risk of JIA (16).

HLA class II genes play essential roles in the susceptibility to JIA and even in the course and prognosis of this condition. Therefore, performing further studies on the susceptibility of patients with particular HLA allele polymorphisms to JIA could advance our knowledge regarding the genetic basis of this childhood disorder. Therefore, we carried out this study to compare the HLA-DQB1 allelic sequence variations between JIA patients and controls and also to investigate the contribution of this gene to the genetic susceptibility to JIA in a sample consisting of Iranian patients.

Methods

Patients and Controls

Sixty JIA *patients* under 16 years old referred to the Rheumatology Department of Mofid Hospital (affiliated with Shahid Beheshti University of Medical Sciences, Tehran, Iran) were enrolled in this study, of whom 36 subjects were female, and 24 were male, with a mean age of nine years old (ranging from 3 to 15 years old). A pediatric rheumatologist had confirmed the diagnosis of JIA in terms of the ILAR criteria. The cohort was limited to subjects diagnosed either with oligoarticular JIA (32 patients) or systemic-onset JIA (28 patients). Since the sampling location of this study, i.e., Mofid Children Hospital is known as a major tertiary referral center in the capital of Iran, the included patients were residents of different geographical areas of the country. This study has also included 50 randomly selected age-matched children with no autoimmune or hereditary disorders history.

DNA Extraction

Venous blood samples were collected from patients and controls, then transferred into anticoagulant tubes containing EDTA (17). The genomic DNA was extracted from the obtained peripheral blood samples through a modified salting-out procedure (18-20). Subsequently, the quality and the quantity of the extracted DNA samples were evaluated using a NanoDrop™ spectrophotometer and 1% agarose gel electrophoresis.

HLA-DQB1 Genotyping

Polymerase chain reaction with sequence-specific primers (PCR-SSP) was employed for the genotyping of HLA-DQB1 alleles, as it discriminates between completely-matched primers and those with mismatches introduced at the 3' terminal. A touchdown PCR protocol was exploited to enhance the specificity of primer-target binding in PCR-SSP (21, 22). Briefly, it was carried out on 100-150 ng genomic DNA with a final reaction volume of 20 µl. Specific primer sequences retrieved from a study by Olerup et al. (21) were used in eight PCR reactions to identify alleles of the DQ2,4-7 serotypes (Table 1). The cycling conditions were as follows: initial denaturation at 94 °C for 2 min, and 10 initial cycles (highly-specific binding) comprised of 94 °C for 10 sec, 65 °C for 60 sec, and then 20 cycles for the particular amplification process at 94 °C for 10 sec, 61 °C for 50 sec, and 72 °C for 30 sec. The PCR products were visualized using 2% agarose gel electrophoresis for amplification analysis (sample results are illustrated in Figure 1), followed by staining with cyber green.

Validation of Genotyping Results

Marginally higher than eight percent of the samples, i.e., five, were randomly selected, and sequencing was performed to confirm the obtained genotypes (ABI sequencer) (23). In order to determine the resulting subtypes with a high resolution, BLAST (Basic Local Alignment Search Tool) was used at https://www.ebi.ac.uk. Representatives of the genotypes are shown in Figure 2.

Statistical Analysis

The statistical analysis was performed using the SPSS

Table 1. Polymerase chain reaction primers used for amplification of HLA-DQB1

Subtypes	Length (bp)	Primer mixes (5′->3′)
DQB1*02:01	206	F:GTGCGTCTTGTGAGCAGAAG R:TGCAGGATCCCGCGGTACC
DQB1*(04:01-04:02)	211	F: GTGCTACTTCACCAACGGGACC R: CTGGTAGTTGTGTCTGCATACG
DQB1*(05:01-05:04)	216	F: GACGGAGCGCGTCCGGGG R: TGCAGGATCCCGCGGTACG
DQB1*(06:01-06:09)	218/219	F: GGGACGGAGCGCGTGCGTTA F: GGGACGGAGCGCGTGCGTCT R: CTGCAAGATCCCGCGGAACG R: TGCAGGATCCCGCGGTACC
DQB1*(03:01,03:04)	126	F: GGGACGGAGCGCGTGCGTTA R: CAGTACTCGGCGGCAGGCG R: CCAGTACTCGGCGTCAGGCG
DQB1 *(02:01,03:02,03:05)	132/147	F: GGGACGGAGCGCGTGCGTCT R: GTGCTACTTCACCAACGGGACC R: GCTGTTCCAGTACTCGGCGG
DQB1*(03:01,03:03)	123	F: GCCGCTGGGGCCGCCTGA R: TGCAAGGTCGTGCGGAGCT
DQB1 *(03:02,03:03,03:05)	125/140	F: GGGACGGAGCGCGTGCGTCT F: GTGCTACTTCACCAACGGGACC R: CAGTACTCGGCGTCAGGCG

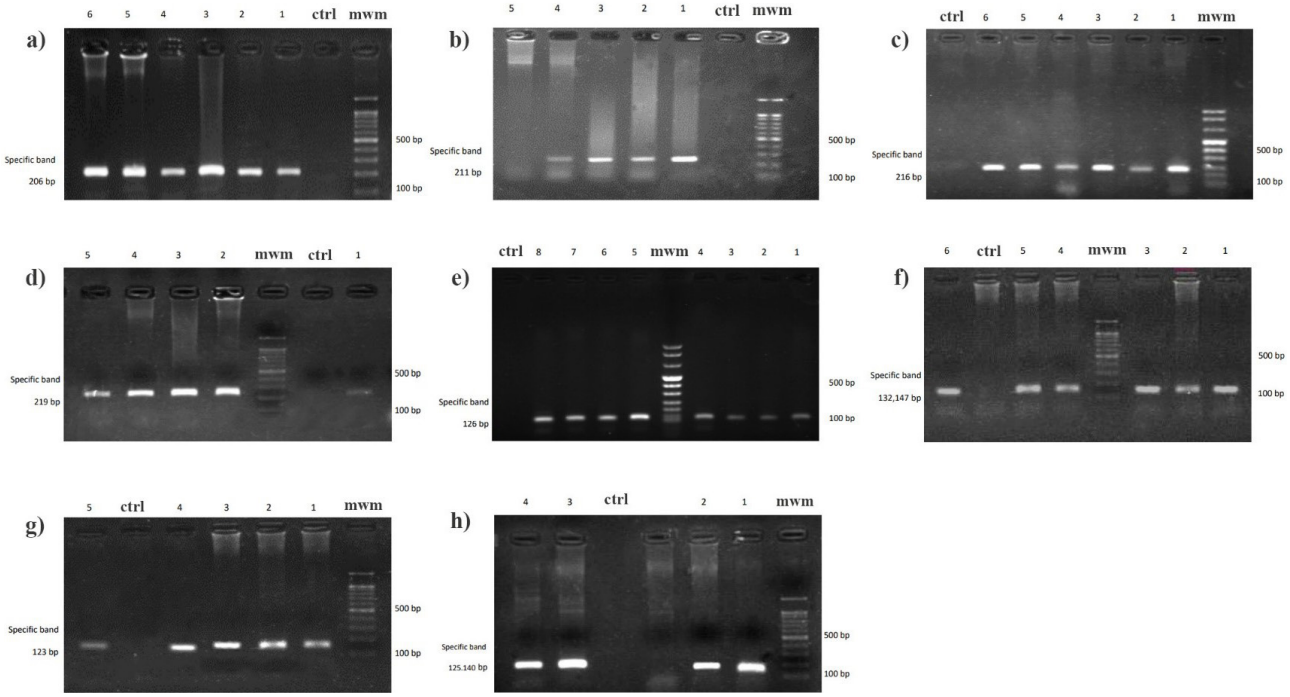

Figure 1. Gel electrophoresis result of genotyping of a) DQ2, b) DQ4, c) DQ5, d) DQ6, e) DQ7, f) DQ2/8, g) DQ7/9, and h) DQ8/9 alleles.

Figure 2. Representative of genotypes confirmed by sequencing. Results of the BLAST search were used to confirm that of PCR-SSP.

software package version 20 (SPSS, Chicago, IL, USA). The association between HLA-DQB1 alleles and JIA was analyzed using the chi-squared test. The odds ratio (OR) and its 95% confidence intervals (95% CI) were also calculated. P value < 0.05 were considered statistically significant. In order to account for multiple testing corrections and the associated bias for the relatively small sample size, Plink software was employed, and the empirical P value

was generated using the adaptive permutation procedure (EMP1). The maximum permutation number of 1000000 times was set for the analysis.

Ethics

The study was in accordance with the code of ethics of the World Medical Association (WMA). The Ethical Committee of Shahid Beheshti University of Medical Sciences approved the study's protocol. Prior to sampling, the objectives of this study were explained to the children's parents, and informed consent was obtained from them.

Results

Sixty JIA patients (36 female and 24 male) were included in this study. Of them, 32 patients had oligoarticular JIA, and 28 had systemic-onset JIA. Table 2 displays the frequency distribution of gender among JIA patients and the controls and their mean age values.

JIA Versus Control

Phenotypic differences among JIA patients, JIA subtypes, and controls are presented in Table 3. Moreover, among the tested alleles, in case of presence, a significant association of DQ2 (*02:01) ($P = 0.028$; OR = 1.909, CI = 1.067 - 3.415) and DQ7 (*03:01, *03:04) ($P = 0.046$; OR = 1.778, CI = 1.009 - 3.131) alleles was found with JIA. The empirical *P* values obtained from the adaptive permutation procedure were 0.048 and 0.063 for DQ2 and DQ7, respectively. None of the tested alleles demonstrated protective effects.

Oligoarticular JIA

The presence frequencies of DQ7 (*03:01, *03:04) (EMP1 < 0.001, $P < 0.001$; OR = 2.901, CI = 1.633 to 5.151) and DQ5 (*05:01- *05:04) (EMP1 = 0.024, $P = 0.030$; OR = 1.884, CI = 1.060 to 3.349) alleles were significantly higher in patients with oligoarticular-JIA compared to the controls.

Table 2. Gender distribution and the mean age of subjects in the studied groups

	Female No.	Female Age	Male No.	Male Age
All JIA	36	6.5	24	6.5
Oligoarticular JIA	21	6	11	6.4
Systemic-onset JIA	15	7	13	6.7
Control	30	8.5	20	8

Systemic-Onset JIA

The DQ2 (*02:01) subtype was more frequent in systemic-onset JIA patients in comparison to the control group (EMP1 < 0.001, $P < 0.001$; OR = 3.093; CI = 1.727 to 5.540).

Discussion

As an immune system disorder, JIA is majorly influenced by the patient's genetic background (24). In this regard, the evaluation and clarification of the genes associated with JIA would shed light on the ways of controlling and preventing this disorder. Several genes, including HLA and non-HLA ones, are involved in the onset and progression of JIA and the increased susceptibility of individuals to the disease (25). HLA proteins are known to have more significant associations with JIA compared to non-HLA factors. Accordingly, the role of HLA class II in the development of JIA has been extensively investigated so far. HLA-DQ, HLA-DP, and HLA-DR molecules are encoded by a cluster of highly polymorphic genes, showing relatively low recombination levels (26-28). Correspondingly, this characteristic of HLA proteins consequently results in higher rates of preferential linkage, which more often occur between HLA-DRB1 and HLA-DQB1 loci. Ultimately, they lead to the systematic development of the common MHC haplotypes (12, 13). Based on our findings, two HLA-DQB1 alleles (DQ2 (*02:01) and DQ7 (*03:01, *03:04)) had significant associations with JIA. However, it is essential to mention that the empirical *P* value of DQ7 was higher than the 0.05 cutoff threshold, implying the need for further research with sufficient JIA cases. Furthermore, some alleles showed susceptibility to the subtypes of JIA (i.e., oligoarticular and systemic-onset JIA forms).

In a study performed in Britain, a statistically significant association was found between the expression of DQB1*04 alleles and oligoarticular JIA. Thereafter, it was

Table 3. Frequency of HLA-DQB1 alleles in the juvenile idiopathic arthritis patients, JIA subtypes, and control group

S	Alleles	All JIA patients				OA-JIA				SO-JIA				Control Group
		N. (%)	*P*	OR	95% CI	N. (%)	*P*	OR	95% CI	N. (%)	*P*	OR	95% CI	N. (%)
DQ2	*02:01	27(45)	**0.028**[#]	1.909	1.067-3.415	11(34.4)	0.544	1.202	0.663-2.179	16(57)	**<0.001**[#]	3.093	1.727-5.540	15(30)
DQ4	*04:01-*04:02	4(6.6)	0.774	1.179	0.382-3.641	3(9.4)	0.421	1.549	0.530-4.528	1(3.6)	0.516	0.653	0.178-2.387	3(6)
DQ5	*05:01-*05:04	27(45)	0.059	1.739	0.977-3.093	15(46.8)	**0.030**[#]	1.884	1.060-3.349	12(42.8)	0.108	1.603	0.900-2.855	16(32)
DQ6	*06:01-*06:09	13(21.6)	0.327	0.725	0.381-1.381	6(19)	0.133	0.603	0.311-1.171	7(25)	0.631	0.857	0.457-1.607	14(28)
DQ7	*03:01-*03:04	30(50)	**0.046**[#]	1.778	1.009-3.131	20(62.5)	**<0.001**[#]	2.901	1.633-5.151	10(35.7)	1	1	0.561-1.782	18(36)
DQ8	*03:02-*03:05	7(11.6)	1	1	0.426-2.347	3(9.4)	0.489	0.725	0.291-1.806	4(14.3)	0.674	1.194	0.522-2.728	6(12)
DQ9	*03:03	4(6.6)	0.778	0.866	0.302-2.485	2(6.2)	0.579	0.734	0.245-2.198	2(7.1)	0.788	0.866	0.302-2.485	4(8)

Abbreviations: S, Serotype; N, Number; *P*, *P*-value; OR, Odds ratio; CI, Confidence interval; JIA, Juvenile idiopathic arthritis; OA, Oligoarticular; SO, Systemic onset. [#]$P < 0.05$ was considered statistically significant.

demonstrated that DQB1*03 and DQB1*06 haplotypes and specific alleles obtained from DRB1 and DQA1 loci might aggravate the oligoarticular and systemic subtypes of JIA. In contrast, it was shown that a particular haplotype of DQ2 diminishes the risk of oligoarticular JIA development (16).

Similar studies further confirmed the contribution of specific HLA haplotypes to the genetic susceptibility to Juvenile idiopathic arthritis. A previous study on the Finnish population reported a correlation between the DQB1*04:02 allele and JIA (29). Alsaeid et al. have also demonstrated the association of DQB1*03:04 and DQB1*05:01 alleles with oligoarticular JIA (30). Similarly, in a study on the American population, a strong association was reported between HLA-DR alleles and JIA (10).

The present study was a novel investigation of the association between HLA-DQB1 and JIA in Iranian patients. DQ2 (*02:01) and DQ7 (*03:01, *03:04) are the two most common alleles among JIA patients, regardless of their disease subtypes. Moreover, it was indicated that the oligoarticular subtype had a strong association with DQ7 and, to a lesser extent, with DQ5 (*05:01-*05:04) allele. Furthermore, it was observed that patients with systemic-onset JIA had a higher rate of DQ2 allele expression. DQ9, DQ8, DQ6, and DQ4 alleles were also evaluated in this study, but no significant alterations were found in JIA patients compared to the controls.

The results of our study are in agreement with those reported by Alsaeid et al. (30). Indeed, in both Kuwaiti and Iranian patients with oligoarticular JIA, some associations were observed among DQ7 and DQ5 alleles and JIA. Moreover, the substantial contribution of DQ2 (*02:01) in systemic-onset JIA was in accordance with the results of a study performed in the UK (16). To the best of our knowledge, this was the only study describing an association between HLA-DQB1 haplotypes and systemic JIA. It is noteworthy that we found DQ4 (*04:02) as one of the rarest alleles in both patient and control groups, in contrast to the American, British, and Finnish populations.

Conclusion

In conclusion, allelic polymorphisms in the HLA-DQB1 loci could confer genetic susceptibility to JIA and its subtypes in Iranian children. Understanding the interaction between distinct HLA alleles and JIA would be helpful for further investigating the severity of JIA and the assessment of its drug-response status. The reported HLA alleles associated with oligoarticular and systemic-onset JIA will also have diagnostic implications for the correct classification of JIA subtypes if studies performed in other populations report consistent data. Finally, the reported association between HLA-DQB1 and JIA would be applicable for developing more accurate diagnostic, preventive, and therapeutic approaches.

Acknowledgments

The authors highly appreciate the Rheumatology division of Mofid Children's Hospital for providing samples and also the blood donors and their parents for participating in this study.

Authors' Contribution

Conceptualization: Shirin Farivar.
Data curation: Mahsa Kazerani.
Formal analysis: Reza Shiari.
Investigation: Shirin Farivar, Reza Shiari.
Methodology: Mahsa Kazerani, Shirin Farivar.
Project administration: Shirin Farivar.
Resources: Shirin Farivar.
Software: Mahsa Kazerani, Shervin Afzali, Shirin Farivar.
Supervision: Shirin Farivar.
Validation: Reza Sinaei.
Visualization: Shirin Farivar.
Writing–original draft: Mahsa Kazerani, Reza Shiari, Shervin Afzali.

Competing Interests

The authors declare that they do not have any conflict of interest.

Ethical Approval

This study was approved by the ethics commity of Kerman university of medical sceiences.(IR.KMU.REC.1390.535).

Funding

This research received no specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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