

Rheumatoid Arthritis Susceptibility Is Associated with the *KIR2DS4-Full* of Killer-Cell Immunoglobulin-Like Receptor Genes in the Lur Population of Iran

Bijan Ansari-Moghaddam¹, Ali Asghar Kiani¹, Ali Sheikhian¹, Mehdi Birjandi², Seyyed Amir Yasin Ahmadi³, Nazanin Mousavi³, Hamzeh Ali Torang⁴, Farhad Shahsavar*¹

Abstract

Background: The pathophysiology underlying the progression and development of autoimmune conditions, such as Rheumatoid Arthritis (RA), is a result of dysregulations of the immune system. Research has explored the genetic alterations present in RA; however, limited studies have examined the role of Killer cell Immunoglobulin-like Receptors (*KIR*) and Human Leukocyte Antigen (*HLA*) molecules in RA. Therefore, the aim of this study was to examine *KIR* genes, their *HLA* ligands, and *KIR-HLA* compounds in patients with RA.

Methods: In this case-control study, a total of 50 patients with RA and 100 healthy individuals were enrolled. DNA samples were evaluated using PCR with sequence specific Primers (PCR-SSP). Odds ratio (OR) with a 95% confidence interval (CI) were reported.

Results: Among the *KIR* genes examined, *KIR2DLA* ($p=0.0255$, OR= 0.389, 95% CI= 0.210-0.722) and *KIR2DS4-full* ($p<0.0001$, OR= 6.163, 95% CI= 3.174-11.968) were observed to have a statistically significant correlation with disease susceptibility to RA. As an inhibitory gene, *KIR2DLA* was observed to have a protective effect against RA while *KIR2DS4-full* as an activating gene, was found to increase risk for RA. No significant associations were found between any of the other *KIR* genotypes, *HLA* ligands, or *KIR-HLA* compounds examined in this study to RA susceptibility.

Conclusions: In this study of RA in the Lur population of Iran, *KIR2DS4-full* was observed to increase susceptibility to RA, while *KIR2DL5A* was found to act as a protecting factor based on both the cross Table and regression analyses. Further research should focus on repeating this study in additional populations.

Keywords: *HLA*, *KIR*, NK cells, Rheumatoid Arthritis.

Introduction

Rheumatoid Arthritis (RA) is a common chronic autoimmune disorder characterized by synovitis, chronic hyperplasia, bone erosion, progressive joint damage, irreversible loss of bone shape and mobility problems (1, 2). It is considered a systemic autoimmune disease with

multiorgan involvement (3). An estimated 0.5-1 percent of the world's population are affected by RA (4). The prevalence of RA depends on age and gender, occurring three times more often in women than in men (5).

1: Department of Immunology, Lorestan University of Medical Sciences, Khorramabad, Iran.

2: Department of Epidemiology and Biostatistics, Lorestan University of Medical Sciences, Khorramabad, Iran.

3: Student Research Committee, Lorestan University of Medical Sciences, Khorramabad, Iran.

4: Department of Internal Medicine, Lorestan University of Medical Sciences, Khorramabad, Iran.

*Corresponding author: Farhad Shahsavar; Tel: +98 09369158091; E-mail: shahsavarfarhad@yahoo.com.

Received: 21 Nov, 2020; Accepted: 13 Dec, 2020

A growing body of literature has found a range of genetic and environmental factors contributing to the incidence of RA including, cigarette smoke, certain ethnicities, age, epigenetic factors, and immunological factors (6). It has been shown that approximately 60% of the genetic contribution to RA susceptibility is present in populations of different ethnicities (2). Human leukocyte antigen (*HLA*)-DR appears to hold the most significant role among the potential predisposing genetic factors for RA as the example of a gene cluster associated with ethnicities. Some of the most important genes involved in RA have been found to be *PTPN22*, *PADI4*, *EGFR*, *MHTFR*, *IL-23R*, *CD226*, *HLA-DR*, *STST4*, *TRAF1/C5*, *CCR6*, *HLA-G*, *BACH2*, etc. (7). Among the different genes, *HLA-DRB1* has the strongest relationship with RA with a sequence of specific amino acids in areas 70-74 of the third variable region called the Shared Epitope (8). Other mechanisms involved in the development and progression of RA include molecular imitation by certain microbial peptides and the activation of inflammatory pathways by T-cell antigen detection and the innate immune response. Among the proposed factors involved in RA, natural killer (NK) cells have been observed to have an important role in disease development and pathogenesis (9).

As a critical part of the immune system, NK cells originate from bone marrow precursors. They have a critical role in the first line of defense against pathogens and inhibiting tumor growth. Two major NK cell subsets exist: CD16⁺CD56^{dim} with high killing ability and CD16⁻CD56^{bright} with high cytokine secretion (10, 11). A sharp increase in the CD56^{bright} subtype has been observed in the synovial fluid of patients with RA (12). The activity of NK cells is regulated by cell surface receptors, which can exhibit either inhibitory or activating functions. Two families of NK cells receptors include the immunoglobulin superfamily and the large family of C-type lectins located on chromosomes 12 and 19, respectively (13, 14).

The immunoglobulin superfamily has several members including the Killer cell Immunoglobulin-like Receptors (*KIR*), Natural

Cytotoxicity Receptors (NCR), and the Fc epsilon receptor (FCεR). The *KIR* holds a key role in the ability of NK cells to recognize self from non-self-ligands (15-20). These receptors are regulatory molecules found on the surface of NK cells and some T cell subgroups, including *TCD8+* and *TCD4CD28 null*. They are located on the 19q13.4 chromosome in the LRC locus (21, 22), which includes a centromeric region and a telomeric region separated by *KIR2DL4* in all haplotypes. This cluster is surrounded by *KIR3DL2* at the end of the telomere and *KIR3DL3* at the end of the centromere (23). The vast diversity of *KIR* gene loci is a result of gene duplication (24), non-allelic homologous recombination, diverse alleles due to the exchange of amino acids in the polymorphic region, nucleotide mutations, and haplotype variety. These variations create a range of genotypes within the population. This polymorphism of the *KIR* gene makes it such that it is extremely rare for two unrelated people have the same *KIR* genes. Some of these *KIR* variants contribute to the pathogenesis of autoimmune disorders (25, 26). Thus far, fourteen *KIR* genes and two pseudo genes have been identified in humans including: seven inhibitory genes, six activating genes, and one gene with both inhibitory and activating functions (*KIR2DL4*). The genes are named based on the structure of the molecules they encode for (27). After binding to their respective ligands, inhibitory *KIRs* (*iKIRs*) signal through their Immunoreceptor Tyrosine-based Inhibition Motifs (*ITIMs*) in their cytoplasmic sequence. Activating *KIRs* (*aKIR*) do not carry any signal motifs. They function through the use of membrane-bound signaling adaptor proteins, *DAP10* and *DAP12* (28). After binding to the receptor through the immunoreceptor tyrosine-based activation motif (ITAM), these molecules trigger an activation signal (29). The most common *KIR* haplotype in humans is relatively simple and carries five inhibitory genes (*KIR2DL1*, *KIR2DL3*, *KIR3DL1*, *KIR3DL2*, *KIR3DL3*), one activating gene (*KIR2DS4*), one gene with both activating and inhibitory functions (*KIR2DL4*), and two pseudo genes, commonly

known as the group A *KIR* haplotype (30). The *KIR-HLA* interactions are involved in the pathogenesis of many diseases, including both autoimmune and inflammatory diseases (31, 32). Both *KIR* and *HLA* clusters have a high diversity among different populations that this variation is attractive in bioinformatics and anthropology (33-35).

Previous studies exploring the relationship between *KIR* and RA have conflicting and ambiguous results. Due to the inconsistent findings, the limited number of studies examining *KIR-HLA* and RA, and the diverse range of ethnicities in Iran, the aim of this study was to explore the relationship between *KIR* and their respective *HLA* ligands in RA susceptibility among the Lur population of Iran.

Materials and Methods

Design and patients

In this case-control study, a total of 50 patients with RA (35 women and 15 men) were enrolled. Participant demographics included an average age of 46.5 years, in which the mean age of women was 44 years and men was 52.4 years. Patients with RA were initially diagnosed according to the criteria of the American Rheumatology Association and referred to the hospitals of Shahid Rahimi and Shohadaye Ashayer of Khorramabad, Lorestan Province, Iran. Exclusion criteria included patients who were not of Lur heritage and those with additional comorbidities, such as malignancies. Genomic DNA of the participants was extracted from the white blood cells of peripheral blood samples. A total of 100 healthy controls were selected from a previous study by Shahsavar *et al.* (36). All controls were of Lur heritage and have lived in Lorestan.

KIR genotyping

The *KIR*-TYPE kit (BAG Health Care GmbH, Germany) was used to determine the expression of *KIR* genes using polymerase chain reaction technology and sequence-specific primers (PCR-

SSP). Our PCR products included 20 *KIR* genes, *2DL1*, *2DL2*, *2DL3*, *2DL4*, *2DL5* (with two form *2DL5A* and *2DL5B*), *2DS1*, *2DS2*, *2DS3*, *2DS4-full*, *2DS4-del*, *2DS4-del(*008)*, *2DS5*, *3DL1*, *3DL2*, *3DL3*, *3DS1*, *2DP1*, *3DP1-full*, and *3DP1-del* for patients with RA and healthy controls according to the kit's brochure. In order to prepare the mixture of reaction, 180 µl of molecular grade water, 26 µl of master mix (10 x PCR buffer) and 2.1 µl of Taq DNA polymerase enzyme (500 U) was transferred to a 2 ml microtube. Then, 10 µl of the prepared mixture was transferred to strip number 1 (negative control). Next, 52 µl of DNA (25-40 ng/µl) was added to the solution prepared in the microtube. After a few seconds of microfusion, 10 µl of the prepared mixture was transferred to strip numbers 2-22.

HLA genotyping

The EPITOP-TYPE kit (BAG Health Care GmbH, Germany) based on the PCR-SSP method was used to determine the presence of *HLA* alleles. In this study, we evaluated 5 *HLA*-ligand alleles including *HLA-C1*, *HLA-C2*, *HLA-Bw4T*, *HLA-Bw4I*, and *HLA-Bw4A* according to the kit brochure. In order to prepare the mixture of reaction, 55 µl of ordinary grade water, 8 µl of master mix (10 x buffer) and 0.64 µl of Taq DNA polymerase enzyme (500 U) was transferred to a 2 ml microtube. Then, 10 µl of the prepared mixture was transferred to strip number 6 (negative control). Next, 16 µl of DNA (25-40 ng/µl) was added to the solution prepared in the microtube (first stage). After a few seconds of microfusion, 10 µl of the prepared mixture was transferred to strip numbers 1-5.

Gel electrophoresis

Following PCR, gel electrophoresis was performed using horizontal electrophoresis and immersion of the gel in 1 X TBE buffer and Erythrogel dye solution. The results were reviewed and recorded using the GelDoc device (Fig. 1).



Fig. 1. Gel electrophoresis of the PCR products. A) *KIR* genes including 1: 2DL1, 2: 2DL2, 3: 2DL3, 4: 2DL4, 5: 2DL5A, 6: 2DL5B, 7: 2DS1, 8: 2DS2, 9: 2DS3, 10: 2DS4-full, 11: 2DS4-del, 12: 2DS4-del(008), 13: 2DS5, 14: 3DL1, 15: 3DL2, 16: 3DL3, 17: 3DS1, 18: 2DP1, 19: 3DP1-full, 20: 3DP1-del. B) *HLA* genes including 1: C1, 2: C2, 3: Bw4T, 4: Bw4I, 5: A Bw4.

Statistical analysis

Chi-square test with Yate's correction and multivariable logistic regression were used to analyze the data. After calculating the odds ratio (OR) with 95% confidence interval (CI), the results were reported at a significance level of 0.05.

Ethical considerations

Approval of the methods for this study was provided by the ethics committee of Lorestan University of Medical Sciences (registration number IR.LUMS.REC.1396.375). Informed consent was obtained from each participant prior to taking part in the study.

Results

In our study population, a total of 22 genotypes were observed in the case and control groups. As expected, most of the participants had the AA genotype (cAcA-tAtA) (28% of the cases and 29% of the controls). No significant differences were observed among the genotypes (Table 1).

Our findings show that the inhibitor gene, *KIR2DL5A* ($p = 0.0255$, OR = 0.389, 95% CI = 0.210-0.722), and the activating gene, *KIR2DS4-full* ($p < 0.0001$, OR = 6.163, 95%

CI = 3.174-11.968), had a significant association with RA susceptibility. No significant relationship was observed between other *KIR* genes and susceptibility to RA. After Bonferroni correction, only the relationship between *KIR2DS4-full* and increased risk for RA remained statistically significant (Table 2). For the examination of the *HLA* ligand alleles, our findings showed no significant relationship between any of the examined *HLA* alleles and RA susceptibility (Table 3). No significant differences were found for any of the examined combinations of *HLA-KIR* (Table 4). Haplotypes of *KIR* were studied according to previously reported methods (37). Protection against RA was found to only be associated with the centromeric A haplotype, however, after Yate's correction, this difference did not remain statistically significant (Table 5). The mean of B score (the count of B haplotype in a genotype from 0 to 4) was observed to be higher in the group with RA (1.40 vs 1.17), although this was not statistically significant ($p = 0.201$, independent t test) (Table not shown).

Multivariable logistic regression was also used to reach the adjusted effects of *KIR* genes on disease susceptibility without confounding

effects of other *KIR* genes. Our findings show that *KIR2DS4-full* exhibited the strongest risk association with RA ($p < 0.001$, adjusted OR= 18.531, 95% CI= 5.453-62.972) followed by *KIR2DS4-del* ($p = 0.034$, adjusted OR= 3.894, 95% CI= 1.107-13.690). *KIR3DL1* showed the strongest protecting association with RA ($p = 0.037$, adjusted OR= 0.073, 95% CI= 0.006-

0.850) followed by *KIR2DL3* ($p = 0.013$, adjusted OR= 0.136, 95% CI= 0.028-0.657) and *KIR2DL5A* ($p = 0.021$, adjusted OR= 0.195, 95% CI= 0.049-0.783). After Bonferroni correction, only the association of *KIR2DS4-full* and increased risk for RA remained significant (Table 6 and Fig. 2).

Table 1. Genotypes of *KIR* among the participants.

Genotype group	Genotype number	Genotype	A Score	B score	KIR genes																Frequency in Controls N (%)	Frequency in Cases N (%)	p Value	
					Inhibitory								Activating					Pseudo						
					1D12	2D12	2D13	2D14	2D15	3D11	3D12	3D13	2DS1	2DS2	2DS3	2DS4	2DS5	3DS1	2DP1	3DP1				Inhibitory
AA	1	cAcA-tAtA	4	0														6	1	29	14(28)	0.8984		
BX	2	cAcA-tAtB	2	1														7	4	12	4(8)	0.6401		
	3	cAcB-tAtA	3	0														7	2	10	5(10)	1		
	4	cAcB-tAtA	3	1														8	3	7	2(4)	0.7154		
	5	cAcB-tAtB	2	2														8	6	7	2(4)	0.7154		
	6	cAcB-tAtB	2	1														8	5	7	4(8)	0.8247		
	7	cAcB-tAtB	2	1														8	5	6	2(4)	0.4651		
	8	cBcB-tAtA	1	1														7	3	4	4(8)	0.5206		
	9	cBcB-tAtB	1	2														7	6	3	3(6)	0.6585		
	10	cAcB-tAtB	2	0														8	0	2	0(0)	0.8013		
	11	cBcB-tAtA	1	0														6	4	2	0(0)	0.8013		
	12	cAcB-tBtB	1	3														7	5	1	0(0)	0.478		
	13	cBcB-tBtB	0	3														6	5	1	3(6)	0.2097		
	14	cAcA-tBtB	1	2														6	3	1	0(0)	0.478		
	15	cAcB-tBtB	1	2														7	4	1	1(2)	0.6147		
	16	cAcB-tAtB	2	1														8	4	1	1(2)	0.6147		
	17	cAcA-tAtB	2	0														7	4	1	0(0)	0.478		
	18	cAcA-tAtB	2	1														7	5	1	1(2)	0.6147		
	19	cAcA-tAtB	2	1														7	5	1	0(0)	0.478		
	20	cAcA-tAtB	2	0														7	3	1	1(2)	0.6147		
	21	cBcB-tAtB	1	1														7	5	1	1(2)	0.6147		
	22	cBcB-tAtA	1	0														7	4	1	2(4)	0.5362		

Role of *KIR* Gene In Rheumatoid Arthritis

Table 2. Distribution of *KIR* genes and their associations with RA.

<i>KIR</i> gene	Frequency in cases	Frequency in controls	Odds ratio	95% CI	p value ¹
2DL1	50 (100%)	98 (98%)	5.101	0.242-107.624	0.8013
2DL2	31 (62%)	54 (54%)	1.389	0.791-2.442	0.4489
2DL3	37 (74%)	88 (88%)	0.388	0.183-0.822	0.0528
2DL4	50 (100%)	100 (100%)	1	0.019-50.893	1
2DL5	30 (60%)	61 (61%)	0.959	0.544-1.691	0.9059
2DL5A	11 (22%)	42 (42%)	0.389	0.210-0.722	0.0255*
2DL5B	23 (46%)	31 (31%)	1.896	1.064-3.380	0.1044
3DL1	47 (94%)	96 (96%)	0.653	0.178-2.387	0.8911
3DL2	50 (100%)	100 (100%)	1	0.019-50.893	1
3DL3	50 (100%)	100 (100%)	1	0.019-50.893	1
2DS1	23 (46%)	48 (48%)	0.923	0.529-1.608	0.9539
2DS2	31 (62%)	55 (55%)	1.334	0.759-2.346	0.5209
2DS3	19 (38%)	34 (34%)	1.19	0.667-2.121	0.7627
2DS4	46 (92%)	96 (96%)	0.479	0.139-1.646	0.5206
2DS4full	42 (84%)	46 (46%)	6.163	3.174-11.968	0.0001**
2DS4del	38 (76%)	72 (72%)	1.231	0.654-2.320	0.7441
2DS5	20 (40%)	40 (40%)	1	0.568-1.761	1
3DS1	23 (46%)	45 (45%)	1.041	0.597-1.817	0.9077
2DP1	50 (100%)	98 (98%)	5.101	0.242-107.624	0.8013
3DP1	50 (100%)	100 (100%)	1	0.019-50.893	1
3DP1full	25 (50%)	39 (39%)	1.564	0.892-2.742	0.2674
3DP1del	48 (96%)	90 (90%)	2.667	0.807-8.806	0.3382

Chi-square with Yate's correction. * Significant at 0.05. ** Significant at 0.0023 (Bonferroni correction).

Table 3. Distribution of *HLA* ligand alleles and their associations with RA.

<i>HLA</i> ligand	Frequency in cases	Frequency in controls	Odds ratio	95% CI	p value ¹
HLA-C1	41 (82%)	75 (75%)	1.518	0.768-3.000	0.4482
HLA-C2	33 (66%)	71 (71%)	0.793	0.436-1.1440	0.6612
HLA-BW4	39 (78%)	73 (73%)	1.311	0.682-2.504	0.6422
HLA-BW4 T	8 (16%)	9 (9%)	1.926	0.808-4.592	0.3165
HLA-BW4 I	25 (50%)	51 (51%)	0.961	0.552-1.673	0.9081
HLA-A-BW4	18 (36%)	33 (33%)	1.142	0.637-2.047	0.9665

Chi-square with Yate's correction.

Table 4. Distribution of *HLA-KIR* combinations and their associations with RA.

<i>HLA-KIR</i> combination	Frequency in cases	Frequency in controls	Odds ratio	95% CI	p value ¹
<i>HLA-C1</i> and <i>KIR2DL2/3</i>	41 (82%)	75 (75%)	1.518	0.768-3.003	0.4482
<i>HLA-C2</i> and <i>KIR2DL1</i>	33 (66%)	69 (69%)	0.872	0.482-1.577	0.8527
<i>HLA-Bw4</i> and <i>KIR3DL1</i>	40 (80%)	69 (69%)	1.797	0.940-3.435	0.2184
<i>HLA-C1</i> and <i>KIR2DS2</i>	28 (56%)	43 (43%)	1.687	0.964-2.951	0.1836
<i>HLA-C2</i> and <i>KIR2DS1</i>	14 (28%)	36 (36%)	0.691	0.380-1.257	0.4260
<i>HLA-Bw4</i> and <i>KIR3DS1</i>	18 (36%)	35 (35%)	1.044	0.585-1.864	0.9039

Chi-square with Yate's correction.

Table 5. Distribution of *KIR* haplotypes and their associations with RA.

Haplotype	Included genes	Frequency in cases	Frequency in controls	Odds ratio	95% CI	p-value	p-value (Yate's correction)
Centromeric A	<i>2DL3</i> <i>2DL1</i>	37 (74%)	88 (88%)	0.388	0.183-0.822	0.0301*	0.0528
Centromeric B	<i>2DS2</i> <i>2DL2</i> <i>2DS3</i> <i>2DL5</i> <i>2DL1</i>	30 (60%)	54 (54%)	1.278	0.729-2.239	0.4853	0.6007
Telomeric A	<i>3DL1</i> <i>2DS4</i>	46 (92%)	96 (96%)	0.479	0.139-1.646	0.3041	0.5206
Telomeric B	<i>3DS1</i> <i>2DL5</i> <i>3DS3</i> <i>2DS1</i> <i>2DS5</i>	24 (48%)	45 (45%)	1.128	0.647-1.967	0.7282	0.8621

*Significant at 0.05.

Table 6. Prediction of RA through complete profile of *KIR* using multivariable logistic regression.

<i>KIR</i> genes	Adjusted odds ratio	95% CI	SE	Z	p value	-log(p)
<i>KIR2DL4</i>	NA					
<i>KIR3DL2</i>	NA					
<i>KIR3DL3</i>	NA					
<i>KIR2DP1</i>	NA					
<i>KIR2DL1</i>	NA					
<i>KIR2DL2</i>	5.284	0.292-95.64	7.807	1.13	0.260	0.585202
<i>KIR2DL3</i>	0.136	0.028-0.657	0.109	-2.48	0.013*	1.884818
<i>KIR2DL5A</i>	0.195	0.049-0.783	0.138	-2.31	0.021*	1.674194
<i>KIR2DL5B</i>	0.83	0.311-2.212	0.415	-0.37	0.709	0.149176
<i>KIR3DL1</i>	0.073	0.006-0.850	0.091	-2.09	0.037*	1.435487
<i>KIR2DS1</i>	0.696	0.041-11.707	1.002	-0.25	0.801	0.096277
<i>KIR2DS2</i>	0.301	0.016-5.798	0.454	-0.8	0.426	0.370124
<i>KIR2DS3</i>	0.593	0.116-3.021	0.492	-0.63	0.529	0.276502
<i>KIR2DS4full</i>	18.531	5.453-62.972	11.565	4.68	<0.001***	5.537358
<i>KIR2DS4del</i>	3.894	1.107-13.69	2.498	2.12	0.034*	1.467455
<i>KIR2DS5</i>	0.91	0.116-7.131	0.956	-0.09	0.929	0.032058
<i>KIR3DS1</i>	6.873	0.625-75.614	8.409	1.58	0.115	0.938657
<i>KIR3DP1full</i>	1.575	0.621-3.991	0.747	0.96	0.338	0.470567
<i>KIR3DP1del</i>	1.343	0.173-10.412	1.404	0.28	0.778	0.109256

Nagelkerke R square= 0.429. NA: not applicable because of collinearity. * Significant at 0.05.

*** Significant at 0.001.

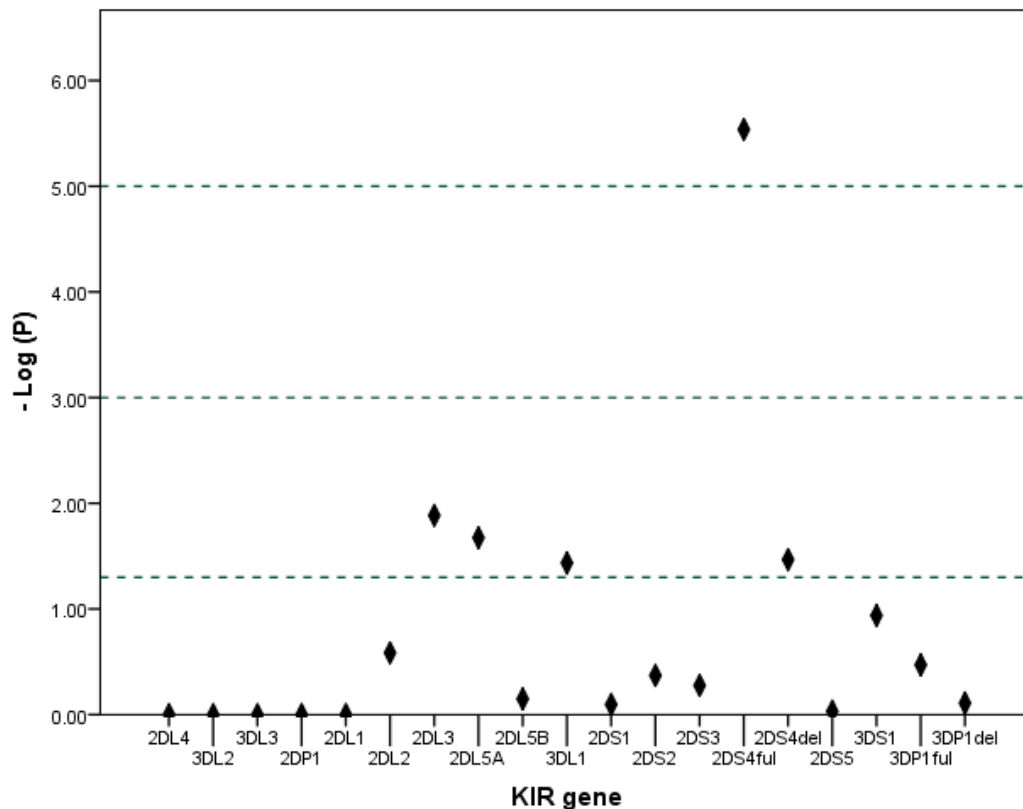


Fig. 2. Manhattan plot of the logistic regression model showing $-\log_{10}$ of P value for each gene. The reference lines indicate significance levels 0.05, 0.001 and 0.00001.

Discussion

This genetic association study was designed to determine the role of *KIR* genes in susceptibility to RA in the Lur population of Iran. Autoimmune diseases result from the failure of immune tolerance mechanisms to prevent reactions to self-antigens or unregulated activity of the immune system, both leading to tissue damage (38). NK cells detect class I *HLA* through their *KIR* cell surface receptors which, upon binding, can have different effects on the immune system in different individuals due to *KIR* gene polymorphism. RA is one of the most common autoimmune diseases in which overactive NK cells play a major part in the susceptibility and pathogenesis of RA. However, the exact role of NK cells and whether they are beneficial or harmful in disease progression remains controversial (39).

In the present study, *KIR2DL3* was observed to be less prevalent in the patient group and, before Yate's correction, have a statistically significant protective relationship with RA decreasing susceptibility to RA. Although the

frequency of *KIR2DL5* was the same in both the control and patient groups, one of its subtypes, *KIR2DL5A*, was significantly lower in the patient group, and statistically, it had a significant protective affect against the development of RA. Among the activating genes, despite the similar presence of *KIR2DS4* in both the patient and control groups, one of its subtypes, *KIR2DS4-full*, had a much higher frequency in the patient group than the control group. This finding indicates a significant relationship between *KIR2DS4-full* and increased risk for RA. The results of our study suggest that a reduction in the frequency of *KIR2DL3* and *KIR2DL5A* genes and an increase in the frequency of *KIR2DS4-full* may predispose an individual to RA. Our logistic regression model showed a similar result.

In a study by Yen et al. (2001) in Finland, they observed an increase in the activating genes, *KIR2DS2* and *KIR2DS4-full*, in patients with RA, corroborating our findings (40). Nevertheless, Kogure et al. (2007) found no

correlation between the expression of *KIR* and disease activity when examining RA in Japanese patients (41). The results of our study were consistent with findings from Yen et al. (2006) in which they observed *KIR2DS4* to be a risk factor for RA (42). Our findings were also consistent with a meta-analysis by Xiaona Li et al. (2015) about the protective effect of *KIR2DL3* (43). In an Iranian case-control study conducted by Nazari et al. (2015), *KIR2DL5A*, *KIR2DL5B*, and *KIR2DL2*, as well as the activating *KIRs*, *KIR2DS5* and *KIR3DS1*, were found to have a protective effect against RA (9). A meta-analysis conducted by Aghaei et al. (2019) found that there were significant relationships between *KIR2DL3*, *KIR2DL5*, *KIR2DS5*, and *KIR3DL3* and a reduced risk for RA. However, the findings among the different studies were not entirely consistent (2).

With respect to the *KIR* haplotypes, even though the centromeric A haplotype was more abundant, its association with RA did not remain statistically significant following Yate's correction. Regarding *HLA*, our study did not show a significant relationship between any of the examined *HLA* ligands and RA. In previous research studies, Nishimura et al. (2015) in Brazil, observed an association between the presence of *HLA-DRB1*1402* and *HLA-DRB1*0101* and cutaneous ulcers in patients with arthritis vasculitis (44). Regarding *KIR-HLA* compounds, although our study found *HLA-C1* and *KIR2DS2* to be more common in patients with RA, which was consistent with the results of a study by Mc Geough et al. in 2010 in Northern Ireland (45), no statistically significant correlation was observed between these genes and the degree of RA risk in our study. This discrepancy could be due to differences in the type of the study and the type of ethnicity examined. Among different ethnicities, some genes have both haplotypes and genotypes together, resulting in the effects of single genes being hidden under one another. In this study, we overcame this limitation with multiple logistic regression. With respect to the

KIR2DS4-full gene after adjustment, the OR almost tripled. In our study, the *KIR2DL3* and *KIR3DL1* genes were both observed to have protective effects against RA. However, this protection was partially counteracted due to the simultaneous presence of *KIR2DS4* in the AA genotype, which may confirm the phenomenon of natural selection during evolution (i.e., the individuals with harmful genes survived if they had some other protecting genes). Our study was consistent with the findings from Yen et al. (2006) related to the presence of *KIR2DS4* as a risk factor for RA.

Due to financial limitations, this study had a relatively low sample size and used control samples from previous work. Regardless of the low sample size, our study was able to reveal significant associations among groups, indicating sufficient power. Despite the limitations, this study added this finding that in the Lur population of Iran *KIR2DS4-full* was the most dominant risk factor for RA among the *KIR* genes.

In this study, our findings show *KIR2DS4-full* to increase susceptibility to RA whereas *KIR2DL5A* was found to be a protective factor based on both cross Table (unadjusted) and regression (adjusted) analyses. Furthermore, *KIR2DL3* and *KIR3DL1* was observed to have a protective role and *KIR2DS4-del* was found to increase the risk of RA based on the regression model. However, only the role of *KIR2DS4-full* remained statistically significant multiple comparison wise. Since the association of these genes with susceptibility to RA is ethnicity dependent, further research should explore these relationships in other populations.

Acknowledgements

We would like to thank Lorestan University of Medical Science for approving this dissertation with grant number 574 as a MSc thesis of Bijan Ansari-Moghaddam under the supervision of Farhad shahsavari.

The authors declare no conflicts of interest in this study.

References

- McInnes IB, Schett G. Cytokines in the pathogenesis of rheumatoid arthritis. *Nat Rev Immunol*. 2007;7(6):429-42.
- Aghaei H, Mostafaei S, Aslani S, Jamshidi A, Mahmoudi M. Association study between *KIR* polymorphisms and rheumatoid arthritis disease: an updated meta-analysis. *BMC Med Genet*. 2019;20(1):24.
- Deane KD, Holers VM. The natural history of rheumatoid arthritis. *Clin Ther*. 2019;41(7):1256-1269.
- Guo Q, Wang Y, Xu D, Nossent J, Pavlos NJ, Xu J. Rheumatoid arthritis: pathological mechanisms and modern pharmacologic therapies. *Bone research*. 2018;6(1):15.
- Gerlag DM, Raza K, van Baarsen LG, Brouwer E, Buckley CD, Burmester GR, et al. EULAR recommendations for terminology and research in individuals at risk of rheumatoid arthritis: report from the Study Group for Risk Factors for Rheumatoid Arthritis. *Ann Rheum Dis*. 2012;71(5):638-41.
- Abbasi Z, Kazemi Nezhad SR, Pourmahdi-Broojeni M, Rajaei E. Association of PTPN22 rs2476601 Polymorphism with rheumatoid arthritis and celiac disease in Khuzestan province, southwestern Iran. *Iran Biomed J*. 2017;21(1):61-6.
- Sánchez-Hernández P, Muñoz-Valle J, Palafox-Sánchez C, Rosales-Rivera L, García-Iglesias T, Daneri-Navarro A, et al. Associations of killer cell immunoglobulin-like receptor genes with rheumatoid arthritis. *Dis Markers*. 2012;33(4):201-6.
- Cappelli LC, Dorak MT, Bettinotti MP, Bingham III CO, Shah AA. Association of HLA-DRB1 shared epitope alleles and immune checkpoint inhibitor-induced inflammatory arthritis. *Rheumatology*. 2018;58(3):476-480.
- Nazari M, Mahmoudi M, Rahmani F, Akhlaghi M, Beigy M, Azarian M, et al. Association of killer cell immunoglobulin-like receptor genes in Iranian patients with rheumatoid arthritis. *PloS one*. 2015;10(12):e0143757.
- Campbell JJ, Qin S, Unutmaz D, Soler D, Murphy KE, Hodge MR, et al. Unique subpopulations of CD56+ NK and NK-T peripheral blood lymphocytes identified by chemokine receptor expression repertoire. *J Immunol*. 2001;166(11):6477-82.
- Fehniger TA, Cooper MA, Nuovo GJ, Cella M, Facchetti F, Colonna M, et al. CD56bright natural killer cells are present in human lymph nodes and are activated by T cell-derived IL-2: a potential new link between adaptive and innate immunity. *Blood*. 2003;101(8):3052-7.
- Dalbeth N, Callan MF. A subset of natural killer cells is greatly expanded within inflamed joints. *Arthritis Rheum*. 2002;46(7):1763-72.
- Pende D, Parolini S, Pessino A, Sivori S, Augugliaro R, Morelli L, et al. Identification and molecular characterization of NKp30, a novel triggering receptor involved in natural cytotoxicity mediated by human natural killer cells. *J Exp Med*. 1999;190(10):1505-16.
- Sivori S, Carlomagno S, Pesce S, Moretta A, Vitale M, Marcenaro E. TLR/NCR/*KIR*: which one to use and when?. *Front Immunol*. 2014;5:105.
- Lanier LL. On guard—activating NK cell receptors. *Nat Immunol*. 2001;2(1):23-7.
- McQueen KL, Parham P. Variable receptors controlling activation and inhibition of NK cells. *Curr Opin Immunol*. 2002;14(5):615-21.
- Lanier LL. NK cell receptors. *Annual review of immunology*. 1998;16(1):359-393.
- Long EO, Barber DF, Burshtyn DN, Faure M, Peterson M, Rajagopalan S, et al. Inhibition of natural killer cell activation signals by killer cell immunoglobulin-like receptors (CD158). *Immunol Rev*. 2001;181(1):223-33.
- Moretta A, Bottino C, Vitale M, Pende D, Cantoni C, Mingari MC, et al. Activating receptors and coreceptors involved in human natural killer cell-mediated cytotoxicity. *Annu Rev Immunol*. 2001;19(1):197-223.
- Vilches C, Parham P. *KIR*: diverse, rapidly evolving receptors of innate and adaptive immunity. *Annu Rev Immunol*. 2002;20:217-51.
- Lanier LL. NK cell recognition. *Annu Rev Immunol*. 2005;23:225-74.
- Kumar V, McNerney ME. A new self: MHC-class-I-independent natural-killer-cell self-tolerance. *Nat Rev Immunol*. 2005;5(5):363-74.
- Carrington M, Norman P. The *KIR* gene cluster. 2003.

24. Martin AM, Kulski JK, Gaudieri S, Witt CS, Freitas EM, Trowsdale J, et al. Comparative genomic analysis, diversity and evolution of two *KIR* haplotypes A and B. *Gene*. 2004;335:121-31.
25. Shilling HG, Guethlein LA, Cheng NW, Gardiner CM, Rodriguez R, Tyman D, et al. Allelic polymorphism synergizes with variable gene content to individualize human *KIR* genotype. *J Immunol*. 2002;168(5):2307-15.
26. Berinstein J, Pollock R, Pellett F, Thavaneswaran A, Chandran V, Gladman DD. Association of variably expressed *KIR3DL1* alleles with psoriatic disease. *Clin Rheumatol*. 2017;36(10):2261-2266.
27. Marsh SG, Parham P, Dupont B, Geraghty DE, Trowsdale J, Middleton D, et al. Killer-cell immunoglobulin-like receptor (*KIR*) nomenclature report, 2002. *Hum Immunol*. 2003;64(6):648-54.
28. Lanier LL, Bakker AB. The ITAM-bearing transmembrane adaptor DAP12 in lymphoid and myeloid cell function. *Immunol Today*. 2000;21(12):611-4.
29. Rajalingam R. Killer cell immunoglobulin-like receptors influence the innate and adaptive immune responses. *Iran J Immunol*. 2007;4(2):61-78.
30. Uhrberg M, Valiante NM, Shum BP, Shilling HG, Lienert-Weidenbach K, Corliss B, et al. Human diversity in killer cell inhibitory receptor genes. *Immunity*. 1997;7(6):753-63.
31. Shahsavari F, Mapar S, Ahmadi SAY. Multiple sclerosis is accompanied by lack of *KIR2DS1* gene: A meta-analysis. *Genom Data*. 2016;10:75-78.
32. Shahsavari F, Mousavi T, Entezami K, Azargoon A R. Association of *KIR-HLA* interactions with diseases. *Yafteh*. 2011;13(3):82-96.
33. Shapouri-Moghaddam A, Mohammadi M, Rahimi HR, Esmaeili H, Mahmoudi M, Modaghegh M-HS, et al. The Association of HLA-A, B and DRB1 with Buerger's Disease. *Rep Biochem Mol Biol*. 2019;8(2):153-160.
34. Shahsavari F, Varzi A-M, Ahmadi SAY. A genomic study on distribution of human leukocyte antigen (HLA)-A and HLA-B alleles in Lak population of Iran. *Genom Data*. 2017;11:3-6.
35. Gonzalez-Galarza FF, McCabe A, Santos EJMd, Jones J, Takeshita L, Ortega-Rivera ND, et al. Allele frequency net database (AFND) 2020 update: gold-standard data classification, open access genotype data and new query tools. *Nucleic Acids Res*. 2020;48(D1):D783-D788.
36. Shayanrad B, Ghanadi K, Varzi A-M, Birjandi M, Ahmadi SAY, Shahsavari F. Association of *KIR* genes and their *HLA* ligands diversity with colorectal cancer in Lur population of Iran. *Meta Gene*. 2019;22:100603.
37. Leung W. Infusions of allogeneic natural killer cells as cancer therapy. *Clin Cancer Res*. 2014;20(13):3390-400.
38. Toubi E, Vadasz Z. Think autoimmunity, breath autoimmunity, and learn autoimmunity. *Clin Rheumatol*. 2019;38(5):1227-1230.
39. Shegarfi H, Naddafi F, Mirshafiey A. Natural killer cells and their role in rheumatoid arthritis: friend or foe?. *ScientificWorldJournal*. 2012;2012:491974.
40. Yen J-H, Moore BE, Nakajima T, Scholl D, Schaid DJ, Weyand CM, et al. Major histocompatibility complex class I-recognizing receptors are disease risk genes in rheumatoid arthritis. *J Exp Med*. 2001;193(10):1159-67.
41. Kogure T, Tatsumi T, Niizawa A, Fujinaga H, Ito T, Shimada Y, et al. No correlation exists between disease activity and the expression of killer-cell immunoglobulin-like receptors in patients with rheumatoid arthritis. *Mediators Inflamm*. 2007;2007:65179.
42. Yen JH, Lin CH, Tsai WC, Wu CC, Ou TT, Hu CJ, et al. Killer cell immunoglobulin-like receptor gene's repertoire in rheumatoid arthritis. *Scand J Rheumatol*. 2006;35(2):124-7.
43. Li X, Xia Q, Fan D, Cai G, Yang X, Wang L, et al. Association between *KIR* gene polymorphisms and rheumatoid arthritis susceptibility: a meta-analysis. *Human Immunology*. 2015;76(8):565-570.
44. Nishimura WE, Sachetto Z, Costallat LTL, Yazbek MA, Londe ACS, Guariento EG, et al. The role of *KIR2DL3/HLA-C* 0802* in Brazilian patients with rheumatoid vasculitis. *Clinics*. 2015;70(6):408-12.
45. McGeough CM, Berrar D, Wright G, Mathews C, Gilmore P, Cunningham RT, et al. Killer immunoglobulin-like receptor and human leukocyte antigen-C genotypes in rheumatoid arthritis primary responders and non-responders to anti-TNF- α therapy. *Rheumatology international*. 2012;32(6):1647-53.