

ORIGINAL RESEARCH PAPER

Role of KIR gene cluster in susceptibility to rheumatoid arthritis

Kakati Pankaj¹, Misra Dhritiman², Choudhury MK³, Talukdar KL⁴, Deka Roonmoni⁵, Baruah Chitralekha⁶

Received on April 12, 2019; editorial approval on June 01, 2019

ABSTRACT

Introduction: Rheumatoid Arthritis (RA) is an autoimmune and chronic inflammatory disease of unknown etiology whose pathogenesis is not fully understood. Small joints in the hands and feet are involved the most. Genetic risk association of RA with HLA-DRB1 gene is the most significant. Women are more affected than men. Natural killer cells and CD28 null T-cells present in synovial membranes of joints of RA patients express Killer cell immunoglobulin-like receptors on its surface. KIR gene cluster has a strong association with autoimmunity as found in various studies. **Objective:** To investigate the role of various genes of KIR gene cluster in the pathogenesis of RA. **Materials and methods:** Blood samples from 80 cases (following ACR/ EULAR criteria-2010) and 80 controls were collected in EDTA vials using standard venipuncture procedure. DNA was extracted from each of the collected blood samples and KIR genotyping was done by molecular techniques using SSP kits. **Results:** The presence of KIR2DS1, KIR2DS3, KIR2DS4, KIR2DS5 and KIR3DL1 genes among RA patients showed risk association. Using standard statistical tools results were validated. **Conclusion:** Some of the KIR genes have risk association with occurrence of RA. Individuals carrying these genes are suspected to be more susceptible to develop RA.

Keywords: Pathogenesis; genotyping; risk association; susceptible.

INTRODUCTION

Rheumatoid Arthritis (RA) is an auto-inflammatory disease whose progression is chronic in nature. It mainly attacks synovial joints but extra-articular involvement can also be seen. Pain, swelling, and stiffness of the joints are the most common signs and symptoms. Most often small joints in the hands and feet are involved, although larger joints may also be involved. Typically joints are affected in a symmetrical pattern; for example, if joints in the right hand are affected,

left hand also tends to be involved. It affects approximately 1% of the population distributed worldwide. The prevalence of RA is higher in women as compared to men.¹ Depending on the age of onset, it reduces the lifespan of the patient by 5-10 years.² Pathogenesis of RA has not been fully elucidated till now, even though many researches have been done. As the immune system attacks the body's own tissues and organs, and, due to the presence of autoantibodies like rheumatoid factor (RF) and anti-cyclic citrullinated antibodies (Anti-CCP), it is considered to be an autoimmune disease. As both genetic and environmental factors are involved, it is considered a multifactorial disease. Etiology of the disease comprises of 60% of genetic factors.³ Variations in human leukocyte antigen (HLA) genes, especially the HLA-DRB1 gene is the most significant genetic risk factors for rheumatoid arthritis. It has been documented that shared epitope (SE) alleles, such as HLA-DRB1*01 and DRB1*04, some HLA alleles like HLA-DRB1*13 and DRB1*15 are connected to RA susceptibility.⁴

Address of correspondence:

¹Senior Research Fellow and Ph.D. Scholar

Mobile: +919401156293

Email: pankajkakati1@gmail.com

²Ph.D. Research Scholar

Mobile: +918761068646

Email: dhriti.dhriti@gmail.com

HLA Laboratory, Department of Anatomy

³Professor (**Corresponding author**)

Department of Physiology

Mobile: +919864034231

Email: drmanojchoudhury@gmail.com

⁴Former Professor and Head of Anatomy, Gauhati Medical College & Hospital (GMCH), Guwahati, Assam,

⁵Professor and Head of Anatomy, Tezpur Medical College, Tezpur

⁶Professor of Medicine, GMCH, Assam, India

Cite this article as: Kakati Pankaj, Misra Dhritiman, Choudhury MK, Talukdar KL, Deka Roonmoni, Baruah Chitralekha. Role of KIR gene cluster in susceptibility to rheumatoid arthritis. *Int J Health Res Medico Leg Prae* 2019 JJuly;5(2):38-42. DOI 10.31741/ijhrmlp.v5.i2.2019.9

The Immune system distinguish the body's own proteins from proteins made by foreign invaders with the help of the proteins produced from HLA genes which is estimated to be 11–37 %.⁵ Other genetic risk factors for RA are PTPN22, CD40, CTLA4 and also genes coding elements of NF- κ B signaling pathway like TNFAIP3 and TRAF1.⁶ Natural killer (NK) cells, which are bone marrow derived large granular lymphocytes, mount early immune responses in an antigen independent manner by direct cytotoxicity.⁷ NK cells display Killer cell immunoglobulin-like receptors (KIRs). Located on chromosome 19q13.4, the KIR gene cluster spans about 150–200 kb in the Leukocyte Receptor Complex. By interacting with MHC class I molecules, which are expressed on all cell types, KIRs regulate the killing functions. This interaction allows them to detect virally infected cells or tumor cells that have a characteristic low level of Class I MHC on their surface. The KIRs comprise a multigene family of receptors. KIR gene cluster consists of inhibitory genes, viz., KIR2DL1, KIR2DL2, KIR2DL3, KIR3DL1, KIR3DL2, KIR3DL3, KIR2DL4, KIR2DL5; activating genes, viz., KIR2DS1, KIR2DS2, KIR2DS3, KIR2DS4, KIR2DS5, KIR3DS1 and pseudo genes, viz., KIR2DP1 and KIR3DP1.⁸ KIR genes like KIR2DL2, KIR2DS2, KIR3DS1 and KIR2DS4 are found to be associated with RA in Iranian, Mexican, Polish, Caucasian, Taiwanese and North Indian populations.⁸⁻¹³ Studies on the relationship of KIR and RA are inconsistent and contradictory.¹⁴

In the Northeastern parts of India, only a few studies related to association of genetic factors related to RA, have been done. A recent study found TNF- α –308 variant GA genotype was higher in RA (46.03%) than in control (25%). The presence of TNF- α –308 variant A allele was associated with increased risk of RA susceptibility.¹⁵ Till date, not a single study examining the KIR gene cluster in relation to RA, has been done in this part of India i.e., the Northeastern parts. Hence, the present, study aims to examine the role of various KIR genes in the susceptibility to rheumatoid arthritis among the population of Assam, which is one of the most important states of North East India.

MATERIALS AND METHODS

The study was conducted in a tertiary care government hospital situated in Guwahati, which is the gateway to Northeast India and where the capital of Assam (Dispur) is situated. This hospital caters to a large number of patients from every region of the state. A total number of 80 cases were taken from the Rheumatology OPD, Department of Medicine, Gauhati Medical College and Hospital, Guwahati, Assam, following the ACR/EULAR criteria (2010), during the period from 2017 to 2019. Four to six milliliters of blood were obtained, from patients diagnosed with RA as per the standard venipuncture procedure and transferred into EDTA vials. Similarly blood samples were also collected from 80 controls who were individuals neither having any history of RA nor any other autoimmune diseases. DNA was extracted from all samples using standard (Manatis, et al) technique.¹⁶ Quality and quantity was checked using Multiscan Go

(Spectrophotometer/Nanodrop). Purity ratio of DNA samples were found to be between 1.8- 2.0 using wavelength 260/ 280 nm.¹⁷ Concentrations were found to be above 200 ng/ μ L. PCR amplification of DNA samples of both patients and controls groups for KIR genes were performed using Sequence-Specific Primer (SSP) technique. The amplified products were subsequently loaded in 2% agarose gel submersed in 0.5X Tris buffer in a gel electrophoresis system. After running the electrophoresis for the required period, the 2% agarose gels were documented under gel documentation system. Intercalating agent ethidium bromide was used to tag the DNA in the gels viewed under UV rays using gel documentation system. KIR genes were identified, using appropriate tools, for the KIR genes responsible for inhibitory signals (KIR2DL1, KIR2DL2, KIR2DL3, KIR3DL1, KIR3DL2, KIR3DL3, KIR2DL4 and KIR2DL5), activating signals (KIR2DS1, KIR2DS2, KIR2DS3, KIR2DS4, KIR2DS5 and KIR3DS1) and two pseudo genes (KIR2DP1 and KIR3DP1). Results were validated using statistical tools like Fisher's exact test and Chi square test for independence and the findings less than P value- 0.05 were considered as statistically significant.

RESULTS

After analyzing the data of the study group it has been found that the frequency of female RA patients was higher (**Figure 1**) than male patients (P value<0.0001, OR- 9.0, 95% CI- 4.7450-17.0706). Females were found to be more predominant in terms of disease acquirement.

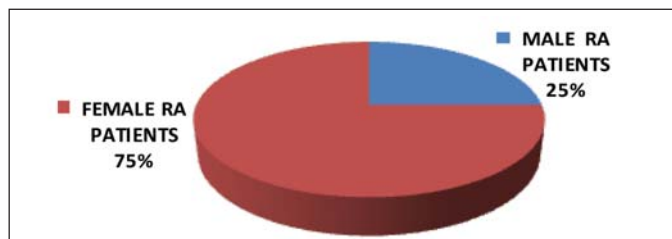


Figure 1 Sex distribution among RA patients

Associations of 16 KIR genes have been portrayed in **Table 1**. Considerable difference in the occurrence of KIR2DS3 gene between the patient and the control group was found during the study. From the univariate analysis comparing RA patient with healthy controls it was found that KIR2DS3 may have a significant role in increasing the susceptibility to RA (P value- < 0.0001, OR- 4.4211, CI- 2.1912 to 8.9200). Apart from that, few other genes from the KIR cluster were found to have risk associations with RA. Those activating genes were KIR2DS1 (P value- 0.0372, OR- 2.0636, 95% CI- 1.0904 to 3.9056), KIR2DS4 (P value- .0072, OR- 4.7500, 95% CI- 1.5115 to 14.9269) and KIR2DS5 (P value- 0.0092, OR- 2.6667, 95% CI- 1.3147 to 5.4091). Moreover, it was found that the incidence of inhibitory gene KIR3DL1 was higher in frequency among the RA patients than the controls in the study population (P value- 0.0446, OR- 2.8141, 95% CI- 1.0965 to 7.2222). The protective functionality of KIR genes in RA as reported in different populations⁸⁻¹³ was not observed in this study group.

Table 1 Distribution of the KIR genes in patient and control group

Genes	Patients (n)	Controls (n)	P value	Odds ratio	Confidence interval
KIR2DL1	78	75	0.4426	2.6000	0.4893 to 13.8145
KIR2DL2	64	56	0.1074	1.7143	0.8285 to 3.5473
KIR2DL3	60	64	0.5704	0.7500	0.3558 to 1.5811
KIR2DL4	80	80	-	-	-
KIR2DL5	72	64	0.1198	2.2500	0.9029 to 5.6069
KIR2DS1	53	39	0.0372*	2.0636	1.0904 to 3.9056
KIR2DS2	68	65	0.6734	1.3077	0.5692 to 3.0043
KIR2DS3	64	38	< 0.0001*	4.4211	2.1912 to 8.9200
KIR2DS4	76	64	0.0072*	4.7500	1.5115 to 14.9269
KIR2DS5	64	48	0.0092*	2.6667	1.3147 to 5.4091
KIR3DL1	73	63	0.0446*	2.8141	1.0965 to 7.2222
KIR3DL2	80	80	—	—	—
KIR3DL3	80	80	—	—	—
KIR3DS1	73	71	0.7930	1.3219	0.4671 to 3.7414
KIR2DP1	80	80	—	—	—
KIR3DP1	77	75	0.7194	1.7111	0.3949 to 7.4144

*Statistically significant.

Distribution of the KIR genes in patient and control group in terms of frequency of occurrences has been illustrated in **Figure 2**.

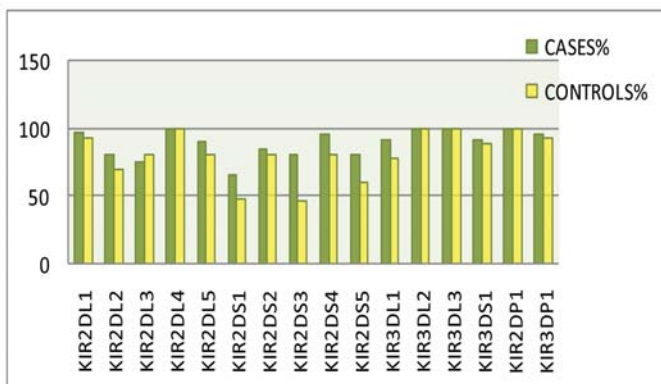


Figure 2 Distribution of the KIR genes in patient and control group in terms of frequency of occurrences

DISCUSSION

RA is an autoimmune disease with immunocomplex mediated hypersensitivity. It drastically involves the synovial joints and has a prevalence of approximately 1%. It affects generally the people of age group 30 to 60 years. Though its etiology is not fully understood still polygenic involvement is suspected to be associated. Whereas KIR has been seen to be associated with various autoimmune diseases and viral complications. The multiallelic diversity of KIR has made it the prime candidate for disease association studies. In this study, an approach has been made to elucidate the relationship between KIR and RA. After investigating the cases and controls drawn from homogenous population of Assam, it has been seen that the incidence of RA is higher in females as compared to

males (P value- < 0.0001, OR- 9.0, 95% CI- 4.7450-17.0706), such findings based on gender variation are also reported in studies conducted on other populations.¹

Upon univariate analysis of individual KIR genes in the study group, it has been found that KIR2DS1 (P value- 0.0372, OR- 2.0636, 95% CI- 1.0904 to 3.9056), KIR2DS3 (P value- < 0.0001, OR- 4.4211, CI- 2.1912 to 8.9200), KIR2DS4 (P value- 0.0072, OR- 4.7500, 95% CI- 1.5115 to 14.9269), KIR2DS5 (P value- 0.0092, OR- 2.6667, 95% CI- 1.3147 to 5.4091) and KIR3DL1 (P value- 0.0446, OR- 2.8141, 95% CI- 1.0965 to 7.2222) genes have risk associations with RA. Out of these five genes, KIR2DS3 has been found to be highly associated with susceptibility to RA in terms of frequency. A similar finding from Taiwanese population has also been reported where KIR2DS4 was found to be associated with RA.¹² In contrary to our findings, risk association and protective functions of KIR genes are contradictory and inconsistent in different populations. In Mexican population, KIR2DL2 and KIR 2DS2 have risk associations with RA whereas KIR2DL3 seems to confer protection against RA.⁹ In Polish population,¹⁰ it has been found that frequencies of KIRs in patients with RA are similar to the frequencies in controls whereas in Iranian population KIR2DL2, KIR2DL5, KIR2DS5 and KIR3DS1 are found to be protective against RA.⁸ In North Indian population also KIR association with RA has been studied where they observed KIR3DS1 and KIR2DS2 have risk association however KIR2DL2, KIR2DL3 and KIR3DL1 have protective function.¹³

Another observation was made where KIR3DL1, which is normally classified under inhibitory signaling genes,⁸ has been found to be higher in patients than in controls, thus contraindicating its inhibitory role in the population in Assam. Other KIR genes viz., KIR2DL1, KIR2DL2, KIR2DL3, KIR2DL4, KIR2DL5, KIR2DS2, KIR3DL2, KIR3DL3, KIR3DS1, KIR2DP1, KIR3DP1 were found to have similar frequencies in both cases and controls and their relationship with RA could not be statistically proven.

CONCLUSION

In this study it has been seen that there is an activating role of KIR2DS1, KIR2DS3, KIR2DS4, KIR2DS5 and KIR3DL1 in RA and the individuals with these genes are more susceptible to develop RA. The actual mechanism behind their role in susceptibility is through various cascades of pathways involving the KIR receptors in NK cells, further to validate the findings comparison and confirmation from various independent cohorts is needed. Despite of everything, irreconcilability has been seen in the effort of replicating the result.

Acknowledgement: We would like to acknowledge DBT, Govt. of India funded DBT Healthcare Laboratory for HLA Tissue Typing and Transplant Immunology, Department of Anatomy, Gauhati Medical College and Hospital for providing the infrastructure facilities and Rheumatology OPD, Department of Medicine, Gauhati Medical College and Hospital

for providing diagnosed cases of RA. Further, we would like to thank Miss Kritanjali Dutta, Laboratory Technician, DBT Healthcare Laboratory for HLA Tissue Typing and Transplant Immunology, Department of Anatomy, Gauhati Medical College and Hospital and Dr. Devika Barman for helping us in sample collection.

Conflict of interest: None declared.

Ethical clearance: Taken.

Source of funding: Nil.

Author Disclosure: (1) The article is original with the author(s) and does not infringe any copyright or violate any other right of any third party. (2) The article has not been published (whole or in part) elsewhere, and is not being considered for publication elsewhere in any form, except as provided herein. (3) The first, second and third authors are co-authors and have equal contribution in planning, designing and executing the study. All other author(s) in the article have contributed immensely in their respective speciality to take public responsibility for it and (4) all author(s) have reviewed the final version of the above manuscript and approved it for publication.

REFERENCES

1. Alamanos Y, Drosos AA. Epidemiology of adult rheumatoid arthritis. *Autoimmun Rev* 2005;4(3):130-6.
2. Wolfe F, Mitchell DM, Sibley JT, Fries JF, Bloch DA, Williams CA, et al. The mortality of rheumatoid arthritis. *Arthritis Rheum* 1994; 37:481-94.
3. Orozco G, Goh CL, Olama AA, Benlloch GS, Govindasami K, Guy M, et al. Common genetic variants associated with disease from genome-wide association studies are mutually exclusive in prostate cancer and rheumatoid arthritis. *BJU international* 2013;111(7):1148-55.
4. Karami J, Aslani S, Jamshidi A, Garshasbi M, Mahmoudi M. Genetic implications in the pathogenesis of rheumatoid arthritis; an updated review. *Gene* 2019 Jun 20;702:8-16.
5. Kurko Julia, Besenyei imea, Laki Judit, Glant TT, Mikecz Katalin, Szekanecz Zoltan. Genetics of rheumatoid arthritis - a comprehensive review. *Clin Rev Allergy Immunol* October 2013;45(2):170-9.
6. McInnes IB, Schett G. The pathogenesis of rheumatoid arthritis. *The New England J of medicine* 2011;365(23):2205-19.
7. Trinchieri G. Biology of natural killer cells. *AdvImmunol* 1989;47:187-376.
8. Masoumeh Nazari, Mahdi Mahmoudi, Farzaneh Rahmani, Masoumeh Akhlaghi, Maani Beigy, Maryam Azarian, et al. Association of killer cell immunoglobulin-like receptor genes in Iranian patients with rheumatoid arthritis. *PLOS One* 2015;10(12):e0143757.
9. Ramirez Santos, Sanchez Hernandez, Munoz Valle, Palafox Sanchez, Rosales Rivera, García Iglesias.

- Associations of Killer Cell Immunoglobulin-Like Receptor Genes with Rheumatoid Arthritis. *Disease Markers* Volume 2012;33(4):201-6.
10. Majorczyk E, Pawlik A, Luszczek W, Nowak I, Wiśniewski A, Jasek M, et al. Associations of killer cell immunoglobulin-like receptor genes with complications of rheumatoid arthritis. *Genes Immun* 2007 Dec;8(8):678-83.
 11. Jeng-Hsien Yen, Brenda EM, Takako Nakajima, Dirk Scholl, Daniel JS, Cornelia M, et al. Major histocompatibility complex class I – recognizing receptors are disease risk genes in rheumatoid arthritis, *J Exp Med* 2001 May 21;193(10):1159-68.
 12. 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 Mar-Apr;35(2):124-7.
 13. Prakash Swayam, Alam Shahnawaz, Bharadwaj Uddalak, Aggarwal Amita, Mishra RN, Agrawal Suraksha. Associations of killer cell immunoglobulin like receptors with rheumatoid arthritis among North Indian population. *Human Immunology* Jan 1 2014;75(8):802-7.
 14. Hamideh Aghaei, Shayan Mostafaei, Saeed Aslani, Ahmadrza Jamshidi, Mahdi Mahmoudi. Association study between KIR polymorphisms and rheumatoid arthritis disease: an updated meta-analysis. *BMC Med Genet* 2019 Jan 29;20(1):24.
 15. Das Somdatta, Baruah Chitrakleha, Saikia AK, Tiwari Diptika, Bose Sujoy. Genetic and expression changes in TNF- α as a risk factor for rheumatoid arthritis pathogenesis in northeast India. *Journal of Genetics* 2019;98:3.
 16. Maniatis, Sambrook, Fritsch. *Molecular cloning: Part 3*. 2nd ed. p 9.18.
 17. Desjardins P, Conklin D. Nanodrop microvolume quantitation of nucleic acids. *J Vis Exp* 2010 Nov 22;45:2565.