Human Immunology 75 (2014) 802–807

Contents lists available at ScienceDirect

journal homepage: www.elsevier.com/locate/humimm

Associations of killer cell immunoglobulin like receptors with rheumatoid arthritis among North Indian population

Swayam Prakash [a], Shahnawaz Alam [a], Uddalak Bharadwaj [b], Amita Aggarwal [c], Ram Nath Mishra [c], Suraksha Agrawal [a,*]

[a] Department of Medical Genetics, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Raibareily Road, Lucknow, Uttar Pradesh, India
[b] Margaret M. and Albert B. Alkek Department of Medicine, Section of Infectious Diseases, Baylor College of Medicine, Houston, TX, United States
[c] Department of Immunology, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Raibareily Road, Lucknow, Uttar Pradesh, India

ARTICLE INFO

Article history:
Received 19 November 2013
Accepted 29 May 2014
Available online 6 June 2014

Keywords:
Killer immunoglobulin like receptor (KIR)
Rheumatoid arthritis (RA)
Autoimmune disease
Genetic association
Polymerase chain reaction-sequence specific primer (PCR-SSP)

ABSTRACT

Introduction: Rheumatoid arthritis (RA) is an autoimmune and chronic inflammatory disease of unknown etiology. Killer cell immunoglobulin-like receptors (KIR) expressed on surface of natural killer cells and CD28 null T-cells which are present in synovial membrane of RA. The present study has evaluated associations of KIR genes with RA among North Indian population from Uttar Pradesh.
Materials and methods: KIR genotypes were determined in 100 RA cases and 100 healthy controls using sequence specific primer polymerase chain reaction (PCR-SSP) method.
Results: RA cases positive for KIR3DS1 (OR = 1.17, p-value = 0.0498) and KIR2DS2 (OR = 2.21, p-value = 0.0120) showed risk associations. While, KIR2DL2 (OR = 0.40, p-value = 0.0026), KIR2DL3 (OR = 0.44, p-value = 0.0283) and KIR3DL1 (OR = 0.32, p-value = 0.0012) showed protective associations. Increased incidence of BB genotype (45%) was revealed among cases. Risk association was noted against telomeric region (OR = 2.12, p = 0.0120) genes for RA. Pair-wise linkage disequilibrium (LD) analysis among RA cases revealed KIR2DS1-2DL1 (D' = 0.83, r^2 = 0.36), KIR3DL1-3DS1 (D' = 1, r^2 = 0.58) and KIR2DL1-2DL2 (D' = 1, r^2 = 0.61) to be in significant LD. KIR3DS1 and KIR2DS3 genes showed significant risk associations among RA patients with extra-articular manifestations (OR = 5.14, p-value = 0.0018; OR = 3.79, p-value = 0.0106) and in limited range of motion in affected joints (OR = 14.91, p-value = 0.0001; OR = 2.95, p-value = 0.0126).
Conclusion: The KIR activating genes have risk association with RA in the present study.

1. Introduction

Rheumatoid arthritis (RA) is an autoimmune disease affecting 1% of the world population, in which chronic inflammatory response develops [1]. RA is determined by an inflammation of the synovial membrane leading to destruction of cartilage and bone [2]. Several studies [3–5] have investigated the potential involvement of Natural Killer (NK) cells in RA. NK cell-like lymphocytes have been demonstrated in the affected joints in RA patients. It is proposed that NK cells may have a regulatory role through the release of cytokines and cross-talk with dendritic cells [3]. It is also apparent that cytokines play a major role in RA. Reduced NK cell activity has been shown to occur in patients with RA [6–9].

The NK cells display killer cell immunoglobulin-like receptors (KIRs). The KIRs comprise a multigene family of receptors. Highly polymorphic KIR haplotypes are located on chromosome 19q13.4 and are classified into group A or B. The classification is based on the number and type of genes which encode inhibitory and activating KIRs. Group B haplotypes are determined due to the presence of one or more of the KIR2DL5, KIR2DS1, KIR2DS2, KIR2DS3, KIR2DS5 and KIR3DS1 genes, where-else, group A haplotypes are characterized by the absence of all these genes [10,11]. KIRs are expressed on the surface of CD8 + T cells, CD4 + CD28 null T-cells, and natural killer (NK) cells. Activatory KIR functions as a co-stimulatory molecule on CD4 + CD28 null T-cells, which may lead to the clonal expansion of these cells [4]. RA patients show expanded CD4 + CD28 null T-cells, which play a direct role in acute coronary syndrome [12] like vascular injury. Therefore, KIR may be involved

* Corresponding author. Address: Department of Medical Genetics, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Raebareli Road, Lucknow, UP 226014, India. Fax: +91 522 6680973/6680017.
E-mail address: sur_ksha_agrawal@yahoo.co.in (S. Agrawal).

http://dx.doi.org/10.1016/j.humimm.2014.05.014
0198-8859/

in the pathogenesis of RA. In contrast, CD56 bright KIR-negative cells have been shown to accumulate in the synovial fluid of patients with RA [13].

Previous studies revealing the involvement of KIR receptors in RA have presented a debatable picture. KIR2DS2 is reported to be a risk factor among Caucasian patients with RA [9] and KIR2DS4 was shown to be increased in patients with RA from Taiwan [14]. However, a study among the Japanese population has not found any significant change in KIR gene frequencies in patients with RA as compared to controls [15].

In addition to studies on RA, association of activating KIR receptors KIR2DS1 [16,17] and KIR2DS2 [16] have been reported in psoriatic arthritis. While higher prevalence of KIR2DL5 [18] and KIR2DS1 [18,19] have been reported among psoriasis vulgaris cases. The conflicting evidence, in the frequency of KIR receptors, led us to examine the KIR frequency in a cohort of patients and controls with chronic RA from north India.

2. Materials and methods

2.1. Genomic DNA samples

A total of 100 North Indian patients with chronic RA (Female = 67 and Male = 33) fulfilling the criteria of the American College of Rheumatology were examined. Age range was 29–65 years, with an average age of 46 years, and all patients were treated with at least one disease-modifying anti-rheumatic drug. We further categorized the RA cases into three broad groups i.e., (i) Cases with all extra articular manifestations ($N = 21$) placing the cases who had systemic manifestations like vasculitis, visceral nodules, sjogren's syndrome and pulmonary fibrosis, (ii) RA limited to joints ($N = 34$) and (iii) Other extra articular manifestations ($N = 45$) placing the cases who had destructive polyarthritis along with extra articular organ involvement and assessed the association of various KIR gene content in these categories. The normal healthy controls consisted of 100 unrelated north Indian individuals without any history of RA or any other autoimmune diseases (Female = 60 and Male = 40) and were from the same ethnic background. 5 ml of whole blood from RA cases and healthy controls was collected in EDTA coated collection vials and DNA was extracted using Quiagen kits (Brand GMbH and Co KG, Cat # 51104). The study was approved by the institute ethics committee and was performed as per the ethical standards laid down by the Declaration of Helsinki. Informed written consent was obtained from all individuals prior to their inclusion in the study.

2.2. KIR Genotyping

KIR typing was performed using a sequence-specific primer (SSP) approach (Supplementary Table 1). PCR-SSP method was implemented to study the KIR complement of human genome. The DNA samples of controls as well as RA patients were typed for the KIR genes responsible for inhibitory signals (2DL1, 2DL2, 2DL3, 3DL1, 3DL2, 3DL3, 2DL4 and 2DL5), those for activating signals (2DS1, 2DS2, 2DS3, 2DS4, 2DS5 and 3DS1), and two pseudo genes (2DP1 and 3DP1), based on the primers described earlier [20,21] Positive and negative controls were included in every reaction.

2.3. Statistical analysis

Gene and genotype carrier frequency of KIR was determined by direct counting. Frequency differences between the RA patients and control groups for the individual inhibitory and activating KIR genes as well as for the haplogroups was tested for significance at 95% confidence limits using Fisher's exact test. Bonferroni correction was applied keeping in view the multiple comparisons. The significance levels of the inhibitory or activating KIR genes between RA patients and controls were tested and a linear model with a logistic link was used to test the association between increasing/decreasing extent of activating/inhibiting interactions and the prevalence of RA. p-value $\leqslant 0.05$ were considered significant. The Linkage Disequilibrium has been calculated as per the Lewontin's principle; strong positive LD has been assigned to the KIR genes having a LD score ranging between 0.8 and 1. Further r^2 values for all the KIR genes were calculated according to the Cramer's principle to strengthen the genetic association No significant differences occurred in the number of females vs. the number of males having each of the KIR genes. Statistical power of the study and the sample size estimation was carried out using G*Power version 2 (Heinrich Heine, Universitat Dusseldorf, Germany). .

3. Results

Significant statistical power was calculated against patient and control groups (RA-Control: 0.96, RA limited to joints-control: 0.80, other extra articular manifestations-control: 0.87) which justified the sample size for the present study (Supplementary Table 2). However, for all extra articular manifestations the sample size was inadequate as it was evident from the insignificant statistical power of the study.

3.1. Variation in KIR gene content

Decreased incidence of KIR2DL2, 2DL3 and 3DL1 genes were observed among RA patients. Odds of occurrence of RA in patient group were linearly related to the increased incidence of activating KIR2DS2 ($p = 0.0120$, OR = 2.21, 95%CI = 1.22–3.99) and KIR3DS1 ($p = 0.0039$, OR = 1.53, 95%CI = 0.29–1.96). On the other hand there was a significant protective effect of inhibitory KIR2DL2 ($p = 0.0026$, OR = 0.40, 95%CI = 0.22–0.71), 2DL3 ($p = 0.0283$, OR = 0.44, 95%CI = 0.22–0.88) and 3DL1 ($p = 0.0012$, OR = 0.32, 95%CI = 0.16–0.63) gene among patients and controls. Other KIR inhibitory (KIR2DL1 and 2DL5) and activating (KIR2DS2, 2DS3, 2DS4 and 2DS5) genes revealed no statistical significance against case and control groups.

3.2. Genotype and haplo-group variation

Genotypic data obtained for the RA patients and controls was classified into group A and Bx haplogroups (Table 1). The A haplogroup carries a fixed set of nine genes including a single activating gene (2DS4), and various inhibitory genes, and is designated as inhibitory haplogroup. On the other hand haplogroup-Bx is characterized by the presence of additional activating genes and absence of group-A specific variable inhibitory KIR genes (KIR2DL2, 2DL3 and 3DL1). The total KIR profiles among patients and controls are shown in Table 2. We have observed significant difference for the KIR BB and AB genotypes. However, an increased incidence of BB genotype among patients (45.0%) as compared to controls (34.0%) was found. AB genotype was found in higher frequency among the controls (54.0%) in comparison to RA (42.0%) cases. The telomeric region (T_4) comprising of KIR2DL5, KIR3DS1, KIR2DS5 and KIR2DS1 genes showed susceptibility ($p = 0.0120$, OR = 2.12, 95%CI = 1.22–3.99) to RA. Among the four KIR Bx Subgroups namely C_4T_4, C_4T_x, C_xT_4, C_xT_x; C_4T_x showed protective association ($p = 0.0001$, OR = 0.24, 95%CI = 0.12–0.48) for RA.