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Association of *NKG2D* gene variants with susceptibility and severity of rheumatoid arthritis

C. M. Mariaselvam,*† R. Tamouza,† R. Krishnamoorthy,† D. Charron,† D. P. Misra,† V. K Jain† and V. S. Negi†

**Department of Clinical Immunology, Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Puducherry, India, and †INSERM, UMRS, U1160, Saint Louis Hospital, Paris, France*

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Correspondence: V. S. Negi, Department of Clinical Immunology, Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Pondicherry 605006, India.
E-mail: vsnegi22@yahoo.co.in

Summary

NKG2D *(KLRK1)* is a C-type lectin receptor present on natural killer (NK) cells, γδ, $CD8^+$ and $CD4^+$ T cells. Upon ligand binding, NKG2D mediates activatory and co-stimulatory signals to NK cells and activated $CD4^+$ T cells, respectively. Polymorphisms in *NKG2D* predispose to infectious diseases, cancer, transplantation and autoimmune disorders. We studied the influence of this NK receptor polymorphism on predisposition to and modification of the disease phenotype in patients with rheumatoid arthritis (RA). Eight different single nucleotide polymorphisms (SNP) in the *NKG2* gene were genotyped in 236 patients with RA and 187 controls using Taqman 5' nuclease assays. *NKG2D* genotype/allele frequency did not differ between patients and controls. Subgroup analysis showed that the frequency of A allele of *NKG2D9* and T allele of *NKG2D10* was significantly higher in patients with deformities (a marker of severe disease) [11 *versus* 5%, Pc = 0·03, odds ratio (OR) = 2·44, 95% confidence interval (CI) = 1·09-5·98 and 10 *versus* 4%, Pc = 0·04, OR = 2·45, 95% CI = 1·05-6·39, respectively], while the frequency of alleles G of *NKG2D9* and A of *NKG2D10* was greater in patients without deformities (Pc = 0·03, OR = 0·41, 95% CI = 0·17-0·91 and Pc = 0·04, OR = 0·41, 95% CI = 0·16-0·96). Similar trends of association were observed with deforming phenotype of RA in female patients and deforming young onset RA subgroups. Haplotype analysis revealed that the frequency of haplotype *G-C-A-G-A-T-C-C* was higher in patients than in controls (12 *versus* 8%, *P* = 0·04, OR = 1·61, 95% CI = 1·01-2·55), suggesting that it may predispose to RA. Our study suggests that the *NKG2D* gene polymorphisms may modify the risk of development and severity of RA.

Keywords: MHC class I-related chain A, NKG2D receptor, rheumatoid arthritis, haplotypes, single nucleotide polymorphism

Introduction

Rheumatoid arthritis (RA), a systemic autoimmune disease, is characterized by irreversible joint damage and deformities. It affects 0·5–1·0% of adults in developed countries [1]. Synovial inflammation and hyperplasia, autoantibody production, cartilage and bone destruction over time characterize RA. The accompanying disabilities in RA impact significantly upon quality of life and socio-economic status of the patient, the family and society at large [2]. The most significant risk factors for RA include female gender, advanced age and a family history of such disorders. The presence of familial tendency suggests a genetic predisposition to the development of RA. Genetic predisposition is thought to account for approximately one-half to two-thirds of the risk of developing RA [3]. The precise mechanism through which genetic factors act is still unclear. It is likely to be triggered by complex and stochastic interactions between genetic and environmental factors [2].

Human and mouse natural killer (NK) cells express both activating and inhibitory cell surface receptors. Fine-tuning of immune surveillance (activated or quiescent state of NK cells) involves the integration of both activating and inhibitory receptor signalling [4]. Among many NK cell

receptors, the most studied receptors are the killer-cell immunoglobulin-like receptors (KIR). Several polymorphisms in the KIR genes have been identified, and the association of *KIR* genes with RA have been reported in Caucasian [5], Polish [6], Taiwanese [7], Mexican [8] and Iranian [9] populations. In a case–control study involving 100 North Indian subjects with RA the presence of *KIRDS1* and *KIRDS2* increased the risk of RA, whereas *KIRDL1, KIRDL2* and *KIRDL3* were protective. The presence of *KIRDS1* and *KIRDS3* increased the risk of extra-articular complications and deforming disease in patients with RA [10]. *KIR* gene polymorphisms are reported to influence disease severity and treatment response to methotrexate [11,12].

In humans, the *NK* gene complex on chromosome 12p12–p13 harbours 19 genes that encode for C-type lectins NKG2 and CD94. *NKG2D* lies amid this cluster of genes, referred to as 'NK complex' (NKC). Several genes expressed by NK cells, including *KLRD1 (CD94)* on the centromeric side and *KLRC4 (NKG2F)*, *KLRC3 (NKG2E)*, *KLRC2 (NKG2C)* and *KLRC1 (NKG2A)* on the telomeric side, are located within this complex. NKG2D (KLRK1) encoded by killer cell lectin-like receptor subfamily K is an activatory C-type lectin receptor on NK cells, gamma delta T (γδ T) cells, $CD8^+$ and $CD4^+$ T cells. The NKG2D receptor binds to a variety of ligands, including major histocompatibility complex (MHC) class-I polypeptide sequence A (MICA), MHC class-I polypeptide sequence B (MICB), UL16 binding protein (ULBP)−1, ULBP-2, ULBP-3, ULBP-4 and retinoic acid early transcript 1 G (RAET1G). Ligand binding of NKG2D mediates activatory signals to NK cells and co-stimulatory signals to activated $CD4^+$ T cells. NKG2D expressed on the surface of most cytotoxic $CD8^+$ T cells acts as a co-stimulatory receptor for naive $CD8^+$ T cells, enabling their transformation to activated $CD8^+$ T cells. Regulation of ligand expression is important for immune homeostasis, as inappropriate expression in normal tissues favours autoimmune processes. Conversely, failure to up-regulate ligand expression in pathological conditions favours the development of cancers or dissemination of intracellular infections [13].

Most of the published literature related to NKG2D in autoimmune diseases concerns MIC molecules, MICA and MICB. Genetic analyses have revealed a predisposition to develop RA in Tunisian patients bearing the A/A genotype of the single nucleotide polymorphism (SNP) *MICA-250*. The same study also revealed increased risk of seropositive RA in those individuals carrying the G allele and G/G genotype of MICA-250 SNP and the (val) allele and (val/val) genotype of *MICAmet129val* SNP [14]. An autoimmune response is triggered if MIC molecules are induced non-specifically by an inflammatory process or expressed inappropriately on the cells in predisposed individuals [15]. Dysregulated receptor/ligand expression was first reported in RA for *NKG2D/MICA* [16]. High levels of interleukin (IL)−15 and tumour necrosis factor (TNF)-α found in the serum and inflamed joints of RA patients induce expansion of autoreactive $CD4^+CD28^-NKG2D^+$ cells in peripheral blood and synovial tissue [16]. MICA and MICB up-regulated in RA synoviocytes are capable of activating these autoreactive autologous T cells in an NKG2D-dependent manner [17]. While an abundance of soluble MICA (sMICA) has been found in the serum and synovial fluid of RA patients, it is unable to down-regulate the activating NKG2D receptors on NK cells due to massive *NKG2D* up-regulation driven by elevated levels of IL-15 and TNF-α. These reports suggest that dysregulation of *NKG2D* and abnormal expression of MIC in the local tissue environment can cause autoreactive T cell stimulation, contributing to the pathogenesis of RA [15]. There is a paucity of data on the role of *NKG2D* polymorphism on disease susceptibility, severity and outcomes in RA, with the exception of one study from Korea [18]. Hence, this study was carried out to identify the contribution of polymorphisms in *NKG2D* gene towards risk of the development of RA and their influence on disease characteristics and response to methotrexate-based synthetic disease-modifying anti-rheumatic drug (DMARD) therapy in South Indian Tamil patients.

Methods

Study design

This was a case–control immunogenetic study conducted at the Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Puducherry, a major tertiary care referral center in South India.

Study subjects

Ethnic Tamil South Indian patients fulfilling the 2010 modified American College of Rheumatology Criteria (ACR) [19] for rheumatoid arthritis were recruited for the study. A total of 236 patients (215 females and 21 males) were enrolled. Patients with inactive disease or other co-morbid conditions were excluded. The mean age of patients at enrolment was $40{\cdot}9 \pm 11{\cdot}1$ years (range = 18–72 years) and age of onset was $37{\cdot}3 \pm 11{\cdot}6$ years (range = 17–72 years). Their clinical characteristics are presented in Table 1. All patients received methotrexate (MTX) as initial therapy up to a maximum of 25 mg per week. In case of inadequate response, sulphasalazine (SSZ) up to 2 g per day and hydroxychloroquine (HCQ) up to 400 mg per day, with or without low-dose prednisolone ($< 7{\cdot}5$ mg per day) was added. Treatment response was assessed using European League Against Rheumatism (EULAR) response criteria [20] at the end of 6 months or after 8 continuous weeks of a stable combination of DMARD therapy, whichever was later. Clinical

Table 1. Characteristics of the patients with rheumatoid arthritis

Characteristics	n = 236 (%)
Clinical features	
Female RA	215 (91)
YORA	206 (87)
Erosive deforming disease	142 (60)
Extra articular manifestations	53 (22)
Autoantibody status	
IgM-, RF-positive	177 (75)
ACPA-positive	137 (58)
Treatment response	
Good responders	54 (23)
Moderate responders	158 (67)
Non-responders	24 (10)

Ig = immunoglobulin; RA = rheumatoid arthritis; YORA = young onset RA; RF = rheumatoid factor; ACPA = anti-citrullinated peptide antibody.

characteristics assessed were age of onset (young onset RA, or YORA – onset before 55 years); presence or absence of deformities; and presence or absence of extra-articular features related to RA (rheumatoid nodules, sicca symptoms, lung involvement, neuropathy and vasculitis).

A total of 187 (157 females and 30 males) unrelated age-, sex- and ethnicity-matched healthy individuals without family history of autoimmune disorders were included as healthy controls (HC). Written informed consent was obtained from all study participants before enrolment. The study was reviewed and approved by the Institutional Ethics Committee.

Autoantibody measurement

Immunoglobulin (Ig)M rheumatoid factor (RF) in the serum of patients was measured by Nephelometry (Seimens Health Care, Marburg, Germany N Prospec®; Dade Behring, Germany) using kits supplied by Siemens Healthcare Diagnostics Inc. (Marburg, Germany). RF titres above 10 IU/ml were considered positive. ACPA status was determined by sandwich enzyme-linked immunosorbent assay (ELISA) method using a second-generation commercial ELISA kit (Biosystems, Barcelona, Spain). Patients with values of ACPA above 15 IU/ml were labelled as ACPA-positive.

DNA extraction

Genomic DNA from patients and controls was extracted from peripheral blood leucocytes using a standard protocol. The quality and quantity of DNA were analysed by spectrophotometry (Nanodrop, ThermoScientific, Fremont, CA, USA).

NKG2D and *NKG2A* genotyping

We evaluated seven single nucleotide polymorphisms (SNPs) in *NKG2D* gene (rs1049174: *NKC-3*, rs2255336: *NKC-4*, rs2617160: *NKC-7*, rs2246809: *NKC-9*, rs2617169: *NKC-10* and rs2617170: *NKC-11*, rs2617171: *NKC-12*) and one SNP in *NKG2A* gene (rs1983526: *NKC-17*) using a pre-designed TaqMan 5' nuclease assay (Applied Biosystems, Foster City, CA, USA) with allele-specific fluorogenic oligonucleotide probes allowing discrimination of each pair of alleles under investigation. Briefly, 50 ng of DNA was amplified in a 10 µl reaction containing 5 µl of TaqMan genotyping master mix, 0·25 µl of ×40 allelic discrimination mix and 3·75 µl of DNase and RNase free water using the following polymerase chain reaction (PCR) cycling conditions: preread at 60°C for 1 min, hold at 50°C for 2 min, 95°C for 10 min followed by 40 cycles of 95°C for 15 s, 60°C for 1 min and postread at 60°C for 1 min for all the above polymorphisms of NK receptors except *NKG2D12*. PCR conditions for *NKG2D12* was preread at 60°C for 1 min, hold at 50°C for 2 min, 95°C for 10 min followed by 40 cycles of 95°C for 15 s, 60°C for 1 min 30 s and postread at 60°C for 1 min [21]. PCR plates were run and read in the ABI StepOne Plus™ Sequence Detection System (Applied Biosystems). Results were analysed using allelic discrimination software.

Statistical analysis

Sample size was calculated using the CaTS power calculator for genetic studies [22], taking into account the described minor allele frequency with significance level of 0·05 and power of 0·8. Allele and genotype frequencies of the studied SNPs were compared between patients and controls using the χ^2 test with Yates' correction. *P*-values less than 0·05 were considered significant. Both odds ratio (OR) and 95% confidence interval (95% CI) were calculated to assess the relative risk conferred by a specific allele or genotype. Gender, age of onset, disease activity, erosive/deforming disease, extra-articular manifestations, autoantibody (RF, ACPA) positivity and response to DMARD therapy were the characteristics included in the analysis. Deviation from Hardy–Weinberg equilibrium was analysed using χ^2 testing. The statistical software GraphPad InStat was used for carrying out the statistical analysis. Haplotype and linkage disequilibrium (LD) analysis between the three SNPs was performed with the Haploview program version 4.2 (www.broadinstitute.org/haploview). Lewontin's D' measure was used to estimate the intermarker coefficient of LD. Two markers with a D' value greater than 0·7 were defined to be part of the same haplotype block.

Results

Distribution of *NK* receptor genotypes/alleles

Eight different SNPs clustered in the *NK* complex (rs1049174, rs2255336, rs2617160, rs2246809, rs2617169, rs2617170, rs2617171 and rs1983526) were genotyped in

Table 2. Distribution of natural killer (*NK*) receptor alleles and genotypes in study subjects

	RA, $n = 236$, %	HC, $n = 187$, %	*P*	Pc	OR (95% CI)
NK3 (rs1049174)					
CC	86 (36)	75 (40)	n.s.		
CG	97 (41)	83 (44)	n.s.		
GG	53 (23)	29 (16)	n.s.		
NK4 (rs2255336)					
GG	197 (84)	157 (84)	n.s.		
GA	36 (15)	30 (16)	n.s.		
AA	3 (1)	0 (0)	n.s.		
NK7 (rs2617160)					
AA	49 (21)	26 (14)	n.s.		
AT	105 (44)	86 (46)	n.s.		
TT	82 (35)	75 (40)	n.s.		
NK9 (rs2246809)					
AA	2 (1)	0 (0)	n.s.		
AG	35 (15)	30 (16)	n.s.		
GG	199 (84)	157 (84)	n.s.		
NK10 (rs2617169)					
AA	202 (85·5)	158 (84·5)	n.s.		
TA	33 (14)	28 (15)	n.s.		
TT	1 (0·5)	1 (0·5)	n.s.		
NK11 (rs2617170)					
CC	85 (36)	75 (40)	n.s.		
CT	100 (42)	84 (45)	n.s.		
TT	51 (22)	28 (15)	n.s.		
NK12 (rs2617171)					
CC	53 (22)	28 (15)	0·05	0·07	1·64 (0·97–2·84)
CG	101 (43)	86 (46)	n.s.		
GG	82 (35)	73 (39)	n.s.		
NK17 (rs1983526)					
CC	90 (38)	54 (29)	0·05	0·06	1·52 (0·99–2·34)
CG	102 (43)	98 (52)	0·06	0·08	0·69 (0·46–1·04)
GG	44 (19)	35 (19)	n.s.		

Frequencies were determined by χ^2 test. RA = rheumatoid arthritis; HC = healthy controls; Pc = Yates' corrected P; OR = odds ratio; 95% CI = 95% confidence interval; n.s. = not significant. Pc values $< 0{\cdot}05$ were considered significant. Values of Pc and OR mentioned only in those cases where Pc was statistically significant or approaching statistical significance.

236 patients and 187 controls. The genotype/allele frequency did not differ between patients and controls for all the eight different polymorphisms analysed in this study. However, the CC genotype of *NKC-12* (rs2617171) (22 *versus* 15%, Pc = 0·07, OR = 1·64, 95% CI = 0·97–2·84) and the CC genotype of *NKC-17* (rs1983526) (38 *versus* 29%, Pc = 0·06, OR = 1·52, 95% CI = 0·97–2·34) were higher in patients compared to controls, albeit not to a level of statistical significance, suggesting a possible role in susceptibility to RA (Table 2).

Association of *NK* receptor polymorphism with clinical phenotypes

Analysis of the association of the *NK* receptor polymorphisms with clinical characteristics (namely age at onset, presence or absence of deformities, extra-articular manifestations and autoantibody status) was performed. We observed that the frequency of GG genotype and G allele of *NK9* (rs2246809) (91 *versus* 80%, Pc = 0·04, OR = 0·41, 95% CI = 0·16–0·96 and 95 *versus* 89%, Pc = 0·03, OR = 0·41, 95% CI = 0·17–0·91, respectively) and AA genotype and A allele of *NK10* (rs2617169) (92 *versus* 81%, Pc = 0·04, OR = 0·40, 95% CI = 0·15–0·97 and 96 *versus* 90% Pc = 0·04, OR = 0·41, 95% CI = 0·16–0·95, respectively) was significantly higher in individuals without deformities compared to those with deformities. We observed that the frequencies of A allele of *NK9* (rs2246809) and the T allele of *NK10* (rs2617169), respectively, were significantly higher in patients with deformities (11 *versus* 5%, Pc = 0·03, OR = 2·44, 95% CI = 1·09–5·98 and 10 *versus* 4%, Pc = 0·04, OR= 2·45, 95% CI = 1·05–6·39). A similar trend of association was observed in female patients and those with YORA having deformities (Table 3).

Association of *NK* receptor polymorphism with treatment response

Polymorphisms in the *NK* receptor complex were not found to be associated with response to treatment with methotrexate-based synthetic DMARD therapy.

Association of *NK* receptor haplotypes with RA susceptibility

Linkage analysis was performed for the eight bi-allelic SNPs of various genes in the NK cell receptor complex, including *NKG2D3*, *NKG2D4*, *NKG2D7*, *NKG2D9*, *NKG2D10*, *NKG2D11*, *NKG2D12* and *NKG2A17* on chromosome 12. Haplotypes were constructed for 236 patients and 187 controls. We observed that the frequency of haplotype *G-C-A-G-A-T-C-C* was higher in patients than in controls (12 *versus* 8%, $P = 0{\cdot}04$, OR = 1·61, 95% CI = 1·01–2·55) (Fig. 1, Table 4), suggesting that the presence of this haplotype predisposed to the development of RA.

Discussion

NKG2D dysregulation and abnormal MIC expression can cause autoreactive T cell stimulation and promote pathogenic process in RA [15]. It is likely that polymorphisms in *NK* cell receptors such as *NKG2D* may play a major role in modulating NK cell activation. To address this issue, eight different SNPs in the *NK* gene complex (rs1049174, rs2255336, rs2617160, rs2246809, rs2617169, rs2617170, rs2617171 and rs1983526) were genotyped in RA patients and healthy controls. We observed that the

Table 3. Association of NK receptor polymorphism with deformities

	Total RA				Female RA				YORA			
Deformities (%)	+ (%)	– (%)	Pc	OR (95% CI)	+ (%)	– (%)	Pc	OR (95% CI)	+ (%)	– (%)	Pc	OR (95% CI)
NK9												
A	11	5	0·03	2·44 (1·09–5·98)	11	5	0·05	2·26 (1·01–5·57)	10	4	0·04	2·50 (1·07–6·51)
G	89	95	0·03	0·41 (0·17–0·91)	89	95	0·05	0·44 (0·18– 0·99)	90	96	0·04	0·40 (0·15–0·94)
AA	1	0	n.s.		1	0	n.s.		1	0	n.s.	
AG	19	9	n.s.		20	11	n.s.		18	9	n.s.	2·21 (0·89– 5·99)
GG	80	91	0·04	0·41 (0·16–0·96)	79	89	0·07	0·45 (0·17–1·05)	81	91	0·05	0·41 (0·15–0·99)
NK10												
A	90	96	0·04	0·41 (0·16–0·95)	90	95	0·06	0·44 (0·17–1·03)	90	96	0·04	0·38 (0·14–0·93)
T	10	4	0·04	2·45 (1·05–6·39)	10	5	0·04	2·28 (0·97–5·95)	10	4	0·04	2·64 (1·08–7·38)
AA	81	92	0·04	0·40 (0·15–0·97)	80	90	0·07	0·43 (0·16–1·05)	82	92	0·04	0·37 (0·13–0·95)
TA	18	8	0·06	2·39 (0·98–6·42)	19	10	0·09	2·22 (0·91–5·99)	18	8	0·06	2·56 (0·997–7·37)
TT	1	0	n.s.		1	0	n.s.		1	0	n.s.	

RA = rheumatoid arthritis; YORA = young onset RA; Pc = Yates' corrected *P*-value; OR = odds ratio; 95% CI = 95% confidence interval. Pc values < 0·05 were considered significant.

genotype/allele frequency did not differ between patients and controls for all the eight polymorphisms analysed. However, the *G-C-A-G-A-T-C-C* haplotype was more frequent in patients with RA than in healthy controls, suggesting that *NKD2D* may play a role in predisposing to development of RA.

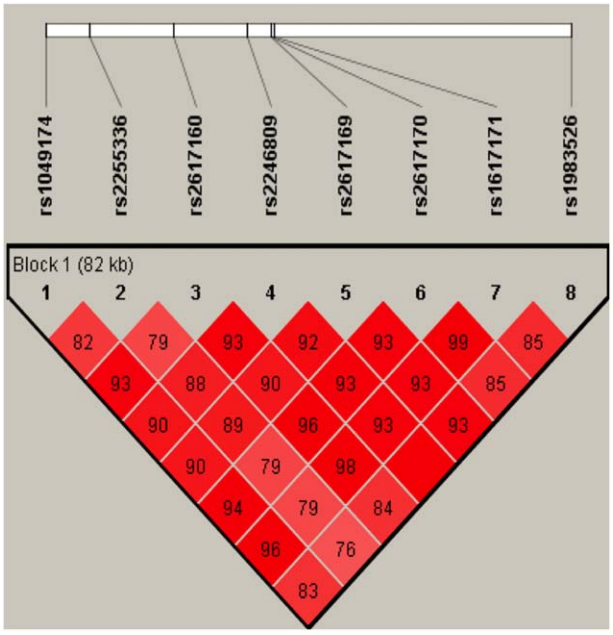

Fig. 1. Linkage disequilibrium of natural killer (*NK*) cell receptor polymorphisms. Haplotypes of various single nucleotide polymorphisms (SNPs) in *NK* cell receptors were constructed using the Haploview software version 4.2. Colour scheme of the linkage disequilibrium map is based on the standard D'/LOD option in the software. Dark squares indicate high r^2 and bright squares indicate low r^2 values. Values in squares are D' between single markers. [Colour figure can be viewed at wileyonlinelibrary.com]

In our study, we observed that the frequency of allele A (rs2246809) and T (rs2617169) was significantly higher in patients with deformities, while that of the other alleles G (rs2246809) and A (rs2617169) was higher in patients without deformities. A similar trend of association was observed with the deforming disease in female patients and in those with young age of onset. However, *NKG2D* polymorphisms did not appear to influence the phenotype related to RF and ACPA autoantibodies.

Park *et al.*, from Korea, reported that the *NKG2D* (rs225536) thr/thr genotype conferred risk of developing RA. They also reported that the increased risk was noted in individuals with *NKG2C* (rs1141715) ser/ser and *NKG2D* (rs225536) thr/thr genotypes and that the presence of these two polymorphisms together increase RA risk by 12-fold [18]. No association was noted with other variants of *NKG2D* (rs1049174 and rs1049172). Contrary to their report that NK cell receptor polymorphism did not affect the antibody production and joint erosions, we observed that *NKG2D* polymorphisms were associated with the development of deformities in South Indian Tamil patients. The differences in our findings could be explained by the larger number of SNPs studied by us in a different population. Therefore, it is quite possible that different polymorphisms of NKG2D influence different disease characteristics in RA.

Our findings compare favourably with studies in other autoimmune disorders, such as diabetes mellitus, paediatric inflammatory bowel disease (IBD) and systemic lupus erythematosus (SLE). In these diseases, *NKG2D* has been reported to play a major role in disease severity and progression by altering the distribution and signal strength of receptors [23–26]. Non-depleting anti-NKG2D treatment during the prediabetic stage of non-obese diabetic (NOD) mice prevented the development of diabetes by impairing

Table 4. Frequency of natural killer (*NK*) receptor haplotypes

1-2-3-4-5-6-7-8	RA, n = 472 (%)	HC, n = 374 (%)	*P*-value	OR (95% CI)
G-C-A-G-A-T-C-C	58 (12)	30 (8)	0·04	1·61(1·01-2·55)
G-C-T-G-A-T-G-C	32 (6·8)	16 (4·3)	n.s.	
G-C-T-G-A-T-G-G	42 (8·9)	46 (12·3)	n.s.	
C-C-A-G-A-C-C-C	78 (16·5)	56 (15)	n.s.	
C-C-T-G-A-C-G-C	44 (9·3)	39 (10·4)	n.s.	
C-C-T-G-A-C-G-G	113 (24)	105 (28)	n.s.	

1-rs1049174, 2-rs2255336, 3-rs2617160, 4-rs2246809, 5-rs2617169, 6-rs2617170, 7-rs2617171, 8-rs1933526. RA = rheumatoid arthritis; HC = healthy control; OR = odds ratio; 95% CI = 95% confidence interval. *P*-values $< 0{\cdot}05$ are considered significant.

the expansion and function of autoreactive $CD8^+$ T cells, suggesting that *NKG2D* is essential for disease progression [23]. In paediatric IBD, prophylactic anti-NKG2D therapy attenuated the development of colitis. It also reduced severity when administered to animals with mild but not severe colitis [24,25]. In the case of systemic autoimmune diseases such as SLE, the *NKG2D* rs2255336 (NKC-4) ala/ala genotype was found to have a high prevalence in German and Spanish patients [26], while the Thr allele (rs2255336) was reported to be protective in SLE patients from Poland [27]. Kabalak *et al*. hypothesized that this SNP does not affect the cell surface expression of *NKG2D* but changes the binding affinity to its adapter DAP10, thus altering the signal strength. In the presence of the ala/ala genotype of NKC-4, activation of $CD4^+CD28^-NKG2D^+$ cells is reduced, leading to severe disease in SLE [26]. Similar mechanisms may be operative in RA, responsible for early onset of the disease and a severe phenotype with deformities reported by us.

Hayashi *et al*. reported the presence of two different haplotype blocks in the NK gene complex. Within these two blocks, allele combinations had either weak cytotoxic activity or high NK activity, which predisposed these patients to develop malignancy differentially [21]. In our study, we identified the *G-C-A-G-A-T-C-C* haplotype as a possible risk haplotype for RA. The alleles of this haplotype were found to be associated with high NK cell activity (HNK) in the study referred to above. Assessment of the degree of cytotoxic activity exhibited by this haplotype in patients with rheumatoid arthritis could form the basis of future investigation.

To conclude, our study shows that *G-C-A-G-A-T-C-C* haplotype of the *NKG2D* gene may predispose to RA. In addition, *NK* cell receptor polymorphisms may also play a role in influencing disease expression, with certain variants causing severe deforming disease. Given the reported abundance of TNF and IL-15 in RA synovium and their ability to activate autoreactive autologous T cells in an NKG2D-dependent manner, it is plausible that polymorphisms in *MICA* and *NKG2D* influence the threshold of effector cell activation in autoimmune disorders such as RA, leading to a poor outcome of the disease [16]. Further functional studies are required to understand the underlying cellular interactions/mechanisms influenced by NK receptor polymorphisms in modifying the phenotype of RA.

Key messages

- Individual *NKG2D* receptor polymorphisms are not risk factors for RA.
- The *G-C-A-G-A-T-C-C* haplotype of NKG2D increases the risk for RA.
- Polymorphisms in *NKC-9* and *NKC-10* are risk modifiers, and may be responsible for a severe disease phenotype of RA.

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Author contributions

V. S. N., R. T., R. K. and D. C. contributed to the conception and design of the work. V. S. N., D. P. M. and V. K. J. contributed to acquisition of clinical data. Laboratory data were generated by C. M. M.; C. M. M., R. T., R. K., D. C., D. P. M., V. K. J. and V. S. N. contributed to analysis and interpretation of the data, C. M. M., D. P. M. and V. K. J. drafted the paper and V. S. N., R. T., R. K. and D. C. critically revised the paper for important intellectual content. C. M. M., R. T., R. K., D. C., D. P. M., V. K. J. and V. S. N. gave approval of the version to be published and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Disclosures

The authors declare they have no disclosures.

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