Influence of activating and inhibitory killer immunoglobulin-like receptors on predisposition to recurrent miscarriages

R.M. Faridi, V. Das, G. Tripathi, S. Talwar, F. Parveen, and S. Agrawal

1Department of Medical Genetics, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Raebareli Road, Lucknow, Uttar Pradesh 226014, India
2Department of Obstetrics and Gynaecology, Queen Mary Hospital, CSM Medical University, Lucknow, Uttar Pradesh, India
3Correspondence address. Tel: +91-522-6680048 ext. 2338, 2346, 2347, 2339; Fax: +91-522-6680973/6680017; E-mail: suraksha@sgpgi.ac.in

BACKGROUND: Understanding of the immune events and mechanisms occurring at the feto–maternal interface is likely to help in understanding the ability of the fetus to survive within the maternal body. Evidence supporting extensive roles of natural killer cells during pregnancy gives rise to a possibility that these NK cells can be mis-regulated and involved in fetal allograft rejection. Killer immunoglobulin-like receptors (KIR) play an important role in regulating the NK cell activity through their activating and inhibiting isoforms. Since there exists a considerable, genetically determined variation in the repertoire of KIR receptors between different individuals, a particular maternal KIR repertoire may predispose to recurrent miscarriages (RMs).

METHODS: Gene-specific PCR amplification (PCR-SSP) was used to determine the individual KIR genotypes in women experiencing RM and controls.

RESULTS: A higher prevalence of activating KIR genes was seen in patients than in controls. Among women experiencing RM, the BB genotypes were more prevalent (P < 0.0001, OR = 4.4, 95% CI = 2.89–6.69) compared with controls.

CONCLUSIONS: Our results indicate that the balance between inhibitory and activating receptor-mediated signals present in natural killer cells is inclined toward a more activating state that may contribute to pregnancy loss.

Key words: recurrent miscarriages / natural killer cells / trophoblast / activating and inhibitory killer immunoglobulin-like receptors

Introduction

Spontaneous miscarriage is defined as the loss of pregnancy prior to the 20th gestational week. The definite cause for many pregnancy losses still remains to be ascertained. It is well recognized that the definition of recurrent pregnancy loss includes three or more consecutive spontaneous miscarriages, taking into account the fact that the risk of miscarriage increases proportionately to the number of previous miscarriages. Despite the paucity of supporting evidence (Porter et al., 2006; Rai and Regan, 2006), the idea of recurrent miscarriage (RM) having an immunological etiology is widespread. Subsets of natural killer cells which populate the decidua during the first and second trimesters are the most debated candidate cells in this context. During early pregnancy, the uterine NK (uNK) cells accumulate as a dense infiltrate around the trophoblast cells, but they progressively disappear from mid-gestation onwards and are absent at term. Therefore, their presence is coincident with trophoblast invasion, because placentaion is complete at approximately the 20th week of gestation. The fetal trophoblast does not express the classical HLA-A and HLA-B antigens and this may protect it from attack from maternal T cells, whereas on the other hand, the lack of classical class I HLA antigens might render it susceptible to attack from NK cells. The possibility that uNK cells interact with the fetal trophoblast is suggested by the observations that uNK cells have a series of receptors including the killer immunoglobulin-like receptor (KIR) family and the CD94/NKG2 family (Hiby et al., 1997; Verma et al., 1997, Wilson et al., 2000; Mcqueen and Parham, 2002). The ligands for these receptors include HLA-C, HLA-E and HLA-G, which are expressed on the trophoblast cells (King et al., 2000a, b; Moffet and Loke, 2002). Of these, only HLA-C is highly polymorphic and interacts with the KIRs expressed...
on the NK cell surface. While the precise role of the uNK cells is not clear, there is evidence to suggest their involvement in maternal blood vessel remodeling (Ashkar et al., 2000; Croy et al., 2003). Several studies have shown that women with RM have elevated NK cell numbers and activity in the peripheral blood (Aoki et al., 1995; Yamada et al., 2003) as well as in the endometrium (Clifford et al., 1999). There is, however, a considerable debate concerning this issue with conflicting reports. Moffet al. (2004) and Rai al. (2005) reviewed at length the fundamental flaws in the methodologies used in measuring the number and activity of both peripheral as well as uNK cells. Currently, the role of both peripheral blood and uNK cells in RM remains unknown, which means that understanding the function of uNK cells is still a major challenge in human reproduction.

The KIR family of receptors contributes to the regulation of uNK cell function through their activating and inhibitory isoforms. The KIR haplotypes vary greatly in the presence or absence of a particular gene and are categorized into two groups designated as A and B. The classification is based on the number and type of genes encoding inhibitory and activating KIRs. The simpler group-A KIR haplotype comprises a fixed gene content of KIR3DL1,3DL2,DLA1,2DP1,3DP1,DLA4,3DL1,2DS4,2DL2. Haplotypes carrying any other combination of KIR loci are classified as group-B haplotypes. Although both haplogroups have comparable genes for inhibitory KIR, they differ in the genes encoding activating KIR. Generally, the group-B KIR haplotypes encode more activating KIRs (Rajalingam et al., 2008). Likewise, KIR-mediated NK cell responses in individuals with two copies of A haplotypes (AA) are mainly inhibitory in nature. The KIR-HLA-C receptor—ligand interactions conform to the dimorphisms in the HLA-Cw a1 domain, which are characterized by Ser77/Asn80 and Asn77/Lys80, and define serologically distinct allotypes of HLA-Cw, viz. group I and group 2, respectively (Colonna et al., 1993; Parham, 2005). C1 allotypes are the ligands for KIR2DL2/3 (inhibitory) and possibly KIR2DS2 (activating), whereas C2 allotypes are bound by inhibitory KIR2DL1 and activating KIR2DS1 receptors. Other activating KIR2DS receptors (KIR2DS3, 2DS4 and 2DS5) with very similar sequences may also bind to HLA-C molecules (Carrington and Norman, 2003).

It is imperative, however, that the purpose of these receptor—ligand interactions on the NK cell—trophoblast interface is either to activate the NK cells (through activating receptors) thereby stimulating them to secrete blood vessel modifying cytokines, and/or to inhibit them (through inhibiting receptors) thereby protecting the trophoblast from NK-mediated lysis. Therefore, fine tuning between the fetal antigens and the maternal NK receptors is believed to be a crucial feature of the fetal–maternal interaction. Nonetheless, it could be hypothesized that as there exists a considerable genetic variation in the repertoire of KIR receptors between different individuals, a particular KIR repertoire might predispose an individual to RM. Till date, there have been only a few reports pertaining to KIR gene polymorphisms with RM, with conflicting conclusions. Witt et al. (2004) reported that the maternal KIR repertoire was not associated with RM, whereas Varla-Leftherioti et al. (2005) reported fewer appropriate inhibitory KIRs in RM women in a study of 26 patients. Likewise, Wang et al. (2007) and Hiby et al. (2008) reported an association between the maternal KIR repertoire and HLA-Cw alleles in 67 and 73 RM couples, respectively. Therefore, in order to investigate whether or not the outcome of pregnancy depends on the maternal KIR gene repertoire, we have analyzed the KIR genotypes of a well-characterized RM group of women who have suffered three or more miscarriages with unknown etiology. We have compared our results with a panel of ethnically matched parous control women with at least two live births and no history of previous miscarriages.

Materials and Methods

Genomic DNA samples

All the samples were collected from patients attending the Out Patients’ Department of Sanjay Gandhi Post Graduate Institute of Medical Sciences (SGPGIMS), Lucknow, and Queen Mary Hospital of CSM Medical University, Lucknow, Uttar Pradesh, India, for the evaluation of RM. All had a history of at least three spontaneous miscarriages (mean 4, range 3–7) and no previous successful pregnancy. All the patients were screened for factors relating to various known causes of miscarriages, including parental chromosomes, Day 2 hormone levels of follicle-stimulating hormone (3–11 U/l), leutinizing hormone (3–12 U/l) and testosterone (0.5–3 mol/l), antiphospholipid antibodies including lupus anticoagulant (positive likelihood ratio, 0.8–1.05) and anticardiolipin antibodies (IgG 0–12 GPL units, IgM 0–5 MPL units) and prothrombotic risk factors including activated protein-C resistance (2.6–4.36 ratio), factor V Leiden and prothrombin mutations, leutal phase insufficiency, prolactin dosage, glycosylated curve, thyroid hormone levels, Toxoplasmosis, Cytomegalovirus, Rubella, HIV, group B streptococci, Chlamydia trachomatis, hepatitis B and C and bacterial vaginosis. The uterine cavity was investigated for cervical incompetence by hysteroscopy, hysterosalpingography and serial ultrasound. Of all the initially screened individuals, 39% (n = 205) had no known cause of RM were included in this study. All patients selected were primary aborters, not having any live child and belonged to four different caste groups: upper caste Hindus (Brahmin, Vaiysha, Kayastha and Kshatriya), backward class (OBC and SC/ST), Muslims (Shiyah and Sunni) and others (Siddhi, Sikh, Jain and Christians) Patients’ detailed clinical information was recorded prior to inclusion in this study. The control group consisted of 224 healthy parous women of the same ethnic distribution as that of RM patients (Table S1, Supplementary data), and having at least two live births and with no history of miscarriage, pre-eclampsia, ectopic pregnancy or preterm delivery. From both controls and RM women, 5 ml of blood was collected in EDTA-coated collection vials and DNA was extracted using Qiagen kits. This investigation was approved by the Ethics Committees of SGPGIMS and Queen Mary Hospital, CSM medical University, Lucknow, Uttar Pradesh, India. Written informed consent to participate in this study was obtained from all the individuals.

KIR genotyping

All the DNA samples of controls as well as RM patients were typed for the KIR genes responsible for inhibitory signals (2DL1, 2DL2, 2DL3, 3DL1, 3DL2, 3DL3, 2DL4 and 2DL5) and for activating signals (2DS1, 2DS2, 2DS3, 2DS4, 2DS5 and 3DS1), as well as two pseudo-genes 2DP1 and 3DP1, based on the primers described by Vilchis et al. (2007) and Murdoch et al. (2006). Briefly, the KIR genes were typed for the presence or absence by using 17 PCR-SSP reactions, each containing between two and four KIR-specific primers. Every combination of sense and antisense primers used was specific for a single gene (Table I). All reactions contained an internal positive control consisting of an additional pair of primers specific for non-polymorphic sequences of the HLA-DRA gene: FDRasa360 (5’-gaggtacatctgctcagcagc-3’) and RDRasa955 (5’-ggttcttacacccttcgctg-3’) for reactions 1–16 (product length: 283 bp), and FDRasa360 and RDRasa633 (5’-cagctgctcgtcctcgtg-3’) for reaction 17 (product length: 608 bp). In each reaction, 50 ng of genomic DNA was

---

**Materials and Methods**

**Genomic DNA samples**

All the samples were collected from patients attending the Out Patients’ Department of Sanjay Gandhi Post Graduate Institute of Medical Sciences (SGPGIMS), Lucknow, and Queen Mary Hospital of CSM Medical University, Lucknow, Uttar Pradesh, India, for the evaluation of RM. All had a history of at least three spontaneous miscarriages (mean 4, range 3–7) and no previous successful pregnancy. All the patients were screened for factors relating to various known causes of miscarriages, including parental chromosomes, Day 2 hormone levels of follicle-stimulating hormone (3–11 U/l), leutinizing hormone (3–12 U/l) and testosterone (0.5–3 mol/l), antiphospholipid antibodies including lupus anticoagulant (positive likelihood ratio, 0.8–1.05) and anticardiolipin antibodies (IgG 0–12 GPL units, IgM 0–5 MPL units) and prothrombotic risk factors including activated protein-C resistance (2.6–4.36 ratio), factor V Leiden and prothrombin mutations, leutal phase insufficiency, prolactin dosage, glycosylated curve, thyroid hormone levels, Toxoplasmosis, Cytomegalovirus, Rubella, HIV, group B streptococci, Chlamydia trachomatis, hepatitis B and C and bacterial vaginosis. The uterine cavity was investigated for cervical incompetence by hysteroscopy, hysterosalpingography and serial ultrasound. Of all the initially screened individuals, 39% (n = 205) had no known cause of RM were included in this study. All patients selected were primary aborters, not having any live child and belonged to four different caste groups: upper caste Hindus (Brahmin, Vaiysha, Kayastha and Kshatriya), backward class (OBC and SC/ST), Muslims (Shiyah and Sunni) and others (Siddhi, Sikh, Jain and Christians) Patients’ detailed clinical information was recorded prior to inclusion in this study. The control group consisted of 224 healthy parous women of the same ethnic distribution as that of RM patients (Table S1, Supplementary data), and having at least two live births and with no history of miscarriage, pre-eclampsia, ectopic pregnancy or preterm delivery. From both controls and RM women, 5 ml of blood was collected in EDTA-coated collection vials and DNA was extracted using Qiagen kits. This investigation was approved by the Ethics Committees of SGPGIMS and Queen Mary Hospital, CSM medical University, Lucknow, Uttar Pradesh, India. Written informed consent to participate in this study was obtained from all the individuals.

**KIR genotyping**

All the DNA samples of controls as well as RM patients were typed for the KIR genes responsible for inhibitory signals (2DL1, 2DL2, 2DL3, 3DL1, 3DL2, 3DL3, 2DL4 and 2DL5) and for activating signals (2DS1, 2DS2, 2DS3, 2DS4, 2DS5 and 3DS1), as well as two pseudo-genes 2DP1 and 3DP1, based on the primers described by Vilchis et al. (2007) and Murdoch et al. (2006). Briefly, the KIR genes were typed for the presence or absence by using 17 PCR-SSP reactions, each containing between two and four KIR-specific primers. Every combination of sense and antisense primers used was specific for a single gene (Table I). All reactions contained an internal positive control consisting of an additional pair of primers specific for non-polymorphic sequences of the HLA-DRA gene: FDRasa360 (5’-gaggtacatctgctcagcagc-3’) and RDRasa955 (5’-ggttcttacacccttcgctg-3’) for reactions 1–16 (product length: 283 bp), and FDRasa360 and RDRasa633 (5’-cagctgctcgtcctcgtg-3’) for reaction 17 (product length: 608 bp). In each reaction, 50 ng of genomic DNA was
amplified in 10 μl of PCR buffer [67 mM Tris–HCl, pH 8.8, 16 mM (NH₄)₂SO₄, 2 mM MgCl₂, 0.01% Tween 20 and 100 mM dNTPs] containing 0.5 U of Taq DNA polymerase. All reactions were conducted in an oil-free thermal cycler (PTC 200 Thermal Cyclers, Bio-Rad Inc.). The amplification products were electrophoresed on 2% agarose gels containing ethidium bromide with a migrating distance of 3 cm, and the product bands were visualized under ultraviolet light. The presence of each gene was determined by a band of the expected size (Figure 1). Individuals were determined negative for a KIR gene when a band of the expected size was absent in the presence of the control band. For easier size discrimination of KIR2DS4 full length and deletion variants, aliquots of PCR products were run separately on the gel, and individuals were assigned positive when either or both the variants gave signals, whereas when both the variants were absent, the individual was labeled as KIR2DS4 negative. The data were verified and validated by Dr Raja Rajalingam of University of California, Los Angeles (UCLA), CA, USA. In cases of unique profiles or previously unreported profiles, genotyping was repeated at least twice.

### Statistical analysis

Gene and genotype frequency was determined by direct counting. Frequency differences between the RM and control groups were tested for significance using the χ² test. The magnitude of the effect was estimated by odds ratios and their 95% confidence intervals (Windows 11.0.0.2001; SPSS Inc.). The different significances of average inhibitory or activating KIR numbers between RM patients and control subjects were tested and a linear model with a logistic link was used to test the association between increasing/decreasing numbers of activating/inhibiting KIR and the prevalence of RM. P-values < 0.05 were considered significant. Yates’ corrections were applied wherever required.

### Results

Using gene-specific PCR amplifications, we have analyzed the presence/absence of 17 KIR genes in a panel of 205 RM patients and compared them with that of 224 ethnically matched healthy parous women. The observed genotypes, their designations and frequencies are included in the Supplementary data (Tables S2 and S3).

### Varied gene content incidences among patients and controls

We observed significant differences in the prevalence of individual genes for 2DL1, 3DL1, 2DS2, 2DS3, 2DS4 and 3DS1 in patients when compared with controls. It was observed that there was a decrease in the occurrence of inhibitory KIR2DL1 and KIR3DL1 genes in the RM patients in association with an increased incidence of activating KIR2DS2, 2DS3 and 3DS1 genes when compared with the control group (Table II). The only activating KIR (2DS4) which is associated with the group-A haplotypes was found to be significantly higher in the control group. Gene frequencies for other inhibitory KIRs (2DL2, 2DL3, and 2DL5) did not reach statistical significance, whereas genes comprising framework loci (3DL2, 3DL3, 2DL4 and 3DP1) were found in all the individuals. We observed that the odds of occurrence of RM in the patient’s group were linearly related to the increased incidences of activating KIR3DS1 (~3.5-fold), 2DS2 (~1.9-fold) and 2DS3 (~2-fold). There was significant protective effect against RM associated with inhibitory KIR2DL1 and 3DL1 gene contents among patients and controls (P < 0.0001, OR = 0.09, 95% CI = 0.04–0.19; P < 0.0001, OR = 0.27, 95% CI = 0.17–0.44.

| Table 1 Sequence-specific primers for KIR genotyping |
|---|---|---|---|
| S. No. | Gene | Sense primer | Antisense primer | Product (bp) |
| 1 | 2DL1 | GGTTGTCAGATGTCATGTGTGAA | CTCGCAGAGTCTTGGCGA | 142 |
| 2 | 2DL2 | AACACTTCCTCTCAGCCCA | GCCCTGCAGAGAACTACA | 142 |
| 3 | 2DL3 | AAGACCCTCAGGGAGTTG | CAGGAGAACCCTTTGTGATCA | 156 |
| 4 | 3DL1 | TCATCCGGTCCCCATGATGGT | CCGAGATGTCCAGGGGA | 109 |
| 5 | 3DL2 | CATGACGTAGGCTCAG | GACCAACCGCAGGGAC | 131 |
| 6 | 2DS1 | TCTCCATCTAGTCGATGAA | GTGCAGGGCAGCTGAC | 96 |
| 7 | 2DS2 | TGGCAGAACAGGGGAAGTGA | CTCGCAGAGTCTTGGCGA | 110 |
| 8 | 2DS3 | CTTGTGCTGACTTGCCT | GCTACTGGTGCTTGCCT | 158 |
| 9 | 2DS4 | GGTGACAGGGAGAGAAT | CTGGAATGTCGCTTGATG | 133/111 |
| 10 | 2DS5 | AGAGAGGGGACGGTATAC | CTGATAGGGGAGTGTGAT | 147 |
| 11 | 3DS1 | CATCGGTTCTCATGATGCG | CCACGGTGCACCGGA | 107 |
| 12 | 3DS1b | CATCGGTTCTAGATGCG | 107 |
| 13 | 2DP1 | CGACATTTGGACCTCACC | GGGAGCTGACAACGTGAT | 141 |
| 14 | 3DL1 | AATGGTGTGCAGATGTCAG | GCGCACAATCTCAGGGTA | 196 |
| 15 | 3DX1 | TTCTTGTTGCGCCCGTCACA | GTCCTGGGCGCTTAGAT | 88 |
| 16 | 2DL1 | TCAGGACAGCCCTTCTGC | GGGAGGGACCCCCATCCTTC | 131 |
| 17 | 3DP1 | GTACGTACCTCCCTCATGATGTA | GAAACGGTGTTCGGAATA | 398 |
Genetic variability of KIR and recurrent miscarriage

Haplotypic and genotypic variability among patients and controls

We further characterized the genotypic profiles of patients as well as controls in group-A and -B haplotypes based on their gene contents (Fig. 1). Individuals who carried a fixed gene set of nine genes consisting of KIR2DL3-2DL1-2DL3-2DL3-2DL1-2DS5-2DS2-2DS3-2DS4, characteristic of group-A haplotypes, were considered as having two copies of group-A KIR haplotypes (AA genotypes). On the other hand, individuals lacking any of the four variable genes (KIR2DL1, 2DL3, 3DL1, and 2DS4) were regarded as carrying two copies of group-B haplotypes (BB genotypes). All the remaining combinations were regarded as heterozygotes carrying both the haplogroups, i.e. AB genotypes. Of the patient group, 3.9% of individuals were homozygous for group A (AA), 76.1% for group B (BB), whereas 20.0% had the AB genotype, when compared with 8.5% for AA, 43.3% for BB and 48.2% for AB genotypes, respectively, in the control population (Fig. 2). There was a significant association of the increased activating gene content with RM patients, which is evident from the increased prevalence of BB (P < 0.0001, OR = 4.4, 95% CI = 2.89–6.69) genotypes among the patient group when compared with the control group.

Discussion

The present study is the first report demonstrating the association of maternal uNK cell KIR gene repertoire with RM among North Indian women. Our results support the view of the cumulative effects of activating and inhibiting signals on the modulation of the effector function of uNK cells. We found a shift toward the over activation of NK cells based on the individual’s KIR gene content, comprising of a greater number of activating and reduced number of inhibitory KIR2DL1 genes in the patient group compared with the control group. The RM patients group showed a higher prevalence of B haplotypes when compared with A haplotypes. Individuals having BB genotypes were at a greater risk for spontaneous miscarriages (~4.4-fold) than those having AA or AB genotypes. There was a significant association of RM patients with B haplotypes.

Several factors have been proposed to explain or contribute to maternal tolerance to the potentially allogenic fetus, although our understanding of the immunobiology of normal pregnancy and its implications for pregnancy-related pathologies is still limited (Pearson, 2002). One prominent feature of pregnancy is that human decidua has a large number of NK cells, which constitute 70% of resident lymphocytes (Moffett-King, 2002). Thus, human uNK cells have been thought to play a role in the implantation and pregnancy, at least in early gestation. Although the precise functions of uNK cells in vivo are still unknown, their proximity to the invading trophoblasts, which lack expression of classical HLA-A and -B antigens (Faulk and Temple, 1976) but selectively express HLA-C and the non-classical HLA-G, HLA-E and CD1d molecules (King et al., 1996), has led to the hypothesis that these MHc antigens on trophoblasts interact with NK cell receptors (Boyson et al., 2002). The KIR locus is polygenic and polymorphic, giving rise to variable KIR repertoires that are expressed on subsets of NK cells among individuals. It is unclear if and which receptors on uNK cells interact with trophoblast-expressed HLA molecules, and whether such interactions inhibit NK cell lysis, or lead to production of cytokines that favor normal placental development and maintenance of pregnancy.

In the present report, we have demonstrated that the number of activating KIR genes were higher in patients with unexplained RM when compared with control subjects. Hence, we hypothesized that the number of activating KIR genes per phenotype may influence disease susceptibility through a gene dosage effect, which may lead to the rejection of a potentially allogenic fetus. Although the potentially deleterious effects of activating KIRs are certainly not lethal, these genes may remain and continue to induce or modulate pathogenesis of RM. In the findings of Hiby et al. (2004), the maternal AA genotype was associated with increased risk of pre-eclampsia, another pathological condition believed to be having similar pathogenicity with RM. They, however, implicated the strong inhibition of NK cell activity through increased inhibitory receptor-mediated signaling...
in incomplete remodeling of spiral arteries. Our findings reflected a shift toward the over activation of NK cells through increased activating KIRs. The effect is not directly lethal, but it can contribute to the alteration of the cytokine milieu which could have important effects on trophoblast behavior by affecting functions, for example, integrin expression and metalloproteinase production (Norwitz et al., 2001), which are important for their survival, growth and differentiation.

The genetic predisposition caused by the maternal KIR gene content is subject to the contribution of HLA-C genotype. For example, individuals who are 2DL1−2DS1+ were found to be more prevalent in the patients group (14.7%) when compared with controls (2.4%). This reflects a particular risk group if the fetus carries HLA-C2 genotype. In the normal situation, the uNK cells are proposed to mediate a mucosal immunological balancing act that prevents trophoblast overinvasion, but also allows degree of placental access to maternal blood supply. A compromise is reached and both maternal and fetal polymorphic gene systems and NK cell KIR genes may affect this compromise.

There are only a few studies to date where maternal KIR gene repertoires have been investigated for RM, and these have conflicting conclusions. Witt et al. (2004) reported, in a study including 51 Brazilian Caucasian women, that maternal KIR repertoire was not associated with RM, whereas Varla-Leftherioti et al. (2005) reported fewer appropriate inhibitory KIR in RM women in a study of 26 patients. Flores et al. (2007) conducted similar studies in 30 RM couples and their findings support that in RM patients, the balance between inhibitory and activating receptors present in natural killer cells is inclined toward an activating state that may contribute to pregnancy loss. They, however, found an increased prevalence of the inhibitory AA genotypes among the patients’ cohort. Likewise, Wang et al. (2007), in a study with 67 RM couples from a Chinese Han Population, demonstrated that RM is associated with increased frequency of activating KIR genes. Yet recently, Hiby et al. (2008), in a study on 73 RM patients, reported an increased prevalence of the maternal AA genotype. It is important to appreciate the influence of different ethnic groups, while conducting such studies on the outcome of KIR frequencies, as KIR genotypes have a wide geographical distribution.
This is exemplified by the increased prevalence of AA genotypes reported by Hiby et al. (2008) where all the participants were Caucasians. The frequency of B haplotypes is higher and the frequency of A haplotypes is lower in North Indians than in other Eurasian populations including Caucasian, Palestinian, Thai and Vietnamese panels (Uhrberg et al., 1997; Witt et al., 1999; Crum et al., 2000; Norman et al., 2001; Toneva et al., 2001; Rajalingam et al., 2002). We conducted this study on a relatively larger sample size when compared with others in order to investigate whether or not the outcome of pregnancy depends on the maternal KIR gene repertoire, and revealed that there exists an increased prevalence of maternal BB genotypes among patients when compared with controls. This demonstrates an inclination of the NK cell activity toward a more activating state.

Understanding the basis for the observed genetic associations is complicated due to the large repertoire of receptors used by NK cells to interpret their environment. There is extensive polymorphism among KIR haplotypes, which differ not only in nucleotide sequence but also in gene content (Parham 2005). This genetic complexity echoes the complications confronted clinically in defining and diagnosing RM, which can be considered not so much a disease or disorder but ‘simply the extreme end of a continuum of characteristics common to all pregnancies.’ Analysis of KIR genotype and phenotype in repeated miscarriages may have a practical application as a marker supporting the diagnosis of the alloimmune etiology in miscarriages.

**Author’s role**

R.M.F. was involved in the genotyping of the samples, analyzed the results and drafted the manuscript. V.D. helped with the clinical evaluation of the RM patients. G.T. was involved in the statistical calculations. S.T. was involved in KIR genotyping. F.P. collected blood samples and extracted DNA. S.A. designed the study, provided intellectual input and helped in finalizing the manuscript.

**Supplementary data**

Supplementary data are available at http://humrep.oxfordjournals.org/.

**Acknowledgements**

We are thankful to Dr Raja Rajalingam (UCLA Immunogenetics Center, University of California at Los Angeles, Los Angeles, USA) for his kind and generous help in verification of KIR genotyping and frequency data. R.M.F. is receiving his Doctoral Fellowship from the Department of Biotechnology, Government of India, New Delhi, India.

**Funding**

We are indebted to the Department of Science and Technology, New Delhi, India for financial support.

**References**


Hiby SE, King A, Sharkey AM, Loke YW. Human uterine NK cells have a similar repertoire of killer inhibitory and activatory receptors to those found in blood, as demonstrated by RT-PCR and sequencing. *Mol Immunol* 1997;34:419–430.


Submitted on July 18, 2008; resubmitted on January 31, 2009; accepted on February 4, 2009