ID Design 2012/DOOEL Skopje, Republic of Macedonia
South East European Journal of Immunology
Volume 2015; Article ID 20002, 5 pages
http://dx.doi.org/10.3889/seejim.2015.20002
Immunogenetics

KIR Gene Frequencies in Women with Infertility Problems

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Citation: Djulejic E, Petlichkovski A, Trajkov D, Dimitrov G, Alabakovska S. KIR Gene Frequencies in Women with Infertility Problems. SEE J Immunol. 2015 Feb 04; 2015:20002. http://dx.doi.org/ 10.3889/seejim.2015.20002

Key words: Killer immunoglobulin-like receptors (KIRs); women; infertility.

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Received: 18-Nov-2014; **Revised:** 25-Dec-2014; **Accepted:** 21-Jan-2015; **Published:** 04-Feb-2015

Competing Interests: The author have declared that no competing interests exist.

Abstract

BACKGROUND: Natural killer (NK) cells are the predominant lymphocyte population in the decidua. Being the most abundant leucocytes, the activity of NK cells is important in different immuno-pathological conditions, such as recurrent spontaneous abortions, infertility and problems in implantation. The NK cells recognize HLA class I molecules on trophoblasts trough killer immunoglobulin-like receptors (KIRs) found on their surface. The KIRs are classified as either activating or inhibitory, regarding the effect they produce on NK cells upon interaction with corresponding ligand. Since KIR genes exhibit extensive polymorphism and individuals differ in both the number and kind (activating vs. inhibitory) of KIR genes, it is hypothesized that the KIR gene content might influence the pregnancy outcome.

AIM: The aim of this pilot study is to analyze the frequency of different KIR genes in women with infertility problems, and compare them to healthy women.

MATERIAL AND METHODS: Total of 122 healthy women (Control) and 25 women with reproductive problems (MISSC) participated in this study. After signing of written consent DNA was isolated from peripheral blood using phenol/chloroform method. The genotyping of 16 KIR genes was performed using commercially available kit from Dynal Biotech, (Pel-Freez Clinical Systems, Brown Deer, WI, USA), based on SSP method.

RESULTS: We found that inhibitory KIR are present in similar observed frequency in both control and patients with MISSC, except KIR2DL5 which was found in lower frequency in patients with MISSC. There are no significant differences of all noninhibitory KIR between control and patients with MISSC. The number of inhibitory KIR genes in patients with MISSC was lover, except for seven inhibitory KIR genes which was almost doubled. The number of noninhibiotry (stimulatory) KIR genes was lower in patients with MISSC, except for those with three KIR genes which were almost four times more frequent. We found significantly bigger percentage of 0.34 – 0.60 activating/inhibitory KIR gene number ratio in the patients with MISSC.

CONCLUSION: In conclusion, there are differences in the KIR gene distribution, gene number, and activating/inhibitory KIR gene number ratio between control and Macedonian patients with MISSC. Further analysis of frequencies of corresponding KIR genotypes or in the ratio of activating/inhibiting genes content in two groups are needed.

Introduction

Natural killer (NK) cells are the predominant lymphocyte population in the decidua. Being the most abundant leucocytes, the activity of NK cells is important in different immuno-pathological conditions, such as recurrent spontaneous abortions, infertility and problems in implantation. The NK cells recognize HLA class I molecules on trophoblasts trough killer immunoglobulin-like receptors (KIRs) found on their surface. The KIRs are classified as either activating or inhibitory, regarding the effect they produce on NK cells upon interaction with corresponding ligand. Since KIR genes exhibit extensive polymorphism and individuals differ in both the number and kind (activating vs. inhibitory) of KIR genes, it is hypothesized that the KIR gene content might influence the pregnancy outcome.

Natural killer (NK) cell receptors (NKRs) have been suggested to protect trophoblast, but their function at the fetomaternal interface remains

unknown. To investigate if the outcome of pregnancy depends on women's NKRs, we studied the NKR repertoire in couples with recurrent spontaneous abortions (RSA). Less aborters than controls were found to have all three inhibitory KIRs, some of them had only one inhibitory KIR and most of them were lacking inhibitory KIRs possessed by their husbands. It was concluded that women with alloimmune abortions have a limited inhibiting KIR repertoire and such miscarriages may occur because trophoblastic HLA class I molecules are recognized by decidual NK cells lacking the appropriate inhibitory KIRs [1].

Another study from a total population of 158 recurrent spontaneous abortion (RSA) couples, 40 couples with repeated implantation failures (IVF) and 81 control couples, reported by five different laboratories, analysis was performed. RSA couples were divided into alloimmune aborter (RSAallo) and autoimmune aborter (RSAauto). The ratio of inhibitory to activating KIR (actKIR) was slightly lower in RSAallo and IVF women, while in a high percentage of these women, the standard receptors of the KIR A haplotype were combined with actKIR/s of the haplotype B. This may suggest a possible involvement of actKIRs in embryo implantation and the maintenance of pregnancy and also requires further investigation [2].

Published results from China showed that gene frequency of KIR2DS1 was higher in patients with RSA compared to that of control subjects. Increased numbers of activating KIR genes was observed in patients. Women who possessed more than two activating KIR genes were found more frequently in patients than those in control subjects. From a cohort of husband and wife couples, the women with a KIR2DS1 gene, and with a decreased group 2 HLA-C allele for the homologous inhibitory receptor KIR2DL1, had a tendency to fall into the RSA group. The results suggest that a genetic variation at the KIR locus influences the susceptibility to unexplained RSA in the Chinese Han population. Moreover, decreased ligands for inhibitory KIRs could potentially lower the threshold for NK cell activation, mediated through activating receptors, thereby contributing to pathogenesis of RSA [3].

Hiby et al., 2008 obtained DNA from the male and female partners of couples with three or more spontaneous miscarriages and genotyped for HLA-C groups and 11 KIR genes using the PCR-sequence-specific primer method (SSP). The frequency of the HLA-C2 group was increased in both parents compared with a parous control population. The KIR gene frequencies of the male partners were similar to controls, but the women had a high frequency of KIR AA haplotypes that lack activating KIR. In particular, the activating KIR for HLA-C2 groups (KIR2DS1) was significantly lower in these women. This was the first report to identify a genetic male factor that confers risk in RM. These findings support the idea that successful placentation depends on the correct balance of NK cell inhibition and activation in response to trophoblast [4].

The aim of this pilot study is to analyze the frequency of different KIR genes in women with infertility problems, and compare them to healthy women.

Methods

Investigated groups

The total studied sample consists of 147 examinees composed of two different groups: healthy individuals (n=122) (Control) and patients with infertility problems (including spontaneous miscarriages) (MISSC) (n=25).

Each individual was interviewed on a one-to-one basis; his/her genealogy was recorded for the last three generations. Admixture, if any, was recorded for each individual. Individuals with only one Albanian parent were excluded from the study. After signing of written consent, genomic DNA was extracted from the peripheral blood leukocytes using standard phenol/chloroform procedure, described elsewhere [5], and stored in the anthropology project field of the Macedonian Human DNA Bank (hDNAMKD) [6] until processing.

PCR amplification

For *KIR* genotyping, commercially available PEL-FREEZ *KIR* genotyping SSP kit (Dynal Biotech, Brown Deer, WI) was used. It is a PCR-based method (using sequence-specific priming approach) designed to detect the presence or absence of 16 *KIR* genes and pseudogenes defined by the International nomenclature committee of WHO [7, 8]. In brief, locus specific primer sets, dispensed in a 96 well thermal tray were used for amplification of genomic DNA. After the amplification, the PCR products are loaded and separated by electrophoresis onto a 2% agarose gel stained with ethidium bromide, after which the results are interpreted using a worksheet for the specific amplification patterns. The presence of each *KIR* gene was determined by the presence of a band of DNA of the expected size (Fig. 1).

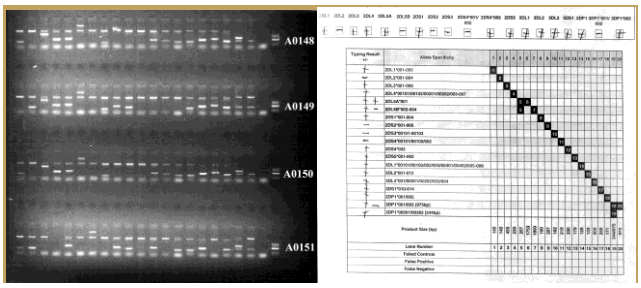

Figure1: Electrophoresis of PCR products (left) and KIR genotyping documentation form (right).

All PCRs contained an internal positive control consisting of an additional pair of primers specific for the growth hormone (GH) gene and a negative control [9]. Individuals were determined negative for a particular *KIR* gene when a band of expected size was absent in the presence of a band for the GH gene. We have used external quality control consisting of cell lines from Immunogenetics and Histocompatibility Worskshop Conferences and Centre d' Etude du Polymorphisme Humain.

Statistical analysis

The occurrence of *KIR* genes in individuals (frequency = F) was obtained by direct counting. Gene frequencies (GF) were calculated using the formula GF=1-√(1-F), being aware of the limitation in its ability to detect *KIR* genes present at low frequency. For analysis of the molecular polymorphism of the locus studied, the Arlequin software version 3.0 [10] was used.

Linkage disequilibrium (LD) values for two locus associations were calculated using 2×2 tables [11]. Because LD is not independent of allele frequencies, normalized LD was calculated as described previously [12, 13]. Comparisons of different genotypes for two groups were tested by the χ2 test. Crude odds ratios (OR) were calculated within 95% CI.

Results

Observed and estimated KIR gene frequencies for 25 women with infertility problems (including recurrent spontaneous miscarriages) (MISSC) and 122 healthy controls are shown in Table 1. We can see that KIR pseudogenes are present in all investigated women, except in one of the controls and one in the group with MISSC. Inhibitory KIR are present in similar observed frequency in both control and patients with MISSC, except KIR2DL5 which was found in lower frequency in patients with MISSC (0.41 versus 0.16, respectively; Pearson's P = 0.018). The most frequent noninhibitory KIR was KIR2DS4 with estimated frequency of 0.80 in controls and 1 in patients with MISSC followed by KIR2DS2 with the estimated frequency of 0.35 in controls and 0.34 in women with MISSC. There are no significant differences of all noninhibitory KIR between control and patients with MISSC.

The content of inhibitory and noninhibitory genes in controls and women with infertility problems is shown in Table 2. The number of inhibitory KIR genes in patients with MISSC was lover, except for seven inhibitory KIR genes which was almost doubled. The number of noninhibiotry (stimulatory) KIR genes was lower in patients with MISSC, except for those with three KIR genes which were almost four times more frequent.

Table 1: Observed and estimated KIR gene frequencies for 25 women with infertility problems (including recurrent spontaneous miscarriages) (MISSC) and 122 healthy controls.

		Pseudogenes		Inhibitory KIR								Noninhibitory KIR					
		KIR 2DP1	KIR 3DP1	KIR 2DL1	KIR 2DL2	KIR 2DL3	KIR 2DL4	KIR 2DL5	KIR 3DL1	KIR 3DL2	KIR 3DL3	KIR 2DS1	KIR 2DS2	KIR 2DS3	KIR 2DS4	KIR 2DS5	KIR 3DS1
MISSC	OF	0.96	1	0.96	0.68	0.96	0.96	0.16 *	0.96	1	1	0.32	0.56	0.44	1	0.24	0.28
	S.D. of	0.04	0	0.04	0.095	0.04	0.04	0.075	0.04	0	0	0.095	0.101	0.101	0	0.087	0.092
	EF	0.80	1	0.80	0.43	0.80	0.81	0.08	0.80	1	1	0.18	0.34	0.25	1	0.13	0.15
Control	OF	0.98	1	0.94	0.59	0.90	1	0.41 *	0.96	1	1	0.48	0.57	0.33	0.96	0.30	0.38
	S.D. of	0.01	0	0.02	0.04	0.03	0	0.04	0.018	0	0	0.045	0.04	0.04	0.018	0.04	0.04
	EF	0.87	1	0.76	0.36	0.69	1	0.23	0.80	1	1	0.28	0.35	0.18	0.80	0.17	0.22

MISSC, women with infertility problems (including recurrent spontaneous miscarriages); * Pearson's P = 0.018; OR = 0.274; WALD 95% CI = 0.089 – 0.848.

The ratios of number of activating and inhibitory KIR genes in control and patients with MISSC are shown in Table 3. Activating/inhibitory KIR gene number ratio was classified in three groups: those with ratio less than 0.33; ratio between 0.34 and 0.60; and ratio above 0.60. Significantly bigger percentage of 0.34 – 0.60 ratio was found in the patients with MISSC (Pearson's P = 0.035; OR = 0.398; WALD 95%CI = 0.166 – 0.956).

Table 2: The content of inhibitory and noninhibitory (activating) genes in controls and women with infertility problems.

Group	Number of inhibitory KIR genes					Number of activating KIR genes					
	4	5	6	7	8	1	2	3	4	5	6
Control (N=122)	0.8 (1)	1.6 (2)	38.5 (47)	34.4 (42)	24.6 (30)	24.6 (30)	19.7 (24)	9.8 (12)	26.2 (32)	13.1 (16)	6.5 (8)
MISSC (N=25)	0	4.0	32.0	56.0	8.0	12.0	28.0	36.0	12.0	12.0	0

Table 3: Ratios of number of noninhibitory (activating) and inhibitory KIR genes.

Group	Activating/inhibitory KIR gene number ratio (number)		
	< 0.33	0.34 – 0.60	> 0.60
Control (N=122)	43.4 (53)	33.6 (41) *	23.0 (28)
MISSC (N=25)	32.0 (8)	56.0 (14) *	12.0 (3)

* Pearson's P = 0.035; OR = 0.398; WALD 95% CI = 0.166 – 0.956

Discussion

In this pilot study we present differences in the KIR gene frequencies between control and patients with MISSC. We found that inhibitory KIR are present in similar observed frequency in both control and patients with MISSC, except KIR2DL5 which was found in lower frequency in patients with MISSC. There are no significant differences of all noninhibitory KIR between control and patients with MISSC. The number of inhibitory KIR genes in patients with MISSC was lover, except for seven inhibitory KIR genes which was almost doubled. The number of noninhibiotry (stimulatory) KIR genes was lower in patients with MISSC, except for those with three KIR genes which were almost four times more frequent. We found significantly bigger percentage of 0,34 – 0,60 activating/inhibitory KIR gene number ratio in the patients with MISSC.

In the frame of International HLA and Immunogenetics Workshop there is reproductive immunology component which summarize the results from several scientific groups dealing with KIR and recurrent spontaneous abortion (RSA). They published several papers in which investigate if the outcome of pregnancy depends on women's NKRs. They concluded that women with alloimmune abortions have a limited inhibiting KIR repertoire and such miscarriages may occur because trophoblastic HLA class I molecules are recognized by decidual NK cells lacking the appropriate inhibitory KIRs [1]. In another study they performed analysis from a total population of 158 recurrent spontaneous abortion (RSA) couples, 40 couples with repeated implantation failures (IVF) and 81 control couples, reported by five different laboratories. RSA couples were divided into alloimmune aborter (RSAallo) and autoimmune aborter (RSAauto). It was suggested a possible involvement of actKIRs in embryo implantation and the maintenance of pregnancy [2].

Published results from China suggest that a genetic variation at the KIR locus influences the susceptibility to unexplained RSA in the Chinese Han population. Moreover, decreased ligands for inhibitory KIRs could potentially lower the threshold for NK cell activation, mediated through activating receptors, thereby contributing to pathogenesis of RSA [3]. Hiby et al., 2008 reported for the first time that genetic male factor confers risk in RM. These findings support the idea that successful placentation depends on the correct balance of NK cell inhibition and activation in response to trophoblast [4]. Several studies suggest that the imbalance of inhibitory and activating KIRs in uterine NKs might confer susceptibility to the occurrence of pregnancy loss. The maternal inhibitory/activating KIRs-HLA-C polymorphism expressed on trophoblast cells from decidual tissues seems to play a limited role in abortion [14-17]. In addition, the results suggest for the first time that sporadic and recurrent spontaneous abortions as well as miscarriage in the presence or absence of autoantibodies may have different KIR genotypic backgrounds [18].

The results from the 15th International Histocompatibility Workshop oppose the suggestion that increased HLA-DQA1*0505 sharing predispose to RSA or RIF. The KIR2DL3-C1 combination (or lack of the KIR2DL1-C2 one) is associated with implantation failure [19].

Several papers published association of HLA*C with KIR in patients with MISSC. They suggest that among KIR AA women who have HLA-C C2C2 partners, HLA-C heterozygous females show a trend towards an increased chance of successful pregnancy [20-22].

In conclusion, there are differences in the KIR gene distribution, gene number, and activating/inhibitory KIR gene number ratio between control and Macedonian patients with MISSC. Further analysis of frequencies of corresponding KIR genotypes or in the ratio of activating/inhibiting genes content in two groups are needed.

Acknowledgements

The authors thank Elena Cvetkovska (Institute of Immunobiology and Human Genetics, Faculty of Medicine, St Cyril and Methodius University of Skopje, Republic of Macedonia) for isolation of genomic DNA, and taking care of the Macedonian Human DNA Bank (http://www.hdna.mk).

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