Recurrent Spontaneous Abortion (RSA) and Maternal KIR Genes: A Comprehensive Meta-Analysis

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ABSTRACT

Natural killer cells (NKs) are the most important cells in the fetomaternal immune tolerance induced through interaction of maternal killer-cell immunoglobulin-like receptors (KIR) and fetal human leucocyte antigens (HLA). Hence, we intend to perform a meta-analysis on the role of maternal KIR genes diversity in recurrent spontaneous abortion (RSA). The present paper is a meta-analysis of previous genetic association studies and our previous original study. The results showed that KIR3DL1 was a significantly protecting factor for RSA (p=0.044; OR=0.833 [0.698-0.995]; fixed effect model). KIR2DS2 (p=0.034; OR=1.195 [1.013-1.408]; fixed effect model) and KIR2DS3 (p=0.013; OR=1.246 [1.047-1.483]; fixed effect model) were significantly risk factors for RSA. For KIR2DS1 there was a high heterogeneity and publication bias. Briefly, the inhibitory gene KIR3DL1 was a protecting factor, and the activating genes KIR2DS2 and KIR2DS3 were risk factors for RSA. However, the effect sizes were not suitable. We suggest further studies on different causes of pregnancy loss, to find the role of KIR2DS1.

Keywords: recurrent spontaneous abortion, killer-cell immunoglobulin-like receptor, human leukocyte antigen, meta-analysis

INTRODUCTION

Rationale

Recurrent spontaneous abortion (RSA) and pregnancy loss have different pathogeneses, consisting of genetic and chromosomal abnormalities (Hume & Chasen, 2015), environmental toxicities and oxidative stress (Gupta et al., 2007), infectious agents (Ambühl et al., 2016), hormonal causes, etc. Among them, immunological causes and their involving molecules are still controversial and unknown topics. The immune system is a fascinating system, one that does not normally reject the semi-allograft fetus. The immune system has two roles in implantation and pregnancy; preventing the formation of abnormal embryos, and protecting the fetomaternal interaction by releasing angiogenic factors, cytokines and adhesive molecules. The fascinating point is how a system can have two mutually exclusive features; protection and rejection. Indeed, the immune system is the bodyguard of the body through self- and non-self-recognition. However, pregnancy is a semi-allograft transplantation. So the question is what the immune system does in this situation; rejection or protection (Akbari et al., 2018; Würfel, 2016)?!

Immune tolerance is the best answer for the above question (Akbari et al., 2018; Würfel, 2016). Natural killer cells (NKs), which name is self-explanatory, are one of the most important lymphocytes in immune tolerance. They identify self-cells through their killer-cell immunoglobulin-like receptors (KIRs) expressed on their surface. The KIRs interact with their ligands, the human leukocyte antigens (HLAs) - the identification cards of self-cells. These interactions usually result in immune tolerance under normal conditions. Both KIR and HLA genes in human genome have loci (not locus), inherited as haplotypes. Thus, interaction of different KIR molecules with different HLA molecules results in different outcomes consisting of inhibitory and activating responses. KIR gene cluster is located on chromosome 19. This cluster has two types of genes, including 8 inhibitory and 6 activating genes, and 2 pseudogenes. Some of these genes exist in all individuals, like the KIR2DL4. From the viewpoint of medical anthropology, different people from different ethnicities have different KIR-HLA interactions (Alecsandru et al., 2014; Ashouri et al., 2016; Middleton et al., 2008; Norman et al., 2016; Solgi et al., 2011).

HLA has two classes, I and II, and the class I can be further divided into classical and non-classical HLA. KIR2DL4 is an inhibitory KIR binding to the trophoblast HLA-G, which is a non-classical HLA. The combination KIR2DL4+HLA-G triggers the immune tolerance. Both KIR2DL4 and HLA-G are polymorphic genes. Therefore, anthropological variations can contribute to implantation success and pregnancy maintenance. For example, HLA-G*01:03:01 is a risk factor for implantation failure; because its connection with KIR2DL4 is not sufficient to trigger inhibitory signals (Nardi et al., 2012).

NKs may have the CD16 marker, which is the weapon of antibody-dependent cell-mediated cytotoxicity (ADCC). Usually CD56dim NKs are CD16+. So CD16+CD56dim NKs are known as cytotoxic NKs, whereas CD16CD56bright NKs are known as immune-regulatory NKs (Ghafari-an et al., 2015). About 90% of uterine NKs (UNks) are immune-regulatory. In conclusion, UNks are not usually cytotoxic for the embryo (Ghafari-an et al., 2015; Sacks, 2015).

Objectives

As we mentioned above, KIR and HLA have different genes and interactions. KIR has 8 inhibitory (2DL1, 2DL2, 2DL3, 2DL4, 2DL5, 3DL1, 3DL2 and 3DL3) and 6 activating
genes (2DS1, 2DS2, 2DS3, 2DS4, 2DS5 and 3DS1). Since the involving NKS in implantation of embryo are maternal, we intend to perform a meta-analysis on the role of maternal KIR genes diversity in RSA. Previously, Pereza et al. (2017) carried out a meta-analysis on different genes, including the KIR. Nevertheless, their studies were few and therefore our study can serve as an update for that meta-analysis.

MATERIALS AND METHODS

Study selection
For the present meta-analysis, we searched in scientific databases such as Web of Science, PubMed, Scopus, Google Scholar, etc. Our keywords were searched only among the titles. After exclusion of duplicates, all the eligible studies were used for qualitative systematic review.

Eligibility criteria
Among the studies imported for qualitative systematic review, only the studies with available and enough numerical data were imported for the quantitative meta-analysis. Our original paper on this topic was manually added (Table 1) (Akbari et al., 2018). Performing KIR typing was the most important criterion.

Statistical analysis
To perform the present meta-analysis, we used the comprehensive meta-analysis version 2 software (BioStat, US). The analyses were carried out through a p value and individual sample size using fixed-effect and random-effect models. Since the p values were calculated using Yate's correction (or Fisher's exact test if necessary), the odds ratios (OR) (effect sizes) achieved from these p values were underestimated. This statistical protocol has been previously published (Anbari & Ahmadi, 2017).

Heterogeneity and publication bias
We used the I² scale and I²<50 was considered as homogeneity. In the cases of heterogeneity, we used the random-effect model. In order to find publication bias, we used funnel plots. If a study were to be find outside the funnel, it meant that its effect size was outside the expected 95% confidence interval (CI). In other words, its difference with other studies is statistically significant at p=0.05. Hence, a publication bias does not have necessarily a negative connotation. In the present study, a funnel plot p value < 0.05 means that the mentioned individual study is outside the funnel of 95% CI.

Additional analyses
In order to cluster the studies for meta-analysis, we designed a dendrogram using the STATA14 software (StataCorp LLC, US). This cluster analysis involved the complete linkage of binary variables (Table 2, Figure 1).

RESULTS

Eligible studies
Table 1 depicts the findings from the selected studies, in addition to our original case-control study, this table includes 11 studies. The p values were analyzed through Yate's correction (or fisher's exact test when necessary). Positive effect directions show each gene as a risk factor and negative effect directions show each gene as a protecting factor. Our cluster analysis showed that the study by Dambaeva et al. (2016) had a different design in comparison to other studies (Figure 1). Hence, it was excluded from the meta-analysis. At the end, 10 studies remained.

Meta-analysis
The role of KIR2DL1 in RSA was not statistically significant (p=0.051; OR=0.849; fixed). Faridi et al. (2009) showed a significantly more protective effect of this gene in comparison to other studies ( funnel plot p value <0.05 ) (Figures 2 and 3).

The role of KIR2DL2 in RSA was not statistically significant (p=0.325; OR=1.091; fixed). Hong et al. (2008) showed a significantly higher risk of this gene's effect in comparison to other studies ( funnel plot p value <0.05 ) (Figures 4 and 5). The role of KIR2DL3 in RSA was not statistically significant (p=0.448; OR=1.062; fixed).

No publication bias was found based on the funnel plot (Figures 6 and 7). The role of KIR2DL5 in RSA was not statistically significant (p=0.767; OR=0.960; random). Hibi et al. (2008) showed a significantly more protective effect of this gene in comparison to other studies ( funnel plot p value <0.05 ) (Figures 8 and 9).

The role of KIR3DL1 in RSA was statistically significant (p=0.044*; OR=0.833; fixed). Faridi et al. (2009) showed a significantly more protective effect of this gene in comparison to other studies (p<0.05; based on funnel plot) (Figures 10 and 11). The role of KIR2DS1 in RSA was not statistically significant (p=0.726; OR=1.056; random). Inconclusive publication bias was found for this analysis based on the funnel plot (Figures 12 and 13). The role of KIR2DS2 in RSA was statistically significant (p=0.034*; OR=1.195; fixed).

Faridi et al. (2009) study showed significantly more risk effect of this gene in comparison to other studies ( funnel plot p value <0.05 ) (Figures 14 and 15). The role of KIR2DS3 in RSA was statistically significant (p=0.013*; OR=1.246; fixed).

Faridi et al. (2009) showed significantly more risk effect of this gene in comparison to other studies ( funnel plot p value <0.05 ) (Figures 16 and 17).

The role of KIR2DS4 in RSA was not statistically significant (p=0.094; OR=0.762; fixed). Faridi et al. (2009) showed significantly more protective effect of this gene in comparison to other studies ( funnel plot p value <0.05 ) (Figures 18 and 19). The role of KIR2DS5 in RSA was not statistically significant (p=0.642; OR=1.042; fixed). Hibi et al. (2008) showed a significantly more protective effect of this gene in comparison to other studies ( funnel plot p value <0.05 ) (Figures 20 and 21). The role of KIR3DS1 in RSA was not statistically significant (p=0.851; OR=1.037; random). Hibi et al. (2008) and Faridi et al. (2009) showed significantly more protective and risk effect of this gene in comparison to other studies, respectively ( funnel plot p value <0.05 ) (Figures 22 and 23).

DISCUSSION

Summary of evidence
NKS are lymphocytes that participate in the innate immune system. They have 2 subtypes: CD16+CD56dim and CD16-CD56bright that are called as cytotoxic and immune-regulatory NKS, respectively. In the implantation site, the NKS are mainly CD56bright. Hence, the immune system has a positive and protecting role in implantation and early pregnancy. Embryo implantation and pregnancy are a type of transplantation called semi-allograft. Thus, we need immune tolerance to have a successful pregnancy. The NKS play their roles with their KIRs interacting with the HLAs expressed on trophoblasts (Würfel, 2016). Because of the important roles of NKS in the implantation process, this meta-analysis aimed to investigate the role of maternal KIR genes diversity in RSA.

Among the investigated genes, only the results of 3DL1, 2DS2 and 2DS3 were statistically significant
| Study | Witt et al., 2004 | Wang et al., 2007 | Hong et al., 2008 | Hibi et al., 2008 | Vargas et al., 2009 | Faridi et al., 2009 | Khoreshavifar et al., 2011 | Oztekin et al., 2012 | Djuletic et al., 2015 | Dambaeva et al., 2016 | Our original study |
|-------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Gene  | RSA N=52        | RSA N=73        | RSA N=16        | RSA N=95        | RSA N=68        | RSA N=205       | RSA N=100       | RSA N=40        | RSA N=25        | RSA N=139       | RSA N=100       |
|       | Control N=55    | Control N=68    | Control N=41    | Control N=269   | Control N=68    | Control N=224   | Control N=100   | Control N=90   | Control N=122  | Control N=195   | Control N=100   |
| 2DL1  | 52              | 55              | 73              | 68              | 8               | 21              | 92              | 258            | 63              | 64              | 141             | 215             | 97              | 95              | 40              | 89              | 24              | 115             | 135             | 189             | 93              | 95              |
|       | p value (ED) a  | 1 (FET) b       | 1 (FET)        | 0.841 (-)       | 0.769 (FET) (-) | 0.999 (FET) (-) | 0.0001 (-)      | 0.720 (+)      | 1 (FET)        | 1 (FET)        | 1 (FET)         | 1 (FET)         | 1 (FET)         | 1 (FET)         | 0.764 (-)       |
| 2DL2  | 29              | 23              | 22              | 22              | 16              | 22              | 45              | 137            | 43              | 37              | 110             | 111             | 52              | 58              | 26              | 41              | 17              | 72              | 69              | 69              |
|       | p value (ED)    | 0.211 (+)       | 0.777 (-)      | 0.002 (+)       | 0.361 (-)       | 0.383 (+)       | 0.446 (+)       | 0.475 (-)      | 0.632 (+)      | 0.537 (+)      | 1                |
| 2DL3  | 47              | 47              | 72              | 67              | 6               | 18              | 88              | 245            | 58              | 58              | 169             | 187             | 87              | 85              | 37              | 74              | 24              | 110             | 124             | 172             |
|       | p value (ED)    | 0.631 (+)       | 1 (FET)        | 0.887 (-)       | 0.806 (+)       | 1 (FET)        | 0.887 (-)       | 0.841 (+)      | 0.207 (+)      | 0.469 (FET) (+) | 0.920 (+)       |
| 2DL4  | 16              | 20              | 35              | 28              | 5               | 12              | 36              | 148            | 37              | 33              | 127             | 151             | 32              | 56              | 4               | 50              | 79              | 103             | 58              | 60              |
|       | p value (ED)    | 0.680 (-)       | 0.521 (+)      | 1 (FET)        | 0.005 (-)       | 0.610 (+)       | 0.238 (+)       | 0.072 (+)      | 0.032 (-)      | 0.537 (+)      | 0.887 (-)       |
| 3DL1  | 50              | 48              | 73              | 67              | 88              | 256            | 64              | 63             | 120             | 191             | 36              | 81              | 24              | 117             | 125             | 185             | 93              | 95              |
|       | p value (ED)    | 0.162 (FET) (+) | 1 (FET)        | 0.502 (-)       | 0.999 (FET) (+) | 0.0001 (-)     | 1 (FET)        | 1 (FET)       | 0.131 (-)      | 0.764 (-)       |
| 3DL2  | 205             | 181             | 72              | 65              | 8               | 24              | 90              | 255            | 62              | 64              | 109             | 163             | 36              | 82              | 25              | 117             | 130             | 185             | 95              | 95              |
|       | p value (ED)    | 0.862 (-)       | 0.352 (FET) (+) | 0.777 (-)      | 1 (FET)        | 0.740 (-)       | 0.0001 (-)     | 1 (FET)       | 0.588 (FET) (+) | 0.777 (-)       |
| 2DS5  | 10              | 18              | 38              | 26              | 4               | 8               | 23              | 102            | 30              | 19              | 122             | 122             | 22              | 35              | 6               | 37              | 53              | 70              | 35              | 34              |
|       | p value (ED)    | 0.171 (-)       | 0.139 (+)      | 0.722 (FET) (+) | 0.021 (-)       | 0.074 (+)       | 0.337 (+)       | 0.129 (+)      | 0.698 (-)      | 0.764 (+)       |
| 3DL6  | 17              | 20              | 38              | 32              | 24              | 121             | 34              | 23             | 162             | 116             | 16              | 37              | 7               | 46              | 62              | 77              | 41              | 40              |
|       | p value (ED)    | 0.590 (-)       | 0.761 (+)      | 0.001 (-)       | 0.082 (+)       | 0.0001 (+)     | 0.920 (-)       | 0.488 (-)      | 0.409 (+)      | 1                |

**Table 1.** Data summary of the found articles.

- **Study design:** Case-control, Case-control, Case-control, Case-control, Case-control, Case-control, Case-control, Case-control, Cohort for KIR2DS1, Case-control
- **Genotyping method:** PCR-SSP, PCR-SSP, PCR-SSP, PCR-SSP, PCR-SSP, PCR-SSP, PCR-SSP, PCR-SSP, PCR-SSP, PCR-SSP
- **RSA definition:** 3 spontaneous abortion, 3 spontaneous abortion, 3 spontaneous abortion, 3 spontaneous abortion, 3 spontaneous abortion, 3 spontaneous abortion, Any primiparous woman, 2 history of normal delivery, Not mentioned, Not mentioned
- **Place:** Brazil, China, China, London, Brazil, India, Iran, Mediterranean, Albania, America, Iran
- **Ethnicity:** Caucasian, Chinese, Chinese, Caucasian, Indian, Caucasian, Caucasian, Caucasian, Caucasian, Caucasian
- **Study number in dendrogram:** 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11

a) ED stands for effect direction; the positive ones show risk factors and the negative ones show protecting factors. b) FET stands for Fisher’s exact test.
Table 2. Dissimilarity matrix of studies’ characteristics based on the below of Table 1

|   | Witt et al., 2004 | Wang et al., 2007 | Hong et al., 2008 | Hibi et al., 2008 | Vargas et al., 2009 | Faridi et al., 2009 | Khosravifar et al., 2011 | Ozturk et al., 2012 | Djulejic et al., 2015 | Dambaeva et al. 2016 | Our study |
|---|------------------|-------------------|-------------------|------------------|-------------------|-------------------|------------------------|-------------------|-----------------------|---------------------|-----------|
| 1 | Witt et al., 2004 | 0                 | 0.33              | 0.33             | 0.33              | 0.33              | 0.50                   | 0.50              | 0.83                  | 0.16                |
| 2 | Wang et al., 2007 | 0.33              | 0                 | 0.50             | 0.50              | 0.50              | 0.66                   | 0.66              | 1                     | 0.33                |
| 3 | Hong et al., 2008 | 0.33              | 0                 | 0.50             | 0.50              | 0.50              | 0.66                   | 0.66              | 0.83                  | 0.33                |
| 4 | Hibi et al., 2008 | 0.33              | 0.50              | 0.50             | 0                 | 0.50              | 0.33                   | 0.66              | 0.83                  | 0.33                |
| 5 | Vargas et al., 2009 | 0.16            | 0.50              | 0.50             | 0                 | 0.50              | 0.33                   | 0.66              | 0.66                  | 0.33                |
| 6 | Faridi et al., 2009 | 0.33            | 0.33              | 0.33             | 0.50              | 0                 | 0.50                   | 0.66              | 0.66                  | 0.33                |
| 7 | Khosravifar et al., 2011 | 0.33          | 0.50              | 0.50             | 0.33              | 0.50              | 0                      | 0.66              | 0.83                  | 0.33                |
| 8 | Ozturk et al., 2012 | 0.50            | 0.66              | 0.66             | 0.33              | 0.66              | 0                      | 0.66              | 0.66                  | 0.50                |
| 9 | Djulejic et al., 2015 | 0.50            | 0.66              | 0.66             | 0.50              | 0.66              | 0.50                   | 0                 | 0.66                  | 0.50                |
| 10 | Dambaeva et al., 2016 | 0.83           | 1                 | 0.83             | 0.66              | 1                 | 0.83                   | 0.66              | 0.66                  | 0.83                |
| 11 | Our original study | 0.16           | 0.33              | 0.33             | 0.33              | 0.33              | 0.33                   | 0.50              | 0.50                  | 0.83                |

Figure 1. Cluster analysis of Table 2 based on complete linkage method. The numbers of studies are based on Tables 1 and 2.
with protective, risk and risk effect impacts, respectively (Table 3). If we adjust multiple test correction for these findings, none of them would remain significant. It shows that there is no specific KIR gene predicting RSA. The funnel plot analyses showed that Faridi et al. (2009), in India, had more publication bias in comparison to the others. In our original study we showed that maternal KIR2DS1 in combination with paternal HLA-C2 can be a risk factor (Akbari et al., 2018).

**Literature review**

This concern in reproductive immunology dates back to 2004. Witt et al. (2004) found no significant association of maternal KIR genes with the risk of RSA in a Brazilian population. Lack of paternal or fetal

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**Figure 2.** KIR2DL1 Funnel plot showing a significant bias for Faridi et al. (2009).

**Table 3.** Odds ratio and 95% CI for KIR2DL1 (fixed). Kovacic et al. (2017) showed that maternal KIR2DS1 in combination with paternal HLA-C2 can be a risk factor (Akbari et al., 2018).

**Figure 3.** Forest plot of KIR2DL1 (fixed). Favours A shows protecting effect and favours B shows harmful effect (in all figures).
evaluation of HLA-C was their study limitation. Yamada et al. (2004) evaluated different immune markers such as CD94, CD158 (the very KIR) and CD161 through flow cytometry in 20 RSA women and 15 fertile controls. They found a lower level of CD158a (the very KIR2DL1) in the RSA group. Their low sample size was a limitation in their study (Yamada et al., 2004). Because of their quantitative approach and different aims and protocols, we excluded that study from our meta-analysis. Varla-Leftherioti et al. (2005) evaluated only KIR2DL1, 2DL2 and 2DL3 among the KIR genes in a small sample size. Wang et al. (2007) found a risk association for KIR2DS1 in a Chinese population. They evaluated HLA-C in couples, similar to our original experience. Conversely, our original study and some studies before, e.g. Hiby et al. (2008), found a
strongly protecting association for KIR2DS1 in a Caucasian population. However, since their control group criteria was to be a first-birth woman, this might be the reason of their publication bias. Vargas et al. (2009) found a risk association for the number of maternal activating KIR genes. Faridi et al. (2009) found that RSA was more associated with activating, and more protected with inhibitory KIR genes. Nowak et al. (2009) found that RSA could be associated with KIR genotypes. Conversely, other studies found that RSA was more frequent in patients with genotypes bearing 6 inhibitory genes. Because we did not have access to the frequencies of KIR genes, we excluded this study from our meta-analysis. Nowak et al. (2011) found
that female heterozygosity for HLA-C in combination with AA KIR genotype could be a protecting factor for RSA. Khosravifar et al. (2011) investigated the role of maternal KIR and parental HLA-C in an Iranian population. They found that RSA was associated with maternal HLA-C2. Ozturk et al. (2012) found a protecting role for the KIR AA genotype. A small sample size and one miscarriage episode in the RSA group were the negative points of their study. Alecsandru et al. (2014) found that maternal AA genotype was a risk factor affecting the success of double embryo transformation. Djulejic et al. (2015) evaluated the role of KIR genes on women with any fertility problem. Hence, we excluded it from our meta-analysis. Nowak et al. (2016)
investigated the role of KIR2DL4 and HLA-G polymorphisms in RSA. Dambaeva et al. (2016) showed that maternal KIR2DS1 is not a risk factor for RSA by itself, rather its combination with maternal HLA-C2 could be associated.

**Interpretation**

As we observe above, there are many paradoxical findings for the role of maternal KIR genes in RSA. This can be justified through reasons like different ethnicities, different sample sizes, different RSA group criteria, different control criteria, and so on. In all the studies in Table 1, the genotyping method used was polymerase chain reaction with sequencing specific primers (PCR-SSP), and PCR with sequence specific oligonucleotides (PCR-SSO). Therefore, the genotyping method cannot be a reason for such paradoxes. Other features likely
to be involved with this paradox are shown as a cluster analysis (Tables 1 and 2, Figure 1).

The results of KIR2DS1 had more publication bias based on funnel plots than the present meta-analysis. A paradoxical piece of evidence is that in early pregnancy KIR2DS1 is a helping factor (contrary to some studies), because its activating role (especially in combination with trophoblast HLA-C2) results in higher cytokine releasing of UNKs (Xiong et al., 2013). Hence, it seems that this receptor has a protecting role for implantation and placentation, and is a risk factor for late pregnancy maintenance. For instance, Alecsandru et al. (2014) found that maternal AA genotype was a risk factor for the success of assisted reproduction. AA is the most
inhibitory genotype and therefore it supports this hypothesis that NK activation is necessary in early pregnancy. Pregnancy loss has numerous causes, in particular embryo genetic and chromosomal abnormalities. Therefore, the immune system’s theoretical role is to reject such malformed embryos. Therefore, this risky role of activating KIRs is in fact a protecting role! Of course, it is remarkable that the lack of genetic evaluation of the lost embryos was a limitation for the studies imported to this meta-analysis. It is suggested that this variable should be adjusted in future studies.

**Limitations**

Although we found significant associations involving 3 genes in the meta-analysis (Table 3), but these findings would not be reliable, because, 1) the odds ratios
Figure 16. KIR2DS3 Funnel plot showing a rather significant bias for Faridi et al. (2009).

| Study name       | Odds ratio | Lower limit | Upper limit | Z-Value | p-Value |
|------------------|------------|-------------|-------------|---------|---------|
| Witt et al. 2004 | 1/082      | 0/539       | 2/173       | 0/222   | 0/824   |
| Wang et al. 2007 | 1/164      | 0/635       | 2/134       | 0/492   | 0/623   |
| Hong et al. 2008 | 1/283      | 0/486       | 3/383       | 0/503   | 0/615   |
| Hibi et al. 2008 | 0/924      | 0/636       | 1/344       | 0/412   | 0/680   |
| Vargas et al. 2009 | 1/056    | 0/570       | 1/957       | 0/173   | 0/862   |
| Faridi et al. 2009 | 1/821   | 1/284       | 2/582       | 3/365   | 0/001   |
| Ozturk et al. 2012 | 1/351  | 0/717       | 2/544       | 0/931   | 0/352   |
| Gjulejc et al. 2015 | 1/290  | 0/712       | 2/337       | 0/841   | 0/400   |
| Our study        | 1/123      | 0/676       | 1/864       | 0/448   | 0/654   |
|                  | 1/246      | 1/047       | 1/483       | 2/477   | 0/013   |

Figure 17. KIR2DS3 Forest plot (fixed).

are not large enough to show a remarkable effect size; 2) the paper selection and homogenizing process of meta-analyses are different and customized among researchers; 3) there were a lot of missed data even in the cited studies; 4) pregnancy loss has a number of definitions such as abortion, stillbirth (Gold et al., 2010) and assisted reproduction failure (Mitra & Boroujeni, 2015), and happens because due to conditions such as the anti-phospholipid syndrome (APS) (Rand et al., 1997), and there might be confusion involving these.
concepts. Adjusting models in future studies help researchers solve these limitations.

CONCLUSION
The role of maternal KIR gene diversity in RSA is still in unclear, although our meta-analysis showed 3 genes as associated factors. KIR3DL1 was a protecting factor, and KIR2DS2 and KIR2DS3, which proved to be risk factors for RSA. For KIR2DS1 there was a high heterogeneity. It seems that its role is different among different causes of pregnancy loss. Our previous case-control original investigation showed a significant relation with maternal KIR2DS1 in combination with paternal HLA-C2 as a risk factor. In order to clarify this
role we have some suggestions for future studies, such as investigations of this combination concerning the success rate of assisted reproduction, for early first trimester abortions occurring after implantation and early placentation, for stillbirth groups, for abortions secondary to APS, and for successful and unsuccessful pregnancies of malformed embryos and fetuses. We would also like to suggest adjusting models and cohort studies.
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**CONFLICT OF INTEREST**

None to declare.
| Gene   | I2 | p value | Odds ratio | I2 | p value | Odds ratio |
|--------|----|---------|------------|----|---------|------------|
| 2DL1   | 20.92 | 0.051 | 0.849 | - | - | - |
| 2DL2   | 36.59 | 0.325 | 1.091 | - | - | - |
| 2DL3   | 0.00  | 0.448 | 1.069 | - | - | - |
| 2DL4   | 53.79 | 0.325 | - | - | - | - |
| 3DL1   | 47.16 | 0.044* | 0.833 | 0.00 | 0.767 | 0.960 |
| 3DL2   | 70.31 | 0.990 | 0.999 | 0.00 | 0.726 | 1.058 |
| 3DL3   | 25.97 | 0.034* | 1.195 | - | - | - |
| 3DS1   | 40.36 | 0.094 | 0.862 | - | - | - |
| 3DS2   | 48.52 | 0.642 | 1.042 | - | - | - |
| 3DS3   | 75.82 | 0.525 | 1.059 | 0.00 | 0.851 | 1.037 |
| 2DP1   | 70.31 | 0.990 | 0.999 | 0.00 | 0.726 | 1.058 |

* significant at 0.05.

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