JBRA Assisted Reproduction 2019;**24**(2):197-213
doi: 10.5935/1518-0557.20190067

Review

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

Soheila Akbari[1], Farhad Shahsavar[2], Reza Karami[1], Fatemeh Yari[3], Khatereh Anbari[4], Seyyed Amir Yasin Ahmadi[5]

[1]Department of Obstetrics and Gynecology, Lorestan University of Medical Sciences, Khorramabad, Iran
[2]Department of Immunology, Lorestan University of Medical Sciences, Khorramabad, Iran
[3]Department of Reproductive Health, Lorestan University of Medical Sciences, Khorramabad, Iran
[4]Social Determinants of Health Research Center, Lorestan University of Medical Sciences, Khorramabad, Iran
[5]Student Research Committee, Iran University of Medical Sciences, Tehran, Iran

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. In addition, each gene in their loci is polymorphic. 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-depended cell-mediated cytotoxicity (ADCC). Usually $CD56^{dim}$ NKs are $CD16^{+}$. So $CD16^{+}CD56^{dim}$ NKs are known as cytotoxic NKs, whereas $CD16^{-}CD56^{bright}$ NKs are known as immune-regulatory NKs (Ghafourian *et al*., 2015). About 90% of uterine NKs (UNKs) are immune-regulatory. In conclusion, UNKs are not usually cytotoxic for the embryo (Ghafourian 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

Received April 21, 2019
Accepted November 01, 2019

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 I^2 scale and I^2<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). Hiby *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 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). Hiby *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). Hiby *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: $CD16^{+}CD56^{dim}$ and $CD16^{-}CD56^{bright}$ that are called as cytotoxic and immune-regulatory NKs, respectively. In the implantation site, the NKs are mainly $CD56^{bright}$. 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

Table 1. Data summery of the found articles.

Study	Witt *et al.*, 2004		Wang *et al.*, 2007		Hong *et al.*, 2008		Hiby *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 original study	
Gene	**RSA N=52**	**Control N=55**	**RSA N=73**	**Control N=68**	**RSA N=16**	**Control N=41**	**RSA N=95**	**Control N=269**	**RSA N=68**	**Control N=68**	**RSA N=205**	**Control N=224**	**RSA N=100**	**Control N=100**	**RSA N=40**	**Control N=90**	**RSA N=25**	**Control N=122**	**RSA N=139**	**Control N=195**	**RSA N=100**	**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)		0.764 (-)	
2DL2	29	23	22	23	16	22	45	137	43	37	110	111	52	58	26	41	17	72	69	96		
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																						
2DL5	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																						
3DL3																						
2DS1	21	25	44	28	1	4	24	121	32	26	92	88	35	48	21	31	8	58	63	73	49	40
p value (ED)	0.740 (-)		0.035 (+)		1 (FET)		0.001 (-)		0.386 (+)		0.283 (+)		0.084 (-)		0.005 (+)		0.228 (-)		0.182 (+)		0.254 (+)	
2DS2	27	26	22	18	1	3	46	140	45	39	104	72	50	58	26	41	14	69	69	97	59	54
p value (ED)	0.777 (+)		0.764 (+)		1 (FET)		0.624 (-)		0.377 (+)		0.001 (+)		0.319 (-)		0.063 (+)		0.806 (+)		1		0.565 (+)	
2DS3	16	15	25	20	2	3	22	70	22	24	94	66			17	29	11	40	44	55	38	34
p value (ED)	0.824 (+)		0.622 (+)		0.613 (+)		0.680 (-)		0.862 (-)		0.0007 (+)				0.350 (+)		0.399 (+)		0.577 (+)		0.654 (+)	
2DS4	18	21	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 (-)		1	
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 (+)		1	
3DS1	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	
2DP1																						
3DP1																						
Study design	Case-control		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-SSO		PCR-SSP		PCR-SSP		PCR-SSO		PCR-SSP		PCR-SSO		PCR-SSP	
RSA definition	3 spontaneous abortion		3 spontaneous abortion		3 spontaneous abortion		3 spontaneous abortion		3 spontaneous abortion		3 spontaneous abortion		3 spontaneous abortion		A history of miscarriage		Any fertility problem		2 spontaneous abortion		3 spontaneous abortion	
Control definition	2 history of normal delivery		2 history of normal delivery		2 history of normal delivery		Any primiparous woman		2 history of normal delivery		2 history of normal delivery		1 history of normal delivery		2 history of normal delivery		Not mentioned		Not mentioned		2 history of normal delivery	
Place	Brazil		China		China		London		Brazil		India		Iranian		Mediterranean		Albania		America		Iran	
Ethnicity	Caucasian		Chinese		Chinese		Caucasian		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**	**Hiby *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	0.16	0.33	0.33	0.50	0.50	0.83	0.16
2	Wang *et al.*, 2007	0.33	0	0	0.50	0.50	0.33	0.50	0.66	0.66	1	0.33
3	Hong *et al.*, 2008	0.33	0	0	0.50	0.50	0.33	0.50	0.66	0.66	0.83	0.33
4	Hiby *et al.*, 2008	0.33	0.50	0.50	0	0.50	0.50	0.33	0.66	0.50	0.83	0.33
5	Vargas *et al.*, 2009	0.16	0.50	0.50	0.50	0	0.50	0.50	0.33	0.66	0.66	0.33
6	Faridi *et al.*, 2009	0.33	0.33	0.33	0.50	0.50	0	0.50	0.66	0.66	1	0.33
7	Khosravifar *et al.*, 2011	0.33	0.50	0.50	0.33	0.50	0.50	0	0.66	0.50	0.83	0.33
8	Ozturk *et al.*, 2012	0.50	0.66	0.66	0.66	0.33	0.66	0.66	0	0.66	0.66	0.50
9	Djulejic *et al.*, 2015	0.50	0.66	0.66	0.50	0.66	0.66	0.50	0.66	0	0.66	0.50
10	Dambaeva *et al.*, 2016	0.83	1	0.83	0.83	0.66	1	0.83	0.66	0.66	0	0.83
11	Our original study	0.16	0.33	0.33	0.33	0.33	0.33	0.33	0.50	0.50	0.83	0

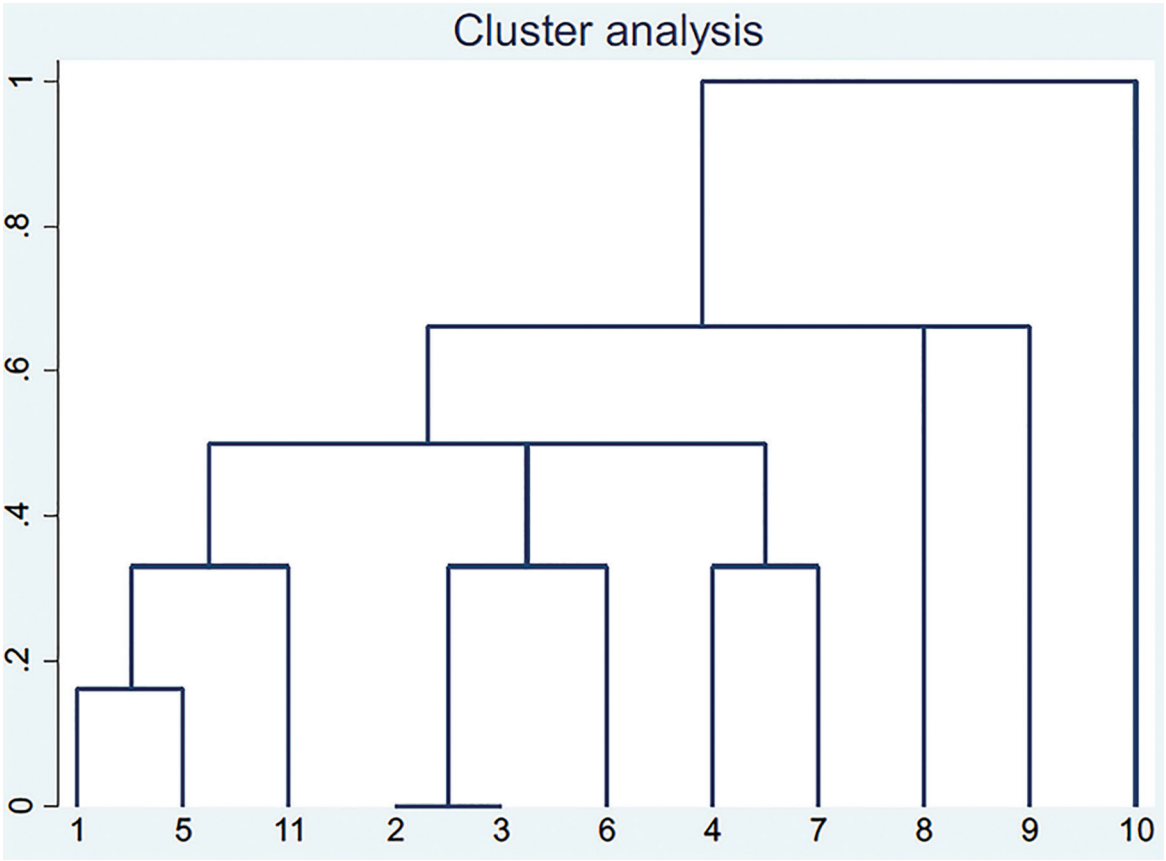

Figure 1. Cluster analysis of Table 2 based on complete linkage method. The numbers of studies are based on Tables 1 and 2.

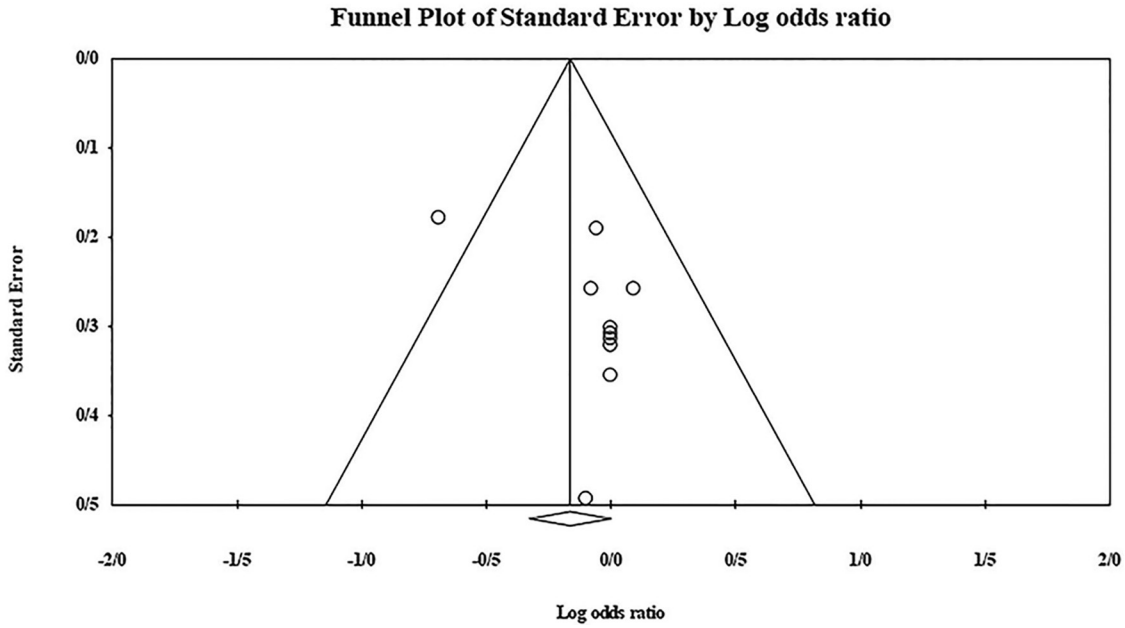

Figure 2. KIR2DL1 Funnel plot showing a significant bias for Faridi *et al*. (2009).

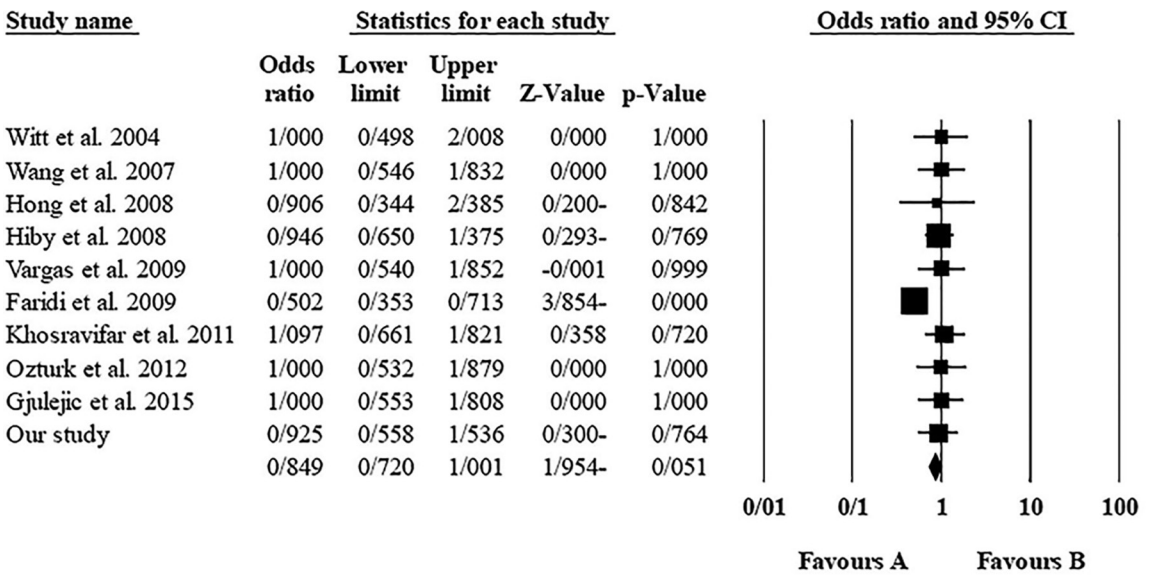

Figure 3. Forest plot of KIR2DL1 (fixed). Favours A shows protecting effect and favours B shows harmful effect (in all figures).

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

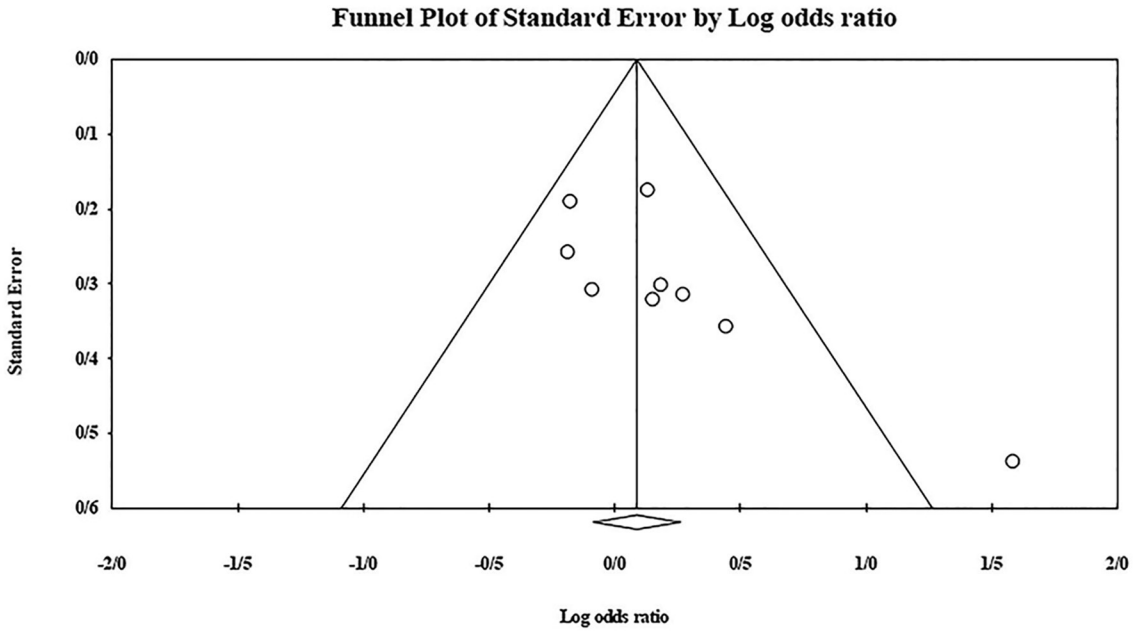

Figure 4. KIR2DL2 Funnel plot showing a significant bias for Hong *et al*. (2008).

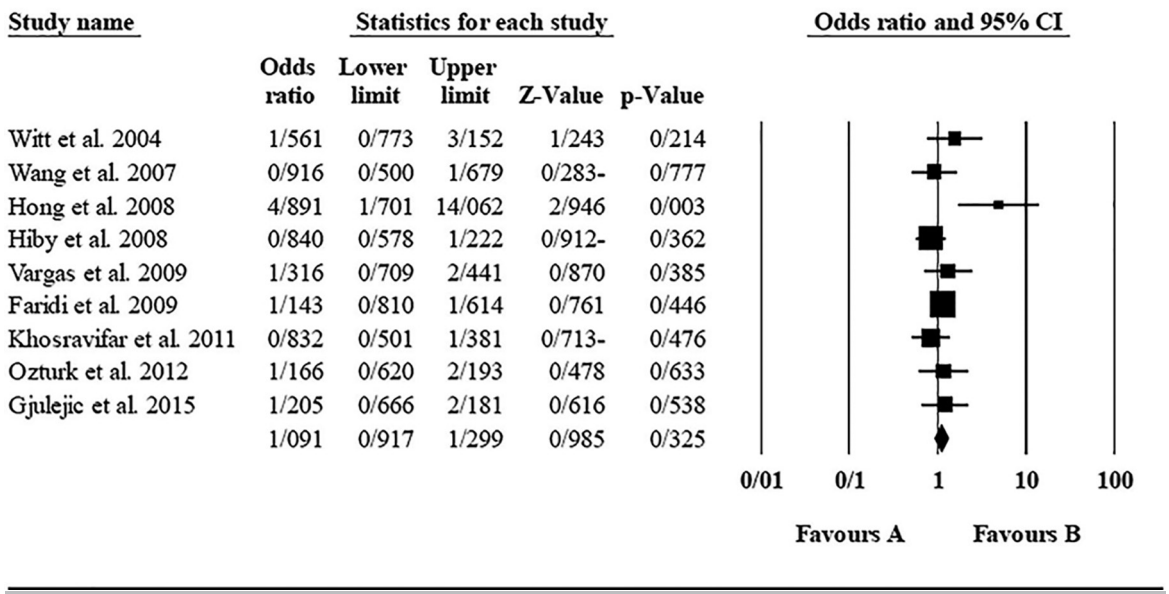

Figure 5. Forest plot of KIR2DL2 (fixed).

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

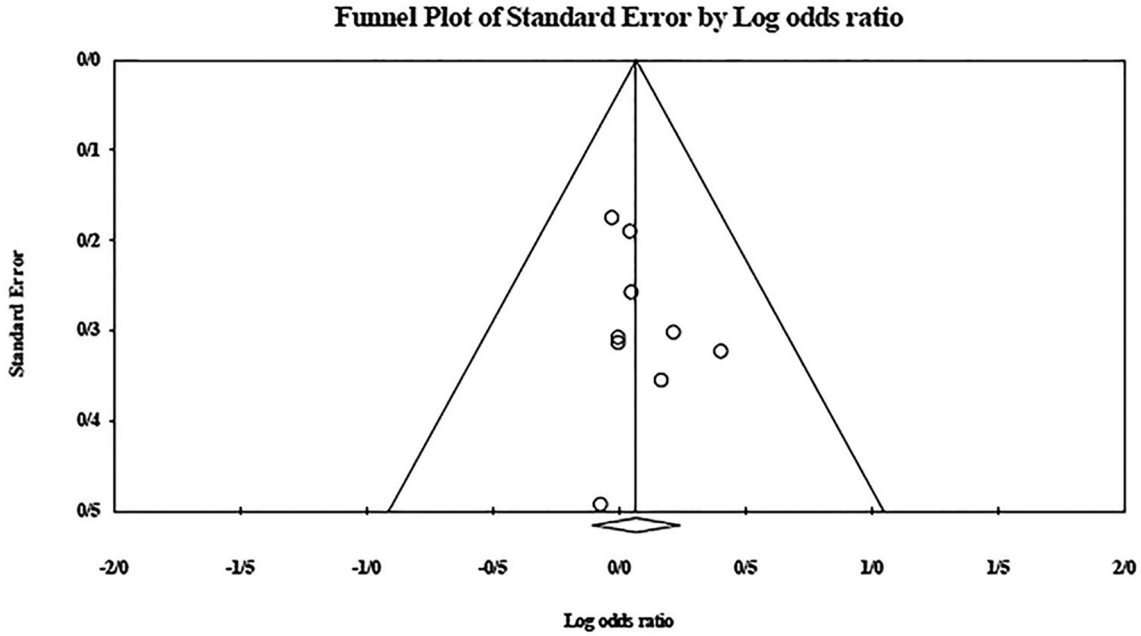

Figure 6. Funnel plot of KIR2DL3 shows no publication bias.

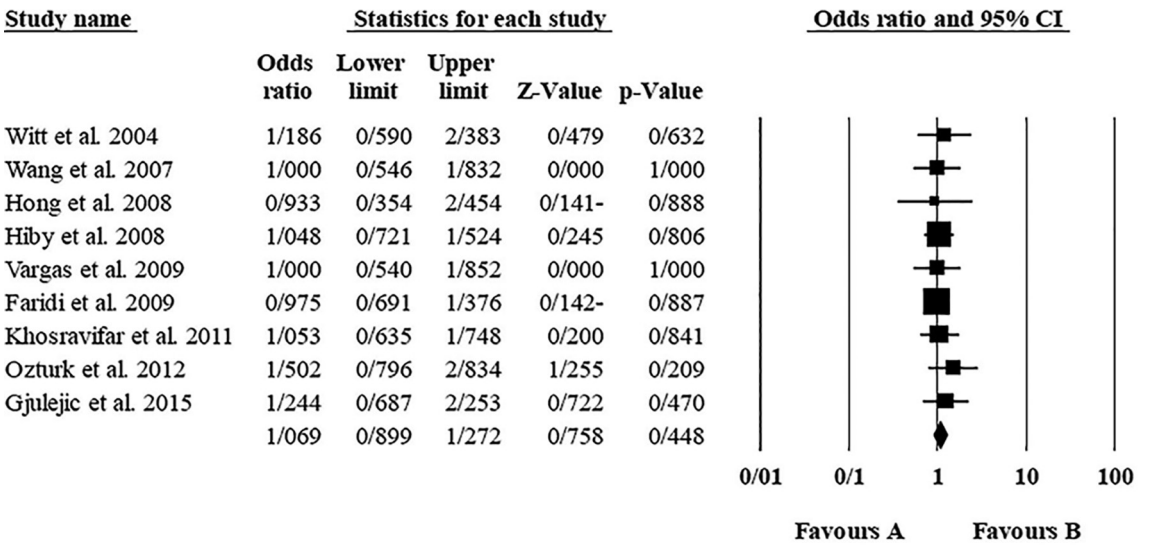

Figure 7. KIR2DL3 Forest plot (fixed).

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

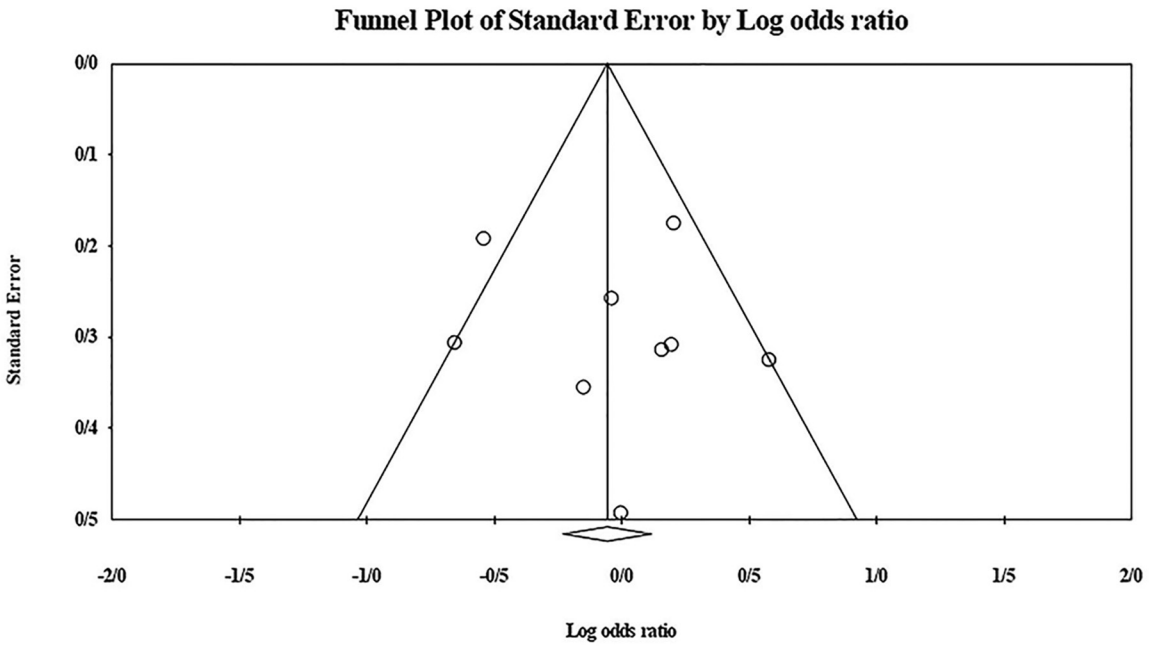

Figure 8. KIR2DL5 Forest plot showing a significant bias for Hiby et al. (2008) study.

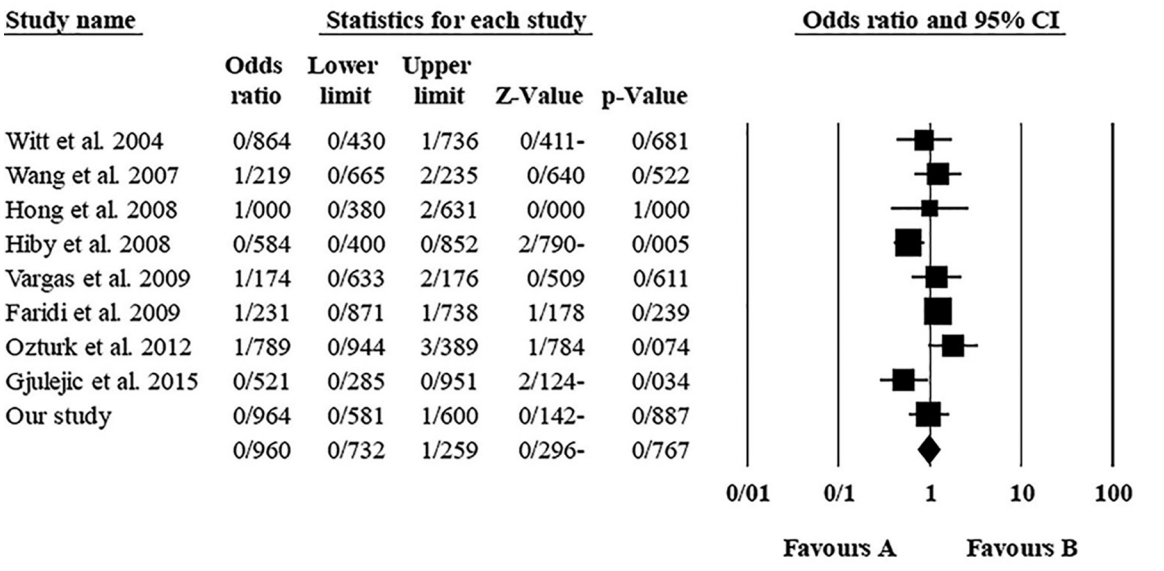

Figure 9. KIR2DL5 Forest plot (random).

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)

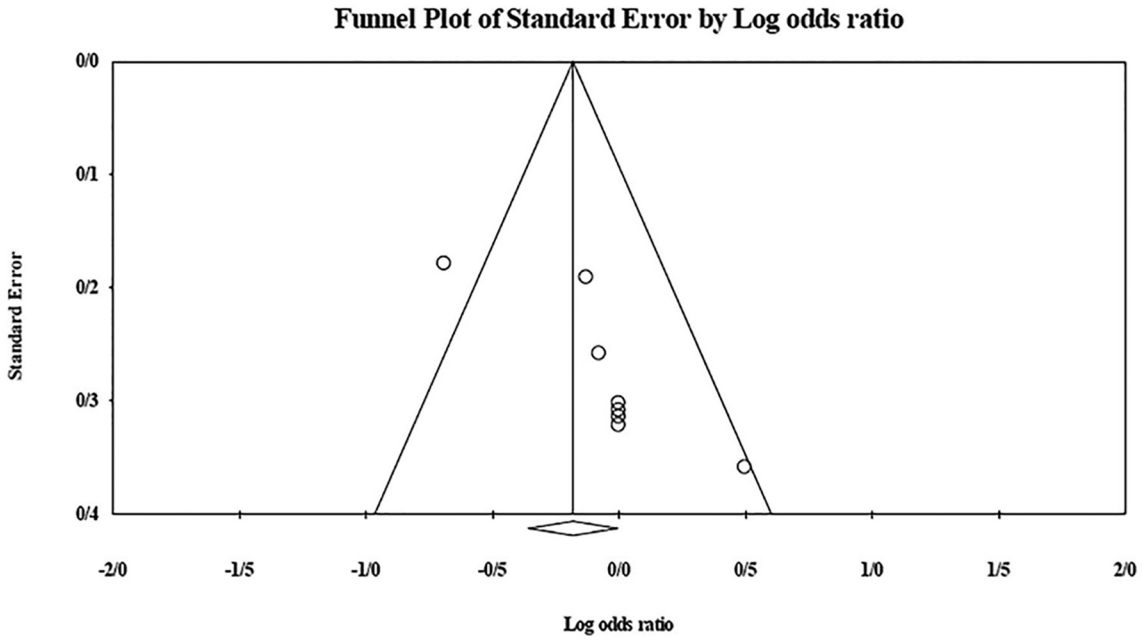

Figure 10. KIR3DL1 Funnel plot showing a significant bias for Faridi et al. (2009).

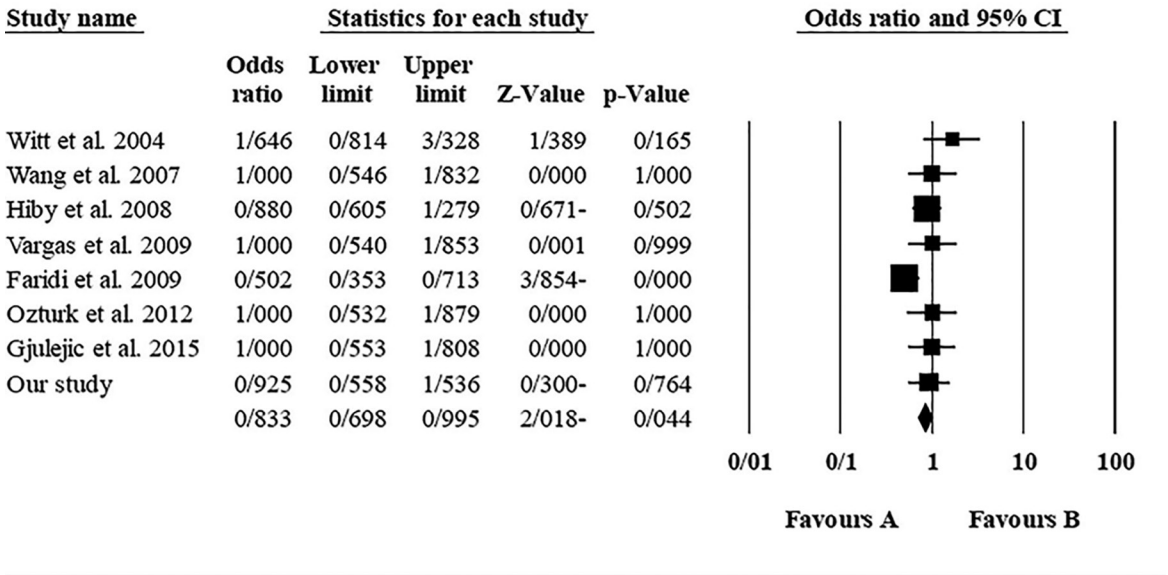

Figure 11. KIR3DL1 Forest plot (fixed).

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

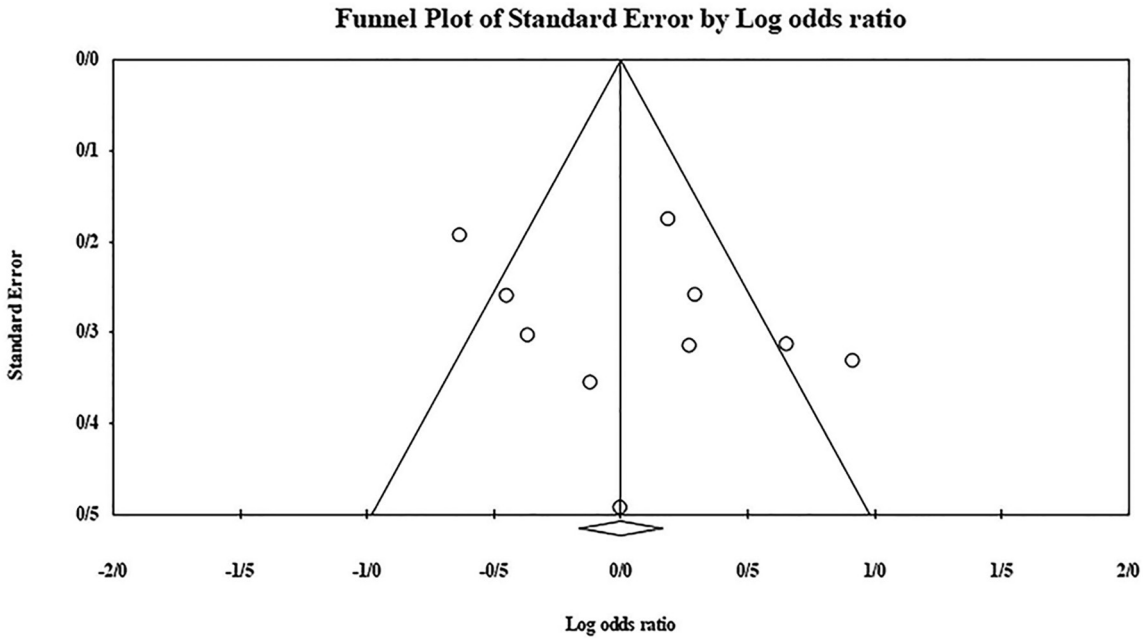

Figure 12. KIR2DS1 Funnel plot showing a huge publication bias which is inconclusive.

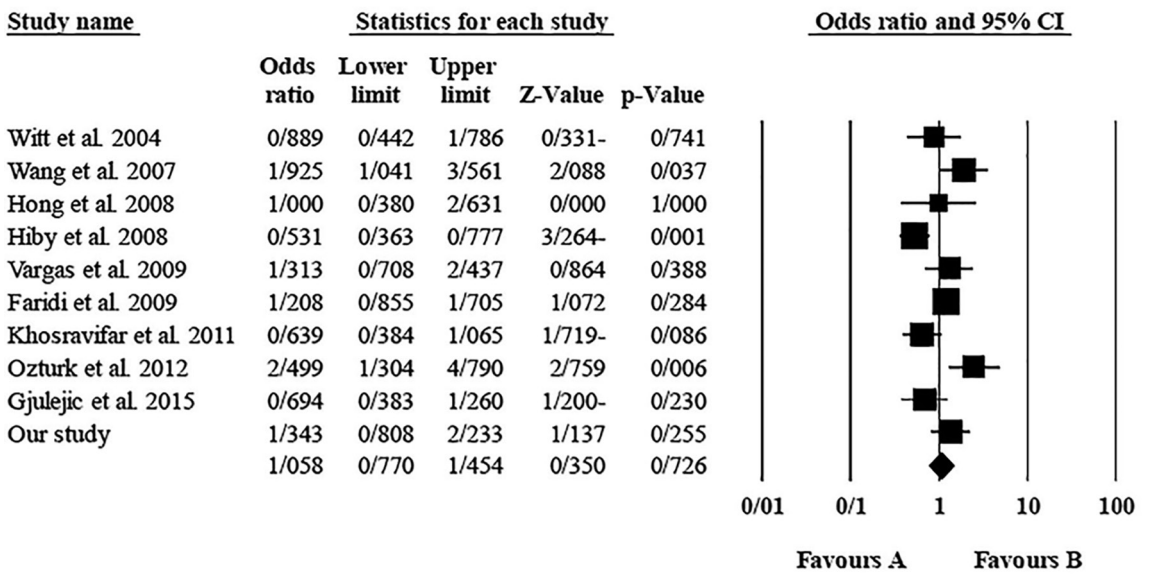

Figure 13. KIR2DS1 Forest plot (random).

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

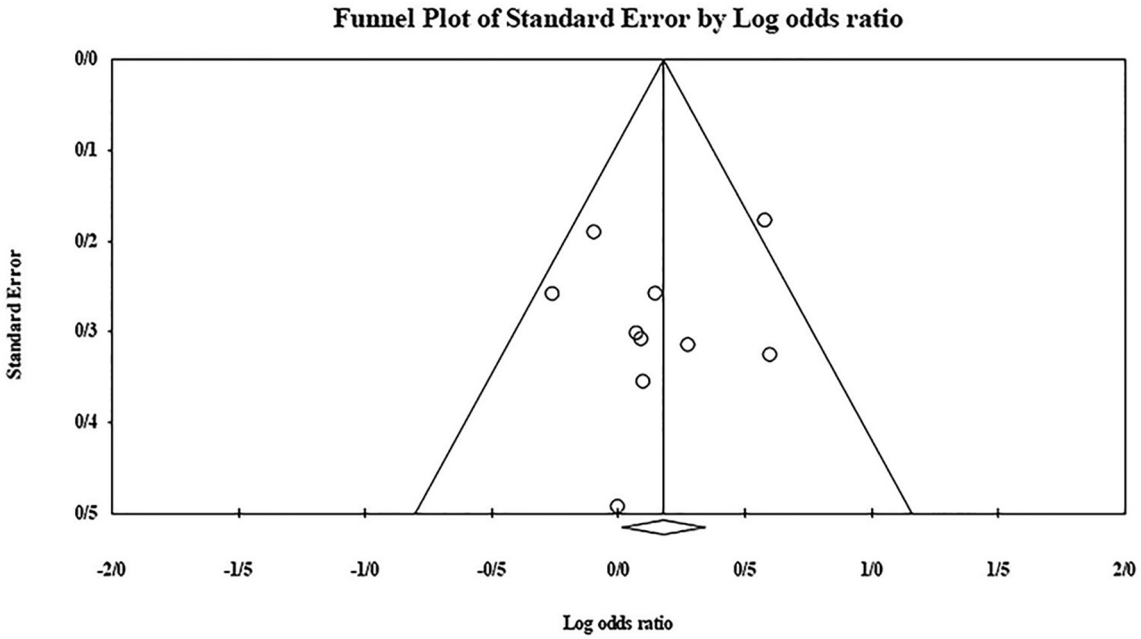

Figure 14. KIR2DS2 Funnel plot showing a significant bias for Faridi et al. (2009).

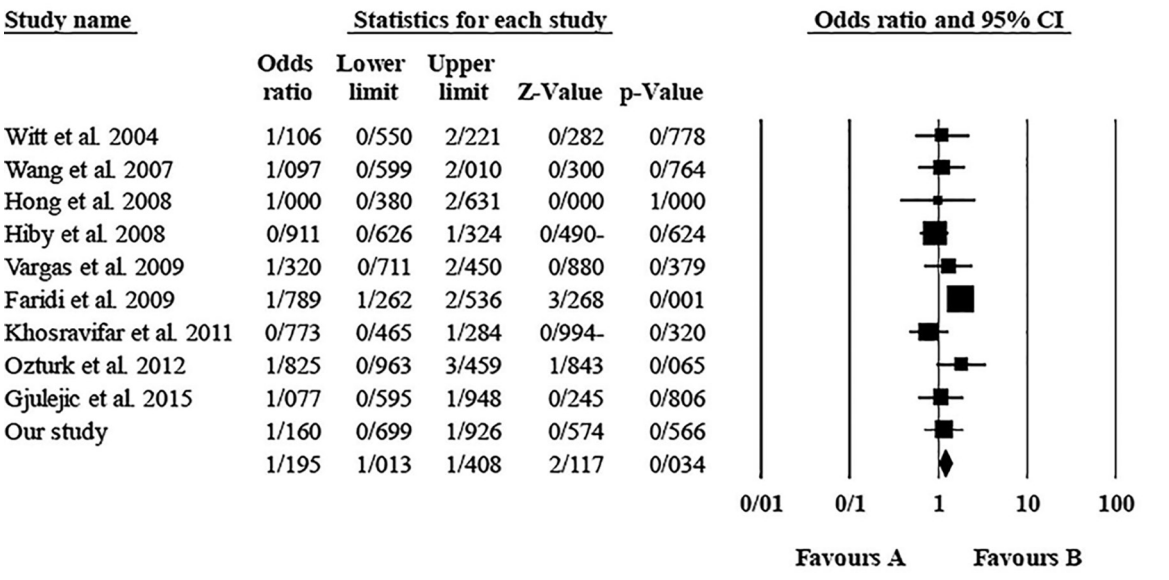

Figure 15. KIR2DS2 Forest plot (fixed).

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

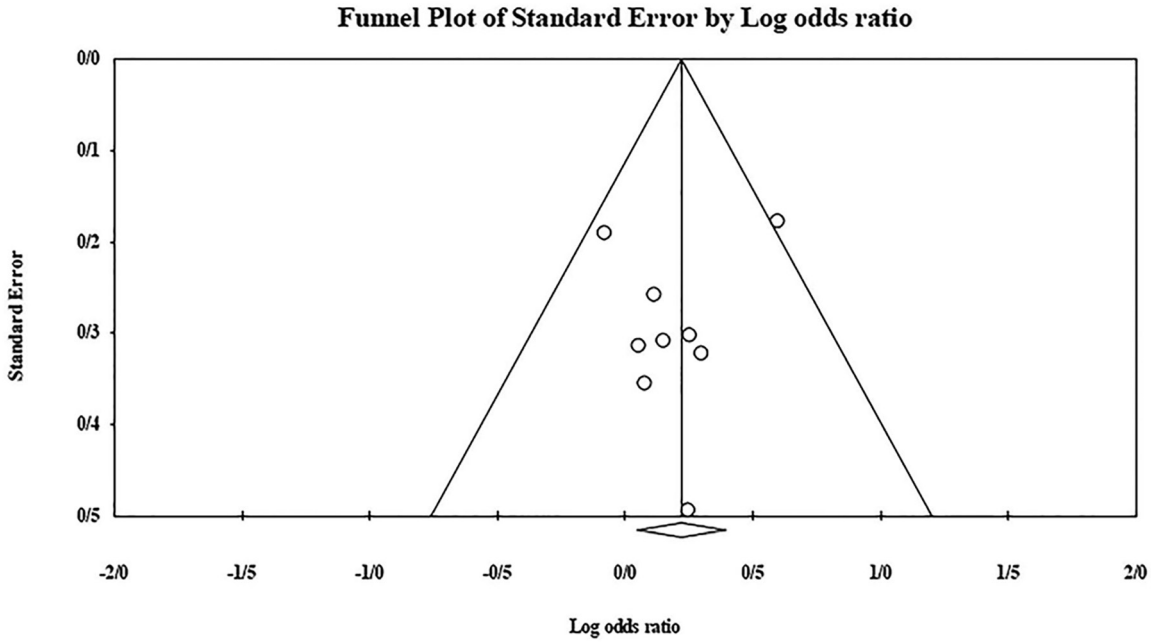

Figure 16. KIR2DS3 Funnel plot showing a rather significant bias for Faridi et al. (2009).

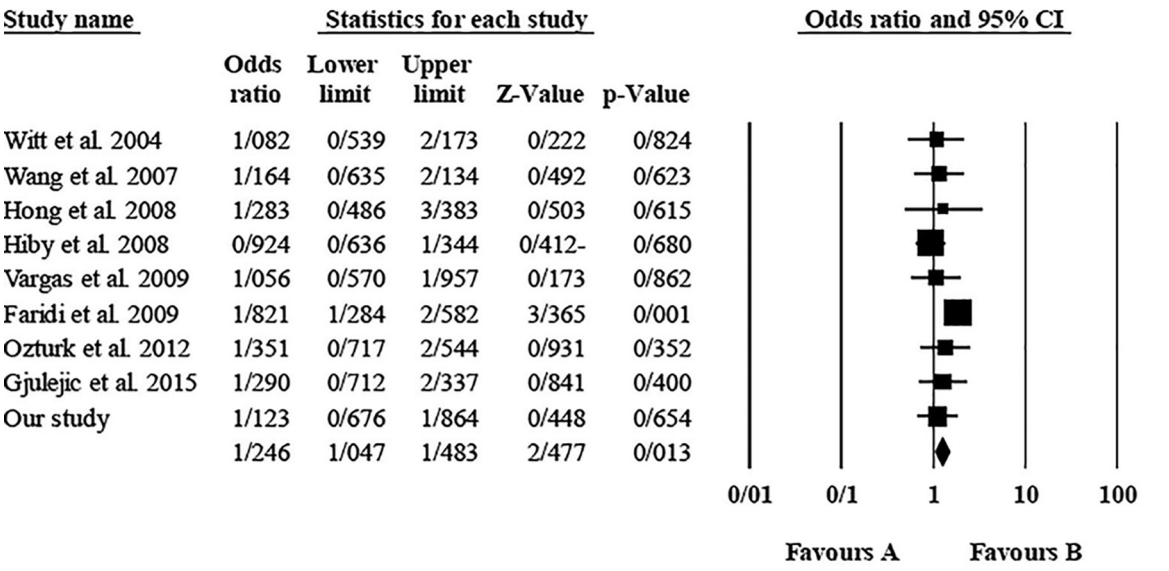

Figure 17. KIR2DS3 Forest plot (fixed).

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

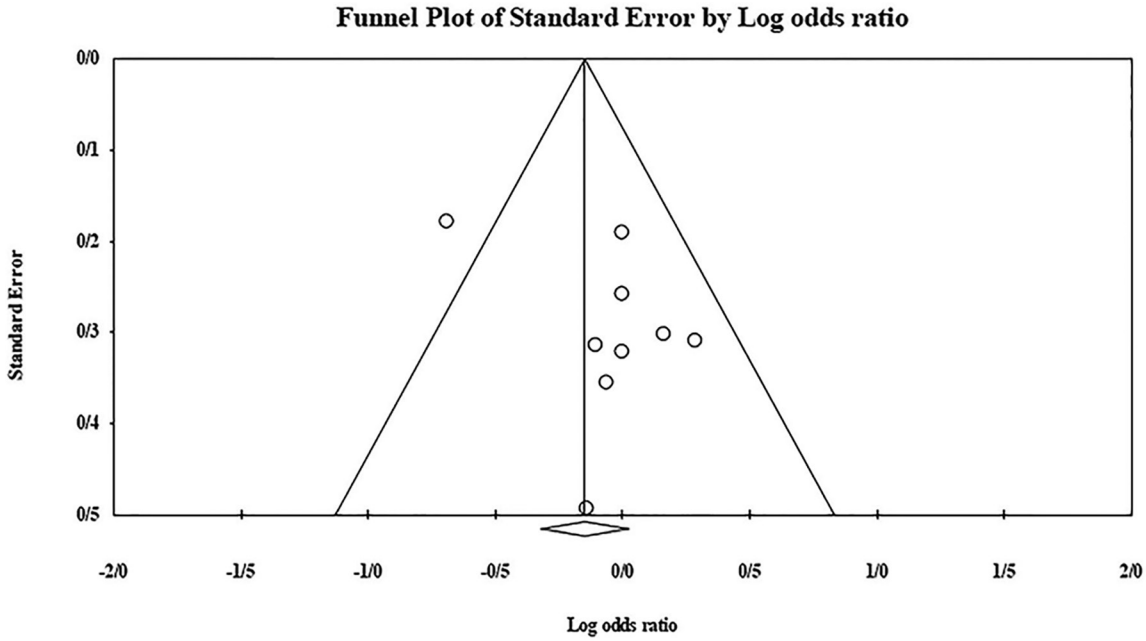

Figure 18. Funnel plot of KIR2DS4 shows a significant bias for Faridi et al. (2009).

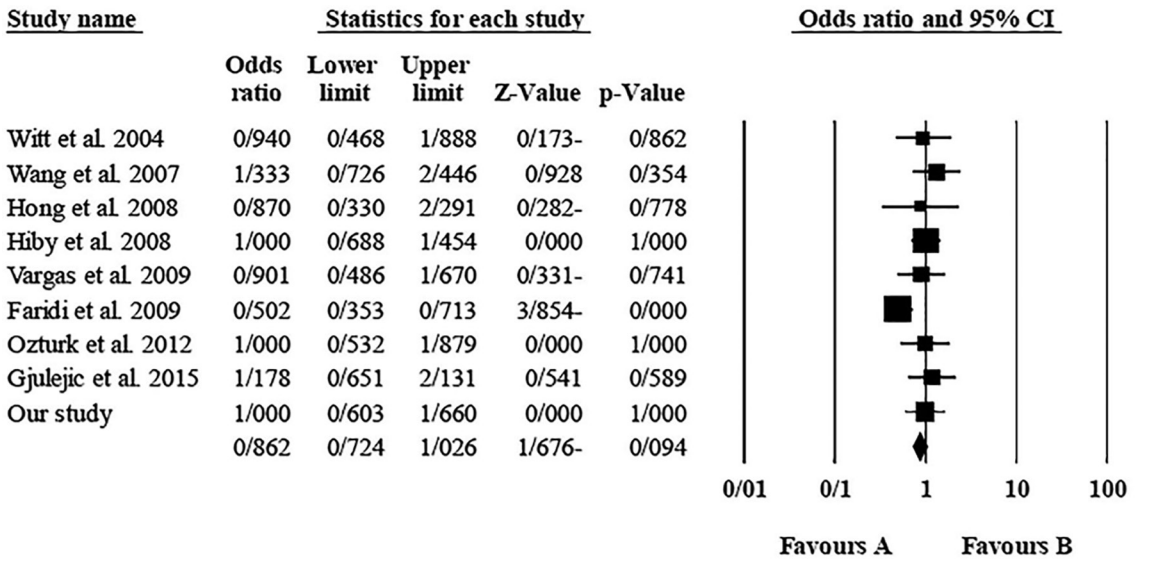

Figure 19. KIR2DS4 Forest plot (fixed).

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

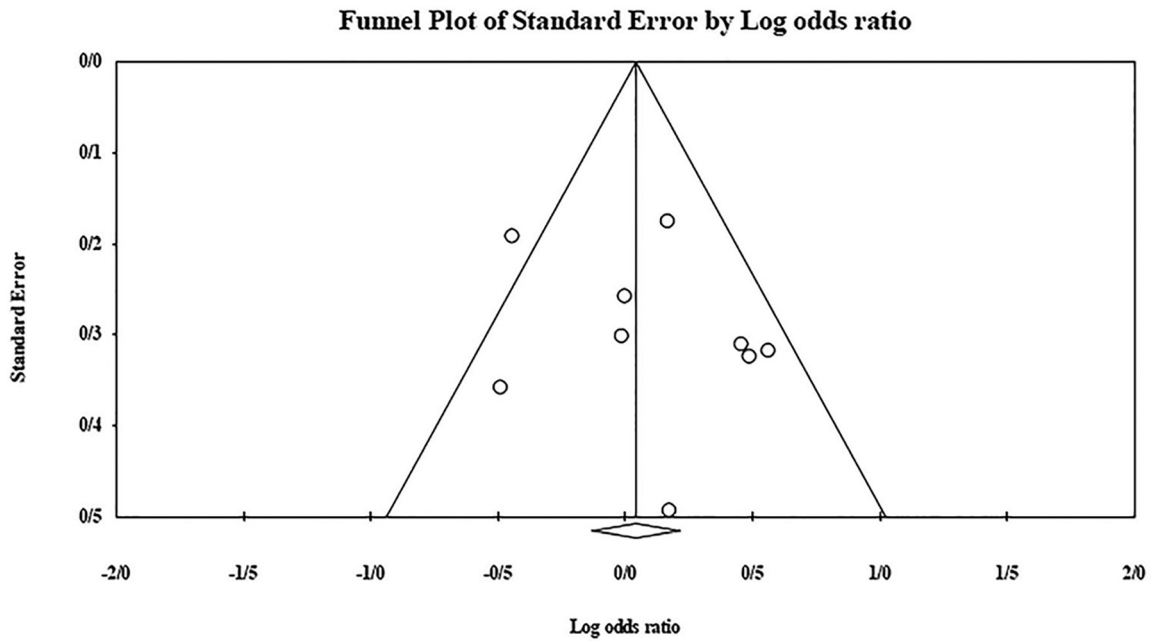

Figure 20. KIR2DS5 Funnel plot showing a significant bias for Hiby et al. (2008).

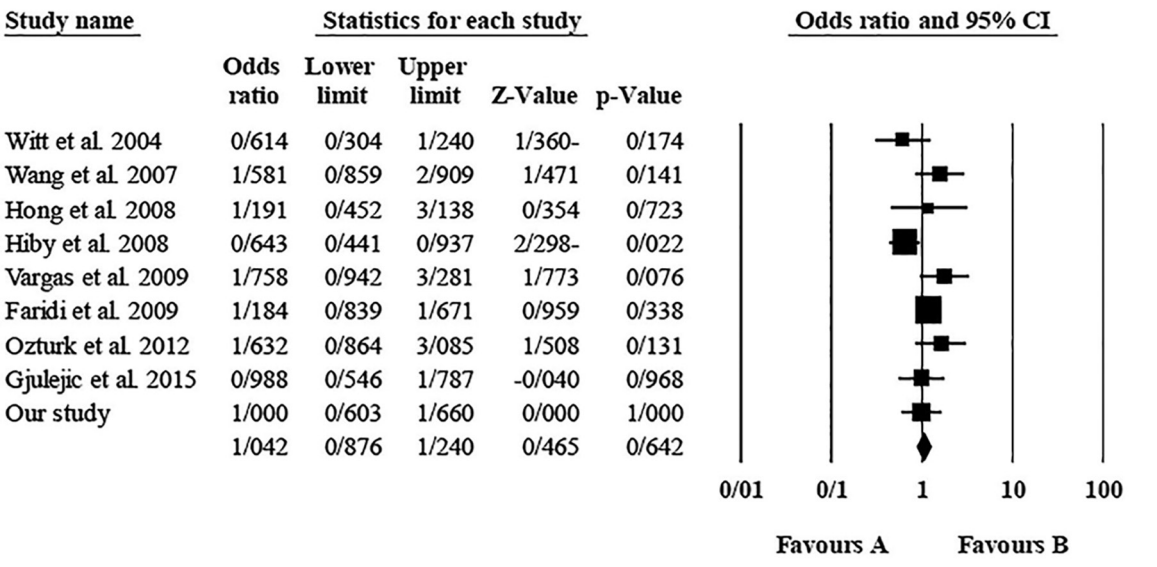

Figure 21. KIR2DS5 Forest plot (fixed).

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.

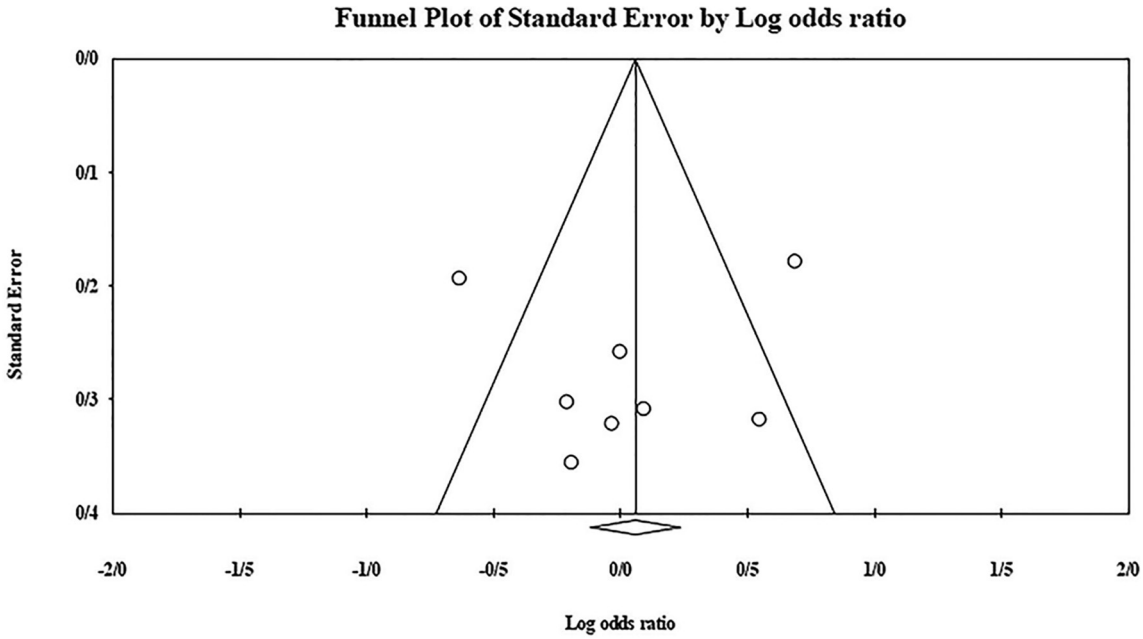

Figure 22. KIR3DS1 Funnel plot showing a significant bias for Hiby et al. (2008) and Faridi et al. (2009).

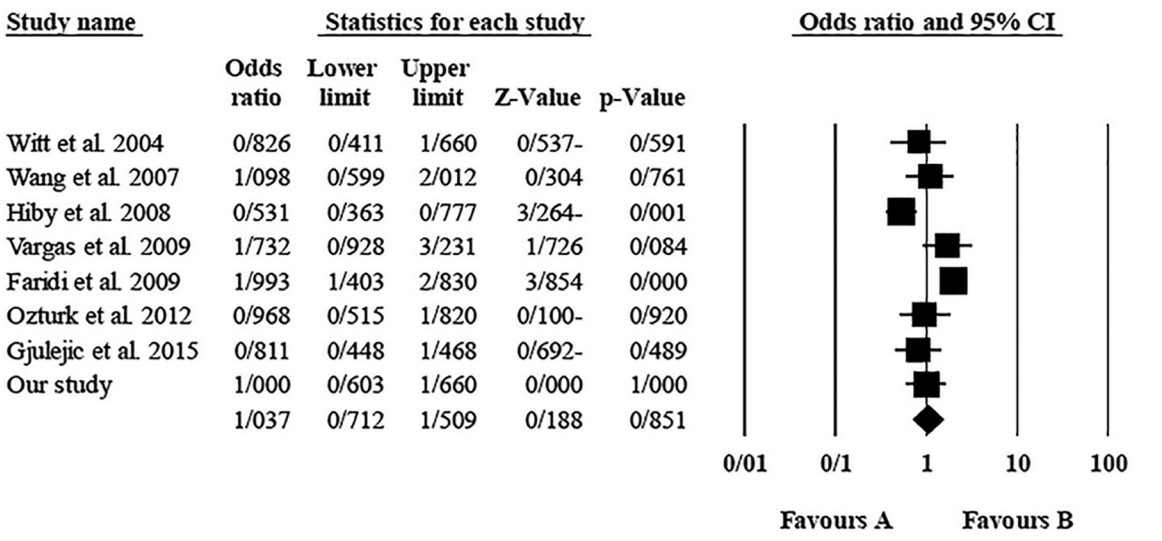

Figure 23. KIR3DS1 Forest plot (random).

ACKNOWLEDGEMENTS

The original case-control version of this study received support by the Lorestan University of Medical Sciences, with the following registration number from the Ethics Committee "lums.rec.1394.10". The corresponding author SAY Ahmadi is a member of the Research Committee; IUMS is the research mentor for the medical students.

CONFLICT OF INTEREST

None to declare.

Table 3. The pooled results of the meta-analsis. In the cases I2>50 random effect model has also been performed.

Pooled	Fixed effect			Random effect		
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						
2DL5	53.79	0.521	0.945	0.00	0.767	0.960
3DL1	47.16	0.044*	0.833	-	-	-
3DL2						
3DL3						
2DS1	70.31	0.990	0.999	0.00	0.726	1.058
2DS2	25.97	0.034*	1.195	-	-	-
2DS3	0.00	0.013*	1.246	-	-	-
2DS4	40.36	0.094	0.862	-	-	-
2DS5	48.52	0.642	1.042	-	-	-
3DS1	75.82	0.525	1.059	0.00	0.851	1.037
2DP1						
3DP1						

* significant at 0.05.

Corresponding author:
Seyyed Amir Yasin Ahmadi
Student Research Committee, Iran University of Medical Sciences
Tehran, Iran.
E-mail: yasin_ahmadi73@yahoo.com

REFERENCES

Akbari S, Ahmadi SAY, Shahsavar F. The Relationship of Maternal KIR and Parental HLA-C Genes With Risk of Recurrent Spontaneous Abortion: A Regional Study in Lorestan Province, Iran. Crescent J Med Biol Sci. 2018;5:194-7.

Alecsandru D, Garrido N, Vicario JL, Barrio A, Aparicio P, Requena A, Garcia-Velasco JA. Maternal KIR haplotype influences live birth rate after double embryo transfer in IVF cycles in patients with recurrent miscarriages and implantation failure. Hum Reprod. 2014;29:2637-43. PMID: 25316448 DOI: 10.1093/humrep/deu251

Ambühl LMM, Baandrup U, Dybkær K, Blaakær J, Uldbjerg N, Sørensen S. Human Papillomavirus Infection as a Possible Cause of Spontaneous Abortion and Spontaneous Preterm Delivery. Infect Dis Obstet Gynecol. 2016;2016:3086036. PMID: 27110088 DOI: 10.1155/2016/3086036

Anbari K, Ahmadi SAY. The Software Comprehensive Meta-Analysis Needs to Be Upgraded Further: Letter to the Editors. Epidemiol Biostat Pub Health. 2017; 14: e12522. DOI: 10.2427/12522

Ashouri E, Norman PJ, Guethlein LA, Han AS, Nemat-Gorgani N, Norberg SJ, Ghaderi A, Parham P. HLA class I variation in Iranian Lur and Kurd populations: high haplotype and allotype diversity with an abundance of KIR ligands. HLA. 2016:88:87-99. PMID: 27558013 DOI: 10.1111/tan.12852

Dambaeva SV, Lee DH, Sung N, Chen CY, Bao S, Gilman-Sachs A, Kwak-Kim J, Beaman KD. Recurrent Pregnancy Loss in Women with Killer Cell Immunoglobulin-Like Receptor KIR2DS1 is Associated with an Increased HLA-C2 Allelic Frequency. Am J Reprod Immunol. 2016;75:94-103. PMID: 26589762 DOI: 10.1111/aji.12453

Djulejic E, Petlichkovski A, Trajkov D, Dimitrov G, Alabakovska S. KIR Gene Frequencies in Women with Infertility Problems. SEE J Immunol. 2015;2015:20002. DOI: 10.3889/seejim.2015.20002

Faridi RM, Das V, Tripthi G, Talwar S, Parveen F, Agrawal S. Influence of activating and inhibitory killer immunoglobulin-like receptors on predisposition to recurrent miscarriages. Hum Reprod. 2009;24:1758-64. PMID: 19279038 DOI: 10.1093/humrep/dep047

Ghafourian M, Band NA, Pour AF, Kooti W, Rad MF, Badiee M. The role of CD16+, CD56+, NK (CD16+/CD56+) and B CD20+ cells in the outcome of pregnancy in women with recurrent spontaneous abortion. Int J Womens Health Reprod Sci. 2015;3:61-6. DOI: 10.15296/ijwhr.2015.10

Gold KJ, Sen A, Hayward RA. Marriage and cohabitation outcomes after pregnancy loss. Pediatrics. 2010;125:e1202-e7. PMID: 20368319 DOI: 10.1542/peds.2009-3081

Gupta S, Agarwal A, Banerjee J, Alvarez JG. The role of oxidative stress in spontaneous abortion and recurrent pregnancy loss: a systematic review. Obstet Gynecol Surv. 2007;62:335-47. PMID: 17425812 DOI: 10.1097/01.ogx.0000261644.89300.df

Hiby SE, Regan L, Lo W, Farrell L, Carrington M, Moffett A. Association of maternal killer-cell immunoglobulin-like receptors and parental HLA-C genotypes with recurrent miscarriage. Hum Reprod. 2008;23:972-6. PMID: 18263639 DOI: 10.1093/humrep/den011

Hong Y, Wang X, Lu P, Song Y, Lin Q. Killer immunoglobulin-like receptor repertoire on uterine natural killer cell subsets in women with recurrent spontaneous abortions. Eur J Obstet Gynecol Reprod Biol. 2008;140:218-23. PMID: 18572300 DOI: 10.1016/j.ejogrb.2008.04.011

Hume H, Chasen ST. Trends in timing of prenatal diagnosis and abortion for fetal chromosomal abnormalities. Am J Obstet Gynecol. 2015;213:545.e1-4. PMID: 26070711 DOI: 10.1016/j.ajog.2015.06.008

Khosravifar M, Sanati MH, Gourabi H, Habibi R, Chaparzadeh N. Influence of maternal killer-cell immunoglobulin-like receptors and parental HLA-C genotypes on predisposition to recurrent miscarriage. Iran J Biol. 2011;24:477-86.

Middleton D, Meenagh A, Serrano-Vela JI, Moscoso J, Arnaiz-Villen A. Different evolution of inhibitory and activating killer immunoglobulin receptors (KIR) in worldwide human populations. Open Immunol J. 2008;1:42-50. DOI: 10.2174/1874226200801010042

Mitra A, Boroujeni MB. Application of gel-based proteomic technique in female reproductive investigations. J Hum Reprod Sci. 2015;8:18-24. PMID: 25838744 DOI: 10.4103/0974-1208.153121

Nardi Fda S, Slowik R, Wowk PF, da Silva JS, Gelmini GF, Michelon TF, Neumann J, Bicalho MG. Analysis of HLA-G polymorphisms in couples with implantation failure. Am J Reprod Immunol. 2012;68:507-14. PMID: 23009094 DOI: 10.1111/aji.12001

Norman PJ, Hollenbach JA, Nemat-Gorgani N, Marin WM, Norberg SJ, Ashouri E, Jayaraman J, Wroblewski EE, Trowsdale J, Rajalingam R, Oksenberg JR, Chiaroni J, Guethlein LA, Traherne JA, Ronaghi M, Parham P. Defining KIR and HLA Class I Genotypes at Highest Resolution via High-Throughput Sequencing. Am J Hum Genet. 2016;99:375-91. PMID: 27486779 DOI: 10.1016/j.ajhg.2016.06.023

Nowak I, Malinowski A, Tchorzewski H, Barcz E, Wilczynski JR, Grybos M, Kurpisz M, Luszczek W, Banasik M, Reszczynska-Slezak D, Majorczyk E, Wisniewski A, Senitzer D, Yao Sun J, Kusnierczyk P. Frequencies of killer immunoglobulin-like receptor genotypes influence susceptibility to spontaneous abortion. J Appl Genet. 2009;50:391-8. PMID: 19875891 DOI: 10.1007/BF03195699

Nowak I, Malinowski A, Tchórzewski H, Barcz E, Wilczyński JR, Banasik M, Gryboś M, Kurpisz M, Luszczek W, Majorczyk E, Wiśniewski A, Senitzer D, Sun JY, Kuśnierczyk P. HLA-C C1C2 heterozygosity may protect women bearing the killer immunoglobulin-like receptor AA genotype from spontaneous abortion. J Reprod Immunol. 2011;88:32-7. PMID: 21134695 DOI: 10.1016/j.jri.2010.11.001

Nowak I, Malinowski A, Barcz E, Wilczyński JR, Wagner M, Majorczyk E, Motak-Pochrzęst H, Banasik M, Kuśnierczyk P. Possible Role of HLA-G, LILRB1 and KIR2DL4 Gene Polymorphisms in Spontaneous Miscarriage. Arch Immunol Ther Exp (Warsz). 2016;64:505-14. PMID: 26973020 DOI: 10.1007/s00005-016-0389-7

Ozturk OG, Sahin G, Karacor ED, Kucukgoz U. Evaluation of KIR genes in recurrent miscarriage. J Assist Reprod Genet. 2012;29:933-8. PMID: 22669573 DOI: 10.1007/s10815-012-9811-1

Pereza N, Ostojić S, Kapović M, Peterlin B. Systematic review and meta-analysis of genetic association studies in idiopathic recurrent spontaneous abortion. Fertil Steril. 2017;107:150-9.e2. PMID: 27842992 DOI: 10.1016/j.fertnstert.2016.10.007

Rand JH, Wu XX, Andree HA, Lockwood CJ, Guller S, Scher J, Harpel PC. Pregnancy loss in the antiphospholipid-antibody syndrome-a possible thrombogenic mechanism. N Engl J Med. 1997;337:154-60. PMID: 9219701 DOI: 10.1056/NEJM199707173370303

Sacks G. Enough! Stop the arguments and get on with the science of natural killer cell testing. Hum Reprod. 2015;30:1526-31. PMID: 25954038 DOI: 10.1093/humrep/dev096

Solgi G, Ghafari H, Ashouri E, Alimoghdam K, Rajalingam R, Amirzargar A. Comparison of KIR gene content profiles revealed a difference between northern and southern Persians in the distribution of KIR2DS5 and its linked loci. Hum Immunol. 2011;72:1079-83. PMID: 21867738 DOI: 10.1016/j.humimm.2011.08.002

Vargas RG, Bompeixe EP, França PP, Marques de Moraes M, da Graça Bicalho M. Activating killer cell immunoglobulin-like receptor genes' association with recurrent miscarriage. Am J Reprod Immunol. 2009;62:34-43. PMID: 19527230 DOI: 10.1111/j.1600-0897.2009.00709.x

Varla-Leftherioti M, Spyropoulou-Vlachou M, Keramitsoglou T, Papadimitropoulos M, Tsekoura C, Graphou O, Papadopoulou C, Gerondi M, Stavropoulos-Giokas C. Lack of the appropriate natural killer cell inhibitory receptors in women with spontaneous abortion. Hum Immunol. 2005;66:65-71. PMID: 15620464 DOI: 10.1016/j.humimm.2004.10.005

Wang S, Zhao YR, Jiao YL, Wang LC, Li JF, Cui B, Xu CY, Shi YH, Chen ZJ. Increased activating killer immunoglobulin-like receptor genes and decreased specific HLA-C alleles in couples with recurrent spontaneous abortion. Biochem Biophys Res Commun. 2007;360:696-701. PMID: 17617375 DOI: 10.1016/j.bbrc.2007.06.125

Witt C, Goodridge J, Gerbase-Delima MG, Daher S, Christiansen FT. Maternal KIR repertoire is not associated with recurrent spontaneous abortion. Hum Reprod. 2004;19:2653-7. PMID: 15333596 DOI: 10.1093/humrep/deh483

Würfel W. Reproductive Immunology? More Important than Ever Before. Reprod Immunol. 2016;1:3.

Xiong S, Sharkey AM, Kennedy PR, Gardner L, Farrell LE, Chazara O, Bauer J, Hiby SE, Colucci F, Moffett A. Maternal uterine NK cell-activating receptor KIR2DS1 enhances placentation. J Clin Invest. 2013;123:4264-72. PMID: 24091323 DOI: 10.1172/JCI68991

Yamada H, Shimada S, Kato EH, Morikawa M, Iwabuchi K, Kishi R, Onoé K, Minakami H. Decrease in a specific killer cell immunoglobulin-like receptor on peripheral natural killer cells in women with recurrent spontaneous abortion of unexplained etiology. Am J Reprod Immunol. 2004;51:241-7. PMID: 15209394 DOI: 10.1111/j.1600-0897.2004.00139.x