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Association of Interleukin-33 with Recurrent Pregnancy Loss: A Systematic Review and Meta-Analysis Study

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Abstract

Background: Recurrent pregnancy loss (RPL) is a condition in which a woman experiences two or more consecutive miscarriages before reaching 20 weeks of gestation. The present study was designed to explore the potential link between the interleukin (IL)-33 and RPL. To achieve this goal, we conducted a comprehensive search across the ISI, PubMed, Embase, and Scopus databases.

Materials and Methods: We reviewed studies that examined the relationship between specific IL-33 polymorphisms and RPL using the appropriate keywords. The comparator in our study consisted of a control group that included women without RPL. This group was used to assess the differences in genetic polymorphisms and IL-33 serum levels compared to women who experienced RPL. We analyzed the collected data using a random-effects model in STATA (version 14). Five case-control studies were eligible, yielding a total sample size of 1,831 participants (891 cases and 940 controls).

Results: The results revealed a significant association between the GG genotype of IL-33 polymorphisms rs16924159 and rs1929992 [odds ratio (OR)=0.72, 95% confidence intervals (CI)=0.51-1.02, I^2=58.2, P=0.068] and RPL. The presence of the GG genotype of IL-33 polymorphisms rs16924159 and rs1929992 in women was associated with a 0.72-fold increased risk of RPL. Additionally, no statistically significant correlation was noted between IL-33 GA (OR=1.19, 95% CI=75-1.88, I^2=80.7, P=0.001) and AA (OR=1.02, 95% CI=0.81-1.29, I^2=71.8, P=0.029) genotypes and RPL. Analysis of IL-33 serum levels indicated a significant association with RPL (OR=-0.45, 95% CI=-0.67-(-0.23), I^2=57.3, P=0.128).

Conclusion: Our findings demonstrate an association between the GG genotype of IL-33 polymorphisms and an increased risk of RPL. Moreover, higher serum levels of IL-33 are likely protective against RPL.

Keywords: IL-33, Polymorphism, Recurrent Pregnancy Loss, rs16924159, rs1929992

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Introduction

Recurrent pregnancy loss (RPL), as a significant reproductive health concern, is characterized by the experience of two or more consecutive miscarriages occurring before the 20th week of gestation (1). RPL affects about 1-2% of women of reproductive age and poses a substantial clinical challenge due to its multifactorial etiology. While genetic, anatomical, endocrine, immunological, and environmental factors have been implicated, the cause remains unidentified in nearly 50% of cases. This gap highlights an urgent need for a deeper exploration of potential contributing factors and biomarkers (2, 3).

Recent advances in immunology have illuminated the significant role of the immune system in pregnancy, highlighting the essential balance between immune tolerance and defense that is critical for successful gestation (4).

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Among the various immune components implicated in pregnancy, cytokines have emerged as pivotal regulators. Interleukin-33 (IL-33), a cytokine family member of IL-1, has garnered considerable attention owing to its dual function as both an extracellular cytokine and a nuclear factor (5). IL-33 is widely expressed in multiple tissues, particularly the reproductive system, and is known to regulate immune responses, tissue repair, and homeostasis (6).

IL-33 functions through its receptor, ST2, which exists in two forms: a transmembrane form (ST2L) and a soluble form (sST2). The former form signals intracellularly, while the latter form acts as a decoy receptor, modulating the availability of IL-33 (7). This cytokine-receptor interaction is crucial in maintaining immune equilibrium at the maternal-fetal boundary (8). During a healthy pregnancy, IL-33 contributes to establishing immune tolerance towards the semi-allogenic fetus. It facilitates the expansion of regulatory T cells and enhances the production of anti-inflammatory cytokines while limiting the activation of pro-inflammatory pathways (9). However, there is a connection between the dysregulation of IL-33 signaling and various pathological conditions, including inflammatory and autoimmune diseases (10). Research has also exhibited a relationship between this interleukin (IL) and pregnancy outcomes, although findings have been mixed (11-14). Some studies have suggested that elevated IL-33 serum levels are linked to adverse pregnancy outcomes, including RPL, pre-eclampsia, and preterm birth, while others indicate a protective role (14). Genetic polymorphisms in cytokine genes can influence their expression and function, potentially affecting pregnancy outcomes. Specifically, single nucleotide polymorphisms in the IL-33 gene variants, such as rs16924159 and rs1929992, may alter IL-33 expression levels or activity, impacting its role in maintaining pregnancy (15). Investigating these polymorphisms provides an opportunity to understand the genetic basis of RPL and identify potential biomarkers for early diagnosis and targeted interventions (15).

The inconsistency in findings across studies necessitates a comprehensive evaluation to clarify the relationship between IL-33 and RPL. The present study aimed to critically appraise and synthesize existing evidence regarding the association of IL-33 polymorphisms rs16924159 and rs1929992 with RPL by examining both gene expression and IL-33 serum levels.

Materials and Methods

This research was carried out as per the guidelines suggested in the preferred reporting items for systematic reviews and meta-analyses (PRISMA) (16).

Search strategies

A comprehensive search was conducted to identify published studies that report the link of IL-33 polymorphisms rs16924159 and rs1929992 with recurrent pregnancy loss. Keywords used included "rs16924159", "rs1929992", "polymorphism", "IL13", "recurrent pregnancy loss", "recurrent miscarriage", "spontaneous abortion", "habitual miscarriage", "RPL", "genotype", and "frequency". The keywords were subsequently combined using Boolean operators ("OR" and "AND") to conduct searches in ISI, PubMed, Embase, and Scopus databases. A search was conducted on Google Scholar to identify studies not indexed in the aforesaid databases. Following this, the references of the studies extracted were examined to identify relevant articles. The records were ultimately imported into the EndNote, and duplicates were removed.

Study selection

This study focuses on women who have experienced RPL, i.e. the occurrence of three or more consecutive miscarriages prior to 20 weeks of gestation. The control group consisted of women without RPL. The intervention was polymorphisms of IL-33 (rs16924159 and rs1929992) and IL-33 serum levels. The primary outcome was the association between IL-33 polymorphisms (rs16924159 and rs1929992), the occurrence of RPL, and the relationship between serum IL-33 levels and RPL. After removing duplicate studies, the titles and abstracts of the remaining articles were examined to determine the eligibility of studies in conformity with the specified inclusion and exclusion criteria. The inclusion criteria consisted of: i. Original case-control studies that investigated the relationship between the genotypes of interest and RPL with extractable data, ii. Studies measured the gene expression levels of IL-33 in relevant tissues (e.g. endometrial or peripheral blood cells) and/or IL-33 serum levels, and iii. The methodology for measuring gene expression and serum levels should be clearly described [e.g., reverse transcription-polymerase chain reaction (RT-PCR) and Enzyme-linked immunosorbent assay (ELISA)]. The criteria excluded were meta-analyses, review articles, conference abstracts, non-English articles, animal studies, and in vitro studies. Two authors initially selected the eligible studies, which were then reviewed and verified for accuracy by all authors.

Data extraction and quality assessment

Two authors independently extracted data from the selected studies. The extracted information entailed authors' names, study locations, publication dates, genotyping methods, the age and number of participants, and details on the IL-33 polymorphisms rs16924159 and rs1929992 and IL-33 serum levels in both study groups. All extracted data were examined for possible bias by additional authors and subsequently validated by all authors. The Newcastle-Ottawa Scale was employed to assess the methodology and quality of the studies. Studies were classified as low, medium, and high quality, with scores of 0-3, 4-6, and 7-9, respectively. Studies scoring less than 4 are indicated in Table 1.

Data synthesis and analysis

After combining the mean sample sizes and standard

deviations of the reported data, we weighted the studies using the inverse-variance method. We also used the Q test and I^2 index to evaluate the heterogeneity of the included studies, with a significance level set at α <10%. Using the random-effects model, we analyzed the heterogeneous data. Finally, the statistical analysis of all data was accomplished using STATA version 14 (StataCorp LLC, USA).

Risk of bias between studies

To assess the presence of publication bias in the data, we used Begg's funnel plots and Egger's test. P<0.05 were deemed to be statistically significant.

Results

The current meta-analysis included five original case-control articles, which investigate the association between IL-33 and RPL (Table 1). The process of selecting a study is depicted in Figure 1. The total number of samples consisted of 1,831 individuals, including 891 cases (with a mean age of 30.79 ± 5.26 years) and 940 controls (with a mean age of 30.33 ± 6.5 years). The genotypes of the participants were classified for statistical analysis into four categories as follows: wild type (AA), homozygotes (GG), heterozygotes (GA), and mutant homozygotes (GG) (Table 2). This table shows the frequency of IL-17A rs2275913 genotypes and alleles in the RPL and the control groups. The results from the random-effects model showed a statistically significant association between the GG genotype of IL-33 polymorphisms rs16924159 and rs1929992 (OR=0.72, 95% CI=0.51-1.02, I^2=58.2, P=0.068) with RPL. The presence of the GG genotype of the same polymorphisms in women was associated with a 0.72-fold increased risk of PRL. The I2 statistic, which indicates heterogeneity, was 58.2%, suggesting heterogeneity among the included studies (Fig.2). Based on the random-effects model, there was no statistically significant relationship between the GA genotype of IL-33 polymorphisms rs16924159 and rs1929992 (OR=1.19, 95% CI=75-1.88, I^2=80.7, P=0.001) and RPL. The I^2 statistic was notably high at 80.7%, indicating substantial heterogeneity among the studies (Fig.3). Additionally, fix-effects model showed no statistically significant association between the AA genotype of IL-33 polymorphisms rs16924159 and rs1929992 (OR=1.02, 95% CI=0.81-1.29, I^2=71.8, P=0.029) with RPL. Our study found a significant relationship between IL-33 serum levels and RPL. In other words, lower serum levels of IL-33 were significantly associated with RPL. The negative OR indicated that decreasing the serum levels of IL-33 increases the risk of RPL (OR=-0.45, 95% CI=-0.67-(-0.23), I^2=57.3, P=0.128).

Table 1: Characterizations of articles reviewed in the present study

First author (reference)	Publication year	Location	Methods	Polymorphism	Sample size		Mean age ± SD		Quality assessment
					Case	Control	Case	Control	
Yue et al. (17)	2016	China	PCR-DNA sequencing, ELISA	rs16924159	321	384	30.17 ± 4.58	29.77 ± 4.66	6
Soheilyfar et al. (18)	2018	Iran	PCR-DNA sequencing	rs1929992	300	300	28 ± 6.1	28.2 ± 5.1	6
Kamrani et al. (19)	2020	Iran	PCR-RFLP	rs16924159	100	100	31.1 ± 4. 67	29.18 ± 2.21	8
Zidan et al. (20)	2018	Egypt	PCR-RFLP, ELISA	rs1929992	142	123	33.9 ± 5.7	34.2 ± 4.3	5
Zhao et al. (14)	2022	China	Flow cytometry	-	28	33	-	-	6

PCR; Polymerase chain reaction, RFLP; Restriction fragment length polymorphism, ELISA; Enzyme-linked immunosorbent assay, and SD; Standard deviation.

Table 2: The frequency of IL-17A rs2275913 genotypes and alleles in recurrent pregnancy loss (RPL) and control group

First author (reference)	Cases				Controls				Cases	Controls
	Total	GG	GA	AA	Total	GG	GA	AA	IL33 serum level (pg/ml)	IL33 serum level (pg/ml)
Yue et al. (17)	321	104	152	65	384	151	184	49	160.57 ± 100.03	196.9 ± 102.12
Soheilyfar et al. (18)	300	42	150	108	300	46	125	129	-	-
Kamrani et al. (19)	100	38	62	0	100	53	47	0	-	-
Zidan et al. (20)	142	46	61	35	123	44	52	27	155.2 ± 72. 3	201 ± 84.9
Zhao et al. (14)									2.58 ± 0.55	1.1 ± 0.34

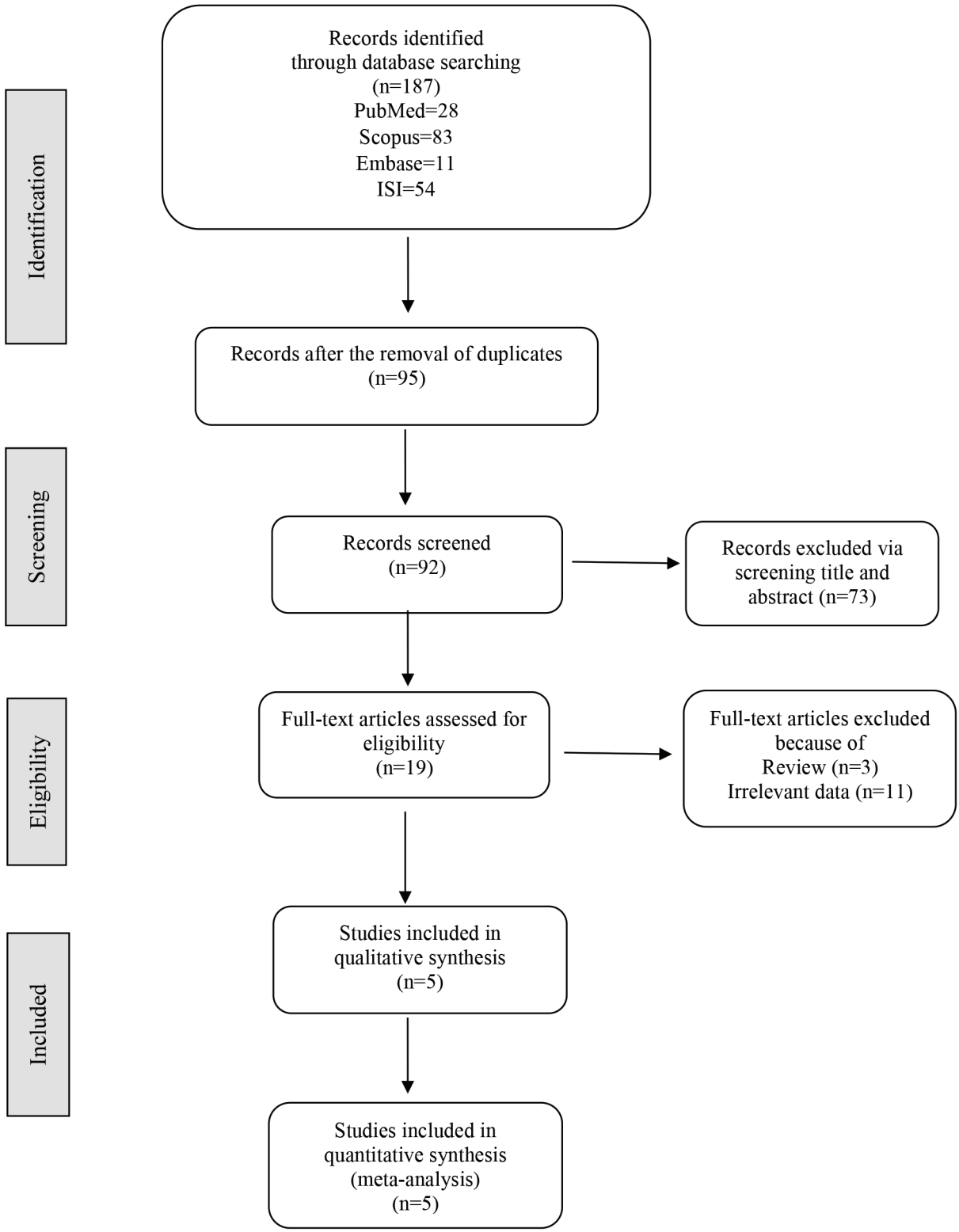

Fig.1: Preferred reporting items for systematic reviews and meta-analyses (PRISMA) flow diagram illustrating selection of articles.

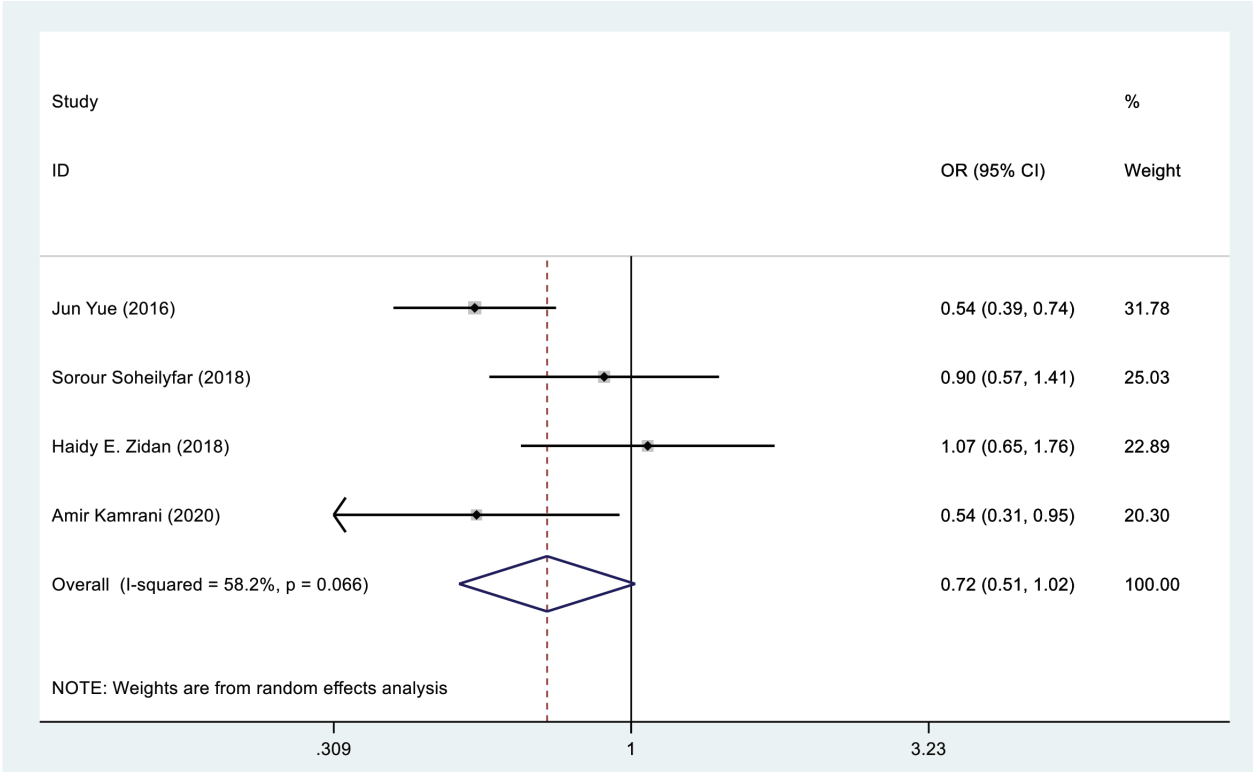

Fig.2: Forest plots for the GG genotype show the relationship of IL-33 polymorphisms with RPL (random effect model). Studies are ordered by publication date and authors' names based on a fixed-effects model. The square represents the effect estimate of individual studies with more than 95% confidence intervals (CI), with the size of the squares proportional to the weight assigned to the study in the meta-analysis. The diamond denotes the overall estimation. RPL; Recurrent pregnancy loss and OR; Odds ratio.

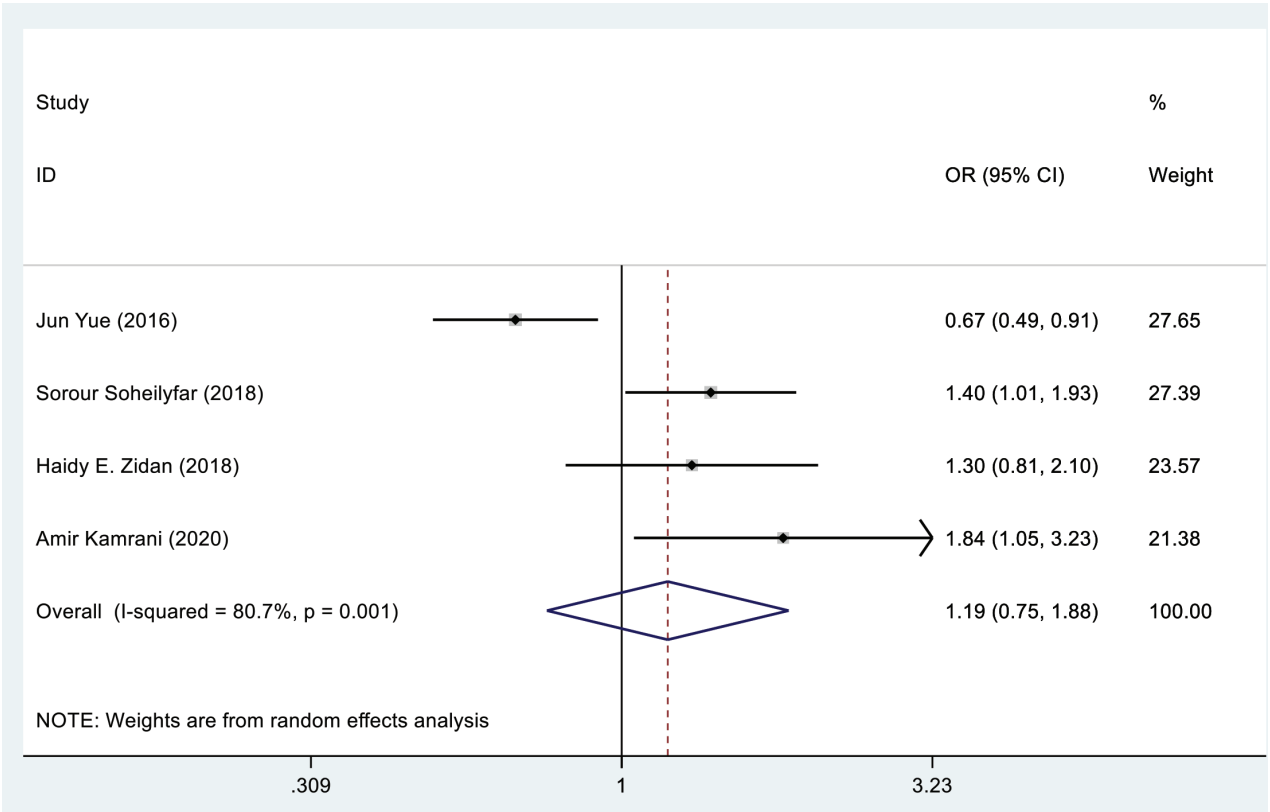

Fig.3: Forest plots for the GA genotype show the relationship of IL-33 polymorphisms with RPL (random effect model). Studies are ordered by publication date and authors' names based on a fixed-effects model. The square represents the effect estimate of individual studies with more than 95% confidence intervals (CI), with the size of squares proportional to the weight assigned to the study in the meta-analysis. Diamond denotes the overall estimation. RPL; Recurrent pregnancy loss and OR; Odds ratio.

Risk of bias between studies

According to the results from Begg's (P=0.602) and Egger's (P=0.442) tests, there was no significant publication bias for either outcome. Figure 4 illustrates the potential for publication bias in the studies, as indicated by the results of the two aforementioned.

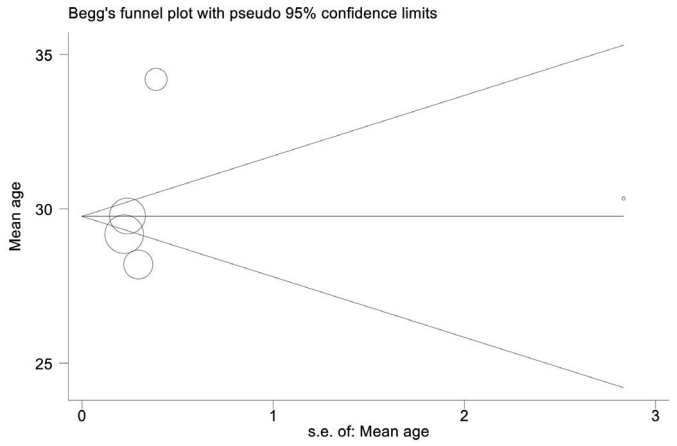

Fig.4: Begg's funnel plot for publication bias diagram in the investigated studies, the circles show the weight of the studies.

Discussion

IL-33 actively participates in the immunological environment necessary for successful pregnancy, and its dysregulation is linked to RPL. The underlying mechanisms involve alterations in immune responses, metabolic reprogramming, and cytokine signaling that disrupt the maternal-fetal interface. Salem et al. (21) have indicated that IL-33 is crucial for the transition from a Th1 to a Th2 cytokine profile, promoting a tolerogenic environment for the fetus. Sheng et al. (22) have also displayed that the IL-33/ST2 axis impacts decidual macrophages, which are essential for clearing apoptotic cells during early pregnancy. To our knowledge, no previous studies have investigated the relationship of IL-33 with RPL. To this end, herein, we investigated the association of IL-33 with RPL. This study included five published articles that report thae relationship between IL-33 and RPL. The findings suggest a nuanced association of IL-33 polymorphisms with RPL. Specifically, the GG genotype of IL-33 polymorphisms rs16924159 and rs1929992 appeared to be a risk factor for RPL, while the GA and AA genotypes did not show significant associations. Also, the results exhibited a significant relationship between the serum levels of IL-33 and RPL.

The studies included in our research presented conflicting results regarding the association of IL-33 polymorphisms rs16924159 and rs1929992, along with its serum levels, with RPL. Yue et al. (17) found that the frequency of the AA genotype of rs16924159 was notably higher in patients compared to controls. In contrast, the research conducted by Soheilyfar et al. (18) indicated significant differences in the frequencies of the GA genotype associated with the IL-33 gene polymorphism (rs1929992) between the case and control groups. The findings of Kamrani et al. (19) showed that the frequency of the GA genotype of the rs16924159 polymorphism was significantly higher in patients than in healthy controls. Three studies from China and Egypt investigated the serum levels of IL-33 in women suffering from RPL (17, 20). Zhao et al. (14) found that IL-33 serum levels were not significantly associated with recurrent spontaneous abortion.

The serum levels of IL-33 are critical for determining the pregnancy outcomes. Research indicates that elevated IL-33 serum levels have a link with RPL in Egyptian women, suggesting that IL-33 could serve as a predictive biomarker for RPL (20). Furthermore, studies involving pregnant mice deficient in the IL-33 gene demonstrate that IL-33 signaling plays a vital role in facilitating various pro-pregnancy processes at the maternal-fetal interface during the early stages of gestation, ultimately influencing favorable outcomes in later phases of pregnancy (23). Additionally, in a rat model, disruption of IL-33 signaling affected placental development and protection against lipopolysaccharide-induced fetal and placental growth restriction, highlighting the regulatory role of IL-33 in pregnancy and its impact on fetal survival and growth (11). In an earlier study, Ren et al. (24) evaluated the association of IL-33 rs3939286 and IL-1RL1 rs13015714 with susceptibility to preeclampsia (PE) in Chinese Han women. Their results indicated that when comparing cases to controls, no notable differences were observed in the genotypic and allelic frequencies of these two polymorphisms. Furthermore, no significant differences were identified in the genetic distributions when comparing mild versus severe PE or early versus late-onset PE with the control group (24). These findings collectively emphasize the importance of IL-33 in modulating immune responses and physiological processes that are crucial for successful pregnancy outcomes.

Like any research, our study has certain limitations. The significant heterogeneity observed in the GA and AA genotype analyses, with I2 values of 80.7 and 71.8%, suggests variability among the study populations, methodologies, or other influencing factors. This heterogeneity could impact the reliability of the pooled estimates. While the total sample size is considerable, the individual studies included may have different sample sizes, which could potentially affect the overall results.

Conclusion

The present meta-analysis reveals a significant relationship between the GG genotype of IL-33 polymorphisms and an increased risk of RPL. Additionally, higher serum levels of IL-33 seem to offer a protective effect against RPL. These findings highlight the importance of genetic factors in RPL and suggest that the GG genotype could serve as a potential marker for assessing RPL risk. However, the substantial heterogeneity observed in some analyses indicates a need for further research to validate these results and explore underlying mechanisms.

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Authors' Contributions

M.A., M.M.; Designed the study's conception. R.B., M.I.Sh., N.J.; Focused on the structural analysis. M.A., M.M., R.B.; Performed technical support and Provided conceptual advice. All authors contributed to drafting the manuscript, revised it critically, and approved the final version.

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