Genetic association between *FXIII* and β -fibrinogen genes and women with recurrent spontaneous abortion: a meta- analysis

Jie Li¹ · Hongbo Wu¹ · Yang Chen² · Huimei Wu¹ · Hong Xu³ · Liuming Li¹

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Abstract

Background *FXIII Val34Leu* (rs5985) and β -fibrinogen -455G/A (rs1800790) genotypes have been reported to be associated with recurrent spontaneous abortion (RSA). However, this topic is controversial. This study aimed to explore whether *FXIII Val34Leu* or β -fibrinogen -455G/A gene polymorphisms are related to RSA.

Methods In this analysis, PubMed, HuGENet and Chinese National Knowledge Infrastructure (CNKI) databases were reviewed. Four models including the dominant model (*Val/Val+Val/Leu* vs. *Leu/Leu*), recessive model (*Val/Val* vs *Val/Leu+Leu/Leu*), co-dominant model (*Val/Val* vs. *Val/Leu*, *Val/Val* vs. *Leu/Leu*) and per-allele analysis (*Val* vs. *Leu*) were applied. The odds ratio (OR) with 95 % confidence interval (CI) was used to assess the association between RSA and *FXIII Val34Leu* and β -fibrinogen -455G/A polymorphisms.

Jie Li and Hongbo Wu contributed equally to this work.

Capsule Our meta-analysis demonstrates that the *FXIII Val34Leu* polymorphism has a close association with RSA and women who carry the Val allele for the *FXIII Val34Leu* polymorphism could have a protective effect against RSA. However, no association is detected between β -fibrinogen -455G/A polymorphisms and the risk of RSA.

✉ Hong Xu
lijie2012@126.com

✉ Liuming Li
liliuming1231@163.com

¹ Guangxi Reproductive Medical Research Center, First Affiliated Hospital of Guangxi Medical University, Nanning, Guangxi Zhuang Autonomous Region 530021, China

² Department of Urology and Nephrology, the First Affiliated Hospital of Guangxi Medical University, Nanning, Guangxi Zhuang Autonomous Region, China

³ Department of Obstetrics and Gynecology, the First Affiliated Hospital of Guangxi Medical University, Nanning, Guangxi Zhuang Autonomous Region 530021, China

Results Nine studies with 10 sets of data were included according to the inclusion criterion. A positive association was detected in the pooled results for the dominant model (*Val/Val+Val/Leu* vs. *Leu/Leu*; OR=0.417, 95 % CI: 0.180–0.965, $I^2=45.60$ %) and co-dominant model (*Val/Val* vs. *Val/Leu*; OR=0.638, 95 % CI: 0.452–0.899, $I^2=36.40$ %) for *FXIII Val34Leu* polymorphisms. However, no statistically significant association between β -fibrinogen -455G/A polymorphisms and RSA was detected in the four different models, including the Asian and Caucasian subgroup analyses.

Conclusions Our meta-analysis demonstrates that the *FXIII Val34Leu* polymorphism has a close association with RSA and women who carry the Val allele for the *FXIII Val34Leu* polymorphism could have a protective effect against RSA. However, no association is detected between β -fibrinogen -455G/A polymorphisms and the risk of RSA. Future well-designed studies are needed to confirm these results.

Keywords *FXIII Val34Leu* · β -fibrinogen -455G/A · Recurrent spontaneous abortion · Polymorphism · Gene

Introduction

Recurrent spontaneous abortion (RSA) is one of the most common complications during pregnancy, especially for early gestation, affecting about 1–5 % of couples [1]. No general consensus can be found on how many consecutive abortions are defined as RSA. Although RSA is defined as three or more consecutive spontaneous abortions before 20 weeks of gestation in some studies [2, 3], two or more consecutive abortions is sometimes considered a way of defining this term [4, 5]. When experiencing consecutive failed pregnancies, couples who want to have children go through a considerable psychological burden. However, the underlying pathophysiological mechanisms of RSA remain unclear and often controversial.

Several identifiable risk factors of RSA have been proposed, such as anatomic, endocrine, immune, infected, and genetic defects, but in up to 30–50 % of cases, the causes of RSA remain unexplained after comprehensive investigation [6, 7]. Inherited thrombophilia has been shown to be a causative factor for reproductive disorders and recurrent pregnancy loss. Thrombophilic gene polymorphisms and mutations, such as *factor V Leiden (FVL)*, *methylenetetrahydrofolate reductase (MTHFR)*, polymorphisms of *plasminogen activator inhibitor-1 (PAI-1)*, *Apolipoprotein E (Apo E)*, and *angiotensin converting enzyme (ACE)* genes have been suspected to be associated with RSA. In addition to above the genetic polymorphisms, *coagulation factor XIII (FXIII)* and *Beta-fibrinogen (BF)* genes have been reported to promote the development of RSA, which appear to be a cause of impaired fibrinolysis and lead to enhanced blood coagulation [8–10].

Coagulation factor XIII (FXIII) is known as fibrin stabilizing factor (FSF), which plays an important role in placental formation and regulates the state of hemostasis and blood coagulation [11, 12]. In the blood, *FXIII* glycoprotein exists as an A2B2 tetramer, including 2A and 2B [3]. The A subunit has catalytic activity which could have an anti-fibrinolytic effect through the early cross-linking of fibrin and the *FXIII* A subunit gene is located on chromosome 6p24–p25, while the B subunit has no catalytic activity and the *FXIII* B subunit gene is located on chromosome 1q31–32.1 [13, 14]. With a high level of polymorphism, nucleotide variations of the *FXIII* A-subunit gene were reported. For *FXIII Val34Leu*, a common G-to-T polymorphism in exon 2 leads to the conversion of *Val* to *Leu*, which could impact on the cross-linking activity and clot stability [14, 15]. *Factor XIII* deficiency could result in abnormal blood clotting and recurrent spontaneous abortion.

The *beta-fibrinogen (BF)* -455G/A substitution in the 5' flanking region is associated with 7–10 % higher plasma fibrinogen levels, which could enhance the physiological increase in fibrinogen concentration driven by enzyme–substrate interactions between thrombin, platelets, and fibrinogen [16]. Thus beta-fibrinogen is possibly associated with arterial complications. During pregnancy, it could increase intravascular fibrin deposition, which is a risk factor for placental thrombosis associated with RPL [17–19].

As thrombophilic gene polymorphisms, both *coagulation factor XIII* and *beta-fibrinogen* variants may associate with spontaneous abortion by impairing fibrinolysis or enhancing blood coagulation. Previous studies have reported that the *FXIII Val34Leu* polymorphisms are related with a reduced risk of arterial and venous thrombosis [20], and the effect of *β-fibrinogen* polymorphisms is related to an increased risk of spontaneous abortion [21]. However, the two suggestions are controversial. In this study, we first carried out a systematic analysis to investigate the real relationship between RSA and the *FXIII Val34Leu* and *β-fibrinogen G-455A* polymorphisms in patients and healthy controls.

Materials and methods

Study selection

In this search, the PubMed, HuGENet and China National Knowledge Infrastructure (CNKI) databases were reviewed, using the following key words: “recurrent pregnancy loss”, “recurrent spontaneous miscarriages”, “RPL”, “RSA”, “RM”, “abortion”, “miscarriage”, “*FXIII*”, “*coagulation factor XIII*”, “*F13A1*”, “*Beta Fibrinogen*”, “*β-fibrinogen*”, “455G>A”, and “polymorphism”. The latest search was performed in January 2015. The languages of articles were not restricted in this analysis. All articles were published in the primary literature and the studies were included only once if the same samples were used for duplicate publications. The eligibility criteria were as follows: (a) case–control designed studies about the relationship of the *FXIII Val34Leu/β-fibrinogen -455G/A* polymorphisms and the risk of recurrent spontaneous miscarriages; (b) samples included patients who had more than two consecutive spontaneous abortions before 20 weeks of gestational age, who were defined as RSA in the included studies; (c) healthy parous women with no history of pregnancy loss were chosen as controls; and (d) genotypes identified by polymerase chain reaction (PCR) analysis. The reasons for exclusion included: (a) studies not providing adequate information, making it difficult to screen cases and controls; and (b) studies were lacking raw data on the distribution of polymorphisms in each group. We also excluded case series, case reports, conference drafts, and review articles. In addition, we assessed the quality of the included studies by using the criteria identified according to the published article [22].

The following characteristics were collected for each eligible study: genotype, authors and year of publication, country and ethnicity, number of cases and controls, genotyping methods, the number of consecutive abortions, source of controls, and the quality assessment (Table 1). The effective data were independently extracted by two investigators and a consensus was reached if there was a disagreement.

Statistical analysis

The odds ratio (OR) and corresponding 95 % confidence interval (95 % CI) were used to assess the association for each study. The dominant model (*Val/Val+Val/Leu* vs. *Leu/Leu*), recessive model (*Val/Val* vs. *Val/Leu+Leu/Leu*), co-dominant model (*Val/Val* vs. *Val/Leu*, *Val/Val* vs. *Leu/Leu*) and per-allele analysis (*Val* vs. *Leu*) were applied to explore the relationship between the *FXIII Val34Leu* genotype and the risk of recurrent spontaneous miscarriages in this analysis. The chi square (χ^2)-based Q statistic was applied when estimating heterogeneity (with $p < 0.10$ as the standard). I^2 values less than 50 % were assigned as low or moderate estimates, otherwise, the estimate was high [23]. Among the statistical analysis, the fixed-effect

Table 1 The essential characteristics of eligible studies in this research

Genotype	Author (Year)	Country (Ethnicity)	No. cases	No. controls	Method	Spontaneous abortion (n)	Source of controls	Quality assessment
<i>FXIII Val34Leu</i> polymorphism (rs5985)	Elmahgoub IR (2014)	Egypt (African)	120	130	PCR	3 or more	Healthy women who had no previous miscarriages and had completed at least two successive pregnancies without complications.	1:adequate;2:adequate;3:adequate;4:adequate;5:unequal
	Poursadegh Zonouzi A (2013)	Iran (Asian)	82	50	ARMS-PCR	2 or more	Healthy women with at least two live births and no miscarriages.	1:adequate;2:adequate;3:adequate;4:adequate;5:unequal
	Bagheri M (2011)	Iran (Asian)	54	46	RFLP-PCR	2 or more	Fertile females who had experienced live births of history of pregnancy loss.	1:adequate;2:adequate;3:adequate;4:adequate;5:adequate
	Lopez Ramirez Y (2006)	Venezuela (Caucasian)	40	40	PCR	2 or more	Healthy women with at least two live births and no miscarriages.	1:adequate;2:adequate;3:adequate;4:adequate;5:unequal
<i>β-Fibrinogen-455G/A</i> polymorphism (rs1800790)	Barbosa HC (2004)	Brazil (Caucasian and Blacks)	86	86	PCR	3 or more	Fertile females who had experienced live births of singletons and no history of pregnancy loss.	1:adequate;2:adequate;3:adequate;4:adequate;5:unequal
	Poursadegh Zonouzi A (2013)	Iran (Asian)	89	50	ARMS-PCR	2 or more	Healthy women with at least two live births and no miscarriages.	1:adequate;2:adequate;3:adequate;4:adequate;5:unequal
	Torabi R (2012)	Iran (Asian)	100	100	PCR-RFLP	2 or more	Healthy women with at least two live births and no miscarriages.	1:adequate;2:adequate;3:adequate;4:adequate;5:unequal
	Ticconi C (2011)	Italy (Caucasian)	98	78	PCR	2 or more	Healthy women with at least two live births and no miscarriages.	1:adequate;2:adequate;3:adequate;4:adequate;5:unequal
	Cai XJ (2008)	China (Asian)	30	30	RFLP-PCR	2 or more	Fertile females who had experienced live births of singletons and no history of pregnancy loss.	1:adequate;2:adequate;3:adequate;4:adequate;5:unequal
	Pihusch R (2001)	Germany (Caucasian)	102	128	PCR	2 or more	Fertile females who had experienced live births of singletons and no history of pregnancy loss.	1:adequate;2:adequate;3:adequate;4:adequate;5:unequal

Quality assessment codes: 1 = including laboratory design and methods; 2 = definition of the RPL; 3 = assessment and validation of cases and controls; 4 = eliminating confounding factors for participants; 5 = Equal assessment for confounding factors for cases and controls

model was used when I^2 values were low or moderate and the random-effect model was considered when I^2 values were high. And the same way was used to evaluate the association between the β -fibrinogen -455G/A polymorphism and the risk of recurrent spontaneous miscarriages.

In order to further explore the source of heterogeneity for β -fibrinogen -455G/A genotypes, the subgroup analysis was performed regarding Caucasian and Asian populations. Based on similar characteristics, four models and similar analyses were performed for subgroup studies. In addition, the susceptibility analysis was carried out for the *FXIII Val34Leu* and β -fibrinogen -455G/A genotypes by excluding the most heterogeneous study to recalculate the pooled OR, which could evaluate the magnitude of influence on the overall summary estimates.

Begg's unweighted regression test and funnel plots were used to assess the publication bias [24]. Genotype data were reported and allele frequencies were calculated. In this statistical analysis, Stata 9.0 (Stata Corporation, USA) was used and all P values were two-tailed.

Results

Characteristics

A total of 662 relevant studies (362 for *FXIII Val34Leu* polymorphisms, 300 for β -fibrinogen -455G/A polymorphisms) were retrieved from the 3 databases (PubMed, HuGENet and CNKI) with keyword searches. After carefully screening the titles and abstracts, 633 reports were removed due to the absence of any discussion of the relationship between *FXIII Val34Leu* or β -fibrinogen -455G/A polymorphisms and the risk of recurrent spontaneous miscarriages or reports being repeated in the 3 database. As a result, 29 full text articles were considered. In detail, 17 closely related studies were focused on the *FXIII Val34Leu* polymorphism and 12 were on the β -fibrinogen -455G/A polymorphism. When scanning the full texts, 12 articles did not provide valid genotype for the *FXIII Val34Leu* polymorphism, 6 articles had no raw data about the association between the β -fibrinogen -455G/A polymorphism and the risk of recurrent spontaneous miscarriages, and 1 report was a duplication with data that have been published in another journal [25]. In addition, one report investigated the association between the two genotypes and RSA [26]. Finally, nine studies were included for further analysis [20, 21, 26–32] (Fig. 1). For analysis of the β -fibrinogen -455G/A polymorphism, three reports investigated Asian populations [26, 30, 31], and the others investigated Caucasian populations [21, 32]. The two populations were from four different countries: Iran, Italy, Germany and China. Women who had suffered from more than two consecutive fetal losses with unexplained causes were defined as the case group. These patients had no history of systemic diseases,

immunological disorders, autoimmunity, thrombophilic disorders, or uterine abnormalities. The controls were limited to the general population who had no history of pregnancy loss and had completed at least one successive pregnancy without any obstetric problems. Polymerase chain reaction (PCR) was mainly used to assess the genotype present. The study characteristics are summarized in Table 1.

FXIII Val34Leu polymorphism

Five studies were included in this analysis. A total of 382 cases and 352 normal controls participated in investigating the association between the *FXIII Val34Leu* polymorphism and recurrent spontaneous miscarriages. The prevalence of the homozygous wild type Val/Val genotype was 64.9 % (248 of 382) in the case group and 75.9 % (267 of 352) in the controls. After the 4 different genotype models were analyzed, a positive association was detected in the pooled results for the dominant model (Val/Val+Val/Leu vs. Leu/Leu; OR=0.417, 95 % CI: 0.180–0.965, I^2 =45.60 %) and the co-dominant model (Val/Val vs. Val/Leu; OR=0.638, 95 % CI: 0.452–0.899, I^2 =36.40 %). However, there was no significant difference detected in the recessive model (Val/Val vs Val/Leu +Leu/Leu, OR=0.617, 95 % CI: 0.361–1.054, I^2 =59.90 %) and allele analysis (Val vs. Leu, OR=0.615, 95 % CI: 0.320–1.180, I^2 =79.60 %). After comprehensive analysis, these results showed that the *FXIII Val34Leu* polymorphism had a positive relationship with RSA and women who carried the Val/Val genotype for the *FXIII Val34Leu* polymorphism could have a protective effect on RSA. The statistical results for the association between the *FXIII Val34Leu* polymorphism and the risk of RSA are summarized in Table 2 and Fig. 2. We did not detect a significant publication bias using Begg's test (P =0.677) and the Egger's funnel plot was estimated to be symmetrical.

β -fibrinogen -455G/A polymorphism

To investigate the association between the β -fibrinogen -455G/A polymorphism and recurrent spontaneous miscarriages, 419 cases and 386 normal controls were included in this analysis. In total, five relevant studies were carefully reviewed. The prevalence of the homozygous wild type GG genotype in the control group was slightly higher than in the case group: 59.9 % (251 of 419) for RSA women and 65.3 % (252 of 386) for controls. However, no statistically significant association between the β -fibrinogen -455G/A polymorphism and recurrent spontaneous miscarriages was detected using the four different genotype models. Also, the pooled results were shown as follows: the dominant model (GG+GA vs. AA; OR=0.660, 95 % CI: 0.346–1.259, I^2 =30.0 %), the recessive model (GG vs GA+AA; OR=0.815, 95 % CI: 0.444–1.496, I^2 =73.3 %), the co-dominant model (GG vs. GA;

Fig. 1 The process flow diagram of selected articles

$OR=0.839$, 95 % CI : 0.455–1.546, $I^2=71.3$ %), the co-dominant model (GG vs. AA ; $OR=0.679$, 95 % CI : 0.353–1.305, $I^2=32.7$ %), and allele analysis (G vs. A ; $OR=0.830$, 95 % CI : 0.477–1.446, $I^2=77.5$ %). In order to further explore

the association, two subgroups were identified based on ethnicity (Asians and Caucasians). In the subgroup analysis, the four different genotype models were used and the β -fibrinogen -455G/A polymorphism did not show a significant

Table 2 Meta-analysis of *FXIII Val34Leu* polymorphisms and RSA

Models	OR (95 % CI)	I^2	P for heterogeneity
Dominant (<i>Val/Val+Val/Leu</i> vs. <i>Leu/Leu</i>)	0.417 (0.180–0.965)	45.60 %	0.159
Recessive (<i>Val/Val</i> vs. <i>Val/Leu+Leu/Leu</i>)	0.617 (0.361–1.054)	59.90 %	0.041
Codominant model (<i>Val/Val</i> vs. <i>Val/Leu</i>)	0.638 (0.452–0.899)	36.40 %	0.179
Codominant model (<i>Val/Val</i> vs. <i>Leu/Leu</i>)	0.433 (0.110–1.706)	51.60 %	0.127
Allele analysis (<i>Val</i> vs. <i>Leu</i>)	0.615 (0.320–1.180)	79.60 %	0.001

Fig. 2 Meta-analysis with fixed effects and codominant model (*Val/Val* vs. *Val/Leu*) for the association between the *FXIII Val34Leu* polymorphisms and recurrent spontaneous abortion

association with RSA in Asian and Caucasian populations. The statistical results for the association between the β -fibrinogen -455G/A polymorphism and the risk of RSA are summarized in Table 3 and Fig. 3. Tests for publication bias did not detect a significant bias; the *p* value was 0.819.

Sensitivity analysis

To further explore heterogeneity among the results of all studies, a sensitivity analysis was carried out for the *FXIII Val34Leu* and β -fibrinogen -455G/A polymorphisms. For the *FXIII Val34Leu* polymorphism, the article with the highest

relative heterogeneity [20] was removed and the analyses were repeated. Significantly, the value of I^2 dropped from 45.6 to 0 % and the results for the odds ratio estimates were 1.017 (95%CI: 0.285–3.624) in the dominant model with fixed effects. In addition, the co-dominant model (*Val/Val* vs. *Val/Leu*) also showed a negative statistical significance and the OR was 0.793 (95%CI: 0.537–1.173) and the value of I^2 dropped from 36.4 to 0 %. After removing the article with the highest relative heterogeneity [31], we still did not detect any statistically significant association between the β -fibrinogen -455G/A polymorphism and recurrent spontaneous miscarriages.

Table 3 Meta-analysis of β -Fibrinogen -455G/A polymorphisms and RSA

Models	Subgroup analysis	OR (95 % CI)	I^2	<i>P</i> for heterogeneity
Dominant (<i>GG+GA</i> vs. <i>AA</i>)	Asian	0.768 (0.259–2.282)	8.50 %	0.335
	Caucasian	0.608 (0.273–1.353)	73.40 %	0.052
	Overall	0.660 (0.346–1.259)	30.00 %	0.222
Recessive (<i>GG</i> vs <i>GA+AA</i>)	Asian	0.695 (0.225–2.148)	81.90 %	0.004
	Caucasian	1.046 (0.676–1.618)	15.60 %	0.276
	Overall	0.815 (0.444–1.496)	73.30 %	0.005
Codominant model (<i>GG</i> vs. <i>GA</i>)	Asian	0.660 (0.217–2.007)	80.00 %	0.007
	Caucasian	1.159 (0.716–1.763)	0.00 %	0.584
	Overall	0.839 (0.455–1.546)	71.30 %	0.007
Codominant model (<i>GG</i> vs. <i>AA</i>)	Asian	0.740 (0.247–2.219)	21.90 %	0.278
	Caucasian	0.647 (0.287–1.460)	74.80 %	0.047
	Overall	0.679 (0.353–1.305)	32.70 %	0.174
Allele analysis (<i>G</i> vs. <i>A</i>)	Asian	0.753 (0.279–2.029)	82.50 %	0.003
	Caucasian	0.970 (0.494–1.904)	75.60 %	0.043
	Overall	0.830 (0.477–1.446)	77.50 %	0.001

Fig. 3 Meta-analysis with fixed effects and dominant model (*GG+GA* vs. *AA*) for the association between the β -fibrinogen -455G/A polymorphisms and recurrent spontaneous abortion

Discussion

Recurrent spontaneous miscarriage is a terrible reproductive disease affecting reproductive-age women. Although serious damage is presented, the accurate pathogenesis of RSA remains unknown in ~50 % of cases [1, 33]. There is a strong belief that RSA patients with unknown etiology have a multifactorial condition and that genetic and environmental elements play a key role. Several meta-analyses have been published focusing on thrombophilic gene mutations and their association with RSA such as *apolipoprotein E* and the angiotensin converting enzyme gene [33, 34]. However, there is no meta-analysis of the prevalence of *FXIII Val34Leu* or β -fibrinogen -455G/A polymorphisms in patients with a history of RSA. This time, we first conducted a meta-analysis to discover the potential association between the SNPs and RSA risk.

A common *G-to-T* polymorphism in exon 2 leads to the conversion of *Val* to *Leu* for *FXIII Val34Leu* polymorphism, which could impact the cross-linking activity and clot stability leading to abnormal blood clotting [14, 15]. However, the relationship between the *FXIII Val34Leu* polymorphism and RSA is controversial. Elmahgoub et al. [20] suggested that the *FXIII Val34Leu* polymorphism had a positive relationship with RSA and carriers of the heterozygous (*Val/Leu*) and homozygous (*Leu/Leu*) factor *XIII* polymorphism might be at an increased risk of RSA. On the contrary, the other studies [26–29] showed that *factor XIII Val34Leu* genetic variation was not associated with recurrent spontaneous abortion. In our analysis, an obvious difference was detected to support

the suggestion that the *FXIII Val34Leu* polymorphism has a significant relationship with RSA in the dominant model and the co-dominant model (*Val/Val* vs. *Val/Leu*). The results showed that the *FXIII Val34Leu* polymorphism may reduce RSA risk. Furthermore, the *FXIII Val34Leu* polymorphism was identified to be associated with myocardial infarction (MI), venous thromboembolism (VTE), and pulmonary embolism [35, 36]. In the two models, the fixed-effect model was used as the value of the heterogeneity was moderate. However, a negative association between the *FXIII Val34Leu* polymorphism and RSA was found after removing the article with the highest relative heterogeneity [20]. In contrast with other researches, Elmahgoub et al. [20] recently suggested that *FXIII Val34Leu* polymorphisms had a positive relationship with RSA, which may result in the higher heterogeneity. In addition, large samples including 120 unexplained primary first trimester RSA patients and 130 healthy controls might also be related with the high heterogeneity.

Several studies suggested that thrombophilic gene mutations might lead to inherited thrombophilia, which was associated with blood clots in the placenta, decreasing oxygen delivery to the fetus, and causing fetal loss [25]. As one of the thrombophilic genes, the β -fibrinogen -455G/A polymorphism was reported to be associated with RSA [21, 31]. However, in our analysis, our results showed that the β -fibrinogen -455G/A polymorphism was not associated with RSA. These results suggested that the β -fibrinogen -455G/A polymorphism was not a risk factor for fetal loss, which was supported by Pihusch et al. [32] and Poursadegh Zonouzi et al.

[33]. With the different outcomes, patient selections have been considered one possible reason. In the subgroup analysis, the subgroups of RSA were considered based on ethnic variations, but there was still no association detected between the subgroup and the β -fibrinogen -455G/A genotype. Considering the moderate or high heterogeneity, sensitivity analyses were also carried out for each model and the results were similar. Combined with these results and our meta-analysis, β -fibrinogen -455G/A might not be a useful prognostic biomarker for fetal loss in the Asian and Caucasian populations. In this meta-analysis, clinical heterogeneity was moderate or high for the β -fibrinogen -455G/A polymorphism in 4 different genotype models which might affect the results of the analysis. Thus, the potential sources of heterogeneity were worth considering. Based on the small population, our study aimed to explore the relationship between the β -fibrinogen -455G/A polymorphism and the risk of RSA; the results were affected by the small sample size. In addition, the results might not be stable. Therefore, the overall conclusions need many more samples for support, especially for Caucasian and Asian populations.

Although comprehensive analyses and tests have been identified in the meta-analysis, there were still some limitations of this study. First, only three databases were searched, which could lead to a selection bias in this analysis. Second, although the similar inclusion and exclusion criteria were adopted in each included study, the sources of heterogeneity could not be ignored. For example, the definition of RSA is debatable. RSA is defined as three or more consecutive spontaneous abortions before 20 weeks of gestation in some studies, but two or more consecutive abortions is also used to define it. In this study, we included women with two consecutive miscarriages in the case group. In addition, the different gestational age used in defining pregnancy loss ranged from 10 weeks to the second trimester in the included studies, which also provided the potential heterogeneity of the sample resources. At the same time, some controls had experienced live births of singletons and had no history of pregnancy loss, while others had at least two normal pregnancies. Therefore, the factors mentioned above might be the major source of heterogeneity resulting from different samples which might also expand the selection bias. Third, as the unknown mechanism of RSA for most patients, the gene–gene, SNP–SNP, and gene–environment interactions should be taken into account. Therefore, the genetic and environmental backgrounds may play a key role the information of fetal loss. Lastly, the I^2 seemed to be high in our meta-analysis which could affect the stability of the results. In addition, there were some factors that were not evaluated in the included studies, such as the number of consecutive abortions, the age of patients, and the lifestyle of the population, which made us explore them further.

Conclusions

Based on the analysis of the present results, our meta-analysis demonstrates that the *FXIII Val34Leu* polymorphism has a positive relationship with RSA and women who carried the Val allele for *FXIII Val34Leu* polymorphisms could have a protective effect against RSA. On the other hand, there was no association detected between the β -fibrinogen -455G/A polymorphism and the risk of RSA, including the Asian and Caucasian subgroup analyses. Considering the effect of the small samples size, more larger-scale, case-controlled, and population-based studies are needed to confirm these results.

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