Factor V Leiden 1691G > A mutation and the risk of recurrent pregnancy loss (RPL): systematic review and meta-analysis

Mohammad Masoud Eslami1, Majid Khalili2,3, Mina Soufizomorrod1, Saeid Abroun1 and Bahman Razi1*

Abstract

Background: Although numerous replication case-control studies have attempted to determine the association between Factor V Leiden (FVL) 1691G > A mutation and susceptibility to Recurrent pregnancy loss (RPL), there have been confliction among the results of various ethnic groups. To address this limitation, here we implemented first meta-analysis to provide with consistent conclusion of the association between FVL 1691G > A mutation and RPL risk.

Methods: After a systematic literature search, pooled odds ratio (OR) and their corresponding 95% confidence interval (CI) were used to evaluate the strength of the association. Additionally, meta-regression analyses were performed to find potential source of heterogeneity.

Results: In this meta-analysis, 62 studies, containing 10,410 cases and 9406 controls, were included in quantitative analysis. Overall population analysis revealed a significant positive association in the dominant (OR = 2.15, 95% CI = 1.84–2.50, \(P < 0.001\)), over-dominant (OR = 1.88, 95% CI = 1.61–2.19, \(P < 0.001\)), allelic (OR = 2.05, 95% CI = 1.79–2.35, \(P < 0.001\)), and heterozygote (OR = 1.97, 95% CI = 1.68–2.30, \(P < 0.001\)) models. Moreover, a significant association of dominant (OR = 3.04, 95% CI = 2.04–4.54, \(P < 0.001\)), over-dominant (OR = 2.65, 95% CI = 1.74–4.05, \(P < 0.001\)), and heterozygote (OR = 2.67, 95% CI = 1.81–4.22, \(P < 0.001\)) models was found in the Iranian population. The subgroup analysis indicated strong significant association in Asian, European, Africa population, and case-control studies but not in South Americans and cohort studies.

Conclusion: The FVL 1691G > A mutation and the risk of RPL confers a genetic contributing factor in increasing the risk of RPL, particularly in Iranians, except for South Americans.

Keywords: Recurrent pregnancy loss, Factor V Leiden, 1691G > A mutation, Meta-analysis, Meta-regression

Introduction

Recurrent pregnancy loss (RPL) is a heterogeneous disorder which affects women of reproductive age. Recently, The American Society of Reproductive Medicine has defined RPL as two or more than two failed pregnancies before the 20th week of pregnancy [1–3]. Overall, 1–5% of women during reproductive ages could be affected [4].

From pathophysiological point of view, RLP might be influenced by various items, such as genetic factors (chromosomal aberrations, genetic polymorphisms), infectious diseases, structural abnormalities of the uterus, coagulative disorders (thrombophilia), endocrinological problems (thyroid disease and diabetes), and immunological disease (autoimmune disorder and inflammatory diseases) [5–7]. With considering these factors, still approximately 40 to 50% of cases remained idiopathic [8].

Although pregnancy as a physiological condition is associated with a hypercoagulable state, and the contact
between placenta and maternal circulation is crucial for the establishment of a successful pregnancy, but any abnormality in this circulation, especially abnormal blood clotting in the small placental blood vessels, may results in RPL [9, 10]. During last decades, thrombophilia attracted a lot of attention as a risk factor for RLP. Thrombophilia is characterized as a hemostatic disorder which leads to an increased tendency of thromboembolic processes. Classically, thrombophilia could be classified into acquired and inherited forms [11, 12]. In this regards, antiphospholipid syndrome is an established acquired thrombophilia factor which increase the risk of RPL. Among inherited factors, mutation in Factor V Leiden (FVL) of the FV gene, G20210A of the FII (prothrombin) gene, and C677T of the methylenetetrahydrofolate reductase (MTHFR) gene are believed to play a key role in pathogenesis of RPL [13, 14].

FVL mutation shows an autosomal dominant pattern which occurs by substitution of guanine by adenine (CGA—> CAA) at the nucleotide 1691 in the exon 10. As a result of this missense mutation, arginine (Arg) at amino acid 506 is substituted with glutamine (Gln), leading to generation of FVL resistant to the activated protein C (APC). APC is a natural anticoagulant which in normal situation cleaves activated factor V at amino acid 506 and makes it inactive [15–20].

Studies have shown that FVL mutation increases the risk of venous thrombosis 7 times in heterozygote and 80 times in homozygote carriers. In addition, it has been reported that this mutation increases the risk of pre-eclampsia in FVL carriers [21, 22]. The exact mechanism that FVL mutation influence the etiology of RPL is a controversial issue and has not yet been divulged thoroughly, but several studies suggested that production of micro thrombosis could sediment in delicate placental blood vessels and cause placental infarction and subsequent maternal and fetal complications [23, 24].

In spite of all findings, still the exact association between FVL mutation and the risk RPL is unclear and several investigators worldwide try to clarify this question. Therefore, here we conducted the first and the most comprehensive meta-analysis on the association between FVL 1691G > A mutation and risk of RPL by exerting 62 studies encompassing 10,410 cases and 9406 health control to achieve more reliable conclusion.

**Methods**

Ethical approval is not necessary for this meta-analysis. The current meta-analysis was conducted according to the Preferred Reporting Items for Systematic reviews and Meta-Analysis (PRISMA) statement [25], including publication search, study selection, inclusion and exclusion criteria, data extraction, quality assessment, and statistical analysis.

**Publication search**

A comprehensive systematic search in the ISI Web of Science, Scopus, and PubMed/Medline databases was conducted to retrieve all publications evaluating the associations between FVL 1691G > A mutation and susceptibility to RPL prior to May 2020. The following combinations of key words were used: (“Miscarriage” OR “abortion” OR “pregnancy loss” OR “habitual abortion” OR “fetal loss” OR “Recurrent Pregnancy Loss”) AND (“Factor V Leiden” OR “FV Leiden” OR “1691G >A” OR “rs6025”) AND (“polymorphism” OR “variant” OR “mutation” OR “genotype” OR “allele” OR “single nucleotide polymorphism” OR “SNP”). In spite of detailed search, a manual cross-check of eligible studies and reviews was carried out to include other potential studies. Original data in English language and human population studies were collected.

**Study selection**

Primary search strategy generates 1266 studies that were exported into Endnote X8 software. The duplicated studies were removed and title & abstract of remaining studies were reviewed by two investigators and irrelevant studies were excluded. Full-text verification was performed if we could not classify studies based on title & abstract. Any disagreements during study selection were discussed and resolved by consensus.

**Inclusion and exclusion criteria**

Studies considered eligible if they met the following inclusion criteria: a) Studies concerning the association between FVL 1691G > A mutation and susceptibility to recurrent pregnancy loss as the main outcome; b) Studies that their case group have recurrent pregnancy loss (two or more times of abortion); c) Studies with case-control and cohort design; d) Studies reporting sufficient data of genotype or allele frequency that could confer feasibility of calculating the odds ratios (ORs) and 95% confidence intervals (CIs). On the other hand, duplicates, case reports, book chapters, reviews, letter to editor, studies with insufficient data, and abstracts were all excluded.

**Data extraction and quality assessment**

According to a standardized extraction form, the following data were independently extracted by two investigators: the first author’s last name, journal and year of publication, country of origin, ethnicity, allele and genotype frequency in cases and controls, mean or range of age, genotyping method, and total sample size of cases and controls. The third investigator finalized the extracted data, and potential discrepancies were resolved by consensus. For quality assessment of the included publications, the Newcastle-Ottawa Scale (NOS) was applied [26]. In this respect, studies with 0–3, 4–6 or 7–9 scores were of, respectively, low, moderate, and high-quality.
Statistical analysis
Deviation from Hardy–Weinberg equilibrium (HWE) for distribution of the genotype frequencies was analyzed by \( \chi^2 \)-test in the control group. The strength of the association between FVL 1691G > A mutation and RPL risk was evaluated by the pooled OR and its corresponding 95% CI. Different comparison models for FVL 1691G > A mutation were as follow: dominant model (AA+GA vs. GG), over-dominant model (GA vs. GG + AA), allelic model (A vs. G), and heterozygote (GA vs. GG). It should be noted that due to the AA genotype frequency of zero in both cases and controls, the recessive and homozygote models were not calculable. Presence of heterogeneity between included studies was estimated by Cochran’s Q-statistic (\( P \) value< 0.10 was considered as statistically significant) [27]. Besides, to report quantitative heterogeneity I-squared (I\(^2\)) tests was used. The fixed-effect model (FEM) was used if P_Q-statistic > 0.10 or I\(^2\) was< 50%; otherwise, the random-effect model (REM) was applied. In order to assessed the predefined sources of heterogeneity among included studies, subgroup analysis and meta-regression analysis based on year of population, the continent of the study population, and genotyping method were performed. Additionally, sensitivity analysis was conducted in presence of heterogeneity [28, 29]. Publication bias was estimated by Begg’s funnel plots and Egger’s regression test (\( P \) value< 0.05 was considered as statistically significant) [30, 31]. The funnel plot asymmetry was assessed with the Egger’s test. Practically, in case of no evidence of publication bias, studies with high precision (large study effects) will be located near the average line, and studies with low precision (small study effects) will be spread equally on both sides of the average line; any deviation from this shape can indicate publication bias. The data analyses were carried out using STATA (version 14.0; Stata Corporation, College Station, TX) and SPSS (version 23.0; SPSS, Inc. Chicago, IL) software.

Results
Study characteristics
The four-phase search and screening process of the literatures based on the PRISMA statement is depicted in the Fig. 1. According to the aforementioned keywords, a
| Study author               | Year | Country | Study design | Ethnicty | Total cases/controls | Age case/control (Mean) | Genotyping method | Quality score |
|---------------------------|------|---------|--------------|----------|----------------------|-------------------------|-------------------|---------------|
| Souza et al. [34]         | 1999 | Brazil  | case-control | South America | 56/384                     | 29.6 / 24.3               | RLFP-PCR            | 7             |
| Brenner et al. [35]       | 1999 | Israel  | case-control | Asia      | 76/106                | 31 ± 5 / 31 ± 6           | RLFP-PCR            | 6             |
| Wrambsy et al. [36]       | 2000 | Sweden  | case-control | Europe    | 62/69                    | 21–39 / 21–39             | RLFP-PCR            | 7             |
| Murphy et al. [37]        | 2000 | Ireland | case-control | Europe    | 41/540                  | 32 ± 0.74 / NR            | RLFP-PCR            | 6             |
| Pihusch et al. [33]       | 2000 | Germany | case-control | Europe    | 102/128                 | 35 / 32                   | RLFP-PCR            | 6             |
| Younis et al.             | 2000 | Israel  | case-control | Asia      | 78/139                  | 30.0 ± 4.4 / 30.7 ± 4.2   | RLFP-PCR            | 6             |
| Foka et al. [14]          | 2000 | Greece  | case-control | Europe    | 80/100                  | 33 / 35                   | RLFP-PCR            | 6             |
| Rai et al.                | 2001 | London  | cohort       | Europe    | 1111/150               | 33.5 / 33                 | RLFP-PCR            | 8             |
| Carp et al.               | 2002 | Israel  | case-control | Asia      | 108/82                  | 31 / 36                   | RLFP-PCR            | 6             |
| Finan et al. [38]         | 2002 | Lebanon | case-control | Asia      | 110/67                 | 32.3 ± 5.3 / 33.9 ± 7.3   | RLFP-PCR            | 6             |
| Hohlagschwandtner et al.  | 2003 | Australia | case-control | Oceania  | 145/101              | 32 / 56                    | Multiplex PCR       | 7             |
| Pauer et al. [39]         | 2003 | Germany | case-control | Europe    | 30/122                  | 31.3 / NR                 | RLFP-PCR            | 6             |
| Mitraou et al.            | 2004 | Tunisia | case-control | Africa    | 146/99                 | 29.0 ± 6.1 / 28.9 ± 5.3   | RLFP-PCR            | 6             |
| Aksoy et al.              | 2005 | Turkey  | case-control | Europe    | 41/50                   | 32 ± 5.54 / 29 ± 4.66     | PCR                | 5             |
| Mahjoub et al. [40]       | 2005 | Tunisia | case-control | Africa    | 200/200                | 28.68 ± 5.61 / 28.24 ± 5.51 | RLFP-PCR            | 8             |
| Ulukus et al.             | 2006 | Turkey  | case-control | Europe    | 10/53                   | 29.1 ± 5.2 / 28.0 ± 4.8   | PCR                | 5             |
| Sotiriadis et al.         | 2006 | Greece  | case-control | Europe    | 99/102                 | 32.2 / 32.2               | RLFP-PCR            | 6             |
| Mohammad et al. [21]      | 2007 | Syria   | case-control | Asia      | 35/45                  | 29.6 ± 6.3 / 28.8 ± 6.8   | Q-PCR              | 5             |
| Alkintas et al. [41]      | 2007 | Turkey  | case-control | Europe    | 114/185                | 30.6 ± 4.4 / 30.5 ± 4.3   | Q-PCR              | 7             |
| Toth et al. [42]          | 2008 | Germany | case-control | Europe    | 151/157               | 33.2 ± 4.6 / 45.2 ± 12.6  | RLFP-PCR            | 7             |
| Pasquier et al.           | 2008 | France  | case-control | Europe    | 311/599               | 32.8 ± 34.3              | Q-PCR              | 8             |
| Biswas et al. [43]        | 2008 | India   | case-control | Asia      | 85/31                  | 27.9 ± 0.3 / 26 ± 0.5     | RLFP-PCR            | 6             |
| Lvanov et al.             | 2009 | Bulgaria | case-control | Europe    | 153/100              | 29.7 / 31.0               | RLFP-PCR            | 7             |
| Mukhopadhyay et al. [44]  | 2009 | India   | case-control | Asia      | 84/80                  | 24.9 ± 3.3 / 24.9 ± 3.3   | RLFP-PCR            | 6             |
| Ciacci et al. [45]        | 2009 | Italy   | case-control | Europe    | 39/72                  | 36.24 ± 8.26 / 30.10 ± 8.6 | Multiplex PCR       | 6             |
| Mohamed et al. [46]       | 2010 | Egypt   | case-control | Africa    | 20/20                  | 29.0 ± 4.80 / 31.4 ± 6.82 | PCR                | 5             |
| Hussein et al. [47]       | 2010 | Palestine | case-control | Asia      | 145/205              | 31.9 / 32                  | ARMS-PCR            | 7             |
| Serrano et al. [17]       | 2011 | Portugal | case-control | Europe    | 100/100               | 32 ± 4.25 / 30.9 ± 5.19   | PCR                | 7             |
| Settin et al.             | 2011 | Egypt   | case-control | Africa    | 72/70                 | 19 to 38 / 19 to 38       | PCR                | 6             |
| Dissanayake et al. [32]   | 2012 | Sri Lanka | case-control | Asia      | 200/200               | 32.1 ± 5.6 / 32.4 ± 4.6   | RLFP-PCR            | 8             |
| Gazi et al.               | 2012 | Turkey  | case-control | Europe    | 57/47                  | 30.12 ± 7.32 / 27.80 ± 6.36 | PCR                | 6             |
| Karata et al.             | 2012 | Turkey  | case-control | Europe    | 84/84                  | 31.6 ± 3.7 / 32.2 ± 3.9   | Q-PCR              | 6             |
| Mierla et al. [48]        | 2012 | Romania | case-control | Europe    | 283/100               | 33.76 / 32.8              | RLFP-PCR            | 7             |
| Ozdemir et al. [49]       | 2012 | Turkey  | case-control | Europe    | 543/106               | 27.8 ± 2.1 / 28.9 ± 2.2   | Q-PCR              | 7             |
| Torabi et al. [50]        | 2012 | Iran    | case-control | Asia      | 100/100               | NR / NR                   | RLFP-PCR            | 6             |
| Kaur et al.               | 2012 | India   | case-control | Asia      | 107/588               | 24.89 / 25.32             | RLFP-PCR            | 7             |
| Parveen et al.            | 2012 | India   | case-control | Asia      | 1000/500              | 28.4 ± 5.9 / 31.9 ± 7.3   | ARMS-PCR            | 8             |
| Ardestani et al.          | 2012 | Iran    | case-control | Asia      | 80/80                  | 28.8 / 23.6               | RLFP-PCR            | 6             |
| Cardona et al. [51]       | 2012 | Colombia | case-control | South America | 93/206                | 34.1 ± 0.9 / 41.6 ± 0.7   | RLFP-PCR            | 7             |
| Kazerooni et al. [52]     | 2013 | Iran    | case-control | Asia      | 60/60                  | 24.8 ± 3.9 / 24.6 ± 4.7   | PCR                | 5             |
| Baumann et al.            | 2013 | Germany | cohort       | Europe    | 641/157               | 32.95 ± 4.94 / 33.16 ± 6.24 | RLFP-PCR            | 8             |
| Parand et al. [53]        | 2013 | Iran    | case-control | Asia      | 90/44                  | 29.21 ± 5.9 / 28.75 ± 5.2 | RLFP-PCR            | 6             |
| Zonouzi et al. [54]       | 2013 | Iran    | case-control | Asia      | 89/50                  | 30.18 ± 4.95 / 31.54 ± 4.81 | ARMS-PCR            | 6             |
A total of 1266 studies were retrieved (PubMed: 254, Scopus: 512, and ISI Web of Science: 500). Subsequently, application of inclusion/exclusion criteria resulted in the exclusion of 1206 studies (324 duplicates studies, 714 and 168 studies excluded according to title & abstract and full-text examination, respectively). Eventually, 62 qualified studies were included in the quantitative analysis, of which two studies were detected by cross-check of eligible studies and reviews [32, 33]. All eligible studies were published between 1999 to 2019 and had an overall good methodological quality with NOS scores ranging from 5 to 8. The Restriction fragment length polymorphism (RFLP)-PCR was the most genotyping methods which used in the included studies. Except two studies which had cohort design, other 60 studies had case-control design. Tables 1 and 2 summarize the characteristics and allele/genotype frequency of the included studies.

### Meta-analysis of FVL 1691G > A mutation and the risk of RPL

### Overall

62 studies with 10,410 cases and 9406 controls included in quantitative analysis of the association between FVL 1691G > A mutation and the risk of RPL. Of those, 25 studies were in Asian countries [21, 22, 32, 35, 38, 43, 44, 47, 50, 52–54, 56, 57, 59, 61, 63–71], 26 studies were conducted in European countries [17, 33, 36, 37, 39, 41, 42, 45, 48, 49, 55, 60, 62, 72–82], 6 studies in South American countries [34, 51, 58, 83–85], 4 studies in African countries [40, 46, 86, 87] and one study in Oceania. The analysis of overall population revealed a significant positive association between FVL 1691G > A mutation and the risk of RPL across all possible genotype models, including dominant model (OR = 2.15, 95% CI = 1.84–2.50, *P* < 0.001, FEM), over-dominant model (OR = 1.88, 95% CI = 1.61–2.19, *P* < 0.001, FEM), allelic model (OR = 2.05, 95% CI = 1.79–2.35, *P* < 0.001, REM), and heterozygote model (OR = 1.97, 95% CI = 1.68–2.30, *P* < 0.001, FEM) (Table 3 and Fig. 2).

### Meta-analysis of FVL 1691G > A mutation and the risk of RPL in Iranian population

Among the included studies, studies performed in Iran with 9 publications (1409 cases and 1160 controls) were in the first rank with respect to sample size and the number of studies, therefore we performed separate analysis. Our results found a significant positive association between FVL 1691G > A mutation and increased risk of RPL in this population under dominant model (OR = 3.04, 95% CI = 2.04–4.54, *P* < 0.001, FEM), over-dominant model (OR = 2.65, 95% CI = 1.74–4.05, *P* < 0.001, FEM), and heterozygote model (OR = 2.67, 95% CI = 1.81–4.22, *P* < 0.001, FEM) (Table 3 and Fig. 2).
### Table 2 Distribution of genotype and allele among RPL patients and controls

| Study author | RPL cases | Healthy control | P-HWE | MAF |
|--------------|-----------|-----------------|-------|-----|
|              | GG | GA | AA | G | A | GG | GA | AA | G | A | GG | GA | AA | G | A |
| Souza et al. [34] | 52 | 4 | 0 | 108 | 4 | 378 | 6 | 0 | 762 | 6 | 0.007 |
| Brenner et al. [35] | 52 | 19 | 5 | 123 | 29 | 95 | 11 | 0 | 201 | 11 | 0.057 |
| Wramsby et al. [36] | 51 | 10 | 1 | 112 | 12 | 67 | 2 | 0 | 136 | 2 | 0.014 |
| Murphy et al. [32] | 39 | 2 | 0 | 80 | 2 | 527 | 13 | 0 | 1067 | 13 | 0.012 |
| Pihusch et al. [33] | 94 | 8 | 0 | 196 | 8 | 117 | 11 | 0 | 245 | 11 | 0.042 |
| Younis et al. | 63 | 12 | 3 | 138 | 18 | 131 | 8 | 0 | 270 | 8 | 0.028 |
| Foka et al. [14] | 65 | 15 | 0 | 145 | 15 | 96 | 4 | 0 | 196 | 4 | 0.032 |
| Rai et al. | 1037 | 72 | 2 | 2146 | 76 | 138 | 12 | 0 | 288 | 12 | 0.04 |
| Carpenter et al. | 104 | 4 | 0 | 212 | 4 | 77 | 5 | 0 | 159 | 5 | 0.03 |
| Finan et al. [38] | 65 | 38 | 7 | 168 | 52 | 56 | 11 | 0 | 123 | 11 | 0.082 |
| Hohlagschwandtner et al. | 130 | 15 | 0 | 275 | 15 | 97 | 4 | 0 | 198 | 4 | 0.019 |
| Pauer et al. [39] | 28 | 2 | 0 | 58 | 2 | 113 | 9 | 0 | 235 | 9 | 0.036 |
| Mtiraoui et al. | 116 | 24 | 6 | 256 | 36 | 93 | 6 | 0 | 192 | 6 | 0.075 |
| Aksoy et al. | 31 | 9 | 1 | 71 | 11 | 45 | 5 | 0 | 95 | 5 | 0.05 |
| Mahjoub et al. [40] | 152 | 40 | 8 | 344 | 56 | 189 | 11 | 0 | 389 | 11 | 0.027 |
| Ulukus et al. | 7 | 3 | 0 | 17 | 3 | 49 | 3 | 1 | 101 | 5 | 0.001 |
| Sotiriadis et al. | 94 | 5 | 0 | 193 | 5 | 99 | 3 | 0 | 201 | 3 | 0.014 |
| Mohammad et al. [21] | 25 | 10 | 0 | 60 | 10 | 41 | 4 | 0 | 86 | 4 | 0.044 |
| Alkintas et al. [41] | 105 | 9 | 0 | 219 | 9 | 172 | 13 | 0 | 357 | 13 | 0.035 |
| Toth et al. [42] | 138 | 13 | 0 | 289 | 13 | 145 | 12 | 0 | 302 | 12 | 0.038 |
| Pasquier et al. | 296 | 15 | 0 | 607 | 15 | 574 | 25 | 0 | 1173 | 25 | 0.02 |
| Biswas et al. [43] | 83 | 2 | 0 | 168 | 2 | 31 | 0 | 0 | 62 | 0 | 0.001 |
| Lvanov et al. | 133 | 19 | 1 | 285 | 21 | 93 | 7 | 0 | 193 | 7 | 0.035 |
| Mukhopadhyay et al. [44] | 80 | 4 | 0 | 164 | 4 | 80 | 0 | 0 | 160 | 0 | 0.001 |
| Ciacci et al. [45] | 38 | 1 | 0 | 77 | 1 | 70 | 2 | 0 | 142 | 2 | 0.013 |
| Mohamed et al. [46] | 6 | 12 | 2 | 24 | 16 | 19 | 1 | 0 | 39 | 1 | 0.025 |
| Hussein et al. [47] | 104 | 36 | 5 | 244 | 46 | 181 | 24 | 0 | 386 | 24 | 0.058 |
| Serrano et al. [17] | 95 | 5 | 0 | 195 | 5 | 95 | 5 | 0 | 195 | 5 | 0.025 |
| Settin et al. | 54 | 17 | 1 | 125 | 19 | 69 | 1 | 0 | 139 | 1 | 0.007 |
| Dissanayake et al. [32] | 196 | 4 | 0 | 396 | 4 | 195 | 5 | 0 | 395 | 5 | 0.012 |
| Gazi et al. | 50 | 6 | 1 | 106 | 8 | 43 | 4 | 0 | 90 | 4 | 0.042 |
| Karata et al. | 66 | 16 | 2 | 148 | 20 | 66 | 18 | 0 | 150 | 18 | 0.07 |
| Mierla et al. [48] | 260 | 21 | 2 | 541 | 25 | 95 | 5 | 0 | 195 | 5 | 0.025 |
| Ozdemir et al. [49] | 433 | 109 | 1 | 975 | 111 | 104 | 2 | 0 | 210 | 2 | 0.009 |
| Torabi et al. [50] | 87 | 12 | 1 | 186 | 14 | 96 | 4 | 0 | 196 | 4 | 0.03 |
| Kaur et al. | 102 | 4 | 1 | 208 | 6 | 573 | 15 | 0 | 1161 | 15 | 0.012 |
| Parveen et al. | 950 | 50 | 0 | 1950 | 50 | 488 | 12 | 0 | 988 | 12 | 0.012 |
| Ardestani et al. | 78 | 2 | 0 | 158 | 2 | 79 | 1 | 0 | 159 | 1 | 0.006 |
| Cardona et al. [51] | 92 | 1 | 0 | 185 | 1 | 205 | 1 | 0 | 411 | 1 | 0.002 |
| Kazerouni et al. [52] | 43 | 12 | 5 | 98 | 22 | 54 | 4 | 2 | 112 | 8 | 0.034 |
| Baumann et al. | 592 | 49 | 0 | 1233 | 49 | 145 | 12 | 0 | 302 | 12 | 0.038 |
| Parand et al. [53] | 72 | 15 | 3 | 159 | 21 | 38 | 6 | 0 | 82 | 6 | 0.068 |
| Zonouzi et al. [54] | 87 | 2 | 0 | 176 | 2 | 50 | 0 | 0 | 100 | 0 | 0.001 |
but not allelic model (OR = 2.09, 95% CI = 0.88–4.94, P = 0.09, REM) (Table 3).

Subgroup analysis by continent
The included studies were performed in Asia (25 studies), Europe (26 studies), South America (6 studies), Africa (4 studies) and Oceania (1 article). Since there was only one study for Oceania, we exclude it from the subgroup analysis. The final results revealed strong significant association between FVL 1691G > A mutation and the risk of RPL in Asian, European, and African population, but not in South Americans (Fig. 3).

The results of pooled ORs, heterogeneity tests, and publication bias tests in different analysis models are shown in the Table 3.

Subgroup analysis by study design
The stratification of studies based on study design caused to the inclusion of two studies with 1752 cases and 307 controls in cohort group, and 60 studies with 8658 cases and 9099 controls in case-control group. The findings demonstrated a statistical significant association between FVL 1691G > A mutation and the risk of RPL in case-control studies across dominant model (OR = 2.33, 95% CI = 1.99–2.74, P < 0.001, REM), over-dominant model (OR = 2.05, 95% CI = 1.74–2.41, P < 0.001, REM), allelic model (OR = 2.18, 95% CI = 1.8–2.52, P < 0.001, FEM), and heterozygote model (OR = 2.16, 95% CI = 1.83–2.55, P < 0.001, FEM). However, no significant association was observed in cohort studies (Table 3).

Heterogeneity and publication bias
To check existence of publication bias, Egger’s linear regression and Begg’s funnel plot test were used. The shape of the funnel plots did not disclose obvious asymmetry under all the genotype model of the FVL 1691G > A mutation (Fig. 4). Additionally, some degree of heterogeneity was detected in overall population. Therefore, we stratified study by continent and study design to find its potential source.

Meta-regression analyses
Meta-regression analyses were performed to explore potential sources of heterogeneity among included studies (Table 4). The findings indicated that none of the expected heterogeneity parameter were the source of heterogeneity (Fig. 5).

Sensitivity analysis
The impact of individual study on pooled OR was evaluated by sequential omission of each studies. The analysis results showed that no individual study significantly affected the pooled ORs under any genotype models of the FVL 1691G > A mutation (Fig. 6).

Table 2 Distribution of genotype and allele among RPL patients and controls (Continued)

| Study author     | RPL cases | Healthy control | P-HWE | MAF |
|------------------|-----------|-----------------|-------|-----|
|                  | GG   | GA | AA | G | A |
| Dutra et al.     | 142  | 3  | 0  | 287 | 3 |
| Isaqgül et al.   | 47   | 13 | 0  | 107 | 13|
| Pietropolli et al. [55] | 168  | 18 | 0  | 354 | 18|
| Lino et al.      | 79   | 4  | 0  | 162 | 4 |
| Sharma et al. [56] | 36  | 40 | 2  | 112 | 44|
| Farahmand et al. | 302  | 28 | 0  | 632 | 28|
| Kashif et al. [57] | 53   | 3  | 0  | 109 | 3 |
| Gonçalves et al. [58] | 133  | 4  | 0  | 270 | 4 |
| Khaniani et al. [59] | 202  | 8  | 0  | 412 | 8 |
| Eldeen et al.    | 0    | 72 | 24 | 72  | 120|
| Wolski et al. [60] | 333  | 26 | 0  | 692 | 26|
| Elgari et al. [61] | 56   | 4  | 0  | 116 | 4 |
| Mahmutbegović et al. [62] | 44  | 7  | 0  | 95  | 7 |
| Wingeyer et al.  | 239  | 8  | 0  | 486 | 8 |
| Jusić et al.     | 51   | 9  | 0  | 111 | 9 |
| Taghi Kardi et al. | 236 | 12 | 2  | 484 | 16|
| Xu et al.        | 426  | 0  | 0  | 852 | 0 |
| Bigdeli et al. [63] | 150  | 30 | 20 | 330 | 70|
| Xu et al.        | 130  | 14 | 3  | 261 | 29|

P-HWE p-value for Hardy–Weinberg equilibrium; MAF Minor allele frequency of control group

0.001, FEM) but not allelic model (OR = 2.09, 95% CI = 0.88–4.94, P = 0.09, REM) (Table 3).

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Overall Dominant 10,410 / 9406 2.15 1.84–2.50 (<0.001) 38.3 0.002 1.49 0.13 1.64 0.11
Over-Dominant 10,410 / 9406 1.88 1.61–2.19 (<0.001) 35.8 0.005 1.33 0.17 1.45 0.14
Allelic model 10,410 / 9406 2.05 1.79–2.35 (<0.001) 48.6 ≤0.001 1.45 0.16 1.59 0.13
GA vs. GG 10,410 / 9406 1.97 1.68–2.30 (<0.001) 28.3 0.03 1.51 0.11 2.01 0.04

Iranian population
Dominant 1409 / 1160 3.04 2.04–4.54 (<0.001) 37.3 0.13 −0.45 0.65 −0.53 0.61
Over-Dominant 1409 / 1160 2.65 1.74–4.05 (<0.001) 0 0.66 −1.05 0.29 −0.64 0.55
Allelic model 1409 / 1160 2.09 0.88–4.94 (0.09) 76.8 0.008 −0.45 0.65 −0.33 0.75
GA vs. GG 1409 / 1160 2.67 1.81–4.22 (<0.001) 0 0.59 −1.05 0.29 −0.67 0.53

Subgroup (continent)
Asia
Dominant 4153 / 3957 2.80 2.20–3.56 (<0.001) 35.4 0.06 −0.80 0.42 −0.44 0.64
Over-Dominant 4153 / 3957 2.22 1.73–2.85 (<0.001) 47.2 0.01 −1.43 0.15 −0.98 0.34
Allelic model 4153 / 3957 2.35 1.92–2.87 (<0.001) 62.6 0.003 −0.45 0.64 0.39 0.7
GA vs. GG 4153 / 3957 2.51 1.95–3.21 (<0.001) 11.9 0.31 −1.10 0.27 −0.35 0.73

Europe
Dominant 4913 / 3929 1.49 1.20–1.84 (0.001) 14.7 0.25 3.06 0.002 3.79 0.001
Over-Dominant 4913 / 3929 1.43 1.15–1.79 (0.002) 16 0.23 2.99 0.003 3.65 0.001
Allelic model 4913 / 3929 1.48 1.19–1.81 (0.001) 9.9 0.32 1.37 0.16 1.58 0.13
GA vs. GG 4913 / 3929 1.44 1.15–1.80 (0.001) 16 0.23 2.96 0.003 3.65 0.001

South America
Dominant 761 / 1030 2.04 0.88–4.74 (0.09) 0 0.76 0.19 0.85 −0.53 0.62
Over-Dominant 761 / 1030 2.04 0.88–4.74 (0.09) 0 0.76 0.19 0.85 −0.53 0.62
Allelic model 761 / 1030 2 0.87–4.60 (0.1) 0 0.76 −0.19 0.85 −0.47 0.66
GA vs. GG 761 / 1030 2.04 0.88–4.74 (0.09) 0 0.76 0.19 0.85 −0.53 0.62

Africa
Dominant 438 / 389 5.65 3.15–10.14 (<0.001) 3.9 0.37 1.36 0.17 2.55 0.12
Over-Dominant 438 / 389 4.44 2.45–8.03 (<0.001) 3.2 0.37 1.36 0.17 2.41 0.13
Allelic model 438 / 389 5.93 3.38–10.40 (<0.001) 0 0.55 1.36 0.17 2.59 0.12
GA vs. GG 438 / 389 4.70 2.59–8.53 (<0.001) 12.3 0.33 1.36 0.17 2.47 0.13

Subgroup (Study design)
Case-Control
Dominant 8658 / 9099 2.33 1.99–2.74 (<0.001) 31.5 0.01 0.28 0.78 0.79 0.43
Over-Dominant 8658 / 9099 2.05 1.74–2.41 (<0.001) 29.3 0.02 1.43 0.15 1.28 0.21
Allelic model 8658 / 9099 2.18 1.8–2.52 (<0.001) 44.9 0.003 0.71 0.47 0.87 0.39
GA vs. GG 8658 / 9099 2.16 1.83–2.55 (<0.001) 18.2 0.13 1.70 0.09 1.78 0.08

Cohort
Dominant 1752 / 307 0.90 0.55–1.49 (0.68) 0 0.69 −1.23 0.47 −1.88 0.11
Over-Dominant 1752 / 307 0.88 0.54–1.46 (0.63) 0 0.64 −1.23 0.47 −0.95 0.38
Allelic model 1752 / 307 0.91 0.56–1.49 (0.71) 0 0.72 −1.23 0.47 −1.27 0.25
GA vs. GG 1752 / 307 0.88 0.54–1.46 (0.63) 0 0.64 −1.23 0.47 −1.68 0.14

Discussion
RPL has been one of the most prevalent obstetric complications, that affect more than 30% of gestations. A remarkable amount of pregnancy losses has been attributed to genetic variations, of which over 50% have been related to chromosomal abnormalities. Several investigations have reported the association of FVL 1691G > A mutation with RPL; that notwithstanding, there have been conflicting results among various ethnicities. The inconsistent results have been attributed to variety in the race of included subjects, different diagnostic criteria of patients, little statistical power, small sample sizes, and the linkage disequilibrium (LD) between various genes and variations [88]. However, meta-analysis strategy provides a pertinent tool to settle the problem of confliction by resolving the limitations of single replication studies, such as limited statistical power and little sample size. Thus, here we conducted
the first meta-analysis to find a valid estimation of the association between FVL 1691G > A mutation and risk of RPL.

The FVL 1691G > A mutation is a G-to-A point mutation at nucleotide 1691 in the factor V gene, that results in the single amino-acid replacement Arg506Gln, leading to resistance to be cleaved and, therefore, inactivation by APC and promoted susceptibility to clotting [89, 90]. This mutation enhances the risk of venous thrombosis up to 50–100 times in homozygote carriers [22].

In this meta-analysis, 62 studies, containing 10,410 cases and 9406 controls, were included in quantitative analysis. The analysis of overall population indicated that all genetic comparisons of the FVL 1691G > A mutation, including dominant model (OR = 2.15), over-dominant model (OR = 1.88), allelic model (OR = 2.05), and heterozygote model (OR = 1.97) significantly increased the risk of RPL susceptibility. In 2015, Sergi et al. [91] by including nine studies, containing a total of 2147 women for the FVL mutation, 1305 women with early RPL, and 842 women with no gestational complications, indicated higher carrier frequency of FVL mutation in women with early RPL (OR = 1.68). Moreover, Marcelo and colleagues [92] in 2019 revealed that there was no association between recurrent miscarriage and inherited thrombophilias in patients with polycystic ovarian syndrome, with respect to FVL (OR = 0.74; 95% CI = 0.38–1.45; \( P = 0.38 \)), among others. On the other hand, a comprehensive systematic review and meta-analysis in 2016 [93], by exerting 369 articles evaluating 124 polymorphisms of 73 genes, to explore the potential genetic biomarkers for recurrent miscarriage identified increased risk of the disease in the recessive and over-dominant models, but a decreased risk in the dominant and allelic models for FVL 1691G > A mutation, both in overall analysis and subgroup analysis in Caucasians. Our analysis is unique of its type, as it included only patients having RPL diagnosis. Moreover, our subgroup analysis based on the continent of the study population divulged a strong association between FVL 1691G > A mutation and the risk of RPL in Asian, European, and Africa populations, but not in South Americans. It should be noted that among the 62 case-control studies included, 25 studies were in Asia, 26 studies in Europe, 6 studies in South America, 4 studies in Africa, and 1 study in Oceania. Although the subgroup analysis of 6 studies in South America indicated an OR < 1 (which was not significant across all genetic models), all other populations (which made large portion of the studies included) had OR > 1, imply that the South America data had little effect on the pooled effect estimation. The other parameter for subgroup analysis was study design. In this regard, a significant positive association between FVL 1691G > A mutation and the risk of
Fig. 3 Pooled OR and 95% CI of individual studies and pooled data for the association between FVL 1691G > A mutation and the risk of RPL in different continents based on subgroup analysis for Over-Dominant model.
RPL was observed in case-control studies, while cohort studies revealed no such association. The result of this subgroup should interpret with caution because of imbalance between included studies in each group (60 vs. 2).

On the other side, the analysis was also performed in the Iranian population, containing 9 publications with 1409 cases and 1160 controls. The previous meta-analysis in Iranian population by Kamali et al. [94] in 2018, by employing 7 studies, indicated significant increased risk of RPL only in the allelic (OR = 2.252) and dominant models (OR = 2.217). However, our analysis indicated that the measured genetic models, including dominant model (OR = 2.97), over-dominant model (OR = 2.58), and heterozygote model (OR = 2.67, 95%) increased the risk of RPL. The difference between our analysis and the previous one was that we included two more study with higher sample size.

There was a degree of heterogeneity during the overall analysis. From statistical perspective, this heterogeneity describes the variability between included studies and may originate from clinical or methodological heterogeneity, from other unreported, unknown study characteristics, or may be due to chance. Therefore, for finding any sources of heterogeneity and attenuating their effects, we conducted subgroup analysis and weighted meta-regression. Collectively, the results of meta-regression showed that none of the parameters, including publication year, the continent of the study population, and genotyping methods were the expected source of heterogeneity. However, subgroup analysis reduced heterogeneity in all groups and explained part of the
Table 4  Meta-regression analyses of potential source of heterogeneity

| Heterogeneity Factor | Coefficient | SE    | T-test | P-value | 95% CI   |
|----------------------|-------------|-------|--------|---------|----------|
|                      |             |       |        |         | UL | LL |
| Publication Year     |             |       |        |         |     |     |
| Dominant             | 0.296       | 0.31  | 0.85   | 0.39    | −0.365 | 0.905 |
| Over-Dominant        | 0.211       | 0.26  | 0.79   | 0.43    | −0.325 | 0.747 |
| Allelic model        | 0.159       | 0.20  | 0.77   | 0.44    | −0.257 | 0.576 |
| GA vs. GG            | 0.253       | 0.29  | 0.86   | 0.39    | −0.341 | 0.848 |
| Continent            |             |       |        |         |     |     |
| Dominant             | 0.879       | 1.92  | 0.46   | 0.65    | −2.99 | 4.74  |
| Over-Dominant        | 0.498       | 1.63  | 0.30   | 0.76    | −2.79 | 3.78  |
| Allelic model        | 0.650       | 1.27  | 0.51   | 0.61    | −1.90 | 3.20  |
| GA vs. GG            | 0.72        | 1.80  | 0.40   | 0.69    | −2.90 | 4.35  |
| Genotyping Methods   |             |       |        |         |     |     |
| Dominant             | −0.04       | 1.35  | −0.04  | 0.97    | −2.76 | 2.66  |
| Over-Dominant        | 0.028       | 1.15  | 0.02   | 0.98    | −2.29 | 2.35  |
| Allelic model        | −0.115      | 0.89  | −0.13  | 0.89    | −1.92 | 1.68  |
| GA vs. GG            | 0.016       | 1.26  | 0.01   | 0.98    | −2.52 | 2.55  |

Fig. 5 Meta-regression plots of the association between FVL 1691G > A mutation and risk of RPL (Dominant model) based on; a: Publication year, b: Continent, c: Genotyping methods
observed heterogeneity expect Asians and studies with cohort design. Furthermore, the other way of dealing with statistical heterogeneity, which we used in our analysis, was to incorporate “Random” term to account for it in a random-effects. Random effect model typically produces more conservative estimates of the significance of a result (a wider confidence interval). As it gives proportionately higher weights to smaller studies and lower weights to larger studies than fixed effect analysis.

To address the limitations in the current meta-analysis, it should be stated that, first our literature search was limited to only studies published in English language. Second, there was a degree of heterogeneity during the overall analysis. But not in all subgroup analyses, indicating the role of genetic diversity and other confounders in susceptibility to RPL. Third, as this meta-analysis a crude estimation of the association between FVL 1691G > A mutation and the risk of RPL,
thus the roles of age, paternal genetic impression, environmental factors, and the effect of gene–gene interactions in conferring the susceptibility risk to RPL were neglected.

Considering all the facts, this meta-analysis, the first one of its type to our best knowledge, retrieved 62 studies, encompassing 10,410 cases and 9406 health controls, to find a consistent result of the association between FVL 1691G > A mutation and risk of RPL. Our results indicated statistically significant increased risk of RPL in the overall analysis. The increased susceptibility to RPL was also observed in Iranian, Asian, European, Africa populations, and studies with case-control design, but not in South Americans and studies with cohort design. Further experiments, alongside with inclusion of additional studies with large sample sizes, should consider the role cofounders in susceptibility to RPL.

Abbreviations
FVL: Factor V Leiden; RPL: Recurrent pregnancy loss; MTHFR: Methylenetetrahydrofolate reductase; CI: Confidence interval; OR: Odds ratio; SNP: Single-nucleotide polymorphism; PRISMA: Preferred reporting items for systematic reviews and meta-analyses; NOS: Newcastle–Ottawa scale; HWE: Hardy–Weinberg equilibrium

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Authors’ contributions
ME and BR originated the study, acquired data. BR and MK performed statistical analysis, interpreted data, drafted the manuscript. SA revised the manuscript. SA and MS approved the manuscript. All authors read and approved the final manuscript.

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Author details
1Department of Hematology, Faculty of Medical Sciences, Tarbiat Modares University, North Kargar Av, Tehran 14117, Iran. 2Department of Basic sciences, Maragheh University of medical sciences, Maragheh, Iran. 3Rahat Breach and Sleep Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.

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