Journal of Assisted Reproduction and Genetics (2023) 40:1533–1558
https://doi.org/10.1007/s10815-023-02823-x

REVIEW

Thrombophilic gene polymorphisms and recurrent pregnancy loss: a systematic review and meta-analysis

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Received: 14 February 2023 / Accepted: 29 April 2023 / Published online: 30 May 2023

Abstract

Purpose Recurrent pregnancy loss (RPL) is affecting 1–4% of women who conceive approximately, and no cause could be found in more than 50% of women suffering from RPL. Inherited thrombophilias have got increasing attention in women with unexplained RPL, so we aim to explore the relationship among these most common thrombophilic polymorphisms and RPL through a literature review and meta-analysis.

Methods Observational studies from PubMed, Embase, Cochrane, and Web of Science from 1997 to 7 April 2022 were searched. For each genetic variant, a fixed or random-effect model was used according to the heterogeneity test to calculate pooled ORs and 95% CIs for both dominant and recessive genetic models. Egger's line regression test was used to assess publication bias. The quality of the included articles was assessed by the Newcastle Ottawa scale.

Results A total of 124 articles comprising 17,278 RPL patients and 16,021 controls were included. Results showed that hyperhomocysteinemia (MTHFR) C677T (dominant model: OR, 1.43; 95% CI, 1.25–1.64; recessive model: OR, 1.60; 95% CI, 1.36–1.87), MTHFR A1298C (dominant model: OR, 1.66; 95% CI, 1.26–2.18; recessive model: OR, 1.79; 95% CI, 1.42–2.26), PAI-1 4G/5G (dominant model: OR, 1.67; 95% CI, 1.36–2.06; recessive model: OR, 1.80; 95% CI, 1.39–2.32), angiotensin-converting enzyme I/D (OR, 1.23; 95% CI, 1.00–1.53), Factor XIII V34L (OR, 1.38; 95% CI, 1.02–1.87), and β-fibrinogen-455G/A (OR, 1.60; 95% CI, 1.02–2.51) were significantly associated with RPL.

Conclusion This study provides potentially useful clinical markers to evaluate the risk of RPL or to help unexplained RPL patients identify possible causes, which may allow for targeted treatment.

Keywords Meta-analysis · Recurrent pregnancy loss · Thrombophilic gene polymorphisms · Clinical marker

Introduction

Although the definition of recurrent pregnancy loss (RPL) is highly controversial in several studies, it is generally accepted that RPL is the spontaneous end of two or more clinically recognized pregnancies, as defined by Guidelines from the American Society for Reproductive Medicine [1]. It is estimated that approximately 1–4% of women who conceive are affected by RPL and the rates may be underestimated due to inconsistencies in definition and classification [2, 3]. The underlying triggers vary among women, but several common risk factors are identified including maternal age, health, genetic abnormalities, environmental, and lifestyle factors [4]. However, more than 50% of women suffering from RPL have no clearly identifiable etiology [1, 5, 6].

Inherited thrombophilia, which increases the risk of forming venous or arterial thromboembolism, could be one of the suspected causes for women with unexplained RPL. The normal growth and development of the fetus depends on adequate blood supply in the placental circulation. The persistent abnormal hypercoagulable states in women with inherited thrombophilias, and the hemostatic balance shifting towards hypercoagulability physiologically in pregnant women, lead to a tendency to form blood clots and, therefore, disrupt this blood supply and impede the growth and development of embryos [7]. Several related genetic risk factors have been discovered including coagulation factors (Factor II,

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Factor V, Factor XIII, and fibrinogenβ), defects of the fibrinolytic system (PAI-1), hyperhomocysteinemia (MTHFR), and angiotensin-converting enzyme (ACE). When the genes that encode these factors are mutated, the risk of thromboembolism is greatly increased. Yet the results of studies researching the association between inherited thrombophilias and RPL were discrepant. Liu et al. had done a thorough and comprehensive assessment on the relationship between RPL and the two common polymorphisms factor V Leiden and G20210A mutation of the prothrombin gene recently [8], but there is still no consensus on other polymorphisms, including MTHFR C677T, MTHFR A1298C, PAI-1 4G/5G, ACE I/D, Factor V R2, Factor XIII V34L, and β-fibrinogen-455G/A. Specific information about these polymorphisms is shown in Table 1. In this case, integrating data from similar research for these polymorphisms would be necessary.

The aim of this study was to perform a literature review and meta-analysis to determine the association between patients diagnosed with RPL and common thrombophilic polymorphisms.

Materials and methods

Information sources, search strategy

This meta-analysis was conducted by two authors independently. We screened studies published from 1997 to 7 April 2022 without any restriction of countries from databases PubMed, Embase, Cochrane, and Web of Science. The major search terms used in the strategy include RPL, MTHFR C677T, MTHFR A1298C, PAI-1 4G/5G, ACE I/D, Factor V R2, Factor XIII V34L, and β-fibrinogen-455G/A. More detailed search terms are listed in Supplemental Data 1. Moreover, references of relative reviews and meta-analyses (other sources) were manually checked to ensure all the eligible studies. Only studies published in English and Chinese were included. We contacted authors via e-mail if needed. The protocol for this review was registered in advance on International Prospective Register of Systematic Reviews with ID CRD42022327937.

Eligibility criteria

Inclusion criteria were as follows: (1) observational studies (cohort or case–control study) searching the relationship between RPL and genetic polymorphisms; (2) at least two groups where one was diagnosed with RPL and the other was healthy population; (3) genotypes involved in the candidate genes; (4) study population only women were included; (5) reliable genetic method to detect genotype; (6) sufficient information of genotyping to calculate ORs and the corresponding 95% CIs. Literature would be excluded in the following cases: (1) reviews, letters, case reports, or abstracts; (2) cases with anatomic, chromosomal, hormonal, autoimmune, infectious, or other known causes. Only one would be included if there were several studies from the same population with the same distribution of genotypes.

Exposure and outcomes

The key exposure variable was the presence of hereditary thrombophilia, including MTHFR, PAI-1, ACE, Factor V, Factor XIII, and β-fibrinogen mutation. C677T and A1298C are the two most common polymorphisms of MTHFR, with C > T substitution at nucleotide 677 and A > C substitution at nucleotide 1298, respectively. The PAI-1 4G/5G mutation results in a common guanosine insertion/deletion 675-bp upstream from the start site of translation. The most studied ACE I/D polymorphism results from an Alu element insertion or deletion in intron 16 of the ACE gene. A novel complex haplotype called R2 with an A to G transition at nucleotide 4070 in exon 13 of the gene is one of the exposures here. Factor XIII Val-34Leu is a common polymorphism where a G-to-T transition (FXIII G103T) is in exon 2 of the gene encoding for FXIIIA. Fibrinogen variants were studied here because the synthesis of the fibrinogen β-chain is considered to be the rate-limiting step in the fibrinogen biosynthesis, especially G–455A substitution in the 5-flanking region.

Table 1 Specific information about thrombophilic gene polymorphisms

Gene	Variant	Minor allele	Consequence	Studies	Cases	Controls
MTHFR	C677T	T	Elevated plasma homocysteine levels	85	11,823	11,776
MTHFR	A1298C	C	Elevated plasma homocysteine levels	40	7121	6739
PAI-1	4G/5G	4G	Elevated plasma PAI-1 levels	34	5294	4254
ACE	I/D	Deletion	Higher serum ACE levels	26	4284	2992
Factor V	R2/HR2	G	Poor response to activated protein C	13	1771	1172
Factor XIII	G103T	T	Interferes with fibrin cross-linking and regulation of fibrinolysis	15	1282	1093
β-Fibrinogen	G455A	A	Elevated the plasma fibrinogen levels	12	1258	891

The main outcome was RPL, including early RPL and late RPL. Early RPL was defined as pregnancy losses before the 12th week of gestation, while late RPL was defined as pregnancy losses after the 12th week of gestation.

Quality assessment

The quality of the included articles was assessed by the Newcastle Ottawa scale by two authors from the following eight major criteria: adequate determination of cases, representativeness of the cases, selection of controls, determination of controls, comparability of cases and controls, ascertainment of exposure, same method of ascertainment for cases and controls, and non-response rate [9]. With the maximum score of 9, the higher the score, the better the quality. Studies with low scores (< 5) will be excluded.

Data extraction

For the articles finally included, the following data were abstracted: the first author's name, publication year, geographic region, sample size, distribution of genotypes, mean age of the population, method of genotyping, and definition of RPL. Any divarication was assessed again by two reviewers or by consulting a third author.

Statistical analysis

The effect magnitude to measure the association between thrombophilic gene polymorphism and RPL was odds ratios (ORs) with 95% CIs. For each genetic variant, a fixed or random-effect model was used according to the heterogeneity test to calculate pooled ORs and 95% CIs for both dominant and recessive genetic models. For a pair of alleles A and a, where A is the major allele and a is the minor allele, the dominant model refers to Aa + aa vs. AA, and the recessive model refers to AA + Aa vs. aa. To qualify the effect of heterogeneity, we used I^2 statistic which describes the percentage of total variation across studies that is due to heterogeneity rather than chance ($I^2 > 50\%$ implied a high degree of heterogeneity, and $I^2 < 50\%$ implied a low degree of heterogeneity) [10]. Publication bias was assessed by Egger's line regression test [11]. Trim and fill method was used in models with significant publication bias to evaluate the robustness of results by correcting for bias [12]. Sensitivity analysis was performed by excluding each included study and repeating the meta-analysis to assess the stability of the results. In addition, in view of the defining differences of RPL and widely distributed geographical regions, we performed the subgroup analyses according to the number of pregnancy loss (2 or more pregnancy losses, 3 or more pregnancy losses), gestational age at pregnancy loss (early RPL, late RPL), and ethnicity (Caucasian, non-Caucasian). $P < 0.05$ was considered statistically significant for all the analyses. All the analyses were conducted using R programming language (R-4.1.3).

Results

Study selection and characteristics

The specific process of literature screening is shown in the flow diagram of articles included in the meta-analysis (Fig. 1). A total of 124 articles published from 1997 to 2022 were included in this meta-analysis. The detailed information of the 124 included articles is shown in Table 2. In total, 120 were in English and 4 were in Chinese. Of the studies included here, 123 were case–control studies, and only one was a cohort study. These studies were conducted in 37 countries worldwide, with the population in 98 studies being Caucasian and in 26 studies being non-Caucasian. After the quality assessment, all studies were rated as having medium or high quality, with scores ranging from 5 to 9. The results of meta-analyses and subgroup analyses are presented in Table 3 and Table 4, respectively.

MTHFR polymorphisms

For C677T, 85 studies consisted of 11,823 patients and 11,776 controls for meta-analysis. Under the dominant model (CT + TT vs. CC), a high degree of among-study heterogeneity was observed ($I^2 = 79\%$, $P < 0.01$). The pooled ORs and 95% CI showed great significance for the relationship between this polymorphism and RPL (OR: 1.43; 95% CI: 1.25–1.64; $P < 0.01$) (Fig. 2A). Under the recessive model (CC + CT vs. TT), a high degree of among-study heterogeneity was observed ($I^2 = 60\%$, $P < 0.01$). The pooled ORs and 95% CI also showed statistical significance (OR: 1.60; 95% CI: 1.36–1.87; $P < 0.01$) (Fig. 2B). As for the sensitivity analysis, the risk estimate did not materially change for both the dominant model and the recessive model. The examination for publication bias via the Egger's line regression test did not show a significant bias for the dominant model ($t = 1.46$, $P = 0.14$); however, there was quite a significant publication bias in the recessive model ($t = 3.47$, $P < 0.01$). After adding 18 studies and dropping out 3 studies via trim and fill method, the pooled ORs and 95% CI were consistent with the original result (OR: 1.27; 95% CI: 1.05–1.54; $P < 0.05$), suggesting the publication bias had no significant impact on the result. Subgroup analyses showed that the number of pregnancy loss, gestational age at pregnancy loss, and ethnicity were

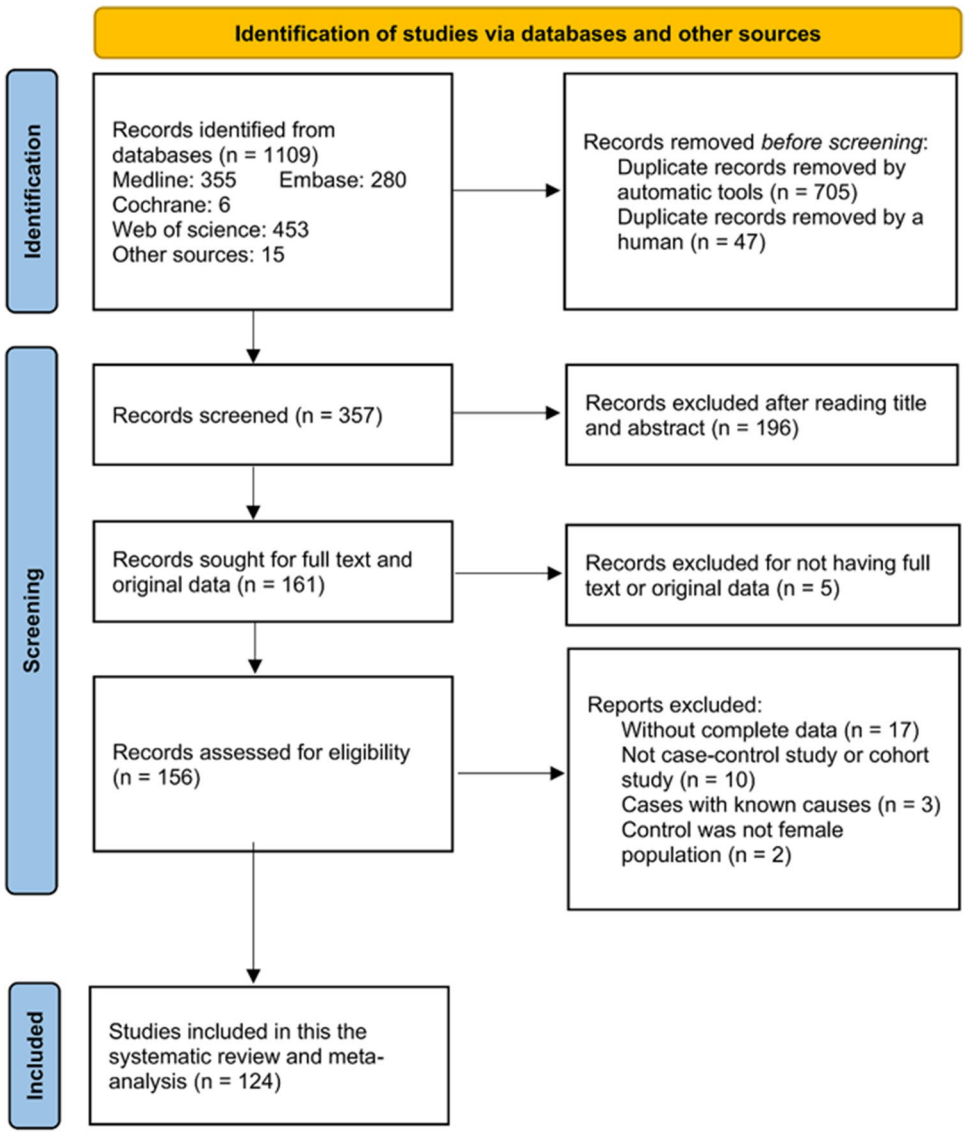

Fig. 1 Flow diagram of articles included in the meta-analysis

not associated with among-study heterogeneity under both the two genetic models.

For A1298C, 40 studies consisted of 7121 patients and 6739 controls for meta-analysis. Under the dominant model (AC + CC vs. AA), an extremely high degree of among-study heterogeneity was observed ($I^2 = 90\%$, $P < 0.01$). The pooled ORs and 95% CI showed significance for the relationship between MTHFR A1298C and RPL (OR: 1.66; 95% CI: 1.26–2.18; $P < 0.01$) (Fig. 3A). Under the recessive model (AA + AC vs. CC), a high degree of among-study heterogeneity was observed ($I^2 = 56\%$, $P < 0.01$). The pooled ORs and 95% CI also showed great statistical significance (OR: 1.79; 95% CI: 1.42–2.26; $P < 0.01$) (Fig. 3B). As for the sensitivity analysis, the risk estimate did not materially change for both the dominant model and the recessive model. The examination for publication bias via the Egger's line regression test showed no significant bias for the dominant model ($t = 1.07$, $P = 0.29$), but a significant bias for the recessive model ($t = 3.28$, $P < 0.01$). After adding 9 studies via trim and fill method, the pooled ORs and 95% CI were consistent with the original result (OR: 1.51; 95% CI: 1.16–1.97; $P < 0.01$), suggesting the publication bias had no significant impact on the result. Subgroup analyses showed that ethnicity under both the two models and the number of pregnancy loss under the recessive model were associated with among-study heterogeneity ($P < 0.05$), while gestational age at pregnancy loss was not.

PAI-1 polymorphisms

Thirty-four studies consisted of 5294 patients and 4254 controls for meta-analysis. Under the dominant model (4G5G + 4G4G vs. 5G5G), a high degree of among-study heterogeneity was observed ($I^2 = 76\%$, $P < 0.01$).

Table 2 The detailed information of included articles

MTHFR (C677T)								
Author	Year	Country	Genotype frequency(C/C:C/T:T/T)		Mean age		Definition of RPL	Genotyping method	Quality
			Case	Control	Case	Control			
W L Nelen [13]	1997	Netherlands	77:79:29	48:59:6	NA	NA	2 or more spontaneous consecutive miscarriages before 17 weeks of gestation from the same partner	PCR–RFLP	8
I Quere [14]	1998	France	28:52:20	32:54:14	NA	NA	Recurrent early miscarriages of unknown cause (≥3 consecutive episodes)	PCR–RFLP	6
Z R Holmes [15]	1999	UK	71:47:11	31:30:6	NA	NA	At least 3 consecutive miscarriages before 12 weeks of gestation	PCR–RFLP	6
A Lissak [16]	1999	Israel	17:20:4	7:7:4	NA	NA	≥2 consecutive first-trimester spontaneous abortions or a total of≥3 first-trimester spontaneous abortions	PCR–RFLP	6
R P Murphy [17]	2000	Ireland	18:19:3	214:270:56	32	25	At least 2 previous and unexplained events at any point during pregnancy	PCR–RFLP	6
M L Wramsby [18]	2000	Sweden	27:32:3	27:35:7	NA	NA	At least 3 spontaneous consecutive miscarriages	PCR–RFLP	6
R Pihusch [19]	2001	Germany	41:47:14	55:61:12	35	32	2 or more unexplained consecutive abortions at 25 weeks of gestation	PCR–RFLP	6
A Dilley [20]	2002	USA	27:25:7	39:45:9	36	33	3 or more fetal losses, regardless of trimester of loss or previous live birth, or any late loss	PCR–RFLP	8
Gertrud Unfried [21]	2002	Austria	64:46:23	46:24:4	32	56	At least 3 spontaneous, consecutive miscarriages before 20 weeks of gestation	PCR–RFLP	7
Wang YW [22]	2002	China	13:33:16	43:53:23	28.23	28.7	2 or more abortions with unexplained causes	PCR–RFLP	8
K S D Kumar [23]	2003	India	18:6:0	22:2:0	26.1	NA	3 or more consecutive pregnancy losses at less than 22 weeks of gestation	PCR-SSCP	8
Maria Hohlagschwandtner [24]	2003	Austria	72:52:21	53:41:7	32	56	3 or more consecutive spontaneous miscarriages before 20 weeks of gestation	PCR-ASO	7
T Buchholz [25]	2003	Germany	74:87:22	55:61:11	35	32.8	At least 2 unexplained consecutive spontaneous miscarriages before 25 weeks of gestation	PCR–RFLP	7
Hans-Ulrich Pauer [26]	2003	Germany	28:32:9	64:51:15	NA	NA	2 or more consecutive miscarriages	PCR–RFLP	6
Aiko Makino [27]	2004	Japan	33:42:10	29:32:15	31	30	2 or more unexplained first-trimester recurrent embryonal losses (before 10 weeks of gestation)	PCR–RFLP	7
Wang XP [28]	2004	China	49:78:20	43:34:5	27.7	32	3 or more consecutive pregnancy losses before 20 weeks of gestation	PCR–RFLP	6
Li XM [29]	2004	China	16:32:9	25:20:5	28.97	27.86	≥2 spontaneous abortions	PCR–RFLP	6
Li-xue Guan [30]	2005	China	13:59:55	19:73:25	27	26	At least 3 spontaneous abortions	PCR–RFLP	7
Gen Kobashi [31]	2005	Japan	15:20:3	67:82:25	NA	29.8	2 or more consecutive spontaneous with unexplained etiological causes	PCR–RFLP	6
Song LY [32]	2005	China	36:2:12	40:12:4	NA	NA	Unexplained repeated spontaneous abortions	PCR–RFLP	6
Egle Couto [33]	2005	Brazil	29:47:12	53:26:9	NA	NA	Recurrent spontaneous abortions	PCR–RFLP	8
Carolyn B Coulam [34]	2006	USA	58:80:12	11:9:0	34.7	39.6	2 or more consecutive abortions	PCR–RFLP	6
N Mtiraoui [35]	2006	Tunisia	92:47:61	156:30:14	28.68	28.24	3 or more consecutive RPLs at 5–30 weeks of gestation	PCR–RFLP	8
Dong, S.Q [36]	2006	China	2:14:20	12:27:18	29.58	27.04	3 or more spontaneous abortions	PCR–RFLP	8

Table 2 (continued)

Alexandros Sotiriadis [37]	2007	Greece	24:61:12	32:57:13	32.2	32.2	3 or more consecutive miscarriages with the same partner in < 15 weeks of gestation	PCR–RFLP	8
Venkatesan Vettriselvi [38]	2008	India	86:15:3	98:19:3	NA	NA	3 or more spontaneous consecutive miscarriages less than 20 weeks of gestation	PCR–RFLP	6
Arijit Biswas [39]	2008	India	74:11:0	23:8:0	27.9	26	With spontaneous recurrent abortions (mean number of recurrent abortions, 3)	PCR–RFLP	6
Bettina Toth [40]	2008	Germany	71:68:12	68:70:19	33.2	45.2	2 or three and more consecutive miscarriages	PCR–RFLP	6
Rupak Mukhopadhyay [41]	2009	India	75:6:3	78:2:0	NA	NA	2 or more than two pregnancy losses	PCR–RFLP	8
Vinukonda Govindaiah [42]	2009	USA	111:25:4	112:28:0	NA	NA	3 or more unexplained recurrent pregnancy losses	PCR–RFLP	7
C Ciacci [43]	2009	Italy	16:15:8	25:35:12	NA	NA	At least 2 pregnancy losses within the first 3 months of pregnancy	PCR-ASO	6
Jeehyeon Bae [44]	2009	Korea	82:104:36	45:63:14	32.6	31.2	More than 2 consecutive abortions	PCR–RFLP	7
Gonca Imir Yenicesu [45]	2010	Turkey	133:109:30	32:24:0	27.2	29.5	2 or more consecutive early RPL at 5–12 weeks of gestation	PCR-ASO	7
Agnieszka Seremak-Mrozikiewicz [46]	2010	Poland	44:49:11	89:67:13	30.15	29.4	3 or more unexplained consecutive recurrent miscarriages in the first trimester of pregnancy (6–13 week of gestation)	PCR–RFLP	7
Mohamed A Mohamed [47]	2010	Egypt	6:9:5	20:0:0	31.4	29	Loss of 3 or more consecutive pregnancies before 20 weeks of pregnancy	PCR-ASO	7
Ahmad Settin [48]	2011	Egypt	40:26:4	67:68:1	NA	NA	2 or more events of fetal loss in the form of abortion, miscarriage, or still birth	PCR–RFLP	6
Mahmood Jeddi-Tehrani [49]	2011	Iran	43:42:15	66:25:9	NA	NA	At least 2 successive pregnancy losses before 20th week of gestation	PCR–RFLP	6
Chan Woo Park [50]	2011	Korea	14:16:9	17:26:7	34.9	38.5	2 or more unexplained pregnancy losses	PCR-DNA sequencing	7
Oztürk Ozdemir [51]	2012	Turkey	231:239:73	76:30:0	27.8	28.9	2 or more consecutive early RPL at 5–12 weeks of gestation	PCR-ASO	8
Rohini R Nair [52]	2012	India	75:26:5	118:21:1	NA	NA	3 or more trimester miscarriages before 12 weeks of gestation	PCR–RFLP	7
Suat Karata [53]	2012	Turkey	6:54:24	40:43:12	31.6	32.2	3 or more consecutive pregnancy losses before 10 weeks of gestation	PCR-ASO	7
Vajira H W Dissanayake [54]	2012	Sri Lanka	158:39:3	169:27:2	32.1	32.4	2 or more consecutive spontaneous abortions with no living children	PCR–RFLP	8
Farah Idali [55]	2012	Iran	61:36:9	66:25:9	30.1	NA	At least 3 pregnancy losses before 20th week of gestation	PCR–RFLP	6
Ahmad Poursadegh Zonouzi [56]	2012	Iran	53:30:6	27:22:1	30.17	31.54	First trimester recurrent spontaneous abortions	PCR–RFLP	6
Raheleh Torabi [57]	2012	Iran	43:42:15	66:25:9	NA	NA	At least 2 recurrent pregnancy losses before the 20th week of gestation	PCR–RFLP	6
Talieh Kazerooni [58]	2013	Iran	50:6:4	54:6:2	24.8	24.6	3 or more consecutive pregnancy losses at less than 20 weeks of gestation	PCR–RFLP	8
Farah Parveen [59]	2013	India	110:70:20	196:90:14	NA	NA	At least 3 spontaneous miscarriages	PCR–RFLP	7
Montserrat Creus [60]	2013	Spain	23:26:11	13:13:4	35	35.8	≥ 3 consecutive spontaneous miscarriages of unknown etiology ≤ 10 weeks of gestation	PCR-FRET	9

Table 2 (continued)

Lovejeet Kaur [61]	2013	India	86:16:5	463:109:21	24.89	25.32	3 or more consecutive unexplained recurrent pregnancy losses before 24 weeks of gestation	PCR–RFLP	8
Kristin Baumann [62]	2013	Germany	279:287:75	66:70:19	33.14	33.16	≥2 consecutive miscarriages	PCR–RFLP	7
Caroline Gross Dutra [63]	2014	Brazil	73:59:13	71:53:11	31.72	29.86	At least 2 pregnancy losses before 24 weeks of gestation with the same partner and with no report of a full-term pregnancy	TaqMan-qPCR	8
Yunlei Cao [64]	2014	China	29:43:10	53:83:30	28.43	28.1	At least 2 consecutive pregnancy losses before 12-week gestational age	PCR-DNA sequencing	9
Elham Yousefian [65]	2014	Iran	96:90:18	63:43:10	29.7	30.4	3 or more consecutive pregnancy losses before the 22nd week of pregnancy, regardless of a previous live birth	PCR-SSOP	9
A Pietropolli [66]	2014	Italy	55:86:45	31:71:27	35.2	40.4	2 or more consecutive spontaneous miscarriages before the 20th week of gestation	PCR-FRET	8
Fabio L Lino [67]	2015	Brazil	53:43:16	46:41:11	30.3	40.2	3 or more idiopathic miscarriages early in pregnancy(≤12 weeks)	PCR-ASO	7
Li Luo [68]	2015	China	40:70:15	60:65:10	30.89	29.4	2 or more consecutive spontaneous abortions	PCR–RFLP	7
Wendell Vilas Boas [69]	2015	Brazil	59:26:4	97:47:6	29.4	23	At least 2 consecutive miscarriages in the first, second or third trimester of gestation, without any successful pregnancy	PCR–RFLP	8
Shiny Vanilla [70]	2015	India	13:2:0	13:2:0	NA	NA	2 or more consecutive miscarriages with or without normal child	PCR–RFLP	6
L Zhu [71]	2015	China	60:40:18	100:72:2	29.8	28.5	Spontaneous abortions that occur ≥2 times in a row	TaqMan-qPCR	8
Somayeh-Sadat Tara [72]	2015	Iran	62:114:49	70:26:4	32.4	35.2	At least 3 successive pregnancy losses below 20th week of gestation	PCR–RFLP	6
Kamelia Farahmand [73]	2016	Iran	180:114:36	230:85:35	30.37	29.88	3 or more consecutive pregnancy losses before 20 weeks of gestation, with no history of full-term pregnancies	PCR-ASO	8
R O Gonçalves [74]	2016	Brazil	80:51:6	59:37:4	32.1	25.8	2 or more consecutive first-trimester abortions (<12 weeks gestation)	PCR–RFLP	7
J J López-Jiménez [75]	2016	México	17:26:13	12:23:15	30	30	3 consecutive pregnancy losses prior to the 20th week of gestation	PCR–RFLP	7
Kyu Ri Hwang [76]	2017	Korea	104:153:45	94:156:65	34.8	50.3	At least 2 unexplained consecutive spontaneous miscarriages before 20 weeks of gestation	TaqMan-qPCR	6
Walid Al-Achkar [77]	2017	Syria	41:41:18	66:39:1	30	31	2 or more miscarriages and diagnosed as RPLs	PCR–RFLP	8
M Chatzidimitriou [78]	2017	Greece	30:18:0	21:3:3	35.5	35.1	2 or more consecutive fetal losses prior to 20 weeks of gestation	PCR-ASO	6
Hubert Wolski [79]	2017	Poland	165:153:41	201:164:35	30.99	30.05	≥2 loss of pregnancy before 22 completed weeks of gestation	PCR–RFLP	7
Alptekin H [80]	2017	Turkey	49:43:14	37:20:5	27.9	29.4	2 or more lost pregnancies(early or late miscarriages or stillbirths) for no reason	PCR-DNA sequencing	7

Table 2 (continued)

Amela Jusić [81]	2018	Bosnian	22:29:9	47:26:7	33.05	34.08	2 or more consecutive miscarriages before 20 weeks of gestation	PCR–RFLP	7
Razieh Bigdeli [82]	2018	Iran	91:76:33	136:58:6	23	25.1	At least 2 pregnancy losses	PCR–RFLP	8
Domenico Dell'Edera [83]	2018	Italy	220:86:74	197:100:90	NA	NA	At least 2 miscarriages	PCR-FRET	6
Hanadi El Achi [84]	2018	Lebanon	11:30:29	76:26:1	32.2	NA	At least 2 consecutive miscarriages	PCR-ASO	5
Anil Kumar Sah [85]	2018	Nepal	28:5:2	35:0:0	NA	NA	2 or more consecutive miscarriages with or without normal child, unexplained cause of losses	PCR–RFLP	6
Kallur Nava Saraswathy [86]	2018	India	64:16:5	66:43:12	NA	NA	3 or more consecutive unexplained pregnancy losses before 24 weeks of gestation	PCR–RFLP	8
Yuanchang Zhu [87]	2018	China	166:157:47	66:59:19	NA	NA	2 or more clinical pregnancy failures	PCR-DNA sequencing	8
E A Trifonova [88]	2019	Russia	129:99:25	210:112:17	29.5	27.3	At least 2 pregnancy losses up to 20 weeks	PCR–RFLP	8
Yajuan Xu [89]	2019	China	26:87:105	40:122:102	31.82	31.16	2 or more spontaneous miscarriages with a diagnosis of recurrent pregnancy loss	TaqMan-qPCR	8
Najmeh Ahangari [90]	2019	Iran	127:95:23	222:22:6	32.16	31.81	2 or more repeated abortions	PCR-DNA sequencing	8
Yalda Zarfeshan Fard [91]	2019	Iran	15:20:15	31:15:4	31.26	33.76	2 or more frequent abortions with normal karyotype and hormone tests	PCR–RFLP	7
Jyoti Mishra [92]	2019	India	13:9:6	25:26:21	NA	NA	Recurrent miscarriages	PCR–RFLP	7
Zhong Lin [93]	2019	China	213:153:37	253:78:11	29.58	29.88	2 or more consecutive spontaneous abortions	TaqMan-qPCR	8
Ivana Joksic [94]	2020	Serbia	35:30:5	12:18:1	33.2	33.2	3 or more consecutive pregnancy losses	PCR-ASO	7
Irem Yengel [95]	2020	Turkey	56:68:21	50:44:11	NA	NA	At least 2 recurrent pregnancy losses before the 12th week of gestation	TaqMan-qPCR	6
Yan Zhang [96]	2020	China	141:85:11	313:262:43	27.85	27.01	Diagnosed with RPL in compliance with the American Society for Reproductive Medicine definitions of infertility and recurrent pregnancy loss	TaqMan-qPCR	7
Mai Mahmoud Shaker [97]	2021	Egypt	48:46:6	58:38:4	26.2	25.7	2 to three consecutive pregnancy losses earlier to the 20th week of gestation	PCR–RFLP	8
MTHFR (A1298C)									
Author	Year	Country	Genotype frequency(A/A:A/C:C/C)		Mean age		Definition of RPL	Genotyping method	Quality
			Case	Control	Case	Control			
Maria Hohlagschwandtner [24]	2003	Austria	63:67:15	35:50:16	32	56	3 or more consecutive spontaneous miscarriages before 20 weeks of gestation	PCR-ASO	7
Wang XP [28]	2004	China	102:35:10	60:20:2	27.7	32	3 or more consecutive pregnancy losses before 20 weeks of gestation	PCR–RFLP	6
Carolyn B Coulam [34]	2006	USA	50:80:20	12:8:0	34.7	39.6	2 or more consecutive abortions	PCR–RFLP	6
N Mtiraoui [35]	2006	Tunisia	108:65:27	130:62:8	28.68	28.24	3 or more consecutive RPLs at 5–30 weeks of gestation	PCR–RFLP	8
Alexandros Sotiriadis [37]	2007	Greece	44:37:7	45:39:6	32.2	32.2	2 or more consecutive miscarriages with the same partner in < 15 weeks gestation	PCR–RFLP	8
C Ciacci [43]	2009	Italy	18:20:1	29:34:9	NA	NA	At least 2 pregnancy losses within the first 3 months of pregnancy	PCR-ASO	6

Table 2 (continued)

Jeehyeon Bae [44]	2009	Korea	144:68:9	74:43:4	32.6	31.2	More than 2 consecutive abortions	PCR–RFLP	7
Agnieszka Seremak-Mrozikiewicz [46]	2010	Poland	40:51:13	78:74:17	30.15	29.4	3 or more unexplained consecutive recurrent miscarriages in the first trimester of pregnancy (6–13 week of gestation)	PCR–RFLP	7
Ahmad Settin [48]	2011	Egypt	15:49:6	36:97:3	NA	NA	2 or more events of fetal loss in the form of abortion, miscarriage, or still birth	PCR–RFLP	6
Mahmood Jeddi-Tehrani [49]	2011	Iran	69:27:4	94:6:0	NA	NA	At least 2 successive pregnancy losses before 20th week of gestation	PCR–RFLP	6
Oztürk Ozdemir [51]	2012	Turkey	201:257:85	71:35:0	27.8	28.9	2 or more consecutive early RPL at 5–12 weeks of gestation	PCR-ASO	8
Farah Idali [55]	2012	Iran	40:46:20	94:6:0	30.1	NA	At least 3 pregnancy losses before 20th week of gestation	PCR–RFLP	6
Ahmad Poursadegh Zonouzi [56]	2012	Iran	35:46:8	13:34:3	30.17	31.54	First trimester recurrent spontaneous abortions	PCR–RFLP	6
Vajira H W Dissanayake [54]	2012	Sri Lanka	74:78:43	72:79:46	32.1	32.4	2 or more consecutive spontaneous abortions	PCR–RFLP	8
Farah Parveen [59]	2013	India	88:92:20	157:127:16	NA	NA	At least 3 spontaneous miscarriages	PCR–RFLP	7
Rohini R Nair [52]	2013	India	48:68:13	116:80:6	26.89	30.76	3 or more miscarriages before 12 weeks of gestation	PCR–RFLP	7
Yunlei Cao [64]	2014	China	49:31:2	132:31:3	28.43	28.1	At least 2 consecutive pregnancy losses before 12-week gestational age	PCR-DNA sequencing	9
Elham Yousefian [65]	2014	Iran	98:81:25	68:39:9	29.7	30.4	3 or more consecutive pregnancy losses before the 22nd week of pregnancy, regardless of a previous live birth	PCR-SSOP	9
Fabio L Lino [67]	2015	Brazil	71:32:9	52:43:3	30.3	40.2	3 or more idiopathic miscarriages early in pregnancy($\leq$12 weeks)	PCR-ASO	7
Li Luo [68]	2015	China	82:40:3	78:54:3	30.89	29.4	2 or more consecutive spontaneous abortions	PCR–RFLP	7
Wendell Vilas Boas [69]	2015	Brazil	57:27:5	80:62:8	29.4	23	At least 2 consecutive miscarriages in the first, second or third trimester of gestation, without any successful pregnancy	PCR–RFLP	8
L Zhu [71]	2015	China	48:58:12	76:88:10	29.8	28.5	Spontaneous abortions that occur $\geq$2 times in a row	TaqMan-qPCR	8
Somayeh-Sadat Tara [72]	2015	Iran	47:116:62	59:32:9	32.4	35.2	At least 3 successive pregnancy losses below 20th week of gestation	PCR–RFLP	6
Kamelia Farahmand [73]	2016	Iran	134:152:44	329:20:1	30.37	29.88	3 or more consecutive pregnancy losses before 20 weeks of gestation, with no history of full-term pregnancies	PCR–RFLP	8
J J López-Jiménez [75]	2016	México	42:13:1	37:13:0	30	30	3 consecutive pregnancy losses prior to the 20th week of gestation	PCR–RFLP	7
M Chatzidimitriou [78]	2017	Greece	36:6:6	19:8:0	35.5	35.1	RPLs	PCR-ASO	6
Kyu Ri Hwang [76]	2017	Korea	209:86:7	210:93:12	34.8	50.3	At least 2 unexplained consecutive spontaneous miscarriages before 20 weeks of gestation	TaqMan-qPCR	6
Hubert Wolski [79]	2017	Poland	152:163:44	179:172:49	30.99	30.05	$\geq$2 loss of pregnancy before 22 completed weeks of gestation	PCR–RFLP	7
Alptekin H [80]	2017	Turkey	26:65:15	38:17:7	27.9	29.4	2 or more lost pregnancies(early or late miscarriages or stillbirths) for no reason	PCR-DNA sequencing	7

Table 2 (continued)

Walid Al-Achkar [77]	2017	Syria	53:40:8	65:40:1	30	31	2 or more miscarriages and diagnosed as RPLs	PCR–RFLP	8
Razieh Bigdeli [82]	2018	Iran	142:48:10	171:28:1	23	25.1	At least 2 pregnancy loss	PCR–RFLP	8
Domenico Dell'Edera [83]	2018	Italy	294:54:32	320:35:32	NA	NA	At least 2 miscarriage	PCR-FRET	6
Yuanchang Zhu [87]	2018	China	243:114:13	83:56:5	NA	NA	2 or more clinical pregnancy failures	PCR-DNA sequencing	8
Hanadi El Achi [84]	2018	Lebanon	9:25:36	55:24:24	32.2	NA	At least 2 consecutive miscarriages	PCR-ASO	5
Yajuan Xu [89]	2019	China	155:58:5	214:44:6	31.82	31.16	2 or more spontaneous miscarriages with a diagnosis of recurrent pregnancy loss	TaqMan-qPCR	8
Najmeh Ahangari [90]	2019	Iran	83:129:33	179:57:14	32.16	31.81	2 or more repeated abortions	PCR-DNA sequencing	8
Zhong Lin [93]	2019	China	231:144:28	221:102:19	29.58	29.88	2 or more consecutive spontaneous abortions were diagnosed as RSA	TaqMan-qPCR	8
Ivana Joksic [94]	2020	Serbia	34:25:11	12:19:0	33.2	33.2	3 or more consecutive pregnancy losses	PCR-ASO	7
Irem Yengel [95]	2020	Turkey	56:64:25	43:47:15	NA	NA	At least 2 recurrent pregnancy losses before the 12th week of gestation	TaqMan-qPCR	6
Yan Zhang [96]	2020	China	127:89:21	381:207:30	27.85	27.01	Diagnosed with RPL in compliance with the American Society for Reproductive Medicine definitions of infertility and recurrent pregnancy loss	TaqMan-qPCR	7

Factor V HR2

Author	Year	Country	Genotype frequency(A/A:A/G:G/G)		Mean Age		Definition of RPL	Genotyping method	Quality
			Case	Control	Case	Control			
A Dilley [20]	2002	USA	53:7:0	79:13:0	36	33	3 or more fetal losses, regardless of trimester of loss or previous live birth, or any late loss	PCR–RFLP	8
Carolyn B Coulam [34]	2006	USA	147:2:1	19:1:0	34.7	39.6	2 or more consecutive abortions	PCR–RFLP	6
Alexandros Sotiriadis [37]	2007	Greece	78:10:0	65:23:2	32.2	32.2	2 or more consecutive miscarriages with the same partner in < 15 weeks gestation	PCR–RFLP	8
C Ciacci [43]	2009	Italy	37:2:0	65:7:0	NA	NA	At least 2 pregnancy losses within the first 3 months of pregnancy	PCR-ASO	6
Oztürk Ozdemir [51]	2012	Turkey	470:73:0	103:3:0	27.8	28.9	2 or more consecutive early RPL at 5–12 weeks of gestation	PCR-ASO	8
Raheleh Torabi [57]	2012	Iran	86:12:2	96:4:0	NA	NA	At least 2 recurrent pregnancy losses before the 20th week of gestation	PCR–RFLP	6
Vajira H W Dissanayake [54]	2012	Sri Lanka	186:9:1	177:11:1	32.1	32.4	2 or more consecutive spontaneous abortions	PCR–RFLP	8
Ahmad Poursadegh Zonouzi [56]	2013	Iran	85:4:0	48:2:0	30.18	31.54	At least 2 consecutive miscarriages	ARMS-PCR	6
Nadia Arabkhazaeli [98]	2016	Iran	95:5:0	91:9:0	NA	NA	2 or more spontaneous consecutive abortions at 5–20 weeks of gestation	PCR–RFLP	6
Mari Izuhara [99]	2017	Japan	74:13:1	84:10:1	33	38.5	3 or more consecutive miscarriages of unexplained cause during the first trimester	PCR-DNA sequencing	6
M Chatzidimitriou [78]	2017	Greece	42:6:0	23:4:0	35.5	35.1	2 or more consecutive fetal losses prior to 20 weeks of gestation	PCR-ASO	6
Razieh Bigdeli [82]	2018	Iran	186:12:2	196:4:0	23	25.1	At least 2 pregnancy loss	PCR–RFLP	8
Ivana Joksic [94]	2020	Serbia	47:23:0	22:9:0	33.2	33.2	3 or more consecutive pregnancy losses	PCR-ASO	7

Table 2 (continued)

Factor XIII V34L									
Author	Year	Country	Genotype frequency (V/V:V/L:L/L)		Mean age		Definition of RPL	Genotyping method	Quality
			Case	Control	Case	Control			
R Anwar [100]	1999	UK	20:15:0	29:13:0	NA	NA	3 or more recurrent miscarriages	PCR-SSCP	5
Astrid Dossenbach-Glaninger [101]	2003	Austria	24:21:4	31:16:1	35.6	36.6	2 consecutive or 3 to 6 nonconsecutive early pregnancy losses	PCR-ASO	8
Helena C L Barbosa [102]	2004	Brazil	53:50:3	55:27:4	30.4	NA	3 or more rms accompanied by vaginal elimination of a fetus weighing less than 0.5 kg, with or without vital signs, and/or a gestational age under 20 weeks	PCR–RFLP	8
Carolyn B Coulam [34]	2006	USA	82:57:11	8:11:1	34.7	39.6	2 or more consecutive abortions	PCR–RFLP	6
Ysabel López Ramírez [103]	2006	Venezuela	25:15:0	24:16:0	28.3	27.3	≥3 recurrent miscarriages of unknown causes	PCR–RFLP	7
C Ciacci [43]	2009	Italy	24:14:1	49:21:2	NA	NA	At least 2 pregnancy losses within the first 3 months of pregnancy	PCR-ASO	6
Mahmood Jeddi-Tehrani [49]	2010	Iran	71:25:4	83:15:2	NA	NA	At least 2 recurrent pregnancy losses before the 20th week of gestation	PCR–RFLP	6
Morteza Bagheri [104]	2011	Iran	35:19:0	34:12:0	NA	NA	2 or more consecutive fetal losses between the 8th and the 12th week of gestation without a known reason	PCR–RFLP	8
Ahmad Poursadegh Zonouzi [56]	2013	Iran	59:27:3	38:11:1	30.18	31.54	At least 2 consecutive miscarriages	ARMS-PCR	6
Iman Rifaat Elmahgoub [105]	2014	Egypt	81:26:13	116:11:3	28.5	29.1	Unexplained, recurrent first trimester miscarriage	PCR–RFLP	8
Fabio L Lino [67]	2015	Brazil	75:34:3	60:29:9	30.3	40.2	3 or more idiopathic miscarriages early in pregnancy(≤12 weeks)	PCR-ASO	7
M Chatzidimitriou [78]	2017	Greece	30:16:2	11:16:0	35.5	35.1	2 or more consecutive fetal losses prior to 20 weeks of gestation	PCR-ASO	6
Razieh Bigdeli [82]	2018	Iran	121:72:7	146:49:5	23	25.1	At least 2 pregnancy loss	PCR–RFLP	8
Hanadi El Achi [84]	2018	Lebanon	57:13:0	77:21:5	32.2	NA	At least 2 consecutive miscarriages	PCR-ASO	5
Ivana Joksic [94]	2020	Serbia	30:37:3	21:10:0	33.2	33.2	3 or more consecutive pregnancy losses	PCR-ASO	7
β-Fibrinogen-455G > A									
Author	Year	Country	Genotype frequency (G/G:G/A:A/A)		Mean age		Definition of RPL	Genotyping method	Quality
			Case	Control	Case	Control			
R Pihusch [19]	2001	Germany	61:33:8	69:48:11	35	32	2 or more unexplained consecutive abortions at 25 weeks of gestation	PCR–RFLP	6
Carolyn B Coulam [34]	2006	USA	121:26:3	15:5:0	34.7	39.6	2 or more consecutive abortions	PCR–RFLP	6
Cai XJ [106]	2008	China	23:7:0	21:7:2	NA	NA	2 or more consecutive spontaneous abortions	PCR–RFLP	6
C Ciacci [43]	2009	Italy	28:9:2	33:37:2	NA	NA	At least 2 pregnancy losses within the first 3 months of pregnancy	PCR-ASO	6
Gonca Imir Yenicesu [45]	2010	Turkey	167:88:17	28:28:0	27.2	29.5	2 or more consecutive early RPL at 5–12 weeks of gestation	PCR-ASO	7
Carlo Ticconi [107]	2011	Italy	58:32:8	50:28:0	35.5	36	≥2 consecutive miscarriages	PCR-DNA sequencing	8

Table 2 (continued)

Mahmood Jeddi-Tehrani [49]	2011	Iran	64:33:3	88:11:1	NA	NA	At least 2 successive pregnancy losses before 20th week of gestation	PCR–RFLP	6
Ahmad Poursadegh Zonouzi [56]	2013	Iran	45:38:6	24:24:2	30.18	31.54	At least 2 consecutive miscarriages	ARMS-PCR	6
Parisa Maziri [108]	2017	Iran	31:17:2	27:20:3	NA	NA	At least 2 consecutive miscarriages	PCR–RFLP	6
M Chatzidimitriou [78]	2017	Greece	36:10:2	15:9:3	35.5	35.1	2 or more consecutive fetal losses prior to 20 weeks of gestation	PCR-ASO	6
Razieh Bigdeli [82]	2018	Iran	131:59:10	163:36:1	23	25.1	At least 2 pregnancy loss	PCR–RFLP	8
Noha Mahmoud Issa [109]	2021	Egypt	47:28:5	43:31:6	30	31	At least 3 RPLs at ≤ 24 weeks of gestation	PCR–RFLP	8
PAI-1 4G/5G									
Author	Year	Country	Genotype frequency (5G/5G:5G/4G:4G/4G)		Mean age		Definition of RPL	Genotyping method	Quality
			Case	Control	Case	Control			
Cornelia E Wolf [110]	2003	Germany	7:25:17	20:50:32	31.9	33.1	At least 2 unexplained early abortions	Allele-specific PCR	6
T Buchholz [25]	2003	Germany	37:75:72	28:58:41	35	32.8	At least 2 unexplained consecutive spontaneous miscarriages before 25 weeks of gestation	PCR–RFLP	7
Astrid Dossenbach-Glaninger [101]	2003	Austria	9:28:12	15:25:8	35.6	36.6	2 consecutive or 3 to 6 nonconsecutive early pregnancy losses	PCR-ASO	8
Li-xue Guan [30]	2005	China	17:52:58	28:69:20	27	26	At least 3 spontaneous abortions	PCR–RFLP	7
Carolyn B Coulam [31]	2006	USA	22:117:11	5:13:2	34.7	39.6	2 or more consecutive abortions	PCR–RFLP	6
Chelsi Goodman [111]	2009	USA	25:57:38	13:48:23	34.7	NA	2 or more consecutive spontaneous abortions	PCR-DNA sequencing	6
C Ciacci [43]	2009	Italy	5:19:15	25:30:17	NA	NA	At least 2 pregnancy losses within the first 3 months of pregnancy	PCR-ASO	6
Gonca Imir Yenicesu [45]	2010	Turkey	28:185:59	12:44:0	27.2	29.5	2 or more consecutive early RPL at 5–12 weeks of gestation	PCR-ASO	7
Rami J Al Sallout [112]	2010	Palestine	40:44:16	36:48:16	28.9	NA	At least 3 unexplained consecutive spontaneous miscarriages before 25 weeks of gestation	allele-specific PCR	8
Mahmood Jeddi-Tehrani [113]	2011	Iran	60:31:9	72:27:1	NA	NA	At least 2 successive pregnancy losses before 20th week of gestation	PCR–RFLP	6
Mahmoud Aarabi [114]	2011	Iran	21:23:10	31:66:2	32.5	32.9	At least 3 unexplained consecutive spontaneous abortions before 25 weeks of gestation	PCR–RFLP	7
Farah Idali [55]	2012	Iran	35:54:17	72:27:1	30.1	NA	At least 3 pregnancy losses before 20th week of gestation	PCR–RFLP	6
Oztürk Ozdemir [51]	2012	Turkey	91:331:121	34:62:10	27.8	28.9	2 or more consecutive early RPL at 5–12 weeks of gestation	PCR-ASO	8
Ivan Subrt [115]	2013	Czech	23:75:59	10:54:10	NA	NA	2 or more consecutive spontaneous abortions	PCR–RFLP	6
Kalthoum Magdoud [116]	2013	Tunisia	139:128:37	257:104:10	32.4	31.9	3 or more pregnancy losses of unknown etiology with the same partner	PCR-SSCP	8
Farah Parveen [59]	2013	India	55:100:45	95:131:74	NA	NA	At least 3 spontaneous miscarriages	PCR–RFLP	7
Young Joo Jeon [117]	2013	Korea	47:132:129	39:117:71	32.94	33.2	At least 2 consecutive pregnancy losses before 20 weeks of gestation	PCR–RFLP	8
Ahmad Poursadegh Zonouzi [56]	2013	Iran	26:49:14	15:28:7	30.18	31.54	At least 2 consecutive miscarriages	ARMS-PCR	6

Table 2 (continued)

Jin Ju Kim [118]	2014	Korea	31:123:73	48:154:102	36	50.3	At least 2 unexplained consecutive spontaneous miscarriages before 20 weeks of gestation	TaqMan-qPCR	6
Iman Rifaat Elmahgoub [105]	2014	Egypt	75:37:8	99:28:3	28.5	29.1	Unexplained, recurrent first trimester miscarriage	PCR–RFLP	8
Farhad Khosravi [119]	2014	Iran	128:208:85	72:27:1	29.5	33	At least 2 recurrent miscarriage	PCR–RFLP	6
A Pietropolli [66]	2014	Italy	57:74:55	58:29:42	35.2	40.4	2 or more consecutive spontaneous miscarriages before the 20th week of gestation	PCR-FRET	8
Fatemeh Shakarami [120]	2015	Iran	33:50:17	45:50:5	NA	NA	At least 2 spontaneous abortions	PCR–RFLP	6
Fabio L Lino [67]	2015	Brazil	37:57:12	42:40:16	30.3	40.2	3 or more idiopathic miscarriages early in pregnancy($\leq$12 weeks)	PCR-DNA sequencing	7
Magdalena Barlik [121]	2016	Poland	27:75:50	32:85:63	30.16	29.46	$\geq$2 consecutive loss of pregnancy before 22 completed weeks of gestation	PCR–RFLP	7
Maria D Salazar Garcia [122]	2016	USA	28:53:32	28:50:14	NA	NA	$\geq$2 spontaneous abortions	allele-specific PCR	cohort study
Grażyna Kurzawińska	2016	Poland	27:75:50	32:85:63	30.16	29.46	At least 2 consecutive pregnancy losses in the first and second trimester	PCR–RFLP	7
J J López-Jiménez [75]	2016	México	21:25:10	25:18:7	30	30	3 consecutive pregnancy losses prior to the 20th week of gestation	PCR–RFLP	7
Alptekin H [80]	2017	Turkey	21:57:28	30:21:11	27.9	29.4	2 or more lost pregnancies(early or late miscarriages or stillbirths) for no reason	allele-specific PCR	7
M Chatzidimitriou [78]	2017	Greece	2:26:20	6:20:1	35.5	35.1	2 or more consecutive fetal losses prior to 20 weeks of gestation	PCR-ASO	6
Razieh Bigdeli [82]	2018	Iran	70:112:18	150:43:7	23	25.1	At least 2 pregnancy loss	PCR–RFLP	8
Amela Jusić [81]	2018	Bosnian	31:22:7	50:28:2	33.05	34.08	2 or more consecutive miscarriages before 20 weeks of gestation	PCR–RFLP	7
E A Trifonova [88]	2019	Russia	38:139:76	58:173:108	29.5	27.3	At least 2 pregnancy losses up to 20 weeks	PCR-ASO	8
Dao Anh Thi Le [123]	2022	Viet Nam	16:14:13	7:12:11	30.1	28.6	At least 2 unexplained RPL before 22 weeks of gestation	ARMS-PCR	8
ACE (intron 16 I/D)									
Author	Year	Country	Genotype frequency(I/I:I/D:D/D)		Mean age		Definition of RPL	Genotyping method	Quality
			Case	Control	Case	Control			
C Fatini [124]	2000	Italy	10:21:28	20:30:20	31	32.5	3 or more first-trimester (7 $\pm$ 12 weeks of gestation) fetal losses	PCR	8
T Buchholz [25]	2003	Germany	42:83:59	26:71:30	35	32.8	At least 2 unexplained consecutive spontaneous miscarriages before 25 weeks of gestation	PCR-AFLP	7
Venkatesan Vettriselvi [38]	2008	India	42:39:23	55:38:27	NA	NA	2 or more spontaneous consecutive miscarriages less than 20 weeks of gestation	PCR	6
Chelsi Goodman [111]	2009	USA	31:55:34	22:34:28	34.7	NA	2 or more consecutive spontaneous abortions	PCR	6
Morteza Bagheri [125]	2010	Iran	7:26:17	12:27:24	28.27	29.58	At least 3 pregnancy losses with unknown etiology before 20 weeks gestational age	PCR	8

Table 2 (continued)

Rami J Al Sallout [112]	2010	Palestine	9:42:49	12:34:54	28.9	NA	At least 3 unexplained consecutive spontaneous miscarriages before 25 weeks of gestation	PCR	8
Yi Seul Choi [126]	2011	Korea	77:130:44	35:50:41	31.97	31.22	At least 3 consecutive spontaneous abortions	PCR	6
Mahmoud Aarabi [114]	2011	Iran	14:30:19	22:47:25	32.5	32.9	At least 3 unexplained consecutive spontaneous abortions before 25 weeks of gestation	PCR	7
Shufang Zhang [127]	2011	China	57:49:21	90:34:8	30.1	28.2	At least 2 consecutive spontaneous abortions in early pregnancy	PCR	7
Oztürk Ozdemir [51]	2012	Turkey	71:260:212	33:54:19	27.8	28.9	2 or more consecutive early RPL at 5–12 weeks of gestation	PCR-ASO	8
Ahmad Poursadegh Zonouzi [56]	2013	Iran	23:31:35	7:28:15	30.18	31.54	At least 2 consecutive miscarriages	ARMS-PCR	6
Jin Ju Kim [118]	2014	Korea	83:110:34	104:148:52	36	50.3	At least 2 unexplained consecutive spontaneous miscarriages before 20 weeks of gestation	PCR	6
Fatemeh Shakarami [120]	2015	Iran	6:60:34	0:48:52	NA	NA	At least 2 spontaneous abortions	PCR–RFLP	6
Grażyna Kurzawińska [128]	2016	Poland	32:80:40	44:84:52	30.16	29.46	At least 2 consecutive pregnancy losses in the first and second trimester	PCR–RFLP	7
Shokoufeh Fazelnia [129]	2016	Iran	23:33:44	31:40:29	NA	NA	2 or more spontaneous consecutive abortions at 5–20 weeks of gestation	PCR	6
J J López-Jiménez [75]	2016	México	11:34:10	21:19:10	30	30	3 consecutive pregnancy losses prior to the 20th week of gestation	PCR	7
Fatimah Basil Al-Mukaynizi [130]	2016	Saudi Arabia	3:18:40	2:25:32	34.1	34.6	3 or more consecutive pregnancy losses before the 20th week of gestation	PCR	8
Nina Pereza [131]	2016	Croatia	31:75:43	32:62:55	NA	NA	≥3 consecutive spontaneous abortions of unknown etiology before the 22nd week of gestation	allele-specific PCR	6
Aisha Mahmood Fageer Hussian [132]	2016	Sudan	3:14:23	0:3:37	32.3	30.9	3 or more consequent abortions with no apparent cause	PCR	6
M Chatzidimitriou [78]	2017	Greece	4:23:21	9:10:8	35.5	35.1	2 or more consecutive fetal losses prior to 20 weeks of gestation	PCR-ASO	6
Parisa Maziri [108]	2017	Iran	1:13:36	2:22:26	NA	NA	At least 2 consecutive miscarriages	PCR	6
Evren Gumus [133]	2018	Turkey	180:477:350	46:75:48	25.88	26.41	≥2 consecutive pregnancy losses	PCR	8
Hanadi El Achi [84]	2018	Lebanon	17:27:26	6:30:27	32.2	NA	At least 2 consecutive miscarriages	PCR-ASO	5
Mohammad Mehdi Heidari [134]	2019	Iran	49:102:51	41:99:70	27.32	29.68	3–9 miscarriages with fetal loss	ARMS-PCR	6
E A Trifonova [88]	2019	Russia	63:129:61	85:176:78	29.5	27.3	At least 2 pregnancy losses up to 20 weeks	PCR	8
Noha Mahmoud Issa [109]	2021	Egypt	11:31:38	18:37:25	30	31	At least 3 RPLs at ≤24 weeks of gestation	PCR–RFLP	8

Table 3 Results of meta-analyses

Genotype	Genetic model	Pooled ORs (95%CI)	*P*	I^2	*P*
MTHFR C677T	Dominant	1.43 (1.25, 1.64)	< 0.01	79%	< 0.01
	Recessive	1.60 (1.36, 1.87)	< 0.01	60%	< 0.01
MTHFR A1298C	Dominant	1.66 (1.26, 2.18)	< 0.01	90%	< 0.01
	Recessive	1.79 (1.42, 2.26)	< 0.01	56%	< 0.01
ACE I/D	Dominant	1.23 (1.00, 1.53)	0.05	62%	< 0.01
	Recessive	1.09 (0.87, 1.36)	0.44	71%	< 0.01
Factor VIII V34L	Dominant	1.38 (1.02, 1.87)	< 0.05	59%	< 0.01
	Recessive	1.28 (0.81, 2.01)	0.28	28%	0.17
Factor V R2	Dominant	1.12 (0.68, 1.83)	0.65	59%	< 0.01
	Recessive	NA			
PAI-1 4G/5G	Dominant	1.67 (1.36, 2.06)	< 0.01	76%	< 0.01
	Recessive	1.80 (1.39, 2.32)	< 0.01	71%	< 0.01
β-Fibrinogen-455G/A	Dominant	0.92 (0.62, 1.37)	0.69	74%	< 0.01
	Recessive	1.60 (1.02, 2.51)	< 0.05	22%	0.23

NA not available

The pooled ORs and 95% CI showed great significance for the relationship between PAI-1 mutation and RPL (OR: 1.67; 95% CI: 1.36–2.06; $P < 0.01$) (Supplementary Fig. 1). Under the recessive model (5G5G + 4G5G vs. 4G4G), a high degree of among-study heterogeneity was observed ($I^2 = 71\%$, $P < 0.01$). The pooled ORs and 95% CI also showed great statistical significance (OR: 1.80; 95% CI: 1.39–2.32; $P < 0.01$) (Supplementary Fig. 2). As for the sensitivity analysis, the risk estimate did not materially change for both the dominant genetic model and the recessive genetic model. The examination for publication bias via the Egger's line regression test showed no significant bias for the dominant model ($t = -0.98$, $P = 0.33$), but a significant bias for the recessive model ($t = 4.54$, $P < 0.01$). After adding 10 studies via trim and fill method, the pooled ORs and 95% CI were inconsistent with the original result (OR: 1.27; 95% CI: 0.90–1.80; $P = 0.17$), suggesting the publication bias for the recessive model of this polymorphism had a significant impact on the result. Subgroup analyses showed that ethnicity and gestational age at pregnancy loss under the dominant model were associated with among-study heterogeneity ($P < 0.05$), while the number of abortions was not.

ACE polymorphisms

Twenty-six studies consisted of 4284 patients and 2992 controls for meta-analysis. Under the dominant model (ID + DD vs. II), the among-study heterogeneity was significant ($I^2 = 62\%$, $P < 0.01$). The pooled ORs and 95% CI showed a weak correlation between this polymorphism and RPL (OR: 1.23; 95% CI: 1.00–1.53; $P = 0.05$) (Supplementary Fig. 3). Under the recessive model (II + ID vs. DD), a high degree of among-study heterogeneity was observed ($I^2 = 71\%$, $P < 0.01$). The pooled ORs and 95% CI showed no statistical significance (OR: 1.09; 95% CI: 0.87–1.36; $P = 0.44$) (Supplementary Fig. 4). As for the sensitivity analysis, the risk estimate did not materially change for the recessive models, but for the dominant model, the result was unstable (Supplementary Figure X). The examination for publication bias via the Egger's line regression test did not show a significant bias for both the two models (dominant model: $t = -0.47$, $P = 0.65$; recessive model: $t = 0.48$, $P = 0.63$). Subgroup analyses showed that gestational age at pregnancy loss under the dominant model was associated with among-study heterogeneity ($P < 0.05$), while the number of pregnancy loss and ethnicity was not.

Factor V polymorphisms

Thirteen studies consisted of 1775 patients and 1183 controls for analysis. Under the dominant model (AG + GG vs. AA), a high degree of among-study heterogeneity was observed ($I^2 = 59\%$, $P < 0.05$). The pooled ORs and 95% CI showed no significant relationship between Factor V R2 polymorphisms and RPL (OR: 1.12; 95% CI: 0.68–1.83; $P = 0.65$) (Supplementary Fig. 5). Owing to very few cases and controls with homozygotic mutation, the recessive model is not suitable for this polymorphism. As for the sensitivity analysis, the risk estimate did not materially change. The examination for bias via the Egger's line regression test did not show a significant bias ($t = 0.36$, $P = 0.73$). Subgroup analyses showed that none of the variables including the gestational age at pregnancy loss, the number of abortions, and ethnicity

Table 4 Results of subgroup analyses

MTHFR C677T					
Subgroup	Genetic model	No. of study	OR(95% CI)	Test for subgroup differences	
				χ^2	*P*
Ethnicity	Dominant			0.99	0.32
Caucasian		67	1.47(1.25, 1.74)		
Non-Caucasian		18	1.28(1.03, 1.59)		
	Recessive			0.33	0.57
Caucasian		67	1.65(1.37, 1.98)		
Non-Caucasian		18	1.47(1.06, 2.05)		
Number of PL	Dominant			0.06	0.8
≥ 2		51	1.46(1.24, 1.73)		
≥ 3		28	1.52(1.18, 1.96)		
	Recessive			0.82	0.37
≥ 2		51	1.54(1.25, 1.90)		
≥ 3		28	1.79(1.39, 2.32)		
Gestational age at PL	Dominant			0.02	0.89
Early pregnancy loss		14	1.39(0.99, 1.95)		
Late pregnancy loss		31	1.43(1.16, 1.67)		
	Recessive			0.12	0.73
Early pregnancy loss		14	1.38(0.98, 1.95)		
Late pregnancy loss		31	1.49(1.17, 1.89)		
MTHFR A1298C					
Subgroup	Genetic model	No. of study	OR(95%CI)	Test for subgroup differences	
				χ^2	*P*
Ethnicity	Dominant			5.09	0.02
Caucasian		30	1.88(1.32, 2.69)		
Non-Caucasian		10	1.15(0.91, 1.46)		
	Recessive			4.13	0.04
Caucasian		30	2.14(1.55, 2.94)		
Non-Caucasian		10	1.36(1.01, 1.82)		
Number of PL	Dominant			1.09	0.3
≥ 2		26	1.51(1.15, 1.98)		
≥ 3		12	2.23(1.12, 4.42)		
	Recessive			3.96	0.05
≥ 2		26	1.48(1.15, 1.90)		
≥ 3		12	2.72(1.58, 4.69)		
Gestational age at PL	Dominant			0.81	0.37
Early pregnancy loss		7	1.35(0.78, 2.33)		
Late pregnancy loss		15	1.96(1.07, 3.60)		
	Recessive			0.08	0.78
Early pregnancy loss		7	1.97(1.29, 3.01)		
Late pregnancy loss		15	2.10(1.20, 3.69)		
ACE I/D					
Subgroup	Genetic model	No. of study	OR(95%CI)	Test for subgroup differences	
				χ^2	*P*
Ethnicity	Dominant			0.07	0.79
Caucasian		22	1.25(0.99, 1.57)		
Non-Caucasian		4	1.13(0.56, 2.29)		
	Recessive			0.87	0.35
Caucasian		22	1.17(0.95, 1.43)		
Non-Caucasian		4	0.63(0.18, 2.24)		

Table 4 (continued)

Number of PL	Dominant			0.15	0.7
≥ 2		16	1.25(0.93, 1.69)		
≥ 3		10	1.15(0.86, 1.55)		
	Recessive			1.76	0.19
≥ 2		16	1.23(0.96, 1.58)		
≥ 3		10	0.88(0.57, 1.35)		
Gestational age at PL	Dominant			4.11	0.04
Early pregnancy loss		2	2.40(1.24, 4.65)		
Late pregnancy loss		14	1.19(1.00, 1.41)		
	Recessive			0.26	0.61
Early pregnancy loss		2	1.62(0.48, 5.53)		
Late pregnancy loss		14	1.17(0.95, 1.43)		
PAI-1 4G/5G					
Subgroup	Genetic model	No. of study	OR(95%CI)	Test for subgroup differences	
				χ^2	*P*
Ethnicity	Dominant			3.89	0.05
Caucasian		30	1.76(1.41, 2.19)		
Non-Caucasian		4	1.22(0.91, 1.63)		
	Recessive			0.32	0.57
Caucasian		30	1.87(1.41, 2.48)		
Non-Caucasian		4	1.50(0.74, 3.05)		
Number of PL	Dominant			0.01	0.94
≥ 2		25	1.67(1.31, 2.14)		
≥ 3		8	1.64(1.06, 2.55)		
	Recessive			0.81	0.37
≥ 2		25	1.60(1.25, 2.04)		
≥ 3		8	2.31(1.07, 4.97)		
Gestational age at PL	Dominant			4.98	0.03
Early pregnancy loss		5	2.05(1.56, 2.68)		
Late pregnancy loss		15	1.33(1.02, 1.73)		
	Recessive			1.69	0.19
Early pregnancy loss		5	2.12(0.96, 4.68)		
Late pregnancy loss		15	1.23(0.98, 1.54)		
Factor VIII V34L					
Subgroup	Genetic model	No. of study	OR(95%CI)	Test for subgroup differences	
				χ^2	*P*
Number of PL	Dominant			0.12	0.73
≥ 2		10	1,31(0.92, 1.86)		
≥ 3		4	1.19(0.75, 1.88)		
	Recessive			5.14	0.02
≥ 2		10	1.55(0.79, 3.05)		
≥ 3		4	0.38(0.14, 1.05)		
Gestational age at PL	Dominant			0.35	0.55
Early pregnancy loss		4	1.58(0.77, 3.21)		
Late pregnancy loss		2	1.35(0.73, 2.50)		
	Recessive			0.32	0.57
Early pregnancy loss		4	1.12(0.17, 7.27)		
Late pregnancy loss		2	2.23(0.50, 10.01)		
Factor V R2					
Subgroup	Genetic model	No. of study	OR(95%CI)	Test for subgroup differences	
				χ^2	*P*

Table 4 (continued)

Ethnicity	Dominant			0.3	0.58
Caucasian		12	1.09(0.63, 1.88)		
Non-Caucasian		1	1.44(0.62, 3.38_		
Number of PL	Dominant			0	1
≥2		11	1.13(0.62, 2.05)		
≥3		2	1.12(0.59, 2.14)		
Gestational age at PL	Dominant			0.73	0.39
Early pregnancy loss		3	1.70(0.50, 5.80)		
Late pregnancy loss		4	0.84(0.28, 2.45)		
β-Fibrinogen-455G/A					
Subgroup	Genetic model	No. of study	OR(95%CI)	Test for subgroup differences	
				χ^2	*P*
Ethnicity	Dominant			0.19	0.66
Caucasian		11	0.93(0.61, 1.43)		
Non-Caucasian		1	0.71(0.22, 2.25)		
	Recessive			1.56	0.21
Caucasian		11	1.37(0.79, 2.39)		
Non-Caucasian		1	0.19(0.01,4.06)		
Number of PL	Dominant			0.26	0.61
≥2		10	0.94(0.58, 1.53)		
≥3		2	0.80(0.53, 1.19)		
	Recessive			1.5	0.22
≥2		10	1.75(0.77, 3.96)		
≥3		2	0.87(0.41, 1.85)		
Gestational age at PL	Dominant			1.3	0.25
Early pregnancy loss		2	0.49(0.27, 0.90)		
Late pregnancy loss		3	1.15(0.31, 4.30)		
	Recessive			1.84	0.18
Early pregnancy loss		2	3.03(0.59, 15.49)		
Late pregnancy loss		3	0.82(0.32, 2.10)		

PL, pregnancy loss

were associated with among-study heterogeneity for this polymorphism.

Factor XIII polymorphisms

Fifteen studies consisted of 1262 patients and 1093 controls for meta-analysis. Under the dominant model (GT + TT vs. GG), a high degree of among-study heterogeneity was observed ($I^2 = 59\%$, $P < 0.05$). The pooled ORs and 95% CI showed significance for the relationship between this polymorphism and RPL (OR: 1.38; 95% CI: 1.02–1.87; $P < 0.05$) (Supplementary Fig. 6). Under the recessive model (GG + GT vs. TT), the statistical heterogeneity was not significant ($I^2 = 28\%$, $P = 0.17$), so a fixed-effect model was chosen. The pooled ORs and 95% CI showed no statistical significance (OR: 1.28; 95% CI: 0.81–2.01; $P = 0.28$) (Supplementary Fig. 7). As for the sensitivity analysis, the risk estimate did not materially change for the recessive models, but for the dominant model, the result was unstable (Supplementary Fig. 11). The examination for publication bias via the Egger's line regression test did not show a significant bias for both models (dominant model: $t = -0.98$, $P = 0.34$; recessive model: $t = 0.06$, $P = 0.95$). Subgroup analyses showed that the number of abortions was associated with among-study heterogeneity under the recessive model ($P < 0.05$), while the gestational age at pregnancy loss was not. We did not analyze the effect of ethnicity because populations in all included studies were Caucasian.

β-Fibrinogen polymorphisms

Twelve studies consisted of 1258 patients and 891 controls for meta-analysis. Under the dominant model (GA + AA vs. GG), a high degree of among-study heterogeneity was observed ($I^2 = 74\%$, $P < 0.01$). The pooled ORs and 95% CI showed no significance (OR: 0.92; 95%

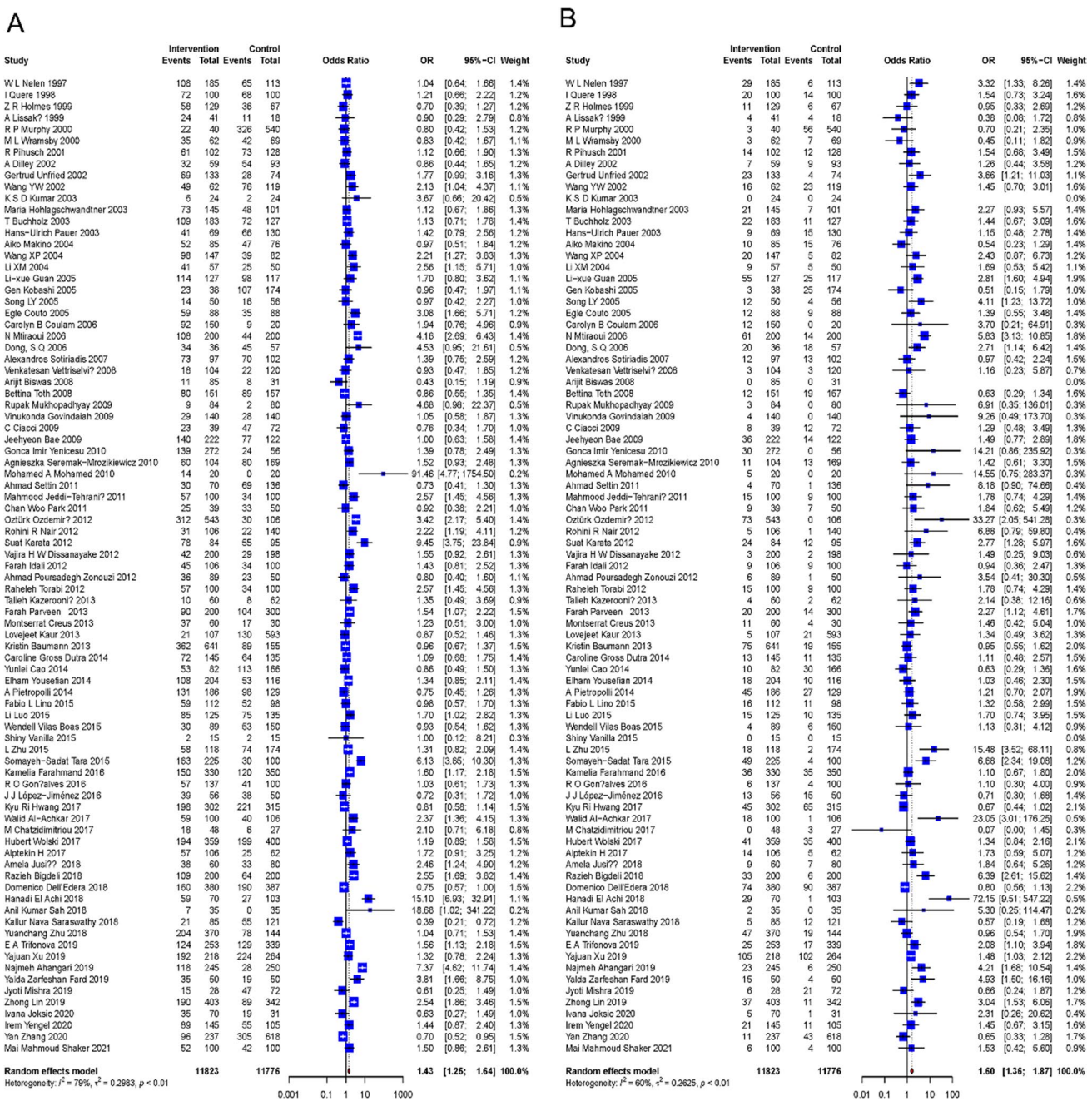

Fig. 2 Forest plot of the association between risk of RPL and MTHFR C677T polymorphism. **A** Forest plot under a dominant model; **B** forest plot under a recessive model. RPL, recurrent pregnancy loss

CI: 0.62–1.37; $P = 0.69$) (Supplementary Fig. 8). Under the recessive model (GG + GA vs. AA), the statistical heterogeneity was not significant ($I^2 = 22\%$, $P = 0.23$), so a fixed-effect model was chosen. The pooled ORs and 95% CI showed statistical significance (OR: 1.60; 95% CI: 1.02–2.51; $P < 0.05$) (Supplementary Fig. 9). As for the sensitivity analysis, the risk estimate did not materially change for the dominant models, but for the recessive model, the result was unstable (Supplementary Fig. 12). The examination for publication bias via the Egger's line regression test did not show a significant bias for both two models (dominant model: $t = -1.49$, $P = 0.17$; recessive model: $t = 1.29$, $P = 0.23$). Subgroup analyses showed that none of the variables mentioned before were associated with among-study heterogeneity.

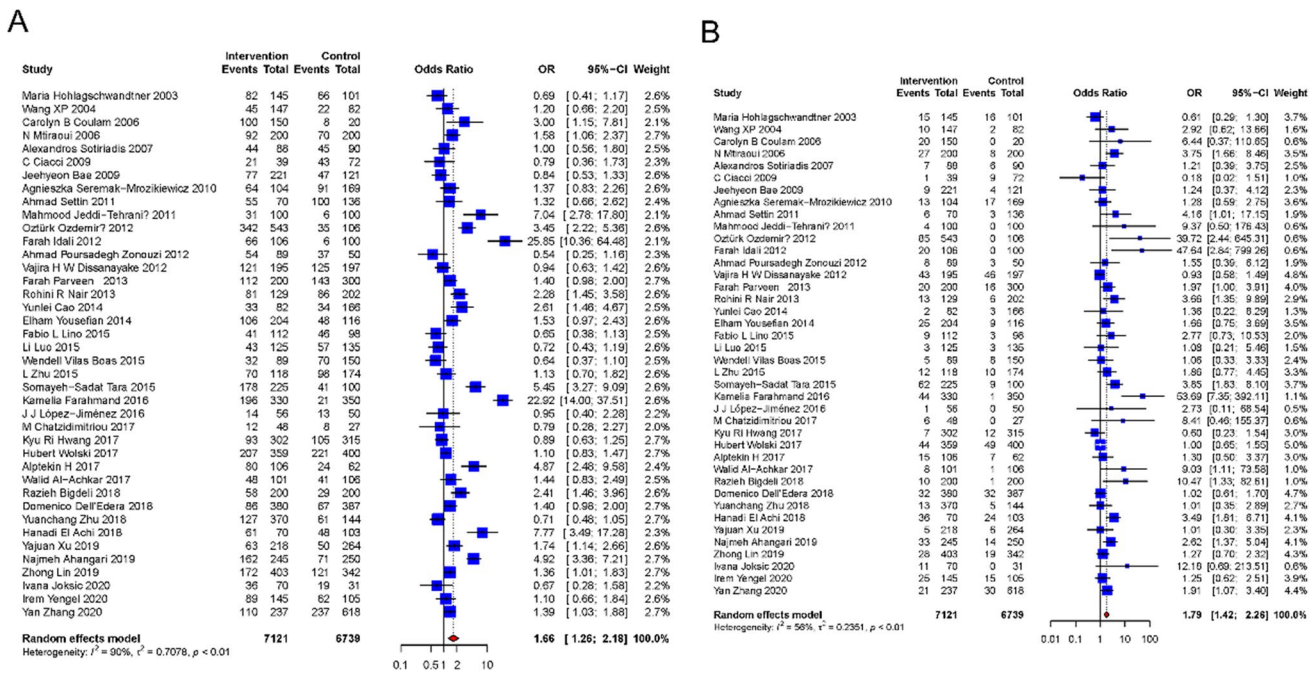

Fig. 3 Forest plot of the association between risk of RPL and MTHFR A1298C polymorphism. **A** Forest plot under a dominant model; **B** forest plot under a recessive model. RPL, recurrent pregnancy loss

Discussion

In this meta-analysis of thrombophilic gene polymorphisms and RPL, 7 polymorphisms in 6 genes related to inherited thrombophilias were involved including MTHFR C677T, MTHFR A1298C, PAI-1 4G/5G, ACE I/D, Factor V R2, Factor XIII V34L, and β-fibrinogen-455G/A. The result of the analysis has shown that MTHFR C677T, MTHFR A1298C, and PAI-1 4G/5G mutations increase the risk of RPL both in the dominant genetic model and in the recessive model. ACE I/D and Factor XIII V34L are positively associated with RPL only in the dominant model, and β-fibrinogen-455G/A is positively associated with RPL only in the recessive model. This may be related to the specific genetic characteristics and their different effects on the disease phenotype. Besides, the evidence in this current study could not support any association between Factor V R2 polymorphisms and RPL significantly.

In light of previous meta-analyses, some studies which analyzed the relation between MTHFR C677T and/or A1298C and RPL were consistent with our findings [135–138]. But no significant association between MTHFR A1298C polymorphism and unexplained RPL was found in several studies [139–141], which was probably due to the relatively small sample size or the selection of literature. There were 3 studies performing meta-analyses about the PAI-1 4G/5G and RPL before, but the findings conflicted possibly due to the limited included studies [142–144]. As for the ACE I/D polymorphism, all had the same findings regardless of the number of included studies, similar to ours [144–146]. Jung et al. explored the association of Factor XIII V34L and RPL, and also found the Val34Leu polymorphism can act as a prognostic factor of RPL [147]. Based on these previous studies, we made a more comprehensive and the most up-to-date analysis. But for the other two polymorphisms, Factor V R2, and β-fibrinogen-455G/A, it is the first time to integrate published data to perform a meta-analysis.

There are two strengths in the present meta-analysis. Our literature search strategy, using MeSH searching in PubMed and Embase, ensured all the related articles were included to the greatest extent under strict inclusion and exclusion criteria, resulting in a relatively large quantity of literature. The integration of sample sizes increased the statistical power, allowing for more accurate risk assessments. On the other hand, the statistical work was done by two reviewers independently, minimizing the omissions and contrived errors to an extreme.

Remarkably, the inclusion criteria of the RPL group were inconsistent in the included studies, including the number of abortions and the gestational age at pregnancy loss. We included all related studies regardless of their definition of RPL. But according to the results of subgroup analyses, we found that the differences in the risk for RPL were associated with both the number of

abortions and the gestational age at pregnancy loss, suggesting that the definition of RPL may have a substantial impact on the relationship between thrombophilic gene polymorphisms and RPL, which might be one of the potential sources of among-study heterogeneity. Furthermore, by performing the subgroup analyses according to ethnicity, most of the results showed different risks of RPL for patients with thrombophilic gene mutations in different ethnicities. The inconsistency of results suggests that these thrombophilic gene polymorphisms may have different functional influences on the etiology of RPL, possibly owing to the varied distribution of genotypes among ethnicities. Notably, non-Caucasian people made up a relatively smaller proportion and may not be powered to address ethnic differences, and more studies are necessary to conduct in non-Caucasian regions to compensate for this limitation. Altogether, when clinicians assess the risk of RPL for patients, they should pay attention to these variables.

From the results of the heterogeneity analysis, different degrees of heterogeneity existed in most of the polymorphisms, and many were at medium or relatively high degrees. Given this limitation, subgroup analyses were performed to explore the sources of heterogeneity, and we found that ethnicity and the criteria for the case group, according to the definition of RPL, might be potential sources. Besides, many of the included studies ignored the comparability between case group and control group, namely the control of confounding factors, such as maternal age, BMI, and smoking. All of these factors may contribute to heterogeneity, particularly the maternal age, which has a great impact on fertility. However, the lack of sufficient information hindered further exploration of these factors. In addition, using the random-effect model could reduce the effect of among-study heterogeneity in our results.

Another limitation is publication bias, which is an inevitable issue for all meta-analyses. Studies with positive results are more likely to be published, thereby omitting those unpublished researches with negative results, exacerbating the effect of genetic polymorphisms. In the present meta-analysis, no publication bias was found by Egger's line regression test in most polymorphisms with $P > 0.05$. However, for PAI-1 4G/5G under the recessive model, conclusions should be adopted with caution considering the significant publication bias.

Of note, sensitivity analyses of some polymorphisms showed unstable results after excluding each included study and repeating the meta-analysis, including ACE I/D polymorphisms under the dominant genetic model, Factor XIII V34L polymorphisms under the dominant genetic model, and β-fibrinogen-455G/A polymorphisms under the recessive genetic model. The instability of the findings is possibly owing to the limited number of included studies, substantial between-study heterogeneity, or some other important potential bias factors, which need to be verified by further studies.

Our meta-analysis provided evidence that thrombophilic gene polymorphisms were positively associated with RPL and improved risk prediction influencing diagnosis and treatments for clinicians. For those patients with a family history of inherited thrombophilia or suffered unexplained RPL, acquiring more insight into genetic risk factors is important, which may allow for targeted treatment. However, inherited thrombophilia testing is still a double-edged sword [148]. Firstly, the testing has some limitations itself. For instance, it is costly and has risks of both false-positive and false-negative results, increasing the psychological and economic burden on patients [148]. Secondly, there was still no solid evidence currently to support the effectiveness of therapeutic options to prevent pregnancy complications in patients with inherited thrombophilia. Several case–control studies explored the effect of anticoagulant therapies using aspirin, heparins, or low molecular weight heparins; however, results remain discrepant [149]. Some large randomized clinical trials are required to provide higher-level evidence for this question. Overall, whether inherited thrombophilia testing is used for risk prediction of pregnancy complications or not requires clinicians to take all other factors into account.

In conclusion, the present meta-analysis showed significant associations between the increased risk of RPL and thrombophilic gene polymorphisms, especially MTHFR C677T, MTHFR A1298C, PAI-1 4G/5G, ACE I/D, Factor XIII V34L, and β-fibrinogen-455G/A, which may be useful clinical markers to evaluate the risk of RPL or to help unexplained RPL patients identify possible causes, allowing for targeted treatment during pregnancy if necessary.

Supplementary information The online version contains supplementary material available at https://doi.org/10.1007/s10815-023-02823-x.

Author contribution All authors contributed to the study conception and design. Literature review, data collection, and analysis were performed by Yuanjia Wen and Haodong He. The first draft of the manuscript was written by Yuanjia Wen. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data availability All the materials and data are available.

Declarations

Ethics approval No ethical approval is required.

Consent No consent is required.

Competing interests The authors declare no competing interests.

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