



A meta-analysis of the association of the ACE I/D and PAI-1 4G/5G polymorphisms with recurrent pregnancy loss in Iranian women: Are the investigations adequate?

İranlı kadınlarda tekrarlayan gebelik kaybı ile ACE I/D ve PAI-1 4G/5G polimorfizmlerinin ilişkisine yönelik bir meta-analiz: Araştırmalar yeterli mi?

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Abstract

The associations of ACE I/D and PAI-1 4G/5G polymorphisms with recurrent pregnancy loss (RPL) in Iranian women have yielded controversial results. Thus, we conducted a meta-analysis to obtain more certain results. A comprehensive literature search was performed in the PubMed, Web of Sciences, Scopus, MedRxiv, SID, and CNKI databases up to January 1st, 2021, using the appropriate terms. All case-control studies were included. Odds ratios (ORs) and 95% confidence intervals (CIs) were used to estimate the strength of associations. A total of 14 studies including eight studies with 783 patients and 761 healthy subjects on ACE I/D and six studies with 1.155 patients and 699 healthy subjects on PAI-1 4G/5G were included. Combined data revealed that ACE I/D polymorphism was significantly associated with RPL risk in Iranian women under three models i.e., allele [OR=0.744, 95% CI: (0.640-0.864); p≤0.001], dominant [OR=0.774, 95% CI: (0.601-0.996); p=0.047], and recessive [OR=0.767, 95% CI: (0.611-0.963); p=0.022]. Moreover, the pooled data showed a significant association between the PAI-1 4G/5G polymorphism and RPL risk under all five models i.e., allele [OR=2.352, 95% CI: (1.623-3.408); p≤0.001], heterozygote [OR=8.364, 95% CI: (4.744-14.756); p≤0.001], homozygote [OR=2.192, 95% CI: (1.093-4.394); p=0.027], dominant [OR=2.354, 95% CI: (1.309-4.235); p=0.004], and recessive [OR=5.208, 95% CI: (3.005-9.025); p≤0.001]. Stratification analysis revealed that these polymorphisms were associated with RPL risk by the number of miscarriages. Our pooled data indicated that ACE I/D and PAI-1 4G/5G polymorphisms were significantly associated with an increased risk of RPL in Iranian women. These significant findings showed that the investigation might be adequate for ACE I/D and PAI-1 4G/5G polymorphisms in the Iranian population.

Keywords: Pregnancy loss, miscarriage, thrombophilia, plasminogen activator inhibitor-1, angiotensin-I-converting enzyme, polymorphism

Öz

İranlı kadınlarda ACE I/D ve PAI-1 4G/5G polimorfizmlerinin tekrarlayan gebelik kaybı (TGK) ile ilişkisi tartışmalı sonuçlar vermiştir. Bu yüzden, daha güvenilir sonuçlar almak için bir meta-analiz gerçekleştirildi. PubMed, Web of Sciences, Scopus, MedRxiv, SID ve CNKI veritabanlarında uygun terimler kullanılarak 01 Ocak 2021 tarihine kadar kapsamlı bir literatür taraması gerçekleştirildi. Tüm olgu kontrol çalışmalarını dahil edildi. İlişkilerin gücünü tahmin etmek için olasılık oranları (OO'lar) ve %95 güven aralıkları (GA) kullanıldı. Meta-analize ACE I/D ile ilgili 783 hasta ve 761 sağlıklı denek içeren 8 çalışma ve PAI-1 4G/5G ile ilgili 1.155 hasta ve 699 sağlıklı denek içeren 6 çalışma dahil olmak üzere toplam 14 çalışma dahil edildi. Birleşik veriler, ACE I/D polimorfizminin; allel (OO=0,744, %95 GA: 0,640-0,864, p≤0,001), dominant (OO=0,774, %95 GA: 0,601-0,996, p=0,047) ve resesif (OO=0,767, %95 GA: 0,611-0,963, p=0,022) olmak üzere üç model altında İranlı kadınlarda TGK riski ile önemli ölçüde ilişkili olduğunu ortaya koymuştur. Ayrıca,

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havuzlanmış veriler, PAI-1 4G/5G polimorfizmi ile TGK riski arasında, beş modelin tümünde, yani allel (OO=2,352, %95 GA: 1,623-3,408, $p \leq 0,001$), heterozigot (OO=8,364, %95 GA: 4,744-14,756, $p \leq 0,001$), homozigot (OO=2,192, %95 GA: 1,093-4,394, $p = 0,027$), dominant (OO=2,354, %95 GA: 1,309-4,235, $p = 0,004$) ve resesif (OO=5,208, %95 GA: 3,005-9,025, $p \leq 0,001$) modellerinde anlamlı ilişkinin varlığını göstermiştir. Tabakalaşma analizi, bu polimorfizmlerin düşük sayısına göre TGK riski ile ilişkili olduğunu ortaya çıkarmıştır. Birleştirilmiş verilerimiz, ACE I/D ve PAI-1 4G/5G polimorfizmlerinin, İranlı kadınlarda artmış TGK riski ile önemli ölçüde ilişkili olduğunu gösterdi. Bu önemli bulgular bizi, İran popülasyonunda ACE I/D ve PAI-1 4G/5G polimorfizmleri açısından araştırmanın yeterli olabileceği kararına götürdü.

Anahtar Kelimeler: Gebelik kaybı, düşük, trombofili, plazminojen aktivatör inhibitörü-1, anjiyotensin-I-dönüştürücü enzim, polimorfizm

Introduction

Recurrent pregnancy loss (RPL) is one of the main public health issues with a rate of 5% among women of reproductive age^(1,2). RPL is defined as the loss of three or more successive pregnancies before viability and includes all pregnancy losses from the time of conception until 24 weeks of gestation⁽³⁻⁵⁾. The most commonly cited causes of miscarriage are structural chromosome abnormalities of one of the partners, uterine abnormalities, elevated random levels of homocysteine, and antiphospholipid syndrome^(6,7). Thrombophilia is described as a susceptibility to arterial or venous thrombotic complications due to hemostatic system defects, which may be acquired, like the antiphospholipid syndrome, or inherited^(8,9). Adverse pregnancy outcomes, such as pregnancy failure (i.e. sporadic and RPL, late fetal loss), pre-eclampsia, and HELLP syndrome, are associated with thrombotic mechanisms and thrombophilia⁽¹⁰⁾. Thrombotic disorders are detectable in 40-50% of RPL cases⁽¹¹⁾. It has been presumed that the etiology of RPL is associated with factors involved in fibrinolysis and coagulation^(3,12). In the past two decades, several investigators suggested that thrombophilia had an impact on susceptibility to RPL⁽¹³⁻¹⁵⁾. Thus, genotyping of genetic variants at thrombophilic genes is useful to describe the etiology of RPL, and improvement our knowledge about the nature of this disease⁽¹⁶⁾.

Currently, there are a limited number of genetic variants as independent risk factors for venous thromboembolism in women with RPL⁽¹⁷⁾. There is growing evidence of a causal relationship of genetic variations at plasminogen activator inhibitor-1 (PAI-1) and angiotensin-converting enzyme (ACE) genes with RPL in different populations^(3,18). PAI-1, a 52 kDa glycoprotein belonging to the serine proteinase inhibitor superfamily, is the principal inhibitor of tissue and urinary plasminogen activators^(3,19,20). PAI-1 is involved in various physiologic functions and associated with many diseases⁽²¹⁻²³⁾. The most commonly studied functional variant in the PAI-1 gene is the 4G/5G polymorphism, which is characterized by a single guanosine nucleotide insertion/deletion variation at -675 bp to the transcription start site of the *PAI-1* gene^(24,25). Moreover, ACE or kininase II, is a dipeptidyl carboxypeptidase that plays an important role in regulating blood pressure and electrolyte balance^(26,27). The most widely studied polymorphism of ACE is an insertion/deletion (I/D), which is characterized by insertion or deletion of a 287-non-coding base pair Alu repeat sequence^(28,29).

RPL is one of the main reproductive health issues in Iran^(30,31). However, there is no consensus regarding the frequency of RPL in Iranian women. In the past decade, several molecular studies have evaluated the association of ACE I/D and PAI-1 4G/5G polymorphisms with RPL risk in Iran^(3,18). Nevertheless, their results were inconsistent and inconclusive. Moreover, the findings provided limited evidence due to relatively small sample sizes and might have been underpowered to estimate the risk. Thus, meta-analysis is a standardized approach to combine the results of different studies on ACE I/D and PAI-1 4G/5G polymorphisms to provide more reliable conclusions. Therefore, we conducted this meta-analysis to obtain a more precise estimation on the association of ACE I/D and PAI-1 4G/5G polymorphisms with RPL risk in Iranian women from all eligible case-control studies published in English and Farsi.

Materials and Methods

Search Strategy

We performed a comprehensive search on the United States National Library of Medicine's PubMed, Scopus, EMBASE, Web of Knowledge, MedRxiv, Cochrane Library, Google Scholar, Scientific Information Database, WanFang, VIP, Chinese Biomedical Database, Scientific Electronic Library Online and China National Knowledge Infrastructure database to find all relevant publications on the association of ACE I/D and PAI-1 4G/5G polymorphisms with RPL in Iranian women up till January 1st, 2021. The following keywords and terms were used for the search: ("Pregnancy Loss" OR "RPL" OR "Recurrent Pregnancy Loss" OR "Recurrent Miscarriage" OR "recurrent spontaneous abortion" OR "Idiopathic/Unexplained Recurrent Pregnancy Loss") AND ("Angiotensin-Converting Enzyme" OR "ACE" OR "SERPINE1") AND ("Insertion/Deletion Polymorphism" OR "ACE I/D" OR "rs4646994") AND ("Plasminogen Activator Inhibitor-1" OR "PAI-1") AND ("4G/5G" OR "rs1799889") AND ("Gene" OR "Genotype" OR "Allele" OR "Polymorphism" OR "Single nucleotide polymorphisms" OR "SNP" OR "Variation" OR "Mutation"). Articles were limited to the English and Farsi languages. Additionally, the reference lists of each eligible study, previous meta-analyses, and review articles were manually checked to find more relevant publications.

Inclusion and Exclusion Criteria

The criteria employed to retrieve publications for this meta-analysis were as follows: (1) studies with case-control or cohort design; (2) studies conducted among Iranian populations; (3)

studies that evaluated the association of ACE I/D and PAI-1 4G/5G polymorphisms with RPL; (4) studies that provided sufficient data on the genotype frequencies of the polymorphisms to calculate the pooled odds ratio (OR) with corresponding 95% confidence interval (CI). The exclusion criteria were as follows: (1) studies not relevant to RPL; (2) case-only studies or no controls; (3) linkage studies and family-based studies (twins and sibling); (4) duplicate studies and incomplete data; (5) abstracts, posters, presentations, letters, case reports, case series, comments, conference editorials, reviews and previous meta-analyses; and (6) unpublished data and studies without extractable data.

Data Extraction

Data were carefully extracted from all eligible studies independently by two authors according to the criteria listed above. Then, to minimize bias and to improve the reliability of the data, the authors checked all potentially relevant studies independently and reached a consensus or a third author was consulted to make a final decision. The following data were collected from each study: name of the first author, year of publication, genotyping method, numbers of patients with RPL and healthy controls, genotypes and alleles frequencies in patients and controls for ACE I/D and PAI-1 4G/5G polymorphisms, minor allele frequency, and p-values for Hardy-Weinberg equilibrium (HWE) tests in control subjects. If a study included more than one case-control group, each studied group was considered as an independent dataset. For studies with overlapping data or samples by the same author, the larger sample size or the study that was published more recently was included in the meta-analysis.

Statistical Analysis

The strength of the association between ACE I/D and PAI-1 4G/5G polymorphisms and RPL risk in Iranian women was calculated using odds ratios (ORs) with 95% confidence intervals (CIs). The significance of the pooled OR was determined using the Z-test, in which a p-value <0.05 was considered significant. The pooled ORs for ACE I/D and PAI-1 4G/5G polymorphisms were estimated under all five genetic comparison models, i.e., allele (A vs B), homozygote (AA vs BB), heterozygote (BA vs BB), dominant (AA+BA vs BB), and recessive (AA vs BA+BB). A Cochrane-based Q statistical test was used to test between-studies heterogeneity, in which p-values <0.1 indicated the absence of indicated heterogeneity. Moreover, we used the inconsistency index (I^2) (range of 0 to 100%) to quantify the proportion of the total variation due to heterogeneity, in which the heterogeneity was considered low, moderate, and high based on I^2 values of 25%, 50%, and 75%, respectively. If heterogeneity was observed among the studies, the random-effects model (the DerSimonian and Laird method) was used to estimate the pooled OR. Otherwise, a fixed-effects model (the Mantel-Haenszel method) was adopted. For each study, the HWE in healthy subjects was estimated using the chi-square

goodness-of-fit test and $p < 0.05$ was considered statistically significant. Sensitivity analysis was performed by sequential omission of individual studies to assess the stability of pooled data in this meta-analysis. Moreover, sensitivity analysis was performed by excluding studies that deviated from the HWE. Both Begg's funnel plot and Egger's weighted regression tests were used to assess publication bias. If publication bias existed, the Duval and Tweedie non-parametric "trim and fill" method was applied to adjust results. All statistical analyses were performed using the Comprehensive Meta-analysis (CMA) software version 2.0 (Biostat, USA). Two-sided probability (p) values of <0.05 were considered statistically significant.

Results

Study Selection and Characteristics

A flow chart detailing the inclusion/exclusion process is shown in Figure 1. The primary online database queries and manual reference searches generated 337 potentially relevant studies that reported the association of ACE I/D and PAI-1 4G/5G polymorphisms with susceptibility to RPL. After the removal of duplicate articles, the search retrieved 218 items. Based on the title, abstract screening, or both, 129 articles were excluded according to the eligibility criteria. Subsequently, 75 publications were excluded because they were reviews, previous meta-analyses, and evaluated the association of RPL with other polymorphism of ACE and PAI-1 genes. Finally, a total of 14 studies involving eight studies with 783 patients and 761 controls on the ACE I/D polymorphism^(6,18,29,32-35) and six studies with 1155 patients and 699 controls on the PAI-1 4G/5G polymorphism^(18,31,34,36-38) were included in this meta-analysis. One study in the present meta-analysis did not state the source of controls. Two genotyping methods were used, including ARMS-PCR and PCR-RFLP. The genotype distributions among the controls in the two studies were not consistent with the HWE on the ACE I/D polymorphism (Table 1).

Quantitative Data Synthesis

ACE I/D Polymorphism

The pooled results on the association of ACE I/D polymorphism with RPL risk in Iranian women are presented in Table 2. When all eligible studies were pooled together, a significant association between ACE I/D polymorphism and increased risk of RPL in Iranian women was found only under three models i.e., allele [D vs I: OR=0.744, 95% CI: (0.640-0.864); $p \leq 0.001$, Figure 2A], dominant [DD+DI vs II: OR=0.774, 95% CI: (0.601-0.996); $p = 0.047$, Figure 2B], and recessive [DD vs DI+II: OR=0.767, 95% CI: (0.611-0.963); $p = 0.022$, Figure 2C]. When stratified by the number of recurrent miscarriages (RM), a significant association between the ACE I/D polymorphism and increased risk of RPL was detected in the group of studies with ≥ 2 RMs under the allele genetic model [D vs I: OR=0.666, 95% CI: (0.539-0.822); $p \leq 0.001$], but not in studies with ≥ 3 RM.



PRISMA 2009 Flow Diagram

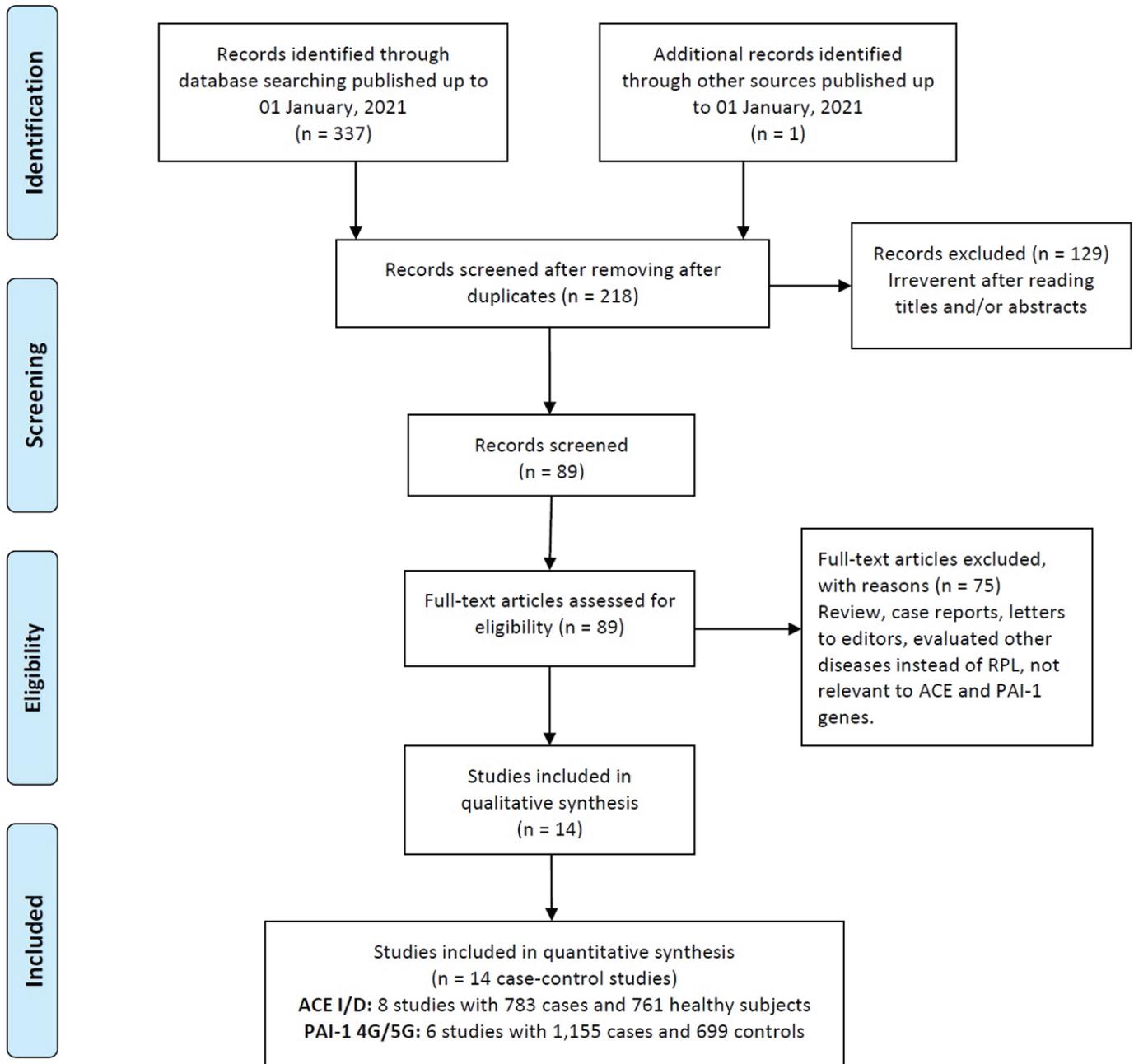


Figure 1. Flow diagram of selection of studies included in the meta-analysis

Moreover, a significant association was found between ACE I/D and RPL in ARMS-PCR group studies under two genetic models i.e., allele [D vs I: OR=0.799, 95% CI: (0.659-0.967); $p=0.022$] and recessive [DD vs DI+II: OR=0.734, 95% CI: (0.544-0.989); $p=0.042$], and PCR-RFLP group studies under the allele model [D vs I: OR=0.667, 95% CI: (0.524-0.850); $p=0.001$].

PAI-1 4G/5G Polymorphism

Table 3 summarizes the main results of the meta-analysis for the PAI-1 4G/5G polymorphism and RPL in Iranian women. Overall pooled data showed that there was a significant association between the PAI-1 4G/5G polymorphism with

Table 1. Main characteristics of studies included in this meta-analysis

First author	Genotyping technique	RM NO.	Case/ Control	Cases					Controls					MAFs	HWE
				Genotype			Allele		Genotype			Allele			
ACE I/D				II	ID	DD	I	D	II	ID	DD	I	D		
Soltanghoreae et al. ⁽³²⁾	PCR-RFLP	≥2	129/94	29	62	38	112	128	22	47	25	108	116	0.484	0.992
Bagheri et al. ⁽³³⁾	ARMS-PCR	≥3	50/63	7	26	17	40	60	12	27	24	51	75	0.404	0.380
Aarabi et al. ⁽³⁴⁾	PCR-RFLP	≥3	63/94	14	30	19	54	62	22	47	25	91	97	0.484	0.992
Poursadegh Zonouzi et al. ⁽³⁵⁾	ARMS-PCR	≥2	89/50	35	31	23	101	77	15	28	7	58	42	0.580	0.135
Shahkarami et al. ⁽¹⁸⁾	PCR-RFLP	≥2	100/100	6	60	34	74	128	0	48	52	48	152	0.240	0.001
Fazelnia et al. ⁽²⁹⁾	ARMS-PCR	≥2	100/100	31	40	29	102	98	23	33	44	79	121	0.395	0.001
Heidari et al. ⁽⁶⁾	ARMS-PCR	≥3	202/210	49	102	51	200	204	41	99	70	181	239	0.431	0.573
Maziri et al. ⁽²⁾	ARMS-PCR	≥2	50/50	36	13	1	85	15	26	22	2	74	26	0.260	0.310
PAI-1 4G/5G				5G5G	5G4G	4G4G	5G	4G	5G5G	5G4G	4G4G	5G	4G		
Arabi et al. ⁽³⁴⁾	PCR-RFLP	≥3	54/99	21	23	10	65	43	31	66	2	128	70	≤0.001	0.354
Jeddi-Tehran et al. ⁽³⁶⁾	PCR-RFLP	≥2	100/100	60	31	9	151	49	72	27	1	171	29	0.373	0.145
Idali et al. ⁽³⁷⁾	PCR-RFLP	≥3	106/100	35	53	18	123	89	72	27	1	171	29	0.373	0.145
Khosravi et al. ⁽³⁸⁾	PCR-RFLP	≥2	595/100	128	208	85	464	375	72	27	1	171	29	0.373	0.145
Shahkarami et al. ⁽¹⁸⁾	PCR-RFLP	≥2	100/100	33	50	17	116	84	45	50	5	140	60	0.300	0.056
Bigdeli et al. ⁽³¹⁾	PCR-RFLP	≥3	200/200	70	112	18	252	148	150	43	7	343	57	0.089	0.143

PCR: Polymerase chain reaction, RFLP: Restriction fragment length polymorphism, RM: Recurrent miscarriage, MAF: Minor allele frequency, HWE: Hardy-Weinberg equilibrium

RPL risk under all five genetic models i.e., allele [4G4G vs 5G5G: OR=2.352, 95% CI: (1.623-3.408); $p \leq 0.001$, Figure 3A], heterozygote [4G4G vs 5G5G: OR=8.364, 95% CI: (4.744-14.756); $p \leq 0.001$, Figure 3B], homozygote [4G5G vs 5G5G: OR=2.192, 95% CI: (1.093-4.394); $p = 0.027$, Figure 3C], dominant [4G4G+4G5G vs 5G5G: OR=2.354, 95% CI: (1.309-4.235); $p = 0.004$, Figure 3D], and recessive [4G4G vs 4G5G+5G5G: OR=5.208, 95% CI: (3.005-9.025); $p \leq 0.001$, Figure 3E]. When stratified by RM, a significant association between PAI-1 4G/5G polymorphism and increased risk of RPL was detected in the group of studies with ≥ 2 RMs under four genetic models i.e., allele [4G4G vs 5G5G: OR=2.083, 95% CI: (1.617-2.682); $p \leq 0.001$], homozygote [4G5G vs 5G5G: OR=8.390, 95% CI: (3.509-20.061); $p \leq 0.001$], dominant [4G4G+4G5G vs 5G5G: OR=2.003, 95% CI: (1.472-2.727); $p \leq 0.001$], and recessive [4G4G vs 4G5G+5G5G: OR=5.871, 95% CI: (2.528-13.631); $p \leq 0.001$], and ≥ 3 under three genetic models i.e., allele [4G4G vs 5G5G: OR=2.653, 95% CI: (1.299-5.418); $p = 0.007$], homozygote [4G5G vs 5G5G: OR=8.345, 95% CI: (3.955-17.606); $p \leq 0.001$], and recessive [4G4G vs 4G5G+5G5G: OR=4.764, 95% CI: (2.305-9.845); $p \leq 0.001$].

Heterogeneity Test and Sensitivity Analysis

There was no between-study heterogeneity found in all five genetic models and thus the fixed-effect model was applied to calculate their combined OR. Moreover, we conducted a sensitivity analysis to investigate whether the absence of each study would alter the pooled ORs and stability of our results. However, we observed no significant change in the association level of ACE I/D and PAI-1 4G/5G polymorphisms with RPL risk in the Iranian population by excluding any of the studies. This suggests that the current meta-analysis results were relatively robust and stable.

Publication Bias

The publication bias of the studies was assessed using a funnel plot and Egger's test (Table 2 and Figure 4A-B). The funnel plot did not indicate any evidence of funnel plot asymmetry for the ACE I/D polymorphism. Moreover, the results of Egger's test revealed no significant publication bias for the ACE I/D polymorphism. However, Begg's funnel plot and Egger's tests showed publication bias for the PAI-1 4G/5G polymorphism under two genetic models i.e., heterozygote (4G4G vs

Table 2. Summary risk estimates for association of ACE I/D polymorphism with RPL in Iranian women

Subgroup	Genetic model	Type of model	Heterogeneity		Odds ratio (OR)				Publication bias	
			I ² (%)	P _H	OR	95% CI	Z _{OR}	P _{OR}	P _{Begg}	P _{Egger}
Overall	D vs I	Fixed	6.65	0.379	0.744	0.640-0.864	-3.863	≤0.001	0.386	0.857
	DI vs II	Fixed	0.00	0.472	0.872	0.657-1.157	-0.952	0.341	0.710	0.778
	DD vs II	Random	52.55	0.039	0.941	0.595-1.487	-0.260	0.795	0.901	0.947
	DD+DI vs II	Fixed	13.69	0.323	0.774	0.601-0.996	-1.988	0.047	0.710	0.368
	DD vs DI+II	Fixed	45.76	0.074	0.767	0.611-0.963	-2.285	0.022	0.265	0.458
RM NO.										
≥2	D vs I	Fixed	27.81	0.245	0.666	0.539-0.822	-3.790	≤0.001	0.734	0.211
	DI vs II	Fixed	28.12	0.243	0.753	0.499-1.136	-1.1351	0.177	0.089	0.209
	DD vs II	Fixed	59.04	0.062	0.781	0.494-1.233	-1.061	0.289	0.734	0.520
	DD+DI vs II	Fixed	22.68	0.275	0.754	0.516-1.101	-1.461	0.144	0.734	0.154
	DD vs DI+II	Random	72.38	0.012	0.827	0.446-1.535	-0.601	0.548	0.089	0.237
≥3	D vs I	Fixed	0.00	0.613	0.838	0.676-1.039	-1.611	0.107	0.296	0.055
	DI vs II	Fixed	0.00	0.560	0.975	0.657-1.448	-0.124	0.901	0.296	0.231
	DD vs II	Fixed	10.49	0.327	0.790	0.513-1.217	-1.070	0.285	1.000	0.186
	DD+DI vs II	Fixed	0.00	0.461	0.896	0.618-1.300	-0.576	0.565	0.296	≤0.001
	DD vs DI+II	Fixed	0.00	0.399	0.796	0.572-1.108	-1.354	0.176	1.000	0.414
Genotyping methods										
ARMS-PCR	D vs I	Fixed	13.73	0.324	0.799	0.659-0.967	-2.298	0.022	0.734	0.441
	DI vs II	Fixed	15.59	0.314	0.831	0.590-1.172	-1.054	0.292	0.734	0.781
	DD vs II	Fixed	22.51	0.276	0.693	0.474-1.011	-1.903	0.057	0.734	0.179
	DD+DI vs II	Fixed	0.00	0.605	0.761	0.554-1.047	-1.680	0.093	0.734	0.414
	DD vs DI+II	Fixed	55.14	0.083	0.734	0.544-0.989	-2.030	0.042	0.308	0.288
PCR-RFLP	D vs I	Fixed	23.37	0.271	0.667	0.524-0.850	-3.275	0.001	1.000	0.625
	DI vs II	Fixed	17.81	0.296	0.932	0.560-1.551	-0.272	0.786	0.296	0.115
	DD vs II	Fixed	53.67	0.115	1.039	0.591-1.827	0.134	0.893	0.296	0.130
	DD+DI vs II	Fixed	37.97	0.199	0.985	0.609-1.590	-0.064	0.949	0.296	0.120
	DD vs DI+II	Fixed	65.79	0.054	0.824	0.578-1.176	-1.067	0.286	1.000	0.555
RM: Recurrent miscarriage, ARMS: Amplification-refractory mutation system, RFLP: Restriction fragment length polymorphism										

5G5G5: P_{Begg}=0.060 and P_{Egger}=0.021) and recessive (4G4G4 vs 4G5G+5G5G5: P_{Begg}=0.132 and P_{Egger}=0.028). One probable explanation is that the results were underpowered and biased by limited sample sizes. Therefore, the Duval and Tweedie non-parametric “trim and fill” method was applied to adjust for publication bias on the PAI-1 4G/5G polymorphism under the heterozygote and recessive models. However, the results with and without “trim and fill” did not draw different results, indicating that the current meta-analysis results are statistically robust and reliable.

Discussion

The etiology of RPL is complicated, and several risk factors are involved in the development of the disease. In addition to fetal and maternal factors, including chromosomal abnormalities, endocrine and metabolic aberrations, and autoimmune abnormalities, genetic single nucleotide polymorphisms at different loci, also play essential roles in RPL^(6,12,39). In this meta-analysis, we evaluated the association of the ACE I/D and PAI-1 4G/5G polymorphisms with RPL risk in Iranian women from all eligible case-control studies.

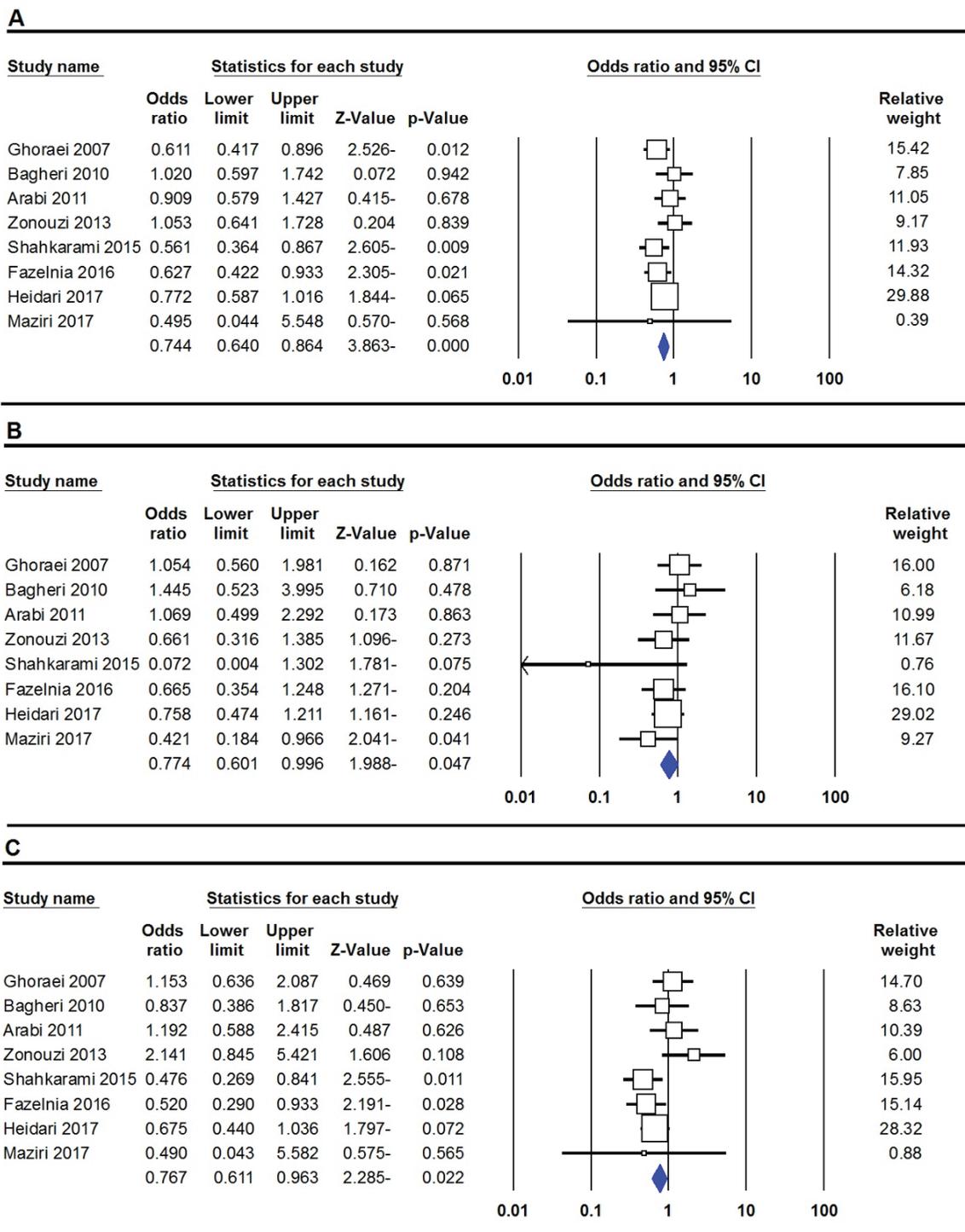


Figure 2. Forest plots for the association of ACE I/D polymorphism with risk of RPL risk in Iranian women. A: allele model; B: dominant model; and C: recessive model

The 4G/5G polymorphism is a major genetic variant determinant of plasma PAI-1 levels. The 4G allele has been reported to increase the risk for different diseases such as atherosclerosis and coronary artery disease⁽⁴⁰⁾. On the other hand, the 5G allele may increase the risk of conditions such as abdominal aortic aneurysm. In this meta-analysis, our combined data based on six studies with 1,155 patients and 699 healthy subjects

revealed that the PAI-1 4G/5G polymorphism was associated with an increased risk of RPL in Iranian women. In 2003, Wolf et al.⁽⁴¹⁾ first reported an increased risk of RPL in Austrian women in association with the PAI-1 4G/5G polymorphism. However, later studies in different ethnicities yielded controversial results^(18,34,42,43). In 2018, Adler et al.⁽⁴⁴⁾ evaluated the associations of the -675 I/D and 4G/5G polymorphisms

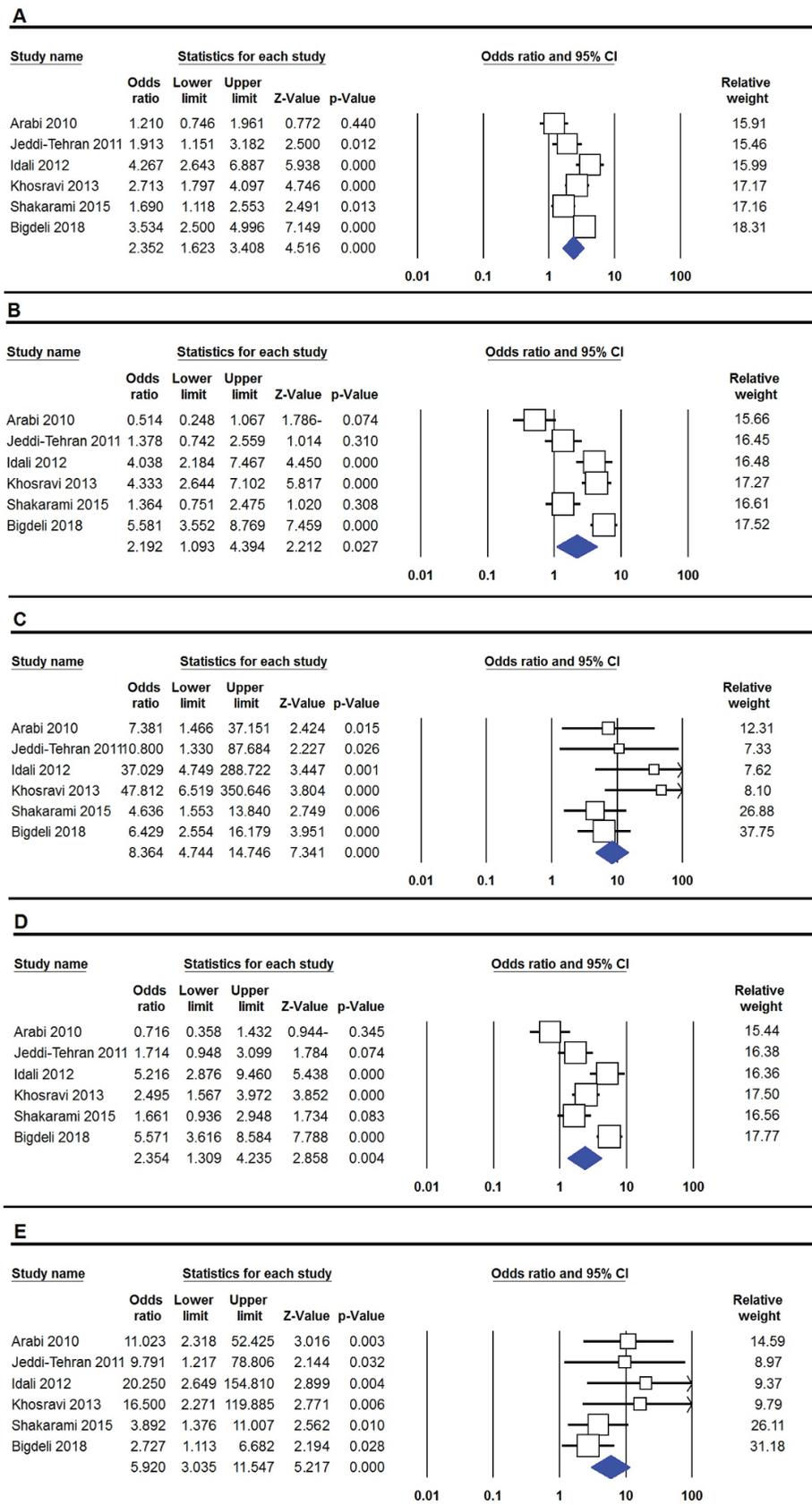


Figure 3. Forest plots for the association of PAI-1 4G/5G polymorphism with risk of RPL risk in Iranian women. A: allele model; B: heterozygote model; C: homozygote model; D: dominant model; and E: recessive model

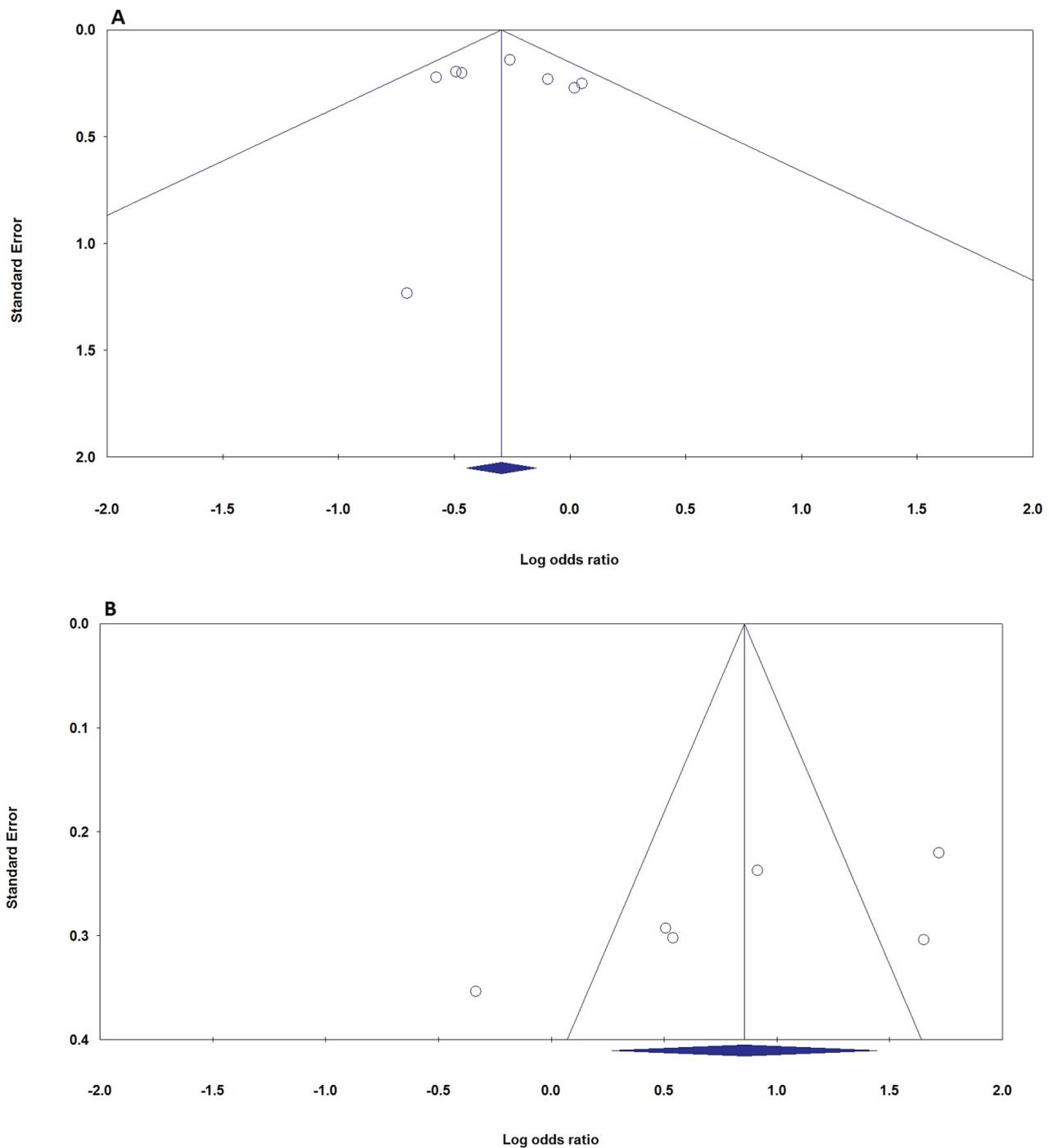


Figure 4. Begg's funnel plot of publication bias test for association of ACE I/D and PAI-1 4G/5G polymorphisms with risk of RPL risk in Iranian women. A: ACE (allele model); B: PAI-1 4G/5G (dominant model)

and PAI-1 with susceptibility to pregnancy loss in European and worldwide populations. Their pooled data revealed that there was no significant relationship between the 4G/5G polymorphism and pregnancy loss both in Europe or elsewhere in the world. However, Huang et al.⁽¹²⁾, in a meta-analysis based on 31 studies with 5617 patients and 3.952 healthy subjects reported that the PAI-1 4G/5G polymorphism might contribute to the susceptibility of RPL; their subgroup

analysis by ethnicity indicated a significantly elevated risk of RPL in Asians, Caucasians, and Africans. In 2015, Liu et al.⁽⁴⁵⁾, in a meta-analysis of 22 studies with 4306 patients and 3076 controls, showed that the PAI-1 4G/5G polymorphism might be associated with RPL in the overall population [OR=1.89; 95% CI: (1.34-2.67); $p<0.001$]. In a subgroup analysis, they found that the PAI-1 4G/5G polymorphism was significantly associated with an increased risk of RPL in Caucasian

Table 3. Summary risk estimates for association of PAI-1 4G/5G polymorphism with RPL in Iranian women

Subgroup	Genetic model	Type of model	Heterogeneity		Odds ratio (OR)				Publication bias	
			I ² (%)	P _H	OR	95% CI	Z _{OR}	P _{OR}	P _{Begg}	P _{Egger}
Overall	4G4G vs 5G5G	Random	77.18	0.001	2.352	1.623-3.408	4.516	≤0.001	0.452	0.379
	4G5G vs 5G5G	Random	88.76	≤0.001	2.192	1.093-4.394	2.212	0.027	0.060	0.021
	4G4G vs 5G5G	Fixed	22.68	0.263	8.364	4.744-14.756	7.341	≤0.001	0.259	0.061
	4G4G+4G5G vs 5G5G	Random	85.59	≤0.001	2.354	1.309-4.235	2.858	0.004	0.259	0.120
	4G4G vs 4G5G+5G5G	Random	23.72	0.256	5.208	3.005-9.025	5.882	≤0.001	0.132	0.028
RM no										
≥2	4G4G vs 5G5G	Fixed	25.21	0.263	2.083	1.617-2.682	5.684	≤0.001	1.000	0.848
	4G5G vs 5G5G	Random	83.16	0.003	2.046	0.920-4.551	1.756	0.079	1.000	0.102
	4G4G vs 5G5G	Fixed	51.41	0.128	8.390	3.509-20.061	4.783	≤0.001	1.000	0.374
	4G4G+4G5G vs 5G5G	Fixed	0.00	0.465	2.003	1.472-2.727	4.416	≤0.001	1.000	0.114
	4G4G vs 4G5G+5G5G	Fixed	0.00	0.392	5.871	2.528-13.631	4.118	≤0.001	1.000	0.207
≥3	4G4G vs 5G5G	Random	87.73	≤0.001	2.653	1.299-5.418	2.678	0.007	1.000	0.698
	4G5G vs 5G5G	Random	93.44	≤0.001	2.318	0.613-8.774	1.238	0.216	0.296	0.329
	4G4G vs 5G5G	Fixed	14.93	0.309	8.345	3.955-17.606	5.570	≤0.001	0.296	0.389
	4G4G+4G5G vs 5G5G	Random	92.39	≤0.001	2.814	0.860-9.202	1.711	0.087	0.296	0.423
	4G4G vs 4G5G+5G5G	Random	55.99	0.103	4.764	2.305-9.845	4.215	≤0.001	0.296	0.067

RM: Recurrent miscarriage

populations [OR=2.23; 95% CI: (1.44-3.46); p<0.001], but they reported that the PAI-1 4G/5G polymorphism was not significantly associated with RPL risk in Asian populations⁽⁴⁵⁾. The current meta-analysis results showed that ACE I/D polymorphism was associated with increased risk of RPL in Iranian women under the allele genetic model [D vs I: OR=0.745, 95% CI: (0.641-0.866); p≤0.001]. Recently, Gumus⁽⁴⁶⁾ in a case-control study, showed that the ACE I/D polymorphism was associated with idiopathic recurrent pregnancy loss (IRPL), and women with DD or ID genotypes had a 72% higher risk of developing IRPL than women with the II genotype. In 2018, Aslebahar et al.⁽³⁹⁾, in a meta-analysis of 26 case-control studies with 3140 patients with RPL and 3.370 controls, showed that ACE I/D polymorphism was associated with increased risk of RPL in the overall population. Similarly, Wang et al.⁽⁴⁷⁾, in a meta-analysis on 11 studies with a total of 3.357 individuals showed that the polymorphism was linked with an increased risk of recurrent miscarriage. By contrast, Perez et al.⁽⁴⁸⁾, in a meta-analysis based on 1,192 patients and 736 healthy subjects, showed no association between the ACE I/D polymorphism and RPL. Moreover, they performed a case-control study on 149 women (with ≥3 spontaneous abortions) and 149 healthy subjects to evaluate the association among Croatian women. Similarly, they showed a negative association between the ACE I/D polymorphism and the risk of RPL⁽⁴⁸⁾. In 2013, Su et al.⁽⁴⁹⁾, in a meta-analysis based on 11 studies, reported a

significant association between the ACE I/D polymorphism and IRPL. However, they found no significant association between the ACE I/D polymorphism and IRPL in Caucasian and non-Caucasian patients⁽⁴⁹⁾.

To our knowledge, this is the first meta-analysis to prove a significant association of the ACE I/D and PAI-1 4G/5G polymorphisms with RPL risk in Iranian women. However, the results presented here should be interpreted with caution because of several potential limitations. First, only published studies were included in this meta-analysis and some unpublished studies may have been missed, which may have biased the observed associations of ACE I/D and PAI-1 4G/5G polymorphisms with RPL in Iranian women. Second, there was relatively high heterogeneity under some genetic models. Third, the number of studies and the sample sizes were relatively small for analysis, thereby having insufficient power to estimate the association of ACE I/D and PAI-1 4G/5G polymorphisms with RPL in Iranian women. Fourth, the pooled estimates were based on unadjusted data, which might have affected the accuracy of the results. This is a meta-analysis with insufficient individual data to stratify results by other risk factors such as maternal age, environmental pollution, smoking; therefore, the association in these factors could not be assessed. Finally, RPL is a multifactorial disease influenced by many compound factors, including single or combined genetic polymorphisms and environmental factors. However, the impact of gene-gene,

gene-environment interactions and also ACE I/D and PAI-1 4G/5G polymorphisms interactions were precluded owing to insufficient original data.

Conclusion

Considering all the results, the pooled data indicated that ACE I/D and PAI-1 4G/5G polymorphisms were associated with an increased risk of RPL in Iranian women. Moreover, these polymorphisms were associated with RPL risk by the number of previous miscarriages. These significant findings suggest that investigation might be adequate for ACE I/D and PAI-1 4G/5G polymorphisms in association with RPL in Iranian women.

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Ethics

Peer-review: Internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: S.A.D., M.K.Z., R.S.T., A.J., M.N., S.R.M., H.N., Concept: R.B., Design: S.A.D., M.K.Z., R.S.T., A.J., M.N., S.R.M., H.N., Data Collection or Processing: S.A.D., H.N., Analysis or Interpretation: S.A.D., M.K.Z., R.S.T., A.J., M.N., S.R.M., H.N., Literature Search: S.A.D., M.K.Z., R.S.T., A.J., M.N., S.R.M., H.N., Writing: S.A.D., M.K.Z., R.S.T., A.J., M.N., S.R.M., H.N.

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