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Review Article

Association of *protamine1* gene c.-190C>A polymorphism with male infertility risk: a meta-analysis

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Abstract: Objective: Many studies have investigated the association of *PRM1* gene c.-190C>A polymorphism with male infertility risk. Previous results have been inconclusive, however. To derive a more precise estimation of the relationship, a meta-analysis was performed. Methods: A search of PubMed, Embase, Web of Science, and Chinese National Knowledge Infrastructure (CNKI) was conducted up through Nov 30, 2017. Odds ratios (ORs) with 95% confidence intervals (CIs) were used to assess strength of association in the allele comparison model, dominant model, recessive model, and codominant model. Sensitivity analysis was used to confirm the reliability and stability of the meta-analysis. Results: A total of 8 studies, involving 1,891 cases and 1,491 controls, were included in this meta-analysis. Pooled results indicated that *PRM1* c.-190C>A polymorphism was significantly associated with increased risk of male infertility in the allele model (A vs. C: OR = 1.58, 95% CI = 1.17-2.13), dominant model (AA+CA vs. CC: OR = 1.66, 95% CI = 1.20-2.30), and additive model (AA vs. CC: OR = 1.90, 95% CI = 1.01-3.56). According to subgroup analysis by nationality, c.-190C>A polymorphism was significantly associated with male infertility risk in Caucasians in the allele model (A vs. C: OR = 1.87, 95% CI = 1.14-3.06) and dominant model (AA+CA vs. CC: OR = 2.06, 95% CI = 1.24-3.44). No association was found between *PRM1* gene c.-190C>A polymorphism and male infertility in Asians in any of the genetic models. Conclusions: This meta-analysis suggests that *PRM1* gene c.-190C>A polymorphism can cause male infertility susceptibility, especially in Caucasian populations.

Keywords: Protamine gene, single nucleotide polymorphism, male infertility, meta-analysis

Introduction

Infertility affects 10%~15% of couples that wish to have children. Half of these cases are associated with male factors [1-3]. The etiology of half of male infertility cases is still not well understood. It has been suggested that genetic factors contribute up to 15~30% of male factor infertility [4, 5]. Previous studies have reported that some genetic mutations in *PRM1* and *PRM2* genes, such as *PRM1* gene c.-190C>A and *PRM2* gene 298G>C polymorphisms, may be associated with risk of male infertility. These findings have been supported by subsequent meta-analysis [6-8].

Protamines, major proteins in the sperm nucleus, are involved in the formation of a highly compact package of genomic DNA in the head of the sperm [9, 10]. Sperm nuclear is completely reorganized during spermatogenesis and DNA condensation. Histones are replaced by transition proteins in round spermatids and these are replaced by protamine in elongating spermatids [11, 12]. It has been suggested that protamine defected proteins cause abnormal condensation of sperm chromatin and increase sperm DNA strand breaks and immobility of spermatozoa, leading to male infertility [13, 14]. Several studies have noted that altered expression of protamines and abnormal PRM1/PRM2 ratios have been observed in sperm of infertile patients [15, 16]. Mutations or polymorphisms in protamine protein genes might induce conformational changes of the proteins, affecting DNA condensation and spermatogenesis. In the mouse model, knockout of either protamine gene leads to a reduction of the total amount of protamine formation, DNA damage, and reduced sperm function, resulting in male infertility [17]. Many studies have investigated the association of *PRM1* gene c.-190C>A polymorphism with male infertility risk. However,

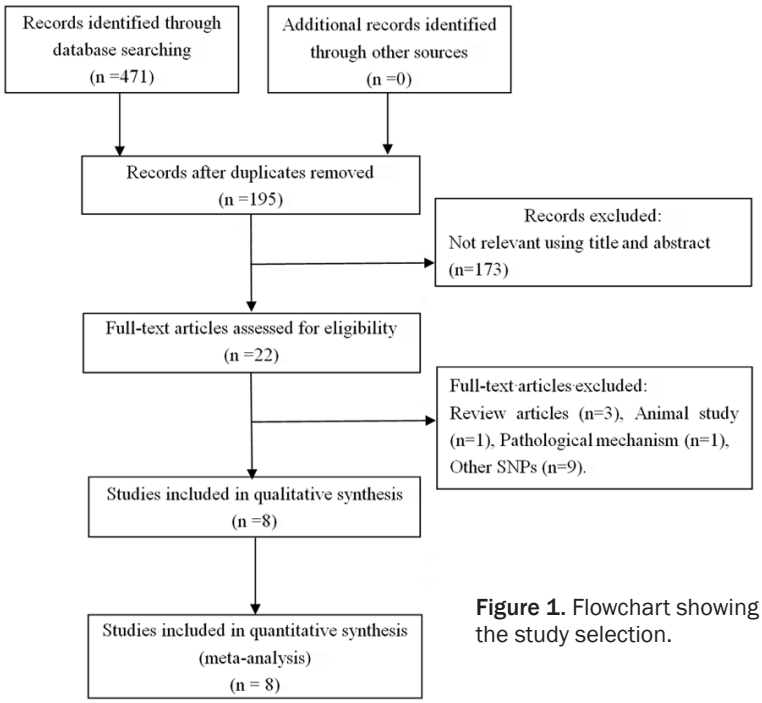

Figure 1. Flowchart showing the study selection.

the majority of these had small patient sample sizes, resulting in inconclusive results. A meta-analysis based on 5 case-control studies, including 1025 cases and 819 controls, was performed in 2015. Sample sizes of included published articles were small, however. Subsequently, a series of novel studies have been performed, thus an updated meta-analysis based on 8 studies of *PRM1* gene c.-190C>A polymorphism (1,891 cases and 1,491 controls) was performed to derive a more precise estimation of association.

Methods

Search strategy

A comprehensive search of studies in PubMed, Embase, Web of Science, and CNKI was conducted up through November 30, 2017. Included studies evaluated the association of *PRM1* gene c.-190C>A polymorphism with male infertility in humans. The search strategy provided use of the following terms: "Protamine gene" or "*PRM* gene" and "SNP" or "polymorphism" or "mutation" or "variant" and "male infertility". In addition, reference lists were screened of all cited articles and relevant reviews to identify other eligible studies that may have been missed by the search. A search strategy flowchart is shown in **Figure 1**.

Inclusion and exclusion criteria

Inclusion criteria of literature were as follows: 1) Full text of the article was available; 2) Case-control studies investigating association between *PRM1* gene c.-190C>A polymorphism and male infertility; 3) Genotype distributions were available for both cases and controls; 4) There were no duplicate data. For studies that considered partially or fully duplicate data and were by the same authors, the study with the most subjects was selected; 5) Published language was English or Chinese; and 6) Genotypic distributions were available for estimation of odds ratios (ORs) and 95% confidence intervals (CIs). Exclusion criteria included: 1) Studies not concerning association between *PRM1* gene c.-190C>A polymorphism and male infertility risk; and 2) Articles that were animal studies, review articles, meta-analysis, and conference abstracts or editorial articles.

Quality assessment

Newcastle-Ottawa Scale (NOS) was used to assess the quality of included studies [18]. NOS contains eight items for both cohort and case-control studies. This scale assesses the quality of case-control studies based on three areas: selection, comparability, and exposure. A star rating system was used to judge methodological quality. Selection had a maximum of 4 stars, comparability had a maximum of 2 stars, and exposure had a maximum of 3 stars. Total scores ranged from 0 stars (worst) to 9 stars (best). The quality of each study was graded as low (0±3), moderate (4±6), or high (7±9). Discrepant opinions were resolved by discussion and consensus.

Data extraction strategy

Two investigators independently extracted data, in compliance with inclusion criteria using a standardized data-collection form. Disagreements were resolved by discussion and con-

Table 1. Characteristics of studies included in meta-analysis

Author	Year	Country	Method	Case	Control	Case					Control					HWE
						CC	CA	AA	C	A	CC	CA	AA	C	A	
Aydos et al.	2018	Turkey	PCR	100	100	58	38	4	154	46	92	8	0	192	8	0.564
Gazquez et al.	2008	Spain	PCR sequence	220	101	114	90	16	318	122	68	30	3	166	36	0.887
He et al.	2012	China	MassArray	304	369	187	100	17	474	134	241	112	16	594	144	0.523
Imken et al.	2009	Morocco	PCR sequence	135	160	85	45	5	215	55	113	42	5	268	52	0.658
Jamli et al.	2016	Iran	PCR-RFLP	130	130	80	39	11	199	61	109	20	1	238	22	0.120
Jiang et al.	2017	China	MassArray	636	442	378	229	29	985	287	277	144	21	698	186	0.684
Jodar1 et al.	2010	Spain	PCR sequence	156	102	88	55	13	231	81	60	38	4	158	46	0.492
Jodar2 et al.	2010	Sweden	PCR sequence	53	50	25	27	1	77	29	26	17	7	69	31	0.153
Yu et al.	2012	China	MassArray	157	37	61	70	26	192	122	17	19	1	53	21	0.086

Table 2. Quality assessment for all included studies

Author	Publishing year	Selection	Comparability	Exposure	Total
Aydos et al.	2018	★★★	★★	★★	7
Gazquez et al.	2008	★★	★	★★	5
He et al.	2012	★★★	★	★★	6
Imken et al.	2009	★★	★	★★	5
Jamli et al.	2016	★★	★★	★★	6
Jiang et al.	2017	★★	★	★★	5
Jodar1 et al.	2010	★★★	★★	★★	5
Jodar2 et al.	2010	★★★	★★	★★	7
Yu et al.	2012	★★	★	★★	5

sensus. The following information was extracted: 1) First author's name, year of publication, country, and genotyping method; 2) Number of cases and controls; 3) Genotype and allele frequencies; and 4) Results of Hardy-Weinberg equilibrium tests.

Statistical analysis

Meta-analysis of association studies between *PRM1* gene c.-190C>A polymorphism and male infertility were estimated by pooled ORs with 95% CI. To perform the meta-analyses, data were entered and analyzed using Reviewer Manager 5.3 and STATA 12.0. Pooled ORs were performed in the allele comparison model, dominant model, recessive model, and codominant model. Statistical heterogeneity among studies was estimated using Q-test and I^2 statistics. Also, I^2 statistics was used to measure the degree of heterogeneity (I^2 = 0%-20%, no heterogeneity; I^2 = 20%-50%, moderate heterogeneity; I^2>50%, obvious heterogeneity). A random-effects model was used to estimate pooled ORs and 95% CIs, as heterogeneity was found with P<0.10 or I^2>50. Potential publication bias was estimated using funnel plots and Egger's regression test. Sensitivity analysis was performed to evaluate the stability of results.

Results

Study characteristics

A total of eight case-control articles, considering 1,891 cases and 1,491 controls, were included in this meta-analysis. Five studies were conducted in Caucasian populations [19-23] and three involved Asian populations [24-26]. These studies were published between 2008 and 2018. Hardy-Weinberg test (HWE) was performed on all included studies. Results showed that *PRM1* gene genotype frequencies of nine studies were in HWE in the controls. Detailed characteristics of all included studies are shown in **Table 1**. Quality of studies based on the NOS scores is presented in **Table 2**.

Association of PRM1 gene c.-190C>A polymorphism with male infertility

A total of 8 studies, including 3,382 individuals, evaluated the influence of *PRM1* gene c.-190C>A polymorphisms on risk of male infertility. **Figures 2**-**5** show meta-analysis results for the allele model (A vs. C), additive model (AA vs. CC), dominant model (AA+CA vs. CC), and recessive model (AA vs. CA+CC). I^2 values, representing the among-study heterogeneity, were 83%, 57%, 78%, and 57%, respectively. Thus, random-effects models were applied. Overall, results indicate that significant

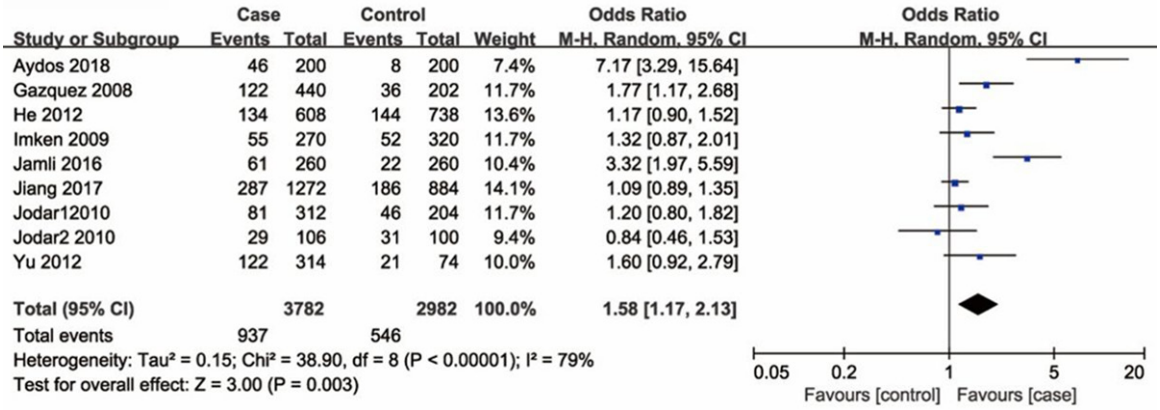

Figure 2. Forest plot of studies assessing association between *PRM1* gene c.-190C>A polymorphism and male infertility. (Allelic model: A vs. C).

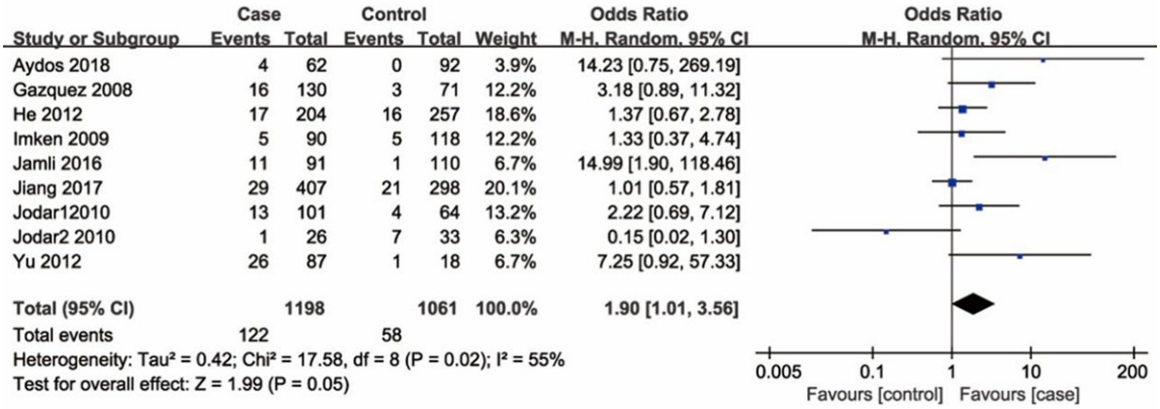

Figure 3. Forest plot of studies assessing association between *PRM1* gene c.-190C>A polymorphism and male infertility. (Additive model: AA vs. CC).

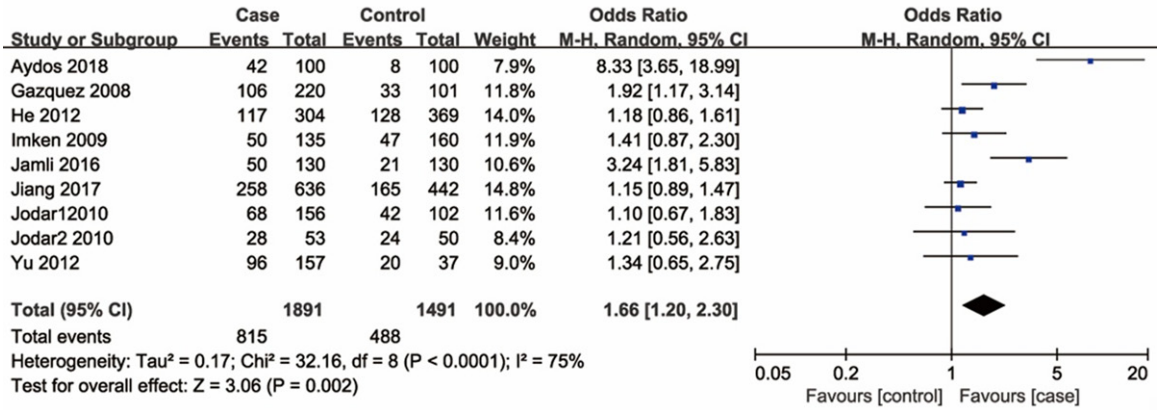

Figure 4. Forest plot of studies assessing association between *PRM1* gene c.-190C>A polymorphism and male infertility. (Dominant model: AA+CA vs. CC).

association was observed between *PRM1* gene c.-190C>A polymorphism and male infertility risk (A vs. C: OR = 1.58, 95% CI = 1.17-2.13; AA vs. CC: OR = 1.90, 95% CI =1.01-3.56; AA+CA vs. CC: OR = 1.66, 95% CI = 1.20-2.30; AA vs. CA+CC: OR = 1.95, 95% CI = 0.71-5.34).

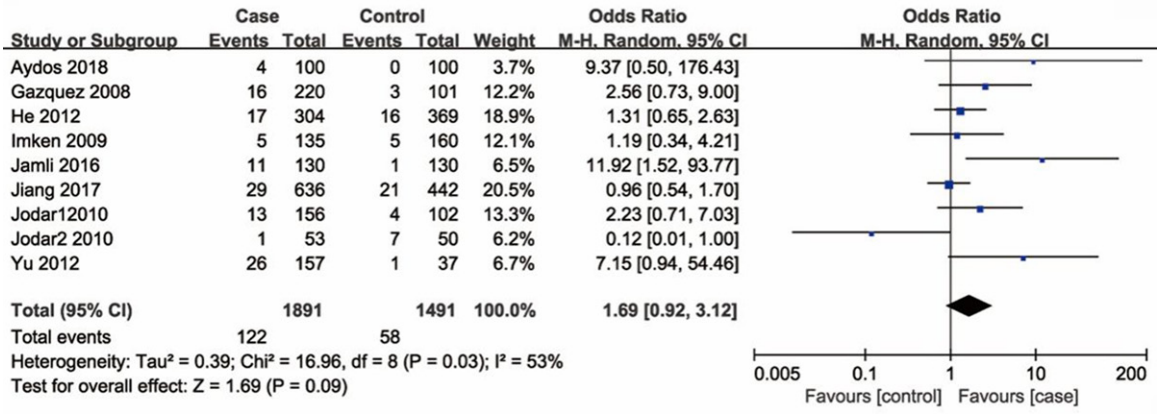

Figure 5. Forest plot of studies assessing association between *PRM1* gene c.-190C>A polymorphism and male infertility. (Recessive model: AA vs. CA+CC).

Table 3. Meta-analysis of association of *PRM1* gene c.-190C>A polymorphism with male infertility

	Genetic model	Type of model	I^2	$P_{Heterogeneity}$	OR, 95% CI	P_{OR}
Caucasian	A vs. C	Random	83%	<0.001	1.87 [1.14, 3.06]	0.010
	AA vs. CC	Random	57%	0.040	2.31 [0.83, 6.48]	0.110
	AA+CA vs. CC	Random	78%	<0.001	2.06 [1.24, 3.44]	0.006
	AA vs. CA+CC	Random	57%	0.040	1.95 [0.71, 5.34]	0.200
Asian	A vs. C	Fixed	0%	0.450	1.15 [0.99, 1.35]	0.070
	AA vs. CC	Fixed	42%	0.180	1.37 [0.71, 2.64]	0.350
	AA+CA vs. CC	Fixed	0%	0.920	1.17 [0.97, 1.41]	0.100
	AA vs. CA+CC	Fixed	47%	0.150	1.30 [0.85, 1.98]	0.230
Overall	A vs. C	Random	79%	<0.001	1.58 [1.17, 2.13]	0.003
	AA vs. CC	Random	55%	0.020	1.90 [1.01, 3.56]	0.050
	AA+CA vs. CC	Random	75%	<0.001	1.66 [1.20, 2.30]	0.002
	AA vs. CA+CC	Random	53%	0.030	1.69 [0.92, 3.12]	0.090

Sub-group analyses were performed on data stratified by ethnicity. Significant association was observed between *PRM1* gene c.-190C>A polymorphism and male infertility risks in Caucasians. There were no significantly elevated infertility risks associated with *PRM1* gene c.-190C>A polymorphism and male infertility in Asians. Results of sub-group analyses for all genetic models are listed in detail in **Table 3**.

Sensitivity and publication bias

Publication bias was assessed for *PRM1* gene c.-190C>A polymorphism by funnel plots, Begg's test, and Egger's test, under all contrast models. The shape of the funnel plot did not indicate any evidence of obvious asymmetry in any contrast model for *PRM1* gene c.-190C>A polymorphism (**Figure 6**). In addition, Egger's linear regression analysis suggested no evidence of publication bias (P = 0.054 for an allelic contrast model, P = 0.146 for an additive model, P = 0.200 for a recessive model, and P = 0.069 for a dominant model) (**Table 4**). Sensitivity analyses were conducted to calculate pooled ORs by omitting one study each time. Results showed that no individual study influenced overall pooled ORs (**Figure 7**), indicating that the results of this meta-analysis are relatively stable.

Discussion

This meta-analysis and systemic review investigated the association of *PRM1* gene c.-190C>A polymorphism and male infertility. This novel data demonstrated that *PRM1* gene c.-190C>A polymorphism was correlated with male infertility risk in Caucasians, but not in Asians. Patients with the A allele of c.-190C>A gene polymorphism have a higher risk for male infertility. Significant heterogeneity was found between individual studies under the four genetic models ($P_{heterogeneity}$<0.05). Subgroup analysis showed that the ethnicity could partially explain the heterogeneity. In subgroup analysis

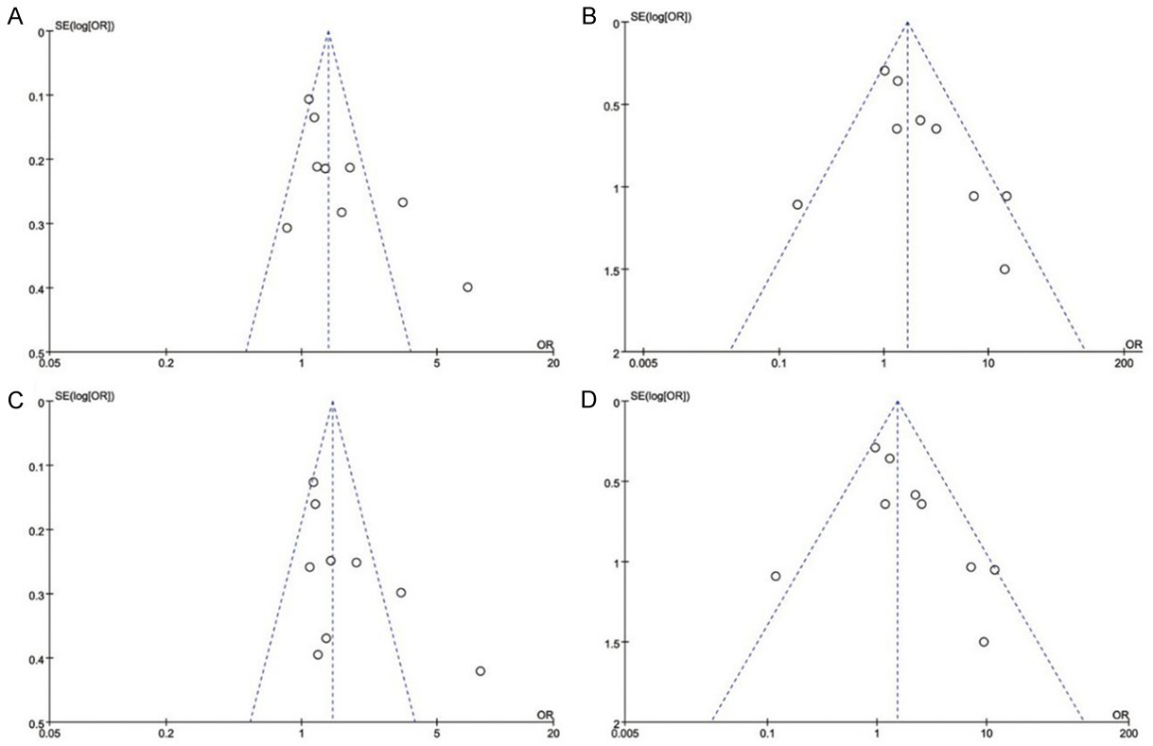

Figure 6. Funnel plots for *PRM1* gene c.-190C>A polymorphism and male infertility risk. (A. Allelic model: A vs. C; B. Additive model: AA vs. CC; C. Dominant model: AA+CA vs. CC, D. Recessive model: AA vs. CA+CC).

Table 4. Publication bias test for *PRM1* gene c.-190C>A polymorphism

Comparisons	Egger test			Begg test
	Coefficient	*P* value	95% CI	*P* value
A vs. C	3.514	0.054	-0.723 7.101	0.076
AA vs. CC	1.532	0.146	-0.682 3.746	0.251
AA+CA vs. CC	3.073	0.069	-0.319 6.466	0.076
AA vs. CA+CC	1.347	0.200	-0.906 3.600	0.251

stratified by ethnicity, heterogeneity still existed in the Caucasian subgroup ($P_{heterogeneity}$< 0.0001) but disappeared in the Asian subgroup ($P_{heterogeneity}$ = 0.31). This heterogeneity among studies may be due to study differences in genotyping method, population substructure, or other factors.

There is considerable experimental evidence that protamines are essential for male infertility. *PRM1* and *PRM2* play a pivotal role in sperm chromatin condensation and spermatogenesis [27, 28]. Single nucleotide polymorphisms (SNPs) of *PRM* genes can impair nuclear condensation, leading to male infertility [29, 30]. Recent studies have revealed that *PRM1* gene c.-190C>A polymorphism was associated with an increased risk of male infertility. However, due to different inclusive criteria and uneven sample sizes, these reports have presented different conclusions. Although most of them indicated that *PRM1* gene c.-190C>A polymorphism might be a risk factor for male infertility, the effects of the polymorphism on different ethnic groups have not been fully clarified. Jamli et al. studied *PRM1* gene c.-190C>A polymorphism in 130 infertile and 130 fertile men in Iran. Results showed that the c.-190C>A transversion may involve in susceptibility for oligozoospermia. Similarly, Aydos et al. found that *PRM1* c.-190C>A polymorphism was associated with sperm DNA fragmentation, possibly impacting male infertility in the Turkish population. However, He et al. found no evidence of an association of this polymorphism with male infertility risk in the Chinese population. This present study revealed that *PRM1* c.-190C>A polymorphism significantly associated with male infertility risk in Caucasians, but not in Asian populations. Inconsistency between the

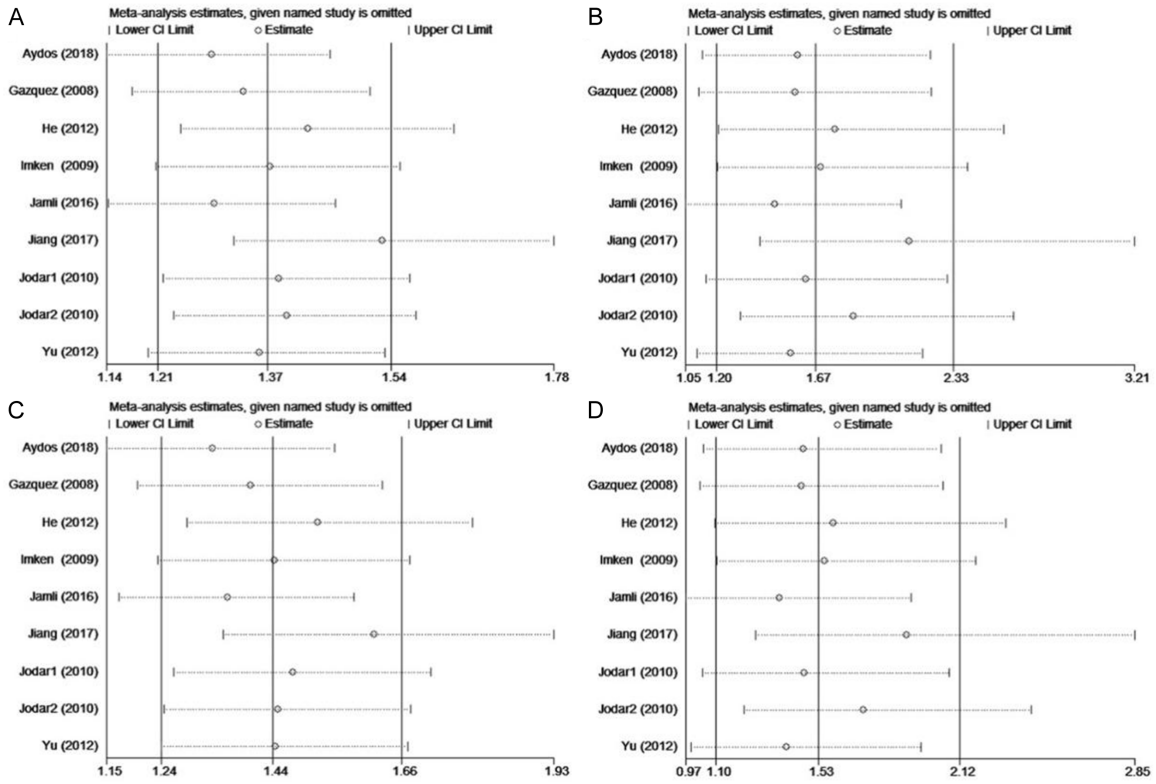

Figure 7. Sensitivity analysis diagram for each study used to assess relative risk estimates for *PRM1* gene c.-190C>A polymorphism and male infertility in all included studies. (A. Allelic model: A vs. C; B. Additive model: AA vs. CC; C. Dominant model: AA+CA vs. CC, D. Recessive model: AA vs. CA+CC).

studies could arise from geographic variations, racial, and ethnic differences in the distribution of polymorphisms in *PRM1* genes. However, only 9 studies were included in the meta-analysis. Three studies reported a relationship between *PRM1* c.-190C>A polymorphism and male infertility risk, but sample sizes were small. Thus, high-quality studies with larger sample sizes are needed to further investigate the potential relationship of *PRM1* c.-190C>A polymorphism with male infertility risk.

There were some limitations to the present meta-analysis. First, only nine studies were included. Sample sizes of included published articles were small. Thus, sufficient data was unavailable. Second, other clinical data such as source of control, age, semen quality, and so forth, were not analyzed due to lack of information. Third, this meta-analysis did not estimate potential interactions among gene-gene and gene-environment due to lack of information in the original studies. Finally, only studies published in English or Chinese language were included. Unpublished studies and those in other languages were likely missed.

Conclusion

In summary, this meta-analysis provides evidence that *PRM1* gene c.-190C>A polymorphism may contribute to genetic susceptibility to male infertility risk in Caucasians, but not in Asians. To reach a more definitive conclusion, large-scale, well-designed, and population-based studies are necessary.

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Disclosure of conflict of interest

None.

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