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Association Between *TLR4* Gene Polymorphisms and Risk of Preeclampsia: Systematic Review and Meta-Analysis

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Background: Toll-like receptor 4 (TLR4) plays a pivotal role in the innate immune response and is hyperactivated in preeclampsia (PE). Several researchers have published conflicting evidence for *TLR4* rs4986790 and rs4986791 single nucleotide polymorphisms (SNPs) as risk factors for PE. The present meta-analysis was conducted to obtain a more definitive conclusion about the effects of these SNPs on PE susceptibility.

Material/Methods: To determine the correlation between rs4986790 and rs4986791 polymorphisms in the *TLR4* gene and susceptibility to PE, the PubMed, Web of Science, EMBASE, Chinese National Knowledge Infrastructure, and Chinese WANFANG databases were searched for eligible articles. Statistical analysis was performed with STATA software, version 12.0. Pooled odds ratios with corresponding 95% confidence intervals (CIs) were extracted for assessment of correlation strength.

Results: We identified 5 studies including 578 cases and 631 controls for the rs4986790 SNP and 4 studies including 469 cases and 457 controls for the rs4986791 SNP, mainly from a White population. The pooled analyses showed no statistical relationship between the polymorphisms rs4986790 and rs4986791 and PE susceptibility in 5 genetic models (all $P>0.05$). Moreover, the allelic and dominant gene models of rs4986790 and the allelic, heterozygous, and dominant gene models of rs4986791 had high heterogeneity. The sensitivity analysis explored potential sources of heterogeneity and confirmed the findings of this meta-analysis.

Conclusions: *TLR4* rs4986790 and rs4986791 polymorphisms may not be implicated in PE susceptibility, primarily in a White population. More high-quality studies of genetic associations with PE are warranted.

Keywords: Meta-Analysis • Pre-Eclampsia • Toll-Like Receptor 4

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Background

Preeclampsia (PE), a multisystem, pregnancy-related syndrome that is associated with significant maternal and perinatal morbidity and mortality, occurs in 2% to 8% of pregnancies globally. It is typically characterized by new-onset hypertension and significant proteinuria after the 20th week of gestation and can lead to maternal multiple organ dysfunction or fetal growth restriction and premature delivery, potentially resulting in adverse lifelong outcomes for the mothers and their offspring [1,2]. Although PE is currently being intensively investigated, its underlying pathogenic mechanism has not yet been completely elucidated, because it is a complex process driven by multiple factors that involve interaction between genetic predisposition and environmental risk factors [3-5]. A family history of PE and PE in a previous pregnancy have been found by several researchers to increase risk in subsequent pregnancies; many genetic variants also have been found to be strongly associated with increased susceptibility to the syndrome [6-9]. Overall, the genetic predisposition to PE is important for its occurrence and development [10].

The Toll-like receptor (TLR) family includes 13 TLRs identified in mammals. They are the main type of pattern recognition receptors (PRRs) involved in the innate (non-specific) immune system that recognize specific pathogen-associated molecular patterns (PAMPs) and trigger signaling events to induce the expression of cytokines and chemokines [11]. Previous studies have suggested that proper functioning of TLRs at the maternal-fetal interface may play a key role in successful placentation, but activation of the innate immune system through alterations in the expression of TLRs may be related to defective placentation in PE [12,13]. Because of the polymorphic nature of the TLR genes, functional single nucleotide polymorphisms (SNPs) in DNA sequences that encode TLRs affect TLR expression, which contributes to individual susceptibility to various immune-related diseases [14]. Some recent studies have investigated the correlation between genetic variations in TLR family genes, particularly TLR4, and susceptibility to PE [15].

TLR4 was the first mammalian TLR to be discovered. The *TLR4* gene (Entrez Gene ID: 7099) is located on chromosome 9q32-33 and belongs to the subfamily of cell surface TLRs. It recognizes and interacts with the gram-negative bacterial outer membrane component lipopolysaccharide (LPS) and chlamydia heat shock protein 60 (cHSP60) as cognate PAMPs [16-18]. Following ligation, TLR4 signals the inflammatory cascade mainly through the adapter molecule myeloid differentiation protein 88 (MyD88) to activate the nuclear factor (NF)-κB pathway and further promote transcription of pro-inflammatory cytokines such as tumor necrosis factor-α, interleukin-1 (IL-1), IL-6, and IL-8 [11,19]. TLR4 expression is significantly increased in interstitial trophoblasts at the feto-maternal interface of patients with PE [20]. The serum level of TLR4 in women with PE is significantly higher than in healthy pregnant women, and TLR4 may be a serum biomarker for the prediction and diagnosis of PE [20]. LPS treatment of isolated cytotrophoblast cells increases TLR4 and pro-inflammatory cytokine expression and impairs the motility of trophoblast cells [21,22]. In addition, after the administration of a low dose of LPS, an animal model developed typical PE-like features and had high expression of TLR4 in the placenta [23]. Taken together, these findings indicate that activation of TLR4 may contribute to development of PE by creating a locally altered cytokine environment.

Several polymorphisms in the *TLR4* gene have been described, and rs4986790 (https://www.ncbi.nlm.nih.gov/snp/rs4986790) and rs4986791 (https://www.ncbi.nlm.nih.gov/snp/rs4986791) are 2 of the most frequently investigated co-segregating SNPs of the *TLR4* gene in functional and genetic association studies. The rs4986790 SNP is characterized as an adenine (A) to guanine (G) transition at nucleotide 896, which results in an aspartate acid (Asp) to glycine amino acid (Gly) substitution at position 299, while the rs4986791 SNP substitutes a thymine (T) for a cytosine (C) at nucleotide 1196 that changes threonine (Thr) to isoleucine (Ile) at codon 399 [24]. These 2 common variants affect the extracellular domain of TLR4 and might impair ligand binding [25]. Moreover, polymorphisms in the *TLR4* gene are related to the risks of multiple autoimmune disorders and inflammatory diseases, including rheumatoid arthritis [26], chronic psoriasis [27], inflammatory bowel disease [28], and infection with *Plasmodium falciparum* [29] or respiratory syncytial virus [30]. Recent observational studies of these 2 polymorphisms in the *TLR4* gene revealed their effect on the predisposition to PE. For instance, studies by Vamvakopoulou and Xie found an association between the rs4986790 and rs4986791 variants of the *TLR4* gene and PE [31,32]. In contrast, discrepant conclusions have been drawn in studies by both Mohammadpour-Gharehbagh and Franchim [33,34]. Considering the limitations of the design and size of these studies, the real impact may not have been evaluated because of insufficient statistical power. To date, few studies have systematically tested whether genetic variants in the *TLR4* gene contribute to susceptibility to PE.

In the present study, a meta-analysis of eligible studies was conducted to systematically evaluate the relationship between the functional allelic variants rs4986790 and rs4986791 in the *TLR4* gene and risk of PE. In addition, we checked for publication bias and estimated between-study heterogeneity. Also, sensitivity analyses were applied to explore potential sources of heterogeneity and assess the robustness of results.

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Material and Methods

The protocol for our meta-analysis followed PRISMA guidelines (see S1 PRISMA checklist).

Literature Search Strategy

Potentially eligible studies published through June 30, 2020 were retrieved from the following online databases: PubMed, Web of Science, EMBASE, Chinese National Knowledge Infrastructure, and Chinese WANFANG. The exhaustive search string included the following MeSH terms and keywords: ("Pre-Eclampsia" [MeSH] or "Preeclampsia" or "Pregnancy Toxemia*" or "Edema Proteinuria Hypertension Gestosis" or "Hypertension Edema Proteinuria Gestosis" or "Toxemia of Pregnancy") and ("Toll-Like Receptor 4" [Mesh] or TLR4) and ("Genetic" [Mesh]" or "Genetic Polymorphism" or "Polymorphism" or "mutation"). Review articles and other related studies from reference lists were further investigated by performing a manual search to identify additional publications that qualified for the present analysis.

Eligibility Criteria

Articles that met the following criteria were selected: (1) case-control, cohort, or cross-sectional studies reporting the association of *TLR4* polymorphisms (rs4986790 or rs4986791) with susceptibility to PE; (2) a confirmed diagnosis of PE in patients in the case group, but not in the control group; (3) studies with clear data on the genotype and allele frequency of investigated polymorphisms; (4) studies published in English or Chinese; and (5) data and full text available. Studies with the following characteristics were excluded: (1) studies with designs other than case-control, cohort, or cross-sectional; (2) family-based, sibling, twin, and linkage studies; (3) studies without detailed information for data extraction; (4) comments, letters to the editor, case reports, reviews, conference abstracts, and animal studies; and (5) repetitive data from previous publications.

Data Extraction

Two authors independently screened the literature according to the selection criteria and then extracted the following items from each included article: name of the first author, publication year, country, race, genotyping method, sample size, type of specimen, and genotype distribution. The "mixed" group refers to White and non-White participants. The probability value (*P* value) of the Hardy-Weinberg equilibrium (HWE) test in the control group from each study was calculated using a chi-square test, with $P<0.05$ indicating a significant deviation from HWE [35]. Any disagreement was settled by discussion with a third author until a consensus was achieved.

Quality Assessment

The Newcastle-Ottawa Scale (NOS) for non-randomized studies was used by 2 independent investigators to evaluate the methodological quality of the identified articles [36]. The quality of each study was scored based on selection, comparability, and outcome, with a range from 1 to 9 stars and a score of >7 stars considered a high-quality study.

Statistical Analysis

The strength of the correlation between the *TLR4* SNP and PE risk, as assessed using 5 possible genetic models (allelic, dominant, recessive, homozygote, and heterozygote), was presented as odds ratios (ORs) with corresponding 95% confidence intervals (95%CIs). $P<0.05$ indicated that the difference was statistically significant. The between-study heterogeneity was analyzed with a *Q* test and I^2 test [37]. $P \leq 0.10$ for the *Q* test and $I^2 \geq 50\%$ for the I^2 test indicated statistically significant heterogeneity in the included studies. A random effects model was applied; a fixed effects model was used for the summarized OR if the heterogeneity met the criteria of $P>0.1$ or $I^2 <50\%$.

In addition, when significant heterogeneity existed, a sensitivity analysis was conducted to further ascertain the underlying sources of heterogeneity and confirm the robustness of our primary outcome by omitting each individual dataset sequentially and recalculating the effect after adjustment. An Egger's linear regression analysis was used to assess potential publication bias of the included studies, which was considered statistically significant if $P<0.05$ [38]. All of the aforementioned statistical analyses were performed using STATA software, version 12.0 (STATA Corporation, College Station, Texas, United States).

Results

Literature Screening Process and Results

Two hundred eighty-eight records were retrieved during the primary inspection. After initial screening of the titles and abstracts, 280 documents that were obviously irrelevant, duplicates, reviews, or abstracts were eliminated. After searching and reading the full text, 1 article with repeated data [39] and 2 articles with incomplete data were excluded [40,41]. For the rs4986790 SNP, 5 remaining articles met our selection criteria, involving 578 patients with PE and 631 controls [31-34,42]. For the rs4986791 SNP, 4 remaining articles were included in our meta-analysis, with 469 PE patients and 457 controls [31-33,42]. A study flowchart detailing the selection and exclusion process is shown in **Figure 1.**

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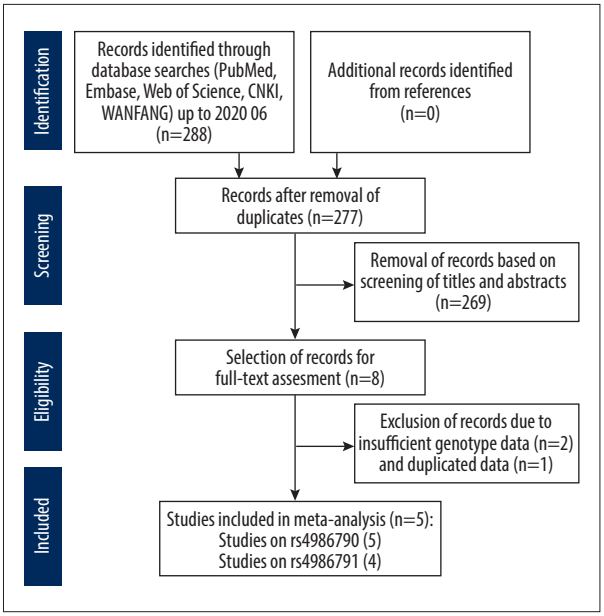

Figure 1. Flowchart of the literature search and study selection process.

Basic Characteristics of Included Studies

The characteristics of the included studies are summarized in **Table 1**. Published between 2008 to 2019, they were all case-control studies conducted in Greece [31], Iran [33], Brazil [34], Canada [32], and Hungary [42]. The controls in the study by Vamvakopoulou et al [31] were healthywomen with a history of ≥1 uneventful pregnancies; the controls in the remaining included studies were healthy pregnant women. No significant deviations from HWE were observed for any genotype in the control group ($P>0.05$). The quality of studies was rated using the NOS; all of the enrolled studies were of good quality, scoring 7 or 8 stars. Blood and placenta samples were collected in the included studies. Diverse genotyping techniques used to detect the polymorphism in all eligible studies included direct sequencing, polymerase chain reaction (PCR)-restriction fragment length polymorphism, and real-time PCR. The sample sizes in the included studies ranged from 84 to 180 for patients and from 76 to 174 for controls. Four studies assessed populations of White origin [31-33,42] and 1 assessed a mixed-race population [34]. The distribution of *TLR4* genotypes is shown in **Table 2**.

Meta-Analysis of Associations of TLR4 Alleles and Genotypes with PE

As shown in **Table 2**, the pooled analyses revealed no statistically significant association between *TLR4* (rs4986790 and rs4986791) gene polymorphisms and susceptibility to PE in any of the genetic models. The results for the individual models were as follows: allelic – G vs A, $P=0.887$, OR=0.96, 95%CI=0.51-1.78 for rs4986790 and T vs C, $P=0.871$, OR=1.07, 95%CI=0.48-2.38 for rs4986791; homozygous – GG vs AA, $P=0.740$, OR=1.22, 95%CI=0.38-3.90 for rs4986790 and TT vs CC, $P=0.922$, OR=1.08, 95%CI=0.03-4.63 for rs4986791; heterozygous - GA vs AA, $P=0.527$, OR=0.89, 95%CI=0.62-1.28 for rs4986790 and TC vs CC, $P=0.850$, OR=1.07, 95%CI=0.54-2.09 for rs4986791; dominant genetic – GG+GA vs AA, $P=0.826$, OR=0.94, 95%CI=0.53-1.67 for rs4986790 and TT+TC vs CC, $P=0.854$, OR=1.07, 95%CI=0.50-2.29 for rs4986791; and recessive inheritance – GG vs GA+AA, $P=0.743$, OR=1.22, 95%CI=0.38-3.93 for rs4986790 and TT vs TC+CC, $P=0.953$, OR=1.05, 95%CI=0.24-4.57 for rs4986791.

Analysis of Publication Bias

Based on the results of Egger's regression test shown in **Table 3**, no significant evidence of publication bias was detected for the allelic model of rs4986790 (G vs A, $P=0.289$) and rs4986791(T vs C, $P=0.474$), the homozygous model of rs4986790 (GG vs

Table 1. Characteristics of the included studies.

First Author	Published Year	Country	Race	Genotyping method	Cases/ controls	Specimen	Source of cases	Source of controls	HWE (*P*)	NOS score
Vamvakopoulou	2019	Greece	Caucasian	Automated cycle sequencing	84/94	Blood	EOP	Heathy women	>0.05	7
Mohammadpour-Gharehbagh	2019	Iran	Caucasian	PCR-RFLP	111/115	Placenta	PE	Healthy pregnancies	>0.05	7
Franchim	2011	Brazil	Mixed	PCR-RFLP	109/174	Blood	PE	Healthy pregnancies	>0.05	8
Xie	2010	Canada	Caucasian	RT-PCR	94/76	Blood	PE	Healthy pregnancies	>0.05	8
Molvare	2008	Hungary	Caucasian	PCR-RFLP	180/172	Blood	PE	Healthy pregnancies	>0.05	8

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Table 2. Distribution of *TLR4* genotypes and alleles among patients and controls.

First Author (year)	Cases							Controls						
rs4986790	GG	GA	AA	G	A	GG+GA	GA+AA	GG	GA	AA	G	A	GG+GA	GA+AA
Vamvakopoulou (2019)	2	12	70	16	152	14	82	0	6	88	6	182	6	94
Mohammadpour-Gharehbagh (2019)	0	12	99	12	210	12	111	1	16	98	18	212	17	114
Franchim (2011)	0	7	102	7	211	7	109	1	18	134	20	286	19	152
Xie (2010)	1	12	81	14	174	13	93	0	18	158	18	334	18	176
Molvare (2008)	0	15	165	15	345	15	180	1	21	150	23	321	22	171
rs4986791	TT	TC	CC	T	C	TT+TC	TC+CC	TT	TC	CC	T	C	TT+TC	TC+CC
Vamvakopoulou (2019)	2	14	68	18	150	16	82	0	6	88	6	182	6	94
Mohammadpour-Gharehbagh (2019)	0	7	104	7	215	7	111	1	9	105	11	219	10	114
Xie (2010)	0	8	86	8	180	8	94	0	14	162	14	338	14	176
Molvare (2008)	0	15	165	15	345	15	180	1	22	149	24	320	23	171

Table 3. Main results for pooled ORs in the meta-analysis.

Genetic model	Test of association			Test of heterogeneity		Test of publication bias (Egger)		Type of model
	OR	95% CI	*P*	I^2 (%)	*P*	T	*P*	
rs4986790								
G vs A	0.96	0.51-1.78	0.887	67.9	0.014	1.28	0.289	Random
GG vs AA	1.22	0.38-3.90	0.740	0.0	0.438	-1.53	0.225	Fixed
GG vs GA	1.18	0.32-4.34	0.802	0.0	0.814	-0.53	0.630	Fixed
GG+GA vs AA	0.94	0.53-1.67	0.826	59.8	0.041	1.40	0.256	Random
GG vs GA+AA	1.22	0.38-3.93	0.743	0.0	0.479	-1.40	0.255	Fixed
rs4986791								
T vs C	1.07	0.48-2.38	0.871	71.5	0.015	0.87	0.474	Random
TT vs CC	1.08	0.03-4.63	0.922	17.8	0.296	-40.8	0.016	Fixed
TT vs TC	0.82	0.15-4.47	0.818	0.0	0.727	-2.17	0.275	Fixed
TT+TC vs CC	1.07	0.50-2.29	0.854	65.9	0.032	0.95	0.441	Random
TT vs TC+CC	1.05	0.24-4.57	0.953	8.7	0.334	-41.69	0.015	Fixed

AA, *P*=0.225), the heterozygous model of rs4986790 (GA vs AA, *P*=0.406) and rs4986791 (TC vs CC, *P*=0.593), the dominant genetic model of rs4986790 (GG+GA vs AA, *P*=0.256) and rs4986791(TT+TC vs CC, *P*= 0.441), or the recessive inheritance model of rs4986790 (GG vs GA+AA, *P*=0.255). However, obvious publication bias was noted for the homozygous model of rs4986791 (TT vs CC, *P*=0.016) and the recessive model of rs4986791 (TT vs TC+CC, *P*=0.015), likely because of the limited number of studies involved.

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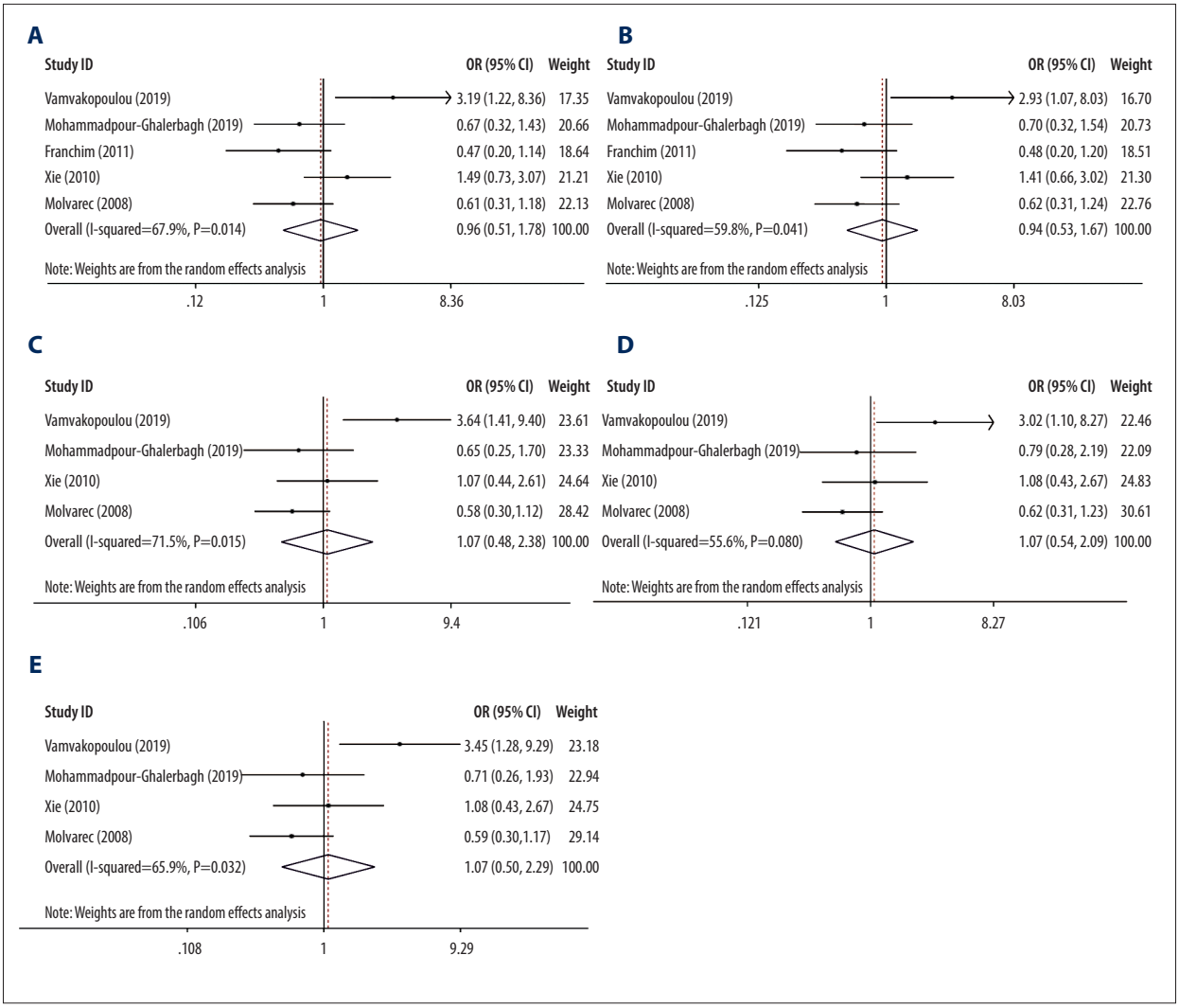

Figure 2. Forest plots for associations between *TLR4* polymorphisms and preeclampsia risk in the (**A**) allelic model of rs4986790: G vs A; (**B**) dominant model of rs4986790: GG+GA vs AA; (**C**) allelic model of rs4986791: T vs C; (**D**) heterozygous model of rs4986791: TC vs CC; and (**E**) dominant model of rs4986791: TT+TC vs CC.

Heterogeneity

As shown in **Table 3**, the estimation of heterogeneity using *Q* and I^2 tests was significant in some models, including the allelic and dominant genetic models of rs4986790 (G vs A, I^2=67.9%, *P*=0.014; GG+GA vs AA, I^2=59.8%, *P*=0.041) and the allelic, heterozygous, and dominant genetic models of rs4986791 (T vs C, I^2=71.5%, *P*=0.015; TC vs CC, I^2=55.6%, *P*=0.080; TT+TC vs CC, I^2=65.9%, *P*=0.032). Thus, the random effects model was applied to merge data. The derived forest plots are shown in **Figure 2**. Otherwise, none of the remaining models of these 2 SNP loci showed noticeable heterogeneity, and thus, the fixed effects model was employed.

Sensitivity Analysis

We performed a sensitivity analysis to further identify the possible origins of heterogeneity in different models of rs4986790 and rs4986791. The results are shown in **Table 4**. We used the leave-one-out method and repeated the meta-analysis of remaining studies. When the data from the study by Vamvakopoulou et al [31] were removed, no significant heterogeneity was observed in the allelic and dominant genetic models of rs4986790 or the allelic, heterozygous, and dominant genetic models of rs4986791. When the data from the study by Molvarec et al [42] were removed, no significant heterogeneity was observed in the heterozygous model of rs4986791. The forest plots of sensitivity analysis are shown in **Figure 3**. However, heterogeneity still existed after removal of the data from the other reports. The pooled ORs were

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Table 4. Results of the sensitivity analysis.

Except First Author (year)	Test of association			Test of heterogeneity	
	OR	95% CI	*P*	I^2 (%)	*P*
rs4986790 allelic model (G vs A)					
Vamvakopoulou (2019)	0.74	0.52-1.08	0.118	40.1	0.171
Mohammadpour-Gharehbagh (2019)	0.99	0.68-1.44	0.972	74.3	0.009
Franchim (2011)	1.05	0.73-1.51	0.808	69.9	0.019
Xie (2010)	0.81	0.56-1.18	0.271	70.5	0.017
Molvarec (2008)	1.06	0.72-1.56	0.765	71.5	0.015
rs4986790 dominant gene model (GG+GA vs AA)					
Vamvakopoulou (2019)	0.75	0.51-1.10	0.135	22.2	0.278
Mohammadpour-Gharehbagh (2019)	0.96	0.65-1.42	0.847	68.2	0.024
Franchim (2011)	1.02	0.70-1.51	0.905	61.5	0.051
Xie (2010)	0.80	0.54-1.19	0.278	63.5	0.042
Molvarec (2008)	1.03	0.69-1.55	0.882	64.3	0.038
rs4986791 allelic model (T vs C)					
Vamvakopoulou (2019)	0.70	0.44-1.12	0.137	0.0	0.542
Mohammadpour-Gharehbagh (2019)	1.11	0.72-1.12	0.643	79.4	0.008
Xie (2010)	1.00	0.64-1.55	0.980	80.9	0.005
Molvarec (2008)	1.42	0.85-2.35	0.179	70.1	0.035
rs4986791 heterozygous model (TC vs CC)					
Vamvakopoulou (2019)	0.76	0.47-1.24	0.271	0.0	0.630
Mohammadpour-Gharehbagh (2019)	1.06	0.67-1.70	0.803	69.3	0.039
Xie (2010)	1.00	0.61-1.60	0.914	70.2	0.035
Molvarec (2008)	1.38	0.80-2.37	0.249	47.0	0.152
rs4986791 dominant gene model (TT+TC vs CC)					
Vamvakopoulou (2019)	0.73	0.45-1.74	0.192	0.0	0.582
Mohammadpour-Gharehbagh (2019)	1.09	0.69-1.72	0.721	75.9	0.016
Xie (2010)	1.00	0.62-1.58	0.969	77.1	0.013
Molvarec (2008)	1.41	0.83-2.39	0.209	62.2	0.071

not significantly altered by the exclusion of individual datasets from the sensitivity analysis, which validated the stability and reliability of our results.

Discussion

Abnormalities in the immune response related to the uteroplacental environment may be implicated in some pregnancy-associated disorders, such as PE [43]. Innate immunity, a primary component of the immune system, plays a crucial role in host defenses against invading microorganisms, which are recognized by a set of PRRs, including TLRs, retinoic acid-inducible gene I-like receptors, and nucleotide-binding oligomerization domain-like receptors expressed on innate immune cells such as dendritic cells, monocytes, macrophages, natural killer (NK) cells, and γδ T cells [16].

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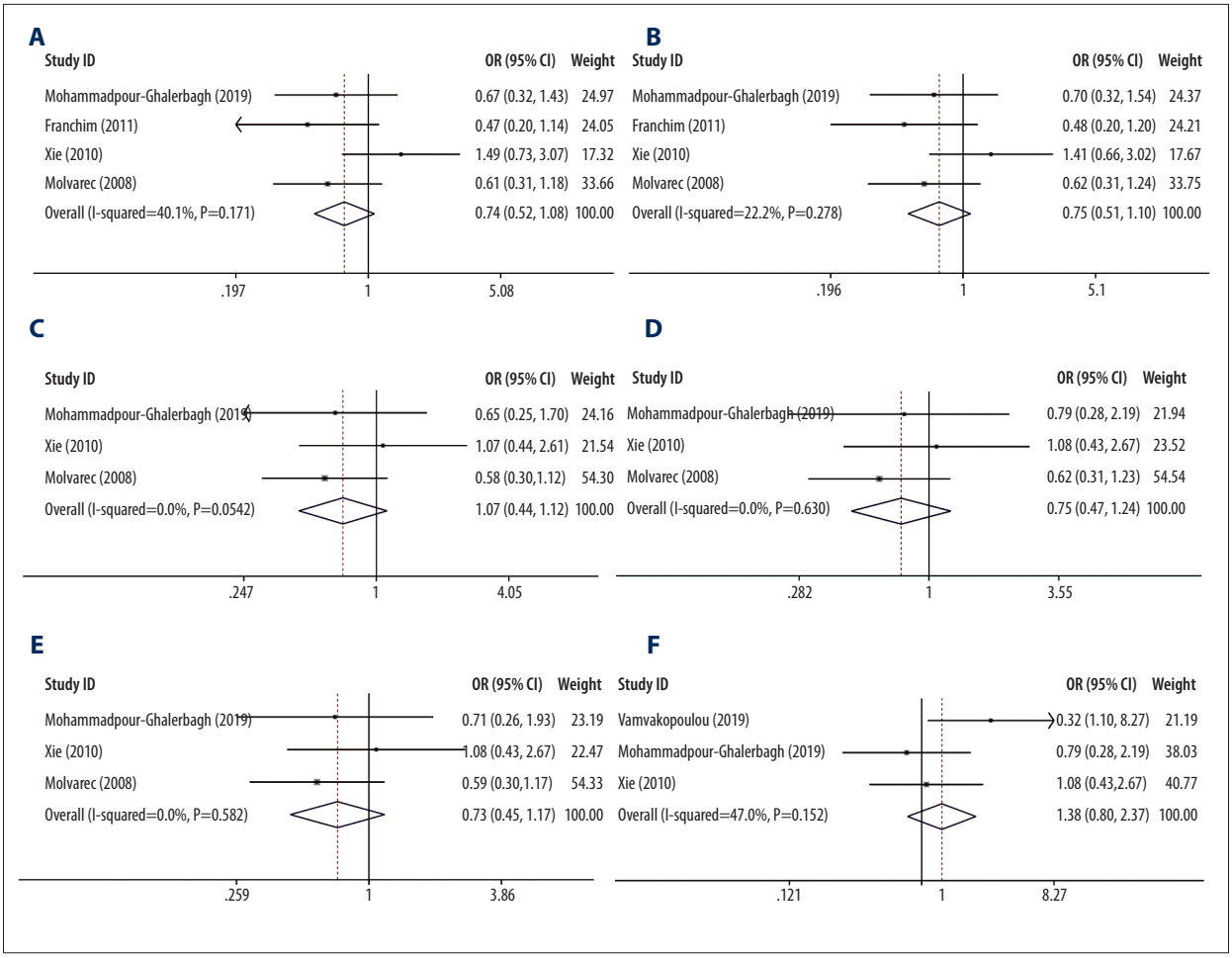

Figure 3. Forest plots of sensitivity analysis after removal of data from the study by Vamvakopoulou et al under the: (**A**) allelic model of rs4986790: G vs A; (**B**) dominant model of rs4986790: GG+GA vs AA; (**C**) allelic model of rs4986791: T vs C; (**D**) heterozygous model of rs4986791: TC vs CC; (**E**) dominant model of rs4986791: TT+TC vs CC or by removing the data from the study by Molvarec et al under (**F**) heterozygous model of rs4986791: TC vs CC.

Activation of the innate immune response mediates a protective response in the host by promoting the production of type I and III interferons, chemokines, and pro-inflammatory cytokines and subsequently triggering adaptive immunity [44,45]. Innate immune cells are responsible for protecting the mother from pathogens throughout pregnancy and are believed to be essential for the process of blastocyst implantation in the endometrium, trophoblast invasion, spiral artery remodeling, placentation, and fetal development [46]. Excess activation of the maternal immune response, as manifested in elevated neutrophil infiltration, macrophage polarization from M2 to M1, increased levels of γδ T cells, and a lack of decidual NK cells, can result in local and systemic inflammation, impaired angiogenesis, placental ischemia, organ dysfunction, and ultimately, the development of PE [47].

As a family of transmembrane glycoprotein receptors expressed on all innate immune cells, TLRs are more specifically and dynamically expressed on placental immune cells and trophoblasts during the progression of pregnancy [48]. TLR4 expression is detected in trophoblasts in the first trimester, and with advancing gestation, increases in syncytiotrophoblasts and villous vascular endothelium [49,50]. TLR4 also is expressed in the extravillous trophoblasts, including endovascular and interstitial trophoblasts in the placenta [21]. Theseresults indicate that TLR4 may play a fundamental role in normal placentation and successful pregnancy outcomes. Alteration in TLR4 expression and function at the maternal-fetal interface may be associated with the development of PE as a result of unnecessary activation of the innate immune system. To date, a growing number of researchers have investigated the role of polymorphisms in *TLR* gene sequences in susceptibility to PE, which is helpful for identifying promising marker candidates for early prediction and diagnosis of this disease. Xie et al have reported that the allelic variant rs5743708 in the *TLR2* gene appears to be associated with a lower threshold for development of

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early-onset PE [32]. The *TLR3* rs3775291 and rs3775296 SNPs affect the expression and location of TLR3, thereby influencing activity in the NF-κB signaling pathway. Chen et al did not observe significant differences in these 2 functional variants between patients with PE and healthy pregnant women [51]. Regarding the association between the 2 SNPs in the *TLR4* gene – rs4986790 and rs4986791 – and PE, previous studies have reported inconsistent results. Thus, the present meta-analysis aimed to resolve this issue by summarizing the data from related articles published to date.

Overall, the rs4986790 and rs4986791 polymorphisms in the *TLR4* gene were not significantly correlated with PE risk. Significant heterogeneity was observed for the allelic and dominant gene models of rs4986790 and the allelic, heterozygous, and dominant gene models of rs4986791. A subsequent sensitivity analysis identified the source of these heterogeneities and verified the robustness and reliability of our results.

Redman and Sargent proposed a 2-stage model for PE: insufficient spiral artery remodeling leads to poor placental perfusion in early pregnancy and subsequently contributes to widespread maternal endothelial damage and clinical manifestations [52]. Based on the time of onset, PE is divided into 2 categories, early-onset PE (EOP) and late-onset PE (LOP), which occur at <34 weeks and ≥34 weeks of gestation, respectively. Impaired placentation in early pregnancy and subsequent growth restriction often is associated with EOP, which is also called placental PE, while LOP, which is also called maternal PE, is associated with maternal endothelial dysfunction that can cause systemic vasoconstriction and ischemia in vital organs [53].

Our study included a predominantly White population; only 1 article, by Franchim [34], was conducted in mixed-race individuals. Thus, our results may apply to White women and the generalizability to other patients with different racial and ethnic backgrounds may be limited. Ethnic variation in the association between the risk of some diseases and the *TLR4* polymorphisms has been suggested. For *TLR4* rs4986790, a significant association has been found with Crohn disease and ulcerative colitis risk in Whites but not in Asians. While *TLR4* rs4986791 was associated with inflammatory bowel disease susceptibility only in Caucasians [54]. Ding et al revealed that the rs4986791 polymorphism decreased the risk of cancer in both Whites and Asians [55]. There are marked racial and ethnic differences in the prevalence of PE. In contrast with White women, Black and American Indian women have higher rates of PE [56]. Therefore, whether race has an influence on the relationship between the *TLR4* polymorphisms and risk of PE requires further study in a larger population that includes patients of other races.

The leave-one-out sensitivity analysis showed that the critical contributor to high heterogeneity was a single study by Vamvakopoulou et al [31], which evaluated the relationship between *TLR4* polymorphisms and EOP susceptibility in White women from central Greece. Carriers of these mutant alleles were more likely to suffer from EOP. Xie et al drew a similar conclusion [32]. No significant heterogeneity was observed when the meta-analysis was conducted without the data from Vamvakopoulou et al Considering the difference in the case populations between this study and the other 4 studies included in our meta-analysis, namely, patients with EOP and PE, respectively, we postulate that the case populations may be the main source of heterogeneity in our analysis. Our results indicate that the *TRL4* gene polymorphisms are not associated with PE susceptibility, but this conclusion may not be applicable to EOP. Nizyaeva et al studied the specific features of placental TLR4 expression in patients with EOP and LOP. Higher expression of TLR4 was detected in the vascular endothelium of placental villi from patients with EOP than patients with LOP, and the authors concluded that the severity of PE was related to the extent of damage to the placental villi [50]. This discrepancy in placental TLR4 expression between patients with EOP and LOP might be interpreted in the context of placental lesions in patients with EOP and maternal endothelial injury in patients with LOP. Therefore, the role of *TLR4* gene polymorphisms in PE risk may be associated with PE subtypes, particularly EOP. However, we were not able to perform a relevant stratified analysis because of the small sample size and lack of information on population subgroups.

Placenta samples were used in the study of the relationship between the *TLR4* polymorphisms and PE susceptibility by Abbas Mohammadpour-Gharehbagh et al [33], whereas blood samples were analyzed in the other 4 eligible studies. Removing the data from this report did not influence our results, indicating that the sample source may not contribute to the heterogeneity. The genetic predisposition to PE is believed to result from the interactions of maternal, fetal, and paternal genetic factors. The effects of maternal genetics on PE liability have been estimated to be 35%, of fetal genetics to be 20%, and the effects of combined maternal and paternal genetic effects to be 13% [57]. Clearly, further studies of fetal and paternal genetic effects are necessary to gain a better understanding of the genetic predisposition to PE; studies of the maternal genome alone are insufficient.

The present meta-analysis has 2 main limitations. First, because of the small number of studies included, a stratified analysis was not performed, and we were unable to assess whether *TLR4* polymorphisms are related to race, PE categories, specimen source, research method, or other factors. Second, the lack of sufficient information in qualified studies prevented us from studying the effects of other parameters, such as age, pregnancy outcome, lifestyle, and the interaction of *TLR4* polymorphisms with other genes or with environmental factors.

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Our meta-analysis provides conclusive evidence that *TLR4* polymorphisms may have no significant correlation with the risk of PE. Nonetheless, we are still unable to exclude the potential role of *TLR4* gene polymorphisms in the development of PE. Considering the limitations of the present study, high-quality, large-scale, prospective studies are needed to further validate our results.

Conclusions

Our findings indicate that the rs4986790 and rs4986791 SNPs in the *TLR4* gene are unlikely to play a vital role in the development of PE, primarily in a White population. Therefore, further high-quality, large-scale studies are warranted to provide a more precise estimate and evaluation of the association between SNPs of the *TLR4* gene and PE. Exploring the roles of other factors, such as race, age, PE categories, and contributing environmental factors, will provide additional insights into the mechanisms that underly development of PE.

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Conflict of Interest

None.

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