Investigation of *FOXP3* (rs3761548) polymorphism with the risk of preeclampsia and recurrent spontaneous abortion: A systemic review and meta−analysis

Govinda Sri Varshini[1#], Sivakumar Harshini[1#], Muhammed Ali Siham[1#], Govindaraj Krishnamurthy Tejaswini[1#], Yasam Santhosh Kumar[1#], Langeswaran Kulanthaivel[2], Gowtham Kumar Subbaraj[1✉]

[1]*Faculty of Allied Health Sciences, Chettinad Hospital and Research Institute, Chettinad Academy of Research and Education (Deemed to be University), Kelambakkam, Tamil Nadu 603 103, India*

[2]*Department of Bioinformatics, Alagappa University, Karaikudi−630 003, Tamil Nadu, India*

ABSTRACT

Objective: To investigate the association between forkhead box P3 (*FOXP3*) (rs3761548) polymorphism and the risk of preeclampsia and recurrent spontaneous abortion.

Methods: Literature on the association of *FOXP3* gene polymorphisms and susceptibility to preeclampsia and unexplained recurrent spontaneous abortion was retrieved by searching databases such as PubMed, Science Direct, Google Scholar and Embase from 2000 to 2021. The association measure was analyzed using an odds ratio (*OR*) and 95% confidence interval (*CI*). All the statistical analyses were executed using RevMan 5.4 software.

Results: In the present meta-analysis, 11 articles were analyzed. The pooled results showed no association between *FOXP3* gene polymorphism (rs3761548) and preeclampsia risk in allelic, recessive, dominant and over dominant contrast models. *FOXP3* gene polymorphism (rs3761548) showed an association with recurrent abortion in allelic, recessive and dominant models (*OR* 1.85, *CI* 1.59-2.14; *OR* 2.02, 95% *CI* 1.56-2.62; *OR* 2.69, 95% *CI* 1.50-4.83, respectively), while no association in the over dominant contrast model (*OR* 1.35, *CI* 0.87-2.10).

Conclusions: In the present study, *FOXP3* gene (rs3761548) polymorphism is associated with risk of recurrent spontaneous abortion but not preeclampsia. However, larger sample size and multiracial studies are needed in the future to confirm the findings.

KEYWORDS: Preeclampsia; *FOXP3* gene; Single nucleotide polymorphism; rs3761548; Unexplained recurrent spontaneous abortion

Preeclampsia is a hypertensive pregnancy condition that causes death and morbidity in both mother and fetus. It affects 5% of all pregnancies in every population; 2%-8% of all pregnancies worldwide (2 to 8 in 100)[2]. It is linked to placental perfusion problems, proteinuria, edema, and multiple organ failure. Its clinical manifestations range from mild hypertension to severe hypertension[3]. Preeclampsia causes cephalgia, unclear vision, light sensitivity, exhaustion, emesis, upper right abdominal pain, dyspnea, and contusions. Normal pregnancy generates unique conditions for the mother's immune system, allowing infection tolerance. Because the fetus expresses paternal antigens, a pregnant woman's immune regulatory competence is critical for a good pregnancy[4]. Although the reasons for this problem are unknown, the pathophysiology has an immunological basis. According to some of the recent researches, a loss of cytokine-mediated endothelium due to elevated maternal inflammation during the gestational period has a significant part in the pathophysiology of preeclampsia. The risk of pregnancy complications rises as the population of regulatory T (Treg) cells decreases. T helper-0 (CD4$^+$) cells give rise to T cells, but Treg cells suppress the immune system. Forkhead box P3 (FOXP3) variations show the progress of preeclampsia through quantitative or functional impacts on Treg CD4$^+$CD25$^+$[5].

#: They are first authors equally.

✉To whom correspondance may be addressed. E-mail: gowtham_phd@yahoo.com

1. Introduction

Preeclampsia is a severe pregnancy complication in which normal women have elevated blood pressure after 5 months of pregnancy[1].

Recurrent spontaneous abortion or unexplained recurrent abortion is a condition where two or more pregnancy miscarriages occur after 20 weeks of gestation[6]. Complex etiological factors like chromosomal (2%-5%), anatomic (15%), endocrine, autoimmune (20%), reproductive tract infections (1%-2%), and hormonal (20%) have been documented as causes of recurrent spontaneous abortion[7]. Women experiencing pregnancy difficulties, such as repeated pregnancy loss and hypertension, have been found to have lower amounts of circulating Treg. The lack of immune tolerance at the decidua is one of the reasons for unexplained recurrent spontaneous abortion. An association between the *FOXP3* gene polymorphism and preeclampsia, recurrent spontaneous abortion risk was previously reported in multiple case-control studies.

Therefore, to investigate these genetic associations, systematic review and meta-analysis are required. The present systematic review and meta-analysis were designed to determine the association between the *FOXP3* gene polymorphism and the risk of diseases such as preeclampsia and recurrent spontaneous abortion.

2. Materials and methods

This systematic review and meta-analysis was reported as per Preferred Reporting Items for Systematic Review and Meta Analysis (PRISMA) guidelines. The review protocol was registered prospectively with PROSPERO (Id No 32617).

2.1. Literature search

In this study, all the articles were retrieved from various databases such as PubMed, Science Direct, Google Scholar and Embase from 2000-2021. The English language was used for the search strategy. Keywords used to retrieve the articles were "*FOXP3*", "rs3761548", "single nucleotide polymorphism", "preeclampsia", "recurrent abortion", "gene polymorphism", and "hypertension". An advanced search was conducted using the wildcard symbol '*' in combination with Boolean operators ('AND', 'OR', 'NOT') to narrow down the search scope. Additional references were manually searched from the lists of references in all related reviews and articles.

2.2. Inclusion and exclusion criteria

We selected the studies based on the following exclusion criteria: i) case-control studies; ii) studies which evaluated the association of the *FOXP3* (rs3761548) gene polymorphism and risk of preeclampsia and recurrent abortion with allelic frequency and genotyping with a 95% confidence level were included. Only English-language articles were considered.

Exclusion criteria were: i) studies that contained only control groups; ii) studies that were reported on animal studies; iii) review articles; iv) studies that did not contain genotypic data.

2.3. Data extraction

In the present meta-analysis, three authors (Govinda Sri Varshini, Sivakumar Harshini, Muhammed Ali Siham) extracted the data from various articles that contained the year of publication, journal and author's name, methodology, study type, allelic frequency, genotyping of 95% confidential interval (*CI*) with odds ratio (*OR*), and the *P*-value of Hardy Weinberg equilibrium. The Newcastle-Ottawa tool was used to assess the risk of bias in observational studies. Studies were classified as high-quality (≥7 stars), medium-quality (4-6 stars) or low quality (0-3 stars). The data were extracted into a table and were analyzed to improve screening adaptability.

2.4. Heterogeneity, sensitive analysis and publication bias

In order to assess the possibility of heterogeneity, the Mantel-Haenszel model was used. Heterogeneity was done by forest plot. For estimating publication bias, we used Egger's linear regression test and visual funnel plots. There can be a publication bias if there was an asymmetric plot; *t*-test can be used for verification. Sensitivity analysis was not performed due to less number of studies.

2.5. Statistical analysis

In the present meta-analysis, 95% *CI* with *OR* was assessed to find the association of *FOXP3* gene polymorphism with the risk of preeclampsia and recurrent abortion. The statistical considerable value of *P* was less than 0.05. A *P* value of less than 0.1 indicated the presence of heterogeneity (I^2) and I^2 value of 25%, 50% and 75% represented low, moderate and high heterogeneity, respectively. When I^2 is less than 50%, fixed effects model was used, and random effects model was used when I^2 is between 50% to 90% as it represents substantial heterogeneity between the studies. For the Hardy Weinberg equilibrium examinations, a *Chi*-square test was executed. Hardy Weinberg equilibrium for random and fixed effetcs models was carried out. The meta-analysis was conducted using the software Review Manager 5.4.

3. Results

3.1. Search results

The preliminary searches in Science Direct, PubMed, Google Scholar, and Embase resulted in the retrieval of 100 articles relevant to the present study. Fifty articles were removed due to replicative and duplicative contents. Articles that were irrelevant to topics and not of human subjects were excluded. After excluding studies with overlapping data, irrelevant data, and other languages, 25 articles were screened, with 11 being eligible for the qualitative analysis. The search strategy of *FOXP3* gene polymorphism is shown in Figure 1.
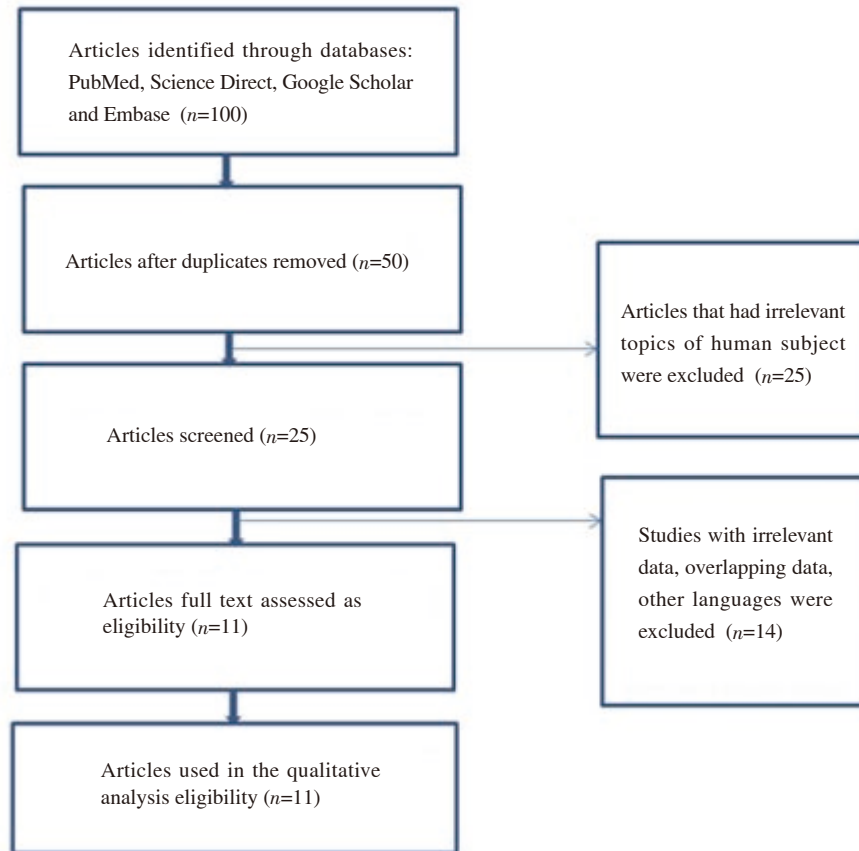
Figure 1. Flow chart of the literature search and selection of *FOXP3* gene polymorphism.

Eventually, in the present meta-analysis, the literature showed a total of 5 studies for the association of *FOXP3* gene with preeclampsia consisting of 1 370 cases and 1 257 controls, and 6 studies for recurrent abortion consisting of 736 cases and 802 controls.

Table 1 illustrates the characterstics features such as ethnicity, genotype (cases/conrols) of the present investigation. The gene polymorphism of *FOXP3* and preeclampsia risk showed four Asian studies[8–10,5] and one Caucasian study[11]. The *FOXP3* gene polymorphism with risk of recurrent abortion showed five Asian studies[12–16] and one Caucasian study[17].

3.2. Quantitative data analysis

A total of five studies were included in this meta-analysis in order to evaluate *FOXP3* gene polymorphism and its association with preeclampsia risk. According to the pooled results, the *FOXP3* polymorphism was not associated with preeclampsia risk in allelic (*OR* 0.78, *CI* 0.54-1.13), recessive (*OR* 0.64, *CI* 0.35-1.15), dominant (*OR* 0.77, *CI* 0.51-1.16), and over dominant contrast models (*OR* 1.17, *CI* 0.99-1.37). The subgroup analysis for heterogeneity was also

assessed and the results showed no association in allelic, recessive, dominant and over dominant contrast models (Figure 2, 3).

A total of 6 studies were examined for the association of the *FOXP3* gene polymorphism and recurrent spontaneous abortion risk. The pooled results revealed that the *FOXP3* polymorphism was associated with recurrent abortion risk in allelic (*OR* 1.85, *CI* 1.59-2.14), recessive (*OR* 2.02, *CI* 1.56-2.62) and dominant models (*OR* 2.69, *CI* 1.50-4.83), while no association in the over dominant contrast model (*OR* 1.35, *CI* 0.87-2.10). The subgroup analysis for heterogeneity was also assessed and the results showed an association in dominant, allelic and recessive models, while no association in the over dominant contrast model (Figure 4, 5).

3.3. Heterogeneity, sensitive analysis and publication bias

Sensitivity analysis for individual studies was not done due to small number of studies. Heterogeneity was done by forest plot and publication bias was analyzed using funnel plot. The funnel plot displayed the y-axis as sample size and the x-axis as effect size. All the results were constant, suggesting that the present study results were statistically stable (Figure 6, 7).

Table 1. Characteristics of the studies for the association of *FOXP3* polymorphisms with preeclampsia and recurrent abortion risk.

Polymorphism	Name of the author and year	Ethnicity	Genotype cases/Controls			Total cases/Controls
			AA	AC	CC	
FOXP3 pre-eclampsia	Cekin *et al* 2020	Caucasian	145/165	270/265	85/70	500/500
	Chen *et al* 2019	Asian	5/5	86/95	112/143	203/243
	Chen *et al* 2013	Asian	6/2	62/40	184/114	252/156
	Gholami *et al* 2018	Asian	17/33	69/63	47/47	133/143
	Jahan *et al* 2013	Asian	78/117	134/79	70/19	282/215
FOXP3 recurrent abortion	Dirsipam *et al* 2021	Asian	35/18	102/60	13/72	150/150
	Farhan *et al* 2018	Caucasian	14/14	24/16	2/10	40/40
	Saxena *et al* 2015	Asian	45/33	87/128	68/139	200/300
	Jaber *et al* 2017	Asian	30/14	37/42	33/44	100/100
	Wu *et al* 2012	Asian	75/42	56/45	15/25	146/112
	Mishra *et al* 2018	Asia	13/7	52/46	35/47	100/100
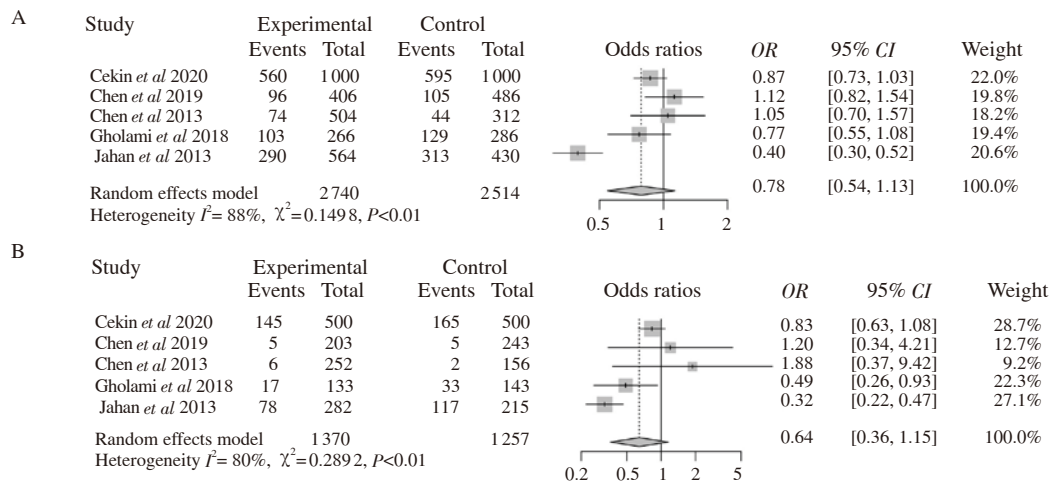
Figure 2. Forest plots for the association of *FOXP3* gene polymorphism with preeclampsia risk under allelic (A), recessive variants (B).

Figure 3. Forest plots for the association of *FOXP3* gene polymorphism with preeclampsia risk under dominant (A) and over dominant variants (B).

A

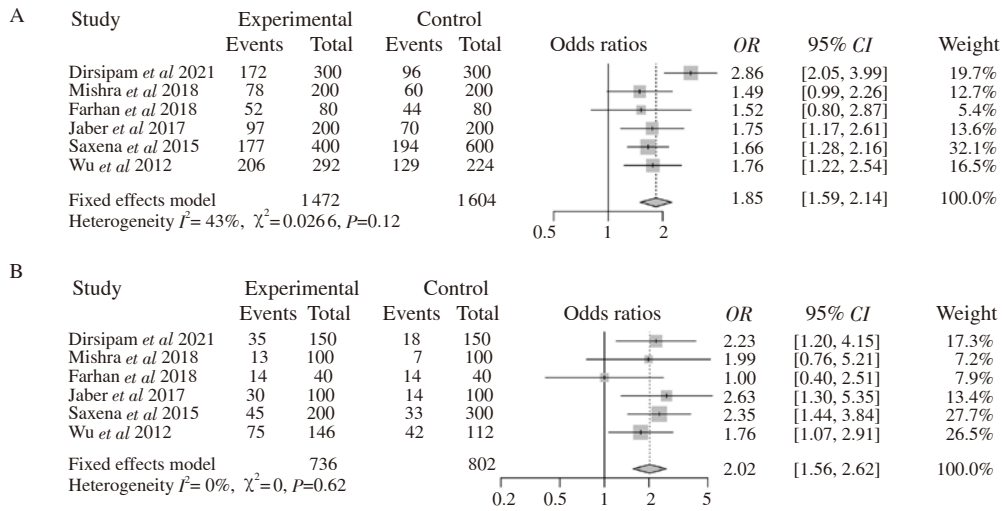| Study	Experimental		Control		Odds ratios	OR	95% CI	Weight
	Events	Total	Events	Total				
Dirsipam *et al* 2021	172	300	96	300		2.86	[2.05, 3.99]	19.7%
Mishra *et al* 2018	78	200	60	200		1.49	[0.99, 2.26]	12.7%
Farhan *et al* 2018	52	80	44	80		1.52	[0.80, 2.87]	5.4%
Jaber *et al* 2017	97	200	70	200		1.75	[1.17, 2.61]	13.6%
Saxena *et al* 2015	177	400	194	600		1.66	[1.28, 2.16]	32.1%
Wu *et al* 2012	206	292	129	224		1.76	[1.22, 2.54]	16.5%
Fixed effects model		1472		1604		1.85	[1.59, 2.14]	100.0%

Heterogeneity I^2= 43%, χ^2=0.0266, P=0.12

B

Study	Experimental		Control		Odds ratios	OR	95% CI	Weight
	Events	Total	Events	Total				
Dirsipam *et al* 2021	35	150	18	150		2.23	[1.20, 4.15]	17.3%
Mishra *et al* 2018	13	100	7	100		1.99	[0.76, 5.21]	7.2%
Farhan *et al* 2018	14	40	14	40		1.00	[0.40, 2.51]	7.9%
Jaber *et al* 2017	30	100	14	100		2.63	[1.30, 5.35]	13.4%
Saxena *et al* 2015	45	200	33	300		2.35	[1.44, 3.84]	27.7%
Wu *et al* 2012	75	146	42	112		1.76	[1.07, 2.91]	26.5%
Fixed effects model		736		802		2.02	[1.56, 2.62]	100.0%

Heterogeneity I^2= 0%, χ^2=0, P=0.62

Figure 4. Forest plots for the association of *FOXP3* gene polymorphism with recurrent abortion risk under allelic (A), recessive variants (B).

A

Study	Experimental		Control		Odds ratios	OR	95% CI	Weight
	Events	Total	Events	Total				
Dirsipam *et al* 2021	137	150	78	150		9.73	[5.06, 18.69]	17.4%
Mishra *et al* 2018	65	100	53	100		1.65	[0.93, 2.91]	18.4%
Farhan *et al* 2018	38	40	30	40		6.33	[1.29, 31.11]	8.4%
Jaber *et al* 2017	67	100	56	100		1.60	[0.90, 2.83]	18.4%
Saxena *et al* 2015	132	200	161	300		1.68	[1.16, 2.43]	20.5%
Wu *et al* 2012	131	146	87	112		2.51	[1.25, 5.03]	16.9%
Random effects model		736		802		2.69	[1.50, 4.83]	100.0%

Heterogeneity I^2= 81%, χ^2=0.3980, P<0.01

B

Study	Experimental		Control		Odds ratios	OR	95% CI	Weight
	Events	Total	Events	Total				
Dirsipam *et al* 2021	102	150	60	150		3.19	[1.98, 5.12]	18.0%
Mishra *et al* 2018	52	100	46	100		1.27	[0.73, 2.22]	16.6%
Farhan *et al* 2018	24	40	16	40		2.25	[0.92, 5.50]	11.7%
Jaber *et al* 2017	37	100	42	100		0.81	[0.46, 1.43]	16.5%
Saxena *et al* 2015	87	200	128	300		1.03	[0.72, 1.48]	19.7%
Wu *et al* 2012	56	146	45	112		0.93	[0.56, 1.53]	17.5%
Random effects model		736		802		1.35	[0.87, 2.10]	100.0%

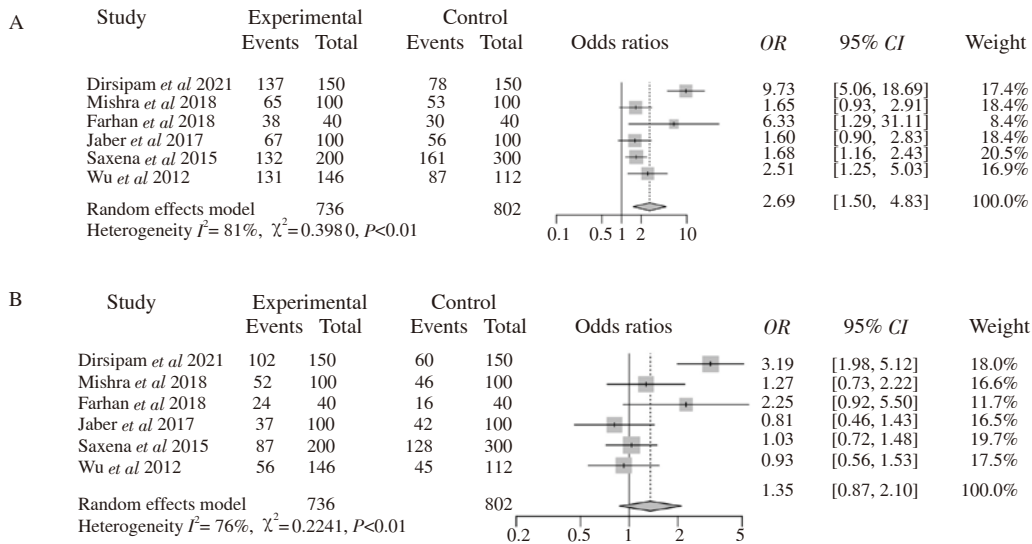Heterogeneity I^2= 76%, χ^2=0.2241, P<0.01

Figure 5. Forest plots for the association of *FOXP3* gene polymorphism with recurrent abortion risk under dominant (A) and over dominant models (B).
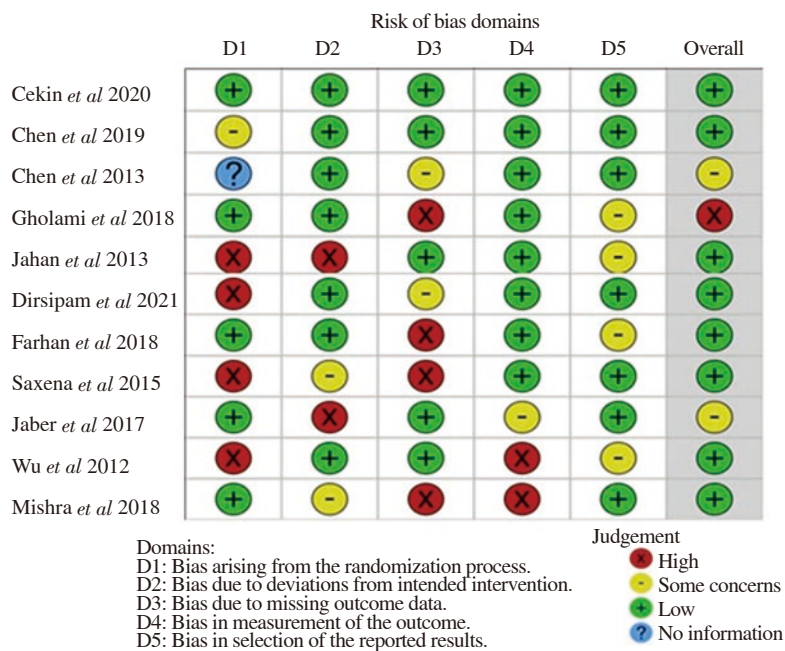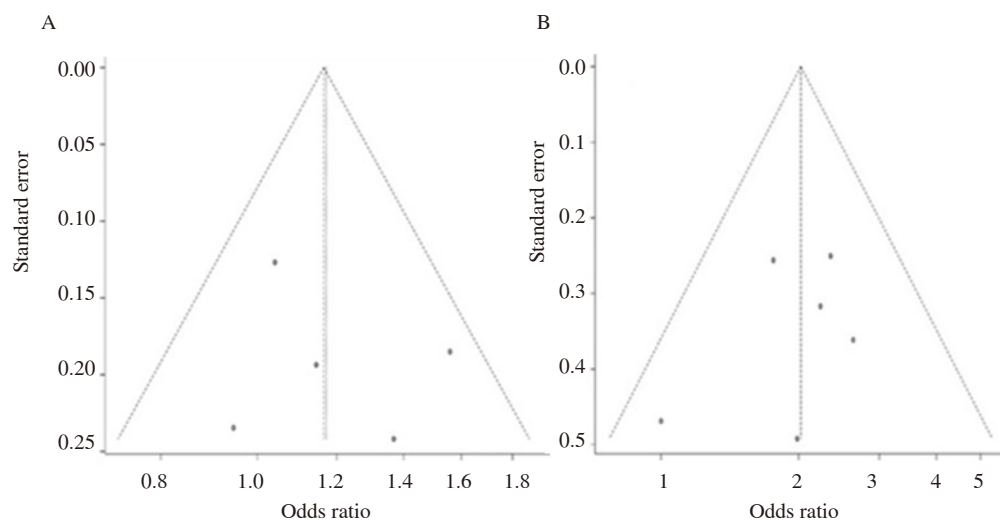

Figure 6. Risk of bias summary.

Domains:
D1: Bias arising from the randomization process.
D2: Bias due to deviations from intended intervention.
D3: Bias due to missing outcome data.
D4: Bias in measurement of the outcome.
D5: Bias in selection of the reported results.

Judgement
High
Some concerns
Low
No information

Figure 7. Publication bias analyzed by the funnel plot association of *FOXP3* gene polymorphism with various diseased conditions: A) Preeclampsia; B) Recurrent abortion.

4. Discussion

Meta-analysis is a formal, epidemiological, quantitative design that methodically assesses the outcomes of previous studies to determine the conclusions about that body of research[18]. The current meta-analysis includes a total of 2 106 cases and 2 059 controls gathered from different databases. Xp11.23 encodes FOXP3 protein, a member of the forkhead/winged-helix transcription factor family. It functions as a transcriptional regulator in the low-cytokine production of Tregs[10]. There are four potential functional domains: an *N*-terminal repressor, a zinc finger, a leucine zipper, and a *C*-terminal fork. At the *N*-terminus of FOXP3, a repressor domain is present, which inhibits the nuclear factor of activated T-cells mediated transcription process. The FKH domain is required for both DNA binding and nuclear localization. The fork-head domain is the most frequently attacked in patients with immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome. The FOXP3 protein is transcribed by regulatory T cells[12].

FOXP3 is also assiciated with allergic rhinitis, endometrial cancer, parasitic and viral infection, HIV, autoimmune diseases, transplantation tolerance. Chen *et al*[19] reported the *FOXP3* gene polymorphism association with cancer risk in the Chinese population and the results of pooled data showed an increased risk of cancer in the AA genotype particularly in the A allele. Zhang *et al*[20] reported the *FOXP3* gene polymorphism with multiple sclerosis risk and the results revealed that dominant and allelic variants showed a strong association while recessive and over dominant variants were associated with multiple sclerosis susceptibility. Li *et al*[21] reported

the *FOXP3* gene polymorphism and risk of Graves' disease and the results revealed that the *FOXP3* gene polymorphism was associated with the risk of Graves' disease among the Asian population, while *FOXP3* gene polymorphism in Caucasia people cannot be determined due to less number of studies. Pan *et al*[22] reported the case-control study of *FOXP3* rs2232365 gene polymorphism with the risk of preeclampsia and the results of pooled data showed an increased risk of preeclampsia in CC genotype particularly in C. Zhang *et al*[23] reported *FOXP3* gene polymorphism with the risk of allergic rhinitis and the results revealed that *FOXP3* gene polymorphism was not associated with the risk of allergic rhinitis. He *et al*[24] reported the association of *FOXP3* gene polymorphism with the risk of autoimmune disorders and the results showed autoimmune disorders risk in the AA genotype particularly in the A allele.

Karimian *et al*[25] reported the meta-analysis of *FOXP3* rs3761548 gene polymorphism with the risk of preeclampsia. The results revealed that *FOXP3* rs3761548 gene polymophism may be a protective risk factor against preeclampsia. Hosseini Teshnizi *et al*[26] reported the meta-analysis of *FOXP3* rs3761548 gene polymorphism with the outcome of pregnancy. The results showed that *FOXP3* rs3761548 gene polymorphism was associated with the immune related pregnancy complication. In the present investigation, we perfomed meta-analysis to reveal the association of *FOXP3* gene polymorphism with the risk of recurrent spontaneous abortion. The results of preeclampsia showed no association in allelic, recessive, dominant and over dominant contrast models. The results of recurrent spontaneous abortion showed an association in dominant,

allelic and recessive models, while no association was observed in over dominant contrast model.

In the present study, all the outcomes of *FOXP3* gene polymorphism were fit for Hardy Weinberg equilibrium. We analyzed the publication bias for *FOXP3* gene polymorphism and all the results were statistically significant and stable.

There are some limitations in the study. The results regarding association of *FOXP3* gene polymorphism with the risk of diseases such as preeclampsia and recurrent spontaneous abortion were based on combining unadjusted findings of eligible studies due to the lack of raw data. The *FOXP3* gene polymorphism may also be affected by environmental factors. Unfortunately, we did not perform comparison between different ethnicities (Asian and Caucasian) due to the limited number of studies.

In conclusion, our meta-analysis shows that *FOXP3* gene (rs3761548) polymorphism is associated with risk of recurrent spontaneous abortion but not preeclampsia. The forest plot is plotted using the random and fixed effects models; publication bias is analyzed using funnel plot. This article discusses preeclampsia, a polygenic disease that can be influenced by a variety of factors, with *FOXP3* being an important factor that should not be ignored. The study further explains the relationship between the functional polymorphism rs3761548 A/C and recurrent spontaneous abortion. However, more extensive investigations are needed in future to explore the *FOXP3* gene polymorphism with other ethnic groups.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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Authors' contributions

Govinda Sri Varshini, Sivakumar Harshini, Muhammed Ali Siham, Govindaraj Krishnamurthy Tejaswini and Yasam Santhosh Kumar performed data analysis and wrote the manuscript. Langeswaran Kulanthaivel involved in the data validation and revison. Gowtham Kumar Subbaraj designed the study and approved the final version of the manuscript.

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