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Association of maternal *AGT* rs699 polymorphism and preeclampsia: meta-analysis

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Abstract

Background: Preeclampsia (PE), a hypertensive disorder associated with pregnancy, is a leading cause of both maternal and neonatal morbidity and mortality. Ongoing research into the genetic factors underlying this condition is significant, but the role of the renin-angiotensin system gene polymorphism (rs699), has yielded varied and inconclusive results, with discrepancies in findings across different geographical and ethnic populations. The aim of this study: This meta-analysis is aimed to establish a more robust and dependable correlation between *AGT* (rs699) polymorphism and the risk of PE. Materials and methods: A comprehensive literature search for relevant studies was performed in electronic databases between January 2017 and December 2024. A total of 13 studies were included in this meta-analysis. Odds ratios (ORs) and 95 % confidence intervals (CIs) were calculated using five genetic models to assess the strength of the association, and a heterogeneity test was performed. Results: The results revealed a statistically significant association between the *AGT* rs699 polymorphism and the risk of developing PE in all genetic models, including dominant (OR = 1,73, 95 % CI [1,45–2,05], p <0,00001), recessive (OR = 1,46, 95 % CI [1,14–1,87], p = 0,03), homozygote (OR = 2,03, 95 % CI [1,37–3,00], p = 0,0004), and heterozygote (OR = 1,26, 95 % CI [1,05–1,52], p <0,00001) models. No heterogeneity was observed in two genetic models (dominant and heterozygote, I^2 = 18 % and 0 %, respectively). For subgroup analysis, a number of significant associations were identified. However, the analysis of subgroups based on race revealed an absence of significant associations in the mongoloid and negroid subgroups. It should be noted that only a single study was analysed in these specific subgroups, which may limit the robustness of the findings. No evidence of

publication bias Egger's test p-value > 0,05 in all genetic models. Conclusion: Our results indicate that the *AGT* rs699 polymorphism is significantly associated with an increased risk of PE. This increased risk was also observed in all subgroups analysis, except for Negroid and Mongoloid in racial subgroups.
Keywords: Pre-eclampsia; polymorphism; rs699; renin-angiotensin system.

Ассоциация материнского полиморфизма *AGT* rs699 и преэклампсии: мета-анализ

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Аннотация

Введение: Преэклампсия (ПЭ), гипертензивное расстройство, связанное с беременностью, является одной из основных причин материнской и неонатальной заболеваемости и смертности. Проводимые исследования генетических факторов, лежащих в основе этого заболевания, имеют большое значение, однако изучение роли полиморфизма гена ренин-ангиотензиновой системы (rs699) дало разнообразные и неубедительные результаты, а также расхождения в результатах в различных географических и этнических популяциях. Цель исследования: Данный мета-анализ направлен на установление более надежной и достоверной корреляции между полиморфизмом *AGT* (rs699) и риском развития ПЭ. Материалы и методы: Всесторонний поиск релевантных исследований был проведен в электронных базах данных в период с января 2017 года по декабрь 2024 года. Всего в мета-анализ было включено 13 исследований. Для оценки силы ассоциации с помощью пяти генетических моделей были рассчитаны коэффициенты вероятности (OR) и 95 % доверительные интервалы (CI), а также проведен тест на гетерогенность. Результаты: Полиморфизм *AGT* rs699 был значительно связан с риском ПЭ во всех генетических моделях (доминантный OR = 1,73, 95 % CI [1,45-2,05], p<0,00001; аллельный OR = 1,48, 95 % CI [1,25-1,74], p<0,0001; рецессивный OR = 1,46, 95 % CI [1,14-1,87], p = 0,03; гомозиготный OR = 2,03, 95 % CI [1,37-3,00], p = 0,0004; гетерозиготный OR = 1,26, 95 % CI [1,05-1,52], p <0,00001). В двух генетических

моделях (доминантной и гетерозиготной, I^2 = 18 % и 0 % соответственно) гетерогенности не наблюдалось. При анализе подгрупп был выявлен ряд значимых ассоциаций. Однако анализ подгрупп по расовому признаку показал отсутствие значимых ассоциаций в монголоидной и негроидной подгруппах. Следует отметить, что в этих конкретных подгруппах было проанализировано только одно исследование, что может ограничить надежность полученных результатов. Отсутствие признаков смещения публикаций Тест Эггера p-значение > 0,05 во всех генетических моделях. Выводы: Полученные нами результаты свидетельствуют о том, что полиморфизм *AGT* rs699 достоверно ассоциирован с повышенным риском развития ПЭ. Этот повышенный риск также наблюдался во всех анализируемых подгруппах, за исключением негроидной и монголоидной расовых подгрупп.
Ключевые слова: Преэклампсия; полиморфизмы; OGG1; SOD2.

Introduction

The problem of preeclampsia (PE) is one of the most important problems in modern obstetrics. The frequency of PE according to different estimates is from 3 to 5% of all pregnancies (Giardini et al., 2022; Torres et al., 2024). This condition is primarily characterized by the development of hypertension after the twentieth week of pregnancy, often accompanied by dysfunctions in various organs such as the kidneys, liver, cardiovascular system, brain, and placenta, as reported in studies (Brown et al., 2018; ACOG, 2020). It can be the cause of premature detachment of the normally located placenta (PNRP), massive bleeding during and after delivery, and low birth weight babies (Giardini et al., 2022; de Mendonça et al., 2022; Fondjo et al., 2022). Complications of PE also include placental insufficiency and delayed fetal development syndrome (FDDS) (Alonso-Ventura et al., 2020; Kornacki et al., 2020; Tarca et al., 2022).

The renin-angiotensin system (RAS) is implicated in the pathogenesis of PE (Arthurs et al., 2019). It is thought that the activation of the RAS, which is the body's natural response to low blood pressure and low blood volume, results in increased vascular resistance and increased blood pressure, which can lead to PE (Gathiram et al., 2020). The RAS has been linked to the development of abnormal placental vascularization, which is thought to be a major factor in the development of PE (Haram et al., 2020). Additionally, the compensatory alterations in the RAS contribute to the salt-water balance and sufficient placental perfusion for the mother and fetus (Delforce et al., 2019). The complex metabolic pathway mediated by RAS encompasses a diverse array of genes, with their distinct genetic variants potentially serving as a key determinant in the pathogenesis of

various conditions, including PE. The *AGT* gene is located on the long arm of chromosome 1 and consists of five exons and four introns. This gene has two common polymorphisms, (*AGT*: T704C; M235T; rs699) and (*AGT*: C521T; T174M; rs4762), which are associated with angiotensinogen expression. The second exon of the gene contains the rs699 polymorphism. Studies have shown that this polymorphism is linked to increased AGT levels and may be a risk factor for PE (Chengalvala et al., 2017; Nathaniel et al., 2024). The minor T allele of the rs699 polymorphism has been linked to aberrant remodeling of spiral arteries. This may explain why it is associated with PE. Study by Afshariani et al., (Afshariani et al., 2014) found that the presence of the TT genotype of the rs699 polymorphism was associated with hypertension during pregnancy. Another study by Aung et al., (Aung et al., 2017) revealed that the T allele of the rs699 polymorphism may be implicated in the development of PE. In addition, Shahvaisizadeh et al., (Shahvaisizadeh et al., 2014) showed that this polymorphism affects the likelihood of developing PE at an earlier stage. However, a study by Choi et al (Choi et al., 2004) confirmed that no differences were observed between the control group and PE group for *AGT* rs699. The *AGT* rs699 polymorphism was ultimately included in our meta-analysis due to the imbalance of the RAAS system it causes and various studies focusing on it that show inconsistent results regarding its correlation with PE. To the best of our knowledge, inconsistencies may be attributable to differences in geographic location, ethnic background, and sample size.

Materials and methods

1. Systematic search strategy

A meta-analysis was performed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines (Moher et al., 2009). The search for original articles was conducted from January 2017 to December 2024, with the aim of investigating the association between *AGT* rs699 and the risk of PE. The systematic search encompassed various databases, including PubMed, Google Scholar, ScienceDirect, Web of Science, the Cochrane Library, and CyberLeninka. The search was conducted in both English and Russian, employing terms related to polymorphisms and PE, such as "preeclampsia" OR "pre-eclampsia" OR "gestosis," as well as "polymorphism" OR "genetic variant" OR "SNP" AND "rs699" OR "T704C" OR "Met235Thr" OR "M235T". Additionally, more articles were identified through reference lists of relevant studies.

2. Eligibility criteria

In order to minimize bias, we subjected the titles and abstracts of all the research articles that emerged to analysis, subsequently evaluating them based on the inclusion and exclusion criteria. A study was deemed eligible for inclusion if it met the following criteria: 1. The study sought to assess the relationship between *AGT* rs699 and PE; 2. It was a human case-control study; 3. Genotyping data were available to calculate the odds ratios (ORs) with a 95 % confidence interval (CI). The exclusion criteria were as follows: 1; Meta-analyses. 2; Review articles; 3. Animal studies; 4. Cohort designed studies; 5. Studies with insufficient data.

3. Data extraction

After eliminating duplicates, the essential data from each included study were extracted. These data included, but were not limited to: the first author, publication year, country, geographical location, ethnicity, age, gestation weeks, degree of PE, genotyping method, and any other available statistical or clinical information. In the meta-analysis, all controls were assessed for Hardy–Weinberg equilibrium (HWE) using the chi-square test, with the exception of two studies, whose p-values were less than 0,05. Departures from HWE may be attributed to various factors, such as population stratification, selective bias, or errors in genotyping. Despite this, we included these two studies in our further analysis due to their significance in subgroup analysis.

4. Statistical analysis

All analyses were performed using Review Manager 5.3 software, with the odds ratio (OR) and 95 % confidence interval (95 % CI) being utilised to investigate the effect strength of the associations between *AGT* rs699 polymorphism and PE risk. The HWE was assessed by the chi-squared test for every study in the control group. The genetic models employed in our meta-analysis encompass the allelic model (C vs. T), the dominant model (CC + CT vs. TT), the recessive model (CC vs. CT + TT), the heterozygous model (CT vs. TT), and the homozygous model (CC vs. TT). Subsequently, the Cochrane's Q-statistic test was employed to ascertain heterogeneity in the meta-analysis, and the I^2 statistic (I^2 value > 50 % or P value < 0,10 was deemed to indicate significant heterogeneity) was utilised to quantify it. In instances where the I^2 value was significant (> 50 %), the random-effect model (REM) was employed; conversely, the fixed-effect model (FEM) was used in cases where the I^2 value was not significant. Subgroup analysis was conducted, stratifying by geography (Asian, African, and European), ethnicity (Caucasoid, Mongoloid, Negroid, and Mixed race), gestational week (early-onset PE "EOPE", late-onset PE "LOPE", and mixed), and case sample size (< 100, ≥ and < 200, ≥ 200). The presence of publication bias was evaluated through visual

inspection of funnel plots and the Egger's test p-value (Niemeyer et al., 2013). In the event that publication bias was identified, the 'trim and fill' method (Wang et al., 2017) was employed. This method involves the conservative estimation of hypothetical negative unpublished studies to mirror the positive studies that cause funnel plot asymmetry, thereby facilitating an assessment of the potential effect of publication bias. A p-value less than 0,05 was considered to be statistically significant.

Results

1. Inclusion strategy

Upon meticulous examination of all citations retrieved from scientific databases, a comprehensive meta-analysis encompassing 13 papers was conducted, involving 15 distinct datasets pertaining to *AGT* rs699. Through data mining, 88 publications were initially identified that explored the genetic polymorphism *AGT* rs699 in relation to PE. Following the elimination of duplicate citations, 52 articles were subject to rigorous screening and evaluation against predetermined eligibility criteria, ultimately resulting in the selection of the final number of studies for inclusion. Fig. 1 illustrates the meticulous process of exclusion and selection, adhering to the principles outlined in PRISMA guidelines.

2. Included studies characteristics

Table 1 presents a comprehensive overview of the key features of the studies that were incorporated into the meta-analysis of *AGT* rs699 polymorphism. A total of 15 data sets were employed for the analysis, which were conducted using dominant, recessive, allelic, homozygote, and heterozygote genetic models, encompassing a sample size of 4,010 individuals, comprising 1,801 cases and 2,209 controls, representing diverse ethnic backgrounds.

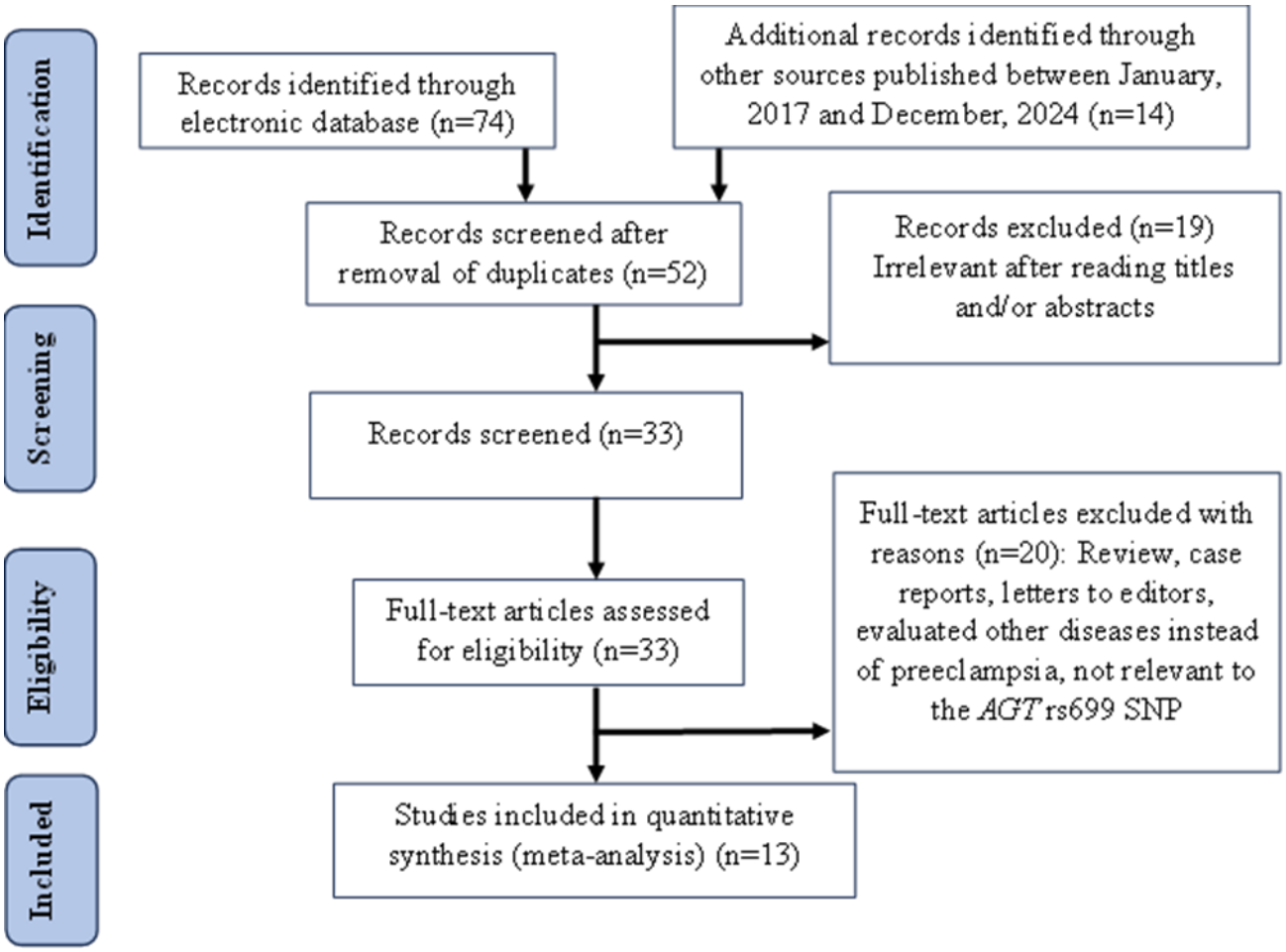


Fig. 1 - Flow chart of inclusion and exclusion in meta-analysis according to PRISMA guidelines.

3. Meta-analysis results

In all of the investigated genetic models — dominant, recessive, allelic, homozygote, and heterozygote — *AGT* rs699 exhibited a significant correlation with an increased risk of PE, with overall P-values of <0,03 in all genetic models. The findings are presented in Table 2 and in the form of forest plots depicted in Fig. 2. The extensive

research undertaken on this polymorphism explains the substantial heterogeneity observed in three of the models, with statistically significant moderate heterogeneity detected in allelic, recessive, and homozygote models with (I^2 = 54 %, 45 %, 50 %, respectively) and corresponding p-values (p = 0,007, 0,03, 0,02, respectively). As a result, REM analysis was employed for meta-analysis across these three models: recessive, allelic, and homozygote, while FEM analysis was applied in the two other models: dominant and heterozygote, due to the absence of heterogeneity.

Table 1 - Main characteristics of included studies regarding the associations between AGT rs699 polymorphism and PE risk.

Country	Geography	Ethnicity	Gestation weeks	PE degree	Total cases	Total controls	PE genotypes			Controls genotypes			G
							MM genotype cases, n	MT genotype cases, n	TT genotype cases, n	MM genotype controls, n	MT genotype controls, n	TT genotype controls, n	
Pakistan	South Asia	Mixed race	Mixed	Not mentioned	100	50	8 (8 %)	72 (72 %)	20 (20 %)	13 (26 %)	35 (70 %)	2 (4 %)	A
China	East Asia	Mongoloid race	Mixed	Not mentioned	168	204	96 (57,1 %)	65 (38,7 %)	7 (4,2 %)	133 (65,2 %)	62 (30,4 %)	9 (4,4 %)	H
Russia	East Europe	Caucasian race	Mixed	Not mentioned	95	54	21 (22,1 %)	51 (53,7 %)	23 (24,2 %)	13 (24,1 %)	27 (50 %)	14 (25,9 %)	H
Thailand	South-east Asia	Mixed race	Mixed	Not mentioned	61	142	0 (0 %)	10 (16,4 %)	51 (83,6 %)	3 (2,1 %)	32 (22,5 %)	107 (75,4 %)	
Russia	East Europe	Caucasian race	Mixed	Severe	100	100	20 (20 %)	55 (55 %)	25 (25 %)	21 (21 %)	45 (45 %)	34 (34 %)	
Romania	South-east Europe	Caucasian race	EOPE	Not mentioned	33	130	7 (21,2 %)	18 (54,5 %)	8 (24,2 %)	70 (53,8 %)	45 (34,6 %)	15 (11,5 %)	R
Romania	South-east Europe	Caucasian race	LOPE	Not mentioned	54	130	17 (31,5 %)	23 (42,6 %)	14 (25,9 %)	70 (53,8 %)	45 (34,6 %)	15 (11,5 %)	R
Iran	West Asia	Caucasian race	Mixed	Not mentioned	178	240	27 (15,1 %)	100 (56,17 %)	51 (28,6 %)	51 (21,25 %)	129 (53,75 %)	60 (25 %)	SS M
Russia	East Europe	Caucasian race			30	30	9 (30 %)	14 (46,7 %)	7 (23,3 %)	10 (33,3 %)	10 (33,3 %)	10 (33,3 %)	
Tunisia	North Africa	Caucasian race	Mixed	Not mentioned	272	278	137 (50 %)	109 (40 %)	26 (10 %)	176 (63 %)	90 (32 %)	12 (4 %)	RI
Uzbekistan	Central Asia	Caucasian race	Mixed	Not mentioned	50	110	1 (2 %)	10 (20 %)	39 (78 %)	9 (8,2 %)	30 (27,3 %)	71 (64,5 %)	
Russia	East Europe	Caucasian race			153	99	42 (27,4 %)	78 (51 %)	33 (21,6 %)	33 (33,3 %)	43 (43,4 %)	23 (23,2 %)	M
South Africa	South Africa	Negroid race	EOPE	Not mentioned	187	246	0 (0 %)	22 (12 %)	165 (88 %)	3 (1 %)	39 (16 %)	204 (83 %)	H
South Africa	South Africa	Negroid race	LOPE	Not mentioned	170	246	0 (0 %)	14 (8 %)	156 (92 %)	3 (1 %)	39 (16 %)	204 (83 %)	H
India	South Asia	Mixed race	Mixed	Not mentioned	150	150	13 (8,7 %)	58 (38,6 %)	79 (52,7 %)	29 (19,3 %)	61 (40,7 %)	60 (40 %)	R

AS-PCR: Allele-specific polymerase chain reaction; SSP-PCR: sequence specific primer-polymerase chain reaction; MS-PCR: methylation-specific; RFLP-PCR: polymerase chain reaction-restriction fragment length polymorphism; RT-PCR: Real Time Polymerase Chain Reaction; HWE: Hardy-Weinberg Equilibrium; N/A: not available; EOPE: Early-onset PE; LOPE: Late-onset PE

Funnel plots constructed for our analysis confirmed the symmetrical distribution of OR values based on standard errors, indicating no evidence of publication bias, as shown

in Fig. 3. The results of the Egger's test for all genetic models do not support the existence of funnel plot asymmetry, with p = 0,098 for dominant model and t-statistic 1,783; in a recessive model P = 0,454 and t-statistic 0,772; in the allelic model p = 0,303 and t-statistic 1,072; in homozygote model p = 0,182 and t-statistic 1,409; and in heterozygote mode p = 0,07 and t-statistic 1,971. Therefore, the Egger's test fails to support the presence of asymmetry in funnel plots with p-values ranging from 0,07 to 0,454.

Table 2 - Overall and subgroup analysis of associations between AGT rs699 polymorphism and PE risk.

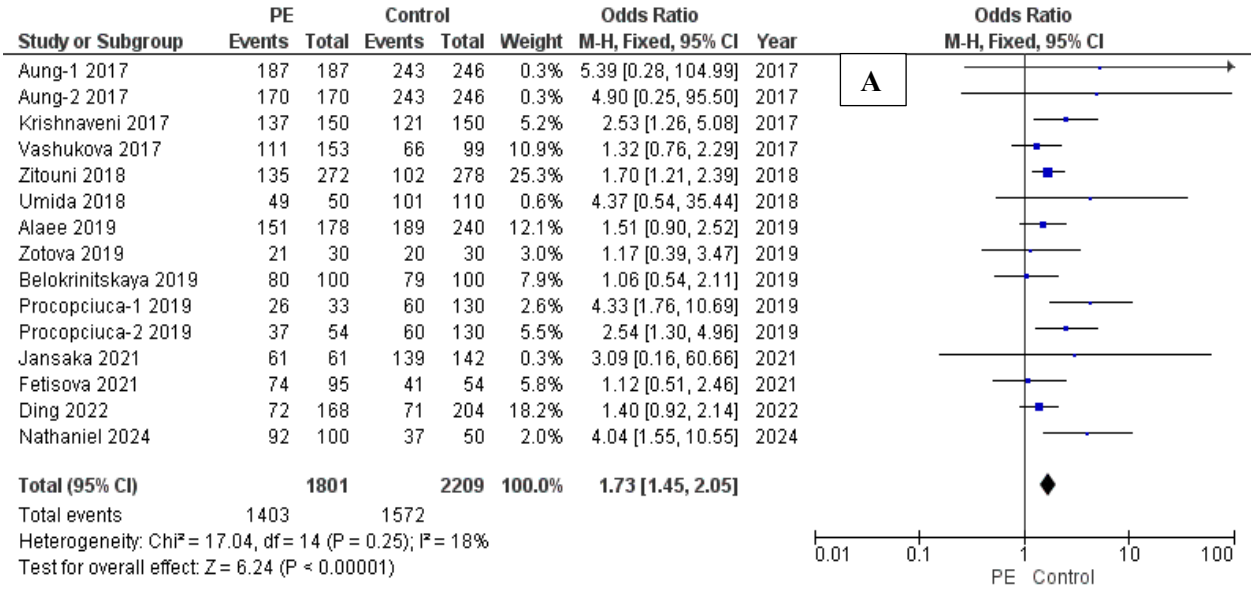
		Dominant model					**Allelic model**				
Subgroup	N	OR [95% CI]	P-value[a]	Effect model	I^2	P#	OR [95% CI]	P-value[a]	Effect model	I^2	P#
Overall-1	15	**1,73 [1,45, 2,05]**	<0,00001	Fixed	18	0,25	**1,48 [1,25, 1,74]**	<0,0001	Random	54	0,007
Overall-2	13	**1,66 [1,40, 1,98]**	<0,00001	Fixed	7	0,37	**1,42 [1,19, 1,70]**	0,0001	Random	56	0,007
Race											
Mixed	3	**2,95 [1,69, 5,15]**	0,0001	Fixed	0	0,74	**1,78 [1,37, 2,31]**	<0,0001	Fixed	0	0,86
Caucasian	9	**1,65 [1,35, 2,02]**	<0,00001	Fixed	24	0,23	**1,37 [1,07, 1,74]**	0,01	Random	65	0,003
Mongoloid	1	1,40 [0,92, 2,14]	0,11	Random	NA	NA	1,26 [0,89, 1,79]	0,20	Random	NA	NA
Negroid	2	5,14 [0,63, 41,97]	0,13	Fixed	0	0,96	**1,90 [1,28, 2,84]**	0,002	Fixed	0	0,36
Geography											
Asia	6	**1,79 [1,36, 2,35]**	0,0001	Fixed	20	0,29	**1,47 [1,25, 1,72]**	<0,00001	Fixed	13	0,33
Europe	6	**1,62 [1,06, 2,47]**	0,02	Random	47	0,09	1,29 [0,89, 1,87]	0,18	Random	73	0,002
Africa	3	**1,78 [1,27, 2,48]**	0,0008	Fixed	0	0,59	**1,72 [1,37, 2,16]**	<0,00001	Fixed	0	0,56
Gestation weeks											
Mixed	11	**1,58 [1,32, 1,90]**	<0,00001	Fixed	0	0,46	**1,32 [1,12, 1,56]**	0,001	Random	44	0,06
EOPE	2	**4,44 [1,87, 10,54]**	0,0007	Fixed	0	0,89	**2,01 [1,37, 2,95]**	0,0004	Fixed	36	0,21
LOPE	2	**2,66 [1,39, 5,10]**	0,003	Fixed	0	0,67	**2,26 [1,56, 3,28]**	<0,0001	Fixed	0	0,88
Case sample size											
<100	6	**2,18 [1,48, 3,22]**	<0,0001	Fixed	28	0,23	**1,64 [1,13, 2,37]**	0,009	Random	58	0,04
≥100 and <200	8	**1,59 [1,27, 2,01]**	<0,0001	Fixed	19	0,28	**1,37 [1,12, 1,68]**	0,03	Random	53	0,04
≥200	1	**1,70 [1,21, 2,39]**	0,002	Random	NA	NA	**1,63 [1,24, 2,15]**	0,0005	Random	NA	NA
Subgroup		**Recessive model**					**Homozygote model**				
Overall-1	15	**1,46 [1,14, 1,87]**	0,03	Random	45	0,03	**2,03 [1,37, 3,00]**	0,0004	Random	50	0,02
Overall-2	13	**1,38 [1,08, 1,77]**	0,01	Random	44	0,05	**1,79 [1,24, 2,57]**	0,002	Random	41	0,06

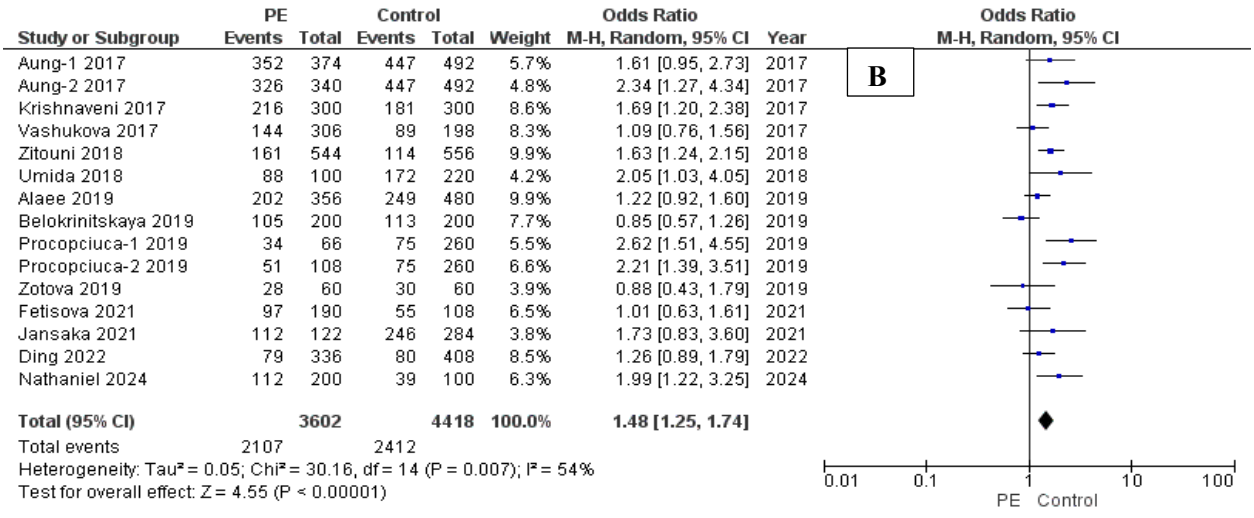
Race											
Mixed	3	**1,89 [1,30, 2,76]**	0,0009	Fixed	25	0,26	**3,95 [2,08, 7,53]**	<0,0001	Fixed	39	0,19
Caucasian	9	1,30 [0,91, 1,84]	0,15	Random	54	0,03	**1,74 [1,11, 2,71]**	0,02	Random	54	0,03
Mongoloid	1	0,94 [0,34, 2,59]	0,91	Random	NA	NA	1,08 [0,39, 2,99]	0,89	Random	NA	NA
Negroid	2	**1,84 [1,21, 2,80]**	0,004	Fixed	0	0,36	5,51 [0,67, 45,00]	0,11	Fixed	0	0,98
Geography											
Asia	6	**1,56 [1,21, 2,01]**	0,0007	Fixed	14	0,32	**2,52 [1,31, 4,83]**	0,006	Random	48	0,09
Europe	6	1,11 [0,68, 1,82]	0,67	Random	57	0,04	1,52 [0,81, 2,87]	0,19	Random	63	0,02
Africa	3	**1,96 [1,37, 2,81]**	0,0002	Fixed	0	0,55	**3,07 [1,56, 6,04]**	0,001	Fixed	0	0,83
Gestation weeks											
Mixed	11	1,27 [0,95, 1,71]	0,10	Random	46	0,05	**1,68 [1,10, 2,55]**	0,02	Random	50	0,03
EOPE	2	**1,71 [1,05, 2,78]**	0,03	Fixed	0	0,41	**5,40 [1,79, 16,32]**	0,003	Fixed	0	97
LOPE	2	**2,42 [1,46, 4,01]**	0,0006	Fixed	0	0,77	**4,01 [1,69, 9,55]**	0,002	Fixed	0	83
Case sample size											
<100	6	**1,57 [1,11, 2,21]**	0,01	Fixed	32	0,19	**2,24 [1,37, 3,66]**	0,001	Fixed	46	0,10
≥100 and <200	8	1,33 [0,96, 1,85]	0,09	Random	55	0,03	**1,79 [1,04, 3,10]**	0,04	Random	56	0,03
≥200	1	**2,34 [1,16, 4,74]**	0,02	Random	NA	NA	**2,78 [1,36, 5,72]**	0,005	Random	NA	NA

			Heterozygote model			
Subgroup	N	OR [95% CI]	P-value[a]	Effect model	I^2	P#
Overall-1	15	**1,67 [1,39, 2,00]**	<0,00001	Fixed	0	0,83
Overall-2	13	**1,62 [1,34, 1,95]**	0,00001	Fixed	0	0,88
Race						
Mixed	3	**2,49 [1,39, 4,45]**	0,02	Fixed	0	0,76
Caucasian	9	**1,62 [1,30, 2,01]**	<0,0001	Fixed	0	0,67
Mongoloid	1	1,45 [0,94, 2,25]	0,09	Random	NA	NA
Negroid	2	3,23 [0,38, 27,20]	0,28	Fixed	0	0,84
Geography						
Asia	6	**1,70 [1,27, 2,26]**	0,0003	Fixed	0	0,65
Europe	6	**1,70 [1,24, 2,33]**	0,001	Fixed	4	0,39
Africa	3	**1,60 [1,13, 2,27]**	0,009	Fixed	0	0,79
Gestation weeks						
Mixed	11	**1,56 [1,28, 1,89]**	<0,00001	Fixed	0	0,93
EOPE	2	**3,55 [1,48, 8,53]**	0,005	Fixed	0	0,93
LOPE	2	**2,56 [1,22, 5,36]**	0,01	Fixed	0	1,00
Case sample size						
<100	6	**2,11 [1,38, 3,23]**	0,0002	Fixed	0	0,59
≥100 and <200	8	**1,60 [1,25, 2,04]**	0,001	Fixed	0	0,78
≥200	1	**1,56 [1,09, 2,22]**	0,02	Random	NA	NA

P-value[a]: p-value for overall test; P#: p-value for heterogeneity test; NA: not available
Overall-1: Overall analysis with control groups, which deviates from the HWE
Overall-2: Overall analysis without control groups, which deviates from the HWE

A sensitivity test was conducted on all models, revealing that the exclusion of three articles (Vashukova et al., 2017; Belokrinitskaya et al., 2019; Fetisova et al., 2021) significantly reduced heterogeneity, lowering it to a depressed level ($p = 0,14$; $I^2 = 32$ %). It is thus proposed that the genotyping data from these studies constitutes the primary source of heterogeneity within the allelic, recessive, and homozygote models.





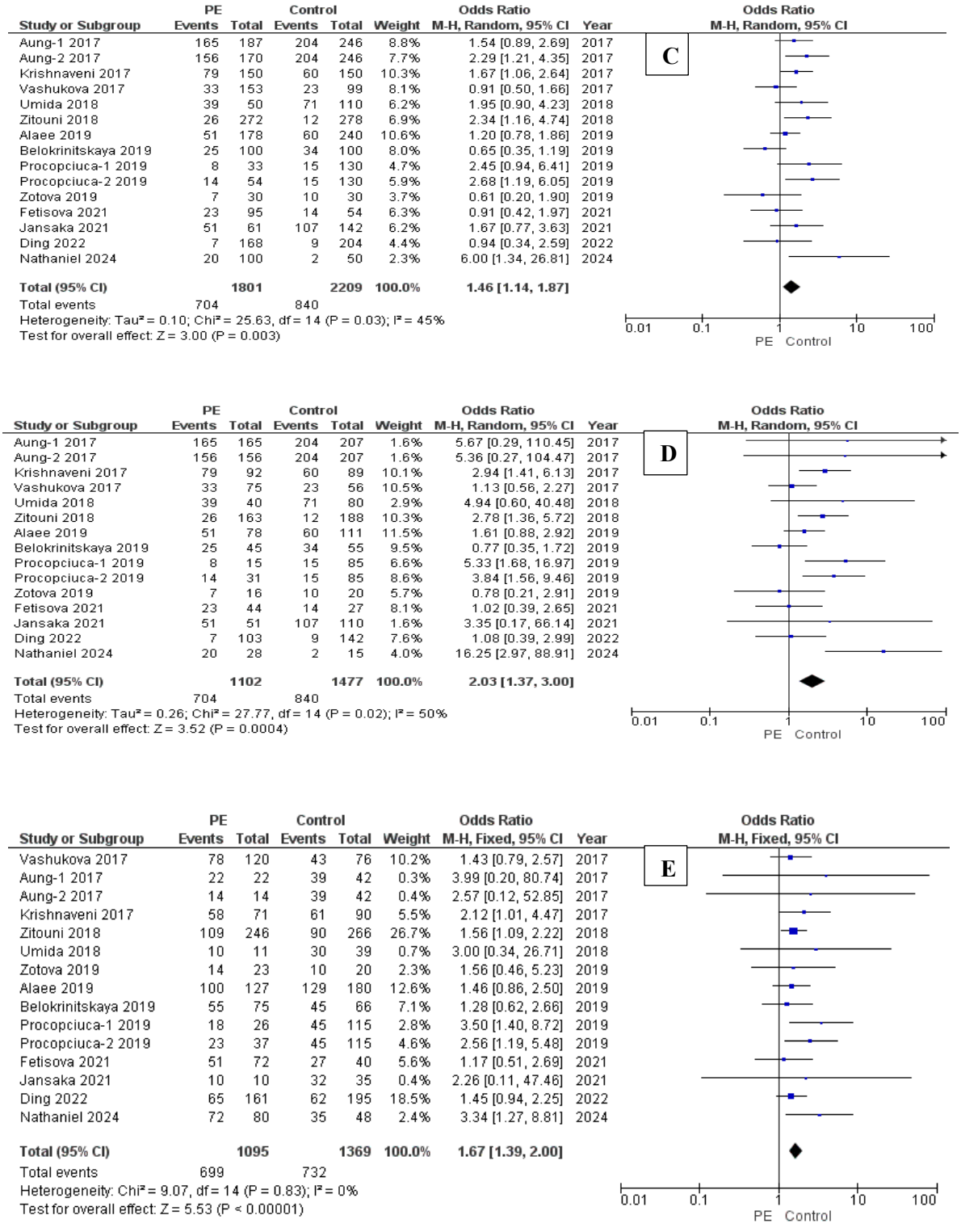


Fig. 2 - Forest plot of meta-analysis the association of AGT rs699 with PE. A) dominant model; B) allelic model; C) recessive model; D) homozygote model; E) heterozygote model

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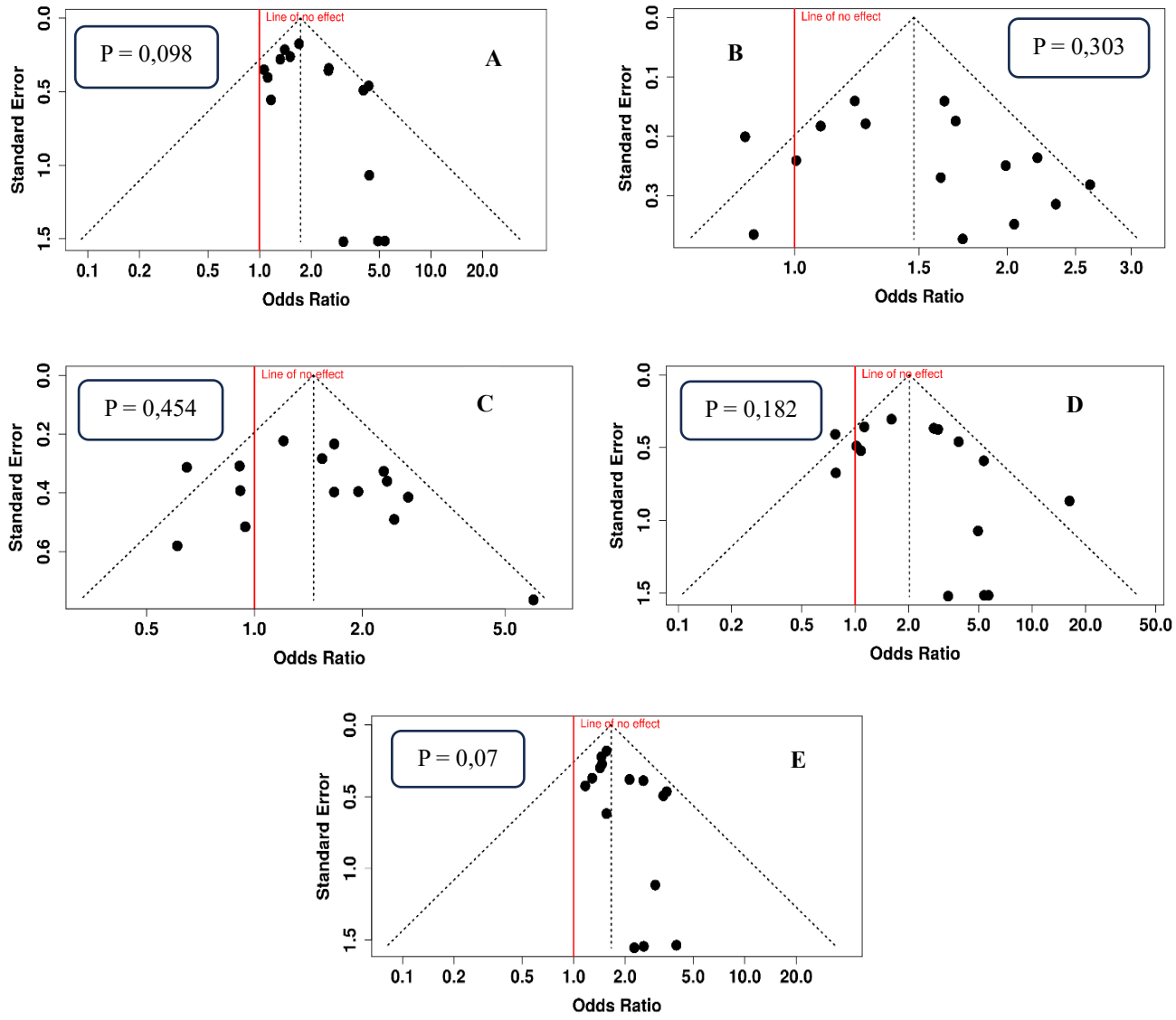

Fig. 3 - Forest plot of meta-analysis the association of AGT rs699 with PE. A) dominant model; B) allelic model; C) recessive model; D) homozygote model; E) heterozygote model

4. Diagnosis-based subgroups analysis

In order to establish a correlation between the *AGT* rs699 polymorphism and PE, the citations were initially categorized based on factors such as race, geographical location, gestational weeks, and sample size of cases. Subsequently, subgroup analysis was performed. With regard to racial classification, the findings suggest that this genetic variant may be associated with an increased risk of PE in individuals of Caucasian race

(OR = 1,65, 95 % CI [1,35–2,02], p<0,00001) and Mixed race (OR = 2,95, 95 % CI [1,69–5,15], p = 0,0001). However, no significant association was observed in individuals of Negroid or Mongoloid descent (ORs: 5,14 and 1,40, respectively, 95 % CIs [0,63–41,97] and [0,92–2,14], respectively). This may be attributable to the small number of studies conducted within these groups.

When considering geographical distribution, the analysis revealed a potential link between this polymorphism and PE in Asian (OR = 1.79, 95 % CI [1.36–2.35]), European (OR = 1.62, 95 % CI [1.21-2.17]), and African (OR = 1.78, 95 % CI [1.27-2.48]) populations, with statistical significance p-values (p = 0,001, 0,02 and 0,0008, respectively). For subgroup analysis stratified by gestational week classification, a statistically significant association was observed in EOPE (OR = 4,44; 95 % CI [1,87–10,54]; p = 0,0007), LOPE (OR = 2,66; 95 % CI [1,39–5,10]; p = 0,003), and Mixed (OR = 1,58; 95 % CI [1.32–1.90]; p<0,00001).

When stratifying by case sample size, the findings demonstrated a significant association for <100 cases (OR = 2,18; 95 % CI [1,48–3,22]; p<0,0001), ≥100 to <200 cases (OR = 1,59; 95 % CI [1,27–2,01]; p<0,0001), and ≥200 cases (OR = 1,70; 95 % CI [1,21–2,39]; p = 0,002).

Discussion

The mechanistic explanation for the involvement of *AGT* rs699 polymorphism in the development of PE has previously been proposed to occur via the localized increase in angiotensin II (Ang II) levels, leading to aberrant physiological remodeling of the uterine spiral arteries (Morgan et al., 1999). Studies have found that increased levels of the RAS component angiotensin II, have been linked to an increased risk of PE (Gintoni et al., 2021). Additionally, increased levels of Ang II have been linked to an increased severity of PE (Leaños-Miranda et al., 2018). It is thought that the RAS increases the production of pro-inflammatory cytokines, which can lead to increased vascular permeability and decreased blood flow to the placenta, leading to placental ischemia and the development of PE (Lu et al., 2019).

The findings of this study suggest that the *AGT* rs699 T allele is associated with an increased risk of PE. This conclusion was drawn based on several models. In order to obtain more accurate results, we excluded studies that were not in HWE and recalculated the odds ratios (OR). The recalculation showed a slight decrease in the pooled OR, accompanied by narrower 95 % confidence intervals (CIs). However, there were no significant changes in the statistical significance of the results. This suggests that the studies that deviated from HWE had a negligible impact on the overall findings. Therefore, we decided to include these studies in our further analysis. Our findings differ

from those of two out of the eight previous meta-analyses that focused on *AGT* rs699 and were unable to detect a correlation with PE. The other six meta-analyses reached a positive conclusion based on their overall analysis (Medica et al., 2007; Zafarmand et al., 2008; Lin et al., 2012; Ni et al., 2012; Buurma et al., 2013; Zhang et al., 2016; Wang et al., 2020, Wang et al., 2023).

For *AGT* rs699, when stratified by race, the presence of the T allele has been associated with an elevated risk of PE among individuals of mixed race across all genetic models examined in our study ($p<0,05$). Among Caucasians, there was an increase in the risk of PE under partial genetic models. Conversely, in Negroids, this risk was only elevated in two specific models: the allelic model and the recessive model ($p<0,05$). Due to the limited number of studies on Negroid and Mongoloid individuals in our meta-analysis, these correlations should be approached with circumspection.

In a stratified analysis based on geographic regions, our findings diverge from those presented by Wang et al., (Wang et al., 2020), who failed to detect any association in Asia and Europe subgroups, while they found an association in Africa subgroup. Our study revealed that in Asia and Africa, the T allele augments the risk of PE under all genetic models, whereas in Europe, it elevates the risk only under two models: dominant and heterozygous ($p<0,05$). These results diverge from the findings of Wang's meta-analysis (Wang et al., 2020), but our results for Africa subgroup are consistent with their findings. It is important to note that both studies have a very small number of studies, therefore, when evaluating the association between *AGT* rs699 polymorphisms and the risk of PE in this geographical region, it is crucial to consider sample size and number of studies collectively.

Furthermore, when stratified based on gestational weeks, we observed that the T allele increases the likelihood of PE in subgroups (mixed, EOPE, and LOPE), under all models with statistical significance ($p<0,05$). However, in the mixed group, the association was not significant under the recessive model (OR = 1,27; 95 % CI [0,95–1,71]; p = 0,10). Nonetheless, given the limited number of studies incorporated into EOPE and LOPE subgroups, these correlations should be also interpreted with circumspection. Previously, a meta-analysis conducted by Wang et al., (Wang et al., 2020) and Wang et al., (Wang et al., 2023) failed to investigate the correlation between the T allele and PE in gestational week subgroups for the rs699 polymorphism. Therefore, our meta-analysis represents the first attempt to incorporate this subgroup into a meta-analytic framework.

Furthermore, when stratified by sample size of cases, the previous meta-analysis by Wang et al., (Wang et al., 2020) did not detect any association in samples with fewer than 100 or between 100 and 200 individuals. Conversely, our meta-analysis revealed an elevated risk in all genetic models for samples comprising less than 100 participants

($p<0,05$). For samples ranging from 100 to 200 individuals, an increased risk was observed across all genetic models except for the recessive model (OR =1,33, 95 % CI [0,96, 1,85], p = 0,09). In the subgroup with more than 200 individuals, the correlation was evident across all genetic configurations. Nonetheless, considering the limited number of studies encompassed in this subgroup, these correlations should be approached with caution.

This meta-analysis was constrained by several limitations. Firstly, the language restriction was limited to English and Russian publications. Secondly, in some subgroups, the sample sizes of studies included in the meta-analysis were comparatively small, indicating that our findings should be interpreted with caution. Lastly, it is important to consider the potential impact of environmental factors on the association between genotype and PE.

Conclusion

AGT rs699 minor T allele is linked to an increased risk of PE in all genetic models. This association has also been observed in subgroup analyses for all subgroups except for Negroid and Mongoloid racial subgroups. It should be noted that only one study was analysed for these specific subgroups, which may limit the reliability of the findings. For pregnant women carrying the *AGT* rs699 T allele, special attention and intensive prenatal care should be provided, considering race and geographic location, to prevent and detect PE early.

Ethical approval: The research received approval from the Ethics Committee of Southern Federal University, Academy of Biology and Biotechnology. Consent was acquired from all subjects participating in the research.

Declaration of competing interest: The authors declare no conflict of interests.

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