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Original Article

Investigation of the *rs722503* Polymorphism in the *FLT1* Gene and Its Association with Preeclampsia Susceptibility in the East Azerbaijan Province, Iran

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Abstract

Background: Genetic predisposition plays a crucial role in the development of preeclampsia, with the Fms-like tyrosine kinase 1 (*FLT1)* gene implicated in angiogenesis and endothelial dysfunction, both central to the disease's pathophysiology. This study aimed to investigate the relationship between the *rs722503* polymorphism of the *FLT1* gene and susceptibility to preeclampsia in pregnant women in East Azerbaijan Province, Iran.

Method: In this case-control study, 24 fetal cord blood samples from pregnant women, including 13 with preeclampsia and 11 healthy controls, were recruited from healthcare centers in East Azerbaijan Province. Genomic DNA was extracted from blood samples, and the *rs722503* polymorphism in the *FLT1* gene was analyzed using polymerase chain reaction (PCR). The PCR products were then subjected to electrophoresis to observe the desired polymorphism. Statistical analysis was performed to assess the association between the *rs722503* polymorphism and preeclampsia risk.

Results: A significant association was found between the *rs722503* polymorphism of the *FLT1* gene and the risk of preeclampsia. Women with the TT genotype had a higher risk of developing preeclampsia compared to those with the CC genotype (*P-value* < 0.05). The frequency of the T allele was also significantly higher in the preeclampsia group compared to controls.

Conclusion: The *rs722503* polymorphism of the *FLT1* gene may be a genetic risk factor for preeclampsia in the population of East Azerbaijan Province. These findings could contribute to early identification of at-risk individuals and potential development of targeted therapeutic strategies.

Keywords: FLT1; Genetic Association Studies; Preeclampsia; Single Nucleotide Polymorphism

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Introduction

Preeclampsia is a pregnancy-specific condition characterized by hypertension and organ dysfunction, typically occurring after 20 weeks of gestation. This disorder is a leading cause of maternal and perinatal morbidity and mortality worldwide. It is defined by the presence of hypertension (blood pressure ≥140/90 mmHg) and proteinuria (≥300 mg in a 24-hour urine sample) or signs of end-organ dysfunction (1). Preeclampsia presents a significant challenge in obstetric care due to its potential complications and the necessity for timely management and intervention (2). The prevalence of preeclampsia varies globally, affecting approximately 2-10% of all pregnancies (3, 4). Several risk factors contribute to the development of preeclampsia, including maternal age (both advanced and young), obesity, multiple gestations, and a history of preeclampsia in previous pregnancies. Conditions such as diabetes, renal disease, and autoimmune disorders increase the likelihood of preeclampsia (5). Genetic predispositions and environmental factors, including inadequate prenatal care and certain lifestyle choices, also play a role in the etiology of this condition (4).

Preeclampsia is characterized by impaired angiogenesis and endothelial dysfunction, processes in which the Fms-like tyrosine kinase 1 (FLT1) gene plays a central role (6, 7). The *FLT1* gene encodes the vascular endothelial growth factor receptor 1 (VEGFR-1), a key regulator of angiogenesis. VEGFR-1 functions as a receptor for vascular endothelial growth factor (VEGF) and placental growth factor (PlGF), playing a crucial role in maintaining vascular homeostasis. Genetic variants in the *FLT1* gene can influence the expression and activity of VEGFR-1, potentially predisposing individuals to preeclampsia (8). Studies have identified specific single nucleotide polymorphisms (SNPs) within the *FLT1* gene that are associated with altered levels of soluble Flt-1 (sFlt-1) and an increased risk of preeclampsia (9). For example, certain *FLT1* variants have been linked to higher circulating levels of sFlt-1 and an earlier onset of preeclampsia, suggesting a genetic predisposition to the disorder (10). The genetic predisposition to preeclampsia was first recognized in the early 1960s (11), with the heritability of the condition estimated to be around 55%, involving both maternal and fetal contributions to risk (approximately 35% and 20%, respectively) (12). The placenta is essential for the development of preeclampsia, and its delivery remains the only cure. Both fetal genome-wide association studies (GWAS) and earlier research indicate that disruptions in the placental *FLT1* pathway may explain the critical role of the placenta in this disease (13, 14). Epidemiological studies have shown that increased circulating placental sFlt-1 levels are linked to several risk factors for preeclampsia, including multiple gestations (due to increased placental mass), trisomy 13 (with *FLT1* encoded on chromosome 13), nulliparity (which shows higher sFlt-1 levels compared to multiparous women), antiphospholipid antibody syndrome, pre-existing diabetes, and molar pregnancies (associated with increased chromosomal dosage)(15). The exposure of maternal blood vessels to excess sFlt-1 and other anti-angiogenic factors may also contribute to long-term changes that heighten the risk of cardiovascular disease later in life (16).

The fetal preeclampsia GWAS now offers an opportunity to explore how specific dysregulation at the *FLT1* locus leads to preeclampsia at the gene, cellular, placental, and fetal-maternal interface levels (12). The identification of *FLT1* gene variants as risk factors for preeclampsia has important implications for understanding the molecular mechanisms underlying the disease and for developing potential therapeutic interventions. The present study aims to investigate the relationship between the *rs722503* polymorphism of the *FLT1* gene in the fetuses of pregnant mothers with preeclampsia in East Azerbaijan Province, Iran.

Materials and Methods

In the current case-control study, 13 fetal cord blood samples from women with preeclampsia as the case group and 11 fetal cord blood samples from healthy women as the control group were collected. All the participants were Iranian and shared a common ethno-geographic origin. Inclusion criteria for preeclampsia encompassed women who had a resting systolic blood pressure exceeding 140 mmHg and diastolic blood pressure above 90 mmHg after 20 weeks of gestation, accompanied by significant and newly developed proteinuria occurring after the same gestational period. Written informed consent was obtained

from all participants. Blood samples were collected from the study participants in EDTA tubes, and the genomic DNA was extracted by using the Thermo Fisher, USA kit according to the manufacturer's protocol. Then the quality and quantity of the extracted DNA were examined. The quality of the extracted DNA samples was assessed through electrophoresis on a 1% agarose gel. The size of the bands formed was examined using a Gel Documentation device (Sartorius, Germany). The quality assessment of the extracted DNA samples on the 1% agarose gel indicated a single band. A single, sharp band typically suggests that the DNA is intact and of high molecular weight, with minimal degradation. The quantity of the extracted DNA samples was assessed using a NanoDrop device (Thermo Fisher, USA). The NanoDrop spectrophotometer was used to measure the optical absorbance at wavelengths of 260 nm and 280 nm to evaluate protein contamination, as well as the optical absorbance at wavelengths of 260 nm and 230 nm to assess contamination with polysaccharides. The quantification of the extracted DNA samples by NanoDrop indicated that the absorbance ratios (260/280) and (260/230) of the extracted samples ranged between 1.6 and 2, which suggests a high level of purity and the absence of contamination by proteins and polysaccharides. Additionally, the concentration of the extracted DNA samples was over 350 ng/µl, which is considered acceptable.

Genotyping of samples for the rs722503 polymorphism was performed using the Tetra-ARMS PCR method and specific primers of the *FLT1* gene **(Table 1)**. PCR amplification was carried out in a total volume of 25 µL reaction containing genomic DNA (1 µl), 0. 5 µl of each primer, and 12.5 µl Master Mix (Biofact, South Korea). The thermal cycling protocol used included the following steps: primary denaturation (1 cycle at 95°C for 5 minutes), denaturation (35 cycles at 95°C for 60 seconds), primer annealing (35 cycles at 62.5°C for 55 seconds), extension (35 cycles at 72°C for 30 seconds), and final extension (1 cycle at 72°C for 5 minutes). Then the PCR products were electrophoresed to observe the desired polymorphism.

Statistical analysis

To analyze the differences in the distribution of genotypes of the *FLT1* gene polymorphism and allele frequencies between the case and control groups, the Chi-square test was used. Additionally, the odds ratio (OR) was employed as a measure of the association between allele frequencies and preeclampsia. The T-test was used to assess differences between the two groups for demographic data. All p-values were calculated with a 95% confidence interval (CI), and p-values of less than 0.05 were considered statistically significant.

Table 1. Specific primers of the FLT1 gene.

Primer	Primer Sequence
Forward Outer	AAGACTGTCCTTGCCCTATCCTCTC
Reverse Outer	CTTCACGTTCCGCCTGCATTTTG
Forward Inner	TTTCCATTCCACAGAGAAGGTCA
Reverse Inner	TACAGGACTGGAGGGAAGGG

Results

Demographic data of the studied groups are described in Table 1. The results showed that the body mass index (BMI) and age were higher in the case group compared to the control group; however, this difference was not statistically significant. Additionally, there was no significant difference between the case and control groups in other characteristics, including gestational age at birth, smoking, diabetes, cesarean section, and birth outcomes. However, a significant difference was observed regarding the gender of the fetus. Specifically, the incidence of female fetuses was significantly higher in the case group than in the control group ($P<0.05$) (Table 1). The electrophoresis results of the PCR product showed that the expected length of the amplified fragment in the wild-type homozygous state (CC wild-type homozygous) was a single band of 88 base pairs, while the expected length of the amplified fragment in the mutant homozygous state (TT) was a single band of 230 base pairs. The determination of genotype frequency for the rs722503 polymorphism of the *FLT1* gene in the case group showed that 3 individuals (23.08%) had the CC genotype, and 10 individuals (76.92%) had the TT genotype, while the heterozygous CT genotype was not observed in any of the samples in the case group. Similarly, in the control group, 8 individuals (72.72%) had the CC genotype, and 3 individuals (27.27%) had the TT genotype, with the heterozygous CT genotype not being observed in any of the samples in the control group. The re-

sults of the statistical analysis indicated that the TT genotype was significantly more frequent in the case group (76.92%) compared to the control group (27.27%) ($P<0.05$). This significant difference was also observed in other inheritance models (dominant and recessive) **(Table 3)**.

Based on the results, the observed frequency for the CT, CC, and TT genotypes were 3 (23.08%), 0 (0.00%), and 10 (76.92%) individuals, respectively. Meanwhile, the expected frequency for the CT, CC, and TT genotypes were 1 (7.70%), 4 (30.80%), and 8 (61.50%) individuals, respectively. The observed and expected genotype frequencies are shown in Table 5. The results obtained from the statistical analysis indicated that the genotypic frequency in this study does not conform to Hardy-Weinberg equilibrium **(Table 4, and Table 5)**.

Table 2. Demographic characteristics of participants

Characteristics		Cases (N=13)	Controls (N=11)
Age (years)		36/28 ± 5/23	29/93 ± 6/14
Fetus gender (%)	Female	8 (61/53%)	3 (27.27)
	Male	5 (38.46%)	8 (72.72)
Gestational age at birth (weeks)		32/67 ± 3/49	38/50 ± 1/16
Body mass index (BMI)		29/39 ± 3/06	27/79 ± 3/86
History of Diabetes (%)		5 (35.46)	3 (27.27)

Table 3. Genotypic frequency of the *rs722503* polymorphism

Genotype Models	Genotype	Cases (N=13)	Controls (N=11)
Homozygous	CC	3 (23.8%)	8 (72.72%)
	CT	0 (0%)	0 (0%)
	TT	10 (76.92%)	3 (27.28%)
Dominant	CC	3 (23.8%)	8 (72.72%)
	CT + TT	10 (76.92%)	3 (27.28%)
Recessive	TT	10 (76.92%)	3 (27.28%)
	CC + CT	3 (23.8%)	8 (72.72%)
Over dominant	CT	0 (0%)	0 (0%)
	CC + TT	13 (100%)	11 (100%)

Table 4. Allelic frequency of the *rs722503* polymorphism

Allele	Cases (N=13)	Controls (N=11)
C	3 (23.08%)	8 (72.72%)
T	10 (76.92%)	6 (27.28%)

Table 5. Frequencies of observed and expected genotypes in Hardy-Weinberg equilibrium

Genotype	Observed Frequency (%)	Expected Frequency (%)
CC	3 (23.08%)	1 (7.70%)
CT	0 (0%)	4 (30.80%)
TT	10 (76.92%)	8 (61.50%)

Discussion

This study explored the association between the *rs722503* polymorphism in the *FLT1* gene and the risk of preeclampsia in the population of East Azerbaijan Province, Iran. The findings indicate a significant association between the TT genotype and the presence of preeclampsia, suggesting that this polymorphism may contribute to the susceptibility of this condition. The role of *FLT1* in preeclampsia is well-established, particularly its involvement in angiogenesis and endothelial dysfunction, which are critical processes in the pathophysiology of preeclampsia. Elevated levels of soluble Flt-1 (sFlt-1) have been implicated in

the anti-angiogenic state observed in preeclampsia, leading to placental ischemia and subsequent maternal systemic effects, such as hypertension and proteinuria (8, 17).The significant association observed in this study aligns with previous research suggesting that specific genetic variants in *FLT1* may predispose individuals to preeclampsia (16, 18-20). The presence of the TT genotype was notably higher in the case group, emphasizing the potential genetic predisposition conferred by this polymorphism. However, it is important to consider the relatively small sample size in this study, which may limit the generalizability of the findings. A study by Mona Amin Beidokhti et al. (21) conducted in Iran, investigated the relationship between the *rs722503* polymorphism and preeclampsia. Similar to our findings, this study reported a significant association between *rs722503* polymorphism in the *FLT1* gene and increased preeclampsia risk, further supporting the role of this polymorphism in the Iranian population (19). In China, Zhang et al. (2015) identified a similar link between *FLT1* gene variants and preeclampsia, specifically noting that *rs722503* was associated with an increased risk of early-onset preeclampsia (22). In contrast, some studies from other countries have produced varying results. For instance, a similar study in the UK by Johnson et al. (2018) found a modest association between *FLT1* polymorphisms, including *rs722503*, and preeclampsia risk, though the association was not as strong as that observed in Asian populations (23). The consistency of results from studies within Iran suggests a shared genetic predisposition, which may offer valuable insight into personalized medicine strategies for managing preeclampsia. Future research should focus on larger, multi-ethnic cohorts to better understand the genetic and environmental interactions that contribute to the variability in preeclampsia susceptibility across different populations.

Conclusion

This study provides evidence supporting the association between the *rs722503* polymorphism in the *FLT1* gene and preeclampsia. The results contribute to the growing understanding of the genetic factors underlying this complex condition and highlight the need for further research in larger, diverse populations to fully elucidate the role of *FLT1* in preeclampsia.

Ethics approval

This study was approved by the ethical committee of Tabriz Islamic Azad University with the code IR.IAU.TABRIZ.REC.1401.277.

Conflict of Interest

The authors declared that they have no conflict of interest.

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Authors Contributions

Conceptualization, M.M. and H.J; methodology, M.M. and H.J.; software, H.J. and S.A.; validation, M.M. and H.J.; formal analysis, M.M. and H.J.; investigation, M.M. and H.J.; resources, M.M. and H.J.; data curation, M.M. and H.J.; writing—original draft preparation, H.J.; writing—review and editing, S.A.; supervision, M.M.; project administration, M.M. and H.J.

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