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**ORIGINAL ARTICLE**

# Platelet Protease Activated Receptor 4 Receptor Genotype is associated with an increased Risk of Preterm Birth

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## ABSTRACT

**Objective:** To investigate the association between platelet protease-activated receptor 4 (PAR4) receptor genotypes and the risk of preterm birth.

**Study Design:** Prospective cohort study.

**Place and Duration of Study:** Liaquat University Hospital, Hyderabad and Department of Pathology, Liaquat University of Medical & Health Sciences, Jamshoro from 1st January 2022 to 30th June 2023.

**Methodology:** A total number of 80 patients were enrolled. Blood of mothers was obtained on admission for genotyping the PAR4 gene. The principal outcome was preeclampsia or gestational hypertension at rate among patients having Thr/Thr genotype as against those with Ala/Thr and Ala/Ala genotypes. Severe preeclampsia was diagnosed before 37 weeks, and delivery before 37 wks due to preeclampsia and/or fetal growth restriction. Exposure was defined as the presence of the homozygous Thr/Thr genotype for the PAR4 Thr/Ala polymorphic site group 1. The comparator group consisted of non-homozygous women for the Thr allele, heterozygous group 2.

**Results:** Preterm birth <37 weeks of Group I & Group II was 6 (15.0%) and 4 (14.0%), respectively. (p=0.499). Spontaneous preterm birth <37 weeks in Group I & Group II was 5 (12.5%) and 2 (5.0%), respectively. (p=0.235). Indicated preterm birth <37 weeks of Group I and Group II was 3 (7.5%) and 2 (5.0%), respectively. (p=0.644). Preeclampsia in Group I & Group II was 10 (25.0%) and 5 (12.5%), respectively. (p=0.152).

**Conclusion:** The PAR4 receptor genotype as a significant risk variable for premature delivery, underscoring the need for genetic and mechanistic studies to develop targeted therapies. Integrating genetic screening into prenatal care could transform the management of high-risk pregnancies, improving maternal and neonatal outcomes.

**Keywords:** Preterm birth, Platelet, PAR4, Preeclampsia, Genetics

## INTRODUCTION

Preterm birth, whether doctors recommend it or it happens on its own, is one of the main reasons for sickness and death in newborns.[1] High blood pressure problems during pregnancy (HDP), which include preeclampsia and pregnancy-related high blood pressure, play a big part in making mothers sick or even causing death.[2] These issues also lead to babies being born and the problems that come with it. On top of that, there's a connection to blood clots and inflammation. Platelets in the blood start to get active, which is linked to preeclampsia.[3] This condition begins in the first three months of pregnancy when blood vessels form in the placenta, but people notice it in the last three months.[4]

Inflammation have double role in pregnancy. Firstly, it is Important for implantation and labor; Secondly, excessive inflammation is a strong risk factor for early or preterm delivery, main cause of neonatal complications (morbidity and mortality).[5] Numerous previous studies have demonstrated that PAF and PAR4 involved in the pathophysiology of preterm birth through mechanisms such as activation of the maternal-fetal immune interface, stimulation of uterine contractions, and promotion of membrane rupture.[6,7]

It has shown a connection between platelet activation and spontaneous preterm birth. Issues like uteroplacental ischemia and vascular problems play a part in this happening.[8,9] Evidence backs up the idea that platelet activation has a role in both preterm birth and preeclampsia. Drugs that stop platelets from activating can help prevent these conditions. PAR4, on the other hand starts a signal chain that wakes platelets and forms blood clots. One type of PAR4 has threonine (Thr) at position 120 (PAR4 Thr120) instead of alanine (Ala). This version boosts GPCR signaling and makes platelets more active.[10]

The purpose to evaluate the PAR4 Thr120 variant and its possible association with the risk involved of developing preeclampsia and other effects perinatal outcomes. It will attempt to establish whether there is an increased risk of preeclampsia and delivery before 37 wks gestation in singleton pregnancies associated with homozygous carriage of the PAR4 Thr120 allele compared with non-carriers.

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## MATERIALS AND METHODS

This prospective cohort study enrolled singleton parturients delivered at Liaquat University Hospital, Hyderabad & Department of Pathology, LUMHS Jamshoro. It spanned 18 months, from January 2022 to June 2023. After detailed description of study purpose and methodology consent was obtained for maternal blood collection at the time of admission for genotyping of PAR4. Exclusions included multifetal pregnancies, individuals who consented but did not provide a sufficient maternal blood sample for genotyping, and those with inadequate DNA extraction. Baseline recoding of study characteristics like body mass index, risk factors og preterm delivery, parity, diabetes, hypertension, gestational age and delivery outcomes were monitored.

Severe preeclampsia was diagnosed before 37 weeks, and delivery before 37 weeks due to preeclampsia and/or limitation of foetal growth. Exposure was defined as the presence of the homozygous Thr/Thr genotype for the PAR4 Thr/Ala polymorphic site group 1. The comparator group consisted of non-homozygous women for the Thr allele, heterozygous group 2. Detailed analysis investigating the dose-response relationship of the alleles by comparing the effects of different genotypes' outcomes in preterm births: More detailed outcome analyses of births delivered were carried out in nulliparas as a subgroup as most related biases arise from previous history end.

The data was entered and analyzed through SPSS-24. To evaluate the association between two categorical variables, a Chi-square test of significance was employed. For comparisons between two numerical variables, an independent Student's t-test was utilized. A p-value of ≤0.050 was deemed statistically significant for all evaluations.

## RESULTS

The mean age of Group I and Group II mothers was 24.65±4.12 years and 27.32±4.16 years, respectively (p=0.005). The mean BMI of Group I & Group II mothers was 27.34±2.18 kg/m2 and 26.71±2.73 kg/m2, respectively. (p=0.261). The gestational age of the patients was 38.68±1.54 weeks and 38.05±1.64 weeks, respectively. (p=0.084). The average weight at birth of babies for Group I and Group II was 3.21±0.68 kg and 3.17±0.84 kg, respectively. (p=0.804). Hypertension was 11 (27.5%) and 8 (20.0%) in Group I & II, respectively. (p=0.431). Diabetes was 4 (10.0%) and 9 (22.5%) in Group I & Group II, respectively. (p=0.130). Prior full-term delivery in Group I & Group II was 20 (50.0%) & 22 (55.0%), respectively. (p=0.654). Previous preterm births in Group I & Group II were 5 (12.5%) & 8 (20.0%), respectively. (p=0.363). In Group I & Group II, Nulliparous were 16 (40.0%) and 20 (50.0%), respectively. (p=0.369). History of preeclampsia in Group I & Group II was 4 (10.0%) & 7 (17.5%), respectively. (p=0.330). Whereas the increased risk of preeclampsia in Group I & Group II was 11 (27.5%) and 12 (30.0%), respectively. (p=0.805) [Table 1].

Preterm birth <37 weeks of Group I & Group II were 6 (15.0%) & 4 (14.0%), respectively. (p=0.499). Spontaneous preterm birth <37 weeks in Group I & Group II was 5 (12.5%) & 2 (5.0%), respectively. (p=0.235). Indicated preterm birth <37 weeks of Group I & Group II was 3 (7.5%) & 2 (5.0%), respectively. (p=0.644) [Fig. 1]. Preeclampsia in Group I & Group II was 10 (25.0%) & 5 (12.5%), respectively. (p=0.152) [Fig. 2).

Table 1: Demographics and baseline characteristics

| Characteristic | Group I (n=40) | Group II (n=40) | Test of significance |
|---|---|---|---|
| Age of mother (years) | 24.65±4.12 | 27.32±4.16 | t=-2.88, d.f=78, p=0.005 |
| BMI of mother (kg/m$^2$) | 27.34±2.18 | 26.71±2.73 | t=1.13, d.f=78, p=0.261 |
| Gestational age (weeks) | 38.68±1.54 | 38.05±1.64 | t=1.75, d.f=78, p=0.084 |
| Weight at birth (kg) | 3.21±0.68 | 3.17±0.84 | t=0.245, d.f=78, p=0.804 |
| Hypertension | 11 (27.5%) | 8 (20%) | $\chi^2$=0.62, d.f=1, p=0.431 |
| Diabetes mellitus | 4 (10%) | 9 (22.5%) | $\chi^2$=2.29, d.f=1, p=0.130 |
| Prior full-term delivery | 20 (50%) | 22 (55%) | $\chi^2$=0.20, d.f=1, p=0.654 |
| Prior preterm birth | 5 (12.5%) | 8 (20%) | $\chi^2$=0.82, d.f=1, p=0.363 |
| Nulliparous | 16 (40%) | 20 (50%) | $\chi^2$=0.80, d.f=1, p=0.369 |
| History of preeclampsia | 4 (10%) | 7 (17.5%) | $\chi^2$=0.95, d.f=1, p=0.330 |
| Increased risk of preeclampsia | 11 (27.5%) | 12 (30%) | $\chi^2$=0.061, d.f=1, p=0.805 |

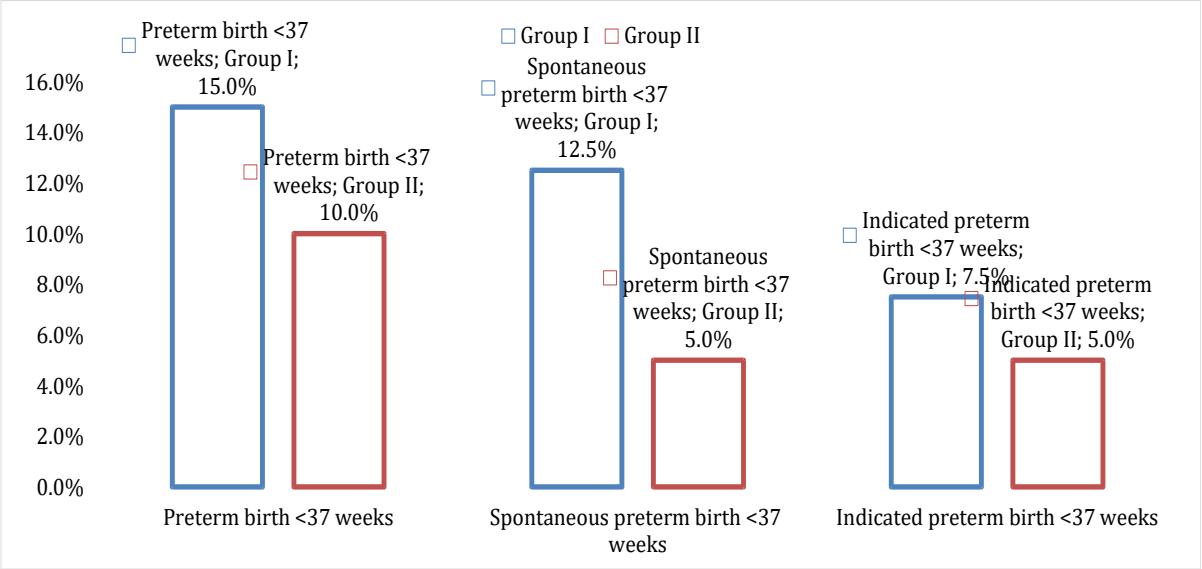


Fig. 1: Perinatal outcomes distribution between two groups

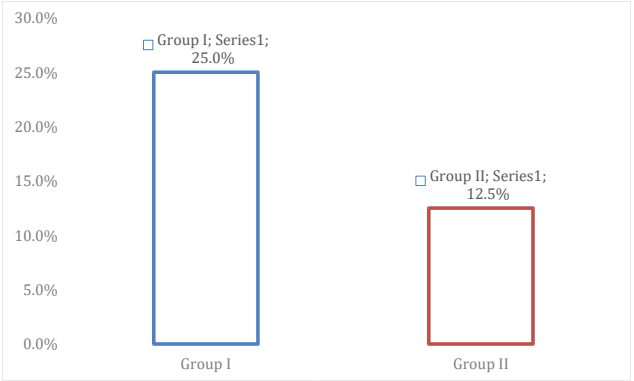


Fig. 2: Preeclampsia distribution between two groups

## DISCUSSION

PAR4 is a G-protein-coupled receptor primarily activated by thrombin, a central mediator in hemostasis and inflammation. Prior research indicates that platelets contribute to the inflammatory milieu within the uteroplacental interface, a key driver in preterm labor.[11] Specific genetic variants in the PAR4 receptor may alter platelet activation thresholds, amplifying thrombin-driven inflammatory pathways, thereby increasing susceptibility to preterm birth.[12]

The mean BMI of Group I (Thr/Thr genotype) and Group II (Thr allele) mothers was 27.34±2.18 kg/m2 and 26.71±2.73 kg/m$^2$, respectively. A study was conducted by Boelig et al[13] on 320 singleton births, and 52 (16.3%) of these were identified as PAR4 Thr/Thr. Individuals with the PAR4 Thr/Thr genotype were significantly more likely to be Black (67.3% compared to 29.5%), younger (28±6 years versus 31±6 years), and possess an elevated

body mass index (35.2±6.8 compared to 33.1±7.4)

Konijnenberg et al[14] and Theilen et al[15] have demonstrated that increased platelet activity is associated with both preeclampsia and preterm birth spontaneously. Furthermore, aspirin therapy, while well-established for its role in reducing the preeclampsia risk, has also been shown to lower the preterm delivery risk, including preterm birth spontaneously, in pregnant women regardless of whether they are classified as low-risk or high-risk singletons. These findings underscore the potential dual benefit of aspirin in mitigating adverse pregnancy outcomes linked to platelet hyperactivity.

Individuals carrying the PAR4 Thr-encoding variant homozygously do not demonstrate such a significant overall increase in the risk of preeclampsia or gestational hypertensive disease. Still, they pose a risk for preterm delivery sooner than others.[16] Recognizing that preterm and term preeclampsia is separate conditions with different underlying mechanisms is essential. Epidemiological studies show that early-onset preeclampsia has a notable genetic component.[17,18] Our findings support this idea, but the limited number of preterm preeclampsia cases restricts our ability to make definitive conclusions, as preeclampsia in Group I and Group II was 10 (25.0%) and 5 (12.5%).

Research on the PAR4Thr120 genetic variant in non-pregnant adults has revealed a diminished response to standard antiplatelet therapies, potentially influencing their efficacy in preventing thrombotic events.[19] This finding highlights the need for prospective pharmacogenomic studies to determine the optimal formulation and dosage of antiplatelet therapy tailored explicitly for homozygous individuals for the PAR4Thr120 variant. Such studies could provide critical insights into personalized treatment strategies to mitigate the increased thrombotic risk associated with this genetic profile while ensuring safety and effectiveness.[20]

## CONCLUSION

The PAR4 receptor genotype as a significant risk factor for preterm birth, underscoring the need for genetic therapies required. Integrating genetic screening into prenatal care could transform the management of high-risk pregnancies, improving maternal and neonatal outcomes.

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