



Research article

Progens progesterone receptor gene polymorphism as a risk factor for preterm delivery

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ABSTRACT

The study's aim and the objective were to evaluate the association between progesterone receptor PROGENS and preterm delivery by correlating with clinical data. The study had begun with 150 preterm delivery cases (PTD) among which it has classified into 3 cohorts as extreme preterm i.e. 24 - 28 gestation weeks, n= 37, very preterm i.e. 28 to <32 gestation weeks, n =36; and late preterm i.e. 32 to <37 gestation weeks, n =77. 150 cases of term delivery were evaluated for progesterone receptor PROGENS gene polymorphism. Software R version 3.6.3 was used for the statistical analysis. PTD cases were having higher PR mutation. The mutant allele of T2 frequencies were .23 in preterm delivery women and .01 in controls with odds ratio 18.0 (CI, 4.25- 76.1). Homozygous for T2 allele was present in 2.6% of preterm delivery women. PROGENS polymorphisms had showed association in preterm labor and are evidently enhancing risky genetic factors in terms of the susceptibility of PTD.

Keywords: Preterm delivery, mutation, genotype, homozygous, heterozygous.

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INTRODUCTION

World health organization (WHO) has defined that if birth occurs before 37 weeks of gestation is preterm delivery. ^[1,2] PTD plays a major role in contributing to increased global neonatal morbidity and mortality ^[3], with prolonged negative effect for health and poor intellectual development as well as financial support for health. ^[4-6]

The gestational period acts as the most important clinical marker for determining the health and survival of an infant. Statistically on an average 80-85% number of preterm babies born between 32 to < 37 weeks of gestation (moderate/late preterm); on the other hand, 10% of premature babies are born in between 28 to 32 weeks and 5% of babies born prematurely in < 28 gestation weeks. It has also resulted in fetal mortality cases, which are found in alarming numbers in countries with low income. Additionally, the global prevalence rate shows the highest number of preterm birth cases where India occupies the first position. ^[7]

Genetic findings have proved to be an associated factor of preterm birth since a couple of decades. Due to the complexity of the disorder, the causes of the disorder are still unclear. Moreover, it is involved with several other factors such as pathological, genetic and environmental factors. ^[4,8] There are a few pathways which assume a crucial part in the support of a pregnancy which is fruitful. Progesterone pathway and folate pathway are the two most

fundamental pathways related with fruitful pregnancy are. So any disturbance in these fundamental pathways prompts the intricacy in pregnancy. ^[4,8,9]

Progesterone hormones has an important role in pregnancy maintenance particularly in later gestation.^[10] Progesterone is a composite hormone of Carbon 21 a steroid hormone it is produced in brain, ovaries, placenta and the adrenal glands which help in menstrual cycle in females, embryogenesis and of pregnancy that is found in several mammalian groups. ^[11] Progesterone receptors are found in many pregnancy-associated tissues including fetal amniotic and chorionic membranes.^[12] In progesterone receptor (PR) gene (PROGENS) polymorphisms has been identified, with the insertion of 306 base pair PV/HS-1 (an Alu subfamily in intron G).^[13]

In this paper, we assessed the role of genetic variation in progesterone receptor qualities to distinguish ladies who might be at sequential danger of preterm conveyance contrasted and everybody hazard. Uncovering hereditary variations in the progesterone receptor that are related to a high danger of preterm birth could prompt the designated utilization of progesterone interventional treatments later on. Also, one of the genuine worries that the western locale of India is a zone with an exceptionally large number of instances of preterm conveyance cases. Shockingly, no logical examinations have been embraced to date, to recognize the basic sub-atomic mechanism(s) or

hereditary inclination related to preterm conveyance and related inconveniences from this space. [9,14]

MATERIALS AND METHODS

Study Group and Design

Mothers who had undergone labor (n=300) were selected for the study. Pregnant women of the suburban population who had preterm labor (n = 150) had selected on the bases of the gestation period (>37 weeks of gestation). Three cohort's gestation age were considered under the preterm delivery cases as: extremely preterm i.e. 24 to 28 weeks, very preterm i.e. 28 to <32 weeks, and moderately or late pre-term i.e. 32 to <37. Additionally, term delivery cases (n = 150) were selected as a control group, with all the clinical findings for both pre as well as term deliveries from MGM Medical College and Hospital, Kalamboli, Navi Mumbai following the guidance of a practitioner who is registered for medical practice with the informed consent and necessary clinically important data along with in this mothers with Tuberculosis, HIV, Jaundice, Infection of HPV, HBV, UTI or urinary tract infection, eclampsia, pre-eclampsia, miscarriage, pregnant female's twin fetus, a fetus with congenital anomalies and IUGR had been excluded. The Institutional Ethics Committee of MGMIHS, Navi Mumbai had approved the study.

PCR genotypic investigation of PR (PROGIN) Gene mutation: 3 ml of Peripheral blood was collected from MGM Hospitals in EDTA vacutainer. The collected blood was additionally prepared for extraction of DNA utilizing NucleoSpin Dx Blood DNA, RNA and protein refinement Kit (Macherey-Nagel, Germany). The DNA was put away at 4°C until investigated. For the polymerase chain reaction (PCR), we utilized the accompanying oligonucleotide introductions for in vitro intensification of explicit progesterone receptor fragment of gene: 5' GGC AGA AAG CAA AAT AAA AAG A 3' (forward) and 5' AAA GTA TTT TCT TGC TAA ATG TC 3' (reverse).^[15] The wild sort of the PR was characterized as the insertion shortfall. At the point when this PCR technique was utilized, the wild-type created a 149-base pair section and the freak produced a base pair of 455. PCR items were settled by using 2% concentration of agarose gel. Alleles were recorded on the basis of the individual band molecular weight.

Statistical analysis

The frequencies of Allele were compared with genotype in the control groups and patient group were compared and analyzed with the Fisher exact test. One-way ANOVA and Post hoc test were used for the demographic tests. The strength of the association was measured using the odds ratio (OR) between the frequencies of allele, genotype, and preterm. Statistical analysis was done using the software R version 3.6.3. All p-values were two-tailed, and 95% CIs were calculated. p-value less than .05 was considered statistically significant.

RESULT

Clinical Demographic patient's profile: In the current study, we had enrolled women who had underwent labor (n=300). The details of which are represented in the table:1

Table 1: Demographic profile

Cases Term	Maternal Age (in range)	Parity					Birth Status		Birth Weight
		1	2	3	4	5	Live	Death	
Extremely Preterm (24-28 weeks)	28.01±6.04	56	64	24	5	1	137	13	2.75±0.58
Very Preterm (28-<32 weeks)	28.02±6.92	10	16	10	0	1	4	28	1.16±0.66
Late Preterm (32-<37 weeks)	28.36±6.98	14	16	3	2	1	22	12	2.04±0.57
	28.14±6.88	24	40	9	3	1	80	4	1.80±0.71

The subjects were enlisted with complete details which were clinically important of the baby and the mother along with the weight at birth of the baby (table 1). In our investigation we had discovered that childbirth weight was fundamentally lower in all the preterm cases of the delivery as compared with the term cases of the delivery (p-value 0.0001); just as in extremely preterm delivery cases contrasted with moderately preterm cases of the delivery. The birth weight of the baby was also significantly lower to very preterm delivery cases compared to the moderately preterm delivery cases. Hence it signifies that gestational age is proportional to birth weight of the infant. Extremely premature infant and extremely low birth weight infant (ELBW) are at high risk for disability and death with mortality rate 30–50% and 20–50% at least risk of morbidity in survivors.^[16]

In our findings, we had found that there is a difference of 1.83-1.51 kg of weight in extremely newborn and term infants, and therefore there is the highest number of deaths seen in extremely premature infants n=28 as compared to term infants n=13. Therefore, survival rate among the term babies is higher compared to preterm babies (Table 1). Similar patterns were expressed in other parts of the world that in the USA the Preterm birth complicates 12.5% of all deliveries, prominently describing itself as the main cause of perinatal mortality and morbidity, accounting for as many as 75% of perinatal deaths.^[17-20] In recent years, preterm delivery incidence has increased leading to different clinical risk factors and epidemiological factors. But it is not just epidemiological or clinical factors which can cause preterm labor, but also genetic aspect is vital and remains relatively unexplored.^[21]

The wild type allele (T1) is of 149bp and polymorph allele (T2) band is of 455bp. Lane (L) 1 represents 50 base pair (bp) ladder; L 2,4,6,8-10 represents

heterozygous polymorph; L 3 represents wild type; L 5 & 7 represents homozygous polymorph Distribution of PR mutation and its association with preterm delivery

Figure 1: Representation of 2% Agarose gel showing 306bp intron G insertion of Progesterone Receptor Gene PROGINS polymorphisms

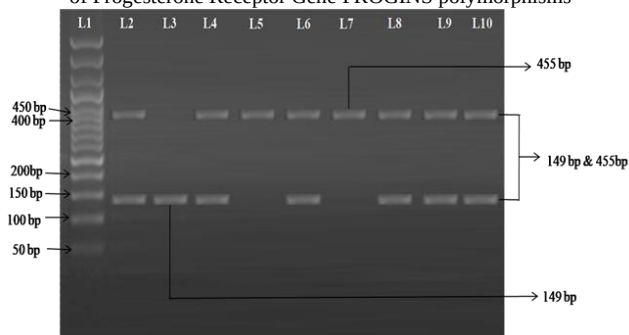


Table 2: Allele and Genotype frequencies among mothers with preterm and term deliveries

	Preterm (n=150)	Term (n=150)	ODDS ratio (95% CI)	P value
Genotypes				
T1/T1	118(78.66%)	148(98.67%)	20.06 (4.71 – 85.45)	0.0001 ^a b*
T1/T2	28(18.67%)	2(1.33)		
T2/T2	4 (2.66)	0		
T1/T2 & T2/T2	32(22.6%)	2 (1.3%)		
Alleles				
T1	150(100%)	150(100%)	18 (4.25- 76.1)	0.0001
T2	36 (24%)	2 (1.33)		

Data stated otherwise are number of patients in percentage

^a T1/T2 and T2/T2 Vs T1/T1

^b Fisher's Test

Genotype Analysis

Genotype analysis was conducted in all enrolled cases for the detection of wild type T1 and mutant T2 alleles shown in Figure 1. Women underwent preterm deliveries and term resulted significantly difference, in the distribution of genotype frequency for allele T2 (T1/T2 and T2/T2 Vs T1/T1 or 20.06 {95% CI 4.71-85.45}; p- 0.0001) and in the frequency of the mutant T2 allele (0.23-0.01; or 18 {95% CI: 4.25 – 76.1; p- 0.0001}). Four women from the study group (preterm) and none in term (control) group were found homozygous mutant for allele T2 (Table 2).

Preterm and its incidence by parity

Albeit, numerous interventions have been assessed, few have a moderate-to-high proof for diminishing preterm birth: smoking end and progesterone treatment in ladies with a high danger of preterm birth in low to average-pay nations and cervical cerclage for those in big-time salary nations. Risk partum and post-pregnancy mediations (eg, antepartum maternal steroid organization, or kangaroo mother care) to improve preterm neonatal endurance after birth have been exhibited to be compelling yet have not been broadly executed.^[22]

In our study cohort, heterozygous variation was seen in 2 patients from the control group and 28 patients from preterm group (study group). In the control group both the babies were born alive. But from the preterm group it was seen that only 5 (17.85%) neonates

survived and 23 (82.14%) of them were either still born or died immediately after birth. Additionally, the birth weight of 23 neonates who had died and 5 neonates who had survived from the study group were found in the range of 0-1 kg and 1-2 kg respectively. Similarly, in preterm cases the homozygous variation was 4, among which 3 neonates were still born and 1 was alive. Neonates who survived weighted 1.2kg and the still born weighted 0.80kg or less. This shows how in both variations (heterozygous and homozygous) the neonates who were still born were comparatively lower in weight as compared to the neonates who survived.

As it is known, the age of the mother during pregnancy plays another vital aspect for the outcome of the pregnancy and is strongly linked with genetic variations we also found that 100% of our study group mothers had access to full ANC and PNC care which is very vital for proper monitoring and management of the pregnancy outcomes. Therefore, it shows that the cause of preterm birth and stillborn neonates seen in mentioned 23 preterm cases with heterozygous variation and 3 stillborn neonates seen in preterm cases with homozygous variation occurred due to progesterone receptor PROGINS polymorphisms.

Women who had received early prenatal care appears to have a lower incidence of preterm birth than women who receive late care or no prenatal care, and some researcher believes that this decreased incidence results directly from some beneficial influence of prenatal care.^[23] The continuing elucidation of the mechanisms that regulate preterm labor, combined with rigorous clinical assessment, offer hope for future solutions.^[20] To conclude, in this study the variation in genetic factors, plays a significant role in preterm delivery. However, this study resembles the studies where genetics associations are found between SNP and clinically associated factors. The major drawbacks in these studies are lack of evaluations of SNP, small sample size, patient's demographical data etc. However, our finding puts a light on the SNP, whose research needed to be taken into consideration to avoid the adverse pregnancy outcome in future. With this current study, it has been analyzed that genetic factors are involved with different clinical classification of Preterm deliveries occurred in Navi Mumbai Population. Moreover, including the cohorts from various regions of Navi Mumbai population is important to correlate the findings from other regional populations that contribute expanded knowledge for the cause of preterm delivery

DISCUSSION

Progesterone receptor gene altogether has seven introns and eight exons (A-G). One of the progesterone receptor variant polymorph consists of PV/HS-1 of 320/306bp Alu gets insert at intron G and their [15, 24-27]. PR involves in the physiological effects of the progesterone and PR gene polymorphism, mostly in

PROGINS polymorphism which is associated with the Alu insertion that decreases the progesterone activity during pregnancy [28]. In current study we had found association between PROGINS and preterm delivery. It was observed that intron G insertion had shown significant in preterm delivery cases (22.6%) and in term it was observed (1.3%) of genotype distribution. The association of PROGINS gene polymorphism had also shown significant (OR=20.06[95% CI: 4.71-85.45]; $p=0.0001$) and has shown 20 folds and in the variant genotype of PROGINS polymorph had resulted as (OR=18 [95% CI: 4.25-76.1]; $p=0.0001$) which had also shown as statistically significant with 18 folds. Later, the analysis was done between the term groups and sub-cohorts of preterm our data had given a pleasant results, when term was compared with extremely preterm (OR=14.32, [95% CI: 2.67-74.31], $p=0.0015$) that resulted statistically significant, next term was compared with very preterm (OR=21.14, [95% CI: 4.26-104.85], $p=0.0002$) which also significant, and lastly term was compared to late preterm (OR=22.57 [95% CI: 5.07- 100.34], $p=0.0001$) that too resulted statistically significant. Our data had shown correlating results with a study which was conducted in north-eastern part of India (OR=2.652[95% CI: 0.830-8.474] $p=0.115$) [9]. Our study also resembled a study which was conducted by Langmia et.al., 2015 in Malaysia had found association between progesterone receptor gene polymorph and preterm delivery (OR 2.3, [95 % CI: 1.2–4.5] $P=0.011$) where they had found it significant different [29]. On the other hand, some studies did not match our analysis and these are the studies which had proved that progesterone receptor does not play a key role in any manifestation. A study which was held to detect the polymorphism of progesterone receptor and its association with recurrent spontaneous abortion (RSA) in pooled (OD: 1.82 [95% CI: 0.98-3.37] $p=0.056$) but the data did not show any significant difference [30]. Progesterone plays a vital role in sustaining pregnancy and has been successfully used to prevent preterm labor reoccurrence. By analyzing both maternal and fetal effects we examined the role of genetic mutations in the progesterone receptor (PGR) gene in altering risks to preterm labor. With enormous availability of data and studies there is still vast gap between the associations of PROGINS with preterm or related complications. Although our data has given a descriptive path where the research could be continued but the actual results will be only state if large sample size is been presented.

CONCLUSION

PROGINS polymorphisms had showed association in preterm labor and are evidently enhancing risky genetic factors in terms of the susceptibility of PTD.

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CONFLICT OF INTEREST

Authors contributes no conflicts of interests.

ETHICS APPROVAL

The study is approved by institutional ethical committee

AUTHORS CONTRIBUTIONS

MT and SS conceived and planned the experiments. SP carried out the experiments. MT and SP, SJ planned and carried out the simulations. SP and SS contributed to sample preparation. SP, and MT contributed to the interpretation of the results. MT, SP and SJ took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.

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