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Genetic variants of progesterone receptor in etiology of preterm delivery

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ABSTRACT

Objectives: Preterm delivery (PTD) accounts for around 11% of pregnancies worldwide. Unfortunately, no diagnostic indicator, specific mechanism or genetic predisposition has yet been identified. One of the hypotheses suggest local or functional progesterone decrease as a potential reason for preterm uterine contractions leading to preterm delivery. It is believed that any change in progesterone receptor DNA may be crucial for higher risk of preterm delivery due to abnormal response to prostaglandins, normally inhibited by properly built progesterone. The aim of this study was to determine whether there is an association between progesterone gene polymorphisms (PROGINS and +331G/A) and preterm birth.

Material and methods: A total of 230 women were enrolled, including 115 cases of preterm deliveries (between 22 and 36 weeks of gestation) and 115 healthy mothers of full-term infants. Genomic DNA was isolated from the blood sample. Polymerase chain reaction (PCR) amplification was carried out in a final volume of 25 µL. Genotyping was assayed by PCR. Statistical analysis of the results was conducted with $p < 0.05$ accepted as statistically significant.

Results: For both PROGINS (Alu ins/del) and +331G/A (rs10895068) polymorphisms were equally frequent in case and control group. The prevalence of PGR alleles in both groups was also comparable.

Conclusions: The results of our study showed no association between progesterone gene polymorphisms (PROGINS and +331G/A) and risk of preterm delivery. Identifying mechanisms to prolong the length of gestation, particularly in women at risk for preterm delivery, will improve both maternal and fetal outcomes.

Key words: genetic polymorphism; preterm delivery; progesterone receptor gene

INTRODUCTION

Preterm delivery (PTD), defined as birth occurring prior to 37 weeks of gestation, is one of the greatest challenges in modern perinatal medicine, accounting for 11% of pregnancies worldwide [1]. Preterm births concerns about 75% of perinatal morbidity which ranks PTD as fifth in the leading causes of disease burden over time [2, 3]. Unfortunately, effective diagnostic indicators of PTD and effective treatment are yet to be identified. To date

no specific mechanisms leading to PTD or genetic predisposition has been discovered and accumulating evidence suggests multiple attributable causes [4].

Studies indicate that prophylactic administration of progesterone, beginning at 16 to 20 weeks of gestation and continued to delivery or 36 weeks of gestation, decreases significantly the incidence of preterm birth in high risk pregnant women [5]. Considering that the levels of circulating progesterone levels are not decreasing with the onset of human labor, presumably local or functional progesterone decrease is of significance in pathogenesis of preterm birth [6].

The physiological effects of progesterone are mainly controlled by binding to specific progesterone receptors (PGR) or by changing the expression level of the PGR [7]. Polymorphic variants of human progesterone receptor have been described [8–11]. "PROGINS", firstly described as a 306bp insertion in intron G of the T2 allele of the PGR [12], is found to be linked to other polymorphisms (G to T substitution in exon 4, causing a Valine to Leucine change (V660L) and a C to T substitution in exon 5 (H770H)) and therefore have been referred in literature as PROGINS complex [13]. It has been suggested that its polymorphism results in abnormal transcription of progesterone receptor gene [14]. Other widely described polymorphism, known as +331G/A, is of more functional significance and is able to affect the ratio of two main progesterone receptor isoforms: A and B [15].

The role of progesterone in early pregnancy is critical to its maintenance until the placenta takes over its function. However, the role of progesterone in later stages of pregnancy remains unclear. One of the hypotheses proposed that progesterone may be responsible for limiting the production of stimulatory prostaglandins and inhibiting the expression of contraction-associated protein genes and prostaglandin receptors within the myometrium [16, 17]. Therefore, it is believed that any change in PGR DNA may be crucial for higher risk of preterm delivery due to abnormal response to prostaglandins, normally inhibited by properly built progesterone.

The aim of this study was to determine whether there is an association between progesterone gene polymorphisms (PROGINS and +331G/A) and preterm birth.

MATERIAL AND METHODS

Ethics statement

This study was approved by the Bioethics Committee of Poznan University of Medical Sciences, Poland (No: 490/21). All participants were voluntary recruited and provided written informed consent before taking part in this research.

Patient recruitment

A total of 230 women were enrolled, including 115 cases of preterm pregnancy termination (between 22 and 36 weeks of gestation from the date of the last menstrual period) and 115 healthy mothers of full-term infants who born in the Division of Perinatology and Women's Diseases of University of Medical Sciences in Poznan, Poland between February 2015 and April 2020. Each patient donated a onetime 10 ml of the whole blood drawn into a K_2EDTA tube for genetic testing. Preterm is defined as babies born alive before 37 weeks of pregnancy are completed. Exclusion criteria included: maternal age of less than 18 years, history of drug abuse, pregnancy acquired through assisted reproduction techniques, past terminations of pregnancy, hypertension, preeclampsia, bleedings during present pregnancy, preterm premature rupture of membranes, intrauterine infection, multiple pregnancy, detected fetal or placental abnormalities, cholestasis of pregnancy, cervical insufficiency. Controls were recruited during a routine check-up in uncomplicated pregnancy with a history of a previous at least two healthy gestations. All subjects were obliged to sign a written consent to participate in the study. Medical records of the participants were obtained for more detailed information about the patients.

Genotyping

Genotyping was performed in the Molecular Biology Laboratory of Poznan Medical Science University. Genomic DNA was isolated from the blood sample, using a Blood Mini Kit (Qiagen, Germany) according to the manufacturer's instructions. DNA concentrations were determined by spectrophotometric measurement of absorbance at 260 nm and the purities were calculated by A260/A280 ratio using NanoDrop 2000 spectrophotometer (Thermo Scientific, USA). and the DNA was stored at –20°C until analyzed. PCR amplification was carried out using DNA Engine Dyad Thermocycler (Bio-Rad Laboratories, Inc.) in a final volume of 25µL. Genotyping was assayed by polymerase chain reaction (PCR) using isolated genomic DNA, deionized water, 200 mM dNTP Mix (Thermo Fisher Scientific, USA), 0.25 µM forward and reverse primers (TiBMolBiol, Germany), 1U of Taq DNA polymerase and respective buffer (DreamTaq Green DNA polymerase, Thermo Fisher

Scientific, USA). For each analyzed polymorphism, 10% of the total samples were randomly retested with 100% concordance.

Alu insertion/deletion variant (PROGINS)

The following primers were used: 5'-GGC AGA AAG CAA AAT AAA AAG A-3' in the forward direction and 5'-AAA GTA TTT TCT TGC TAA ATG TC-3' in the reverse direction, as reported by Lancaster et al. [18], the DNA was amplified by initial denaturation at 95ºC for 10 min, followed by 30 cycles of denaturation at 95ºC for 30 s, annealing at 52ºC for 30 s and extension at 72ºC for 45 s, with a final extension at 72ºC for 10 min. The PCR products of the PGR gene were visualized on a 2% agarose gel with Midori Green staining (Nippon Genetics, Europe GmbH). Alleles for this variant are named traditionally as described by Rowe et al. [12]. The *T1* allele was defined as the absence an *Alu* insertion in intron G and generated a 149 base pair fragment in agarose gel, while the polymorphic allele (allele *T2*) generated a 455-base pair.

+331G>A (rs10895068)

The *PGR* gene rs10895068 variant were detected by using PCR–RFLP technique as previously described by Li et al. [19]. Primers with the following sequences were used for the PCR reaction: forward primer 5'-CAC TAC TGG GAT CTG AGA TC-3' and reverse primer 5'-CAC AAG TCC GGC ACT TGA GT-3' PCR conditions consisted of an initial denaturation at 95°C for 10 minutes, followed by 30 cycles of 95°C for 30 s, 55°C for 30 s, 72°C for 30 s with a final extension at 72°C for 10 minutes. The 262 base pair PCR products were digested with *BspL*I (*Nla*IV) (Thermo Scientific, USA) in a volume of 20 µL at 37°C overnight. Products were electrophoresed in a 2.5% agarose gel with Midori Green (Nippon Genetics, Europe GmbH) and visualized through the UV transilluminator. A band of 156, 56, 50 bp represented *GG* (wild type) genotype; two bands of 212 bp and 50 bp represented *AA* (polymorphic).

Statistical methods

All analyses were performed using R version 4.0.3 [20] and R package "SNPassoc." [21]. Continuous variables without skewness were estimated via means ± standard derivation (SD) and compared with the student's t tests. Categorical variables were used through frequency counts and percentage. Data were tested for goodness of fit between observed and

expected genotype frequencies according to Hardy–Weinberg equilibrium. Associations between the genotypes and the susceptibility of PTD were assessed via odds ratio (OR) with 95% confidence interval (CI) by logistic regression analyses. The most appropriate inheritance model was selected based on Akaike information criteria (AIC) and was adjusted by pre-pregnancy BMI. Linkage disequilibrium between loci was assessed by Haploview version 4.0 (Daly Lab at the Broad Institute, Cambridge, MA, USA). Statistical significance was defined as a p value < 0.05 with a two-tailed test.

RESULTS

Characteristics of the study population

A hospital-based case-control study was conducted, involving 115 women with preterm birth and 115 controls. Cases and controls were evenly matched by age. Preterm is defined as babies born alive before 37 weeks of pregnancy are completed. Preterm women were divided into three groups based on their gestational age: extremely preterm (less than 28 weeks), very preterm (28 to 31 + 6 weeks) and moderate to late preterm (32 to 36 + 6 weeks). The characteristics of the cases and controls are summarized in Table 1. The majority of the PTD women gave birth moderate to late preterm (80.00%) followed by very preterm (15.65%) and extremely (4.35%). The mean ages of the PTD patients and full-term controls were 29.18 (5.14) and 30.14 (4.60) years, respectively (p = 0.14). Cases were more probably to have higher blood pressures, pre-pregnancy BMI and more likely to have caesarian section then controls. All the control patients were multiparous, while this was the first delivery for 38.26% of the PTD women. Interestingly, the women from the study group had a higher BMI at the end of pregnancy (without statistical significance), even though their pregnancies were on average shorter than in the control group (33.65 (2.74) weeks vs 38.90 (1.09) in controls, p < 0.001). When analyzing the clinical data of newborns from the studied groups, statistically significant differences were observed between the Apgar scores, newborn weights and placental weights. There were no differences in the frequency distributions of newborns gender between the PTD women and the controls (p = 0.28).

PGR gene polymorphisms and the susceptibility of PTD

As shown in Table 2, the genotype distributions of PGR polymorphisms in the case and control groups were in Hardy-Weinberg equilibrium (p > 0.05). However, there were no

differences in allele frequencies between the women with preterm delivery and controls. A frequency of minor alleles in the cases (*T2* — 16.9% and *A* — 6.1%) were similar to the frequency observed in controls (17.3% and 5.6% respectively).

The genotype distributions of the PGR variants (ins/del and rs10895068) in PTD women and controls are presented in Table 3. There were no differences in frequency of each of the three genotypes, neither for ins/del (T1T1: 68.7% vs 67.8%, T1T2: 27.8% vs 30.4%, T2T2: 3.5% vs 1.7%) nor rs10895068 (GG: 89.6% vs 87.8%. GA: 9.6 vs 12.2%, AA: 0.9% vs 0.0%). Moreover, no significant differences were observed for these two polymorphisms in dominant or recessive models even after adjusting for pre-pregnancy BMI.

An analysis of the linkage disequilibrium was also performed by using Haploview software. Analyzed variants of the *PGR* gene (ins/del and rs10895068) are in a linkage equilibrium with D' = 1.0 and $r^2 = 0.01$.

Stratified analysis of PGR polymorphisms and the risk of PTD

We carried out stratified analysis to assess the relationship between the *PGR* variants and the risk of PTD by the subtypes distinguished according to the week of pregnancy termination (Tab. 4). As the group of women whose pregnancy ended before 28 weeks (extremely preterm delivery, EPTD) was very small (n = 5), we connected it with the group very preterm delivery (VPTD). Both crude and adjusted subgroup analyses revealed no significant differences for genotype frequencies of studied variants in any genetic models.

Effect of PGR ins/del and +331G > A genotypes on week of pregnancy termination

We found no significant difference in week of pregnancy termination before and after adjusted for pre-pregnancy BMI among the genotypes of study polymorphisms in either the patients or the control group (Tab. 5).

DISCUSSION

The hypothesis of the present study assumed that genetic variations in the progesterone receptor gene would be more frequent in PTD in comparison to the control group. Progesterone is responsible for the maintenance of the pregnancy thus any deviations in both progesterone receptor and progesterone itself might be responsible for loss of its physiological function and in consequence result in induction of preterm uterine contractions [16].

A recent metanalysis and systematic review considering association of estrogen and progesterone receptor with recurrent pregnancy loss (RPL) proved an association between those two determinants [22]. The authors evaluated six studies from years 1993–2011 which investigated the PR polymorphism, from which two presented positive association [23, 24], whereas the other four did not suggest the risk. However, the meta-analysis showed that PROGINS polymorphism had no impact on higher rate of RPL.

Present research focuses mostly on impact of progesterone receptor polymorphisms on cancer risk. A few authors proved that PROGINS polymorphism might be associated with an increased risk of ovarian cancer [11, 25–27] and endometrial carcinoma [28], however the results are not consistent and further studies are required. Similarly, the progesterone receptor gene +331 G/A polymorphism might increase breast cancer risk [8, 15]. A recent metanalysis suggested that this effect appears to be more prominent in American rather than in Europeans and Australians [9].

Until present four different studies investigated the association between progesterone gene receptor and preterm delivery. In Portuguese population Oliveira et al. on a group of 114 women did not prove that the presence of PROGINS polymorphism constitute a risk factor for premature birth [29]. Similarly, Gouyang et al. found that maternal carriage of minor alleles of +331G/A, +770C/T, and +600G/T single nucleotide polymorphisms in the PR gene is not associated with spontaneous preterm birth. Interestingly, the carriage of +770T and +660T was confirmed to be linked with preterm birth in women with a low body mass index (< 18.5 kg/m^2) [30]. On the contrary a recent study by Tiwari et al. showed that the distribution of the PROGINS mutation was higher in preterm delivery cases compared to controls but the increase was not statistically significant ($p = 0.09$). Even if divided into three different cohorts (extremely — less than 28 weeks, very — between 28 and 32 weeks and moderately preterm — between 32 and 37 weeks) and analysed against term deliveries, the presence of PR mutation was not associated with a higher risk of preterm birth. However, it was significantly related to negative pregnancy outcomes (intrauterine death) in moderately preterm group ($p = 0.03$) and lower baby birth weight in both term and preterm cases ($p = 0.04$) [31]. Another interesting study focused on evaluation of both fetal and maternal genetic variation in the progesterone receptor gene for contributions to preterm birth [32]. Ehn et al. suggested that genetic variation in the PGR gene of either the mother or the fetus may trigger preterm delivery. Another study conducted to determine whether increase frequency of mutant alleles of the progesterone receptor gene was associated with preterm birth in a population of

Hispanic women proved that PROGINS and +331G/A polymorphisms are not associated with preterm birth [33]. The findings of the present study on Polish women are similar to those on Hispanic population, which suggests that the genetic variations of those specific locus in the PGR gene do not contribute to this pathology.

Limitations of the study include relatively small sample size and single-center character. Moreover, the study examined the association of only a few PGR variants while possibly the presence of additional PGR polymorphisms may influence the risk of PTD. Lastly, the study population was only Polish women therefore the results cannot be generalized to other nationalities and ethnicities. We recognize that further studies are necessary to determine whether progesterone gene polymorphisms are associated with preterm birth in a more diverse and bigger group.

In conclusion, the results of our study showed no association between progesterone gene polymorphisms (PROGINS and +331G/A) and risk of preterm delivery. Identifying mechanisms to prolong the length of gestation, particularly in women at risk for preterm delivery, will improve both maternal and fetal outcomes.

Conflict of interest

The authors have no conflict of interest to disclosure. The manuscript has not been and will not be submitted to any other journal while it is under consideration by the *Polish Archives of Internal Medicine*. Also, there are no prior publications or submissions with any overlapping information.

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Table 1. Demographic and clinical characteristics of the study population

Characteristics	PTD (n = 115)	Controls (n = 115)	p
Maternal age, years	29.18 (5.14)	30.14 (4.60)	0.14
Gestational age at delivery, weeks	33.65 (2.74)	38.90 (1.09)	< 0.001
— extremely preterm, n, %	5 (4.35)	—	
— very preterm, n, %	18 (15.65)	—	
Systolic blood pressure, mmHg	110.83 (15.63)	107.26 (11.28)	0.049
Diastolic blood pressure, mmHg	70.17 (11.12)	67.00 (8.35)	0.02
Pre-pregnancy BMI [kg/m^2]	21.43 (2.89)	20.71 (1.76)	0.02
Post-pregnancy BMI, kg/m^2]	26.15 (3.66)	25.87 (2.38)	0.50
Caesarean section, n (%)	39 (33.91)	24 (20.87)	0.04[1]
Primipara, n (%)	44 (38.26)	0 (0.00)	—
Birth weight [g]	2375.30 (714.36)	3473.130 (403.79)	< 0.001
Placenta weight [g]	552.01 (149.32)	626.80 (112.03)	< 0.001
Infant male, n (%)	65 (56.52)	74 (64.35)	0.28[1]
1-min Apgar score	7.83 (2.68)	9.86 (0.54)	< 0.001
5-min Apgar score	8.97 (1.44)	9.98 (0.13)	< 0.001

mean ± SD; p — Two Sample t-test; p[1] — Pearson's Chi-squared test

Table 2. Prevalence of PGR alleles in cases and controls

Variants	Alleles	PTD n = 230		Controls n = 230		p
		MAF n (frequency)	HWE p	MAF n (frequency)	HWE p	
Ins/del	T1 > T2	39 (0.169)	0.69	40 (0.173)	0.94	0.90
Rs10895068	G > A	14 (0.061)	0.79	13 (0.056)	0.54	0.84

HWE — Hardy-Weinberg equilibrium; χ2 p — Pearson's Chi-squared test

Table 3. Genotype distributions of the studied polymorphisms in cases and controls, and their risk prediction for preterm delivery under three genetic models of inheritance

Polymorphism	Controls (n = 115)	PTD (n = 115)	Crude OR (95%CI)	p-value	AIC	Adjusted (95%CI)
Ins/del						
T1T1	79 (68.7)	78 (67.8)	1.00	0.66	324.0	1.00
T1T2	32 (27.8)	35 (30.4)	1.11 (0.62–1.96)			1.06 (0.59–1.90)
T2T2	4 (3.5)	2 (1.7)	0.51 (0.09–2.84)			0.44 (0.08–2.56)
Dominant	36 (31.3)	37 (32.2)	1.04 (0.60–1.81)	0.89	322.8	0.99 (0.56–1.74)
Recessive	111 (96.5)	113 (98.3)	0.49 (0.09–2.74)	0.40	322.2	0.43 (0.07–2.49)
+331G > A (rs10895068)						
GG	103 (89.6)	101 (87.8)	1.00	0.67	323.1	1.00
GA	11 (9.6)	14 (12.2)	1.30 (0.56–2.99)			1.40 (0.60-3.26)
AA	1 (0.9)	0 (0.0)	—			—
Dominant	12 (10.4)	14 (12.2)	1.19 (0.52–2.70)	0.68	322.7	1.30 (0.57–2.98)
Recessive	114 (99.1)	115 (100.0)	—	1.00	321.5	—

AIC — Akaike information criteria, adjusted for pre-pregnancy BMI; CI — confidence interval; OR — odds ratio