

A polymorphism of the Corticotropin-releasing hormone receptor 2 (CRHR2) and preterm birth

Maximilian Schmid^{a,*}, Christoph Grimm^a, Heinz Leipold^b, Martin Knöfler^a, Peter Haslinger^a and Christian Egarter^a

^aDepartment of Obstetrics and Feto-maternal Medicine, Medical University of Vienna General Hospital, Austria

^bDepartment of Obstetrics and Gynecology, Landeskrankenhaus Klagenfurt, Austria

Abstract. Our aim was to investigate whether a genetic variation in the corticotropin-releasing hormone receptor 2 gene might be associated with preterm birth. In this case-control study we evaluated the G/A polymorphism (rs2267717) in intron 2 of the corticotropin-releasing hormone receptor 2 gene in one hundred women with preterm birth and one hundred healthy women with at least one uncomplicated full term pregnancy and no history of preterm birth. No significant correlation was found between the presence of the investigated polymorphism and preterm birth ($p = 0.9$, odds ratio 0.9 [Confidence interval 0.5–1.7]). A dose dependent association of the investigated polymorphism, in women with preterm birth, with gestational age at delivery ($p = 0.003$) and birth weight was observed ($p = 0.0001$). However, no association between IUGR ($n = 10$) with either one of the investigated genotypes ($p = 0.3$) was found. Stratified analysis within case group (i.e. PPROM vs. non-PPROM) revealed no significant difference in genotype distribution ($p = 0.6$). In conclusion, the investigated polymorphism does not increase the risk for preterm birth overall but might modulate the length of pregnancy in a dose dependent fashion in a series of Caucasian women.

Keywords: Corticotropin-releasing hormone receptor 2, polymorphism, preterm birth

1. Introduction

Preterm birth (PTB) remains a major cause of perinatal mortality and long term handicap in surviving infants. In industrialized countries, 5–11% of infants are born preterm (< 37 weeks' gestation), and the rate has been increasing continuously. PTB accounts for 70% of neonatal deaths and up to 75% of neonatal morbidity, and contributes to long-term neurocognitive deficits, pulmonary dysfunction and ophthalmologic disorders [1].

Genetic factors play a strong role in PTB [2]. One of the best predictors for preterm delivery is the woman's

history of a previous preterm birth [3]. In addition, a family history of preterm delivery [4,5] strongly supports an underlying genetic component. In twin studies, the heritability of preterm delivery has been suggested to be as high as 40% [6].

Evidence exists that corticotropin-releasing hormone (CRH) plays a role in the pathophysiology of preterm birth [7]. CRH, a 41-amino acid peptide, is the principal regulator of the hypothalamic-pituitary-adrenal axis. CRH activates the two G-protein-coupled corticotropin-releasing hormone receptors (CRHRs), CRHR1 and CRHR2, each with unique splicing patterns and remarkably distinct pharmacological properties, but similar signaling properties. The physiological effects of CRH and CRH-related agonists in target tissues depend on sufficient expression of functional CRHRs [8]. CRH is produced by fetomaternal tissues and secreted into the maternal circulation, so that dur-

*Corresponding author: Maximilian Schmid, MD, Department of Obstetrics and Feto-maternal Medicine, Medical University of Vienna General Hospital, Waehringer Guertel 18-20, A-1090 Vienna, Austria. Tel.: +43 1 40400 2822; Fax: +43 1 40400 2995; E-mail: maximilian.schmid@meduniwien.ac.at.

ing pregnancy the maternal plasma levels of this hormone increase while the corresponding levels of binding protein (CRHBP) decrease [9]. Patients at risk for preterm birth have elevated plasma levels of CRH and lower CRHBP levels [10]. CRHR1 and CRHR2, are expressed in human placenta, decidua, fetal membranes, endometrium, cervix and myometrium [11]. CRH is synthesized and secreted by the human placenta and might act as a “placental clock” that regulates the onset of human labor, most likely by modulating myometrial contractility [12,13]. Of note, the usefulness of maternal plasma CRH as a predictor of preterm birth remains controversial [14,15].

To the best of our knowledge the association between corticotropin-releasing hormone receptor (CRHR) gene polymorphisms and preterm birth has not been investigated. We evaluated the role of the common maternal CRHR2 G/A gene polymorphism (rs2267717) in intron 2 to identify women who may be at higher or lower risk of preterm delivery compared with women with at least one uncomplicated pregnancy who delivered at term.

2. Materials and methods

2.1. Patients

Blood samples of 100 women with singleton pregnancies who delivered preterm consecutively were obtained from the serum bank of the Medical University of Vienna, Department of Obstetrics and feto-maternal Medicine. This included women with spontaneous PTB due to preterm labor (26/100) or preterm premature rupture of membranes (PPROM) (55/100) as well as cases where preterm delivery was indicated for medical reasons such as intrauterine infection after PPROM (19/100). Preterm delivery was considered as delivery before 37 completed weeks of gestation. 100 women with at least one uncomplicated full term singleton pregnancy and no history of PTB, preeclampsia or stillbirth were asked to serve as controls. Gestational age was estimated from the first day of the last menstrual period and was confirmed by early ultrasound. Clinical data were obtained from files at the Medical University of Vienna. All patients were of Caucasian origin. Blood samples of cases and controls were drawn 2.4 (standard deviation 1.8) and 2.2 (1.6) days after delivery. Written informed consent was obtained from mothers before collection of biological materials. Approval was obtained by the institutional review board of the Medical University of Vienna (Reference EK Nr. 385/2004).

2.2. Genotyping

Genomic DNA was isolated from anticoagulated blood by using the QiAmp Blood Midi Kit, as described by the manufacturer (Qiagen, Hilden, Germany), and stored at -20°C. The G/A polymorphism (rs2267717) in intron 2 of the CRHR2 gene was genotyped by polymerase chain reaction – restriction fragment length polymorphism (PCR-RFLP). The PCR amplification was conducted with primers flanking the gene region containing the polymorphism (5'-CAGAAAGCCTCCCAGGAATG-3' and 5'-GGCTTTCTCCCTGTCTCCATC-3'). The reaction was performed in a volume of 25 µl, consisting of 1xPCR buffer, 200 µmol of dNTP, 1,5 nmol of MgCl₂, 0,5U TaqDNA Polymerase (Fermentas, Germany) and 40ng of genomic DNA. The concentration of each primer was 20 pmol/µl. Cycle conditions were 94°C for 4 minutes; 37 cycles at 94°C for 30 seconds, 58°C for 30 seconds, 72°C for 3 minutes, and 72°C for 5 minutes. For allele detection, the PCR product was digested with the restriction endonuclease NlaIII (New England BioLabs, Beverly, MA). Fragments were separated by electrophoresis on an ethidium bromide-stained agarose gel (2%) and analyzed under UV light. The individual genotype had characteristic bands at the following molecular weights other than four common bands (257, 163, 96, and 47 bp): G/G, 392 and 225 bp; G/A, 617, 392, and 225 bp; A/A, 617 bp.

2.3. Statistical analysis

After testing for normality using Kolmogorov-Smirnov test, values are given as medians (interquartile range [IQR]) or means (standard deviation [SD]) where appropriate. Groups were compared using Student's t-tests and chi-square tests where appropriate. Statistics have been performed accordingly. P-values and Odds Ratios (OR) with 95% Confidence Intervals (95% CI) were calculated wild-type/wild-type (wt/wt) vs. wild-type/mutant (wt/mt) and mutant/mutant (mt/mt), i.e., wild-type versus presence of at least one mutant allele. P-values < 0.05 were considered statistically significant. Study's power was calculated under the assumption of a 5% type I error, 100 cases, 1/1 ratio cases/controls, 25% probability of exposure in controls, and a 2.0 OR. This calculation revealed a power of 56.2% to detect an OR of 2.0. The given sample size allows for detection of an OR greater than 3 with a power of 80.2%. Statistical software SPSS 11.0 for Windows (SPSS 11.0, SPSS Inc., Chicago, IL) was

Table 1
Patients' characteristics

Characteristics	Women with preterm birth	Women with full term pregnancy	P-value	OR (95%CI) ¹
Total number of patients	100	100	—	—
Maternal age (years)	29.1 (6.6) ²	28.8 (6.4) ²	0.8 ³	—
History of previous preterm birth	15	0	< 0.001 ⁴	2.2 (1.9–2.5)
Gestational age at delivery (wks.)	29.6 (2.9) ²	39.9 (1.3) ²	< 0.001 ³	—
Birth weight (grams)	1286.6 (553.7) ²	3421.9 (465.4) ²	< 0.001 ³	—
First pregnancy	48	39	0.3 ⁴	1.4 (0.8–2.5)
Delivery mode				
Caesarean section	90	27	< 0.001 ⁴	24.3 (11.1–53.5)
Spontaneous delivery	10	73		
Antenatal bacterial vaginosis	7	1	0.07 ⁴	7.5 (0.9–61.7)
LLETZ ⁵ conisation	3	1	0.6 ⁴	3.1 (0.3–30.0)
Smoking	9	1	0.02 ⁴	9.8 (1.2–78.8)

¹Odds Ratio (95% Confidence Interval).

²Mean (Standard deviation).

³Student's t-test.

⁴Chi-square test.

⁵LLETZ: large loop excision of the transformation zone.

Table 2
Genotype and allele frequencies of the CRHR2 gene polymorphism in women with preterm birth and women with full term pregnancy

	Women with preterm birth	Women with full term pregnancy	P-value	OR (95% CI)
<i>CRHR2</i>				
wt/wt	77 (77%)	75 (75%)	0.9 ¹	0.9 (0.5–1.7) ¹
wt/mt	22 (22%)	22 (22%)	0.6	
mt/mt	1 (1%)	3 (3%)		
Alleles				
wt	176 (88%)	172 (86%)	0.7	0.8 (0.5–1.5)
mt	24 (12%)	28 (14%)		

¹P-value and Odds Ratio (95% Confidence Interval) were calculated wt/wt vs. wt/mt and mt/mt.

wt: wild type; mt: mutant.

used for statistical analysis. Power/ sample size was calculated by PS Power and Sample Size Calculations version 2.1.30 [16].

3. Results

Patient characteristics are shown in Table 1. Distribution of genotypes in women with preterm birth ($p = 0.9$) and term controls ($p = 0.9$) was in Hardy-Weinberg equilibrium. No significant association was found between the investigated CRHR2 gene polymorphism and preterm birth ($p = 0.9$, odds ratio 0.9 [Confidence interval 0.5–1.7] (Table 2). In women with preterm birth, a dose dependent association of the investigated polymorphism with gestational age at delivery ($p = 0.003$, wt/wt: 29.4 [2.8] weeks [wks.], wt/mt: 30.0 [3.1] wks., and mt/mt: 37.0 wks.) and birth weight was found ($p = 0.0001$, wt/wt: 1287.3 [531.2] grams

[g.], wt/mt: 1214.4 [550.5] g, and mt/mt: 2820.0 g (Figs 1 and 2). No association between IUGR ($n = 10$) with either one of the investigated genotypes ($p = 0.3$) was found. Stratified analysis within case group (i.e. PPROM vs. non-PPROM) revealed no significant difference in genotype distribution ($p = 0.6$).

4. Discussion

A considerable body of evidence points to a crucial role of genetics in the pathogenesis of preterm birth [2–5]. Several associations between polymorphisms in maternal genes and preterm birth have been described: Single nucleotide polymorphisms (SNP) in tumor necrosis factor, interleukin (IL)-1 β receptor antagonist, IL-4, matrix metalloproteinase 9, β 2-adrenergic receptor, vascular endothelial growth factor, and factor V Leiden are all associated with PTB [17].

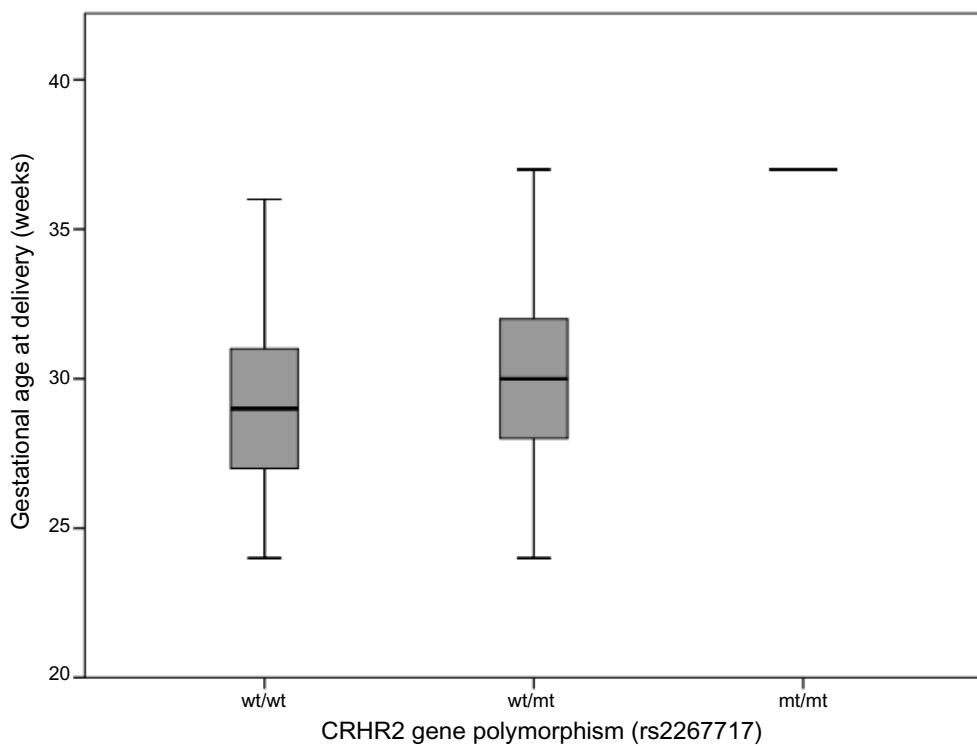


Fig. 1. CRHR2 gene polymorphism (rs2267717) and gestational age at delivery.

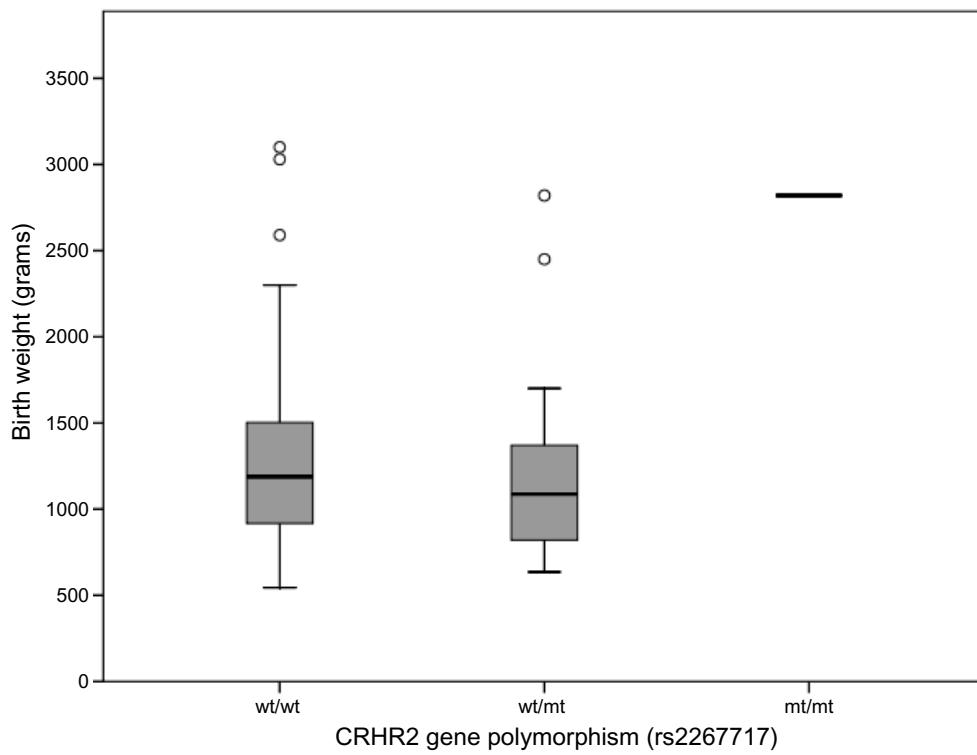


Fig. 2. CRHR2 gene polymorphism (rs2267717) and birth weight.

Recent advances in the physiology of human pregnancy have implicated CRH and its receptors as important endocrine mediators of parturition and possibly also of fetal development [7,13]. To the best of our knowledge the role of CRHR gene polymorphisms in PTB have not been evaluated. We speculated that a common polymorphism within the CRHR2 gene might be associated with PTB. However, there was no significant correlation between the presence of the investigated polymorphism and PTB in our study. Interestingly we observed a dose dependent association of the investigated polymorphism with gestational age at delivery and birth weight in women with PTB. One could speculate that presence of the mutant allele influences the effect of CRH as endocrine mediator of parturition or perhaps fetal development. However, this most likely represents a random finding since further analysis revealed no association between IUGR with either one of the investigated genotypes.

However not all cases of preterm birth are related to changes in placental CRH production; in particular, intrauterine infection, a relatively frequent cause of preterm birth, is not associated with elevated placental CRH production. PTB is likely the result of various different underlying causes [18]. A single gene disorder model for PTB seems unlikely because human families with a Mendelian pattern of inheritance (i.e. autosomal dominant, autosomal recessive) for preterm delivery are difficult to identify [19]. Polymorphisms in the TNF alpha, interleukin 6, and interleukin 4 genes as well as other inflammatory mediators have [20] been successfully associated with preterm birth. However also stratified analysis within case group (e.g. PPROM vs. non-PPROM) revealed also revealed no significant difference in genotype distribution. It seems that for the majority of preterm deliveries, a genetic predisposition is more likely to occur through many genes and gene-environment interactions. Given the current methodology and knowledge of biology, the ideal genetic study of PTB may not yet be possible. Further genome-wide and candidate gene studies have been proposed to a gain a better etiologic and biologic understanding of the hereditary components of PTB [21].

To conclude, we are the first to report on a genetic polymorphism within the CRHR 2 gene and PTB in a series of Caucasian women. Our data, however, does not support the assumption that the common G/A polymorphism within the CRHR2 gene is associated with PTB. To understand the role of CRHR polymorphisms in PTB further studies investigating additional polymorphisms of the CRHR 2 gene and other components of the CRH signaling system are needed.

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