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Interactions between environmental factors and maternal–fetal genetic variations: strategies to elucidate risks of preterm birth

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ABSTRACT

Context: Preterm birth (PTB) is a complex disease in which medical, social, cultural, and hereditary factors contribute to the pathogenesis of this adverse event. Interactions between genes and environmental factors may complicate our understanding of the relative influence of both effects on PTB. To overcome this, we combined data obtained from a cohort of newborns and their mothers with multiplex analysis of inflammatory-related genes and several environmental risk factors of PTB to describe the environmental–genetic influence on PTB.

Objective: The study aimed to investigate the association between maternal and fetal genetic variations in genes related to the inflammation pathway with PTB and to assess the interaction between environmental factors with these variations.

Study design: We conducted a case–control study at the Pereira Rossell Hospital Center, Montevideo, Uruguay. The study included 143 mother–offspring dyads who delivered at preterm (gestational age < 37 weeks) and 108 mother–offspring dyads who delivered at term. We used real-time PCR followed by a high-resolution melting analysis to simultaneously identify gene variations involved in inflammatory pathways in the context of environmental variables. The genes analyzed were: Toll-like receptor 4 (TLR4), Interleukin 6 (IL6), Interleukin 1 beta (IL1B) and Interleukin 12 receptor beta (IL12RB).

Results: We detected a significant interaction between IL1B rs16944 polymorphism in maternal samples and IL6 rs1800795 polymorphism in newborns, emphasizing the role of the interaction of maternal and fetal genomes in PTB. In addition, smoke exposure and premature rupture of membranes (PROM) were significantly different between the premature group and controls. IL1B and IL6 polymorphisms in mothers were significantly associated with PTB when controlling for smoke exposure. TLR4 polymorphism and PROM were significantly associated with PTB when controlling for PROM, but only in the case of severe PTB.

Conclusions: Interactions between maternal and fetal genomes may influence the timing of birth. By incorporating environmental data, we revealed genetic associations with PTB, a finding not found when we analyzed genetic data alone. Our results stress the importance of studying the effect of genotype interactions between mothers and children in the context of environmental factors because they substantially contribute to phenotype variability.

Introduction

Preterm birth (PTB), defined as a live birth occurring between 20 and 37 weeks of gestation, complicates 12.2% of pregnancies [1], contributes to more than one-third of infant deaths in the United States annually [2], and is associated with serious medical complications, including neurodevelopmental delay, hearing disabilities, retinopathy, and chronic lung disease [3,4]. PTB is etiologically heterogeneous [5,6] but epidemiological evidence indicates that genetic factors play a significant role in the etiology of spontaneous PTB [7,8]. A number of candidate gene studies, almost exclusively using case–control design, have identified some genes that associate with PTB [9–13]. However, the results have rarely been replicated. Of importance for this phenotype are the possible effects of two genomes, maternal and fetal [13–15]. In addition,

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interactions between maternal and fetal genomes may affect PTB risk [13]. As a multifactorial trait, maternal stress, multiple pregnancies, and exposure to toxics during pregnancy could interact with genetic predisposition and develop in PTB [5,6,16]. Interactions between genes and environmental factors may make it difficult to elucidate and discriminate both effects; for example, some maternal genotypes modify the association between maternal cigarette smoking and infant birth weight [17], as well as the presence of bacterial vaginosis in Tumor Necrosis Factor-2 carriers predisposes to spontaneous PTB [18]. Not considering environmental factors when performing a case–control study of genetic association and PTB may lead to misinterpretation of the results. Genome-wide association studies (GWAS) are promising but require a very large number of well-characterized subjects to overcome the challenge of multiple statistical comparisons. Given the practical limitations of assembling very large sample sizes in complex phenotypes, such as PTB, there is a need for creative research strategies that seek to resolve the tension between the hypothesis-directed candidate gene and the relatively unbiased GWAS approach. It is generally accepted that inflammation in pregnancy plays an important role [10,11,19–22]. Inflammatory pathways involve hundreds of mediators, and each of them could present several single nucleotide polymorphisms (SNPs). We previously reported a high resolution melting (HRM) analysis to simultaneously identify mutations in four genes involved in inflammatory pathways Toll-like receptor 4 (TLR4), Interleukin 6 (IL6), Interleukin 1 beta (IL1B), and Interleukin 12 receptor beta (IL12RB) [23].

In this study, our first aim was to look for associations between PTB and four SNPs of inflammatory genes TLR4, IL6, IL1B, and IL12RB in a relatively small cohort of dyads of newborns and their mothers, using a case–control study design. Secondly, we aimed to combine this genotype data with an analysis of environmental risk factors of PTB to enlighten the environmental–genetic influence on PTB.

Materials and methods

Subjects

A case–control study was conducted. Subjects were mothers and their offspring, receiving obstetrical care at the Pereira Rossell Hospital Center (CHPR), Montevideo, Uruguay, collected between February 2012 and March 2014. CHPR is the main gynecologic and obstetric center in Uruguay, and it has both secondary and tertiary care units. Cases were dyads ($n = 143$) of mothers and their neonates from pregnancies complicated by spontaneous PTB (gestational age < 37 weeks). Controls ($n = 108$) were mothers and their neonates delivered at term (gestational age ≥ 37 weeks), who experienced uncomplicated term vaginal delivery or elective cesarean. Exclusion criteria included multiple births, drug consumption, chronic pathologies and/or infections, fetal malformations, and failure to give consent to participate in the study. Infections included here were urinary tract infections, lower genital infections, and syphilis but not HIV because patients with HIV infections were excluded from the study.

Mothers were questioned regarding sociodemographic characteristics and obstetric history through a specifically designed questionnaire, including clinically diagnosed PROM, tobacco consumption, previous PTB, and education level. Medical data from mothers and newborns were collected in the perinatal informatics system [24]. Sample size was estimated at the beginning of the study, taking into consideration the frequency of minor allele of SNPs that have a possible inflammatory effect in European populations (approximately 20–40%). The odds ratio (OR) was estimated to be approximately 1.5–2 between cases and controls.

Sample collection, DNA extraction, real-time PCR

Whole blood samples from newborns and cheek swabs from mothers were collected, and DNA was isolated using DNeasy Blood and Tissue kit (Qiagen, Hilden, Germany). HRM analysis was performed on the Rotor-Gene 6000 real-time instrument (Corbett Life Science, Sydney, Australia) with a saturating dye technology (Type-it HRM PCR Kit, Qiagen, Hilden, Germany), as previously described in which SNPs rs4986790 (TLR4), rs1800795 (IL6), rs16944 (IL1B), and rs375947 (IL12RB) were typed [23]. DNA from individuals with heterozygous and both homozygous genotypes were included as controls in all experiments. HRM curves were normalized, and genotypes were assigned according to HRM curve shape by the Rotor-Gene software and visual inspection. Melting curves were analyzed separately for each amplicon.

Statistical analyses

Genotypes, allele frequencies, and Hardy–Weinberg (H–W) equilibrium exact tests were calculated in PLINK v1.07 [25]. Comparisons of allele frequencies were performed using the χ^2 test or Fisher's exact test and means using Student's *t*-test. Association between genotypes and environmental factors was examined by logistic regression analysis. Differential effects of genotypes on the risk of PTB were explored by the inclusion of interaction or conditional terms using Epi Info 2000 software (Centers for Disease Control and Prevention, Atlanta, GA, USA). Probability ≤ 0.05 was considered significant.

Ethics

The study protocol conforms to the Declaration of Helsinki and was approved by the School of Medicine Ethics Committee of the Republic University, Uruguay, in September 2011. Informed consent was obtained from the mothers in all cases.

Results

A total of 251 dyads of mothers and their newborn babies were included in the study. The main characteristics of the study population are shown in Table 1. No differences were detected in both groups regarding mother's age and parity. Mean ± standard

Table 1
Sociodemographic and medical characteristics of mothers and their newborns in study population.

Condition	Term (%) $n = 108$	Preterm (%) $n = 143$	OR (95%CI)	*p*-value
Maternal age (<19 and >35)	16	22	1.6 (0.8–2.9)	0.2
Education level[a]	35	43	0.7 (0.4–1.2)	0.2
Marital status[b]	17	25	1.6 (0.8–3.2)	0.1
Male newborn	51	54	1.15 (0.6–1.9)	0.6
Smoke exposure	44	65	2.6 (1.5–4.3)	**<0.01**
Hypertension	7	4	0.4 (0.14–1-4)	0.2
Pregnacy bleeding	1	4	4.7 (0.5–39)	0.2
Infections	19	27	1.6 (0.8–3)	0.2
IURG	2	1	2.3 (0.6–8.9)	0.2
PROM	13	45	5.5 (2.9–10.6)	**<0.01**
Anemia	14	12	0.8 (0.4–1.7)	0.5

ORs: odds ratios, CI: confidence interval, *p*-values between term and preterm births.
[a] At least 1 year of high school vs. less than 1 year of high school.
[b] Single mothers vs. married or cohabiting mothers.
PROM: premature rupture of membranes; IUGR: intrauterine growth restriction.
Statistical significance is marked as bold values.