Placenta 36 (2015) 1480–1486

Contents lists available at ScienceDirect

Placenta

journal homepage: www.elsevier.com/locate/placenta

Circadian clock-related genetic risk scores and risk of placental abruption

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ARTICLE INFO

Article history:
Received 16 June 2015
Received in revised form
6 October 2015
Accepted 11 October 2015

Keywords:
Circadian clock
Genetic risk score
Placental abruption
SNPs
Pregnancy

ABSTRACT

Introduction: The circadian clock plays an important role in several aspects of female reproductive biology. Evidence linking circadian clock-related genes to pregnancy outcomes has been inconsistent. We sought to examine whether variations in single nucleotide polymorphisms (SNPs) of circadian clock genes are associated with PA risk.

Methods: Maternal blood samples were collected from 470 PA case and 473 controls. Genotyping was performed using the Illumina Cardio-MetaboChip platform. We examined 119 SNPs in 13 candidate genes known to control circadian rhythms (e.g., *CRY2, ARNTL, and RORA*). Univariate and penalized logistic regression models were fit to estimate odds ratios (ORs); and the combined effect of multiple SNPs on PA risk was estimated using a weighted genetic risk score (wGRS).

Results: A common SNP in the *RORA* gene (rs2899663) was associated with a 21% reduced odds of PA ($P < 0.05$). The odds of PA increased with increasing wGRS ($P_{trend} < 0.001$). The corresponding ORs were 1.00, 1.83, 2.81 and 5.13 across wGRS quartiles. Participants in the highest wGRS quartile had a 5.13-fold (95% confidence interval: 3.21–8.21) higher odds of PA compared to those in the lowest quartile. Although the test for interaction was not significant, the odds of PA was substantially elevated for pre-eclamptics with the highest wGRS quartile (OR = 14.44, 95%CI: 6.62–31.53) compared to normotensive women in the lowest wGRS quartile.

Discussion: Genetic variants in circadian rhythm genes may be associated with PA risk. Larger studies are needed to corroborate these findings and to further elucidate the pathogenesis of this important obstetrical complication.

1. Introduction

Placental abruption (PA), the premature separation of the placenta, is a life threatening obstetrical condition that complicates approximately 1% of all pregnancies. Pathophysiologic mechanisms involved in PA include utero-placental ischemia, underperfusion, oxidative stress, chronic hypoxia, and infarctions. On this basis, investigators have begun to conceptualize abruption as an "ischemic placental disorder" characterized by acute and chronic pathophysiological features [1]. As a multi-factorial disorder of complex origin, PA aggregates in families of women with the

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http://dx.doi.org/10.1016/j.placenta.2015.10.005
0143-4004/

condition [2], suggesting a strong role for genetic predisposition, a thesis supported by a number of candidate gene studies [3,4]. Findings from recent PA-related genome-wide association studies (GWAS) and candidate gene association studies (mitochondrial biogenesis and oxidative phosphorylation pathway genes) in the maternal genome by our group provided suggestive evidence supporting associations of variation in maternal cardio-metabolic genes with risk of PA [5–7]. On balance, findings from family studies suggest that the heritability of PA is approximately 16% [8]. Despite considerable effort, however, the precise genetic factors that predispose to PA remain unknown.

The circadian clock plays an important role in several aspects of female reproductive biology, including ovulation, embryonic implantation, and parturition. For example, in premenopausal women the luteinizing hormone (LH) surge generally occurs immediately prior to the start of the active period, while the onset of parturition generally occurs during inactive period as a result of the circadian secretion of the pineal hormone melatonin [9–11]. Investigators have reported that circadian rhythm disruption attributable to rotating and night shiftwork or jetlag is associated with an increase in the frequency of irregular, extended menstrual cycles, alterations in serum LH and follicle stimulating hormone (FSH) concentrations, and reduced fecundity [12–14]. However, evidence linking circadian clock-related genes to pregnancy outcomes has been inconsistent [15,16]. Notably, polymorphisms in *ARNTL* and *NPAS2* have been associated with the risk of miscarriages [16]. Furthermore, we recently noted some evidence of diurnal circadian periodicity among PA cases [17]. Given these findings indicative of the importance of circadian rhythm disruption in reproductive biology and data suggesting genetic susceptibility factors in miscarriages and preterm delivery [15,17], we hypothesized that genetic variations in the maternal genome, and particularly those variants in circadian clock gene pathways are associated with PA risk. Furthermore, given that available evidence suggest individual genetic variants are likely to contribute small effects and/or be weakly associated with PA, we also assessed weighted genetic risk scores to evaluate the influence of accumulation of variants in genes regulating circadian rhythms on PA risk.

2. Materials and methods

2.1. Study setting and population

The current analyses were conducted using data from two case–control studies completed in the setting of the Peruvian Abruptio Placentae Epidemiology (PAPE) study group. The studies have been previously described [5,7]. Briefly, PAPE study participants were recruited and enrolled among patients admitted for obstetrical services to the Hospital Nacional Dos de Mayo, Instituto Especializado Materno Perinatal, and Hospital Madre-Niño San Bartolomé in Lima, Peru. There were two enrollment periods (August 2002 and May 2004 and September 2006 and September 2008). Study protocols are the same for the two study periods. Hospital admission and delivery logs were monitored daily to identify PA cases among new admissions to antepartum, emergency room, and labor and delivery wards of participating hospitals. PA was diagnosed based on evidence of retro-placental bleeding (fresh blood) entrapped between the decidua and the placenta or blood clots behind the placental margin and accompanied by any two of the following: (i) vaginal bleeding in late pregnancy not due to placenta previa or cervical lesions; (ii) uterine tenderness and/or abdominal pain; and, (iii) non-reassuring fetal status or fetal death. Controls were randomly selected from among pregnant women who delivered at participating hospitals during the study period and did not have a diagnosis of PA in the current pregnancy. PA cases and controls were not matched on any maternal characteristics. A total of 517 PA cases and 524 controls provided maternal blood specimens.

Ethical approval for the study (both study periods) was granted by the Institutional Review Boards (IRB) of Hospital Nacional Dos de Mayo, Instituto Especializado Materno Perinatal, Hospital Madre-Niño San Bartolomé in Lima, Peru and the IRB of Swedish Medical Center, Seattle, WA. All participants provided written informed consent in accordance with the principles of the declaration of Helsinki.

2.2. Data collection, DNA extraction and genotyping

Standardized structured questionnaires administered by trained research personnel were used to collect information on socio-demographic characteristics, and medical history. Medical records were reviewed to abstract information on course and outcomes of the pregnancy. The Gentra PureGene Cell kit for DNA preparations (Qiagen, Hilden, Germany) was used to extract DNA from blood specimens. Genotyping was conducted using the Illumina Cardio-MetaboChip (Illumina Inc., San Diego, CA) platform [5], a high-density custom array designed to include 217,697 SNPs that represent DNA variations at regions previously related to diseases and traits relevant to metabolic and atherosclerotic-cardiovascular endpoints [18]. During the assay manufacturing process 20,972 SNPs (9.6%) failed, resulting in 196,725 SNPs available for genotyping, downstream quality control and statistical analyses [18].

2.3. Candidate gene, SNP selection & data quality control

For the candidate association study, 13 genes that were involved in circadian clock gene regulation (based on literature review) and a total of 119 SNPs belonging to these genes and found in the Cardio-MetaboChip were included in the candidate gene association analyses. Quality control and preprocessing were performed on the genotype data as described previously [5,7]. A total of 470 PA cases and 473 controls with genotyping data that passed quality control tests were included in the present study.

2.4. Statistical analysis

Univariate logistic regression model was used to estimate odds ratio (OR) and 95% confidence interval (95%CI) relating each SNP with risk of PA. For multiple testing corrections, a false discovery rate (FDR) procedure was used [19]. In multivariable analyses, we used a penalized logistic regression model to identify sets of SNPs that are jointly associated with the odds of PA [20]. The number of selected variables was guided by a penalty parameter: the larger the parameter, the smaller the selected subset. A 20-fold cross-validation approach was performed to select the penalty parameter and the value yielding the smallest prediction error was used. A group penalty approach was also used to account for the membership in a gene [21]. Furthermore, we considered a bi-level selection approach that uses a composite minimax concave penalty [22] to select candidate genes associated with PA as well as relevant SNPs within those genes. These penalized regression methods do not accommodate missing values; hence we used the BEAGLE software version 3.3.2 [23] to impute missing genotypes.

Weighted genetic risk scores (wGRS) were computed by multiplying the number of risk alleles for each locus by its associated effect size. Once the wGRS were obtained for all individuals, the subjects were categorized into four groups defined by the quartiles in the control. We fitted a logistic regression model to derive odds ratios (OR) and 95% confidence intervals (95%CI) for the odds of PA using the lowest wGRS quartile as reference group. In