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Original Article

Placental genetic variations in circadian clock-related genes increase the risk of placental abruption

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Abstract: The genetic architecture of placental abruption (PA) remains poorly understood. We examined variations in SNPs of circadian clock-related genes in placenta with PA risk. We also explored placental and maternal genomic contributions to PA risk. Placental genomic DNA samples were isolated from 280 PA cases and 244 controls. Genotyping was performed using the Illumina Cardio-MetaboChip. We examined 116 SNPs in 13 genes known to moderate circadian rhythms. Logistic regression models were fit to estimate odds ratios (ORs). The combined effect of multiple SNPs on PA risk was estimated using a weighted genetic risk score. We examined independent and joint associations of wGRS derived from placental and maternal genomes with PA. Seven SNPs in five genes (ARNTL2, CRY2, DEC1, PER3 and RORA), in the placental genome, were associated with PA risk. Each copy of the minor allele (G) of a SNP in the RORA gene (rs2899663) was associated with a 30% reduced odds of PA (95% CI 0.52-0.95). The odds of PA increased with increasing placental-wGRS (P_{trend}<0.001). The ORs were 1.00, 2.16, 3.24 and 4.48 across quartiles. Associations persisted after the maternal-wGRS was included in the model. There was evidence of an additive contribution of placental and maternal genetic contributions to PA risk. Participants with placental- and maternal-wGRS in the highest quartile, compared with those in the lowest quartile, had a 15.57-fold (95% CI 3.34-72.60) increased odds of PA. Placental variants in circadian clock-related genes are associated with PA risk; and the association persists after control of genetic variants in the maternal genome.

Keywords: Circadian gene, placental abruption, pregnancy, placentae, SNPs

Introduction

Circadian rhythms are present in virtually all organs including those of the reproductive system [1]. In mammals, the suprachiasmatic nucleus (SCN) of the hypothalamus is the master clock, which can synchronize other peripheral tissue clocks via endocrine, autonomic and behavioral cues. In both the SCN and peripheral tissues, the circadian oscillation depends on a transcription/translation feedback loop of a group of genes collectively named "clock genes". This family of clock genes include the transcription factors BMAL1 (also known as ARNTL) and CLOCK and proteins encoded by genes PER1-3, CRY1-2 and enzyme casein kinase 1 epsilon (CSNK1E) [2]. Mutations in any of the clock genes can cause severe disruptions in circadian rhythm [3]. The heterodimer composed of CLOCK-BMAL1 protein is a positive regulator and binds to the E-box sequences (CACGTG) of the promoters of PER and CRY, inducing their expression. The negative regulator is a complex of the proteins PER and CRY which translocates to the nucleus and by protein-protein interaction with CLOCK-ARNTL

inhibits the transcription of PER and CRY. Translocation to the nucleus PER and CRY requires the formation of a complex with CSNK1E and provides a delay in the system to achieve a period of 24 hours [4].

Emerging evidence indicates that the placenta may have a functional circadian rhythm and that clock genes are expressed across the maternal-placental interface [5-9]. For example, the existence of the functional placental circadian clock is supported by a study that showed that HTR/SVneo human primary cells are synchronized *in vitro* by serum shock [5]. Furthermore, investigators have documented a circadian rhythm pattern of expression of CLOCK and BMAL1 (ARNTL) genes in full-term human placental explants [9]. These findings, together with observations linking circadian disruption with preterm delivery [10] and placental abruption [11] suggests that variants in clock-related genes in placental tissues - the specific target organ involved in placental abruption - may play an independent etiological role in the pathogenesis of this complication of pregnancy. In a recent study, we documented the cumulative association of maternal circadian rhythm related gene variants, in the maternal genome, with an increased risk of placental abruption [12].

We hypothesized that variations in single nucleotide polymorphisms (SNPs) of circadian clock-related genes in the placental genome may be independently associated with placental abruption risk. Furthermore, given the unique biological interactions and microenvironment at the maternal-placental interface, we also explored potential interactions of variations in circadian clock-related genes in both placental and maternal genomes.

Materials and methods

Study setting and population

The current analyses were conducted using data from two case-control studies completed by the Peruvian Abruptio Placentae Epidemiology (PAPE) study group. The studies have been previously described [12-14]. Briefly, hospital admission and delivery logs were monitored daily to identify placental abruption cases among new admissions to antepartum, emergency room, and labor and delivery wards of participating hospitals. Placental abruption 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 placental abruption in the current pregnancy. A total of 280 abruption cases and 244 controls provided placental specimens. A subset of these cases and controls that also provided maternal DNA samples (222 placental abruption cases and 198 controls) were also included in exploratory analyses designed to assess the independent and joint contributions of maternal and placental genetic variants to placental abruption risk.

Ethical approval for both studies was granted by the Institutional Review Boards (IRB) of all hospitals in Lima, Peru and the IRB of Swedish Medical Center, Seattle, WA. All participants provided written informed consent.

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 the course and outcomes of the pregnancy. Placental tissue was collected immediately after delivery. The chorionic plate, including overlying membranes, was removed and tissue biopsies were taken from the villous tissue, which consists of the intervillous tissues and chorionic villi on the fetal side of the placenta. Biopsies of approximately 0.5 cm^3 were taken from a lateral position, approximately one-third the distance from the placental edge. Biopsies were placed in cryotubes containing RNAlater (Qiagen Inc., Valencia, CA) at 10 ml per 1 mg of tissue and stored at -80°C. The GentraPureGene Cell kit for DNA preparations (Qiagen, Hilden, Germany) was used to extract DNA from placental specimens. Genotyping was conducted using the Illumina

Table 1. Maternal characteristics of placental abruption cases and controls

Characteristics	Study Groups				*P*-value[2]
	Placental Abruption (N=280)		Controls (N=244)		
	n	%	n	%	
Maternal age at delivery (years)[1]	27.0 ± 6.5		27.3 ± 6.6		0.59
<20	35	12.5	32	13.1	0.75
20-34	204	72.8	168	68.9	
≥35	40	14.3	43	17.6	
Gravidity					
1	110	39.3	95	38.9	0.24
2-3	118	42.1	116	47.5	
≥4	52	18.6	33	13.5	
Nulliparous	115	41.1	95	38.9	0.66
Maternal education ≤ high school	220	78.6	186	762	0.64
Single marital status	49	17.5	38	15.6	0.32
Employed during pregnancy	126	45.0	108	44.3	0.93
Planned pregnancy	114	40.7	99	40.6	0.97
Received no prenatal care	37	13.2	18	7.4	0.03
Did not take prenatal vitamins	82	29.3	65	26.6	0.78
Smoked during pregnancy	12	4.3	5	2.1	0.22
Pre-pregnancy body mass index (kg/m^2)[1]	23.5 ± 3.5		23.9 ± 3.9		0.22
Pre-pregnancy body mass index (kg/m^2)					
Lean (<18.5)	14	5.0	8	3.3	0.49
Normal (18.5-24.9)	178	63.6	149	61.1	
Overweight (25-29.9)	57	20.4	56	22.9	
Obese (≥30.0)	13	4.6	18	7.4	
Gestational age at delivery	35.0 ± 4.3		37.8 ± 3.5		<0.001
Preterm delivery (<37 weeks)	164	58.6	51	20.9	<0.001
Infant birthweight (grams)	2357 ± 889		3058 ± 825		<0.001
Stillbirth	68	24.3	2	0.8	<0.001
Perinatal death	92	32.6	8	3.3	<0.001

[1]Mean ± Standard deviation (SD); [2]*P*-value are from Student's *t* test for continuous variables and chi-square test/Fisher's Exact test for categorical variables.

Cardio-MetaboChip (Illumina Inc., San Diego, CA) platform, 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 [15]. 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.

Candidate gene, SNP selection & data quality control

For this candidate gene association study, 13 genes (PER2, PER3, CLOCK, ARNTL, ARNTL2, CRY1, CRY2, CSNK1E, RORA, RORB, RORC, NAPS2, DEC1) that were involved in circadian clock gene regulation (based on literature review) were identified and a total of 116 SNPs belonging to these genes and represented 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 [14].

Statistical analysis

We examined general characteristics of the study population using mean (standard deviation, SD) for continuous variables and numbers (%) for categorical variables. We compared maternal characteristics of placental abruption cases and controls using Student's t-test (for

Table 2. Top 20 SNPs in univariate analyses of circadian rhythm candidate genes from placental tissue in relation to risk of placental abruption

Genes	Chromosome	SNPs	Minor Allele	MAF	OR (95% CI)	Empirical *P*-value
ARNTL2	12	rs16931937	G	0.01	2.36 (0.92-6.09)	0.06674
ARNTL2	12	rs1256955	C	0.23	1.21 (0.91-1.61)	0.1845
CRY2	11	chr11:45829415	A	0.03	2.33 (1.29-4.21)	0.003994
CRY2	11	chr11:45849748	G	0.02	0.21 (0.06-0.76)	0.008928
CRY2	11	chr11:45835169	A	0.02	0.17 (0.04-0.79)	0.01022
CRY2	11	rs11038695	A	0.02	0.17 (0.04-0.79)	0.01022
CRY2	11	chr11:45833192	A	0.02	0.17 (0.04-0.79)	0.01022
CRY2	11	chr11:45831281	G	0.02	0.17 (0.04-0.79)	0.01022
CRY2	11	chr11:45859770	G	0.02	0.29 (0.09-0.89)	0.02157
CRY2	11	chr11:45825589	A	0.20	0.71 (0.52-0.98)	0.03701
Dec1	9	rs11794627	A	0.16	0.66 (0.46-0.94)	0.01987
PER3	1	rs875994	G	0.27	0.78 (0.59-1.04)	0.08814
RORA	15	rs2899663	G	0.27	0.66 (0.49-0.88)	0.004592
RORA	15	rs1370433	A	0.42	1.26 (0.99-1.61)	0.06303
RORA	15	rs17303530	C	0.31	0.80 (0.61-1.05)	0.1051
RORA	15	rs12908671	A	0.01	1.89 (0.76-4.68)	0.1611
RORA	15	rs341397	A	0.01	2.20 (0.69-7.06)	0.1742
RORA	15	rs726955	A	0.31	0.83 (0.64-1.09)	0.1802
RORA	15	rs340025	A	0.43	0.87 (0.68-1.11)	0.2661
RORA	15	rs8036966	C	0.20	1.18 (0.88-1.59)	0.2698

MAF=Minor Allele Frequency in controls.

continuous variables) and chi-square tests or Fisher's exact tests (for categorical variables). Univariate logistic regression model was used to estimate odds ratio (OR) and 95% confidence interval (95% CI) relating each SNP with risk of placental abruption. For multiple testing corrections, a false discovery rate (FDR) procedure was used [16]. In multivariable analyses, we used a lasso logistic regression model to identify sets of SNPs that are jointly associated with the odds of placental abruption [17]. 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. For weighted genetic risk score (WGRS) analyses, a 10-fold cross-validation procedure was implemented to protect against model over-fitting, which arises from using the same data to estimate the regression parameters used in computing WGRS and to evaluate the association between placental abruption risk and WGRS [18]. This 10-fold cross-validation procedure was repeated 1,000 times to account for the variability in randomly partitioning the data into subsamples. The procedure consisted of randomly partitioning the data into 10 equal size subsamples, using nine of the subsamples as training set and the left-out one as validation set, with each subsample being used in turn as a test set. For each fold, a multivariate logistic regression model was fit on the training set using the SNPs selected from multivariate analyses. The receiver operating characteristics (ROC) curve for each of the replicates was evaluated. The estimated effect sizes and AUCs over the 1,000 replicates were used to obtain the respective point estimates and confidence intervals. Weighted genetic risk scores (wGRS) were computed by multiplying the number of selected risk alleles for each locus by its associated effect size.

Once the wGRS were obtained for all individuals, study subjects were categorized into four groups defined by the quartiles in the controls. We fitted a logistic regression model to derive ORs and 95% CIs for placental abruption corresponding to wGRS quartiles, with the first quartile as a referent. In multivariable analyses, we evaluated linear trends in risk by treating wGRS as ordinal variables after assigning a score (i.e., 1, 2, 3, and 4) to each quartile.

We derived maternal circadian clock-related candidate genes weighted genetic risk scores (herein after referred to as maternal-wGRS) as previously described [12]. Briefly, the maternal-wGRS was computed using 65 SNPs identified from among the 119 SNPs included in the circadian rhythm candidate genes pathway that were associated with placental abruption

Table 3. Multiple logistic regression based on SNPs selected from candidate circadian rhythm genes in placental tissue using lasso regression

Genes	Chromosome	SNPs	Minor Allele	MAF	OR (95% CI)	Empirical *P*-value
ARNTL2	12	rs16931937	G	0.01	2.85 (1.03-7.90)	0.044
CRY2	11	chr11:45849748	G	0.02	0.22 (0.06-0.75)	0.015
CRY2	11	chr11:45829415	A	0.03	2.09 (1.13-3.87)	0.019
CRY2	11	chr11:45825589	A	0.20	0.68 (0.48-0.96)	0.027
DEC1	9	rs11794627	A	0.16	0.64 (0.44-0.94)	0.022
PER3	1	rs875994	G	0.27	0.76 (0.57-1.02)	0.069
RORA	15	rs2899663	G	0.27	0.70 (0.52-0.95)	0.020

MAF=Minor Allele Frequency in controls.

[please see reference 12]. To assess the effect of placental-wGRS on placental abruption risk while controlling for maternal-wGRS, we included both placental-wGRS and maternal-wGRS in a fully adjusted model, (i.e., models that included maternal age, use of prenatal care services, preeclampsia status and gestational age at delivery). Finally, we examined the extent to which the association of placental abruption with placental-wGRS is modified by maternal-wGRS. For these analyses, we fitted another fully adjusted model that include placental-wGRS and maternal-wGRS along with the interaction terms for both maternal and placental genomes. The global test for effect modification was evaluated using a likelihood ratio test. Statistical analyses were conducted using gPLINK (version 2.050), R (version i386 3.1.2) and STATA (Version 13).

Results

Characteristics of placental abruption cases and controls are summarized in **Table 1**. Cases and controls were similar with regards to maternal age, gravidity, nulliparity and a number of other characteristics. As expected, cases were more likely to deliver preterm and neonates of cases, as compared with controls, were smaller and had a higher level of perinatal mortality. **Table 2** presents the top 20 individual SNPs (in genes in the circadian rhythm candidate pathway) in the placental genome that are associated with placental abruption, in univariate logistic regression analyses. Using lasso logistic regression procedures, we identified 7 SNPs in, placental DNA, among the circadian rhythm candidate genes pathway (**Table 3**). Of note, 6 of these SNPs had empirical *P*-values<0.05 (rs16931937 (ARNTL2), rs11794627 (DEC1), rs-875994 (PER3) and rs-2899663 (RORA)). For example, a common SNP (minor allele frequency= 27%) in the RORA gene (rs2899663) G^+ allelic status was found to be associated with a 30% reduced odds of placental abruption (95% CI 0.52-0.95). The cross-validated area under the curve (AUC) was estimated to be 0.627 (95% CI 0.626-0.627) for the selected model including 7 SNPs, confirming that the models has relatively good predictive ability for placental abruption risk.

We computed a placental wGRS (i.e., placental-wGRS) using the 7 SNPs in, placental DNA, involved in the circadian rhythm candidate genes pathway. Mean placental-wGRS values were higher for placental abruption cases as compared with controls (5.63 vs. 5.35, P<0.001). As shown in **Table 4**, the odds of placental abruption increased across each successive quartile of the placental-wGRS (P_{trend}<0.001). Multivariable-adjusted ORs for abruption were 1.00, 2.16, 3.24 and 4.48 across successive quartiles of the placental-wGRS. Furthermore, we noted that women with very high placental-wGRS (i.e., those with a wGRS≥5.99, the upper decile) had a 6.60-fold (95% CI 2.96-14.70) increased odds of placental abruption as compared with women who had a placental-wGRS<4.98 (i.e., the lowest quartile).

To determine associations of placental circadian rhythm candidate genes variants with placental abruption risk, controlling for maternal circadian rhythm candidate gene variants, we included both the placental-wGRS and maternal-wGRS in fully logistic regression models that included adjustment for possible confounding by maternal age, utilization of prenatal care, preeclampsia status and gestational age at delivery. The ORs for placental abruption for successive placental-wGRS quartiles were: 1.00, 2.44, 3.06, and 5.02 (P_{trend}<0.001). The corresponding ORs for successive maternal-wGRS quartiles were: 1.00, 1.37, 2.36, and

Table 4. Odds ratio (OR) and 95% confidence interval (CI) for placental abruption in relation to categories of placental weighted genetic risk score (placental-wGRS) computed from placental candidate circadian rhythm genes SNPs selected in multivariable analyses

Placental Circadian Rhythm Genes Weighted Genetic Risk Score (wGRS)	Median	Placental Abruption (N=280)	Controls (N=244)	Unadjusted OR (95% CI)	Adjusted OR* (95% CI)
		n (%)	n (%)		
Quartile 1 (<4.98)	4.79	28 (10.0)	65 (26.6)	1.00 (referent)	1.00 (referent)
Quartile 2 (4.98-5.35)	5.27	60 (21.4)	64 (26.2)	2.18 (1.24-3.83)	2.16 (1.17-3.98)
Quartile 3 (5.36-5.66)	5.59	71 (25.4)	54 (22.1)	3.05 (1.73-5.38)	3.24 (1.77-5.94)
Quartile 4 (≥5.67)	5.98	121 (43.2)	61 (25.0)	4.60 (2.68-7.90)	4.48 (2.51-7.98)
P-value for linear trend				<0.001	<0.001
Quartile 1 (<4.98)	4.79	28 (10.0)	65 (26.6)	1.00 (referent)	1.00 (referent)
≥90% decile (≥5.99)	6.33	50 (17.9)	18 (7.4)	6.45 (3.21-12.95)	6.60 (2.96-14.70)

*Adjusted for maternal age, received prenatal care, preeclampsia status and gestational age at delivery.

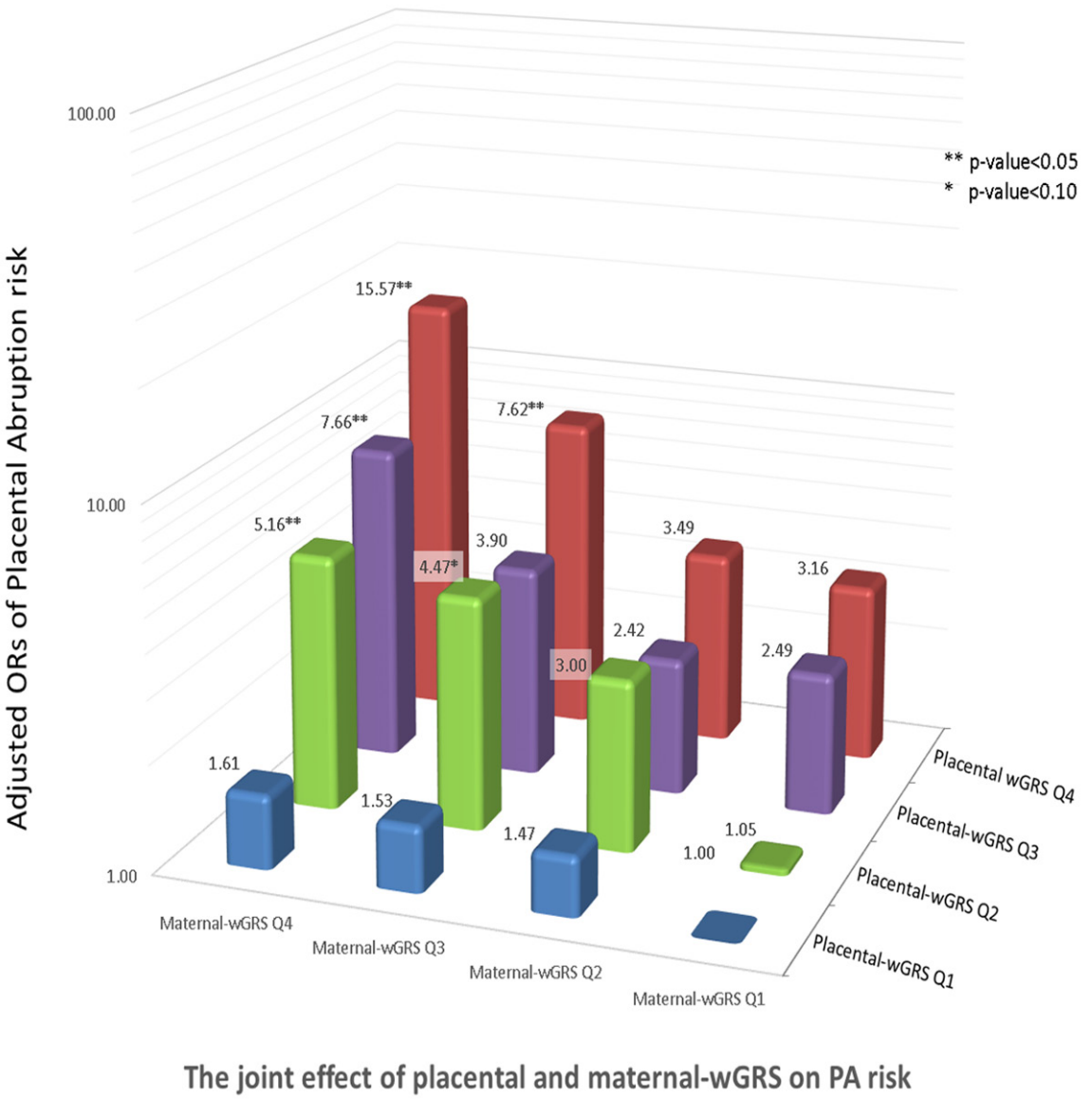

Figure 1. The joint effect of placental and maternal weighted genetic risk scores computed from candidate circadian rhythm genes SNPs on placental abruption (PA) risk. Bars indicate the odds ratios (OR).

3.62 (P_{trend}<0.001). The findings indicate that variants in both placental and maternal genomes contribute to placental abruption risk. We also fitted another model with interaction terms for both placental and maternal wGRS. We observed that odds of placental abruption was greatest for subjects in the highest quartiles of both the placental and maternal wGRS (OR=15.57; 95% CI 3.34-72.60) as compared with those in the lowest quartiles for both scores (the reference group) (**Figure 1**). However, given our relatively small sample size, we observed no evidence of a statistically significant interaction (*P*-value=0.884).

Discussion

The current study adds to the sparse literature on the role of variations in circadian clock-related genes in the pathogenesis of placental abruption. We identified some SNPs in clock-related genes (e.g., rs2899663 in the RORA gene) in the placental genome that are associated with placental abruption risk, and these associations remained when genetic variations in clock-related genes in the maternal genome are accounted for in multivariable logistic regression models. These results are consistent with and extend findings that we reported concerning placental abruption risk in relation to genetic variations in clock-related genes in the maternal genome [12]. For example, the common SNP (minor allele frequency=27%) in the RORA gene (rs2899663) G^+ allelic status was associated with a 30% reduced odds of placental abruption (OR=0.70, 95% CI 0.52-0.95) in the current study, while the same SNP (in the maternal genome) was associated with 20% reduced risk of placental abruption (OR=0.80, 95% CI 0.61-1.04) [12]. The RORA gene encodes RAR-related orphan receptor A, which is a member of the NR1 subfamily of nuclear hormone receptors. RORA is a novel gene that is related to autism disorder [19]. Two other SNPs in RORA (rs1482057 and rs12914272) were previously associated with breast cancer risk, possibly due to the interaction of circadian clock gene variants and night-shift work with reproductive hormones [20]. A SNP in CRY2 chr11:45829415 (A^+ allelic status) was associated with 2.09-fold (*P*-value=0.019) increased risk of placental abruption in the current study, while the same SNP in the maternal genome was associated with 1.72-fold (*P*-value=0.088) increased odds of placental abruption [12]. The CRY2 gene encodes cryptochrome circadian clock 2, a flavin adenine dinucleotide-binding protein that is a key component of the circadian core oscillator complex, which regulates the circadian clock. The CRY2 gene is up-regulated by CLOCK/ARNTL heterodimers and is known to represses this up-regulation in a feedback loop using PER/CRY heterodimers to interact with CLOCK/ARNTL [2]. Of note, altered expression level of the CRY2 gene has been observed in pregnancies affected by medically complicated pregnancies including gestational diabetes mellitus [21].

Our observation adds to the extant literature documenting the importance of clock-related genes in placental function and disorders [5-7]. For example, Frigato et al. investigated the expression clock genes in human placenta, and demonstrated a circadian expression pattern for PER2 and DEC1 transcripts in *in vitro* synchronized HTR/SVneo cells established from a human first trimester extra villous trophoblast. Notably, the authors reported that circadian oscillations persisted and were even enhanced in cells experimentally rendered hypoxic [5]. Of note, hypoxemia is considered important in the pathogenesis of placental abruption.

To the best of our knowledge this is the first study to explore the joint and independent contributions of variants in clock-related genes in both placental and maternal genomes on placental abruption risk. Despite our relatively small sample size, given the unique biological intersection of two distinct genomes at the maternal-placental interface, we also explored potential interactions of variations in circadian clock-related genes in both placental and maternal genomes. We found that the cumulative association of placental circadian clock-related gene variants, in the placental genome, was associated with increased odds of placental abruption, even after adjusting for variations in the circadian clock-related gene pathway ways in the maternal genome. Further, we found that the odds of placental abruption was particularly high among subjects in the highest quartiles of both placental- and maternal wGRS. Larger studies are needed to corroborate these preliminary findings and to further elucidate the independent and joint contribu-

tions of genetic variants in maternal and fetal genomes to the pathogenesis of placental abruption.

Our findings must be interpreted while considering some additional limitations. First, we used the same dataset to identify SNPs that constitute the wGRS and test associations of wGRS with placental abruption. Although we attempted to assess the predictive validity of wGRS by conducting repeated ten-fold cross-validation, we recognize that inferences from our analyses will be enhanced when we are able to validate our findings and evaluate the predictive power of both the placental and maternal wGRS in independent samples of placental abruption cases and controls [22]. Second, we used a research operational definition of placental abruption which may have led to some degree of misclassification. For instance, sub-clinical cases of placental abruption (i.e., those not presenting with abnormal vaginal bleeding) may be missed or misclassified among controls. Consequently, observed associations may be conservative. Lastly, the generalizability of our findings should be confirmed in studies that are conducted in other geographically and ethnically diverse populations of obstetric patients.

In conclusion, genetic variants in circadian clock-related genes in placenta may contribute to the pathogenesis of placental abruption, and this contribution is independent and possibly additive to the contributions of variants in clock-related genes in the maternal genome. Larger molecular epidemiology studies that interrogate the independent and joint contributions of genomes at the maternal-placental interface are needed to confirm our findings and to further elucidate the pathogenesis of placental abruption.

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Disclosure of conflict of interest

None.

Authors' contribution

Conceived and designed the study: CQ, BG, and MAW. Analyzed the data: CQ, and MD. Contributed to the writing of the manuscript: CQ, BG, MD, MGT, DAG, CVA, PNP, MS, SES and MAW.

Abbreviations

ARNTL, Aryl hydrocarbon receptor nuclear translocator-like; ARNTL2, Aryl hydrocarbon receptor nuclear translocator-like 2; CLOCK, Circadian Locomotor Output Cycles Kaput; CRY1, Cryptochrome 1; CRY2, Cryptochrome 2; CSNK1E, casein kinas 1 epsilon; DEC1, deleted in esophageal cancer 1; NAPS2, DEC1; PER2, Period 2; PER3, Period 3; RORA, RAR-related orphan receptor A; RORB, RAR-related orphan receptor B; RORC, RAR-related orphan receptor C.

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