Abruptio placentae risk and genetic variations in mitochondrial biogenesis and oxidative phosphorylation: replication of a candidate gene association study

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Abstract

BACKGROUND: Abruptio placentae is a complex multifactorial disease that is associated with maternal and neonatal death and morbidity. Abruptio placentae's high recurrence rate, high prevalence of heritable thrombophilia among women with abruptio placentae, and aggregation of cases in families of women with the disease support the possibility of a genetic predisposition. Previous genome-wide and candidate gene association studies have identified single nucleotide polymorphisms in mitochondrial biogenesis and oxidative phosphorylation genes that potentially are associated with abruptio placentae risk. Perturbations in mitochondrial biogenesis and oxidative phosphorylation, which results in mitochondrial dysfunction, can lead to the impairment of differentiation and invasion of the trophoblast and to several obstetrics complications that include abruptio placentae.

OBJECTIVE: The purpose of this study was to determine whether the results of a candidate genetic association study that indicated a link between DNA variants (implicated in mitochondrial biogenesis and oxidative phosphorylation) and abruptio placentae could be replicated.

STUDY DESIGN AND METHODS: The study was conducted among participants (507 abruptio placentae cases and 1090 control subjects) of the Placental Abruption Genetic Epidemiology study. Weighted genetic risk scores were calculated with the use of abruptio placentae risk-increasing alleles of 11 single nucleotide polymorphisms in 9 mitochondrial biogenesis and oxidative phosphorylation genes (*CAMK2B, NR1H3, PPARG, PRKCA, THRB, COX5A, NDUFA10, NDUFA12,* and *NDUFC2*), which previously was reported in the Peruvian Abruptio Placentae Epidemiology study, a study with similar design and study population to the Placental Abruption Genetic Epidemiology study. Logistic regression models were fit to examine associations of weighted genetic risk scores (quartile 1, <25th percentile; quartile 2, 25–50th percentile; quartile 3, 50–70th percentile, and quartile 4, >75th percentile) with risk of abruptio placentae, adjusted for population admixture (the first 4 principal components), maternal age, infant sex, and preeclampsia. The weighted genetic risk score was also modeled as a continuous predictor. To assess potential effect modification, analyses were repeated among strata that were defined by preeclampsia status, maternal age (35 vs 18–34 years), and infant sex.

RESULTS: Abruptio placentae cases were more likely to have preeclampsia, shorter gestational age, and lower infant birthweight. Participants in quartile 2 (score, 12.6–13.8), quartile 3 (score, 13.9–15.0) and quartile 4 (score, 15.1) had a genetic risk score of 1.45-fold (95% confidence interval, 1.04–2.02; P=.03), a 1.42-fold (95% confidence interval, 1.02–1.98; P=.04), and a 1.75-fold (95% confidence interval, 1.27–2.42; P=7.0E-04) higher odds of abruptio placentae, respectively, compared with those in quartile 1 (score,<12.6; P-for trend=.0003). The risk of abruptio placentae was 1.12-fold (95% confidence interval, 1.05–1.19; P=3.0×10^{04}) higher per 1-unit increase in the score. Among women with preeclampsia, those in quartile 4 had a 3.92-fold (95% confidence interval, 1.48–10.36; P=.01) higher odds of abruptio placentae compared with

women in quartile 1. Among normotensive women, women in quartile 4 had a 1.57-fold (95% confidence interval, 1.11–2.21; P=.01) higher odds of abruptio placentae compared with those in quartile 1 (P-for interaction=.12). We did not observe differences in associations among strata defined by maternal age or infant sex.

CONCLUSIONS: In this study, we replicated previous findings and provide strong evidence for DNA variants that encode for genes that are involved in mitochondrial biogenesis and oxidative phosphorylation pathways, which confers risk for abruptio placentae. These results shed light on the mechanisms that implicate DNA variants that encode for proteins in mitochondrial function that are responsible for abruptio placentae risk. Therapeutic efforts to reduce risk of abruptio placentae can be enhanced by improved biologic understanding of maternal mitochondrial biogenesis/oxidative phosphorylation pathways and identification of women who would be at high risk for abruptio placentae.

Keywords

abruptio placentae; genome; mitochondria; placenta

INTRODUCTION

Abruptio placentae (AP) is an obstetric complication that is characterized by the premature separation of an implanted placenta. AP is a significant cause of global maternal and neonatal death and morbidity.[1] As a complex multifactorial disease, pathophysiologic mechanisms of AP include uteroplacental under perfusion, chronic hypoxemia, placental ischemia, and infarctions.[2–8]

The role of genetic factors in AP has garnered increasing attention over the past decade. Previous findings for high recurrence of AP,[9] high prevalence of heritable thrombophilia among women with AP,[10] and aggregation of AP in families of women with an abruption[11] support the possibility of a genetic predisposition.[12–14] Particularly, there is evidence that shows the role of perturbations in mitochondrial biogenesis (MB) and oxidative phosphorylation (OP) in the pathogenesis of AP from candidate gene studies.[15,16] The mitochondria control subjects many critical cell functions, which include the production of cellular energy, adenosine triphosphate, by the coupling of OP to cell respiration.[17–19] Oxidative stress-induced damage to mitochondrial structural elements (eg, lipid membrane) alters mitochondrial gene expression and promotes a deficiency in OP,[20] which results in mitochondrial dysfunction. Hundreds of nuclear DNA genes across the chromosome regulate MB and maintain mitochondrial structure and function by regulating OP.[21]

Mitochondrial dysfunction can lead to the impairment of differentiation and invasion of the trophoblast and lead to several obstetric complications that includes AP.[22] Epidemiologic and experimental studies have highlighted the roles of MB/OP genes in pregnancy complications that involve the placenta.[23–26] For instance, *PPARG*, a master regulator gene of MB, mediates defective placentation that results from oxidized low-density lipoproteins in cytotrophoblasts of villous and extravillous cells.[23] Expression of this gene was shown to be reduced in placentae of women with gestational diabetes mellitus.[25] Another MB gene, *NR1H3* (Liver X alpha),[27] which plays a key role in cholesterol metabolism[28] and cell

signaling,[29] is important in normal trophoblast invasion during placental implantation.[24,26] In addition to assessment of genetic variations in the whole population, subgroup analyses can help to identify members of the population whose genetic background makes them more susceptible to disease.[30] However, such analyses are largely nonexistent in the context of MB/OP genetic variations and AP risk.

On the basis of this emerging literature, we previously conducted 2 candidate single nucleotide polymorphism (SNP) studies to investigate variations in MB/OP genes and AP risk[16,31] in the Peruvian Abruptio Placentae Epidemiology (PAPE) study. Using a weighted genetic risk score (wGRS) that was computed based on the maternal SNPs selected from MB (peroxisome proliferator-activated receptor gamma [*PPARG*], thyroid hormone receptor beta [*THRB*], calcium/calmodulin dependent protein kinase II beta [*CAMK2B*], nuclear receptor subfamily 1 group H member 3 [*NR1H3*], and protein kinase C alpha [*PRKCA*]) and OP (cytochrome c oxidase subunit 5A [*COX5A*], NADH:ubiquinone oxidoreductase subunit A10 [*NDUFA10*], NADH: ubiquinone oxidoreductase subunit A12 [*NDUFA12*] and NADH:ubiquinone oxidoreductase subunit C2 [*NDUFC2*]) genes, we found associations between increased MB/OP wGRS and AP risk. Similarly, increased MB/OP wGRS that was computed based on placental SNPs that were assessed from the fetal-side was also associated with AP risk in the latter study.[31] In this new study, we conducted a replication candidate gene study to examine SNPs in MB/OP genes and risk of AP in the Placental Abruption Genetic Epidemiology (PAGE) study. Of note, the PAGE study had similar design and study population drawn from the same catchment area as the PAPE study but did not include participants from any of the previously published studies. In addition, we examined the extent to which the association of wGRS with AP risk is modified by known and potential risk factors of AP: preeclampsia,[32] advanced maternal age,[33] and infant sex.[34] These analyses could have important clinical and public health implications by highlighting potential gene-gene or gene-environment interactions, enhancing the biologic understanding of the mechanisms that lead to AP, and facilitating therapeutic efforts to reduce impact of AP.[35]

MATERIALS AND METHODS

Study settings and study populations

The study was conducted among participants of the PAGE study, a case-control study of AP conducted in Lima, Peru. Study participants included women who were admitted for obstetrics services to antepartum wards, emergency room, and labor and delivery wards of participating hospitals between March 2013 and December 2015. Participating hospitals were Instituto Nacional Materno Perinatal, Hospital Edgardo Rebagliati Martins, Hospital San Bartolome, Hospital Hipolito Unanue, Hospital Arzobispo Loayza, Hospital Dos de Mayo, and Hospital Maria Auxiliadora. Participants who were <18 years old, had delivered a multifetal pregnancy, had medical records that were insufficient to determine the presence or absence of AP (described later), and reported taking anticoagulants were excluded from the study. Participants with other diagnoses that were associated with third-trimester bleeding (eg, placenta previa) were also excluded. The total number of participants remained 522 AP cases and 1147 control subjects. The study protocol was approved by the

Institutional Review Boards of participating institutions and the Swedish Medical Center, Seattle, WA, where the study was administratively based. All participants provided written informed consent.

Data collection

Study participants were interviewed by trained personnel who used standardized structured questionnaires to collect information on sociodemographic characteristics and risk factors that included maternal age, marital status, employment status during pregnancy, medical history, smoking, and alcohol consumption (both current and before pregnancy). Maternal medical records were reviewed to obtain information on the course and outcomes of the pregnancy and to ascertain AP case-control status. A diagnosis of AP was based on fulfilling ≥1 of the following 4 criteria noted in the participants' medical record: (1) antepartum emorrhage after 20 weeks gestation, (2) uterine pain or tenderness (localized or diffuse), (3) fetal distress or death, and (4) retroplacental blood clot. Retroplacental blood clot was determined based on ultrasound scans or examination of the delivered placenta. Not all cases were cesarean deliveries. Data collection protocol also included ascertainment of differential diagnoses for AP that included all causes of abdominal pain and bleeding, such as placenta previa, appendicitis, urinary tract infection, preterm labor, fibroid degeneration, ovarian disease, and muscular pain.[36] Control subjects were selected randomly from eligible pregnant women who delivered at the same participating hospitals as the AP cases during the study period and who did not have a diagnosis of AP in the current pregnancy. Maternal saliva was collected, plated, and stored with the use of the Oragene saliva cell collection kits (OGR500 and OGR250; DNA Genotek, Ottawa, Canada).

DNA extraction, genotyping, data quality control, and candidate gene/SNP selection

Genomic DNA was extracted with the use of Qiagen DNAeasy system and manufacturer protocols (Qiagen, Valencia, CA). Genotyping to characterize genome-wide variation (>300,000 SNPs) was conducted with the use of the Illumina HumanCore-24 BeadChip platform (Illumina Inc, San Diego, CA). Genotype data quality control procedures were applied before data analyses. SNPs were excluded if they had excessive missing genotype (SNPs with genotype call rate of <95%), deviated from Hardy-Weinberg equilibrium (P<1e-05), and had low minor allele frequency (P<0.05). Individuals (n=27) were excluded if they were duplicates or related (identity by decent value, >0.9), had >5% of genotyping failure rate (n=16), had excess heterozygosity/homozygosity rate (outside the range of mean ±3 standard deviations of heterozygosity rate; n=6), had genotype data that were inconclusive regarding sex (n=8), and did not pass test of divergent ancestry (if the first 2 principal components were outside the range of [−0.02, 0.02]; n=6; Supplementary Figure 1). The total number of 1597 individuals remained for further analysis (507 cases and 1090 control subjects). After the quality control step, SNP imputation was conducted to infer unobserved genotypes. The genotype data were phased with the use of Shape-IT[37] to infer haplotypes and improve imputation accuracy with the 1000 Genomes. Phased haplotypes were then used to impute our non-typed SNPs using IMPUTE2.[38]

A total of 11 SNPs in 9 MB and OP genes (peroxisome proliferator-activated receptor gamma [*PPARG*], thyroid hormone receptor beta [*THRB*], calcium/calmodulin dependent

protein kinase II beta [*CAMK2B*], nuclear receptor subfamily 1 group H member 3 [*NR1H3*], protein kinase C alpha [*PRKCA*], cytochrome c oxidase subunit 5A [*COX5A*], NADH:ubiquinone oxidoreductase subunit A10 [*NDUFA10*], NADH:ubiquinone oxidoreductase subunit A12 [*NDUFA12*] and NADH:ubiquinone oxidoreductase subunit C2 [*NDUFC2*]; Table 1) previously reported in the PAPE study[16] were evaluated in the current analyses. The MB and OP genes were selected from previously published studies based on hypothesized functional and biologic significance and known associations with phenotypes that are related to placental function and/or perinatal outcomes in mammals.[16,28,39–43]

Genetic risk score calculation

The wGRSs were calculated by multiplying the number of risk alleles for each MB and OP SNP by externally derived effect size estimates. It previously has been shown that the use of weights derived from the same data under analysis resulted in bias, compared with the use of externally derived estimated effect sizes as weights.[44] The corresponding externally derived effect sizes were obtained from the previously reported PAPE study,[16] a candidate gene study of AP. We assumed an additive genetic risk model, which corresponded to a linear increase of AP risk per unit increase in dosages of risk alleles (or the presence of 0, 1, and 2 risk alleles for directly typed SNPs). The weights (effect sizes) were multiplied by the number of respective risk alleles and summed across the SNPs to create a single score for each individual.

Statistical analyses

Mean and standard deviations for continuous variables and proportions for categoric variables were used to compare the characteristics of AP cases and control participants. Adjustment factors as confounders included in the models were principal components (the first 4 principal components represented population stratification), maternal age, infant sex, and a diagnosis of preeclampsia in the current pregnancy. The logistic regression models that included AP as the dependent variable, wGRS of SNPs in MB/OP genes as the independent variable, and adjustment factors were fit.

Participants were categorized into quartile groups defined by the 25th, 50th, and 75th percentile wGRS scores among control participants. The odds ratios (ORs) of AP and their corresponding 95% confidence intervals (CIs) and probability values were estimated from the logistic regression models. The corresponding ORs of each of the upper 3 wGRS quartiles were used to compare with the first quartile (<25th percentile) as the reference group. We assessed the test for linear trend across increasing quartiles of wGRS. The wGRS was also evaluated as a continuous variable to estimate the OR of AP per 1-unit increase in wGRS. In stratified analyses, multivariable adjusted logistic regression models were fit separately among groups defined by the diagnosis of preeclampsia in the current pregnancy, infant sex, and advanced maternal age (35 vs 18–34 years). The likelihood ratio test was used to report effect modification. To determine statistical significance, P<.05 was used as a cut-off. Statistical analyses were performed with R software (version i386 3.1.2) and SAS software (version 13; SAS Institute Inc, Cary, NC).

RESULTS

Sociodemographic and medical/obstetric characteristics of the study participants are shown in Table 2. AP cases and control subjects were similar with respect to maternal age, education, marital status, employment, prepregnancy body mass index, planned pregnancy, infant sex, alcohol use, and vitamin use. Compared with control subjects, AP cases were more likely to deliver earlier (ie, shorter gestational age), deliver infants with lower birthweight, have a diagnosis of preeclampsia in the current pregnancy, and differ in population admixture captured by the first principal component.

Eleven previously reported SNPs in 9 MB and/or OP genes and corresponding effect size estimates were used to compute the wGRS for a total of 507 cases (median, 14.2; range, 7.9–19.0) and 1090 control subjects (median, 13.9; range, 7.5–18.5; Table 1). Compared with control subjects, the mean wGRS was higher for AP cases (14.1 [SD=1.8] vs 13.7 [SD=1.9]). In models that accounted for covariates (Table 3 and Supplementary Figure 2), participants in the second quartile (25–50th wGRS percentile; score, 12.6–13.8) had a 1.45-fold (95% CI, 1.04–2.02; P=.03) higher odds of AP compared with those in the lowest quartile (<25th wGRS percentile; score, <12.6). Participants in the third quartile (50–75th wGRS percentile; score, 13.9–15.0) had a 1.42-fold (95% CI, 1.02–1.98; P=.04) higher odds of AP compared with those in the lowest percentile. Participants in the highest quartile (>75th wGRS percentile; score, >15.1) had a 1.75-fold (95% CI, 1.27–2.42; P=7.0E-04) higher odds of AP compared with those in the lowest percentile. Significant linear trend in association between wGRS and AP risk was observed in this replication study (P-for-trend=.0003). When wGRS was entered in the model as a continuous predictor, the risk of AP was 1.12-fold (95% CI, 1.05–1.19; P=3.0E-04) higher per 1-unit increase in wGRS.

In stratified analyses, among women with preeclampsia, the odds of AP were 3.92 (95% CI, 1.48, 10.36; p=0.01), 3.50 (95% CI, 1.27, 9.65; p=0.02), and 2.96 (95% CI, 1.15, 7.65; p=0.02) for participants in the highest, third, and second wGRS quartiles, respectively, compared with participants in the lowest wGRS quartile (P-for-trend=0.03). The odds of AP was 1.27 (95% CI, 1.06–1.52; p=0.01) per one-unit increase in wGRS among women with preeclampsia (Table 4 and Supplementary Figure 3). Among normotensives, similar corresponding estimates were 1.57 (95% CI, 1.11, 2.21; p=0.01), 1.27 (95% CI, 0.89, 1.80; p=0.18), and 1.32 (95% CI, 0.93, 1.87; p=0.12) (P-for-trend=0.08). The interaction test p-value for wGRS and preeclampsia status suggests effect modification of the wGRS-AP associations by preeclampsia (interaction p-value=0.12). The odds of AP was 1.10 (95% CI, 1.03, 1.17; p=0.01) per one-unit increase in wGRS among normotensives.

Among women 18–34 years, the odds ratios of AP were 1.68 (95% CI, 1.17, 2.41; P=.01), 1.49 (95% CI, 1.02–2.15; P=.04), and 1.46 (95% CI, 1.01–2.11; P=.05), respectively, for women in the fourth, third, and second quartiles of wGRS compared with women in the lowest quartile (P-for-trend=.04). The corresponding odds ratios among women 35 years old were 2.22 (95% CI, 1.05–4.69), 1.31 (95% CI, 0.61–2.80), and 1.56 (95% CI, 0.72–3.33; P-for-trend=.19; P-for interaction=.73). Women who were 18–34 years old had a 1.10-fold (95% CI, 1.03–1.18; P=.004) higher odds of AP per 1-unit increase in wGRS. Women who were 35 years old had a 1.18-fold (95% CI, 1.03–1.36) higher odds of AP per 1-unit

increase in wGRS (Table 4 and Supplementary Figure 4). Similarly, among participants with male infants, the odds of AP were 1.95 (95% CI, 1.24–3.07; P=8.0E-04), 1.36 (95% CI, 0.78–2.00; P=0.18), and 1.83 (95% CI, 1.16–2.87; P=0.01), respectively, for women in the fourth, third, and second quartiles of wGRS compared with women in the reference group (lowest quartile; P-for-trend=.004); corresponding odds ratios among participants with female infants were 1.37 (95% CI, 0.85–2.20; P=.20), 1.47 (95% CI, 0.91–2.39; P=.11), and 1.11 (95% CI, 0.68–1.81; P=.67; P-for-trend=.35; P-for-interaction=.44). Women who carried a male infant had a 1.14-fold (95% CI, 1.05–1.24; P=.002) higher odds of AP per 1-unit increase in wGRS. Women who carried a female infant had a 1.09-fold (95% CI, 1.00–1.19; P=.06) higher odds of AP per 1-unit increase in wGRS (Table 4 and Supplementary Figure 5).

COMMENT

Principal findings

In this candidate gene association study of AP, we provide strong evidence that genetic variants in MB (*CAMK2B*, *NR1H3*, *PRKCA*, *PPARG* and *THRB*) and OP pathways (*COX5A, NDUFA10, NDUFA12,* and *NDUFC2*) influence AP risk. Women in the highest wGRS quartile for MB/OP variants had 1.75-fold (95% CI, 1.27–2.42; P=7.0E-04) higher odds of AP compared with those in the lowest quartile. Women also had a 1.12-fold (95% CI, 1.05–1.19; P=3.0E-04) higher odds of AP per 1-unit increase in wGRS. We also observed evidence suggestive of possible effect modification (P-for-interaction=.12) of the association between MB/OP wGRS and risk of AP by preeclampsia. Women who had preeclampsia and were in the highest quartiles for MB/OP wGRS had a 3.92-fold higher odds of AP (95% CI, 1.48–10.36; P=.01) compared with women who had preeclampsia and were in the lowest quartile for MB/OP wGRS. Preeclamptic women also had a 1.27-fold (95% CI, 1.06–1.52; P=0.01) higher odds of AP per 1-unit increase in wGRS.

Research in context of other results

Our candidate gene association study allowed investigation of genetic variants that may not account individually for large effects in complex traits when assessed with the use of an underpowered genome-wide association study, which provides a more effective and economical hypothesis-driven method to assess the role of genetic variations.[45] Other previous candidate gene association studies of AP included investigations of genes in thrombophilia, rennin-angiotensin system, folate metabolism, and interleukin 1 receptor antagonist-related and oxidative stress pathways.[11,12,46–48] However, these studies were small in sample size that showed modest effects and did not validate the findings with the use of either SNPs or genetic risk scores in an independent study. Using SNPs in MB/OP genes and wGRS analysis, our team previously reported that participants (470 AP cases and 473 control subjects) in the highest quartiles of the risk score (10.0) had 1.9-fold (95% CI, 1.2–3.1) higher odds of AP (8.0) compared with participants in the lowest risk score group.[16] Using SNPs in MB/OP genes from placenta (fetal side) biopsy samples (280 AP cases and 244 control subjects), another study reported that participants in the highest quartiles of wGRS had a 4.5-fold (95% CI, 2.9–6.7) higher odds of AP compared with participants in the lowest risk score group.[31] This study was also limited in sample size, but it allowed for other

mechanistic investigations such as effects of imprinting on AP. Placental growth and development, which are key underlying pathways that may later lead to the occurrence of AP, may be under the control of fetal genes that are inherited from the father.[5,49] In the current study, we were able to replicate the associations of maternal MB/OP wGRS that we reported before with risk of AP. This independent replication study will minimize concerns of failure to replicate, which is a recurring problem with candidate gene association studies.[45,50,51]

Clinical implications

MB and OP genes that were evaluated in our study have been known to influence phenotypes that are related to placental function and/or perinatal outcomes. For instance, protein kinase C-alpha (*PRKCA*), which is an MB gene among family of serine- and threonine-specific protein kinases that can be activated by calcium and the second messenger diacylglycerol, is critical in many cellular processes that include cell signaling through phosphorylation of variety of proteins.[52] A body of literature suggests *PRKCA* affects contractility[53] in cardiac myocytes,[54,55] vascular,[56] and myometrial cells,[57,58] whose abnormal mechanisms can trigger AP.[59] Peroxisome proliferator-activated receptor gamma (*PPARG*), which is a master regulator of MB and highly expressed in the placenta, mediates defective placentation (eg, inhibition of trophoblast invasion) through oxidized low-density lipoproteins in cytotrophoblasts of villous and extravillous cells, which are involved in uterus invasion.[23,60] Defective invasion of the uterine spiral arteries is involved directly in preeclampsia,[60] which is a common risk factor of AP.[32] Trophoblast dysfunction because of the failure of spiral artery physiologic transformation in the placental basal plate may be responsible for AP.[61] Therapeutic efforts to reduce the risk of AP can be enhanced by improved biologic understanding of these maternal MB/OP pathways and the identification of women who would be at high risk for AP.

Research implications

Genetic risk scores for the prediction of risk are particularly advantageous because they summarize risk-associated variation across the genome, and they are robust to issues of imperfect linkage and relatively uncommon individual risk alleles for a single SNP.[62,63] In the current study, we identified and evaluated the same SNPs and used the previously reported estimated effect sizes. Interestingly, we found stronger a trend in association between wGRS and higher odds of AP in the current study (P-for-trend=.0003) compared with the previous report (P-for-trend=.01), because of the larger sample size in the current study.

Our stratified wGRS-AP analyses findings may allow the identification of subgroups in the population who are more susceptible to the deleterious effects of genetic risk factors.[64] This approach has been suggested when standard univariate tests (ie, evaluation of each SNP for interaction independently) fail to identify any interactions.[65] We found suggestive evidence that support higher AP risk conferred by MB/OP genetic variants among women with preeclampsia, and vice versa. Although, the global test for interaction between wGRS and preeclampsia was not significant, among women with preeclampsia, the odds of AP were higher for successively increasing quartiles of wGRS, compared with normotensive women

in the lowest quartile of wGRS. A systematic review showed that patients with preeclampsia had 1.73-fold (95% CI, 1.47–2.04) increased odds of AP compared with normotensive patients.[66] Maternal and fetal genetic factors contribute to 35% and 20% of the variance in preeclampsia, respectively.[67] Reduced placental perfusion is thought to interact with preexisting maternal factors such as hypertension, renal disease, obesity, gestational diabetes mellitus, insulin resistance, and lipid abnormalities,[68] which contribute to susceptibility to preeclampsia.[68] As a result, the observed potential interaction in our study may be a reflection of potential gene-environment interaction and warrants further investigation of the roles of MB/OP genes in preeclampsia.

Strengths and limitations

Our study is the largest candidate gene study of AP that has the potential to enhance our understanding of genetic variations in maternal genome that contribute to a multifactorial heritable disorder such as AP. A key strength of our study is that we replicated the association of a wGRS of MB/OP with AP in an independent dataset. We studied Peruvians, which is a population with high prevalence of pregnancy complications, including AP. However, limitations of our study include potential misclassification of subclinical AP, which may introduce bias in the interpretation of our study results or reduce power of our study. Comparing severe AP with mild abruption and/or nonabruption cases may minimize this limitation and facilitate epidemiologic and genetic research.[36] Our study evaluated genetic variations of the mother. Future studies should also investigate genetic variations in the fetus and of the placenta, where abnormal vasculature, thrombosis, lesions, and reduced perfusion all culminate in the eventual occurrence of AP. In addition, findings from our study population may not be generalizable to other populations that differ in genetic and other characteristics, such as smoking.

CONCLUSION

In summary, findings reported herein provide strong evidence for DNA variants that encode for genes involved in the MB and OP pathways, conferring risk for AP. Future studies that will examine whether the identified variants contribute to AP risk in other populations are warranted. Similar genetic studies that will involve MB and OP, or other potential pathways underlying AP, can inform preventative and therapeutic efforts to reduce risk of AP. In addition, they could facilitate identification of individuals who have an elevated risk for AP, which is a significant public health problem.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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AJOG at a Glance:

Why was this study conducted?

We conducted the study to replicate results of a candidate genetic association study that indicated a link between DNA variants that are implicated in mitochondrial biogenesis and oxidative phosphorylation and abruptio placentae.

Key Findings

Genetic risk score calculated with the use of abruptio placentae risk-increasing alleles of genes that is involved in mitochondrial biogenesis and oxidative phosphorylation was associated with abruptio placentae risk; this association was stronger among women with preeclampsia.

What does this add to what is known?

The findings reported herein provide strong evidence for DNA variants encoding for genes that are involved in the mitochondrial biogenesis and oxidative phosphorylation pathways that confer risk for abruptio placentae.

Table 1.

Selected characteristics of the Placental Abruption Genetic Epidemiology (PAGE) Study Population.

Characteristics*	Study Participants		P-value[2]
	Cases (N=507)	Controls (N=1090)	
	% or mean±SD	% or mean±SD	
Maternal age at delivery (years)[1]	28.4±6.7	27.5±6.6	0.93
Maternal age at delivery (years)	-	-	0.22
18–19	6.8	11.7	-
20–29	51.0	50.7	-
30–34	20.8	19.9	-
35	21.4	17.7	-
Education high school	67.3	73.5	0.03
Married/living with partner	86.1	87.1	0.56
Employed during pregnancy	55.0	53.9	0.69
Pre-pregnancy body mass index (BMI) (kg/m^2)	25.0±4.6	25.4±4.6	0.61
Pre-pregnancy BMI (kg/m^2)	-	-	0.53
Lean (< 18.5)	2.8	2.0	-
Normal (18.5–24.9)	56.1	55.6	-
Overweight (24.9–30.0)	10.9	12.8	-
Obese (30.0)	30.2	29.6	-
Planned pregnancy	38.5	32.8	0.03
Smoked during pregnancy	1.0	1.0	0.96
Alcohol use during pregnancy	3.9	2.8	0.20
Drug abuse during pregnancy	0.6	0.3	0.34
Preeclampsia	21.4	6.3	1.0E-04
Vitamins use during pregnancy	84.6	86.1	0.47
Gestational age at delivery[1]	34.3±4.4	39.0±1.2	1.0E-04
Male infant	55.7	52.9	0.30
Population admixture quantified by principal components (PC)	-	-	-
PC 1	−8.8e-4±0.03	4.1e-4±0.02	0.03
PC 2	−4.4e-4±0.02	2.0e-4±0.03	0.52
PC 3	6.7e-4±0.03	−3.2e-4±0.02	0.31
PC 4	9.7e-4±0.03	−4.5e-4±0.02	0.69
Infant birthweight (grams)[1]	2390±939	3418±484	1.0E-04

[1] mean ± standard deviation;

[2] p-values are from Chi-square test/Fisher's exact test for the categorical variables and student t-test for continuous variables.

*Data were complete except for the following variables: maternal age (7 cases, 12 controls), education (9 cases, 6 controls), marital status (5 cases, 3 controls), employment status (5 cases, 3 controls), pre-pregnancy BMI (10 cases, 22 controls), planned pregnancy (5 cases, 9 controls), smoking status (2 cases, 6 controls), drug abuse during pregnancy (3 cases, 6 controls), preeclampsia status (7 cases, 15 controls), vitamin use during

pregnancy (7 cases, 19 controls), gestational age at delivery (77 cases, 135 controls), infant sex (6 cases, 10 controls), and infant birthweight (7 cases, 13 controls)

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Table 2.

Characteristics of SNPs in MB/OP candidate genes and risk of abruptio placentae

Gene	SNP±	chr:position±	Imputation Score£	Risk allele	Risk allele frequency¥	Association estimates from Workalemahu et al[16] study			Association estimates from PAGE study		Function	Nomenclature
						OR (95% CI)	P-value	FDR	OR (95% CI)	P-value		
Mitochondrial Biogenesis												
CAMK2B		7:44255034	0.96	T	0.7471	1.30 (1.05,1.58)	0.01	0.02	1.13 (0.96,1.34)	0.14	3downstream	Calcium/calmodulin-dependent protein kinase (CaM kinase) II beta
NR1H3	rs11039155	11:47280762	0.77	A	0.1235	1.31 (1.02,1.68)	0.04	0.04	1.24 (0.96,1.66)	0.10	intronic	liver X receptor, alpha/liver X receptor-alpha
PPARG	rs6782178	3:12334555	0.97	C	0.8706	1.44 (1.11,1.84)	0.005	0.02	1.22 (0.99,1.49)	0.06	intronic	Estrogen-related receptor alpha
PPARG	rs10865711	3:12361385	1	C	0.5882	1.19 (0.99,1.44)	0.07	0.04	1.07 (0.92,1.24)	0.41	intronic	Estrogen-related receptor alpha
PPARG	rs1175540	3:12465243	0.98	A	0.1824	1.30 (1.02,1.62)	0.03	0.04	1.04 (0.85,1.27)	0.70	intronic	Estrogen-related receptor alpha
PRKCA	rs4328478	17:64307982	1	T	0.7	1.22 (0.98,1.49)	0.06	0.04	1.13 (0.95,1.33)	0.16	intronic	protein kinase C, alpha
THRB	rs9814223	3:24362252	0.99	G	0.6882	1.20 (1.01,1.47)	0.05	0.04	1.02 (0.86,1.19)	0.85	intronic	Thyroid hormone receptor beta
Oxidative Phosphorylation												
COX5A	rs12437831	15:75226086	0.99	A	0.8647	1.32 (1.00,1.69)	0.05	0.04	1.06 (0.86,1.31)	0.60	intronic	cytochrome c oxidase subunit Va
NDUFA10	rs4149549	2:240931266	1	C	0.7059	1.23 (0.98,1.54)	0.07	0.04	1.00 (0.83,1.21)	0.99	intronic	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 10, 42kDa
NDUFA12	rs11107847	12:95386791	1	G	0.5	1.20 (0.99,1.43)	0.05	0.04	1.21 (1.04,1.40)	0.02	intronic	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 12
NDUFC2_KCTD14	rs627297	11:77763789	0.96	T	0.8	1.35 (1.05,1.69)	0.01	0.02	1.11 (0.90,1.35)	0.33	intronic	NADH:ubiquinone oxidoreductase subunit C2

± Build 37 hg19 dbSNP and chromosome:position

¥ Risk allele frequency among Peruvians obtained from the Phase 3 1000 Genomes Browser (https://www.ncbi.nlm.nih.gov/variation/tools/1000genomes/)

£ Imputation quality score

Table 3.

Association between genetic risk score generated using candidate SNPs (11 SNPs in 9 genes) and risk of abruptio placentae

	Genetic Risk Score (GRS)					
	Quartile 1	Quartile 2	Quartile 3	Quartile 4	Trend test	Continuous score
Replication study (507 AP cases and 1,090 controls)[1]						
Weighted Score Intervals	<12.6	12.6–13.8	13.9–15.0	15.1	-	7.5–19.0
Cases, Number (%)	89 (18.2)	127 (26.0)	122 (25.0)	150 (30.7)	-	488 (100.0)
Controls, Number (%)	270 (25.6)	257 (24.4)	264 (25.0)	263 (25.0)	-	1054 (100.0)
OR (95% CI)	1.00	**1.45 (1.04–2.02)**	**1.42 (1.02–1.98)**	**1.75 (1.27–2.42)**	-	**1.12 (1.05, 1.19)**
P-value	-	0.03	0.04	7.0E-04	1.6E-03	3.0E-04
Workalemahu et al 2013 study (470 AP cases and 473 controls)[2]						
Weighted Score Intervals	<8.0	8.0–8.9	9.0–9.9	10.0	-	-
Cases, Number (%)	34 (8.0)	72 (17.0)	113 (27.0)	197 (47.0)	-	-
Controls, Number (%)	58 (14.0)	80 (19.0)	103 (25.0)	175 (42.0)	-	-
OR (95% CI)	1.00	1.55 (0.91–2.64)	**1.88 (1.14–3.11)**	**1.91 (1.20–3.06)**	0.01	-

Statistically significant estimates are highlighted in bold

[1] The effective sample size for the fully-adjusted model was 488 cases and 1054 controls

[2] The effective sample size for the fully-adjusted model was 416 cases and 416 controls

Table 4.

Association between genetic risk score generated using candidate single nucleotide polymorphisms (11 SNPs in 9 genes) and risk of abruptio placentae stratified by preeclampsia, advanced maternal age, and infant sex characteristics.

	Genetic Risk Score (GRS)*					
	Quartile 1	Quartile 2	Quartile 3	Quartile 4	Trend test	Continuous Score
Weighted Score Intervals	<12.6	12.6–13.8	13.9–15.0	15.1		7.5–19.0
Preeclamptics						
AP Cases, Number (%)	12 (11.5)	32 (30.8)	26 (25.0)	34 (32.7)	-	104 (100.0)
Controls, Number (%)	19 (28.8)	19 (28.8)	13 (19.7)	15 (22.7)	-	66 (100.0)
OR (95% CI); P-value	1.00	**2.96 (1.15–7.65); 0.02**	**3.50 (1.27–9.65); 0.02**	**3.92 (1.48–10.36); 0.01**	**0.03**	**1.27 (1.06–1.52); 0.01**
Normotensives						
AP Cases, Number (%)	77 (20.1)	95 (24.7)	96 (25.0)	116 (30.2)	-	384 (100.0)
Controls, Number (%)	251 (25.4)	238 (24.1)	251 (25.4)	248 (25.1)	-	988 (100.0)
OR (95% CI); P-value	1.00	1.32 (0.93–1.87); 0.12	1.27 (0.89–1.80); 0.18	**1.57 (1.11–2.21); 0.01**	0.08	**1.10 (1.03, 1.17); 0.01**
Maternal age 35						
AP Cases, Number (%)	19 (17.9)	28 (26.4)	25 (23.6)	34 (32.1)	-	106 (100.0)
Controls, Number (%)	46 (24.3)	44 (23.3)	53 (28.0)	46 (24.3)	-	189 (100.0)
OR (95% CI); P-value	1.00	1.56 (0.72–3.33); 0.26	1.31 (0.61–2.80); 0.49	**2.22 (1.05–4.69); 0.04**	0.19	**1.18 (1.03–1.36); 0.02**
Maternal age 18–34						
AP Cases, Number (%)	70 (18.3)	99 (25.9)	97 (25.4)	116 (34.8)	-	382 (100.0)
Controls, Number (%)	224 (25.9)	213 (24.6)	211 (24.4)	217 (25.1)	-	865 (100.0)
OR (95% CI); P-value	1.00	**1.46 (1.01–2.11); 0.05**	**1.49 (1.02–2.15); 0.04**	**1.68 (1.17–2.41); 0.01**	**0.04**	**1.10 (1.03–1.18); 0.004**
Male infant						
AP Cases, Number (%)	47 (17.3)	73 (26.8)	63 (23.2)	89 (32.7)	-	272 (100.0)
Controls, Number (%)	145 (26.3)	128 (23.2)	145 (26.3)	134 (24.3)	-	552 (100.0)
OR (95% CI); P-value	1.00	**1.83 (1.16–2.87); 0.01**	1.36 (0.78–2.00); 0.18	**1.95 (1.24–3.07); 8.0E-04**	**0.004**	**1.14 (1.05–1.24); 0.002**
Female Infant						
AP Cases, Number (%)	42 (19.4)	54 (25.0)	59 (27.3)	61 (28.2)	-	216 (100.0)
Controls, Number (%)	125 (24.9)	129 (25.7)	119 (23.7)	129 (25.7)	-	502 (100.0)
OR (95% CI); P-value	1.00	1.11 (0.68–1.81); 0.67	1.47 (0.91–2.39); 0.11	1.37 (0.85–2.20); 0.20	0.35	1.09 (1.00–1.19); 0.06

*
For each characteristic, normotensive women with wGRS in the lowest quartile, women with advanced maternal age in the lowest wGRS quartile, and women with female infant in the lowest wGRS quartile, respectively, served as the single common reference group.

P-for-interaction for wGRS and preeclampsia, wGRS and advanced maternal age status, and wGRS and infant sex were 0.12, 0.73 and 0.44, respectively. P-for-interaction estimates did not change when wGRS was entered in the model as a continous variable†

Statistically significant estimates are highlighted in bold