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Original Article

Genome-wide and candidate gene association studies of placental abruption

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Abstract: Placental abruption (PA), a pregnancy-related vascular disorder, is a leading cause of maternal and perinatal morbidity and mortality. The success of identifying genetic susceptibility loci for PA, a multi-factorial heritable disorder, has been limited. We conducted a genome-wide association study (GWAS) and candidate gene association study using 470 PA cases and 473 controls from Lima, Peru. Genotyping for common genetic variations (single nucleotide polymorphisms, SNPs) was conducted using the Illumina Cardio-Metabo Chip platform. Common variations in 35 genes that participate in mitochondrial biogenesis (MB) and oxidative phosphorylation (OS) were selected for the candidate gene study. Regression models were fit to examine associations of each SNP with risk of PA. In pathway analyses, we examined functions and functional relationships of genes represented by the top GWAS hits. Genetic risk scores (GRS), based on top hits of the GWAS and candidate gene analyses, respectively, were computed using the risk allele counting method. The top hit in the GWAS analyses was rs1238566 (empirical *P*-value=1.04e-4 and FDR-adjusted *P*-value=5.65E-04) in FLI-1 gene, a megakaryocyte-specific transcription factor. Networks of genes involved in lipid metabolism and cell signaling were significantly enriched by the 51 genes whose SNPs were among the top 200 GWAS hits (*P*-value <2.1e-3). SNPs known to regulate MB (e.g. CAMK2B, NR1H3, PPARG, PRKCA, and THRB) and OP (e.g., COX5A, and NDUF family of genes) were associated with PA risk (*P*-value <0.05). GRS was significantly associated with PA risk (trend *P*-value <0.001 and 0.01 for GWAS and candidate gene based GRS, respectively). Our study suggests that integrating multiple analytical strategies in genetic association studies can provide opportunities for identifying genetic risk factors and novel molecular mechanisms that underlie PA.

Keywords: Placental abruption, genome-wide association study, pathway analyses, candidate gene, genetic risk score

Introduction

Placental abruption (PA) is the separation of the placenta from the uterus prior to delivery of the fetus [1-3]. This pregnancy-related vascular disorder complicates about 1% of all births and is associated with significant complications in the mother and her offspring [1-3]. The success of identifying genetic susceptibility loci for PA, a multi-factorial heritable disorder, has been limited [4-13]. Findings from the only PA-related genome-wide association study (GWAS) from our team provided suggestive evidence supporting associations of variations in maternal cardiometabolic genes with risk of PA. We previously identified variations in SNPs in novel (SMAD2 MIR17HG, and DGKB) and candidate (AGT, KDR, F2, and THBD) genes that may be associated with PA [14]. However, the previous GWAS study was conducted among 253 PA cases and 258 controls, limiting the study's statistical power to identify significant associa-

tions. The need to conduct larger PA-related GWAS studies and the opportunities they provide to investigate genetic variations in recently recognized molecular pathways that lead to PA, such as mitochondrial biogenesis and oxidative stress, motivated the current study.

Impaired placental function and oxidative phosphorylation, pathways implicated in the pathogenesis of PA, have their origins extending to mitochondrial dysfunction [15, 16]. Mitochondria are semi-autonomous cytoplasmic organelles of the eukaryotic system that produce adenosine triphosphate by the coupling of oxidative phosphorylation to respiration, providing a major source of energy to the cell [15, 16]. Approximately 1,500 proteins encoded by nuclear DNA (nDNA) regulate mitochondrial biogenesis and maintain mitochondrial structure and function by regulating oxidative phosphorylation, apoptosis, and mitochondrial DNA replication, transcription, and translation [17]. While most previous work on genetic variations and mitochondrial dysfunction has focused on variations in mitochondrial DNA (mtDNA), recently, investigators have noted that single nucleotide polymorphisms (SNPs) in nDNA may be associated with mitochondrial dysfunction (and related disease conditions) through subtle changes in encoded proteins that alter mitochondrial biogenesis and/or oxidative phosphorylation activity [18]. On the basis of this emerging literature and our recent observation that PA risk is associated with increased mtDNA copy number abundance [19], a marker of mitochondrial dysfunction, we hypothesized that variations in mitochondrial biogenesis- and oxidative phosphorylation-related genes are associated with PA risk.

The current study extends our previous GWAS study by doubling the size of study participants (470 PA cases and 473 controls), testing of specific hypothesis linking genetic variation in mitochondrial biogenesis and oxidative phosphorylation-related genes with PA risk, and employing multiple analytical approaches to investigate genetic risk factors of PA.

Methods

Study setting and study population

This study was conducted as part of the Peruvian Abruptio Placentae Epidemiology (PAPE) study. The study setting and study population were described before [14]. Briefly, study participants were recruited and enrolled among women who were admitted for obstetrical services to the Hospital Nacional Dos de Mayo, Instituto Especializado Materno Perinatal, and Hospital Madre-Niño San Bartolomé in Lima, Peru, between August 2002 and May 2004 and between September 2006 and September 2008.

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 retroplacental bleeding (fresh blood) entrapped between the decidua and the placenta or blood clots behind the placenta and 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; (iii) fetal distress or death. Controls were 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.

Institutional Review Boards of participating institutions approved the project protocol. All participants provided written informed consent.

Data collection

Trained research personnel conducted participant interviews using standardized structured questionnaires to collect information on sociodemographic characteristics and risk factors including maternal age, marital status, employment status during pregnancy, medical history, and smoking and alcohol consumption (both before and during the current pregnancy). A brief physical examination was conducted to measure maternal height, weight, and mid-arm circumference. Medical records were reviewed to abstract information on course and outcomes of the pregnancy [14]. Blood specimens collected from 470 PA cases and 473 controls were processed for assessment of genetic variation as described below.

DNA extraction and genotyping

The Gentra PureGene Cell kit for DNA preparations (Qiagen, Hilden, Germany) was used to extract DNA from blood specimens collected

from 470 PA cases and 473 controls. Genotyping to characterize genome-wide variation in cardiovascular and metabolism genes was conducted using the Illumina Cardio-Metabochip (Illumina Inc, San Diego, CA) platform [14]. Briefly, the Cardio-Metabo Chip is a high-density custom array that captures DNA variation at regions previously related to diseases and traits relevant to metabolic and atherosclerotic-cardiovascular endpoints using 217,697 SNPs.

Data quality control and preprocessing

Rigorous quality control procedures were applied to the DNA before analyses [20]. Individuals were excluded (n=48) if they had genotyping failure for more than 10% of the sites. SNPs were excluded if the minor allele frequency was less than 1%, if the SNP failed to be genotyped in more than 10% of the study population, or if the SNP was not in Hardy-Weinberg equilibrium among controls (critical P=0.0001). After these QC procedures, a total of 124,499 SNPs and 470 PA cases and 473 controls remained for analyses. Population stratification was assessed using the genomic inflation factor [21] and adjustment for the first four principal components in regression models [22].

Candidate gene/SNP selection

A total of 35 genes that were involved in mitochondrial biogenesis and oxidative phosphorylation were selected based on literature (Supplementary Table 1). There were a total of 322 SNPs representing these genes in the Cardio-Metabo Chip. Of these 322 SNPs, 310 SNPs were excluded because they were rare (12 SNPs with MAF <1%) and/or in significant linkage disequilibrium (298 SNPs with R^2 >0.8) with another SNP in the set. A total of 11 SNPs in 9 candidate genes remained for further analysis.

Statistical analyses

Logistic regression models were fit to evaluate associations between each SNP and risk of PA in both the genome-wide and candidate gene analyses. Bonferroni correction was used to account for multiple testing (*P*-value threshold 0.05/124,499 SNPs=8.03e-6) in overall genome-wide level analyses. In addition to assessment of the empirical *P*-value, we also computed false discovery rate (FDR) for a less conservative assessment of statistical significance [23]. For the secondary candidate SNP-PA association analyses, we used P <0.05 as a cut-off to determine statistical significance.

Ingenuity Pathway Analyses (IPA, Ingenuity, Redwook, CA) was used to evaluate functions and functional relationships of genes represented by top 200 hits from our current PA GWAS analysis. In IPA, each gene identifier was mapped to its corresponding gene object in the Ingenuity Pathways Knowledge Base (IPKB). These genes were overlaid onto a global molecular network developed from information contained in the IPKB. Gene-enrichment of networks was assessed using network score based on a modified Fisher's exact test. The network score, negative log of *P*-values of Fisher tests, was used to rank biological significance of gene function networks in relation to PA.

Using risk allele counting method, we computed genetic risk scores (GRS) for top hits from the GWAS and candidate gene analyses [24]. The top 10-15 risk alleles that were common (higher MAF) and not in LD with other SNPs in the set were selected from each analysis. We assumed the additive genetic risk model corresponding to a linear increase of PA risk to the presence of 0, 1, and 2 risk alleles. In addition, we assumed equal risk of PA conferred by each locus. Participants were categorized into four groups defined by the 25th, 50th, and 75th percentile GRS scores among control participants (i.e., Group 1: 0-25%ile, Group 2: 25-50%ile, Group 3: 50-75%ile, and Group 4: 75-100%ile). Regression models were then fit to examine associations of the genetic score with PA risk using Group 1 (0-25%ile group) as a referent.

Statistical analyses were conducted using PLINK v1.07, SAS, and R software. Pathway analyses were conducted using IPA v6 software [25].

Results

Selected socio-demographic and medical/obstetric characteristics of participants are shown in **Table 1**. Average maternal age of PA cases and controls was 28 years. PA cases and controls were similar with respect to their fre-

Table 1. Socio-demographic and reproductive characteristics and infant outcomes in the study population, Lima, Peru

Maternal Characteristics	Placental Abruption	
	Cases (n=470)	Controls (n=473)
Maternal age at delivery, years*	27.7 (6.7)	27.7 (6.6)
<35	380 (11.2)	388 (10.8)
≥35	90 (18.0)	85 (17.4)
Nulliparous	41 (8.4)	12 (2.41)
Less than High school education	353 (72.3)	356 (71.3)
Employed during pregnancy	213 (43.5)	223 (44.8)
Planned pregnancy	195 (40.5)	207 (42.2)
No prenatal vitamin	154 (31.6)	148 (29.7)
Smoked during pregnancy	18 (3.7)	9 (1.8)
Alcohol use during pregnancy	21 (4.2)	26 (5.3)
Pre-pregnancy BMI, kg/m^{2}*	23.8 (3.8)	23.7 (3.9)
<18.5	24 (5.3)	15 (3.1)
18.5-24.9	288 (64.0)	322 (67.5)
25.0-29.9	111 (24.7)	103 (21.6)
≥30.0	27 (6.0)	37 (7.8)
Chronic hypertension	22 (4.6)	12 (2.4)
Preeclampsia or Eclampsia	134 (27.9)	37 (7.4)
History of placental abruption	5 (1.1)	0 (0)
Infant birthweight, grams*	2430 (863)	3158 (690)
Gestational age at delivery, weeks*	35.4 (4.1)	38.3 (2.7)

*Mean (standard deviation), otherwise number (%).

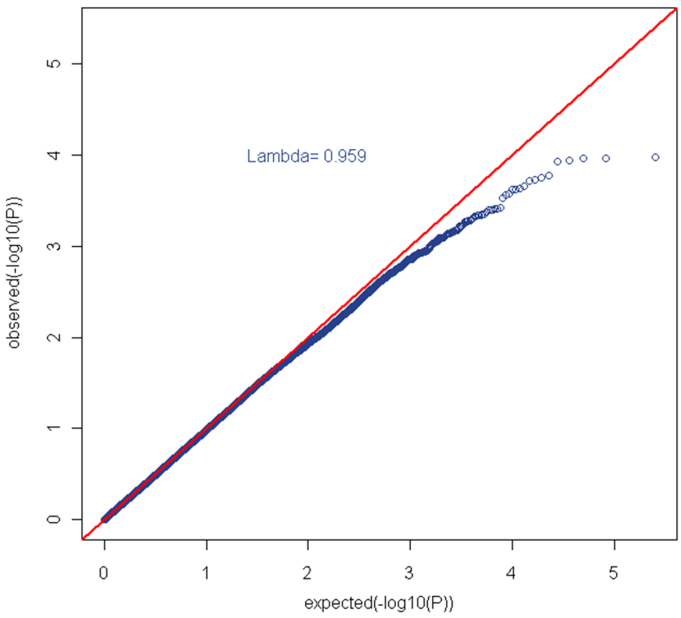

Figure 1. Q-Q plot (λ=0.96).

quency of high school completion and employment during pregnancy. They also had comparable mean pre-pregnancy BMI (average 24 kg/m^2 for both). However, compared with controls, PA cases were more likely to be smokers during pregnancy, and have a history of preeclampsia, eclampsia or PA. As expected, PA cases delivered low birth weight infants more frequently and had shorter gestational lengths, compared with controls.

We did not observe significant genomic inflation (λ=0.96) (**Figure 1**). In genome-wide analyses, none of the *P*-values for SNP-PA associations surpassed the stringent criteria for significant associations (Bonferroni corrected *P*-value 8.03e-6) (**Figure 2**). FLI-1 gene (rs1238566, *P*-value=1.04e-4, FDR=5.65e-4), a megakaryocyte-specific transcription factor, was the top hit in the GWAS analyses. Genes represented by the top GWAS hits are shown in **Table 2**. A total of 51 genes were represented by the top 200 GWAS hits (with *P*-values <2.1e-3, FDR <2.0e-3); and, these were evaluated for functions and functional relationships using IPA. The top networks enriched by these genes included networks of lipid metabolism (score=40, *P*-value=4.25e-18) and cell signaling (score=26, *P*-value=5.11e-12) (**Table 3** & **Figure 3**). In additional to these 51 genes, the top networks included other well-described genes in lipid metabolism (e.g., FLI-1, CETP, LIPC, and THRB) and cell signaling (e.g., Akt, NFKB, and PI3K) (**Figure 3**). In candidate gene analyses, SNPs in genes related to mitochondrial biogenesis (e.g., CAMK2B, NR1H3, PPARG, PRKCA, and THRB) or oxidative phosphorylation (e.g., COX5A, and NDUF family of genes) were significantly associated with PA risk (*P*-values <0.05) (**Table 4**).

Two GRS were computed using 14 SNPs identified in the GWAS analyses and 11 SNPs in 9 genes identified in the candidate gene association analyses, respectively (Supplementary Table 2). Both GRS were significantly associat-

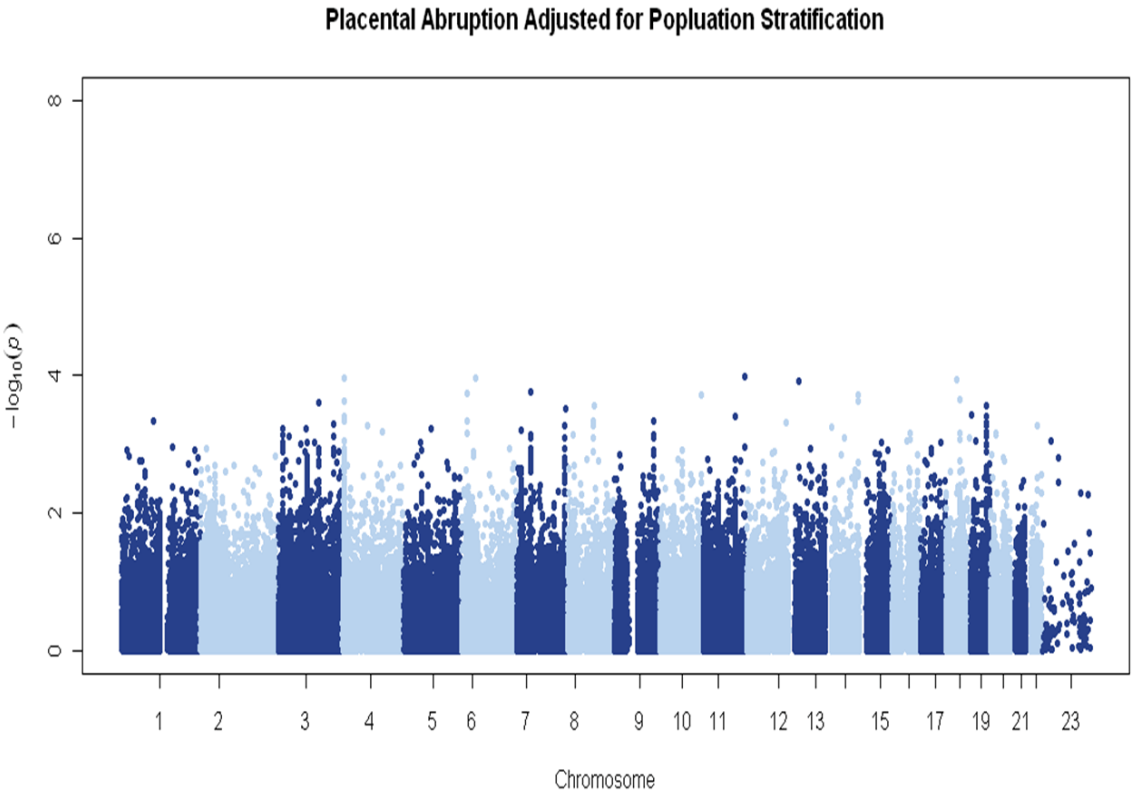

Figure 2. Manhattan plot of genome-wide association study of placental abruption.

Table 2. Top hits of analyses examining genome-wide genetic variations and placental abruption risk

Gene	SNP	Minor Allele	MAF	Odds Ratio	Empirical *P*-value	FDR
FLI1	rs1238566	G	0.34	1.46 (1.21-1.77)	1.05E-04	5.65E-04
C6orf108	rs7832	A	0.07	2.14 (1.48-3.18)	1.08E-04	5.65E-04
LOC647946	rs10502722	C	0.41	0.7 (0.59-0.85)	1.13E-04	5.65E-04
ZAR1L	rs206136	G	0.11	0.56 (0.41-0.74)	1.19E-04	5.65E-04
CAP2	rs1320995	C	0.35	0.67 (0.56-0.83)	1.77E-04	5.65E-04
TECPR2	rs4900536	G	0.35	0.70 (0.57-0.83)	1.89E-04	5.65E-04
DPP6	rs2024366	G	0.14	1.65 (1.27-2.17)	2.97E-04	7.55E-04
PLIN3	rs3760950	A	0.38	1.42 (1.18-1.72)	3.74E-04	7.55E-04
CAP2	rs1320994	G	0.37	0.71 (0.58-0.85)	4.48E-04	7.55E-04
PSMD5	rs10760117	A	0.18	1.52 (1.18-1.88)	4.53E-04	7.55E-04
FRRS1	rs951125	G	0.21	0.67 (0.53-0.84)	4.59E-04	7.55E-04
NCOR2	rs12582168	G	0.43	0.72 (0.60-0.87)	4.77E-04	7.55E-04
EDIL3	rs350477	A	0.24	0.67 (0.55-0.86)	6.03E-04	7.76E-04
KIF16B	rs8117456	C	0.22	1.48 (1.16-1.81)	6.71E-04	7.76E-04
CAP2	rs9383287	G	0.38	0.72 (0.59-0.86)	6.73E-04	7.76E-04
CETP	rs891144	A	0.18	0.66 (0.51-0.83)	6.94E-04	7.76E-04
MYO5B	rs17716496	C	0.17	0.66 (0.52-0.84)	7.04E-04	7.76E-04
KIF16B	rs1028540	A	0.39	1.38 (1.15-1.66)	7.35E-04	7.76E-04
LOC253039	rs4837796	G	0.19	1.48 (1.16-1.83)	8.10E-04	8.10E-04

Abbreviations: MAF = Minor Allele Frequency in Controls.

ed with risk of PA (linear trend *P*-values <0.001 and 0.01 for the genome-wide GRS and the candidate GRS, respectively) (**Table 5**). Participants in the highest GRS Group (Group

Table 3. SNPs in candidate genes and risk of placental abruption

Gene	SNP	Minor Allele	MAF	Odds Ratio	Empirical *P*-value	FDR
CAMK2B	Chr7:44221559	G	0.27	0.77 (0.63-0.95)	0.01	0.02
NR1H3	Chr11:47237338	A	0.16	1.31 (1.02-1.68)	0.04	0.04
PPARG	Chr3:12309555	A	0.16	1.44 (1.11-1.84)	0.005	0.02
PPARG	Chr3:12336385	A	0.47	1.19 (0.99-1.44)	0.07	0.04
PPARG	Chr3:12440243	A	0.18	1.30 (1.02-1.62)	0.03	0.04
PRKCA	Chr17:61738444	G	0.29	0.82 (0.67-1.02)	0.06	0.04
THRB	rs9814223	A	0.35	0.83 (0.68-0.99)	0.05	0.04
COX5A	Chr15:73013139	G	0.14	0.76 (0.59-1.00)	0.05	0.04
NDUFA10	Rs4149549	A	0.21	1.23 (0.98-1.54)	0.07	0.04
NDUFA12	Rs11107847	A	0.43	0.83 (0.70-1.01)	0.05	0.04
NDUFC2	Rs627297	C	0.17	0.74 (0.59-0.95)	0.01	0.02

Abbreviations: MAF = Minor Allele Frequency in Controls.

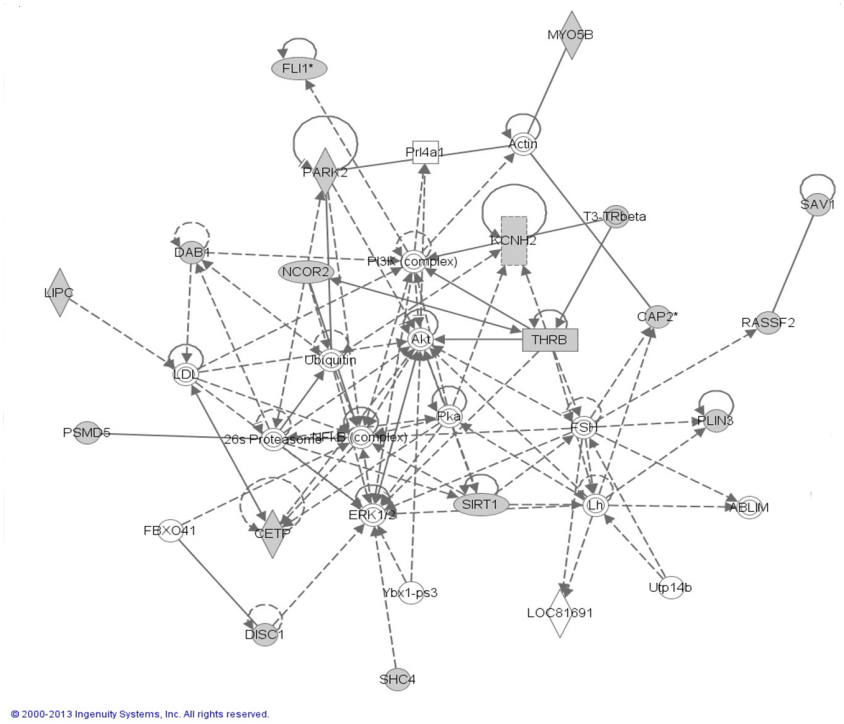

Figure 3. Top network represented by genes represented by top hits of GWAS analysis.

4), GRS >17.1 in the GWAS analysis and GRS >10.0 in the candidate gene analysis, had 5.45 (95% CI: 3.68-8.06) and 1.91 (95% CI: 1.20-3.06) fold higher risk of PA, respectively, compared with participants in the lowest GRS Group, Group 1 (GRS <10.9 in the GWAS analysis and GRS <8.0 in the candidate gene analysis) (**Table 5**).

Discussion

In genome-wide and candidate gene association analyses, we identified several SNPs, genes, and pathways that are related to PA risk. In the GWAS analysis among PA cases and controls, the top hit was rs1238566 (*P*-value =1.04e-4) in FLI-1 gene. None of the SNPs reached statistical significance after correction for multiple testing using the conservative Bonferroni correction. Networks enriched by 51 genes represented by the top 200 GWAS hits (*P*-values <2.1e-3) included networks of lipid metabolism (e.g., FLI-1, CETP, LIPC, and THRB) and cell signaling (e.g., Akt, NFKB, and PI3K). In candidate gene analysis, SNPs in genes participating in mitochondrial biogenesis (e.g. CAMK2B, NR1H3, PPARG, PRKCA, and THRB) or oxidative phosphorylation (e.g., COX5A, and NDUF family of genes) were significantly associated with PA risk (*P*-values <0.05). GRS computed using risk alleles identified from both the GWAS and candidate gene analyses were associated with risk of PA.

Most previous studies of common genetic variations and risk of PA [26-30] were small candi-

Table 4. Significant networks represented by top GWAS hits

Molecules in Network	Score	Focus Molecules (#)	Top Functions	*P*-value
26s Proteasome, ABLIM, Actin, Akt, **CAP2**, **CETP**, **DAB1**, **DISC1**, ERK1/2, FBXO41, **FLI1**, FSH, **KCNH2**, LDL, Lh, *LIPC*, LOC81691, ***MY058,*** **NCOR2**, NFKB, **PARK2**, PI3K, **PLN3**, Prl4a1, **PSMD5**, **RASSF2**, **SAV1**, **SHC4**, **SIRT1**, T3-Trbeta, **THRB**, Ubiquitin, Utp14b, Ybx1-ps3	40	17	Small Molecule Biochemistry, Lipid Metabolism, Cellular development	4.25E-18
AMICA1, BTN2A2, BTN3A1, BTN3A2, C12orf29, **C6orf108**, **CC2D2A**, DDX60L, **DPP6**, **EML2**, FAM108B1, **FSTL4**, GABARAP, GSDMD, IFNG, KCTD7, **KIF16B**, **LUZP1**, MORC2, NLN, PHB, **PHLDB2**, **PITPNC1**, PLEKHO2, RAB12, RAB20, RAB43, RNASE7, **SCAPER**, SLC16A13, SMAGP, **TECPR2**, TGM2, UBC, ZFX	26	12	Cell Death and Survival, Cell-to-Cell Signaling and Interaction, Cellular Function and Maintenance	5.11E-12

The networks were generated through the use of Ingenuity Pathways Analysis (Ingenuity® Systems, www.ingenuity.com). Each gene identifier was mapped to its corresponding gene object in the Ingenuity Pathways Knowledge Base (IPKB) and overlaid onto a global molecular network developed from information contained in the IPKB. Scores, corresponding to degree of enrichment, are negative log of *p*-values of Fisher test. Genes in bold (focus molecules) are genes that are represented by top hit SNPs in our genome-wide association study of placental abruption.

Table 5. Genetic risk score (GRS) and risk of placental abruption

	Genetic Risk Score (GRS)*				
	Group 1 0-25%ile	Group 2 25-50%ile	Group 3 50-75%ile	Group 4 75-100%ile	P
	Genome-wide Association Analysis				
Intervals	<10.9	10.9-13.9	14.0-17.0	≥17.1	
Cases, n (%)	49 (10)	45 (10)	140 (30)	236 (50)	
Controls, n (%)	142 (30)	69 (15)	138 (29)	124 (26)	
OR (95% CI)**	1.00	1.85 (1.12-3.05)	2.90 (1.97-4.33)	5.45 (3.68-8.06)	<0.001
	Candidate Gene Analysis				
Intervals	<8	8-8.9	9-9.9	≥10	
Cases, Number (%)	34 (8)	72 (17)	113 (27)	197 (47)	
Controls, Number (%)	58 (14)	80 (19)	103 (25)	175 (42)	
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

*GRS computed from 14 SNPs that are top hits from GWAS analyses, and 11 SNPs that are top hits from the candidate gene association analyses. **Odds ratios (and 95% confidence intervals) from logistic regression models adjusted for age, sex, and population admixture, linear trend *P*-values.

date gene studies. The only other GWAS by our group [14] identified several novel and candidate SNPs that are potentially related to PA risk. Several of the top SNPs identified in the previous study were significantly associated with PA risk, at the P <0.05 level, in our expanded sample. These included SNPs in the DGKB (chr7:14154194, *P*-value=6e-04), SMAD2 (chr18:453999981, *P*-value=2e-04), and MIR17HG (rs4773624, *P*-value=0.003). In addition, a SNP in previously identified candidate gene, the CETP gene (rs891144, *P*-value=6.94E-04), was among the top hits in the GWAS analyses in our study. Ali et al. [31], in their case-control study among women with idiopathic PA, placenta previa, and normal pregnancies, found that the B2 allele frequency in idiopathic PA was higher than its frequency in placenta previa or normal pregnancy (*P*-value <0.05).

The top GWAS hit in our study, rs1238566, is a SNP in the FLI-1 gene, a megakaryocyte-specific transcription factor. The A allele of this SNP is weakly (P=0.08) associated with increased expression of the FLI-1 gene in lymphoblastoid cell lines [32]. Coagulation defects, where

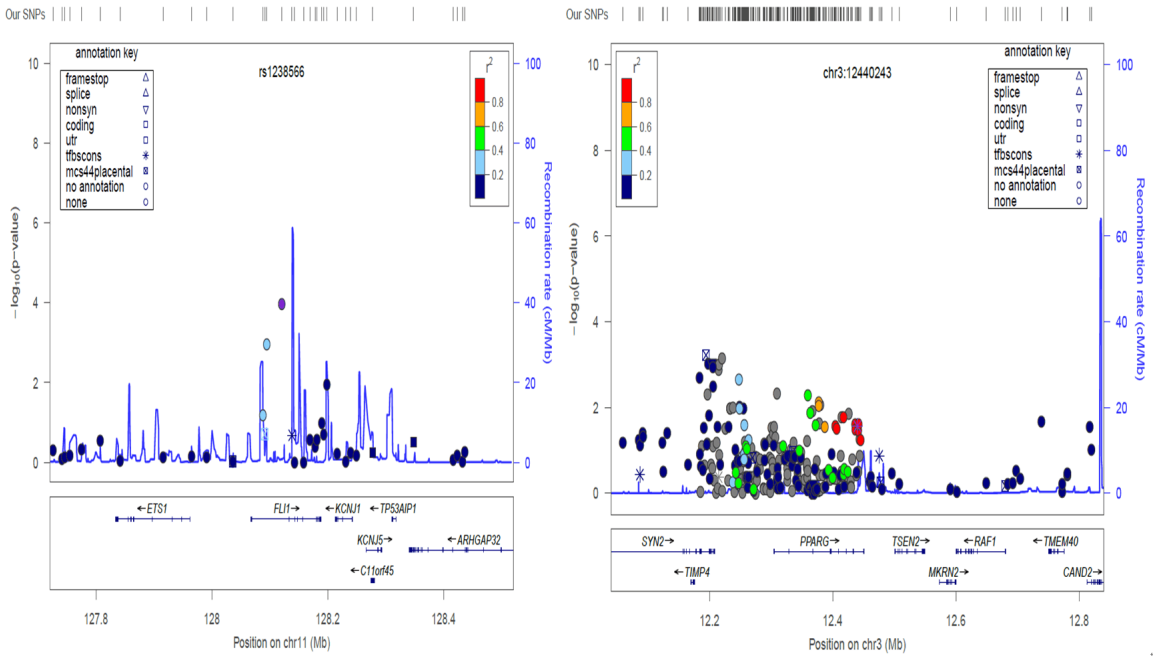

Figure 4. Regional SNP association plot for the top GWAS SNP rs1238566 and candidate SNP chr3:12440243. Estimated local recombination rate and −log10 *P*-values are plotted in blue on the right and left side of the y-axis, respectively. The genomic position is shown on the x-axis. Linkage disequilibrium (r^2) of a nearby SNP with the index SNP is shown by purple color.

megakaryocytes play key roles, such as decreased platelets, elevated prothrombin time, decreased fibrinogen levels, and elevated fibrin split products, have closely been associated with the clinical events of PA. Therefore, the finding that a variation in the FLI-1 gene may be associated with PA risk is of high significance.

We assessed recombination rates and LD of SNPs near putative associated variants identified in the genome-wide and candidate gene association analyses. The regional SNP association plots illustrating the complex genetic architecture for rs1238566 (from the genome-wide analysis) and chr3:12440243 (from the candidate gene analysis) are shown in **Figure 4**. Other genes in the neighborhood of these SNPs, such as the ETS1 gene that has been shown to be involved in connective tissue metabolism, fibrosis, inflammation, and related disease conditions (e.g. SLE), are putative candidates as genetic susceptibility factors of PA [33, 34].

In pathway analyses of genes represented by top hits of the GWAS analyses, lipid metabolism and cell signaling networks were identified. Dyslipidemia has been associated with a number of pregnancy complications that include preeclampsia, a hypertensive disorder of pregnancy that has been closely related to PA [35]. Recent evidence also points to lipid-related high risk profiles (e.g., higher cholesterol, LDL, and lower HDL) for cardiovascular diseases among women with history of PA, supporting potential risk similarities of PA with other cardiovascular diseases in the general population [36]. However, significant gap remains in our understanding of direct relationships between lipid abnormalities, or genetic factors that underlie these abnormalities and risk of PA. Cell signaling which is a broad category that encompasses a diverse set of cellular interactions is a critical component of cellular function. As described in our previous report, and supported by current findings, several candidates (such as the SMAD family of genes and the DPP6 gene) in cell-signaling pathways may have roles in risk of PA [14]. Further research into the extent of their roles and the specific signaling pathways that are important is warranted.

Several mechanisms may explain why variation in mitochondrial biogenesis and oxidative phosphorylation related genes might contribute to mitochondrial dysfunction and PA. Oxidative

stress-induced damage to mitochondrial structural elements (e.g., lipid membranes) may alter mitochondrial gene expression and promote a deficiency in oxidative phosphorylation [37]. Variation in genes related to mitochondrial biogenesis may confer susceptibility to damage from oxidative stress and variation in genes related to oxidative phosphorylation may accentuate the deficiency in oxidative phosphorylation that results from mitochondrial dysfunction. In the current candidate gene association analyses, we found that several SNPs in mitochondrial biogenesis (e.g., CAMK2B, NR1H3, PPARG, PRKCA, and THRB) and oxidative phosphorylation (e.g., COX5A, and NDUF family of genes) related genes are associated with risk of PA. Of these, the PPARG and NDUF genes were represented by several SNPs. The role of PPARG in placentation has been well documented [38, 39]. Interestingly, investigators have put forth thesis indicating that PPARG mediates defective placentation (e.g., inhibition of trophoblast invasion) that results from oxidized LDL in cytotrophoblasts of villous and extravillous cells [40]. The NDUF gene, encoding the NADH:ubiquinone oxidoreductase protein, is part of the enzyme complex in the electron transport chain of mitochondria [41]. While the gene and other genes in the family, and their variants have been reported in relation to other diseases such as AIDS and prostate cancer, to our knowledge, no previous study reported variation in this gene in relation to pregnancy complications [42, 43]. Future studies are needed to further explore the role of these sets of genes in placentation and PA risk.

GRS based on risk allele genotypes of multiple SNPs have been used to summarize genetic effects among ensemble of markers that do not individually achieve significance in association studies [44]. In the current study, both GRS computed using top hits of SNPs in the GWAS and candidate gene analyses were significantly associated with risk of PA. One potential limitation of our GRS-based analyses is the fact that we used the same data set for training and testing. We conducted cross-validation to assess the utility of the GRS [45]. Based on that test, we observed that the GWAS based GRS had an agreement of 64% with the true value (PA cases and controls), while the candidate-based GRS score has an agreement of 55% with the true value. However, sensitivity of the GWAS-based GRS score was 82% while sensitivity of the candidate-based GRS score was 71%. Specificity for the two scores, respectively, was 46% and 34%. A significant overestimation of the PA risk was observed. Based on previous suggestions by Thomsen et al. [45], the validity of our GRS scores should be examined in other study populations with different indices of diseases.

Other limitations of our study merit consideration. Although our study is the largest to date on the topic, we may still be underpowered to detect significant associations between genetic variations, particularly less common variations, and risk of PA. This is particularly true for small effect sizes. Potential misclassifications of sub-clinical PA may further limit our study power. Absence of a replication cohort and lack of follow-up functional studies are other limitations of our study. Finally, generalizability of our findings should be confirmed by studies that are conducted in study populations that differ in genetic and other characteristics from ours.

Some strengths of our study deserve mention. We used multiple (GWAS, pathway, candidate, and GRS) analytic approaches to examine associations between genetic susceptibility and risk of PA. These different approaches provided us with the opportunity to identify genetic risk that may be associated with PA, a multigenic complex disorder. In our candidate gene analyses, we have specifically drawn attention to mitochondrial biogenesis and oxidative phosphorylation, an emerging little explored pathway that is significant in placental pathophysiology, pregnancy complications, and outcomes. Our study was conducted among a high-risk underinvestigated study population and relatively less problem of population stratification are other strengths of the study.

In summary, our study suggests that integrating multiple genomic analytical strategies provides opportunities for identifying novel biological pathways for exploring the underlying molecular mechanisms for PA. In addition, our GRS analyses support the promise of using genetic association studies in PA risk prediction. Future studies that involve larger discovery and replication samples, functional follow-up of confirmed PA-associated variants, and risk-prediction, among others, may enhance efforts to understand PA pathomechanisms

and facilitate the development of prevention strategies.

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Disclosure of conflict of interest

The authors have no conflicts of interest to disclose.

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Supplementary Table 1. Candidate genes implicated in mitochondrial biogenesis and oxidative phosphorylation and their association with placental abruption

Gene abbreviation	Number of SNPs in Gene	Minimum Observed *P*-value	SNP With Minimum Observed *P*-value	SNP with Minimum Observed *P*-value Minor Allele	MAF for SNP with Minimum Observed *P*-value	Odds ratio† for SNP with Minimum Observed *P*-value	Chr for SNP with Minimum Observed *P*-value	sample size	proxy for SNP with Minimum Observed *P*-value	LD (Dprime) between proxy and SNP	Rsquared between proxy and SNP
SNPs implicated in Mitochondrial Biogenesis											
CAMK2B	25	0.0012	chr7:44221640	A	0.02044	4.40	7	934	chr7:44221639	1.00	1.00
CAMK2D	2	0.4804	rs4141007	G	0.03557	1.20	4	941	rs4834348	0.32	0.01
CAMK4	1	0.0669	rs11748647	A	0.1865	1.20	5	940	N/A	N/A	N/A
CREB1	1	0.1239	rs17811997	C	0.01501	1.80	2	938	N/A	N/A	N/A
NR1H3	23	0.0381	chr11:47237338	A	0.1594	1.30	11	918	N/A	N/A	N/A
PPARA	2	0.0338	rs4253728	A	0.05573	0.64	22	941	rs4253776	0.90	0.34
PPARG	127	0.0047	chr3:12309555	A	0.1573	1.40	3	938	N/A	N/A	N/A
PPARGC1A	1	0.1436	rs10018239	G	0.07299	0.77	4	932	N/A	N/A	N/A
PPARGC1B	1	0.3404	rs26124	A	0.3953	1.10	5	940	N/A	N/A	N/A
PRKCA	65	0.0215	chr17:61730083	A	0.01086	0.30	17	939	N/A	N/A	N/A
SP1	1	0.1670	rs7131938	A	0.2204	0.85	12	941	N/A	N/A	N/A
THRB	10	0.0017	rs7609948	A	0.2341	1.40	3	925	rs17014418	1.00	0.17
TRNT1	1	0.1627	rs7629889	A	0.2755	0.87	3	941	N/A	N/A	N/A
SNPs implicated in Oxidative Phosphorylation											
COX5A	17	0.0461	chr15:73013139	G	0.1357	0.76	15	941	N/A	N/A	N/A
COX10	2	0.1680	rs17616591	G	0.06043	0.76	17	941	rs12943936	1.00	0.18
COX4I1	1	0.2943	rs2733954	A	0.4066	0.91	16	940	N/A	N/A	N/A
COX7A2	1	0.4326	rs436898	A	0.1412	0.90	6	939	N/A	N/A	N/A
CPX7A2L	1	0.3998	rs8162	G	0.2234	0.91	2	937	N/A	N/A	N/A
COX7B2	2	0.0557	rs1512128	A	0.02786	1.70	4	941	rs4395475	0.69	0.01
LRPPRC	9	0.2314	rs4953042	G	0.3859	1.10	2	938	rs6723119	1.00	0.33
NDUFA10	2	0.0710	rs4149549	A	0.2058	1.20	2	929	rs6437237	1.00	0.22
NDUFA12	5	0.0180	rs7300945	G	0.4036	0.80	12	937	rs11107847	1.00	0.42
NDUFA3	2	0.2959	rs7253859	G	0.02066	1.40	19	940	N/A	N/A	N/A
NDUFB1	1	0.1511	rs6575231	A	0.4582	0.87	14	941	N/A	N/A	N/A
NDUFC1	1	0.1830	rs12642647	G	0.07453	0.79	4	941	N/A	N/A	N/A
NDUFC2_KCTD14	1	0.0125	rs627297	C	0.1663	0.74	11	940	N/A	N/A	N/A
NDUFS4	2	0.2217	rs256108	G	0.1692	0.86	5	941	rs2637030	1.00	0.56
PPA2	3	0.2656	rs2074396	A	0.3345	1.10	4	940	rs959086	1.00	0.24
SDHB	3	0.0997	rs2235928	G	0.3531	1.20	1	936	rs2647209	0.40	0.00
TSFM	1	0.2785	rs10783848	A	0.4137	1.11	12	941	N/A	N/A	N/A
TUFM	4	0.3121	chr16:28761497	A	0.2199	0.90	16	935	N/A	N/A	N/A
NDUFS3	3	0.2675	chr11:47562003	A	0.2552	1.10	11	937	N/A	N/A	N/A

Supplementary Table 2. Single nucleotide polymorphisms included in the genetic risk score (GRS) analyses

Gene	SNP	Minor Allele	MAF	Odds Ratio	*P*-value
GWAS based GRS					
FLI1	rs1238566	G	0.3366	1.46 (1.21-1.77)	1.00E-04
CAP2	rs1320995	C	0.3482	0.69 (0.56-0.83)	2.00E-04
CC2D2A	rs10939621	A	0.2421	0.71 (0.57-0.88)	0.002
LOC647946	rs10502722	C	0.4144	0.70 (0.59-0.85)	1.00E-04
TECPR2	rs4900536	G	0.3462	0.70 (0.57-0.83)	2.00E-04
SIRT1	rs10997860	G	0.4597	1.33 (1.11-1.59)	0.002
ADCY5	chr3:124618938	A	0.4559	1.41 (1.16-1.68)	2.00E-04
DPP6	rs2024366	G	0.1409	1.65 (1.27-2.17)	3.00E-04
PHLDB2	rs6808720	A	0.4427	1.36 (1.12-1.61)	9.00E-04
PLIN3	rs3760950	A	0.3789	1.42 (1.18-1.72)	4.00E-04
EML2	chr19:50840077	G	0.2717	1.43 (1.17-1.73)	4.00E-04
CAP2	rs1320994	G	0.3681	0.71 (0.58-0.85)	4.00E-04
NCOR2	rs12582168	G	0.4263	0.72 (0.61-0.87)	5.00E-04
EDIL3	rs350477	A	0.2448	0.67 (0.55-0.56)	6.00E-04
Candidate gene analyses based GRS					
CAMK2B	chr7:44221559	G	0.2652	0.77 (0.63-0.95)	0.013
NR1H3	chr11:47237338	A	0.1594	1.31 (1.02-1.68)	0.038
PPARG	chr3:12309555	A	0.1573	1.44 (1.11-1.84)	0.005
PPARG	chr3:12336385	A	0.4687	1.19 (0.99-1.44)	0.067
PPARG	chr3:12440243	A	0.1834	1.30 (1.02-1.62)	0.027
PRKCA	chr17:61738444	G	0.2898	0.82 (0.67-1.02)	0.059
THRB	rs9814223	A	0.3479	0.83 (0.68-0.99)	0.048
COX5A	chr15:73013139	G	0.1357	0.76 (0.59-1.00)	0.046
NDUFA10	rs4149549	A	0.2058	1.23 (0.98-1.54)	0.071
NDUFA12	rs11107847	A	0.4292	0.83 (0.70-1.01)	0.051
NDUFC2_KCTD14	rs627297	C	0.1663	0.74 (0.59-0.95)	0.013