Genetic Variations and Risk of Placental Abruption

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Abstract

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Background: Placental abruption (PA) is a premature separation of an implanted placenta due to the rupture of the maternal vessels in the decidua basalis prior to delivery of the fetus. PA complicates approximately 1 in 100 pregnancies leading to significant maternal and perinatal morbidity and mortality worldwide. The etiology of PA is not fully known. Suggested pathophysiologic mechanisms that lead to PA include uteroplacental ischemia, underperfusion, chronic hypoxia, infarctions and thrombosis. Further, abnormal mitochondrial biogenesis (MB) and oxidative phosphorylation (OP), important in placental physiology, contribute to PA risk. Emerging evidence from genome-wide (GWAS) and candidate gene association studies, including several by our group, support potential roles of genetic variations, characterized by

single nucleotide polymorphisms (SNPs), in PA. However, these studies were small in size and results have been inconsistent across studies. Further, prior investigations did not address maternal-fetal genetic interactions and imprinting effects in PA risk, potential contributors for the missing heritability of PA. The motivation of this dissertation research was to address these limitations.

Methods: The research was conducted using data and genomic DNA samples collected from participants of the previously described Peruvian Abruptio Placentae Epidemiology (PAPE) study and the Placental Abruption Genetic Epidemiology (PAGE) study (REF), case-control studies of PA conducted in Lima, Peru. PAPE participants were genotyped using the Illumina Cardio-Metabochip. PAGE participants were genotyped using the Illumina HumanCore-24 BeadChip platform. Genotypes were imputed using the 1000 genomes reference panel, and >4.9 million SNPs that passed quality control were available. The project had three specific aims. Aim #1 was to conduct a GWAS on PAGE participants (507 PA cases and 1,090 controls) and a GWAS meta-analysis in PAPE and PAGE participants (959 PA cases and 1,553 controls) using population stratification-adjusted logistic regression models and fixed-effects meta-analyses using inverse variance weighting, respectively. Aim #2 was to conduct a replication analysis in the PAGE study population examining associations of previously reported (in the PAPE study) weighted genetic risk scores (wGRS) of 11 SNPs in nine MB/OP genes with risk of PA using multivariable-adjusted logistic regression models. Aim #3 was to investigate (in the combined PAPE and PAGE study) maternal-fetal genetic interaction on PA risk for 78 independent (linkage disequilibrium <0.80) SNPs in MB/OP genes using multinomial models, and imprinting (parent-of-origin effect) effect on PA risk for 2713 independent SNPs in 73 imprinted genes using a likelihood ratio test.

Results: We found 174 independent loci suggestively associated with PA in the PAGE GWAS (P-value<5e-5) including rs4148646 and rs2074311 in *ABCC8*, rs7249210, rs7250184, rs7249100 and rs10401828 in *ZNF28*, rs11133659 in *CTNND2*, and rs2074314 and rs35271178 near *KCNJ11*. Similarly, we found 119 independent loci suggestively associated with PA in the GWAS meta-analysis, including rs76258369 near *IRX1*, and rs7094759 and rs12264492 in *ADAM12*. Functional analyses of these genes revealed trophoblast-like cell interaction, as well as networks involved in endocrine system disorders, cardiovascular disease and cellular function. PAGE participants in the 25-50th (score:12.6-13.8), 50-75th (score:13.9-15.0) and 75-100th (score ≥15.1) wGRS percentiles had 1.45-fold (95% confidence interval [CI]:1.04, 2.02), 1.42-fold (95% CI: 1.02-1.98), and 1.75-fold (95% CI: 1.27, 2.42) higher odds of PA, respectively, compared to those in the lowest wGRS percentile (score <12.6; P-for-trend <0.001). We observed maternal-fetal interaction effects for rs12530904 (log-likelihood=-1874.6; P-value=1.2e-04) in *CAMK2B*, and rs73136795 (log-likelihood=-1644.5; P-value=1.9e-04) in *PPARG*, both MB genes. We identified 311 SNPs in 35 imprinted genes (including *KCNQ1*, *NPM*, and, *ATP10A*) with parent-of-origin effects on PA risk (with P-value<1.8e-5). Among these, top hits included rs8036892 (P-value=2.3e-15) in *ATP10A*, rs80203467 (P-value=6.7e-15) and rs12589854 (P-value=1.4e-14) in *MEG8*, and rs138281088 in *SLC22A2* (P-value=1.7e-13).
Conclusion: Using the largest GWAS of PA, to date, we identified several candidate and novel genetic loci and related functions that may play a role in PA risk. We also replicated previous findings of genetic variants in MB/OP that influence PA risk. Lastly, we identified novel maternal-fetal MB gene interactions and imprinting effects of SNPs in imprinted regions in relation to PA risk. Elucidating genetic factors that underlie pathophysiological mechanisms of PA may facilitate prevention and early diagnostic efforts.

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DEDICATION

This dissertation is dedicated to my father Workalemahu Habtemariam who firmly believed in education, helping others, and providing for so many.

Chapter 1. Background and Introduction

Placental abruption (PA), also known as abruptio placentae, is the premature separation of an implanted placenta. It is typically accompanied by vaginal bleeding but clinical presentation varies with the extent of separation of the placenta from the uterus (i.e., partial versus complete separation)[1-3]. Worldwide, the prevalence of PA is estimated to be 1%[3-6], with considerable geographic variation[7]. This obstetric complication is a significant cause of global maternal and neonatal mortality and morbidity. PA is associated with 3- to 4-fold increased risk of premature cardiovascular mortality and morbidity in the mother[4 5] and 10-fold higher risk of perinatal and infant mortality[8 9]. In addition, several other pregnancy disorders, such as preterm delivery and preeclampsia, are related to PA[10 11].

Risk factors of PA include maternal hypertensive disorders[1], increased maternal age[2], grand-multiparity[8], thrombophilia[3], cigarette smoking[12], cocaine use[13] and external trauma to abdomen[14]. Other risk factors highlighted by recent studies include surgical disruption of the uterine cavity[15], short inter-pregnancy interval[15], maternal uterine fibroids[16], maternal iron deficiency[17], hyperhomocyst(e)inemia[18], low maternal pre-pregnancy body mass index (BMI)[12] and maternal infection and/or inflammation[11 19]. Pathophysiologic mechanisms of PA include uteroplacental underperfusion, chronic hypoxemia, placental ischemia and infarctions[8 10 20-24]. Mitochondrial dysfunction and dysfunction in oxidative phosphorylation (OP), pathways implicated in impaired placental function, have been related to the pathogenesis of PA. Mitochondria are semi-autonomous cytoplasmic organelles of the eukaryotic system that produce adenosine triphosphate by coupling OP to respiration, providing a major source of energy to the cell[25 26]. Epidemiologic and experimental studies have highlighted the roles of mitochondrial biogenesis (MB) and/or OP genes in pregnancy complications that involve the placenta[27-30]. In

particular, MB/OP genes have been implicated in the impairment of differentiation and invasion of the trophoblast[31], mediation of defective placentation[27], and normal trophoblast invasion during placental implantation[28 30], which are all important in PA etiology.

Increasingly, the role of genetic factors in PA has garnered increasing attention over the past decade. High recurrence of PA and high prevalence of heritable thrombophilia among women with PA support the possibility of a genetic contribution. In addition, PA aggregates in families of women with an abruption[14], suggesting a strong genetic predisposition[32-35]. Emerging evidence from GWAS and candidate gene studies[36-40] also supports potential roles for genetic susceptibility factors in PA. However, there has been limited success in identifying genetic susceptibility loci for a multi-factorial heritable disorder such as PA. Of note, available studies, in general, were small and reported inconsistent findings. Moreover, the studies did not fully interrogate the depth of available genetic data; and findings were not replicated in most instances. The missing heritability of PA can also be due to un-assessed maternal and fetal genetic interactions and imprinting effects. In particular, assessment of the complex interplay between maternal and fetal genes, given the importance of both maternal and fetal related (paternal) contributions to the placenta[41 42], is critical to unravel the underlying genetic susceptibility factors of PA, a placenta phenotype.

This dissertation research addresses limitations of previous studies. The research represents the largest genetic study of PA to date (959 PA cases and 1,553 controls). It was conducted among study participants of the Peruvian Abruptio Placentae Epidemiology (PAPE) and Placental Abruption Genetic Epidemiology (PAGE) studies, case-control studies of PA conducted in Lima, Peru. Work done to address the three aims of the dissertation research are reported in separate chapters describing the corresponding, detailed background, methods,

results, and discussion. For Aim # 1, we sought to conduct GWAS of PA among PAPE study participants (521 cases and 520 controls), and meta-analysis of GWAS data from PAPE and PAGE studies (991 cases and 993 controls) (Chapter 2). For Aim #2, we, sought to replicate associations of OP and MB-related genetic variations with PA risk, reported before in the PAPE study, among PAGE study participants (Chapter 3). For Aim # 3, we examined maternal-fetal genetic interactions of MB and OP-related genetic variations on risk of PA as well as imprinting effects of variations in imprinted regions on risk of PA among PAGE and PAPE participants (503 PA case and 1,052 mother-infant pairs) (Chapter 4). In Chapter 5, we provide an overall summary of major research findings, strengths and limitations of the project, its implications, and recommendations for future research.

The dissertation research has the potential to enhance our understanding of genetic variations in maternal and fetal genomes that contribute to pathophysiological mechanisms that underlie PA. Further, it has the potential to facilitate the effort to identify pregnant women at higher risk for PA. These will accelerate preventative and early diagnostic efforts to reduce the burden of PA, an important public health problem.

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Chapter 2. Genome-Wide Association Study and Meta-analysis of Genome-Wide Association Studies of Placental Abruption

2.1 Abstract

Introduction: Accumulating epidemiological evidence points to strong genetic susceptibility to placental abruption (PA). However, characterization of genes associated with PA remain incomplete. We conducted a genome-wide association study (GWAS) of PA and a meta-analysis of GWAS.

Methods: Participants of the Placental Abruption Genetic Epidemiology (PAGE) study, a population based case-control study of PA conducted in Lima, Peru, were genotyped using the Illumina HumanCore-24 BeadChip platform. Genotypes were imputed using the 1000 genomes reference panel, and >4.9 million SNPs that passed quality control were analyzed. We performed a GWAS in PAGE participants (507 PA cases and 1,090 controls) and a GWAS meta-analysis in 2,512 participants (959 PA cases and 1,553 controls) that included PAGE and the previously reported Peruvian Abruptio Placentae Epidemiology (PAPE) study. We fitted population stratification-adjusted logistic regression models and fixed-effects meta-analyses using inverse-variance weighting.

Results: Independent loci (linkage-disequilibrium<0.80) suggestively associated with PA (P-value<5e-5) included rs4148646 and rs2074311 in *ABCC8*, rs7249210, rs7250184, rs7249100 and rs10401828 in *ZNF28*, rs11133659 in *CTNND2*, and rs2074314 and rs35271178 near *KCNJ11* in the PAGE GWAS. Similarly, independent loci suggestively associated with PA in the GWAS meta-analysis included rs76258369 near *IRX1*, and rs7094759 and rs12264492 in

ADAM12. Functional analyses of these genes revealed trophoblast-like cell interaction, as well as networks involved in endocrine system disorders, cardiovascular disease and cellular function. **Conclusions**: We identified several genetic loci and related functions that may play a role in PA risk. Understanding genetic factors underlying pathophysiological mechanisms of PA may facilitate prevention and early diagnostic efforts.

2.2 Introduction

Placental abruption (PA) is the premature detachment of an implanted placenta from the uterus due to the rupture of maternal vessels in the decidua basalis prior to delivery of the fetus [3]. It is one of the leading causes of maternal and neonatal morbidity and mortality [43 44]. Worldwide, the prevalence of PA is estimated to be 1% [3 4], with considerable geographic variation [7]. Pathophysiologic mechanisms of PA, also shared by other perinatal disorders such as preeclampsia [44] and preterm delivery [10], include chronic hypoxemia [45], uteroplacental ischemia and infarctions [23].

Etiologic factors related to PA have not been fully described. To date, non-genetic risk factors associated with increased risk of PA include hypertensive disorders [23], advanced maternal age [3], grand-multiparity, thrombophilia, cigarette smoking [1], illicit drug use (particularly cocaine) and trauma to the abdomen [1 12 13 23 46]. However, most PA cases do not exhibit these known risk factors [47]. PA tends to aggregate in first degree relatives of women with PA [9 48], suggesting a role for genetic predisposition [15 34 49]. Accumulating evidence from GWAS and candidate gene studies also suggest that there are underlying genetic risk factors in the pathogenesis of PA [36-39 42]. Our group previously reported several loci (including SNPs in *SMAD2 MIR17HG, DGKB, FLI-1, CETP, LIPC, Akt, NFKB, PI3K, THRB, CTNNA2,*

TNFRSF1A, and *ZNRF3*) that are associated with PA [36-39]. However, previous candidate gene and GWAS studies were sparse and small in size.

As a multi-factorial disease, characterizing genetic susceptibility for PA requires comprehensive investigations of genetic variations at the genome-wide level. We performed a new GWAS and a GWAS meta-analysis of PA, the largest GWAS of PA to date. We also examined functions and functional relationships of genes represented by association signals using canonical pathway analyses and functional annotation tools.

2.3 Methods

Study Settings and Study Populations

The study was conducted among participants of the Placental Abruption Genetic Epidemiology (PAGE) and the Peruvian Abruptio Placentae Epidemiology (PAPE) studies, case-control studies of PA conducted in Lima, Peru. Both PAGE and PAPE studies were independently recruited, had similar study objectives and study designs. Description of the PAPE study and findings of the PA GWAS among PAPE study participants have been previously reported [36-39]. PAGE study participants were recruited among women who were admitted for obstetrical 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 Rebagliati, Hospital San Bartolome, Hospital Hipolito Unanue, Hospital Arzobispo Loayza, Hospital Dos de Mayo, and Hospital Maria Auxiliadora. Participants who were less than 18 years of age, delivered multiple (non-singleton) infants, had medical records that were insufficient to determine the presence or absence of PA (described below), and reported taking blood thinning medications were excluded from the

study. Participants with other diagnoses associated with third trimester bleeding (e.g. placenta previa) were excluded. Participants from PAGE study included 522 PA cases and 1147 controls. The meta-analysis included PAGE study participants and participants of the previously reported PAPE GWAs (490 PA cases and 500 controls) for a total of 1012 PA cases 1647 controls. Study protocols of both studies were approved by the Institutional Review Boards (IRBs) of participating institutions and the Swedish Medical Center, Seattle, WA, where the studies were administratively based. All participants of both studies provided written informed consent in accordance with the principles of the declaration of Helsinki. There was no overlap in participants across the two studies.

Data Collection

PAGE study participants were interviewed by trained personnel using standardized structured questionnaires to collect information on sociodemographic characteristics and risk factors including maternal age, marital status, employment status during pregnancy, medical history, smoking, and alcohol consumption (both current and pre-pregnancy). Maternal medical records were abstracted to obtain information on the course and outcomes of the pregnancy, and to ascertain PA case/control status. PA cases were identified through daily review of emergency room admission logbooks, labor and delivery admission logbooks, and the surgery report book (where post-operative diagnoses are registered). Controls were randomly selected from eligible pregnant women who delivered at participating hospitals during the study period and who did not have a diagnosis of PA in the current pregnancy. Maternal saliva was collected, plated and stored using the Oragene™ saliva cell collection kits (OGR500 and OGR250, DNA Genotek Ottawa, Canada).

DNA Extraction and Genotyping

Genomic DNA were extracted using Qiagen DNAeasy™ system and manufacturer protocols (Qiagen, Valencia, CA). Genotyping to characterize genome-wide variation (>300,000 SNPs) was conducted using the Illumina HumanCore-24 BeadChip platform (Illumina Inc., San Diego, CA).

Data Quality Control and Imputation

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 (HWE) (p<1e-05), and had low minor allele frequency (MAF<0.05). The total number of SNPs, directly genotyped, that remained for further analysis in PAGE and the combined (meta-analysis) PAGE and PAPE studies were 232,960 and 205,100, respectively. Individuals (N=53) were excluded if they were duplicates or related (Identity by Decent [IBD] value>0.9), had more than 5% of genotyping failure rate (N=67), had excess heterozygosity rate (outside the range of mean ± 3 standard deviations of heterozygosity rate; N=6), had genotype data that was inconclusive regarding sex (N=8), and failed test of divergent ancestry (if principal components were outside the range of [-0.02, 0.02]; N=12). The total number of individuals that remained for further analysis for PAGE GWAS and the GWAS meta-analysis (combined PAGE and PAPE studies) were 1,597 (507 cases and 1090 controls) and 2,512 (959 cases, and 1553 controls), respectively.

After quality control, SNP imputation was conducted to infer unobserved genotypes. The genotype data were phased using SHAPEIT [50] to infer haplotypes and improve imputation accuracy using the 1000 Genomes haplotypes. Phased haplotypes were then used to impute our non-typed SNPs using IMPUTE2 [51]. After imputation and further quality control (filtering SNPs with imputation certainty score (Info)<0.3, HWE<0.00001, genotyping call rate<0.05, and

MAF<0.05), a total of 5,400,957 and 4,983,952 SNPs were evaluated in the PAGE study and the meta-analysis, respectively.

Statistical Analyses

Mean and standard deviations for continuous variables and proportions (percentages) for categorical variables were used to compare the characteristics of PA cases and controls across PAGE and the combined PAGE and PAPE study populations. Study-specific GWAS analyses were conducted in PAGE using logistic regression models, with PA as the dependent variable, and, each SNP and adjustment factors (population stratification) as independent variables using SNPTEST v2 [52]. Adjustment for population stratification was conducted by including principal components (PCs) in the models and examining the degree of genetic variability due to admixture, assessed using scree plots [53]. PCs were computed using the 1000 genomes population reference [54]. Adjusted odds ratios (OR), corresponding 95% confidence intervals (CIs), and their genomic control corrected p-values (λ_{GC}) corresponding to each copy of the risk allele of the SNPs were estimated. We assumed additive genetic risk models with estimates corresponding to a linear increase of PA risk per unit increase in dosages of risk alleles.

In the meta-analysis, individually analyzed PAGE and PAPE study results were combined after study specific standard error values were transformed to correspond to the logarithm of the ORs [55]. Fixed effects meta-analysis was conducted using the inverse variance weighting method implemented in METAL [56]. GWAS meta-analysis results were additionally corrected for λ_{GC} based on all SNPs, as described above [55]. The Q-statistic and I^2 measures were calculated to estimate between-study heterogeneity. SNPs with pronounced heterogeneity (I^2 >75%) were identified and further analyzed using the alternative random-effects meta-analysis approach recommended in previous studies [55 57]. These sensitivity analyses were conducted using

GWAMA [58]. Statistical analyses software used in these analyses included R (version i386 3.1.2) and SAS (Version 13).

Pathway and Functional Analyses

Genes represented by PAGE GWAS and GWAS meta-analysis signals with suggestive significance ($p<5e-5$) were further interrogated for functional relationships using analytical tools - Ingenuity Pathway Analysis (IPA, Ingenuity, Redwood, CA) [59], online databases assisted by FUMA (Functional Mapping and Annotation of GWAS) [60], and the human protein atlas [61]. In the IPA analysis based on the Ingenuity Pathways Knowledge Base (IPKB), gene-enrichment of networks was assessed using network score, negative log of P-values of a modified Fisher's exact test.

In FUMA, SNPs with suggestive significance were queried against the 1000 genomes Admixed American (AMR) reference panel for any SNPs flanking 250kb of the index SNP and in linkage disequilibrium (LD) with the index SNP ($r^2 \geq 0.6$). Gene-set and functional effect annotations were examined using ANNOVAR [62]. Combined Annotation Dependent Depletion (CADD) score, a deleteriousness score of variants computed by integrating 63 functional annotations was reported for most relevant functional variants [63]. In addition, FUMA summarized chromatin interaction mapping using 15-core chromatin state predicted by ChromHMM15 [64] for 127 tissue/cell types [65]. SNPs (top hits associated with PA) were queried using FUMA evaluate their biological functionality as expression quantitative trait loci (eQTL) and involvement in chromatin interaction. Information on eQTL were obtained from GTEx v6 [60] that includes gene expression database of 53 tissue types in >70 samples.

2.4 Results

Socio-demographic, medical and obstetric characteristics of PA cases and controls of the PAGE study and the combined PAGE/PAPE studies are shown in **Table 2.7.1**. PA cases were more likely to deliver earlier (i.e., shorter gestational age), deliver infants with lower birth weight, and have a diagnosis of preeclampsia in the current pregnancy as compared with controls. Compared with controls, PA cases tended to report smoking and illicit drug use during pregnancy.

We did not observe significant genomic inflation or deviation from expectation when examining the QQ plots of the PAGE GWAS and the GWAS meta-analysis (λ_{GC} PAGE=1.00; λ_{GC} meta-analysis=0.99; **Figure 2.7.1**). The top independent signals of the PAGE GWAS with suggestive statistical significance (p<5e-5), included rs4148646 (odds ratio [OR]=0.67; p=1e-6; effect allele frequency [EAF]=0.63) and rs2074311 (OR=0.68; p=2e-6; EAF=0.64) in *ABCC8*, rs2074314 (OR=0.68; p=1e-6; EAF=0.64) and rs35271178 (OR=0.69; p=4e-6; EAF=0.62) near *KCNJ11*, rs7249210 (OR=2.11; p=3e-6; EAF=0.09), rs7250184 (OR=2.09; p=4e-6; EAF=0.09), rs7249100 (OR=2.08; p=4e-6; EAF=0.09) and rs10401828 (OR=2.05, p=4e-6; EAF=0.09) in *ZNF28*, and rs11133659 (OR=2.12; p=4e-6; EAF=0.09) in *CTNND2* genes (**Figure 2.7.2, Table 2.7.2 and Table 2.7.5**).

In the GWAS meta-analysis, the top independent SNPs that were suggestively associated with PA, included rs76258369 (OR=1.56; p=3e-6; EAF=0.16) near *IRX1*, rs7094759 (OR=0.74; p=4e-6; EAF=0.48) and rs12264492 (OR=0.73; p=4e-6; EAF=0.40) in *ADAM12* gene, rs30080 (OR=0.73; p=5e-6; EAF=0.42) and rs7704841 (OR=0.73; p=6e-6; EAF=0.42) in *DOCK2*, rs11995662 (OR=0.61; p=5e-6; EAF=0.10) in *PDGFRL*, rs4867606 (OR=1.82; p=6e-6; EAF=0.10) in KCNIP1, rs2291228 (OR=1.37; p=8e-6; EAF=0.42) near *FAM196B*, rs799758

(OR=1.47; p=9e-6; EAF=0.18) in *GALNT13*, and rs17837210 (OR=1.80; p=9e-6; EAF=0.07) near *FAM124A* (**Figure 2.7.2, Table 2.7.2 and Table 2.7.6)**.

In IPA analysis, networks (scores>15) enriched by 27 genes represented by 174 PAGE GWAS hits (with p<5e-5) included networks for developmental disorder, endocrine system disorders, organismal injury and abnormalities, molecular transport, cardiac arrhythmia, and cardiovascular disease (**Table 2.7.3**). The functional annotation and mapping of PAGE GWAS hits in *ABCC8*, *KCNJ11*, *ZNF28*, and *CTNND2* genes identified trophoblast-like cell chromatin interactions (**Table 2.7.4**). Rs5215 in *KCNJ11* (CADD score=12.4) had the highest deleteriousness score. Significant networks represented by the top 27 genes are displayed in **Figure 2.7.2a**, highlighting molecules implicated in cardiovascular disease and cardiac arrhythmia pathway. Networks (scores>24) enriched by 36 genes represented by the top 149 GWAS meta-analysis hits (with p<5e-5) included networks for cellular function and maintenance, cell-to-cell signaling and interaction, and lipid metabolism (**Table 2.7.3**). Trophoblast-like cell chromatin interactions were also identified for genes *ADAM12*, *DOCK2*, *PDGFRL*, *LOC105374318*, and *FAM124A* represented by GWAS meta-analysis hits. Among the top hits, rs72841199 in *DOC2* had the highest deleteriousness score (CADD score=17.3) (**Table 2.7.4**). Significant networks represented by the top 36 genes are displayed in **Figure 2.7.2b**, highlighting molecules implicated in cell signaling/cell-cell interaction and lipid metabolism pathway. Significant networks represented by the genes from top hits include cellular movement and cell morphology (**Table 2.7.8**).

2.5 Discussion

While we did not find genome-wide significant hits (p<5e-8), we identified several SNPs and networks that are potentially associated with increased PA risk. These include SNPs in/near *ABCC8, KCNJ11, ZNF28, CTNND2, IRX1, ADAM12, DOCK2, PDGFRL, KCNIP1, FAM196B, GALNT13* and *FAM124A* genes as well as networks involved in endocrine system disorders, cardiovascular disease, and cellular function and maintenance. Several SNPs in these genes were mapped to trophoblast-like cell chromatin interaction, suggesting potential pregnancy related cell-type-specific regulatory activity.

Previous candidate gene and GWA studies of PA have reported several genetic loci associated with PA. A systematic review (483 cases and 1476 controls) of candidate gene studies identified that SNPs in Factor V Leiden 1691 G→A (*F5*) gene, also linked with heritable thrombophilia, are potentially associated with PA [42]. Inferences from these earlier studies, however, are limited in part because of statistical imprecision of relative risk estimates attributable to small sample sizes. More recent studies identified SNPs in genes *AGT, KDR, F2* and *THBD* that are involved in coagulation, rennin-angiotensin, angiogenesis, inflammation, and B-vitamin metabolism [37]. SNPs in *CAMK2B, NR1H3, PPARG, PRKCA, THRB, COX5A, NDUF* family and *COX10* genes, involved in mitochondrial biogenesis and oxidative phosphorylation [39], that are associated with PA have also been reported. In addition, genes known to control circadian rhythms (e.g., *CRY2, ARNTL*, and *RORA*) were also associated with increased risk of PA [38]. The first three GWAS studies of PA, conducted by our group, [36 37 39] suggest SNPs in *SMAD2, MIR17HG, DGKB, FLI-1, CTNNA2, TNFRSF1A*, and *ZNRF3* genes, as well as networks of lipid metabolism and cell signaling represented by *CETP, LIPC, COX10, THRB, Akt, NFKB, PI3K* genes are associated with PA risk. Most of the previously described genes

were not represented by the SNPs in our list of top GWAS hits with statistically suggestive association. In the current GWAS meta-analysis, rs10919196 and rs9332544 in *F5* gene were potentially associated with increased risk of PA (OR=1.20 [95%CI:1.03-1.40] and OR=1.19 [95%CI:1.00-1.43], respectively). SNPs of previously reported candidate genes that were also potentially associated with PA in the current GWAS meta-analysis include rs2009705 (OR=1.18 [95% CI: 1.02-1.36]) in *CAMK2B*, rs4328478 (OR=1.19 [95%CI:1.04-1.36]) in *PRKCA*, and rs11107847 (OR=0.84 [95%CI:0.74-0.94]) in *NDUFA12*. In the current GWAS meta-analysis, we identified the following SNPs in genes with known functional significance in PA that were associated with PA before multiple testing correction: rs10919196 (OR=1.20 [95%CI:1.03-1.40]) and rs9332544 (OR=1.19 [95%CI:1.00-1.43]) in F5 gene [23], rs2009705 (OR=1.18 [95% CI: 1.02-1.36]) in CAMK2B [22], rs4328478 (OR=1.19 [95%CI:1.04-1.36]) in PRKCA [22], and rs11107847 (OR=0.84 [95%CI:0.74-0.94]) in NDUFA12 [22]. In **Table 2.7.7**, we provide a list of nine SNPs in four genes (PCSK6, GALNT13, LINC01019, and NEDD4L) that were suggestively associated with PA in both the PAGE study and the meta-analysis.

Notable findings from this study include common protein coding variants that are associated with PA. For instance, in our study, the C allele of rs757110, a coding SNP near *ABCC8*, was potentially associated with increased risk of PA (OR=1.47 [95%CI:1.27-1.72]). *ABCC8*, ATP binding cassette subfamily C member 8 gene, has been associated with GDM, type-2 diabetes, and hyperglycemia-cardiovascular risk [66]. The ATP-sensitive potassium (KATP) channel is one of the most abundant potassium channels in myometrium [67]. Functional KATP channels, which are expressed in human pregnant myometrium, may contribute to enhanced uterine contractility associated with the onset of labor [68], common clinical findings in PA. The

network analyses showed that network of genes including *ABCC8* and *KCNJ11* were among genes involved in organismal injury and abnormalities, and endocrine system disorder diseases.

The associations we found between PA and two common exonic variants (rs5219 and rs5215) in *KCNJ11* (ATP sensitive inward rectifier potassium channel, subfamily J, member 11) are noteworthy because the SNPs have already been recognized to be clinically relevant in the development of gestational and type-2 diabetes [69]. A systematic review of GWAS studies that evaluated SNPs in relation to gestational diabetes mellitus (GDM) identified that the T allele of rs5219 is associated with an increased risk of GDM (pooled OR=1.15 [95%CI:1.06–1.26]) [70]. Several other studies highlight the roles of *KCNJ11* in the etiology of GDM, neonatal diabetes and maternal metabolism [71 72]. Although the link between GDM and PA is unknown, maternal hypertensive disorders of pregnancy, particularly maternal history of chronic hypertension, risk factors of GDM, have been among the most consistently noted risk factors for PA [23]. Our findings may signal a genetic link between PA and GDM through the regulatory action of biological pathways involving potassium channels.

This GWAS meta-analysis identified 10 SNPs in *ADAM12* (ADAM metallopeptidase domain 12), a highly expressed gene in the placenta and implicated in cellular function and maintenance. *ADAM12* regulates the migration and invasion of trophoblasts into the lining of the uterus, a critical step in normal placental development [73 74]. The two *ADAM12* SNPs, rs7094759 and rs12264492 among our top GWAS meta-analysis hits mapped to trophoblast-like cell chromatin interaction in functional analyses. *ADAM12* SNPs and their potential roles in PA risk through trophoblast regulation is particularly intriguing because trophoblastic invasion is thought to lead to vascular malformations and PA [75]. *ADAM12* is also associated with other risk factors of PA such as preeclampsia that have shared etiology and pathophysiology with PA including

trophoblast invasion [44 73]. In addition, *ADAM12* is primarily expressed in the placenta [61], highlighting the potential clinical significance of our findings. Five strongly correlated SNPs (LD>0.8) in *GALNT13* suggestively associated in PAGE GWAS were also suggestively associated in meta-analysis GWAS (P-value<5e-5) (**Table 2.7.7**). *GALNT13*, polypeptide N-acetylgalactosaminyltransferase 13 gene, is among a list of differentially expressed genes in preeclamptic tissue samples compared with normotensive tissue samples [76].

Our study identified several significantly enriched pathways (e.g. including organismal injury and abnormalities, lipid metabolism and cardiovascular disease) involving genes represented by top hit SNPs. Previous studies with similar observations highlighted placental ischemia and infarctions as risk factors of PA [23 32]. Our observation of a lack of a strong signal for a specific disease or function is in line with the current understanding of PA as a complex disorder with potentially multiple underlying pathways. The 27 genes representing our top PAGE GWAS hits and highlighted in our pathway analysis were significantly involved in a function cluster in gene ontology (GO) terms of G-protein coupled glutamate receptor signaling pathway that are important for downstream cellular processes, such as transcription. The 36 genes representing our top meta-analysis GWAS hits also highlighted in our pathway analysis were significantly involved in a function cluster in GO terms of regulation of metabolic and cellular processes.

We queried the top SNP findings in our PAGE GWAS and meta-analysis GWAS using dbPTB, PESNPdb, and SNPedia databases. None of the SNPs we identified have previously been associated with preeclampsia or preterm birth. However, *ADAM12*, a gene represented by SNPs suggestively associated with PA (rs7094759, rs12264492) and *FTO*, a gene represented by

SNPs suggestively associated with PA (rs28637326, rs16953154, rs12598570, rs12934459, rs28613919, and rs12445575), were associated with preeclampsia.

Several strengths of our study deserve mention. This study is, to date, the largest GWAS study of PA that has the potential to enhance our understanding of genetic variations in maternal genome that contribute to a multi-factorial heritable disorder such as PA. We studied Peruvians, a relatively understudied population. In addition, we performed imputation to comprehensively characterize genome-wide variation and additional functional pathway analyses, utilizing state-of-the-art bioinformatics tools, to highlight the biological functions of our genetic findings.

Some limitations of the study merit attention. This study is underpowered to evaluate small effects. Although our current study is the largest GWAS on PA to date, it is still underpowered to evaluate small effects. We did not find associations for SNPs that reached genome-wide significance. Another limitation is the potential misclassification of sub-clinical PA (i.e. those not presenting with abnormal vaginal bleeding). These may either introduce bias in the interpretation of our study results or reduce power of our study. Comparing severe placental abruption with mild abruption and/or non-abruption cases may minimize this limitation and facilitate epidemiologic and genetic research [77]. To assess the role of term/preterm delivery and preeclampsia status on observed associations, we conducted independent sensitivity analyses excluding term PA cases and controls as well as excluding preeclampsia cases and controls. Findings from these sensitivity analyses were in general similar to what we report in the current manuscript, with similar estimates (odds ratios), although 95% confidence intervals were wider as expected (attributable to smaller sample size for these sensitivity analyses). Finally, findings from our study population may not be generalizable to other populations.

Findings from this study lend evidence for several genetic loci that may influence PA. These genetic loci included clinically-relevant protein-coding variants (e.g. *ABCC8* and *KCNJ11*), as well as genes that are known to be highly expressed in the placenta (e.g. *ADAM12*) and myometrium (e.g. *ABCC8*) [61]. Understanding these pathophysiological mechanisms may help accelerate preventative and early diagnostic efforts to reduce the burden of PA, an important public health problem.

2.6 References

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2.7 Tables and Figures

Table 2.7.1. Selected characteristics of the study populations

Characteristics	Placental Abruption Genetic Epidemiology (PAGE) Participants			Meta-analysis		
	Cases (N=507)	**Controls (N=1090)**	**P-value**	**Cases (N=959)**	**Controls (N=1553)**	**P-value**
	%	**%**		**%**	**%**	
Maternal age at delivery (years)[1]	28.4±6.7	27.5±6.6	0.93	28.1±6.6	27.6±6.6	0.79
Maternal age at delivery (years)			0.22			0.83
18-19	6.8	11.7		8.2	10.7	
20-29	51.0	50.7		51.4	51.1	
30-34	20.8	19.9		20.8	20.3	
≥35	21.4	17.7		19.5	18	
Education ≤high school	67.3	73.5	**0.03**	69.6	72.6	0.62
Married/living with partner	86.1	87.1	0.56	84.7	87	0.12
Employed during pregnancy	55.0	53.9	0.69	49.8	51.3	0.74
Pre-pregnancy body mass index (BMI) (kg/m^2)	25.0±4.6	25.4±4.6	0.61	24.4±4.3	24.9±4.5	0.65
Pre-pregnancy BMI (kg/m^2)			0.53			0.16
Lean (< 18.5)	2.8	2.0		4.0	2.3	
Normal (18.5-24.9)	56.1	55.6		59.6	59.3	
Overweight (24.9-30.0)	10.9	12.8		27.8	27.2	
Obese (≥30.0)	30.2	29.6		8.7	11.2	
Planned pregnancy	38.5	32.8	**0.03**	38.7	35.4	0.42
Smoked during pregnancy	1.0	1.0	0.96	2.3	1.2	**0.03**
Alcohol use during pregnancy	3.9	2.8	0.20	4.8	3.2	0.33
Drug abuse during pregnancy	0.6	0.3	0.34	0.6	0.2	0.08
Preeclampsia	21.4	6.3	**<0.001**	24.6	6.6	**<0.001**
Vitamins use during pregnancy	84.6	86.1	0.47	77.9	81.4	0.62
Gestational age at delivery[1]	34.3±4.4	39.0±1.2	**<0.001**	34.8±4.3	38.8±1.8	**<0.001**
Infant birthweight (grams)[1]	2390±939	3418±484	**<0.001**	2398±902	3343±561	**<0.001**

[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.

Figure 2.7.1. Quantile-Quantile plots of associated p-values and their genomic control inflation factor for PAGE and meta-analysis studies.

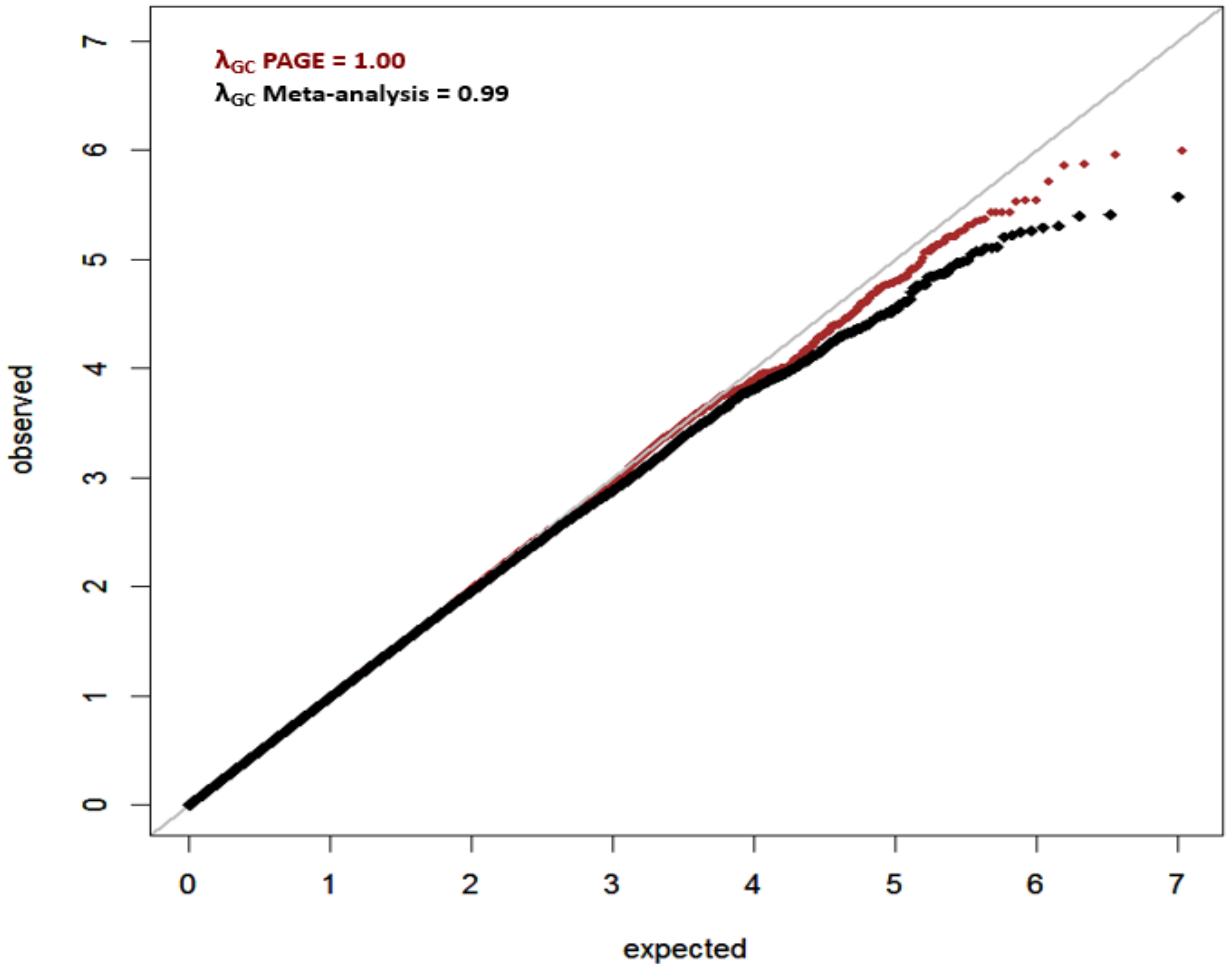


Figure 2.7.2. Manhattan plot associated p-values by chromosomal location. Top: PAGE; Bottom: Meta-analysis. Genome-wide significance p-values are indicated by red dots, and suggestive significance p-values are indicated by black dots.

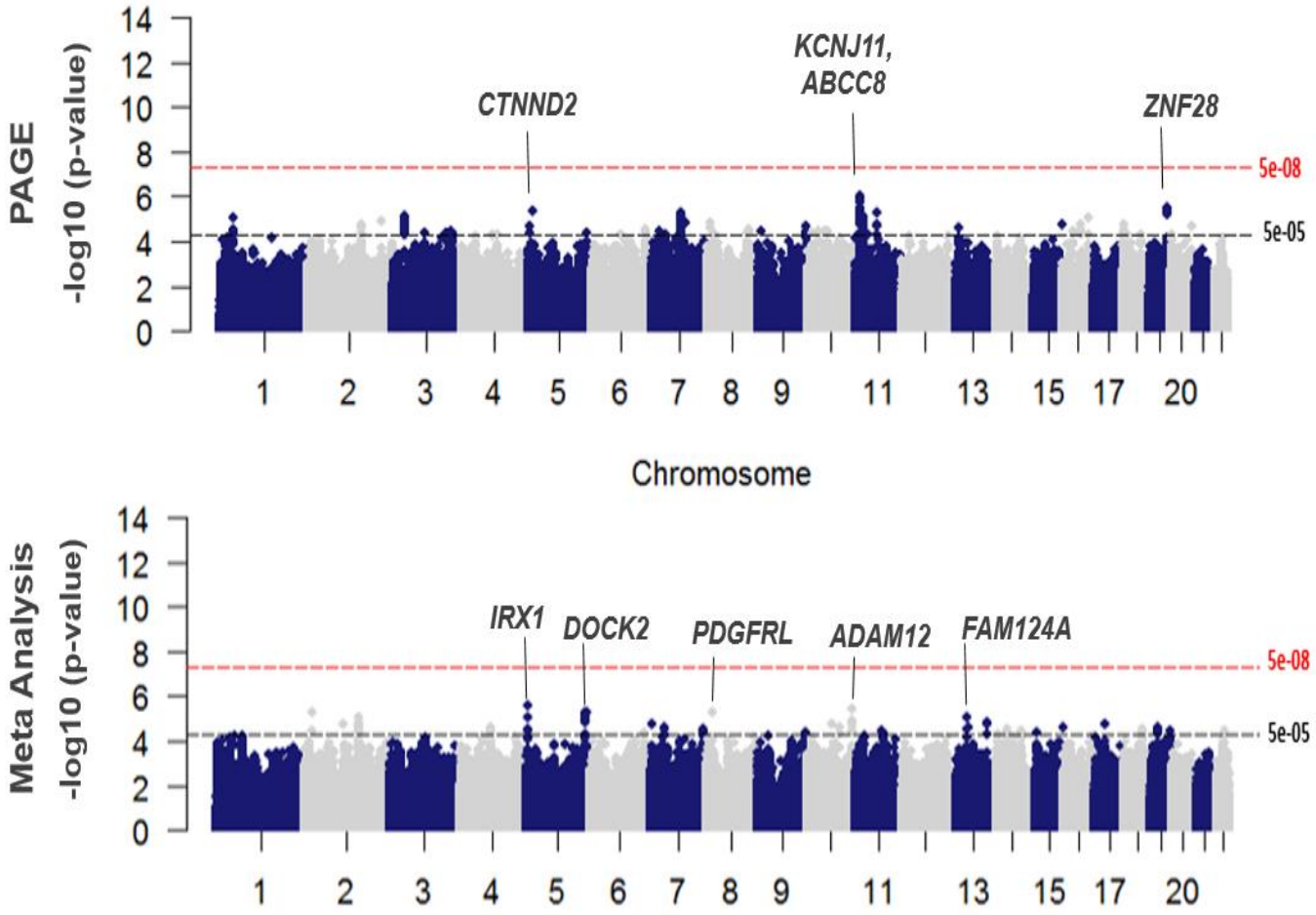


Table 2.7.2. Top 10 independent SNPs that have the lowest association p-values for analyses examining genome-wide genetic variations and placental abruption risk among PAGE and Meta-analysis studies.

Gene	Chr	SNP*	Effect Allele	Effect Allele Frequency	Odds Ratio (95% Confidence Interval)	Empirical p-value
			PAGE Study			
ABCC8	11	rs4148646	G	0.633	0.67 (0.58,0.79)	1.00E-06
KCNJ11	11	rs2074314	T	0.635	0.67 (0.58,0.79)	1.40E-06
ABCC8	11	rs2074311	G	0.623	0.68 (0.58,0.80)	1.90E-06
ZNF28	19	rs7249210	A	0.09	2.11 (1.54,2.87)	3.00E-06
KCNJ11	11	rs35271178	T	0.623	0.69 (0.59,0.81)	3.80E-06
ZNF28	19	rs7250184	C	0.091	2.09 (1.53,2.84)	3.80E-06
ZNF28	19	rs7249100	G	0.091	2.08 (1.53,2.84)	3.80E-06
ZNF28	19	rs10401828	C	0.093	2.05 (1.51,2.78)	4.30E-06
CTNND2	5	rs11133659	A	0.088	2.12 (1.54,2.92)	4.40E-06
ZNF28	19	rs146312	T	0.072	2.00 (1.49,2.70)	5.30E-06
			Meta-analysis			
IRX1	5	rs76258369	C	0.164	1.56 (1.30,1.88)	2.80E-06
ADAM12	10	rs7094759	T	0.482	0.74 (0.65,0.84)	4.00E-06
ADAM12	10	rs12264492	G	0.404	0.73 (0.64,0.83)	4.10E-06
DOCK2	5	rs30080	C	0.424	0.73 (0.63,0.83)	5.00E-06
PDGFRL	8	rs11995662	C	0.1	0.61 (0.49,0.75)	5.20E-06
KCNIP1	5	rs4867606	A	0.099	1.82 (1.41,2.36)	5.50E-06
LOC105374318	2	rs219551	T	0.142	1.64 (1.33,2.04)	5.80E-06
DOCK2	5	rs7704841	G	0.422	0.73 (0.64,0.84)	6.40E-06
FAM196B	5	rs2291228	G	0.424	1.37 (1.19,1.56)	7.90E-06
GALNT13	2	rs799758	C	0.176	1.47 (1.24,1.74)	8.60E-06
FAM124A	13	rs17837210	C	0.072	1.80 (1.39,2.33)	8.60E-06

*hg19 build 37 dbSNP; Chr: chromosome

Table 2.7.3. Significant networks represented by top 27 genes from top PAGE GWAS hits and 36 genes from top GWAS meta-analysis hits (p<1e-5).

Molecules in Network	Score	Number of Genes from GWAS	Top Disease and Functions	P-value
		PAGE Study		
2'-fucosyllactose,**ABCC8**,Akt,ANGPT4,ANGPTL1,basic calcium phosphate crystal,beta-hydroxyisovaleric acid,Cyp2j9,DNMT1,ERBB4,ERK,ERK1/2,INSRR,Insulin,**ITGA9,KCNJ11**,L-leucine,**NEDD4L**,NFkB (complex),NRG3,NRG4,NUCB2,Proinsulin,Ptprv,SLC22A8,SLC3A1,**SLIT3,SNCA,SOX5**,squalene,STYX,Tardbp,**TFAP2E,VAV2**,Ybx1-ps3	26	11	Developmental Disorder, Endocrine System Disorders, Gastrointestinal Disease	1.00E-25
7S NGF,ADH7,BMP2,BMP8B,C16orf87,Cd1,CIPC,Gm8587/Snrpa,GNA14,GPR161,GPX2,**GRM3**,Groucho,GSTT2/GSTT2B,HDAC2,HDAC3,Hdac1l,IKBKG,IL1,IL4,**MACROD1**,MEPE,MOV10,**NCR3LG1**,NFE2L2,**PCSK6**,PRDM4,PRDM6,**PSMB2**,QPRT,SAP30L,SLC7A8,**SS18L1,XPO7,ZMYM4**	20	9	Dermatological Diseases and Conditions, Organismal Injury and Abnormalities, Cancer	1.00E-38
5,6,7,8-tetrahydrobiopterin,AGT,Calmodulin,Camkk,CNGA3,CTNNAL1,**CTNND2**,CYTH3,**DLGAP1**,DMD,GALNT9,**GALNT13**,IQSEC3,ITIH4,KCNA4,KCNAB1,KCNJ4,KCNJ10,KCNJ12,KCNK3,KCNN3,LRRC7,miR-7a-5p (and other miRNAs w/seed GGAAGAC),NOS1,NPR3,**PAMR1**,RGN,**RIMBP2**,SCN5A,SCN7A,SGCD,**SNTB1**,SNTG1,**SYNE1**,WBSCR17	15	7	Molecular Transport, Cardiac Arrythmia, Cardiovascular Disease	1.00E-14
		Meta-analysis		

ADAM12,APP,beta-estradiol,**BRD3**,Brd4,CALML4,**CMSS1,CRTC1,DOCK2,ERICH3,FAM124A,FILIP1L,GALNT13**,GPR88,HTT,IGSF6,KRAS,MDM2,**mir-548**,MPV17L,**MTUS1,PALD1,PDGFRL**,PP2D1,PTPN20,PTTG2,PXN,ROCK1,SLC13A3,SRF,TGFB1,THSD4,TMEM44,**VPS4**1,ZNF706	32	14	Cell Morphology, Cellular Assembly and Organization, Cellular Function and Maintenance	1.00E-31
AJAP1,ATP5A1,**ATP5S,CAMK1D**,CSRNP3,**FAM196B**,Gαgust/Gβ3/Gγ13,ITPKA,JPH3,L-dopa,MPPE1,Na+,**NEDD4L**,Pde,PDE1C,**PDE6C**,PDE7B,PLD6,PLPP6,PPP1CA,PRPF18,PRR16,**RAP1GAP2**,REM1,REM2,**RHBDL3**,SLC10A4,SLC24A2,**SLC24A4**,SLC8B1,**SNCA,SYNE1,TMEM132D**,VAT1L,YWHAZ	24	11	Psychological Disorders, Cell-To-Cell Signaling and Interaction, Drug Metabolism	1.00E-23
ALB,AMT,**ATP10A**,Ca2+,**CACNA1I**,Ces2e,**COX16**,CTNNB1,**DPP6**,FAIM2,FAS,**FBXO31**,FCAMR,GPR160,HNF4A,HPR,HRC,IGDCC4,INHBC,**KCNIP1,L2HGDH,MSRA**,MSRB2,**MYO7A**,NEURL2,NFYB,PAQR5,**PCSK6**,PITPNM3,PRND,PRRG2,QTRT2,**TMEM87B**,ZCCHC9,ZNF345	24	11	Dermatological Diseases and Conditions, Immunological Disease, Lipid Metabolism	1.00E-23

Table 2.7.4. Functional annotation of top 10 independent SNPs that have the lowest association p-values for analyses examining genome-wide genetic variations and placental abruption risk among PAGE and Meta-analysis studies.

Gene	Gene Description	SNP*	Chromosome :Position*	Functional Annotation Analysis (FUMA)			Ingenuity Pathway Analysis (IPA)
				SNP Function	CADD Score	Chromatin Interaction Mapped	Disease Function of Gene
PAGE Study							
ABCC8	ATP binding cassette subfamily C member 8	rs4148646	11:17415190	intronic	2.15	Trophoblast-like cell; Mesendoderm	Developmental Disorder, Endocrine System Disorders, Gastrointestinal Disease
		rs2074311	11:17421860	intronic	2.04	-	
KCNJ11	Potassium inwardly rectifying channel, subfamily J, member 11	rs2074314	11:17411821	5upstream	1.42	-	Developmental Disorder, Endocrine System Disorders, Gastrointestinal Disease
		rs35271178	11:17411020	5upstream	8.38	-	
ZNF28	Zinc finger protein 28	rs7249210	19:53337340	intronic	4.52	Trophoblast-like cell; Mesendoderm	-
		rs7250184	19:53337426	intronic	0.18	-	
		rs7249100	19:53337326	intronic	0.01	-	
		rs10401828	19:53336367	intronic	-	-	
CTNND2	Catenin delta 2	rs11133659	5:11628509	intronic	1.66	-	Molecular Transport, Cardiac Arrythmia, Cardiovascular Disease
Meta-analysis							

ADAM12	ADAM metallopeptidase domain 12	rs7094759	10:127852106	intronic	4.92	Trophoblast-like cell; Mesendoderm	Cell Morphology, Cellular Assembly and Organization, Cellular Function and Maintenance
		rs12264492	10:127854246	intronic	4.28	Trophoblast-like cell; Mesendoderm	
DOCK2	Dedicator of cyto-kinesis 2	rs30080	5:169273557	intronic	1.21	Mesendoderm	Cell Morphology, Cellular Assembly and Organization, Cellular Function and Maintenance
		rs7704841	5:169281214	intronic	3.87	-	
PDGFRL	Platelet derived growth factor receptor like	rs11995662	8:17498730	intronic	1.9	Trophoblast-like cell; Mesendoderm	Cell Morphology, Cellular Assembly and Organization, Cellular Function and Maintenance
KCNIP1	Potassium voltage-gated channel interacting protein 1	rs4867606	5:169924695	intronic	11.68	-	Dermatological Diseases and Conditions, Immunological Disease, Lipid Metabolism
LOC105374318	-	rs219551	2:21540711	ncRNA_intronic	1.83	Trophoblast-like cell; Mesendoderm	-
FAM196B	Family with sequence similarity 196 member B	rs2291228	5:169288732	3downstream	0.49	-	Psychological Disorders, Cell-To-Cell Signaling and Interaction, Drug Metabolism

GALNT13	Polypeptide N-acetylgalactosaminyltransferase 13	rs799758	2:155262144	intronic	1.36	-	Cell Morphology, Cellular Assembly and Organization, Cellular Function and Maintenance
FAM124A	Family with sequence similarity 124 member A	rs17837210	13:51856010	3utr	3.03	Trophoblast-like cell; Mesendoderm	Cell Morphology, Cellular Assembly and Organization, Cellular Function and Maintenance
IRX1	Iroquois homeobox 1	rs76258369	5:3545547	intergenic	2.25	Mesendoderm	-

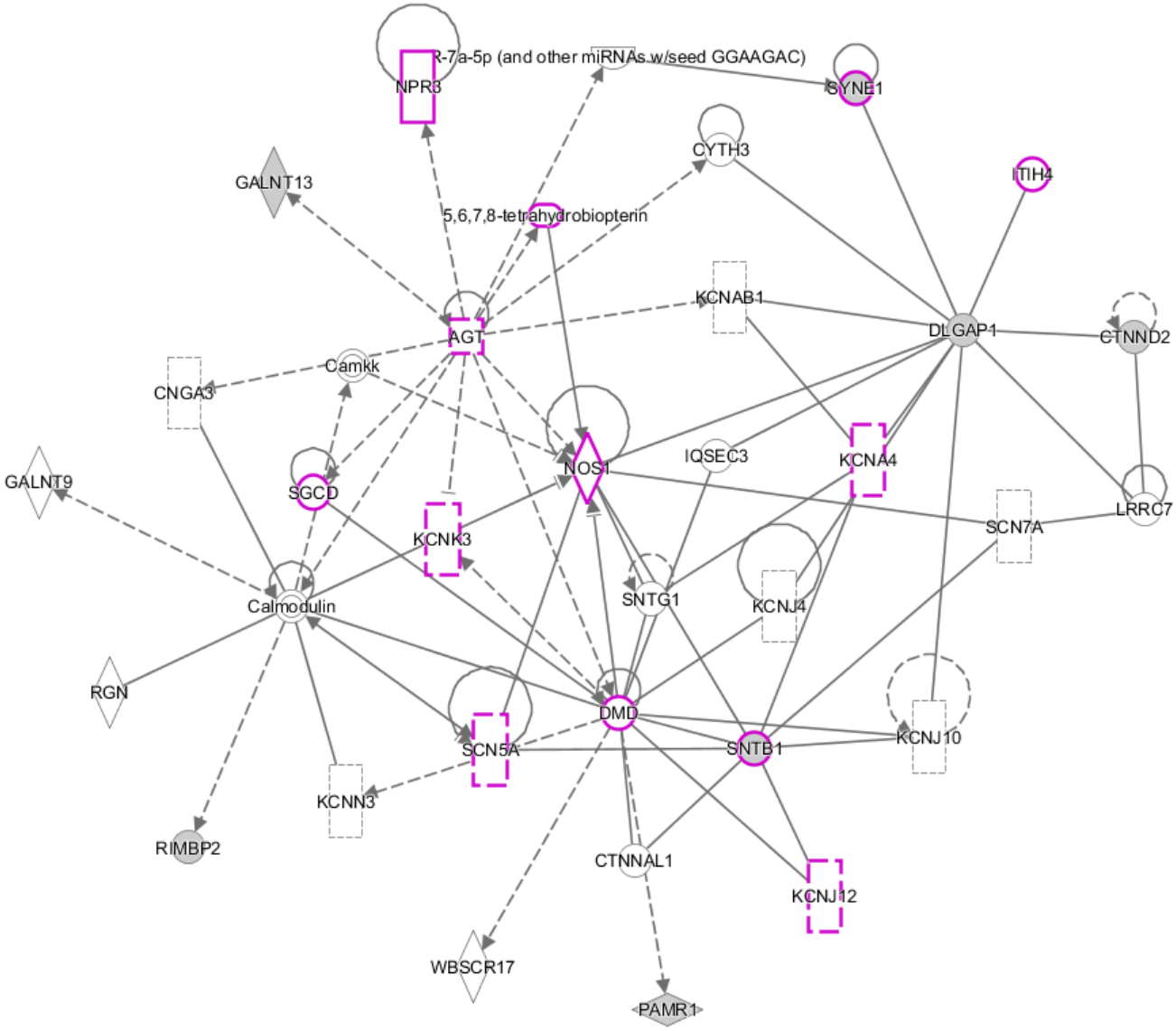
*hg19 build 37 dbSNP

Supplementary Table 2.7.1. Top 15 SNPs that have the lowest association p-values for analyses examining genome-wide genetic variations and placental abruption risk among PAGE and Meta-analysis studies.

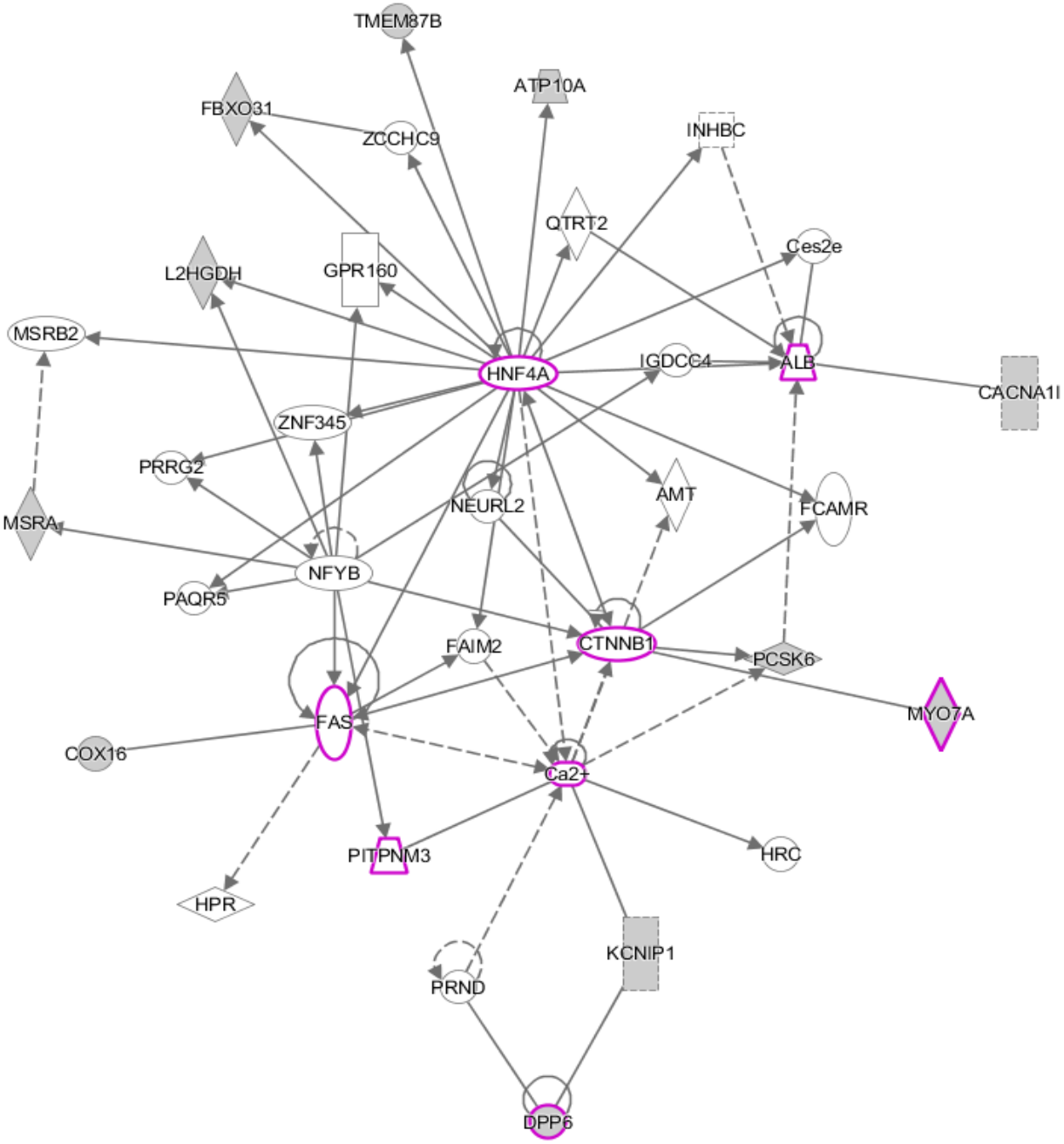
Gene	Chromosome	SNP*	Effect Allele	Effect Allele Frequency	Odds Ratio (95% Confidence Interval)	Empirical p-value
			PAGE Study			
ABCC8	11	rs4148646	G	0.633	0.67 (0.58,0.79)	1.00E-06
ABCC8	11	rs2074310	C	0.635	0.67 (0.58,0.79)	1.10E-06
ABCC8	11	rs757110	A	0.632	0.68 (0.58,0.79)	1.40E-06
KCNJ11	11	rs2074314	T	0.635	0.67 (0.58,0.79)	1.40E-06
ABCC8	11	rs2074311	G	0.623	0.68 (0.58,0.80)	1.90E-06
KCNJ11	11	rs5215	C	0.632	0.69 (0.59,0.80)	2.90E-06
KCNJ11	11	rs5219	T	0.633	0.69 (0.59,0.80)	2.90E-06
ZNF28	19	rs7249210	A	0.09	2.11 (1.54,2.87)	3.00E-06
KCNJ11	11	rs5213	T	0.634	0.69 (0.59,0.81)	3.80E-06
KCNJ11	11	rs35271178	T	0.623	0.69 (0.59,0.81)	3.80E-06
ZNF28	19	rs7250184	C	0.091	2.09 (1.53,2.84)	3.80E-06
ZNF28	19	rs7249100	G	0.091	2.08 (1.53,2.84)	3.80E-06
ZNF28	19	rs10401828	C	0.093	2.05 (1.51,2.78)	4.30E-06
CTNND2	5	rs11133659	A	0.088	2.12 (1.54,2.92)	4.40E-06
ABCC8	11	rs7124355	G	0.643	0.69 (0.59,0.81)	4.60E-06
			Meta-analysis			
IRX1	5	rs76258369	C	0.164	1.56 (1.30,1.88)	2.80E-06
ADAM12	10	rs7094759	T	0.482	0.74 (0.65,0.84)	4.00E-06
ADAM12	10	rs12264492	G	0.404	0.73 (0.64,0.83)	4.10E-06
DOCK2	5	rs30080	C	0.424	0.73 (0.63,0.83)	5.00E-06
PDGFRL	8	rs11995662	C	0.1	0.61 (0.49,0.75)	5.20E-06
KCNIP1	5	rs4867606	A	0.099	1.82 (1.41,2.36)	5.50E-06
LOC105374318	2	rs219551	T	0.142	1.64 (1.33,2.04)	5.80E-06
DOCK2	5	rs1680563	C	0.423	0.73 (0.64,0.84)	6.20E-06
DOCK2	5	rs7704841	G	0.422	0.73 (0.64,0.84)	6.40E-06
FAM196B	5	rs2291228	G	0.424	1.37 (1.19,1.56)	7.90E-06
FAM196B	5	rs10866641	C	0.423	1.36 (1.19,1.56)	8.10E-06
DOCK2	5	rs72841199	G	0.423	1.36 (1.19,1.56)	8.10E-06
GALNT13	2	rs799758	C	0.176	1.47 (1.24,1.74)	8.60E-06
FAM124A	13	rs17837210	C	0.072	1.80 (1.39,2.33)	8.60E-06
IRX1	5	rs115047740	A	0.17	1.52 (1.26,1.82)	9.20E-06

*hg19 build 37 dbSNP

Supplementary Figure 2.7.1a. Significant networks represented by top 27 genes from top PAGE GWAS hits (p<5e-5). Molecules highlighted in purple represent cardiovascular disease and cardiac arrhythmia pathway.



Supplementary Figure 2.7.1b. Significant networks represented by top 36 genes from top GWAS meta-analysis hits (p<5e-5). Molecules highlighted in purple represent cell signaling/cell-cell interaction and lipid metabolism pathway.


TMEM87B
ATP10A
FBXO31
ZCCHC9
INHBC
QTRT2
Ces2e
L2HGDH
GPR160
MSRB2
HNF4A
IGDCC4
ALB
CACNA1I
ZNF345
PRRG2
AMT
FCAMR
NEURL2
MSRA
NFYB
PAQR5
FAIM2
CTNNB1
PCSK6
MYO7A
FAS
COX16
Ca2+
PITPNM3
HRC
HPR
KCNIP1
PRND
DPP6

Supplementary Table 2.7.2. SNPs suggestively associated with PA in PAGE GWAS (P-value<5e-5).

Gene	SNP *	Chromosome: position *	Effect Allele	Odds Ratio (95% Confidence Interval)	Empirical P-value	Function
ABCC8	rs4148646	11:17415190	G	0.67 (0.58, 0.79)	1.0E-06	intronic
ABCC8	rs2074310	11:17421886	C	0.67 (0.58, 0.79)	1.1E-06	intronic
ABCC8	rs757110	11:17418477	A	0.68 (0.58, 0.79)	1.4E-06	coding
KCNJ11	rs2074314	11:17411821	T	0.68 (0.58, 0.79)	1.4E-06	5upstream
ABCC8	rs2074311	11:17421860	G	0.68 (0.58, 0.8)	1.9E-06	intronic
KCNJ11	rs5215	11:17408630	T	0.69 (0.59, 0.8)	2.9E-06	coding
KCNJ11	rs5219	11:17409572	C	0.69 (0.59, 0.8)	2.9E-06	coding
ZNF28	rs7249210	19:53337340	A	2.11 (1.54, 2.87)	3.0E-06	intronic
KCNJ11	rs5213	11:17408404	T	0.69 (0.59, 0.81)	3.8E-06	3utr
KCNJ11	rs35271178	11:17411020	T	0.69 (0.59, 0.81)	3.8E-06	5upstream
ZNF28	rs7250184	19:53337426	C	2.09 (1.53, 2.84)	3.8E-06	intronic
ZNF28	rs7249100	19:53337326	G	2.08 (1.53, 2.84)	3.8E-06	intronic
ZNF28	rs10401828	19:53336367	C	2.05 (1.51, 2.78)	4.3E-06	intronic
CTNND2	rs11133659	5:11628509	A	2.12 (1.54, 2.92)	4.4E-06	intronic
ABCC8	rs7124355	11:17412960	G	0.69 (0.59, 0.81)	4.6E-06	3downstream
ZNF28	rs7248983	19:53337197	T	2.07 (1.52, 2.81)	4.8E-06	intronic
ZNF28	rs7249481	19:53337539	T	2.06 (1.52, 2.81)	4.9E-06	intronic
ZNF28	rs146312	7:86249396	G	0.5 (0.37, 0.67)	5.4E-06	intronic
KCNJ11	rs1002226	11:17405617	T	0.69 (0.59, 0.81)	5.6E-06	3downstream
MACROD1	rs2701545	11:63848527	T	0.59 (0.47, 0.74)	5.7E-06	intronic
ZNF28	rs7249875	19:53337198	C	2.05 (1.5, 2.79)	6.2E-06	intronic
ZNF28	rs10426640	19:53336491	T	2.05 (1.5, 2.79)	6.3E-06	intronic
ZNF28	rs10402232	19:53336513	C	2.05 (1.5, 2.79)	6.3E-06	intronic
KCNJ11	rs10734252	11:17404839	A	0.69 (0.59, 0.81)	6.5E-06	3downstream
NA	rs11027398	11:23640527	C	2.1 (1.52, 2.89)	7.1E-06	NA
NA	rs116573025	11:23638712	C	2.1 (1.52, 2.89)	7.2E-06	NA

NA	rs73431963	11:23635947	T	2.09 (1.52, 2.88)	7.4E-06	NA
ITGA9	rs3821909	3:37695192	A	0.65 (0.54, 0.78)	7.4E-06	intronic
GRM3	rs274628	7:86265855	C	0.5 (0.37, 0.68)	7.8E-06	intergenic
GRM3	rs274618	7:86272016	A	0.5 (0.37, 0.68)	7.8E-06	5upstream
NA	rs4888463	16:76027137	A	0.66 (0.55, 0.79)	8.5E-06	NA
LOC107984316	rs1557765	11:17403639	C	0.7 (0.6, 0.82)	8.5E-06	ncRNA_exonic
TFAP2E	1:36041087	1:36041087	A	2.17 (1.55, 3.04)	8.6E-06	intronic
ITGA9	rs4678984	3:37687941	A	0.65 (0.54, 0.79)	8.7E-06	intronic
NA	rs11027397	11:23639325	T	2.09 (1.51, 2.89)	9.8E-06	NA
GRM3	rs274637	7:86253638	T	0.51 (0.38, 0.69)	1.1E-05	intergenic
ITGA9	rs4678985	3:37688152	A	0.66 (0.54, 0.79)	1.1E-05	intronic
ERBB4	rs143471745	2:212852000	T	1.65 (1.32, 2.07)	1.2E-05	intronic
NCR3LG1	rs12146652	11:17384542	A	0.7 (0.6, 0.82)	1.2E-05	intronic
NCR3LG1	rs12146443	11:17384498	C	0.7 (0.6, 0.82)	1.2E-05	intronic
GRM3	rs802436	7:86286171	G	0.51 (0.38, 0.69)	1.2E-05	intronic
NCR3LG1	rs7928810	11:17372443	A	0.7 (0.6, 0.82)	1.2E-05	5upstream
ITGA9	rs6798191	3:37694036	C	0.66 (0.55, 0.8)	1.3E-05	intronic
NA	rs61234029	11:23651756	C	2.06 (1.49, 2.85)	1.4E-05	NA
NA	rs17168354	7:96974137	T	2.05 (1.49, 2.83)	1.4E-05	NA
NUCB2	rs757081	11:17351683	G	1.43 (1.22, 1.67)	1.5E-05	coding
NCR3LG1	rs10832776	11:17385224	A	0.7 (0.6, 0.82)	1.5E-05	intronic
GRM3	rs2660971	7:86258736	C	0.51 (0.37, 0.69)	1.5E-05	intergenic
NCR3LG1	rs11024268	11:17387982	G	0.7 (0.6, 0.82)	1.5E-05	intronic
ZNF705D	rs199638308	8:11970688	G	1.72 (1.35, 2.2)	1.5E-05	3utr
ITGA9	rs2000494	3:37671379	G	0.66 (0.54, 0.79)	1.6E-05	intronic
NA	rs78178967	11:23649387	A	2.05 (1.48, 2.83)	1.6E-05	NA
NA	rs73431987	11:23648701	T	2.05 (1.48, 2.83)	1.6E-05	NA
NCR3LG1	rs11024271	11:17395540	C	0.71 (0.6, 0.83)	1.6E-05	3utr
NCR3LG1	rs10832778	11:17394073	G	0.71 (0.6, 0.83)	1.6E-05	3utr
GRM3	rs274631	7:86268908	G	0.51 (0.38, 0.69)	1.6E-05	intergenic
ITGA9	rs4678981	3:37672777	T	0.66 (0.54, 0.79)	1.7E-05	intronic

MACROD1	rs11231690	11:63845355	G	1.68 (1.33, 2.12)	1.7E-05	intronic
PCSK6	rs117000886	15:101907493	C	0.66 (0.55, 0.8)	1.7E-05	intronic
DLGAP1	rs17437778	18:4448512	A	1.52 (1.26, 1.84)	1.7E-05	intronic
AC009227.2	rs799770	2:155291579	G	1.61 (1.3, 2)	1.7E-05	3downstream
NCR3LG1	rs4439492	11:17386733	T	0.71 (0.6, 0.83)	1.7E-05	intronic
ITGA9	rs146062203	3:37677693	A	0.66 (0.55, 0.8)	1.7E-05	intronic
NA	rs112034136	11:23638111	T	2 (1.46, 2.73)	1.7E-05	NA
FTO	rs28637326	16:54197187	C	0.7 (0.59, 0.82)	1.8E-05	intergenic
NA	rs11027411	11:23663861	T	2.06 (1.49, 2.86)	1.8E-05	NA
NCR3LG1	rs2051772	11:17389850	A	0.71 (0.6, 0.83)	1.8E-05	intronic
GRM3	rs58882293	7:86239031	G	1.89 (1.42, 2.53)	1.8E-05	intergenic
GRM4	rs2204638	7:86234727	G	1.89 (1.41, 2.52)	1.9E-05	intergenic
GRM5	rs1405876	7:86237581	G	1.89 (1.41, 2.52)	1.9E-05	intergenic
GRM3	rs62488405	7:86234000	A	1.89 (1.41, 2.52)	1.9E-05	intergenic
NA	rs11027415	11:23671985	C	2.12 (1.51, 2.98)	1.9E-05	NA
GRM3	rs2107691	7:86317671	T	0.52 (0.39, 0.7)	2.0E-05	intronic
GRM3	rs724226	7:86325374	G	0.52 (0.38, 0.7)	2.0E-05	intronic
LINC01019	rs76258369	5:3545547	C	1.68 (1.33, 2.13)	2.0E-05	intergenic
GRM3	rs2526952	7:86258737	T	0.51 (0.38, 0.69)	2.1E-05	intergenic
GALNT13	rs707063	2:155285303	A	1.6 (1.29, 1.99)	2.1E-05	intronic
ITGA9	rs2282485	3:37670876	C	0.66 (0.54, 0.8)	2.1E-05	intronic
GRM3	rs802475	7:86323502	A	0.52 (0.38, 0.7)	2.1E-05	intronic
MACROD1	rs12224583	11:63846444	A	1.66 (1.32, 2.1)	2.1E-05	intronic
SS18L1	rs6121945	20:60753390	A	0.59 (0.47, 0.75)	2.1E-05	intronic
MACROD1	rs58449700	11:63846803	T	1.66 (1.32, 2.1)	2.2E-05	intronic
VAV2	rs7032391	9:136655994	G	0.7 (0.6, 0.83)	2.3E-05	intronic
GRM3	rs10241183	7:86230375	T	1.9 (1.41, 2.55)	2.3E-05	intergenic
GRM3	rs724225	7:86325382	G	0.52 (0.39, 0.7)	2.4E-05	intronic
GRM3	rs802434	7:86285544	A	0.52 (0.39, 0.7)	2.4E-05	intronic
AC009227.2	rs707068	2:155297402	G	1.59 (1.28, 1.97)	2.5E-05	intronic
AC009227.2	rs707069	2:155297453	C	1.59 (1.28, 1.97)	2.5E-05	intronic

GRM3	rs802450	7:86297150	T	0.52 (0.39, 0.7)	2.5E-05	intronic
GRM3	rs802421	7:86291446	A	0.53 (0.39, 0.71)	2.5E-05	intronic
GRM3	rs802427	7:86296335	C	0.52 (0.39, 0.7)	2.5E-05	intronic
NA	rs113319378	11:23621775	C	1.93 (1.42, 2.61)	2.5E-05	NA
GRM3	rs802426	7:86295063	T	0.52 (0.39, 0.7)	2.6E-05	intronic
ITGA9	rs10510696	3:37679547	G	0.66 (0.55, 0.8)	2.6E-05	intronic
ZNF705D	rs200672574	8:11975125	A	1.7 (1.33, 2.18)	2.6E-05	3downstream
LINC00544	rs73159324	13:30521041	G	1.67 (1.32, 2.12)	2.6E-05	non-coding
GRM3	rs701338	7:86308889	A	0.52 (0.39, 0.71)	2.7E-05	intronic
GRM3	rs802459	7:86308038	G	0.52 (0.39, 0.71)	2.7E-05	intronic
GRM3	rs802449	7:86304183	A	0.52 (0.39, 0.71)	2.7E-05	intronic
PSMB2	rs182344059	1:36082070	T	2.12 (1.49, 3)	2.8E-05	intronic
SYNE1	rs7382254	6:152544371	A	0.7 (0.59, 0.82)	2.8E-05	intronic
GRM3	rs274624	7:86274289	C	0.52 (0.39, 0.71)	3.0E-05	intronic
GRM3	rs274611	7:86247861	G	0.53 (0.39, 0.71)	3.0E-05	intergenic
ITGA9	rs4678982	3:37683513	T	0.67 (0.55, 0.81)	3.0E-05	intronic
L3MBTL4	rs16949213	18:5999727	C	0.72 (0.61, 0.84)	3.1E-05	intronic
L3MBTL4	rs11875911	18:6016063	T	0.72 (0.62, 0.84)	3.1E-05	intronic
SNTB1	rs4242317	8:121701132	T	0.71 (0.61, 0.83)	3.1E-05	intronic
GRM3	rs802420	7:86291177	C	0.52 (0.39, 0.71)	3.2E-05	intronic
FTO	rs16953154	16:54190017	A	1.41 (1.2, 1.65)	3.2E-05	intergenic
FTO	rs12598570	16:54189497	C	1.4 (1.2, 1.65)	3.2E-05	intergenic
NA	rs11027410	11:23663588	A	1.99 (1.44, 2.74)	3.3E-05	NA
SNTB1	rs4503138	8:121703493	C	0.71 (0.61, 0.83)	3.3E-05	intronic
EGFEM1P	rs62275741	3:168362700	G	1.38 (1.19, 1.6)	3.3E-05	non-coding
SYNE1	rs148924795	6:152545268	G	0.66 (0.54, 0.8)	3.3E-05	intronic
DLGAP1	rs112769787	18:4454872	A	1.4 (1.2, 1.64)	3.3E-05	intronic
SNTB1	rs9643147	8:121705780	C	0.71 (0.61, 0.84)	3.4E-05	intronic
L3MBTL4	rs11876631	18:6017279	T	0.72 (0.62, 0.84)	3.5E-05	intronic
NA	rs9416455	10:57839964	A	0.71 (0.6, 0.83)	3.5E-05	NA
RP11-476F14.1	rs12263033	10:33782231	T	0.5 (0.36, 0.69)	3.5E-05	intergenic

SNTB1	rs6990640	8:121706501	T	0.71 (0.61, 0.84)	3.5E-05	intronic
SNTB1	rs9643148	8:121706867	A	0.71 (0.61, 0.84)	3.5E-05	intronic
C10orf53	rs7099850	10:50907535	T	1.68 (1.32, 2.15)	3.5E-05	intronic
NA	rs7787893	7:22774437	G	0.62 (0.5, 0.78)	3.6E-05	NA
VAV2	rs7039067	9:136656435	C	0.71 (0.6, 0.83)	3.6E-05	intronic
NA	rs73431960	11:23629079	T	1.97 (1.43, 2.7)	3.7E-05	NA
QPRT	rs9940532	16:29697369	T	0.54 (0.4, 0.72)	3.7E-05	intronic
NA	rs10809153	9:10675794	A	0.65 (0.53, 0.8)	3.7E-05	NA
L3MBTL4	rs12373202	18:6019001	T	0.72 (0.62, 0.84)	3.7E-05	intronic
FTO	rs12934459	16:54189238	T	1.4 (1.19, 1.64)	3.8E-05	intergenic
NA	rs1404006	7:22795077	C	0.62 (0.5, 0.78)	3.8E-05	NA
VAV2	rs75443864	9:136657234	G	0.71 (0.6, 0.83)	3.8E-05	intronic
VAV2	rs3824559	9:136657774	G	0.7 (0.6, 0.83)	3.9E-05	intronic
SNTB1	rs7836660	8:121709770	T	0.71 (0.61, 0.84)	3.9E-05	intronic
NA	rs140142711	3:94498812	G	1.9 (1.4, 2.57)	3.9E-05	NA
ITGA9	rs79795980	3:37707767	C	0.67 (0.56, 0.81)	4.0E-05	intronic
VAV2	rs10761399	9:136656383	G	0.71 (0.6, 0.83)	4.0E-05	intronic
VAV2	rs7032952	9:136656394	T	0.71 (0.6, 0.83)	4.0E-05	intronic
EGFEM1P	rs16852525	3:168363205	G	1.37 (1.18, 1.6)	4.0E-05	non-coding
NA	rs79052996	11:23629063	G	1.97 (1.43, 2.71)	4.0E-05	NA
NA	rs73431956	11:23628540	C	1.97 (1.43, 2.71)	4.0E-05	NA
NA	rs73431955	11:23628037	C	1.97 (1.43, 2.71)	4.0E-05	NA
GRM3	rs802423	7:86293799	A	0.53 (0.39, 0.72)	4.0E-05	intronic
LOC105374165	rs6772162	3:153130402	T	0.54 (0.4, 0.72)	4.0E-05	ncRNA_intronic
EIF3H	rs73320568	8:117623479	T	1.41 (1.2, 1.66)	4.0E-05	intergenic
EIF3H	rs113498759	8:117625845	C	1.41 (1.2, 1.66)	4.0E-05	intergenic
NA	rs113562946	3:94397161	A	1.82 (1.37, 2.42)	4.0E-05	NA
OR7E115P	rs11528728	10:15054098	A	1.46 (1.22, 1.75)	4.0E-05	intergenic
LINC01019	rs115047740	5:3553396	A	1.64 (1.3, 2.08)	4.1E-05	intergenic
FTO	rs28613919	16:54196963	A	0.68 (0.56, 0.82)	4.1E-05	intergenic
SLIT3	rs907430	5:168581829	C	1.57 (1.27, 1.94)	4.1E-05	intronic

GALNT13	rs799758	2:155262144	C	1.54 (1.26, 1.89)	4.2E-05	intronic
LOC105374165	rs4417842	3:153141237	T	0.54 (0.4, 0.72)	4.2E-05	ncRNA_intronic
L3MBTL4	rs2212523	18:6000965	A	0.72 (0.62, 0.84)	4.2E-05	intronic
ITGA9	rs76224068	3:37685915	G	0.67 (0.55, 0.81)	4.3E-05	intronic
ZMYM4	rs202202226	1:35797398	A	1.94 (1.42, 2.67)	4.3E-05	intronic
L3MBTL4	rs114730635	18:6013999	C	0.72 (0.62, 0.84)	4.4E-05	intronic
AC009227.2	rs707087	2:155311309	G	1.49 (1.23, 1.8)	4.4E-05	intronic
NUCB2	rs12577815	11:17266868	A	1.41 (1.2, 1.67)	4.5E-05	intronic
NA	rs55692301	3:172191204	G	0.57 (0.44, 0.75)	4.6E-05	NA
PAMR1	rs34190626	11:35494135	C	2.01 (1.44, 2.81)	4.6E-05	intronic
MACROD1	rs2096734	11:63836749	A	1.74 (1.34, 2.28)	4.6E-05	intronic
NUCB2	rs17473243	11:17266259	A	1.41 (1.2, 1.66)	4.6E-05	intronic
LUZP2	rs11028240	11:24897668	C	1.37 (1.18, 1.6)	4.7E-05	intronic
VAV2	rs3824560	9:136657869	T	0.71 (0.6, 0.83)	4.7E-05	intronic
NEDD4L	rs182383	18:55895755	C	0.73 (0.63, 0.85)	4.7E-05	intronic
NUCB2	rs12577525	11:17260116	A	1.41 (1.2, 1.66)	4.7E-05	intronic
FTO	rs12445575	16:54192217	A	1.4 (1.19, 1.64)	4.7E-05	intergenic
PRNT	rs6037941	20:4716479	G	1.55 (1.26, 1.91)	4.7E-05	non-coding
SNCA	rs35409299	4:90685979	T	1.96 (1.42, 2.7)	4.8E-05	intronic
RP11-476F14.1	rs722545	10:33787978	C	0.5 (0.36, 0.69)	4.8E-05	intergenic
ITGA9	rs9790107	3:37708382	G	0.67 (0.56, 0.81)	4.8E-05	intronic
RP1-90L14.1	rs142116635	6:85160231	T	2.64 (1.66, 4.21)	4.8E-05	intronic
VAV2	rs116252966	9:136657348	G	0.71 (0.6, 0.84)	4.9E-05	intronic
L3MBTL4	rs1940618	18:6001284	A	0.72 (0.62, 0.85)	5.0E-05	intronic

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Supplementary Table 2.7.3. SNPs suggestively associated with PA in meta-analysis GWAS (P-value<5e-5).

Gene	SNP *	Chromosome: Position*	Effect Allele	Odds Ratio (95% Confidence Interval)	Empirical P-value	SNP Function
NA	rs76258369	5:3545547	T	1.56 (1.3, 1.88)	2.7E-06	NA
ADAM12	rs7094759	10:127852106	A	0.74 (0.65, 0.84)	3.9E-06	intronic
ADAM12	rs12264492	10:127854246	A	0.73 (0.64, 0.83)	4.0E-06	intronic
DOCK2	rs30080	5:169273557	C	0.73 (0.63, 0.83)	4.9E-06	intronic
PDGFRL	rs11995662	8:17498730	T	0.61 (0.49, 0.75)	5.1E-06	intronic
KCNIP1	rs4867606	5:169924695	A	1.82 (1.41, 2.36)	5.4E-06	intronic
ENSG00000231204	rs219551	2:21540711	T	1.64 (1.33, 2.04)	5.7E-06	ncRNA_intronic
DOCK2	rs1680563	5:169281508	T	0.73 (0.64, 0.84)	6.1E-06	intronic
DOCK2	rs7704841	5:169281214	A	0.73 (0.64, 0.84)	6.3E-06	intronic
FAM196B	rs2291228	5:169288732	A	1.37 (1.19, 1.57)	7.8E-06	3downstream
FAM196B	rs10866641	5:169289206	T	1.36 (1.19, 1.56)	8.0E-06	3downstream
DOCK2	rs72841199	5:169284998	A	1.36 (1.19, 1.56)	8.0E-06	intronic
GALNT13	rs799758	2:155262144	T	1.47 (1.24, 1.74)	8.4E-06	intronic
FAM124A	rs17837210	13:51856010	A	1.8 (1.39, 2.33)	8.5E-06	3utr
NA	rs115047740	5:3553396	A	1.52 (1.26, 1.82)	9.0E-06	NA
DOCK2	rs1482333	5:169288136	A	1.36 (1.19, 1.56)	1.0E-05	intronic
DOCK2	rs30083	5:169274460	T	0.74 (0.64, 0.84)	1.0E-05	intronic
DOCK2	rs30084	5:169275842	T	0.74 (0.64, 0.84)	1.1E-05	intronic
DOCK2	rs1366225	5:169280065	A	0.74 (0.64, 0.84)	1.1E-05	intronic
FAM196B	rs11134593	5:169289635	C	1.36 (1.18, 1.56)	1.1E-05	3downstream
DOCK2	rs1680571	5:169279005	A	0.74 (0.64, 0.85)	1.2E-05	intronic
ADAM12	rs11244854	10:127850629	T	1.35 (1.18, 1.55)	1.3E-05	intronic
ADAM12	chr10:127831629	10:127831629	T	0.72 (0.62, 0.84)	1.4E-05	intronic
ADAM12	rs116240837	10:127831630	A	0.72 (0.62, 0.84)	1.4E-05	intronic
NA	rs9587141	13:107113715	A	1.33 (1.17, 1.52)	1.4E-05	NA
ADAM12	rs2169076	10:127849127	T	1.33 (1.17, 1.52)	1.4E-05	intronic

FAM196B	rs11134594	5:169293379	C	1.35 (1.18, 1.55)	1.4E-05	intronic
AC009227.2	rs799770	2:155291579	A	1.52 (1.26, 1.83)	1.4E-05	3downstream
NA	rs60782217	13:107113505	C	1.33 (1.17, 1.52)	1.4E-05	NA
NA	rs57341162	13:107113714	T	1.33 (1.17, 1.52)	1.5E-05	NA
NA	rs7320042	13:107114134	A	0.75 (0.66, 0.86)	1.7E-05	NA
RHBDL3	rs4794913	17:30615244	A	0.71 (0.61, 0.83)	1.7E-05	intronic
TMEM87B	rs116046835	2:112816365	A	1.63 (1.31, 2.05)	1.7E-05	intronic
ADAM12	rs6597740	10:127852721	T	0.75 (0.66, 0.85)	1.7E-05	intronic
GALNT13	rs707063	2:155285303	A	1.51 (1.25, 1.82)	1.8E-05	intronic
PALD1	rs871870	10:72297411	T	1.84 (1.39, 2.42)	1.8E-05	intronic
NA	chr7:4366642	7:4366642	T	2.04 (1.47, 2.84)	1.8E-05	NA
AC009227.2	rs707068	2:155297402	T	1.5 (1.25, 1.81)	2.0E-05	intronic
AC009227.2	rs707069	2:155297453	T	1.5 (1.25, 1.81)	2.0E-05	intronic
NA	rs6944495	7:38961962	A	1.44 (1.22, 1.71)	2.3E-05	NA
PCSK6	rs117000886	15:101907493	T	0.71 (0.6, 0.83)	2.4E-05	intronic
NA	rs6561866	13:56324143	T	1.34 (1.17, 1.53)	2.5E-05	NA
PDE6C	rs11187575	10:95414150	T	1.73 (1.34, 2.23)	2.5E-05	intronic
AC009227.2	rs2696024	2:155302852	T	1.5 (1.24, 1.82)	2.5E-05	intronic
NA	rs6944869	7:38961943	A	1.44 (1.22, 1.71)	2.5E-05	NA
PDE6C	rs10882294	10:95412215	T	1.71 (1.33, 2.19)	2.6E-05	intronic
SNCA	rs10516845	4:90684278	A	0.77 (0.68, 0.87)	2.6E-05	intronic
CRTC1	rs12610636	19:18814528	T	1.74 (1.35, 2.26)	2.6E-05	intronic
C14orf183	rs150037646	14:50550557	T	1.52 (1.25, 1.85)	2.8E-05	coding
NEDD4L	rs292447	18:55896826	T	0.75 (0.66, 0.86)	2.8E-05	intronic
ADAM12	rs1676742	10:127851757	A	1.32 (1.16, 1.51)	2.8E-05	intronic
NA	rs6946948	7:38957254	A	1.44 (1.21, 1.7)	2.9E-05	NA
DPP6	rs10227166	7:153604079	C	1.55 (1.26, 1.9)	2.9E-05	intronic
AC009227.2	rs707064	2:155293869	T	1.5 (1.24, 1.81)	3.1E-05	3utr
SNCA	rs3775427	4:90687119	A	0.77 (0.68, 0.87)	3.1E-05	intronic
SNCA	rs3796661	4:90687507	T	0.77 (0.68, 0.87)	3.1E-05	intronic
NEDD4L	rs182383	18:55895755	T	0.75 (0.66, 0.86)	3.1E-05	intronic

FAM124A	rs1475377	13:51857647	A	1.71 (1.33, 2.21)	3.1E-05	3utr
LINC01019	rs111557503	5:3486277	A	1.51 (1.24, 1.83)	3.2E-05	non-coding
FAM124A	rs17252152	13:51858706	T	1.75 (1.34, 2.28)	3.2E-05	3downstream
CRTC1	rs112046750	19:18815476	T	1.74 (1.34, 2.25)	3.2E-05	intronic
MYO7A	rs12793619	11:76921358	A	0.73 (0.63, 0.85)	3.3E-05	intronic
ADAM12	rs1674927	10:127852395	T	1.32 (1.16, 1.51)	3.3E-05	intronic
AC009227.2	rs707067	2:155295547	A	1.49 (1.24, 1.81)	3.3E-05	intronic
SNCA	rs10433953	4:90698964	T	0.77 (0.68, 0.87)	3.3E-05	intronic
SNCA	rs72503734	4:90699779	T	0.77 (0.68, 0.87)	3.3E-05	intronic
AC009227.2	rs707065	2:155294058	T	1.49 (1.24, 1.81)	3.4E-05	3utr
AC009227.2	rs799822	2:155298880	A	1.49 (1.24, 1.8)	3.4E-05	intronic
CACNA1I	rs5757746	22:40000442	A	1.85 (1.38, 2.47)	3.4E-05	intronic
NA	rs6545815	2:21579163	T	1.39 (1.19, 1.63)	3.5E-05	NA
CRTC1	rs12610610	19:18814331	T	1.73 (1.33, 2.25)	3.6E-05	intronic
SLC24A4	rs112223482	14:92808889	A	0.53 (0.39, 0.72)	3.6E-05	intronic
ZNF761	rs75739523	19:53942084	A	1.72 (1.33, 2.23)	3.6E-05	intronic
LINC01019	rs924611	5:3510541	T	1.5 (1.24, 1.82)	3.7E-05	non-coding
ZNF761	rs74319504	19:53939710	A	1.74 (1.33, 2.25)	3.8E-05	intronic
CRTC1	rs77697529	19:18818527	A	1.73 (1.33, 2.24)	3.8E-05	intronic
CRTC1	rs77253968	19:18824709	T	1.73 (1.33, 2.24)	3.8E-05	intronic
CRTC1	rs75907904	19:18829977	T	1.73 (1.33, 2.24)	3.9E-05	intronic
CRTC1	rs113922020	19:18829929	A	1.72 (1.33, 2.24)	4.0E-05	intronic
NA	rs3004625	9:136941780	A	1.4 (1.19, 1.64)	4.0E-05	NA
NEDD4L	rs292457	18:55887990	T	0.76 (0.66, 0.86)	4.0E-05	intronic
SLC24A4	rs4600402	14:92803916	T	0.58 (0.45, 0.75)	4.1E-05	intronic
ATP10A	rs11632500	15:25999198	T	1.35 (1.17, 1.56)	4.1E-05	intronic
SYNE1	rs13195723	6:152562967	T	1.37 (1.18, 1.6)	4.1E-05	intronic
DPP6	rs10261158	7:153603763	T	1.53 (1.25, 1.88)	4.1E-05	intronic
NA	rs12417895	11:80736539	T	1.37 (1.18, 1.6)	4.2E-05	NA
DPP6	rs12673076	7:153608121	A	1.54 (1.25, 1.89)	4.2E-05	intronic
ATP5S	rs150255722	14:50777634	T	1.51 (1.24, 1.84)	4.3E-05	5upstream

NA	rs2005661	9:136937191	T	1.39 (1.19, 1.64)	4.3E-05	NA
GALNT13	rs707051	2:155248839	A	1.38 (1.18, 1.6)	4.3E-05	intronic
NA	rs2506711	9:136937604	T	1.39 (1.19, 1.64)	4.3E-05	NA
SNCA	rs356168	4:90674431	A	1.28 (1.14, 1.45)	4.3E-05	intronic
SNCA	rs356204	4:90663542	T	1.28 (1.14, 1.45)	4.4E-05	intronic
SNCA	rs356225	4:90643757	C	1.28 (1.14, 1.45)	4.4E-05	3downstream
NA	rs72937988	11:80736067	T	1.37 (1.18, 1.6)	4.4E-05	NA
NA	rs356215	4:90636561	A	1.28 (1.14, 1.45)	4.4E-05	NA
NA	rs12419847	11:80743719	A	1.37 (1.18, 1.6)	4.4E-05	NA
ADAM12	rs1676743	10:127854048	T	1.32 (1.16, 1.51)	4.5E-05	intronic
NA	rs356219	4:90637601	A	1.28 (1.13, 1.43)	4.6E-05	NA
SYNE1	rs13210209	6:152562965	A	1.37 (1.18, 1.59)	4.6E-05	intronic
NA	rs12420363	11:80732367	A	1.37 (1.18, 1.6)	4.6E-05	NA
NA	rs2905063	9:136941846	A	1.39 (1.19, 1.64)	4.7E-05	NA
NA	rs2319210	9:136942303	A	1.39 (1.19, 1.64)	4.7E-05	NA
CRTC1	rs74253294	19:18821770	T	1.71 (1.32, 2.22)	4.7E-05	intronic
SNCA	rs34806123	4:90684122	A	0.78 (0.69, 0.88)	4.7E-05	intronic
SNCA	rs35495602	4:90684123	A	0.78 (0.69, 0.88)	4.7E-05	intronic
SNCA	rs356165	4:90646886	A	1.28 (1.13, 1.43)	4.7E-05	3utr
NA	rs356209	4:90635606	T	1.28 (1.14, 1.44)	4.7E-05	NA
NA	rs17141367	11:80737997	T	1.37 (1.18, 1.6)	4.7E-05	NA
PDE6C	rs11187569	10:95406729	A	1.66 (1.3, 2.12)	4.8E-05	intronic
NA	rs3004623	9:136938262	A	1.39 (1.19, 1.63)	4.8E-05	NA
NA	rs2905067	9:136938261	T	1.39 (1.19, 1.63)	4.8E-05	NA
NA	rs356220	4:90641340	T	1.27 (1.13, 1.43)	4.9E-05	NA
NA	rs1498331	11:80731375	A	1.37 (1.18, 1.6)	4.9E-05	NA
SLC24A4	rs76030827	14:92808475	C	0.54 (0.4, 0.73)	4.9E-05	intronic
FAM196B	rs6872720	5:169299685	A	1.33 (1.16, 1.52)	4.9E-05	intronic
NA	rs369653636	14:23922644	A	1.32 (1.15, 1.51)	5.0E-05	NA
NA	rs373566161	14:23922645	A	1.32 (1.15, 1.51)	5.0E-05	NA
NA	rs202096969	14:23922646	A	1.32 (1.15, 1.51)	5.0E-05	NA

* hg19 build 37 chromosomal positions and rsID SNP names.

Supplementary Table 2.7.4. Overlap of SNPs suggestively associated with PA in PAGE GWAS and meta-analysis GWAS (P-value<5e-5).

Gene	Chr.	SNP*	Effect Allele	Effect Allele Frequency	PAGE		Meta-analysis	
					OR (95% CI)	Empirical P-value	OR (95% CI)	Empirical P-value
PCSK6	15	rs117000886	C	0.249	0.66 (0.55,0.80)	1.70E-05	0.71 (0.60, 0.83)	2.40E-05
GALNT13	2	rs799770	G	0.150	1.61 (1.30, 2.00)	1.72E-05	1.52 (1.26, 1.83)	1.50E-05
LINC01019	5	rs76258369	C	0.249	1.68 (1.33, 2.13)	2.03E-05	1.56 (1.30, 1.88)	2.80E-06
GALNT13	2	rs707063	A	0.152	1.60 (1.29, 1.99)	2.07E-05	1.51 (1.25, 1.82)	1.80E-05
GALNT13	2	rs707068	G	0.152	1.59 (1.28, 1.97)	2.48E-05	1.50 (1.25, 1.81)	2.01E-05
GALNT13	2	rs707069	C	0.152	1.59 (1.28, 1.97)	2.49E-05	1.50 (1.25, 1.81)	2.04E-05
LINC01019	5	rs115047740	A	0.175	1.64 (1.30, 2.08)	4.06E-05	1.52 (1.26, 1.82)	9.20E-06
GALNT13	2	rs799758	C	0.175	1.54 (1.26, 1.89)	4.15E-05	1.47 (1.24, 1.74)	8.60E-06
NEDD4L	18	rs182383	C	0.530	0.73 (0.63, 0.85)	4.70E-05	0.75 (0.66, 0.86)	3.10E-05

* hg19 build 37 rsID SNP names.

Supplementary Table 2.7.5. Significant networks of function represented by genes from our top PAGE GWAS and meta-analysis GWAS hits (20 SNPs in 14 genes) (P-value<1.e-5).

Molecules in Network	Score	Focus Molecules	Top Diseases and Functions	P-value
1-O-hexadecyl-2-N-methylcarbamol-sn-glycerol-3-phosphocholine,1-oleoyl-lysophosphatidic acid,9530018H14Rik, **ABCC8**, ABLIM3, **ADAM12**, BLID, C9orf3, Ca2+, CDH1, Collagen type VII, **CTNND2**, Dcc dimer, *DOCK2*, elaidic acid, **FAM124A**, FXYD5, **GALNT13**,goralatide, KCNG1, **KCNIP1**, **KCNJ11**,MFAP4,miR-491-5p (and other miRNAs w/seed GUGGGGA),MMP2, MRVI1, NPS, **PDGFRL**, PRKG1, PTK2, PZP, TENM4, TGFB1, WFIKKN2, WISP3	25	9	Cellular Movement, Cell Morphology, Hair and Skin Development and Function	1e-24
FAM196B, L-dopa	3	1	Cell-To-Cell Signaling and Interaction, Cellular Assembly and Organization, Developmental Disorder	0.01
MAPK6, TRIM28, **ZNF28**	3	1	Cell Death and Survival, Cellular Function and Maintenance, Reproductive System Development and Function	0.01

Chapter 3. Genetic Variations in Mitochondrial Biogenesis and Oxidative Phosphorylation and Risk of Placental Abruption: Replication of a Candidate Gene Association Study

3.1 Abstract

Background: Perturbations in mitochondrial biogenesis (MB) and oxidative phosphorylation (OP) contribute to placental abruption (PA). Previous genome-wide and candidate gene association studies have identified single nucleotide polymorphisms (SNPs) in MB/OP genes that are potentially associated with PA risk.

Objective: To replicate a candidate gene association study of genetic variations in MB/OP and risk of PA.

Study Design and Methods: The study was conducted among participants (507 PA cases and 1,090 controls) of the Placental Abruption Genetic Epidemiology (PAGE) study. Weighted genetic risk scores (wGRS) were calculated using PA risk-increasing alleles of 11 SNPs in nine MB/OP genes (*CAMK2B, NR1H3, PPARG, PRKCA, THRB, COX5A, NDUFA10, NDUFA12* and *NDUFC2*), that were previously reported in the Peruvian Abruptio Placentae Epidemiology (PAPE) study, a study with similar design and study population to the PAGE study. Logistic regression models were fit to examine associations of wGRS with risk of PA adjusted for population stratification, maternal age, and infant sex, and preeclampsia. Analyses were repeated among strata defined by associations by preeclampsia status, maternal age (≥35 versus 18-34 years), and infant sex to assess potential effect modification.

Results: PA cases were more likely to have preeclampsia, shorter gestational age and lower infant birthweight. Participants in the 25-50th (score:12.6-13.8), 50-75th (score:13.9-15.0) and

75-100th (score ≥15.1) wGRS percentiles had 1.45-fold (95% confidence interval [CI]:1.04, 2.02), 1.42-fold (95% CI: 1.02-1.98), and 1.75-fold (95% CI: 1.27, 2.42) higher odds of PA, respectively, compared to those in the lowest wGRS percentile (score <12.6; P-for-trend <0.001). Among women with preeclampsia, those in the highest wGRS percentile had 3.92-fold (95%CI:1.48, 10.36) higher odds of PA compared with women in the lowest wGRS percentile. Among normotensives, women in the highest wGRS percentile had 1.57-fold (95%CI:1.11, 2.21) higher odds of PA compared with those in the lowest wGRS percentile (p-value for interaction=0.12). We did not observe differences in associations among strata defined by maternal age or infant sex.

Conclusion: We replicated previous findings of genetic variants in MB/OP that influence PA risk. Future studies to examine whether identified SNPs contribute to PA risk in other populations are warranted. Findings may inform efforts to identify participants who are at elevated risk for PA.

3.2 Introduction

Mitochondrial biogenesis (MB) and oxidative phosphorylation (OP) are among several molecular pathways that have been implicated in the pathogenesis of placental abruption (PA)[39 41], a multifactorial polygenic disease associated with significant maternal and neonatal morbidity and mortality. The mitochondria controls many critical cell functions, including production of cellular energy, adenosine triphosphate (ATP), by coupling of OP to cell respiration[25 26 78]. Oxidative stress-induced damage to mitochondrial structural elements (e.g., lipid membrane) alters mitochondrial gene expression and promotes a deficiency in OP[79], resulting in

mitochondrial dysfunction. Hundreds of nuclear DNA genes across the chromosome regulate MB and maintain mitochondrial structure and function by regulating OP[80].

Mitochondrial dysfunction can lead to the impairment of differentiation and invasion of the trophoblast, leading to several obstetrical complications including PA[31]. Epidemiologic and experimental studies have highlighted the roles of MB/OP genes in pregnancy complications that involve the placenta[27-30]. For instance, *PPARG,* a master regulator gene of MB, mediates defective placentation that results from oxidized LDL in cytotrophoblasts of villous and extravillous cells[27]. Expression of this gene was shown to be reduced in placentae of women with gestational diabetes mellitus (GDM)[29]. Another MB gene, *NR1H3* (Liver X alpha)[81], which plays a key role in cholesterol metabolism[82] and cell signaling[83], is important in normal trophoblast invasion during placental implantation [28 30]. In addition to assessing genetic variations in the whole population, sub-group analyses can help to identify members of the population whose genetic background makes them more susceptible to disease[84]. However, such analyses are largely non-existent in the context of MB/OP genetic variations and PA risk.

On the basis of this emerging literature, we previously conducted two candidate single nucleotide polymorphisms (SNP) studies and reported that variations in MB/OP genes influence PA risk[36 39] in the Peruvian Abruptio Placentae Epidemiology (PAPE) study. Using a weighted genetic risk score (wGRS), computed based on the SNPs selected from MB (*PPARG, THRB, CAMK2B, NR1H3,* and *PRKCA*) and OP (*COX5A, NDUFA10, NDUFA12* and *NDUFC2*) genes, the two studies found associations between increased MB/OP wGRS and PA risk. In this new independent study, we conducted a replication candidate gene study examining SNPs in MB/OP genes and risk of PA in the Placental Abruption Genetic Epidemiology (PAGE) study, a study with similar design and study population to the PAPE study. In addition, we examined the extent

to which the association of wGRS with PA risk is modified by known and potential risk factors of PA: preeclampsia[44], advanced maternal age[12], and infant sex[85]. These analyses could have important clinical and public health implications by highlighting potential gene-gene or gene-environment interactions and promoting personalized precision medicine in the context of obstetrical complications [86].

3.3 Methods

Study settings and study populations

The study was conducted among participants of the PAGE study, a case-control study of PA conducted in Lima, Peru. Study participants were recruited among women who were admitted for obstetrical 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 Rebagliati, Hospital San Bartolome, Hospital Hipolito, Hospital Loayza, Hospital Dos de Mayo, and Hospital Maria Auxiliadrosa. Participants who were less than 18 years of age, delivered multiple (non-singleton) infants, had medical records that were insufficient to determine the presence or absence of PA (described below), and reported taking blood thinning medications were excluded from the study. Participants with other diagnoses associated with third trimester bleeding (e.g., placenta previa) were also excluded. The total number of participants included in the study were 522 PA cases and 1147 controls. The study protocol was approved by the Institutional Review Boards (IRBs) 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 using standardized structured questionnaires to collect information on sociodemographic characteristics and risk factors including maternal age, marital status, employment status during pregnancy, medical history, smoking, and alcohol consumption (both current and pre-pregnancy). Maternal medical records were reviewed to obtain information on the course and outcomes of the pregnancy, and to ascertain PA case-control status. PA cases were identified through daily review of emergency room admission logbooks, labor and delivery admission logbooks, and the surgery report book (where post-operative diagnoses are registered). A diagnosis of PA was determined based on evidence of retro-placental bleeding (fresh blood) entrapped between the decidua and the placenta or blood clots behind the placental margin, accompanied by any two of the following: (i) vaginal bleeding at ≥20 weeks in gestation that is not due to placenta previa or cervical or cervical lesions; (ii) uterine tenderness and/or abdominal pain (without other causes, such as those due to hyperstimulation from pitocin augmentation); and, (iii) non-reassuring fetal status or fetal death[77]. Controls were randomly selected from eligible pregnant women who delivered at the same participating hospitals as PA cases during the study period, and who did not have a diagnosis of PA in the current pregnancy. Maternal saliva was collected, plated and stored using 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 were extracted using Qiagen DNAeasy™system and manufacturer protocols (Qiagen, Valencia, CA). Genotyping to characterize genome-wide variation (>300,000 SNPs) was conducted using 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 (HWE) ($p < 1e-05$), and had low minor allele frequency (MAF <0.05). Individuals (n=27) were excluded if they were duplicates or related (Identity by Decent [IBD] value >0.9), had more than 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 was inconclusive regarding sex (n=8), and failed test of divergent ancestry (if principal components were outside the range of [-0.02, 0.02]; n=6). The total number of individuals that remained for further analysis were 1,597 (507 cases and 1090 controls). After the quality control step, SNP imputation was conducted to infer unobserved genotypes. The genotype data were phased using SHAPEIT[50] to infer haplotypes and improve imputation accuracy using the 1000 Genomes. Phased haplotypes were then used to impute our non-typed SNPs using IMPUTE2[51].

A total of 11 SNPs in 9 MB and OP genes (*CAMK2B, NR1H3, PPARG, PRKCA, THRB, COX5A, NDUFA10, NDUFA12* and *NDUFC2*) (**Table 3.7.2**) previously reported in the PAPE study[39] were evaluated in the current analyses. The MB and OP genes were selected from previously published studies based on hypothesized functional and biological significance, and known associations with phenotypes related to placental function and/or perinatal outcomes in mammals[39 82 87-91].

Genetic risk score calculation

Weighted genetic risk scores (wGRS) were calculated by multiplying the number of risk alleles for each MB and OP SNP by externally derived effect size estimates. It has been previously shown that use of weights derived from the same data under analysis resulted in bias,

compared to the use of externally derived estimated effect sizes as weights[92]. The corresponding externally derived effect sizes were obtained from the previously reported Peruvian Abruptio Placentae Epidemiology (PAPE) study[39], a candidate gene study of PA. We assumed an additive genetic risk model, corresponding to a linear increase of PA 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 categorical variables were used to compare the characteristics of PA cases and control participants. Adjustment factors included in the models were principal components (principal components representing population stratification), maternal age, infant sex and a diagnosis of preeclampsia in the current pregnancy. The logistic regression models which included PA as the dependent variable, wGRS of SNPs in MB/OP genes as the independent variable, and adjustment factors were fit.

Participants were categorized into four quartile groups defined by the 25th, 50th, and 75th percentile wGRS scores among control participants. Odds ratios (OR) of PA and corresponding 95% confidence intervals (CI) that correspond to the upper three wGRS quartiles were estimated using confounder-adjusted logistic regression models and the lowest wGRS quartile (0-25% percentile) as a reference group. To test for linear trends in PA risk, we used ordinal variable form of wGRS assigning values 1, 2, 3, and 4 to each quartile. In stratified analyses, multivariable adjusted logistic regression models were also fit separately among groups defined by the diagnosis of preeclampsia in the current pregnancy, infant sex and advanced

maternal age (≥35 versus 18-34 years). The likelihood ratio test was used to report effect modification. To determine statistical significance, p-value <0.05 was used as a cut-off. Statistical analyses were performed using R (version i386 3.1.2) and SAS (Version 13) software.

3.4 Results

Socio-demographic and medical/obstetric characteristics of the study participants are shown in **Table 3.7.1**. PA cases and controls were similar with respect to maternal age, education, marital status, employment, pre-pregnancy body mass index, planned pregnancy, alcohol use and vitamin use. Compared to controls, PA cases were more likely to smoke and use illicit drug during pregnancy. PA cases were also more likely to deliver earlier (i.e., shorter gestational age), deliver infants with lower birth weight, and have a diagnosis of preeclampsia in the current pregnancy as compared to controls.

Eleven previously reported SNPs in nine 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 controls (median: 13.9, range:7.5-18.5) (**Table 3.7.2**). In multivariable adjusted models (**Table 3.7.3 and Figure 3.7.1**), participants in the second quartile, 25-50th wGRS percentile (score: 12.6, 13.8), had 1.45-fold (95% CI: 1.04, 2.02) higher odds of PA compared to 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 1.42-fold (95% CI: 1.02, 1.98) higher odds of PA compared to 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) higher odds of PA compared to those in the lowest percentile. Significant linear trend in association between wGRS and PA risk was observed in this replication study (P-for-trend <0.001).

In stratified analyses, among women with preeclampsia, the odds of PA were 3.92 (95% CI: 1.48, 10.36), 3.50 (95% CI: 1.27, 9.65), and 2.96 (95% CI: 1.15, 7.65) for participants in the highest, third, and second wGRS quartiles, respectively, compared with participants in the lowest wGRS quartile (P-for-trend <0.03) (**Table 3.7.4 and Supplementary Figure 3.7.2**). Among normotensives, similar corresponding estimates were 1.57 (95% CI: 1.11, 2.21), 1.27 (95% CI: 0.89, 1.80), and 1.32 (95% CI: 0.93, 1.87) (P-for-trend=0.08). The interaction test p-value for wGRS and preeclampsia status suggests effect modification of the wGRS-PA associations by preelcampsia (interaction p-value=0.12).

Among women 18-34 years, the odds (95% CI) of PA were 1.68 (1.17, 2.41), 1.49 (1.02, 2.15), and 1.46 (1.01, 2.11), respectively, for women in the fourth, third and second quartiles of wGRS compared with women in the lowest quartile (P-for-trend=0.04). The corresponding odds ratios (95% CI) among women ≥35 years were 2.22 (1.05, 4.69), 1.31 (0.61, 2.80), 1.56 (0.72, 3.33) (P-for-trend=0.19; P-for-interaction=0.73) (**Table 3.7.4 and Supplementary Figure 3.7.3**). Similarly, among participants with male infants, the odds (95% CI) of PA were 1.95 (1.24, 3.07), 1.36 (0.78, 2.00), and 1.83 (1.16, 2.87), respectively, for women in the fourth, third and second quartiles of wGRS compared with women in the reference group (lowest quartile) (P-for-trend=0.004), while corresponding odds ratios (and 95%CIs) among participants with female infants were 1.37 (0.85, 2.20), 1.47 (0.91, 2.39), and 1.11 (0.68, 1.81) (P-for-trend=0.35; P-for-interaction=0.44) (**Table 3.7.4 and Supplementary Figure 3.7.4**).

3.5 Discussion

In this candidate gene association study of PA, we confirm that genetic variants in MB (*CAMK2B, NR1H3, PPARG* and *THRB*) and OP pathways (*COX5A, NDUFA10, NDUFA12*, and

NDUFC2) influence PA risk. Women in the highest wGRS quartile for MB/OP variants had 1.75-fold (95%CI:1.27, 2.42) higher odds of PA compared to those in the lowest quartile. We also observed evidence suggestive of possible effect modification (P-for-interaction=0.12) of the association between MB/OP wGRS and risk of PA by preeclampsia. Women who had preeclampsia and were in the highest quartiles for MB/OP wGRS had a 3.92-fold higher odds of PA (95% CI: 1.48, 10.36) compared with women who had preeclampsia and were in the lowest quartile for MB/OP wGRS.

Candidate gene association studies have proven to be extremely powerful for studying the genetic architecture of complex traits such as PA, providing a more effective and economical hypothesis driven method to assess the role of genetic variations, compared to genome-wide association approaches[93]. Other previous candidate gene association studies of PA included investigations of genes in thrombophilia, rennin-angiotensin system, folate metabolism, and interleukin receptor related and oxidative stress pathways[9 42 94 95]. However, these studies were small in sample size showing modest effects and did not validate the findings using 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 PA cases and 473 controls) in the highest quartiles of the risk score (≥10.0) had 1.9-fold (95% CI: 1.2, 3.1) higher odds of PA compared with participants in the lowest risk score group (≤8.0)[39]. In the current study, we were able to replicate the associations of MB/OP wGRS we reported before with risk of PA. This independent replication study will minimize concerns of failure to replicate, a recurring problem with candidate gene association studies[96 93 97].

Genetic risk scores for 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[98 99]. In the current study, we identified and evaluated the same SNPs and used the previously reported estimated effect sizes. Interestingly, we found stronger trend in association between wGRS and higher odds of PA in the current study (P-for-trend <0.001) compared with the previous report (P-for-trend=0.01).

Our stratified wGRS-PA analyses findings may allow the identification of subgroups in the population who are more susceptible to the deleterious effects of genetic risk factors[100]. This approach has been suggested when standard univariate tests (i.e. evaluating each SNP for interaction independently) fail to identify any interactions[101]. We found suggestive evidence supporting higher PA 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 preeclamptics, the odds of PA were higher for successively increasing quartiles of wGRS, compared with normotensives in the lowest quartile of wGRS. A systematic review showed preeclamptic patients had 1.73-fold (95%CI: 1.47, 2.04) increased odds of PA compared to normotensives[2]. Maternal and fetal genetic factors contribute to 35% and 20% of the variance in preeclampsia, respectively[102]. 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[103], contributing to susceptibility to preeclampsia [103]. As a result, the observed potential interaction in our study may be a reflection of potential gene-environment interaction.

MB and OP genes evaluated in our study have been known to influence phenotypes related to placental function and/or perinatal outcomes. For instance, the MB gene *PRKCA* (protein kinase C-alpha) is critical in many cellular processes including cell signaling through

phosphorylation of variety of proteins[104]. A body of literature suggests *PRKCA* effects contractility[105] in cardiac myocytes[106 107], vascular[108], and myometrial cells[109 110], whose abnormal mechanisms can trigger PA[111]. *PPARG*, peroxisome proliferator-activated receptor gamma, a master regulator of MB and highly expressed in the placenta, mediates defective placentation (e.g., inhibition of trophoblast invasion) through oxidized LDL in cytotrophoblasts of villous and extravillous cells, which are involved in uterus invasion[27 112]. Defective invasion of the uterine spiral arteries is directly involved in preeclampsia[112], a common risk factor of PA[44].

Our study is the largest candidate gene study of PA that has the potential to enhance our understanding of genetic variations in maternal genome that contribute to a multi-factorial heritable disorder such as PA. A key strength of our study is that we replicated association of a wGRS of MB/OP with PA in an independent dataset. We studied Peruvians, a population with high prevalence of pregnancy complications, including PA. However, limitations of our study include potential misclassification of sub-clinical PA, which may introduce bias in the interpretation of our study results or reduce power of our study. Comparing severe placental abruption with mild abruption and/or non-abruption cases may minimize this limitation and facilitate epidemiologic and genetic research[77]. In addition, findings from our study population may not be generalizable to other populations that differ in genetic and other characteristics.

In summary, our findings confirm the role of genetic variants in MB and OP pathways in PA risk. Future studies to examine whether identified SNPs contribute to PA risk in other populations are warranted. Similar genetic studies involving MB and OP, or other potential pathways underlying PA, can inform molecular mechanistic investigations to identify potential

preventative or therapeutic targets. In addition, they could facilitate identification of individuals who have an elevated risk for PA, a significant public health problem.

3.6 References

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3.7 Tables and Figures

Table 3.7.1. Selected characteristics of the Placental Abruption Genetic Epidemiology Study Population.

Characteristics	**Study Participants**		**P-value**
	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	**<0.001**
Vitamins use during pregnancy	84.6	86.1	0.47
Gestational age at delivery[1]	34.3±4.4	39.0±1.2	**<0.001**
Male infant	279 (55.7)	571 (52.9)	0.24
Infant birthweight (grams)[1]	2390±939	3418±484	**<0.001**

[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.

Table 3.7.2. Characteristics of SNPs in MB/OP candidate genes and risk of placental abruption

Gene	SNP±	chr:position±	Imputation Score£	Risk Allele	Risk allele frequency¥	OR (95% CI)*	Empirical P-value*	FDR*	Function	Nomenclature
Mitochondrial Biogenesis										
CAMK2B	rs2009705	7:44255034	0.96	T	0.747	1.30 (1.05,1.58)	0.01	0.02	3downstream	Calcium/calmodulin-dependent protein kinase (CaM kinase) II beta
NR1H3	rs11039155	11:47280762	0.77	A	0.124	1.31 (1.02,1.68)	0.04	0.04	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	intronic	Estrogen-related receptor alpha
PPARG	rs10865711	3:12361385	1.00	C	0.5882	1.19 (0.99,1.44)	0.07	0.04	intronic	Estrogen-related receptor alpha
PPARG	rs1175540	3:12465243	0.98	A	0.1824	1.30 (1.02,1.62)	0.03	0.04	intronic	Estrogen-related receptor alpha
PRKCA	rs4328478	17:64307982	1.00	T	0.7000	1.22 (0.98,1.49)	0.06	0.04	intronic	protein kinase C, alpha
THRB	rs9814223	3:24362252	0.99	G	0.6882	1.20 (1.01,1.47)	0.05	0.04	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	intronic	cytochrome c oxidase subunit Va
NDUFA10	rs4149549	2:240931266	1.00	C	0.7059	1.23 (0.98,1.54)	0.07	0.04	intronic	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 10, 42kDa
NDUFA12	rs11107847	12:95386791	1.00	G	0.5000	1.20 (0.99,1.43)	0.05	0.04	intronic	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 12

NDUFC2_KCTD14	rs627297	11:77763789	0.96	T	0.8000	1.35 (1.05,1.69)	0.01	0.02	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/)
* Association estimates from Workalemahu et al 2013
£ Imputation quality score

Table 3.7.3. Association between genetic risk score generated using candidate SNPs (11 SNPs in 9 genes) and risk of placental abruption.

	Genetic Risk Score (GRS)				
	Quartile 1	**Quartile 2**	**Quartile 3**	**Quartile 4**	P-for-trend
Replication study (507 PA cases and 1,090 controls)					
Weighted Score Intervals	<12.6	12.6-13.8	13.9-15.0	≥15.1	
Cases, Number (%)	97 (19.1)	102 (20.1)	139 (27.4)	169 (33.3)	
Controls, Number (%)	273 (25.0)	272 (24.9)	274 (25.1)	272 (24.9)	
OR (95% CI)	1.00	**1.45 (1.04-2.02)**	**1.42 (1.02-1.98)**	**1.75 (1.27-2.42)**	< 0.001
Workalemahu et al 2013 study (470 PA cases and 473 controls)					
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

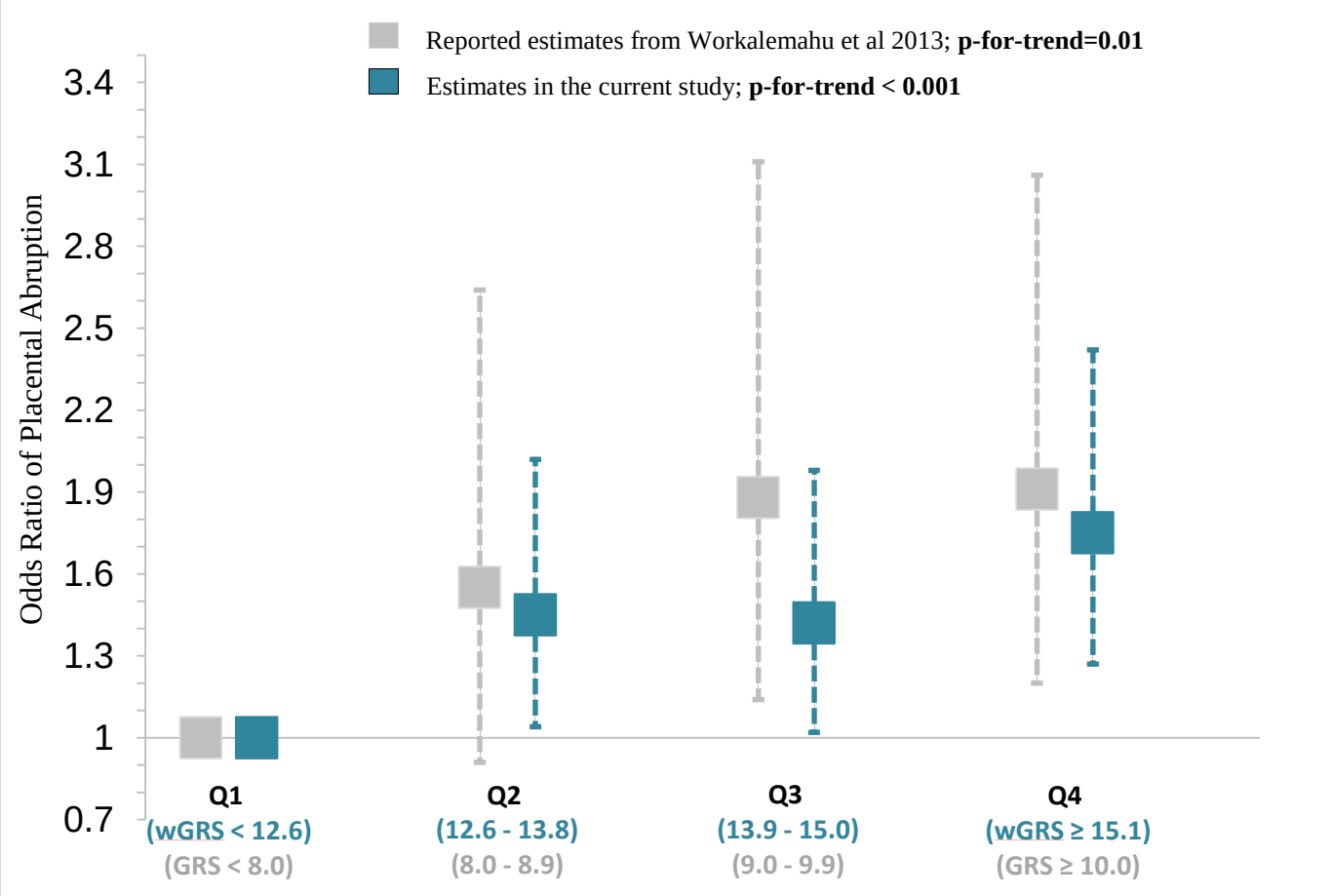
Table 3.7.4. Association between genetic risk score generated using candidate SNPs (11 SNPs in 9 genes) and risk of placental abruption stratified by preeclampsia, advanced maternal age, and infant sex characteristics.

	Genetic Risk Score (GRS)*				
	Quartile 1	**Quartile 2**	**Quartile 3**	**Quartile 4**	P-for-trend
Weighted Score Intervals	<12.6	12.6-13.8	13.9-15.0	≥15.1	
		Preeclamptics			
PA Cases, Number (%)	12 (38.7)	34 (63.0)	26 (66.7)	35 (68.6)	
Controls, Number (%)	19 (61.3)	20 (37.0)	13 (33.3)	16 (31.4)	
OR (95% CI)	1.00	**2.96 (1.15-7.65)**	**3.50 (1.27-9.65)**	**3.92 (1.48-10.36)**	**0.03**
		Normotensives			
PA Cases, Number (%)	80 (24.0)	98 (28.3)	97 (27.6)	118 (31.9)	
Controls, Number (%)	253 (76.0)	248 (71.7)	254 (72.4)	252 (68.1)	
OR (95% CI)	1.00	1.32 (0.93-1.87)	1.27 (0.89-1.80)	**1.57 (1.11-2.21)**	0.08
		Maternal age ≥ 35			
PA Cases, Number (%)	19 (29.2)	28 (37.8)	26 (32.9)	34 (42.5)	
Controls, Number (%)	46 (70.8)	46 (62.2)	53 (67.1)	46 (57.5)	
OR (95% CI)	1.00	1.56 (0.72-3.33)	1.31 (0.61-2.80)	**2.22 (1.05-4.69)**	0.19
		Maternal age 18-34			
PA Cases, Number (%)	71 (23.8)	104 (32.1)	98 (31.2)	120 (35.0)	
Controls, Number (%)	228 (76.3)	220 (67.9)	216 (68.8)	223 (65.0)	

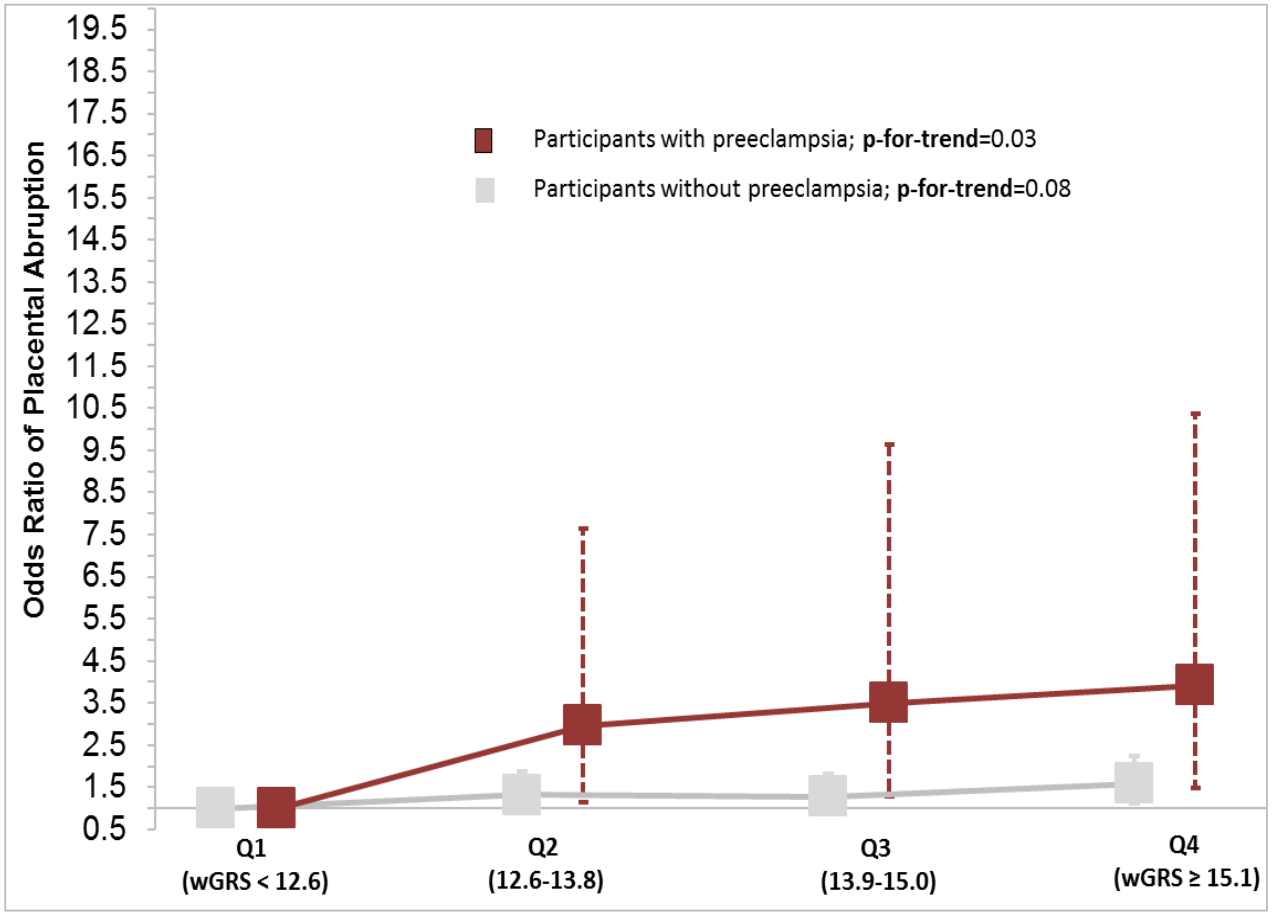
OR (95% CI)	1.00	**1.46 (1.01-2.11)**	**1.49 (1.02-2.15)**	**1.68 (1.17-2.41)**	**0.04**
		Male infant			
PA Cases, Number (%)	47 (24.0)	78 (37.1)	64 (29.8)	90 (39.3)	
Controls, Number (%)	149 (76.0)	132 (62.9)	151 (70.2)	139 (60.7)	
OR (95% CI)	1.00	**1.83 (1.16-2.87)**	1.36 (0.78-2.00)	**1.95 (1.24-3.07)**	0.004
		Female Infant			
PA Cases, Number (%)	44 (26.0)	55 (29.9)	60 (33.0)	63 (32.1)	
Controls, Number (%)	125 (74.0)	129 (70.1)	122 (67.0)	133 (67.9)	
OR (95% CI)	1.00	1.11 (0.68-1.81)	1.47 (0.91-2.39)	1.37 (0.85-2.20)	0.35

***** 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.
Statistically significant estimates are highlighted in **bold**

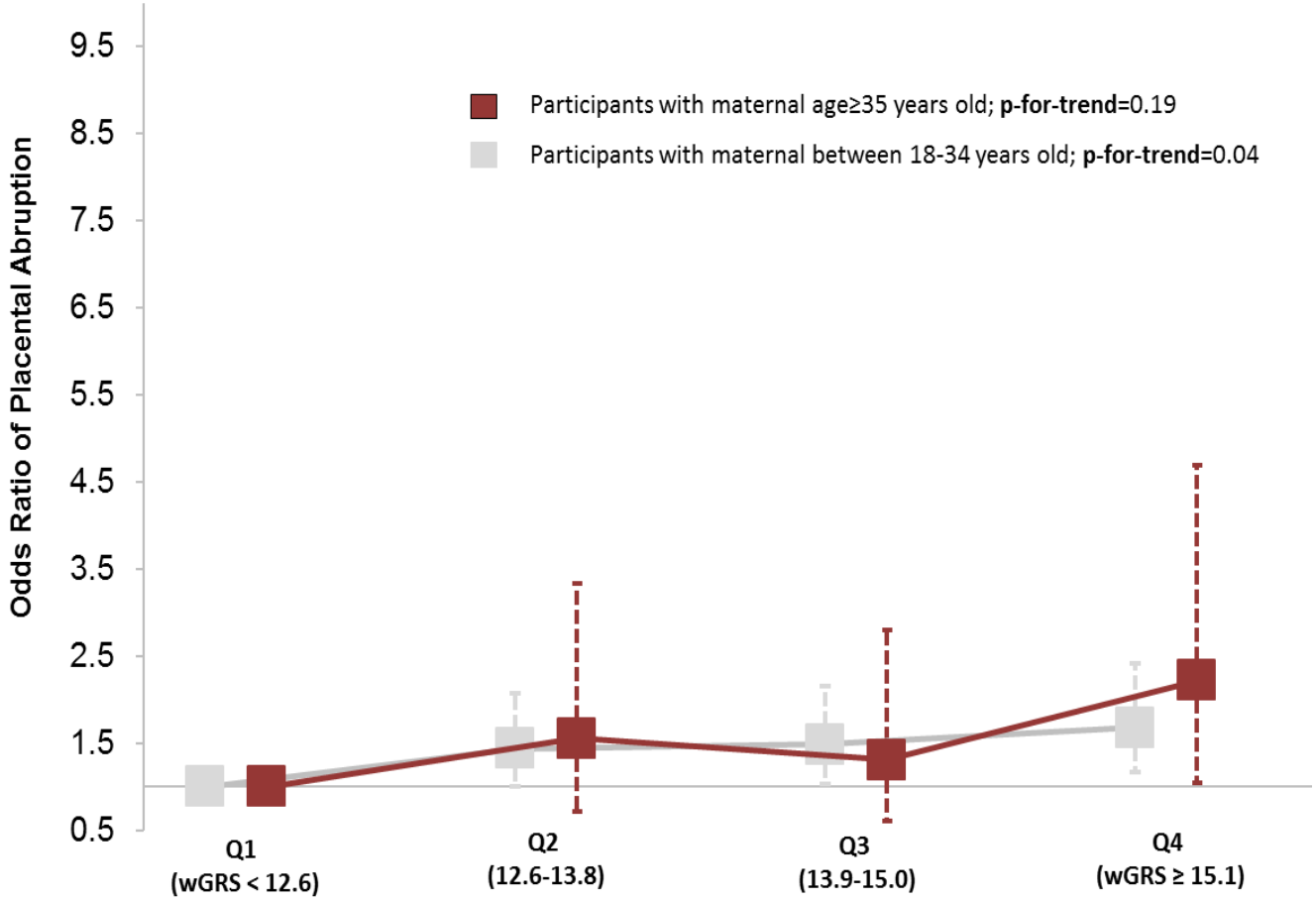
Supplementary Figure 3.7.1. Association between genetic risk score generated using candidate SNPs (11 SNPs in 9 genes) and risk of placental abruption



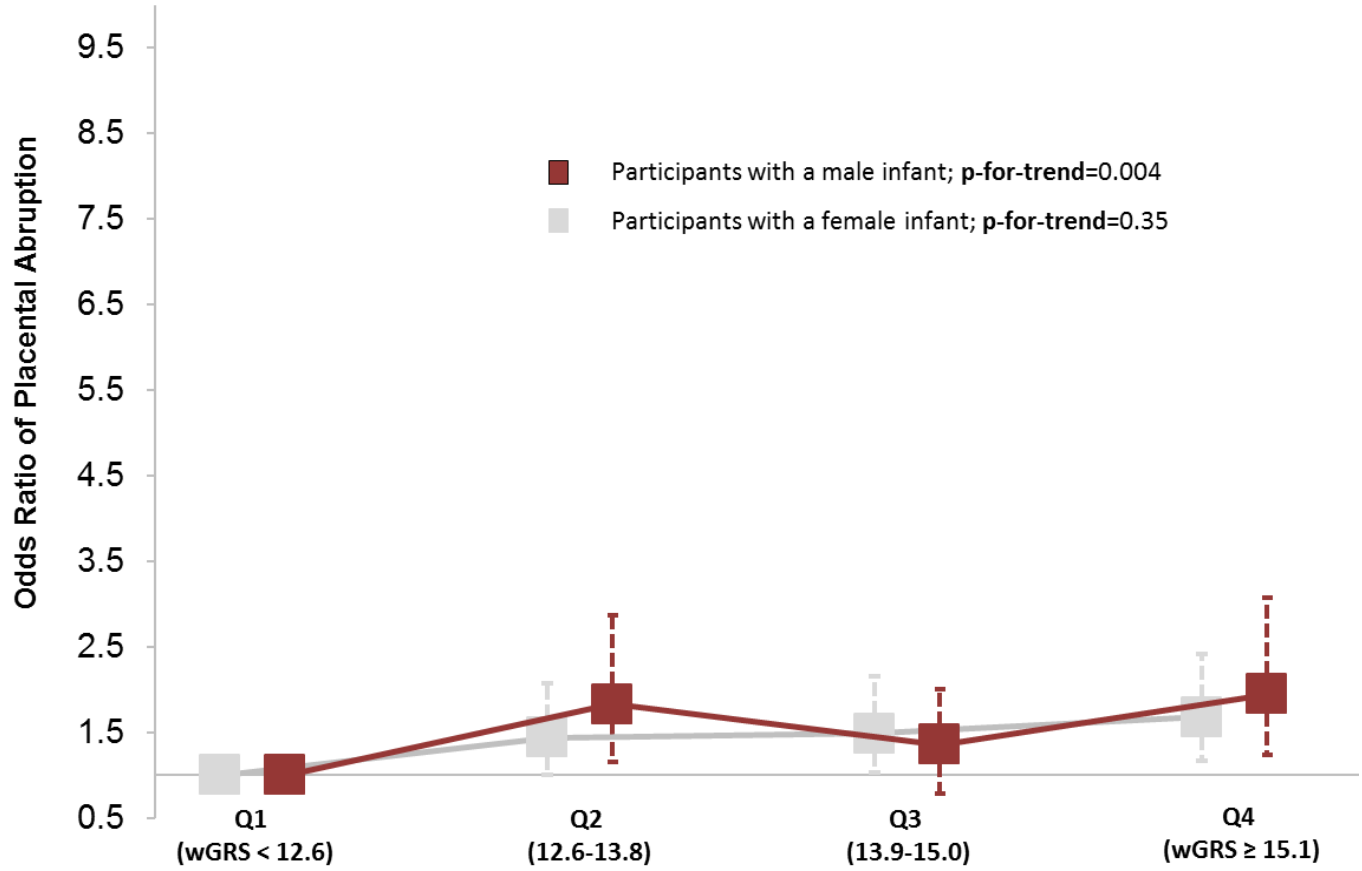
Supplementary Figure 3.7.2. Odds ratios and 95% confidence intervals for placental abruption risk in relation to quartiles of weighted-genetic risk scores (wGRS) computed from selected mitochondrial biogenesis and/or oxidative phosphorylation SNPs according to preeclampsia status. Lowest quartiles served as the reference group.



Supplementary Figure 3.7.3. Odds ratios and 95% confidence intervals for placental abruption risk in relation to quartiles of weighted-genetic risk scores (wGRS) computed from selected mitochondrial biogenesis and/or oxidative phosphorylation SNPs according to maternal age status. Lowest quartiles served as the reference group.



Supplementary Figure 3.7.4. Odds ratios and 95% confidence intervals for placental abruption risk in relation to quartiles of weighted-genetic risk scores (wGRS) computed from selected mitochondrial biogenesis and/or oxidative phosphorylation SNPs according to infant sex status. Lowest quartiles served as the reference group.



Chapter 4. Maternal-Fetal Genetic Interactions, Imprinting and Risk of Placental Abruption

4.1 Abstract

Background: Obstetrical complications such as placental abruption (PA) are consequences of a complex interplay of maternal and fetal genetic, epigenetic, metabolic, and environmental factors. While maternal genetic variations, including variations in mitochondrial biogenesis (MB) and oxidative phosphorylation (OP), have been associated with PA, the role of maternal-fetal genetic interactions and parent-of-origin effects (imprinting) in PA remain unknown.

Objective: We investigated interactions between maternal and fetal genes in MB-OP, and, imprinting effects in relation to risk of PA.

Methods: Among Peruvian women and infants (503 PA cases and 1,052 control mother-infant pairs) who participated in two genome-wide association studies of PA, with similar study design and study populations, independent single nucleotide polymorphisms (SNPs), with linkage-disequilibrium coefficient <0.80, were selected to characterize genetic variations in MB-OP (78 SNPs in 24 genes) and imprinted regions (2713 SNPs in 73 genes). For each MB-OP SNP, four multinomial models corresponding to fetal allele effect, maternal allele effect, maternal and fetal allele additive effect, and maternal-fetal allele interaction effect were fit under Hardy-Weinberg Equilibrium (HWE), random mating, and rare disease assumptions. The Bayesian Information Criterion (BIC) was used for model selection. For each SNP in imprinted regions, imprinting (parent-of-origin) effect on PA risk was tested using a likelihood ratio test. Bonferroni corrections were used to determine statistical significance (P-value <6.4e-4 for 78 maternal-fetal interaction tests and P-value <1.8e-5 for 2713 imprinting tests).

Results: Case mother-infant pairs were more likely to experience preeclampsia, have shorter gestational age and deliver infants with lower birthweight compared with control mother-infant pairs. Models with maternal-fetal interaction effects fitted better than models with only maternal and fetal genotype main effects for SNP rs12530904 (log-likelihood=-1874.6; P-value=1.2e-04) in *CAMK2B*, and, SNP rs73136795 (log-likelihood=-1644.5; P-value=1.9e-04) in *PPARG*, both MB genes. We identified 311 SNPs in 35 imprinted genes (including *KCNQ1*, *NPM*, and, *ATP10A*) with parent-of-origin effects on PA risk (with P-value<1.8e-5). Top hits included rs8036892 (P-value=2.3e-15) in *ATP10A*, rs80203467 (P-value=6.7e-15) and rs12589854 (P-value=1.4e-14) in *MEG8*, and rs138281088 in *SLC22A2* (P-value=1.7e-13).

Conclusion: We identified novel maternal-fetal MB gene interactions and imprinting effects of SNPs in imprinted regions in relation to PA risk. Current findings, along with those from similar studies, highlight the role of the fetus in PA risk development and can inform mechanistic investigations to understand the pathogenesis of PA.

4.2 Introduction

Placental abruption (PA), the premature separation of the placenta from the wall of the uterus, is a complex multifactorial and polygenic disease associated with significant maternal and neonatal morbidity and mortality[41 42]. Disturbances that involve mitochondrial biogenesis (MB) and the process of oxidative phosphorylation (OP), to generate cellular energy, underlie pathologic mechanisms leading to PA[25]. Genome-wide association (GWA) and candidate single nucleotide polymorphism (SNP) association studies have identified common maternal SNPs in several MB and OP genes that are associated with PA risk[36 39]. However, findings from previous

studies were inconsistent, in line with other previous reports of genetic associations in complex diseases[113 114].

Investigators have suggested that assessment of maternal-fetal genetic interactions and assessment of effects of imprinted genes, where risk is conferred depending on the parent-of-origin, may explain the missing heritability of complex diseases, including diseases with perinatal origin[113-115]. This is particularly important in the case of PA, a consequence of complex interplay of maternal and fetal genetics, epigenetics, and metabolic factors. For instance, the fetal genome influences placental growth and development, placental implantation and vascularization[14], all of which have been related to PA risk. In addition, many known imprinted genes affect embryonic or trophoblast growth[116] and have been implicated in preeclampsia[117], a known risk factor of PA[44]. Interactions between maternal and fetal genetic variations have previously been demonstrated in preterm delivery – another complex pregnancy complication[118]. However, only one prior study from our group examined maternal-fetal genetic interactions in relation to risk of PA[36]. Using 222 PA case 198 control maternal-placental pairs, Denis *et al* reported maternal-fetal genetic interactions on PA risk for two SNPs in the *PPARG* gene (chr3:12313450 and chr3:12412978) and imprinting effects for multiple SNPs in the *C19MC* and *IGF2/H19* regions[36]. Using the largest assembled mother-infant dyad of PA cases and controls (503 PA case and 1,052 control mother-infant pairs) to date, that includes participants from the previous report[4] and an expanded set of SNPs (using imputation) in MB-OP genes and imprinted regions, we investigated maternal-fetal genetic interactions and imprinting effects in relation to PA risk.

4.3 Methods

Study setting and study populations

The study was conducted among participants of the Peruvian Abruptio Placentae Epidemiology (PAPE) and Placental Abruption Genetic Epidemiology (PAGE) studies, case-control studies of PA conducted in Lima, Peru. Both PAPE and PAGE studies had similar study objectives and study designs. Details of the studies and findings of PAPE and PAGE (manuscript, under review) have been reported before[36-39]. Briefly, participants were recruited among women who were admitted for obstetrical services to antepartum wards, emergency room, and labor and delivery wards of participating hospitals. PAPE participants were recruited in August 2002-May 2004 and September 2006-September 2008. PAGE participants were recruited between March 2013 and March 2015. Participating hospitals of the PAPE study were Hospital Nacional Dos de Mayo, Instituto Especializado Materno Perinatal, and Hospital Madre-Niño San Bartolomé. Participating hospitals of the PAGE study include Instituto Nacional Materno Perinatal, Hospital Rebagliati, Hospital San Bartolome, Hospital Hipolito, Hospital Loayza, Hospital Dos de Mayo, and Hospital Maria Auxiliadrosa.

Participants who were less than 18 years of age, delivered nonsingleton infants, had medical records that were insufficient to determine the presence or absence of PA (described below), had other diagnoses associated with third trimester bleeding (e.g. placenta previa), or reported taking blood thinning medications were excluded from the studies. PAPE participants who provided maternal blood and placental samples at delivery were included in the current analyses. PAGE participants who provided maternal saliva and newborn buccal cells at delivery were included in the current analyses. The total number of participants included in the current analyses, after exclusions (described above) and sample quality control steps (described below),

were 176 PA case and 185 control maternal-infant pairs from the PAPE study and 327 PA case and 867 control mother-infant pairs from the PAGE study. A total of 503 PA case and 1,052 control mother-infant pairs were included in the current analyses. Study protocols of both studies were approved by the Institutional Review Boards (IRBs) of participating institutions and the Swedish Medical Center, Seattle, WA, where the studies were based. All participants provided written informed consent.

Data collection

PAPE and PAGE study participants were interviewed by trained personnel using standardized structured questionnaires to collect information on sociodemographic characteristics and risk factors including maternal age, marital status, employment status during pregnancy, medical history, smoking, and alcohol consumption (both current and pre-pregnancy). Information on the course and outcomes of the pregnancy and ascertainment of PA case/control status were abstracted from maternal medical records. A diagnosis of PA in both studies was determined through review of emergency room admission logbooks, labor and delivery admission logbooks, and the surgery report book, based on evidence of retro-placental bleeding (fresh blood) entrapped between the decidua and the placenta or blood clots behind the placental margin, accompanied by any two of the following: (i) vaginal bleeding at ≥20 weeks in gestation that is not due to placenta previa or cervical lesions; (ii) uterine tenderness and/or abdominal pain (without other causes, such as those due to hyperstimulation from pitocin augmentation); and, (iii) non-reassuring fetal status or fetal death. The corresponding control participants, who did not have a diagnosis of PA in the current pregnancy, were randomly selected from eligible pregnant women who delivered at the participating hospitals during the respective study periods.

In PAPE, maternal blood was obtained, and placentas were collected immediately after delivery, weighed, double bagged and transported in coolers. Tissue biopsies (approximately 0.5 cm^3 each) were obtained from 8 sites (4 maternal and 4 fetal) by stripping the chorionic plate and overlying membranes. The biopsy samples were taken from the fetal side and sampled for genomic DNA extraction by placing them in cryotubes, snap frozen in liquid nitrogen, and stored at −80°C until analysis. In PAGE, maternal saliva and newborn buccal cells were collected, plated and stored using the Oragene™ saliva cell collection kits (OGR500 and OGR250, DNA Genotek Ottawa, Canada), for DNA extraction and genotyping.

DNA extraction, genotyping, data quality control and imputation

Genomic DNA extraction in the PAPE study were conducted using Gentra PureGene Cell kit (Qiagen, Hilden, Germany). SNP genotyping to characterize genome-wide variations were performed using Illumina Cardio-Metabochip (Illumina Inc, San Diego, CA) platform. In the PAGE study, genomic DNA were extracted using the Qiagen DNAeasy™ system and manufacturer protocols (Qiagen, Valencia, CA). SNP genotyping to characterize genome-wide variations were performed using Illumina HumanCore-24 BeadChip (Illumina Inc., San Diego, CA) platform.

Maternal and fetal SNP data quality control procedures were applied using identical criteria in PAPE and PAGE studies before data analyses. SNPs were excluded if they had excessive missing genotype (SNPs with genotype call rate of <95%), deviated from Hardy-Weinberg equilibrium (HWE; p<1e-05), and had low minor allele frequency (MAF<0.05). The total number of SNPs, directly genotyped, that remained for further analysis in PAPE and PAGE study were 128,371 and 241,301, respectively. Maternal-fetal pairs (PAPE n=23; PAGE n=10) were excluded if they were duplicates or related (Identity by Decent [IBD] value>0.9), had more

than 5% of genotyping failure rate (PAPE n=45; PAGE n=51), and had excess heterozygosity rate (outside the range of mean ± 3 standard deviations of heterozygosity rate; PAPE n=5; PAGE n=15). The total number of individuals that remained for further analysis were 503 cases and 1052 control maternal-placental pairs. PAGE and PAPE genotype data were then imputed using identical criteria to infer unobserved genotypes. The data were phased using SHAPEIT [50] to infer haplotypes and improve imputation accuracy using the 1000 Genomes haplotypes. Phased haplotypes were then used to impute the non-typed SNPs using IMPUTE2 [51]. After imputation and further quality control (filtering SNPs with imputation certainty score (Info) <0.3, HWE <0.00001, genotyping call rate <0.05, and MAF <0.05), a total of 5,553,176 and 5,314,631 SNPs were available for selection of genes and SNPs (described below) in the PAPE and PAGE studies, respectively.

Candidate and imprinted genes/SNP selection

Candidate genes with described functions in MB and OP were selected from previously published studies[39 82 87-91].Among 785 (in 101 MB-OP) and 359 SNPs (in 26 MB-OP genes) that were genotyped/imputed in the PAPE and PAGE study, respectively, 322 overlapping SNPs (in 24 MB-OP genes) were selected for the current analyses. Pair-wise linkage disequilibrium (LD) was assessed between SNPs within the set of genes using SNAP[119]. A total of 78 independent SNPs (LD<0.80 in the set) in the 24 MB-OP genes that overlap between PAPE and PAGE (see **Supplementary Table 4.7.1**) were selected for maternal-fetal interaction analysis. Similarly, a total of 12,459 SNPs in 83 imprinted genes from PAPE study and 10,030 SNPs in 78 imprinted genes from PAGE study were identified using online database[120]. Out of 9,666 SNPs in 73 imprinted genes that overlap between PAPE and PAGE, a total of 2,713 independent SNPs were selected for imprinting analyses.

Statistical analyses

Mean and standard deviations for continuous variables and proportions for categorical variables were used to compare the characteristics of PA cases and control participants. We used EMIM[121] and PREMIM[122], to estimate and test parental and child genetic effects including maternal-fetal genetic interaction and parent-of-origin effects. Biological assumptions, such as Hardy-Weinberg equilibrium (HWE), random mating, and rare disease were made.

For each SNP, similar to Denis et al.[36], four models corresponding to allele effects operating only at fetal level (Model F), allele effects operating at maternal level (Model M), an additive model of maternal and fetal effects (Model M+F), and a model that includes a maternal-fetal interaction effect (Model I) were considered. For the latter, we applied a parametrization that introduces two interaction terms capturing incompatibility between maternal and fetal genotypes; the interaction effects operate when the infant has one copy and the mother has either zero or two copies of the risk allele. The Bayesian information criterion (BIC) was used for model selection. In addition to the maternal and fetal genotype effects, we estimated the risk ratio (RR) of disease when the infant has 1 copy and the mother has zero copies of the risk allele. The reference groups were mother-infant pairs carrying zero copies of the risk allele. Imprinting/parent-of-origin effect, which corresponds to the factor multiplying the disease risk if the infant inherits a risk allele from the mother, was tested using a likelihood ratio test. The Bonferroni correction was applied to correct for multiple testing (P-value <6.4e-4 for the 78 maternal-fetal interaction tests, and P-value <1.8e-5 for 2,713 imprinting effect tests). Statistical analyses software used in these analyses included PREMIM[122], EMIM[122], R (version i386 3.1.2) and SAS (Version 13).

4.4 Results

Overall PAPE and PAGE study participants (cases and controls) were similar in socio-demographic characteristics and medical/obstetric history (**Table 4.7.1**). PAPE and PAGE PA-case mother-infant pairs were similar to control mother-infant pairs with respect to maternal age, marital status, employment, planned pregnancy, infant sex, alcohol use, drug use and vitamin use. Compared to control mother-infant pairs, PA case mother-infant pairs were more likely to smoke during pregnancy, with lower educational attainment, have lower pre-pregnancy body mass index, deliver earlier (i.e., shorter gestational age), deliver infants with lower birth weight, and have a diagnosis of preeclampsia in the current pregnancy.

Among the 78 SNPs in 24 MB-OP genes evaluated, we identified evidence for maternal-fetal genetic interactions on PA risk for two SNPs in two MB genes (**Tables 4.7.2** and **4.7.3**). For these SNPs, BIC for Model I was the smallest (**Table 4.7.2**), suggesting models with maternal-fetal interaction effects fitted better than models with additive maternal and fetal genotype main effects. The SNPs were rs12530904 in *CAMK2B* (log-likelihood=-1874.6; P-value=1.2e-04) and rs73136795 in *PPARG* (log-likelihood=-1644.5; P-value=1.9e-04). The risk of PA associated with maternal-fetal genotype combination of GG/AG at the rs12530904 locus was 1.79 (95%CI: 1.19, 2.69) relative to the maternal-fetal genotype combination of GG/GG. Similarly, the risk of PA associated with maternal-fetal genotype combination of GG/AG at the rs73136795 locus was 2.58 (95%CI: 1.64, 4.07) relative to the maternal-fetal genotype combination of GG/GG. Two other SNPs showing Model I as best fitting model (**Tables 4.7.2 and 4.7.3**), although the p-values were not statistically significant after Bonferroni correction, are rs12535537 in *CAMK2B* (log-likelihood=-1965.8; P-value=1.6e-3) and rs35812816 in *PPARG* (log-likelihood=-1752.2; P-value=0.01). The risk of PA associated with maternal-fetal genotype combination of GG/AG at

rs12535537 locus was 1.87 (95%CI: 1.26, 2.77) relative to GG/GG maternal-fetal genotype combination of rs12535537. The risk of PA associated with maternal-fetal genotype combination of GG/CG at rs35812816 was 1.69 (95%CI: 1.15, 2.50) relative to its GG/GG maternal-fetal genotype combination.

We identified 310 SNPs in 31 imprinted genes with parent-of-origin effects (224 maternally expressed, 79 paternally expressed, 3 isoform-dependent, and 4 random) on PA risk that reached statistical significance after Bonferroni correction (**Table 4.7.4 and Supplementary Table 4.7.2**). These imprinted genes included *KCNQ1* (103 SNPs), *NTM* (30 SNPs), and, *ATP10A* (24 SNPs). Top hits in these analyses were rs8036892 (P-value=2.3e-15) in *ATP10A*, rs80203467 (P-value=6.7e-15) and rs12589854 (P-value=1.4e-14) in *MEG8,* and rs138281088 in *SLC22A2* (P-value=1.7e-13) (**Table 4.7.4**).

4.5 Discussion

In the current study, we identified several novel maternal-fetal MB gene interactions and imprinting effects on PA risk. Maternal-fetal interactions were observed for SNPs in *CAMK2B* (rs12530904) and *PPARG* (rs73136795). Maternal-fetal interactions were observed for two other SNPs in the same genes (rs12535537and rs35812816 in *CAMK2B* and *PPARG*, respectively), although interactions were not statistically significant after Bonferroni correction. Parent-of-origin effects were observed for 310 SNPs in imprinted genes including *KCNQ1, NTM, ATP10A, MEG8,* and *SLC22A2*.

In the only other similar published study related to PA, our team reported maternal-fetal interaction for two *PPARG* SNPs (chr3:12313450 and chr3:12412978) on PA risk, and imprinting effect on PA risk for six SNPs in the *C19MC* region and two SNPs in *IGF2-H19*[36].

While we found maternal and infant interactions on PA risk for *PPARG* SNP rs73136795 (chr3:12468410), the two previously reported *PPARG* SNPs were not in the set of SNPs we evaluated in the current study as they failed imputation quality (Info<0.3) in the PAGE study. Similarly, we identified imprinting effect on PA risk for *IGF2* SNP rs11564732 (chr1:2150895, p-value=9.3e-06); however, the previously reported SNPs in *C19MC* or *IGF2-H19* genes were not evaluated in the current study because they were not genotyped/imputed in the PAGE study. Other studies have previously investigated interactions between maternal and fetal genetic variations on maternal and infant outcomes[118 123 124]. For instance, interaction between maternal and fetal genetic variations at the G308A locus of *TNF-alpha* gene on risk of preterm delivery (PTD) risk has been reported in Han Chinese families of 250 PTD cases and 247 controls[118]. The combined maternal-fetal genotype GA/GA at the locus was associated with reduced risk of PTD (risk ratio=0.20 [95%CI: 0.07, 0.58])[118]. Lupo et al[123] identified interaction between two SNPs in metabolic genes, maternal rs1044498 in *ENPP1* and fetal rs6785233 in *SLC2A2*, on risk of neural tube defects (odds ratio=3.65 [95%CI: 2.32, 5.74]). Their findings suggest that maternal metabolic genes associated with hyperglycemia and insulin resistance and fetal metabolic genes involved in glucose homeostasis may interact to increase risk of neural tube defects on the fetus. Goddard et al[124] observed evidence for maternal-fetal interaction at the rs5742620 loci in *IGF1* gene on preeclampsia risk. Maternal-fetal pairs with AC genotype at this locus had a 2.4-fold (p-value=0.0035) increased risk of preeclampsia compared to maternal-fetal pairs where both individuals had the CC genotype[124]. None of the SNPs investigated by the previous studies were evaluated in our study. However, findings support possibility of maternal-fetal genetic interactions in pregnancy complications.

Our finding for *PPARG* maternal-fetal interaction on PA risk is noteworthy, not only because we found similar, although in different SNPs, maternal-fetal interaction in Denis *et al* study, but also because the gene has been well-described in relation to placental growth, development, and function[27 112]. *PPARG* (peroxisome proliferator-activated receptor gamma) belongs to the PPAR-family of genes and is a master regulator of MB and highly expressed in the placenta. *PPARG* mediates abnormal placentation (e.g., inhibition of trophoblast invasion) through oxidized LDL in cytotrophoblasts of cells involved in invasion of the uterus[27 112] directly involved in the development of preeclampsia[112], established risk factor of PA[44]. The other gene where we found significant maternal-fetal interactions was *CAMK2B* (calcium/calmodulin-dependent protein kinase [CaM kinase] II beta), a CaMK family gene implicated in contraction-induced regulation of calcium handling in skeletal muscle and MB[125 126]. SNPs chr7:44226231, rs2075076 and rs1127065 in *CAMK2B* suggestively showed maternal-placental genetic interaction on PA risk as best fitting model by our group[36]. Only rs1127065 was available in the current study and did not show maternal-fetal genetic interaction on PA risk as the best fitting model. Another candidate gene study conducted by our group showed *CAMK2B* may influence PA risk[39]. In addition, *CAMK2B* is among several genes in myometrial relaxation and contraction pathways that are either transcribed in myometrial muscle cells or act upon the myometrium to regulate contraction[127]. Another study demonstrated that *CAMK2B* is involved in smooth muscle contractions through oxytocin receptor activation[128].

We found strong evidence for parent-of-origin effect of several imprinted genes in the current study. Imprinted genes may affect maternal-fetal interactions that contribute to imbalances and disruption of placental development[10]. For instance, imprinted maternal alleles are required for the development of the embryo, and imprinted paternal alleles regulate formation

of the placenta and the surrounding membranes of the embryo[8 116]. Imprinted genes with parent-of-origin effect on PA risk that were highly represented in our study include *KCNQ1, NTM,* and *ATP10A. KCNQ1* encodes a voltage-gated potassium channel required for repolarization phase of the cardiac action potential[129]. *KCNQ1* is expressed in the placenta and implicated in embryonic and placental growth[130]. In mice, maternally inherited target deletion of *ASCL2*, a gene that resides in *KCNQ1* cluster, is demonstrated to be embryonic lethal at E10.5 due to failure of placental formation, further evidencing the role of *KCNQ1* on placental development [131 132]. SNP rs2237892 in *KCNQ1* was associated with increased risk of gestational diabetes mellitus (GDM) (OR=1.99 [95%CI: 1.26, 3.15]) in a case-control study of Chinese 562 GDM cases and 453 controls[133]. *NTM* and its potential role in pregnancy-related outcomes has not been described before. However, a genome-wide expression analysis found that *NTM* (neurotrimin), involved in neuronal cell adhesion, is expressed in the placenta[134]. *ATP10A*, ATPase phospholipid transporting 10A gene, is expressed in the placenta[61 135]. *ATP10A* was among several top PA GWAS hits representing networks of energy production, molecular transport, and nucleic acid metabolism functions[36]. The imprinting/parent-of-origin effects of *KCNQ1*, *NTM,* or *ATP10A* on pregnancy-related outcomes have not been reported before. We found a parent-of-origin effect for rs11564732 in *H19,* a maternally imprinted gene near *IGF2,* for which we previously reported similar parent-of-origin effects. *H19/IGF2* regulates the development of the embryo and differentiation of cytotrophoblast cells[136] , and was implicated in preeclampsia[137 138], a known risk factor of PA[44].

In post-hoc exploratory analyses, we examined functions and functional relationships of the 35 imprinted genes that were represented by SNPs with significant parent-of-origin effects using ingenuity Pathway Analysis (IPA, Ingenuity, Redwood, CA)[59]. In the IPA analysis based

on the Ingenuity Pathways Knowledge Base (IPKB), gene-enrichment of networks was assessed using network score, negative log of P-values of a modified Fisher's exact test. Based on these analyses, the top two enriched gene networks were a network (Score=29) of cell cycle, cell morphology (**Supplementary Table 4.7.3 and Supplementary Figure 4.7.1**), and a network (Score=29) of cardiovascular disease and free radical scavenging (**Supplementary Table 4.7.3 and Supplementary Figure 4.7.2**). Both of these networks align well with what is known about PA pathogenesis.

The underlying genetic architecture of PA has been examined by previous GWA[36 37 39] and candidate gene studies[36-39 42], which reported predominantly common, non-coding variants with modest effects and limited replication. Important strategies to address subsequent missing heritability include family studies that assess gene-gene interactions and parent-of-origin effects[113 114]. Family studies permit investigation of gene-gene interaction in families because affected relatives are more likely to share two nearby epistatic loci in LD that would be unlinked in unrelated individuals[114]. Additionally, family studies also permit investigations of parent-of-origin effects, where ignoring such effects can mask true associations and diminish the proportion of heritability explained[114]. Other sources of missing heritability may be low frequency variants of intermediate effect. These should be tractable through larger sized studies and imputation of GWA data[114]. Using the largest family study of PA (mothers and their offspring), our study is the most comprehensive investigation, to date, of maternal-fetal interactions and imprinting effects on PA risk.

This study has the potential for enhancing our understanding of genetic variations in maternal and fetal genome that contribute to PA, a multi-factorial heritable disorder. The study was conducted using study population of the PAPE study, reported before, along with a new

study population, participants of the PAGE study, addressing the potential limitations of sample size in previous studies. By conducting 1000 genomes genotype imputations, we analyzed a comprehensive set of SNPs in MB-OP and imprinted genes. However, in the current study, SNPs that did not overlap between PAPE and PAGE were excluded, not allowing us to examine some previously reported SNPs. Other limitations of our study include potential misclassification of sub-clinical PA (i.e. those with less placental disrupter and consequently bleeding), which may limit the interpretation of the study results or reduce statistical power. We also did not distinguish between severe and mild cases of PA, which may have different risk factors or underlying mechanism[77]. We assessed maternal-fetal genetic interaction on PA risk using MB-OP candidate SNPs. There could be similar interactions in other metabolic functions. This study may still be underpowered for small effects and rare genotypes. Finally, findings from the current study population may not be generalizable to other populations with different population genetic structure or PA risk pattern. Therefore, replication studies in different population are critical to fully understand maternal-fetal genetic interactions and imprinting effects on PA risk.

In sum, findings in this study confirm the role of interactions between maternal and fetal genetic variations in mitochondrial biogenesis and imprinting in PA. These findings highlight the potential of understanding the complex interplay between maternal and fetal genetic factors in explaining the missing heritability of PA and PA-related risk stratification. Studies that incorporate mitochondrial biogenesis and oxidative phosphorylation related metabolomics, epigenetic, or proteomic investigations may inform potential preventative or therapeutic targets of placental abruption.

4.6 References

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4.7 Tables and Figures

Table 4.7.1. Selected characteristics of study participants

	PAPE mother-infant pairs		PAGE mother-infant pairs		Combined mother-infant pairs	
	Cases (N=176)	Controls (N=185)	Cases (N=327)	Controls (N=867)	Cases (N=503)	Controls (N=1052)
Characteristics	% or mean±SD	% or mean±SD	% or mean±SD	% or mean±SD	% or mean±SD	% or mean±SD
Maternal age at delivery (years)[1]	26.6±6.4	27.4±6.5	28.4±6.8	27.3±6.6	27.8±6.7	27.3±6.6
Maternal age at delivery (years)						
18-19	13.9	11.1	8.4	12.4	10.3	12.1
20-29	54.9	51.1	48.8	52.1	50.9	51.9
30-34	17.9	20	20.5	19	19.6	19.2
≥35	13.3	17.8	22.4	16.6	19.2	16.8
Education ≤high school	80.1	77.7	68	75.2	72.3	75.6
Married/living with partner	79.6	83.7	85.8	87.3	83.6	86.6
Employed during pregnancy	44.9	48.7	57.1	53.8	52.8	52.9
Pre-pregnancy body mass index (BMI) (kg/m^2)	23.4±3.5	23.9±4.0	24.9±4.6	25.3±4.6	24.9±4.3	25.1±4.5
Pre-pregnancy BMI (kg/m^2)						
Lean (< 18.5)	6.1	4	2.8	2	3.9	2.3
Normal (18.5-24.9)	68.3	62.7	56.9	55.9	60.7	57.1
Overweight (24.9-30.0)	19.5	25.4	29.4	29	26	28.4
Obese (≥30.0)	6.1	7.9	10.9	13.2	9.3	12.2
Planned pregnancy	40	45.6	38.8	32.4	39.2	34.7
Smoked during pregnancy	4.7	1.1	1.2	1	2.4	1.1
Alcohol use during pregnancy	1.7	0	3.4	2.8	2.8	2.3
Drug abuse during pregnancy	0.6	0	0.6	0.2	0.6	0.2
Vitamins use during pregnancy	70.3	69.2	85.5	85.9	80.1	82.9
Preeclampsia	20	13	19.8	5.6	19.9	6.9
Gestational age at delivery[1]	35.0±4.3	37.9±3.3	34.8±4.3	39.1±1.2	34.9±4.3	38.8±1.9
Male infant	58.3	51.4	54.9	52.1	56.1	52.0
Infant birthweight (grams)[1]	2337.3±874.6	3075.8±807.4	2437.6±937.1	3427.6±473.6	2402.1±915.9	3365.6±563.1

[1] mean ± standard deviation

Table 4.7.2. SNPs selected with maternal-fetal interaction as best fitting model.

		Log Likelihood					Likelihood Ratio Test					Bayesian Information Criterion (BIC)			
Gene	**rsID**	**Model Null**	**Model F**	**Model M**	**Model M+F**	**Model I**	**Model F vs Model Null**	**Model M vs Model Null**	**Model M+F vs Model M**	**Model M+F vs Model F**	**Model I vs Model M+F**	**Model F**	**Model M**	**Model M+F**	**Model I**
CAMK2B	rs12535537	-1975.1	-1975.0	-1972.3	-1972.3	-1965.8	0.30	5.66	0.10	5.46	12.84	3964.7	3959.3	3959.2	3946.4
CAMK2B	rs12530904	-1883.9	-1883.9	-1879.4	-1878.8	-1874.6	6.0E-05	8.89	1.31	10.19	18.50	3782.4	3773.5	3772.2	3763.9
PPARG	rs73136795	-1655.3	-1655.3	-1653.3	-1653.1	-1644.5	0.02	4.12	0.43	4.54	21.72	3325.3	3321.2	3320.8	3303.6
PPARG	rs35812816	-1789.5	-1766.1	-1788.5	-1756.7	-1752.2	46.86	2.02	63.56	18.72	8.96	3546.8	3591.7	3528.1	3519.1

Likelihood ratio test: 2 x (Log Likelihood of the 1st model – Log likelihood of the 2nd model)
BIC = -2 x Log Likelihood + #of estimated parameters x ln (#of observations)

Table 4.7.3. Association estimates of SNPs selected with maternal-fetal interaction as best fitting model.

						Main Effect Estimates		Interaction Effect Estimates		
Gene	**chr:pos**	**rsID**	**Risk Allele**	**Other Allele**	**Risk Allele Frequency**	**R1 (95% CI)**	**S1 (95% CI)**	**γ_{01} (95% CI)**	**γ_{21} (95% CI)**	**P-value**
CAMK2B	7:44376785	rs12535537	A	G	0.16	1.05 (0.87, 1.27)	1.25 (1.04, 1.51)	1.87 (1.26, 2.77)	1.47 (0.85, 2.55)	1.6E-03
CAMK2B	7:44377171	rs12530904	A	G	0.14	1.00 (0.82, 1.22)	1.34 (1.11, 1.61)	1.79 (1.19, 2.69)	1.18 (0.66, 2.12)	1.2E-04*
PPARG	3:12468410	rs73136795	A	G	0.12	0.99 (0.79, 1.23)	0.79 (0.63, 0.99)	2.58 (1.64, 4.07)	0.77 (0.27, 2.24)	1.9E-04*
PPARG	3:12439348	rs35812816	C	G	0.17	0.51 (0.42, 0.62)	1.16 (0.94, 1.45)	1.69 (1.15, 2.50)	1.47 (0.42, 5.00)	1.1E-02

chr:pos : dbSNP build 37 hg19 chromosome:position
R1: Risk of PA associated with a copy of fetal risk allele (Fetal genotype effect)
S1: Risk of PA associated with a copy of maternal risk allele (Maternal genotype effect)
γ_{01}: Risk of PA associated with 1 copy of risk allele from fetus and zero copy of the risk allele from the mother
γ_{21}: Risk of PA associated with 1 copy of risk allele from fetus and 2 copies of the risk allele from the mother
* statistically significant after Bonferroni correction for multiple testing

Table 4.7.4. SNPs of imprinted genes with parent-of-origin effect on PA risk (top 15 SNPs out of 311 that were significant after Bonferroni correction [p-values<1.5e-5])

Gene	chr:pos	rsID	Risk Allele	Risk Allele Frequency	Expressed Allele	P-value
ATP10A	15:25973151	rs8036892	G	0.100	Maternal	2.3E-15
MEG8	14:101365390	rs80203467	T	0.150	Maternal	6.7E-15
MEG8	14:101362418	rs12589854	G	0.200	Maternal	1.4E-14
SLC22A2	6:160656533	rs138281088	C	0.150	Maternal	1.7E-13
SLC22A18	11:2942799	rs384898	C	0.110	Maternal	2.5E-13
SNRPN	15:25169683	rs2736696	A	0.230	Paternal	1.9E-12
SNORD115_10	15:25434275	rs1977035	T	0.200	Paternal	2.4E-12
SGK2	20:42193900	rs3827067	G	0.150	Paternal	6.3E-12
SGK2	20:42195446	rs73618902	A	0.120	Paternal	9.2E-12
MAGI2	7:77703542	rs3807754	G	0.110	Maternal	1.1E-11
SLC22A2	6:160656532	rs200258104	T	0.150	Maternal	1.4E-11
SLC22A18	11:2942798	rs426359	G	0.110	Maternal	2.7E-11
KCNQ1	11:2584917	rs12221520	T	0.170	Maternal	4.1E-11
SNRPN	15:25175735	rs377264185	T	0.110	Paternal	5.0E-11
KCNQ1	11:2739521	rs10832572	G	0.160	Maternal	5.5E-11

chr:pos: build 37/hg19 chromosome position

Supplementary Table 4.7.1. Mitochondrial biogenesis and/or oxidative phosphorylation pathway genes evaluated in the current study

Gene	Chromosome: Position	rsID	Risk Allele	Risk Allele Frequency
ACP2	11:47271039	rs16938581	A	0.316
APOH	17:64226140	rs3760292	G	0.256
APOH	17:64231775	rs17769776	C	0.126
APOH	17:64239807	rs12952866	G	0.230
APOH	17:64257044	rs55908798	T	0.246
APOH	17:64280153	rs56152251	A	0.290
APOH	17:64280503	rs62069916	T	0.350
APOH	17:64286494	rs9897002	A	0.504
ATP5G2	12:54073069	rs7976793	T	0.497
C15orf17	15:75207729	rs146341141	T	0.143
CAMK2B	7:44259871	rs1127065	T	0.275
CAMK2B	7:44261978	rs732360	A	0.372
CAMK2B	7:44263456	rs1003573	T	0.336
CAMK2B	7:44374927	rs11976245	G	0.211
CAMK2B	7:44375318	rs4074784	T	0.188
CAMK2B	7:44376785	rs12535537	A	0.157
CAMK2B	7:44376833	rs12535503	T	0.127
CAMK2B	7:44377171	rs12530904	G	0.144
CAMK2B	7:44405210	rs79653744	T	0.114
CAMK2D	4:114439090	rs17531026	C	0.333
CAMK2D	4:114678642	rs10006113	A	0.098
CAMK2D	4:114742249	rs7659107	G	0.321
COX5A	15:75224193	rs78132788	A	0.150
COX5A	15:75224385	rs74718433	G	0.150
COX5A	15:75225415	rs8042694	G	0.133
COX5A	15:75226086	rs12437831	G	0.143

COX5A	15:75230502	rs6495131	A	0.134
COX5A	15:75242041	rs2415250	A	0.452
COX6B1	19:36142187	rs7991	T	0.197
COX7A1	19:36652185	rs11665903	A	0.340
COX7A1	19:36654529	rs474995	A	0.153
FST	5:52783356	rs6450138	G	0.227
LRPPRC	2:44135314	rs13387221	A	0.326
LRPPRC	2:44191555	rs11124953	A	0.249
LRPPRC	2:44354495	rs896986	A	0.222
NDUFA12	12:95386791	rs11107847	A	0.434
NDUFA12L	5:60348046	rs42437	G	0.153
NDUFS3	11:47606865	rs12287076	G	0.196
NDUFS3	11:47607912	rs77465292	C	0.229
NOA1	4:57838583	rs17087335	T	0.285
PPA2	4:106390734	rs2298733	C	0.172
PPA2	4:106394380	rs2074396	A	0.336
PPARG	3:12329758	rs17029009	T	0.112
PPARG	3:12334416	rs2972164	T	0.114
PPARG	3:12334555	rs6782178	T	0.184
PPARG	3:12377344	rs4518111	A	0.285
PPARG	3:12383599	rs75512179	C	0.110
PPARG	3:12413339	rs2120825	G	0.255
PPARG	3:12414290	rs73025259	T	0.247
PPARG	3:12439348	rs35812816	G	0.170
PPARG	3:12455034	rs709152	G	0.450
PPARG	3:12468410	rs73136795	A	0.125
PPARGC1A	4:23801187	rs12650562	T	0.263
PPARGC1A	4:23968295	rs10517031	G	0.145
PPARGC1A	4:23976060	rs1316862	G	0.105
PPARGC1A	4:24028621	rs630902	C	0.232
PPARGC1A	4:24268934	rs1511358	T	0.447

PPARGC1A	4:24379666	rs3857106	C	0.279
PRKCA	17:64300114	rs12936396	T	0.310
PRKCA	17:64300281	rs12945884	C	0.342
PRKCA	17:64312983	rs78510726	A	0.316
PRKCA	17:64323714	rs7207103	C	0.348
PRKCA	17:64326021	rs11651447	T	0.433
PRKCA	17:64331601	rs9903921	T	0.161
PRKCA	17:64438011	rs1005651	C	0.291
PRKCA	17:64482151	rs7225164	A	0.473
PRKCA	17:64526395	rs1806448	G	0.102
PRKCA	17:64535138	rs10512513	G	0.396
PRKCA	17:64563520	rs7220007	A	0.358
THRB	3:24166164	rs7609948	T	0.231
THRB	3:24267842	rs12491199	G	0.231
THRB	3:24306302	rs826230	A	0.452
TMCC3	12:95201306	rs10859756	G	0.098
TMCC3	12:95355541	rs7306455	A	0.217
TUFM	16:28863451	rs72793815	G	0.216
TUFM	16:28871860	rs11861132	A	0.216
UCP1	4:141481581	rs6536991	C	0.348
UQCRFS1	19:29730014	rs11670999	A	0.449

chr:pos: build 37/hg19 chromosome position

Supplementary Table 4.7.2. SNPs of imprinted genes with parent-of-origin effect on PA risk (311 that were significant after Bonferroni correction [p-values<1.5e-5])

Gene	chr:pos	rsID	Risk Allele	Risk Allele Frequency	Expressed Allele	P-value
AIM1	6:106938669	rs79814366	G	0.10	Paternal	2.7E-09
AIM1	6:106935602	rs12661391	G	0.35	Paternal	2.6E-08
AIM1	6:106908018	rs12195920	T	0.17	Paternal	2.7E-07
AIM1	6:106977067	rs62423286	T	0.25	Paternal	4.6E-07
AIM1	6:106822717	rs75122500	G	0.16	Paternal	9.4E-07
AIM1	6:106820859	rs76160079	T	0.16	Paternal	1.5E-06
AIM1	6:106823292	rs201813595	A	0.15	Paternal	2.1E-06
AIM1	6:106820080	rs78872951	T	0.16	Paternal	3.0E-06
AIM1	6:106820150	rs115845447	T	0.16	Paternal	3.1E-06
AIM1	6:106822613	rs115549098	C	0.16	Paternal	3.3E-06
AIM1	6:106809947	rs181155724	C	0.16	Paternal	3.9E-06
AIM1	6:106965180	rs1084698	T	0.27	Paternal	4.3E-06
AIM1	6:106884263	rs6929314	T	0.37	Paternal	4.4E-06
AIM1	6:106819853	rs75668324	G	0.16	Paternal	5.9E-06
AIM1	6:106814910	rs79763115	T	0.16	Paternal	6.2E-06
AIM1	6:106820747	rs77465272	T	0.16	Paternal	8.0E-06
AIM1	6:106928100	rs76416001	A	0.17	Paternal	9.1E-06
AIM1	6:106839843	rs75354751	C	0.19	Paternal	9.6E-06
AIM1	6:106822226	rs79510158	T	0.16	Paternal	1.0E-05
AIM1	6:106825251	rs9373856	A	0.18	Paternal	1.1E-05
AIM1	6:107006117	rs116110353	C	0.26	Paternal	1.4E-05
AIM1	6:106820418	rs117799561	C	0.16	Paternal	1.5E-05
ANO1	11:69968002	rs11234813	G	0.16	Maternal	1.8E-09
ANO1	11:69983292	rs2509180	A	0.13	Maternal	3.8E-09

ANO1	11:69965075	rs183079903	C	0.14	Maternal	3.2E-08
ANO1	11:69955982	rs7927723	A	0.16	Maternal	4.7E-07
ANO1	11:69967703	rs12807045	T	0.29	Maternal	1.7E-06
ANO1	11:69952289	rs948170	T	0.15	Maternal	7.1E-06
ANO1	11:69947141	rs7929748	G	0.08	Maternal	7.2E-06
ATP10A	15:25973151	rs8036892	G	0.10	Maternal	2.3E-15
ATP10A	15:26064325	rs12437810	A	0.17	Maternal	3.5E-09
ATP10A	15:26030475	rs78663370	A	0.19	Maternal	1.2E-08
ATP10A	15:25969509	rs11161210	A	0.22	Maternal	3.4E-08
ATP10A	15:26080744	rs146225594	G	0.20	Maternal	6.2E-08
ATP10A	15:26074857	rs140426022	A	0.10	Maternal	6.5E-08
ATP10A	15:26093286	rs12442754	A	0.11	Maternal	6.7E-08
ATP10A	15:26035081	rs11852996	A	0.13	Maternal	7.7E-08
ATP10A	15:25966731	rs188497582	C	0.16	Maternal	2.8E-07
ATP10A	15:26017478	rs11855095	A	0.16	Maternal	2.9E-07
ATP10A	15:26025138	rs58461136	T	0.32	Maternal	4.8E-07
ATP10A	15:25972182	rs28405373	T	0.21	Maternal	6.2E-07
ATP10A	15:26027738	rs61991471	T	0.33	Maternal	6.2E-07
ATP10A	15:26096921	rs142881075	G	0.18	Maternal	7.2E-07
ATP10A	15:25993394	rs72705812	T	0.19	Maternal	9.0E-07
ATP10A	15:26021969	rs59821497	T	0.13	Maternal	2.2E-06
ATP10A	15:26091259	rs146932698	A	0.19	Maternal	2.3E-06
ATP10A	15:26028154	rs8029959	C	0.39	Maternal	5.3E-06
ATP10A	15:26021387	rs56406103	T	0.16	Maternal	5.8E-06
ATP10A	15:26033764	rs8042764	A	0.30	Maternal	6.4E-06
ATP10A	15:26062983	rs12442386	A	0.09	Maternal	6.6E-06
ATP10A	15:25971754	rs4906756	A	0.25	Maternal	8.5E-06
ATP10A	15:26027888	rs17116135	C	0.33	Maternal	9.7E-06
ATP10A	15:26016653	rs59239372	C	0.13	Maternal	1.7E-05
CPA4	7:129936803	rs3778856	G	0.30	Maternal	1.7E-05

DDC	7:50612850	rs11575280	C	0.20	Isoform Dependent	1.4E-05
DGCR6	22:18896605	rs3890992	T	0.10	Random	1.3E-07
DGCR6	22:18896464	rs2005883	T	0.10	Random	6.2E-07
DGCR6	22:18897795	rs56190723	G	0.16	Random	9.4E-06
DGCR6	22:18896362	rs2077009	C	0.07	Random	1.6E-05
GNAS_AS1	20:57420273	rs117615061	G	0.18	Paternal	5.6E-09
GNAS_AS1	20:57394781	rs117917416	T	0.18	Paternal	1.3E-08
GNAS_AS1	20:57478448	rs2295583	T	0.10	Paternal	4.1E-06
GPR1	2:207053416	rs16838023	T	0.27	Paternal	2.5E-06
GRB10	7:50670036	rs192922398	T	0.12	Isoform Dependent	3.7E-07
GRB10	7:50695975	rs73113022	A	0.16	Isoform Dependent	1.3E-05
IGF2	11:2150895	rs11564732	T	0.22	Paternal	9.3E-06
INPP5F	10:121504511	rs116233776	T	0.33	Paternal	4.6E-08
KCNQ1	11:2584917	rs12221520	T	0.17	Maternal	4.1E-11
KCNQ1	11:2739521	rs10832572	G	0.16	Maternal	5.5E-11
KCNQ1	11:2740734	rs79266660	C	0.15	Maternal	1.9E-10
KCNQ1	11:2757228	rs72844254	G	0.15	Maternal	1.8E-09
KCNQ1	11:2741327	rs2283205	T	0.11	Maternal	2.5E-09
KCNQ1	11:2656731	rs143733399	A	0.14	Maternal	3.1E-09
KCNQ1	11:2751979	rs72844252	T	0.15	Maternal	4.4E-09
KCNQ1	11:2739180	rs231887	T	0.13	Maternal	5.8E-09
KCNQ1	11:2760409	rs1079715	C	0.14	Maternal	6.3E-09
KCNQ1	11:2731245	rs75591462	T	0.14	Maternal	7.9E-09
KCNQ1	11:2728212	rs75143583	T	0.15	Maternal	8.7E-09
KCNQ1	11:2736026	rs4930149	A	0.17	Maternal	9.3E-09
KCNQ1	11:2708454	rs2283187	C	0.15	Maternal	1.0E-08
KCNQ1	11:2737902	rs117591077	T	0.14	Maternal	1.2E-08
KCNQ1	11:2725315	rs2283195	T	0.14	Maternal	1.5E-08

KCNQ1	11:2722119	rs56134303	T	0.14	Maternal	1.8E-08
KCNQ1	11:2686697	rs2283181	C	0.14	Maternal	2.3E-08
KCNQ1	11:2731558	rs78282031	C	0.15	Maternal	3.1E-08
KCNQ1	11:2678627	rs76463248	T	0.14	Maternal	3.3E-08
KCNQ1	11:2751334	rs2283215	A	0.16	Maternal	3.9E-08
KCNQ1	11:2650021	rs79848425	G	0.14	Maternal	4.7E-08
KCNQ1	11:2686442	rs16928523	A	0.14	Maternal	5.2E-08
KCNQ1	11:2694711	rs11827207	A	0.14	Maternal	5.4E-08
KCNQ1	11:2660138	rs140472405	T	0.14	Maternal	5.4E-08
KCNQ1	11:2729340	rs2283200	T	0.16	Maternal	5.5E-08
KCNQ1	11:2580063	rs2283167	A	0.23	Maternal	6.0E-08
KCNQ1	11:2710624	rs7127825	A	0.15	Maternal	7.9E-08
KCNQ1	11:2738696	rs10766311	T	0.12	Maternal	8.0E-08
KCNQ1	11:2692738	rs16928533	C	0.14	Maternal	8.8E-08
KCNQ1	11:2685820	rs75352146	T	0.14	Maternal	1.2E-07
KCNQ1	11:2585230	rs11600454	T	0.13	Maternal	1.2E-07
KCNQ1	11:2819753	rs11024137	A	0.28	Maternal	1.2E-07
KCNQ1	11:2728917	rs2283199	C	0.14	Maternal	1.4E-07
KCNQ1	11:2704056	rs2283185	C	0.15	Maternal	1.4E-07
KCNQ1	11:2584903	rs7101739	A	0.28	Maternal	1.6E-07
KCNQ1	11:2735470	rs2283201	T	0.15	Maternal	1.9E-07
KCNQ1	11:2697029	rs2073954	T	0.13	Maternal	2.0E-07
KCNQ1	11:2660124	rs151246266	C	0.14	Maternal	2.2E-07
KCNQ1	11:2692979	rs78075808	G	0.13	Maternal	2.2E-07
KCNQ1	11:2718011	rs74984745	C	0.14	Maternal	2.3E-07
KCNQ1	11:2751315	rs78599729	A	0.12	Maternal	2.3E-07
KCNQ1	11:2712286	rs7939976	G	0.15	Maternal	2.5E-07
KCNQ1	11:2724212	rs78607138	T	0.15	Maternal	2.9E-07
KCNQ1	11:2685804	rs74667646	T	0.14	Maternal	3.1E-07
KCNQ1	11:2659158	rs145518302	G	0.14	Maternal	3.3E-07
KCNQ1	11:2593648	rs12574935	G	0.14	Maternal	3.9E-07

KCNQ1	11:2668256	rs117258871	G	0.14	Maternal	4.7E-07
KCNQ1	11:2721157	rs3782064	A	0.14	Maternal	5.2E-07
KCNQ1	11:2718313	rs78344341	G	0.14	Maternal	6.0E-07
KCNQ1	11:2680958	rs75589036	C	0.14	Maternal	6.5E-07
KCNQ1	11:2665936	rs77048839	G	0.14	Maternal	6.9E-07
KCNQ1	11:2604437	rs2012323	A	0.12	Maternal	6.9E-07
KCNQ1	11:2644344	rs190960644	C	0.14	Maternal	7.1E-07
KCNQ1	11:2709741	rs2283188	C	0.15	Maternal	7.5E-07
KCNQ1	11:2677645	rs41517049	G	0.14	Maternal	7.6E-07
KCNQ1	11:2704540	rs76341569	G	0.15	Maternal	7.8E-07
KCNQ1	11:2668988	rs77835752	C	0.14	Maternal	7.8E-07
KCNQ1	11:2650004	rs77507212	C	0.14	Maternal	8.0E-07
KCNQ1	11:2711870	rs2283191	C	0.14	Maternal	8.5E-07
KCNQ1	11:2715843	rs78874320	G	0.15	Maternal	9.9E-07
KCNQ1	11:2650230	rs77769573	G	0.15	Maternal	1.0E-06
KCNQ1	11:2669664	rs76659035	A	0.14	Maternal	1.1E-06
KCNQ1	11:2720927	rs12360708	T	0.15	Maternal	1.2E-06
KCNQ1	11:2698280	rs16928535	G	0.14	Maternal	1.2E-06
KCNQ1	11:2699723	rs16928541	C	0.14	Maternal	1.2E-06
KCNQ1	11:2737905	rs2283202	A	0.29	Maternal	1.3E-06
KCNQ1	11:2747905	rs2283210	G	0.12	Maternal	1.4E-06
KCNQ1	11:2654797	rs79596465	C	0.14	Maternal	1.4E-06
KCNQ1	11:2861578	rs10741726	T	0.13	Maternal	1.4E-06
KCNQ1	11:2657141	rs199767559	G	0.14	Maternal	1.5E-06
KCNQ1	11:2732971	rs80069256	C	0.15	Maternal	1.5E-06
KCNQ1	11:2667108	rs80003280	G	0.14	Maternal	1.7E-06
KCNQ1	11:2649057	rs117143634	C	0.15	Maternal	1.8E-06
KCNQ1	11:2720835	rs364930	A	0.16	Maternal	1.9E-06
KCNQ1	11:2859570	rs72847584	A	0.23	Maternal	2.1E-06
KCNQ1	11:2737977	rs79753560	T	0.14	Maternal	2.6E-06
KCNQ1	11:2819620	rs151316	T	0.27	Maternal	2.8E-06

KCNQ1	11:2686694	rs2283180	C	0.14	Maternal	3.0E-06
KCNQ1	11:2658712	rs139061123	A	0.14	Maternal	3.4E-06
KCNQ1	11:2660007	rs147725435	C	0.14	Maternal	3.4E-06
KCNQ1	11:2688705	rs16928527	A	0.16	Maternal	3.8E-06
KCNQ1	11:2645794	rs80127491	C	0.14	Maternal	4.4E-06
KCNQ1	11:2707564	rs117438519	C	0.14	Maternal	4.5E-06
KCNQ1	11:2691824	rs2283184	T	0.13	Maternal	4.6E-06
KCNQ1	11:2767262	rs149658560	A	0.14	Maternal	4.6E-06
KCNQ1	11:2723367	rs55786794	T	0.12	Maternal	5.2E-06
KCNQ1	11:2692562	rs77981756	C	0.14	Maternal	5.3E-06
KCNQ1	11:2711018	rs2283189	A	0.14	Maternal	6.2E-06
KCNQ1	11:2686716	rs2283182	A	0.14	Maternal	6.6E-06
KCNQ1	11:2659025	rs7102138	C	0.18	Maternal	7.9E-06
KCNQ1	11:2644544	rs78695585	A	0.14	Maternal	9.5E-06
KCNQ1	11:2856428	rs11819853	T	0.20	Maternal	9.8E-06
KCNQ1	11:2660580	rs7936778	C	0.23	Maternal	1.0E-05
KCNQ1	11:2762998	rs112908040	T	0.16	Maternal	1.0E-05
KCNQ1	11:2654508	rs77336468	T	0.14	Maternal	1.1E-05
KCNQ1	11:2696397	rs2073956	T	0.14	Maternal	1.1E-05
KCNQ1	11:2696889	rs2073955	T	0.13	Maternal	1.6E-05
KCNQ1	11:2685641	rs16928522	G	0.14	Maternal	1.6E-05
KCNQ1	11:2691484	rs2283183	T	0.14	Maternal	1.7E-05
KCNQ1	11:2679565	rs76615498	C	0.14	Maternal	1.7E-05
KCNQ1	11:2859541	rs234869	G	0.22	Maternal	1.8E-05
KCNQ1	11:2652867	rs76489785	A	0.14	Maternal	1.8E-05
KCNQ1	11:2743859	rs2283208	A	0.16	Maternal	1.8E-05
L3MBTL1	20:42140842	rs73618895	T	0.16	Paternal	2.2E-08
LIN28B	6:105517445	rs221632	T	0.23	Paternal	6.6E-08
LIN28B	6:105440616	rs11156432	A	0.18	Paternal	3.8E-07
LIN28B	6:105425731	rs4945714	A	0.16	Paternal	3.1E-06
LRRTM4	2:80526239	rs9941541	A	0.15	Paternal	8.2E-08

LRRTM5	2:80526874	rs78743886	C	0.15	Paternal	1.8E-07
MAGI2	7:77703542	rs3807754	G	0.11	Maternal	1.1E-11
MAGI2	7:77914343	rs798336	C	0.08	Maternal	5.5E-08
MAGI2	7:77727872	rs148552561	G	0.11	Maternal	8.9E-08
MAGI2	7:77689571	rs6970942	G	0.22	Maternal	2.2E-07
MAGI2	7:77737225	rs370466425	T	0.10	Maternal	9.5E-06
MAGI2	7:77838642	rs10264610	T	0.20	Maternal	9.7E-06
MAGI2	7:77674023	rs7780163	T	0.18	Maternal	1.0E-05
MEG3	14:101325712	rs45598032	A	0.14	Maternal	3.8E-10
MEG3	14:101293528	rs10134980	A	0.10	Maternal	3.4E-08
MEG3	14:101299000	rs3742390	T	0.10	Maternal	3.5E-07
MEG3	14:101325962	rs10144253	C	0.15	Maternal	5.8E-06
MEG8	14:101365390	rs80203467	T	0.15	Maternal	6.7E-15
MEG8	14:101362418	rs12589854	G	0.20	Maternal	1.4E-14
MEG8	14:101369427	rs144301368	T	0.20	Maternal	2.5E-07
MEG8	14:101361878	rs7146460	T	0.17	Maternal	9.1E-06
NAA60	16:3521049	rs12597473	A	0.33	Maternal	4.5E-06
NLRP2	19:55487066	rs62124626	T	0.13	Maternal	2.1E-08
NLRP2	19:55477723	rs149150614	T	0.14	Maternal	3.7E-08
NLRP2	19:55487932	rs4806628	T	0.13	Maternal	3.1E-07
NLRP2	19:55477483	rs72489176	G	0.14	Maternal	1.6E-06
NLRP2	19:55490072	rs62124641	A	0.13	Maternal	2.3E-06
NLRP2	19:55488606	rs4806463	A	0.14	Maternal	3.5E-06
NLRP2	19:55487997	rs4806462	C	0.13	Maternal	5.0E-06
NLRP2	19:55500215	rs12609974	A	0.44	Maternal	5.3E-06
NLRP2	19:55489862	rs28376680	G	0.13	Maternal	9.1E-06
NTM	11:131265423	rs57328473	A	0.17	Maternal	3.4E-09
NTM	11:131269980	rs57712994	T	0.18	Maternal	9.6E-09
NTM	11:131659530	rs140094444	G	0.08	Maternal	1.4E-08
NTM	11:132002071	rs7483328	T	0.13	Maternal	3.9E-08
NTM	11:131550623	rs202037285	G	0.21	Maternal	5.1E-08

NTM	11:132036752	rs74954142	G	0.08	Maternal	6.7E-08
NTM	11:131497585	rs180756977	A	0.15	Maternal	9.2E-08
NTM	11:131269982	rs59037764	T	0.17	Maternal	1.1E-07
NTM	11:132069635	rs137867769	G	0.13	Maternal	1.4E-07
NTM	11:132077296	rs144681721	C	0.19	Maternal	2.9E-07
NTM	11:131863760	rs148122659	A	0.19	Maternal	3.6E-07
NTM	11:131733969	rs7108867	A	0.13	Maternal	5.9E-07
NTM	11:131815942	rs10791184	T	0.17	Maternal	6.2E-07
NTM	11:131378017	rs12575288	T	0.23	Maternal	9.6E-07
NTM	11:131914022	rs1793622	A	0.16	Maternal	1.0E-06
NTM	11:131532328	rs188693718	C	0.22	Maternal	1.1E-06
NTM	11:131859638	rs73031531	C	0.31	Maternal	1.1E-06
NTM	11:132174215	rs4937691	G	0.15	Maternal	1.3E-06
NTM	11:132191989	rs144199973	T	0.20	Maternal	1.4E-06
NTM	11:131381911	rs141807129	G	0.21	Maternal	1.5E-06
NTM	11:131567596	rs141702444	C	0.13	Maternal	2.2E-06
NTM	11:131378001	rs372825809	C	0.08	Maternal	2.4E-06
NTM	11:131378744	rs4993931	C	0.13	Maternal	2.8E-06
NTM	11:131603036	rs150966016	A	0.16	Maternal	3.1E-06
NTM	11:131268928	rs12272290	T	0.24	Maternal	3.2E-06
NTM	11:131430127	rs11601156	A	0.11	Maternal	4.6E-06
NTM	11:131282496	rs1992895	G	0.19	Maternal	7.1E-06
NTM	11:131430275	rs11222693	A	0.27	Maternal	8.8E-06
NTM	11:132069803	rs77837580	G	0.17	Maternal	9.8E-06
NTM	11:131659587	rs12282501	A	0.19	Maternal	1.2E-05
OSBPL5	11:3124948	rs9736518	G	0.11	Maternal	2.4E-07
OSBPL5	11:3125037	rs74048798	T	0.11	Maternal	3.0E-06
OSBPL5	11:3139648	rs12287346	T	0.20	Maternal	4.2E-06
OSBPL5	11:3140627	rs11025457	T	0.21	Maternal	4.8E-06
OSBPL5	11:3177700	rs34820287	A	0.31	Maternal	5.4E-06
OSBPL5	11:3125043	rs74048799	T	0.11	Maternal	5.8E-06

OSBPL5	11:3138503	rs58462333	G	0.22	Maternal	1.2E-05
OSBPL5	11:3185093	rs4525212	A	0.28	Maternal	1.5E-05
OSBPL5	11:3142477	rs2056423	A	0.45	Maternal	1.7E-05
OSBPL5	11:3125015	rs77374463	T	0.12	Maternal	1.8E-05
PLAGL1	6:144362359	rs59176596	G	0.38	Paternal	7.5E-07
PLAGL1	6:144332295	rs76311854	G	0.41	Paternal	1.1E-06
PLAGL1	6:144332068	rs143146853	A	0.40	Paternal	3.9E-06
PLAGL1	6:144330759	rs146665755	T	0.35	Paternal	4.9E-06
PLAGL1	6:144367133	rs9399470	C	0.38	Paternal	5.0E-06
PLAGL1	6:144276469	rs76539418	A	0.19	Paternal	6.4E-06
PLAGL1	6:144365488	rs140602014	A	0.34	Paternal	7.2E-06
PLAGL1	6:144362698	rs138437628	C	0.33	Paternal	7.2E-06
PLAGL1	6:144331438	rs59090158	A	0.39	Paternal	7.3E-06
PLAGL1	6:144367344	rs4621647	T	0.34	Paternal	7.8E-06
PLAGL1	6:144342818	rs7773278	C	0.39	Paternal	1.0E-05
PLAGL1	6:144374364	rs145704507	T	0.37	Paternal	1.4E-05
SGK2	20:42193900	rs3827067	G	0.15	Paternal	6.3E-12
SGK2	20:42195446	rs73618902	A	0.12	Paternal	9.2E-12
SGK2	20:42191661	rs6017117	G	0.20	Paternal	2.2E-07
SGK2	20:42195863	rs4812721	C	0.24	Paternal	8.2E-06
SLC22A18	11:2942799	rs384898	C	0.11	Maternal	2.5E-13
SLC22A18	11:2942798	rs426359	G	0.11	Maternal	2.6E-11
SLC22A18	11:2938242	rs3764892	T	0.17	Maternal	1.1E-07
SLC22A18	11:2943912	rs2411773	G	0.10	Maternal	2.8E-06
SLC22A18	11:2930134	rs2286659	T	0.18	Maternal	5.3E-06
SLC22A18	11:2931554	rs151047818	C	0.14	Maternal	7.2E-06
SLC22A18	11:2937801	rs77170028	G	0.13	Maternal	1.5E-05
SLC22A2	6:160656533	rs138281088	C	0.15	Maternal	1.6E-13
SLC22A2	6:160656532	rs200258104	T	0.15	Maternal	1.4E-11
SLC22A2	6:160679400	rs624249	A	0.10	Maternal	4.8E-08
SLC22A2	6:160661007	rs143485708	T	0.27	Maternal	1.1E-06

SLC22A2	6:160669718	rs3912161	C	0.14	Maternal	1.4E-05
SNORD115_1	15:25417676	rs2739839	T	0.24	Paternal	1.0E-08
SNORD115_10	15:25434275	rs1977035	T	0.20	Paternal	2.4E-12
SNORD115_40	15:25489376	rs2719919	T	0.18	Paternal	6.9E-06
SNORD115_44	15:25496633	rs8192281	A	0.14	Paternal	9.8E-08
SNRPN	15:25169683	rs2736696	A	0.23	Paternal	1.9E-12
SNRPN	15:25175735	rs377264185	T	0.11	Paternal	5.0E-11
SNRPN	15:25107905	rs72693658	C	0.25	Paternal	1.2E-10
SNRPN	15:25171908	rs77979767	A	0.13	Paternal	2.2E-10
SNRPN	15:25164228	rs61999143	A	0.28	Paternal	5.1E-10
SNRPN	15:25163771	rs184066674	A	0.21	Paternal	2.7E-09
SNRPN	15:25168114	rs113200397	A	0.13	Paternal	4.6E-09
SNRPN	15:25168634	rs78272280	A	0.17	Paternal	1.1E-08
SNRPN	15:25179795	rs671362	C	0.17	Paternal	2.4E-08
SNRPN	15:25171150	rs11638959	A	0.19	Paternal	4.7E-08
SNRPN	15:25174385	rs75465312	T	0.14	Paternal	3.8E-07
SNRPN	15:25153191	rs71461570	T	0.44	Paternal	3.8E-07
SNRPN	15:25112817	rs12595469	A	0.10	Paternal	4.1E-07
SNRPN	15:25145127	rs2201840	A	0.37	Paternal	6.3E-07
SNRPN	15:25134444	rs4307928	T	0.45	Paternal	1.0E-06
SNRPN	15:25143943	rs12901775	T	0.18	Paternal	1.1E-06
SNRPN	15:25175206	rs2647358	T	0.35	Paternal	3.2E-06
SNRPN	15:25151839	rs4906693	T	0.13	Paternal	5.5E-06
SNRPN	15:25174629	rs2736705	T	0.21	Paternal	6.4E-06
SNRPN	15:25143679	rs79988046	A	0.13	Paternal	1.0E-05
SNRPN	15:25138694	rs111700206	A	0.28	Paternal	1.1E-05
SNRPN	15:25118603	rs112641902	T	0.25	Paternal	1.2E-05
SNRPN	15:25123576	rs7172901	G	0.44	Paternal	1.5E-05
SNRPN	15:25118733	rs12050504	C	0.09	Paternal	1.7E-05
TP73	1:3571755	rs60453085	G	0.19	Maternal	2.2E-10
TP73	1:3572821	rs75284414	C	0.22	Maternal	1.4E-09

TP73	1:3571317	rs10910001	C	0.24	Maternal	1.4E-09
TP73	1:3590425	rs6671482	A	0.24	Maternal	6.0E-08
TP73	1:3574756	rs2368544	C	0.25	Maternal	8.3E-08
TP73	1:3629849	rs3765759	A	0.10	Maternal	2.0E-07
TP73	1:3625548	rs3765754	C	0.35	Maternal	2.5E-07
TP73	1:3604189	rs78030280	G	0.27	Maternal	1.0E-06
TP73	1:3610580	rs71634356	C	0.32	Maternal	1.4E-06
TP73	1:3599473	rs200152559	G	0.17	Maternal	1.3E-05
UBE3A	15:25652326	rs8179187	G	0.22	Maternal	9.0E-11
UBE3A	15:25639185	rs2340625	G	0.13	Maternal	6.9E-07
ZIM2	19:57326077	rs56115175	G	0.19	Paternal	7.7E-11

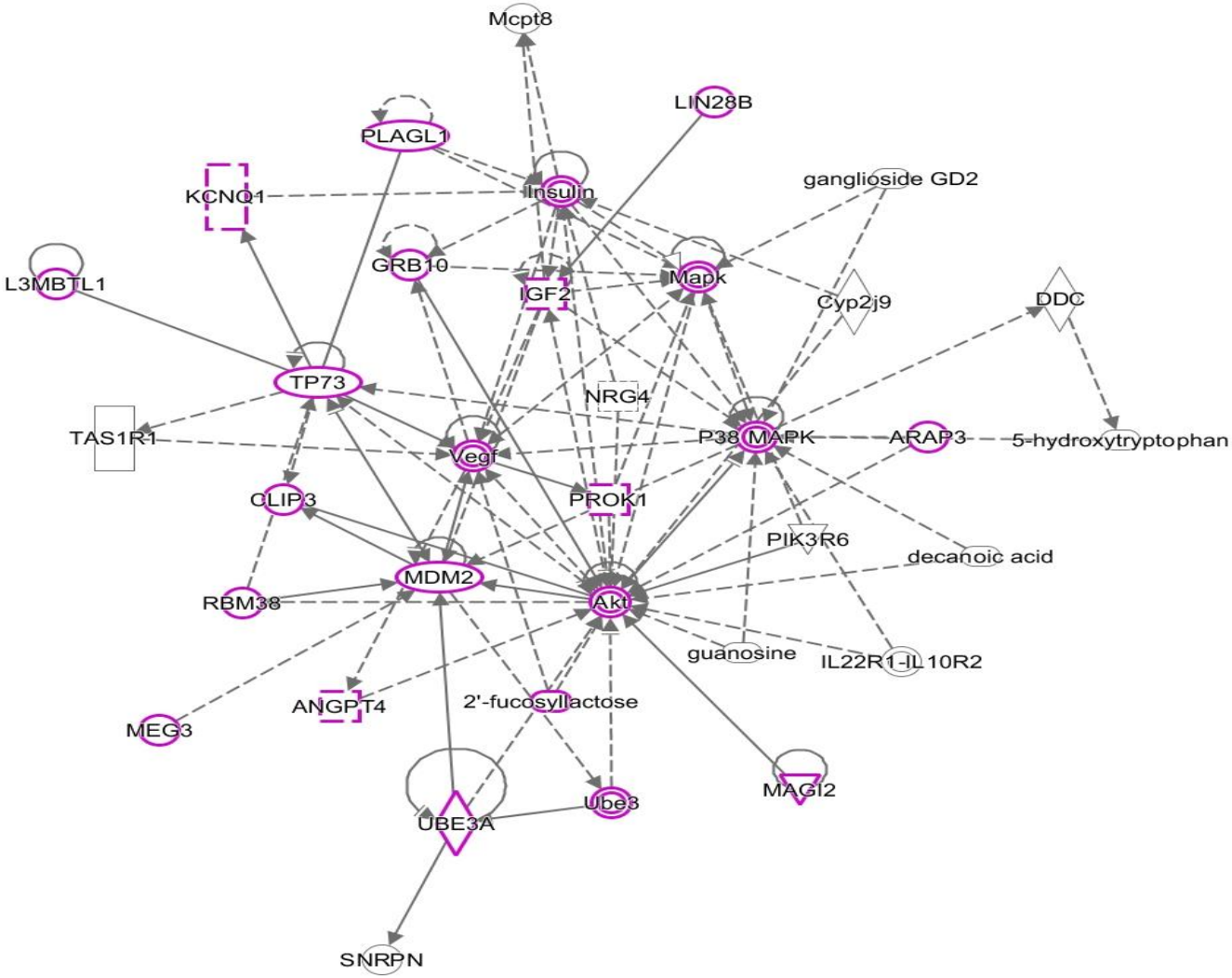
chr:pos: build 37/hg19 chromosome position

Supplementary Table 4.7.3. Networks represented by 35 imprinted genes identified for imprinting/parent-of-origin effect on PA risk.

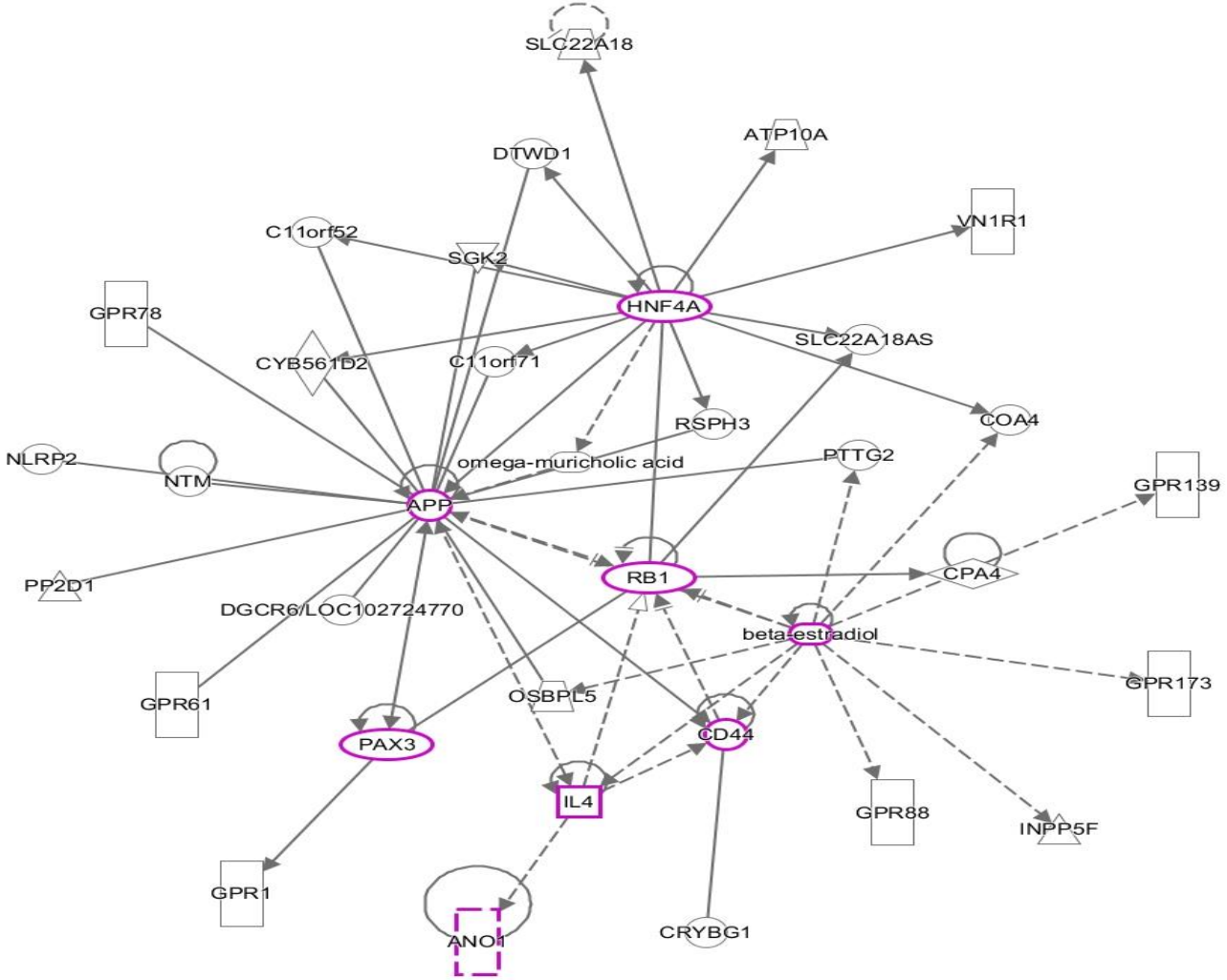
Molecules in Network	Score	Focus Molecules	Top Diseases and Functions	P-value
2'-fucosyllactose,5-hydroxytryptophan,Akt,ANGPT4,ARAP3,CLIP3,Cyp2j9,DDC,decanoic acid,ganglioside GD2,GRB10,guanosine,IGF2,IL22R1-IL10R2,Insulin,KCNQ1,L3MBTL1,LIN28B,MAGI2,Mapk,Mcpt8,MDM2,MEG3,NRG4,P38 MAPK,PIK3R6,PLAGL1,PROK1,RBM38,SNRPN,TAS1R1,TP73,Ube3,UBE3A,Vegf	29	12	Gene Expression, Cell Cycle, Cell Morphology	1.00E-28
ANO1,APP,ATP10A,beta-estradiol,C11orf52,C11orf71,CD44,COA4,CPA4,CRYBG1,CYB561D2,DGCR6/LOC102724770,DTWD1,GPR1,GPR61,GPR78,GPR88,GPR139,GPR173,HNF4A,IL4,INPP5F,NLRP2,NTM,omega-muricholic acid,OSBPL5,PAX3,PP2D1,PTTG2,RB1,RSPH3,SGK2,SLC22A18,SLC22A18AS,VN1R1	29	12	Cardiovascular Disease, Cell Morphology, Free Radical Scavenging	1.00E-29
Histone h4,NAA60,peptide alpha-N-acetyltransferase	3	1	Cellular Function and Maintenance, Cellular Assembly and Organization, DNA Replication, Recombination, and Repair	0.01

APPBP2,SAV1,STK3,TRIM24,TRIM28,TRIM33,TRIM39,ZCWPW1,ZIM2	2	1	Gene Expression, Cancer, Gastrointestinal Disease	0.1
DLG4,EGFR,GPC1,GPC2,GPC3,GPC4,GPC5,GRIA1,LRRTM4,PILRA,PTPRS,REST,SDC2	2	1	Developmental Disorder, Endocrine System Disorders, Hereditary Disorder	0.1
AGO3,androstenedione,corticosterone,CPT2,CPT1A,CPT1B,CST9L,ETV5,EXT2,GTF2B,HEXDC,HYAL3,LIPE,LSM4,mir-210,SLC22A2,SLC22A5,SUMO2,USF1	2	1	Lipid Metabolism, Molecular Transport, Small Molecule Biochemistry	0.1

Supplementary Figure 4.7.1. Significant networks (P-value=1.0e-28) represented by 12 imprinted genes with imprinting/parent-of-origin effect on PA risk. Molecules highlighted in purple represent cell cycle and cell morphology pathway.



Supplementary Figure 4.7.2. Significant networks (P-value=1.0e-29) represented by 12 imprinted genes with imprinting/parent-of-origin effect on PA risk. Molecules highlighted in purple represent cardiovascular disease, cell morphology, and free radical scavenging pathway.



Chapter 5. Conclusion and Discussion

5.1 Overall Findings

We found 174 independent loci suggestively associated with PA in the PAGE GWAS (P-value<5e-5) including rs4148646 and rs2074311 in *ABCC8*, rs7249210, rs7250184, rs7249100 and rs10401828 in *ZNF28*, rs11133659 in *CTNND2*, and rs2074314 and rs35271178 near *KCNJ11*. Similarly, we found 119 independent loci suggestively associated with PA in the GWAS meta-analysis, including rs76258369 near *IRX1*, and rs7094759 and rs12264492 in *ADAM12*. Functional analyses of these genes revealed trophoblast-like cell interaction, as well as networks involved in endocrine system disorders, cardiovascular disease and cellular function. Further, our findings confirm the role of genetic variants in MB and OP pathways (11 SNPs in 9 MB/OP genes) in PA risk using a replication candidate gene study of 507 PA cases and 1,090 controls. Participants in the 25-50th (score:12.6-13.8), 50-75th (score:13.9-15.0) and 75-100th (score ≥15.1) weighted genetic risk score (wGRS) percentiles had 1.45-fold (95% confidence interval [CI]:1.04, 2.02), 1.42-fold (95% CI: 1.02-1.98), and 1.75-fold (95% CI: 1.27, 2.42) higher odds of PA, respectively, compared to those in the lowest wGRS percentile (score <12.6; P-for-trend <0.001). Lastly, we reported interactions between maternal and fetal genetic variations in MB and imprinting on placental abruption risk using 503 PA case and 1,052 mother-infant pairs. SNP rs12530904 (log-likelihood=-1874.6; P-value=1.2e-04) in *CAMK2B*, and, SNP rs73136795 (log-likelihood=-1644.5; P-value=1.9e-04) in *PPARG*, both MB genes, showed better model fit for maternal-fetal interaction effects. Parent-of-origin effects on PA risk were observed for 311 SNPs in 35 imprinted genes (including *KCNQ1*, *NPM*, and, *ATP10A*).

5.2 Conclusion

Findings from this dissertation lend evidence for associations of genetic variants with PA, a complex multifactorial trait associated with maternal and neonatal mortality and morbidity. In this largest GWAS to date, several clinically-relevant protein-coding variants, as well as genes known to be highly expressed in the placenta were associated with PA risk. Further, our findings confirm the role of genetic variants in mitochondrial biogenesis and oxidative phosphorylation pathways in PA. Lastly, we found strong evidence for interactions between maternal and fetal genetic variations in mitochondrial biogenesis and imprinting effects on PA risk.

5.3 Limitations

Although this dissertation research was based on the largest GWAS studies of PA to date, it was still underpowered to identify genome-wide significant associations between SNPs and PA risk, particularly for SNPs that are not common. In addition, analyses in general, and particularly, the interaction analyses, may be underpowered to evaluate small effects or interactions among rare SNPs. Other limitations of this dissertation include potential misclassification of sub-clinical PA (i.e. those with less placental disrupter and consequently bleeding), which may limit the interpretation of the study results or reduce power of the study. This dissertation also did not distinguish between severe and mild cases of PA, which may have different risk factors or underlying mechanism[77]. Furthermore, the imprinting effects assessed in this dissertation were model based and did not incorporate paternal genetic data. The genotypes used for imputation were based on information from direct genotyping using two different platforms. We had a number of SNPs that were neither directly genotyped or imputed in one of the cohorts. This limited some of the analyses on the combined population. Finally, findings

from this dissertation research may not be generalizable to other populations with different population genetic structure or PA risk pattern to those included in the current study.

5.4 Strengths and Innovations

Our project was the largest genetic study on placental abruption to date. The project was conducted among well-characterized study populations. We conducted a comprehensive genetic variation profiling using the state-of-the-art 1000 genomes genotype imputation. Our aims included a successful replication study, which addresses a significant limitation in previous studies. Finally, our assessment of both maternal and fetal genomes, critical in understanding PA pathophysiology, is important in uncovering the missing heritability of PA.

Our findings are of great interest to a broad array of biomedical researchers and clinicians interested in obstetrical complications and consequent health outcomes. Findings can motivate and inform similar genetic studies, either GWAS or those involving MB and OP, or other potential pathways, important in PA. Findings can inform mechanistic investigations to identify potential preventative or therapeutic targets. Finally, findings may facilitate efforts to identify individuals who have an elevated risk for placental abruption for preventative or early diagnostic efforts.

5.5 Future Directions of Research Recommendations

Our findings warrant replications in large, diverse study populations. Future research studies that include functional analyses on potentially clinically-relevant protein-coding variants identified in the current study, including those in *ABCC8* and *KCNJ11*, as well as genes that are known to be highly expressed in the placenta (e.g. *ADAM12*) are needed. Studies should also

investigate mitochondrial biogenesis and oxidative phosphorylation related metabolomics, epigenetics, or proteomics to inform molecular mechanistic investigations and identify potential preventative or therapeutic targets of PA. Other pathways such as the folate and vitamin D metabolism pathways, coagulation pathways, and pathways involved in placental ageing and related biomarkers (e.g. telomeres) are also important potential targets for future investigations. Rare variant analyses, analyses of variations in the X chromosome, as well as other platforms (e.g. exome sequencing) can also be used to leverage current developments in genetics and statistical genetics to investigate genetic susceptibility to PA. Investigations of potential gene-environment interactions between environmental risk factors and maternal genetic variations, as well as potential modifiers (e.g. preeclampsia) of associations of genetic variations with PA. Finally, large family studies that include maternal, fetal, and paternal genotype data to fully understand maternal-fetal genetic interactions and imprinting effects on PA risk are also warranted.

VITA

I am currently a pre-doctoral fellow at the Epidemiology Branch of the Division of Intramural Population Health Research at Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health (NIH). I received my Bachelors of Science in Mathematics with Honors from Saint Francis University and Masters of Science in Mathematics with Scientific Computing from Georgia State University. My PhD training focused on the epidemiology and prevention of maternal, perinatal and childhood diseases. My research interest involves identification of genetic risk factors and underlying mechanisms related to vascular and cardiometabolic disorders of pregnancy. I work in collaboration with investigators including my current mentor at the University of Washington, NIH and Harvard T.H. Chan School of Public Health.