Int J Mol Epidemiol Genet 2012;3(4):305-313
www.ijmeg.org /ISSN:1948-1756/IJMEG1209003

Original Article

A genome-wide association study of variations in maternal cardiometabolic genes and risk of placental abruption

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Received September 14, 2012; Accepted October 21 2012; Epub November 15, 2012; Published November 30, 2012

Abstract: Accumulating evidence suggests that placental abruption has a complex multifactorial pathogenesis that involves cardiovascular risk and metabolic dysfunction. However, comprehensive assessment of variations in genes involved in cardiometabolic traits associated with the risk of placental abruption is lacking. We conducted a case-control study investigating associations of variations in maternal cardiometabolic genes (characterized using 217,697 SNPs on the Illumina Cardio-Metabo Chip) with risk of placental abruption. A total of 253 abruption cases and 258 controls were selected from among participants enrolled in the Peruvian Abruptio Placentae Epidemiology Study in Lima, Peru. In the genome-wide association analyses, top hits did not surpass genome-wide significance. However, we observed suggestive associations of placental abruption with several SNPs, including SNPs in SMAD2 (*P*-value=1.88e-6), MIR17HG (*P*-value=7.8e-6], and DGKB (*P*-value=8.35e-6] loci. In candidate gene analyses, we observed associations of variations in *a priori* selected genes involved in coagulation, rennin-angiotensin, angiogenesis, inflammation, and B-vitamin metabolism with the risk of abruption. Our study suggests that variations in maternal cardiovascular and metabolic genes may be associated with risk of placental abruption. Future studies with large sample sizes are warranted.

Keywords: Placental abruption, pregnancy complications, Cardio-Metabo chip, genetic association, single nucleotide polymorphism, genome-wide association study

Introduction

The premature separation of placenta from the uterus, otherwise known as placental abruption, is an adverse obstetrical complication that is associated with substantial global maternal and infant morbidity and mortality [1-3]. Accumulating evidence suggests that placental abruption has a complex, multifactorial pathogenesis that involves cardiovascular risk and metabolic dysfunction [4-13]. Observations of high recurrence rates and familial clustering of placental abruption cases suggest the existence of a genetic predisposition to placental abruption [7, 8].

Previously identified genetic variations that have been associated with placental abruption include polymorphisms in genes involved in coagulation, the renin-angiotensin system, angiogenesis, inflammation, tissue remodeling, and homocysteine metabolism [14-18]. Polymorphisms in these genes have also been associated with increased risk of cardiovascular and cardiometabolic diseases [19, 20]. The overlap in genetic mechanisms between placental abruption and cardiovascular diseases suggests that both conditions may share common etiologic underpinnings. While genome-wide association studies (GWAS) provide an opportunity to comprehensively examine associations

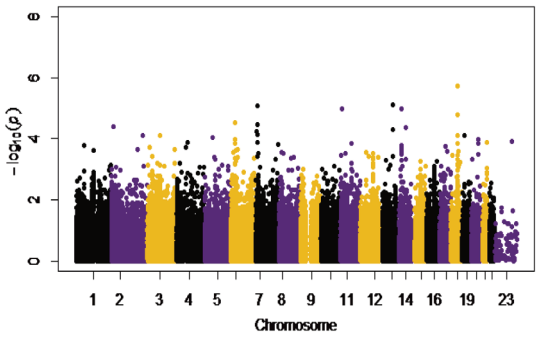

Figure 1. Manhattan plot of genome-wide association study of placental abruption.

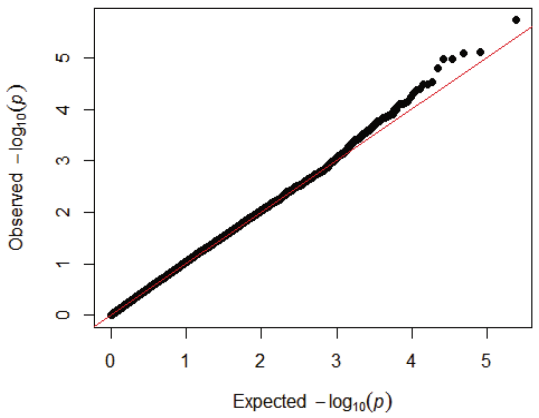

Figure 2. Q-Q plot (λ = 1.07).

of genetic variation with disease phenotypes, no previous GWAS has been published in relation to placental abruption.

Based on available evidence that supports common pathologic mechanisms underlying cardiometabolic traits, cardiometabolic diseases, and placental abruption, we hypothesized that variations in cardiometabolic genes may predict the risk of placental abruption. We tested this hypothesis in the rigorous framework of a GWAS of placental abruption in a case-control study in Lima, Peru.

Methods

Study setting

This study was conducted among participants enrolled in the Peruvian Abruptio Placentae Epidemiology (PAPE) Study, at the Hospital Nacional Dos de Mayo, Instituto Especializado Materno Perinatal, and Hospital Madre-Niño San Bartolomé in Lima, Peru, between August 2002 and May 2004. Participants were recruited from new admissions to antepartum, emergency room, and labor and delivery wards of participating hospitals. Institutional Review Boards of participating institutions approved the project protocol. All participants provided written informed consent.

Study population

Hospital admission and delivery logs were monitored daily to identify placental abruption cases among pregnant women who delivered at participating institutes. Placental abruption cases were selected based on evidence of retroplacental bleeding (fresh blood) entrapped between the decidua and the placenta or blood clots behind the placenta and two of the following: (i) vaginal bleeding in late pregnancy not due to placenta previa or cervical lesions; (ii) uterine tenderness and/or abdominal pain; (iii) fetal distress or death. Controls were selected from eligible pregnant women who delivered at participating hospitals during the study period and who did not have a diagnosis of placental abruption in the current pregnancy.

Data collection

Participants were interviewed by trained research personnel using standardized structured questionnaires. Information was collected on socio-demographic characteristics and risk factors including maternal age, marital status, employment status during pregnancy, medical history, and smoking and alcohol consumption (both current and pre-pregnancy). A brief physical examination was conducted to measure maternal height, weight, and mid-arm circumference. Blood specimens from 253 cases and 258 controls were processed and analyzed for the current GWAS.

DNA extraction and genotyping

DNA was extracted from collected blood specimens using the Gentra PureGene Cell kit for DNA preparations (Qiagen, Hilden, Germany). Variants in cardiovascular and metabolism genes were characterized using 217,697 Single Nucleotide Polymorphisms (SNPs) represented on the Illumina Cardio-Metabo Chip (Illumina

Table 1. Socio-demographic and reproductive characteristics and infant outcomes in the study population, Lima, Peru, 2002-2004

	Placental Abruption			
Maternal Characteristics	Cases (n=235)		Controls (n=232)	
Maternal age at delivery, years (mean, sd)	27.4	7	27.3	7
< 20 (n, %)	25	11	31	13
20-29 (n, %)	126	54	118	51
30-34 (n, %)	46	20	42	18
≥ 35 (n, %)	38	16	41	18
Parity[1] (mean, sd)	1.1	2	0.95	1
0 (n, %)	109	47	108	47
1 (n, %)	63	27	65	28
2-4 (n, %)	53	22	54	23
≥ 5 (n, %)	9	4	4	2
≤ High school education (n, %)	190	81	195	84
Employed during pregnancy (n, %)	93	40	85	37
Planned pregnancy (n, %)	107	46	114	50
No prenatal care (n, %)	40	17	24	10
No prenatal vitamin (n, %)	106	45	109	47
Smoked during pregnancy (n, %)	9	4	4	2
Alcohol use during pregnancy (n, %)	0	0	0	0
Illicit drug use during pregnancy (n, %)	1	0	0	0
Pre-pregnancy BMI, kg/m^2 (mean, sd)	23.4	3	23.8	4
< 18.5 (n, %)	17	8	9	4
18.5-24.9 (n, %)	141	64	151	67
25.0-29.9 (n, %)	53	24	45	20
≥ 30.0 (n, %)	8	4	21	9
Chronic hypertension (n, %)	8	3	4	2
Preeclampsia or eclampsia (n, %)	71	31	26	11
History of placental abruption (n, %)	4	2	0	0
Infant Outcomes				
Birthweight[2], grams (mean, sd)	2396	876	3033	798
Low (<2500 grams) birthweight infant[2] (n, %)	116	49	40	17
Gestational age at delivery[2], weeks (mean, sd)	34.5	7	37.9	3
Preterm (< 37 weeks) delivery[2] (n, %)	146	62	63	27
Stillbirth (n, %)	59	25	3	1

sd = standard d eviation, [1] = number of previous live births (> 28 weeks), [2] = among live births.

Inc, San Diego, CA). Briefly, the Cardio-Metabo Chip is a high-density custom array that captures DNA variation at regions identified by well-powered GWAS meta-analyses for diseases and traits relevant to metabolic and atherosclerotic-cardiovascular endpoints, constructed using the Illumina iSelect technology. The meta-analysis studies that contributed to the identification of variants represented on the Cardio-Metabo ChipInclude CARDIoGRAM (coronary artery disease), DIAGRAM (type 2 diabetes), GIANT (height and weight), MAGIC (glycemic traits), Lipids (lipids), ICBP-GWAS (blood pressure), and QT-IGC (QT interval).

Data quality control and preprocessing

Rigorous quality control procedures were applied to the DNA before analyses [21]. Individuals were excluded if they had genotyping failure for more than 10% of the sites. SNPs were excluded if the minor allele frequency was less than 1%, if the SNP failed to be genotyped in more than 10% of the study population, or if the SNP was not in Hardy-Weinberg equilibrium among controls (critical *p*-value=0.0001). Population stratification was assessed using the genomic inflation factor [22].

Statistical analyses

Study participant characteristics were described using mean (standard deviation) and number (%, percent) for continuous and categorical variables, respectively, for strata defined by case-control status. Independent Chi-square tests were performed to evaluate genetic associations between SNPs and placental abruption. The Bonferroni correction was used to adjust the critical *p*-value threshold (p-value=4.16e-7) to account for multiple testing in overall genome-wide level analyses.

Due to the modest study size, using available evidence in the literature, we conducted secondary data analyses to evaluate associations of genetic variations in genes previously implicated in the pathogenesis of placental abrupti-

Table 2. Top genome-wide metabochip SNP associations with placental abruption

Gene	SNP	MAF	Odds Ratio	*P*-value
SMAD2	Ch18:45399981	0.33	1.90	1.88E-06
MIR17HG	rs4773624	0.31	1.84	7.80E-06
DGKB	Chr7:14154194	0.08	2.51	8.35E-06
SRP54	rs12433712	0.32	0.51	1.04E-05
Between LOC100288365 and GALNTL4	rs4910266	0.08	2.51	1.05E-05
SMAD2	Chr18:45395311	0.45	0.55	1.56E-05
MICA	rs2523454	0.21	1.87	3.00E-05
DGKB	Chr7:14153346	0.07	2.50	3.31E-05
DGKB	Chr7:14154048	0.07	2.50	3.31E-05
C2orf48	rs4668669	0.05	2.85	4.04E-05
RAD51B	rs11628885	0.04	0.05	4.17E-05
MIR17HG	rs6492538	0.27	1.77	4.88E-05
SDK1	rs2880371	0.18	0.45	5.88E-05
DGKB	Chr7:14154456	0.12	2.05	7.22E-05
SMAD2	Chr18:45396171	0.29	0.54	7.56E-05
Between POU1F1 and KRT8P25	rs1497411	0.00	18.82	7.71E-05
ERBB4	rs11887047	0.36	0.56	7.76E-05
MYO9B	rs2279006	0.01	7.91	7.97E-05
FBXL13	rs12523475	0.01	5.60	8.83E-05

Bonferroni-corrected threshold for statistical significance *P*-value of 4.16E-07, MAF = Minor Allele Frequency in Controls.

on [14-18]. Associations between placental abruption and genes involved in coagulation, the renin-angiotensin system, angiogenesis, inflammation, and B-vitamin metabolism were analyzed using 50 SNPs in candidate genes (MTHFR, FS, AGT, MTR, AGTR1, KDR, VEGFC, MTRR, BHMT, MAT2B, VEGFA, PLG, NOS3, ANGPT1, F2, MTHFD1, MMP2, ACE, THBD, MMP9) that were represented on the Cardio-Metabo Chip. For these analyses a *p*-value threshold of 0.05 was used to determine statistical significance. All analyses were conducted using PLINKv1.07 [23].

Results

In general, placental abruption cases and controls had similar demographic and medical characteristics (**Table 1**), with some exceptions. The average age of both cases and controls was about 27 years. Cases and control had similar parity, education, employment, and pre-pregnancy BMI distributions. Abruption cases were more likely to have a history of pre-eclampsia/eclampsia (31% versus 12%), placental abruption (2% versus 0%), or lack of prenatal care during the index pregnancy (16% versus 9%). As expected, adverse perinatal outcomes were more common among cases than controls.

After applying quality control filters for minor allele frequency, genotyping failure, and Hardy-Weinberg Equilibrium, 120,087 SNPs remained for evaluation of associations with placental abruption among 235 cases and 232 controls. A genomic inflation factor of 1.07 indicated minimal effect of population stratification on results; therefore, no adjustment for ancestry was performed.

In the genome-wide analyses, none of the SNPs met the stringent criteria (critical *p*-value 4.16e-7) for association with placental abruption. However, we observed suggestive associations of placental abruption with several SNPs including SNPs in SMAD2 (Ch18:45399981, p=1.88e-6), MIR17HG (rs4773624, p=7.8e-6), and DGKB (Chr7:14154194, p=8.35e-6) loci. A Manhattan plot of the genome-wide results is presented in **Figure 1**. A Q-Q plot of the p-values shows minimal departure from the expected values (**Figure 2**). Using a less conservative false discovery based threshold, two top hits in the SMAD2 and MIR17HG genes, with p < 8.3e-06 (1/120,087; below the *p*-value threshold) where we expect only one computed probability value corresponding to a 1/120,087 false discovery rate. Two of the five top hits in named SNPs were in the SMAD2 gene (Ch18:45399981 and Ch18:45395311) (**Table 2**).

In a secondary analysis, we evaluated the associations of placental abruption with variations in previously implicated placental abruption candidate genes [14-18] represented on the

Table 3. Results of candidate gene analysis for associations between SNPs and placental abruption

Gene	SNP	Chromosome	Odds Ratio	*P*-value
MTHFR	rs1537514	1	1.10	0.704
MTHFR	rs1801131	1	0.91	0.641
MTHFR	rs17421462	1	0.86	0.776
MTHFR	rs1572151	1	0.76	0.597
MTHFR	rs17037397	1	1.10	0.723
MTHFR	rs17367504	1	0.96	0.859
FS	rs4656687	1	1.11	0.464
FS	rs2298908	1	1.08	0.571
FS	rs3753305	1	0.88	0.481
AGT	rs11122577	1	1.09	0.625
AGT	rs2493134	1	1.48	0.010
MTR	rs10495387	1	1.42	0.479
AGTR1	rs5183	3	1.28	0.632
KDR	rs2125489	4	2.15	0.090
KDR	rs11732292	4	1.42	0.007
KDR	rs2305948	4	0.84	0.668
VEGFC	rs2333496	4	1.19	0.192
VEGFC	rs3775202	4	1.01	0.947
MTRR	rs3776464	5	1.05	0.763
MTRR	rs16879334	5	1.03	0.835
BHMT	rs567754	5	0.94	0.610
MAT2B	rs10515862	5	0.93	0.831
VEGFA	rs3025035	6	0.93	0.837
PLG	rs9295131	6	0.75	0.054
PLG	rs4252109	6	0.59	0.086
PLG	rs4252120	6	0.56	0.051
PLG	rs4252159	6	1.59	0.214
NOS3	rs1799983	7	1.14	0.574
NOS3	rs3918188	7	1.06	0.784
NOS3	rs3730006	7	0.39	0.101
NOS3	rs743507	7	0.88	0.649
ANGPT1	rs17295051	8	1.00	0.981
ANGPT1	rs4133395	8	1.12	0.480
ANGPT1	rs1433189	8	1.63	0.170
ANGPT1	rs11776085	8	0.70	0.257
F2	rs3136435	11	1.08	0.863
F2	rs3136441	11	1.34	0.027
MTHFD1	rs17751556	14	1.24	0.653
MTHFD1	rs3818239	14	0.70	0.391
MMP2	rs243840	16	0.88	0.349
ACE	rs4324	17	1.04	0.813
THBD	rs3176123	20	1.44	0.040
MMP9	rs3918253	20	0.82	0.395
MMP9	rs17576	20	0.97	0.882
MMP9	rs2236416	20	0.87	0.727

Cardio-Metabo Chip (i.e., 43 SNPs in 20 candidate genes that met quality control criteria described earlier (**Table 3**). Among these, SNPs in AGT (rs2493134), KDR (rs11732292), F2 (rs3136441), and THBD (rs317623) were associated with placental abruption (*p*-value <0.05).

Discussion

In this GWAS, we provide suggestive evidence supporting associations of variations in maternal cardiometabolic genes with risk of placental abruption. We identified several SNPs in novel (SMAD2 MIR17HG, and DGKB) and candidate (AGT, KDR, F2, and THBD) genes that may be targets for future genetic and downstream functional genomic investigations related to placental abruption. Below, we will underscore our findings within the context of existing literature, and we provide a brief synopsis of hypotheses that merit consideration in future research.

SMAD2, part of the TGF-Beta signaling pathway, is one of the top hits observed in our current study. This finding is consistent with some, but not all, previous studies which have linked TFG-Beta to preeclampsia [24-26], a risk factor for placental abruption. In addition, SMAD2 is also a putative candidate gene for association with placental abruption due to its functional role in placental development [27].

The MIR17HG locus, whose variations were also among the top hits in our GWAS, has been implicated in TGF-Beta signaling [28, 29]. Phenotypic associations with this gene have mostly been with various cancers [30-32], with germline deletion of the locus causing abnormal growth and skeletal development in humans [33, 34]. While this locus has not previously been related to placental abruption, it may contribute to placental abruption pathogenesis through its influence in angiogenesis, cell growth, cell development, and apoptosis [28-34].

Results from the candidate gene-based analyses indicate associations of SNPs in AGT, KDR, F2, and THBD with placental abruption. These genes are involved in the rennin-angiotensin system, angiogenesis, and coagulation pathways. While associations between these genes and placental abruption have been described before [14-18], none of the identified SNPs have been previously associated with placental abruption or other adverse outcomes of pregnancy. One of these SNPs is rs3176123 in the 3'UTR region of the THBD (Thrombomodulin) gene. Thrombomodulin and its protein product are involved in a variety of inflammatory and coagulation processes and related phenotypes (e.g., myocardial infarction and recurrent miscarriage) [35-38]. For instance, the rs3176123 SNP has previously been associated with acute myocardial infarction [36, 37]. Further, in a case-control study, reduced placental expression of thrombomodulin was observed among women experiencing recurrent spontaneous miscarriages compared with those undergoing elective termination [38].

A polymorphism in the prothrombin (F2) gene, rs3136441, was one of the top candidate hits in our secondary analyses. While this particular polymorphism has not been previously associated with placental abruption, the G20210A (rs1799963) mutation in the F2 gene has been studied extensively [18]. The SNP was associated with a greater than 6-fold higher risk of placental abruption in a meta-analysis (OR=6.7; 95% CI: 3.2-13) [18]. Our finding along with these previous reports indicates the potential role of other variations in the F2 gene.

The AGT gene codes for the protein angiotensin II, part of the renin-angiotensin-aldosterone system, which is well-established in its role in cardiovascular diseases [39]. It contains a number of well-studied polymorphisms including the Thr235 mutation (rs699) [40]. This mutation has been associated with placental abruption in some, but not all, studies [18, 40-41]. While this particular SNP was not represented on the Cardio-Metabo Chip, our top hit in the candidate gene analysis, rs2493134 is in perfect linkage disequilibrium (r^2 = 1.0) with rs699.

KDR, also known as vascular endothelial growth factor receptor-2 (VEGFR-2) is a receptor tyrosine kinase involved in the migration, permeability, proliferation, and survival of endothelial cells [42]. Differential expression of KDR has been associated with both preeclampsia [43] and spontaneous abortion [44]. The functional SNP rs1870377 in KDR has been associated with recurrent pregnancy loss in a Taiwanese Han population [45], but this SNP was not covered in the Cardio-Metabo Chip.

The top hit in KDR in the current analyses, rs11732292, has not been previously associated with placental abruption.

Our study is the first genome-wide assessment of genetic variations in Cardio-Metabolic pathways in relation to placental abruption risk. The emphasis on variations in genes participating in cardiometabolic pathways that have been shown to play roles in placental abruption pathogenesis is a particular strength of the study. Population stratification, a common concern in GWAS based on case-control designs, was minimal in our study (genomic inflation factor lambda=1.07). Finally, our study population, Peruvian women, is relatively understudied, particularly in areas of genetic variations that may influence pregnancy outcomes.

Some limitations of our study deserve mention. We used a research operational definition of placental abruption which may have lead to some misclassification. For instance, sub-clinical cases of placental abruption (i.e., those not presenting with abnormal vaginal bleeding) may be missed or misclassified among controls. Furthermore, based on lessons from previous GWAS in other research areas, small effects are more common for SNP related outcomes. Our modest-sized case-control study is underpowered to evaluate small effects. Subsequent larger studies and/or pooled analyses of existing studies will provide more stable estimates of putative associations.

In summary, our suggestive findings, despite the modest sample size of this case-control study and the underlying heterogeneity of placental abruption, justify further larger studies investigating the role of maternal genetic variations in the pathogenesis of placental abruption. These investigations of genetic susceptibility to placental abruption have the potential to enhance understanding of mechanisms for disease development as well as identification of high risk individuals for prevention and/or early antepartum and perinatal intervention.

Acknowledgements

This study was supported by grants from the National Institute of Health, the Eunice Kennedy Shriver National Institute of Child Health and Human Development (5 R01-HD059827; T32HD052462) and the National Heart Lung and Blood Institute (K01HL10374).

Conflict of interest statement

The authors have no conflicts of interest to disclose.

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