OBSTETRICS

Genetic associations with placental and pregnancy proteins in maternal serum identify biomarkers for hypertension in pregnancy

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BACKGROUND: Preeclampsia is a complex syndrome that accounts for considerable maternal and perinatal morbidity and mortality. Despite its prevalence, no effective disease-modifying therapies are available. Maternal serum placenta-derived proteins have been in longstanding use as markers of risk for aneuploidy and placental dysfunction, but whether they have a causal contribution to preeclampsia is unknown.

OBJECTIVE: We aimed to investigate the genetic regulation of serum placental and pregnancy proteins in early pregnancy and their relationships with preeclampsia and gestational hypertension.

STUDY DESIGN: This study used a nested case-control design with nulliparous women enrolled in the Nulliparous Pregnancy Outcomes Study: Monitoring Mothers-to-Be study from 8 clinical sites across the United States between 2010 and 2013. The first-trimester and second-trimester serum samples were collected, and 9 proteins were measured, including vascular endothelial growth factor, placental growth factor, endoglin, soluble fms-like tyrosine kinase-1, a disintegrin and metalloproteinase domain-containing protein 12, pregnancy-associated plasma protein A, free beta-human chorionic gonadotropin, inhibin A, and alpha-fetoprotein. This study used genome-wide association studies to discern genetic influences on these protein concentrations, treating proteins as outcomes. Furthermore, Mendelian randomization was used to evaluate the effects of these proteins on preeclampsia and gestational hypertension, and their further relationship with long-term hypertension, treating proteins as exposures.

RESULTS: A total of 2352 participants were analyzed. We discovered significant associations between the pregnancy zone protein locus and concentrations of a disintegrin and metalloproteinase domain-containing protein 12 (rs6487735, $P=3.03\times10^{-22}$) and between the vascular endothelial growth factor A locus and concentrations of both vascular endothelial growth factor (rs6921438, $P=7.94\times10^{-30}$) and soluble fms-like tyrosine kinase-1 (rs4349809, $P=2.89\times10^{-12}$). Our Mendelian randomization analyses revealed an association between first-trimester a disintegrin and metalloproteinase domain-containing protein 12 concentrations and gestational hypertension (odds ratio=0.78, $P<.001$). We also found that preeclampsia (odds ratio=1.75, $P=8.3\times10^{-3}$) and gestational hypertension (odds ratio=1.84, $P=.005$) during the index pregnancy were associated with an increased risk of hypertension 2 to 7 years later.

CONCLUSION: Our study discovered significant genetic associations with placental proteins a disintegrin and metalloproteinase domain-containing protein 12, vascular endothelial growth factor, and soluble fms-like tyrosine kinase-1, offering insights into their regulation during pregnancy. Mendelian randomization analyses demonstrated the associations between the serum concentrations of proteins, particularly a disintegrin and metalloproteinase domain-containing protein 12, and gestational hypertension, potentially informing future prevention and treatment investigations.

Key words: a disintegrin and metalloproteinase domain-containing protein 12, genome-wide association studies, gestational hypertension, long-term postpartum hypertension, Mendelian randomization, preeclampsia, pregnancy zone protein, soluble fms-like tyrosine kinase-1, vascular endothelial growth factor, vascular endothelial growth factor A

Introduction

Preeclampsia (PE) is a complex syndrome of widespread maternal endothelial activation and intravascular inflammation with a range of contributing factors arising from a final common pathway of dysfunction at the maternal–placental interface.[1] Progressive clinical deterioration may occur, unless placental delivery is achieved. Despite considerable etiologic complexity, the placenta appears to be central to the development of PE, either as the primary pathological source or as a secondary affected organ in the setting of other insults.[2] Because many of the vascular perturbations leading to PE occur in early pregnancy, prior efforts have focused on early, noninvasive detection of placental dysfunction as a means for clinical prediction.[3–6] We previously analyzed clinical and biospecimen data from the Nulliparous Pregnancy Outcomes Study: Monitoring Mothers-to-Be (nuMoM2b) cohort, in which we found that the maternal serum concentrations of 9 placenta-derived proteins collected during the first and second trimesters (n=2352) were associated with PE.[7] Other studies also have found that circulating concentrations of proteins, particularly angiogenic factors produced by the placenta, are associated with PE.[3,4,8–10]

Beyond the short-term implications of PE, there is increasing recognition of its long-term maternal associations with

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AJOG at a glance

Why was this study conducted?

Placental proteins in maternal serum have been used for preeclampsia (PE) prediction and risk stratification, but the lack of therapeutic options remains. We investigated the genetic regulation of 9 placental and pregnancy proteins in early pregnancy serum samples to understand their relationships with PE and gestational hypertension (gHTN). We also sought to determine whether PE and gHTN contribute to long-term maternal hypertension.

Key findings

We found that the pregnancy zone protein gene locus is associated with concentrations of a disintegrin and metalloproteinase domain-containing protein 12 (ADAM-12) ($P=3.03\times10^{-22}$). Similarly, the vascular endothelial growth factor A gene locus is associated with both vascular endothelial growth factor ($P=7.94\times10^{-30}$) and soluble fms-like tyrosine kinase-1 ($P=2.89\times10^{-12}$) serum concentrations. Mendelian randomization analyses revealed an association between ADAM-12 concentrations and gHTN (odds ratio=0.78, $P<.001$), and further analysis indicated a separate association between gHTN and long-term maternal hypertension (odds ratio=1.84, $P=.005$).

What does this add to what is known?

ADAM-12, a key matrix metalloproteinase for early placental implantation and development, may influence hypertensive disorders of pregnancy. ADAM-12 therefore warrants further investigation as both a biomarker and therapeutic target hypertensive disorders of pregnancy.

later-life hypertension, cardiovascular (CV) disease, and renal disease.[11–15] Thus, the development of PE or gestational hypertension (gHTN) serves as an indicator of future CV risk, but it remains unclear whether the PE/gHTN is causal or is merely an indicator of underlying genetic predisposition to CV disease.

Addressing these knowledge gaps in PE may be possible through interrogation of the maternal genome using an approach called Mendelian randomization.[16] This approach, which uses genetic variants randomly assigned at conception as proxies for risk factors, has become increasingly popular in clinical research, mimicking a randomized clinical trial.[17–19] Such an approach has the potential to identify a biomarker as a therapeutic target or clarify the possibility that PE may be part of the pathway leading to CV disease rather than merely predicting future CV risk.[20–22]

To fill the knowledge gap, we first set out to clarify the genetic influences on placental and pregnancy protein concentrations during early pregnancy, which had previously not been done during pregnancy.[23–29] Thus, we performed genome-wide association studies (GWAS) on 9 placental and pregnancy proteins measured in nuMoM2b participant maternal serum during the first (visit 1) and second (visit 2) trimesters and the change between the 2 time points (visit 2–1). Identifying protein–genetics associations can pinpoint disease-linked proteins influenced by genetic variations, thereby facilitating novel therapeutic target identification.[30,31] We then carried out Mendelian randomization, based on the GWAS findings for these 9 proteins and the most recent large GWAS on PE and gHTN,[32] to investigate whether maternal serum placental and pregnancy protein concentrations in early pregnancy are associated with PE and gHTN. This analysis leverages human genetic variation using Mendelian randomization to examine potential causal relationships between placental and pregnancy proteins and PE/gHTN. Observational studies usually cannot determine causality, and preclinical pregnancy models have limitations, underscoring the potential of human genetics to provide additional insights into causal mechanisms. Finally, we used Mendelian randomization to examine the relationship between PE/gHTN and long-term maternal hypertension (HTN).

Materials and methods

This study is a secondary analysis using a nested case-control design with samples and data from the nuMoM2b pregnancy cohort study. The nuMoM2b study was designed as a prospective cohort study aimed at investigating the underlying causes and pathophysiological pathways associated with adverse pregnancy outcomes (APOs) in nulliparous pregnant individuals. The objective of this current secondary analysis is to identify the associations between placental and pregnancy protein concentrations and genetic variations and to further use Mendelian randomization to explore the proteins' potential roles in causing PE, gHTN, and long-term HTN. A subsample of the nuMoM2b cohort was used to perform the GWAS of placental and pregnancy proteins. Leveraging external large-scale GWAS data on PE/gHTN,[32] combined with nuMoM2b data, Mendelian randomization was employed to investigate the relationships. Details of the data and analysis are described in the sections that follow.

Cohorts

Briefly, the nuMoM2b study enrolled 10,038 nulliparous individuals from 8 geographically disparate clinical sites in the United States (Supplemental Table 1).[33] The study participants were longitudinally followed and underwent 4 study visits, from the first trimester to after birth. Biosamples were obtained at study visits (visit 1 [6–13 weeks], visit 2 [16–21 weeks], visit 3 [22–29 weeks], and at delivery) and pregnancy outcomes were obtained by trained and certified chart abstractors (Supplemental Table 2). The methods of

the nuMoM2b study have been described in detail elsewhere,[33] and the study was approved by the Institutional Review Boards at all participating centers.[33–35] Genome-wide genotyping of 9757 women with sufficient material was performed with whole blood samples collected at visit 1, using the Infinium Multi-Ethnic Global D2 BeadChip (Illumina, Miami). The nuMoM2b Heart Health Study was carried out as a sequel study to gain a better understanding of the influence of pregnancy outcomes on subsequent health,[35] and 4484 women completed the laboratory assessments at the 2-year to 7-year postpartum in-person visit (Supplemental Table 2).

Maternal serum samples were collected at visit 1 and visit 2 to measure the concentrations of 9 placental and pregnancy proteins, including vascular endothelial growth factor (VEGF) (pg/mL), placental growth factor (PlGF) (pg/mL), endoglin (ng/mL), soluble fms-like tyrosine kinase-1 (sFlt-1) (pg/mL), a disintegrin and metalloproteinase domain-containing protein 12 (ADAM-12) (ng/mL), pregnancy-associated plasma protein A (mU/mL), free beta-human chorionic gonadotropin (ng/mL), inhibin A (pg/mL), and alpha fetal protein (AFP) (IU/mL).[7] Detailed information on the measurement of protein concentrations is presented in supplemental text. These proteins fall into 3 categories that reflect different aspects of placental function: (1) angiogenesis (VEGF, PlGF, endoglin, and sFlt-1)[4,8,36–41]; (2) placental implantation and development (ADAM-12 and pregnancy-associated plasma protein A)[42,43]; and (3) biomarkers for fetal chromosomal abnormalities (free beta-human chorionic gonadotropin, inhibin A, and AFP).[44,45] While AFP does not originate from the placenta, its increased concentrations in maternal serum during the second trimester are associated with APOs, likely linked to excessive placental permeability.[46,47] The analysis was conducted in a subsample of the nuMoM2b cohort with available genotypes (n=2352), which included participants who experienced any of the following APOs (n=1463): delivery prior to 37 weeks' gestation, PE or eclampsia, birthweight for gestational age <fifth percentile, or stillbirth. It also included 889 controls who delivered at term without complications. The protein concentrations were log-transformed for subsequent analyses.

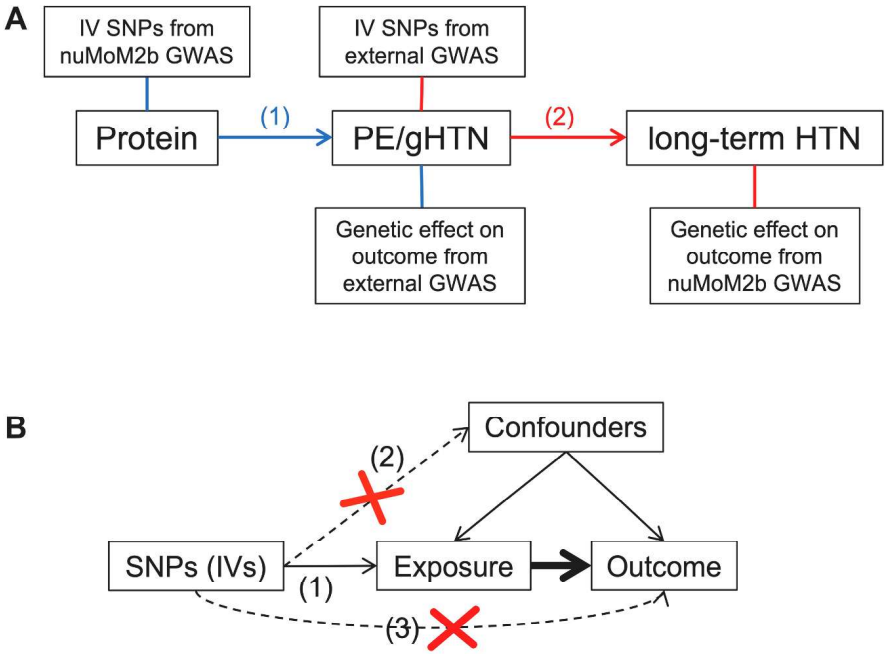
Analytical workflow for quality control and genome-wide association study with multiethnic data

In multiethnic GWAS, it is common to split the total sample into separate populations, perform genotype data quality control, imputation, and GWAS in each population separately, followed by a meta-analysis. However, in our study, the sample size for GWAS of protein concentrations is relatively small, at around 2000 individuals. Further splitting the data into separate populations would make GWAS unreasonable for certain populations with limited sample sizes. The principal component analysis revealed a continuous population structure in the nuMoM2b cohort (Supplemental Figure 1). Consequently, we have developed an analytical workflow for quality control and GWAS with multiethnic data that maximizes the sample size while minimizing bias due to population stratification (Supplemental Figure 2). Genotype imputation was performed using the TOPMed Imputation Server,[48] retaining genotyped and imputed single nucleotide polymorphisms (SNPs) with imputation quality $r^2>0.3$. Our refined nuMoM2b cohort included 9742 women. In the protein GWAS, 2263, 2134, and 2045 women were analyzed for visit 1, visit 2, and visit 2−1 analyses, respectively. Given our sample size, we concentrated on SNPs with minor allele frequency (MAF) >0.05. To control for population stratification, we used the GENESIS R/Bioconductor package[49,50] to fit linear mixed models that integrated a random effect to control for genetic relatedness. The covariates included age and age-squared at visit 1, first 10 principal components calculated by genotypic data, self-reported race, clinical sites, and status for any APOs. Additionally, we conducted supplementary genome-wide interaction studies to remove SNPs with different effects across ancestries. We also ensured no impact from collider bias in our analyses. A genome-wide significance threshold was set at $P<5.6\times10^{-9}$ (Bonferroni-adjusted for 9 proteins: $5\times10^{-8}/9=5.6\times10^{-9}$). This analytical workflow is detailed in the supplemental text.

Mendelian randomization

Mendelian randomization uses SNPs as instrumental variables (IVs) to estimate the effect of a risk factor on the outcome of interest, while removing unmeasured confounding bias. The design of our Mendelian randomization study is depicted in Figure 1, A, which includes 2 analyses: (1) proteins → PE/gHTN and (2) PE/gHTN → long-term postpartum HTN. Several large-scale GWAS have been conducted on PE.[32,51,52] Our study leverages the most recent large GWAS,[32] analyzing PE with 17,150 cases and 451,241 controls and gHTN with 8961 cases and 184,925 controls. As with any Mendelian randomization analysis, some assumptions, such as horizontal pleiotropy, are untestable. To address this, employing multiple approaches with different assumptions and achieving consistent results can enhance confidence in effect estimates. In this study, we considered 3 commonly used approaches to ensure result robustness. Our primary method was the Mendelian randomization-robust adjusted profile scoring[53] given its capability to adjust for weak instrument bias which is particularly relevant in our study with a limited number of strong IV SNPs. We also used random-effect inverse variance weighting[54] and Mendelian randomization pleiotropy residual sum and outlier (MR-PRESSO)[55] as sensitivity analyses. We set the threshold at $P<5.6\times10^{-3}$, Bonferroni-adjusted for 9 proteins. Details of Mendelian randomization analyses can be found in the supplemental text.

FIGURE 1
Mendelian randomization analysis



(**A**) Flowchart for 2-sample Mendelian randomization. (1) Relationship between placental and pregnancy protein concentrations and PE/gHTN and (2) relationship between PE/gHTN and long-term postpartum HTN. (**B**) Illustrative diagram of Mendelian randomization. Its validity relies on 3 assumptions: IVs need to be (1) associated with the exposure, (2) not associated with any confounder of the exposure-outcome association, and (3) independent of the outcome conditional on the exposure and confounders (the horizontal pleiotropy assumption).

gHTN, gestational hypertension; *GWAS*, genome-wide association study; *HTN*, hypertension; *IV*, instrumental variable; *nuMoM2b*, Nulliparous Pregnancy Outcomes Study: Monitoring Mothers-to-Be; *PE*, preeclampsia; *SNP*, single nucleotide polymorphism.

Results

Genome-wide association study of placental and pregnancy protein concentrations

Tables 1 and 2 show the characteristics of the 2352 nulliparous women included in the GWAS of placental and pregnancy protein concentrations. The heritability estimates from Genome-wide Complex Trait Analysis[56] indicated that genome-wide SNPs accounted for approximately 14.5% to 31% of the variance in the 9 proteins (Supplemental Table 3). The quantile-quantile plots (Supplemental Figure 3) for all GWAS (Supplemental Figure 4) showed no inflation of the test statistics. A summary of all GWAS SNPs with $P<5\times10^{-8}$ can be found in Supplemental Table 4.

The SNP rs6487735, located near the pregnancy zone protein (*PZP*) gene, exhibited the strongest association (MAF=0.47, effect=−0.1, $P=3.03\times10^{-22}$; Figure 2, C) with ADAM-12 concentrations at visit 2. A missense variant, rs2277413 in the *PZP* gene, independent of rs6487735 (linkage disequilibrium r2=0.003, Figure 2, C), was also associated with ADAM-12 concentrations at visit 2 (MAF=0.3, effect=0.07, $P=3.32\times10^{-11}$) and showed the strongest association after additional adjustment for rs6487735 ($P=2.37\times10^{-10}$). The GWAS of ADAM-12 at visit 1 also identified the same *PZP* locus, association (Figure 2, B). The results suggested that *PZP* was associated with ADAM-12 concentrations in maternal serum, and this association increased as pregnancy progressed. In sensitivity analyses, we repeated the GWAS of ADAM-12 using only those of self-reported as White, most of whom clustered tightly in the principal component analysis plot (Supplemental Figure 1). We obtained similar but less marked results compared to using all available individuals (Supplemental Figure 5 and Supplemental Table 5).

Our GWAS analysis of VEGF identified rs6921438, located near the vascular endothelial growth factor A (*VEGFA*) gene, as the variant with the strongest association with VEGF concentrations at visit 1 (MAF=0.45, effect=−0.36, $P=7.94\times10^{-30}$) and visit 2 (MAF=0.47, effect=−0.32, $P=2.49\times10^{-28}$) (Figure 3, A and D). This locus has consistently been replicated in multiple studies as being associated with circulating VEGF concentrations in nonpregnant populations.[26–29] Our study confirms the presence of *cis*-acting genetic associations with VEGF concentrations in nulliparous pregnant women. We subsequently investigated whether SNPs previously linked to VEGF concentrations in nonpregnant populations at the *VEGFA* and very low-density lipoprotein receptor loci were also linked to VEGF concentrations in pregnant women. Our analysis confirmed an association with SNPs at *VEGFA*, but not with very low-density lipoprotein receptor (Supplemental Table 6). These findings suggest that the genetic regulation of VEGF concentrations may differ between early pregnancy and nonpregnant periods.

In the GWAS of sFlt-1, rs4349809 at the same *VEGFA* locus was found to be associated with sFlt-1 concentrations at visit 1 (MAF=0.49, effect=−0.09, $P=2.89\times10^{-12}$), but this association was not observed at visit 2 (Figure 3, B and C). Furthermore, rs4349809 was also associated with VEGF concentrations in the same direction at visit 1 (effect=−0.33, $P=7.91\times10^{-25}$) and visit 2 (effect=−0.3, $P=2.34\times10^{-25}$) as with sFlt-1. VEGF stimulates blood vessel growth crucial for supporting fetal development, while sFlt-1 inhibits VEGF by binding to it.[57] Despite their opposing roles in regulating angiogenesis during pregnancy, both were

TABLE 1
Summary of women characteristics included in the GWAS of placental and pregnancy protein concentrations

Demographic characteristics	Total (n=2352)
Age at visit 1 (mean±SD)	26.77 ± 5.84
Self-reported race	
White	1356 (57.65%)
Black	389 (16.54%)
Hispanic	394 (16.75%)
Asian	80 (3.4%)
Other	133 (5.65%)
Adverse pregnancy outcome status	1463 (62.2%)
Preeclampsia or eclampsia	552 (23.47%)
Preterm birth	776 (32.99%)
Spontaneous preterm birth	451 (19.18%)
Stillbirth	48 (2.04%)
Small gestational age	396 (16.84%)

GWAS, genome-wide association studies; *SD*, standard deviation.

influenced by the *VEGFA* locus, which showed a stronger association with VEGF than with sFlt-1. We performed sensitivity analyses by repeating the GWAS analysis of VEGF and sFlt-1 using only self-reported White. The results were similar to those obtained using all individuals, but less marked (Supplemental Figures 6 and 7, and Supplemental Table 5).

All the relevant genetic associations persisted even after further adjustment for gestational age at the time of blood collection (Supplemental Figure 8). To further explore the relationship between VEGF and sFlt-1, we conducted additional GWAS focusing on the VEGF/sFlt-1 ratio at visit 1, visit 2, and visit 2−1. The same *VEGFA* locus was found to be associated with the ratio at visit 1 and visit 2 (Supplemental Figure 9).

Mendelian randomization: placental and pregnancy protein concentrations, preeclampsia/gestational hypertension, and long-term postpartum hypertension

Our Mendelian randomization-robust adjusted profile scoring analyses revealed a substantial effect between ADAM-12 at visit 1 and gHTN (odds ratio [OR]=0.78, $P<.001$) (Figure 4 and Supplemental Figure 10). Both inverse variance weighting[54] and MR-PRESSO[55] analyses confirmed this association (Supplemental Figures 11 and 12). Although ADAM-12 concentrations did not meet the significant association threshold with PE, the consistent direction and similar ORs for ADAM-12 concentrations at both visits for PE and gHTN (Figure 4) suggest that a similar relationship to PE remains possible. This is consistent with clinical evidence that gHTN is on the spectrum of hypertensive disorders with PE, as it has similar clinical features and frequently progresses to PE

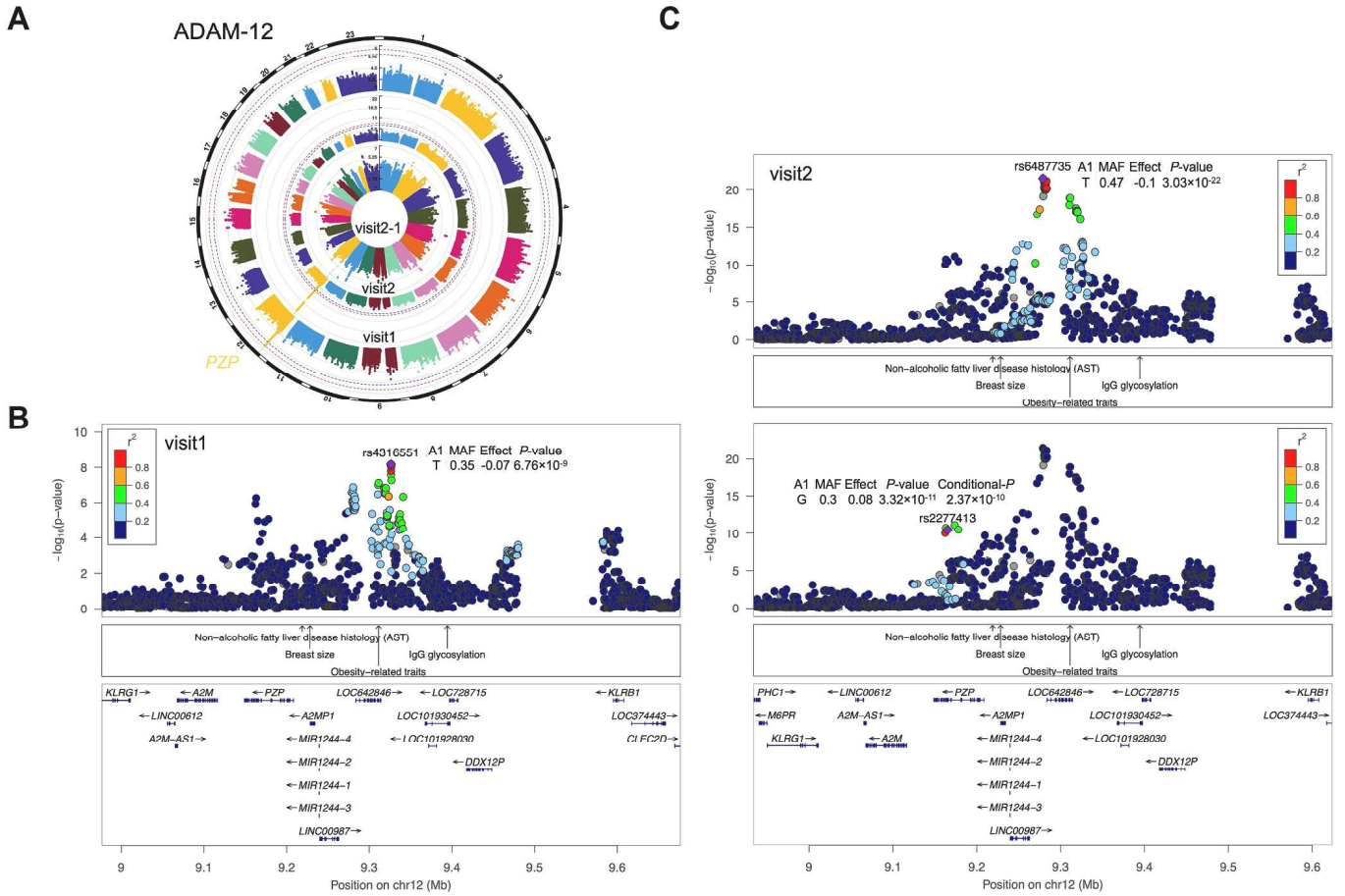
TABLE 2
Summary of the serum concentrations of 9 placental and pregnancy proteins (mean±SD) included in GWAS

Protein	Visit 1 (n=2263)	Visit 2 (n=2134)	Visit 2−1 (n=2045)	*P* value[a] (visit 2−1)
ADAM-12 log (ng/mL)	1.49 ± 0.43	2.25 ± 0.34	0.77 ± 0.36	<.0001
AFP log (IU/mL)	2.53 ± 0.71	3.81 ± 0.48	1.29 ± 0.69	<.0001
ENG log (ng/mL)	1.83 ± 0.22	1.7 ± 0.23	−0.14 ± 0.17	<.0001
fβHCG log (ng/mL)	2.99 ± 0.62	1.39 ± 0.75	−1.6 ± 0.59	<.0001
INHA log (pg/mL)	5.75 ± 0.49	5.35 ± 0.44	−0.39 ± 0.44	<.0001
PAPP-A log (mU/mL)	6.74 ± 1.05	9.05 ± 0.8	2.33 ± 0.9	<.0001
PIGF log (pg/mL)	3.68 ± 0.56	5.18 ± 0.66	1.5 ± 0.61	<.0001
sFlt-1 log (pg/mL)	6.77 ± 0.43	6.77 ± 0.48	0 ± 0.33	.57
VEGF log (pg/mL)	0.18 ± 0.96	0.27 ± 0.88	0.17 ± 0.77[b]	<.0001

ADAM-12, a disintegrin and metalloproteinase domain-containing protein 12; *AFP*, alpha fetal protein; *ENG*, endoglin; *fβHCG*, free beta-human chorionic gonadotropin; *GWAS*, genome-wide association studies; *INHA*, inhibin A; *PAPP-A*, pregnancy-associated plasma protein A; *PIGF*, placental growth factor; *SD*, standard deviation; *sFlt-1*, soluble fms-like tyrosine kinase-1; *VEGF*, vascular endothelial growth factor.

[a] *P* value represents the difference between visit 2 and visit 1 on the logarithm scale using *t*-test (ie, H_0: visit 2−1=0); [b] The discrepancy between the difference in mean VEGF concentrations at visit 1 and visit 2 and the visit 2−1 is attributed to the fact that individuals who had VEGF concentrations measured at both visits had a much higher mean VEGF concentrations at visit 2 (mean=0.37) compared with all individuals who had their VEGF concentrations measured at visit 2 (mean=0.27).

FIGURE 2
GWAS of ADAM-12



(**A**) Circular Manhattan plots. Manhattan plot displays the associations between SNPs across the genome and a specific trait, with the spikes indicating regions of significant associations. This circular format presents results from multiple GWAS simultaneously. The chromosomal position of each single SNP is displayed along the circle and the negative log10 of the association *P* value is displayed on the radius. The red line represents the genome-wide significance level ($P<5.6\times10^{-9}$) and blue line represents the suggestive significance level ($P<5\times10^{-8}$). Results for visit 1 are displayed on the outer circle, visit 2 on the middle circle, and visit 2−1 on the inner circle. (**B**) Regional plot for SNP rs4316551 from the visit 1 analysis. A regional plot provides a detailed view of a specific genomic region, showing the association between SNPs and a specific trait. The lower portion of the figure displays the relative location of genes and the direction of transcription, while the middle portion shows known GWAS associations at the locus from the GWAS catalog. The x-axis displays the chromosomal position and the y-axis shows the significance of the associations. The purple diamond shows the *P* value for the reference SNP. The circles show the *P* values for all other SNPs and are color-coded according to the level of linkage disequilibrium with the reference SNP using the nuMoM2b cohort. (**C**) Regional plots for SNPs rs6487735 and rs2277413 from the visit 2 analysis.

A1, minor allele; *ADAM-12*, a disintegrin and metalloproteinase domain-containing protein 12; *chr*, chromosome; *Conditional-P*, conditional *P* value after adjusting for the top SNP rs6487735; *Effect*, the genetic effect of minor allele; *GWAS*, genome-wide association study; *MAF*, minor allele frequency; *nuMoM2b*, Nulliparous Pregnancy Outcomes Study: Monitoring Mothers-to-Be; *PZP*, pregnancy zone protein; r^2, squared correlation between SNPs; *SNP*, single nucleotide polymorphism.

when expectantly managed.[58,59] Our results also revealed effects of PE (OR=1.75, $P=8.3\times10^{-3}$) and gHTN (OR=1.84, $P=.005$) on the development of long-term postpartum HTN (Figure 5 and Supplemental Figure 13). In all Mendelian randomization analyses, we observed no heterogeneity, as indicated by Cochran's Q, and the MR-PRESSO global pleiotropy test revealed no evidence of horizontal pleiotropy in the IV SNPs.

Discussion

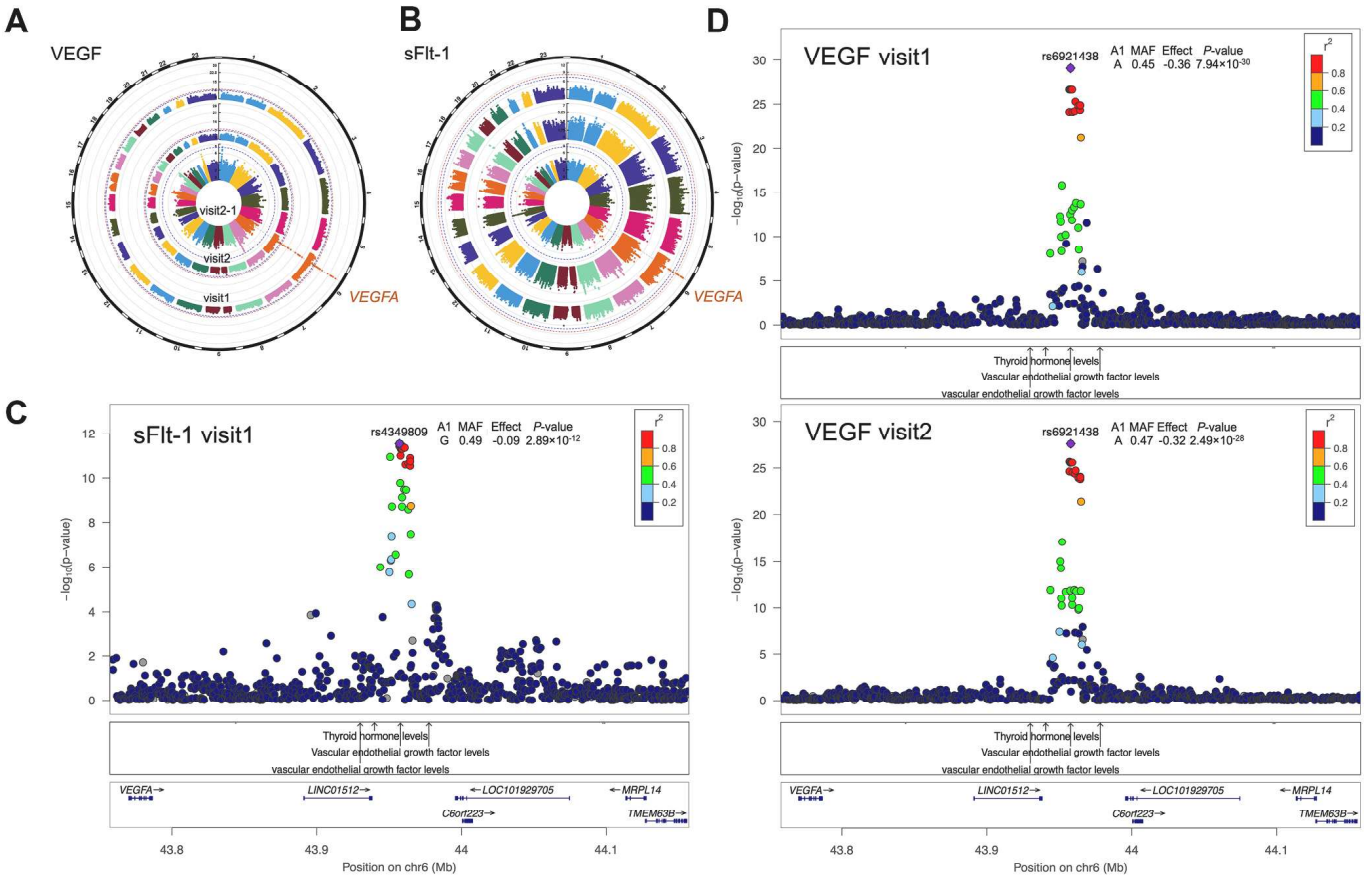
Principal findings

In our GWAS, we identified associations between the *PZP* gene locus and ADAM-12 concentrations and the *VEGFA* locus with concentrations of both VEGF and sFlt-1 during pregnancy. Mendelian randomization analyses revealed associations between ADAM-12 at visit 1 and gHTN and between PE/gHTN in the first pregnancy and HTN occurring 2 to 7 years postpartum.

Results in the context of what is known

Our identification of *PZP* as being important for ADAM-12 concentrations

FIGURE 3
GWAS of sFlt-1 and VEGF

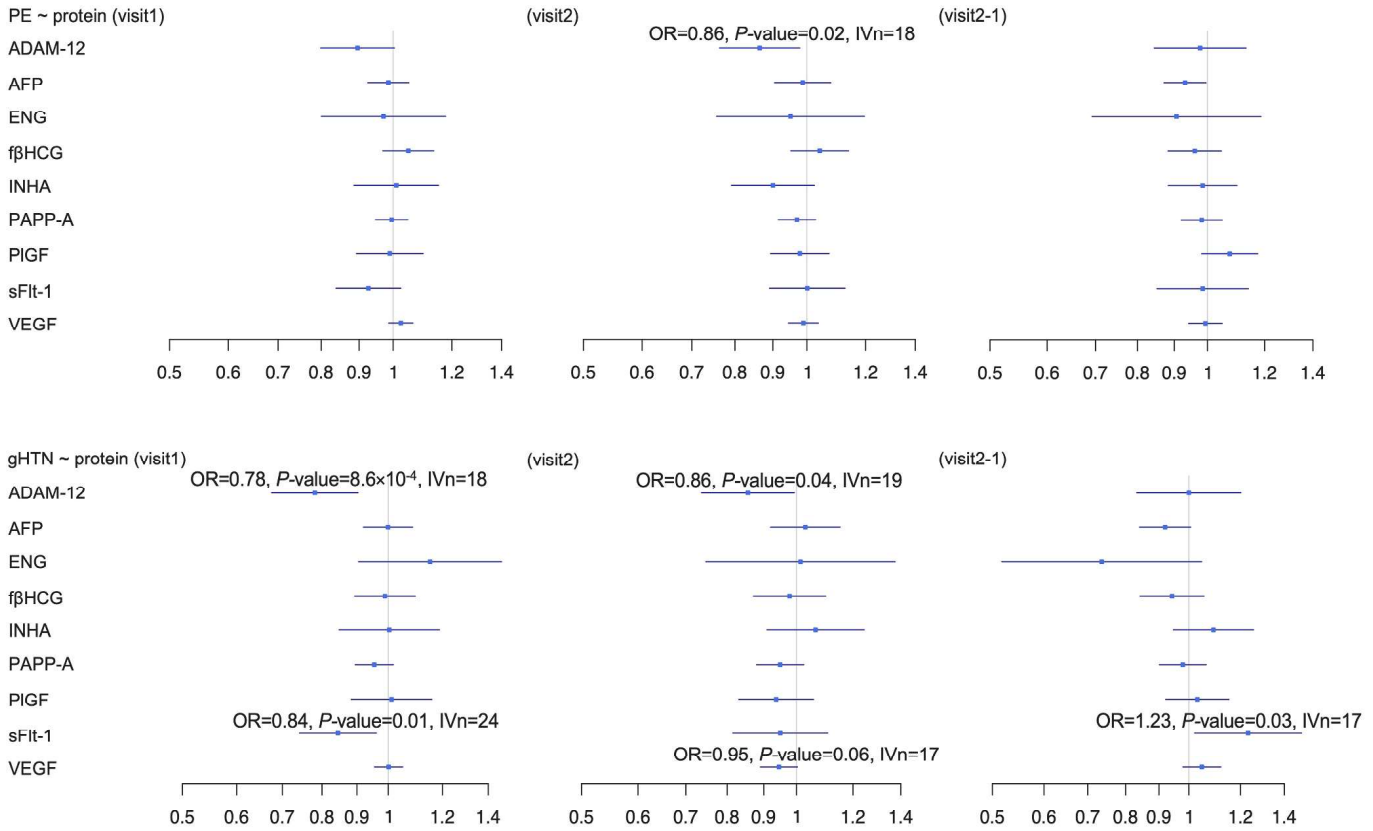


(**A** and **B**) Circular Manhattan plots for sFlt-1 and VEGF. Manhattan plot displays the associations between SNPs across the genome and a specific trait, with the spikes indicating regions of significant associations. This circular format presents results from multiple GWAS simultaneously. The chromosomal position of each single SNP is displayed along the circle and the negative log10 of the association *P* value is displayed on the radius. The red line represents the genome-wide significance level ($P<5.6\times10^{-9}$) and blue line represents the suggestive significance level ($P<5\times10^{-8}$). Results for visit 1 are displayed on the outer circle, visit 2 on the middle circle, and visit 2—1 on the inner circle. (**C**) Regional plot for SNP rs4349809 from the sFlt-1 visit 1 analysis. A regional plot provides a detailed view of a specific genomic region, showing the association between SNPs and a specific trait. The lower portion of the figure displays the relative location of genes and the direction of transcription, while the middle portion shows known GWAS associations at the locus from the GWAS catalog. The x-axis displays the chromosomal position and the y-axis shows the significance of the associations. The purple diamond shows the *P* value for the reference SNP. The circles show the *P* values for all other SNPs and are color-coded according to the level of linkage disequilibrium with the reference SNP using the nuMoM2b cohort. (**D**) Regional plots for SNP rs6921438 from the VEGF visit 1 and visit 2 analyses.

A1, minor allele; *chr*, chromosome; *Effect*, the genetic effect of minor allele; *GWAS*, genome-wide association study; *MAF*, minor allele frequency; *nuMoM2b*, Nulliparous Pregnancy Outcomes Study: Monitoring Mothers-to-Be; r^2, squared correlation between SNPs; *sFlt-1*, soluble fms-like tyrosine kinase-1; *SNP*, single nucleotide polymorphism; *VEGF*, vascular endothelial growth factor; *VEGFA*, vascular endothelial growth factor A.

is consistent with existing literature on the biological effects of *PZP*.[60–64] While we are aware of prior work finding ADAM-12 may be a potential early biomarker for PE, we are unaware of other literature linking PZP and ADAM-12.[65] *PZP* encodes a protein (PZP) that plays a critical protective role during pregnancy by managing inflammation and oxidative stress.[60,61] It assists in clearing misfolded proteins and proinflammatory cytokines, both enhanced by oxidative stress, to prevent inflammatory responses that could impair placental function.[61] Additionally, it modulates immune activity by inhibiting T-helper 1 cells in conjunction with placental protein-14, helping maintain a pregnancy-friendly immune environment.[60–62] Low concentrations of *PZP* can lead to uncontrolled inflammation, contributing to placental dysfunction and the onset of conditions like PE.[61,66] ADAM-12, a metalloproteinase secreted by the placenta, cleaves insulin-like growth factor binding proteins.[42,67] It promotes cell invasion and direct column outgrowth in early placental development.[64] ADAM-12 has been localized to anchoring trophoblast columns of first-trimester placentas and to highly invasive trophoblasts within placental villous explants that degrade the

FIGURE 4
Estimates of the serum concentrations of 9 placental and pregnancy proteins on PE and gHTN



P values were determined by the 2-sample MR-RAPS method. The squares represent the estimates on the odds ratio (OR) scale and the whiskers show the corresponding 95% confidence intervals.

ADAM-12, a disintegrin and metalloproteinase domain-containing protein 12; *AFP*, alpha fetal protein; *ENG*, endoglin; *fβHCG*, free beta-human chorionic gonadotropin; *gHTN*, gestational hypertension; *INHA*, inhibin A; *IVn*, number of instrumental variable SNPs used for the estimation of the effects; *MR-RAPS*, Mendelian randomization-robust adjusted profile scoring; *PAPP-A*, pregnancy-associated plasma protein A; *PE*, preeclampsia; *PlGF*, placental growth factor; *sFlt-1*, soluble fms-like tyrosine kinase-1; *SNP*, single nucleotide polymorphism; *VEGF*, vascular endothelial growth factor.

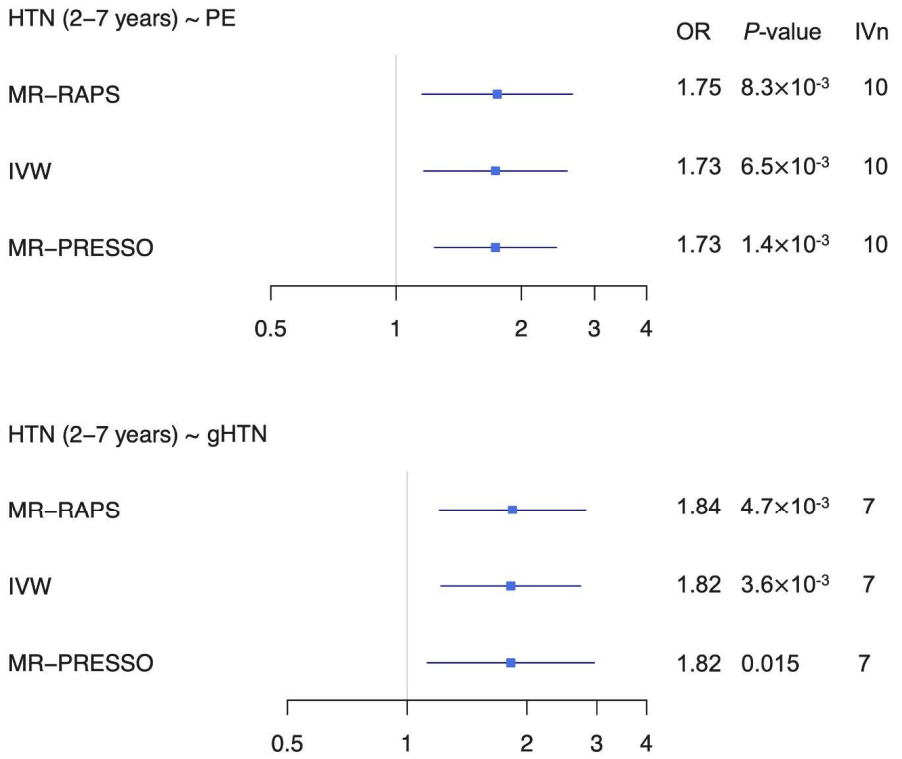
extracellular matrix.[63,64] Recent studies further highlight the role of its shorter variant, ADAM12S, in regulating the migration and invasion of trophoblasts into the uterine lining.[42] Moreover, the placenta expresses ADAM-12 at high concentrations, leading to elevated concentrations in the maternal circulation during pregnancy.[67] Low first-trimester concentrations of ADAM-12 in maternal circulation have been consistently associated with the development of PE.[42,68,69] These known functions of ADAM-12 as a key regulator of early placental implantation and development are consistent with our finding of statistical evidence for its role in gHTN and possibly PE.

Our finding that the *VEGFA* locus was associated with VEGF concentrations at visits 1 and 2 is in line with previous reports in nonpregnant populations.[26–29] Our study extends these findings to confirm the presence of *cis*-acting genetic associations with VEGF concentrations during pregnancy. Surprisingly, *VEGFA*'s association with sFlt-1 concentrations at visit 1 showed the same effect direction as for VEGF concentrations despite their antagonistic functions during pregnancy.[57] Both VEGF and sFlt-1 are known to be crucial for the development of the placenta, and their balance is essential for normal placental angiogenesis.[70–72] VEGF, a potent angiogenic factor, stimulates the formation of new blood vessels and supports endothelial cell function.[70,71] sFlt-1, a soluble receptor of VEGF, acts as an antiangiogenic protein by antagonizing the actions of VEGF and PlGF.[70–72] An imbalance, characterized by increased concentrations of sFlt-1 and decreased concentrations of both VEGF and PlGF, is believed to contribute to the development of PE.[4,8,73–77]

Clinical implications

Our results suggest the potential utility of early pregnancy ADAM-12 serum concentrations as a biomarker and potential therapeutic target for gHTN and PE, similar to conclusions in a 2005 paper.[65] ADAM-12's established role in early placental development and trophoblast invasion,[63,64] a process that is known to be incomplete in PE and other placenta-mediated conditions,[1] makes it an attractive candidate for

FIGURE 5
Estimates of PE and gHTN on long-term postpartum HTN



P values were determined by the 2-sample MR-RAPS, IVW, and MR-PRESSO methods. The squares represent the estimates on the odds ratio (OR) scale and the whiskers show the corresponding 95% confidence intervals.

gHTN, gestational hypertension; *HTN*, hypertension; *IVn*, number of instrumental variable SNPs used for the estimation of the effects; *IVW*, inverse variance weighting; *MR-PRESSO*, Mendelian randomization pleiotropy residual sum and outlier; *MR-RAPS*, Mendelian randomization-robust adjusted profile scoring; *PE*, preeclampsia; *SNP*, single nucleotide polymorphism.

early pregnancy disease modification. The Mendelian randomization results that ADAM-12 is potentially in the pathway of these hypertensive disorders of pregnancy (HDP) further strengthen its potential as a target, more than if the findings were from a simple association study. While we did not find SNPs in the ADAM-12 gene to be associated with the ADAM-12 concentration, we did identify that *PZP* has a role in regulating ADAM-12 concentrations. These findings highlight ADAM-12 and *PZP* as potential therapeutic targets for preventing HDP. Modulating these pathways may also have relevance to preventing progression to chronic hypertension. Our magnetic resonance findings are intended to prioritize potential biological targets[78] rather than assert therapeutic efficacy. Mendelian randomization and human genetic studies have previously helped prioritize several drug targets, including IL6R signaling for coronary disease[79,80] and PCSK9 for low-density lipoprotein lowering,[81] both later translated into clinical or therapeutic evaluation. Similarly, our results highlight ADAM-12 as a candidate for further mechanistic and translational investigation.

Research implications

Further work is needed to confirm both the predictive and causal roles of ADAM-12 for HDP. ADAM-12 administration and the importance of *PZP* can be investigated in animal models, while confirmation of ADAM-12's utility as a biomarker requires validation in large human cohorts. Future research should focus on validating the predictive value of ADAM-12, elucidating its biological mechanisms, and exploring aspects of its practical application in clinical settings. While ours was a targeted approach, additional opportunities for biomarker and therapeutic target discovery are available via untargeted proteomics techniques, such as Olink or SomaScan.[82] Establishing a comprehensive profile of genetically regulated proteins during pregnancy may identify additional biomarkers and therapeutic targets and provide a unique resource for further Mendelian randomization studies examining pregnancy and postpartum outcomes. Finally, investigating the fetal genetic influences on placental proteins is essential, as many circulating proteins (eg, VEGF and sFlt-1[3,4,9]) are predominantly derived from the placenta. Future studies incorporating fetal genotypes will also enable researchers to distinguish between genetic contributions originating from the fetus or the mother.

Strengths and limitations

We analyzed the changes in placental and pregnancy proteins from maternal serum during early pregnancy. These early pregnancy proteins may serve as predictors for outcomes during and after pregnancy. This study is the first to systematically examine multiple protein concentrations during pregnancy using a GWAS approach. Our findings highlight ADAM-12 as a key factor in the development of HDP and point to *PZP* as having a role in regulating ADAM-12 concentrations. These findings highlight both ADAM-12 and *PZP* as potential therapeutic targets. Another strength of our methodology is that we employed the 2-sample Mendelian randomization, a method recognized for its conservative and unbiased approach to potential causal inference,[83] and took advantage of a recent large PE/gHTN GWAS[32] in Mendelian randomization analyses.

We recognize several study limitations. First, the sample size was limited, although this is the first GWAS examining the specific phenotypes. Second, while our analytical workflow was designed to minimize bias due to

Glossary

ADAM-12: A disintegrin and metalloproteinase domain-containing protein 12, involved in cell adhesion, migration, proliferation, and placental function.
AFP: Alpha fetal protein, produced by liver and yolk sac of fetus.
Bonferroni adjustment: A method to correct for multiple comparisons in GWAS. It reduces the chance of false positives by lowering the threshold for statistical significance. It is usually set at *P* value=5×10^{-8} for a single GWAS. When multiple GWAS are performed, further adjustment may be needed.
Circular Manhattan plot: A variant of the traditional Manhattan plot, which is presented in a linear format with chromosomes laid out along the x-axis and the -log10 of the *P* value on the y-axis. In contrast, a circular Manhattan plot arranges this information in a circular format, allowing multiple Manhattan plots to be displayed within the same circle.
Collider bias: A potential bias in regression models (eg, Y=X+covariate), where if Y and X independently influence the covariate, collider bias may occur.
ENG: Endoglin, a cell surface glycoprotein involved in vascular development.
fβHCG: Free beta-human chorionic gonadotropin, produced by the placenta and can be a marker for adverse pregnancy outcomes.
Genome-wide association study (GWAS): A study assessing the association of SNPs across the genome with a specific trait.
Genome-wide interaction studies (GWIS): A study assessing interactions between SNP-by-exposure and a specific trait across the genome.
Genotype: In the context of GWAS, genotypes are often coded as 0 (homozygous for the reference allele), 1 (heterozygous), or 2 (homozygous for the alternative allele) to reflect the number of alternative alleles carried by an individual.
Genotype imputation: The process of inferring genotypes that are not directly measured.
Horizontal pleiotropy: In Mendelian randomization, horizontal pleiotropy occurs when instrumental variable SNPs influence the outcome through pathways other than the exposure, as shown in pathway (3) in Figure 1, B. It is assumed that horizontal pleiotropy is absent. The existence of horizontal pleiotropy can be assessed by statistical tests, such as Cochran's Q and the MR-PRESSO global pleiotropy test.
INHA: Inhibin A, produced by the placenta and can be a marker for adverse pregnancy outcomes.
Instrumental variables: In Mendelian randomization, an instrumental variable is an SNP used as a proxy for the exposure to estimate its effect on the outcome.
Linear mixed model: A statistical model that can account for both fixed effects and random effects. In this study, it is used to control for genetic relatedness in association studies.
Manhattan plot: A graphical representation of GWAS results that displays the -log10 of the *P* value for each SNP across the genome. Each dot represents a single SNP, with its height indicating the level of significance of its association with the outcome. This plot helps to highlight genomic regions significantly associated with the outcome.
Mendelian randomization: A statistical method using SNPs as instrumental variables to estimate the effect of a risk factor on an outcome, aiming to minimize unmeasured confounding bias. Multiple approaches are recommended for consistency due to untestable assumptions like horizontal pleiotropy.
PAPP-A: Pregnancy-associated plasma protein A, produced by the placenta and can be a marker for adverse pregnancy outcomes.
PlGF: Placental growth factor, expressed by the placenta and is a key factor in vascular development.
Population stratification: Confounding in genetic studies due to sampling from different ancestries.
Principal component analysis (PCA): In the context of GWAS, PCA reduces the dimensionality of genetic data, summarizing genetic variations into principal components that represent genetic ancestry.
PZP: Genetic locus of pregnancy zone protein, which encodes a protein thought to inhibit T-cell function during pregnancy.
Regional plot: A detailed visualization of GWAS results focused on a specific genomic region, displaying the association *P* value relative to genomic positions, local linkage disequilibrium reflecting SNP correlations, and the genes located in the region.
sFlt-1: Soluble fms-like tyrosine kinase-1, a circulating antagonist to VEGF and PlGF.
Single nucleotide polymorphism (SNP): A variation at a single position in the DNA sequence among individuals, which is labeled by the "rs" number, a unique identifier assigned to a specific SNP. The "rs" stands for "reference SNP".
VEGF: Vascular endothelial growth factor, required for regulating the proliferation, migration, and survival of embryonic endothelial cells during the female reproductive cycle.
VEGFA: Genetic locus of vascular endothelial growth factor A, which encodes the VEGF protein and is a key factor in vascular development.
VLDLR: Genetic locus of very low-density lipoprotein receptor.

population stratification in our multiethnic cohort, it is possible that this approach inadvertently biased the results toward SNPs more common or with stronger effects in White given their larger proportion. Additionally, this method may have eliminated SNPs with genetic effects specific to a single ancestry. Third, only 9 placental and pregnancy proteins were examined. Untargeted proteomics data may identify additional genetically regulated proteins during pregnancy. We also only measured these proteins at the first 2 study visits. Fourth, the lack of available data concerning the same questions prevented the replication of the GWAS of proteins in other cohorts. Fifth, fetal SNPs, which could be important, are lacking. The use of fetal SNPs as IVs might be a more effective approach in Mendelian randomization analysis to avoid horizontal pleiotropy. Sixth, the correlation between maternal and fetal genotypes makes it challenging to discern the source of the genetic effects.

Conclusion

Our study identified genetic associations with placental proteins ADAM-12, VEGF, and sFlt-1, providing insights into their regulation during pregnancy. Mendelian randomization analyses revealed associations between the serum concentrations of proteins, particularly ADAM-12, and PE/gHTN, which could lead to potential prevention and treatment strategies for HDP. Further research is needed to understand the biological mechanisms underlying these associations and confirm their causal relationships with PE/gHTN. ■

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Measurement of protein concentrations

During 3 study visits in pregnancy, which occurred at 6 to 13 weeks, 16 to 21 weeks, and 22 to 29 weeks of gestation, peripheral maternal blood samples were collected using serum separating tubes. The collected blood samples were then centrifuged, and 0.5 cc serum aliquots were stored at −70 C within 2 hours of collection. The samples were transported on dry ice to analytical laboratories for batch analyses. Placental and pregnancy protein concentrations in maternal serum samples collected at the first 2 study visits were measured for adverse pregnancy outcome (APO) prevention studies as earlier pregnancy biomarkers may provide effective strategies for preventing APOs.

Maternal serum concentrations of proteins were measured at 2 laboratories: Translational Core Laboratory at Children's Hospital of Philadelphia (Philadelphia, PA) and Eurofins NTD, LLC (Melville, NY). The Children's Hospital laboratory used enzyme-linked immunosorbent assays (ELISAs) to measure ENG (human endoglin assay; R&D Systems, Minneapolis, MN) and ADAM-12 (human ADAM-12 ELISA; R&D Systems, Minneapolis, MN), and electrochemiluminescence assays (ECLs) to measure VEGF (human VEGF-A ECL; Merck Sharp & Dohme, Kenilworth, NJ) and sFlt-1 (human Flt-1 ECL; Merck Sharp & Dohme, Kenilworth, NJ). The Eurofins NTD laboratory used lanthanide-based time-resolved fluorometry (TRF) to measure 5 proteins: PlGF, PAPP-A, INHA, fβHCG, and AFP. For more details, please refer to our previous paper.[1]

Analytical workflow for quality control and genome-wide association studies with multiethnic data

Genotype data quality control and imputation

To conduct preimputation QC, we initially excluded SNPs and women with a missing rate more than 10% in the nuMoM2b cohort. Subsequently, we identified SNPs with Hardy-Weinberg equilibrium (HWE) $P<1\times10^{-6}$ or minor allele frequency (MAF) <0.01 in self-reported White, Black, or Hispanic populations, which constitute the 3 major ethnic groups. These SNPs were then removed from the full dataset. We subsequently performed principal component analysis (PCA) using PC-AiR[2] to account for related individuals, with kinship coefficients estimated by KING.[3] Next, we assessed sex concordance and autosomal heterozygosity, accounting for population structure with the first 6 principal components (PCs), following the approach described in Bycroft et al. S3.5.2.[4] In brief, we fitted the following linear regression model for the raw sex concordance or autosomal heterozygosity value, F,

$$F = F_0 + \sum_{i=1}^{6} \beta_i PC_i + \sum_{i=1}^{6}\sum_{j=i}^{6} \beta_{ij} PC_i PC_j + \varepsilon$$

where the fitted F_0 is the PC-adjusted (ie, ancestry-corrected) sex concordance or autosomal heterozygosity F-value. We then excluded women from the data who violated sex concordance (adjusted F-value >0.2) or autosomal heterozygosity (adjusted |F-value| >0.15) (Supplemental Figure 14). As a result, a few individuals were removed from the data. We then repeated the previously mentioned steps for HWE and MAF filters and recalculated the PCA (Supplemental Figure 1).

Next, we used the TOPMed Imputation Server[5] to perform genotype imputation, using the TOPMed (Version R2 on GRC38) as the reference panel, which includes all populations in our cohort. Joint imputation was applied to all individuals, as it is expected to perform and splitting the reference panel to match the target population.[6] We retained genotyped and imputed SNPs with imputation quality $r^2>0.3$ and repeated HWE and MAF filters. Following QC, our nuMoM2b cohort comprised 9742 women. For protein genome-wide association studies (GWAS), we analyzed 2263, 2134, and 2045 women for visit 1, visit 2, and visit 2−1 analyses, respectively. Due to our moderate sample size, we focused on SNPs with MAF>0.05.

GWAS adjusting for population structure

To account for population stratification and genetic relatedness among individuals, we employed a mixed-model approach to conduct GWAS of protein concentrations. This approach is advantageous because it includes all individuals regardless of familial and ancestral relatedness and can provide greater statistical power by controlling for the variance attributed to genetic relatedness.[7] We used the GENESIS R/Bioconductor package[8,9] to fit linear mixed models that integrated a random effect to control for genetic relatedness, with the genetic relationship matrix (GRM) computed by PC-Relate.[10] The covariates considered in the analysis included age and age-squared at visit 1, first 10 PCs calculated by genotypic data, self-reported race, clinical sites, and status for any APOs. To model the continuous log-transformed concentrations of each protein, y, we used a linear mixed model:

$$y = \beta_0 + \beta_1 \mathrm{SNP} + \beta_{\mathrm{cov}} \mathrm{covariates} + u + \varepsilon$$

where u is the random variable accounting for genetic relatedness and ε is the random error. Although mixed models are advantageous for controlling population stratification, their effectiveness may be limited in a diverse cohort. This is because the GRM, which is estimated based on genome-wide data, assumes that all SNPs have similar population-level deviations. However, some SNPs may have larger or smaller deviations between populations than the genome-wide average, leading to inadequate control of false positive rates.[11] As a result, further adjustments are necessary to address this issue.

Hence, we conducted supplementary genome-wide interaction studies

(GWIS) to explore the association between SNP-by-PCs and protein concentrations,

$$y = \beta_0 + \beta_1 \text{SNP} + \beta_{\text{int}} \text{SNP} \times \text{PCs} + \beta_{\text{cov}} \text{covariates} + u + \varepsilon$$

where **PCs** are the first 10 PCs that capture the ancestry information and thus $\boldsymbol{\beta}_{\text{int}}$ includes 10 parameters. We then used a 10 degree of freedom test to evaluate the null hypothesis H_0: $\boldsymbol{\beta}_{\text{int}} = 0$. A significant $\boldsymbol{\beta}_{\text{int}}$ indicates that the effects of a particular SNP differ across ancestries. The GWIS were also performed using the GENESIS package.[8,11] To ensure the retention of only SNPs with consistent effects, we removed SNPs with a P value of $\boldsymbol{\beta}_{\text{int}}$ <0.01 from the GWAS results. It is worth noting that the threshold of 0.01 is conservative and excludes any potentially ancestry-specific SNPs.

Collider bias consideration

The GWAS of protein concentrations were based on a combination of all women with APOs and a random subset. As a result, the protein concentrations might not accurately reflect the distributions in the general population. Although women in the nuMoM2b cohort had not progressed to APOs during visit 1 and visit 2, we adjusted for the APO status in our GWAS of protein concentrations. However, if both the protein and SNP are independent causes of APO status, there is a possibility of collider bias (as illustrated in Supplemental Figure 15). To address this issue, we performed a GWAS of APO status and examined the association between protein concentrations and APO status, while adjusting for the same covariates as before except for the APO status, which was the binary outcome in this analysis. We identified the protein−SNP pair as a potential collider bias and excluded it when the P value was less than 0.01 in the GWAS of APO status and when the P value of the association between the protein and APO status was less than 0.05.

Using the proposed GWAS pipeline, we examined genetic associations with the serum concentrations of 9 placental and pregnancy proteins, which were measured during visit 1, visit 2, and visit 2−1. We set the genome-wide significance level at $P<5.6\times10^{-9}$ (Bonferroni-adjusted for 9 proteins: $5\times10^{-8}/9=5.6\times10^{-9}$) to identify significant SNPs, which were annotated using ANNOVAR.[12] Additionally, we performed sensitivity analyses by restricting the GWAS to self-reported White only. We conducted the analyses using PLINK2[13] without considering a random effect that controls for genetic relatedness.

Mendelian randomization

Figure 1 illustrates the design of the Mendelian randomization analysis. To investigate the relationships between protein concentrations and both preeclampsia (PE) and gestational hypertension (gHTN), as well as PE/gHTN and long-term postpartum HTN, we used a 2-sample Mendelian randomization framework. Two-sample Mendelian randomization has a major advantage over one-sample Mendelian randomization as it only requires GWAS summary statistics rather than individual-level data. Additionally, 2-sample Mendelian randomization is typically considered more conservative and unbiased than one-sample Mendelian randomization because it allows for separate cohorts for exposure and outcome data, while in one-sample Mendelian randomization, both exposure and outcome are from the same cohort.[14]

Proteins → PE/gHTN

We used independent SNPs with a $P<1\times10^{-5}$ from the GWAS of protein concentrations as instrumental variables (IVs). Our rationale for using this threshold was to identify more independent IV SNPs, as this could promote balanced pleiotropy, which helps mitigate bias due to horizontal pleiotropy. Previous studies have shown that this liberal threshold can result in better performance than a conservative threshold of 5×10^{-91}.[15] To select independent IV SNPs, we used a stepwise selection strategy for each chromosome. This approach involved: (1) selecting SNPs with a GWAS $P<1\times10^{-5}$, ordering them by P values, and selecting the top SNP with the lowest P value; (2) running the genetic regression models again on the remaining SNPs, with additional adjustment on the saved top SNP; (3) selecting SNPs with a conditional $P<1\times10^{-5}$, ordering them by P values, and saving the top conditional SNP; (4) rerunning the genetic regression models again on the remaining SNPs, with additional adjustment on the last saved top conditional SNP; and (5) repeating steps (3) and (4) until no SNPs remained in the remaining set.

We obtained genetic association estimates for PE and gHTN from a recent multiancestry meta-analysis of GWAS, which included 17,150 PE cases and 451,241 controls and 8961 gHTN cases and 184,925 controls in the discovery analysis. It's important to note that the nuMoM2b cohort was not included in the discovery analysis but rather treated as a follow-up cohort in that study. A detailed description of the study design and participant characteristics can be found in the original publication.[16] After extracting the genetic effect estimates from the GWAS of PE/gHTN, we switched the effect directions and test alleles to ensure consistency with the results from the GWAS of protein concentrations. Ideally, both studies in a 2-sample Mendelian randomization should include the same populations, as some SNPs are expected to have ancestry-specific effects. However, since we excluded SNPs from the GWAS of protein concentrations that had ancestry-specific effects on protein concentrations, the genetic results were considered to be generic, and therefore, there were fewer concerns about population compatibility with a second study when conducting a 2-sample Mendelian randomization in this present study.

We used MR-robust adjusted profile scoring (MR-RAPS)[17] as the primary method, with squared error loss, as it can account for weak instrument bias which is particularly relevant in our study with a limited number of strong IV SNPs. We also used the commonly used Mendelian randomization

methods, including random-effect inverse variance weighting (IVW),[18] and Mendelian randomization pleiotropy residual sum and outlier (MR-PRESSO),[19] which corrects pleiotropy via outlier IV removal. However, we did not use Mendelian randomization-Egger (MR-Egger) in this study, as it is a conservative method,[20] especially when only a few genetic loci are associated with protein concentrations in our GWAS. The reason for using these different methods is that they make different assumptions, and if they produce similar effect estimates, this provides greater confidence in any causal claims. We also assessed the robustness of our results by conducting analyses to identify potential violations of Mendelian randomization assumptions, including heterogeneity measured by Cochran's Q statistic for IVW analyses[18] and horizontal pleiotropy measured by the MR-PRESSO global pleiotropy test.[19] To ensure statistical significance, we set the threshold at $P < 5.6 \times 10^{-3}$ for the primary analysis, Bonferroni-adjusted for 9 proteins ($0.05/9 = 5.6 \times 10^{-3}$).

PE/gHTN → long-term postpartum HTN

We used SNPs with a $P < 5 \times 10^{-8}$ from a recent multiancestry meta-analysis of GWAS of PE/gHTN as IVs. To investigate genetic associations with long-term postpartum HTN, we conducted a GWAS of HTN occurring 2 to 7 years after the first pregnancy (972 cases and 3409 controls) using the nuMoM2b Heart Health Study cohort according to the proposed GWAS pipeline. Long-term HTN was defined as SBP/DBP ≥130/80 mmHg or use of antihypertensive medication 2 to 7 years after the first pregnancy. Subsequently, we conducted a 2-sample Mendelian randomization analysis using MR-RAPS, IVW, and MR-PRESSO to assess the associations between PE/gHTN during the first pregnancy and long-term postpartum HTN.

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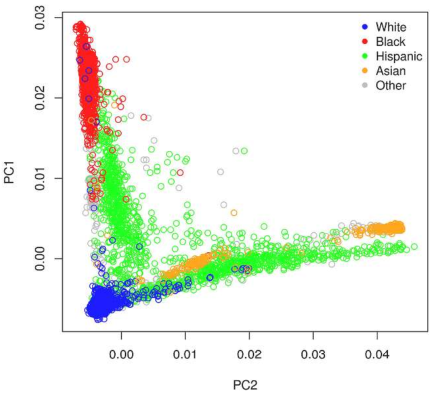

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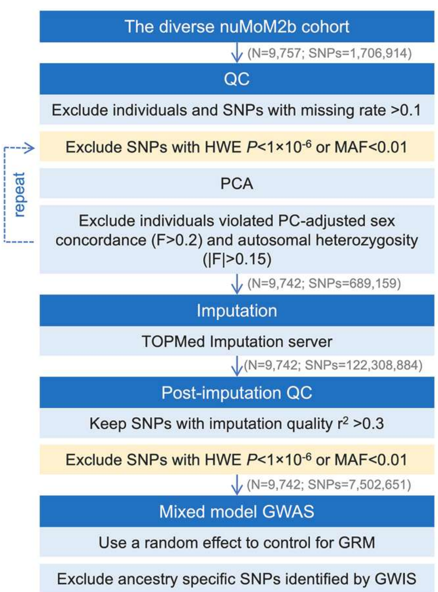

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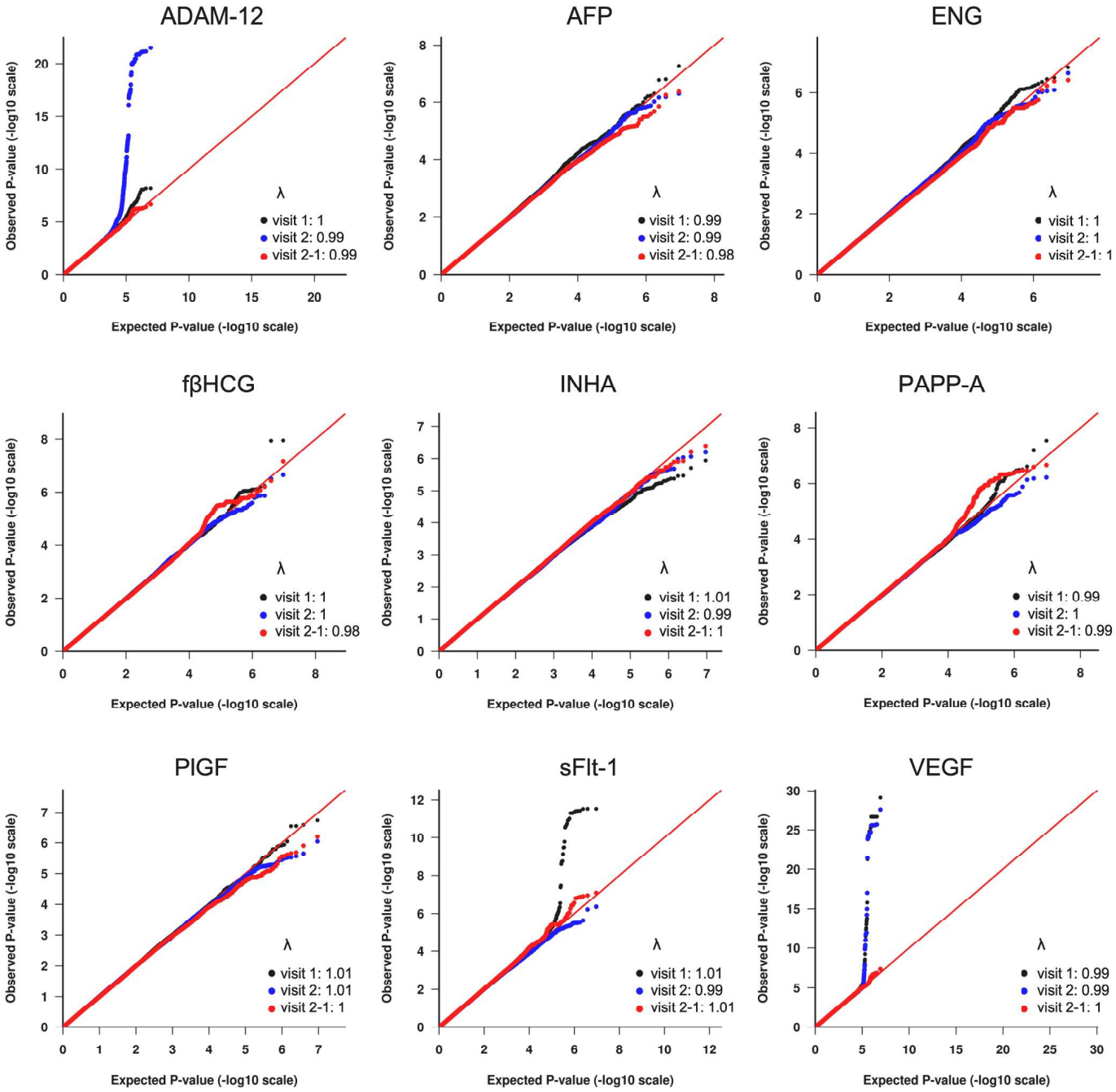
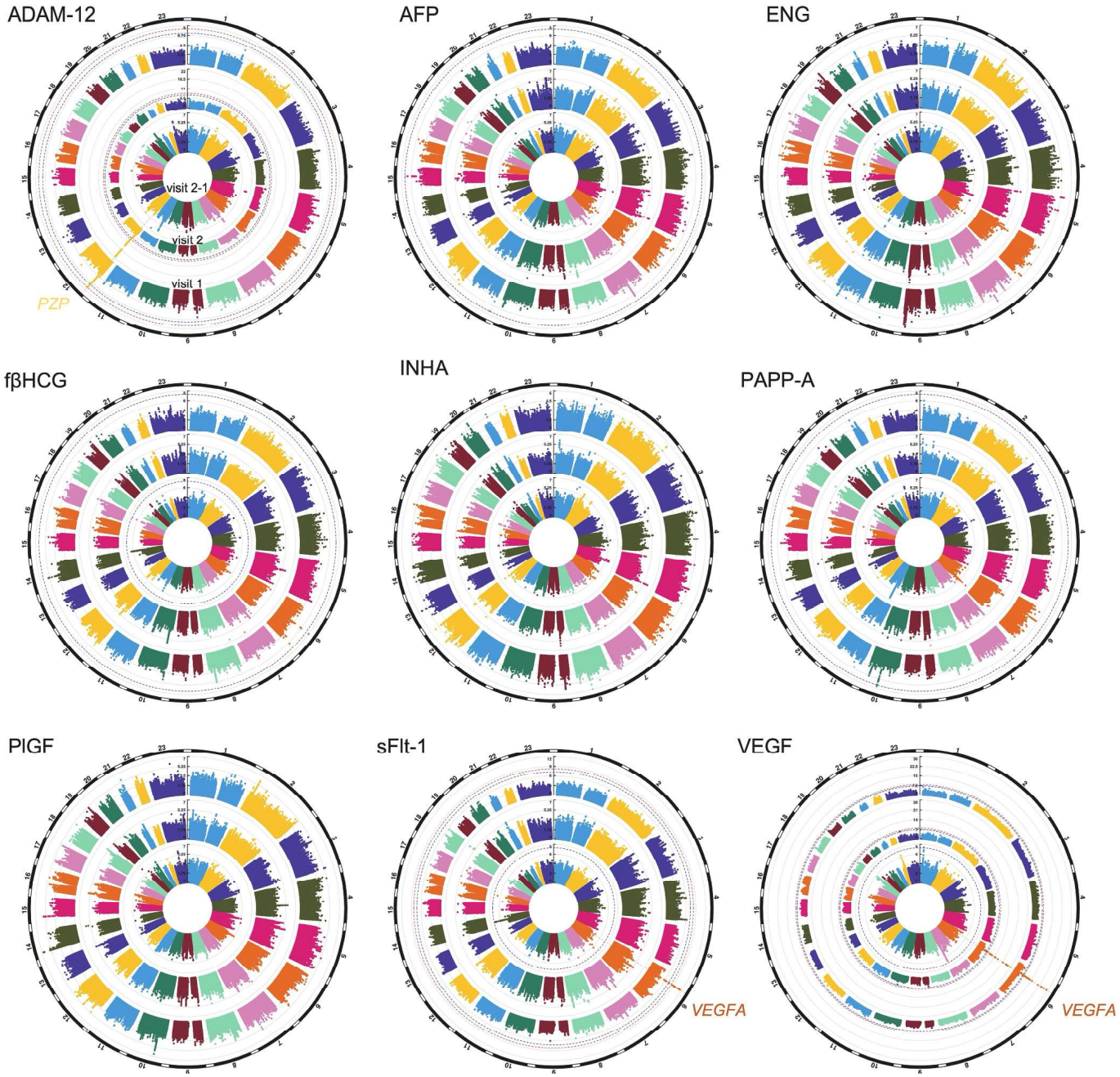
SUPPLEMENTAL FIGURE 3
The quantile-quantile plots



λ is the genomic control value. The plot displays the distribution of *P* values against a theoretical distribution, under the null hypothesis of no association. Points (representing SNPs) that align with the diagonal line suggest adherence to the null hypothesis, with a genomic control value near one indicating no inflation. Upward deviations from this line at the higher end indicate SNPs significantly associated with the trait beyond random chance.

ADAM-12, a disintegrin and metalloproteinase domain-containing protein 12; *AFP*, alpha fetal protein; *ENG*, endoglin; *fβHCG*, free beta-human chorionic gonadotropin; *INHA*, inhibin A; *PAPP-A*, pregnancy-associated plasma protein A; *PIGF*, placental growth factor; *sFlt-1*, soluble fms-like tyrosine kinase-1; *VEGF*, vascular endothelial growth factor.

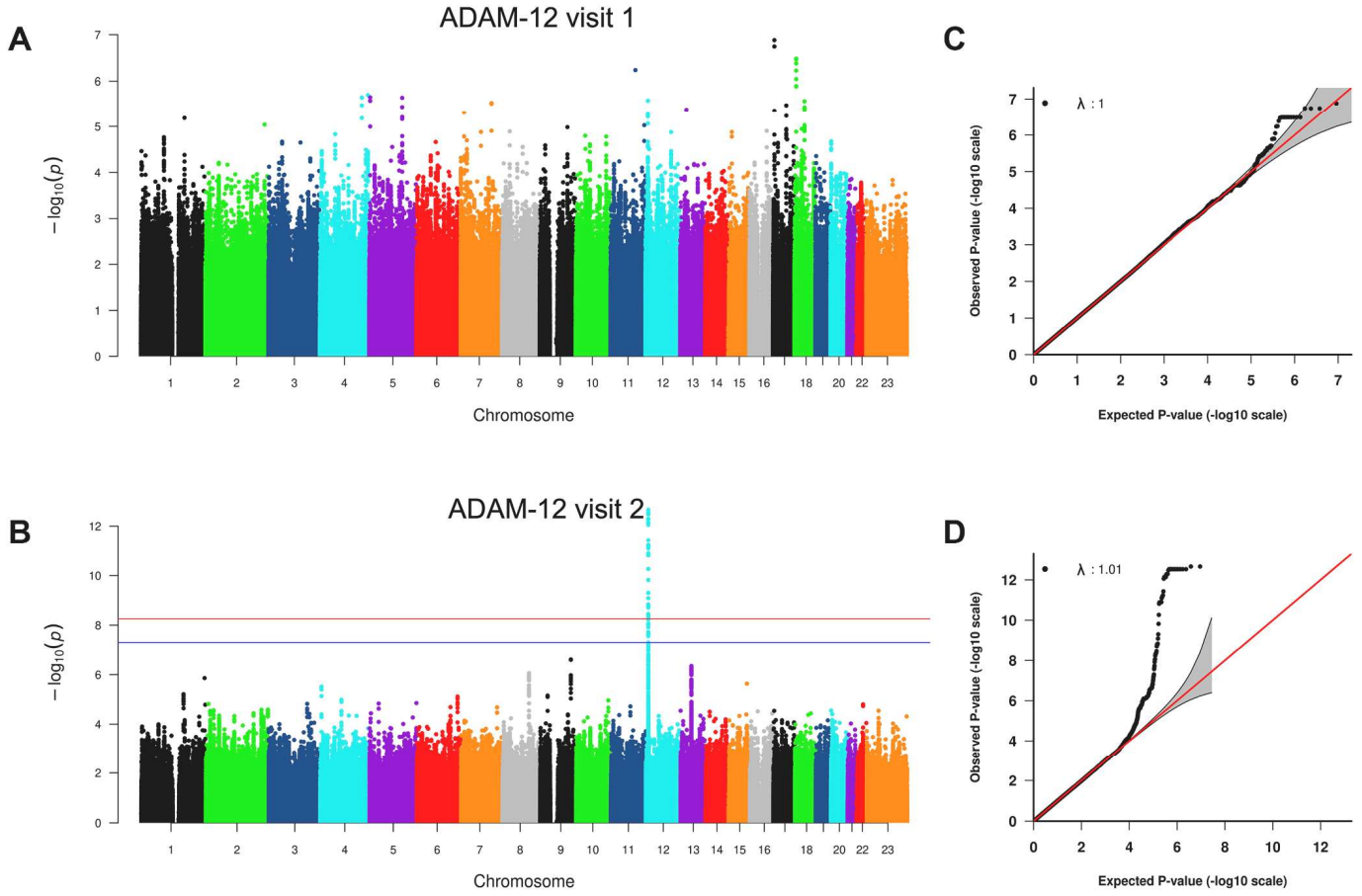
SUPPLEMENTAL FIGURE 4
Circular Manhattan plots



Manhattan plot displays the associations between SNPs across the genome and a specific trait, with the spikes indicating regions of significant associations. This circular format presents results from multiple GWAS simultaneously. The chromosomal position of each single SNP is displayed along the circle and the negative log10 of the association *P* value is displayed on the radius. The red line represents the genome-wide significance level ($P < 5.6 \times 10^{-9}$) and blue line represents the suggestive significance level ($P < 5 \times 10^{-8}$). Results for visit 1 are displayed on the outer circle, visit 2 on the middle circle, and visit 2–1 on the inner circle.

ADAM-12, a disintegrin and metalloproteinase domain-containing protein 12; *AFP*, alpha fetal protein; *ENG*, endoglin; *fβHCG*, free beta-human chorionic gonadotropin; *INHA*, inhibin A; *PAPP-A*, pregnancy-associated plasma protein A; *PIGF*, placental growth factor; *PZP*, pregnancy zone protein; *sFlt-1*, soluble fms-like tyrosine kinase-1; *VEGF*, vascular endothelial growth factor; *VEGFA*, vascular endothelial growth factor A.

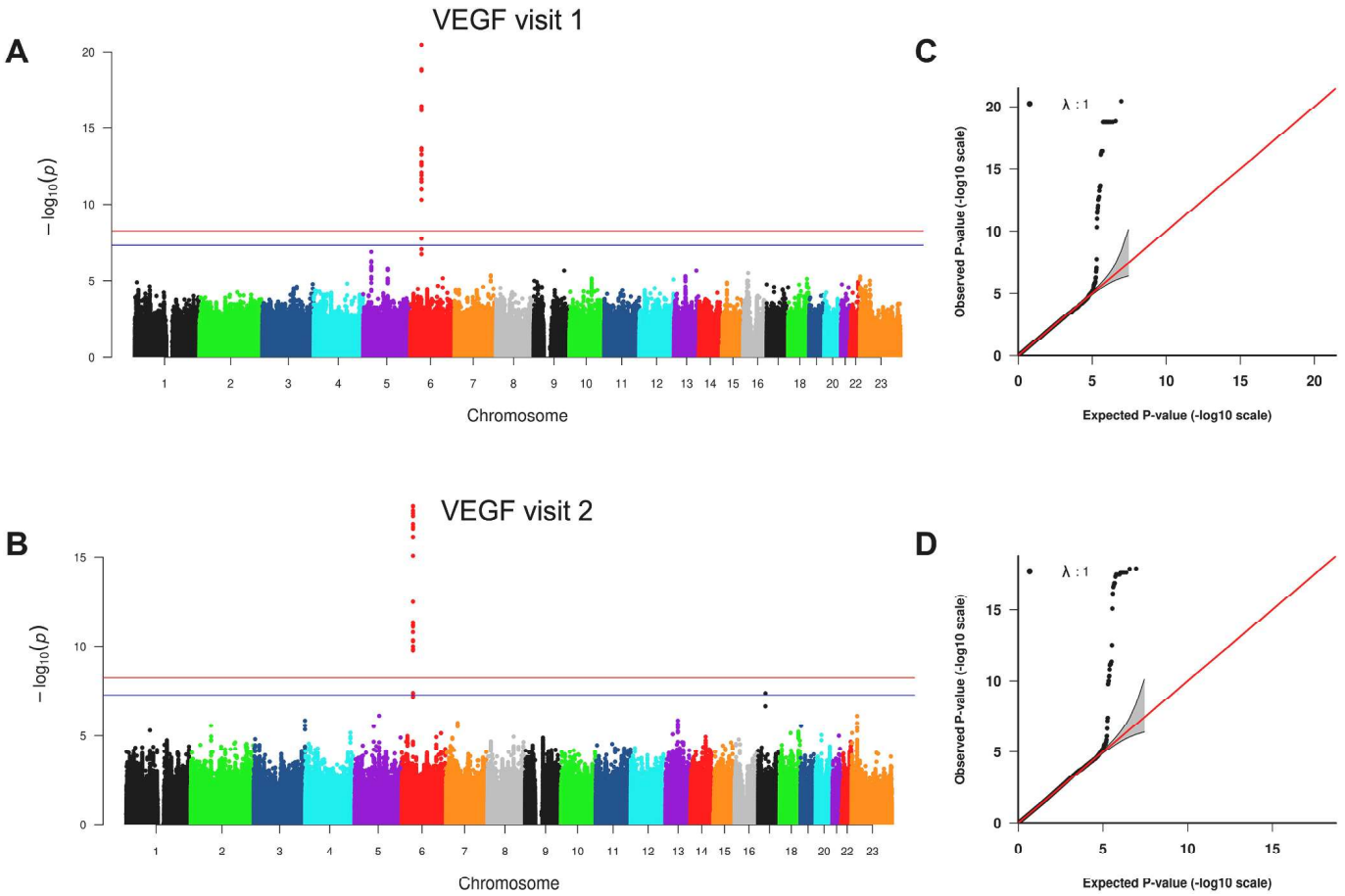
SUPPLEMENTAL FIGURE 5
GWAS of ADAM-12 using individuals of white ancestry



Manhattan plot displays the associations between SNPs across the genome and a specific trait, with the spikes indicating regions of significant associations. (**A** and **B**) Manhattan plots for visit 1 and visit 2 analyses. The chromosomal position of each SNP is displayed along the x-axis and the negative log10 of the association *P* value is displayed on the y-axis. The red line represents the genome-wide significance level ($P < 5.6 \times 10^{-9}$) and blue line represents the suggestive significance level ($P < 5 \times 10^{-8}$). (**C** and **D**) The quantile-quantile plots for visit 1 and visit 2 analyses. λ is the genomic control value. The plot displays the distribution of *P* values against a theoretical distribution, under the null hypothesis of no association. Points (representing SNPs) that align with the diagonal line suggest adherence to the null hypothesis, with a genomic control value near one indicating no inflation. Upward deviations from this line at the higher end indicate SNPs significantly associated with the trait beyond random chance.

ADAM-12, a disintegrin and metalloproteinase domain-containing protein 12; *GWAS*, genome-wide association study; *SNP*, single nucleotide polymorphism.

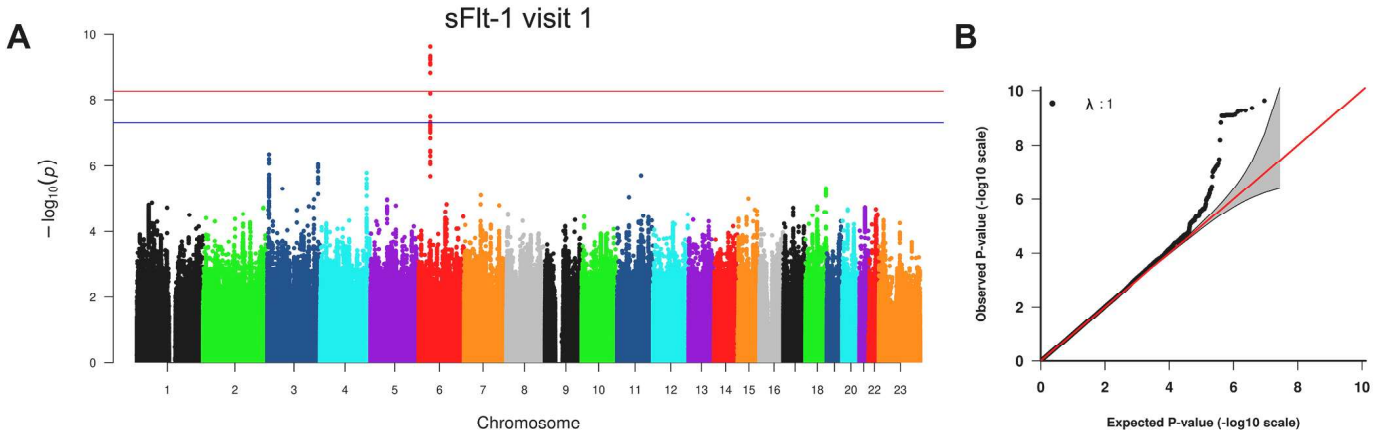
SUPPLEMENTAL FIGURE 6
GWAS of VEGF using individuals of white ancestry



Manhattan plot displays the associations between SNPs across the genome and a specific trait, with the spikes indicating regions of significant associations. (**A** and **B**) Manhattan plots for visit 1 and visit 2 analyses. The chromosomal position of each SNP is displayed along the x-axis and the negative log10 of the association *P* value is displayed on the y-axis. The red line represents the genome-wide significance level ($P < 5.6 \times 10^{-9}$) and blue line represents the suggestive significance level ($P < 5 \times 10^{-8}$). (**C** and **D**) The quantile-quantile plots for visit 1 and visit 2 analyses. λ is the genomic control value. The plot displays the distribution of *P* values against a theoretical distribution, under the null hypothesis of no association. Points (representing SNPs) that align with the diagonal line suggest adherence to the null hypothesis, with a genomic control value near one indicating no inflation. Upward deviations from this line at the higher end indicate SNPs significantly associated with the trait beyond random chance.

GWAS, genome-wide association study; *SNP*, single nucleotide polymorphism; *VEGF*, vascular endothelial growth factor.

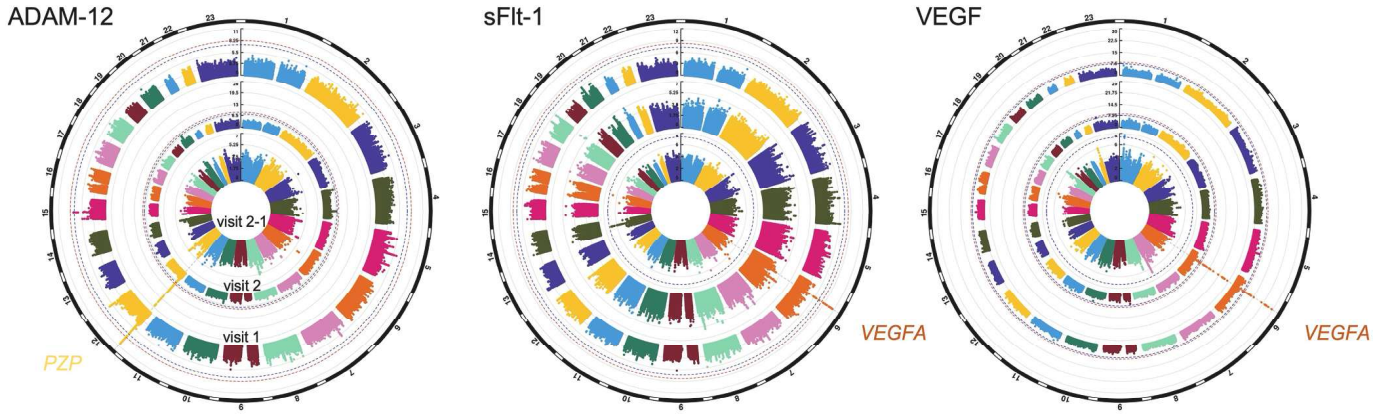
SUPPLEMENTAL FIGURE 7
GWAS of sFlt-1 using individuals of white ancestry



Manhattan plot displays the associations between SNPs across the genome and a specific trait, with the spikes indicating regions of significant associations. (**A**) Manhattan plot for visit 1 analysis. The chromosomal position of each SNP is displayed along the x-axis and the negative log10 of the association *P* value is displayed on the y-axis. The red line represents the genome-wide significance level ($P < 5.6\times10^{-9}$) and blue line represents the suggestive significance level ($P < 5\times10^{-8}$). (**B**) The quantile-quantile plot for visit 1 analysis. λ is the genomic control value. The plot displays the distribution of *P* values against a theoretical distribution, under the null hypothesis of no association. Points (representing SNPs) that align with the diagonal line suggest adherence to the null hypothesis, with a genomic control value near one indicating no inflation. Upward deviations from this line at the higher end indicate SNPs significantly associated with the trait beyond random chance.

GWAS, genome-wide association study; *sFlt-1*, soluble fms-like tyrosine kinase-1; *SNP*, single nucleotide polymorphism.

SUPPLEMENTAL FIGURE 8
Circular Manhattan plots of ADAM-12, sFlt-1, and VEGF after additional adjustment for gestational age at the time of blood collection



Manhattan plot displays the associations between SNPs across the genome and a specific trait, with the spikes indicating regions of significant associations. This circular format presents results from multiple GWAS simultaneously. The chromosomal position of each single SNP is displayed along the circle and the negative log10 of the association *P* value is displayed on the radius. The red line represents the genome-wide significance level ($P < 5.6\times10^{-9}$) and blue line represents the suggestive significance level ($P < 5\times10^{-8}$). Results for visit 1 are displayed on the outer circle, visit 2 on the middle circle, and visit 2—1 on the inner circle.

ADAM-12, a disintegrin and metalloproteinase domain-containing protein 12; *GWAS*, genome-wide association study; *PZP*, pregnancy zone protein; *sFlt-1*, soluble fms-like tyrosine kinase-1; *SNP*, single nucleotide polymorphism; *VEGF*, vascular endothelial growth factor; *VEGFA*, vascular endothelial growth factor A.

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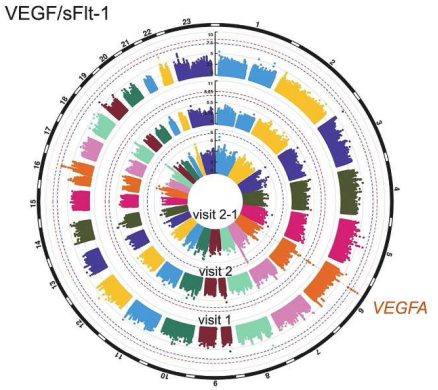

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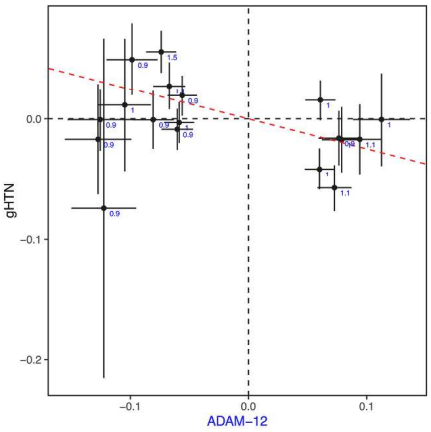

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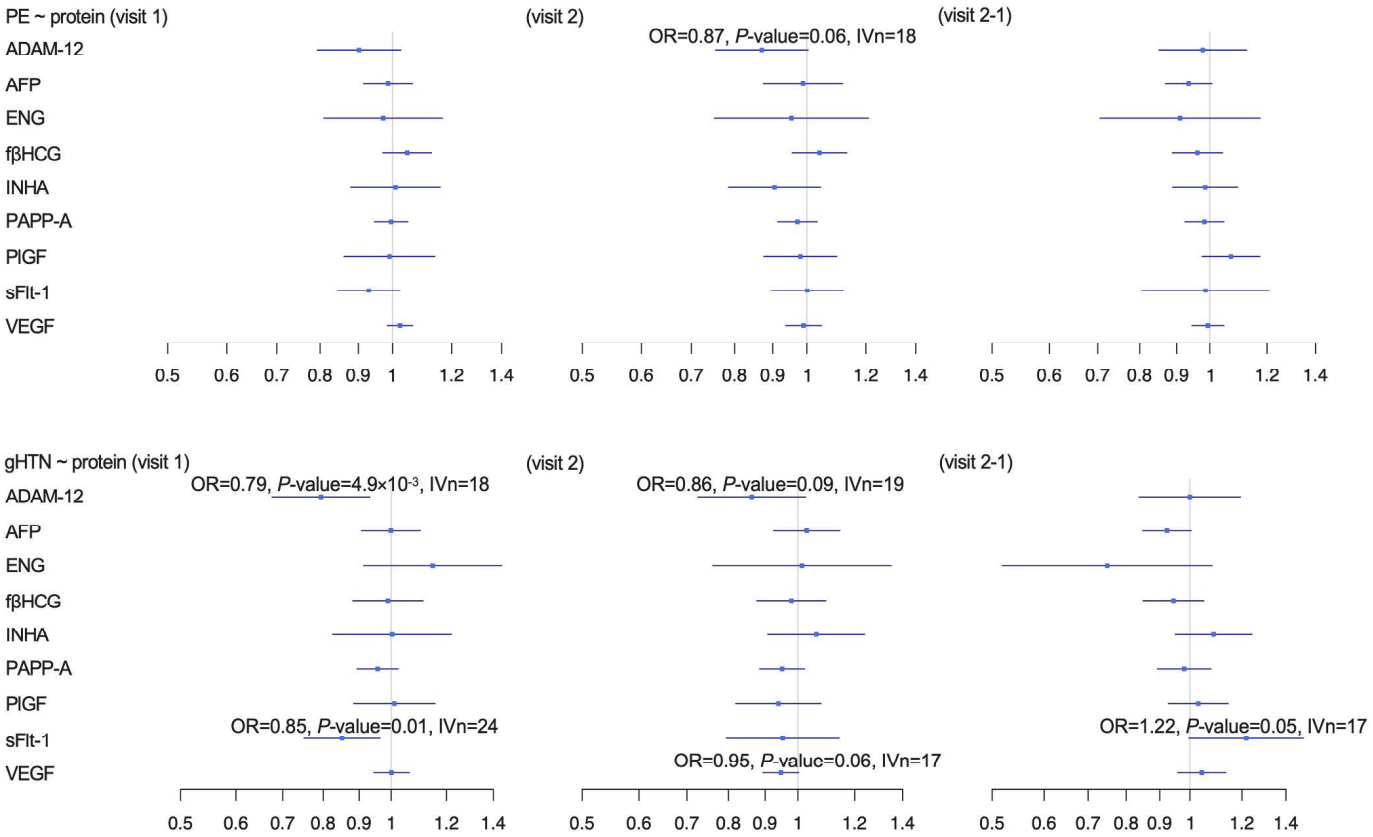
SUPPLEMENTAL FIGURE 11

Estimates of the serum concentrations of 9 placental and pregnancy proteins on PE and gHTN

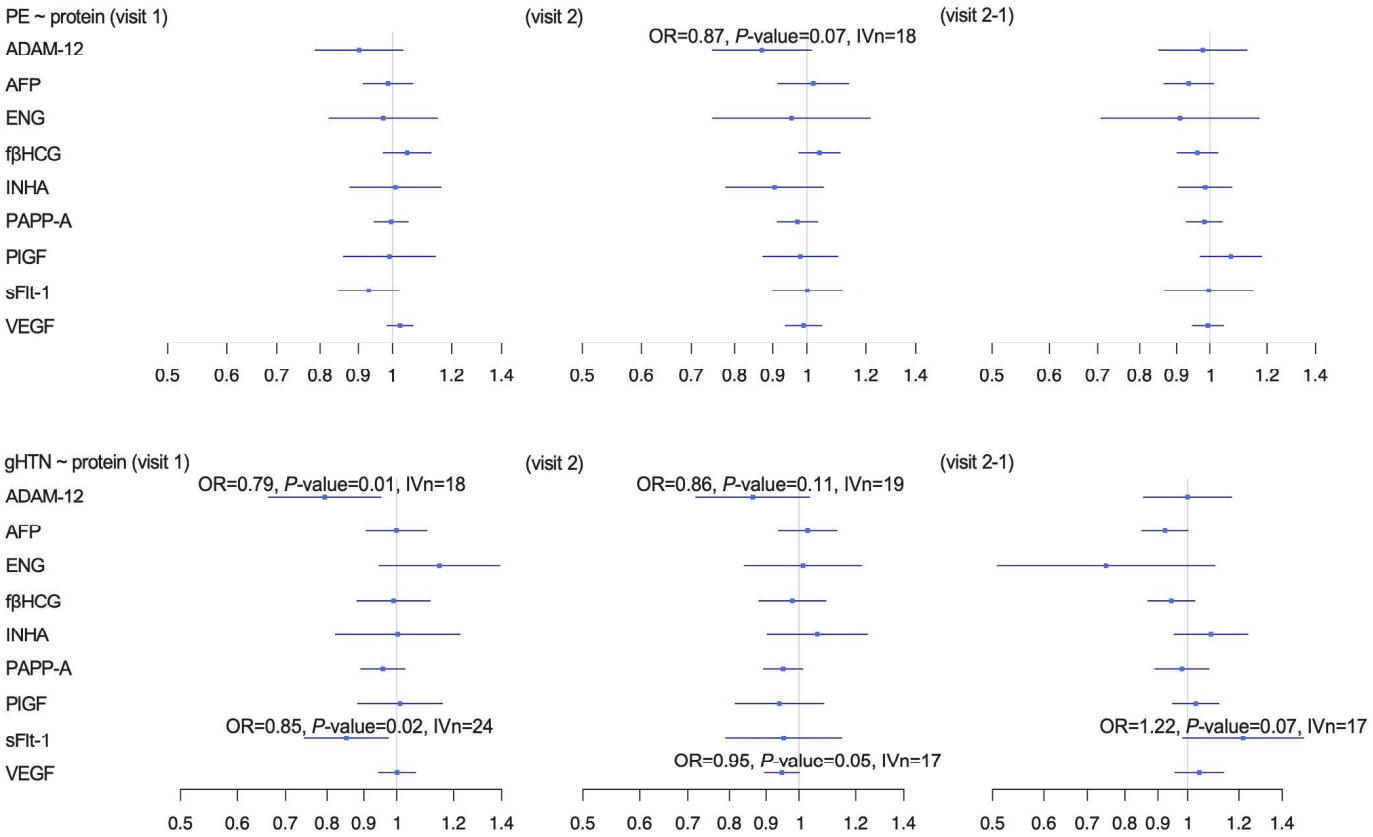


P values were determined by the 2-sample IVW method. The squares represent the estimates on the odds ratio (OR) scale and the whiskers show the corresponding 95% confidence intervals.

ADAM-12, a disintegrin and metalloproteinase domain-containing protein 12; *AFP*, alpha fetal protein; *ENG*, endoglin; *fβHCG*, free beta-human chorionic gonadotropin; *gHTN*, gestational hypertension; *INHA*, inhibin A; *IVn*, number of instrumental variable SNPs used for the estimation of the effects; *IVW*, inverse variance weighting; *PAPP-A*, pregnancy-associated plasma protein A; *PE*, preeclampsia; *PIGF*, placental growth factor; *sFlt-1*, soluble fms-like tyrosine kinase-1; *SNP*, single nucleotide polymorphism; *VEGF*, vascular endothelial growth factor.

SUPPLEMENTAL FIGURE 12

Estimates of the serum concentrations of 9 placental and pregnancy proteins on PE and gHTN

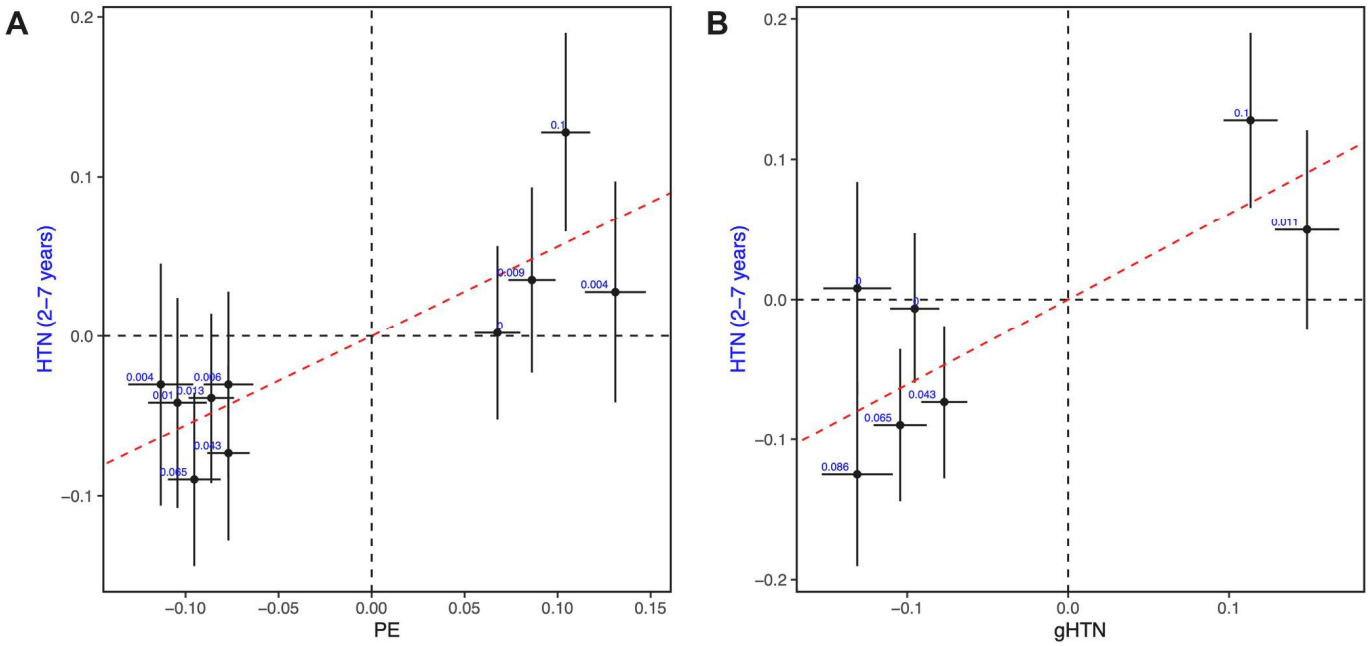


P values were determined by the 2-sample MR-PRESSO method. The squares represent the estimates on the odds ratio (OR) scale and the whiskers show the corresponding 95% confidence intervals.

ADAM-12, a disintegrin and metalloproteinase domain-containing protein 12; *AFP*, alpha fetal protein; *ENG*, endoglin; *fβHCG*, free beta-human chorionic gonadotropin; *gHTN*, gestational hypertension; *INHA*, inhibin A; *IVn*, number of instrumental variable SNPs used for the estimation of the effects; *MR-PRESSO*, Mendelian randomization pleiotropy residual sum and outlier; *PAPP-A*, pregnancy-associated plasma protein A; *PE*, preeclampsia; *PIGF*, placental growth factor; *sFlt-1*, soluble fms-like tyrosine kinase-1; *SNP*, single nucleotide polymorphism; *VEGF*, vascular endothelial growth factor.

SUPPLEMENTAL FIGURE 13

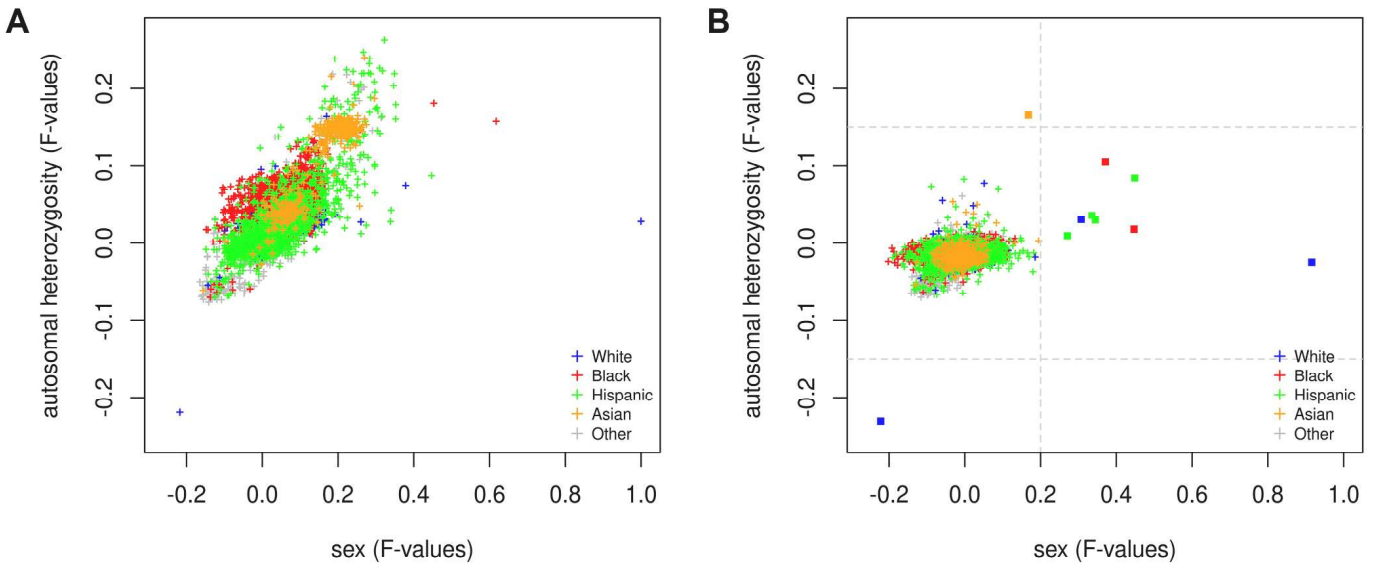
Scatter plots of genetic effects on long-term postpartum HTN (2–7 years) plotted against genetic effects on PE and gHTN



(**A**) PE as the exposure and (**B**) gHTN as the exposure. Each black dot represents an SNP used as an instrumental variable (IV). The selected IV SNPs, which are independent, have $P < 5 \times 10^{-8}$ from a recent multiancestry meta-analysis of GWAS of PE/gHTN. The whiskers represent standard errors of estimated genetic effects, and the red dashed line shows the estimated effects from MR-RAPS. The blue values are the proportions (%) of long-term postpartum HTN variance explained by individual IV SNPs.

gHTN, gestational hypertension; *GWAS*, genome-wide association study; *HTN*, hypertension; *MR-RAPS*, Mendelian randomization-robust adjusted profile scoring; *PE*, preeclampsia; *SNP*, single nucleotide polymorphism.

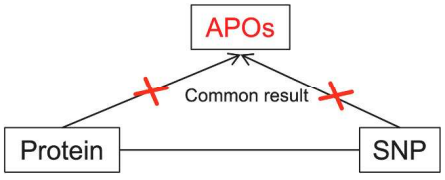
SUPPLEMENTAL FIGURE 14
Assessment of sex concordance and autosomal heterozygosity in the nuMoM2b cohort



(**A**) No principal component (PC) adjustment. (**B**) PC-adjusted results. Each dot represents an individual, and the dots are color-coded according to self-reported race.

nuMoM2b, Nulliparous Pregnancy Outcomes Study: Monitoring Mothers-to-Be.

SUPPLEMENTAL FIGURE 15
Illustration of collider bias



Adjusting for the adverse pregnancy outcome (APO) status in the protein ~ SNP regression model may introduce collider bias if both the protein and SNP are independent causes of APO status.

APO, adverse pregnancy outcome; *SNP*, single nucleotide polymorphism.

SUPPLEMENTAL TABLE 1
Race of nuMoM2b individuals and nuMoM2b-HHS individuals

Baseline characteristics	nuMoM2b (n=10,038)	nuMoM2b-HHS (n=4484)
Maternal race, n (%)		
White non-Hispanic	5989 (59.7%)	2786 (62.1%)
Black non-Hispanic	1418 (14.1%)	618 (13.8%)
Hispanic	1700 (16.9%)	735 (16.4%)
Asian	407 (4.1%)	135 (3.0%)
Other	524 (5.2%)	210 (4.7%)

nuMoM2b, Nulliparous Pregnancy Outcomes Study: Monitoring Mothers-to-Be; *nuMoM2b-HHS*, Nulliparous Pregnancy Outcomes Study: Monitoring Mothers-to-Be Heart Health Study.

SUPPLEMENTAL TABLE 2
Timing of data collection in nuMoM2b and nuMoM2b-HHS[33,35]

	Pregnancy trimester (nuMoM2b)[a]				Postpartum (nuMoM2b-HHS)	
Question domains, samples, and clinical evaluations	Visit 1	Visit 2	Visit 3	Delivery	Interval contact	In-person visit
Demographic characteristics	X	X	X	X	X	X
Medical history	X	X	X	X	X	X
Psychological factors	X	X	X			
Biometric measurements	X	X	X			X
Ultrasound	X	X	X			
Biospecimens						
Urine	X	X	X			X
Blood (whole blood, plasma, serum)	X	X	X	X		X
Cervicovaginal fluid	X	X	X	X		
Cord blood (whole blood, plasma, serum), neonatal saliva				X		
Placenta, fetal membranes, umbilical cord segment				X		
Symptoms or diagnoses between the visit 3 and the admission for delivery				X		
Participant assessment of delivery route/reasons				X		

nuMoM2b, Nulliparous Pregnancy Outcomes Study: Monitoring Mothers-to-Be; *nuMoM2b-HHS*, Nulliparous Pregnancy Outcomes Study: Monitoring Mothers-to-Be Heart Health Study.

[a] Study visits were during the following gestational age intervals: first trimester, 6 weeks 0 days to 13 weeks 6 days; second trimester, 16 weeks 0 days to 21 weeks 6 days; and third trimester, 22 weeks 0 days to 29 weeks 6 days.

SUPPLEMENTAL TABLE 3
The heritability estimates in percentage (%) from Genome-wide Complex Trait Analysis (GCTA)

Protein	Visit 1	Visit 2	Visit 2−1
ADAM-12	NA[a]	21.7 ± 12.4	NA
AFP	NA	NA	NA
ENG	30.2 ± 12.8	16.7 ± 11.8	NA
fβHCG	14.5 ± 9.2	NA	NA
INHA	19.6 ± 11.9	NA	NA
PAPP-A	NA	21.4 ± 9.9	NA
PIGF	24.6 ± 12.8	28 ± 12.7	NA
sFlt-1	29 ± 12.4	20.7 ± 10.3	31 ± 13.9
VEGF	16.3 ± 11.3	NA	19.9 ± 14

The values represent the variance explained by genome±standard error (%).

ADAM-12, a disintegrin and metalloproteinase domain-containing protein 12; *AFP*, alpha fetal protein; *ENG*, endoglin; *fβHCG*, free beta-human chorionic gonadotropin; *INHA*, inhibin A; *PAPP-A*, pregnancy-associated plasma protein A; *PIGF*, placental growth factor; *sFlt-1*, soluble fms-like tyrosine kinase-1; *VEGF*, vascular endothelial growth factor.

[a] NA denotes that the analysis was not properly converged.

SUPPLEMENTAL TABLE 4

SNPs achieving suggestive genome-wide significance (*P* value $<5\times10^{-8}$) in the GWAS of placental and pregnancy protein concentrations

SNP	Chr	Position	A1	A2	A1_freq	Effect	*P* value
ADAM-12 (visit 1)							
rs4316551	12	9,326,362	T	G	0.35	−0.07	6.8E-9
rs7952890	12	9,325,722	G	C	0.35	−0.07	6.9E-9
rs4316550	12	9,326,244	A	G	0.35	−0.07	8.8E-9
rs4262771	12	9,326,722	C	G	0.36	−0.07	8.9E-9
rs4589351	12	9,326,275	T	C	0.35	−0.07	1.6E-8
rs4370983	12	9,326,310	G	C	0.23	−0.08	2.7E-8
ADAM-12 (visit 2)							
rs6487735	12	9,278,806	T	C	0.47	−0.10	3.0E-22
rs7960104	12	9,283,043	C	T	0.47	−0.10	6.8E-22
rs10743634	12	9,280,392	T	G	0.47	−0.10	7.2E-22
rs10743636	12	9,281,464	C	T	0.47	−0.10	7.6E-22
rs10771464	12	9,280,254	G	A	0.47	−0.10	1.0E-21
rs1549428	12	9,282,256	A	G	0.49	0.10	1.3E-21
rs1549429	12	9,282,246	T	C	0.49	0.10	1.3E-21
rs2113899	12	9,280,887	T	C	0.49	0.10	1.3E-21
rs7135251	12	9,282,219	T	G	0.49	0.10	1.3E-21
rs7300369	12	9,281,942	A	G	0.47	−0.10	1.5E-21
rs2113900	12	9,280,830	A	T	0.47	−0.10	3.3E-21
rs10743632	12	9,280,307	A	G	0.49	−0.10	3.6E-21
rs10771463	12	9,280,193	C	G	0.47	−0.10	3.7E-21
rs61916194	12	9,283,487	T	C	0.49	−0.10	5.8E-21
rs61916193	12	9,283,485	T	C	0.49	−0.10	6.7E-21
rs10743633	12	9,280,356	G	A	0.49	−0.10	6.9E-21
rs1549426	12	9,282,648	T	C	0.49	−0.10	7.6E-21
rs6487747	12	9,282,978	C	T	0.49	−0.10	7.6E-21
rs6487748	12	9,283,172	G	A	0.49	−0.10	7.6E-21
rs1549427	12	9,282,344	A	G	0.49	−0.09	1.1E-20
rs1549430	12	9,281,009	G	A	0.49	−0.09	1.1E-20
rs4514480	12	9,281,387	T	C	0.49	−0.09	1.1E-20
rs7300172	12	9,281,827	A	G	0.49	−0.09	1.1E-20
rs397850695	12	9,279,174	A	AT	0.49	−0.09	7.2E-20
rs12366847	12	9,311,372	A	G	0.40	−0.09	1.2E-19
rs7960183	12	9,310,761	C	T	0.40	−0.09	1.2E-19
rs12317441	12	9,310,388	G	A	0.33	−0.10	9.5E-19
rs7957287	12	9,318,173	G	A	0.43	−0.09	2.7E-18
rs6487734	12	9,274,924	G	C	0.37	−0.09	3.8E-18
rs10843408	12	9,319,861	T	C	0.43	−0.09	5.3E-18

(continued)

SUPPLEMENTAL TABLE 4

SNPs achieving suggestive genome-wide significance (*P* value $<5\times10^{-8}$) in the GWAS of placental and pregnancy protein concentrations *(continued)*

SNP	Chr	Position	A1	A2	A1_freq	Effect	*P* value
rs10843404	12	9,318,619	C	G	0.43	−0.09	5.4E-18
rs10843400	12	9,318,235	T	C	0.42	−0.09	7.0E-18
rs4271436	12	9,320,019	A	G	0.43	−0.09	7.1E-18
rs4271437	12	9,320,047	A	G	0.41	−0.09	7.7E-18
rs11050218	12	9,320,023	A	G	0.43	−0.09	8.3E-18
rs10743661	12	9,319,428	T	C	0.41	−0.09	1.1E-17
rs10743660	12	9,319,419	T	C	0.41	−0.09	1.2E-17
rs34337	12	9,271,712	G	A	0.38	−0.09	1.9E-17
rs1059171	12	9,323,351	G	A	0.42	−0.09	8.7E-17
rs1059172	12	9,323,348	T	C	0.42	−0.09	8.7E-17
rs7952890	12	9,325,722	G	C	0.35	−0.08	7.1E-14
rs4370983	12	9,326,310	G	C	0.23	−0.09	9.5E-14
rs4316551	12	9,326,362	T	G	0.35	−0.08	1.5E-13
rs4316550	12	9,326,244	A	G	0.35	−0.08	1.6E-13
rs12369816	12	9,254,686	C	T	0.31	−0.09	1.7E-13
rs10843422	12	9,324,656	T	G	0.32	−0.08	1.9E-13
rs11049845	12	9,260,846	T	C	0.31	−0.08	2.7E-13
rs11049846	12	9,261,011	G	A	0.31	−0.08	2.7E-13
rs4262771	12	9,326,722	C	G	0.36	−0.08	3.8E-13
rs4589351	12	9,326,275	T	C	0.35	−0.08	5.0E-13
rs7298028	12	9,303,444	C	T	0.49	0.07	6.4E-13
rs10743654	12	9,305,929	A	G	0.50	−0.07	6.6E-13
rs2911825	12	9,307,302	T	G	0.50	−0.07	1.2E-12
rs9971685	12	9,307,258	C	G	0.50	−0.07	1.2E-12
rs11049781	12	9,247,292	T	C	0.31	−0.08	1.3E-12
rs10492110	12	9,340,896	A	G	0.29	−0.08	2.0E-12
rs12298908	12	9,173,646	T	A	0.23	0.08	7.7E-12
rs10771539	12	9,326,861	C	G	0.43	0.07	8.4E-12
rs2377762	12	9,313,503	T	C	0.50	−0.07	8.9E-12
rs12321232	12	9,163,072	T	G	0.22	0.08	1.9E-11
rs4322447	12	9,326,791	T	G	0.27	−0.08	2.0E-11
rs201046098	12	9,304,044	A	C	0.38	0.07	3.1E-11
rs10843050	12	9,178,009	C	T	0.23	0.08	3.3E-11
rs2277413	12	9,165,188	G	A	0.30	0.07	3.3E-11
rs397973429	12	9,324,515	C	CT	0.47	0.07	4.7E-11
rs2059759	12	9,244,342	G	A	0.31	−0.08	4.7E-11
rs252024	12	9,269,572	A	G	0.39	−0.07	5.6E-11
rs7299515	12	9,322,198	G	A	0.47	0.07	8.5E-11

(continued)

SUPPLEMENTAL TABLE 4
SNPs achieving suggestive genome-wide significance (*P* value $<5\times10^{-8}$) in the GWAS of placental and pregnancy protein concentrations *(continued)*

SNP	Chr	Position	A1	A2	A1_freq	Effect	*P* value
rs7311982	12	9,162,261	T	C	0.32	0.07	8.8E-11
rs6487821	12	9,321,124	T	C	0.46	0.07	9.9E-11
rs10771532	12	9,321,808	C	T	0.47	0.07	1.1E-10
rs61917373	12	9,302,746	G	A	0.38	0.07	1.4E-10
rs7954451	12	9,243,525	T	G	0.23	−0.08	1.7E-10
rs4883237	12	9,324,123	A	G	0.50	−0.07	1.8E-10
rs6487824	12	9,321,286	G	T	0.47	0.07	1.9E-10
rs35276849	12	9,244,269	C	CT	0.29	−0.07	2.0E-10
rs1035848	12	9,209,508	G	T	0.26	−0.08	2.1E-10
rs10771531	12	9,321,709	C	A	0.48	0.07	2.1E-10
rs7954383	12	9,321,608	T	C	0.48	0.07	2.1E-10
rs4883238	12	9,324,245	A	G	0.47	0.07	2.6E-10
rs10843222	12	9,241,470	A	T	0.22	−0.08	3.2E-10
rs7311758	12	9,321,129	A	G	0.47	0.06	3.3E-10
rs11049626	12	9,225,517	G	A	0.26	−0.07	4.4E-10
rs12303039	12	9,237,702	A	G	0.25	−0.07	7.5E-10
rs55809356	12	9,311,178	T	G	0.36	0.07	8.6E-10
rs10843223	12	9,241,903	T	C	0.22	−0.08	8.9E-10
rs7971371	12	9,326,586	G	A	0.36	0.07	1.5E-9
rs3741848	12	9,242,426	C	T	0.27	−0.07	1.9E-9
rs4353323	12	9,211,628	G	A	0.27	−0.07	2.2E-9
rs10843160	12	9,215,694	A	G	0.27	−0.07	2.5E-9
rs7137569	12	9,219,539	T	C	0.27	−0.07	3.5E-9
rs71656520	12	9,331,369	C	CTGGAGCAGG	0.45	0.06	3.5E-9
rs2377747	12	9,217,768	T	C	0.27	−0.07	4.0E-9
rs7137281	12	9,227,282	A	G	0.27	−0.07	4.8E-9
rs76611603	12	9,228,049	C	CACTT	0.27	−0.07	4.8E-9
rs12580730	12	9,310,189	G	C	0.39	0.06	5.5E-9
rs2195208	12	9,207,884	A	G	0.27	−0.07	5.7E-9
rs2003610	12	9,337,211	T	A	0.35	−0.06	6.4E-9
rs11612935	12	9,355,559	C	T	0.33	0.06	6.5E-9
rs7959473	12	9,333,129	A	G	0.46	0.06	7.4E-9
rs4636721	12	9,334,981	T	C	0.36	−0.06	8.6E-9
rs7958717	12	9,332,510	A	G	0.46	0.06	8.8E-9
rs11050312	12	9,339,311	G	T	0.35	−0.06	8.8E-9
rs7974095	12	9,332,511	T	A	0.46	0.06	9.9E-9
rs3741847	12	9,242,430	T	C	0.26	−0.07	1.1E-8
rs2003859	12	9,337,154	C	A	0.36	−0.06	1.1E-8

(continued)

SUPPLEMENTAL TABLE 4

SNPs achieving suggestive genome-wide significance (*P* value $<5\times10^{-8}$) in the GWAS of placental and pregnancy protein concentrations *(continued)*

SNP	Chr	Position	A1	A2	A1_freq	Effect	*P* value
rs34331	12	9,256,128	T	C	0.18	0.08	1.5E-8
rs4141479	12	9,335,707	C	T	0.36	−0.06	2.0E-8
rs397775325	12	9,336,794	TA	T	0.36	−0.06	2.2E-8
rs12366431	12	9,364,768	A	G	0.33	−0.06	2.3E-8
rs7972572	12	9,336,366	A	C	0.37	−0.06	2.5E-8
rs4883241	12	9,340,686	T	C	0.35	−0.06	2.7E-8
rs11050283	12	9,336,026	A	G	0.37	−0.06	2.9E-8
rs9788250	12	9,336,207	G	A	0.37	−0.06	2.9E-8
rs12828464	12	9,325,142	A	G	0.38	0.06	3.7E-8
rs34723854	12	9,203,265	TA	T	0.27	−0.06	4.3E-8
VEGF (visit 1)							
rs6921438	6	43,957,870	A	G	0.45	−0.36	7.9E-30
rs12205248	6	43,958,482	C	T	0.42	−0.35	2.2E-27
rs13206436	6	43,958,041	A	G	0.42	−0.35	2.2E-27
rs4349808	6	43,957,037	C	T	0.42	−0.35	2.2E-27
rs4513773	6	43,957,789	G	A	0.42	−0.35	2.2E-27
rs4637627	6	43,957,590	A	G	0.42	−0.35	2.2E-27
rs7763440	6	43,958,971	A	G	0.42	−0.35	2.2E-27
rs9472168	6	43,961,248	G	A	0.41	−0.34	5.2E-26
rs11757888	6	43,964,582	T	C	0.42	−0.34	1.3E-25
rs11757868	6	43,964,496	T	C	0.42	−0.34	1.4E-25
rs9472172	6	43,963,248	T	C	0.42	−0.34	1.6E-25
rs11757903	6	43,964,486	A	G	0.42	−0.33	4.9E-25
rs4320361	6	43,960,774	T	G	0.46	−0.33	6.3E-25
rs4349809	6	43,957,093	G	T	0.46	−0.33	7.9E-25
rs4413611	6	43,957,026	A	G	0.46	−0.33	7.9E-25
rs7767396	6	43,959,313	G	A	0.46	−0.33	7.9E-25
rs13206012	6	43,965,026	A	G	0.36	−0.32	5.9E-22
rs9472159	6	43,951,958	A	C	0.39	−0.27	1.7E-16
rs9472170	6	43,961,684	G	C	0.39	0.25	1.6E-14
rs4714719	6	43,964,875	C	T	0.47	0.24	2.2E-14
rs4481426	6	43,960,371	C	T	0.39	0.25	4.6E-14
rs7745184	6	43,958,901	T	G	0.41	0.24	9.9E-14
rs4382251	6	43,957,636	T	C	0.39	0.24	2.9E-13
rs9369434	6	43,950,670	T	C	0.34	−0.25	4.9E-13
rs7745183	6	43,958,898	T	G	0.43	0.22	1.4E-12
rs9472158	6	43,951,160	G	A	0.49	0.23	2.2E-12
rs9654590	6	43,969,052	C	T	0.18	0.29	2.9E-12

(continued)

SUPPLEMENTAL TABLE 4
SNPs achieving suggestive genome-wide significance (*P* value $<5\times10^{-8}$) in the GWAS of placental and pregnancy protein concentrations *(continued)*

SNP	Chr	Position	A1	A2	A1_freq	Effect	*P* value
rs9472171	6	43,963,225	A	G	0.41	0.22	1.0E-11
rs943075	6	43,954,468	A	G	0.36	0.22	6.4E-11
rs6916314	6	43,951,425	G	A	0.48	0.21	1.0E-10
rs73422214	6	43,954,866	G	A	0.21	0.26	6.0E-10
rs9462949	6	43,963,610	G	A	0.35	0.21	2.7E-9
rs6916540	6	43,951,679	C	T	0.49	0.19	4.1E-9
rs7739450	6	43,943,861	A	G	0.41	−0.19	7.6E-9
rs7017991	8	18,549,017	G	C	0.05	0.41	2.7E-8
rs58397113	8	18,540,457	C	G	0.05	0.40	4.4E-8
VEGF (visit 2)							
rs6921438	6	43,957,870	A	G	0.47	−0.32	2.5E-28
rs4349808	6	43,957,037	C	T	0.44	−0.31	2.1E-26
rs4513773	6	43,957,789	G	A	0.44	−0.31	2.5E-26
rs12205248	6	43,958,482	C	T	0.44	−0.31	2.6E-26
rs13206436	6	43,958,041	A	G	0.44	−0.31	2.6E-26
rs7763440	6	43,958,971	A	G	0.44	−0.31	2.6E-26
rs4637627	6	43,957,590	A	G	0.44	−0.31	2.8E-26
rs9472168	6	43,961,248	G	A	0.43	−0.30	1.9E-25
rs4349809	6	43,957,093	G	T	0.47	−0.30	2.3E-25
rs4413611	6	43,957,026	A	G	0.47	−0.30	2.3E-25
rs7767396	6	43,959,313	G	A	0.47	−0.30	3.0E-25
rs4320361	6	43,960,774	T	G	0.47	−0.30	4.1E-25
rs11757903	6	43,964,486	A	G	0.44	−0.30	8.7E-25
rs11757888	6	43,964,582	T	C	0.44	−0.30	1.0E-24
rs9472172	6	43,963,248	T	C	0.44	−0.30	1.2E-24
rs11757868	6	43,964,496	T	C	0.44	−0.30	1.6E-24
rs13206012	6	43,965,026	A	G	0.37	−0.29	4.0E-22
rs9472159	6	43,951,958	A	C	0.41	−0.25	8.9E-18
rs9369434	6	43,950,670	T	C	0.36	−0.24	1.0E-15
rs9472158	6	43,951,160	G	A	0.47	0.23	5.8E-15
rs4481426	6	43,960,371	C	T	0.38	0.21	1.1E-12
rs7739450	6	43,943,861	A	G	0.42	−0.21	1.2E-12
rs4382251	6	43,957,636	T	C	0.38	0.21	1.3E-12
rs4714719	6	43,964,875	C	T	0.47	0.20	1.4E-12
rs9472170	6	43,961,684	G	C	0.38	0.21	1.6E-12
rs943075	6	43,954,468	A	G	0.36	0.22	1.9E-12
rs7745184	6	43,958,901	T	G	0.40	0.20	9.3E-12
rs6916314	6	43,951,425	G	A	0.46	0.20	1.0E-11

(continued)

SUPPLEMENTAL TABLE 4
SNPs achieving suggestive genome-wide significance (*P* value $<5\times10^{-8}$) in the GWAS of placental and pregnancy protein concentrations *(continued)*

SNP	Chr	Position	A1	A2	A1_freq	Effect	*P* value
rs7745183	6	43,958,898	T	G	0.42	0.19	4.7E-11
rs6916540	6	43,951,679	C	T	0.48	0.19	5.8E-11
rs9462949	6	43,963,610	G	A	0.35	0.20	1.1E-10
rs9472171	6	43,963,225	A	G	0.41	0.19	1.5E-10
rs9381268	6	43,966,158	T	C	0.44	0.17	1.2E-8
rs5951549	X	22,349,075	C	T	0.07	0.32	1.9E-8
rs729391	6	43,950,155	C	T	0.32	0.17	4.0E-8
rs9472167	6	43,960,924	A	G	0.06	0.34	4.9E-8
sFlt-1 (visit 1)							
rs4349809	6	43,957,093	G	T	0.49	−0.09	2.9E-12
rs4413611	6	43,957,026	A	G	0.49	−0.09	2.9E-12
rs4637627	6	43,957,590	A	G	0.46	−0.09	3.0E-12
rs4513773	6	43,957,789	G	A	0.46	−0.09	3.7E-12
rs4349808	6	43,957,037	C	T	0.46	−0.09	3.7E-12
rs7767396	6	43,959,313	G	A	0.49	−0.09	3.9E-12
rs4320361	6	43,960,774	T	G	0.49	−0.09	4.4E-12
rs12205248	6	43,958,482	C	T	0.46	−0.09	5.0E-12
rs13206436	6	43,958,041	A	G	0.46	−0.09	5.0E-12
rs7763440	6	43,958,971	A	G	0.46	−0.09	5.0E-12
rs6921438	6	43,957,870	A	G	0.48	−0.09	9.9E-12
rs9369434	6	43,950,670	T	C	0.36	−0.09	1.1E-11
rs11757868	6	43,964,496	T	C	0.45	−0.09	1.2E-11
rs11757888	6	43,964,582	T	C	0.45	−0.09	1.8E-11
rs9472172	6	43,963,248	T	C	0.45	−0.09	2.2E-11
rs9472168	6	43,961,248	G	A	0.44	−0.09	2.5E-11
rs11757903	6	43,964,486	A	G	0.45	−0.09	2.9E-11
rs4382251	6	43,957,636	T	C	0.37	0.09	1.7E-10
rs4481426	6	43,960,371	C	T	0.37	0.08	3.3E-10
rs9472170	6	43,961,684	G	C	0.37	0.08	3.4E-10
rs7745183	6	43,958,898	T	G	0.41	0.08	7.4E-10
rs13206012	6	43,965,026	A	G	0.39	−0.08	1.8E-9
rs9472159	6	43,951,958	A	C	0.42	−0.08	1.9E-9
rs7745184	6	43,958,901	T	G	0.39	0.08	1.9E-9
rs9472171	6	43,963,225	A	G	0.39	0.08	2.6E-9
rs4714719	6	43,964,875	C	T	0.45	0.07	3.3E-8
rs6916540	6	43,951,679	C	T	0.47	0.07	4.0E-8
VEGF (visit 2—1)							
rs72886119	1	24,594,227	C	G	0.43	0.16	4.2E-8

(continued)

SUPPLEMENTAL TABLE 4
SNPs achieving suggestive genome-wide significance (*P* value $<5\times10^{-8}$) in the GWAS of placental and pregnancy protein concentrations *(continued)*

SNP	Chr	Position	A1	A2	A1_freq	Effect	*P* value
fβHCG (visit 1)							
rs981087	6	87,099,684	C	T	0.48	−0.10	1.1E-8
rs981086	6	87,100,023	T	A	0.49	−0.10	1.2E-8
PAPP-A (visit 1)							
rs10458657	10	74,021,178	C	A	0.39	0.19	2.9E-8

A1, minor allele; *A2*, major allele; *A1_freq*, minor allele frequency; *ADAM-12*, a disintegrin and metalloproteinase domain-containing protein 12; *Chr*, chromosome; *Effect*, the genetic effect of minor allele; *fβHCG*, free beta-human chorionic gonadotropin; *GWAS*, genome-wide association study; *PAPP-A*, pregnancy-associated plasma protein A; *SNP*, single nucleotide polymorphism; *sFlt-1*, soluble fms-like tyrosine kinase-1; *VEGF*, vascular endothelial growth factor.

SUPPLEMENTAL TABLE 5
Comparison of the top GWAS SNPs using all individuals vs using only individuals of white ancestry

				White				Multiethnic			
SNP	Chr	Position	A1	n	MAF	Effect	*P* value	n	MAF	Effect	*P* value
ADAM-12 (visit 1)											
rs6487735	12	9,278,806	T	1307	0.49	−0.06	9.5×10^{-5}	2259	0.47	−0.06	2.6×10^{-7}
rs2277413	12	9,165,188	G	1307	0.29	0.06	1.9×10^{-4}	2259	0.3	0.06	1.1×10^{-5}
rs4316551	12	9,326,362	T	1307	0.33	−0.07	5.9×10^{-6}	2259	0.35	−0.07	6.8×10^{-9}
VEGF (visit 1)											
rs6921438	6	43,957,870	A	1053	0.45	−0.35	3.6×10^{-21}	1831	0.45	−0.36	7.9×10^{-30}
rs4349809	6	43,957,093	G	1053	0.44	−0.34	1.7×10^{-19}	1831	0.46	−0.33	7.9×10^{-25}
sFlt-1 (visit 1)											
rs6921438	6	43,957,870	A	1308	0.48	−0.1	4.3×10^{-10}	2262	0.48	−0.09	9.9×10^{-12}
rs4349809	6	43,957,093	G	1308	0.47	−0.1	8.4×10^{-10}	2262	0.49	−0.09	2.9×10^{-12}
ADAM-12 (visit 2)											
rs6487735	12	9,278,806	T	1241	0.49	−0.1	5.1×10^{-13}	2085	0.47	−0.1	3×10^{-22}
rs2277413	12	9,165,188	G	1241	0.3	0.09	5.9×10^{-9}	2085	0.3	0.07	3.3×10^{-11}
rs4316551	12	9,326,362	T	1241	0.33	−0.08	5×10^{-9}	2085	0.35	−0.08	1.5×10^{-13}
VEGF (visit 2)											
rs6921438	6	43,957,870	A	1135	0.47	−0.31	1.4×10^{-18}	1942	0.47	−0.32	2.5×10^{-28}
rs4349809	6	43,957,093	G	1135	0.45	−0.31	2.4×10^{-18}	1942	0.47	−0.3	2.3×10^{-25}

A1, minor allele; *ADAM-12*, a disintegrin and metalloproteinase domain-containing protein 12; *Chr*, chromosome; *Effect*, the genetic effect of minor allele; *GWAS*, genome-wide association study; *MAF*, minor allele frequency; *sFlt-1*, soluble fms-like tyrosine kinase-1; *SNP*, single nucleotide polymorphism; *VEGF*, vascular endothelial growth factor.

SUPPLEMENTAL TABLE 6

Association of SNPs previously published for circulating VEGF concentrations in the nuMoM2b cohort

Gene SNP	Chr	Position	A1	MAF[a]	Effect[a]	*P* value[a]	Ref
VEGFA							
rs6921438	6	43,957,870	A	0.45/0.47/0.47	−/−/-	$7.94\times10^{-30}/2.49\times10^{-28}/2.09\times10^{-171}$	26
rs6921438	6	43,957,870	A	0.45/0.47/0.46	−/−/-	$7.94\times10^{-30}/2.49\times10^{-28}/7.4\times10^{-1467}$	27
rs6921438	6	43,957,870	A	0.45/0.47/0.49	−/−/-	$7.94\times10^{-30}/2.49\times10^{-28}/6.11\times10^{-506}$	28
rs7767396	6	43,959,313	G	0.46/0.47/0.48	−/−/-	$7.91\times10^{-25}/3.01\times10^{-25}/8.35\times10^{-105}$	29
VLDLR							
rs7030781	9	2,686,273	T	0.48/0.48/0.42	−/−/-	$0.41/0.49/2.57\times10^{-15}$	26
rs2375981	9	2,692,583	C	0.49/0.49/0.54	+/+/+	$0.21/0.25/1.5\times10^{-100}$	27
rs10738760	9	2,681,186	A	0.48/0.48/0.49	+/+/+	$0.3/0.46/1.96\times10^{-34}$	28
rs7030781	9	2,686,273	T	0.48/0.48/0.37	−/−/-	$0.41/0.49/1.57\times10^{-13}$	29

A1, minor allele; *Chr*, chromosome; *Effect*, the genetic effect of minor allele; *MAF*, minor allele frequency; *nuMoM2b*, Nulliparous Pregnancy Outcomes Study: Monitoring Mothers-to-Be; *Ref*, reference; *SNP*, single nucleotide polymorphism; *VEGF*, vascular endothelial growth factor; *VEGFA*, vascular endothelial growth factor A; *VLDLR*, very low-density lipoprotein receptor.

[a] The results of visit 1 are displayed first, followed by the results of visit 2, and finally, the referenced study is presented.