Arteriosclerosis, Thrombosis, and Vascular Biology

ORIGINAL RESEARCH

Clinical and Molecular Differences of Hypertensive Disorders During Pregnancy

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BACKGROUND: Hypertensive disorders of pregnancy (HDP) comprise a spectrum of 4 subtypes: chronic hypertension (cHTN), gestational hypertension (gHTN), preeclampsia, and superimposed preeclampsia (siPE). Although often characterized as a spectrum of disease severity, there have been limited comparative studies of detailed clinical and molecular characteristics of these disorders. We hereby evaluate HDP subtypes using clinical, placental histopathologic, and molecular data to compare similarities and differences between HDP subtypes.

METHODS: We used data from an over 10-year-long pregnancy cohort with detailed clinical and placental pathology, as well as placental tissue RNA-sequencing, to compare findings between HDP subtypes using a nested case-control design. Clinical diagnosis was based on current ACOG criteria, and placental gross and histological examination was based on the Amsterdam consensus statement.

RESULTS: Clinical data analysis showed cHTN and gHTN to be more likely to have normal placental pathology, while preeclampsia and siPE were more enriched in maternal vascular malperfusion. RNA-seq showed distinct gene expression signatures and pathway activation across HDP subgroups. We could not identify any molecular evidence that preeclampsia (preeclampsia or siPE) was an advanced stage of hypertensive disorder (gHTN or cHTN), but rather identified distinct gene expression profiles between these entities, suggesting preeclampsia (preeclampsia or siPE) and hypertension (gHTN or cHTN) are distinct pathophysiological conditions. Finally, we found that, in the presence of maternal vascular malperfusion, siPE and preeclampsia share significant gene expression profiles and pathway activation.

CONCLUSIONS: Our findings suggest that maternal vascular malperfusion specifically differentiates pregnancies that progress to preeclampsia and siPE. Maternal vascular malperfusion is thought to initiate in early gestation, indicating the cascade to preeclampsia/siPE may be differentiated from gHTN/cHTN early in pregnancy. Incorporating placental histopathologic evaluation is an essential future avenue in probing the cause of HDP.

Key Words: blood pressure ■ obesity ■ placenta ■ proteinuria ■ transcriptome

Hypertensive disorders of pregnancy (HDP) have been recategorized over the years due to new understanding of the disease, leading to improved clinical diagnosis and focused research on its pathophysiology. The most recent classification of HDP includes 4 major hypertensive disorders that occur in pregnant women: chronic hypertension (cHTN), gestational hypertension (gHTN), preeclampsia, and superimposed preeclampsia (siPE). The differential diagnosis within HDP is based on the timing of hypertension onset (before or after 20 weeks of gestation) and the severity of the disease (presence or absence of preeclampsia symptoms, such as proteinuria or other end-organ dysfunctions).[1] HDP prevalence has been rising over the years, likely due to increased maternal age, obesity, and the higher prevalence of underlying medical conditions, leading to increased maternal and

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Nonstandard Abbreviations and Acronyms

AK4	adenylate kinase 4
BMI	body mass index
cHTN	chronic hypertension
CITED2	Cbp/p300 interacting transactivator with Glu/Asp rich carboxy-terminal domain 2
DEA	differential expression analysis
DEG	differentially expressed genes
FBXO7	F-box protein 7
FOXO3	forkhead box O3
FVM	fetal vascular malperfusion
GA	gestational age
gHTN	gestational hypertension
GSEA	gene set enrichment analysis
GSTA3	glutathione S-transferase alpha 3
GSVA	gene set variation analysis
HDP	hypertensive disorder of pregnancy
HIF	hypoxia inducible factor
MVM	maternal vascular malperfusion
NDRG1	N-myc downstream-regulated gene 1
OPTN	optineurin
PGK1	phosphoglycerate kinase 1
siPE	superimposed preeclampsia
STC1	stanniocalcin-1
VEGFA	vascular endothelial growth factor A
VUE	villitis of unknown cause

What Are the Clinical Implications?

Our study has several important clinical implications in understanding hypertensive disorders of pregnancy. Our placental histopathology analysis found that maternal vascular malperfusion, which originates in early gestation, is significantly elevated in preeclampsia (preeclampsia and superimposed preeclampsia) compared with hypertension (gestational hypertension and chronic hypertension). Transcriptome data showed that preeclampsia (preeclampsia and superimposed preeclampsia) is a distinct condition rather than a severe form of hypertension (gestational hypertension and chronic hypertension). Thus, our data suggest that preeclampsia and hypertension-only subtypes likely have distinct pathophysiologic origins rather than existing on a disease continuum. These findings indicate that the incorporation of placental histopathologic findings can help elucidate the underlying disease cause, refine diagnosis, and develop targeted treatment approaches in the future. This work provides a foundation for future hypertensive disorders of pregnancy management, including the development of early diagnostic biomarkers to enable subtype-specific prevention and treatment strategies.

fetal morbidity and mortality.[2] Thus, there is an increasing need for improved early detection markers and fundamental interventions to improve the long-term health of both mothers and infants, as well as to reduce the burden on the health care system.

There have been multiple epidemiological and clinical studies focused on understanding HDP and its associated risk factors and clinical management, as well as long-term cardiovascular implications for the pregnant person, and impact on their offspring, including their elevated risk of hypertension and metabolic disorders in later life.[3] It has been reported that up to 50% of gHTN and cHTN cases show progression to the clinical symptoms of preeclampsia and siPE, respectively,[4,5] with blood pressure returning to a prepregnancy baseline postpartum.[1,6] Risk factor analyses have shown that, although similar factors are present across the various subtypes of HDP, their relative strengths differ between subtypes.[6] This has fueled an ongoing debate regarding whether HDP represents a continuum of a single pathological process or comprises distinct entities, with no definitive consensus thus far.

Biomarker development studies provide some insight in this respect. There have been significant efforts in developing early preeclampsia detection markers, with the antiangiogenic marker s-Flt and PlGF (and the sFlt:PlGF ratio) effectively detecting early onset cases within 2 weeks of occurrence.[7] This was a significant milestone for the establishment of early detection markers in preeclampsia; however, this marker is only useful after 20 weeks of gestation and is primarily effective for early onset preeclampsia near the time of onset, but not for late-onset preeclampsia or other HDP.[8–10] Thus, it has been suggested that HDP is a complex multifactorial syndrome, and, as a consequence, this biomarker fails to detect all cases. It is, therefore, unlikely that a single biomarker will capture the full spectrum of HDP subtypes, emphasizing the importance of understanding the pathophysiology of each.[1,7,10–12] To date, the majority of studies utilizing placental tissue RNA-seq have focused on subclassifying preeclampsia, without attention to other HDP subtypes. One recent study compared RNA-seq data from placental tissue of patients diagnosed with preeclampsia (n=16), siPE (n=7), cHTN (n=13), and nonhypertensive controls (n=12).[13] Although this study did not contain gHTN samples, the authors identified a unique molecular signature for siPE, suggesting that this may represent a distinct disease entity compared with preeclampsia or cHTN.[13]

Our group previously conducted an in-depth characterization of primary preeclampsia at the clinical, pathological, cellular, and molecular levels, and identified histopathologic findings, cellular abnormalities,

and molecular signatures that further subclassify this entity. Importantly, incorporating established criteria for the 4 main patterns of placental injury,[14] based on the Amsterdam Workshop guidelines, enabled us to dissect preeclampsia further based on the placental pathology.[15] These 4 patterns of placental injury include: maternal vascular malperfusion (MVM), fetal vascular malperfusion (FVM), villitis of unknown cause (VUE), and acute chorioamnionitis.[14,16–18] Among these patterns, MVM is most commonly associated with preeclampsia. MVM is thought to arise early in pregnancy, resulting from abnormal remodeling of maternal spiral arterioles by extravillous trophoblast, manifesting as decidual arteriopathy and leading to insufficient maternal blood flow to the placenta, and resulting in compensatory changes in the chorionic villi, including accelerated villous maturation and infarction.[14,17–19] By leveraging these placental histopathologic findings in our previous study, we found that, while MVM is found in preeclampsia presenting at any gestational age (GA), early onset and late-onset preeclampsia are differentially characterized by combinations of MVM with other distinct patterns of injury, namely with FVM in early onset preeclampsia and VUE in late-onset preeclampsia.[15] This study clearly demonstrated the utility of placental pathology and molecular signatures in further understanding the cause of these placenta-associated pregnancy disorders. Here, we apply a similar strategy, using our more than 10-year-long pregnancy cohort and placental tissue RNA-sequencing data to compare the subtypes of HDP at the clinical, histological, and molecular levels to identify distinguishing characteristics of HDP subtypes.

METHODS

Data and Code Availability

RNA-seq data have been deposited in the Gene Expression Omnibus database under accession numbers GSE186257, GSE234729, PRJNA1027377, and GSE303463. This article does not report original code. The final code used for data processing and analysis has been deposited on GitHub (https://github.com/mahorii/HDP-paper.git).

Ascertainment of Clinical Data and Identification of the Clinical Study Population

As part of an ongoing effort to examine the underlying causes of pregnancy complications, both low- and high-risk patients delivering at a UC San Diego-affiliated hospital are asked to consent to the collection of clinical data and biospecimens (including placental tissue), for contribution to the obstetric registry and biobank at the Center for Perinatal Discovery. For all consented participants, specific demographic and clinical data are extracted from the medical record, including: maternal age, race and ethnicity, gravidity and parity, maternal height and weight (to calculate body mass index), GA at delivery, fetal sex, birthweight, mode of delivery, blood pressure measurements, and laboratory tests conducted during the pregnancy. Using this information, clinical diagnoses of HDP and diabetes are adjudicated by 2 practicing obstetricians. For the present analyses, sex-specific birthweight percentiles for GA were calculated using Fenton growth curves.[20] Small for GA was categorized as a birthweight <10th percentile for GA.

The present study is a nested case-control study of participants with HDP from our obstetric registry, who delivered between January 2010 and December 2023. Patients were included in the present study if their adjudicated hypertensive diagnosis included any of the following: (1) cHTN, defined as documented hypertension (≥140/90 mm Hg) before 20 weeks of pregnancy with no signs or symptoms of preeclampsia during pregnancy (per ACOG definitions)[4]; (2) gHTN, documented hypertension (≥140/90 mm Hg) after 20 weeks of pregnancy through delivery with no signs or symptoms of preeclampsia; (3) siPE, defined as cHTN with evidence of proteinuria (≥300 mg), impaired liver function (twice the transaminase upper limit), low platelets (≤1.0×10^5 cells/μL), or renal insufficiency (Cr ≥1.2 mg/dL) after 20 weeks gestation; or (4) PE, new onset hypertension (≥140/90 mm Hg) after 20 weeks gestation, with evidence of proteinuria, impaired liver function, low platelets, or renal insufficiency after 20 weeks gestation. Both mild and severe hypertension and preeclampsia were included. Participants were excluded from analysis if they had a multiple gestation pregnancy or were missing birth outcome data, body mass index (BMI) before 20 weeks of gestation, placental pathology, or adjudicated diabetes diagnosis. The final analytic samples included 266 cHTN, 259 gHTN, 281 siPE, and 497 preeclampsia patients.

Histopathologic Examination and Classification

Placental examination, including gross and histological examinations, was performed either through the hospital (for patients with a clinical indication for placental exam, for example, diabetes, preeclampsia, or fetal growth restriction, etc) or through the research core (for patients without an exam indication). Regardless of the route of examination, all placentas had histopathologic examination performed by one of 2 perinatal pathologists, based on the Amsterdam Consensus Statement.[18] Minimal obstetric information was available at the time of histological evaluation, including GA, mode of delivery, and indication for placental examination, if applicable. The trimmed weight of the unfixed placental disc without the attached cord and membranes was measured and categorized as small (≤10th percentile for GA), normal (11th–89th percentile), or large (≥90th percentile).[21]

For the present investigation, 3 primary lesions most often associated with HDP were considered. MVM was defined as a small placenta with at least one of 4 vascular findings (accelerated villous maturation, infarction, decidual vasculopathy, or retroplacental/marginal hematoma), consistent with the traditionally used definition of MVM.[15] An additional definition of MVM that included any 2 vascular findings, regardless of placental size, was also considered. Any MVM captured either the traditional or secondary definition. The traditional definition was used for primary analyses, including RNA-seq analysis. FVM was defined as the presence of fetal vascular thrombosis or avascular villi in the absence of villitis. VUE was defined as focal, patchy, or extensive villitis (infiltration of chorionic villi with

chronic inflammatory cells), while severe VUE was defined as villitis associated with avascular villi (FVM) or perivillous fibrin. Pure VUE describes VUE without evidence of MVM (traditional definition). Normal pathology was therefore defined as any case not meeting the criteria for MVM (traditional definition), FVM, or VUE.

Clinical Data Analysis

To examine clinical and pathological differences between subtypes of HDP, clinically and biologically relevant pairwise comparisons were specified a priori: (1) examination of nonpreeclamptic hypertension (cHTN versus gHTN); (2) preexisting hypertension, with or without progression to preeclampsia (cHTN versus siPE); (3) new onset hypertension, with or without progression to preeclampsia (gHTN versus preeclampsia); and (4) preeclampsia subtypes (siPE versus preeclampsia). These comparisons were chosen to elucidate differences between chronic and new-onset hypertension, and hypertension and preeclampsia via clinically relevant pathways. Demographic, clinical, and pathological differences were compared between hypertensive disorder subgroups using χ^2, pairwise *t* test, or pairwise Wilcoxon-rank-sum, as appropriate. Adjustment for BMI and GA was considered in subanalyses, as appropriate, using generalized linear or logistic models. Given that the directionality of the association between placental lesions and hypertensive outcomes is unknown, and that pathological findings can only be determined at the time of delivery, placental lesions were subsequently treated as the outcome and compared across hypertensive groups using log-binomial regression, adjusting for maternal age, BMI, nulliparity, and diabetes, to estimate prevalence ratios with 95% CIs. FVM and VUE models were additionally adjusted for GA at delivery. Models that did not converge were re-run using log-Poisson regression with robust standard errors, as noted. All analyses were conducted using SAS 9.4; a *P* value of <0.05 was considered significant.

RNA Isolation From Placental Tissue and RNA Sequencing

Placental villous tissue, halfway between the chorionic and basal plates, avoiding calcification, infarction, and other abnormal macroscopic findings, was preserved in RNAlater (Thermo Fisher) and banked in our biorepository as previously described.[22] Placental tissue from singleton pregnancies with diagnosis of cHTN (n=13), gHTN (n=11), siPE (n=54), preeclampsia (n=67), and normal, nonhypertensive control (n=129) was used for RNA-sequencing. Total RNA was isolated using the mirVana RNA Isolation Kit (Thermo Fisher). RNA concentration was measured using the Qubit RNA BR assay kit (Thermo Fisher). RNA integrity was checked using RNA 6000 Nano chip, read by a 2100 bioanalyzer (Agilent). RNA samples included in this data set had RIN values ranging from 5.5 to 9.2, with a median of ≈7.8, indicating high RNA integrity across the data set. RNA-seq libraries were prepared using either the TruSeq Stranded Total RNA Sample preparation kit with Ribo-Zero Gold (Illumina) by IGM Genomics Center at UC San Diego, or the KAPA RNA HyperPrep Kit with RiboErase (Roche). TruSeq libraries were pooled and sequenced on NovaSeq 6000, and the HyperPrep libraries on NovaSeq X 25B Flow Cell (Illumina) with PE150 to an average depth of 35 to 40 million reads per sample through the IGM Genomics Center at UC San Diego. Quality control was performed using multiQC (v1.17, RRID:SCR_014982).[23] Strand specificity analysis using RSeQC[24] confirmed that the RNA-seq libraries were stranded, with the majority of reads aligning to the antisense strand.

Adapter trimming and quality filtering were performed using BBDuk (BBMap suite, RRID:SCR_016965) with the following parameters: ktrim=r, k=16, mink=11, hdist=1, tpe, tbo, qtrim=rl, and trimq=20, using a custom adapter reference file. Reads were mapped to the human genome (GRCh38, GENCODE release 44 with ERCC spike-in sequences added, RRID:SCR_014966) using STAR (v.2.7.11, RRID:SCR_004463)[25] with the following parameters: --runMode alignReads, --outSAMtype BAM SortedByCoordinate, --outSAMunmapped Within, and --quantMode GeneCounts. Feature quantification was performed using featureCounts (subread v.2.0.1, RRID:SCR_012919)[26] with parameters -p --countReadPairs -t exon -T 13 -g gene_id. Only Ensembl genes with ≥10 counts in at least 25% of samples were retained for downstream analysis. Normalization and differential expression analysis were performed using the R (v. 4.4.1) package DESeq2 (v. 1.44.0, RRID:SCR_000154).[27] BiomaRt (v. 2.60.1) was used to convert Ensembl gene IDs to HUGO gene names,[28] and gene set enrichment analysis (GSEA) was done using the R (v. 4.4.1) package FGSEA (v. 1.30.0), and gene set variation analysis (GSVA) was done using GSVA (v. 1.4.4).[29] Gene ontology analysis was done through Enrichr.[30] Statistical analysis for GSVA was done using SPSS (v. 29.0.2.0, RRID:SCR_002865).

Study Approval

Clinical data as well as placental samples were collected under the protocol for our Perinatal Biorepository, approved by the Human Research Protections Program Committee of the University of California, San Diego institutional review board (IRB number: 181917X).

RESULTS

Clinical Characteristics and Outcomes by Hypertensive Group

To better understand the clinical signatures of HDP, we characterized HDP subgroups in our cohort (266 cHTN, 259 gHTN, 281 siPE, 497 preeclampsia). Clinical characteristics by hypertensive sub-group, as well as birth outcomes, are presented in Table 1. Minor differences were noted by subgroup in regard to maternal age, BMI, and parity, as patients with cHTN and siPE were slightly older, had higher BMIs, and higher gravidity and parity than those with gHTN and preeclampsia, respectively. Patients with siPE were also more likely than cHTN and preeclampsia patients to have pregestational diabetes. As for the birth outcomes by hypertensive subgroup, GA at delivery, birthweight, and birthweight percentile were lower, while

Table 1. Clinical Characteristics and Birth Outcomes by Hypertensive Outcome Group

Characteristic	Hypertensive outcome group				Pairwise comparisons of interest (*P* value)*			
	cHTN, 266 (20.4%)	gHTN, 259 (19.9%)	siPE, 281 (21.6%)	PE, 497 (38.1%)	cHTN vs gHTN	cHTN vs siPE	gHTN vs PE	siPE vs PE
Maternal demographics								
Age (mean, SD)	32.9 (5.1)	31.8 (5.7)	33.2 (6.1)	31.5 (6.2)	0.04†	0.52	0.52	<0.001†
Race/ethnicity, n (%)					0.33	0.19	0.21	0.29
NH White	95 (37.5)	110 (45.4)	82 (30.5)	178 (36.9)				
NH Black	24 (9.5)	14 (5.8)	19 (7.1)	26 (5.4)				
Hispanic	98 (38.7)	85 (35.1)	132 (49.1)	206 (42.7)				
Asian/PI	30 (11.9)	27 (11.2)	29 (10.8)	55 (11.4)				
Mixed	6 (2.4)	6 (2.5)	7 (2.6)	17 (3.5)				
BMI (mean, SD)	34.7 (10.8)	30.3 (8.2)	34.3 (8.9)	29.4 (7.9)	<0.001†	0.60	0.21	<0.001†
Pregnancy characteristics								
Gravidity (median, IQR)	2 (1–4)	2 (1–3)	2 (1–4)	2 (1–3)	0.003†	0.57	0.77	0.003†
Parity (median, IQR)	1 (0–2)	1 (0–1)	1 (0–2)	0 (0–1)	0.008†	0.47	0.33	0.003†
Nulliparous, n (%)	100 (37.6)	126 (48.6)	126 (45.0)	280 (56.3)	0.01†	0.08	0.04†	0.002†
Fetal sex, male, n (%)	141 (53.0)	137 (52.9)	155 (55.2)	242 (48.7)	0.98	0.61	0.27	0.08
Diabetes, n (%)					0.69	0.008†	0.84	<0.001†
None	183 (68.8)	187 (72.2)	166 (59.1)	349 (70.2)				
Pregestational (T1 or T2)	54 (20.3)	46 (17.8)	90 (32.0)	96 (19.3)				
Gestational diabetes	29 (10.9)	26 (10.0)	25 (8.9)	52 (10.5)				
Birth outcomes								
Gestational age (mean, SD)	38.0 (2.2)	38.8 (1.8)	35.3 (3.8)	36.7 (3.3)	0.003†	<0.001†	<0.001†	<0.001†
Preterm birth, <37 wk, N (%)	50 (18.8)	26 (10.0)	158 (56.2)	192 (38.6)	0.004†	<0.001†	<0.001†	<0.001†
Birthweight, g (mean, SD)	3257 (650)	3389 (530)	2619 (996)	2747 (913)	0.07	<0.001†1	<0.001†	0.04†
Birthweight percentile (mean, SD)	63.9 (24.1)	61.2 (25.9)	54.0 (32.7)	46.7 (32.0)	0.29	<0.001†	<0.001†	<0.001†
Small for gestation age, n (%)	4 (1.5)	5 (1.9)	36 (12.9)	88 (17.7)	0.75	<0.001†	<0.001†	0.08
Mode of delivery‡					0.06	<0.001†	<0.001†	0.002†
C-section	133 (50.0)	108 (41.7)	185 (66.1)	272 (54.7)				
Vaginal	133 (50.0)	151 (58.3)	95 (33.9)	225 (45.3)				

BMI indicates body mass index; cHTN, chronic hypertension; gHTN, gestational hypertension; IQR, interquartile range; PE, preeclampsia; and siPE, superimposed preeclampsia.

*P value based on χ^2, t test (continuous variables reported as mean, SD), or Wilcoxon-rank-sum (variables reported as median, IQR), as appropriate.

‡One participant (siPE) needed a late D&E.

rates of preterm birth (GA <37 weeks at delivery), small for GA, and cesarean section were higher, for preeclampsia groups (preeclampsia and siPE) compared with their hypertension-only counterparts (gHTN and cHTN). GA at delivery was lower, while preterm birth rate, birthweight percentile, and rates of cesarean section were higher in siPE compared with preeclampsia. Furthermore, GA at delivery was slightly lower, and rates of preterm birth were slightly increased in cHTN compared with gHTN, though no other material differences in clinical outcomes were noted within the hypertension-only group (gHTN and cHTN).

Overall, the differences in clinical characteristics between preeclampsia and siPE, as well as gHTN and cHTN, were minor, suggesting similarities in clinical signatures within preeclampsia (preeclampsia and siPE) and hypertension (gHTN and cHTN).

Placental Pathology Differentiates Hypertensive Subgroups

Our previous study,[15] and others,[14,31] have pointed to the importance of incorporating placental pathology to subclassify and understand the cause of pregnancy complications. We leveraged the placental histopathologic data from our database to identify differences in pathological lesions among HDP subgroups.

Notably, no differences in placental pathology were identified between cHTN and gHTN subgroups (Table 2). Both siPE and preeclampsia patients were less likely to have normal placental pathology (35.9% and 43.7%) than cHTN (58.3%, $P<0.001$) and gHTN (53.7%, $P=0.009$) subgroups, respectively, while siPE was slightly less likely to have normal pathology compared with preeclampsia ($P=0.04$). The presence of MVM was significantly

Table 2. Placental Pathology by Hypertensive Outcome Group

Pathology lesion, n (%)	Hypertensive outcome group				Pairwise comparisons of interest (*P* value)			
	cHTN, 266 (20.4%)	gHTN, 259 (19.9%)	siPE, 281 (21.6%)	PE, 497 (38.1%)	cHTN vs gHTN	cHTN vs siPE	gHTN vs PE	siPE vs PE
Normal*	155 (58.3)	139 (53.7)	101 (35.9)	217 (43.7)	0.29	<0.001†	0.009†	0.04†
Any MVM (definition 1 or 2 below)	57 (21.6)	48 (19.7)	158 (56.4)	211 (42.5)	0.41	<0.001†	<0.001†	<0.001†
MVM1 (small placenta+vascular)	45 (17.1)	31 (12.1)	118 (42.3)	159 (32.2)	0.11	<0.001†	<0.001†	0.005†
MVM2: ≥2 vascular findings	25 (9.4)	30 (11.6)	109 (38.8)	141 (28.4)	0.41	<0.001†	<0.001†	0.003†
FVM (no VUE)	33 (12.4)	43 (16.7)	56 (19.9)	104 (20.9)	0.17	0.02†§	0.16	0.74
Pure FVM (no MVM)	26 (9.8)	39 (15.1)	24 (8.5)	65 (13.1)	0.06	0.62	0.45	0.05
VUE (any)	49 (18.4)	57 (22.1)	50 (17.8)	73 (14.7)	0.30	0.85	0.01†‖	0.25
Pure VUE (no MVM)	38 (14.3)	47 (18.3)	36 (12.8)	53 (10.7)	0.22	0.60	0.004†¶	0.37
Severe VUE‡	23 (8.7)	36 (13.9)	24 (8.5)	36 (7.2)	0.05	0.96	0.003†#	0.51

cHTN indicates chronic hypertension; FVM, fetal vascular malperfusion; GA, gestational age; gHTN, gestational hypertension; MVM, maternal vascular malperfusion; PE, preeclampsia; siPE, superimposed preeclampsia; and VUE, villitis of unknown cause.

*No MVM (1), FVM, or VUE.

‡Villitis associated with FVM or perivillous fibrin deposition.

§*P*=0.26 controlling for GA at delivery; GA at delivery *P*=0.004.

‖*P*=0.12 controlling for GA at delivery; GA at delivery *P*=0.02.

¶*P*=0.10 controlling for GA at delivery; GA at delivery *P*=0.003.

#*P*=0.01 controlling for GA at delivery; GA at delivery not significant (*P*=0.45).

increased in both preeclampsia groups (siPE, 56.4% and preeclampsia, 42.5%) compared with their hypertension-only counterparts (cHTN, 21.6% and gHTN, 19.7%; all *P*<0.001). This pattern was consistent regardless of the MVM definition used. VUE was slightly more common in gHTN compared with preeclampsia, though differences were attenuated following adjustment for GA at delivery.

Following adjustment for clinical factors, MVM remained significantly more common in preeclampsia (Figure 1; Table S1): MVM was 2.67× more likely to be seen in siPE compared with cHTN (95% CI, 2.08–3.43) and 2.28× more likely in preeclampsia compared with gHTN (95% CI, 1.74–3.01). Similar patterns were noted for MVM subtypes. Severe VUE was less likely to be noted in cHTN (aPR, 0.58 [95% CI, 0.35–0.96]) and preeclampsia (aPR, 0.58 [95% CI, 0.37–0.92]) compared with gHTN. No other differences in placental lesions were observed within the hypertension-only subgroups or for FVM findings.

Since MVM likely originates in early gestation, secondary to abnormal spiral artery remodeling, our data suggest that the pathophysiology of preeclampsia (preeclampsia and siPE) and hypertension (gHTN and cHTN) is not on a continuum, but rather, diverges early in pregnancy (before 20 weeks GA).

Distinct Molecular Differences Within Preeclampsia (preeclampsia Versus siPE) and Within Hypertension (gHTN Versus cHTN) Subtypes

Given that clinical and histopathologic data analyses revealed similar disease characteristics between preeclampsia and siPE within the preeclampsia subgroup and between gHTN and cHTN within the hypertension-only subgroup, we next investigated whether these similarities extended to the molecular level, using both our previously obtained as well as newly obtained placental RNA-seq data.

To directly assess the molecular differences between hypertensive subgroups in an unsupervised manner, as well as to account for the batch effects of sequencing data, we first conducted GSVA using the Hallmark gene sets from human MSigDB to identify activated pathways, which provides scores of each pathway within a single sample.[29] When comparing gene sets within hypertension (gHTN and cHTN), we identified a total of 9 out of 50 statistically enriched gene sets in gHTN compared with cHTN. Among these 9 gene sets, 5 were immune-associated pathways (Figure 2A), consistent with previous findings suggesting gHTN is associated with increased immune activation.[32] In contrast, when comparing preeclampsia and siPE, there were 7 gene sets significantly enriched, 2 in preeclampsia, and 5 in siPE. Interestingly, siPE showed enrichment in epithelial-mesenchymal transition, angiogenesis, KRAS signaling, and DNA damage-associated gene sets, and preeclampsia showed enrichment in the ER stress pathway and the heme metabolism gene set (Figure 2B). These data suggest that, despite similar clinical manifestations, preeclampsia and siPE (as well as gHTN and cHTN) exhibit distinct molecular profiles.

We next aimed to identify key genes involved in the activated pathways in HDP subtypes by leading-edge gene analysis. To do this, we first conducted differential expression analysis (DEA) and identified a total of 452 differentially expressed genes (DEGs; 144 upregulated, and 308 downregulated genes) in cHTN versus gHTN, and a total of 338 DEGs (137 upregulated and

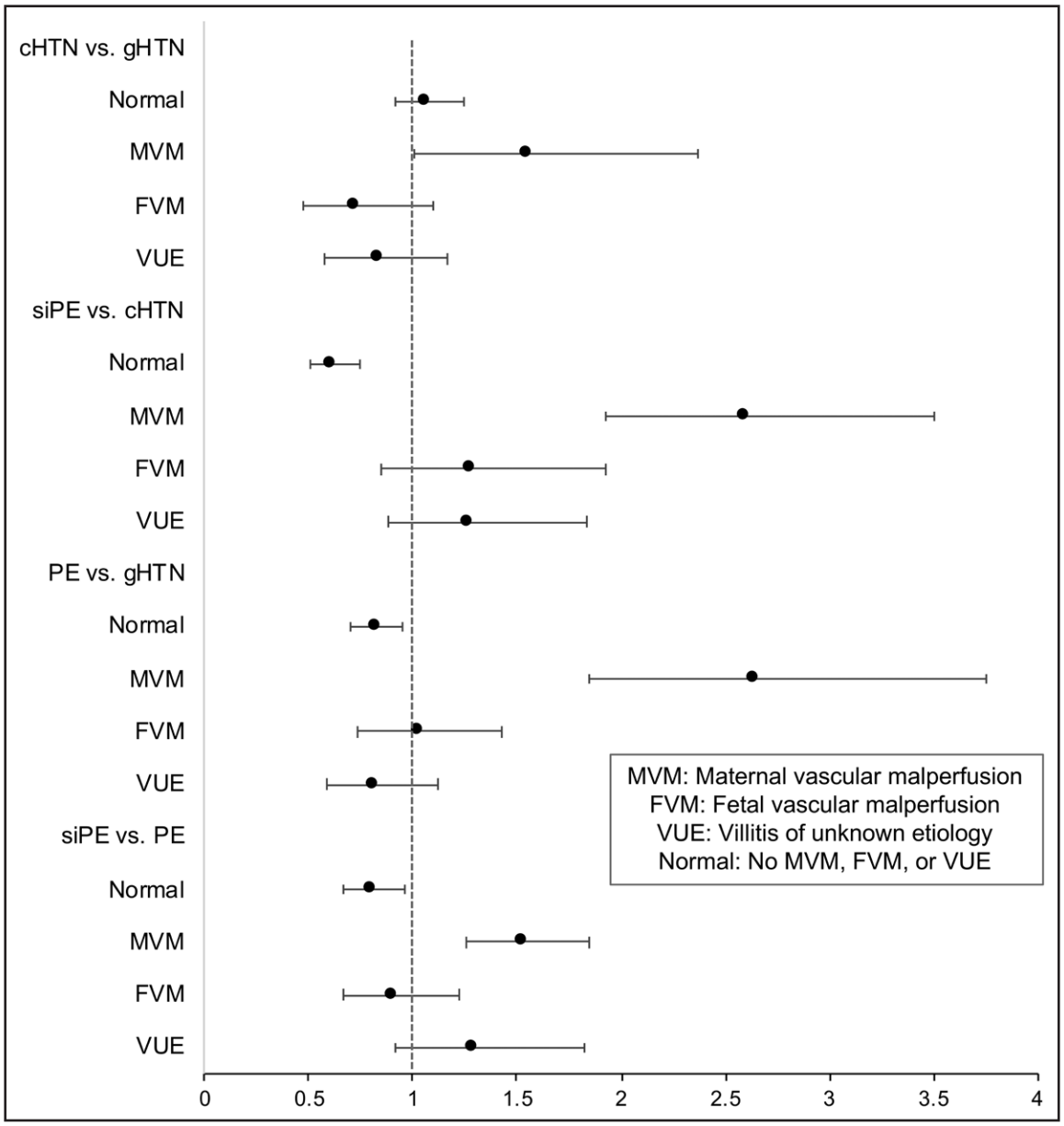

Figure 1. Comparison of specific placental lesions by hypertensive disorders of pregnancy diagnosis: prevalence ratios and 95% CIs from log-binomial regression models adjusted for maternal age, body mass index (BMI), nulliparity, and diabetes; villitis of unknown cause (VUE) additionally adjusted for gestational age (GA).

cHTN indicates chronic hypertension; FVM, fetal vascular malperfusion; gHTN, gestational hypertension; MVM, maternal vascular malperfusion; PE, preeclampsia; and siPE, superimposed preeclampsia.

201 downregulated genes) in preeclampsia versus siPE (Figure 2C; Table S2). We then conducted GSEA using the Hallmark gene sets from human MSigDB to identify the signatures of the activated pathways in a supervised manner, and identify the leading-edge genes. We identified a total of 25 gene sets (23 enriched in gHTN and 2 enriched in cHTN) in gHTN versus cHTN comparisons (Figure S1A), and a total of 31 gene sets (23 enriched in preeclampsia and 8 enriched in siPE) in preeclampsia versus siPE comparisons (Figure S1B). Interestingly, we identified STC1 (stanniocalcin-1) in the hypoxia gene set to be significantly reduced in cHTN, both in cHTN versus gHTN (Log_2-fold-change, −1.31) and siPE versus cHTN (Log_2-fold-change, 0.99) comparisons, pointing to a consistent reduction of STC1 in cHTN placenta. Related to this finding, we also found cHTN placentas had increased expression of GSTA3 (glutathione S-transferase alpha 3; cHTN versus gHTN: Log_2-fold-change, 1.03), which plays a role in detoxifying reactive oxygen species and protecting cells from oxidative damage.[33] However, STC1 and GSTA3 were not statistically different in preeclampsia versus siPE comparisons. Similar results connecting the cHTN subgroup and its response to hypoxia were noted when comparing cHTN to control placentas, those

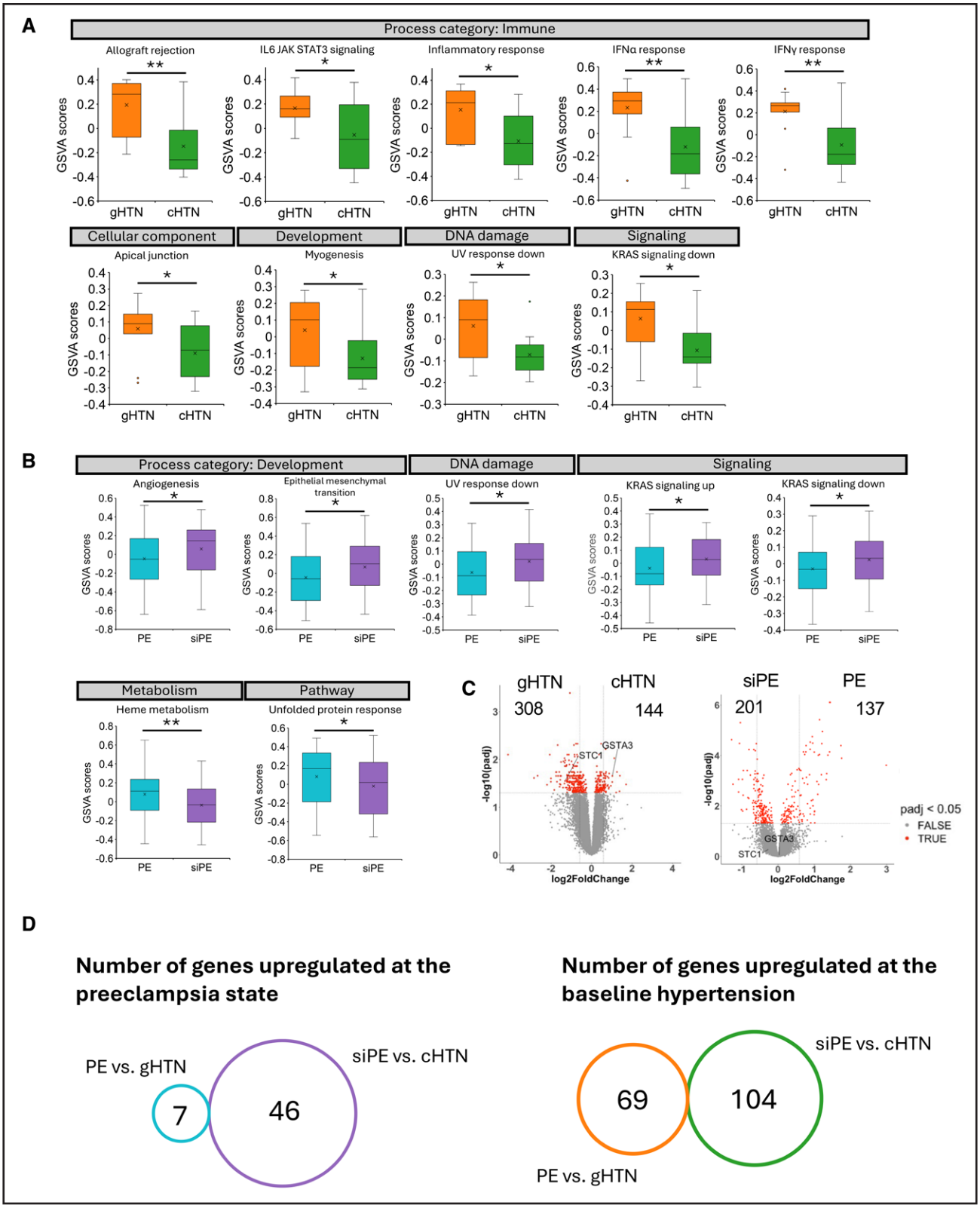

Figure 2. Box plots showing the significantly different gene set variation analysis (GSVA) MSigDB hallmark gene sets between (A) gestational hypertension (gHTN) vs chronic hypertension (cHTN), (B) preeclampsia (PE) vs superimposed preeclampsia (siPE).

The line within the box represents the median, the X mark indicates the mean, top and bottom of the box indicate the interquartile range, with the **bottom** and **top** edge marking the first and third quartiles, respectively. gHTN vs cHTN: Mann-Whitney *U* test was used for allograft rejection, inflammatory response, IFNα response, and IFNγ response; *t* test was used for IL6 JAK STAT3 signaling, apical junction, myogenesis, UV response down, and KRAS signaling down based on the data distribution. PE vs siPE: (*Continued*)

without any associated hypertension. Of the 4 HDP subtypes, only cHTN did not show statistically significant enrichment in the hypoxia pathway induction compared with our normal control group (Figure S1C). These data suggest that the placentas in the cHTN subgroup may not experience the same low oxygen tension as the other HDP subgroups or may be responding to the hypoxic environment in a different way.

Lastly, we hypothesized that if there were similarities within hypertension-only (gHTN and cHTN) or the preeclampsia (preeclampsia and siPE) subgroups, we would see similar gene expression changes and activated pathways in the respective comparisons (gHTN versus preeclampsia and cHTN versus siPE). Thus, we conducted a DEA of gHTN versus preeclampsia and cHTN versus siPE and compared the upregulated genes of preeclampsia and siPE, as well as the upregulated genes of gHTN and cHTN. There were a total of 76 DEGs (7 upregulated and 69 downregulated genes) in preeclampsia versus gHTN, and a total of 150 DEGs (46 upregulated and 104 downregulated genes) in siPE versus cHTN, but no overlapping DEGs between these conditions (Figure 2D; Table S3). We also conducted GSEA on these gene lists using the Hallmark gene sets from human MSigDB to identify the activated pathways in preeclampsia development. Again, we hypothesized that if there are similarities within hypertension (gHTN and cHTN) or within preeclampsia (preeclampsia and siPE), we would see similar pathways activated in our respective comparisons (gHTN versus preeclampsia and cHTN versus siPE). Again, we found no overlapping gene sets significantly enriched between gHTN versus preeclampsia and cHTN versus siPE (Figure S1D).

Overall, these data suggest that there are significant molecular differences in hypertension (gHTN and cHTN) as well as in preeclampsia (preeclampsia and siPE).

Molecular Profiling Suggests That gHTN and cHTN Do Not Transition to Preeclampsia or siPE

As stated previously, it has been reported that up to 50% of cases that manifest as hypertension (gHTN and cHTN) progress to preeclampsia (preeclampsia and siPE).[4,5] However, our clinical data suggest that the progression to preeclampsia may not simply be a continuum of disease severity, but rather a distinct pathological onset. This hypothesis is supported by the finding that MVM, a pathological change that begins early in gestation, is significantly elevated in preeclampsia (preeclampsia and siPE) compared with hypertension (gHTN and cHTN). Thus, the pathways that lead to preeclampsia (preeclampsia and siPE) may differ from those that drive hypertension (gHTN and cHTN). To test this hypothesis, we analyzed placental transcriptome profiles from 2 perspectives. We reasoned that if disease progression follows a continuum, we would expect to see progressively larger gene expression changes from nonhypertensive controls to hypertensive cases (gHTN and cHTN), and then to preeclamptic cases (preeclampsia and siPE). Alternatively, if the disease subtypes arise from distinct causes, their gene expression patterns and activated pathways should differ.

Thus, we first conducted DEA, controlling for GA, BMI, fetal sex, and the sequencing batch, comparing preeclampsia (preeclampsia or siPE) or hypertension (gHTN or cHTN) against our nonhypertensive controls. Common DEGs between the 2 comparisons were filtered out. We found larger gene expression differences between the nonhypertensive control group and the hypertension (gHTN and cHTN) group compared with those of preeclampsia (preeclampsia or siPE; Figure 3A; Table S4). We also conducted a DEA between our nonhypertensive controls and the hypertension (gHTN and cHTN) group, and between our hypertension (gHTN and cHTN) group and our preeclampsia (preeclampsia or siPE) group; however, there were no common DEGs within the respective comparisons.

We next compared gene set enrichment differences between preeclampsia (preeclampsia or siPE) or hypertension (gHTN or cHTN) and nonhypertensive control, using GSVA scores (Figure 3B and 3C; Figure S2A and S2B). There was a total of 22 gene sets significantly enriched in the comparisons between nonhypertensive control, gHTN, and preeclampsia. Within those activated pathways, there were no gene sets showing a continuous increase in the GSVA scores from nonhypertensive control to gHTN, and then to preeclampsia. Instead, gHTN showed immune-associated gene sets to be significantly highly activated. Likewise, there were a total of 19 gene sets significantly enriched in the comparisons between nonhypertensive control, cHTN, and siPE. Again, there were no gene sets showing a continuous increase in the GSVA scores from nonhypertensive control to cHTN, and then to siPE.

Together, these data suggest that hypertension-only disease (gHTN and cHTN) does not show strong transcriptomic evidence of continuously progressing to preeclampsia (preeclampsia and siPE).

Figure 2 Continued. Mann-Whitney *U* test was used for angiogenesis, epithelial-mesenchymal transition, UV response down, heme metabolism, and unfolded protein response; *t* test was used for KRAS signaling down and KRAS signaling up, based on the data distribution. **C**, Volcano plot displaying both upregulated and downregulated differentially expressed genes (DEGs) from comparison of cHTN vs gHTN (**left**) and siPE vs PE (**right**). Adjusted $P<0.05$ is highlighted in red. **D**, Venn diagram shows that there are no overlapping DEGs in upregulated or downregulated genes in these comparisons. *$P<0.05$ and **$P<0.01$.

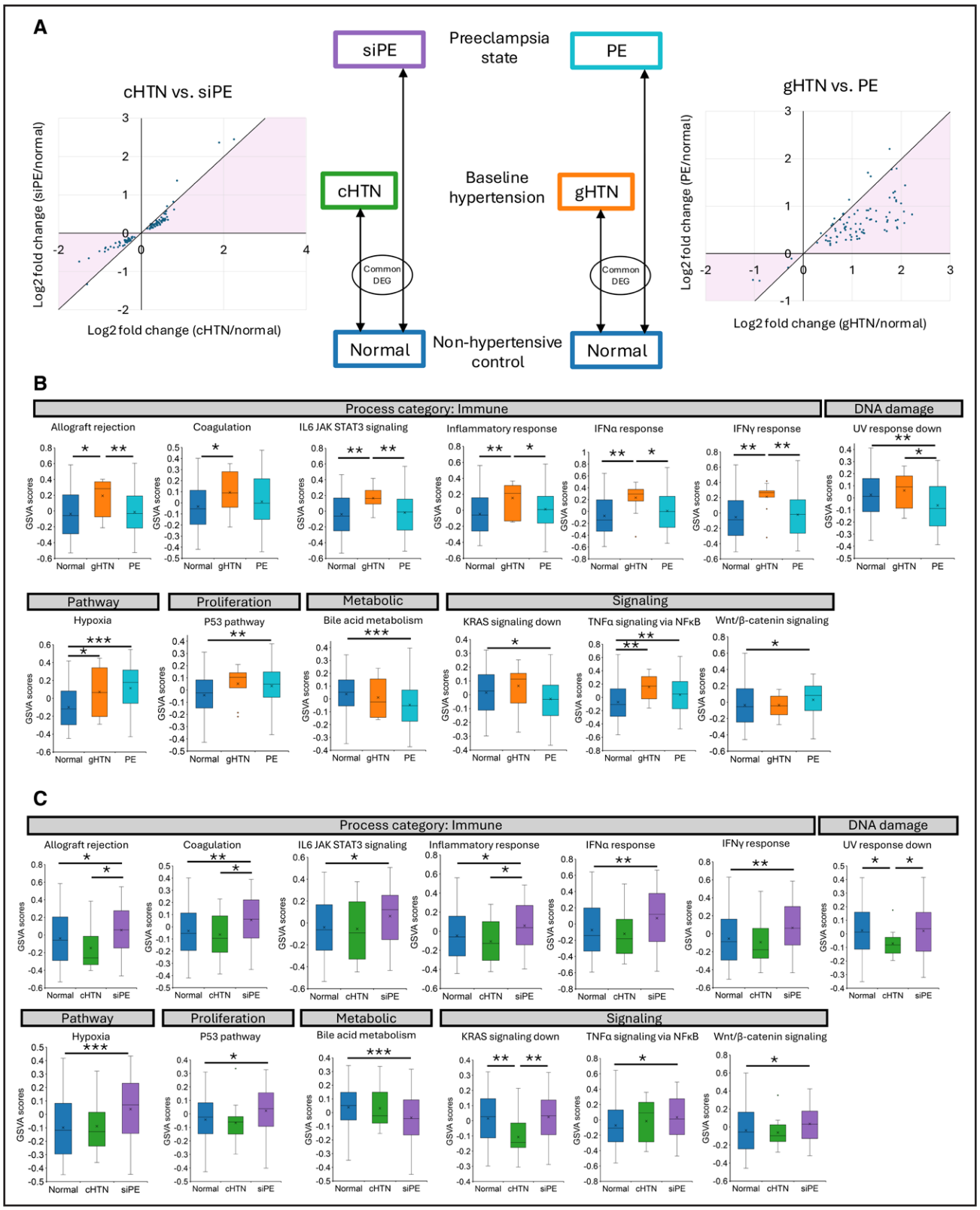

Figure 3. A, Scatter plot displaying the Log2-fold change of the common differentially expressed genes contrasted from nonhypertensive controls to hypertension (gestational hypertension [gHTN] or chronic hypertension [cHTN]) in the *x*-axis, or to preeclampsia (PE or superimposed preeclampsia [siPE]) in the *y*-axis.
The pink shaded area indicates higher gene expression in the hypertension subgroup compared with the preeclampsia subgroup. **B**, Box plots showing the significantly different gene set variation analysis (GSVA) MSigDB hallmark gene sets between normal (nonhypertensive control), gHTN, and PE, and (**C**) between normal (nonhypertensive control), cHTN, and siPE. The line within the box represents the median, the X mark indicates the mean, and the **top** and **bottom** of the box indicate the interquartile range, with the **bottom** and **top** edge (*Continued*)

Placental Tissue RNA-seq Reveals Molecular Signature of MVM to be Similar in preeclampsia and siPE

Since MVM lesions were predominantly found in the placenta from preeclampsia (preeclampsia and siPE), we next questioned whether there were any transcriptomic differences between placentae with and without MVM in preeclampsia or siPE subgroups that may point to the specific disease pathway, using the same approach as the previous section.

We first assessed differences in an unsupervised manner using GSVA scores to identify enriched pathways. Strikingly, within MVM placentas (preeclampsia: n=40, siPE: n=22), GSVA did not show any differences in preeclampsia versus siPE; however, within non-MVM placentas (preeclampsia: n=27, siPE: n=32), we noted significant enrichment in epithelial-mesenchymal transition, heme metabolism, KRAS signaling, PI3 kinase signaling via AKT to mTORC1, TGFβ signaling, hypoxia, and unfolded protein response pathways in preeclampsia versus siPE (Figure 4A). Additionally, DEA was again done by controlling for GA, BMI, fetal sex, and the sequencing batch, and we did not see any DEGs when comparing the siPE and preeclampsia groups within MVM (Figure 4B; Table S5), suggesting that preeclampsia and siPE are similar in molecular signature when MVM is noted in the placenta. In contrast, we found 2051 DEGs when comparing siPE and preeclampsia within non-MVM placentas (n=791 highly expressed in siPE, and n=1260 highly expressed in preeclampsia; Figure 4B; Table S5). We again conducted GSEA to identify significant leading-edge genes. Compared with non-MVM preeclampsia, we found non-MVM siPE placenta showed significantly enriched gene expression in extracellular matrix organization associated genes in the epithelial mesenchymal transition pathway, while non-MVM preeclampsia placenta were significantly enriched in genes BNIP3L (BCL2-interacting protein 3 like), CITED2 (Cbp/p300 interacting transactivator with Glu/Asp rich carboxy-terminal domain 2), PGK1 (phosphoglycerate kinase 1), AK4 (adenylate kinase 4), NDRG1 (N-myc downstream-regulated gene 1), and VEGFA (vascular endothelial growth factor A), which are associated with cellular response to hypoxia (Table S6).[34–42]

Finally, we assessed differences between MVM and non-MVM within the preeclampsia subtypes. We found that epithelial-mesenchymal transition, KRAS signaling, and reactive oxygen species were significantly different between MVM and non-MVM preeclampsia placentas (Figure 4C); however, no differences were noted in siPE by GSVA. We next conducted the DEA and found that there were 282 (153 upregulated and 129 downregulated) and 154 (131 upregulated and 23 downregulated) DEGs in preeclampsia and siPE, respectively (Figure 4D; Table S7). GSEA identified notable differences in pathways with several interesting leading-edge genes. Specifically, leading-edge genes in heme metabolism were significantly enriched with autophagy-associated genes, FBXO7 (F-box protein 7), BNIP3L, FOXO3 (forkhead box O3), and OPTN (optineurin), in placentas from non-MVM preeclampsia when compared with MVM preeclampsia (Table S8). On the contrary, MVM placentas from siPE patients were significantly enriched in hypoxia gene set, including AK4 and NDRG1; Table S9), genes that are known to be upregulated in response to low oxygen tension.[34–36] These findings suggest that placentae with and without MVM show distinct molecular signatures in both preeclampsia and siPE, with larger changes observed in preeclampsia than in siPE.

Overall, these findings indicate that, although the presence or absence of MVM seems to have a somewhat larger influence on preeclampsia than siPE, MVM demonstrates a common molecular signature regardless of the preeclampsia subtype, whereas in the absence of MVM, distinct molecular signatures are seen. Thus, placental histopathologic findings, particularly the presence

Figure 3 Continued. marking the first and third quartiles, respectively. Pathways shown here are common pathways between **B** and **C**. Other pathways are listed in Figure S2. Normal vs gHTN: Mann-Whitney *U* test was used for allograft rejection, coagulation, IL6 JAK STAT3 signaling, inflammatory response, IFNα response, IFNγ response, UV response down, hypoxia, bile acid metabolism, KRAS signaling down, TNFA signaling via NFκB, and Wnt/β-catenin signaling; *t* test was used for p53 pathway, based on the data distribution. Normal vs PE: Mann-Whitney *U* test was used for allograft rejection, coagulation, IL6 JAK STAT3 signaling, inflammatory response, IFNα response, IFNγ response, UV response down, hypoxia, bile acid metabolism, KRAS signaling down, TNFα signaling via NFκB, and Wnt/β-catenin signaling; *t* test was used for p53 pathway, and UV response up, based on the data distribution. gHTN vs PE: Mann-Whitney *U* test was used for allograft rejection, inflammatory response, IFNα response, IFNγ response, UV response down, hypoxia, and Wnt/β-catenin signaling; *t* test was used for coagulation, IL6 JAK STAT3 signaling, p53 pathway, bile acid metabolism, KRAS signaling down, and TNFA signaling via NFκB, based on the data distribution. Normal vs cHTN: Mann-Whitney *U* test was used for allograft rejection, coagulation, IL6 JAK STAT3 signaling, inflammatory response, IFNα response, IFNγ response, UV response down, hypoxia, bile acid metabolism, KRAS signaling down, TNFα signaling via NFκB, and Wnt/β-catenin signaling; *t* test was used for p53 pathway, based on the data distribution. Normal vs siPE: Mann-Whitney *U* test was used for allograft rejection, coagulation, IL6 JAK STAT3 signaling, inflammatory response, IFNα response, IFNγ response, UV response down, hypoxia, bile acid metabolism, KRAS signaling down, TNFα signaling via NFκB, and Wnt/β-catenin signaling; *t* test was used for up p53 pathway, based on the data distribution. cHTN vs siPE: Mann-Whitney *U* test was used for allograft rejection, IL6 JAK STAT3 signaling; *t* test was used for coagulation, inflammatory response, IFNα response, IFNγ response, UV response down, hypoxia, p53 pathway, bile acid metabolism, KRAS signaling down, TNFα signaling via NFκB, and Wnt/β-catenin signaling, based on the data distribution. **P*<0.05, ***P*<0.01, and ****P*<0.001.

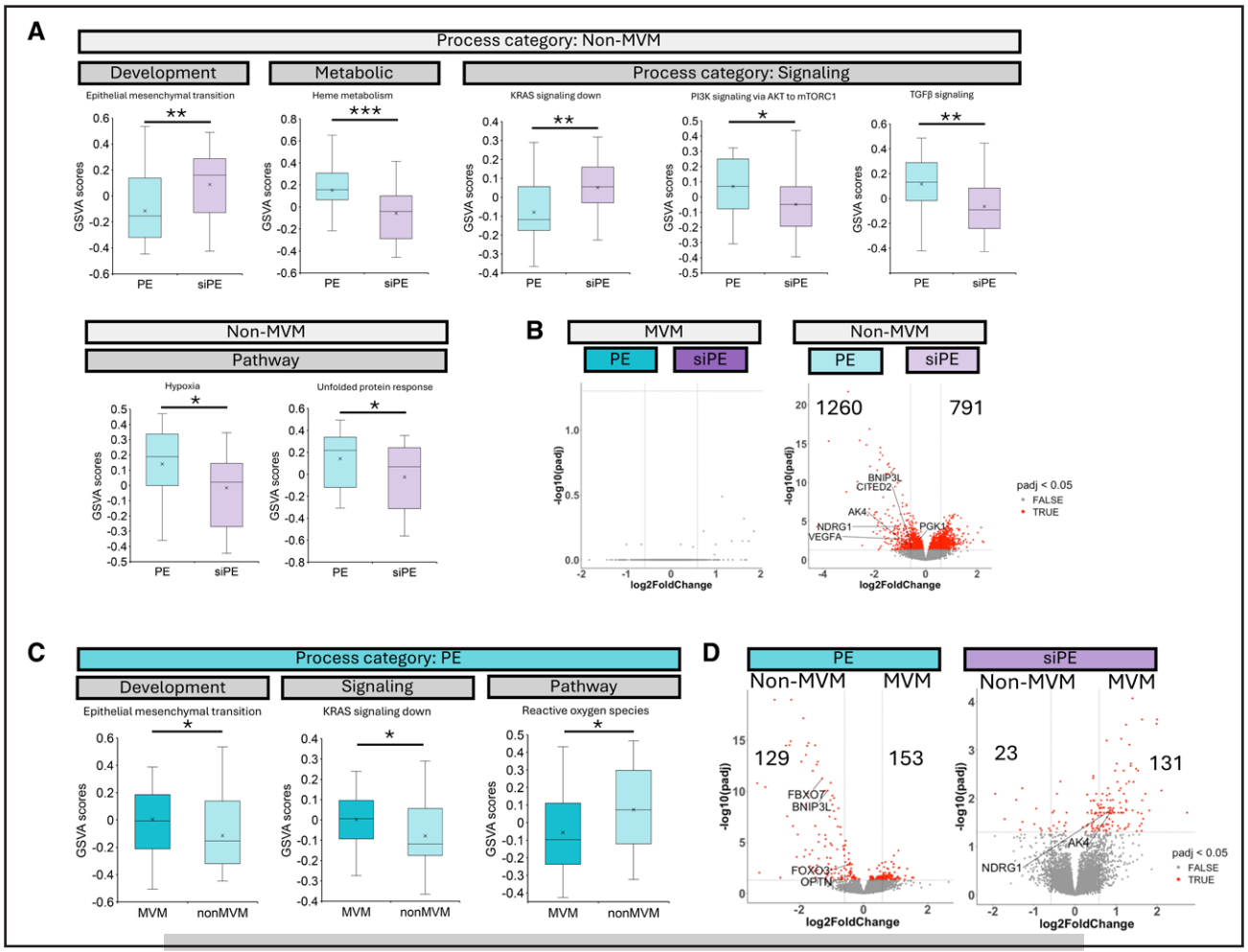

Figure 4. A, Box plots showing the significantly different gene set variation analysis (GSVA) MSigDB hallmark gene sets between preeclampsia (PE), nonmaternal vascular malperfusion (MVM), and superimposed preeclampsia (siPE)-non-MVM. There were no significant gene sets between PE-MVM and siPE-MVM. The line within the box represents the median, the X mark indicates the mean, **top** and **bottom** of the box indicate the interquartile range, with the **bottom** and **top** edge marking the first and third quartiles, respectively. The Mann-Whitney *U* test was used for epithelial-mesenchymal transition (EMT), unfolded protein response; *t* test was used for heme metabolism, KRAS signaling down, PI3K signaling via AKT to mTORC1, TGFβ signaling, and hypoxia, based on the data distribution. **B**, Volcano plot displaying both upregulated and downregulated differentially expressed genes (DEGs) from comparison of PE vs siPE of MVM (**left**) and non-MVM (**right**). Adjusted *P*<0.05 is highlighted in red. Dotted lines indicate adjusted *P* value at 0.05 and fold change at 1.5. **C**, Box plots showing the significantly different GSVA MSigDB hallmark gene sets between PE-MVM and PE-nonMVM. There were no significant gene sets between siPE-MVM and siPE-nonMVM. The line within the box represents the median, the X mark indicates the mean, **top** and **bottom** of the box indicate the interquartile range, with the **bottom** and **top** edges marking the first and third quartiles, respectively. Mann-Whitney *U* test was used for EMT; *t* test was used for KRAS signaling down and reactive oxygen species, based on the data distribution. **D**, Volcano plot displaying both upregulated and downregulated DEGs from comparison of MVM vs non-MVM of PE (**left** panel) and siPE (**right** panel). Adjusted *P*<0.05 is highlighted in red. Dotted lines indicate adjusted *P* value at 0.05 and fold change at 1.5. **P*<0.05, ***P*<0.01, and ****P*<0.001.

of MVM, could better inform the molecular pathophysiology of the HDP subtypes.

DISCUSSION

We previously reported that incorporating placental histopathologic findings is important for dissecting primary preeclampsia, a multifactorial pregnancy syndrome.[15] Thus, in the present study, we applied a similar evaluation to a broader group of patients with HDP to enhance our current understanding of this disease. Using our >10-year cohort of over 1300 patients with HDP, we analyzed maternal clinical features and found only minor differences between HDP subtypes, including maternal age, BMI, and parity. These findings may reflect older maternal age and higher BMI influencing preexisting hypertensive conditions. The only significant difference we noted was a higher prevalence of pregestational diabetes in siPE compared with patients with cHTN and preeclampsia. Clinically meaningful observations were made regarding birth outcomes, which were overall significantly worse in preeclampsia (preeclampsia and siPE)

than in hypertension (gHTN and cHTN), as expected. Thus, we conclude that, within preeclampsia (preeclampsia and siPE) and hypertension-only (gHTN and cHTN) groups, clinical characteristics and birth outcomes are similar, but that birth outcomes are worse in preeclampsia (preeclampsia and siPE) compared with the hypertension-only (gHTN and cHTN) group.

Subsequently, we investigated the pathological characteristics of placental injury in the 4 HDP subtypes, characteristics that have been evaluated less commonly within these distinct subtypes. We found that MVM was significantly increased in preeclampsia (preeclampsia and siPE), compared with their respective hypertension (gHTN and cHTN) subtypes. Further, normal placental pathology was more common in hypertension-only (gHTN and cHTN) cases, which did not ultimately progress to preeclampsia (preeclampsia and siPE). This finding reflects previous reports suggesting that the worst birth outcomes, such as preterm birth and small for GA, are associated with a high prevalence of MVM.[19,31]

We next conducted placental RNA-seq to test whether the absence of significant clinical differences within preeclampsia (preeclampsia and siPE) and within hypertension (gHTN and cHTN) correlated with similar molecular signatures in the placenta. Interestingly, when comparing gHTN versus cHTN or preeclampsia versus siPE, we noted different pathways activated, different DEGs between the subtypes, and no overlapping DEGs or pathways between the preeclampsia and HTN groups, suggesting that the 4 HDP subtypes have unique molecular signatures. One interesting finding was the lack of a gene expression response to hypoxia in cHTN-associated placentas. As noted earlier, we found reduced expression of STC1, a downstream gene turned on in response to the activation of the HIF (hypoxia inducible factor) pathway, in cHTN. STC1 is known to be upregulated in BeWo cells cultured under hypoxia through the activation of HIF.[43] We also noted GSTA3, a gene involved in detoxifying reactive oxygen species and protecting cells from oxidative damage,[33] to be significantly upregulated in cHTN. The gene expression profiles of these hypoxia-related genes, along with our finding that the GSVA hypoxia scores of cHTN are similar to nonhypertensive controls, suggest cHTN placentas may not be experiencing hypoxia or respond to hypoxia in a distinct manner compared with gHTN.

We then asked whether we could see the molecular footprint of the progression of hypertension (gHTN and cHTN) to preeclampsia (preeclampsia and siPE), or whether these HDP subtypes have distinct origins. We first tested the assumption that disease progression would likewise show a mirrored gene expression continuum. We contrasted our nonhypertensive controls to both our hypertension-only subtypes (gHTN or cHTN), as well as to our preeclampsia subtypes (preeclampsia and siPE). Instead of a gene expression continuum, we noted that, compared with our nonhypertensive controls, both our hypertension-only subtypes (gHTN or cHTN) had larger gene expression changes than either of the preeclampsia subtypes (preeclampsia or siPE). Additionally, we used GSVA scores to investigate the activated pathways in an unsupervised manner. If HDP disease subtypes existed on a continuum, we would expect a linear increase in GSVA scores compared with the nonhypertensive state. However, there were no gene sets showing a linear increase in GSVA scores, suggesting no pathways were being activated in a linear fashion. Considering the fact that MVM is thought to start at an early stage of pregnancy, secondary to abnormal vascular remodeling,[14,17,18] and that MVM is significantly more highly enriched in placentas with preeclampsia (preeclampsia and siPE) compared with hypertension (gHTN and cHTN), as well as the transcriptomic data presented here, we propose that preeclampsia (preeclampsia and siPE) and hypertension (gHTN and cHTN) have distinct pathophysiologic origins.

Finally, since we noted MVM as the key pathological feature associated with preeclampsia (preeclampsia and siPE), to identify key pathways that distinguish each condition, we set out to compare the transcriptional changes between these placentas, comparing those with or without MVM. Strikingly, we found similarities in the transcriptomes of preeclampsia and siPE placentas with MVM; however, significant differences were noted in non-MVM placentas between preeclampsia and siPE. These data are intriguing and suggest that preeclampsia and siPE have a common molecular signature in the presence of MVM, but diverge in the absence of MVM. Our current data, as well as previous reports,[13] noted distinct molecular signatures of preeclampsia and siPE when the placental histopathologic findings are not considered, suggesting the importance of incorporating placental histopathologic findings to further elucidate the molecular pathophysiology of the disease.

Together, our clinical and placental transcriptomic data suggest that the factors leading to preeclampsia may not be on a continuum with hypertension, but rather separate in origin at disease onset. Considering the key feature of MVM origin as abnormal spiral artery remodeling, which is mediated by extravillous trophoblast, and given that extravillous trophoblasts play a crucial role in early placentation, we believe that incorporation of placental examination and identification of distinct patterns of placental injury are key to further delineate HDP disease.

Although we believe our findings are meaningful, several limitations should be acknowledged. Our sample collection was selective as recruitment was limited to patients delivering at our university-affiliated hospital, which is a tertiary care center that treats patients with high-risk pregnancies. In addition, within the high-risk patients, clinical coordinators prioritize the recruitment of patients with a higher risk for adverse effects,

as reflected by the older maternal age and higher C-section rate in our study participants. Although this recruitment strategy may introduce biases and limit generalizability to broader study populations, it also provides the opportunity to capture and study a broad range of HDP subtypes in populations at higher risk for developing these conditions. Our cohort did not contain a large number of RNA-seq samples from cHTN and gHTN with MVM to enable a comparison of hypertension placentas with and without MVM. We are continuing to collect placental tissue samples from patients with hypertension and project that our follow-up studies will be sufficiently powered to compare the differences among the MVM-containing HDP subtypes. We concede that our hypothesis of a linear progression from hypertension to preeclampsia is influenced by the availability of placental tissue representing only the terminal stage of the disease. We acknowledge that the best way to test disease progression would be to acquire real-time data throughout pregnancy. However, currently, the only feasible method to evaluate the progression of disease during pregnancy is through maternal blood profiling. We believe future studies involving not only the assessment of placental tissue, but also using longitudinal maternal blood samples as well as placental imaging throughout pregnancy, are necessary to better evaluate disease progression and elucidate disease cause.

To date, in-depth studies of the cause of HDP have been limited to preeclampsia and have generally not included other HDP subtypes. Although existing data suggest that abnormal trophoblast differentiation contributes to disease onset, these studies lack in-depth mechanistic insight into the specific cell types and pathways driving its development. Because the clinical characteristics are similar between preeclampsia and siPE, as well as between gHTN and cHTN, it is challenging to distinguish between these HDP causes without pathological or molecular data. Thus, to fully understand the disease, it is necessary to evaluate not only the clinical phenotype, but also to probe the placenta at the tissue, cellular, and molecular levels. We believe our study confirms previous findings, but also contributes novel findings, highlighting the need for using placental pathology to understand the cause of specific HDP subtypes.

ARTICLE INFORMATION

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Author Contributions

M. Horii and M.B. Jacobs designed the research; A. Edlabadkar, T.N. Liu, S. La Belle, S. Adkins, M. Meads, and V. Stanley recruited patients and collected clinical information; M.B. Jacobs collected and managed database; L. Lamale-Smith, R.B. Wolf reviewed the data within the obstetrics registry and adjudication of hypertensive disease of pregnancy; O. Aisagbonhi reviewed placental pathology data and coding of data into the obstetrics registry; J.N. Chousal, A. Hakim, and A. Edlabadkar performed RNA extraction and preprocessing/mapping of the RNA-seq data, M. Horii and M.B. Jacobs performed clinical data analysis of clinical and pathological data; M. Horii, M.B. Jacobs, and R. Morey performed computational data analysis; M. Horii, M.B. Jacobs, and R. Morey wrote the article text, and all the authors reviewed the final version of the article.

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Disclosures

None.

Supplemental Material

Tables S1–S9
Figures S1 and S2

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