ORIGINAL ARTICLE

Maternal Hypertension-Related Genotypes and Congenital Heart Defects

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BACKGROUND:
Maternal hypertension has been associated with congenital heart defect occurrence in several studies. We assessed whether maternal genotypes associated with this condition were also associated with congenital heart defect occurrence.

METHODS:
We used data from the National Birth Defects Prevention Study to identify non-Hispanic white (NHW) and Hispanic women with (cases) and without (controls) a pregnancy in which a select simple, isolated heart defect was present between 1999 and 2011. We genotyped 29 hypertension-related single nucleotide polymorphisms (SNPs). We conducted logistic regression analyses separately by race/ethnicity to assess the relationship between the presence of any congenital heart defect and each SNP and an overall blood pressure genetic risk score (GRS). All analyses were then repeated to assess 4 separate congenital heart defect subtypes.

RESULTS:
Four hypertension-related variants were associated with congenital heart defects among NHW women (*N* = 1,568 with affected pregnancies). For example, 1 intronic variant in *ARHGAP2*, rs633185, was associated with conotruncal defects (odds ratio [OR]: 1.3, 95% confidence interval [CI]: 1.1–1.6). Additionally, 2 variants were associated with congenital heart defects among Hispanic women (*N* = 489 with affected pregnancies). The GRS had a significant association with septal defects (OR: 2.1, 95% CI: 1.2–3.5) among NHW women.

CONCLUSIONS:
We replicated a previously reported association between rs633185 and conotruncal defects. Although additional hypertension-related SNPs were also associated with congenital heart defects, more work is needed to better understand the relationship between genetic risk for maternal hypertension and congenital heart defects occurrence.

Keywords: blood pressure; congenital heart defects; genetic risk score; hypertension; maternal genotype

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Congenital heart defects affect 1% of live births worldwide and are the most common type of congenital malformation.[1,2] Approximately 4% of all neonatal deaths in the United States are attributable to these defects.[3] In the majority of affected individuals, the etiology is unknown and likely complex. While there are some etiologic differences across congenital heart defect subtypes (e.g., conotruncal vs. left ventricular outflow tract defects), some risk factors have been associated with many different subtypes.

Maternal hypertension is present in around 10% of pregnancies and has been associated with several congenital heart defect subtypes.[4] A recent review and meta-analysis reported a significant association between hypertension and any congenital heart defect across 15 studies (meta relative risk: 1.8, 95% confidence interval [CI]: 1.5–2.2).[5] This association persists even after accounting for antihypertensive medication use,[6] but the mechanisms that underlie this association remain unclear.

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For example, overt maternal hypertension may have a direct effect on *in utero* cardiac development (e.g., hypertension-induced changes to the placental barrier). Additionally, pathways related to the onset of the maternal phenotype may also have independent effects on cardiogenesis during development in pregnancy (e.g., genes with pleiotropic effects).[7]

Common genetic variants have been extensively studied with regard to hypertension risk. A hypertension/elevated blood pressure genetic risk score (GRS) based on 29 single nucleotide polymorphisms (SNPs) was established by the International Consortium for Blood Pressure and has been validated in diverse populations by several large-scale studies.[8–11] The 29 variants were selected for the current study based on their associations with hypertension and performance at hypertension risk prediction in genome-wide association studies among populations with European ancestry and Hispanic ancestry.[8–11] A maternal hypertension GRS has also been associated with increased risk for conotruncal heart defects in 1 study.[12] To further investigate the association of maternal hypertension with congenital heart defects, we evaluated maternal genotypes associated with these conditions and risk for congenital heart defects, using data from a nationally representative, population-based study of birth defects.

METHODS

Study subjects

Data collected through the National Birth Defects Prevention Study (NBDPS) were used for this study. Specifically, we used data from pregnant women with (cases) and without (controls) a pregnancy in which isolated, simple heart defects were present (described below) and with an estimated delivery date between 1 January 1999 and 31 December 2011. Pregnancies before this period were excluded (i.e., years before mandatory folic acid fortification of food products). The NBDPS recruited subjects identified through birth defects surveillance programs among 10 states in the United States (Arkansas, California, Georgia, Iowa, Massachusetts, New Jersey, New York, Texas, and Utah). A detailed explanation of NBDPS recruitment and data collection has been previously described.[13] Information pertaining to maternal characteristics and exposures during pregnancy was collected through computer-assisted telephone interviews with women with and without a pregnancy in which birth defects were present. Maternal hypertension status was self-reported during this interview. Maternal race/ethnicity was also collected, and infant sex and date of birth were abstracted from medical records.

For these analyses, cases were women with a pregnancy (liveborn, stillborn, or terminated) diagnosed with select heart defects (conotruncal, left ventricular outflow tract, right ventricular outflow tract, and septal defects), based on review and classification by NBDPS pediatric cardiologists, using a standardized protocol.[14] These defects were selected based on being common and potentially having serious clinical consequences. Diagnostic tests and medical records were used to confirm the phenotype, and to limit heterogeneity. Analyses were conducted among cases with isolated heart defects (i.e., no additional diagnosed major cardiac or noncardiac birth defects was present, as defined by the NBDPS[14]). Women with index pregnancies in which a chromosomal abnormality or genetic syndrome was present were excluded from the NBDPS. Controls were women with a pregnancy which resulted in a live birth without any birth defects, identified from birth certificates or medical records within each of the 10 NBDPS sites' surveillance areas. Given limited genotyping resources, NBDPS controls with DNA samples (see below) were randomly selected with a 1:1 control: case ratio, based on conotruncal defects.

Genotyping

We evaluated the 29 SNPs that comprise a previously established hypertension/blood pressure GRS.[15,16] DNA samples used for genetic analysis were previously collected by NBDPS laboratories using maternal buccal brushes. The ligation detection reaction assay was used to genotype the 29 SNPs in 1 ligation reaction.[17,18] Two multiplex polymerase chain reaction (PCR) reactions were designed to amplify all 29 SNP loci. Qiagen multiplex PCR kit (Cat#: 206145) was used for the multiplex PCR following the manufacture's protocol. The PCR products were equally mixed and purified by digestion with 1 U of shrimp alkaline phosphatase at 37 °C for 1 hour, followed by deactivation of the phosphatase at 75 °C for 15 minutes. Ligation chain reaction was performed as following: the 20 μl ligation reaction contained 1× ligation buffer, 0.5 μl HiFi Taq DNA Ligase (NEB Cat#: M0647), 1 μl of labeling oligo mixture, 2 μl of probe mixture, and 5 μl of purified PCR product mixture. The cycling program for the ligation reaction was 95 °C for 2 minutes followed by 38 cycles of 94 °C for 1 minute and 56 °C for 4 minutes and a hold at 4 °C. Ligation product (0.5 μl) was loaded into an ABI 3730 DNA analyzer and the raw data were analyzed by GeneMapper 4.1. All primers, probes, and labeling oligos (Supplementary Tables S3 and S4 online) were ordered from Integrated DNA Technologies. Cases or controls with >10% missing genotype data were excluded. SNPs with <5% minor allele frequencies for variants were excluded. SNPs that were found to be out of Hardy–Weinberg equilibrium ($P < 0.002$, based on a Bonferroni adjustment for 29 total SNPs) among the non-Hispanic white (NHW) or Hispanic control group were excluded from the NHW or Hispanic SNP-level analyses, respectively. SNPs with <95% genotype call rates among the full analytic group were excluded from all SNP-level analyses. Participants with any missing genotype data were excluded from the GRS analysis (described below).

Statistical analysis

To limit the potential for population stratification, all analyses were limited to Hispanic and NHW women, and were conducted separately for each of these groups. Characteristics of cases and controls were tabulated using counts and proportions.

We used unconditional logistic regression to evaluate the association between risk for heart defects and the maternal genotype for each of the 29 hypertension-related SNPs. In addition to evaluating associations with any heart defect, we also separately assessed 4 specific defect subtypes: conotruncal, left ventricular outflow tract obstructions (LVOTO), right ventricular outflow tract obstructions (RVOTO), and septal defects. We computed odds ratios (ORs) for the association with each SNP. We used an additive genetic model and treated the allele that was associated with an increased risk for hypertension in previous studies as the risk allele.[8,19] In other words, the hypertension risk allele was hypothesized to be positively associated with heart defects.

A hypertension/blood pressure GRS for each participant was constructed based on the maternal genotype for each of the 29 hypertension-related SNPs. Each genotype contributed a 0, 1, or 2 to the risk score, based on the number of risk alleles present, multiplied by the previously reported beta-coefficient between that SNP and hypertension/blood pressure.[8] This was done to weight the contribution of the genotype to the risk score by the expected magnitude of association with hypertension/blood pressure.[9] This risk score was assessed as a continuous variable (to evaluate for linear effects) and a dichotomous categorical variable (to evaluate threshold effects for an extreme exposure status). The continuous GRS was used to compare mean scores between cases and controls of both the NHW and Hispanic groups. The dichotomous GRS was defined using a threshold of above or below the 95th percentile. The categorical GRS was modeled after a previously published score examining a similar genetic risk for the outcome of interest.[12] The top 5th percentile of risk was selected as a cutoff value to represent the highest genetic risk.[12] Categorization was based on the distributions of the GRS in NHW control mothers. This analysis assessed whether control mothers in the highest percentile for case mothers would have an inflated risk compared with control mothers in the bottom 95th percentile of both groups. Subanalyses were also performed using an unweighted GRS.

To evaluate potential effects related to delivery center, we also repeated analyses, further adjusting for this variable. Among SNPs associated with at least 1 heart defect phenotype in the main analysis (P value <0.05) and for all GRS comparisons, we conducted a sensitivity analysis excluding women with hypertension (i.e., report of having hypertension treated with medications during the pregnancy, chronic hypertension diagnosed prior to the pregnancy, or gestational hypertension during the pregnancy). Analyses were completed using SAS version 9.4 (SAS Institute, Cary, NC). Quality control analyses and exclusions were performed using PLINK v1.07.[20] Approval for the study was granted by the Institutional Review Board at each participating NBDPS site and the Centers for Disease Control and Prevention.

RESULTS

After our quality control procedures were implemented, genotype data were available for 2,057 cases (76.2% NHW) and 541 controls (78.2% NHW, Table 1). Septal defects were the most commonly reported heart defect subtype (n = 695, 33.8%), and there were differences in the distributions of heart defect subtypes between NHWs and Hispanics. No SNP deviated from Hardy-Weinberg equilibrium in controls (P < 0.002 after a Bonferroni adjustment for multiple comparisons).

Hypertension-related SNPs

Of the 29 hypertension-related SNPs evaluated, there were 5 associated with heart defect phenotypes among NHWs and/or Hispanics. Among NHWs (n = 1,568 cases), there were 2 SNPs with significant, positive associations and 2 with significant, negative associations (Table 2 and Supplementary Table S1 online). One SNP (rs932764) was positively associated with any heart defect (OR: 1.2, 95% CI: 1.1–1.4), and this SNP was also positively associated with conotruncal, LVOTO, and septal heart defects. One additional hypertension-related SNP (rs633185) was positively associated with conotruncal defects (OR: 1.3, 95% CI: 1.1–1.6) but not with other phenotypes. Negative associations were observed for 2 SNPs (rs12940887 with conotruncal defects and rs13107325 with septal defects).

Among Hispanics (n = 489 cases), there were 2 associated SNPs, 1 with a significant, positive association and 1 with a significant negative association (Table 2 and Supplementary Table S1 online). One hypertension-related SNP, rs17367504, was negatively associated with the presence of any heart defect (OR: 0.5, 95% CI: 0.2–0.8), and additional negative associations were also observed with conotruncal and septal defects. The other significant hypertension-related SNP (rs12940887) was positively associated with septal defects (OR: 1.6, 95% CI: 1.0–2.4), but not other phenotypes. The directionality of hypertension-related SNPs rs633185 and rs932764 was the same as in NHWs, although the associations for these SNPs were not statistically significant.

Hypertension GRS

To assess aggregate effects of multiple SNPs in combination, we evaluated whether a hypertension GRS was associated with heart defect phenotypes. Because genotype information was missing for 1 or more SNPs from 59 NHW women and 16 Hispanic women, data from these women were excluded from these hypertension GRS analyses. The distribution of the GRS was similar between NHW and Hispanic controls (data not shown). One significant association was found between the hypertension GRS and septal heart defects among NHW women (OR: 2.1, 95% CI: 1.2–3.5) (Table 3). The results were similar for the subanalyses of the unweighted GRS (data not shown).

Sensitivity analyses

To evaluate potential effects related to delivery center, we repeated analyses, further adjusting for this variable. We also conducted sensitivity analyses, repeating comparisons after excluding 49 controls (10.0%) and 262 cases (14.6%) who reported having hypertension. For both of these sensitivity analyses, results were similar to those from the main results (Supplementary Tables S5 and S6 online).

Subanalyses were also performed to examine the risk of congenital heart defects based on the continuous maternal

Table 1. Characteristics of cases and controls, NBDPS, 1999–2011

	Non-Hispanic white (*N* = 1,996)		Hispanic (*N* = 602)	
	Cases[a] (*N* = 1,568, 78.6%)	Controls (*N* = 428, 21.4%)	Cases[a] (*N* = 489, 81.2%)	Controls (*N* = 113, 18.8%)
Characteristic	*N* (%)	*N* (%)	*N* (%)	*N* (%)
Infant defect				
Conotruncal	416 (26.5)	—	127 (26.0)	—
LVOTO	336 (21.4)	—	75 (15.3)	—
RVOTO	323 (20.6)	—	85 (17.4)	—
Septal	493 (31.5)	—	202 (41.3)	—
Infant sex[b]				
Male	835 (53.3)	227 (53.0)	275 (56.4)	60 (53.1)
Female	732 (46.7)	201 (47.0)	213 (43.6)	53 (46.9)
Delivery center[b]				
Arkansas	336 (21.6)	60 (14.2)	39 (8.0)	4 (3.6)
California	57 (3.7)	19 (4.5)	110 (22.6)	23 (20.5)
Georgia	88 (5.6)	28 (6.6)	28 (5.7)	9 (8.0)
Iowa	236 (15.2)	74 (17.5)	13 (2.7)	4 (3.6)
Massachusetts	194 (12.5)	56 (13.2)	24 (4.9)	6 (5.4)
New York	93 (6.0)	25 (5.9)	9 (1.8)	2 (1.8)
North Carolina	141 (9.1)	54 (12.8)	28 (5.8)	10 (8.9)
Texas	56 (3.6)	10 (2.4)	198 (40.7)	46 (41.1)
Utah	352 (22.7)	97 (22.9)	38 (7.8)	8 (7.1)
Maternal age				
<20	78 (5.0)	25 (5.8)	66 (13.5)	14 (12.4)
20–24	346 (22.1)	77 (18.0)	138 (28.2)	40 (35.4)
25–29	488 (31.1)	121 (28.3)	147 (30.0)	27 (23.9)
30–34	423 (27.0)	136 (31.8)	89 (18.2)	19 (16.8)
35–39	192 (12.2)	62 (14.5)	34 (7.0)	9 (8.0)
>40	41 (2.6)	7 (1.6)	15 (3.1)	4 (3.5)
Parity[b]				
Nulliparous	485 (31.0)	138 (32.2)	140 (28.6)	32 (28.3)
Multiparous	1,081 (69.0)	290 (67.8)	349 (71.4)	81 (71.7)
Prior pregnancy with congenital heart defect				
Yes	18 (1.2)	0 (0.0)	1 (0.2)	0 (0.0)
No	1,550 (98.8)	428 (100.0)	488 (99.8)	113 (100.0)

Abbreviations: LVOTO, left ventricular outflow tract obstruction; NBDPS, National Birth Defects Prevention Study; RVOTO, right ventricular outflow tract obstruction.

[a]Women with offspring with isolated, simple heart defects.

[b]Data missing for variable.

hypertension GRS. These results were similar to the main results (e.g., OR for septal defects in NHWs: 2.4, 95% CI: 1.1–4.2). The distribution of the unweighted GRS score is reported in Supplementary Table S7 online. In the subanalysis performed for the unweighted GRS, results were similar to the main results for most comparisons (Supplementary Table S6 online). Of note, the association with septal defects among NHWs was attenuated (and no longer significant), whereas associations among Hispanics with any heart defect and with septal defects were strengthened (and significant). However, the 95% CIs for all 3 of these results overlapped with those from the main analysis.

DISCUSSION

This study evaluated the relationships between maternal genotypes for 29 hypertension-associated SNPs and congenital heart defects. We identified a relatively small number of

Table 2. Maternal hypertension SNPs significantly associated with heart defects in non-Hispanic Whites or Hispanics, NBDPS, 1999–2011

SNP[a]	Gene/flanking genes[b]	Risk allele[c]	Risk allele frequency	OR[d] (95% CI)				
				Any heart defect[e]	Conotruncal	LVOTO	RVOTO	Septal
Non-Hispanic whites								
Hypertension SNPs								
rs633185	*ARHGAP42*	C	.697	1.12 (0.95–1.31)	**1.31 (1.06–1.61)**	1.08 (0.87–1.35)	1.05 (0.85–1.31)	1.06 (0.87–1.29)
rs932764	*PLCE1*	G	.423	**1.22 (1.05–1.43)**	**1.29 (1.06–1.56)**	**1.30 (1.06–1.59)**	1.04 (0.85–1.28)	**1.24 (1.03–1.50)**
rs12940887	*ZNF652*	T	.367	0.87 (0.74–1.02)	**0.78 (0.64–0.96)**	0.86 (0.70–1.06)	1.02 (0.83–1.26)	0.86 (0.71–1.04)
rs13107325	*SLC39A8*	C	.925	0.79 (0.59–1.07)	0.81 (0.56–1.18)	0.80 (0.55–1.17)	1.00 (0.66–1.50)	**0.68 (0.48–0.95**
rs17367504	*MTHFR*	A	.855	1.15 (0.94–1.42)	1.12 (0.86–1.45)	1.11 (0.83–1.47)	1.19 (0.89–1.60)	1.20 (0.93–1.56)
Hispanic								
Hypertension SNPs								
rs633185	*ARHGAP42*	C	.477	1.25 (0.93–1.67)	1.37 (0.95–1.96)	0.99 (0.66–1.49)	1.38 (0.94–2.02)	1.19 (0.87–1.65)
rs932764	*PLCE1*	G	.564	1.17 (0.88–1.55)	1.02 (0.71–1.45)	1.30 (0.87–1.95)	1.30 (0.85–1.99)	1.18 (0.87–1.61)
rs12940887	*ZNF652*	T	.199	1.34 (0.91–1.98)	1.22 (0.78–1.91)	0.98 (0.57–1.67)	1.32 (0.79–2.20)	**1.57 (1.02–2.42)**
rs13107325[f]	*SLC39A8*	C	.966	1.64 (0.88–3.05)	1.54 (0.68–3.50)	2.34 (0.70–7.81)	1.23 (0.53–2.88)	1.76 (0.82–3.78)
rs17367504	*MTHFR*	A	.901	**0.45 (0.24–0.83)**	**0.38 (0.19–0.77)**	0.55 (0.24–1.22)	0.55 (0.24–1.26)	**0.41 (0.21–0.81)**

Significant values are indicated in bold.

Abbreviations: CI, confidence interval; LVOTO, left ventricular outflow tract obstruction; NBDPS, National Birth Defects Prevention Study; OR, odds ratio; RVOTO, right ventricular outflow tract obstruction; SNP, single nucleotide polymorphism.

[a]Data not shown for SNPs that were not associated among non-Hispanic Whites or among Hispanics.

[b]For intergenic variants, the nearest up/downstream genes are listed. Additional annotation details are presented in Supplementary Table S2 online.

[c]Allele positively associated with hypertension.
[d]Crude odds ratio for carrying 1 copy of the high-risk allele compared with no copies.
[e]Conotruncal, LVOTO, RVOTO, or septal.
[f]SNP with Hardy–Weinberg equilibrium *P* value = 0.03 among Hispanic controls.

Table 3. Association between continuous maternal hypertension GRS and congenital heart defects, NBDPS, 1999–2011[a]

	OR[b] (95% CI)				
Comparison	Any heart defect[c]	Conotruncal	LVOTO	RVOTO	Septal
Non-Hispanic whites	1.47 (0.92–2.35)	1.05 (0.57–1.93)	1.38 (0.75–2.51)	1.23 (0.66–2.29)	**2.09 (1.24–3.53)**
Hispanic	0.93 (0.31–2.85)	0.44 (0.08–2.42)	1.15 (0.25–5.28)	2.50 (0.71–8.83)	0.56 (0.14–2.29)

Significant values are indicated in bold.

Abbreviations: CI, confidence interval; GRS, genetic risk score; LVOTO, left ventricular outflow tract obstruction; NBDPS, National Birth Defects Prevention Study; OR, odds ratio; RVOTO, right ventricular outflow tract obstruction.

[a]The analysis of cases vs. controls with risk scores greater than the 95th percentile, compared with NHW control scores. Non-Hispanic white and Hispanic 95th percentile GRS cutoff: 1.34.

[b]Crude odds ratio.

[c]Conotruncal, LVOTO, RVOTO, or septal.

significant positive associations among our GRS analyses and our SNP-level analyses that seemed consistent with our hypothesis that these genotypes would be positively associated with heart defects. Specifically, 1 strong positive GRS association as well as several positive and negative significant associations with specific SNPs were observed. Further, several of these SNP associations were similar across multiple heart defect phenotypes. There were also similar associations observed between Hispanic and NHW groups for several of the same SNP-defect comparisons, although statistical significance varied (i.e., rs633185 and conotruncal defects, rs932764 and any heart defect, rs932764 and LVOTO, and rs932764 and septal defects).

One study conducted by Kaplinski *et al.* evaluated the association between a similar hypertension GRS (including 25 of the 29 SNPs we assessed) and conotruncal heart defects in a different NHW study population.[12] Four of the 5 variants with significant associations observed in our analyses were assessed by Kaplinski *et al.*, as described below. We are unaware of other studies that have evaluated maternal hypertension-related genes and heart defect risk.

The association between the variant for *PLCE1* (rs932764) with any heart and LVOTO defects were nearly identical in magnitude and directionality between Hispanic and NHW groups in our analyses. Among NHW women, this variant was associated with any heart, LVOTO, and septal defects. This variant was not associated with conotruncal defects in the study by Kaplinski *et al.*, though other defects were not assessed in those analyses. *PLCE1* is involved in the regulation of glomerular filtration in the kidneys through its role in podocyte development, and *PLCE1* mutations have been associated with proteinuria.[21] Preeclampsia may also be linked to the dysregulation of podocytes, with regulation controlled by phospholipase enzyme producing genes such as *PLCE1*.[22] Further research may be helpful to understand more about the potential role of this gene in human cardiogenesis.

The maternal genotype for an intronic variant in *ARHGAP42*, rs633185, was positively associated with conotruncal heart defects in pregnancies among NHW women (OR: 1.3, 95% CI: 1.1–1.6) and Hispanic women (OR: 1.4, 95% CI: 1.0–2.0) in our study. This association was also reported among NHWs in the study (OR: 1.2, 95% CI: 1.0–1.5) by Kaplinski *et al.*[12] *ARHGAP42* modulates vascular resistance, though its role during pregnancy is not well-studied.[23] *ARHGAP42* (also commonly identified as *GRAF3*) is a Rho-specific GTPase activating protein (GAP) which, when inhibited in animal models, is associated with a significant increased risk of hypertension,[23] and RhoGAP has been reported to be positively correlated with functional cardiac development in mice.[24] The exact mechanisms by which *ARHGAP42* hypertensive-associated variants might affect RhoGAP function and subsequent cardiac development in humans are unclear. Considering the recent findings surrounding the role of RhoGAP in animal models and replication of this association in 2 independent studies in humans, further investigation of this gene may be warranted.

In the present analyses, the categorical, weighted GRS (based on the 95th percentile of the GRS) was positively associated with septal defects (OR: 2.1, 95% CI: 1.2–3.5) among NHWs. A recent meta-analysis also reported significant associations between overt maternal hypertension and ventricular septal defects specifically (relative risk: 1.3, 95% CI: 1.1–1.6), as well as other heart defect subtypes.[5] Kaplinski *et al.* also reported a significant association between a categorical, weighted hypertension GRS (also based on the 95th percentile of the GRS) and conotruncal defects (OR: 1.7, 95% CI: 1.0–2.8),[12] though septal defects were not assessed. Based on these prior results and our findings, further investigation of cumulative effects of hypertension-associated variants may be useful.

Our other variant-level results among NHW women either were not comparable with or differed somewhat from those reported in the assessment of conotruncal heart defects among a NHW population by Kaplinski *et al.*[12] Specifically, Kaplinski *et al.* reported *P* values suggestive of associations with conotruncal defects for 2 additional maternal hypertension-related SNPs that were not associated with heart defects in our study (rs13139571 and rs1801253).[12] Two SNPs in our analyses, rs932764 (OR: 1.2, 95% CI: 1.1–1.4) and rs12940887 (OR: 1.6, 95% CI: 1.0–2.4) had significant associations with conotruncal defects among NHWs. These SNPs were not associated with conotruncal defects in the analyses by Kaplinski *et al.* (rs932764, OR: 1.0, 95% CI: 0.9–1.2; rs12840887, OR: 1.0, 5% CI: 0.9–1.2). Some of the differences in results between the 2 studies may reflect type I or type II errors in either study (e.g., related to multiple comparisons or underpowered analyses for some comparisons). Differences may also be related to systematic differences between the studies. For instance, the study by Kaplinski *et al.* implemented a mother–father case–control design; included only NHW participants; did not evaluate LVOTO, RVOTO, or septal defects; and used a clinically recruited population. Their population may have been subject to some selection bias (e.g., due to potential differences in defect severity in a clinical sample vs. a population-based sample). The discrepancy between the SNPs used by each study (e.g., there were 25 SNPs in both GRS, 4 SNPs only in our GRS and 6 SNPs only in their GRS) to create the weighted GRS may also account for the varying results.

In our study, an intronic variant in *MTHFR* (rs17367504) was negatively associated with 3 heart phenotypes among pregnancies in Hispanic women but not NHW women. This variant is located within a DNase1 hypersensitivity cluster and a transcription factor binding site.[25] *MTHFR* is involved in a number of metabolic processes, including folic acid metabolism, and other maternal variants in MTHFR have been negatively associated with heart defects in offspring in prior studies, including studies among predominately Hispanic populations.[26–28] Although our results were not similar among NHW (OR: 1.2, 95% CI: 0.9–1.4) and Hispanic (OR: 0.5, 95% CI: 0.2–0.8) women for this SNP, differences in associations between racial/ethnic groups may suggest effect measure modification,[29] and/or differences in linkage patterns between race/ethnicity groups may also play a role.[30] In fact, the GRS used here has been more strongly associated with high blood pressure among European populations than among Hispanic populations (e.g., *P* values: 3.6E−153 and 1.7E−3, respectively[12]). Additionally, some of the individual SNPs that we observed to be associated with heart defects only among NHW women have been reported to be not associated with high blood pressure (i.e., *P* value >0.05) among Hispanics (e.g., rs633185, rs12940887, rs13107325, and rs17367504).[10] This may explain some of the differences in our results between NHW and Hispanic women. Further, there were differences in the distributions of heart defect subtypes between NHWs and Hispanics. If these observed differences are replicated, they might support the notion that some maternal genetic effects involved in heart defects risk could vary by race/ethnicity, as similar effects have been described in other work.[31]

We observed similar results between participants in the full group and in sensitivity analyses among women who did not report having hypertension, and this consistency might be related to several factors. The assessed maternal variants may have contributed to an intermediate, subclinical phenotype in the mother before overt hypertension would be recognizable, which might have had a clinical effect on the uterine environment that contributed toward subsequent cardiac maldevelopment. Additionally, there could be some pleiotropic effects at play (e.g., related to effects of variants on pathways that are independent of hypertension).

Our results should be interpreted with consideration of the potential limitations and strengths of the study. Several comparisons were made (e.g., multiple heart defect subtypes) and we did not consider conservative corrections for multiple comparisons because these comparisons were not completely independent. Maternal data on race/ethnicity were collected through self-report; however, previous studies have shown high concordance between self-reported race/ethnicity and genetic race/ethnicity based on ancestry informative markers (which were not available for the present analyses) among mothers of infants with birth defects.[32] Some of the observed associations may be related to random chance, although this may be less likely for associations that were consistent across multiple phenotypic groups. In the Hispanic study population, 1 SNP, rs13107325, had a minor allele frequency of <5% (3.4%) and Hardy–Weinberg equilibrium *P* value = 0.03 but was included in analysis. Results for this SNP should therefore be interpreted with caution. We analyzed associations with maternal, not infant, genotypes, and ruling out the possibility of infant genetic effects may be helpful in future work. Due to data availability, there were a limited number of Hispanic controls included in the study population; results in this subpopulation should be interpreted with caution. Data on maternal blood pressure measurements were also not available. Because control selection was based on case subgroup counts, the number of cases in the any heart defect group was higher than the number of controls. Major strengths of the study include the use of NBDPS data collected from multiple states, including a large, population-based sample, well-defined case classification, assessment of heart defect subtypes, and analysis of the 2 largest race/ethnicity groups in the United States.[13,33]

Our study is among the first reports on the relationship between maternal genotypes for hypertension-related variants and the occurrence of heart defects in pregnancies of Hispanic women. Considering that maternal hypertension is becoming increasingly recognized as a risk factor for heart defects,[5] a better understanding of the mechanisms between genetic risk for maternal conditions and congenital heart defects is needed. Future research could involve the assessment of additional maternal genotypes, which continue to be identified in genomic studies; a focus on additional comparisons by race/ethnicity; and further assessment of maternal genetic effect pathways.

SUPPLEMENTARY MATERIAL

Supplementary data are available at *American Journal of Hypertension* online.

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DISCLOSURE

The authors declared no conflict of interest.

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