

Research Article

Biological Pathways of Long-Term Visit-to-Visit Blood Pressure Variability in the American Population: Cardiovascular Health Study and Women's Health Initiatives

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Studies reported a positive relationship between visit-to-visit blood pressure variability (VVBPV) and cardiovascular morbidity and mortality independently of the mean arterial blood pressure across clinical visits. The literature is scarce on the genes and biological mechanisms that regulate long-term VVBPV. We sought to identify biological pathways that regulate visit-to-visit blood pressure variability. We used phenotypic and genotype data from the Women's Health Initiatives and Cardiovascular Health Studies. We defined VVBPV of systolic and diastolic blood pressure phenotypes as the standard deviation about the participant's regression line with systolic and diastolic blood pressure regressed separately across visits. We imputed missing genotypes and then conducted a genome-wide association analysis to identify genomic variants related to the VVBPV and detect biological pathways. For systolic VVBPV, we identified a neurological pathway, the GABAergic pathway (P values = $1.1E-2$), and a vascular pathway, the RAPI signaling pathway (P values = $5.8E-2$). For diastolic VVBPV, the hippo signaling (P values = $4.1E-2$), CDO myogenesis (P values = $7.0E-2$), and O-glycosylation of TSR domain-containing protein pathways (P values = $9.0E-2$) were the significant pathways. Future studies are warranted to validate these results. Further understanding of the roles of the genes regulating the identified pathways will help researchers to improve future pharmacological interventions to treat VVBPV in clinical practice.

1. Introduction

Epidemiological studies show that blood pressure varies; it oscillates over days (short term), as well as months and years (long term) [1]. The variation reflects the degree of blood pressure control and its long-term burden on the cardiovascular system [2]. Visit-to-visit blood pressure variability (VVBPV) is associated with organ damage [2]. Several observational studies documented a relationship

between blood pressure variability and cardiovascular morbidity and mortality independently of the absolute mean arterial blood pressure across clinical visits.

In the Women's Health Initiative (WHI) study, the researchers compared the highest quintile of VVBPV versus the lowest quintile. The hazard ratio (HR) for stroke and its 95% confidence interval (CI) were 1.46 (1.15–1.85). In the Cardiovascular Health Study (CHS), a one-unit increase in the VVBPV increased the risk for

myocardial infarction by 11%: HR and its 95% CI were 1.1 (1.0–1.3).

In previous studies, investigators located many genetic variants that are associated with hypertension [3, 4]. Epidemiological studies have shown that hypertension and blood pressure have been considered polygenic genetic traits, which are regulated by many biological pathways. However, the literature has little information on the biological mechanisms that may regulate long-term blood pressure variability. Additional work is warranted to identify these biological mechanisms. In this study, we analyzed genetic data from the WHI and CHS studies to locate SNPs associated with VVBPV and discern the involved biological pathways to provide insight into the architecture of this phenotype.

2. Methods

The Institutional Review Board at the University of Arkansas for Medical Sciences approved this study. We used the genetic data of two cohorts, WHI and CHS. The GWAS of the CHS included 1264 hypertensive patients who had blood pressure. The CHS GWAS study had two subcohorts: the White and African American cohorts. For the GWAS of WHI, the total number of the hypertensive participants was 8889. The WHI cohort has three subcohorts: Garnet (Whites), IMS (Whites), and Share (African Americans). The blood pressure readings of the participants of these two CHS and WHI cohorts were collected over 11 years. Systolic and diastolic blood pressure (SBP and DBP) were measured at each annual study visit by certified staff and according to standardized procedures and instruments. Blood pressure was measured with a mercury sphygmomanometer after the participant was seated and had rested for five minutes. We included participants who had at least four visit-to-visit blood pressure readings. We used the average of at least two within-visit readings to determine the SBP and DBP of each visit. To account for the effect of the antihypertensive medication, we added 10 mmHg and 5 mmHg to the systolic and diastolic blood pressure, respectively, as it was done in previous studies [5, 6]. We defined the two VVBPV of SBP and DBP phenotypes as the standard deviation about the participant's regression line (SDreg); a regression line was fitted for SBP and for DBP, separately, across visits. The estimation of SDreg assumes a linear temporal trend.

For quality control, we excluded SNPs with genotype missingness >5%, samples with low call rates (<95%), and SNPs with genotype frequencies that deviated from Hardy-Weinberg equilibrium (HWE), i.e., $P < 10E - 5$. Because our study was population-based, we only included unrelated individuals. To eliminate the possibility of spurious associations due to population structure, we computed principal components to detect population stratification and used ten components in the statistical analysis to adjust for it. To further assure the quality of samples, we excluded monomorphic SNPs: samples with very low (<4 SDs) heterozygosity would indicate poor DNA quality, and samples with very high (>4 SDs) heterozygosity would suggest sample contamination. We used the LiftOver algorithm to map our

data to genetic assembly GRCH37 [7], and SHAPEIT software package [8] was used to phase our genotype data and detect flipped SNPs. Plink [9] was used to map the forward DNA strand. The Michigan Imputation Server was used to impute untyped and missing. For SNP imputation, the 1000 Genome Project was used as the reference panel for imputation [10]. Following a common variant, common disease hypothesis (CV-CD), we filtered out SNPs that had a minor allele frequency (MAF) < 0.05 (rare and low-frequency SNPs). After imputation, we excluded SNPs that had $r^2 < 0.30$ or $MAF < 0.05$.

2.1. Data Analysis. We conducted GWAS analysis, meta-analysis, gene-based association analysis, and pathway analysis. For GWAS analysis, we converted the genetic data files of the study cohorts into file formats that can be analyzed by the MaCH2qtl software package. We used the MaCH2qtl to fit linear regression models and account for the uncertainty of the imputed data. We analyzed the association of the log (SDreg) of SBP and DBP (separately) with each SNP after adjusting for age, sex (only for CHS), mean arterial blood pressure across visits, number of clinical visits, average time interval between visits, smoking, dyslipidemia, and ten principal components. We assumed an additive effect of allele dosage on blood pressure readings. We set level of significance at $5.0E - 8$ to minimize false-positive results.

For the transethnic meta-analysis, we used random-effect-Han's and Eskin's model (RE-HE) to pool the results of the five cohorts. The RE-HE model has more statistical power than the traditional random-effect or fixed-effect methods to detect a significant association in the presence of interstudy heterogeneity due to differences in the ethnic background of pooled populations [11]. To increase statistical power, we conducted Gene-Based Association Analysis using GCTA64. To compute the gene P values, we used the 1000 Genomes Project panel as a reference population to account for linkage disequilibrium (LD) between SNPs. Figure 1 summarizes the pipeline, which we used to conduct the pathway analysis. We first selected the significant genes based on P values ranging from 10^{-7} to 10^{-6} and analyzed these genes for biological plausibility using DAVID Bioinformatics Resources 6.8 [12]. The detailed process for both the DBP and SBP variability is shown in Figure 1. The pathways were considered significant if the Fisher exact P values were equal to or smaller than 0.05 and the minimum gene count belonging to a pathway was at least two.

3. Results

Table 1 shows the characteristics and number of the WHI and CHS participants. We conducted 5 GWAS analyses (see the supplementary materials: GWAS Analysis, Supplementary Table 1, and Supplementary Figure 1).

3.1. Gene-Based Analysis and Functional Annotation. We used summary-level data from the meta-analysis results of the genome-wide association studies and linkage

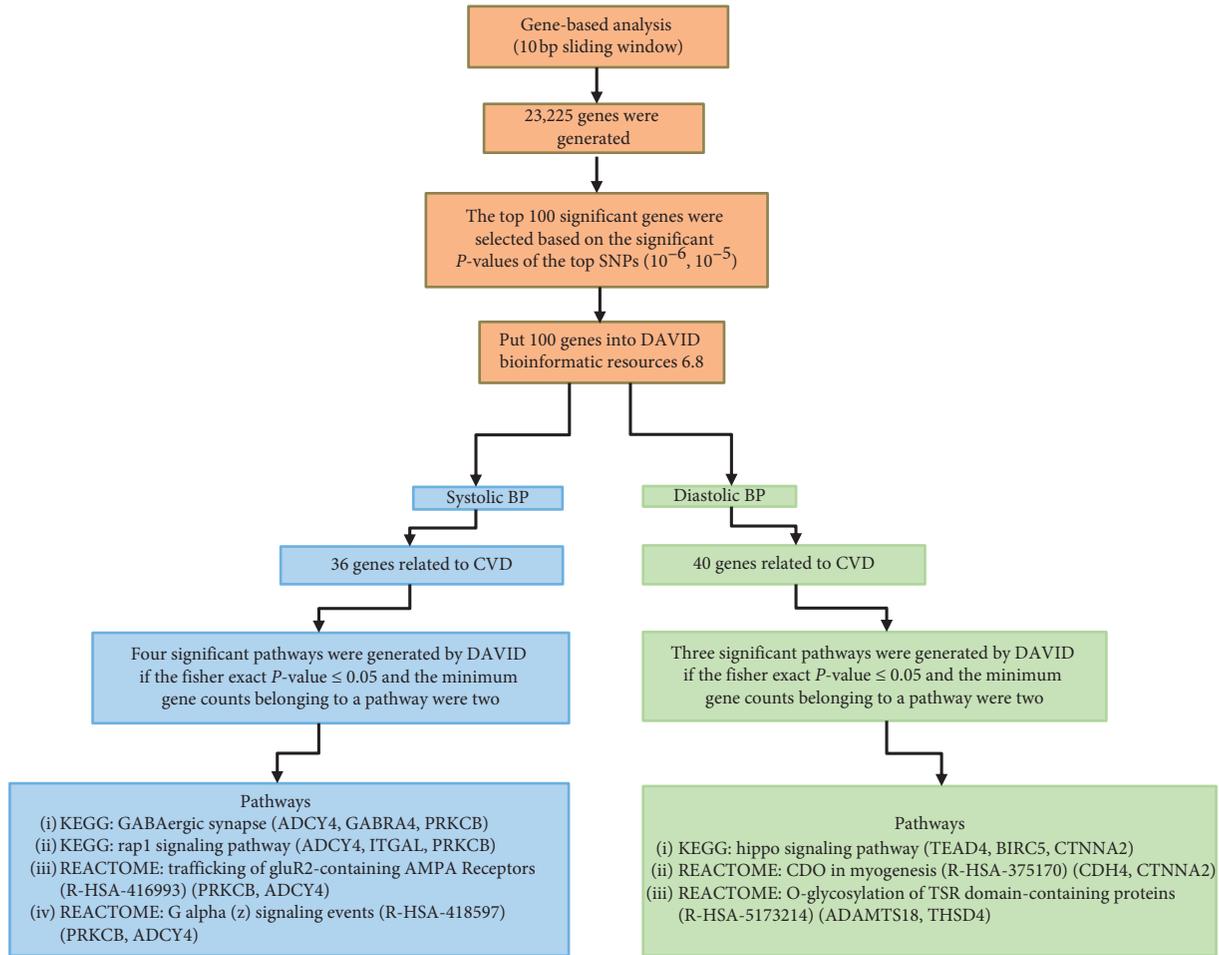


FIGURE 1: Functional annotation analysis.

disequilibrium data from a reference American population to do gene-based association analysis. We identified gene loci that could be associated with VVBPV:

$$P < 1.0E - 6. \tag{1}$$

3.2. SBP Variability. For SBP variability, 36 of the top 100 genes were significant (Table 2). Further genetic analyses located the significant genes that play role in cardiovascular disease pathways (Table 3). These pathways included the GABAergic neurologic pathway, which regulates the sympathetic activity and vascular tone. In the GABAergic pathway (Figure 2), three significant genes were identified *ADCY4*, *PRKCB*, and *GABRA4*. In addition to the neurological pathway, two pathways related to the vasculature were detected (Figure 3). The significant genes *PRKCB* and *ADCY4* can influence the RAP1 protein-signaling pathway that regulates integrin production; integrin production controls normal blood flow. Other important pathways included trafficking of GluR2-containing AMPA receptors and G alpha (z) signaling events, which regulate cardiac function.

3.3. DBP Variability. For DBP variability, 40 of the top 100 genes were significant (Table 2) and the biological pathway analysis generated three significant pathways: the hippo signaling pathway, the CDO myogenesis pathway, and O-glycosylation of TSR domain-containing protein pathway (Table 4 and Figures 4 and 5). The last two pathways have a relation with the cardiac muscle and play a role in DBP variability [13, 14]. Three genes—*CTNNA2*, *TEAD4*, and *BIRC5*—were significant in the gene-based analysis. They were identified in the hippo signaling pathway (Figure 4).

4. Discussion

In the present study, we conducted three types of statistical analyses to explore the genetic background of VVBPV. First, we conducted a GWAS to identify variants that could increase VVBPV among Americans. Second, we conducted gene-based analysis to identify potential genes that influence VVBPV. Third, we conducted pathway analyses to interpret the role of top-scoring SNPs located on the identified genes.

Regarding the GWAS analysis, we observed several suggestive, but not statistically significant, associations (P values $> 5.0E - 8$). We used stringent P value thresholds for significance— $5.0E - 8$ —to control for false-positive results

TABLE 1: Characteristics of the study participants.

Categorical variables N (%)	CHS (EA) N = 1,056	Garnet (EA) N = 2,282	Whims (EA) N = 3,316	CHS (AA) N = 208	Share (AA) N = 3,291
GWAS platform					
		Affymetrix 6.0	Illumina humanomni- quad v1-0B	HumanOmniExpress Exome-8v1B	Affymetrix 6.0
Sex					
Male	385 (36.46)	—	—	50 (24.04)	—
Female	671 (63.54)	2,282 (100.00)	3,316 (100.00)	158 (75.96)	3,291 (100.00)
Race					
European Americans	1,056 (100.00)	2,282 (100.00)	3,316 (100.00)	—	—
African Americans	—	—	—	208 (100.00)	3,291 (100.00)
Diabetes					
Yes	673 (63.73)	886 (38.83)	512 (15.44)	87 (41.83)	1,158 (35.19)
No	383 (36.27)	1,396 (61.17)	2,804 (84.56)	121 (58.17)	2,133 (64.81)
Hyperlipidemia					
Yes	61 (5.78)	837 (36.68)	1,293 (38.99)	14 (6.73)	1,356 (41.21)
No	995 (94.22)	1,445 (63.32)	2,023 (61.01)	194 (93.27)	1,935 (58.80)
Smoking					
Yes	585 (55.40)	170 (7.45)	220 (6.63)	180 (86.54)	367 (11.15)
No	471 (44.60)	2,112 (92.55)	3,096 (93.37)	28 (13.46)	2,924 (88.85)
Antihypertensive medication					
Yes	1,044 (98.86)	2,281 (99.96)	3,314 (99.94)	205 (98.56)	3,291 (100.00)
No	12 (1.14)	1 (0.04)	2 (0.06)	3 (1.44)	—
Age					
Average systolic blood pressure	72.42 (5.14)	65.53 (6.69)	68.54 (5.48)	72.27 (5.06)	61.24 (6.64)
Average diastolic blood pressure	139.94 (16.36)	130.85 (11.22)	132.59 (11.54)	143.01 (16.21)	132.48 (11.74)
Average diastolic blood pressure	71.44 (8.58)	73.35 (6.81)	73.43 (6.79)	74.47 (8.22)	76.57 (7.00)

obtained from the large number of SNPs tested, but we did not observe P values $\leq 5.0E-8$ because of lack of power.

Therefore, we conducted gene-based analyses to overcome the lack of power. The gene-based analysis has several advantages: First, the gene is the functional unit of the human genome. When we use the gene as the unit of analysis, we can conduct functional analyses such as biological pathways. Second, such analyses reduce the multiple-testing burden substantially; they require a correction for thousands of genes rather than millions of SNPs. Finally, we conducted pathways analyses to interpret the gene-based results into functional information, which might identify the pathogenic mechanisms of VVBPV.

4.1. Systolic VVBPV Pathways. In this study, we identified biological pathways that might control SBP variability. The first pathway is the GABAergic pathway, which regulates important neurotransmitters, e.g., GABA.

GABA is an inhibitory neurotransmitter. It is converted to glutamate by a metabolic pathway called the GABA shunt. In the GABAergic pathway (Figure 2), the *ADCY4* gene was significant in our analysis (Table 2). This gene regulates the production of GABA, which can inhibit sympathetic activity [15]. The *ADCY4* also encodes for the adenylyl cyclase enzyme. The adenylyl cyclase is involved in the production of cyclic AMP (c-AMP) and activation of protein kinase A (PKA), which increases GABA release [16]. *GABRA4* [15], a significant gene in our analysis, has a role in the production of

GABA receptors. These receptors are regulated by *PRKCB* [17]. Reduction in GABA will increase the glutamatergic input in the hypothalamic paraventricular nucleus. The increase in glutamate input inhibits the trafficking of the GluR2-containing AMPA receptors [18], which regulates blood pressure [19].

In addition to the neurological pathways, we identified two pathways related to the vasculature (Figure 3). The significant genes related to this pathway were *PRKCB* and *ADCY4*. *ADCY4* forms c-AMP [12, 20] and activates RAP1 (Figure 4). *PRKCB* can activate RAP1 via the protein kinase C (PKC) and phosphorylation of protein kinase-D (PKD) [21]. The RAP1 signaling pathway controls blood pressure variation by the following mechanisms: (1) it modulates the normal function of endothelial cells and angiogenesis [12]; (2) it affects peripheral vascular resistance (i.e., blood flow and pressure); and (3) it induces myocyte growth and hypertrophy [12]. Activation of RAP1 increases integrin activity. Integrin maintains the myogenic activity of blood vessels and regulates blood flow [12].

4.2. Diastolic VVBPV Pathways. Our study showed that 3 pathways might regulate this phenotype (Table 4 and Figures 4 and 5): (1) the hippo signaling pathway, (2) the CDO myogenesis, and (3) the O-glycosylation of TSR domain-containing proteins pathways. Three genes—*TEAD4*, *CTNNA2*, and *BIRC5*—were significant in the hippo signaling pathway (Figure 4). *TEAD4* bears to the

TABLE 2: Genes related to cardiac diseases.

Diastolic BP	Systolic BP
ADAMTS18	ABCG1
ADGRB3	ADCY4
APELA	ANKFN1
ARHGEF3	ARHGAP22
ATF2	BMP2K
BIRC5	C10orf90
CASC15	CCDC85A
CCDC103	CHST15
CCDC149	CMIP
CCDC7	COX7B2
CDH4	EML6
CLSTN2	FAM155A
CRK	FUT8
CTNNA2	GABRA4
DIDO1	GLIPR2
DPF3	GRIP2
ENPP2	HDAC9
EYS	ITGAL
FAM155A	KLF15
FERMT2	NEBL
FGF14	OPCML
FOXP1	PCP4
GAS8	PRKCB
IRF2	PRLR
KCNN3	PTPRD
LBP	PTPRT
LGALS14	RAB3GAP1
MAD1L1	RELN
NAALADL2	SCIN
NFATC2	SORBS1
PITPNA	SORCS3
PLCL2	STK32B
PROKR2	TNIN
RORA	TRPC4
SELP	VEPH1
SLC44A5	XIRP2
TCTE3	
TEAD4	
TG	
THSD4	

TABLE 3: Significant pathways generated from 36 genes related to cardiovascular disease in the SBP variability.

Category	Pathway	P value
KEGG	GABAergic synapse	1.1E-2
KEGG	Rap1 signaling pathway	5.8E-2
REACTOME	Trafficking of GluR2-containing AMPA receptors	2.6E-2
REACTOME	G alpha (z) signaling events	7.0E-2

TEA domain: essential for the heart development. Studies showed that this domain has a role in cardiac-specific gene expression and the hypertrophic response of primary cardiomyocytes to hormonal and mechanical stimuli. *TEAD4* acts as a transcription factor. It increases with the hypertrophy of the heart of rats because of hypertension [22]. *CTNNA2* is a α -catenin expressed in the neural tissues

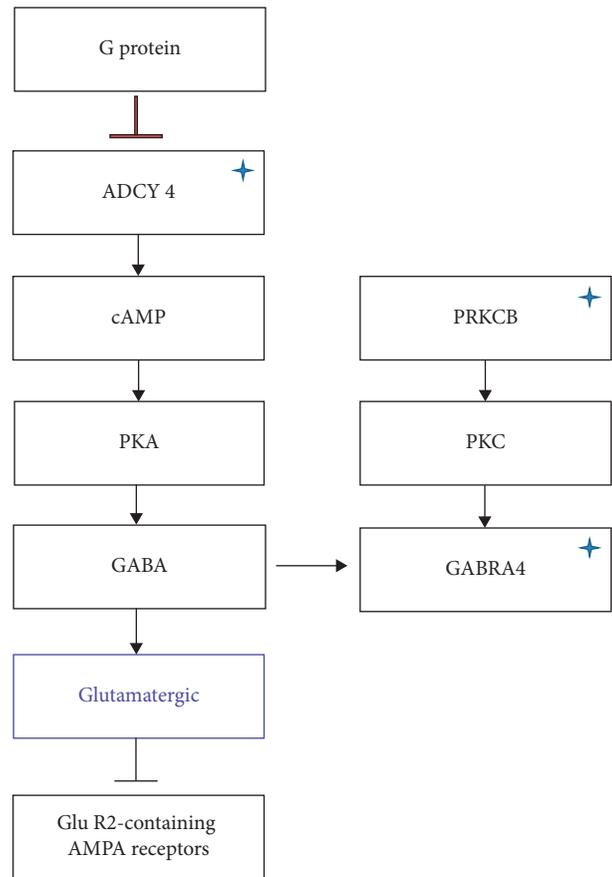


FIGURE 2: Neurological pathway in the SBP variability.

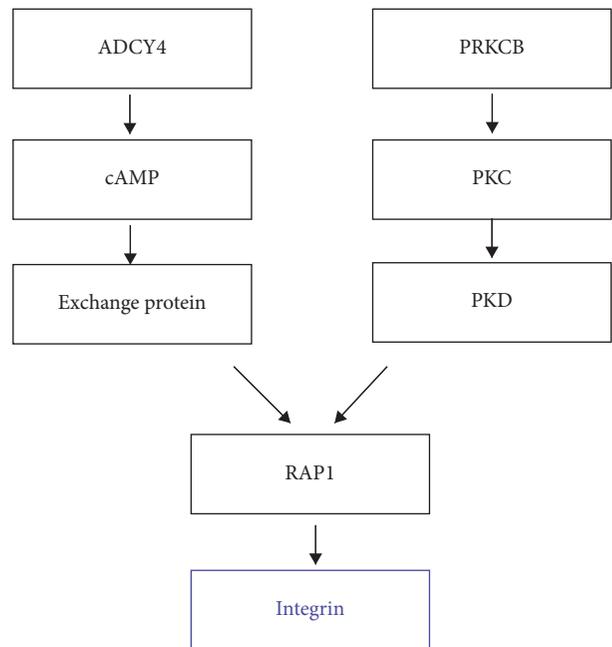


FIGURE 3: Vasculature pathway in the SBP variability.

[23] and the heart [24]. α -Catenin might play a role in left ventricular dilatation and myocardial infarction [25]. Inactivation of α -catenins in mice that have myocardial

TABLE 4: Significant pathways generated from 40 genes related to cardiovascular disease in the DBP variability.

Category	Pathway	P value
KEGG	Hippo signaling pathway	4.1E-2
REACTOME	CDO myogenesis pathway	7.0E-2
REACTOME	O-glycosylation of TSR domain-containing proteins	9.0E-2

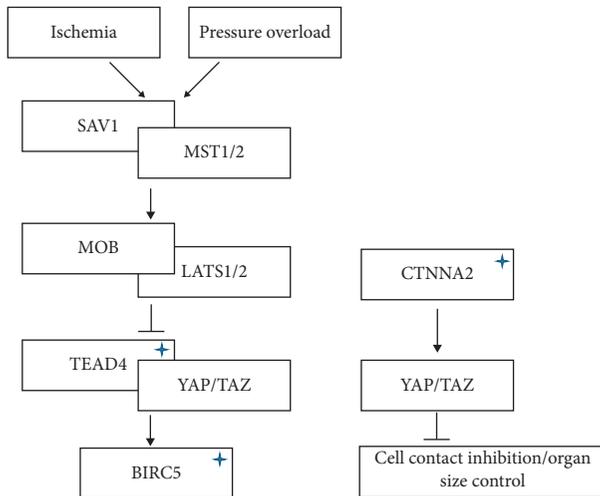


FIGURE 4: Hippo signaling pathway in the DBP variability.

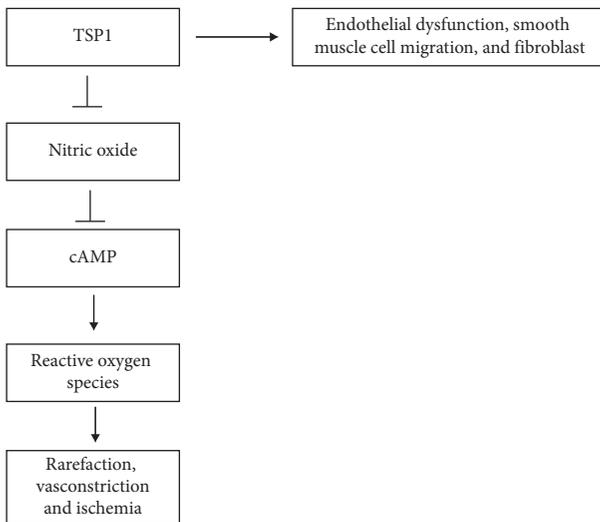


FIGURE 5: O-glycosylation of TSR domain-containing proteins pathway in the DBP variability.

infarction can induce cardiomyocyte regeneration and subsequently improved heart function [26].

The O-glycosylation of TSR domain-containing protein pathways (Figure 5) includes thrombospondin-1 (TSP1): a family of genes that maintain vascular structure and homeostasis [27, 28]. When TSP1 is upregulated, it decreases nitric oxide activity, limits c-AMP signaling, and increases reactive oxygen species, which leads to vasoconstriction and ischemia [28]. In addition, TSP1 leads to endothelial dysfunction, smooth muscle cell migration, and abnormal fibroblast activity [28]. When this gene is downregulated, it

increases angiogenesis, vasodilation, and blood flow [28]. Understanding these roles may help derive sound intervention strategies for controlling visit-to-visit blood pressure variability.

This study should be interpreted in the context of a very important limitation. Although VVBPV could have a genetic component regulating it, the effect of the environment cannot be ignored. We had no chance to evaluate the gene-environment interaction effect because (1) we lacked information on environmental variables, e.g., type and frequency of the antihypertensive medication intake and salt intake, and (2) we had insufficient sample size for evaluating such an effect. This study has many advantages. First, to our knowledge, this is the first study to explore the genetic background of VVBPV in a sample of an American White and Black population. Second, the data of the study cohorts were collected according to comprehensive protocols and thorough quality control measures. Third, the datasets had information on important covariates, which allowed us to adjust for important confounders while conducting the statistical analysis. Fourth, the results of the study may impact the public health field and clinical practice: the discovered pathways could be targeted to control blood pressure variability that leads to adverse cardiovascular outcomes.

In summary, this study shows that VVBPV might have genetic and biological components, which regulate it. Future studies are warranted to complete the following aims: (1) verifying the results of the present study and (2) identifying new pharmacological drugs that target the discovered pathways.

Data Availability

The data we have analyzed are owned by the National Heart, Lung, and Blood Institute (NHLBI) and National Center for Biotechnology Information (NCBI). Investigators need to obtain permission from these two institutes before they use the data.

Disclosure

This work was not prepared in collaboration with investigators of the CHS or WHI and does not necessarily reflect the opinions of these investigators or the NHLBI. The CHS datasets used to prepare this manuscript were obtained from dbGaP at <http://www.ncbi.nlm.nih.gov/sites/entrez?db=gapthrough> dbGaP accession (phs000287.v6.p1).

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

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Supplementary Materials

Supplementary Figure 1: QQ and Manhattan plots of the GWAS of visit-to-visit blood pressure variability. Supplementary Table 1: top 10 SNPs related to visit-to-visit systolic blood pressure variability ordered by *P* values from lowest to highest. (*Supplementary Materials*)

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