Common Genetic Variations in the Vitamin D Pathway in Relation to Blood Pressure

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BACKGROUND
Vitamin D is involved in blood pressure (BP) regulation. Genetic variations may influence the effect of vitamin D on BP, but data from epidemiologic studies remain inconsistent.

METHODS
We conducted a comprehensive genetic association study in the Women's Genome Health Study (WGHS) with genome-wide genotype data among 23,294 women of European ancestry and in the International Consortium of Blood Pressure (ICBP) with genome-wide meta-analysis results from 69,395 men and women of European ancestry.

RESULTS
First, we found none of 5 selected vitamin D–related candidate single nucleotide polymorphisms (SNPs) was associated with systolic BP (SBP) or diastolic BP (DBP). Second, in 61 candidate SNPs involved in vitamin D metabolism and signaling, rs1507023 (in *RBFOX1*) and rs2296241 (in *CYP24A1*) showed significant associations with SBP, DBP, mean arterial pressure, or pulse pressure in the WGHS before, but not after, multiple testing corrections. Nominally significant associations in the ICBP were also not significant after corrections. Third, among 24 candidate genes across vitamin D pathway, associations with BP traits that meet gene-wide significance level were found for *NCOA3* (rs2235734), *RXRA* (rs875444), *DHCR7* (rs1790370), *VDR* (rs2544037), and *NCOR2* (rs1243733, rs1147289) in the WGHS and *NCOR1*, *TP53BP1*, and *TYRP1* in the ICBP. However, none of these associations reached significance threshold in both studies.

CONCLUSIONS
Our study did not replicate previously observed associations of vitamin D–related SNPs with BP. There was suggestive evidence for associations in other vitamin D pathway genes; however, these associations either did not reach the significance threshold or were not replicated.

Keywords: blood pressure; epidemiology; genetics; hypertension; pathway; vitamin D; white population.

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The role of vitamin D in the etiology of hypertension and cardiovascular disease has been increasingly recognized. Experimental studies have suggested multiple mechanisms through which vitamin D may lower blood pressure (BP).[1–5] Prospective observational studies showed inverse association between circulating biomarker of vitamin D status and longitudinal change of BP[6] or risk of developing hypertension.[7] Small, short-term intervention studies reported that vitamin D supplements lowered BP in selected patients,[8,9] although the largest trial of vitamin D, the Women's Health Initiative, found no effect of randomized calcium (1,000 mg/day) plus vitamin D (400 IU/day) supplement on BP change and incident hypertension among 36,282 postmenopausal women over 7 years of treatment.[10]

Genetic variations may modify the effect of vitamin D on BP. Genes responsible for vitamin D synthesis and degradation may determine circulating vitamin D metabolites concentration.[11] Genes coding for vitamin D binding protein, which binds to vitamin D metabolites and facilitates their transport,[12] might affect vitamin D availability. Genes coding for vitamin D receptor (VDR), a nuclear receptor responsive to $1,25(OH)_2$-vitamin D,[13] along with its coactivators and corepressors, could influence the ligand/receptor complex and subsequent target tissue responses. Of note, single nucleotide polymorphisms (SNPs) on the *VDR* gene, including rs1544410[14–16] and rs10735810,[16] have been shown to associate with BP level or risk of hypertension in prior studies. Recently, a cluster of genes involved in vitamin D

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metabolism, transport, and function has been investigated for development of cancer[17,18] and autoimmune disease;[19,20] associations were found in genes other than *VDR*, supporting the hypothesis that genetic variants in vitamin D pathway beyond *VDR* may modify the effect of vitamin D. A comparable study for hypertension has not been reported.

To further address the role of vitamin D in BP regulation and hypertension development, we conducted a comprehensive association study to investigate genetic variants in an expanded vitamin D pathway, including a total of 24 genes, in relation to BP. We conducted parallel analyses to maximize use of data in two independent study samples: the Women's Genome Health Study (WGHS), which includes a homogeneous sample of 23,294 women of European ancestry with genome-wide genotyped data,[20,21] and the International Consortium for Blood Pressure (ICBP) genome-wide association studies (GWASs), which includes 69,395 men and women of European ancestry from 29 studies for a genome-wide meta-analysis on approximately 2.6 million HapMap SNPs in association with BP.[22]

METHODS

Study population of the WGHS

The primary study population is from the Women's Health Study (WHS), a randomized trial evaluating the risks and benefits of low-dose aspirin and vitamin E in primary prevention of cardiovascular disease and cancer among 39,876 US female health professionals aged 45 years and older.[23,24] Overall, 28,345 (70.6%) WHS participants provided baseline blood sample. The WGHS is the subset of 23,294 WHS participants of European ancestry with completed genome-wide genotyping on more than 360,000 SNPs, which can be linked to the extensive epidemiologic databank of the parent WHS.[21]

Determination of BP in the WGHS

In the WHS, baseline systolic BP (SBP) and diastolic BP (DBP) were self-reported in categories (9 for SBP from <110 to ≥180 mm Hg; 7 for DBP from <65 to ≥105 mm Hg). The midpoint of each category was used for analysis. If a participant reported taking antihypertensive medications, 10 and 5 mm Hg were added to self-reported SBP and DBP, respectively, to control for the BP-lowering effect of medications.[25] In health professionals, self-reported BP was highly correlated with measured BP[26] and highly accurate when compared with chart review.[27] The genome-wide significant associations discovered in the ICBP have been successfully replicated in the WGHS, which also indirectly supported the validity of BP phenotype in the WGHS.[22]

Genotyping in the WGHS

Detailed methods of genotyping in the WGHS have been previously described.[21] In brief, genotyping was performed using the Illumina's Infinium II assay[28] applied to the HumanHap300 Duo + platform (Illumina, San Diego, CA), including a genome-wide set of haplotype-tagging SNP markers suitable for populations with European ancestry and custom content to enhance coverage of genomic regions of significance in cardiovascular disease.[29] In the experimental data, all samples were required to have successful genotyping for at least 98% of the SNPs; SNPs were retained with minor allele frequency >1%, successful genotyping in at least 90% of the subjects, and deviations from Hardy–Weinberg equilibrium using an exact test not exceeding $P = 1.0 \times 10^{-6}$ in significance. Finally, a subset of 23,294 participants of self-reported European ancestry verified by a multidimensional scaling procedure in PLINK (http://pngu.mgh.harvard.edu/purcell/plink) had 339,000 genotyped SNPs remaining in the final data after applying quality control filters, and up to a total of 2.6 million SNPs were imputed with MaCH v. 1.0.16 (http://www.sph.umich.edu/csg/abecasis/mach/) using the reference panel from the HapMap2 CEU population.[30] Only genotyped SNPs and imputed SNPs with good quality (R^2 > 0.3) were used for analysis.

Available data in the ICBP

The discovery analyses of ICBP-GWAS included 69,395 individuals of European ancestry.[22] In all studies included in the discovery analysis, BP, height, and weight were directly measured, and sex and age were recorded. All studies performed genotyping using commercially available arrays with >300,000 SNPs and used hidden Markov model approaches[31] and HapMap reference panels[30] to impute genotypes at unmeasured SNPs and excluded SNPs so that a common set of approximately 2.6 million HapMap SNPs were available across the discovery samples. In each study, quality control procedures excluded individual problematic samples and SNPs. After the meta-analysis, the top signals were replicated in up to 133,661 additional individuals of European descent. The WGHS was not included in the ICBP discovery analysis but included in the replication analysis. The publicly available data from the ICBP include *P* values but not effect estimates for SNP associations with SBP and DBP.

Statistical analysis

Analyses of this study were conducted in 3 steps, using both WGHS and ICBP data. In the WGHS, descriptive statistics were conducted using SAS version 9.2 (SAS Institute, Cary, NC), and genetic association study was conducted using PLINK. BP phenotypes included SBP, DBP, mean arterial pressure (MAP, one-third of SBP plus two-thirds of DBP), and pulse pressure (the difference between SBP and DBP). An additive genetic effect model in linear regression was implemented assuming an additive relationship between the number of the minor allele of each SNP (0, 1, or 2; the most likely genotype was used for imputed SNPs) and BP phenotypes. Models were adjusted for age at randomization and population stratification. In the ICBP, genome-wide meta-analysis *P* values of the genotyped and imputed SNPs in association with SBP and DBP after correction for genomic control were evaluated.

First, we selected 5 putative functional SNPs in the vitamin D pathway genes, including those that have previously shown significant associations with BP[14–16] or other

disorders with material BP change such as obesity and insulin resistance,[32,33] and evaluated their associations with BP phenotypes in the WGHS and ICBP. Multiple testing was accounted for by using Bonferroni correction, and thus associations were considered significant if $P < 0.01$ (0.05/5). We also constructed a risk score based on these SNPs, calculated as a total count of the risk alleles with a range from 0 to 10.

Second, we expanded analysis to include 61 SNPs showing associations with vitamin D synthesis, metabolism, transportation, or VDR complex, including 13 SNPs that previous GWASs identified as determinants of circulating vitamin D metabolites. In the WGHS, permutation procedures were performed within the entire set of SNPs, and an empirical P value <0.05 was considered significant. In the ICBP, the genome-wide meta-analysis P values were evaluated, and a Bonferroni correction for the number of effective SNPs (n = 47.5) based on linkage disequilibrium in HapMap 2 was used to control for multiple testing, with a significance threshold of $P < 0.001$ (0.05/47.5).

Third, we *a priori* identified 24 candidate genes across the vitamin D pathway and included SNPs within 50 Kbp before the transcription start site to 10 Kbp beyond the end of transcription in each gene for analyses. In the WGHS, we performed permutations in each gene to control for multiple testing. In the ICBP, we performed a versatile gene-based association study[34] test. We also applied Bonferroni correction for the number of genes tested; thus a gene-based empirical P value <0.002 (0.05/24) was considered significant.

Finally, we searched the database from the Pritchard Lab eQTL resources (http://eqtl.uchicago.edu) for putative expression quantitative trait loci among the SNPs associated with BP traits in either WGHS or ICBP and their close proxies ($r^2 > 0.8$). We also used a gene-set enrichment analysis program, MAGENTA (http://www.broadinstitute.org/mpg/magenta), to investigate pathways enriched for SNPs in the 24 vitamin D–related genes. None of the expression quantitative trait loci or pathways identified from these analyses is directly involved in BP regulation, and therefore the results are not shown.

RESULTS

Analyses in the WGHS included a total of 23,294 women who had both BP data and genome-wide genotyping information (Supplementary Table S1). All women had confirmed European ancestry with mean ± SD age of 54.7 ± 7.1 years. The mean ± SD of SBP and DBP were 123.5 ± 20.5 mm Hg and 76.4 ± 13.2 mm Hg, respectively. Analyses in the ICBP included 69,395 men and women of European ancestry that were previously described.[22]

Candidate SNPs analyses

In the focused set of 5 SNPs selected based on previous associations with BP-related outcomes, none was associated with SBP or DBP in the WGHS and ICBP (Table 1). The risk score constructed from the 5 SNPs was also not associated with SBP (β = −0.017; SE = 0.09 mm Hg/allele; $P = 0.85$) or DBP (β = −0.027; SE = 0.06 mm Hg/allele; $P = 0.65$) in the WGHS. In the expanded set of 61 SNPs that were involved in vitamin D metabolism and signaling pathway, rs1507023 (in *RBFOX1*) was associated with SBP and pulse pressure and rs2296241 (in *CYP24A1*) was associated with SBP, DBP, and MAP at nominal $P < 0.05$ in the WGHS (Table 2; Supplementary Table S2). However, these associations were no longer significant at empirical $P < 0.05$ level after multiple hypotheses correction by permutation and were also not replicated in the ICBP (all genome-wide meta-analysis $P > 0.05$). Similarly, the nominally significant associations of rs2853564, rs1507023, rs9937918, and rs6013897 with SBP and/or DBP observed in the ICBP were no longer significant after Bonferroni correction and were not replicated in the WGHS (Table 2; Supplementary Table S2)

Candidate genes analysis

We examined the associations of genotyped and imputed SNPs in 24 genes on vitamin D pathway with BP phenotype. In the WGHS, after correcting for multiple comparisons by permutation on a gene-wide basis, rs875444 in *RXRA* was associated with pulse pressure ($P = 0.00007$; gene-based $P = 0.02$), rs2544037 in *VDR* was associated with DBP ($P = 0.0003$; gene-based $P = 0.02$) and MAP ($P = 0.0008$; gene-based $P = 0.04$), rs1790370 in *DHCR7* was associated with DBP ($P = 0.001$; gene-based $P = 0.02$), rs2235734 in *NCOA3* was associated with SBP ($P = 0.001$; gene-based $P = 0.03$), rs1147289 in *NCOR2* was associated with DBP ($P = 0.0001$; gene-based $P = 0.01$) and rs1243733 in *NCOR2* was associated with SBP ($P = 0.0003$; gene-based $P = 0.02$) and MAP ($P = 0.00007$; gene-based $P = 0.007$) (Table 3; Supplementary Table S3). However, none of these associations reached significance threshold after further correcting for the number of genes tested, and the associations with SBP and DBP were not replicated in the ICBP (all meta-analysis $P > 0.05$) (Table 3; Supplementary Table S3).

In the ICBP, 3 genes, including *NCOR1* (gene-based $P = 0.02$ for DBP), *TP53BP1* ($P = 0.02$ for SBP and 0.03 for DBP), and *TYRP1* ($P = 0.0008$ for SBP), showed associations at gene-based $P < 0.05$ (Table 4; Supplementary Table S3). After further correcting for the number of genes tested, only the association of *TYRP1* with SBP reached significance threshold. Of the most significant SNP in each gene (rs12899865 in *TP53BP1* and rs10960738 in *TYRP1* for SBP; rs2157990 in *NCOR1* and rs16957715 in *TP53BP1* for DBP), none was associated with BP in the WGHS (all nominal $P > 0.05$) (Table 4).

DISCUSSION

To our knowledge, this is the first comprehensive study of common genetic variations across 24 genes in an extended vitamin D metabolism and signaling pathway in relation to BP. We did not replicate previously observed associations of candidate SNPs with BP in large samples of white population. There is suggestive evidence for associations in 8 genes (*NCOR1, TP53BP1, TYRP1, NCOA3, NCOR2, DHCR7, VDR, RXRA*), but these associations did not reach Bonferroni corrected significance threshold and/or were not replicated.

Table 1. Association of a focused set of candidate single nucleotide polymorphisms with blood pressure phenotypes

				WGHS						ICBP
Index SNP	Chr	Position	Genes	A1/A2	A1F	Genotype	BP phenotype	Beta (SE)	*P* value[a]	*P* value
rs17467825	4	72824381	*GC*	G/A	0.28	Imputed	SBP	−0.11 (0.21)	0.61	0.45
							DBP	−0.11 (0.14)	0.42	0.60
							MAP	−0.13 (0.16)	0.41	
							PP	−0.07 (0.12)	0.56	
rs12785878	11	70845097	*DHCR7*	G/T	0.25	Imputed	SBP	0.068 (0.21)	0.75	0.70
							DBP	−0.081 (0.14)	0.56	0.12
							MAP	−0.029 (0.16)	0.86	
							PP	0.14 (0.12)	0.25	
rs1544410	12	46526102	*VDR*	T/C	0.41	Imputed	SBP	0.052 (0.19)	0.78	0.50
							DBP	−0.037 (0.12)	0.77	0.89
							MAP	−0.0025 (0.14)	0.99	
							PP	0.091 (0.11)	0.41	
rs10735810	12	46559161	*VDR*	A/G	0.38	Genotyped	SBP	0.055 (0.19)	0.77	NA
							DBP	0.00096 (0.13)	0.99	NA
							MAP	−0.0059 (0.14)	0.97	
							PP	0.054 (0.11)	0.63	
rs11568820	12	46588812	*VDR*	T/C	0.20	Imputed	SBP	0.067 (0.23)	0.77	0.34
							DBP	0.0034 (0.15)	0.98	0.97
							MAP	0.022 (0.18)	0.90	
							PP	−0.018 (0.13)	0.90	

Candidate SNPs selected for analysis have previously shown significant associations with blood pressure (BP) or other disorders with material BP change. In the Women's Genome Health Study (WGHS), analysis was adjusted for age at randomization and population stratification; data presented are effect size beta (SE) in millimeters of mercury per coded allele; all imputation $r^2 > 0.80$. In the International Consortium of Blood Pressure (ICBP), *P* for single nucleotide polymorphisms (SNPs) presented are genome-wide meta-analysis *P* values after correction for genomic control.

Abbreviations: A1, coded allele; A2, noncoded allele; A1F, coded allele frequency; Chr, chromosome; DBP, diastolic blood pressure; MAP, mean arterial pressure; NA, not available; PP, pulse pressure; SBP, systolic blood pressure.

[a]Nominal *P* value.

Many lines of evidence suggest that vitamin D is involved in the regulation of BP.[7–9] The postulated mechanisms include downregulation of the renin-angiotensin system,[1] facilitation of calcium homeostasis,[2] improvement in vascular smooth muscle cell[3] and endothelial cell[4] function, and modulation of inflammation.[5] However, the role of genetic variations in the observed association between vitamin D and BP remain largely unknown. To our knowledge, *VDR* is the only vitamin D–related gene that had been directly linked with BP. In *VDR* knockout mice, renin expression in the kidney was increased and BP elevated.[22] In human studies, findings are inconsistent. One study in Korea found that carriers of the B allele of *VDR Bsm*I polymorphism (rs1544410) had a SBP 2.7–3.7 mm Hg higher, a DBP 1.9–2.5 mm Hg higher, and odds of hypertension 2-folds higher than the bb genotype carriers (all $P < 0.05$).[14] One study we conducted in a male cohort showed that carriers of rs1544410 B allele had a hazard ratio (HR) of 1.25 (95% confidence interval (CI) = 1.04–1.51) for incident hypertension compared with carriers of the bb genotype.[16] We also found that the ff genotype of *VDR Fok*I polymorphism (rs10735810) was associated with an increased risk of hypertension (HR = 1.32; 95% CI = 1.03–1.70) compared with the FF and Ff genotypes combined.[16] In the third study, however, the B allele of *VDR* rs1544410 was significantly associated with lower SBP in white men (regression coefficient β per copy of B = −4.15; $P < 0.001$) but was unassociated with SBP or DBP in white women.[15]

Our study did not find evidence to support these previous findings for *VDR* rs1544410 and rs10735810 in very large study samples including the WGHS and the ICBP. The lack of replication may be explained by the small sample size, small number of SNPs examined, unique population characteristics, confounding by environmental factors, or potential publication bias in previous studies. For example, in the 3 studies that had shown associations between *VDR* rs1544410, rs10735810, and BP, one was conducted among 933 Asian men and women lead workers,[14] one was conducted among 590 healthy white men and women in Spain,[15] and the latest was conducted among 1,211 US male physicians.[16] In addition to *VDR* rs1544410 and rs10735810, 3 other SNPs on vitamin D–related genes also showed associations with

Table 2. Association of expanded set of candidate single nucleotide polymorphisms with blood pressure phenotypes

				WGHS							ICBP
Index SNP	Chr	Position	Genes	A1/A2	A1F	Genotype	BP phenotype	Beta (SE)	*P* value[a]	Empirical *P* value[b]	*P* value
Nominal significant associations in the WGHS											
rs1507023	16	7528435	*RBFOX1*	G/A	0.13	Genotyped	SBP	0.55 (0.28)	0.05	0.90	0.14
							PP	0.43 (0.16)	0.0078	0.31	
rs2296241	20	52219626	*CYP24A1*	G/A	0.47	Imputed	SBP	0.42 (0.19)	0.023	0.67	0.08
							DBP	0.28 (0.12)	0.024	0.68	0.11
							MAP	0.32 (0.14)	0.022	0.64	
Nominal significant associations in the ICBP											
rs2853564	12	46564753	*VDR*	G/A	0.40	Genotyped	SBP	−0.069 (0.19)	0.72	1.00	0.045
rs1507023	16	7528435	*RBFOX1*	G/A	0.13	Genotyped	DBP	0.23 (0.18)	0.21	1.00	0.04
rs9937918	16	56159292	*GPR114*	A/G	0.27	Genotyped	SBP	0.22 (0.21)	0.30	1.00	0.02
							DBP	0.12 (0.14)	0.40	1.00	0.049
rs6013897	20	52175886	*CYP24A1*	A/T	0.21	Imputed	SBP	0.023 (0.23)	0.92	1.00	0.045
							DBP	−0.0081 (0.15)	0.96	1.00	0.02

Candidate single nucleotide polymorphisms (SNPs) included those that have previously shown significant associations with blood pressure (BP)–related outcomes, vitamin D metabolism, or vitamin D receptor signaling. Table only shows SNPs that had significant association with any BP phenotype at nominal $P < 0.05$ in the Women's Genome Health Study (WGHS) or International Consortium of Blood Pressure (ICBP). In the WGHS, analysis was adjusted for age at randomization and population stratification; data presented are effect size beta (SE) in millimeters of mercury per coded allele; all imputation $r^2 > 0.80$. In the ICBP, *P* for SNP presented are genome-wide meta-analysis *P* values after correction for genomic control.

Abbreviations: A1, coded allele; A2, noncoded allele; A1F, coded allele frequency; Chr, chromosome; DBP, diastolic blood pressure; MAP, mean arterial pressure; NA, not available; PP, pulse pressure; SBP, systolic blood pressure.

[a]Nominal *P* value.

[b]Empirical *P* value after correction for multiple testing.

Table 3. Association of vitamin D pathway genes with blood pressure observed in the Women's Genome Health Study

				WGHS							ICBP
Gene	Chr.	rs No.	Position	Genotype	A1/A2	A1F	BP phenotype	Beta (SE)	*P* value[a]	Empirical *P* value[b]	*P* value
RXRA	9	rs875444	136435125	Genotyped	G/A	0.41	PP	−0.44 (0.11)	0.00007	0.002	
DHCR7	11	rs1790370	70802569	Imputed	A/G	0.18	DBP	−0.51 (0.15)	0.001	0.02	0.77
VDR	12	rs2544037	46501447	Genotyped	G/A	0.42	DBP	−0.44 (0.12)	0.0003	0.02	0.13
							MAP	−0.48 (0.14)	0.0008	0.04	
NCOR2	12	rs1243733	123522505	Imputed	T/C	0.095	SBP	1.16 (0.32)	0.0003	0.02	0.22
							MAP	0.95 (0.24)	0.00007	0.007	
		rs1147289	123536019	Genotyped	A/G	0.14	DBP	0.67 (0.18)	0.0001	0.01	0.20
NCOA3	20	rs2235734	45725556	Genotyped	C/A	0.35	SBP	−0.64 (0.19)	0.001	0.03	0.24

Table shows the best single nucleotide polymorphisms (SNPs) in each gene that had association with blood pressure (BP) phenotypes in the Women's Genome Health Study (WGHS) at empirical *P* value <0.05 by using gene-based permutation and their replication in the International Consortium of Blood Pressure (ICBP). In the WGHS, analysis was adjusted for age at randomization and population stratification; data presented are effect size beta (SE) in millimeters of mercury per coded allele; all imputation $r^2 > 0.80$. In the ICBP, *P* for SNP presented are genome-wide meta-analysis *P* values after correction for genomic control.

Abbreviations: A1, coded allele; A2, noncoded allele; A1F, coded allele frequency; Chr., chromosome; DBP, diastolic BP; MAP, mean arterial pressure; PP, pulse pressure; SBP, systolic BP.

[a]Nominal *P* value.

[b]Empirical *P* value after correction for multiple testing by using gene-based permutation.

Table 4. Association of vitamin D pathway genes with blood pressure observed in the International Consortium of Blood Pressure

		ICBP					WGHS				
Gene	Chr.	BP phenotype	Gene-based *P* value [a]	Best SNP	Position	*P* value for SNP	Genotype	A1/A2	A1F	Beta (SE)	*P* value[b]
NCOR1	17										
		DBP	0.017	rs2157990	15902718	0.005	Imputed	T/A	0.43	−0.17 (0.19)	0.36
TP53BP1	15										
		SBP	0.018	rs12899865	41528309	0.0004	Imputed	A/G	0.18	−0.027 (0.24)	0.91
		DBP	0.031	rs16957715	41496029	0.005	Imputed	G/T	0.19	−0.027 (0.23)	0.91
TYRP1	9										
		SBP	0.00083	rs10960738	12638831	9.87×10^{-5}	Imputed	C/A	0.31	0.11 (0.20)	0.59

Table shows the genes that had association with systolic blood pressure (SBP) or diastolic blood pressure (DBP) in the International Consortium of Blood Pressure (ICBP) at gene-based *P* value <0.05 and their replication in the Women's Genome Health Study (WGHS). In the ICBP, *P* for single nucleotide polymorphisms (SNPs) presented are genome-wide meta-analysis *P* values after correction for genomic control. In the WGHS, analysis was adjusted for age at randomization and population stratification; data presented are effect size beta (SE) in millimeters of mercury per coded allele; all imputation $r^2 > 0.80$.

Abbreviations: A1, coded allele; A2, noncoded allele; A1F, coded allele frequency; BP, blood pressure; Chr., chromosome.

[a]Corrections for multiple testing were performed by using a versatile gene-based association study.

[b]Nominal *P* value.

BP-related traits in prior studies, including rs1746782 in *GC* with percentage of fat mass[33] and rs1156882 in *VDR* and rs1278587 in *DHCR7* with index of insulin resistance or obesity.[32] Associations of these SNPs with BP were not directly examined in previous studies. In our study, these SNPs were not associated with BP in either the WGHS or the ICBP.

On the other hand, our expanded candidate SNP analysis found suggestive evidence for associations of rs2853564 in *VDR*, rs6013897 and rs2296241 in *CYP24A1* (encoding vitamin D 24-hydroxylase, the major enzyme of $1,25(OH)_2$-vitamin D degradation), rs1507023 in *RBFOX1*, and rs9937918 in *GPR114* (both GWAS-discovered determinants of circulating 25(OH)-vitamin D) with BP traits. In candidate gene analysis, we found marginally significant associations for *VDR* along with *NCOA3*, *NCOR2*, *NCOR1*, *RXRA* (encoding nuclear receptor coactivator 3, corepressor 2, corepressor 1, and retinoid X receptor alpha, respectively, which all interact with *VDR*), *DHCR7, TP53BP1*, and *TYRP1* (encoding 7-dehydrocholesterol reductase, tumor suppressor p53-binding protein 1, and tyrosinase-related protein 1, respectively, which all modify vitamin D synthesis). However, only the association of *TYRP1* with SBP found in the ICBP reached significance thresholds after ultimate multiple testing correction. Furthermore, none of the associations observed in one study was replicated in the other study. Supplemental analyses provided no direct support to functional effect of the observed associations on BP regulation. Future studies will be needed to further explore whether the expression quantitative trait loci and enriched pathways identified in our analyses represent novel biological mechanisms underlying the association between vitamin D and BP.

Strengths of this study include its comprehensive analysis approach and an efficient use of multiple data resources, including one of the largest samples with individual-level GWAS data along with by far the largest international consortium on BP phenotype. One limitation of this study is that the genotyped and imputed SNPs may not cover all variations (e.g., rare variants) on the entire gene region. We have plans for future analyses using 1,000 genome imputed data or exome data when they become available. Second, many SNPs that had moderate associations with BP may not reach the predetermined significance threshold because of the stringency of Bonferroni correction for multiple comparisons. Third, some prior studies including the ICBP did not report beta coefficients for associations with BP. This limited scope of publicly available data restricted our ability to construct a weighted genetic risk score and apply the same analytic approach in the ICBP and WGHS. Fourth, the use of self-reported BP in categories as phenotype in the WGHS would presumably limit our power to detect weak effects and replicate the findings from the ICBP and therefore may underestimate the strength of existing associations. Finally, participants of our study were of European descent; the findings from this study are not generalizable to other ethnic populations.

To date, at least 6 GWASs of BP have undertaken a comprehensive scan in individuals of European ancestry and identified several susceptibility loci across the genome.[22,35–39] None of these detected regions harbor vitamin D pathway genes that were evaluated in our study. Although the effects of individual SNPs in the vitamin D pathway are possibly weak, the consistent associations between vitamin D status and BP and risk of hypertension warrant a closer investigation into whether combined effect of multiple SNPs in 1 gene or all genes in the entire pathway may contribute to the observed associations. Our study specifically evaluated genes in the expanded vitamin D pathway but found no strong or consistent associations with BP. For the SNPs that showed suggestive evidence for associations, the associations did not reach the significance threshold or were not replicated. In addition, the estimated effect size is moderate and may not

be clinically relevant. These findings suggest that common genetic variation in the vitamin D pathway may not substantially influence the association between vitamin D and BP. However, it remains to be seen whether gene–gene or gene–environment interactions play a more prominent role in the link between vitamin D and BP.

SUPPLEMENTARY MATERIAL

Supplementary materials are available at *American Journal of Hypertension* (http://ajh.oxfordjournals.org).

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DISCLOSURE

The authors declared no conflict of interest.

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