Clock Genes Explain a Large Proportion of Phenotypic Variance in Systolic Blood Pressure and This Control Is Not Modified by Environmental Temperature

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BACKGROUND

Diurnal variation in blood pressure (BP) is regulated, in part, by an endogenous circadian clock; however, few human studies have identified associations between clock genes and BP. Accounting for environmental temperature may be necessary to correct for seasonal bias.

METHODS

We examined whether environmental temperature on the day of participants' assessment was associated with BP, using adjusted linear regression models in the Genetics of Lipid Lowering Drugs and Diet Network (GOLDN) ($n = 819$) and the Boston Puerto Rican Health Study (BPRHS) ($n = 1,248$) cohorts. We estimated phenotypic variance in BP by 18 clock genes and examined individual single-nucleotide polymorphism (SNP) associations with BP using an additive genetic model, with further consideration of environmental temperature.

RESULTS

In GOLDN, each additional 1 °C increase in environmental temperature was associated with 0.18 mm Hg lower systolic BP [SBP; $\beta \pm SE = -0.18 \pm 0.05$ mm Hg; $P = 0.0001$] and 0.10 mm Hg lower diastolic BP [DBP; -0.10 ± 0.03 mm Hg; $P = 0.001$]. Similar results were seen in the BPRHS for SBP only. Clock genes explained a statistically significant

proportion of the variance in SBP [$V_G/V_P \pm SE = 0.071 \pm 0.03$; $P = 0.001$] in GOLDN, but not in the BPRHS, and we did not observe associations between individual SNPs and BP. Environmental temperature did not influence the identified genetic associations.

CONCLUSIONS

We identified clock genes that explained a statistically significant proportion of the phenotypic variance in SBP, supporting the importance of the circadian pathway underlying cardiac physiology. Although temperature was associated with BP, it did not affect results with genetic markers in either study. Therefore, it does not appear that temperature measures are necessary for interpreting associations between clock genes and BP.

CLINICAL TRIAL REGISTRATION

Trials related to this study were registered at clinicaltrials.gov as NCT00083369 (Genetic and Environmental Determinants of Triglycerides) and NCT01231958 (Boston Puerto Rican Health Study).

Keywords: blood pressure; circadian clocks; circadian rhythm; clock genes; environmental temperature; hypertension.

doi:10.1093/ajh/hpv082

Blood pressure (BP), heart rate, and other cardiovascular traits show daily oscillations with large daytime and nighttime differences.^{1,2} In humans, BP is generally

lower at night and tends to increase throughout the day.³ Studies suggest that disruptions to daily oscillations in BP may contribute to cardiovascular pathology.⁴ Indeed,

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Initially submitted February 24, 2015; date of first revision April 17, 2015; accepted for publication May 13, 2015; online publication June 4, 2015.

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hypertensive patients with <10% in BP diurnal variation, referred to as non-dippers, are more susceptible to heart and kidney damage and have greater risk for heart failure and stroke.⁴ Shift workers are more likely than others to be non-dippers and are at an elevated risk of coronary heart diseases.^{5,6} Understanding the molecular mechanisms regulating the rhythms in cardiovascular traits is critical to understanding cardiovascular disease resulting from disruptions in rhythmicity.

Diurnal variations in cardiovascular traits are regulated, in part, by an endogenous circadian system that is composed of a central clock in the suprachiasmatic nucleus, and in peripheral clocks in organs such as the heart and kidney.^{7–12} The molecular regulation of the circadian rhythms involves a transcription–translation negative feedback loop composed of clock genes including *CLOCK*, *BMAL1*, *PER(s)*, and *CRY(s)*, regulating downstream clock controlled genes, including up to 10% of genes in the heart.¹¹ Genetic disruptions to the circadian clock have been shown to impact the rhythmicity of cardiovascular traits in experimental animal studies. For example, *Bmal1* deletion in rats abrogated BP rhythmicity through a decrease in catecholamine production,¹² and *Period2* mutant mice have attenuated fluctuation in both BP and heart rate.¹³ In addition, *Clock*, *Per1*, and *Dbp/Tef/Hlf* knockouts in mice show independent associations with lower BPs compared to wild-type mice, possibly as a result of perturbed kidney function which may include altered sodium excretion and reabsorption,^{14–16} whereas *Cry*-null mice exhibit salt-sensitive hypertension.¹⁷ Although animal studies suggest the role of the molecular clock in normal cardiac physiology, evidence remains weak in humans. Genome-wide association studies have not identified clock genes as significant loci associated with BP, heart rate, or other pertinent traits, and few other candidate gene studies have identified associations between clock genes and these traits.^{18–21} As the molecular clock operates as a system, it is possible that an additive genetic effect (i.e., the sum of small genetic contributions of genetic variants rather than individual variants) exists among clock genes variants in explaining the phenotypic variance in cardiovascular traits.

In addition to daily oscillations, cardiac traits also show seasonal changes.^{22,23} There is a general tendency for BP to be higher in winter and lower in summer.²⁴ Seasonal fluctuations in environmental “outdoor” temperature have been strongly implicated in the seasonality of cardiovascular disease mortality with a complex, multifactorial underlying mechanism.^{25–27} Although the diurnal variation in cardiac traits is independent of season,³ accounting for environmental temperature may be necessary to correct for seasonal bias.

In the present study, we hypothesize that both environmental temperature and clock genes independently influence systolic BP (SBP) and diastolic BP (DBP), and that the associations between clock genes and BP are influenced by seasonal changes in environmental temperature. To test this hypothesis, we (i) assessed the association between environmental temperature and BP, (ii) examined whether clock gene variants, first collectively and then separately, explain interindividual variability in BP in humans, and (iii) investigated the role of environmental temperature in modifying the genetic findings.

METHODS

Genetics of Lipid Lowering Drugs and Diet Network population (discovery cohort)

Analyses were conducted using data from the multi-sited family-based Genetics of Lipid Lowering Drugs and Diet Network (GOLDN) study, described in detail in previous publications.^{28,29} Briefly, the study screened 1,327 European American (Caucasian) individuals (aged 18–83 years) from extended pedigrees from 2 sites of the National Heart, Lung, and Blood Institute Family Heart Study in Minneapolis, MN and Salt Lake City, UT. DNA samples used in this study were collected at baseline, while cardiovascular, anthropometric, and other covariates were collected at visit 2. A total of 819 participants with genotype information and other pertinent data were included in the current analysis. Participants provided written informed consent and the institutional review boards at the following institutions approved the study protocol: University of Alabama at Birmingham, University of Minnesota, Tufts University, University of Utah, Washington University in St. Louis, University of Texas, University of Michigan, and Fairview-University of Minnesota Medical Center.

Boston Puerto Rican Health Study population (replication cohort)

Replication analyses were conducted in 1,248 unrelated men and women of Puerto Rican origin in the Boston Puerto Rican Health Study (BPRHS). Participants were aged 45–74 years at baseline and living in the Boston, Massachusetts, metropolitan area.³⁰ Participation eligibility was defined by self-reported Puerto Rican origin, Boston area residence, and the ability to answer interview questions in Spanish or English. Ancestral admixture was estimated using 100 ancestral informative markers and this was included as a covariate in all analyses. The mean ancestral admixtures of BPRHS participants were 57.2% European, 27.4% African, and 15.4% Native American.³¹ Baseline demographic information, medical history, and dietary data were collected between 2004 and 2008 by trained bilingual staff. The institutional review board at Tufts University/New England Medical Center approved the protocol of the current study.

Seasonal variation, anthropometry, and BP

Environmental temperature was estimated in GOLDN by using the average outdoor temperature on the day of participants' visit 2, which ranged from January 2003 to December 2005, and was expressed continuously. Daylight duration was estimated by the difference between sunrise and sunset on the day of the participants' visit 2, and was also expressed continuously. Anthropometric traits and BP were collected between 7:00 am and 11:00 am and height and weight were measured using standard methods. Body mass index (BMI) was calculated as weight (kg) divided by the square of height (m²). Meanwhile, resting BP (SBP and DBP) were estimated

DBP were estimated while seated using standard methods. The average of 2 BP measurements was used in the analysis. Similar methodologies were used to estimate environmental temperature and daylight duration in our replication cohort (BPRHS). Anthropometric data, including height and weight and BP levels, measured in duplicate were consistent with the techniques used by the National Health and Nutrition Surveys.^{32,33}

Genotyping and single-nucleotide polymorphisms selection

In GOLDN, DNA was obtained from blood samples with Gentra Puregene blood kits (Gentra Systems Minneapolis, MN). We used previously described data²⁹ on genome-wide sequence variation as ascertained by the Affymetrix Human Single Nucleotide Polymorphism Array 6.0 and the Birdseed calling algorithm. We imputed untyped single-nucleotide polymorphisms (SNPs) using MACH software (version 1.0.16, Ann Arbor, MI) with Human Genome Build 36 as a reference. After imputation, we created a hybrid dataset of 2,543,887 SNPs, of which 584,029 were originally genotyped.

The following 18 clock genes were included in the present investigation: *BMAL1/2*, *CLOCK*, *CRY1/2*, *DBP*, *DEC1/2*, *NPAS2*, *NR1D1/2*, *PER1–3*, *PPARA*, *PPARG*, *RORA*, and *RORB*. First, a total of 5,004 SNPs were selected from GOLDN genotyped or imputed SNPs located in a region that spans from 100 kb upstream to 100 kb downstream of the above-listed clock genes. Only SNPs with call rate $\geq 96\%$, minor allele frequency $\geq 5\%$, Hardy–Weinberg equilibrium test $P \geq 10^{-6}$, negligible Mendelian error within 1 family, and imputation quality $r^2 \geq 0.3$ (for imputed SNPs) were included in the present analysis. Second, a total of 538 tagging SNPs (tSNPs) that capture variation of the described clock genes were selected by using the web-based service of the “Tagger” option (aggressive tagging approach) within the Haploview software (version 4.2) with parameters based upon linkage disequilibrium in the GOLDN study with an $r^2 \geq 0.80$.

In the BPRHS, DNA was obtained with QIAamp DNA Blood Mini Kits (Qiagen Hilden, Germany) and genome-wide genotyping was performed using Illumina HumanOmni1-Quad genome-wide association study arrays. A total of 4,915 clock genes SNPs were genotyped or imputed and included in the present analysis.

Statistical analyses

In GOLDN, associations between environmental temperature with both SBP and DBP were assessed with multiple linear regression models adjusted for age, sex, BMI, antihypertensive medication use, and study site (model 1); then further adjusted for lifestyle factors: diabetes status, lipid-lowering medication use, smoking status, sodium intake, drinking status, education, and physical activity (model 2); or daylight duration (model 3) to account for additional sources of seasonal bias. In addition, we assessed for effect modification by hypertension status or age group (comparing 1st vs. 4th quartiles of age) by including an interaction term in the adjusted linear regression models. We also examined differences in general characteristics, anthropometry,

and intakes across seasons (Spring: March to May; Summer: June to August; Fall: September to November; Winter: December to February) and visit times (7:00–11:00 am) using one-way analysis of variance.

Next, we implemented the Genome-wide Complex Trait Analysis (GCTA) tool to estimate total variance explained by clock genes variants (N SNPs = 5,004)³⁴ and adjusted for age, sex, BMI, antihypertensive medication use, study site, and population structure (model 1). In addition, we further adjusted for environmental temperature (model 2). Population structure was estimated based on the principal component analysis using SVS (Golden Helix, Bozeman, MT) and 2 principal components were selected as covariates in the analysis in GOLDN.³⁵

Individual tSNP associations with SBP in GOLDN were assessed using an additive genetic model and linear mixed models. The primary model adjusted for age, sex, BMI, antihypertensive medication use, and study site as fixed effects, and family as a random effect through the kinship matrix in R (GWAF package) (model 1), the secondary model further adjusted for the aforementioned lifestyle factors (model 2), and the final model adjusted for environmental temperature in addition to primary covariates (model 3). Furthermore, we conducted sensitivity analyses by excluding individuals with antihypertensive medication use for all the described analyses. All statistical analyses were performed using R statistical software (v. 3.1.0, Vienna, Austria). A prespecified Bonferroni-corrected significance level of ($=0.05/538$ tSNPs) $9.29\text{E-}05$ was used to account for multiple comparisons. The analyses described above were repeated in the BPRHS for the purpose of replication. To account for population admixture in this population, all genetic analyses adjusted for the major ancestry principal component.³¹

RESULTS

General characteristics

The general characteristics of the GOLDN ($n = 821$) and BPRHS ($n = 1,248$) cohorts are presented in [Table 1](#). The prevalence of hypertension was 25.5% in GOLDN with BP means of 115.7 and 68.4 mm Hg for SBP and DBP, respectively. The sampling period in GOLDN ranged from January 2003 to December 2005 and, as such, environmental temperature ranged between -24.4 and 28.9 °C, with a mean of 9.8 °C, and daylight duration ranged between 8.8 and 15.6 hours, with a mean of 12.3 hours. Significant differences in age, SBP, daylight duration, and environmental temperature were observed when comparing individuals assessed at different seasons ($P < 0.05$) ([Supplementary Table 1](#)), and in age, sex, and environmental temperature, but not in BP, when comparing individuals assessed at different times of day ($P < 0.05$) (results not shown).

In BPRHS, the prevalence of hypertension and antihypertensive medication use was 68.0% and 54.2%, respectively, and BP means were 135.6 and 81.0 mm Hg for SBP and DBP, respectively. Sampling period in BPRHS ranged from May 2004 to June 2008 and environmental temperature ranged between -14.4 and 31.1 °C, with a mean of 12.6 °C, and daylight duration ranged between 9.1 and 15.3 hours and had a mean of 12.6 hours.

Table 1. General characteristics of GOLDN (discovery cohort) and BPRHS (replication cohort) participants

Characteristics	GOLDN (n = 819)	BPRHS (n = 1,248)
<i>General characteristics</i>		
Age (years)	48.3 ± 15.9	57.2 ± 7.6
Sex (% female)	50.3	70.4
BMI (kg/m ²)	28.5 ± 5.5	31.9 ± 6.7
Current drinkers, n (%)	407 (49.7)	479 (38.4)
Current smokers, n (%)	67 (8.2)	372 (29.8)
Current antihypertensive Med., n (%)	149 (18.1)	677 (54.2)
<i>Dietary intake</i>		
Total energy (kcal/day)	2136 ± 1262	2315 ± 1228
Sodium (mg)	3330 ± 1876	5101 ± 2876
Potassium (mg)	3180 ± 1773	3035 ± 1643
<i>Cardiovascular traits</i>		
Hypertension, n (%)	209 (25.5)	849 (68.0)
Systolic blood pressure (mm Hg)	115.7 ± 16.3	135.6 ± 19.1
Diastolic blood pressure (mm Hg)	68.4 ± 9.4	81.0 ± 10.7
<i>Seasonal variation</i>		
Temperature, °C	9.8 ± 10.5	12.6 ± 9.4
Daylight duration, hours	12.4 ± 2.1	12.6 ± 2.1

Abbreviations: BPRHS, Boston Puerto Rican Health Study; GOLDN, Genetics of Lipid Lowering Drugs and Diet Network; Med, medication; n, number of observations.

Environmental temperature and BP associations

Environmental temperature was associated with BP in GOLDN (Table 2, Figure 1, Supplementary Table 2): each additional 1 °C increase in temperature was associated with 0.18 mm Hg lower SBP [$\beta \pm SE = -0.18 \pm 0.05$ mm Hg; $P = 0.0001$] after adjusting for age, sex, BMI, antihypertensive medication use, and study site. Further adjustment for life-style factors did not affect the association. In addition, adjustment for daylight duration ($r^2 = 0.77$, daylight duration, and environmental temperature) attenuated the association [$\beta \pm SE = -0.16 \pm 0.08$ mm Hg; $P = 0.03$], and daylight duration was not a significant predictor of SBP in the model. The association between environmental temperature and SBP was modified by hypertension status ($P_{\text{interaction}} = 0.04$): the association was of larger magnitude among hypertensive individuals [normotensive: $\beta \pm SE = -0.14 \pm 0.05$ mm Hg; $P = 0.003$; hypertensive: $\beta \pm SE = -0.35 \pm 0.12$ mm Hg; $P = 0.003$] (Figure 1).

For DBP, each additional 1 °C increase in temperature was associated with a 0.10 mm Hg lower DBP [$\beta \pm SE = -0.10 \pm 0.03$ mm Hg; $P = 0.001$]. Further adjustment by the aforementioned lifestyle factors did not affect the identified associations; meanwhile, further adjustment for daylight duration attenuated the identified association [$\beta \pm SE = -0.06 \pm 0.05$ mm Hg; $P = 0.22$]. The association was not modified by hypertension status ($P_{\text{interaction}} = 0.37$): [normotensive: $\beta \pm SE = -0.08 \pm 0.03$ mm Hg; $P = 0.02$; hypertensive: $\beta \pm SE = -0.13 \pm 0.06$ mm Hg; $P = 0.04$].

In the BPRHS, an association between environmental temperature and BP was seen for SBP, but not for DBP (Table 2): each additional 1 °C increase in temperature was associated with 0.23 mm Hg lower SBP [$\beta \pm SE = -0.23 \pm 0.06$ mm Hg; $P = 0.00004$] after adjusting for age, sex, BMI, antihypertensive medication use, and study site, and further adjustment

Table 2. Associations between environmental temperature and BP in 2 independent populations^a

	GOLDN (n = 819)				BPRHS (n = 1,248)			
	SBP, mm Hg		DBP, mm Hg		SBP, mm Hg		DBP, mm Hg	
	$\beta \pm SE$	P value	$\beta \pm SE$	P value	$\beta \pm SE$	P value	$\beta \pm SE$	P value
Model 1 ^b								
All	-0.18 ± 0.05	0.0001 ^e	-0.10 ± 0.03	0.001 ^f	-0.23 ± 0.06	0.00004	-0.04 ± 0.03	0.20
Normotensive	-0.14 ± 0.05	0.003	-0.08 ± 0.03	0.02	-0.11 ± 0.06	0.06	-0.01 ± 0.04	0.79
Hypertensive	-0.35 ± 0.12	0.003	-0.13 ± 0.06	0.04	-0.24 ± 0.07	0.0003	-0.04 ± 0.04	0.28
Model 2 ^c								
All	-0.09 ± 0.03	0.002	-0.09 ± 0.03	0.002	-0.24 ± 0.06	0.00002	-0.06 ± 0.03	0.08
Model 3 ^d								
All	-0.16 ± 0.08	0.03	-0.06 ± 0.05	0.22	-0.16 ± 0.08	0.04	-0.02 ± 0.05	0.59

Abbreviations: BPRHS, Boston Puerto Rican Health Study; DBP, diastolic blood pressure; GOLDN, Genetics of Lipid Lowering Drugs and Diet Network; SBP, systolic blood pressure.

^aAssociation coefficients are shown as $\beta \pm SE$. β represents the change in SBP or DBP (mm Hg) per each additional 1 °C increase in temperature. ^bModel 1: adjusted for age, sex, BMI, antihypertensive medication use, and study site (GOLDN only). ^cModel 2: adjusted for model 1 covariates and lifestyle factors: diabetes status, lipid-lowering medication use, smoking status, sodium intake, drinking status, education, and physical activity. ^dModel 3: adjusted for model 1 covariates and daylight duration. ^eEffect modification by hypertension status: $P_{\text{interaction}} = 0.04$. ^fEffect modification by hypertension status: $P_{\text{interaction}} = 0.37$.

by lifestyle factors did not influence the identified associations. Sensitivity analyses by the removal of individuals with antihypertensive medication use did not substantially influence these results (Supplementary Table 3).

Phenotypic variance in BP explained by clock genes

We used the GCTA tool to assess how much, if any, of the phenotypic variance in SBP and DBP in GOLDN can be explained by clock genes (V_G/V_P). Collectively, the clock genes variants (N SNPs = 5,004) explained 7.1% of the variance in SBP [$V_G/V_P \pm SE = 0.071 \pm 0.03$; $P = 0.001$], following adjustments for age, sex, BMI, antihypertensive medication use, study site, and population structure (Table 3). Further adjustment for environmental temperature slightly altered the estimated variance [$V_G/V_P \pm SE = 0.069 \pm 0.03$; $P = 0.001$]. Clock genes SNPs did not explain significant variance in DBP [$V_G/V_P \pm SE = 0.02 \pm 0.02$; $P = 0.20$], and further adjustment for environmental temperature did not alter the estimated variance. Moreover, 14 independent clock tSNPs were associated with SBP after adjusting for age, sex, BMI, antihypertensive medication use, study site, and population structure at $P \leq$

0.01, although these associations were not significant following adjustments for multiple comparisons (Table 4). Further adjustment for lifestyle factors or environmental temperature did not influence the tSNP association results with SBP (results not shown). Results from the GCTA analysis and individual tSNP associations were not significant in our replication BPRHS cohort (Tables 3 and 4). However, sensitivity analyses altered results from the GCTA analysis in the BPRHS [$V_G/V_P \pm SE = 0.018 \pm 0.04$; $P = 0.30$] (Supplementary Tables 4 and 5), but not GOLDN (results now shown).

DISCUSSION

In the present analysis, we showed that environmental temperature was associated with SBP in 2 independent populations, GOLDN and BPRHS cohorts, and with DBP in GOLDN. Furthermore, the association with SBP was independent of daylight duration and modified by hypertension status. In addition, we showed that clock genes explained a statistically significant proportion of the phenotypic variance in SBP, but not DBP in a non-Hispanic White population. Environmental temperature did not

Figure 1. Variation in systolic blood pressure (SBP, mm Hg) by day of the year in all participants ($n = 819$) and stratified by hypertension status [normotensive: $n = 610$ (dashed line); hypertensive: $n = 209$ (dotted line)] (A) based on the actual Genetics of Lipid Lowering Drugs and Diet Network (GOLDN) participants' data; (B) based on predicted annual changes in environmental temperature from prediction model adjusted for age, sex, BMI, antihypertensive medication, and study site.

Table 3. Genome-Wide Complex Trait Analysis (GCTA) of systolic and diastolic BP in 2 independent populations^a

	GOLDN ($n = 819$)				BPRHS ($n = 1,248$)			
	SBP, mm Hg		DBP, mm Hg		SBP, mm Hg		DBP, mm Hg	
	$V_G/V_P \pm SE$	P value	$V_G/V_P \pm SE$	P value	$V_G/V_P \pm SE$	P value	$V_G/V_P \pm SE$	P value
Model 1 ^b	0.071 ± 0.03	0.001	0.020 ± 0.02	0.20	0.000001 ± 0.02	0.50	0.001 ± 0.02	0.50
Model 2 ^c	0.069 ± 0.03	0.001	0.020 ± 0.02	0.20	0.000001 ± 0.02	0.50	0.002 ± 0.02	0.50

^aResults are shown as V_G/V_P (phenotypic variance explained by clock genes) and SE. ^bModel 1: adjusted for age, sex, BMI, antihypertensive medication use, study site (GOLDN only), and population structure. ^cModel 2: adjusted for model 1 covariates and environmental temperature.

Table 4. Associations ($P \leq 0.01$) between clock genes tSNPs and SBP (mm Hg) in 2 independent populations^a

Marker name	Chr	Physical location	Nearest clock gene	Allele (minor/major)	GOLDN ($n=819$)			BPRHS ($n=1,248$)		
					MAF	$\beta \pm SE$	P value	MAF	$\beta \pm SE$	P value
rs8109054	19	53774077	<i>DBP</i>	A/G	0.17	2.86 ± 0.95	0.002	0.23	0.54 ± 0.88	0.54
rs2343072	12	26160314	<i>DEC2</i>	T/A	0.18	-2.76 ± 0.97	0.004	0.12	1.73 ± 1.10	0.12
rs10743574	12	26207396	<i>DEC2</i>	G/A	0.28	-2.27 ± 0.81	0.005	0.20	0.25 ± 0.92	0.79
rs2291140	12	26255530	<i>DEC2</i>	C/T	0.20	-2.58 ± 0.92	0.005	0.20	-2.17 ± 0.95	0.02
rs2343870	12	26245766	<i>DEC2</i>	C/G	0.19	-2.62 ± 0.94	0.006	0.08	0.63 ± 1.35	0.64
rs299657	3	12526491	<i>PPARG</i>	G/C	0.50	-1.98 ± 0.74	0.007	0.44	-0.06 ± 0.72	0.93
rs2596829	3	12538639	<i>PPARG</i>	T/C	0.43	1.95 ± 0.74	0.008	0.35	0.30 ± 0.77	0.69
rs9999653	4	56193006	<i>CLOCK</i>	C/T	0.40	-1.91 ± 0.72	0.008	0.54	-0.05 ± 0.73	0.95
rs3027215	17	7959231	<i>PER1</i>	T/C	0.09	3.48 ± 1.33	0.009	0.16	0.02 ± 1.03	0.98
rs7957130	12	106060295	<i>CRY1</i>	G/A	0.41	1.98 ± 0.75	0.009	0.44	0.47 ± 0.72	0.51
rs3760808	19	53769479	<i>DBP</i>	T/C	0.31	2.06 ± 0.78	0.009	0.32	-0.82 ± 0.78	0.30
rs16931815	12	27319184	<i>DEC2</i>	G/A	0.15	2.79 ± 1.08	0.01	0.06	1.54 ± 1.57	0.33
rs2453021	1	7912153	<i>PER3</i>	T/C	0.33	-2.02 ± 0.78	0.01	0.31	-0.84 ± 0.79	0.29
rs16943453	15	59074290	<i>RORA</i>	G/T	0.16	-2.50 ± 0.98	0.01	0.18	-0.48 ± 0.92	0.60

Abbreviations: BPRHS, Boston Puerto Rican Health Study; Chr, chromosome; GOLDN, Genetics of Lipid Lowering Drugs and Diet Network; MAF, minor allele frequency; tSNPs, tagging single-nucleotide polymorphisms.

^aAssociation coefficients are shown as $\beta \pm SE$. β Represents the change in systolic blood pressure (mm Hg) per each additional copy of the minor allele. Analyses adjusted for age, sex, BMI, antihypertensive medication use, study site (GOLDN only), and population structure.

modify the identified genetic results. However, clock genes did not explain a statistically significant proportion of the phenotypic variance in BP levels in an ethnically diverse independent cohort of older Puerto Rican adults. Further, no individual clock genetic tSNPs showed significant association with BP.

As participant recruitment in both GOLDN and BPRHS cohorts were randomly distributed over a 3- and 4-year period, respectively, we were able to examine seasonal differences in BP by day-of-recruitment in cross-sectional analyses. Consistent with previous studies, we identified in these 2 independent populations that seasonal variation in environmental temperature was significantly associated with BP, such that BP is lowest in the summer and highest in winter.³ Unlike the concentrations of 25(OH)D, which could also contribute to BP variability and is seasonally influenced by UV-B radiation,³⁶ the effect of seasonality on SBP seen here appeared to be independent of sunlight. The association between environmental temperature and SBP remained similar after adjusting for daylight duration, despite the high correlation between environmental temperature and daylight duration. The temperature-driven differences in BP may involve the entrainment of clocks in peripheral tissues.³⁷

The association between environmental temperature and SBP was modified by hypertension status. The greater sensitivity of hypertensive individuals who are at an elevated risk for cardiovascular disease to changes in environmental temperature, as suggested by the magnitude difference in the association between environmental temperature and BP, could be of clinical importance, specifically related to the administration of antihypertensive drugs.^{38,39} While studies

have reported seasonal differences in self-reported dietary intake, BMI, and physical activity, all of which could contribute to seasonal variation in BP, we did not detect such differences in GOLDN, reducing the possibility of confounding by these factors.^{3,40}

Heritability studies of BP indicate that genetic factors contribute to a substantial proportion of the phenotypic variance, and is estimated by twin and family studies to range from 44% to 57% for SBP and 46% to 63% for DBP.⁴¹ In this study, we assessed the total contribution of 18 clock genes on BP variance. The total contribution of additive genetic effects tagged by genotyped and imputed SNPs together explained a statistically significant proportion of the variance in SBP, but not DBP in GOLDN, possibly due to differences in the underlying regulatory mechanisms. The significant findings for SBP suggest that clock genes function in concert and each variant contributes a small effect on SBP variance. This finding, however, was not seen in an independent population of older Puerto Ricans adults, which may be due to the higher prevalence of hypertension, differences in genetic architecture related to ancestry or chance. Although the removal of individuals with current antihypertensive medication use and investigating a subset of the BPRHS participants with a lower mean BP did alter the GCTA results, the results remained not significant. Furthermore, while the lack of statistically significant associations between individual clock tSNPs and BP in either cohort following adjustment for multiple comparisons suggests the importance of the cumulative contributions of clock genetic variants rather than individual variants on cardiovascular traits, an investigation in a larger cohort is warranted.

Recent findings also support the composite effects of clock genetic variants on cardiac outputs. Using a genetic risk score composed of 5 tSNPs (in *NPAS2*, *PER2*, *ROR β* , *BMAL1*, and *ROR α*), clock genes were shown to associate with the presence of non-dipper phenotype among young individuals with essential hypertension.⁴² Meanwhile associations between *CRY1* with arterial hypertension and both *CRY1* and *CRY2* with elevated BP were evident only in haplotype analyses factoring in multiple SNPs.²⁰ In addition, 3 *BMAL1* haplotypes were shown to associate with hypertension, suggesting that multiple variants in the *BMAL1* gene contribute to the disease risk.⁴³ As in our study, single SNP associations with the investigated outcomes in the described studies were not evident when accounting for multiple comparisons.^{20,42,43}

Our findings provide further support of the role of clock genes in cardiovascular circadian rhythms in humans, although the underlying mechanism is complex and has not been elucidated.^{44–46} The influence of clock genes on BP may be mediated directly by clock genes' influence on the circadian clock within the vasculature, heart, and kidney. The circadian clock in cardiomyocytes influences the rhythmicity in cardiac physiology, including heart rate and cardiac output and circadian alterations as a result of the clock genetic variants may affect the circadian clock in cardiomyocytes and alter downstream processes.⁴⁷ Alternatively, an indirect influence may be mediated through cardiovascular effectors regulated by the circadian system such as the autonomic nervous system,^{7,8} dopamine,⁴⁸ or melatonin.² In mice, deletion of *Bmal1* abrogated the circadian rhythm of BP in part because of reduced production of catecholamines,¹² for which a robust endogenous circadian rhythm exists in humans.⁸ Also, the hormone melatonin plays an important role in BP diurnal variations^{2,49,50}; however, its role in mediating the present findings cannot be inferred from this epidemiological investigation.

It is conceivable that clock genes may, in part, drive seasonal variation in BP. Genetic variants in core clock genes, *NPAS2*, *PER2*, and *BMAL1*, have been shown to associate with seasonal affective disorder and seasonal variation in behavior.^{51,52} One possible pathway is through the regulation of clock genes via epigenetic mechanisms including cold-induced RNA binding proteins or DNA methylation.^{53–55}

Study limitations should be taken into account. Despite our use of reliable sources for estimating environmental temperature, it is possible that environmental temperature was capturing a dimension of seasonality unaccounted for in our investigation. Therefore, a more accurate assessment method for temperature exposure such as personal-level environmental temperature (PET) may be required to identify participants' true temperature exposure. As our findings for clock genes in a European American cohort were not replicated in an independent population of different genetic ancestry, the role of clock genes in influencing SBP in humans of other ethnic backgrounds is unclear. In addition, as the current cross-sectional analysis cannot infer direct causality, further investigation is warranted to assess the mechanistic link between clock genes and cardiovascular traits.

In conclusion, in 2 independent and diverse population studies, we identified that environmental temperature

was associated with SBP, with a larger magnitude among hypertensive individuals. The effect of environmental temperature on BP is apparent despite well-controlled indoor temperatures common in modern lifestyle, which may have implications related to the administration of antihypertensive drugs. We also identified clock genes that explained a statistically significant proportion of the phenotypic variance in SBP in a non-Hispanic White population, but not in a cohort of older Puerto Rican adults. The inconsistent genetic findings may result from differences in the BP profiles between the investigated cohorts. As our genetic association investigations for BP were not clearly influenced by environmental temperature, it remains unclear that cross-sectional analyses of population-based genetic studies on BP must routinely factor in seasonal variation in environmental temperature. Previous studies also suggest that clock genes associated with hypertension may also show associations with other metabolic syndrome traits, therefore investigation with related metabolic syndrome traits is warranted.⁴³ In addition, further investigation of the link between the circadian clock and cardiovascular traits is needed to understand observed diurnal and seasonal variation in adverse cardiovascular events.^{4,7,10,25}

SUPPLEMENTARY MATERIAL

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

ACKNOWLEDGMENTS

We thank the staff of the GOLDN study for the assistance in data collection and management and Jin Sha for support. The work on the GOLDN study has been funded by the NIH National Heart, Lung, and Blood Institute (NHLBI) (grant U01HL072524-04), National Institute of Diabetes and Digestive and Kidney Diseases, (grant DK075030), and by contracts 53-K06-5-10 and 58-1950-9-001 from the US Department of Agriculture Research. The Boston Puerto Rican Health Study was supported by the National Institutes of Health (grants P01 AG023394 and P50 HL105185). C.E.S. was supported by K08 HL112845. F.A.J.L.S. was supported in part by NHLBI (R01 HL094806).

DISCLOSURE

The authors declared no conflict of interest.

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