Identification, Heritability, and Relation With Gene Expression of Novel DNA Methylation Loci for Blood Pressure

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Abstract—We conducted an epigenome-wide association study meta-analysis on blood pressure (BP) in 4820 individuals of European and African ancestry aged 14 to 69. Genome-wide DNA methylation data from peripheral leukocytes were obtained using the Infinium Human Methylation 450k BeadChip. The epigenome-wide association study meta-analysis identified 39 BP-related CpG sites with $P<1\times10^{-5}$. In silico replication in the CHARGE consortium of 17 010 individuals validated 16 of these CpG sites. Out of the 16 CpG sites, 13 showed novel association with BP. Conversely, out of the 126 CpG sites identified as being associated ($P<1\times10^{-7}$) with BP in the CHARGE consortium, 21 were replicated in the current study. Methylation levels of all the 34 CpG sites that were cross-validated by the current study and the CHARGE consortium were heritable and 6 showed association with gene expression. Furthermore, 9 CpG sites also showed association with BP with $P<0.05$ and consistent direction of the effect in the meta-analysis of the Finnish Twin Cohort (199 twin pairs and 4 singletons; 61% monozygous) and the Netherlands Twin Register (266 twin pairs and 62 singletons; 84% monozygous). Bivariate quantitative genetic modeling of the twin data showed that a majority of the phenotypic

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correlations between methylation levels of these CpG sites and BP could be explained by shared unique environmental rather than genetic factors, with 100% of the correlations of systolic BP with cg19693031 (*TXNIP*) and cg00716257 (*JDP2*) determined by environmental effects acting on both systolic BP and methylation levels. (***Hypertension*. 2020;76:195-205. DOI: 10.1161/HYPERTENSIONAHA.120.14973.)** ● **Data Supplement**

Key Words: blood pressure ■ DNA methylation ■ epigenome ■ hypertension ■ Twin study

Essential hypertension (EH) is a major health problem with global proportions. A report in the Lancet[1] estimated that in 2015 there were 1.13 billion people living with high blood pressure (BP) worldwide. While many pathways involved in the development of EH and corresponding treatment options have been discovered, the elevated BP of 16 million patients with hypertension remains uncontrolled,[2] indicating the need for further understanding of its pathogenesis. Epigenetics has recently been suggested as a massive regulatory machine that cannot be ignored in searching for the molecular understanding of EH.[3,4] In fact, it may explain the late onset, progressive and quantitative nature of this disease better than variations in DNA sequence. Epigenetic alterations of the genes of the renin-angiotensin-aldosterone system, a hormone system that is integral to the physiological regulation of BP, have been extensively tested in hypertensive animal models, providing one line of substantial evidence on the involvement of epigenetic regulation in the development of EH.[5] A recent, genome-wide, peripheral blood DNA methylation study in human by the CHARGE consortium,[6] including a discovery and a replication panel, identified 13 CpG sites in or next to 8 genes that were differentially methylated in relation to BP. A methylation risk score based on these 13 CpG sites explained 1.4% and 2.0% of the interindividual variation in systolic BP (SBP) and diastolic BP (DBP), respectively. Expanding the methylation risk score to include 126 CpG sites that were Bonferroni significant ($P<1\times10^{-7}$) in the overall meta-analysis did not explain additional phenotypic variance, indicating the need for further replication. Moreover, unlike sequence variation, epigenetic variation is influenced both by inherited and environmental factors.[7,8] This is illustrated by the fact that 30% to 100% of the DNA methylation levels of the 13 BP-associated CpG sites described above is explained by heritable factors as estimated by the family data of the Framingham Heart Study.[6] However, the extent to which the link between BP and DNA methylation signatures is driven by inherited versus environmental factors has not been investigated.

In the present meta-EWAS in leukocytes of 4820 individuals of European (EA) and African ancestry (AA) aged 14 to 69, we first identified new DNA methylation signals associated with BP and validated these signals in the CHARGE consortium[6]; next we attempted to replicate the 126 previously identified signals by the CHARGE consortium[6] in our own meta-EWAS data; third, we conducted twin modeling to estimate the heritability of DNA methylation correlated with BP, and finally we assessed the genetic and environmental sources of the correlation between DNA methylation and BP (Figure S1).

Methods

Data Availability

This study involves multiple cohorts. The genome wide DNA methylation data that support the findings of this study are available from the study PI of each cohort upon reasonable request and with permission of the Institutional Review Board of the universities where the participating cohort locates.

Study Populations

The discovery panel included 4820 individuals of EA and AA ancestries from 12 adult cohorts (average age ranges from 26.2 to 63.5 years old) and 2 youth cohorts (average age 16.2 and 17.7 years old; Table 1). Details of each cohort are provided in the Data Supplement. All studies obtained written informed consent from participants and were approved by local institutional review boards and ethics committees.

Blood Pressure Measurements

For all the cohorts, BP was measured after a period of rest and an average of 3 sequential readings was used as the phenotype for each analysis. For 3 cohorts (GSH, EpiGO, and LACHY), BP was measured in a supine position, while for the other 11 cohorts, BP was measured in a sitting position. With the exception of the NTR cohort for which BP was measured within ±2 years from the methylation measurement, all the cohorts had BP measured concurrently with the collection of peripheral leukocytes for DNA methylation profiling. If antihypertensive medication was used, 15 and 10 mmHg were added to the measured SBP and DBP levels, respectively.[23]

DNA Methylation Profiling

For all cohorts, genome-wide DNA methylation data were obtained from peripheral blood using the Illumina Infinium Human Methylation 450K Beadchip (Illumina, Inc). A detailed description on preprocessing and quality control steps for each cohort[24–32] is provided in the Data Supplement. For all cohorts, white blood cell subpopulations were estimated using the approach described by Houseman et al.[33]

Cohort Level Association Analysis

For cohorts only including unrelated subjects, a linear regression model was used to estimate the associations between DNA methylation (ie, β values) and BP with methylation levels used as dependent variables adjusting for age, sex, ancestry (in samples including EAs and AAs), BMI, and white blood cell subpopulations. For cohorts including related subjects, a linear mixed effect model was used to account for sample relatedness.

Meta-Analyses and Cross Validation

Meta-analysis across the 14 cohorts was conducted using METAL[34] by converting the direction of effect and *P*-value observed in each cohort into a signed *Z*-score. CpG sites with $P\leq1\times10^{-5}$ for either SBP or DBP were selected for replication in the CHARGE consortium (n=17 010). Replication was defined as consistent direction of the β-coefficient and FDR (False Discovery Rate) <0.05. Conversely, we also checked whether we could replicate the 126 CpG sites for BP identified in the overall meta-analysis of the CHARGE consortium in our own meta-EWAS results. Replication was again defined as a consistent direction of the β-coefficient and FDR<0.05.

Percent Variance Explained

Percent variance explained by the cross-validated BP associated CpG sites was calculated in the Lifelines DEEP cohort.[35,36] To avoid overestimation of percent variance explained, this cohort was not included in the Meta-analysis. Percent variance explained by the

Table 1. General Characteristics of the Study Cohorts

Cohorts	N	Race*	Age, y; mean (SD)	Female, %	BMI, kg/m^2	SBP, mm Hg	DBP, mm Hg	HTN,† %	AHT,‡ %
Adult cohort									
BHS[9]	968	EA, AA§	43.2 (4.5)	56.5	30.8 (7.5)	127.3 (23.6)	81.2 (14.6)	36.9	26.9
GSH[10]	480	EA, AA‖	27.3 (3.5)	52.4	29.9 (8.2)	114.9 (13.3)	66.6 (8.81)	9.39	4.80
DILGOM[11]	512	EA	51.9 (13.7)	53.7	26.9 (4.8)	143.3 (16.9)	83.4 (10.0)	49.1	37.5
ETS[12]	218	EA	55.7 (3.4)	0	29.4 (4.7)	135.66 (18.4)	84.8 (11.8)	56.3	34.4
EGCUT (Asthma)[13]	173	EA	26.2 (6.9)	64.2	22.8 (3.0)	116.7 (12.0)	73.2 (9.4)	…¶	…¶
EGCUT (Young_Old)[13]	100	EA	52.7 (23.7)	52.0	26.7 (5.1)	129.1 (19.1)	79.6 (10.4)	…¶	…¶
FTC[14]	402	EA	62.3 (3.7)	59.3	26.9 (4.9)	150.2 (18.6)	87.8 (11.0)	58.4	43.5
HBCS[15]	159	EA	63.5 (2.8)	0	27.5 (3.8)	148.1 (19.0)	91.0 (10.3)	75.0	44.7
JHS[16]	96	AA	38.4 (4.3)	50	33.9 (7.1)	127.8 (23.7)	81.5 (14.5)	50.0	34.3
Lifelines[17]	150	EA	50.3 (10.5)	58.7	28.0 (5.1)	124.4 (12.0)	74.1 (8.60)	10.0	0.00
NTR[18,19]	596#	EA	29.4 (10.5)**	66.1	23.6 (3.6)**	126.0 (14.7)	76.3 (10.5)	17.8	1.34
PREVEND[20]	307	EA	46.7 (10.0)	39.7	27 (4.6)	131.1 (20.8)	76.2 (11.2)	48.5	19.5
YFS[21]	188	EA	44.0 (3.3)	38.8	26.3 (4.4)	119.1 (13.2)	73.2 (9.5)	9.57	5.30
Youth cohort									
EpiGO[22]	188	AA	17.7 (1.7)	48.4	29.3 (11.5)	114.8 (15.1)	63.8 (7.7)	9.57	0.00
LACHY[22]	283	AA	16.2 (1.3)	50.0	24.1 (5.6)	112.9 (10.1)	61.4 (6.0)	3.53	0.00

BHS indicates the Bogalusa Heart Study; DILGOM, the Dietary, Lifestyle, and Genetic determinants of Obesity and Metabolic syndrome Study; EGCUT, the Estonian Genome Center of the University of Tartu; EpiGO, the Epigenetic basis of Obesity induced cardiovascular disease and type 2 diabetes study; ETS, the Emory Twin Study; FTC, the Finnish Twin Cohort; GSH, the Georgia Stress and Heart study; HBCS, the Helsinki Birth Cohort Study; JHS, the Jackson Heart Study; LACHY, the Lifestyle, Adiposity, and Cardiovascular Health in Youth study; Lifelines, the Lifelines Cohort Study; NTR, the Netherlands Twin Register; PREVEND, Prevention of Renal and Vascular End stage Disease study; and YFS, the Young Finns Study.

*EA, European Ancestry; AA, African American.

†HTN: hypertension; HTN definition for adults: SBP≥140 mmHg or DBP≥90 mmHg or on antihypertensive medication; HTN definition for youth: SBP≥95th or DBP≥95th percentile for age, sex, and height.

‡AHT, antihypertensive treatment.

§For BHS, there are 70.3% EA and 29.7% AA.

‖For GSH, there are 52.5% EA and 47.5% AA.

¶Only medication adjusted BP data were available.

#This dataset included 499 MZ and 95 DZ twins as well as 2 spouses of twins. The 2 spouses of twins were excluded in the analyses only involving twins.

**Age and BMI at blood sampling time.

cross-validated BP associated CpG sites is reported as the change in the adjusted R^2 from the model including these CpG sites compared with the model only including covariates (ie, age, sex, and BMI).

Pathway Analysis

Pathway enrichment analysis was conducted on the meta-analysis results of the genome-wide DNA methylation data using gene set enrichment analysis.[37] Gene set enrichment analysis was performed on an unfiltered, ranked list of genes (ranked by the *P* values without consideration of directions), and a running-sum statistic was used to determine the enrichment of a priori defined gene sets (pathways) based on the gene ranks. All gene ontology biological process categories (c5.bp.v5.1) were assessed for enrichment at FDR<0.05. The CpG site showing the most significant *P* value within a gene was used to represent the DNA methylation level of the gene.

Associations of DNA Methylation and Gene Expression

Association tests of the cross-validated BP-associated CpGs (Figure S1) with transcripts that were located within 500 kb distance of the corresponding CpGs were performed in the 391 individual twins of the Finnish Twin Cohort for whom both DNA methylation and gene expression data were available. Gene expression data were obtained using the Illumina Human HT-12 V4 expression Beadchip (Illumina, Inc, San Diego, CA).[38] Linear mixed effects regression models were used with gene expression as the dependent variable, DNA methylation as the independent variable, age, sex, and BMI as fixed effects, and family as a random effect. An FDR <0.05 was defined as significant association between DNA methylation and gene expression. BP-associated gene expressions were defined as genes with their expression levels showing significant association with either SBP or DBP at $P<0.05$.

Genetic and Environmental Determinants of DNA Methylation Associated With BP

For all the cross-validated BP-associated CpG sites (Figure S1), we estimated the relative contributions of genetic and environmental factors to the variance of DNA methylation levels in the Finnish Twin Cohort and the Netherlands Twin Register using the R package OpenMx.[39,40] Before analysis, age, sex, and BMI were regressed out, and the DNA methylation residuals were used in the model fitting. Details of this univariate structural equation model for twin data (Figure S2) were described in a previous study.[38] In short, the model allows separation of the observed phenotypic variance into its genetic and environmental variance components including additive genetic variance (A), common environmental variance shared by a twin pair (C), and unique environmental variance specific to individuals (E). The monozygotic twins of each pair (MZ twins) have identical genome sequences, while dizygotic twins (DZ twins) share 50% of their

segregating alleles. Shared environmental factors are exposures and experiences that affect co-twins similarly on average irrespective of zygosity, while unique environmental factors are the effects not shared by cotwins and include measurement error. Significance tests of individual variance components (A or C) were conducted by comparing full models with submodels constraining paths from latent variables to trait values (a, c) to 0 using a χ^2 test; as E contains measurement error, the significance of E is not tested. Statistical significance was defined as $P<0.05$. The analysis was conducted in each twin cohort separately, then a meta-analysis across the 2 twin cohorts (FTC and NTR) was performed to estimate the mean heritability (h^2) using the Meta package in R.[41] Following the approach described by Asefa et al,[42] each h^2 was transformed using a logit function,[43] and a random-effects model was used for the meta-analysis. The pooled h^2 (weighted by sample size) and 95% CIs were back-transformed. Heterogeneity between studies was quantified with Cochran Q test and the I^2-statistic.[44]

Sources Underlying the Associations Between DNA Methylation and BP

For all the cross-validated BP-associated CpG sites (Figure S1), we checked whether they were also significantly associated with BP in the meta-analysis of the Finnish Twin Cohort and the Netherlands Twin Register, that is, a consistent direction of the β-coefficients and $P<0.05$. For those CpG sites significantly associated with BP in the meta-analysis of these 2 twin cohorts, we conducted bivariate structural equation modeling to test the extent to which the link between BP and DNA methylation was driven by genetic or environmental factors. Details of this model (Figure S3) have been described previously.[38] Briefly, the variation of DNA methylation and the variation of BP were decomposed into A, C, and E variance components. The bivariate model allows determination of the sources of the observed covariance between DNA methylation and BP by using a sequence of sub-models that test which genetic, shared environmental or unique environmental paths from DNA methylation to BP can be set to 0. For example, in Figure S3, if a_{21} (genetic path from DNA methylation to BP) cannot be set to 0, it means there is overlap between the genetic factors influencing DNA methylation and BP. The model further allows calculation of genetic and environmental correlations between the traits. Similar to the univariate analysis, the bivariate analysis was conducted in each twin cohort separately, then a meta-analysis was conducted to determine the genetic and environmental contributions to the correlation between DNA methylation and BP. Briefly, for each cohort, genetic (r_g) and environmental (r_e) correlations were calculated based on the variance/covariance matrix estimated from the bivariate twin modeling (Figure S3). The genetic contribution to the observed phenotypic correlation (r_{ph}) is a function of the heritability estimates of the 2 phenotypes and the r_g between them, that is, $\sqrt{h^2_M} \times r_g \times \sqrt{h^2_{BP}}$. Similarly, the environmental contribution to r_{ph} is equal to $\sqrt{e^2_M} \times r_e \times \sqrt{e^2_{BP}}$. Then a random-effects model was used to estimate the meta-genetic and environmental contributions respectively with the 95% CIs.

Results

The general characteristics of the study participants are listed in Table 1. A total of 4820 individuals were included from 14 cohorts with a wide range of mean SBP and DBP values. The prevalence of antihypertensive medication use also varied among the cohorts.

Our meta-analysis identified 39 CpG sites associated with SBP or DBP at $P<1\times10^{-5}$ (Manhattan and QQ plot, Figures S4 and S5 in the Data Supplement; Table S1) with 2 CpG sites showing $P<1\times10^{-7}$. Out of these 39 CpG sites, the heterogeneity test across the cohorts reached significance ($P<0.05$) for 5 sites (cg06500161, cg00508575, cg19693031, cg12555233, and cg02711608, Table S1). Further sensitivity tests by ancestry (EA versus AA) or age (adult cohorts versus youth cohorts) did not support the heterogeneity being due to ancestry or age. Sixteen out of the 39 CpG sites including 3 showing heterogeneity (cg06500161, cg00508575, and cg19693031) could be replicated (FDR<0.05) in the CHARGE consortium (Table 2). Of the 16 replicated CpG sites, only 3 (cg02711608, cg19693031, cg08857797) have previously been reported to be associated with BP (highlighted in gray in Table 2) and the other 13 were novel signals. Conversely, of the 126 CpG sites found to be associated with BP by the CHARGE consortium in the overall sample, 91 sites showed the same direction of effect in our meta-analysis with 21 sites having FDR <0.05 (Table 3). These 21 CpG sites included the 3 CpG sites previously reported to be associated with BP (highlighted in gray in Table 3). In total, 34 CpG sites were cross-validated to be associated with BP by the current meta-analysis and the CHARGE consortium. To assess the impact of antihypertensive medication use, we stratified the meta-analysis of these 34 CpG sites by medication use and provide the results in Table S2. In the individuals reporting no use of antihypertensive medications, the directions of the effects of all the CpG sites remained the same as for the overall sample with 27 out of the 34 retaining their significant associations ($P<0.05$) with BP, rendering it highly unlikely that the differentially methylated CpG sites we identified reflect drug treatment effects.

Inclusion of 33 out of the 34 CpG sites (cg02711608 was filtered out in the quality control step of the Lifelines DEEP cohort) explained an additional 3.31% and 3.99% of the inter-individual variation in SBP and DBP, respectively, beyond the traditional BP covariates of age, sex, and BMI in an additional sample from the Lifelines cohort (the Lifelines DEEP cohort, n=601) not included in the current meta-analysis. Details of this cohort are provided in the Data Supplement. Using the Lifelines DEEP cohort, we further explored whether these 33 CpG sites were individually or collectively associated with EH. A total of 102 out of the 601 (16.97 %) participants were classified as having EH (ie, SBP≥140 mm Hg, or DBP≥90 mm Hg, or taking antihypertensive medication). Out of the 33 CpG sites, 4 CpG sites (cg12593793, cg11376147, cg21766592, and cg06500161) were individually associated with EH with $P<0.05$ in the expected direction (Table S3). Collectively, adding these 33 CpG sites in the model with age, sex, and BMI as covariates increased Nagelkerke pseudo R^2 from 34.3% to 47.7%.

Of the 34 CpG sites, the methylation levels of 6 sites were significantly associated with the expression of 5 genes in cis analysis (FDR <0.05; Table 4). The methylation-gene expression associations did not differ by medication use. For all the CpG sites, increased methylation was associated with decreased gene expression (Figure S6). Furthermore, expression of 2 genes (ie, *ABCG1* and *LMNA*) showed significant association with BP. For both genes, the direction of the association between CpG methylation and gene expression was as expected based on the association of CpG methylation and BP. For example, the methylation level of cg06500161 was negatively associated with *ABCG1* gene expression and positively associated with SBP. This was consistent with the negative association between *ABCG1* gene expression and SBP.

The pathway analyses yielded significant (FDR <0.05) enrichment of 4 biological process pathways for SBP-related DNA methylation changes, and 6 for DBP-related methylation changes in peripheral leukocytes (Table S4). The primary

Table 2. CpG Sites Showing Association With BP in Our Analysis With $P<1\times10^{-5}$ and Replicated by CHARGE Consortium With FDR<0.05

				SBP				DBP			
				META		CHARGE		META		CHARGE	
Probe ID	Chr.	Position	Gene	Direction*	*P* Value	Direction*	*P* Value	Direction*	*P* Value	Direction*	*P* Value
cg19693031	1	145441552	*TXNIP*	–	2.18×10^{-7}	–	3.10×10^{-29}	–	4.65×10^{-5}	–	1.80×10–14
cg01343041	2	24397787	*C2orf84*	+	4.21×10^{-7}	+	2.30×10^{-2}	+	1.01×10^{-4}	+	5.74×10–01
cg19695041	8	38615330	*TACC1*	–	6.26×10^{-6}	–	4.45×10^{-5}	–	3.36×10^{-2}	–	3.26×10^{-3}
cg13696706	9	124396830	*DAB2IP*	+	9.83×10^{-8}	+	3.95×10^{-3}	+	1.16×10^{-3}	+	7.90×10^{-1}
cg11468085	11	67435577	*ALDH3B2*	+	4.16×10^{-6}	+	1.75×10^{-4}	+	1.38×10^{-4}	+	2.27×10^{-2}
cg00508575	12	90050967	*ATP2B1*	+	6.47×10^{-6}	+	1.44×10^{-3}	+	9.20×10^{-4}	+	1.63×10^{-1}
cg05248321	14	20898128	*KLHL33*	+	7.01×10^{-7}	+	1.68×10^{-5}	+	1.88×10^{-3}	+	1.86×10^{-3}
cg02003183	14	103415882	*CDC42BPB*	+	3.66×10^{-7}	+	5.56×10^{-7}	+	1.54×10^{-3}	+	3.09×10^{-2}
cg12555233	15	91455366	*MAN2A2*	+	2.74×10^{-6}	+	6.25×10^{-3}	+	3.50×10^{-3}	+	2.97×10^{-2}
cg07558761	16	87866696	*SLC7A5*	+	8.83×10^{-7}	+	1.46×10^{-5}	+	1.71×10^{-3}	+	3.37×10^{-4}
cg07021906	16	87866833	*SLC7A5*	+	1.38×10^{-6}	+	1.65×10^{-6}	+	3.37×10^{-3}	+	5.73×10^{-3}
cg04583842	16	88103117	*BANP*	+	5.54×10^{-9}	+	4.16×10^{-3}	+	2.99×10^{-6}	+	8.28×10^{-1}
cg08857797	17	40927699	*VPS25*	+	9.64×10^{-6}	+	3.60×10^{-10}	+	4.98×10^{-5}	+	2.30×10^{-6}
cg02711608	19	47287964	*SLC1A5*	–	7.48×10^{-6}	–	2.00×10^{-21}	–	1.41×10^{-3}	–	4.30×10^{-10}
cg06500161	21	43656587	*ABCG1*	+	5.69×10^{-6}	+	1.01×10^{-4}	+	5.06×10^{-5}	+	1.01×10^{-3}
cg01820192	21	44869762	*C21orf125*	+	6.44×10^{-6}	+	1.66×10^{-2}	+	3.96×10^{-2}	+	4.75×10^{-2}

CpG sites previously reported by the CHARGE consortium are highlighted in gray. CpG sites that overlapped between Table 3 and Table 2 are highlighted in gray. BP indicates blood pressure; CHARGE, results of the CHARGE consortium; DBP, diastolic blood pressure; META, meta-analysis results of our analysis; SBP, systolic blood pressure.

*+ indicates that DNA methylation levels increase with BP increase. – indicates that DNA methylation levels decrease with BP increase.

pathway identified by the CHARGE consortium,[6] the transport of neutral amino acids, also showed borderline significance in the current enrichment analyses for both SBP (FDR=0.060) and DBP (FDR=0.074).

The cohort-level results of the univariate structural equation model analysis on the DNA methylation levels of the 34 cross-validated CpG sites are listed in Table S5. For all the CpG sites, the best fitting models were AE models, with heritability estimates ranging from 31% to 83% in the Finnish Twin Cohort and 19% to 81% in the Netherlands Twin Register. The remaining part of the variation for DNA methylation of these CpG sites was attributable to environmental influences that are unique to the individual. Table 5 lists the heritability of the 34 CpG sites from the meta-analysis. The heritabilities ranged from 31% to 78%.

Of the 34 implicated CpG sites, the methylation level of 9 CpG sites showed significant association with BP in the meta-analysis of the Finnish Twin Cohort and the Netherlands Twin Register (Table 6). For these 9 sites, we estimated the relative contributions of genetic and environmental factors to the association between DNA methylation and BP. Given that the AE model has generally been the best fitting model in previous twin studies of BP,[45] which was again confirmed in the current study, the bivariate modeling was conducted using the AE model both for DNA methylation and BP. The cohort-level results are listed in Table S6, and the meta-analysis results are listed in Table 6. For the association of cg19693031 in the *TXNIP* gene and cg00716257 in the *JDP2* gene with SBP, the meta-analysis showed that the correlation due to the environmental contribution cannot be set to 0 while the genetic contribution can be set to 0, suggesting that the phenotypic correlation is determined by unique environmental factors in common to the 2 traits. Similar trends were observed for the association of cg11468085 with SBP and cg19693031 with DBP. For the associations of the other CpG sites with BP, both genetic and environmental contributions can be set to 0, indicating a larger sample size is needed to increase the power to distinguish the relative contributions of genetic and environmental factors to the observed phenotypic correlations.

Discussion

In this epigenome-wide association study, we identified 13 novel CpG sites associated with BP and replicated 21 CpG sites previously identified in the overall meta-analysis of the CHARGE consortium.[6] We also showed that DNA methylation levels from 6 of the 34 cross-validated CpG sites were associated with gene expression. Although all of the 34 CpGs were heritable (31%–78%), further bivariate twin modeling analyses in the Finnish Twin Cohort and the Netherlands Twin Register suggested that, among the 9 CpG sites that were associated with BP, the correlations of cg19693031(*TXNIP*) and cg00716257 (*JDP2*) with SBP were primarily attributable to environmental factors that affect both traits, rather than genetic factors.

The 13 novel CpG sites that were associated with BP were annotated to 10 genes. Among these, only *ABCG1* and *ATP2B1* have previously been implicated in hypertension. For example, newly diagnosed patients with hypertension have been shown to have lower *ABCG1* expression in peripheral blood monocytes in comparison with normotensive controls.[46] This is consistent with

Table 3. Signals Reported by CHARGE Consortium and Replicated by the Current Study (FDR<0.05).

Probe ID	Chr.	Position	Gene	SBP				DBP			
				CHARGE		META		CHARGE		META	
				Direction*	*P* Value	Direction*	*P* Value	Direction*	*P* Value	Direction*	*P* Value
cg18933331	1	110186418	*Intergenic*	–	4.80×10^{-9}	–	7.64×10^{-3}	–	2.40×10^{-8}	–	1.17×10^{-2}
cg16246545	1	120255941	*PHGDH*	–	1.20×10^{-22}	–	4.11×10^{-4}	–	1.10×10^{-9}	–	4.34×10^{-4}
cg14476101	1	120255992	*PHGDH*	–	2.70×10^{-34}	–	4.27×10^{-5}	–	2.10×10^{-21}	–	2.31×10^{-4}
cg19693031	1	145441552	*TXNIP*	–	3.10×10^{-29}	–	2.18×10^{-7}	–	1.80×10^{-14}	–	4.65×10^{-5}
cg19266329	1	145456128	*Intergenic*	–	1.90×10^{-12}	–	3.61×10^{-3}	–	5.70×10^{-5}	–	2.22×10^{-1}
cg24955196	1	154982621	*ZBTB7B*	+	5.00×10^{-8}	+	8.28×10^{-4}	+	6.00×10^{-6}	+	5.09×10^{-2}
cg12593793	1	156074135	*Intergenic*	–	2.60×10^{-12}	–	3.22×10^{-3}	–	3.00×10^{-7}	–	7.49×10^{-2}
cg18119407	2	201980504	*CFLAR*	–	2.00×10^{-9}	–	7.10×10^{-3}	–	4.40×10^{-5}	–	4.51×10^{-3}
cg06690548	4	139162808	*SLC7A11*	–	1.60×10^{-32}	–	1.52×10^{-5}	–	7.90×10^{-26}	–	2.47×10^{-5}
cg18120259	6	43894639	*LOC100132354*	–	2.20×10^{-21}	–	5.58×10^{-3}	–	8.90×10^{-14}	–	4.55×10^{-2}
cg21429551	7	30635762	*GARS*	–	3.40×10^{-16}	–	6.84×10^{-4}	–	8.70×10^{-6}	–	1.34×10^{-2}
cg19390658	7	30636176	*GARS*	–	4.70×10^{-9}	–	2.58×10^{-4}	–	4.40×10^{-6}	–	3.30×10^{-4}
cg00008629	9	115093661	*ROD1*	–	6.50×10^{-8}	–	4.38×10^{-3}	–	8.00×10^{-2}	–	6.45×10^{-2}
cg11376147	11	57261198	*SLC43A1*	–	4.20×10^{-21}	–	6.66×10^{-3}	–	3.40×10^{-12}	–	1.34×10^{-2}
cg00574958	11	68607622	*CPT1A*	–	1.20×10^{-13}	–	1.04×10^{-2}	–	3.00×10^{-10}	–	6.83×10^{-4}
cg00716257	14	75897417	*JDP2*	–	6.00×10^{-8}	–	4.39×10^{-3}	–	4.40×10^{-7}	–	4.28×10^{-1}
cg26916780	15	64889554	*ZNF609*	–	4.50×10^{-6}	–	8.87×10^{-3}	–	3.70×10^{-9}	–	5.77×10^{-2}
cg08857797	17	40927699	*VPS25*	+	3.60×10^{-10}	+	9.64×10^{-6}	+	2.30×10^{-6}	+	4.98×10^{-5}
cg22304262	19	47287778	*SLC1A5*	–	1.40×10^{-17}	–	1.97×10^{-5}	–	9.60×10^{-11}	–	1.05×10^{-2}
cg02711608	19	47287964	*SLC1A5*	–	2.00×10^{-21}	–	7.48×10^{-6}	–	4.30×10^{-10}	–	1.41×10^{-3}
cg21766592	19	47288066	*SLC1A5*	–	2.60×10^{-8}	–	5.65×10^{-4}	–	1.10×10^{-1}	–	1.88×10^{-2}

CpG sites that overlapped between Table 3 and Table 2 are highlighted in gray.

*+ indicates that DNA methylation levels increase with BP increase; CHARGE: results of the CHARGE consortium; DBP, diastolic blood pressure; META, meta-analysis results of our analysis; SBP, systolic blood pressure.

our results in which we also observed that peripheral leukocyte *ABCG1* expression was negatively correlated with both SBP and DBP levels. Several genetic variants in *ATP2B1* have been associated with BP and hypertension in multiple GWA studies,[47–49] and animal studies[50] have demonstrated that mice lacking *ATP2B1* in vascular smooth muscle cells had higher BP than wild-type mice. In the current study, we observed higher methylation level of cg00508575 in *ATP2B1* associated with higher SBP level; however, the methylation status of this CpG site was not associated with *ATP2B1* expression levels in peripheral blood leukocytes. Further studies in other tissues such as vascular smooth muscle cells would be needed to clarify the functional role of this CpG site. The potential involvement of the other 8 genes in the pathogenesis of hypertension has not been directly addressed in the literature although some evidence is available on their involvement in cardiovascular diseases. For example, *TACC1* has been linked to inappropriate smooth muscle and endothelial cell proliferation in pulmonary arterial hypertension.[51] Several genome-wide association studies[52,53] have reported variants in *DAB21P*, which encodes an inhibitor of cell growth and survival, which were associated with abdominal aortic aneurysm and atherosclerotic vascular diseases. *ALDH3B2*, encoding one member of the

Table 4. Cross-Validated CpG Sites That Show Association With Gene Expression (±500 kb) at FDR<0.05 in the Finnish Twin Cohort

DNAm ProbeID	DNAm Annotation	GX ProbID	GX Annotation	DNAm-GX			GX-SBP		GX-DBP		DNAm-SBP		DNAm-DBP	
				Dir.	*P* Value	FDR	Dir.	*P* Value	Dir.	*P* Value	Dir.	*P* Value	Dir.	*P* Value
cg14476101	*PHGDH*	240086	*PHGDH*	–	2.33×10^{-11}	1.19×10^{-8}	+	1.08×10^{-1}	+	5.62×10^{-1}	–	1.33×10^{-2}	–	2.88×10^{-2}
cg16246545	*PHGDH*	240086	*PHGDH*	–	2.94×10^{-10}	7.47×10^{-8}	+	1.08×10^{-1}	+	5.62×10^{-1}	–	5.23×10^{-2}	–	9.29×10^{-2}
cg06500161	*ABCG1*	6060377	*ABCG1*	–	1.37×10^{-4}	1.39×10^{-2}	–	2.75×10^{-3}	–	4.49×10^{-4}	+	4.74×10^{-2}	+	2.51×10^{-1}
cg12593793	*Intergenic*	6020424	*LMNA*	–	3.60×10^{-4}	2.28×10^{-2}	+	2.57×10^{-4}	+	1.78×10^{-5}	–	2.10×10^{-1}	–	2.53×10^{-1}
cg26916780	*ZNF609*	5960682	*RBPMS2*	–	8.61×10^{-4}	4.56×10^{-2}	+	1.88×10^{-1}	+	1.43×10^{-1}	–	6.84×10^{-1}	–	6.64×10^{-1}
cg02711608	*SLC1A5*	7610433	*SLC1A5*	–	8.98×10^{-4}	4.56×10^{-2}	+	4.62×10^{-1}	+	2.88×10^{-1}	–	4.34×10^{-2}	–	1.07×10^{-1}

Table 5. Heritability of the 34 Cross-Validated CpG Sites From the Meta-Analysis

Probeid	chr.	Position	Gene	h^2	95% CI
cg18933331	1	110186418	*Intergenic*	0.72	0.62–0.81
cg16246545	1	120255941	*PHGDH*	0.77	0.69–0.84
cg14476101	1	120255992	*PHGDH*	0.74	0.63–0.82
cg19693031	1	145441552	*TXNIP*	0.56	0.53–0.60
cg19266329	1	145456128	*Intergenic*	0.39	0.31–0.49
cg24955196	1	154982621	*ZBTB7B*	0.41	0.22–0.63
cg12593793	1	156074135	*Intergenic*	0.65	0.37–0.86
cg01343041	2	24397787	*C2orf84*	0.65	0.62–0.68
cg18119407	2	201980504	*CFLAR*	0.36	0.33–0.39
cg06690548	4	139162808	*SLC7A11*	0.36	0.27–0.46
cg18120259	6	43894639	*LOC100132354*	0.63	0.56–0.69
cg21429551	7	30635762	*GARS*	0.68	0.65–0.71
cg19390658	7	30636176	*GARS*	0.35	0.32–0.38
cg19695041	8	38615330	*TACC1*	0.51	0.43–0.59
cg00008629	9	115093661	*ROD1*	0.78	0.65–0.87
cg13696706	9	124396830	*DAB2IP*	0.58	0.55–0.61
cg11376147	11	57261198	*SLC43A1*	0.41	0.25–0.59
cg11468085	11	67435577	*ALDH3B2*	0.54	0.51–0.57
cg00574958	11	68607622	*CPT1A*	0.44	0.30–0.58
cg00508575	12	90050967	*ATP2B1*	0.48	0.38–0.59
cg05248321	14	20898128	*KLHL33*	0.65	0.54–0.75
cg00716257	14	75897417	*JDP2*	0.31	0.11–0.62
cg02003183	14	103415882	*CDC42BPB*	0.62	0.59–0.65
cg26916780	15	64889554	*ZNF609*	0.40	0.34–0.46
cg12555233	15	91455366	*MAN2A2*	0.56	0.44–0.67
cg07558761	16	87866696	*SLC7A5*	0.53	0.38–0.68
cg07021906	16	87866833	*SLC7A5*	0.61	0.58–0.64
cg04583842	16	88103117	*BANP*	0.63	0.58–0.67
cg08857797	17	40927699	*VPS25*	0.48	0.21–0.75
cg22304262	19	47287778	*SLC1A5*	0.70	0.47–0.87
cg02711608	19	47287964	*SLC1A5*	0.69	0.61–0.75
cg21766592	19	47288066	*SLC1A5*	0.58	0.52–0.63
cg06500161	21	43656587	*ABCG1*	0.61	0.50–0.71
cg01820192	21	44869762	*C21orf125*	0.37	0.32–0.42

ALDH family of proteins that play a role in cell proliferation, differentiation, and responsiveness to environmental stress, has been suggested as a candidate gene for bisoprolol (a β blocker) responsiveness.[54] CpG sites in *MAN2A2* have been associated with fasting insulin.[55] Further experimental validation of the role of these genes in BP regulation is warranted.

Similar to the CHARGE consortium,[6] which involved cohorts from different ancestries (European, African American, and Hispanic) and a broad age range (18–80 years), the current study also included individuals from European and African American ancestry with an age range of 14 to 69 years. The fact that the signals could be cross-validated between these 2 studies, and that both studies showed the effect of the majority of BP-related CpG sites to be homogeneous across the cohorts, ancestral groups, and different age groups, indicates that these BP-related CpG sites may be ethnicity- and age-independent. However, a clearer picture of the role of DNA methylation in the pathogenesis of EH in various age and population groups will require even larger EWASs spanning multiple age ranges and ancestry groups.

Interestingly, the majority of the 34 cross-validated CpG sites have been linked with other metabolic phenotypes including obesity, lipids, CRP, insulin resistance, and type 2

Table 6. Meta-Analysis Results of the Bivariate SEM Analysis for BP and Its Associated CpG Sites in FTC and NTR

	Trait	Gene	Association		Bivariate SEM Analysis				
Probeid			Direction	*P* Value	r_{ph}	G contribution	E contribution	$P_{G=0}$	$P_{E=0}$
cg19693031	SBP	*TXNIP*	−−	0.0185	−0.047	0.023 (−0.040 TO 0.085)	−0.070 (−0.132 to −0.008)*	0.477	0.026
cg13696706	SBP	*DAB2IP*	++	0.0120	0.059	0.036 (−0.026 TO 0.098)	0.023 (−0.039 to 0.085)	0.254	0.464
cg11468085	SBP	*ALDH3B2*	++	0.0148	0.026	−0.029 (−0.138 TO 0.079)	0.055 (−0.007 to 0.116)	0.595	0.084
cg05248321	SBP	*KLHL33*	++	0.0117	0.056	0.034 (−0.028 TO 0.096)	0.022 (−0.04 to 0.084)	0.279	0.492
cg00716257	SBP	*JDP2*	−−	0.0317	−0.050	0.013 (−0.050 TO 0.075)	−0.063 (−0.124 to −0.001)*	0.692	0.048
cg04583842	SBP	*BANP*	++	0.0132	0.066	0.045 (−0.017 TO 0.107)	0.021 (−0.041 to 0.083)	0.159	0.512
cg08857797	SBP	*VPS25*	++	0.0029	0.070	0.018 (−0.044 TO 0.08)	0.052 (−0.01 to 0.114)	0.568	0.101
cg22304262	SBP	*SLC1A5*	−−	0.0252	−0.050	−0.030 (−0.118 TO 0.058)	−0.020 (−0.082 to 0.042)	0.509	0.535
cg06500161	SBP	*ABCG1*	++	0.0417	0.031	0.003 (−0.098 TO 0.105)	0.028 (−0.034 to 0.090)	0.952	0.369
cg19693031	DBP	*TXNIP*	−−	0.0246	−0.062	−0.006 (−0.068 TO 0.056)	−0.056 (−0.118 to 0.006)	0.857	0.076
cg11468085	DBP	*ALDH3B2*	++	0.0099	0.023	−0.013 (−0.126 TO 0.101)	0.036 (−0.037 to 0.110)	0.828	0.333
cg08857797	DBP	*VPS25*	++	0.0022	0.090	0.065 (−0.016 TO 0.146)	0.025 (−0.037 to 0.087)	0.117	0.432

A detailed explanation is provided in Figure S2 in the Data Supplement. E contribution indicates unique environmental contribution; G contribution, genetic contribution; and r_{ph}, phenotypic correlation.

diabetes mellitus by previous epigenome-wide association studies (Table S7), indicating that DNA methylation may be one of the common factors related to the concurrence of multiple metabolic abnormalities. Indeed, epigenome-wide association studies have identified several CpG sites whose DNA methylation levels are associated with metabolic syndrome (MetS) including cg00574958 in the *CPT1A* gene[56] and cg06500161 in the *ABCG1* gene.[57] *ABCG1* cg06500161 has also been reported to be associated with fasting insulin,[58] blood lipids,[59] adiposity traits,[22,60] and type 2 diabetes mellitus.[17,61] In the current study, we observed for the first time that a higher methylation level of cg06500161 was also associated with higher BP levels. Taken together, these studies show that *ABCG1* cg06500161 is associated with each MetS component, though the causal direction of these associations has not been determined. Furthermore, although the other components of MetS can be viewed as consequences of obesity, the associations of these CpG sites with these MetS components are independent of obesity. Future studies are warranted with multivariate analyses targeting multiple metabolic traits to disentangle the mechanisms involved in the association of DNA methylation with MetS and its components.

Unlike genetic sequence variants, epigenetic variation is influenced by both genetic and environmental factors.[7,8] We first quantified the genetic and environmental sources of the variation in the 34 cross-validated BP associated CpG sites and confirmed that the variance of all these 34 CpG sites was indeed determined by both genetics (31%–78%) and environment (22%–69%). Since BP is also a heritable trait, an interesting question is to what extent the link between BP and DNA methylation is driven by genetic or environmental factors in common to the 2 traits. We tried to answer this question using the Finnish Twin Cohort and the Netherlands Twin Register by conducting a bivariate twin modeling analysis on BP and the 9 CpG sites, which showed association with BP in the meta-analysis of these 2 cohorts. Surprisingly, we observed that 100% of the correlations of BP with cg19693031(*TXNIP*) and cg00716257 (*JDP2*) could be attributed to environmental factors in common to the 2 traits rather than genetic factors, despite evidence for high heritability of both methylation at those CpG sites and SBP. The apparent lack of shared genetic component indicates that the link between the methylation level of these 2 CpG sites and BP may be driven primarily by environmental conditions; the relatively modest sample size should be recognized, however, and further confirmation is needed.

Our study has several limitations. First, it is cross-sectional, thus making it impossible to discern the temporal order between BP and DNA methylation. Second, the bivariate twin modeling analysis was only conducted in the Finnish Twin Cohort and the Netherlands Twin Register which included about 1000 twins. An even larger sample size is required to tease out reliably the relative contribution of genetic or environmental factors to the associations of BP with DNA methylation. Third, we did not conduct in vitro and in vivo functional studies to confirm the impact of these CpG sites on gene expression and subsequently on blood pressure, which is warranted for future research.

Perspectives

In summary, we identified 13 novel CpG sites associated with BP and replicated several previously identified signals. These newly identified signals may aid in annotating the future gene findings by providing a potential molecular mechanism for BP regulation. Our study further provides new insights into the genetic and environmental sources of BP related DNA methylation signatures as well as their associations with BP. The identification of shared unique environmental factors rather than genetic factors between BP and DNA methylation of *TXNIP* and *JDP2* indicates that the environment plays a significant role in creating an association between DNA methylation signatures and BP.

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Disclosures

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Novelty and Significance

What Is New?

- Identified 13 novel CpG sites of which their methylation levels are associated with blood pressure
- Genetic factors contribute to the methylation variations of the blood pressure associated CpG sites
- The phenotypic correlations between CpG sites and SBP are primarily attributable to environmental factors that affect both traits, rather than genetic factors.

What Is Relevant?

- The identification of shared unique environmental factors rather than genetic factors between blood pressure and DNA methylation indicates that the environment plays a significant role in creating an association between DNA methylation signatures and blood pressure.

Summary

In this study of 4820 individuals of European and African ancestry aged 14 to 69, genome-wide DNA methylation data from peripheral leukocytes were obtained using the Infinium Human Methylation 450k BeadChip and blood pressures were measured during clinical visits. Linear regression or mixed models were used to identify differentially methylated CpG sites associated with BP. Univariate and bivariate structural equation modeling was used to further investigate to what extent the genetic and environmental factors influence DNA methylation and blood pressure in the Finnish Twin Cohort and the Netherlands Twin Register. Our study identifies 13 more CpG sites with their methylation levels associated with BP and replicated 21 previously identified signals. Univariate twin modeling showed that genetic factors contributed to the methylation variations of all the 34 CpG sites with heritability estimates ranging from 31% to 78%. Bivariate twin modeling showed that 100% of the correlations of systolic BP with cg19693031 (*TXNIP*) and cg00716257 (*JDP2*) were determined by environmental effects acting on both systolic BP and methylation levels, rather than genetic factors.