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Article

DNA Methylation Mediated the Association of Body Mass Index With Blood Pressure in Chinese Monozygotic Twins

Jie Yao[1,2], Feng Ning[3], Weijing Wang[1] and Dongfeng Zhang[1]

[1]Department of Epidemiology and Health Statistics, Public Health College, Qingdao University, Qingdao, Shandong, China, [2]Jiangsu Health Development Research Center, Nanjing, Jiangsu Province, China and [3]Qingdao Centers for Disease Control and Prevention/Qingdao Institute of Preventive Medicine, Qingdao, Shandong, China

Abstract

Obesity is an established risk factor for hypertension, but the mechanisms are only partially understood. We examined whether body mass index (BMI)-related DNA methylation (DNAm) variation would mediate the association of BMI with blood pressure (BP). We first conducted a genomewide DNA methylation analysis in monozygotic twin pairs to detect BMI-related DNAm variation and then evaluated the mediating effect of DNAm on the relationship between BMI and BP levels using the causal inference test (CIT) method and mediation analysis. Ontology enrichment analysis was performed for CpGs using the GREAT tool. A total of 60 twin pairs for BMI and systolic blood pressure (SBP) and 58 twin pairs for BMI and diastolic blood pressure (DBP) were included. BMI was positively associated with SBP ($\beta = 1.86$, $p = .0004$). The association between BMI and DNAm of 85 CpGs reached $p < 1\times10^{-4}$ level. Eleven BMI-related differentially methylated regions (DMRs) within *LNCPRESS1*, *OGDHL*, *RNU1-44P*, *NPHS1*, *ECEL1P2*, *LLGL2*, *RNY4P15*, *MOGAT3*, *PHACTR3*, and *BAI2* were found. Of the 85 CpGs, 9 mapped to *C10orf71-AS1*, *NDUFB5P1*, *KRT80*, *BAI2*, *ABCA2*, *PEX11G* and *FGF4* were significantly associated with SBP levels. Of the 9 CpGs, 2 within *ABCA2* negatively mediated the association between BMI and SBP, with a mediating effect of −0.24 (95% CI [−0.65, −0.01]). BMI was also positively associated with DBP ($\beta = 0.60$, $p = .0495$). The association between BMI and DNAm of 193 CpGs reached $p < 1\times10^{-4}$ level. Twenty-five BMI-related DMRs within *OGDHL*, *POU4F2*, *ECEL1P2*, *TTC6*, *SMPD4*, *EP400*, *TUBA1C* and *AGAP2* were found. Of the 193 CpGs, 33 mapped to *ABCA2*, *ADORA2B*, *CTNNBIP1*, *KDM4B*, *NAA60*, *RSPH6A*, *SLC25A19* and *STIL* were significantly associated with DBP levels. Of the 33 CpGs, 12 within *ABCA2*, *SLC25A19*, *KDM4B*, *PTPRN2*, *DNASE1*, *TFCP2L1*, *LMNB2* and *C10orf71-AS1* negatively mediated the association between BMI and DBP, with a total mediation effect of −0.66 (95% CI [−1.07, −0.30]). Interestingly, BMI might also negatively mediate the association between the DNAm of most CpG mediators mentioned above and BP. The mediating effect of DNAm was also found when stratified by sex. In conclusion, DNAm variation may partially negatively mediate the association of BMI with BP. Our findings may provide new clues to further elucidate the pathogenesis of obesity to hypertension and identify new diagnostic biomarkers and therapeutic targets for hypertension.

Keywords: BMI; Blood pressure; DNA methylation; Mediation; Twin studies

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Hypertension is a chronic noncommunicable disease, and approximately 90% of cases are considered as essential hypertension without a definitive cause. In China, there is a high prevalence of hypertension (24.7%) in adults aged 18−69 years, but the awareness, treatment and control of hypertension are low (Zhang et al., 2023). Hypertension has now become a considerable risk factor for reducing life expectancy, being a huge burden for the nationwide health system (Wang et al., 2016).

As a complex phenotype, hypertension is controlled by the interplay between genetic and environmental factors mediated by epigenetic modification. At present, although considerable research progress into the genetics on hypertension has been achieved (J. Chen et al., 2021; Franceschini et al., 2013; Ganesh et al., 2013; Kato et al., 2015; Wain et al., 2011), the specific pathogenesis underlying hypertension still needs to be further elucidated. It has been reported that obesity may play an important role in the pathogenesis of hypertension. One of the mechanisms is that the increase of visceral adiposity could lead to a proinflammatory, pro-oxidative milieu that promotes insulin resistance, and the insulin resistance in skeletal muscle tissue could action on the endothelium by vascular rarefaction, reductions in vascular relaxation, and vascular remodeling, thus further influencing blood pressure (BP) levels (Rao et al., 2015). Another mechanism by which obesity may affect the development of hypertension may be related to an imbalance or lack of number of and function of immune cells, such as the neutrophils, in the adipose tissues of obese individuals (J. Li et al., 2023). Although obesity is now an established risk factor for hypertension, the mechanisms are yet partially understood.

In recent years, increasing evidence has supported the significant role of epigenetic modification, such as DNA methylation (DNAm) variation underlying complex phenotypes. Currently, a functional role of DNAm variation in the development of hypertension or

Corresponding author: Dongfeng Zhang; Email: zhangdf1961@126.com

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regulation of BP has gradually been demonstrated (Demura & Saijoh, 2017; Liang, 2018; Wang, Yao, Li et al., 2023). Moreover, the association of DNAm variation with obesity has also been widely studied (van Dijk et al., 2015). Furthermore, obesity could induce insulin resistance via changes in the DNAm profile of insulin pathway genes, and the DNAm profile changed depending on BMI (Małodobra-Mazur et al., 2019), while the relationship between insulin resistance and hypertension has previously been proved (Rao et al., 2015). However, to date, very few studies have investigated whether obesity (or BMI)-related DNAm variation can mediate the association between BMI and BP.

Nowadays, the trait or disease-discordant monozygotic (MZ) twin design has been a powerful tool for epigenetic studies while controlling for individual genetic components (W. Li et al., 2018; Tan et al., 2015). The Chinese population differ in the genetic make-up, environmental exposure and lifestyles from other ethnic populations worldwide. Considering that BMI and BP are separately important indicators of obesity and hypertension, we here aimed to evaluate the mediating effect of BMI-related DNAm on the relationship of BMI and BP by a causal inference test (CIT) and mediation analysis in a sample of Chinese MZ twins.

Materials and Methods

The primary materials and methods of this study were in accord with those reported in our previously published studies (Wang, Li et al., 2023; Wang, Yao, Li et al., 2023; Wang, Yao, Tan et al., 2023).

Participants

Participants' recruitment and collection have been described previously (Duan et al., 2013). The participants who were pregnant or breastfeeding, or who suffered from heart disease, stroke and/or tumor, or who were regularly taking any medications within one month before participation were excluded. Meanwhile, participants who were unable or unwilling to cooperate were also excluded. In the analysis with systolic blood pressure (SBP) as the outcome, we excluded 8 pairs of twins. In the analysis with diastolic blood pressure (DBP) as the outcome, we excluded 10 pairs of twins. A total of 60 SBP-discordant and 58 DBP-discordant MZ twin pairs were finally included, with a median of absolute values of intrapair trait difference of 17 mmHg (95% range: 1, 50) for SBP and 10 mmHg (95% range: 2, 25) for DBP respectively.

All co-twin pairs completed a questionnaire and undertook a health examination after a 10−12 hour overnight fast. The twins' zygosity was determined by a combination of sex, ABO blood types, and DNA testing (Wang, Yao, Li et al., 2023). The measurement of BMI and BP has been described in our previous studies (Z. Li et al., 2021; Wang, Yao, Li et al., 2023). The ethical principles of the Helsinki Declaration were followed, and prior written informed consent was achieved.

Reduced Representation Bisulfite Sequencing Experiment

As described in our previous studies (Wang, Li et al., 2023; Wang, Yao, Li et al., 2023), total DNA extracted from whole blood was tested by the reduced representation bisulfite sequencing (RRBS) experiment (Biomarker Biological Technology in Beijing, China). Briefly, genomic DNA was first digested to generate short fragments that were then chosen and bisulfite-converted. Subsequently, a cDNA library was constructed and sequenced to obtain raw sequencing data that covered 551,447 CpGs across the genome of each individual. The raw sequencing data was mapped to the human GRCh37 by Bismark (Krueger & Andrews, 2011) and then imported to BiSeq to smooth the methylation level (Hebestreit et al., 2013). After quality control, a total of 248,593 CpGs for SBP and 249,300 CpGs for DBP remained for subsequent analyses. The methylation β value was transformed to M value by applying $\log_2$ transformation with $M = \log_2(\beta/[1-\beta])$ (Du et al., 2010).

Since total DNA was extracted from whole blood, the methylation profiles of distinct cell-types might cause false discoveries (Jaffe & Irizarry, 2014). The top five components of *ReFACTor* method were chosen as covariates to correct for the cell-type composition effect on DNAm in this analysis (Rahmani et al., 2016). The top five components of *ReFACTor* method for SBP and DBP are separately provided in Table S1 and Table S2.

Statistical Analysis

Mediation analyses. We first used a partial correlation analysis model to examine the correlation between intrapair BMI difference and intrapair blood pressure difference, with adjusting for sex and age. Then we applied the CIT method and mediation analysis to evaluate the mediating effect of DNAm on the association between BMI and BP levels (Millstein et al., 2009). For each individual CpG, four models were separately fitted in the CIT method: (1) Model 1 assessed the association of BMI and BP by using generalized estimating equation (GEE) through geeglm function in R-package geepack, with BP as the outcome and BMI as the predictor, adjusting for age and sex. (2) Model 2 assessed the association of BMI and DNAm of each CpG by conducting a genomewide DNA methylation analysis. We fitted this model by using the GEE while controlling for BP, with DNAm of each CpG as the outcome and BMI as the predictor, adjusting for age, sex, cell-type composition as well as BP. The Manhattan plot was drawn. To correct for multiple testing, we calculated false discovery rate (FDR) (Benjamini & Hochberg, 2018) and defined FDR < 0.05 as genomewide significance. For CpGs with FDR ≥ 0.05, we defined p value $< 1\times10^{-6}$ as suggestive significance and $1\times10^{-6} \leq p$ value $< 1\times10^{-5}$ as weaker-than-suggestive significance (S. Li et al., 2021). The CpGs with p value $< 1\times10^{-4}$ were reported as top CpGs in our study and were selected for subsequent analysis (Mohammadnejad et al., 2021; Wang, Li et al., 2023). (3) Model 3 assessed the association of DNAm of each CpG with BP by using the GEE while controlling for BMI, with BP as the outcome and DNAm of each CpG as the predictor, adjusting for age, sex, cell-type composition as well as BMI. The CpGs with p values of association $<$ .05 were chosen for subsequent analysis (Wang, Li et al., 2023). (4) This model assessed the independence of BMI and BP by using R-package cit, adjusting for age, sex and cell-type composition as well as DNAm of each CpG identified in Model 3. If the median of all p values was more than .05 after conducting 1000 simulations using the Permutation method, it was considered that the BMI and SBP/DBP was insignificant. In the above four CIT models, in order to address the paired structure of twin data, we included a vector that identified the clusters of twins within a pair into the GEE model.

For the candidate CpG mediators obtained through the CIT method, we further verified their mediating effect on the association of BMI with BP by using R-package *mediation* (Tingley et al., 2014). We used 5000 simulations to calculate estimates and 95% confidence intervals (95% CIs). For each CpG, the average causal mediation effect (ACME), average direct effect (ADE), and proportion mediated (or absolute value of ratio of

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indirect effect to direct effect) were calculated. If the lower limit (LL) to the upper limit (UL) in the 95% CIs for the ACME of a particular CpG did not include zero, this CpG was considered to significantly mediate the association between BMI and BP. Additionally, we also tested the parallel mediating effects of DNAm mediators between BMI and BP by employing a bootstrap method using PROCESS macro in SPSS program and reported the total mediation effect of DNAm mediators. This approach allows researchers to investigate multiple mediator variables simultaneously, rather than focusing on a single mediator's impact. The above analyses were also performed stratified by sex. Moreover, whether BMI could also mediate the association between DNAm of CpGs and BP was further evaluated.

Region-based analysis. As described previously (Wang, Li et al., 2023; Wang, Li, Jiang et al., 2021; Wang, Li, Wu et al., 2021; Wang, Yao, Li et al., 2023), we applied the *comb-p* tool to detect the BMI-associated differentially methylated regions (DMRs) using the CpGs with p values < .05 identified in Model 2 (Pedersen et al., 2012). The significant enriched DMRs were determined by *slk* corrected p value < .05.

Ontology enrichments analysis. We submitted the CpGs with p values < .05 identified in Model 2 and the candidate CpG mediators identified in CIT method to Genomic Regions Enrichment of Annotations Tool (GREAT) online to analyze the functional ontology enrichments (McLean et al., 2010). The significant ontology enrichments were judged by FDR < 0.05.

Study power estimation. We have published a computer simulation study on the power of methylation analysis using twin design (W. Li et al., 2018). According to this study, if one trait or disease had a heritability (h^2) of .6 and there was a low correlation between environmental factors and DNAm ($R^2_{M,\ E} = .1$), the sample size required for statistical power to exceed 80% in a trait/disease-discordant twin design would range from 22 (when the correlation within twin pair due to either shared genetic background or common environment, denoted as $\rho_\varepsilon = .8$) to 63 (when $\rho_\varepsilon = .1$) pairs, which was an immense improvement over the ordinary case-control design. The heritability was about .76 for BMI (Duan et al., 2011) and .60 for BP (Wu et al., 2015) in the same twin population as our study; thus we speculated that our study based on nearly 60 twin pairs would get a statistical power of about 80%.

Results

Characteristics of Participants

As shown in Table 1, a total of 60 twin pairs for SBP and 58 twin pairs for DBP were included in this study, with the medians of 134.00 mmHg (95% range: 102.05, 184.90) for SBP and 80.00 mmHg (95% range: 62.00, 105.03) for DBP respectively. The mean of BMI was all about 25 kg/m^2 (*SD* 3.5). Most of the clinical indicators, including serum uric acid, fasting glucose, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol and triglyceride showed intrapair statistically correlated, implying the co-twin design beneficial.

Mediation Analyses

Mediating effect of DNAm on association between BMI and SBP. *Association of BMI and SBP*

The intrapair BMI difference in twins was positively correlated with intrapair SBP difference ($r = .338$, $p = .010$). BMI was positively associated with SBP levels ($\beta = 1.86$, $p = .0004$) in twins using the GEE in Model 1.

Association of BMI and DNAm while controlling for SBP. The Manhattan plot of genomewide DNA methylation analysis on BMI is shown in Figure S1(a). We identified 85 BMI-related top CpGs ($p < 1\times10^{-4}$), of which 18 CpGs showed weaker-than-suggestive significance ($1\times10^{-6} \le p < 1\times10^{-5}$) and 5 CpGs reached genome-wide significance (FDR < 0.05). In the top 20 CpGs shown in Table S3, the five strongest associations were detected for the CpGs located at *CAMK4* ($\beta = -0.74$) and *OGDHL* ($\beta = 0.04–0.05$). These top 20 CpGs were located at or near six genes, including *CAMK4*, *OGDHL*, *KCNQ1*, *BTBD19*, *FEZF2* and *AXDND1*.

We found 11 DMRs potentially related to BMI (Table 2). As illustrated in Figure 1(a), most DMRs were hypermethylated with increasing BMI, whereas the methylation levels of 3 DMRs (DMR-5, 6, 11) were negatively correlated with BMI. But the associations of another DMR (DMR-9) with BMI were uncertain. Interestingly, one DMR within *OGDHL* also covered the strongest DNAm signals listed in Table S3.

Many GREAT ontology enrichments potentially related to BMI were found for the identified CpGs with p values < .05, including p53 pathway by glucose deprivation, hedgehog signaling pathway, notch signaling pathway and so forth (Figure S2).

Association of DNAm with SBP while controlling for BMI. The DNAm at 9 CpGs mapped to *C10orf71-AS1*, *NDUFB5P1*, *KRT80*, *BAI2*, *ABCA2*, *PEX11G*, and *FGF4* was significantly associated with SBP levels ($p < .05$) in Model 3 (Table S4). These CpGs were mainly involved in voltage-gated chloride channel activity and regulation of cholesterol transport (Figure S3).

Correlation of BMI and SBP while controlling DNAm. The medians of p values for CpGs identified in Model 3 were all more than .05, indicating that BMI was independent of SBP while controlling for DNAm.

Mediation effect of candidate CpG mediators. The results of testing mediation effects are shown in Table 3. We found that two CpGs mapped to *ABCA2* could mediate the association between BMI and SBP by using R-package *mediation*. The DNAm of individual CpG was with an ADE of about 1.97 (95% CI [0.98, 2.96])-mmHg greater SBP levels and an ACME of about 0.24 (95% CI [0.65, 0.01])-mmHg lower SBP levels. However, the total mediation effect (−0.23, 95% CI [−0.66, 0.05]) of these two DNAm mediators did not show statistically significance in the parallel mediation model. Interestingly, BMI also negatively mediated the association between the DNAm of these two CpGs and SBP, with an ACME of −1.6748 (95% CI [−3.6883, −0.2662]) and −2.3493 (95% CI [−4.9707, −0.4547]) respectively (Table S5).

Mediation analyses in different sexes. In male twins, BMI was positively associated with SBP level ($\beta = 1.68$, $p = .002$) in Model 1. The association between BMI and DNAm of 261 CpGs reached $p < 1\times10^{-5}$ level in Model 2, of which the DNAm at 99 CpGs was significantly associated with SBP level ($p < .05$) in Model 3. The CpGs mapped to *ZNF579*, *FOXQ1* and *ENSG00000271788* negatively mediated the association between BMI and SBP, with a total negative mediation effect of −2.18 (95% CI [−3.99, −1.07]) (Table S6).

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Table 1. Basic characteristics of the participants

	BMI-SBP			BMI-DBP		
		Intrapair correlation			Intrapair correlation	
Characteristics	Values	*r*	*p* value	Values	*r*	*p* value
Number of twin pairs	60			58		
Sex, *n* (%)						
Male	32 (53)	–	–	30 (52)	–	–
Female	28 (47)	–	–	28 (48)	–	–
Age, years	52 (40, 66)	–	–	52 (40, 66)	–	–
BMI, kg/m^2	24.92 (3.49)	.571	<.001	24.89 (3.46)	.564	<.001
BP, mmHg	134.00 (102.05, 184.90)	.408	.001	80.00 (62.00, 105.03)	.177	.181
SUA, μmol/L	292.60 (93.57)	.427	.001	277.50 (152.63, 521.68)	.531	<.001
GLU, mmol/L	5.40 (3.60, 10.73)	.608	<.001	5.41 (3.60, 10.81)	.654	<.001
CHOL, mmol/L	4.96 (1.21)	.569	<.001	4.90 (1.18)	.548	<.001
TG, mmol/L	1.15 (0.20, 5.65)	.604	<.001	1.12 (0.32, 5.66)	.582	<.001
HDLC, mmol/L	1.31 (0.67, 2.56)	.769	<.001	1.36 (0.67, 2.71)	.771	<.001
LDLC, mmol/L	2.92 (0.90)	0.494	< 0.001	2.85 (0.86)	0.466	< 0.001

Note: Continuous variables were presented as mean (standard deviation (SD)) or median ($P_{2.5}$, $P_{97.5}$); Categorical variables were presented as numbers with percentages.Intrapair correlations were tested by Pearson or Spearman correlation method.
BMI, body mass index; BP, blood pressure; CHOL, total cholesterol; DBP, diastolic blood pressure; GLU, fasting glucose; HDLC, high-density lipoprotein cholesterol; LDLC, low-density lipoprotein cholesterol; SBP, systolic blood pressure; SUA, serum uric acid; TG, triglyceride

Table 2. The results of annotation to significant differentially methylated regions for BMI

DMR ID	Chromosome	Start (bp)	End (bp)	Length	*slk* corrected *p* value	Gene symbol
BMI-SBP						
A	chr7	100,943,139	100,943,685	12	1.02E-04	*LNCPRESS1*
B	chr10	50,976,601	50,977,082	40	1.31E-04	*OGDHL*
C	chr4	147,558,368	147,558,515	9	1.24E-03	*RNU1-44P*
D	chr12	133,022,301	133,022,591	12	3.68E-03	NA
E	chr19	36,351,441	36,351,997	33	6.90E-03	*NPHS1*
F	chr2	233,251,538	233,252,184	30	1.24E-02	*ECEL1P2*
G	chr17	73,522,435	73,522,746	15	1.28E-02	*LLGL2*
H	chr2	88,583,665	88,584,027	18	2.55E-02	*RNY4P15*
F	chr7	100,841,039	100,841,638	15	2.65E-02	*MOGAT3*
G	chr20	58,180,078	58,180,700	19	3.33E-02	*PHACTR3*
H	chr1	32,196,460	32,196,632	10	3.76E-02	*BAI2*
BMI-DBP						
A	chr10	50,976,601	50,977,082	40	8.72E-07	*OGDHL*
B	chr4	147,558,368	147,558,515	9	1.52E-04	*POU4F2*
C	chr2	233,251,538	233,252,184	30	2.84E-04	*ECEL1P2*
D	chr14	38,080,315	38,080,698	33	1.18E-03	*TTC6*
E	chr2	130,937,526	130,937,631	7	1.31E-03	*SMPD4*
F	chr12	132,553,984	132,554,103	9	1.83E-03	*EP400*
G	chr12	49,659,788	49,659,928	10	3.59E-03	*TUBA1C*
H	chr12	58,120,476	58,120,621	19	8.33E-03	*AGAP2*
F	chr12	133,022,301	133,022,591	12	8.73E-03	NA
G	chr1	32,196,460	32,196,632	10	9.97E-03	*BAI2*
H	chr14	34,269,360	34,270,338	86	1.81E-02	*NPAS3*

(*Continued*)

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Table 2. (*Continued*)

DMR ID	Chromosome	Start (bp)	End (bp)	Length	*slk* corrected *p* value	Gene symbol
I	chr9	99,984,209	99,984,354	9	1.89E-02	*ANKRD18CP*
J	chr21	38,068,931	38,069,683	14	1.92E-02	*SIM2*
K	chr7	100,841,464	100,841,638	14	2.13E-02	*MOGAT3*
L	chr7	35,297,006	35,297,434	28	2.51E-02	*TBX20*
M	chr14	101,035,539	101,035,909	15	2.57E-02	*BEGAIN*
N	chr5	146,888,814	146,888,944	8	2.67E-02	*DPYSL3*
O	chr1	2,987,521	2,987,647	16	3.16E-02	*PRDM16*
P	chr13	36,049,335	36,050,293	28	3.39E-02	*NBEA*
Q	chr13	112,902,186	112,902,357	10	3.69E-02	*LINC01070*
R	chr1	44,399,746	44,400,183	13	3.77E-02	*ARTN*
S	chr16	3,534,860	3,535,154	17	4.09E-02	*NAA60*
T	chr17	76,661,168	76,661,323	11	4.09E-02	*CYTH1*
U	chr19	56,126,297	56,126,437	19	4.70E-02	*ZNF865*
V	chr7	36,007,041	36,007,468	33	4.73E-02	NA

Note: bp, base pair; DMR, differentially methylated region; DBP, diastolic blood pressure; Length, the number of CpGs in each DMR; SBP, systolic blood pressure; *slk*, Stouffer-Liptak-Kechris; NA, not available.

In female twins, BMI was also positively associated with SBP level ($\beta = 2.03$, $p = .021$). The association between BMI and DNAm of 473 CpGs reached $p < 1\times10^{-5}$ level, of which the DNAm of 203 CpGs was significantly associated with SBP level ($p < .05$). The CpGs within *RSPH6A*, *CPZ*, *UFC1*, *RN7SL831P*, *SMAD6* and *TUBB4BP6* could negatively mediate the association between BMI and SBP in female twins, with a total mediation effect of −1.61 (95% CI [−3.54, −0.17]) (Table S6).

Mediating effect of DNAm on association between BMI and DBP. *Association of BMI and DBP*

The intrapair BMI difference in twins was positively correlated with intrapair DBP difference (r =.400, p = .002). BMI was positively associated with DBP levels ($\beta = 0.60$, $p = .0495$) in twins using the GEE in Model 1.

Association of BMI and DNAm while controlling for DBP. The Manhattan plot of genomewide DNA methylation analysis on BMI is shown in Figure S1(b). We identified 193 BMI-related top CpGs ($p < 1\times10^{-4}$), of which 55 CpGs reaching genomewide significance (FDR < 0.05). As shown in Table S3, the strongest associations were detected for the CpGs located at *OGDHL* ($\beta = 0.06$). The top 20 CpGs were located at or near *OGDHL*, *PEX11G*, *RSPH6A*, *RXRA*, *RNF126*, *MED27* and *POU4F2*.

We found 25 DMRs potentially related to BMI (Table 2). As shown in Figure 1(b), most DMRs were hypermethylated with increasing BMI, whereas the methylation levels of seven DMRs (DMR-3, 5, 6, 7, 8, 10, 12) were negatively correlated with BMI. However, the relationship between another five DMRs (DMR-11, 13, 15, 21, 22) and BMI were unclear. Interestingly, two DMRs falling into *OGDHL* and *POU4F2* also covered the top DNAm signals listed in Table S3.

Many GREAT ontology enrichments, such as p53 pathway by glucose deprivation and PI3 kinase pathway, potentially related to BMI, were found for the identified CpGs (p value < .05) (Figure S2).

Association of DNAm with DBP while controlling for BMI. The DNAm at 33 CpGs mapped to *ABCA2*, *ADORA2B*, *CTNNBIP1*, *KDM4B*, *NAA60*, *RSPH6A*, *SLC25A19* and *STIL*, etc. was significantly associated with DBP levels ($p < .05$) in Model 3 (Table S4). These CpGs were mainly involved in regulation of chronic inflammatory response and ABCA transporters in lipid homeostasis. (Figure S3).

Correlation of BMI and DBP while controlling DNAm. The medians of p values for all CpGs identified in Model 3 were more than .05, indicating that BMI was independent of DBP while controlling for DNAm.

Mediation effect of candidate CpG mediators. As shown in Table 3, the DNAm at 12 CpGs could negatively mediate the association between BMI and DBP, with a total mediation effect of −0.66 (95% CI [−1.07, −0.30]) in the parallel mediation model. The DNAm of 3 CpGs mapped at *ABCA2* was with an ACME of about −0.19 (95% CI [−0.45, −0.02]), 2 CpGs mapped at *KDM4B* with an ACME of about −0.17 (95% CI [−0.39, −0.02]), and 2 CpGs mapped at *SLC25A19* with an ACME of about −0.14 (95% CI [−0.34, −0.0006]), and so forth. Interestingly, BMI also negatively mediated the association between the DNAm of most CpGs mentioned above and DBP, with an ACME ranging −0.9111 to −0.3485 (Table S5).

Mediation analyses in different sexes. In male twins, BMI was positively associated with DBP level ($\beta = 1.23$, p =.004) in Model 1. The association between BMI and DNAm of 207 CpGs reached $p < 1\times10^{-5}$ level in Model 2, of which the DNAm at 160 CpGs was significantly associated with DBP level ($p < .05$) in Model 3. Five CpGs mapped to *MAU2* and *CHRNE* negatively mediated the association between BMI and DBP in male twins, with a total negative mediation effect of −0.3 (95% CI [−1.65, −1.42]) (Table S6). However, the association of BMI with DBP level was

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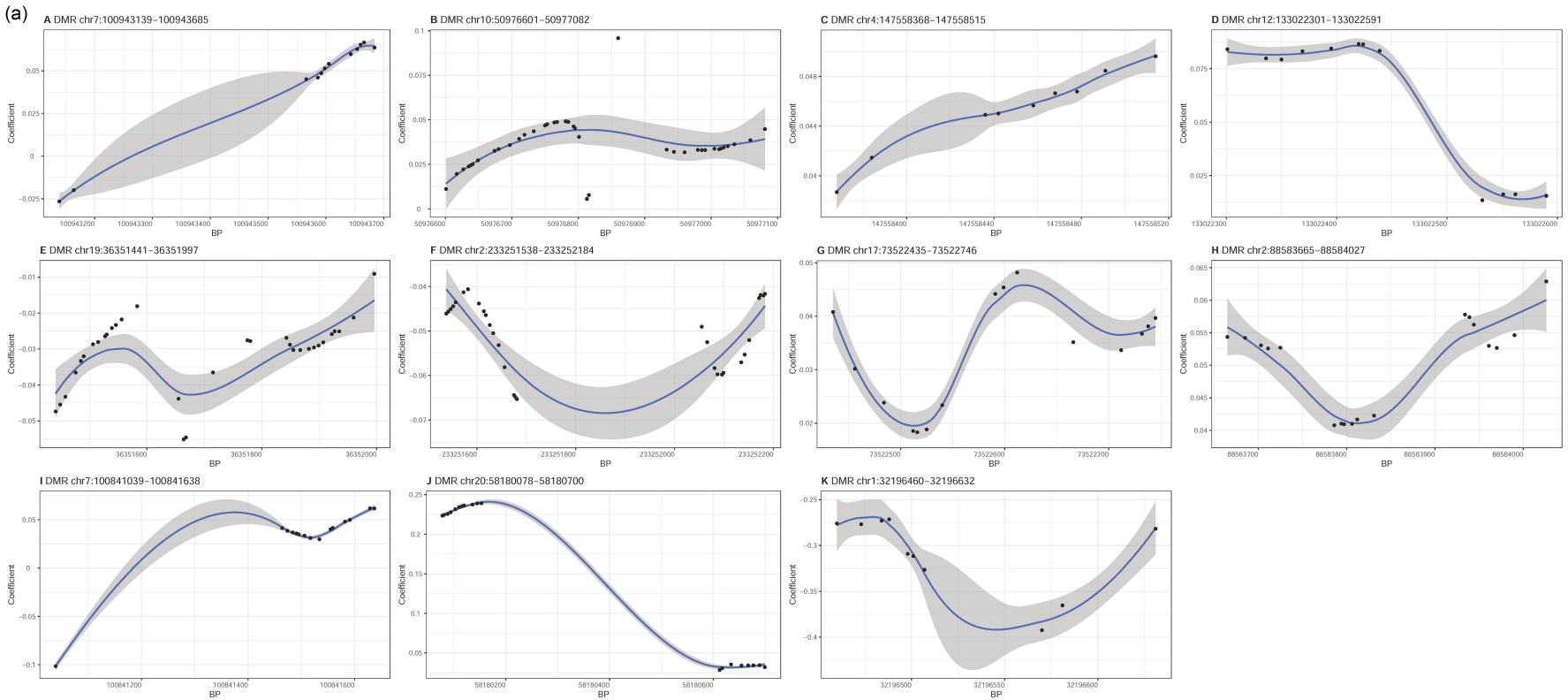

Figure 1. Circular Manhattan plots for genomewide DNA methylation analysis of BMI. (a) BMI-systolic blood pressure; (b) BMI-diastolic blood pressure. The numbers of chromosome and the $-\log_{10}$ of *p* values for statistical significance are shown. The dots represent the observed CpGs.

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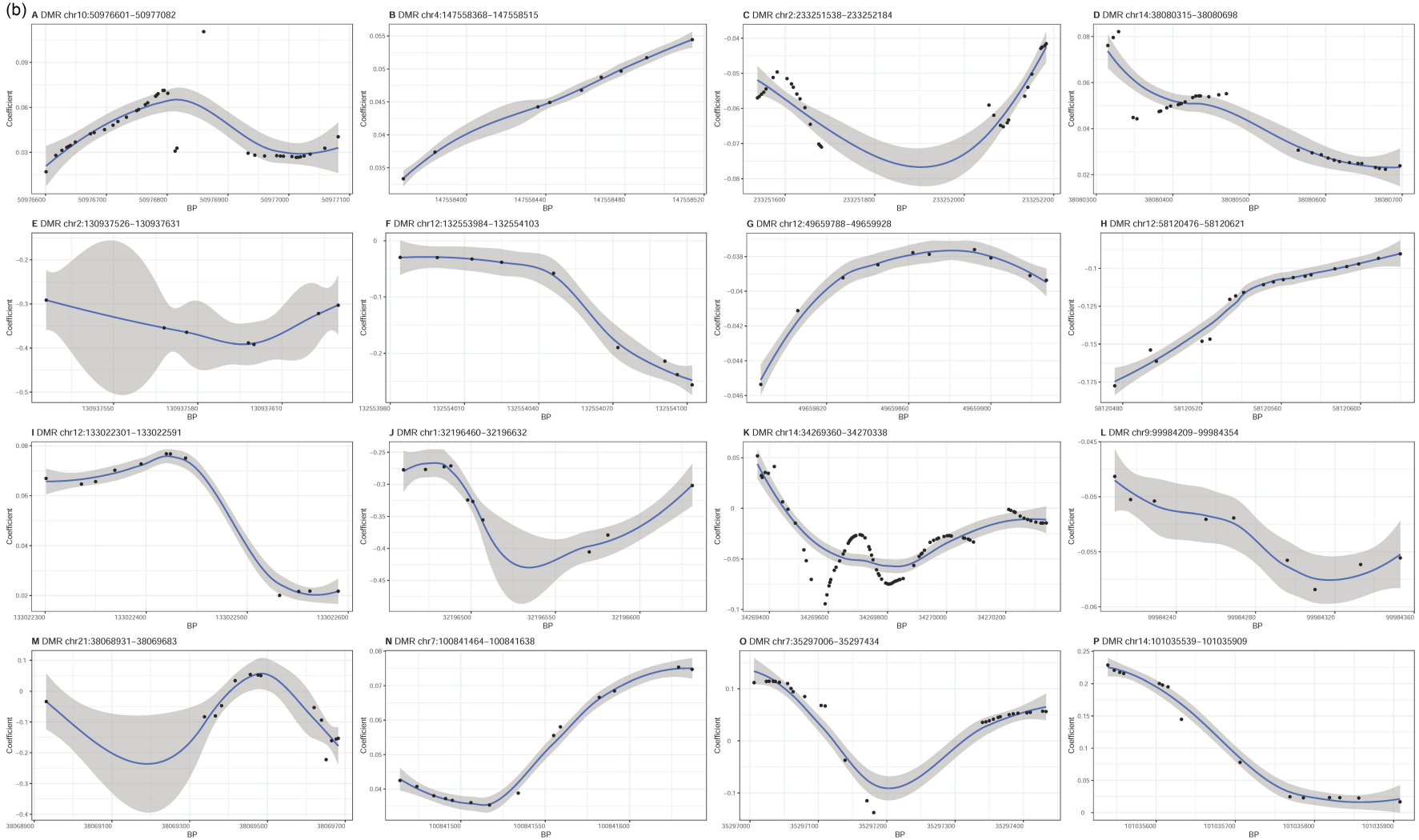

Figure 1. Continued.

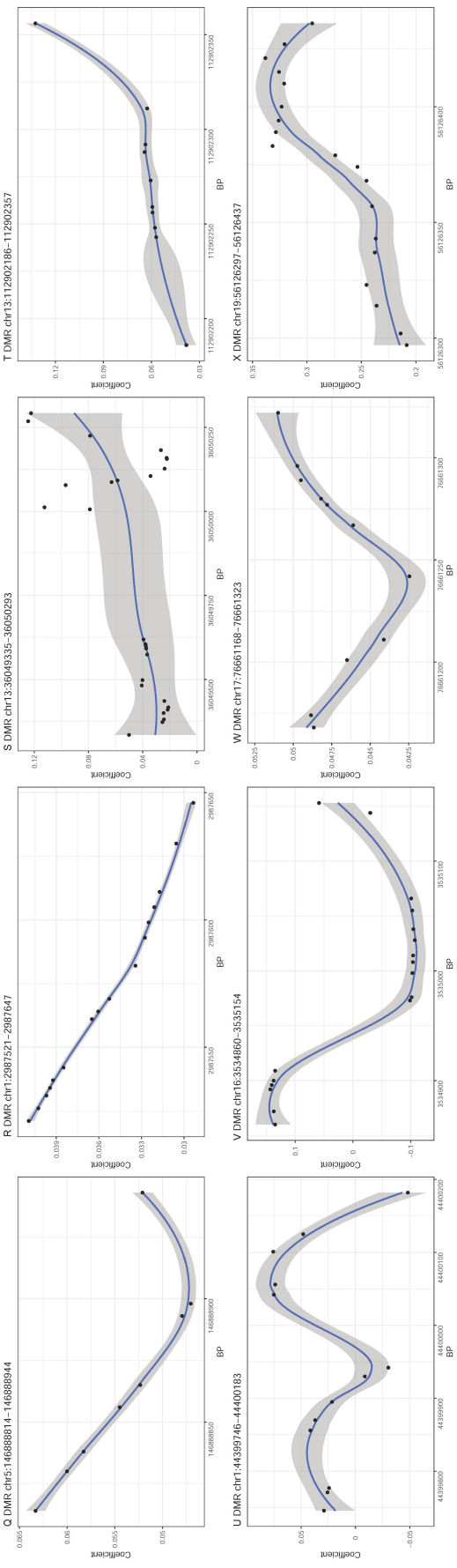

Figure 1. Continued.

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Table 3. DNA methylation mediators for the association of BMI and blood pressure in single and parallel mediation model

CpGs annotation				Direct effects		Average/total mediated effects of DNAm		Absolute value of ratio of indirect effect to direct effect*
CpGs No.	Chromosome	Position (bp)	Gene symbol	β	95% CI	β	95% CI	
BMI→DNAm→SBP								
CpG1#	chr9	139,902,785	*ABCA2*	1.9691	(0.9810, 2.9572)	−0.2437	(−0.6513, −0.0080)	12.38%
CpG2#	chr9	139,902,796	*ABCA2*	1.9742	(0.9790, 2.9695)	−0.2488	(−0.6493, −0.0123)	12.60%
Parallel mediation model	–	–	–	1.9569	(0.9588, 2.9550)	−0.2315	(−0.6627, 0.0499)	11.83%
BMI→DNAm→DBP								
CpG1#	chr19	2,434,321	*LMNB2*	0.6702	(0.1053, 1.2350)	−0.1959	(−0.4070, −0.0357)	29.23%
CpG2#	chr19	5,070,737	*KDM4B*	0.6448	(0.0789, 1.2108)	−0.1706	(−0.4018, −0.0172)	26.46%
CpG3#	chr10	50,489,592	*C10orf71-AS1*	0.7506	(0.1998, 1.3013)	−0.2763	(−0.5326, −0.0822)	36.81%
CpG4#	chr19	5,070,724	*KDM4B*	0.6481	(0.0787, 1.2175)	−0.1738	(−0.3870, −0.0157)	26.82%
CpG5#	chr16	3,706,626	*DNASE1*	0.7150	(0.1497, 1.2803)	−0.2408	(−0.5030, −0.0493)	33.68%
CpG6#	chr2	122,014,456	*TFCP2L1*	0.6778	(0.1174, 1.2382)	−0.2036	(−0.3886, −0.0416)	30.04%
CpG7	chr17	73,279,760	*SLC25A19*	0.6382	(0.0755, 1.2009)	−0.1639	(−0.3731, −0.0034)	25.68%
CpG8	chr17	73,279,753	*SLC25A19*	0.6184	(0.0551, 1.1816)	−0.1441	(−0.3392, −0.0006)	23.30%
CpG9	chr7	158,217,257	*PTPRN2*	0.7062	(0.1468, 1.2656)	−0.2319	(−0.4823, −0.0726)	32.84%
CpG10#	chr9	139,902,796	*ABCA2*	0.6626	(0.0870, 1.2381)	−0.1883	(−0.4476, −0.0166)	28.42%
CpG11#	chr9	139,902,785	*ABCA2*	0.6641	(0.0917, 1.2366)	−0.1899	(−0.4508, −0.0216)	28.60%
CpG12#	chr9	139,902,798	*ABCA2*	0.6479	(0.0728, 1.2230)	−0.1736	(−0.4286, −0.0081)	26.79%
Parallel mediation model†	–	–	–	1.1349	(0.5321, 1.7378)	−0.6607	(−1.0656, −0.3034)	58.22%

Note: DBP, diastolic blood pressure; SBP, systolic blood pressure; β, regression coefficient.
*Given that the direct effects and mediated effects showed the opposite direction, the absolute value of ratio of indirect effect to total effect was calculated.
#BMI also mediated the association between DNAm of CpGs and blood pressure.
†Given the characteristics of PROCESS macro in SPSS program, choosing 10 CpGs in the parallel mediation model on the basis of absolute value of regression coefficients.

not statistically significant (β = 0.02, $p = .953$) in female twins, thus the subsequent mediation analysis was not performed.

Discussion

Obesity is an established risk factor for hypertension. Here, based on a sample of BMI and BP-discordant MZ twins, we observed that the DNAm of 2 and 12 CpGs could separately negatively mediate the association of BMI with SBP and DBP, suggesting the negative mediating role of DNAm variation. Interestingly, we found that BMI could also negatively mediate the association between the DNAm of several CpGs and SBP/DBP, highlighting the need to consider whether exposure and mediator were moderators of each other in epigenetic studies.

We found several CpG mediators annotated to *ABCA2*, *KDM4B*, *SLC25A19*, *LMNB2*, *PTPRN2* and *DNASE1* could partially negatively mediate the relationship between BMI and BP. (1) *ABCA2*. The protein encoded by the *ABCA2* gene was involved in cholesterol metabolism and cholesterol transport (Mack et al., 2007), while dyslipidemia was an independent risk factor for atherosclerotic cardiovascular disease (Miao et al., 2023), thus further influencing BP levels. (2) *KDM4B*. It was reported that the protein encoded by *KDM4B* in adipose tissues played a critical role in energy balance, oxidation, lipolysis and thermogenesis, and might serve as a therapeutic target for treatment of obesity (Cheng et al., 2018). *KDM4B* was also involved in estrogen-mediated signaling pathway that played a role underlying the vasculature during hypertension and other cardiovascular diseases (Visniauskas et al., 2023). (3) *SLC25A19*. The protein encoded by the *SLC25A19* gene was a member of the solute carrier family and was involved in vitamin B_1 metabolism process, while the vitamin B_1 level was reported to be negatively related to obesity (Fu et al., 2023). Moreover, the vitamin B_1 deficiency was also related to some cardiovascular traits, such as elevated cardiac stroke volume, decreased vascular resistance, and elevated pressor responses, suggesting a possible role in influencing BP levels (DiNicolantonio et al., 2018). (4) *LMNB2*. The role of LMNB2 protein in regulating polyploidization of cardiomyocyte nuclei and myocardial regeneration has been reported in an animal experiment (Han et al., 2020). The RNA m^6A methylation of *LMNB2* was reported to be strongly associated with diabetes (C. Chen et al., 2021), but the association of *LMNB2* with obesity is still unknown. (5) *PTPRN2*. This gene encodes a protein with a sequence similarity to receptor-like protein tyrosine phosphatases. It was reported that the *PTPRN2* in the blood of newborn term infants born to mothers with obesity showed differential methylation compared to infants from mothers with a normal BMI (Sasaki et al., 2022). In addition, some DNAm variants of *PTPRN2* might

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be related to the renin-angiotensin-aldosterone system (RAAS), which plays a role in the pathophysiology underlying hypertension (van der Linden et al., 2022). Moreover, the variants at the *PTPRN2* were also suggested to be associated with persistent pulmonary hypertension of the newborn (PPHN) (Nakwan et al., 2023). (6) *DNASE1*. The study on the role of *DNASE1* gene in the pathophysiology underlying obesity or hypertension was limited. It might be that *DNASE1* was involved in regulation of acute inflammatory response (GO:0002673 in AmiGO 2 database) and then induced the occurrence of diseases. However, the association of *TFCP2L1* and *C10orf71-AS1* with obesity or hypertension is still unknown. In sum, the DNAm variation of most of the genes we observed might potentially negatively mediate the association of BMI with BP.

A major strength of our study is the implementation of a trait-discordant MZ twin model that was proven to be a powerful tool for methylation studies in linking the environmental basis of epigenetic modification variation to certain traits while controlling for individual genetic makeup (W. Li et al., 2018; Tan et al., 2015). Although a majority of the participants in our study did not have obesity or hypertension, we still observed certain CpG mediators that effectively associated BMI with BP. Additionally, considering that the genetic constitutions, environmental exposures and a multitude of lifestyles in various ethnic populations differed worldwide, our findings will specifically help elucidate the underlying pathogenesis of increased BP levels and hypertension in the Chinese population.

Several limitations should also be mentioned for this study. First, our sample size was relatively limited due to the challenges of recruiting and identifying qualified twin pairs. However, the trait-discordant twin design we adopted had a greater statistical power over the traditional cross-sectional or case-control design. For BMI and BP with a moderate and high heritability (Duan et al., 2011; Wang et al., 2018), this design would allow for large sample size reductions compared to the traditional designs according to the computer simulation study we published (W. Li et al., 2018). Hence, our EWAS had sufficient power of about 80% for detecting the CpG mediators. We will further validate the CpG mediators, genes and biological pathways in a community population. Second, because we performed this study in BMI- and BP-discordant MZ twins rather than obesity- or hypertension-discordant MZ twins, the DNAm mediators we found potentially played a negative mediating effect between BMI and BP. Third, we did not find any replicated results while comparing the CpGs identified in our study with other similar methylation studies because the latter were very limited in number.

In summary, we observed that BMI might interact with DNAm at specific loci, mediating the association with SBP/DBP. Our findings provide important clues to further elucidate the pathogenesis of elevated BP levels and helped to identify new diagnostic biomarkers and therapeutic targets for hypertension.

Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.1017/thg.2024.3.

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Competing interests. The authors declare no competing financial interests.

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