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NPPA Promoter Hypomethylation Predicts Central Obesity Development: A Prospective Longitudinal Study in Chinese Adults

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Atrial natriuretic peptide · Central obesity · DNA methylation

Abstract
Introduction: Atrial natriuretic peptide plays a potential role in obesity with unclear molecular mechanisms. The objective of this study was to examine the association between its coding gene (*natriuretic peptide A* [*NPPA*]) methylation and obesity. ***Methods:*** Peripheral blood DNA methylation of *NPPA* promoter was quantified at baseline by targeted bisulfite sequencing for 2,497 community members (mean aged 53 years, 38% men) in the Gusu cohort. Obesity was repeatedly assessed by body mass index (BMI) and waist circumference (WC) at baseline and follow-up examinations. The cross-sectional, longitudinal, and prospective associations between *NPPA* promoter methylation and obesity were examined. ***Results:*** Of the 9 CpG loci assayed, DNA methylation levels at 6 CpGs were significantly lower in participants with central obesity than those without (all $p < 0.05$ for permutation test). These CpG methylation levels at baseline were also inversely associated with dynamic changes in BMI or WC during follow-up (all $p < 0.05$ for permutation test). After an average 4 years of follow-up, hypermethylation at the 6 CpGs (CpG2 located at Chr1:11908348, CpG3 located at Chr1:11908299, CpG4 located at Chr1:11908200, CpG5 located at Chr1:11908182, CpG6 located at Chr1:11908178, and CpG8 located at Chr1:11908165) was significantly associated with a lower risk of incident central obesity (all $p < 0.05$ for permutation test). ***Conclusions:*** Hypomethylation at *NPPA* promoter was associated with increased future risk of central obesity in Chinese adults. Aberrant DNA methylation of the *NPPA* gene may participate in the mechanisms of central obesity.

Introduction

Obesity, a leading but modifiable risk factor for metabolic and cardiovascular complications, has become a global public health crisis [1–4]. It still affects about 46%

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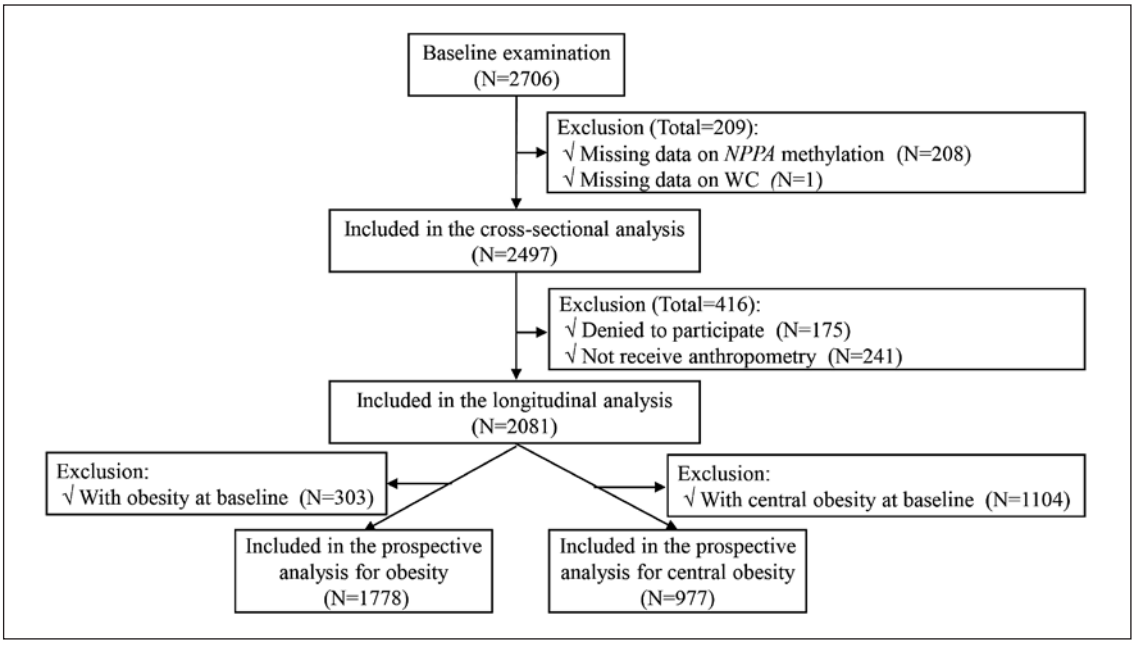

Fig. 1. A flowchart illustrating the selection of study participants.

of adults and 15% of children in China now despite decades of control efforts made, indicating unclear complicated mechanisms of this debilitating disorder [4]. Fundamentally, obesity reflects the imbalance between energy intake and expenditure. Atrial natriuretic peptide (ANP) has been suggested to play a potential role in maintaining weight through enhancing lipolysis, lipid mobilization, oxidation, and inhibiting appetite [5–10]. Bodyweight was significantly increased in ANP knockout mice [11]. In humans, the circulating level of ANP and the polymorphisms of its coding gene – *natriuretic peptide A* (*NPPA*) have been associated with obesity [12–15], and its related complications, e.g., hypertension [16, 17], atherosclerosis [18], diabetes [19, 20], and eating disorders [21]. However, the clinical implications of ANP are still limited [22]. A better understanding of the underlying molecular mechanisms of the energy-regulating effect of ANP may promote its clinical translation. DNA methylation, an important epigenetic modification that links the environment to the fixed genome and regulates gene function and expression [23], may provide some insights into the mechanisms that we are searching for. In fact, DNA methylation status changed dramatically in our lifetime and dysregulated DNA methylation was previously related to obesity in epigenome-wide association studies (EWAS) [24–28]. Although no genome-widely significant CpG locus was found, *NPPA* methylation was found to be associated at gene level with obesity in an EWAS [28]. However, no candidate gene study, to the best of our knowledge, was conducted to examine *NPPA* methylation and obesity. Therefore, we aimed to examine the association, cross-sectional, longitudinal, and prospective, between *NPPA* promoter methylation and obesity in a systematic manner, leveraging a prospective longitudinal cohort of middle-aged and elderly Chinese adults. The prospective analysis may guide causal inference for the role of *NPPA* promoter methylation in obesity.

Methods

Study Participants

The Gusu cohort, initiated in 2010, is a community-based prospective longitudinal study of cardiovascular disease and its risk factors in middle-aged and elderly Chinese adults as described previously [29]. In brief, via cluster random sampling, 8 communities were selected from the total 39 communities in the Gusu District, Suzhou, China. In the selected communities, residents who met the inclusion criteria (i) age: ≥30 years, (ii) ethnicity: Han were recruited and invited to participate in the baseline examination. The research was conducted ethically in accordance with the World Medical Association Declaration of Helsinki. The protocols were approved by the Soochow University Ethics Committee (approval NO. SUDA20100601H02). Written informed consent was obtained from all study participants. Figure 1 describes the selection of study participants included in the current analysis. Of 2,706 individuals participating in the baseline examination, after excluding

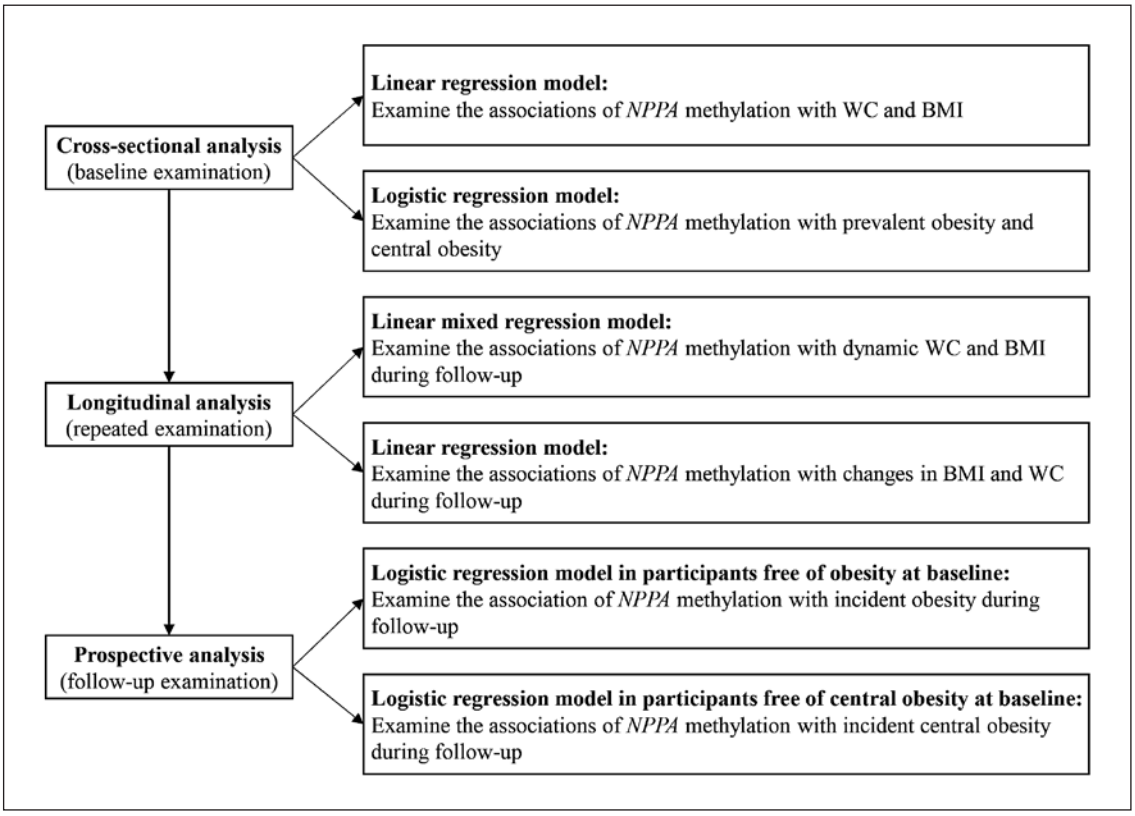

Fig. 2. A flowchart illustrating the statistical plan.

participants with missing data on *NPPA* methylation ($n = 208$) and waist circumference (WC) ($n = 1$), a total of 2,497 participants with complete data on *NPPA* methylation, WC, and body mass index (BMI) included in the cross-sectional analysis. All surviving participants were invited to participate in the follow-up examination 4 years later. After further excluding participants who denied to participate ($n = 175$) or not received anthropometry ($n = 241$) in the follow-up examination conducted in 2014, 2,081 participants were included in the longitudinal analysis. Of these, participants free of obesity ($n = 1,778$) were included in the prospective analysis for the association between *NPPA* methylation and risk of obesity during follow-up and those free of central obesity ($n = 977$) at baseline were included in the prospective analysis for the association between *NPPA* methylation and risk of central obesity during follow-up.

Quantification of NPPA Promoter Methylation

DNA methylation levels in the promoter region of the *NPPA* gene were quantified by target bisulfite sequencing as previously described [30], using genomic DNA isolated from peripheral blood mononuclear cells. In brief, based on the genomic coordinates of the *NPPA* promoter in Genome Reference Consortium Human Build 37 (GRCh37), we carefully designed the primers to detect the maximum CpG loci within the CpG islands. The targeted sequence (Chr1:11908117–11908380, reverse strand, relative to TSS: −540 bp to −277 bp) was illustrated previously [31]. Following primer validation, genomic DNA was bisulfite-treated using the EZ DNA Methylation-Gold Kit (Zymo Research, Inc., Irvine, CA, USA) according to the manufacturer's protocol, which converts unmethylated cytosine into uracil and leaves methylated cytosine unchanged. The treated samples were amplified, barcoded, and sequenced by Illumina Hiseq 2000 (Illumina, Inc., San Diego, CA, USA) using the paired-end sequencing protocol according to the manufacturer's guidelines. Methylation level at each CpG dinucleotides was calculated as the percentage of the methylated alleles over the sum of methylated and unmethylated alleles. For quality control, the samples with bisulfite conversion rate <98% and the cytosine sites with average coverage less than 20× were filtered out. DNA methylation levels were finally quantified at 9 CpG loci in the *NPPA* promoter. The average of the 9 CpG sites was used to represent the methylation level of the targeted region.

Follow-Up Examination and Definition of Obesity/Central Obesity

Bodyweight (kg) and height (cm) were measured when participants wore light clothes and no shoes by trained staff at both baseline and follow-up examinations. BMI was calculated by dividing weight in kilograms by the square of height in meters (kg/m^2). WC was measured at the level of 1 cm above the umbilicus. According to the recommendations of the Working Group on Obesity in China [32], obesity was defined as BMI ≥28 kg/m^2 and central obesity was defined as WC ≥85 cm for men and ≥80 cm for women. Incidences of obesity and central obesity were defined as

participants who were free of obesity and central obesity, respectively, at baseline but had obesity or central obesity at the follow-up examination.

Assessment of Confounding Variables

Data on sociodemographic, lifestyles, and metabolic factors were repeated measured at baseline and follow-up examinations. Demographic data including age, sex, and education level were obtained by questionnaires administered by trained staff. The education level was recorded as years a person stays in the education system and the highest level of educational quantifications they hold. Cigarette smoking was classified as current, former, and never smoking. Current smoking was defined as having smoked at least 100 cigarettes in the subject's entire life, having smoked cigarettes regularly, and smoking currently. Former smoking was defined as having smoked at least 100 cigarettes in the subject's entire life, having smoked cigarettes regularly in the past, and not smoking currently. Never smoking was defined as never smoked or having smoked fewer than 100 cigarettes in their lifetime by the end of examination. Alcohol consumption was classified as current drinkers or not. The current drinkers are those who had drunk any alcoholic beverages more than 12 times in the past year. According to the standard protocol, 3 consecutive sitting blood pressure measurements (30 s between each) were taken by trained staff using a standard mercury sphygmomanometer and a cuff of the appropriate size [33], after the participant had been resting for at least 5 min in a relaxed sitting position. All participants were required to have avoided exercise, drinking, smoking, and tea for at least 30 min before the measurement. The first and fifth Korotkoff sounds were recorded as systolic blood pressure and diastolic blood pressure, respectively. All participants completed 3 blood pressure measurements and the mean of the 3 measurements was used in statistical analyses. According to the Chinese guidelines for the management of hypertension, participants with a systolic blood pressure ≥140 mm Hg and/or a diastolic blood pressure ≥90 mm Hg or under antihypertensive treatment in the last 2 weeks were diagnosed with hypertension [34]. Fasting glucose was measured by standard laboratory methods [29]. Diabetes was defined as fasting glucose ≥7.0 mmol/L or use of hypoglycemic medication in the last 2 weeks [35].

Statistical Analysis

Baseline characteristics of study participants were presented according to obesity and central obesity, respectively. Group differences in these characteristics were compared using a Student's *t* test, Mann-Whitney-U test, or the χ^2 test as appropriate. To examine whether potential selection bias existed, we compared the baseline characteristics between participants excluded and included in the analysis. To carefully examine the association between *NPPA* promoter methylation and obesity in a systemic manner, as illustrated in Figure 2, we tested the cross-sectional, longitudinal, and prospective associations of *NPPA* promoter methylation with obesity and central obesity, respectively. Complete case analysis was used to deal with missing data. Multiple testing was controlled by applying a permutation test which has been widely used in correction for multiple hypotheses [36]. The *p* value generated from the permutation test of less than 0.05 was considered statistically significant. In addition to single CpG associations, the joint association of multiple CpG sites with obesity was also examined by using gene-based analysis approach which was typically applied in genetic and epigenetic studies to test the joint effect of multiple CpGs or SNPs on a complex phenotype. All analyses were conducted using R version 4.0.2.

Cross-Sectional Analysis

As the effect of DNA methylation at a single CpG site could be very small, testing the individual effect of a single CpG site on a complex trait such as obesity may not be efficient. Therefore, we examined both the single CpG and gene-based associations of the 9 CpGs with obesity. The average methylation level of the 9 CpG sites was used as a surrogate for the methylation level of the targeted region. The mean methylation level was presented and compared using *t* test between participants with and without obesity/central obesity. To examine the association between single CpG methylation and obesity, we constructed a linear regression model in which WC and BMI was the dependent variable individually and DNA methylation level at each CpG locus was the independent variable, adjusting for age, sex, education level, cigarette smoking, and alcohol consumption. To examine the gene-based association between DNA methylation at multiple CpG sites at *NPPA* promoter and obesity, we constructed a similar regression model with the average methylation level as the independent variable. We also employed the weighted truncated product method (wTPM) as previously described [37] to combine *p* values of all CpGs that reaches a preselected threshold (raw $p < 0.1$ in this study). The regression coefficient of each individual CpG was included as weight in the wTPM statistics. This method has been evaluated by simulation studies [38] and applied to epigenetic analysis [39]. To facilitate data interpretation, we similarly constructed a logistic regression model with prevalent central obesity and obesity as the dependent variable individually to examine whether *NPPA* promoter methylation was associated with risks of prevalent obesity/central obesity. BMI was additionally adjusted for in the analyses for the association between *NPPA* promoter methylation and WC/central obesity.

Longitudinal Analysis

We further examined whether *NPPA* promoter methylation at baseline could predict the dynamic and the changes in WC and BMI during follow-up. In specific, to further examine the associations of *NPPA* promoter methylation with dynamic WC and BMI during follow-up, we constructed a linear mixed regression model with a random intercept in which repeated measures of WC and BMI were the dependent variables individually (at baseline and follow-up), single CpG methylation at baseline was the independent variable, adjusting for age, sex, education level, cigarette smoking, current drinking at baseline and follow-up examinations, and follow-up time, with participants as the random effect. The mixed model was used here to account for the repeated measurements of WC and BMI in both examinations and performed using the R package "lme4" [40]. To examine the associations of *NPPA* promoter methylation with the changes in BMI and WC during follow-up, we constructed a linear regression model in which WC and BMI change (follow-up minus baseline) was the dependent variable individually, single CpG methylation was the independent variable, adjusting for abovementioned baseline covariates. The gene-based association of *NPPA* promoter methylation with dynamic WC and BMI was similarly examined. BMI was additionally adjusted for in the analyses for the associations between *NPPA* promoter methylation and dynamic or changes in WC.

Table 1. Baseline characteristics of study participants according to exhibition of obesity and central obesity, respectively

Characteristics	*N*	Central obesity		*p* value	Obesity		*p* value
		without	with		without	with	
Participants, *n*	2,497	1,174	1,323		2,126	371	
Age, years	2,497	51 (43–57)	55 (48–60)	<2.20e−16	53 (43–59)	56 (47–60)	3.19e−04
Sex, men (%)	2,497	433 (36.88)	528 (39.91)	1.13e−01	791 (37.21)	170 (45.82)	2.01e−03
Education, *n* (%)	2,485						
Elementary school or below		403 (16.21)	671 (27.00)	4.56e−15	891 (35.86)	183 (7.36)	4.87e−02
Middle school		498 (20.04)	407 (6.38)		791 (31.83)	114 (4.59)	
High school		203 (8.17)	177 (7.12)		324 (13.04)	56 (2.25)	
Bachelor's degree or above		66 (2.66)	60 (2.41)		109 (4.39)	17 (0.68)	
Smoking	2,497						
Current smoking, *n* (%)		274 (23.34)	307 (23.20)	3.71e−01	486 (22.86)	95 (25.61)	3.59e−01
Former smoking, *n* (%)		31 (2.64)	48 (3.63)	–	65 (3.06)	14 (3.77)	–
Current drinking, *n* (%)	2,497	201 (17.12)	263 (19.88)	8.60e−02	384 (18.06)	80 (21.56)	1.27e−01
WC, cm	2,497	75.5 (71.2–79.0)	88.0 (84.3–93.0)	<2.20e−16	81.0 (75.0–86.0)	94.0 (89.5–98.0)	<2.20e−16
BMI, kg/m^2	2,497	22.58 (21.25–24.14)	26.28 (24.68–28.13)	<2.20e−16	23.94 (22.09–25.63)	29.56 (28.70–30.88)	<2.20e−16
Diabetes, *n* (%)	2,497	70 (5.96)	147 (11.11)	7.22e−06	174 (8.18)	43 (11.59)	4.05e−02
Hypertension, *n* (%)	2,497	375 (31.94)	733 (55.40)	<2.20e−16	881 (41.44)	227 (61.19)	2.43e−12
SBP, mm Hg	2,497	124.0 (116.0–136.0)	131.3 (122.0–142.0)	<2.20e−16	128.0 (118.0–138.7)	132.0 (124.0–144.0)	3.06e−10
DBP, mm Hg	2,497	82.0 (78.0–88.0)	86.0 (80.0–92.2)	<2.20e−16	84.0 (80.0–90.0)	86.0 (82.0–93.3)	7.05e−15
Antihypertensive medication, *n* (%)	2,497	177 (15.08)	445 (34.39)	<2.20e−16	462 (21.73)	160 (43.13)	<2.20e−16
Fasting glucose, mmol/L	2,497	5.00 (4.60–5.50)	5.30 (4.80–5.80)	<2.20e−16	5.10 (4.70–5.60)	5.40 (4.90–6.00)	5.40e−11

Data are presented as the medians and first and third quartile (Q1–Q3) or *n* (%). *p* values indicate the differences between 2 groups performed by Mann-Whitney U test and χ^2 test as appropriate. There are 12 missing values in the variable of the education level. SBP, systolic blood pressure; DBP, diastolic blood pressure.

Table 2. The levels of *NPPA* promoter methylation in participants with and without central obesity/obesity

CpG loci	Genomic position, GRCh37	Relative to TSS, bp	Central obesity				Obesity			
			without	with	*p* value	permutation test *p* value	without	with	*p* value	permutation test *p* value
CpG1	Chr1:11908353	−513	28.99±5.21	28.16±5.20	7.21e−05	7.19e−05	28.67±5.22	27.89±5.17	7.76e−03	8.01e−03
CpG2	Chr1:11908348	−508	93.30±2.43	93.04±2.60	1.12e−02	1.15e−02	93.22±2.48	92.84±2.76	1.18e−02	6.48e−03
CpG3	Chr1:11908299	−459	22.97±3.81	22.73±3.86	1.18e−02	1.18e−01	22.85±3.82	22.75±3.96	6.46e−01	6.38e−01
CpG4	Chr1:11908200	−360	68.67±6.37	67.94±6.57	4.85e−03	4.93e−03	68.41±6.36	67.54±7.12	2.79e−02	1.71e−02
CpG5	Chr1:11908182	−342	81.97±4.80	81.41±4.97	4.34e−03	4.42e−03	81.78±4.79	81.08±5.41	1.88e−02	8.66e−02
CpG6	Chr1:11908178	−338	40.45±6.06	39.72±6.16	3.22e−03	3.25e−03	40.15±6.08	39.56±6.35	9.70e−02	1.03e−02
CpG7	Chr1:11908168	−328	50.61±6.39	50.01±6.48	1.95e−02	1.96e−02	50.39±6.41	49.75±6.63	8.66e−02	7.87e−02
CpG8	Chr1:11908165	−325	30.92±6.45	30.47±6.38	8.30e−02	8.27e−02	30.72±6.40	30.45±6.49	4.69e−01	4.65e−01
CpG9	Chr1:11908142	−302	36.67±7.77	36.41±7.72	4.09e−01	4.09e−01	36.54±7.67	36.47±8.15	8.75e−01	8.70e−01
Average*			50.50±4.74	49.99±4.82	7.14e−03	7.20e−03	50.30±4.74	49.81±5.07	8.37e−02	6.93e−02

Methylation level at each CpG site was calculated as the percentage of the methylated alleles over the sum of methylated and unmethylated alleles. GRCh37, Genome Reference Consortium Human Build 37. * The average of the methylation level at the 9 CpG sites was used to represent the methylation level of the targeted region.

Prospective Analysis

In participants free of obesity or central obesity at baseline, we further examined whether *NPPA* promoter methylation at baseline could predict future risk of obesity or central obesity, respectively, by constructing a logistic regression model in which incident obesity/central obesity (yes/no) was the dependent variable and single CpG methylation was the independent variable, adjusting for follow-up time and abovementioned baseline covariates. The gene-based associations of *NPPA* promoter methylation with future risk of central obesity and obesity were similarly examined. BMI was additionally adjusted for in the analyses for the associations between *NPPA* promoter methylation and incident central obesity.

Sensitivity Analysis

To examine whether diabetes and hypertension affect our results, we conducted sensitivity analyses by excluding participants with prevalent diabetes or hypertension at baseline.

Results

Baseline Characteristics of Study Participants

A total of 2,497 participants (median aged 54 [Q1–Q3: 45–59] years, 38.49% of men) were included in the current study. Of them, 371 (14.85%) and 1,323 (52.98%) participants have been diagnosed with obesity and central obesity, respectively. Their clinical characteristics are shown in Table 1. Participants with obesity/central obesity, as expected, were more likely to be older, have lower educational level, and have prevalent diabetes and hypertension than those without ($p < 0.05$). No significant difference in other listed variables was found. Some variables, for example, age and prevalent diabetes seemed to be different between participants included and excluded (online suppl. Table S1; for all online suppl. material, see www.karger.com/doi/10.1159/000521295). Although the main exclusion is missing data on *NPPA* methylation, selection bias cannot be prevented.

Cross-Sectional Associations between NPPA Methylation and Obesity/Central Obesity

Of the 9 CpG sites assayed, DNA methylation levels at 6 and 4 CpG loci were significantly lower in participants with central obesity and obesity, respectively, than those without (all $p < 0.05$, Table 2; Fig. 3). Of them, 6 CpG loci remained to be inversely associated with WC or BMI after multivariable adjustment and correction for multiple testing (all $p < 0.05$ for permutation test). The average methylation level of all CpGs was significantly associated with lower WC (β = −0.28, 95% confidence interval [CI]: −0.54 to 0.02, p = 3.61e−02). The results of the wTPM showed that hypermethylation of the 9 CpGs at the *NPPA*

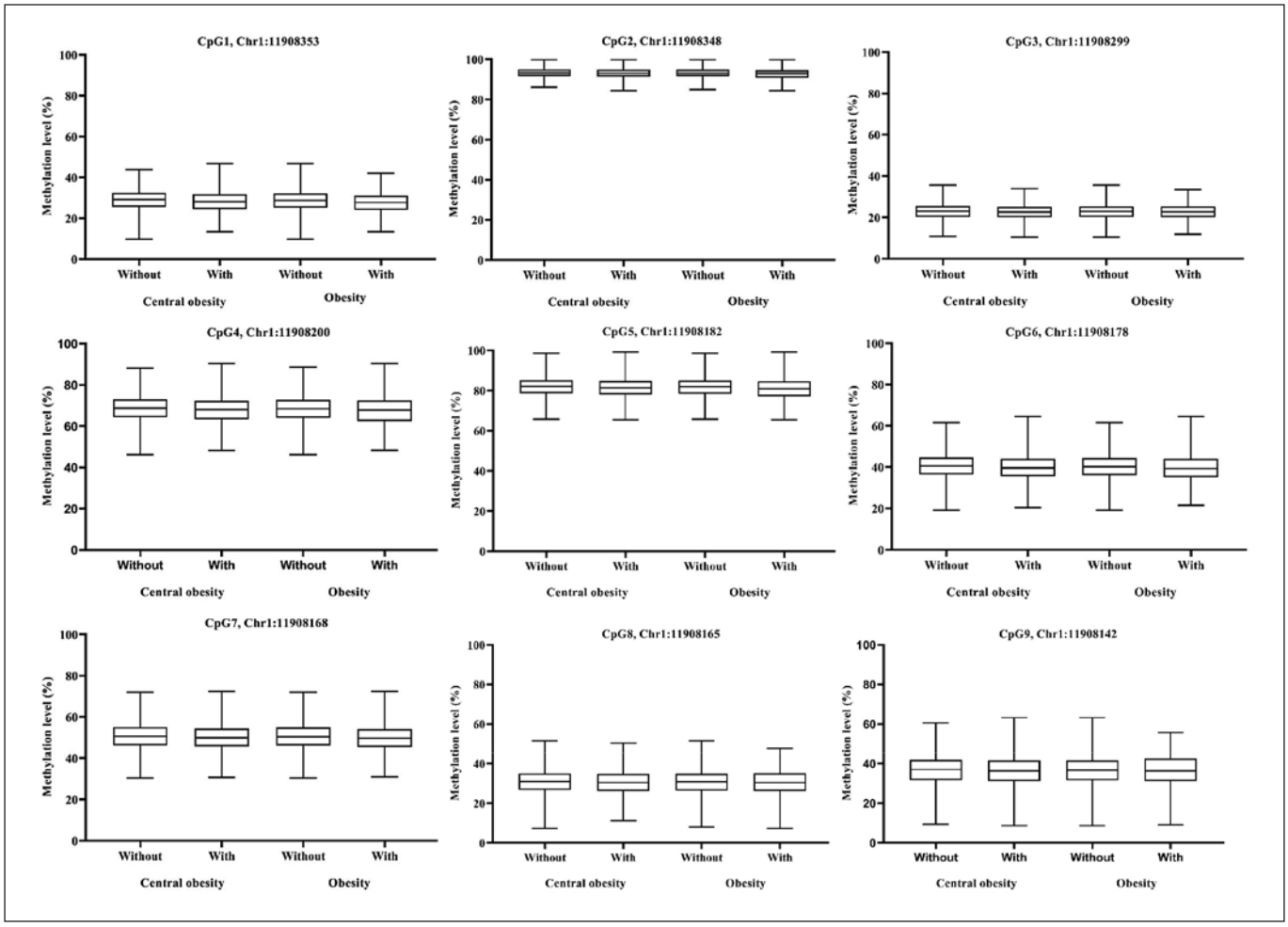

Fig. 3. A box plot showing the levels of *NPPA* promoter methylation in participants with and without obesity/central obesity.

promoter as a whole was significantly associated with WC, BMI, and prevalent obesity (all $p < 0.05$, Table 3). The results of all covariates from the complete models for the 9 CpGs and average methylation level are presented in the online supplementary Tables S2–S11.

Longitudinal Association between Baseline NPPA Promoter Methylation and Dynamic WC and BMI during Follow-Up

After a median of 4 years of follow-up, more participants, as expected, suffered from hypertension and diabetes, but had lower WC (online suppl. Table S12). However, the longitudinal analysis found similar negative associations to the cross-sectional analysis (Table 4). After adjustment for covariates and multiple testing, hypermethylation at 8 CpG loci was significantly associated with lower levels of either WC or BMI (all $p < 0.05$ for permutation test). The average methylation level of all CpGs was also significantly associated with lower levels of WC ($\beta = -0.25$, 95% CI: −0.46 to 0.04, $p = 2.09e{-}02$) and BMI ($\beta = -0.22$, 95% CI: −0.36 to 0.07, $p = 2.94e{-}03$). The results of the wTPM uncovered similar gene-based association (all $p < 0.05$). The results of all covariates from the complete models for the 9 CpGs and average methylation level are presented in the online supplementary Tables S13–S22.

Prospective Associations between Baseline NPPA Promoter Methylation and Obesity/Central Obesity

Of the 977 participants free of central obesity at baseline, 195 participants developed to central obesity after an average 4 years of follow-up. After multivariable adjust-

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Table 3. The cross-sectional associations of *NPPA* promoter methylation with obesity/central obesity

CpG loci	Genomic position, GRCh37	Relative to TSS, bp	Baseline WC			Prevalent central obesity			Baseline BMI			Prevalent obesity		
			β (95% CI)[a]	*p* value	permutation test *p* value	OR (95% CI)[b]	*p* value	permutation test *p* value	β (95% CI)[c]	*p* value	permutation test *p* value	OR (95% CI)[d]	*p* value	permutation test *p* value
Single CpG association														
CpG1	Chr1:11908353	−513	−0.18 (−0.42 to 0.06)	1.41e−01	8.42e−02	0.92 (0.83–1.01)	9.16e−02	8.90e−02	−0.20 (−0.33 to 0.06)	4.59e−03	<2.00e−16	0.88 (0.79–0.98)	2.24e−02	2.60e−02
CpG2	Chr1:11908348	−508	−0.58 (−1.08 to 0.09)	2.09e−02	2.21e−02	0.86 (0.71–1.05)	1.36e−01	1.18e−01	−0.17 (0.45 to 0.11)	2.29e−01	2.42e−01	0.76 (0.61–0.95)	1.60e−02	1.70e−02
CpG3	Chr1:11908299	−459	−0.29 (−0.61 to 0.04)	8.50e−02	7.74e−02	0.93 (0.81–1.06)	2.56e−01	2.41e−01	−0.03 (−0.21 to 0.16)	7.90e−01	4.26e−01	0.96 (0.83–1.11)	6.05e−01	6.11e−01
CpG4	Chr1:11908200	−360	−0.21 (−0.40 to 0.02)	3.45e−02	<2.00e−16	0.94 (0.87–1.01)	1.02e−01	8.90e−02	−0.10 (−0.21 to 0.00)	6.01e−02	<2.00e−16	0.91 (0.83–0.99)	2.94e−02	3.70e−02
CpG5	Chr1:11908182	−342	−0.37 (−0.63 to 0.12)	4.15e−03	<2.00e−16	0.93 (0.84–1.03)	1.70e−01	1.43e−01	−0.17 (−0.31 to 0.02)	2.53e−02	<2.00e−16	0.88 (0.78–0.98)	2.24e−02	2.80e−02
CpG6	Chr1:11908178	−338	−0.22 (−0.43 to 0.02)	3.19e−02	3.40e−03	0.93 (0.86–1.02)	1.09e−01	9.20e−02	−0.07 (−0.19 to 0.04)	2.09e−01	1.25e−01	0.93 (0.85–1.02)	1.37e−01	1.35e−01
CpG7	Chr1:11908168	−328	−0.21 (−0.40 to 0.01)	3.64e−02	9.82e−02	0.95 (0.88–1.03)	2.29e−01	2.10e−01	−0.07 (−0.18 to 0.04)	2.14e−01	1.56e−02	0.94 (0.86–1.02)	1.53e−01	1.58e−01
CpG8	Chr1:11908165	−325	−0.14 (−0.33 to 0.06)	1.72e−01	1.00e + 00	0.96 (0.89–1.04)	3.51e−01	3.46e−01	−0.03 (−0.14 to 0.08)	6.51e−01	8.43e−01	0.98 (0.90–1.07)	6.01e−01	6.02e−01
CpG9	Chr1:11908142	−302	−0.08 (−0.24 to 0.09)	3.53e−01	1.00e + 00	0.97 (0.91–1.04)	3.62e−01	3.65e−01	0.01 (−0.08 to 0.10)	7.81e−01	1.00e + 00	0.99 (0.93–1.07)	8.71e−01	8.75e−01
Gene-based association														
Average			−0.28 (−0.54 to 0.02)	3.61e−02	–	0.92 (0.83–1.02)	1.33e−01	–	−0.10 (−0.25 to 0.05)	1.76e−01	–	0.91 (0.81–1.02)	1.14e−01	–
wTPM				1.14e−02			1.74e−01			1.00e−04			2.00e−04	

The results of all covariates from the complete models for all CpGs and average methylation level were presented in the online supplementary Tables S2–S11. OR, odds ratio. [a] β indicated the change in WC associated with a per 5% increase in DNA methylation level, adjusting for age, sex, education level, cigarette smoking, alcohol consumption, and BMI. [b] OR of having central obesity associated with a per 5% increase in DNA methylation level, adjusting for age, sex, education level, cigarette smoking, alcohol consumption, and BMI. [c] β indicated the change in BMI associated with a per 5% increase in DNA methylation level, adjusting for age, sex, education level, cigarette smoking, and alcohol consumption. [d] OR of having obesity associated with a per 5% increase in DNA methylation level, adjusting for age, sex, education level, cigarette smoking, and alcohol consumption.

Table 4. The longitudinal associations of *NPPA* promoter methylation with dynamic and changes in BMI/WC

CpG loci	Genomic position, GRCh37	Relative to TSS, bp	Dynamic WC			WC changes			Dynamic BMI			BMI changes		
			β (95% CI)	*p* value	permutation test *p* value	β (95% CI)	*p* value	permutation test *p* value	β (95% CI)	*p* value	permutation test *p* value	β (95% CI)	*p* value	permutation test *p* value
Single CpG association														
CpG1	Chr1:11908353	−513	−0.18 (−0.37 to 0.02)	7.70e−02	7.50e−02	−0.30 (−0.55 to 0.06)	1.66e−02	3.80e−03	−0.27 (−0.40 to 0.13)	7.81e−05	<2.00e−16	−0.07 (−0.17 to 0.03)	1.59e−01	1.00e−00
CpG2	Chr1:11908348	−508	−0.54 (−0.94 to 0.14)	8.55e−03	1.00e−02	−0.63 (−1.14 to 0.12)	1.62e−02	2.78e−01	−0.29 (−0.55 to 0.01)	4.60e−02	5.80e−02	−0.24 (−0.44 to 0.04)	2.05e−02	<2.00e−16
CpG3	Chr1:11908299	−459	−0.31 (−0.57 to 0.04)	2.22e−02	2.10e−02	−0.50 (−0.84 to 0.16)	3.48e−03	<2.00e−16	−0.14 (−0.32 to 0.04)	1.31e−01	1.32e−01	−0.19 (−0.33 to 0.06)	3.88e−03	<2.00e−16
CpG4	Chr1:11908200	−360	−0.17 (−0.32 to 0.01)	3.53e−02	3.30e−02	−0.190 (−0.39 to 0.01)	6.36e−02	2.71e−01	−0.18 (−0.29 to 0.08)	6.43e−04	2.00e−03	−0.08 (−0.16 to 0.00)	4.18e−02	<2.00e−16
CpG5	Chr1:11908182	−342	−0.32 (−0.52 to 0.11)	2.89e−03	3.00e−03	−0.27 (−0.54 to 0.01)	4.49e−02	6.60e−01	−0.25 (−0.39 to 0.11)	4.31e−04	2.00e−03	−0.10 (−0.20 to 0.01)	6.22e−02	<2.00e−16
CpG6	Chr1:11908178	−338	−0.17 (−0.34 to 0.01)	4.18e−02	4.50e−02	−0.23 (−0.44 to 0.02)	3.06e−02	1.80e−03	−0.18 (−0.29 to 0.07)	1.86e−03	3.00e−03	−0.13 (−0.21 to 0.04)	2.94e−03	<2.00e−16
CpG7	Chr1:11908168	−328	−0.19 (−0.35 to 0.03)	1.88e−02	1.90e−02	−0.22 (−0.42 to 0.02)	3.24e−02	2.73e−01	−0.14 (−0.24 to 0.03)	1.31e−02	1.50e−02	−0.10 (−0.18 to 0.02)	1.45e−02	<2.00e−16
CpG8	Chr1:11908165	−325	−0.14 (−0.30 to 0.01)	7.58e−02	9.70e−02	−0.20 (−0.40 to 0.00)	4.94e−02	4.00e−03	−0.11 (−0.22 to 0.01)	3.65e−02	4.20e−02	−0.08 (−0.15 to 0.00)	5.85e−02	<2.00e−16
CpG9	Chr1:11908142	−302	−0.07 (−0.20 to 0.07)	3.27e−01	3.32e−01	−0.13 (−0.30 to 0.03)	1.15e−01	1.00e + 00	−0.05 (−0.14 to 0.04)	2.56e−01	2.47e−01	−0.06 (−0.12 to 0.10)	9.25e−02	5.80e−03
Gene-based association														
Average			−0.25 (−0.46 to 0.04)	2.09e−02	–	−0.33 (−0.60 to 0.06)	1.63e−02	–	−0.22 (−0.36 to 0.07)	2.94e−03	–	−0.13 (−0.24 to 0.03)	1.40e−02	–
wTPM				4.10e−03			1.49e−02			1.00e−04			8.30e−03	

For dynamic analysis, we constructed a mixed regression model with the repeated measures of BMI or WC as the dependent variable. For change analysis, we first calculated the changes in BMI or WC using the follow-up values minus the baseline values and then constructed a linear regression model with the changes in BMI or WC as the dependent variable. The results of all covariates from the complete models for all CpGs and average methylation level in association with dynamic WC and BMI were presented in the online supplementary Tables S13–S22. GRCh37, Genome Reference Consortium Human Build 37..

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Table 5. The prospective associations of *NPPA* promoter methylation at baseline with the risks of central obesity and obesity

CpG loci	Genomic position, GRCh37	Relative to TSS, bp	Central obesity						Obesity					
			unadjusted			adjusted[a]			unadjusted			adjusted[b]		
			OR (95% CI)	*p* value	permutation test *p* value	OR (95% CI)	*p* value	permutation test *p* value	OR (95% CI)	*p* value	permutation test *p* value	OR (95% CI)	*p* value	permutation test *p* value
Single CpG association														
CpG1	Chr1:11908353	−513	0.83 (0.72–0.97)	1.77e−02	1.30e−02	0.85 (0.72–1.00)	4.95e−02	6.40e−02	0.91 (0.75–1.11)	3.64e−01	3.62e−01	0.92 (0.76–1.12)	4.18e−01	3.39e−01
CpG2	Chr1:11908348	−508	0.72 (0.52–0.99)	4.47e−02	5.20e−02	0.62 (0.44–0.88)	7.37e−03	3.00e−03	0.83 (0.55–1.26)	3.85e−01	3.84e−01	0.83 (0.55–1.26)	3.80e−01	4.18e−01
CpG3	Chr1:11908299	−459	0.72 (0.58–0.89)	2.07e−03	2.00e−03	0.69 (0.55–0.87)	1.47e−03	5.00e−03	0.81 (0.62–1.06)	1.32e−01	1.39e−01	0.82 (0.62–1.07)	1.43e−01	1.58e−01
CpG4	Chr1:11908200	−360	0.88 (0.78–0.95)	4.11e−02	4.10e−02	0.85 (0.75–0.97)	1.91e−02	2.00e−02	0.91 (0.77–1.06)	2.23e−01	2.26e−01	0.91 (0.78–1.07)	2.55e−01	2.59e−01
CpG5	Chr1:11908182	−342	0.82 (0.70–0.97)	1.85e−02	1.50e−02	0.78 (0.65–0.93)	5.77e−03	4.00e−03	0.94 (0.76–1.16)	5.60e−01	5.58e−01	0.94 (0.76–1.17)	5.85e−01	6.60e−01
CpG6	Chr1:11908178	−338	0.86 (0.75–0.98)	2.05e−02	1.60e−02	0.84 (0.73–0.96)	1.31e−02	1.80e−02	0.93 (0.79–1.10)	4.17e−01	4.21e−01	0.93 (0.79–1.10)	4.07e−01	3.42e−01
CpG7	Chr1:11908168	−328	0.90 (0.80–1.02)	9.88e−02	8.80e−02	0.88 (0.77–1.01)	7.22e−02	7.70e−02	0.92 (0.78–1.08)	2.91e−01	2.83e−01	0.92 (0.79–1.08)	3.32e−01	3.15e−01
CpG8	Chr1:11908165	−325	0.90 (0.80–1.02)	8.32e−02	7.30e−02	0.87 (0.76–0.99)	3.70e−02	4.10e−02	0.93 (0.79–1.09)	3.47e−01	3.36e−01	0.93 (0.79–1.08)	3.38e−01	3.04e−01
CpG9	Chr1:11908142	−302	0.95 (0.86–1.05)	3.28e−01	3.18e−01	0.92 (0.82–1.02)	1.24e−01	1.23e−01	0.90 (0.79–1.02)	1.06e−01	9.30e−02	0.89 (0.78–1.02)	8.95e−02	7.70e−02
Gene-based association														
Average			0.83 (0.70–0.97)	2.37e−02	–	0.79 (0.66–0.95)	1.04e−02	–	0.87 (0.70–1.08)	2.10e−01		0.87 (0.70–1.08)	2.19e−01	–
wTPM				5.00e−03			5.00e−03			2.57e−01			2.14e−01	

OR, odds ratio; GRCh37, Genome Reference Consortium Human Build 37. Unadjusted, not adjusted for other covariates; Adjusted, adjusted for other covariates. The results of all covariates from the complete models for all CpGs and average methylation level are presented in the online supplementary Tables S23–S32. [a] Adjusting for follow-up time, age, sex, education level, cigarette smoking, alcohol consumption, and BMI at baseline. [b] Adjusting for follow-up time, age, sex, education level, cigarette smoking, and alcohol consumption at baseline.

ment, DNA methylation levels at CpG5, which was also cross-sectionally or longitudinally associated with a lower level of WC, were associated with a lower risk of developing central obesity (all $p < 0.05$). After further correction for multiple testing, hypermethylation at 6 CpGs (CpG2 located at Chr1:11908348, CpG3 located at Chr1:11908299, CpG4 located at Chr1:11908200, CpG5 located at Chr1:11908182, CpG6 located at Chr1:11908178, and CpG8 located at Chr1:11908165) were significantly associated with a lower risk of incident central obesity. The average methylation level of all CpGs at baseline predicted a lower risk of developing central obesity, independent of conventional risk factors (odds ratio = 0.79, 95% CI: 0.66–0.95, $p = 1.04e{-}02$). Similarly, the results of the wTPM showed that hypermethylation of the *NPPA* promoter was significantly associated with a lower risk of central obesity ($p < 0.05$, Table 5). The results of all covariates from the complete models for the 9 CpGs and average methylation level were presented in the online supplementary Tables S23–S32.

Of the 1,778 participants free of obesity at baseline, 100 individuals developed new obesity after an average 4 years of follow-up. But we did not find significant prospective association between *NPPA* promoter methylation and obesity (Table 5). The results of all covariates from the complete models for the 9 CpGs and average methylation level are presented in the online supplementary Tables S23–S32.

Results of Sensitivity Analysis

Excluding participants with prevalent diabetes and hypertension did not change our results a lot (online suppl. Tables S33–S38), indicating that the associations of *NPPA* promoter methylation with central obesity and obesity may not be driven by diabetes and hypertension.

Discussion

In a prospective longitudinal cohort of middle-aged and elderly Chinese adults in the Gusu cohort, our study for the first time systemically examined the cross-sectional, longitudinal, and prospective associations of DNA methylation levels of *NPPA* promoter with obesity and central obesity. The results showed that DNA methylation levels of *NPPA* promoter significantly predicted a lower risk of incident central obesity during an average 4 years of follow-up. These identified associations were independent of lifestyles and may not be driven by diabetes or hypertension. Our results may indicate that *NPPA* promoter methylation may possess the potential to serve as a predictor, or even probably a therapeutic target, for central obesity.

In line with our study, the role of the *NPPA* gene in central obesity has been suggested by previous studies [14, 15]. For example, a cross-sectional study including 1,507 individuals revealed that the *NPPA* gene rs5063 and rs198358 polymorphism might contribute to the occurrence of central obesity [14]. Another study including 1,608 individuals revealed that the *NPPA* gene rs5068 polymorphism might contribute to lower WC [15]. Further, Arora et al. [41] speculated that rs5068, which is located in the 3′ untranslated region, could affect transcript stability and result in higher ANP production. These findings suggest that factors regulating the function of the *NPPA* gene may conserve the potential to be the molecular mechanisms emerging to be studied that underneath the association between ANP and obesity observed in ours [42] and other population studies [12, 13]. As a modifiable molecular modification to the genome without changes in the genes' sequence, DNA methylation may affect gene function and repress transcription by altering promoter DNA accessibility and blocking the binding of transcription-activating proteins [43]. We, therefore, examined whether *NPPA* promoter methylation was associated with obesity. Numerous EWAS studies have been conducted and identified many epigenetic markers of obesity [24–27, 44], but no studies found a genome-widely significant CpG located at the *NPPA* gene. However, in line with our study, *NPPA* methylation was found to be associated at the gene level with obesity in an EWAS study [28]. Leveraging an unselected population in the Gusu cohort, we are the first to examine the prospective associations of the *NPPA* promoter methylation with obesity and central obesity and provide initial evidence for the potential role of the *NPPA* promoter methylation in the pathogenesis of central obesity.

In this study, we found that the contribution of an individual CpG methylation to central obesity was in general small (mostly <5%), and statistically most CpG sites could not withstand multiple testing correction. Such a small effect size may not be detected by conventional statistical methods, but their combined effects may be large enough to be useful for risk prediction. Therefore, we tested the joint association of multiple CpG methylations, with central obesity and found that the joint contribution of these CpG sites appeared to be much larger. Our results may unravel a molecular mechanism that the *NPPA* promoter methylation may participate in the pathology of central obesity, and suggest that simultaneously testing

the joint effects of multiple CpG sites is a powerful approach in epigenetic analysis for complicated diseases, e.g., central obesity.

To the best of our knowledge, our study is the first to examine the prospective association of *NPPA* promoter methylation with obesity and central obesity in Chinese adults. The strengths of this study include a prospective longitudinal study design, careful and systemic analyses of the associations of *NPPA* promoter methylation with obesity and central obesity, comprehensive adjustments of many conventional risk factors, and application of a gene-based analytical approach to testing the combined effect of multiple CpG methylations at *NPPA* promoter on central obesity. However, our study also has several limitations. First, as all observational studies, unobserved confounders may exist in our study. Second, we only included middle-aged and elderly Chinese adults in our study. The generalizability of our findings to other age-groups or populations with different ethnic backgrounds is uncertain. Third, we did not have data on circulating ANP levels in our study. Whether and to what extent *NPPA* promoter methylation accounts for the molecular mechanisms underlying the association between ANP and central obesity still needs more investigation, although hypomethylation of the *NPPA* gene was found to be associated with the upregulation of the transcripts of ANP [45]. Fourth, data on medications during follow-up were not comprehensively collected. The influence of drugs impacting weight and alcohol consumption could not be prevented. Fifth, given that DNA methylation is tissue- and cell-type specific, it is unclear whether or to what extent the results derived from peripheral blood could reflect methylation changes in the target organs of central obesity. However, accumulating evidence indicated that epimutations may not be limited to the affected tissue but could also be detected in peripheral blood [30, 46, 47]. For example, the same direction of demethylation of *NPPA* with ANP was also detected in peripheral blood [48]. Sixth, although the main exclusion of study participants is missing data on *NPPA* methylation, selection bias cannot be prevented in our study. The data interpretation should be of caution. Last, data on white blood cell-type proportions were not available in our study; we cannot eliminate the influence of cell type on our results. The association between *NPPA* promoter methylation and obesity should be further studied in more population studies and validated in adipose tissues in the future.

In conclusion, our study demonstrated that hypomethylation at *NPPA* promoter at baseline was not only significantly associated with a higher WC and BMI but also predicted an increased risk of future central obesity in Chinese adults. These findings indicate that *NPPA* promoter methylation could serve as a predictor for the identification of individuals at high risk for central obesity during primary prevention, but more evidence is needed to establish the causality between *NPPA* promoter methylation and central obesity.

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Statement of Ethics

The research was conducted ethically in accordance with the World Medical Association Declaration of Helsinki. The protocols were approved by the Soochow University Ethics Committee (approval NO. SUDA20100601H02). Written informed consent was obtained from all study participants.

Conflict of Interest Statement

None of the authors have financial associations that might pose a conflict of interest in connection with the submitted article.

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Author Contributions

J.L., J.Z., and Q.Z. performed the statistical data analysis and drafted the manuscript. H.P. and L.W. developed the concept of the study design and contributed to the draft of the manuscript. L.C., S.M., B.S., and Y.L. obtained the clinical data and critically

reviewed the manuscript. Y.H., R.Z., and M.Z. contributed to the interpretation of the results. All authors contributed to draft the final versions of the manuscript. All authors read and approved the final manuscript.

Data Availability Statement

The datasets used during the current study are available from the corresponding author on a reasonable request.

References

1 Wang Y, Lobstein T. Worldwide trends in childhood overweight and obesity. Int J Pediatr Obes. 2006;1:11–25.
2 Wang Y, Mi J, Shan XY, Wang QJ, Ge KY. Is China facing an obesity epidemic and the consequences? The trends in obesity and chronic disease in China. Int J Obes. 2007;31:177–88.
3 Ng M, Fleming T, Robinson M, Thomson B, Graetz N, Margono C, et al. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980–2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet. 2014;384:766–81.
4 Wang Y, Wang L, Qu W. New national data show alarming increase in obesity and noncommunicable chronic diseases in China. Eur J Clin Nutr. 2017;71:149–50.
5 Sengenès C, Berlan M, De Glisezinski I, Lafontan M, Galitzky J. Natriuretic peptides: a new lipolytic pathway in human adipocytes. FASEB J. 2000;14:1345–51.
6 Sengenes C, Bouloumie A, Hauner H, Berlan M, Busse R, Lafontan M, et al. Involvement of a cGMP-dependent pathway in the natriuretic peptide-mediated hormone-sensitive lipase phosphorylation in human adipocytes. J Biol Chem. 2003;278:48617–26.
7 Birkenfeld AL, Boschmann M, Engeli S, Moro C, Arafat AM, Luft FC, et al. Atrial natriuretic peptide and adiponectin interactions in man. PLoS One. 2012;7:e43238.
8 Stellar E, Epstein AN. Neuroendocrine factors in salt appetite. J Physiol Pharmacol. 1991;42:345–55.
9 Tarjan E, Denton DA, Weisinger RS. Atrial natriuretic peptide inhibits water and sodium intake in rabbits. Regul Pept. 1988;23:63–75.
10 Franci CR. Possible dual effect of endogenous ANP on water and sodium intake and role of AII. Neurosci Biobehav Rev. 1997;21:525–9.
11 Mori T, Chen YF, Feng JA, Hayashi T, Oparil S, Perry GJ. Volume overload results in exaggerated cardiac hypertrophy in the atrial natriuretic peptide knockout mouse. Cardiovasc Res. 2004;61:771–9.
12 Wang JH, Lee CJ, Hsieh JC, Chen YC, Hsu BG. Serum atrial natriuretic peptide level inversely associates with metabolic syndrome in older adults. Geriatr Gerontol Int. 2014;14:640–6.
13 Hsieh JC, Wang JH, Lee CJ, Chen YC, Liou HH, Hsu BG. Low serum long-acting natriuretic peptide level correlates with metabolic syndrome in hypertensive patients: a cross-sectional study. Arch Med Res. 2013;44:215–20.
14 Zhang H, Mo X, Zhou Z, Zhu Z, Huangfu X, Xu T, et al. Smoking modifies the effect of two independent SNPs rs5063 and rs198358 of NPPA on central obesity in the Chinese Han population. J Genet. 2018;97:987–94.
15 Cannone V, Boerrigter G, Cataliotti A, Costello-Boerrigter LC, Olson TM, McKie PM, et al. A genetic variant of the atrial natriuretic peptide gene is associated with cardiometabolic protection in the general community. J Am Coll Cardiol. 2011;58:629–36.
16 Macheret F, Heublein D, Costello-Boerrigter LC, Boerrigter G, McKie P, Bellavia D, et al. Human hypertension is characterized by a lack of activation of the antihypertensive cardiac hormones ANP and BNP. J Am Coll Cardiol. 2012;60:1558–65.
17 Niu W. The relationship between natriuretic peptide precursor a gene T2238C polymorphism and hypertension: a meta-analysis. Int J Hypertens. 2011;2011:653698.
18 Ichiki T, Izumi R, Cataliotti A, Larsen AM, Sandberg SM, Burnett JC Jr. Endothelial permeability in vitro and in vivo: protective actions of ANP and omapatrilat in experimental atherosclerosis. Peptides. 2013;48:21–6.
19 Magnusson M, Jujic A, Hedblad B, Engström G, Persson M, Struck J, et al. Low plasma level of atrial natriuretic peptide predicts development of diabetes: the prospective Malmo Diet and Cancer study. J Clin Endocrinol Metab. 2012;97:638–45.
20 Jujić A, Nilsson PM, Engström G, Hedblad B, Melander O, Magnusson M. Atrial natriuretic peptide and type 2 diabetes development – biomarker and genotype association study. PLoS One. 2014;9(2):e89201.
21 Frieling H, Bleich S, Otten J, Römer KD, Kornhuber J, de Zwaan M, et al. Epigenetic downregulation of atrial natriuretic peptide but not vasopressin mRNA expression in females with eating disorders is related to impulsivity. Neuropsychopharmacology. 2008;33:2605–9.
22 Volpe M, Rubattu S, Burnett J Jr. Natriuretic peptides in cardiovascular diseases: current use and perspectives. Eur Heart J. 2014;35:419–25.
23 Zhu H, Wu LF, Mo XB, Lu X, Tang H, Zhu XW, et al. Rheumatoid arthritis-associated DNA methylation sites in peripheral blood mononuclear cells. Ann Rheum Dis. 2019;78:36–42.
24 Demerath EW, Guan W, Grove ML, Aslibekyan S, Mendelson M, Zhou YH, et al. Epigenome-wide association study (EWAS) of BMI, BMI change and waist circumference in African American adults identifies multiple replicated loci. Hum Mol Genet. 2015;24:4464–79.
25 Dick KJ, Nelson CP, Tsaprouni L, Sandling JK, Aïssi D, Wahl S, et al. DNA methylation and body-mass index: a genome-wide analysis. Lancet. 2014;383:1990–8.
26 Aslibekyan S, Demerath EW, Mendelson M, Zhi D, Guan W, Liang L, et al. Epigenome-wide study identifies novel methylation loci associated with body mass index and waist circumference. Obesity. 2015;23:1493–501.
27 Al Muftah WA, Al-Shafai M, Zaghlool SB, Visconti A, Tsai PC, Kumar P, et al. Epigenetic associations of type 2 diabetes and BMI in an Arab population. Clin Epigenetics. 2016;8:13.
28 van Dijk SJ, Peters TJ, Buckley M, Zhou J, Jones PA, Gibson RA, et al. DNA methylation in blood from neonatal screening cards and the association with BMI and insulin sensitivity in early childhood. Int J Obes. 2018;42:28–35.
29 Peng H, Zhang Q, Cai X, Liu Y, Ding J, Tian H, et al. Association between high serum soluble corin and hypertension: a cross-sectional study in a general population of China. Am J Hypertens. 2015;28:1141–9.
30 Pu W, Wang C, Chen S, Zhao D, Zhou Y, Ma Y, et al. Targeted bisulfite sequencing identified a panel of DNA methylation-based biomarkers for esophageal squamous cell carcinoma (ESCC). Clin Epigenetics. 2017;9:129.
31 Li J, Zhu J, Ren L, Ma S, Shen B, Yu J, et al. Association between NPPA promoter methylation and hypertension: results from Gusu cohort and replication in an independent sample. Clin Epigenetics. 2020;12:133.
32 Zhou BF. Predictive values of body mass index and waist circumference for risk factors of certain related diseases in Chinese adults – study on optimal cut-off points of body mass index and waist circumference in Chinese adults. Biomed Environ Sci. 2002;15:83–96.
33 Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL Jr, et al. The seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure: the JNC 7 report. JAMA. 2003;289:2560–72.
34 Liu LS. [2010 Chinese guidelines for the management of hypertension]. Zhonghua Xin Xue Guan Bing Za Zhi. 2011;39:579–615.
35 Kerner W, Brückel J. Definition, classification and diagnosis of diabetes mellitus. Exp Clin Endocrinol Diabetes. 2014;122:384–6.
36 Camargo A, Azuaje F, Wang H, Zheng H. Permutation – based statistical tests for multiple hypotheses. Source Code Biol Med. 2008;3:15.

37 Zaykin DV, Zhivotovsky LA, Westfall PH, Weir BS. Truncated product method for combining P-values. Genet Epidemiol. 2002;22:170–85.
38 Sheng X, Yang J. Truncated product methods for panel unit root tests. Oxf Bull Econ Stat. 2013;75:624–36.
39 Peng H, Zhu Y, Strachan E, Fowler E, Bacus T, Roy-Byrne P, et al. Childhood trauma, DNA methylation of stress-related genes, and depression: findings from two monozygotic twin studies. Psychosom Med. 2018;80:599–608.
40 Bates D, Mächler M, Bolker B, Walker S. Fitting linear mixed-effects models using lme4. J Stat Soft. 2015;67(1).
41 Arora P, Wu C, Khan AM, Bloch DB, Davis-Dusenbery BN, Ghorbani A, et al. Atrial natriuretic peptide is negatively regulated by microRNA-425. J Clin Invest. 2013;123:3378–82.
42 Zhu Z, Zhang Q, Peng H, Zhong C, Liu Y, Huangfu X, et al. Plasma proANP (1–98) levels are positively associated with central obesity: a cross-sectional study in a general population of China. Clin Chim Acta. 2017;469:26–30.
43 Marx A, Kahan T, Simon I. Integrative analysis of methylome and transcriptome reveals the importance of unmethylated CpGs in non-CpG island gene activation. Biomed Res Int. 2013;2013:785731.
44 Sayols-Baixeras S, Subirana I, Fernández-Sanlés A, Sentí M, Lluís-Ganella C, Marrugat J, et al. DNA methylation and obesity traits: an epigenome-wide association study. The REGICOR study. Epigenetics. 2017;12:909–16.
45 Rubattu S, Stanzione R, Cotugno M, Bianchi F, Marchitti S, Forte M. Epigenetic control of natriuretic peptides: implications for health and disease. Cell Mol Life Sci. 2020;77:5121–30.
46 Menke A, Binder EB. Epigenetic alterations in depression and antidepressant treatment. Dialogues Clin Neurosci. 2014;16:395–404.
47 Uddin M, Koenen KC, Aiello AE, Wildman DE, de los Santos R, Galea S. Epigenetic and inflammatory marker profiles associated with depression in a community-based epidemiologic sample. Psychol Med. 2011;41:997–1007.
48 Meder B, Haas J, Sedaghat-Hamedani F, Kayvanpour E, Frese K, Lai A, et al. Epigenome-wide association study identifies cardiac gene patterning and a novel class of biomarkers for heart failure. Circulation. 2017;136:1528–44.