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ORIGINAL ARTICLE

The interactions between alcohol consumption and DNA methylation of the *ADD1* gene promoter modulate essential hypertension susceptibility in a population-based, case–control study

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The potential effects of the interactions between DNA methylation (CpG1 and CpG2-5 methylation levels) of the α-adducin (*ADD*1) gene promoter and ADD1 tagSNPs (tag single-nucleotide polymorphisms) or the environmental factors on essential hypertension (EH) risk have not been clarified. Thus, we performed an age- and gender-matched case–control study to investigate the association between ADD1 tagSNPs and EH. A total of 1020 subjects with EH and 1020 normotensive subjects were genotyped by melting temperature shift technology. Logistic regression was used to assess the associations of ADD1 tagSNPs, environmental factors and EH. The generalized multifactor dimensionality reduction (GMDR) method was applied to explore the potential interactions. Under additive, dominant and recessive models, no significant associations were evidenced between EH and rs3755885, rs2071694, rs4963 or rs3775067 with the complete data set or the gender-stratified analysis after adjusting for triglycerides, body mass index and alcohol consumption. However, we observed a significant association between rs4961 and EH under the dominant model after Bonferroni correction when adjusting for confounding factors in the entire sample (odds ratio (OR) = 0.64, 95% confidence interval (CI) = 0.50–0.83, *P* = 0.001). In GMDR, the two-factor interaction model of alcohol consumption and DNA methylation (CpG1 methylation) was the best model, with a maximum cross-validation consistency of 9/10 and testing balance accuracy of 0.63 (*P* = 0.01). Our results indicate that the SNP rs4961 has a protective role in the development of EH. In conclusion, the interactions between alcohol consumption and DNA methylation (CpG1 methylation) of the ADD1 gene promoter have a significant role in modifying EH susceptibility.
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Keywords: *ADD1*; DNA methylation; essential hypertension; gene-environment interaction

INTRODUCTION

Hypertension is a serious global public health burden. In fact, 9.4 million deaths are ascribed to hypertension based on a global burden of disease study in 2010.[1] In China, the prevalence of hypertension is increasing rapidly: a multistage study with a nationally representative sample of 50 171 subjects revealed that the adjusted prevalence of hypertension is 29.6%, which corresponds to 325 million Chinese adults.[2]

Essential hypertension (EH) is a multifactorial disease influenced by genes, environmental factors and their interactions.[3] More than 110 genes linked with EH susceptibility have been identified; however, the identified genes contribute only modestly to the heritability of EH,[4] and a large fraction of the heritability remains unexplained. In addition, the etiology and pathogenesis of EH remain unclear.

To further characterize EH heritability, it is necessary to understand gene–environment interactions as well as epigenetic changes that may contribute to EH susceptibility and EH etiology.[5] Indeed, growing evidence suggests that epigenetics has a critical role in hypertension regulation. The methylation of DNA is an epigenetic process that has a pivotal role in the regulation of gene expression,[6] and environmental factors have been shown to affect DNA methylation.[7,8] In particular, the methylation of CpGs in the promoter region has the potential ability to silence gene expression. Currently, aberrant DNA methylation patterns are not well characterized in subjects with EH. In addition, very little is known regarding the interactions between DNA methylation and gene polymorphisms as well as DNA methylation and environmental factors on EH susceptibility.

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In a previous study, we evaluated the association between tagSNPs (tag single-nucleotide polymorphisms) of the α-adducin (*ADD*1) gene and EH susceptibility. Our results indicated that rs4963 was significantly associated with EH susceptibility in Chinese populations and that the interactions among body mass index (BMI), rs4963 and rs16843452 were involved in EH susceptibility. In addition to BMI, total cholesterol and triglycerides (TG) were significantly associated with EH.[9] We also investigated the contribution of promoter DNA methylation (CpG1 and CpG2-5 methylation levels) of the *ADD*1 gene to the risk of developing EH, demonstrating that lower ADD1 gene promoter DNA methylation increased the risk of developing EH.[10] The above-mentioned studies indicated that genetic and environmental risk factors and DNA methylation had important roles in modulating the individual susceptibility to EH. Therefore, we hypothesize that the potential interactions between ADD1 tagSNPs and DNA methylation of the ADD1 gene promoter as well as the environmental factors and DNA methylation of the ADD1 gene promoter may modify EH susceptibility.

Thus, the main objective of this study was to explore the potential interactions between ADD1 tagSNPs and DNA methylation of the ADD1 gene promoter as well as the environmental factors and DNA methylation of the ADD1 gene promoter on EH risk. The results of this study may be helpful in illustrating the underlying biological mechanisms for EH. In addition, despite numerous human candidate gene studies that show associations between *ADD*1 gene polymorphisms and EH, many cannot be replicated in different ethnicities. Thus, we also replicated the associations that were previously identified between ADD1 tagSNPs (rs4961, rs12503220, rs3755885, rs3775067, rs4963 and rs2071694) and EH in a homogeneous Chinese population with a relatively large sample size.

METHODS

Study population

This was a population-based, case–control study. A total of 1020 patients with EH and 1020 age- and gender-matched controls (category-matching) were recruited from the communities surrounding the city of Ningbo in the Zhejiang province in China. All of the participants were Han Chinese, aged 35–70 years and their families had been living in Ningbo for at least three generations. The details of the inclusion criteria were presented in a previous publication.[9] In brief, hypertensive patients were defined according to the gold standard.[11] As controls, subjects were recruited who had a SBP <120 mm Hg and a DBP <80 mm Hg, did not have a family history of hypertension and did not have a first-degree relative with hypertension. The study protocol was approved by the ethics committee of Ningbo University. Written, informed consent was obtained from each participant.

The following data were collected by well-trained interviewers from each subject: name, age, gender, ethnicity, height, weight, smoking habits, alcohol consumption habits, history of hypertension and other diseases. 'Smoking' and 'alcohol consumption' were considered categorical variables; a 'smoker' was identified when a participant smoked >1 cigarette per day for at least 6 months, and an 'alcohol consumer' was identified when a participant drank at least one serving of alcohol >2 times per week for at least 6 months.

Genotyping of *ADD1* tagSNPs

Blood samples (5 ml) were collected in the morning, after an overnight fast, without stasis into EDTA vacutainers. Fasting blood glucose, TG, total cholesterol, high-density lipoprotein and low-density lipoprotein concentrations were measured using a CX7 biochemistry analyzer (Beckman, Fullerton, CA, USA).

Human genomic DNA was prepared from peripheral blood samples using a nucleic acid extraction automatic analyzer (Lab-Aid 820, Xiamen City, China). DNA was quantified using the PicoGreen double strand DNA (dsDNA) Quantification Kit (Molecular Probes, Eugene, USA). Amplification was performed via PCR on the ABI GeneAmp PCR System 9700 Dual 96-Well Sample Block Module (Applied Biosystems, Foster City, CA, USA). The plates used were standard 96-well plates (Bioplastics, Landgraaf, the Netherlands) sealed with Cyclerseal Sealing Film. The details of the PCR conditions are described elsewhere.[9]

We obtained the tagSNPs (rs4961, rs12503220, rs3755885, rs3775067, rs4963 and rs2071694) of the *ADD*1 gene through the International HapMap Project (http://hapmap.ncbi.nlm.nih.gov/cgi-perl/gbrowse/hapmap24_B36/). The position of the tagSNPs in the *ADD*1 gene is shown in Supplementary Table 1. The primer sequences for the *ADD*1 gene tagSNPs are listed in Supplementary Table 2. we didn't find the suitable primers for tagSNP rs12503220, so this tagSNP was not tested.

Statistical analysis

Continuous variables are expressed as the mean ± s.d. Categorical variables are expressed as absolute numbers. An independent, two-sample *t*-test was applied to continuous variables for comparison of the EH patients and the controls. To test for Hardy–Weinberg equilibrium, a χ^2-test was used. Genotype distribution of ADD1 tagSNPs was assessed by logistic regression assuming additive, dominant and recessive models of inheritance after adjusting for TG concentration, BMI and alcohol consumption. Univariate and multivariate logistic regression analyses were performed to estimate the effects of environmental factors on EH risk. Linkage disequilibrium and haplotypes from the tagSNPs were estimated using Haploview software (http://www.broad.mit.edu/mpg/haploview/). $P<0.05$ was considered statistically significant. All the above statistical analyses were performed with the PASW Statistics 18.0 software (SPSS, Somers, NY, USA).

To reduce the type I errors introduced by multiple tests, the Bonferroni correction was applied to the significance thresholds. Specifically, the formula $1-(1-\partial)1/n$ was employed to adjust the significance level and maintain a type I error rate of 0.05. Thus, $P<0.005$ was adopted as the significant threshold (for Table 2).

To explore potential high-order interactions of ADD1 tagSNPs and DNA methylation of the ADD1 gene promoter, environmental factors and DNA methylation of the ADD1 gene promoter with EH susceptibility, a newly developed generalized multifactor dimensionality reduction (GMDR) method with the ability to account for covariates was applied (www.healthsystem.virginia.edu/internet/addiction-genomics/). ADD1 tagSNPs, DNA methylation of the ADD1 gene promoter (CpG1 and CpG2-5 methylation levels), age, gender, BMI,high-density lipoprotein, low-density lipoprotein, total cholesterol, TG, the distribution of smoking and the distribution of alcohol consumption were included in the GMDR analysis.

GMDR is a nonparametric and genetic model-free alternative to linear or logistic regression for detecting and characterizing nonlinear interactions among discrete genetic and environmental attributes. The data were randomly split into 10 sets: 9 for training and 1 for testing. *N* factors were chosen from the training set and their combinations were demonstrated in *n*-dimensional space. Then, the GMDR reduces high-dimensional data of multiple factors into a one-dimensional variable with two levels (high risk or low risk) by the ratio of patients to controls. Therefore, this approach facilitates the detection of interactions in small sample sizes, permits adjustment for covariates and is applicable to both dichotomous and continuous phenotypes. A number of parameters were provided to estimate each selected interaction model with a testing balance accuracy, sign test *P*-value and a cross-validation consistency. The best model was selected by the maximum testing balance accuracy, a sign test *P*-value of <0.05 and the maximum of cross-validation consistency. Confounding factors, such as BMI, TG and alcohol consumption, were included as covariates in the GMDR models.

RESULTS

This population-based, case–control study included 1020 EH subjects (mean age: 58.5 ± 6.4 years; 339 males and 681 females) and 1020 controls (mean age: 58.3 ± 6.5 years; 350 males and 670 females). The clinical characteristics of the participants with EH and controls are listed in Table 1. No significant differences were observed with respect to age, high-density lipoprotein, low-density lipoprotein, gender or smoking

status between the EH subjects and controls. However, total cholesterol, TG and BMI were significantly higher in the EH group than the controls. Furthermore, there was a statistically significant difference in the drinking status between the two groups (Table 1). Multivariable, logistic regression analysis showed that TG, BMI and alcohol consumption were the risk factors for EH (Supplementary Table 3).

Table 1 Clinical characteristics between the EH and normotensive groups

Variables	*EH*	*Controls*	P*-value*
Number	1020	1020	NA
Gender (M/F)	339/681	350/670	0.61
Age (years)	58.5±6.4	58.3±6.5	0.58
BMI (kg m^{-2})	23.96±2.89	22.94±2.77	<0.01
TC (mmol l^{-1})	5.39±1.01	5.28±1.05	0.02
TG (mmol l^{-1})	1.74±1.40	1.52±0.86	<0.01
HDL (mmol l^{-1})	1.68±0.47	1.70±0.50	0.57
LDL (mmol l^{-1})	3.22±0.80	3.16±0.83	0.30
Smoking (Y/N)	155/865	148/872	0.66
Drinking (Y/N)	161/859	73/947	<0.01

Abbreviations: BMI, body mass index; EH, essential hypertension; F, females; HDL, high-density lipoprotein; LDL, low-density lipoprotein; N, no; NA, not applicable; TC, total cholesterol; TG, triglyceride; Y, yes.

The genotype and allele frequencies for each tagSNPs are in accordance with Hardy–Weinberg equilibrium expectations in the control group (see Table 2). Also in Table 2 are the results of the multivariate logistic regression for each tagSNPs under dominant, recessive and additive genetic models after adjusting for TG, BMI and alcohol consumption. Using the dominant model (GT+TT *vs.* GG) on the whole data set, after adjusting for TG, BMI and alcohol consumption, it was revealed that the SNP rs4961 was a protective factor in the development of EH (OR =0.64, 95% CI=0.50–0.83, *P*=0.001; Table 2). However, no other tagSNPs were identified to be associated with EH in the whole sample or in a gender-stratified analysis (Table 2).

Haplotype frequencies of ADD1 tagSNPs (rs16843452, rs12503220, rs4963, rs3755885, rs2071694 and rs3775067) were estimated using Haploview software. There was a positive linkage disequilibrium among rs3755885(C/G), rs3775067(C/T), rs4961 (G/T) and rs4963 (C/G). The linkage disequilibrium block for five tagSNPs in *ADD*1 gene is shown in Figure 1. Haplotype analysis for *ADD*1 gene tagSNPs are listed in Table 3. Five haplotype sequences were identified. The haplotype GCTG had a protective role in the development of EH (OR=0.86, 95% CI=0.76–0.97), whereas the haplotypes GTGC (OR=1.47, 95% CI=1.20–1.79) and GCGG (OR=3.08, 95% CI=1.93–4.90) were the risk factors for EH.

In our previous study, there was not a statistically significant association between the ADD1 CpG1 level and EH when assessing

Table 2 Distribution of genotype and allele frequencies between cases and controls in the whole sample and stratified by gender

						Additive model		*Dominant model*		*Recessive model*		
	11	*12*	*22*	*Minor allele*	*Major allele*	*OR (95% CI)*[a]	P*-value*	*OR (95% CI)*[a]	P*-value*	*OR (95% CI)*[a]	P*-value*	*HWE*
rs4961												
Control	262	498	260	1018	1022							0.45
Case	331	443	245	933	1105	1.21 (1.04–1.41)	0.01	0.64 (0.50–0.83)	0.001	0.92 (0.71–1.19)	0.54	
M-control	91	184	75	334	366							0.31
M-case	116	128	94	316	360	1.12 (0.87–1.44)	0.37	0.58 (0.38–.89)	0.01	1.26 (0.82–1.95)	0.27	
F-control	171	314	185	684	656							0.10
F-case	215	315	151	617	745	1.28 (1.05–1.55)	0.01	0.58 (0.40–.85)	0.005	0.77 (0.56–1.06)	0.11	
rs3755885												
Control	500	427	92	611	1427							0.95
Case	522	423	75	573	1467	1.08 (0.91–1.29)	0.34	0.96 (0.77–1.20)	0.73	0.71 (0.47–1.08)	0.11	
M-control	168	148	34	216	484							0.86
M-case	179	134	26	186	492	1.18 (0.88–1.57)	0.25	0.84 (0.58–1.23)	0.39	0.69 (0.36–1.32)	0.26	
F-control	332	279	58	395	943							0.95
F-case	343	289	49	387	975	1.01 (0.81–1.27)	0.90	1.03 (0.78–1.36)	0.80	0.80 (0.46–1.38)	0.42	
rs2071694												
Control	508	413	98	609	1429							0.29
Case	522	414	83	580	1458	1.04 (0.87–1.23)	0.63	0.97 (0.78–1.21)	0.82	0.87 (0.58–1.29)	0.49	
M-control	173	143	34	211	489							0.57
M-case	173	135	31	197	481	0.99 (0.75–1.32)	0.98	1.02 (0.70–1.49)	0.89	0.94 (0.50–1.77)	0.86	
F-control	335	270	64	398	940							0.37
F-case	349	279	52	383	977	1.05 (0.84–1.30)	0.63	0.95 (0.72–1.25)	0.74	0.87 (0.52–1.44)	0.60	
rs4963												
Control	243	518	259	1036	1004							0.61
Case	265	503	252	1007	1033	1.05 (0.90–1.23)	0.49	0.88 (0.68–1.15)	0. 37	0.97 (0.75–1.24)	0.81	
M-control	89	185	76	337	363							0.27
M-case	92	152	95	342	336	0.96 (0.74–1.25)	0.78	0.85 (0.55–1.31)	0.46	1.29 (0.84–1.98)	0.24	
F-control	154	333	183	699	641							0.91
F-case	173	351	157	665	697	1.13 (0.92–1.38)	0.22	0.88 (0.63–1.22)	0.45	0.82 (0.60–1.12)	0.22	
rs3775067												
Control	379	491	150	791	1249							0.65
Case	354	507	159	825	1215	0.93 (0.79–1.10)	0.45	1.10 (0.87–1.38)	0.41	1.05 (0.76–1.45)	0.74	
M-control	124	175	51	277	423							0.39
M-case	129	154	56	266	412	0.96 (0.74–1.26)	0.80	0.96 (0.74–1.26)	0.80	1.32 (0.79–2.21)	0.28	
F-control	255	316	99	514	826							0.94
F-case	225	353	103	559	803	0.91 (0.74–1.12)	0.39	1.21 (0.91–1.61)	0.18	0.95 (0.63–1.43)	0.80	

Abbreviations: BMI, body mass index; CI, confidence interval; F, female; HWE, Hardy–Weinberg equilibrium; M, male; OR, odds ratio; TG, triglycerides.
11: wild-type homozygote; 12: heterozygote; 22: variant homozygote.
[a]Adjusted for BMI, TG and drinking.

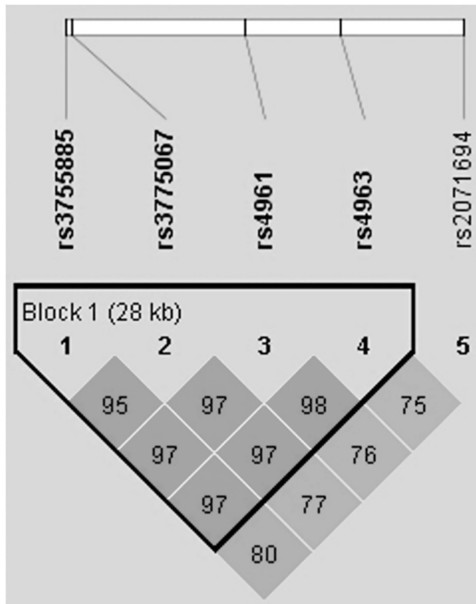

Figure 1 Linkage disequilibrium block for five tagSNPs in α-adducin (ADD1) gene. A full color version of this figure is available at the *Hypertension Research* journal online.

Table 3 Haplotype analysis for *ADD1* gene tagSNPs

Haplotype sequence	*Haplotype frequency*	*Case, control ratios*	P-*value*	*OR (95% CI)*
GCTG	0.47	0.45, 0.49	0.01	0.86 (0.76, 0.97)
CTGC	0.28	0.27, 0.29	0.17	0.91 (0.79, 1.04)
GTGC	0.11	0.13, 0.09	<0.01	1.47 (1.20, 1.79)
GCGC	0.10	0.10, 0.10	0.84	0.98 (0.80, 1.20)
GCGG	0.02	0.04, 0.01	<0.01	3.08 (1.93, 4.90)

Abbreviations: *ADD1* gene, α-adducin gene; CI, confidence interval; OR, odds ratio.

the complete data set (9.52 ± 61.46 for the EH group *vs.* 10.50 ± 62.85 for the control group, $P = 0.091$). However, the ADD1 CpG2-5 methylation level was significantly associated with EH in the total subjects (27.54 ± 67.48 for the EH group *vs.* 31.44 ± 65.30 for the control group, adjusted $P = 0.026$). A breakdown analysis by gender showed that lower levels of ADD1 CpG2-5 methylation were associated with an increased risk of EH in males (cases *vs.* controls: 22.48% *vs.* 31.86%, adjusted $P = 0.008$), although no association of CpG2-5 methylation levels with EH was found in females (adjusted $P = 0.557$). By contrast, the ADD1 CpG1 methylation level was significantly associated with EH in females (cases *vs.* controls: 10.0 ± 1.41 *vs.* 11.36 ± 3.63, adjusted $P = 0.042$), but not in males (adjusted $P = 0.133$).[10]

Next, we used the GMDR method to detect higher-order interactions. The best models of various orders are shown in Table 4. No significant interactions were identified between the ADD1 tagSNPs and DNA methylation of the ADD1 gene promoter. However, for the interaction between environmental factors and DNA methylation of the ADD1 gene promoter, the two-factor model (alcohol consumption and DNA methylation (CpG1 methylation)) had the highest testing balance accuracy (0.63) and best cross-validation consistency (9/10); the corrected *P*-value was 0.01 after permutation testing and the training OR with 95% CI was 5.64(1.13–28.01). Figure 2 shows the

Table 4 GMDR models of high-order interaction on EH risk

Model	*Training balance accuracy*	*Testing balance accuracy*	*Sign test (P)*	*Cross-validation consistency*
Interaction between ADD1 tagSNPs and DNA Methylation[a]				
rs3755885	0.65	0.60	0.17	8/10
cpg1, rs2071694	0.69	0.50	0.62	4/10
cpg1, rs3755885, rs2071694	0.75	0.58	0.054	8/10
Interaction between environmental factors and DNA Methylation				
Smoking	0.63	0.54	0.82	7/10
Drinking, cpg1	0.69	0.63	0.01	9/10
Drinking, cpg1, gender	0.74	0.55	0.37	6/10
Drinking, cpg1, gender, age	0.80	0.65	0.37	8/10
Drinking, cpg1, cpg2-5	0.84	0.60	0.37	7/10

Abbreviations: *ADD1* gene, α-adducin gene; BMI, body mass index; EH, essential hypertension; GMDR, generalized multifactor dimensionality reduction; SNP, single-nucleotide polymorphism; TG, triglycerides.
[a]Adjusted by confounding factors: BMI, TG and drinking.

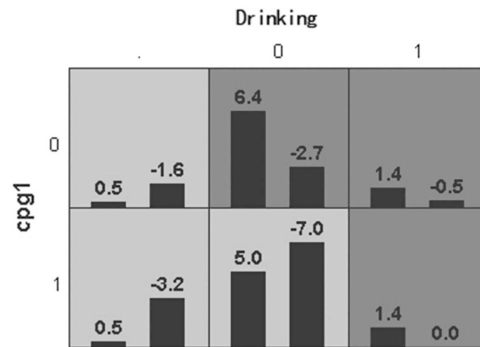

Figure 2 The effect of interactions between drinking and DNA methylation (CpG1 methylation). In each cell, the left bar represents a positive score, and the right bar a negative score. High-risk cells are demonstrated by dark shading, low-risk cells by light shading. Drinking: for the missing value, 0 for not drinking, 1 for drinking; CpG1: 0 for low CpG1 methylation level, 1 for high CpG1 methylation level.

best interaction model. When incorporating all of the ADD1 tagSNPs, environmental factors and DNA methylation patterns of the ADD1 gene promoter into the GMDR analysis, no significant results were found (data not shown).

DISCUSSION

Herein, we explored the potential interactions between DNA methylation of the ADD1 gene promoter and (1) ADD1 tagSNPs and as well as (2) environmental factors in modulating the EH susceptibility. Our findings are the first to show an interaction between alcohol consumption and DNA methylation (CpG1 methylation, specifically). This interaction may be a novel area for EH research, as it might explain the missing heritability of EH susceptibility.

We observed that CpG1 methylation was associated with EH in females in the present study, whereas CpG2-5 methylation was significantly associated with EH in males and in the total subjects in our previous study[10]. Similarly, a study conducted by Philibert *et al.*[12] concluded that recent chronic alcohol intake was associated with significant changes in CpG methylation (especially with increased hypermethylation of CpG islands) in middle-aged female subjects.

Alcohol-induced hypermethylation of CpG islands may indeed explain our observation of the interaction between alcohol consumption and CpG1 methylation.

Recently, nonparametric and genetic model-free approaches have proven to be efficient in identifying high-order gene–environment interactions. Owing to the 'curse of dimensionality', traditional methods are unsuitable. The GMDR approach proposed by Lou *et al.*[13] permits the adjustment for covariates and is suitable for both dichotomous and continuous phenotypes. In addition, GMDR enhances the precision accuracy and allows us to draw more meaningful conclusions in a variety of population-based study designs.

Our GMDR analysis did not show any significant interaction between the ADD1 tagSNPs and DNA methylation of the ADD1 gene promoter. However, our results indicated that there was a significant relationship between alcohol consumption and DNA methylation (CpG1 methylation). Specifically, our analysis suggested a 5.64-fold increased risk for developing EH in individuals with both factors. It is well established that EH is a multifactorial disease and that environmental factors may contribute strongly to EH susceptibility. For example, a study with 50 171 participants suggested that physical inactivity, habitual alcohol consumption, chronic use of non-steroidal anti-inflammatory drugs, high body mass index and central obesity are independently associated with the presence of hypertension.[2]

Wang *et al.*[14] conducted a genome-wide methylation analysis study comparing individuals with hypertension to normotensive controls and demonstrated that the changes in DNA methylation had an important role in the pathogenesis of hypertension. Furthermore, Smolarek *et al.*[15] suggested that the global level of DNA methylation indexed by the genome level of 5-methylcytosine was significantly lower in patients with EH compared with controls.

Traditional risk factors of hypertension, including aging, diet and smoking, have been shown to cause changes in DNA methylation.[16,17] Therefore, when we design a genetic association study, we should not only consider DNA methylation but also take into account environmental risk factors. In addition, Simino *et al.*[18] proposed that the interactions between epigenetic phenomena and environmental factors may be synergistic. The environmental factors may alter gene expression through epigenetic mechanisms in human complex diseases.[19–21] Environmental factors can be proven to affect DNA methylation.[7,8]

In our study, we observed a significant association between the rs4961 tagSNP and EH under a dominant model after Bonferroni correction when adjusting for TG, BMI and alcohol consumption in the whole sample. A meta-analysis with 5562 EH individuals and 4289 controls supported that the G460W polymorphism in ADD1 was associated with the increased risk of hypertension in the Han Chinese population.[22] It is important to note that Kundu *et al.*[23] determined that the mutation from glycine to tryptophan at the residue position 460 in the ADD1 native protein was a potential candidate for the association between hypertension and the *ADD*1 gene. Their silico analysis reported that the rs4961 that expresses the amino-acid variant (G460W) had a significant damaging effect and important functionality. Thus, because the mutation affected the capacity of the tubular epithelial cell to transport sodium and, hence, influence blood pressure, they declared ADD1 to be a 'renal hypertensive gene'.[23]

Our study has many strengths. First, to the best of our knowledge, the present study is the first to evaluate the interactions of the DNA methylation of the ADD1 gene promoter with ADD1 tagSNPs and environmental factors on EH risk. Our study provides novel insight into these interactions and adds to the current evidence that the interaction between alcohol consumption and DNA methylation (specifically, CpG1 methylation) has a significant role in conferring EH susceptibility. Second, by applying the GMDR method to assess high-order interactions, we overcame the 'curse of dimensionality'. Third, as obesity is an important risk factor for EH, we adjusted our models by using BMI as a covariant in the multivariate logistic regression analysis and GMDR interaction analysis. Fourth, we conducted the haplotype analysis, which is often more informative than analyses of single tagSNPs. However, future prospective studies with larger sample sizes are needed to confirm our findings.

In addition to the strengths of this study, there are also limitations that need to be noted. One of the limitations was that only DNA methylation of the ADD1 gene promoter was measured; there is a possibility that DNA methylation of promoters of other genes may confer susceptibility to EH. Another limitation is that there may be other confounding factors that were not included in our models. In addition, the potential biological mechanism underlying the significant interaction model is not known.

Indeed, the interactions between alcohol consumption, DNA methylation and expression of the *ADD*1 gene are biologically plausible. For example, Alegria-Torres *et al.*[24] demonstrated that alcohol consumption alters genes directly and influences the gene's expression through epigenetic mechanisms. Alcohol drinking may alter the DNA methylation levels of EH genes.

Epidemiological studies have established that alcohol consumption is a major risk factor for EH. DNA methylation level in humans is reported to change with changes in environmental factors. A recent study found that habitual consumption of high amounts of alcohol was associated with widespread changes in DNA methylation; the degree of these changes in methylation diminished after only one month of abstinence.[25] Adducin was implicated in the pathogenesis of EH by modulating Na^+–K^+-ATPase activity.[26–28] Moreover, increased gene expression and protein activity of the Na^+–K^+-pump was observed in hypertensive rats.[28] The lower level of DNA methylation in the ADD1 gene promoter in individuals with EH shown herein may result in the higher expression of α-adducin and increased expression of the Na^+–K^+-pump, which might eventually lead to excess sodium reabsorption and EH.

The genetic variants discovered in candidate gene studies and genome-wide association studies only account for a small fraction of the EH heritability; gene–environment and epigenetic–environment interactions may account for the missing factors for heritability of EH. Exploring the potential interactions is helpful in recognizing individuals with a high risk for developing EH and for developing cost-effective prevention strategies.

Moving forward, epigenomic studies (that is, those that investigate DNA methylation, histone modifications and noncoding RNA) may provide novel insights into the mechanisms underlying EH.[29] Indeed, epigenetics provides the link between genetic programming and environmental influence that result in the expressed phenotype.[8] In addition, future large-scale studies should focus on clarifying the causal mechanism of the interactions and discovering more factors in the heritability of EH. Notably, El Shamieh *et al.*[30] proposed a new category of functional genetic biomarkers, eMethSNPs, which could act through DNA methylation mechanisms and predispose individuals to EH.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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