Association of single nucleotide polymorphisms in the 5' upstream region of the *C4BPA* gene with essential hypertension in a northeastern Han Chinese population

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Abstract. A previous study of the authors using microarray analysis indicated that the expression of complement component 4 binding protein (*C4BP*)*A* is upregulated in essential hypertension (EH) patients, but the association between *C4BPA* variations and EH has not yet been clearly demonstrated. Since the 5' upstream region is known to serve important roles in the gene expression regulation, the present study aimed to identify and analyze the association of single nucleotide polymorphisms (SNPs) in the 5' upstream region between the *C4BPA* gene with EH in a case-control study among a northeastern Han Chinese population through direct sequencing as well as genotype detection. A total of 822 unrelated participants were included. The higher expression level of *C4BPA* in the peripheral blood of patients with EH was verified through reverse transcription-quantitative polymerase chain reaction and ELISA. A total of four SNPs, rs73079108, rs74148971, rs77660718 and rs11120211 were identified in the 5' upstream region of *C4BPA*. Association analysis demonstrated that the genotypic frequencies of rs73079108 were significantly different between EH and the control groups (P=0.011), and A allelic frequency was lower in EH (P<0.001). Logistic regression analysis indicated that the rs73079108 polymorphism was closely associated with EH (AA:GA:GG genetic model: P=0.007, odds ratio (OR)=0.604, 95% confidence interval (CI) [0.418-0.873]; AA+GA:GG genetic model: P=0.005, OR=0.806, 95% CI[0.382-0.841]), and the A allele may be a protective factor. Subgroup analysis by sex and BMI presented concordant conclusions in female and non-obese samples. Further analysis indicated that rs73079108 was associated with systolic blood pressure (P<0.001), diastolic blood pressure (P=0.001) and fast blood glucose (FBG) (P=0.021). In addition, rs73079108 GA and GG carriers reported a significant increase in the level of the protein encoded by *C4BPA* than those of AA carriers. The rs73079108 polymorphism in the 5' upstream region of *C4BPA* was associated with EH, and rs73079108-A may be an independent predictor.

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Introduction

Essential hypertension (EH) is a major health burden worldwide, and leads to poor mortality and morbidity from the complications such as myocardial infarction, cerebrovascular diseases, heart failure and renal dysfunctions (1). It is a multi-factorial condition involving interactions among environmental, demographic and genetic disorders (2), among which, heritability accounts for 30-40% of blood pressure changes (3). A previous study of the authors identified 31 upregulated and 18 downregulated genes through microarray analysis in the peripheral blood samples of EH compared to normotensives, among which *CD36* was upregulated by 4.8-fold (4), and the association between the +273A/G single nucleotide polymorphism (SNP) of *CD36* and EH has been identified (1). The complement component 4 binding protein (*C4BP*)*A* gene is indicated to be significantly upregulated by 2.4-fold in EH.

C4BPA, located in the 1q32 chromosome, with 12 exons and 11 introns, encodes the alpha chain of C4BP, a major soluble inhibitor of both the lectin and the classical pathways of complement (5). C4BP exists in three different isoforms, $\alpha_7\beta_1$, $\alpha_7\beta_0$, and $\alpha_6\beta_1$ (6), with $\alpha_7\beta_1$ as the major form and $\alpha_6\beta_1$ as the minor, inhibiting complement activation by binding to the activated complement component C4b through the α chain, and works in the classical and lectin pathway (7). Upon inflammation, the $\alpha_7\beta_0$ isoform is upregulated, so the level of the α-chains of C4BP by and large reflects the total C4BP. The α chain also contains the binding sites for C3b, serum amyloid protein (8), heparin (9), low-density lipoprotein receptor-related protein (LRP) (10) and the surface proteins of some bacteria (11,12), which are key molecules involved in inflammation, lipid

metabolism and coagulation pathways (13,14). It has been reported that the rs11120211-A allele in the 5' upstream sequence is associated with increased *C4BPA* mRNA levels corresponds to the rs3813948-C allele associated with increased plasma levels of C4BPα and $\%\alpha_7\beta_0$. In addition, the variants are associated to the venous thrombosis susceptibility independent of the protein S regulation (15).

5' upstream regions of certain genes contain transcription factor binding sites, which are known as DNA response elements (REs), and regulate the initiation of gene transcription (16). Variations in the 5' upstream regions may alter the RE sequence, regulate the gene transcription and consequently alter the functions of the gene (17). Therefore, the aim of the present study was to identify candidate SNPs in the 5' upstream region of *C4BPA* and analyze the possible associations with EH through a case-control study in a northeastern Han Chinese population.

Materials and methods

Ethics statement. The present study complies with the 1975 Declaration of Helsinki, and was approved by the local ethics committee of China-Japan Union Hospital of Jilin University (Changchun, China). Written informed consent was obtained from each participant.

Subjects. A total of 822 participants for genotyping in the study aged from 25 to 65 were recruited from the Medical Physical Examination Center in China-Japan Union hospital, Jilin University (Changchun, China) during March 2014 to May 2014. Subjects with secondary hypertension, primary renal disease, dyslipidemia, diabetes mellitus, cancer, hepatic disorders and endocrine diseases were excluded. Height, weight, systolic blood pressure (SBP), diastolic blood pressure (DBP), total cholesterol (TC), high density lipoprotein cholesterol (HDL-c), low density lipoprotein cholesterol (LDL-c), triglyceride (TG), fasting blood glucose (FBG), serum creatinine and blood urea nitrogen (BUN) were measured.

A total of 371 EH subjects and 451 control subjects were recruited. Blood pressure (BP) was measured in a seated position using a mercury column sphygmomanometer twice with a 5 min interval according to the common protocol recommended by European Society of Hypertension (2). EH was defined as follows: SBP ≥140 mmHg and/or the average DBP ≥90 mmHg and/or current antihypertensive medication treatment. The controls with by SBP<140 mmHg, DBP<90 mmHg and had never been treated for hypertension. Questionnaires were administered to investigate the family history, smoking and drinking habits. Participants who smoked ^{3}100 cigarettes or drank ≥12 times a year were defined as smokers or drinkers (18,19). Body mass index (BMI)=weight/height^2 (kg/m^2). According to the obesity guidelines of the World Health Organization on Asian people (20), obesity was defined as a BMI ≥25 kg/m^2.

C4BPA gene expression study. A total of 30 EH and 30 controls were randomly selected to test the *C4BPA* expression in the peripheral blood. *C4BPA* gene expression was evaluated by reverse transcription-quantitative polymerase chain reaction (RT-qPCR), and C4BPα expression level in the blood plasma was evaluated with a human C4BPα ELISA test kit purchased from IBL International GmbH, Hamburg, Germany (catalog no. IBATGP1664) according to the manufacturer's protocol. To evaluate the mRNA expression level of *C4BPA*, total RNA was extracted from the peripheral blood using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) and purified using the RNeasy Mini cleanup kit (Qiagen, Inc., Valencia, CA, USA) according to manufacturer's protocol. cDNA was synthesized using the Superscript First Strand Synthesis kit (Invitrogen; Thermo Fisher Scientific, Inc.) following the manufacturer's protocol. *C4BPA* cDNA was subjected to qPCR and amplifications were performed with BioEasy SYBR®-Green I (Hangzhou Bori Technology Co., Ltd., Zhejiang, China) and with the β-actin gene as a control. The primer sequences were as follows: Forward, 5'-CTACGCATACGGCTTTTCTGT-3' and reverse, 5'-CCCATGTGAAACATCTGGCTTG-3' for *C4BPA*; forward, 5'-CCACGAAACTACCTTCAACTCC-3' and reverse, 5'-TCATACTCCTGCTGCTTGCTGATCC-3' for the gene encoding β-actin. qPCR was conducted in a 25 μl reaction volume under the following cycling conditions: An initial predenaturation step at 95˚C for 5 min, followed by 40 cycles of denaturation at 95˚C for 30 sec, annealing at 60˚C for 30 sec, extension at 72˚C for 30 sec and a final extension step at 72˚C for 7 min. For data analysis, the $2^{-\Delta\Delta Cq}$ quantification method was utilized (21).

Genotyping. All blood samples were taken into EDTA-containing receptacles and stored at -20˚C until genomic DNA was extracted using an AxyPrep DNA gel extraction kit (Axygen Scientific; Thermo Fisher Scientific, Inc.). A total off 100 individuals were randomly selected to scan and analyze the initial SNPs with 5 DNA pools of 20 samples in each pool. The 2kb 5' upstream region of *C4BPA* was PCR-amplified and sequence analysis was performed by Sangon Biotech Co., Ltd. (Shanghai, China). A total of four SNPs were identified and sequenced through PCR-sequencing among the 100 samples. Following preliminary analysis, genotype distribution of two SNPs were different between EH and normotensives and were further examined among all the participants through PCR-restriction fragment length polymorphism (RFLP) and PCR-single strand conformation polymorphism (SSCP). The primer sequences for PCR are listed in Table I. Genotyping was performed blindly to all other data.

Statistical analysis. SPSS software (version 19.0, IBM SPSS, Armonk, NY, USA) was used for database management and statistical analyses. Categorical variables were expressed as proportions (%) and continuous variables as mean ± standard deviation. All comparisons between two groups for allelic and genotypic frequencies were performed by chi-squared test and continuous variables by independent t-test. Genetic models (additive, dominant and homozygote comparison) were analyzed by multivariate logistic regression adjusted for covariates to calculate odds ratios (OR) with 95% confidence intervals (CI) to predict the risk of EH. Analyses used two-tailed estimation of significance. Presence of Hardy-Weinberg equilibrium was tested by the chi-squared test. P<0.05 was considered to indicate a statistically significant difference.

Table I. The primer sequences of C4BPA gene.

Fragment	Primer sequence (5' to 3')	Product size (bp)
rs73079108	CATGAAGACATGGAAGCCTTGC TTGAACTCTTCCTCTCCCTCAC	288
rs74148971	TTCCCGAGAACCAGAGGTCAG CCAGTAAGAAGACTAGCCAGCACT	210
rs77660718	TGCTGGCTAGTCTTCTTACTGGT TGTCTGCAGCCTTTGTCACT	262
rs11120211	AAGCAACAGGTGGAGTGATGAATGAG CCACTATGTGCTGAGTTATCTAGAACGT	924

Table II. Clinical characteristics of EH participants and normotensives.

	Total (n)		Male (n)		Female (n)	
	EH (371)	Control (451)	EH (276)	Control (313)	EH (276)	Control (313)
Sex, M/F	214/157	237/214	/	/	/	/
Age (years)	50.66±8.64[b]	48.43±8.60	49.47±7.94	48.64±8.51	52.28±9.29[d]	48.19±8.72
BMI (kg/m^2)	25.49±0.68[b]	23.75±0.36	24.63±6.05[c]	22.84±4.15	24.63±6.05[d]	22.84±4.15
SBP (mmHg)	154.63±14.01[b]	118.93±10.48	152.34±13.65[c]	120.74±9.51	157.75±13.94[d]	116.92±11.13
DBP (mmHg)	94.89±10.03[b]	74.57±8.60	96.60±9.64[c]	77.19±7.48	92.56±10.12[d]	71.65±8.83
HR (bpm)	79.92±13.70[a]	76.05±14.12	79.32±11.48	77.38±15.11	80.74±16.25[d]	74.58±9.72
HDL-C (mmol/l)	1.16±0.31	1.16±0.27	1.11±0.34	1.07±0.27	1.24±0.25	1.28±0.28
LDL-C (mmol/l)	3.00±0.95	2.92±0.77	2.86±0.95	2.94±0.78	3.19±0.92[d]	2.89±0.76
TG (mmol/l)	2.18±1.48[b]	1.66±1.23	2.35±1.53[c]	1.97±1.37	1.93±1.37[d]	1.32±0.94
TC (mmol/l)	5.08±0.92[b]	4.81±0.89	4.96±0.86	4.84±0.86	5.23±0.97[d]	4.76±0.92
FBG (mmol/l)	5.30±0.78[b]	5.02±0.69	5.34±0.74[c]	5.12±0.73	5.24±0.82[d]	4.93±0.63
Height (cm)	168.03±7.32	168.12±7.26	172.81±4.85	172.79±5.35	161.43±4.44	162.95±5.34
Weight (kg)	72.03±14.05[b]	67.67±13.11	76.96±9.90[c]	73.97±10.68	65.22±15.98	60.70±11.99
Cr (μmol/l)	78.87±29.91	72.59±20.82	85.21±16.12	88.30±17.20	75.15±30.11	72.94±20.12
BUN	4.96±1.34	4.94±2.53	5.03±1.29	5.51±1.64	4.93±1.37	4.59±1.32
FH (yes/no)	125/111[b]	107/186	66/67[c]	46/111	59/44	61/75
SH (yes/no)	56/180	78/215	55/78	75/82	1/102	3/133
DH (yes/no)	70/166	74/219	70/63	71/86	0/103	3/133

SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglyceride; TC, total cholesterol; Glu, glucose; Cr, creatinine; HR, heart rate; BUN, blood urea nitrogen; FH, family history; SH, smoking history; DH, drinking history. Values are presented as the mean ± standard deviation. [a]P<0.05, [b]P<0.01 vs. total control group; [c]P<0.01 vs. female control group; [d]P<0.01 vs. female control group.

Results

Characteristics of the participants. Samples comprising 822 unrelated participants comprising 371 hypertensive patients (214 men and 157 women; mean age 50.66±8.64) and 451 normotensive controls (237 men and 214 women; mean age 48.43±8.60) were genotyped for the 5' upstream region. The participants were further divided into two subgroups according to sex. The clinical and laboratory parameters were summarized in Table II. For total subjects, female and male, when compared with the normotensives, the following variables were significant higher in EH: BMI, SBP, DBP, MBP, HR, TG and FBG. Age and TC were higher in EH compared with the controls in total and in female. Significant differences were identified in weight and family history incidence in total and in male.

Expression valuation of C4BPA in peripheral blood. The expression levels of *C4BPA* mRNA and C4BPα protein in peripheral blood in the EH and normotensives were tested (Fig. 1). C4BPA expression was significantly higher in EH than in controls both in the transcriptional level and translational level (P<0.05).

Detection and distribution of the SNPs. Through direct sequencing of the 5' upstream region of *C4BPA*, four SNPs

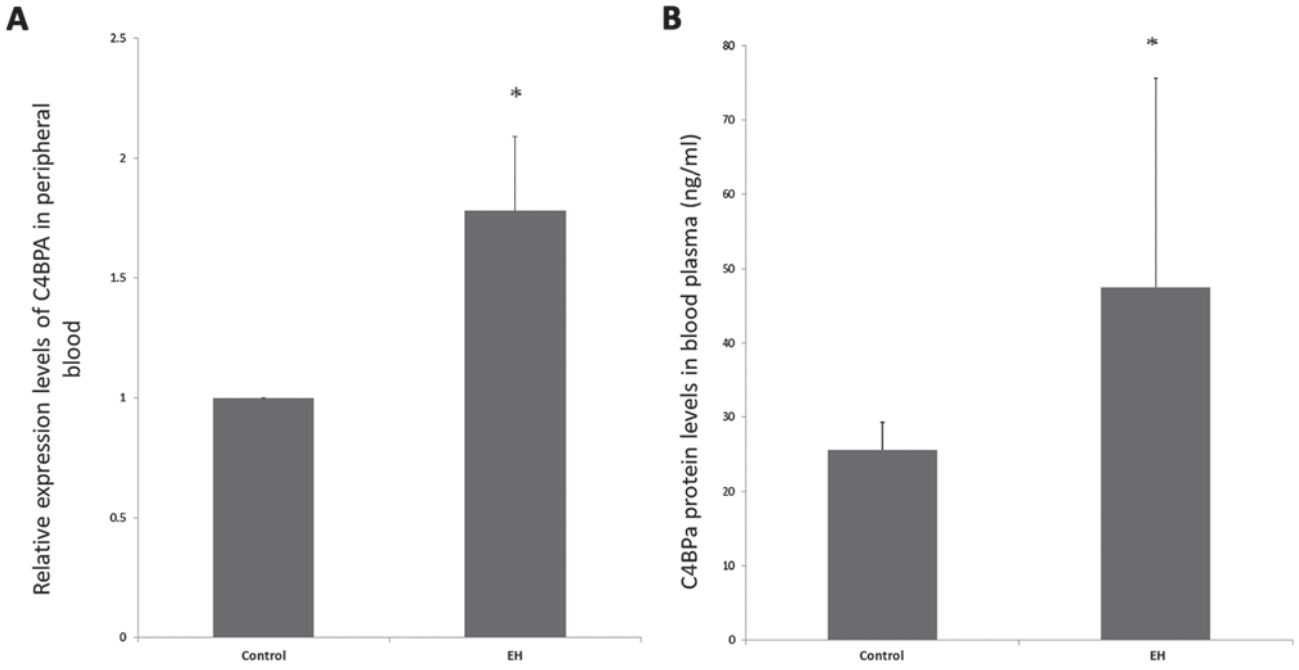

Figure 1. The expression level of C4BPA mRNA and protein in peripheral blood. Both the C4BPA (A) mRNA level and (B) C4BPα protein level were significantly upregulated in EH samples, when compared with controls. *P<0.05 vs. controls. EH, essential hypertension.

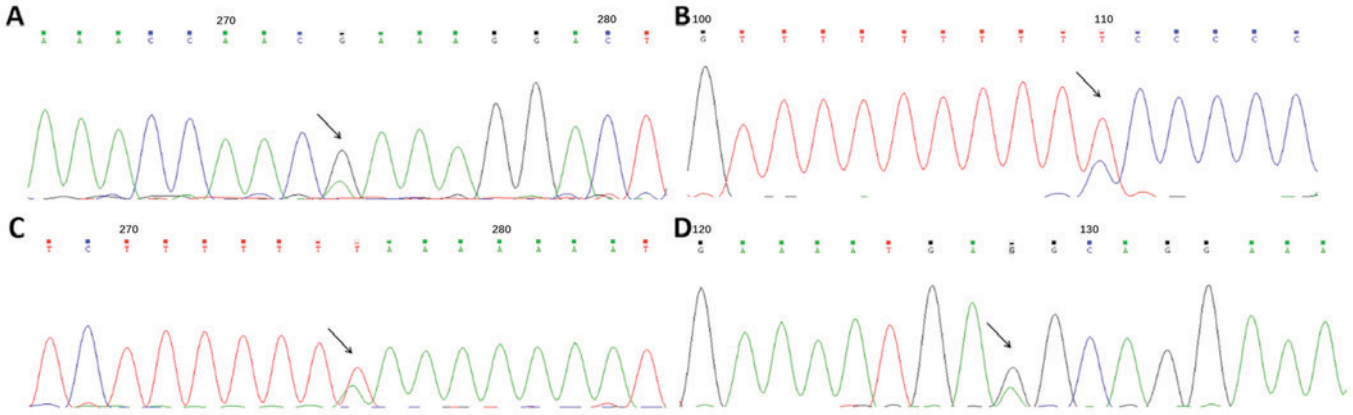

Figure 2. DNA sequencing results of the four SNPs identified in the 5' upstream of C4BPA gene. (A) rs73079108, (B) rs74148971, (C) rs77660718 and (D) rs11120211 respectively. The arrows indicate the SNP sites. SNP, single nucleotide polymorphism.

were identified (Fig. 2), and they were consistent with the SNPs labeled rs73079108, rs74148971, rs77660718 and rs11120211 in the National Center for Biotechnology Information database. Following preliminary analysis of genotype distributions in 100 samples, rs73079108 and rs74148971 presented a different trend in two groups and were further tested through PCR-SSCP and PCR-RFLP, respectively (Fig. 3). No deviation from the Hardy-Weinberg equilibrium expectation was observed for rs73079108 and rs74148971 in either normotensives or hypertensives (P>0.05).

Univariate analysis indicated that the genotype and allele distribution of the rs73079108 polymorphism differed significantly between EH and normotensive subjects (P<0.05). The AA and GA genotypes were significantly more prevalent in the control cases, and the A allele frequency was significantly higher in the normotensives. When subdivided by sex, the difference of genotype distribution was also observed in males and females. The prevalence of G allelic frequencies was significantly higher in the hypertensives than in the normotensives both in total and in female subgroup, but not in male subgroup. For rs74148971, no significant differences in the proportion of genotypes and alleles were found between the two groups whether in total case, in male or in female (Table III).

Age, SBP, DBP, FBG, TC, TG and BMI levels were compared among genotypes of the rs73079108 polymorphism (Table IV). Lipid profiles, age and BMI did not differ significantly among the genotypes, but there was a significantly higher level of SBP, DBP and FBG in the GG genotype.

Association analysis. Logistic regression analysis was performed under different genetic models (additive, dominant and recessive) after adjusting for confounding risk factors, including age, sex, BMI, HDL-c, LDL-c, TG, TC, FBG,

Table III. The frequencies of the C4BPA gene rs73079108 and rs74148971 genotypes.

Group	Genotype (frequency, %)			P-value[a]	Allele (frequency, %)		P-value[b]
	GG	GA	AA		G allele	A allele	
rs73079108							
EH (Total)	324 (87.33)	44 (11.86)	3 (0.81)	0.011	692 (93.26)	50 (6.74)	<0.001
Control (Total)	350 (77.61)	92 (20.40)	9 (2.00)		792 (77.80)	110 (12.20)	
EH (Male)	187 (87.38)	27 (12.62)	0 (0)	0.038	401 (93.69)	27 (6.31)	0.184
Control (Male)	201 (84.81)	31 (13.08)	5 (2.11)		433 (91.35)	41 (8.65)	
EH (Female)	137 (87.26)	17 (10.83)	3 (1.91)	<0.001	291 (92.68)	23 (7.32)	<0.001
Control (Female)	149 (69.63)	61 (28.50)	4 (1.87)		359 (83.88)	69 (16.12)	
rs74148971							
EH (Total)	156 (65.27)	71 (29.71)	12 (5.02)	0.709	383 (80.13)	95 (19.87)	0.517
Control (Total)	128 (61.54)	70 (33.65)	10 (4.81)		326 (78.37)	90 (21.63)	
EH (Male)	72 (65.45)	32 (29.09)	6 (5.45)	0.806	176 (80.00)	44 (20.00)	0.640
Control (Male)	59 (61.46)	32 (33.33)	5 (5.21)		150 (78.13)	42 (21.87)	
EH (Female)	84 (65.12)	39 (30.23)	6 (4.65)	0.828	207 (80.23)	51 (19.77)	0.479
Control (Female)	69 (61.61)	38 (33.93)	5 (4.46)		166 (77.57)	48 (22.43)	

[a]P-value, the comparison of the additive genetic models; [b]P-value, the results of the comparison between the alleles; EH, essential hypertension.

smoking and drinking history. As presented in Table V, for the rs73079108 polymorphism, significant association could be identified in the additive genetic model (AA vs. GA vs. GG, OR=0.604, 95% CI: 0.418-0.873, P=0.007) and dominant genetic model (AA+GA vs. GG, OR=0.567, 95% CI: 0.382-0.841, P=0.005), but not in receive model or homozygote comparison. A significantly lower prevalence of A allelic frequency (P<0.001, OR=0.520, 95% CI: 0.489-0.554) was observed in EH than in the control group, which suggested that the A allele may be a protective factor for the EH in the northeastern Han Chinese population. When subdivided for sex, the situation was the same in the females in total, but not in males. As there was a rare frequency of AA in males, logistic regression analysis was not performed in receive model or homozygote comparison. The P-value of rs73079108 genotype-sex interaction was significant (P=0.015). For the rs74148971 polymorphism, significant association could only be found in recessive model, but no significant association was identified in other genetic models.

Interactive effect of rs73079108 polymorphism on EH. To examine whether there were associations between *C4BPA* rs73079108, obesity and EH, all subjects were subdivided into the obese and non-obese subgroup according to BMI and the analysis was further conducted.

Genotype distributions and allele frequencies of EH in the non-obese cases and non-obese female were significantly different from the control subjects (Table VI). Following logistic regression analysis, rs73079108 was indicated to be significantly related to the prevalence of EH both in the non-obese (P=0.001, OR=0.374, 95%CI [0.205-0.682]) and in the non-obese female (P=0.001, OR=0.251, 95%CI [0.110-0.573]). Whereas in obese group, no significant associations could be identified between rs73079108 and hypertension

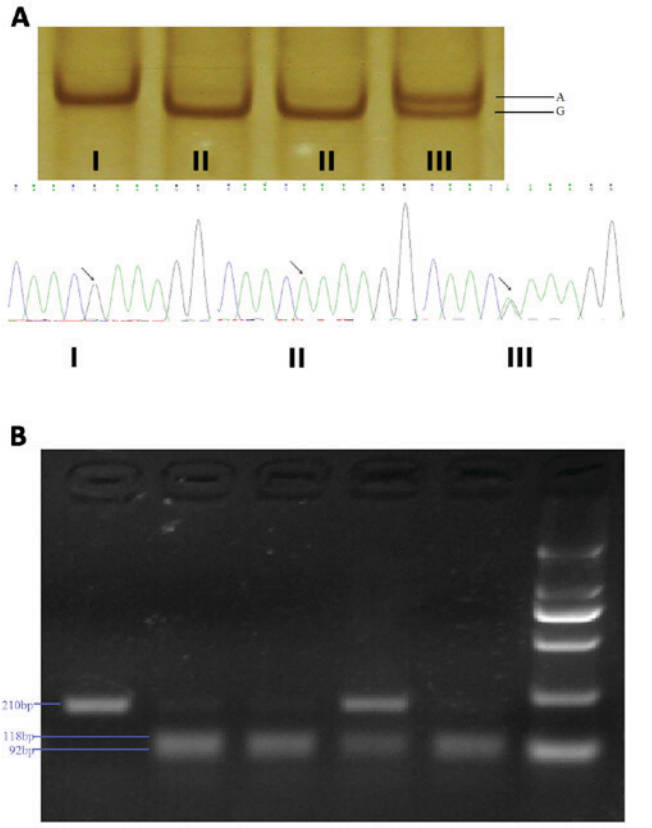

Figure 3. The genotype detection of rs73079108 and rs74148971 polymorphisms. (A) PCR-single strand conformation polymorphism detection and DNA sequencing results of rs73079108. Each of the three genotypes was sequenced. Type I was the AA genotype, type II was the GG genotype and type III was the GA genotype. (B) The electrophoresis of digested products of the rs74148971 polymorphism. Three genotypes were determined by BslI digestion fragments. The AA genotype was not digested and presented a 210 bp fragment. The GG genotype was digested into 118 bp and 92 bp fragments, which did not clearly separate during electrophoresis. One DNA chain was digested and another was not digested in the GA genotype.

Table IV. The association between rs73079108 and clinical features.

	Age	SBP (mmHg)	DBP (mmHg)	FBG (mmol/l)	TG (mmol/l)	TC (mmol/l)	BMI (kg/m^2)
AA	50.00±9.24	121.08±19.95	78.33±1.00	4.69±0.24	1.52±1.00	4.48±0.54	23.34±2.96
AG	50.14±8.22	129.42±22.30	80.07±14.99	5.02±0.59	1.61±1.06	4.76±0.78	24.15±4.65
GG	49.28±8.77	136.42±21.18	84.56±13.30	5.18±0.77	1.95±1.42	4.97±0.94	24.47±3.52
P-value	0.562	<0.001	0.001	0.021	0.276	0.066	0.423

SBP, systolic blood pressure; DBP, diastolic blood pressure; FBG, fasting blood glucose; TG, triglyceride; TC, total cholesterol; BMI, body mass index. Values are presented as the mean ± standard deviation.

Table V. Logistic regression analysis of additive genetic model comparison for each single-nucleotide polymorphism genotype associated with essential hypertension in the northeastern Han Chinese population.

		Overall			Male			Female		
SNP	Contrast	OR	95% CI	P-value	OR	95% CI	P-value	OR	95% CI	P-value
rs73079108	AA:AG:GG	0.604	0.418-0.873	0.007	0.702	0.407-1.210	0.203	0.551	0.328-0.926	0.024
	AA+GA:GG	0.567	0.382-0.841	0.005	0.806	0.461-1.409	0.450	0.410	0.229-0.735	0.003
	AA:GA+GG	0.548	0.142-2.111	0.382	-	-	-	1.605	0.327-7.885	0.560
	AA:GG	3.592	0.765-16.865	0.105	-	-	-	2.004	0.379-10.587	0.413
rs74148971	AA:AG:GG	1.184	0.836-1.677	0.342	1.149	0.699-1.889	0.585	1.175	0.717-1.925	0.522
	AA+GA:GG	1.210	0.798-1.834	0.369	1.123	0.617-2.042	0.704	1.273	0.705-2.298	0.424
	AA:GA+GG	1.508	1.298-1.752	<0.001	1.255	1.033-1.526	0.022	2.105	1.615-2.743	<0.001
	AA:GG	1.328	0.495-3.564	0.573	2.552	0.758-8.591	0.130	0.346	0.063-1.899	0.222

OR, odds ratio; CI, confidence interval; SNP, single nucleotide polymorphism. ORs are of the mutant alleles. Logistic regression analysis was adjusted for sex, age, BMI, total cholesterol, triglyceride levels, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, plasma glucose level, smoking history and drinking history, with sex excluded in the male and female group.

risk (Table VII). The P-value for rs73079108 genotype-BMI interaction was 0.042. The results indicated that there was a significant correlation between rs73079108 genotypes and obesity on EH, especially in women.

rs73079108 affects C4BPα protein level in control samples. To determine the effect of rs73079108 on C4BPα protein level, 100 control samples were randomly selected and the expression of C4BPα in the blood plasma was detected. Through ELISA detection, a significant increase in C4BPα protein level was observed in GA and GG carriers compared to the AA carriers (P<0.05; Fig. 4). In addition, it presented an increase trend in the GG carriers compared to the GA carriers, but the difference was not statistically significant.

Discussion

In the present study, the authors verified the elevated expression of *C4BPA* in the peripheral blood both at the transcriptional and translational level, which was consistent with their previous work through microarray analysis, suggesting a positive relationship between *C4BPA* and EH. A total of four SNPs in the 5' upstream region were identified and genotyping were further performed and analyzed. Univariate analysis demonstrated that elevated age, BMI, HR, TG, TC, FBG and weight may be risk factors of EH. The chi-squared test and logistic regression analysis, by taking the confounding factors together with the SNP into the regression model, were performed and the association of *C4BPA* rs73079108 with EH in the northeastern Han Chinese population was identified. The rs73079108-A allele may be a protective marker for EH in total and in females, and rs73079108 was indicated to affect C4BPα protein level in control samples. In addition, although the distribution of rs74148971 genotypes was not significantly different in both groups, the receive model indicates the association of rs74148971 and AA genotype may be a risk factor. The authors further carried on the stratification analysis by sex and BMI and discussed the possible relationship of EH, *C4BPA*, sex and BMI.

C4BP is an acute phase protein and it increases in concentration upon inflammation (22). The C4BP levels have been proven to be strongly and directly correlated to hs-CRP, which has been proved to be associated with EH in a previous report (23). C4BPA or C4BP have also been reported to be associated with myocardial infarction (23), atherosclerosis of the descending thoracic aorta (24), preeclampsia (25), venous thrombosis (15), schizophrenia (26), non-small cell lung cancer (27), joint hypermobility syndrome (28), triglyceride levels, as well as platelet count and warfarin treatment (29), but no

Table VI. The genotype distributions and allele frequencies of the C4BPA gene rs73079108 polymorphism in obese and non-obese samples.

			Genotype (frequency, %)				Allele (frequency, %)		
rs73079108 samples	Group	n	AA	GA	GG	P-value[a]	A allele	G allele	P-value[b]
Obese n=338	EH (Total)	191	3	26	162	0.931	32	350	0.784
	Control (Total)	147	3	21	123		27	267	
	EH (Male)	127	0	17	110	0.093	17	237	0.307
	Control (Male)	107	3	14	90		20	194	
	EH (Female)	64	3	9	52	0.212	15	113	0.644
	Control (Female)	40	0	7	33		7	73	
Non-obese n=482	EH (Total)	178	0	18	160	<0.001	18	338	<0.001
	Control (Total)	304	6	71	227		83	525	
	EH (Male)	87	0	10	77	0.331	10	164	0.448
	Control (Male)	13	2	17	111		21	239	
	EH (Female)	91	0	8	83	<0.001	8	174	<0.001
	Control (Female)	174	4	54	116		62	286	

[a]P-value, the comparison of the additive genetic models; [b]P-value, the results of the comparison between the alleles.

Table VII. Logistic regression analysis of the rs73079108 polymorphism associated with essential hypertension in obese and non-obese samples.

		Overall			Male			Female		
Sample groups	Contrast	OR	95% CI	P-value	OR	95% CI	P-value	OR	95% CI	P-value
Non-obese	AA:AG:GG	0.374	0.205-0.682	0.001	1.587	0.577-4.369	0.371	0.251	0.110-0.573	0.001
Obese	AA:AG:GG	0.961	0.568-1.628	0.883	0.723	0.359-1.455	0.363	0.737	0.289-1.878	0.522

OR, odds ratio; CI, confidence interval.

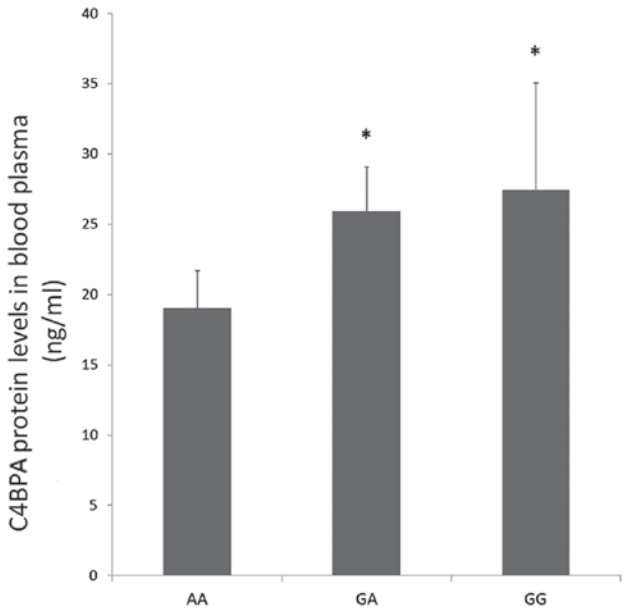

Figure 4. The expression of C4BPα protein in blood plasma of control subjects. The GA and GG carriers demonstrated a significant increase in C4BPα level than those of AA carriers. *P<0.05 vs. AA genotype.

evidence has been proved their direct association with EH. To the best of the authors' knowledge, the present study is the first time to identify the possible positive association between C4BPA and EH. *C4BPA* may contribute to the EH etiology in at least three ways: (a) Inhibition of complement activation by C4BP contributes to the endothelial dysfunction (30), which is now considered as essential element of EH etiology; (b) *C4BPA* may indirectly influence blood pressure through combination with low-density lipoprotein receptor-related protein 6 and regulate glucose metabolism (31). In the current study, the authors further proved the association between glucose and *C4BPA* and that FBG levels varied significantly among different genotypes of rs73079108 (Table IV; (c) The association of atherosclerosis and triglycerides with *C4BPA* also indicated the potential role of *C4BPA* in EH.

In a previous study, the authors already demonstrated that there was upregulated expression of *C4BPA* in EH (4), and the protective factor of rs73079108-A allele. In addition, rs73079108 was identified to affect the C4BPa level in controls. Based on the results, the authors inferred that the mutation from G allele to A allele in the 5'upstream region

may possibly change the DNA response elements and effect the transcription and consequently reduced *C4BPA* expression, which could attenuate the effects of *C4BPA* on EH. The present study then examined C4BPα protein level with different rs73079108 genotypes. A significant increase in C4BPα protein levels was identified in GA and GG carriers relative to the AA carriers. This data suggested that the A allele of rs73079108 influenced C4BPα levels and further proved the association with EH.

Sexual bias has long been recognized in hypertension. Here, stratification analysis by sex indicated that the association of rs73079108 was EH were female-specific. It may be caused by differences in lifestyle, social stress, hormonal system and genetic determinants (32). Conversely, C4BPα may bind efficiently to LRP (10) and effect the LRP functions in regulation of lipid homeostasis, LDL uptake and body fat mass (31). The significant differences of LDL-C and TG between EH and controls in females may partly contribute to the different association in sex-subgroups. Still, the different results between male and female as well as the P-value for genotype-sex interaction should be treated cautiously, as the female subjects were considerably fewer than males, which may be a limiting factor to detect the difference of OR estimates between the subgroups.

Following stratification analysis on obesity, association of rs73079108 and EH risk was shown in the non-obese subjects and non-obese women. Significant association between rs73079108 genotypes and BMI on EH risk could be identified. These findings suggested a potential effect of obesity on the association between hypertension risk and genetic factors.

In the current study, all participants were enrolled from the northeastern Han ethnic group and strict inclusive and exclusive criteria were made to reduce population stratification on some level. However, there were still some limitations, as demonstrated by the following: (a) Given that no clues implying correlation between C4BPA and hypertension biomarkers, the authors did not examine the association between rs73079108 and biomarkers. In the following study, they will focus on the downstream regulators of C4BPA involved in the regulation of EH to see whether the potential biomarkers of hypertension can be identified. (b) Due to relatively small number of the study samples, and strong genetic influences from other populations caused by migration in history, our understanding of the role of C4BPA gene polymorphisms in the development of EH was limited. Additional studies with a larger sample size in more diverse areas are required to further verify the association found in the present study. (c) Four SNPs were preliminarily examined in the present study, and only rs73079108 was further analyzed, whereas other SNPs are still worthy of study.

To the best of the authors' knowledge, the present study is the first study to examine the association between the rs73079108 polymorphism and EH in the northeastern Han Chinese population, to identify rs73079108-A as a protective factor of EH and to verify the association between *C4BPA* with EH. Subgroup analysis by sex and BMI demonstrated specificity in female and non-obese samples. Further functional studies of *C4BPA* in EH and studies in different populations are needed to confirm the discovery.

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