Research Article

Association of single nucleotide polymorphisms of \textit{MTHFR}, \textit{TCN2}, \textit{RNF213} with susceptibility to hypertension and blood pressure

Shan Liu*, Mengwei Liu*, Qian Li, Xiuping Liu, Yue Wang, Michael Mambiya, Kaili Zhang, Luping Yang, Qian Zhang, Mengke Shang, Fanxin Zeng, Fangfang Nie and \(\text{Wanyang Liu}\)

Department of Nutrition and Food Hygiene, School of Public Health, China Medical University, Shenyang, China

Correspondence: Wanyang Liu (wyliu@cmu.edu.cn)

Introduction

Hypertension is a multifactorial disease and is a major life-threatening health concern throughout the world. Approximately 2 million people in China die of diseases directly associated with hypertension each year, and its the prevalence rate is still on the rise. Especially in the middle-aged and old population, high blood pressure (BP) has become the main cause of coronary heart disease, stroke and many other cardiovascular diseases (CVDs). The results of an 8-year follow-up with 170000 people over 40 years old in China showed that coronary heart disease was the first cause factor of death, while hypertension was the first risk factor for total mortality, and the relative risk ratio (RR) was 1.48 [1]. The pathogenesis of hypertension is known as a result of the interaction of lifestyle exposures, such as high dietary sodium, overweight and excess alcohol consumption [2]. However, previous studies have shown that up to 60% of the variation in inducing increased hypertension risk could be due to genetic factors [3].
A number of previous studies have suggested that genetic alterations in the genes controlling homocysteine (Hcy) and folate metabolism were linked to onset of CVDs [4,5]. Hyperhomocysteinemia (HHcy), an important and independent risk factor, which also contributes to endothelial damage and oxidative stress [6], has been linked to hypertension as it induces arteriolar constriction, renal dysfunction and increase in sodium reabsorption [7]. The excessive increase in Hcy level was mainly caused by gene mutation of key enzymes in the metabolic pathways.

Methylenetetrahydrofolate reductase gene (MTHFR), which catalyzes the conversion of 5,10-methylenetetrahydrofolate into 5-methyltetrahydrofolate, is a crucial enzyme in the metabolism of Hcy and folate which both have associated with methylation of genomic DNA [8]. Two common single nucleotide polymorphisms (SNPs), C677T (rs1801133) and A1298C (rs1801131), are particularly reported to be associated with reduced enzyme activity and thermostability, resulting in a relative deficiency in the re-methylation process and interfering with the metabolic pathway. The C/T variant at site 677 is to replace the encoded alanine with valine, and the A/C variant at site 1298, is to convert its encoded glutamic acid into alanine, which leads to elevated plasma Hcy [9–14] and damages the integrity of blood vessels [15]. Furthermore, recent evidence has found that MTHFR rs9651118 was associated with the serum level of Hcy and also contributes to the development of vascular diseases [16], the same is true of transcobalamin II (TCN2) rs117353193 and RNF213 rs9916351. However, rs9651118 is an intron variant that does not cause amino acid changes.

Vitamin B12 is considered as a nutritional factor for regulating Hcy metabolism, the absorption and cellular delivery of which largely depend on the specific plasma transporter, TCN2 [17]. Vitamin B12 and TCN2 combine to form holotranscobalamin (holo-TC) complex, which plays an important role in cells within target tissues [18]. The variant in its loci rs117353193 causes the encoded arginine to be converted into glutamine. Because the biological function of TCN2 is mainly regulated by its own genetic polymorphism, it is also one of the important genetic factors affecting the metabolism of Hcy.

Ring finger protein 213 (RNF213) was originally identified as a susceptibility gene for moyamoya disease (MMD) [19]. MMD is often accompanied by hypertension [20,21], and the incidence of hypertension in MMD patients is significantly higher than that of the general population, suggesting that there may be a common susceptibility gene between the two. Rs9916351, though, is also an intron variant that does not cause amino acid changes. However, little data were so far found concerning the link of hypertension.

The loci of MTHFR, TCN2 and RNF213 have been found to be related to Hcy level in the study of susceptibility genes to MMD, which provides an idea for our study to see if there is actually a susceptibility gene loci between the two diseases. Many genome-wide association studies have been done on hypertension in different ethnic and regional populations [22,23], and the identified novel loci differ, requiring further verification. The present study was conducted to examine the association of MTHFR rs1801133, rs1801131, rs9651118, TCN2 rs117353193 and RNF213 rs9916351 gene polymorphisms with the risk of hypertension and BP in Northeast Chinese population.

Materials and methods
Editorial policies and ethical considerations
Before data collection, all subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by Ethics Committee of China Medical University.

Study population
The sample size was determined using the software Power and Sample Size (PS) for calculation. The relevant parameters of the statistical efficiency calculation in the present study were as follows: (1) the lowest allele frequencies of each SNP in the Chinese population referred to the dbSNP website, where the frequency of MTHFR rs1801131 was set at 0.219, the frequency of rs1801133 was set at 0.333, the frequency of rs9651118 was set at 0.267, the frequency of TCN2 rs117353193 was set at 0.333, the frequency of RNF213 rs9916351 was set at 0.329; (2) their odds ratios (ORs) were estimated based on previous observations [16,24,25], in which the OR value of MTHFR rs1801131 was set to 1.56, the OR value of rs1801133 was set to 1.87, and the OR value of RNF213 rs9916351 was set to 1.96. Among them, MTHFR rs9651118, TCN2 rs117353193 and RNF213 rs9916351 were recently found in the study of genome-wide association for MMD, there is no correlation of hypertension, so the OR value setting adopts their OR value in MMD study; (3) the ratio of sample size of control group to case group was set at 1:1; (4) test level α was set to 0.05. From the sample size calculation: 416 cases were needed for rs1801131 to be able to reject the null hypothesis reaching at least 80% power in the present study, 651 cases for rs1801133, 485 cases for rs9651118, 954 cases for rs117353193 and 145 cases for rs9916351. Meanwhile, the results of power calculation based on our sample size show that: 98.9% power...
for rs1801131, 92.4% power for rs1801133, 97.6% power for rs9651118, 80.0% power for rs117353193, 100% power for rs9916351.

A total of 953 patients with hypertension and 1103 controls were enrolled from Fushun and Panjin City in Liaoning Province, China. The population proportions from the two cities were not significantly different in the case and control groups ($P > 0.05$). There were 894 male (43.5%) and 1162 female (56.5%) in our study. In summary, participants with hypertension who met the following criteria were recruited: (1) systolic BP (SBP) of at least 140 mmHg or diastolic BP (DBP) of at least 90 mmHg were measured three times on different days in resting state; (2) people who had been treated with antihypertensive drugs. The control group was normotensive after medical measurement (SBP < 140 mmHg and DBP < 90 mmHg). Both groups were 18 years of age and older and excluded severe liver, kidney and acute or chronic infectious diseases, hyperthyroidism or hypothyroidism, systemic arteriopathy, various tumors and other cerebrovascular diseases and metabolic diseases.

**Data collection and clinical evaluation**

Clinical data including gender, age, height, weight, body mass index (BMI), waistline and smoking history were recorded in health datasheet. After sitting for 5 min, baseline BP was measured three times using a standardized mercury-gravity monometer with a 30-s interval between replicates, and the mean value of three measurements was taken. Pulse pressure (PP) was calculated as the difference of SBP and DBP. Mean arterial pressure (MAP) was calculated as the sum of one-third SBP and two-thirds DBP. Ten milliliters of peripheral blood of each fasting study individual was collected in EDTA vacutainer. Biochemical profiles, including fasting blood glucose (FBG), total cholesterol (TC) and triglyceride (TG) were done on automated biochemical analyzer (Murray, BS-820).

**DNA isolation and genotyping**

After receiving informed consent, 10 ml peripheral vein blood without centrifugation was extracted from available hypertensive patients and normal control subjects placed in EDTANa4 anticoagulant tubes, and stored in a freezer at $-80^\circ$C until analysis. Genomic DNA was extracted from blood samples with a Blood Genetic DNA Mini Kit (CW-BIO, Beijing, China). The concentration of the 2056 DNA was tested by NanoDrop 2000 (Thermo Fisher Scientific, Waltham, MA, U.S.A.), of which the purity is considered to be up to the requirements of subsequent tests, then stored at $-80^\circ$C for future genotyping.

Genotyping of five SNPs in all participants was conducted using Taqman™ Probe (Taqman™ SNP Genotyping Assays; Applied Biosystems, Foster City, CA, U.S.A.) and a QuantStudio™ 6 Flex Real-Time PCR System (Applied Biosystems, Foster City, U.S.A.) in a single lab. The total system contained 5 μl, 2.0 μl purified genomic DNA, 2.5 μl of TaqPath™ ProAmp™ Master Mixes (Applied Biosystems, Foster City, CA, U.S.A.), 0.1 μl of 40× SNP Genotyping Assay and 0.4 μl deoxyribonuclease-free water. The appropriate PCR thermal cycling conditions were as follows: maintained for 5 min for initial denature/enzyme activation, 40 cycles of 5 s at 95°C for denaturation, and 1 min at 60°C for annealing and extension. After each PCR amplification, an end point plate read was conducted using QuantStudio™ 6Flex Real-Time PCR System. The genotype of each sample was confirmed based on the fluorescence signals. We sampled DNA and repeated genotyping, and the results were consistent with previous experiments.

**Statistical analysis**

The Epidata 3.1 software package was used for database design, data entry and data check, and SBP and DBP were respectively increased by 10 and 5 mmHg in patients with hypertension treated with drugs [26]. Statistical analysis was performed with SPSS 21.0 software. Quantitative variables were tested for normality and homoscedasticity. Those conforming to normal distributions were expressed as mean ± standard deviation, while skewed distributions were expressed as median (P25–P75). The comparison between the two groups was carried out by independent-samples t test or nonparametric test, and one-way analysis of variance (ANOVA) was used in the multi-group comparison. Pairwise comparison was performed by least significant difference (LSD) and student newman keuls (SNK-q) test. Qualitative variables were compared chi-square test or Fisher’s exact test and expressed as counts and proportions. Univariate logistic regression analysis was used to test the association between each SNP and hypertension under the genetic models. Binary regression analysis was used to test the association between environmental factors and hypertension. Furthermore, the multivariate logistic regression analysis was used to test the susceptibility of each SNP to hypertension after correcting the confounding factors. Multiple linear regression analysis was used to see the effects of other covariates and genetic component on BP. The SHEsis [27] software was used for Hardy–Weinberg balance test, allele and genotype correlation analysis with hypertension and haploid analysis. A value of $P < 0.05$ was considered as statistically significant.
Table 1 Clinical and demographic characteristics of the study subjects

| Characteristics | Patients (n=953) | Controls (n=1103) | P-value |
|-----------------|-----------------|------------------|---------|
| Male:Female     | 439:514         | 455:648          | 0.0291  |
| Age (years)     | 66 (49–71)      | 44 (32–66)       | <0.001  |
| Height (cm)     | 162.44 ± 7.56   | 162.52 ± 8.97    | 0.864   |
| Weight (kg)     | 63.15 ± 9.25    | 60.89 ± 8.57     | <0.001  |
| Waistline (cm)  | 82.83 ± 9.01    | 80.03 ± 6.76     | <0.001  |
| BMI (kg/m²)     | 23.92 ± 3.09    | 23.02 ± 2.84     | <0.001  |
| SBP (mmHg)      | 150.68 ± 15.20  | 121.62 ± 11.32   | <0.001  |
| DBP (mmHg)      | 91.05 ± 9.34    | 77.98 ± 6.80     | <0.001  |
| PP (mmHg)       | 59.63 ± 14.87   | 43.84 ± 9.25     | <0.001  |
| MAP (mmHg)      | 110.94 ± 9.28   | 92.51 ± 7.40     | <0.001  |
| Smoking (%)     | 16.80%          | 7.75%            | <0.001  |
| FBG (mmol/l)    | 5.60 ± 1.86     | 5.17 ± 1.20      | <0.001  |
| TC (mmol/l)     | 4.80 ± 1.99     | 4.45 ± 1.20      | <0.001  |
| TG (mmol/l)     | 1.78 ± 1.10     | 1.51 ± 1.20      | <0.001  |

*Significant difference (P<0.05).

Results

Baseline characteristics

The clinical and demographic characteristics of 953 patients and 1103 controls are reported in Table 1. Compared with controls, the patients had significant differences in gender, age, weight, waistline, BMI, smoking frequency and higher levels of SBP, DBP, PP, MAP, FG, TC and TG. All of these foregoing parameters were statistically higher in patients when compared with control subjects (P<0.05). However, no statistically significant differences were observed in height (P=0.864) between the two groups.

The distributions of genotypes, alleles and associations with hypertension

Genotypes and alleles frequencies of five SNPs in patients and controls are shown in Table 2. The observed genotype distributions of five SNPs among controls were in agreement with Hardy–Weinberg equilibrium (P=0.503 for rs1801131; P=0.151 for rs1801133; P=0.707 for rs9651118; P=0.555 for rs117353193; P=0.545 for rs9916351). However, the genotypes’ distributions and the alleles frequencies were not statistically different between the two groups (P>0.05).

Logistic regression analysis of environmental and genetic factors

The results of binary regression analysis of environmental factors, including gender, age, weight, waistline, BMI, smoking frequency, FBG, TC and TG, are shown in Table 3. We found that waistline (P=0.006) and BMI (P=0.016) were risk factors associated with hypertension. The results of logistic regression analysis of genetic factors are shown in Table 4. Univariate logistic regression analysis showed that the five SNPs had no significant differences under the three genetic models. After adjusting for important confounding factors, including gender, age, waistline, BMI, smoking, FBG, TC and TG, the results showed that A allele carriers of TCN2 rs117353193 under the dominant genetic model (AA+GA vs GG) had a significantly protective effect compared with the risk of hypertension [OR = 0.56, 95% confidence interval (CI) (0.32–0.99); P=0.045], and the AA+GA genotype carriers had 0.56-times higher risk than the GG genotype carriers. Additionally, a borderline significant association was observed under the additive model (GA vs GG) after adjustment [OR = 0.59, 95% CI (0.33–1.04); P=0.069]. However, in the adjusted analysis, the effects of waistline and BMI still exist.

Haplotype distribution of three SNPs of MTHFR gene

Haplotype analysis of rs1801131, rs1801133 and rs9651118 polymorphisms of MTHFR gene are represented in Table 5. No significant differences were observed in any of the examined haplotypes (ACC, ACT, ATT, CCT) between hypertensive patients and controls (P>0.05). These findings suggest that the haplotypes of the MTHFR gene are not associated with susceptibility of hypertension in our subjects.
Table 2 Genotypes and alleles frequency of five SNPs in patients with hypertension and controls

| Genotypes and alleles | Patients (%) | Controls (%) | OR (95% CI) | P-value |
|-----------------------|-------------|--------------|-------------|---------|
| rs1801131             |             |              |             |         |
| A                     | 1587 (85.3%)| 1852 (86.2%)| 0.93 (0.78–1.11) | 0.147   |
| C                     | 273 (14.7%) | 296 (13.8%)  |             |         |
| AA                    | 679 (73.0%) | 801 (74.6%)  |             |         |
| AC                    | 229 (24.6%) | 250 (23.3%)  |             |         |
| CC                    | 22 (2.4%)   | 23 (2.1%)    |             |         |
| rs1801133             |             |              |             |         |
| C                     | 839 (44.9%) | 933 (43.4%)  | 1.08 (0.94–1.20) | 0.333   |
| T                     | 1029 (55.1%)| 1217 (56.6%) |             |         |
| CC                    | 200 (21.4%) | 214 (19.9%)  |             |         |
| CT                    | 439 (47.0%) | 505 (47.0%)  |             |         |
| TT                    | 295 (31.6%) | 356 (33.1%)  |             |         |
| rs9651118             |             |              |             |         |
| T                     | 1383 (74.2%)| 1564 (73.2%) | 0.95 (0.83–1.10) | 0.485   |
| C                     | 481 (25.8%) | 572 (26.8%)  |             |         |
| CC                    | 200 (21.4%) | 214 (19.9%)  |             |         |
| CT                    | 349 (37.4%) | 414 (38.8%)  |             |         |
| CC                    | 66 (7.1%)   | 79 (7.4%)    |             |         |
| rs117353193           |             |              |             |         |
| G                     | 1774 (94.9%)| 2041 (94.8%) | 0.98 (0.74–1.29) | 0.873   |
| A                     | 96 (5.1%)   | 113 (5.2%)   |             |         |
| GG                    | 840 (89.8%) | 966 (89.7%)  |             |         |
| GA                    | 94 (10.1%)  | 109 (10.1%)  |             |         |
| AA                    | 1 (0.1%)    | 2 (0.2%)     |             |         |
| rs9916351             |             |              |             |         |
| C                     | 1087 (58.3%)| 1195 (55.6%) | 1.12 (0.98–1.26) | 0.088   |
| T                     | 779 (41.7%) | 955 (44.4%)  |             |         |
| CC                    | 319 (34.2%) | 337 (31.3%)  |             |         |
| CT                    | 449 (48.1%) | 521 (48.5%)  |             |         |
| TT                    | 165 (17.7%) | 217 (20.2%)  |             |         |

P is calculated for carriers of the polymorphism. Abbreviation: CI, confidence interval.

Table 3 Binary regression analysis of environmental factors

| Environmental factors | OR (95% CI) | P-value |
|-----------------------|-------------|---------|
| Gender (%)            | 1.129 (0.747–1.705) | 0.565   |
| Age (years)           | 1.007 (0.978–1.037) | 0.632   |
| Weight (kg)           | 0.988 (0.953–1.025) | 0.528   |
| Waistline (cm)        | 1.033 (1.010–1.058) | 0.0061  |
| BMI (kg/m²)           | 1.139 (1.025–1.267) | 0.0161  |
| Smoking (%)           | 1.556 (0.962–2.520) | 0.072   |
| FBG (mmol/l)          | 1.058 (0.967–1.157) | 0.217   |
| TC (mmol/l)           | 0.938 (0.833–1.057) | 0.294   |
| TG (mmol/l)           | 0.964 (0.863–1.077) | 0.521   |

1Significant difference (P<0.05).

Comparison of BP levels of five SNPs in three genetic models

As shown in Table 6, we found that the average DBP (P=0.044) and MAP (P=0.035) levels of RNF213 rs9916351 were significantly different under the additive model, and the average SBP level had a borderline significant difference (P=0.077). Further pairwise comparison showed that the average SBP level with the homozygous TT genotype carriers were significantly higher than in CC genotype carriers (P=0.024), the average DBP and MAP levels with the homozygous TT genotype carriers were significantly higher than in CT (P=0.044 for DBP, P=0.012 for MAP) and...
Table 4 Logistic regression analysis of five SNPs in three genetic models

| SNP       | Genetic model | Univariate   | Adjusted 1 |
|-----------|---------------|--------------|------------|
|           |               | OR (95% CI)  | P-value    | OR (95% CI)  | P-value    |
| rs1801131 | Additive      | 1.13 (0.62–2.04) | 0.690 | 1.32 (0.45–3.86) | 0.608 |
|           | AC vs AA      | 1.08 (0.88–1.33) | 0.462 | 0.92 (0.64–1.33) | 0.667 |
|           | CC+AC vs AA   | 1.09 (0.89–1.32) | 0.425 | 0.95 (0.67–1.35) | 0.782 |
|           | CC vs AC+AA   | 1.11 (0.61–2.00) | 0.736 | 1.35 (0.47–3.92) | 0.579 |
| rs1801133 | Additive      | 0.89 (0.69–1.14) | 0.340 | 1.24 (0.81–1.92) | 0.324 |
|           | AC vs AA      | 0.93 (0.74–1.17) | 0.540 | 1.32 (0.91–1.93) | 0.143 |
|           | CC vs AC+AA   | 0.91 (0.74–1.17) | 0.405 | 0.81 (0.58–1.21) | 0.196 |
| rs9651118 | Additive      | 0.93 (0.66–1.32) | 0.679 | 0.77 (0.41–1.46) | 0.425 |
|           | CC+TC vs TT   | 0.94 (0.78–1.13) | 0.496 | 1.00 (0.71–1.40) | 0.999 |
|           | CC vs TC+TT   | 0.94 (0.79–1.12) | 0.464 | 0.80 (0.59–1.10) | 0.166 |
| rs117353193| Additive     | 0.93 (0.66–1.32) | 0.955 | 0.96 (0.83–1.11) | 0.576 |
|           | AA vs GG      | 0.96 (0.74–1.31) | 0.910 | 0.56 (0.32–0.99) | 0.045 2 |
|           | GA vs GG      | 0.99 (0.74–1.33) | 0.955 | 0.59 (0.33–1.04) | 0.069 |
| rs9916351 | Additive      | 1.25 (0.97–1.61) | 0.091 | 1.13 (0.68–1.89) | 0.644 |
|           | TT vs CC      | 1.13 (0.89–1.44) | 0.304 | 0.88 (0.61–1.20) | 0.552 |
|           | CT vs CC      | 0.88 (0.73–1.06) | 0.176 | 0.91 (0.66–1.25) | 0.552 |
|           | TT vs CT+CC   | 0.85 (0.68–1.06) | 0.155 | 1.23 (0.76–1.99) | 0.398 |

1 Adjusted for gender, age, waistline, BMI, smoking, FBG, TC and TG.
2 Significant difference (P<0.05).

Table 5 Haplotype distribution of three SNPs of MTHFR gene

| Haplotype | Patients (%) | Controls (%) | OR (95% CI) | P-value |
|-----------|--------------|--------------|-------------|---------|
| ACC       | 462.58 (25.1%) | 548.54 (25.9%) | 0.96 (0.83–1.11) | 0.576 |
| ACT       | 95.85 (5.2%) | 67.92 (4.1%) | 1.09 (0.94–1.21) | 0.115 |
| ATT       | 1000.58 (54.4%) | 1178.35 (56.6%) | 0.95 (0.79–1.21) | 0.412 |
| CCT       | 264.13 (14.4%) | 282.32 (13.3%) | 1.09 (0.79–1.56) | 0.358 |

All those P<0.05 will be ignored in analysis.

CC (P=0.048 for DBP, P=0.010 for MAP) genotypes carriers. In the recessive model, the average SBP, DBP and MAP levels of RNF213 rs9916351 with the homozygous TT genotype carriers were significantly higher than in CT+CC genotype carriers (P=0.043 for SBP, P=0.018 for DBP, P=0.017 for MAP). However, there were no significant differences in BP levels of rs1801131, rs1801133, rs9651118 and rs117353193 under the three genetic models.

Multiple linear regression analysis of five SNPs in genetic models

The results of multiple linear regression analysis of baseline covariates and genetic component on BP are shown in Table 7. In the recessive model, we found that RNF213 rs9916351 had significant effects on SBP (P=0.025), DBP (P=0.017) and MAP (P=0.010) as a risk factor. However, there were no significant associations of rs1801131, rs1801133, rs9651118 and rs117353193 on BP.

Discussion

Hypertension is a complex disease that comes about as a result of the interaction between genetic and environmental factors. Recently, numerous gene polymorphisms have been found to be associated with hypertension. The present study was designed to investigate the association of MTHFR (rs1801133, rs1801131, rs9651118), TCN2 (rs117353193) and RNF213 (rs9916351) gene variants with the susceptibility of hypertension and BP among the population of northeast in China.
In our study, we found that three MTHFR gene polymorphisms were not significantly associated with hypertension. Many genetic studies have shown that genetic variants in the MTHFR gene have been linked to CVDs, such as coronary heart disease [28,29], type 2 diabetes [30,31], ischemic stroke [32,33] and hypertension [34,35], but the clear mechanisms need to be further investigated. Markan et al. [36] reported that MTHFR rs1801133 CT/TT genotype were increased risk of hypertension in the Indian population. Koupepidou et al. [37] also suggested that rs1801133 TT/CT and rs1801131 CC genotypes may be the risk factors for hypertensive renal sclerosis and chronic renal damage in hypertensive patients. Furthermore, a meta-analysis combining 5207 patients and 5383 control subjects indicated a significant association between the rs1801133 gene polymorphism and hypertension, which suggested that carriers of the T allele and TT genotype were more susceptible [38], but no significant association with rs1801131 was found. Additionally, more studies have reported that the MTHFR rs1801133 is an independent factor for hypertension in different ethnic groups [39–41]. A meta-analysis from 114 studies with 15411 cases and 21970 controls shown that the rs1801133 polymorphism was significantly associated with hypertension, and stratified analysis by ethnicity revealed a significant association among East Asians and Caucasians, but not among Latinos, Black Africans, and Indians and Sri Lankans. This shows the effect of ethnicity on the results, the differences in environmental exposures and genetic background among different populations might suggest potentially different pathways of BP regulation. However, for the rs1801131 polymorphism, no significant association was observed either in overall or subgroup analysis under all genetic models [24].

### Table 6 Comparison of BP levels of five SNPs in three genetic models

| SNP      | Genetic model | n   | SBP (Mean ± SD) | DBP (Mean ± SD) | PP (Mean ± SD) | MAP (Mean ± SD) |
|----------|---------------|-----|-----------------|-----------------|----------------|-----------------|
| rs1801131| Additive      | 24  | 138.58 ± 17.63  | 87.38 ± 11.77   | 51.21 ± 13.48  | 104.42 ± 12.50  |
|          | Dominant      | 257 | 136.67 ± 18.22  | 85.01 ± 9.71    | 51.66 ± 14.73  | 102.24 ± 10.90  |
| rs1801133| Additive      | 27  | 138.58 ± 17.63  | 87.38 ± 11.77   | 51.21 ± 13.48  | 104.42 ± 12.50  |
|          | Dominant      | 257 | 136.67 ± 18.22  | 85.01 ± 9.71    | 51.66 ± 14.73  | 102.24 ± 10.90  |

| SNP      | Genetic model | n   | SBP (Mean ± SD) | DBP (Mean ± SD) | PP (Mean ± SD) | MAP (Mean ± SD) |
|----------|---------------|-----|-----------------|-----------------|----------------|-----------------|
| rs9651118| Additive      | 72  | 139.35 ± 20.29  | 86.90 ± 11.78   | 51.44 ± 14.67  | 102.26 ± 12.50  |
|          | Dominant      | 419 | 137.67 ± 20.44  | 84.98 ± 10.21   | 51.69 ± 15.56  | 102.53 ± 12.46  |
| rs11735319| Additive     | 180 | 136.72 ± 19.98  | 85.14 ± 10.39   | 50.57 ± 12.39  | 102.01 ± 12.33  |
|          | Dominant      | 419 | 137.67 ± 20.44  | 84.98 ± 10.21   | 51.69 ± 15.56  | 102.53 ± 12.46  |

| SNP      | Genetic model | n   | SBP (Mean ± SD) | DBP (Mean ± SD) | PP (Mean ± SD) | MAP (Mean ± SD) |
|----------|---------------|-----|-----------------|-----------------|----------------|-----------------|
| rs9915391| Additive      | 123 | 140.91 ± 19.69  | 87.23 ± 11.58   | 53.68 ± 13.59  | 105.11 ± 13.36  |
|          | Dominant      | 419 | 137.69 ± 20.40  | 85.08 ± 10.69   | 51.61 ± 15.37  | 102.62 ± 12.76  |

| SNP      | Genetic model | n   | SBP (Mean ± SD) | DBP (Mean ± SD) | PP (Mean ± SD) | MAP (Mean ± SD) |
|----------|---------------|-----|-----------------|-----------------|----------------|-----------------|
| rs9915391| Additive      | 123 | 140.91 ± 19.69  | 87.23 ± 11.58   | 53.68 ± 13.59  | 105.11 ± 13.36  |
|          | Dominant      | 419 | 137.69 ± 20.40  | 85.08 ± 10.69   | 51.61 ± 15.37  | 102.62 ± 12.76  |

Abbreviation: SD, standard deviation.

1 Significant difference (P<0.05).

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number of studies have revealed that Hcy may be involved in the pathogenesis of hypertension, including that plasma Hcy could induce arteriolar constriction, renal dysfunction and increased sodium reabsorption [7]. Increased plasma Hcy levels contribute to vascular endothelium damage and promote oxidative stress, which lead to endothelial dysfunction and an imbalance of the antioxidant status [42–44]. Our study did not evaluate the level of Hcy. Inversely, Ravera et al. [45] found no association between rs1801133 and BP level among hypertensive patients. It was also reported that there is no significant association between rs1801133 and hypertension in Algerians [46]. In addition, no studies had reported the significant association between rs1801131 and hypertension. These results were consistent with ours. Meanwhile, we found that the statistical efficiency of these loci was above 80% in our power calculations, the results showed that: 98.9% power for rs1801131, 92.4% power for rs1801133, 97.6% power for rs9651118, 80.0% power for rs117353193, 100% power for rs9916351, this excluded the reason of insufficient sample size for the lack of association. We think the difference is more likely due to difference in the degree of ethnic heterogeneity. Regarding rs9651118, little studies on hypertension have been conducted up to date. Similarly vitamin B₁₂ and folate are also involved in the metabolism of Hcy as nutritional factors. The former acts as a coenzyme in the catalytic synthesis of methionine with Hcy. TCN2 is recognized as a specific plasma transporter to facilitate the cellular uptake of vitamin B₁₂ by its receptor-mediated endocytosis [47]. Thus, TCN2 gene polymorphisms have been considered as another genetic trait which may affect Hcy metabolism by modulating the bioavailability of vitamin B₁₂ [48]. A genome-wide association study for MMD found that TCN2 rs117353193 genotype frequency was significant difference between patients with normal Hcy levels and hyperhomocysteine [16], and this applies to MTHFR rs9651118 as well. In our study, we found that the risk of GG genotype carriers of rs117353193 is lower than that of GA+AA genotypes carriers after adjusting for confounding factors, which suggested that it may be a protective factor for hypertension. However, waistline and BMI also play important roles, so our results may be more influenced by environmental and genetic interactions. In addition, the mutant AA genotype carriers are too few to compare with other genotypes carriers. The relevant studies are so few and there is need for it to be further explored.

Regarding RNF213, it has been proved to be a susceptible gene to MMD [49], but it has also been reported to be involved in other vascular disorders such as coronary heart disease, hypertension, aneurysm and heterogeneous intracerebral vasculopathy [50–54]. One study showed a significant association of RNF213 polymorphisms with SBP [53]. Our present study demonstrated that the homozygous TT genotype carriers of rs9916351 had significantly higher SBP, DBP and MAP levels than the CT or CC genotypes carriers, that is to say TT genotype might be a risk factor that is linked to increase the level of BP. Similarly, the results of multiple linear regression analysis also show that rs9916351 had significant effects on SBP, DBP and MAP as a risk factor, this further supports our conclusion. MTHFR rs9651118, TCN2 rs117353193 and RNF213 rs9916351 were recently revealed as the novel susceptibility

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Table 7 Multiple linear regression analysis of five SNPs in three genetic models

| SNP           | Genetic models | SBP β  | P-value | DBP β  | P-value | PP β  | P-value | MAP β  | P-value |
|---------------|----------------|--------|---------|--------|---------|-------|---------|--------|---------|
| rs1801131     | Additive       | 1.238  | 0.347   | 0.079  | 0.193   | 1.159 | 0.275   | 0.468  | 0.570   |
|               | Dominant       | 1.763  | 0.241   | 0.344  | 0.678   | 1.420 | 0.241   | 0.807  | 0.392   |
|               | recessive      | -1.213 | 0.778   | -1.955 | 0.407   | 0.742 | 0.830   | -1.600 | 0.551   |
| rs1801133     | Additive       | -0.627 | 0.503   | -0.295 | 0.565   | -0.332| 0.660   | -0.424 | 0.469   |
|               | Dominant       | -1.954 | 0.230   | -1.009 | 0.258   | -0.945| 0.471   | -1.338 | 0.188   |
|               | recessive      | 0.045  | 0.975   | 0.093  | 0.907   | -0.048| 0.968   | 0.045  | 0.961   |
| rs9651118     | Additive       | 0.806  | 0.457   | 0.492  | 0.412   | 0.314 | 0.719   | 0.617  | 0.365   |
|               | Dominant       | 0.854  | 0.524   | 0.714  | 0.335   | 0.139 | 0.897   | 0.785  | 0.351   |
|               | recessive      | 1.522  | 0.571   | 0.151  | 0.919   | 1.370 | 0.527   | 0.634  | 0.707   |
| rs117353193   | Additive       | 2.698  | 0.266   | 0.227  | 0.865   | 2.471 | 0.207   | 1.026  | 0.500   |
|               | Dominant       | 2.666  | 0.285   | 0.084  | 0.951   | 2.582 | 0.199   | 0.924  | 0.554   |
|               | recessive      | 9.356  | 0.604   | 8.155  | 0.412   | 1.202 | 0.934   | 8.279  | 0.464   |
| rs9916351     | Additive       | 1.137  | 0.258   | 0.851  | 0.124   | 0.287 | 0.724   | 0.939  | 0.135   |
|               | Dominant       | 0.049  | 0.972   | 0.370  | 0.624   | 0.321 | 0.772   | 0.258  | 0.764   |
|               | recessive      | 4.547  | 0.025   | 2.669  | 0.017   | 1.878 | 0.253   | 3.279  | 0.010   |

Abbreviation: β, partial regression coefficient.  
1significant difference (P<0.05).
loci for MMD by a genome-wide association study, no studies were conducted concerning the link to hypertension, and more evidences are needed to support our results.

Some limitations of our study should be mentioned. First, we did not get some of the relevant data in subjects, such as serum Hcy, vitamin B₁₂ and folate levels. Second, the differences in results in different studies of different countries may also attribute to the ethnic differences. Furthermore, hypertension is a complex disease and is affected by both environmental and genetic factors. Some important characteristics were significantly different between the patients and the controls in our study, such as BMI, smoking, FBG and so on. Smoking is particularly known to exacerbate hypertension [55], we speculate that the higher proportion of female in the controls may have led to a higher incidence of smoking in the cases than in controls, which also had an impact on our results. Hypertension is a disease that seriously affects the health of all mankind, but at the same time, hypertension is also a controllable disease. While preventing its various risk factors, gene polymorphism also provides an important direction for us to study its pathogenesis and disease progress. However, our study mainly aimed to determine the association of gene polymorphisms on hypertension, the differences of basic data between the two groups had no effect on genotypes. Our study needs to be further studied on account of the consideration of above limitations.

Conclusion
In summary, our study suggests that the TCN2 rs117353193 gene polymorphism might serve as protective factor in hypertension, and the RNF213 rs9916351 gene polymorphism might be an important risk factor that is linked to increase the level of BP among the population of northeast in China. Considering our relatively small sample size and narrow coverage, further studies are needed to confirm our results in the future.

Acknowledgments
The authors are thankful to all participants in the present study.

Author Contribution
All the authors participated in the whole work. However, S.L. designed the study and wrote the manuscript. M.L., Q.L., Y.W., M.M. and X.L. collected and analyzed the data. K.Z., L.Y. and Q.Z. assisted and involved in the experiment and in the revision of manuscript. M.S., F.Z. and F.N. contributed to select the samples, and the discussion. W.L. is the corresponding author and supervisor of the entire project.

Competing Interests
The authors declare that there are no competing interests associated with the manuscript.

Funding
This work was supported by the National Natural Science Foundation of China [grant number 81573240]. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Abbreviations
BMI, body mass index; BP, blood pressure; CVD, cardiovascular disease; DBP, diastolic BP; FBG, fasting blood glucose; Hcy, homocysteine; LSD, least significant difference; MAP, mean arterial pressure; MMD, moyamoya disease; MTHFR, methylenetetrahydrofolate reductase gene; OR, odds ratio; PP, pulse pressure; RNF213, ring finger protein 213; RR, relative risk; SBP, systolic BP; SNK-q, student newman keuls; SNP, single nucleotide polymorphism; TC, total cholesterol; TCN2, transcobalamin II; TG, triglyceride.

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