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Original Article

Associations between single nucleotide polymorphisms in miR-221, self-reported essential hypertension, and interactions between genetic and environmental factors: a multiethnic study in China

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Abstract: This case-control study explored the relationship between SNPs in miR-221 and self-reported essential hypertension, as well as interactions between genetic and environmental factors, in a multiethnic Chinese cohort. A MassArray analysis was performed to genotype 462 patients with essential hypertension and 442 healthy participants. The association between four SNPs in miR-221 and essential hypertension risk was determined by investigating the differences in allelic and genotypic frequencies between case and control groups using PLINK version 1.07 software. A 4 × 2 table approach was conducted to explore the synergistic effect of SNPs and environmental factors on the risk of essential hypertension. Subjects with the C allele of rs2858060 in miR-221 had a lower risk of essential hypertension than those with the G allele (OR = 0.692; 95% CI = 0.521-0.920; P = 0.011). Logistic regression analysis showed that carriers of the CC genotype had a significantly lower risk of essential hypertension than those with the homozygous GG genotype (OR = 0.679; 95% CI = 0.498-0.925; P = 0.014). Using crossover analyses, we identified significant interactions between rs2858060 and the effect of age, triglycerides, HDL-C, ApoB, and fasting blood glucose on essential hypertension risk. We conclude that rs2858060 in miR-221 is associated with essential hypertension risk in the Chinese population, with a clear interaction between rs2858060 and classical risk factors in predicting the condition. Therefore, rs2858060 in miR-221 could play an important role as a genetic risk factor for the development of essential hypertension in the Chinese population.

Keywords: Self-reported essential hypertension, single nucleotide polymorphisms, interaction, multiethnic study

Introduction

Essential hypertension is the single largest cause of mortality around the world. To reduce its burden on public health, new discoveries are required for the detection and treatment of the condition. However, the cause of essential hypertension remains largely unknown. Hypertension is known to result from the interactions of environmental and genetic factors, with approximately 30% of the interindividual variability in blood pressure regulation determined by genetic factors [1].

Because ethnic differences in cardiovascular disease are well recognized [2], studies have examined the relative importance of genetic, environmental, social, and cultural factors in causing cardiovascular diseases, including studies of racial and ethnic variations within countries and international variations in disease incidence [3]. However, data regarding the differences in hypertensive phenotypes, including the interactions between environmental and genetic factors from different ethnic groups, are lacking. We previously identified substantial ethnic differences in circulating miR-221 [4] and lipoprotein-associated phospholipase A2 [5] levels. As a sensitive regulator in the endothelium, miR-221 may help to identify novel biomarkers and therapeutic targets. Because endothelial dysfunction contributes to

the development of cardiovascular damage, miR-221 produced by endothelial cells is a putative biomarker for a wide range of cardiovascular diseases [6, 7]. MiR-221 might also affect the prevalence of hypertension and the interactions between environmental and genetic factors within multiethnic populations.

In the present study, we compared differences in the prevalence of self-reported essential hypertension and explored the interactions between SNPs in miR-221 and environmental factors in a multiethnic population in China.

Materials and methods

The present study received approval from the Ethics Committee of the First Affiliated Hospital of Nanjing Medical University (Jiangsu Province, China) and the Ethics Committee of the Friendship Hospital of Ili Kazakh Autonomous Prefecture (Xinjiang Uygur Autonomous Regions, China). The methods were carried out in accordance with approved guidelines, and written informed consent was obtained from each subject.

Study subjects

Ili Kazakh Autonomous Prefecture in northernmost Xinjiang is the only Kazakh autonomous prefecture in China. It is located west of Mongolia, south of Russia and east of Kazakhstan in northwestern China. In 2016, the Ili Kazakh Autonomous Prefecture had an estimated population of 466.20 million and was composed of primarily Han Chinese, with minorities of Kazak, Uygur, Hui, Mongol, Siwe, Kyrgyz, Uzbeks, Manju, Daur, and Russian descent within the population (www.stats.gov.cn).

From March 1, 2011 to July 31, 2014, 904 consecutive adult participantssubjects (661 males and 243 females) aged 32-84 years who were hospitalized at the Friendship Hospital of Ili Kazakh Autonomous Prefecture in China were enrolled in this study. The ethnicities of the 904 participantssubjects were Han (n = 572), Uygur (n = 137), Kazakh (n = 91), Hui (n = 67), and other (Mongol [n = 4], Siwe [n = 23], Kyrgyz [n = 2], Uzbeks [n = 2], Manju [n = 2], Daur [n = 2], and Russian [n = 2]).

Definition of self-reported hypertension

The outcome for this study was self-reported hypertension, which was defined as having a past hypertensive diagnosis and current hypertension treatment. Hypertensive screening and diagnosis were estimated by asking participants subjects the following question: “Have you ever been told by a doctor or health professional that you have hypertension, also called high blood pressure?” Among those who answered yes to the above question, they were further asked: “Are you currently taking medicine for your hypertension or undergoing hypertension treatment?” Hypertension treatment referred to any antihypertensive medication used, and when participants answered yes, the participants were asked to identify these medications [8].

Laboratory measurements

Enzymatic procedures were conducted using an automated Hitachi 7600 autoanalyzer (Hitachi Co., Tokyo, Japan) to measure cholesterol (CH, mmol/L), triglycerides (TG, mmol/L), fasting blood glucose (FBG, mmol/L), creatinine (μmol/L), fasting high-density lipoprotein cholesterol (HDL-C, mmol/L), fasting low-density lipoprotein cholesterol (LDL-C, mmol/L), apolipoprotein A (apoA, g/L), and apolipoprotein B (apoB, g/L) levels.

SNPs selection and genotyping

SNPs were selected on the basis of the several principal criteria. First, we selected polymorphisms in noncoding RNAs that are significantly related to cardiovascular disease, namely miR-221. Second, SNPs were selected from functional regulatory regions of genes, including promoter and exon regions. Third, allelic frequencies and linkage disequilibrium were analyzed based on the 1000 Genomes Project Chinese Han Beijing population data. Fourth, we chose a minor allele frequency greater than 0.05 and abandoned the strong linkage disequilibrium SNPs (R^2 = 1). Finally, we selected SNPs in the miR-221 gene using the candidate gene method.

Genomic DNA was extracted from whole blood samples using a whole blood genomic DNA purification mini kit (Bioteke Corporation, Beijing, China, #AU18016, lot number B016-007017) following the manufacturer’s instructions. The purified DNA was free of protein, nucleases, and other contaminants or inhibitors. DNA purity and concentration were estimated by a NanoDropND-2000 spectropho-

tometer (Thermo, Wilmington, DE, USA). The selected SNPs were genotyped by Bio Miao Biological Technology (Beijing, China) Co., Ltd. Primers, and multiplex reactions were designed using Assay Designer 3.1 and the RealSNP website (www.mysequenom.com). MassArray (Sequenom, San Diego, CA, USA) was used for genotyping all markers using allele-specific matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF).

Cigarette smoking and alcohol intake

Nonsmokers were those who reported having never smoked. Former smokers referred to those who previously smoked but quit. Persistent smokers were those who persistently consumed cigarettes. Each subject's smoking status was defined as either "never smoking" or "smoking" (included former and persistent smokers) [9, 10]. Participants who reported consuming alcohol within the previous 12 months at the time of the interview were considered current drinkers. Alcohol intake status was classified as either "never drinking" or "drinking" (included both former and current drinkers) [11].

Statistical analyses

The Hardy-Weinberg equilibrium and association assessments between SNPs and self-reported essential hypertension were performed using PLINK version 1.07 (http://zzz.bwh.harvard.edu/plink). The genotype variable was first analyzed as a categorical variable and then reanalyzed as a dichotomized variable by combining the heterozygous and homozygous genotypes of the variants, using the wild-type genotype as a reference.

Association analysis was performed on SNPs in miR-221 located on chromosome X according to the principles of how PLINK handles this chromosome in association tests.

By default, in the linear and logistic (-linear, -logistic) models for alleles A and B, men were coded as $A \geq 0$ and $B \geq 1$, while women were coded as $AA \geq 0$, $AB \geq 1$, and $BB \geq 2$. Additionally, sex (0 = men; 1 = women) was also automatically included as a covariate. Thus, it was important exclude sex as a separate covariate in a covariate file, and instead, use the special -sex command that tells PLINK to add sex as coded in the PED/FAM file as the covariate (in this way, it was not double entered for X chromosome markers). The basic association tests that are allelic (-assoc, -mh, etc.) do not need any special changes for X chromosome markers; the above only applies to the linear and logistic models where the individual, not the allele, is the unit of analysis. Similarly, the TDT remains unchanged. For the -model test and Hardy-Weinberg calculations, male X chromosome genotypes are excluded [12].

Descriptive statistical analyses were conducted using the Statistics Package for Social Sciences (ver. 16.0; SPSS Incorporated, Chicago, IL, USA). Participants were classified into two groups by self-reported essential hypertension status or five groups by ethnicity. Normally distributed variables, including apoB, were presented as the means ± standard deviation, and the comparisons were analyzed using the independent-samples t-test and analysis of variance. Variables with a skewed distribution, including age, total cholesterol, triglycerides, FBG, HDL-C, LDL-C, apoA, and creatinine levels, were presented as median and quartile ranges. The comparisons were made using the Mann-Whitney U test or the Kruskal-Wallis H test. Categorical variables were compared using chi-square analyses. Significance was assumed if the null hypothesis could be rejected with greater than 95% confidence. All *P*-values are two-tailed.

To further explore the synergistic effect of SNPs and environmental factors on the risk of self-reported essential hypertension, a 4 × 2 table approach was conducted to calculate the odds ratios (ORs), 95% confidence intervals (CIs), and two-tailed *P*-values. Multiple indexes were used to evaluate the synergistic effect between SNPs and environmental factors [13, 14].

Results

Baseline characteristics of participants by ethnicity

Table 1 presents the baseline characteristics of the participants grouped according to ethnicity. Significant differences in age ($P = 0.023$), FBG ($P = 0.029$), LDL-C ($P = 0.009$), apoA ($P = 0.018$), and apoB ($P = 0.003$) concentrations were observed across the various ethnicity groups. In addition, a difference in significance,

Table 1. Baseline subject characteristics by ethnicity

Variable	Ethnicity (n = 904)					F from chi-square test	*P*
	Han (n = 572)	Uygur (n = 137)	Kazakh (n = 91)	Hui (n = 67)	Others (n = 37)		
Age (years)	61.50 (52.00-70.00)	60.00 (53.50-66.00)	57.00 (50.00-64.00)	58.00 (54.00-64.00)	64.00 (56.00-68.50)	11.381	0.023
Sex (M/F)	402/170	102/35	75/16	54/13	26/11	8.466	0.076
Smoking (yes/no)	258/303	54/80	53/37	31/35	16/20	7.750	0.101
Drinking (yes/no)	103/456	16/118	14/76	8/58	4/32	5.284	0.259
Total cholesterol (mmol/L)	4.60 (3.87-5.45)	4.88 (4.09-5.69)	4.61 (3.80-5.28)	4.35 (3.58-5.33)	4.72 (3.91-5.48)	6.968	0.138
Triglycerides (mmol/L)	1.82 (1.24-2.48)	1.69 (1.24-2.43)	1.52 (1.07-2.05)	1.72 (1.08-2.53)	2.03 (1.26-2.74)	8.422	0.077
Glucose (mmol/L)	5.07 (4.62-6.02)	5.08 (4.56-5.95)	4.95 (4.49-5.55)	5.21 (4.75-7.07)	4.99 (4.77-5.62)	10.815	0.029
CR (μmol/L)	71.00 (62.00-81.00)	70.00 (60.00-81.50)	70.00 (64.00-84.00)	69.00 (62.00-76.50)	72.80 (62.00-85.50)	4.632	0.327
HDL-C (mmol/L)	1.35 (1.13-1.62)	1.45 (1.18-1.71)	1.29 (1.06-1.62)	1.32 (1.14-1.61)	1.41 (1.17-1.63)	5.455	0.244
LDL-C (mmol/L)	2.68 (2.14-3.30)	3.01 (2.44-3.76)	2.85 (2.22-3.53)	2.68 (1.97-3.54)	2.82 (2.07-3.42)	13.411	0.009
Apolipoprotein A (g/L)	1.30 (1.17-1.46)	1.26 (1.12-1.44)	1.25 (1.10-1.38)	1.33 (1.19-1.51)	1.26 (1.10-1.49)	11.954	0.018
Apolipoprotein B (g/L)	0.91±0.22	0.98±0.25	0.90±0.22	0.88±0.23	0.89±0.20	4.009	0.003
Self-reported essential hypertension (yes/no)	305/267	64/73	44/47	28/39	21/16	5.256	0.262

Abbreviations: CR, creatinine; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol. Normally distributed variables, including apoB, were presented as the means ± standard deviation, and the comparisons were analyzed using the independent-samples t-test and analysis of variance. Variables with a skewed distribution, including age, total cholesterol, triglycerides, Glucose, HDL-C, LDL-C, apoA, and CR levels, were presented as median and quartile ranges, and the comparisons were made using the Mann-Whitney U test or the Kruskal-Wallis H test. Categorical variables were compared using chi-square analyses. Significance was assumed if the null hypothesis could be rejected with > 95% confidence. All *P*-values are two-tailed.

Table 2. Baseline subject characteristics by self-reported essential hypertension status

Variable	Self-reported essential hypertension cases (n = 462)	Controls (n = 442)	Statistical Parameter	*P*
Age (years)	62.00 (54.00-70.00)	60.00 (51.00-68.00)	-2.732	0.006
Gender (male/female)	306/156	353/89	21.242	0.000
Smoking Status (Yes/No)	177/285	235/207	20.098	0.000
Drinking Status (Yes/No)	66/396	79/363	2.159	0.114
Total cholesterol (mmol/L)	4.62 (3.94-5.43)	4.65 (3.84-5.54)	-0.183	0.855
Glucose (mmol/L)	5.04 (4.62-5.97)	5.07 (4.60-5.85)	-0.296	0.767
Creatinine (μmol/L)	71.00 (61.00-83.00)	71.00 (62.00-80.00)	-0.462	0.644
Triglycerides (mmol/L)	1.84 (1.28-2.57)	1.67 (1.15-2.31)	-2.899	0.004
HDL (mmol/L)	1.36 (1.11-1.62)	1.37 (1.15-1.64)	-0.567	0.571
LDL (mmol/L)	2.75 (2.19-3.35)	2.75 (2.15-3.55)	-0.533	0.594
Apolipoprotein A (g/L)	1.29 (1.14-1.46)	1.29 (1.15-1.45)	-0.673	0.501
Apolipoprotein B (g/L)	0.923±0.231	0.908±0.218	-1.003	0.316

Abbreviations: HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol. Normally distributed variables, including apoB, are presented as the means ± standard deviation, and the comparisons were analyzed using the independent-samples t-test and analysis of variance. Variables with a skewed distribution, including age, total cholesterol, triglycerides, Glucose, HDL-C, LDL-C, apoA, and creatinine levels, are presented as median and quartile ranges, and the comparisons were made using the Mann-Whitney U test or the Kruskal-Wallis H test. Categorical variables were compared using chi-square analyses. Significance was assumed if the null hypothesis could be rejected with > 95% confidence. All *P*-values are two-tailed.

Table 3. Results of allelic association analysis for SNPs in miR-221 with self-reported essential hypertension and control

Chr	Location	SNP	Gene	SNP Function	A1	TEST	HWE	HWE in case	HWE in control	OR (95% CI)	*P*
X	45492134	rs2858059	miR-221	promoter	G	ADD	0.027	0.028	0.426	0.801 (0.613-1.047)	0.105
X	45492134	rs2858059	miR-221	promoter	G	SEX	0.027	0.028	0.426	0.474 (0.346-0.650)	3.498e-006
X	45491952	rs2858060	miR-221	promoter	G	ADD	0.061	0.206	0.259	0.692 (0.521-0.920)	0.011
X	45491952	rs2858060	miR-221	promoter	G	SEX	0.061	0.206	0.259	0.460 (0.335-0.631)	1.467e-006
X	45491076	rs2858061	miR-221	promoter	C	ADD	0.055	0.072	0.574	0.813 (0.618-1.069)	0.138
X	45491076	rs2858061	miR-221	promoter	C	SEX	0.055	0.072	0.574	0.476 (0.348-0.653)	3.933e-006
X	45491660	rs34678647	miR-221	promoter	T	ADD	0.036	0.017	1	0.960 (0.715-1.289)	0.785
X	45491660	rs34678647	miR-221	promoter	T	SEX	0.036	0.017	1	0.485 (0.353-0.665)	7.236e-006

Abbreviations: Chr., chromosome; CI, confidence interval; HWE, Hardy-Weinberg Equilibrium; OR, odds ratio. The HWE and association assessments between SNPs and self-reported essential hypertension were performed in PLINK version 1.07 (http://zzz.bwh.harvard.edu/plink).

regarded as the frequency distribution for self-reported essential hypertension status (*P* = 0.262), was not found across the various ethnicities.

Demographic, clinical, and biochemical characteristics by self-reported essential hypertension status

Table 2 shows the demographic, clinical, and biochemical characteristics of participants according to self-reported essential hypertension status. As shown in **Table 2**, a total of 904 participants were included in the study: 462 in the case group and 442 in the control group. The results showed different distributions for age (*P* = 0.006) and triglyceride (*P* = 0.004) concentrations between the two groups. Additionally, the frequency distributions for gender (*P* < 0.001) and smoking status (*P* < 0.001) were significantly different between the groups.

Allelic association analysis for investigated genes by group

The results of allelic association analyses for SNPs by group are shown in **Table 3**. The SNP rs2858060 showed a strong association with a risk of self-reported essential hypertension in the present population. The minor allele for the

Table 4. Results of allelic association analysis for SNPs in miR-221 and self-reported essential hypertension status by ethnicity

SNP	Ethnicity	Alleles	MAF	MAF in case	MAF in control	HWE	HWE in case	HWE in control	OR (95% CI)	P
rs2858059	Han	G/A	0.203	0.182	0.230	0.001	0.003	0.158	0.749 (0.530-1.058)	0.101
rs2858060	Han	G/C	0.187	0.163	0.218	0.008	0.054	0.075	0.697 (0.486-1.000)	0.050
rs2858061	Han	C/G	0.186	0.170	0.205	0.004	0.012	0.136	0.785 (0.548-1.124)	0.187
rs34678647	Han	T/G	0.206	0.209	0.203	0.078	0.034	1.000	0.997 (0.698-1.424)	0.985
rs2858059	Uygur	G/A	0.419	0.421	0.417	1.000	0.667	0.554	1.093 (0.578-2.067)	0.785
rs2858060	Uygur	G/C	0.353	0.337	0.369	0.712	1.000	0.554	0.907 (0.462-1.778)	0.775
rs2858061	Uygur	C/G	0.419	0.421	0.417	1.000	0.667	0.554	1.093 (0.578-2.067)	0.785
rs34678647	Uygur	T/G	0.111	0.115	0.107	0.362	0.298	1.000	1.068 (0.404-2.822)	0.895
rs2858059	Kazakh	G/A	0.346	0.327	0.365	1.000	0.480	0.333	0.871 (0.377-2.014)	0.747
rs2858060	Kazakh	G/C	0.243	0.200	0.289	1.000	1.000	0.111	0.633 (0.255-1.567)	0.323
rs2858061	Kazakh	C/G	0.321	0.296	0.346	1.000	0.480	0.333	0.786 (0.332-1.862)	0.584
rs34678647	Kazakh	T/G	0.170	0.130	0.212	1.000	1.000	1.000	0.533 (0.176-1.615)	0.266
rs2858059	Hui	G/A	0.141	0.091	0.178	1.000	1.000	1.000	0.399 (0.088-1.816)	0.235
rs2858060	Hui	G/C	0.143	0.091	0.182	1.000	1.000	1.000	0.391 (0.086-1.773)	0.223
rs2858061	Hui	C/G	0.141	0.091	0.178	1.000	1.000	1.000	0.399 (0.088-1.816)	0.235
rs34678647	Hui	T/G	0.231	0.273	0.200	1.000	1.000	0.441	1.432 (0.479-4.280)	0.521
rs2858059	Others	G/A	0.229	0.241	0.211	0.505	1.000	1.000	1.092 (0.226-5.268)	0.913
rs2858060	Others	G/C	0.125	0.103	0.158	1.000	1.000	1.000	0.539 (0.083-3.519)	0.519
rs2858061	Others	C/G	0.229	0.241	0.211	0.505	1.000	1.000	1.092 (0.226-5.268)	0.913
rs34678647	Others	T/G	0.229	0.207	0.263	0.439	0.200	1.000	0.741 (0.195-2.823)	0.661

Abbreviations; MAF, minor allele frequency; HWE, Hardy-Weinberg Equilibrium; OR, odds ratio; CI, confidence interval. The HWE and association assessments for SNPs in miR-221 with self-reported essential hypertension status grouped by ethnicity were performed in PLINK version 1.07 (http://zzz.bwh.harvard.edu/plink).

SNP rs2858060 was associated with a 30.8% decrease (OR = 0.692; 95% CI = 0.521-0.920; P = 0.011) in self-reported essential hypertension risk in an ADD model after adjusting for sex. In addition, no significant allelic associations with self-reported essential hypertension risk and the rs2858059, rs2858061, and rs34678647 SNPs were observed in the present population.

Stratified by ethnicity, the allelic associations between polymorphisms and self-reported essential hypertension risk are presented in **Table 4**. No statistical correlations were found between the groups for the SNPs rs2858059, rs2858060, rs2858061, and rs34678647.

Genotypic association analysis for investigated genes by group

The associations between the genotype frequencies of the investigated polymorphisms and the risks of self-reported essential hypertension are shown in **Table 5**. The associations between the rs2858060 polymorphism and the reduced risk of self-reported essential hypertension were observed in the ADD model (AA *vs.* GG: OR = 0.679; 95% CI = 0.498-0.925; P = 0.014) and the GENO_2DF model (P = 0.038). Additionally, when stratified by ethnicity, the association between the rs2858060 polymorphism and self-reported essential hypertension risk was confirmed in the Han population in the ADD model (AA *vs.* GG: OR = 0.674; 95% CI = 0.455-0.999; P = 0.049) (**Table 6**).

Interactions between rs2858060 in the ADD model and environmental factors

The results of an analysis of a possible synergistic effect of rs2858060 in the ADD model with environmental factors are presented in **Table 7**. No significant interaction was found between rs2858060 and gender, smoking, drinking, total cholesterol, LDL-C, apoA, or creatinine on the risk of self-reported essential hypertension. However, significant interactions were found between rs2858060 and age, triglycerides, HDL-C, apoB, and FBG on the risk of self-reported essential hypertension by a crossover analysis.

Regarding the baseline risk for participants unexposed to classical risk factors or to the rs2858060 GG genotype (reference category, 1.0), the OR estimating the combined effect of

Table 5. Results of genotypic association analysis for SNPs in miR-221 and self-reported essential hypertension status

SNP	A1/A2	Test	OR	95% CI	*P*
rs2858059	A/T	ADD	0.820	(0.617-1.091)	0.173
		DOMDEV	0.867	(0.480-1.566)	0.637
		SEX	0.455	(0.318-0.652)	1.79e-005
		GENO_2DF	NA	NA	0.240
rs2858060	A/G	ADD	0.679	(0.498-0.925)	0.014
		DOMDEV	1.103	(0.596-2.041)	0.756
		SEX	0.472	(0.331-0.673)	3.348e-005
		GENO_2DF	NA	NA	0.038
rs2858061	A/G	ADD	0.823	(0.614-1.104)	0.194
		DOMDEV	0.934	(0.514-1.696)	0.822
		SEX	0.467	(0.327-0.668)	3.06e-005
		GENO_2DF	NA	NA	0.325
rs34678647	A/G	ADD	1.012	(0.731-1.401)	0.942
		DOMDEV	0.776	(0.413-1.458)	0.431
		SEX	0.456	(0.320-0.649)	1.313e-005
		GENO_2DF	NA	NA	0.706

Abbreviations: CI, confidence interval; OR, odds ratio.

age (≥ 64.5 years defined as the age at greater risk), triglycerides (≥ 2.25 mmol/L was defined as the triglycerides at greater risk), HDL-C (< 1.03 mmol/L defined as the HDL-C at greater risk), apoB (≥ 1.10 g/L defined as the apoB at greater risk), and FBG (≥ 6.10 mmol/L defined as the FBG at greater risk) was significantly higher than the ORs estimating the effects of each factor alone.

A positive association was found between the rs2858060 GG genotype and age (Rothman's synergy index for an interaction (SI): 1.367; proportion of disease attributable to an interaction (AP): 0.159; relative excess risk due to an interaction (RERI): 0.391). Additionally, in these groups, as much as 15.9% of self-reported essential hypertension was attributable to the interaction between the rs2858060 GG genotype and age. The same analysis regarding triglycerides found significant results for carriers of the rs2858060 GG genotype (SI: 71.474; AP: 0.515; RERI: 1.080). The rs2858060 GG genotype interacted with HDL-C in predicting coronary artery disease (SI: 4.200; AP: 0.401; RERI: 0.848). An interaction was also found between apoB and the rs2858060 GG genotype (SI: 1.274; AP: 0.106; RERI: 0.208). FBG levels interacted significantly in predicting the risk of self-reported essential hypertension, and this combination accounted for 35% of the self-reported essential hypertension cases (SI: 10.380; AP: 0.350; RERI: 0.572).

Discussion

To the best of our knowledge, this is the first multiethnic study in China to explore the associations between SNPs in miR-221 and self-reported essential hypertension. Our major finding was that people with the C allele of rs2858060 in miR-221 have a lower risk of self-reported essential hypertension than those with the G allele (OR = 0.692; 95% CI = 0.521-0.920; *P* = 0.011). Additionally, logistic regression analysis showed that carriers of the CC genotype have a significantly lower risk of self-reported essential hypertension those with the homozygous GG genotype (*OR* = 0.679; 95% CI = 0.498-0.925; *P* = 0.014). Furthermore, significant interactions on the risk of self-reported essential hypertension were found between rs2858060 and the effect of age, triglycerides, HDL-C, apoB, and FBG by means of the crossover analysis.

As a multiethnic country, China has many ethnic groups that are geographically isolated due to its diverse terrain. Geographic isolation and different traditions may have led to various cultural and social customs of indigenous population groups in China. The distributed characteristics of SNPs in this multiethnic population can be explored to effectively understand the genetic associations among various populations and aid in the diagnosis and treatment of cardiovascular disease. In a recent study, 40 Y-chromosomal STR loci were genotyped in 2018 unrelated males from the following seven ethnic populations in South China: Yao, Zhuang, Gelao, Miao, Maonan, Gin, and Guangxi Han. The results indicated that a high degree of differentiation of 40 Y-STRs exists among ethnic groups [15]. In another study, phylogenetic trees and principal component analyses revealed a clear pattern of population differentiation of the Hui and Uygur ethnic groups in China [16]. To gain more insight into the genetic back-

Table 6. Results of genotypic association analysis for SNPs in miR-221 and self-reported essential hypertension status by ethnicity

SNP	Ethnicity	Test	OR	95% CI	*P*
rs2858059	Han	ADD	0.763	(0.527-1.104)	0.151
		DOMDEV	0.893	(0.417-1.912)	0.771
		SEX	0.534	(0.351-0.815)	0.004
		GENO_2DF	NA	NA	0.250
rs2858060	Han	ADD	0.674	(0.455-0.999)	0.049
		DOMDEV	1.183	(0.545-2.570)	0.670
		SEX	0.566	(0.372-0.862)	0.008
		GENO_2DF	NA	NA	0.135
rs2858061	Han	ADD	0.786	(0.533-1.159)	0.224
		DOMDEV	0.995	(0.458-2.158)	0.989
		SEX	0.559	(0.368-0.849)	0.006
		GENO_2DF	NA	NA	0.418
rs34678647	Han	ADD	1.050	(0.709-1.555)	0.809
		DOMDEV	0.781	(0.369-1.652)	0.518
		SEX	0.539	(0.349-0.831)	0.005
		GENO_2DF	NA	NA	0.811
rs2858059	Uygur	ADD	1.207	(0.610-2.389)	0.589
		DOMDEV	0.373	(0.082-1.699)	0.202
		SEX	0.187	(0.057-0.609)	0.005
		GENO_2DF	NA	NA	0.430
rs2858060	Uygur	ADD	1.039	(0.500-2.158)	0.919
		DOMDEV	0.398	(0.084-1.882)	0.245
		SEX	0.175	(0.054-0.571)	0.004
		GENO_2DF	NA	NA	0.489
rs2858061	Uygur	ADD	1.207	(0.610-2.389)	0.589
		DOMDEV	0.373	(0.082-1.699)	0.202
		SEX	0.187	(0.057-0.609)	0.005
		GENO_2DF	NA	NA	0.430
rs34678647	Uygur	ADD	1.126	(0.371-3.417)	0.834
		DOMDEV	0.805	(0.095-6.850)	0.843
		SEX	0.282	(0.116-0.682)	0.005
		GENO_2DF	NA	NA	0.972
rs2858059	Kazakh	ADD	0.690	(0.286-1.664)	0.409
		DOMDEV	9.272	(0.683-125.8)	0.094
		SEX	0.819	(0.188-3.571)	0.790
		GENO_2DF	NA	NA	0.227
rs2858060	Kazakh	ADD	0.434	(0.157-1.205)	0.109
		DOMDEV	2700000000.000	(0-inf)	0.998
		SEX	0.696	(0.189-2.565)	0.586
		GENO_2DF	NA	NA	0.277
rs2858061	Kazakh	ADD	0.605	(0.241-1.518)	0.284
		DOMDEV	10.270	(0.745-141.700)	0.082
		SEX	0.793	(0.180-3.483)	0.758
		GENO_2DF	NA	NA	0.182
rs34678647	Kazakh	ADD	0.524	(0.1455-1.886)	0.323
		DOMDEV	1.074	(0.082-14.020)	0.957
		SEX	0.318	(0.077-1.315)	0.114
		GENO_2DF	NA	NA	0.539

rs2858059	Hui	ADD	0.711	(0.118-4.281)	0.709
		DOMDEV	0.211	(0.009-5.048)	0.337
		SEX	0.422	(0.090-1.983)	0.275
		GENO_2DF	NA	NA	0.340
rs2858060	Hui	ADD	0.684	(0.113-4.129)	0.679
		DOMDEV	0.219	(0.009-5.246)	0.349
		SEX	0.439	(0.093-2.063)	0.297
		GENO_2DF	NA	NA	0.335
rs2858061	Hui	ADD	0.711	(0.118-4.281)	0.709
		DOMDEV	0.211	(0.009-5.048)	0.337
		SEX	0.422	(0.090-1.983)	0.275
		GENO_2DF	NA	NA	0.340
rs34678647	Hui	ADD	1.245	(0.394-3.934)	0.709
		DOMDEV	4.588	(0.416-50.590)	0.214
		SEX	1.870	(0.301-11.600)	0.502
		GENO_2DF	NA	NA	0.387
rs2858059	Others	ADD	1.650	(0.227-11.990)	0.621
		DOMDEV	0.303	(0.010-9.157)	0.492
		SEX	0.227	(0.0222-2.390)	0.217
		GENO_2DF	NA	NA	0.785
rs2858060	Others	ADD	0.458	(0.036-5.789)	0.547
		DOMDEV	1.455	(0.031-67.750)	0.848
		SEX	0.364	(0.060-2.194)	0.270
		GENO_2DF	NA	NA	0.803
rs2858061	Others	ADD	1.650	(0.227-11.990)	0.621
		DOMDEV	0.303	(0.010-9.157)	0.492
		SEX	0.227	(0.022-2.390)	0.217
		GENO_2DF	NA	NA	0.785
rs34678647	Others	ADD	1.181	(0.234-5.972)	0.840
		DOMDEV	0.063	(0.002-1.960)	0.115
		SEX	0.143	(0.015-1.332)	0.088
		GENO_2DF	NA	NA	0.264

Abbreviations: CI, confidence interval; OR, odds ratio.

ground of self-reported essential hypertension from multiethnic groups, a set of SNPs in miR-221 (rs2858059, rs2858060, rs2858061, and rs34678647) was genotyped in a multiethnic population from China. Our results suggest that a significant difference among the multiethnic groups was observed for the rs2858060 polymorphism in an ADD model. These results support the conclusion that genetic variation exists at this locus among various ethnic groups.

MiR-221 was identified as a specific miRNA in human umbilical vein endothelial cells that participates in the regulation of angiogenesis [17]. Cardiovascular morbidity is associated with the differential expression of a myriad of miRNAs, and miR-221 is one of the top nine most reported miRNAs in hypertension and atherosclerotic disease [18]. A previous study reported an association between miR-221 and hypertension [19]. However, to date, associations between SNPs in miR-221 and hypertension have not been reported in a multiethnic population. In the present study, we systematically explored the relationship between SNPs in miR-221 and self-reported essential hypertension, as well as interactions between genetic and environmental factors, in a multiethnic study in China.

A previous case-control study suggested that the rs2858060 polymorphism is associated with an increased risk of polycystic ovary syn-

Table 7. Synergistic effect of rs2858060 in ADD model and environmental factors in self-reported essential hypertension cases and controls

rs2858060	Classical risk	Cases	Controls	OR (95% CI)	*P*
	Age (years)		*SI*: 1.367; *AP*: 0.159; *RERI*: 0.391		
0 (GG)	0 (< 64.5)	34	58	1.000 (reference)	0.002
0 (GG)	1 (≥ 64.5)	25	31	1.376 (0.700-2.704)	0.355
1 (CC)	0 (< 64.5)	207	209	1.690 (1.061-2.690)	0.027
1 (CC)	1 (≥ 64.5)	144	100	2.456 (1.498-4.027)	0.000
	Sex		SI: -0.449; AP: -0.520; RERI: -0.448		
0 (GG)	0 (Female)	9	8	1.000 (reference)	0.000
0 (GG)	1 (Male)	50	81	0.549 (0.199-1.515)	0.247
1 (CC)	0 (Female)	101	51	1.760 (0.641-4.834)	0.273
1 (CC)	1 (Male)	250	258	0.861 (0.327-2.268)	0.762
	Smoke		SI: -6.913; AP: -0.171; RERI: -0.149		
0 (GG)	0 (No)	32	33	1.000 (reference)	0.000
0 (GG)	1 (Yes)	27	56	0.497 (0.255-0.971)	0.041
1 (CC)	0 (No)	211	143	1.522 (0.895-2.587)	0.121
1 (CC)	1 (Yes)	140	166	0.870 (0.509-1.486)	0.610
	Drink		SI: 0.459; AP: -0.290; RERI: -0.385		
0 (GG)	0 (No)	47	70	1.000 (reference)	0.016
0 (GG)	1 (Yes)	12	19	0.941 (0.418-2.118)	0.883
1 (CC)	0 (No)	302	254	1.771 (1.180-2.656)	0.006
1 (CC)	1 (Yes)	49	55	1.327 (0.778-2.263)	0.299
	TC (mmol/L)		SI: 0.743; AP: -0.134; RERI: -0.218		
0 (GG)	0 (< 5.70)	47	72	1.000 (reference)	0.034
0 (GG)	1 (≥ 5.70)	12	17	1.081 (0.474-2.468)	0.853
1 (CC)	0 (< 5.70)	285	247	1.768 (1.179-2.650)	0.006
1 (CC)	1 (≥ 5.70)	66	62	1.631 (0.984-2.703)	0.058
	TG (mmol/L)		SI: 71.474; AP: 0.515; RERI: 1.080		
0 (GG)	0 (< 2.25)	47	64	1.000 (reference)	0.001
0 (GG)	1 (≥ 2.25)	12	25	0.654 (0.298-1.432)	0.288
1 (CC)	0 (< 2.25)	231	231	1.362 (0.896-2.069)	0.148
1 (CC)	1 (≥ 2.25)	120	78	2.095 (1.306-3.359)	0.002
	HDL-C (mmol/L)		SI: 4.200; AP: 0.401; RERI: 0.848		
0 (GG)	0 (≥ 1.03)	51	73	1.000 (reference)	0.012
0 (GG)	1 (< 1.03)	8	16	0.716 (0.285-1.798)	0.477
1 (CC)	0 (≥ 1.03)	289	267	1.549 (1.044-2.299)	0.030
1 (CC)	1 (< 1.03)	62	42	2.113 (1.243-3.591)	0.006
	LDL-C (mmol/L)		SI: 0.310; AP: -0.322; RERI: -0.377		
0 (GG)	0 (< 4.10)	53	78	1.000 (reference)	0.011
0 (GG)	1 (≥ 4.10)	6	11	0.803 (0.280-2.303)	0.683
1 (CC)	0 (< 4.10)	320	270	1.744 (1.187-2.562)	0.005
1 (CC)	1 (≥ 4.10)	31	39	1.170 (0.651-2.103)	0.600
	ApoA (g/L)		SI: 0.089; AP: -0.879; RERI: -0.962		
0 (GG)	0 (< 1.60)	52	80	1.000 (reference)	0.004
0 (GG)	1 (≥ 1.60)	7	9	1.197 (0.420-3.411)	0.737
1 (CC)	0 (< 1.60)	319	264	1.859 (1.265-2.733)	0.002
1 (CC)	1 (≥ 1.60)	32	45	1.094 (0.617-1.939)	0.758
	ApoB (g/L)		SI: 1.274; AP: 0.106; RERI: 0.208		
0 (GG)	0 (< 1.10)	45	69	1.000 (reference)	0.027

0 (GG)	1 (≥ 1.10)	14	20	1.073 (0.492-2.340)	0.859
1 (CC)	0 (< 1.10)	269	245	1.684 (1.113-2.546)	0.014
1 (CC)	1 (≥ 1.10)	82	64	1.965 (1.194-3.232)	0.008
	FBG (mmol/L)		SI: 10.380; AP: 0.350; RERI: 0.572		
0 (GG)	0 (< 6.10)	53	73	1.000 (reference)	0.020
0 (GG)	1 (≥ 6.10)	6	16	0.517 (0.189-1.408)	0.197
1 (CC)	0 (< 6.10)	268	239	1.544 (1.041-2.291)	0.031
1 (CC)	1 (≥ 6.10)	83	70	1.633 (1.015-2.628)	0.043
	CR (μmol/L)			-	
0 (GG)	0 (< 133)	59	89	1.000 (reference)	0.013
0 (GG)	1 (≥ 133)	0	0	-	-
1 (CC)	0 (< 133)	347	304	1.722 (1.197-2.476)	0.003
1 (CC)	1 (≥ 133)	4	5	1.207 (0.311-4.680)	0.786

Abbreviations: AP, proportion of disease attributable to an interaction; CR, creatinine; FBG, fasting blood glucose; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; RERI, relative excess risk due to an interaction; SI, Rothman's synergy index for an interaction; TC, cholesterol; TG, triglycerides.

drome in Iranian women [20]. In another case-control study, chi-square analysis of genotyping results of rs2858060 located in relation to MIR221 showed no significant differences for genotype and allele frequencies between cases and controls in the Genomics Research Center Breast Cancer population [21]. However, associations between the rs2858060 polymorphism and hypertension have not been reported. In the present study, we found that people with the C allele and the CC genotype of rs2858060 in miR-221 have a lower risk of self-reported essential hypertension than those with the G allele and the homozygous GG genotype. Therefore, rs2858060 in miR-221 could play an important role as a genetic risk factor for the development of self-reported essential hypertension in the Chinese population.

The exact mechanism underlying the association of rs2858060 in miR-221 and self-reported essential hypertension is unknown. The interactions between genetic and environmental factors may shed light on the association. Therefore, we considered the synergistic effect of rs2858060 and environmental factors on the risk of self-reported essential hypertension. Our crossover analysis revealed a significant interaction between rs2858060 and the effect of age, triglycerides, HDL-C, apoB, and FBG on the risk of self-reported essential hypertension. Our study is the first to report an association between rs2858060 in miR-221 and self-reported essential hypertension risk in individuals of a Chinese background. However, to explore the potential significance of the results in the present study, future studies should validate these results by genotyping larger sample cohorts and including individuals of different ethnicities.

Limitations

We are aware that this study has some potential limitations. We did not determine whether the single nucleotide polymorphisms at rs2858060 in miR-221 alter the miR-221 expression levels or processing causing susceptibility of self-reported essential hypertension. Furthermore, we did not explore the potential functional significance of the association between rs2858060 in miR-221 and self-reported essential hypertension risk. Finally, because the present study was conducted at a single center and had a cross-sectional design, a definite cause-effect relationship between rs2858060 in miR-221 and self-reported essential hypertension could not be achieved.

Conclusion

The rs2858060 polymorphism in miR-221 is associated with a risk of self-reported essential hypertension in the Chinese population in an ADD model. Additionally, a clear interaction was found between rs2858060 and classical risk factors of self-reported essential hypertension. Therefore, rs2858060 in miR-221 could play an important role as a genetic risk factor for the development of self-reported essential hypertension in the Chinese population.

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Disclosure of conflict of interest

None.

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