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Abstract

The class A scavenger receptor, which is encoded by the macrophage scavenger receptor 1 (MSR1) gene, is a pattern recognition receptor (PPR) primarily expressed in macrophages. It has been reported that genetic polymorphisms of MSR1 are significantly associated with many cardiovascular events. However, whether it links genetically to essential hypertension (EH) in Chinese is not defined. Here, we performed an independent case-control study in a Chinese population consisting of 617 EH cases and 620 controls by genotyping three single nucleotide polymorphisms (SNPs) of MSR1. We found that rs13306541 and rs3747531 were significantly associated with an increased risk of EH with per allele odds ratio (OR) of 1.63 [95% confidence interval (CI) = 1.27-2.09; $P < 0.001$] and 1.29 (95% CI = 1.09-1.52; $P = 0.003$), respectively. Our results indicate that genetic variants of MSR1 may serve as predictive markers for the risk of EH in combination with traditional risk factors of EH in Chinese population.

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Introduction

Hypertension is a major global public-health problem. The World Health Organization has projected that 29.2% (range, 28.8-29.7%) of the world's population will have hypertension by 2025, and that the number will increase by approximately 60% to a total of 1.56 billion (range, 1.54-1.58 billion) ^[1]. In China, approximately 153 million (18%) Chinese adults were hypertensive in 2002 ^[2]. The pathogenetics of hypertension include both genetic factors and environmental factors, such as dietary sodium intake, obesity, and smoking. More importantly, hypertension is a typical

multifactorial inherited disease in which the combination of small quantitative effects from the variants of many genes, together with several environmental factors, increases the risk of its occurrence. Genome-wide association studies (GWAS) are widely used to identify the common gene variants associated with hypertension or blood pressure (BP), and to explore the genetics of hypertension.

Previous studies have showed that endothelial dysfunction is involved in the occurrence and progression of many cardiovascular pathological conditions, including essential hypertension [3-5]. Xiuping Chen et al have found that lectin-like oxidized low-density lipoprotein (LDL) receptor-1 (OLR-1), a major receptor for oxidized LDL in endothelial cells, has been determined to play a notable role in the initiation and progression of endothelial dysfunction [6]. Furthermore, the polymorphism of OLR-1 has been found associated with susceptibility in Chinese essential hypertensive population [7]. However, another important receptor for oxidized LDL, the class A scavenger receptor (SR-A), has not been researched for its connection with essential hypertension. SR-A is expressed primarily in macrophages, but can also be detected in endothelial, fibroblast, and vascular smooth muscle cells. It is encoded by the gene macrophage scavenger receptor 1 (*MSRI*) that is located on chromosome 8p22. The association of *MSRI* polymorphism with several cardiovascular conditions, e.g. atherosclerosis (AS), acute myocardial infarction (AMI) or coronary artery disease (CAD) has been investigated [8-10]. However, its relationship with essential hypertension remains unknown. In present study, we explored the role of *MSRI* SNPs in a Chinese essential hypertensive population.

Subjects and methods

Subjects

All of the study participants were unrelated ethnic Han Chinese origin residing in or near Jiangsu Province. The participants consisted of 617 patients with essential hypertension (EH) who were recruited from the Second People's Hospital of Wuxi. Hypertension was defined as systolic blood pressure (SBP) >140 mm Hg, or diastolic

blood pressure (DBP) >90 mm Hg in supine position, after 20 min of rest on 2 separate days ^[11]. Secondary forms of hypertension in the patients were ruled out by routine examinations. As a control population, 620 participants unrelated to the patients were selected from among individuals without any history of chronic diseases who were undergoing health examinations at the Second People's Hospital of Wuxi. These individuals had normal BPs (defined as a systolic BP < 140 mm Hg and a diastolic BP < 90 mm Hg) and no history of taking antihypertensive medication.

This study was approved by the Ethics Committee of the Second People's Hospital of Wuxi and informed consent was obtained from each participant.

Clinical measurements

A 5 mL peripheral venous blood sample was obtained from all participants. Part of this blood sample was analyzed for plasma levels of glucose, triglycerides (TG), total cholesterol, HDL-cholesterol (HDL-C), and LDL-cholesterol (LDL-C), all of which were measured using an automated chemistry analyzer (Olympus Au5400, Japan). Genomic DNA was extracted using the Blood Genome DNA Extraction Kit purchased from Takara Biotech Co. (Japan).

Genotyping

Potentially functional SNPs of the *MSR1* gene were identified to meet the following criteria: (a) located in the 5'-flanking regions, 5'-UTR, 3'-UTR, and coding regions with amino acid changes; (b) were shown to be of biological significance according to the literature review; (c) were associated with the gene expression and/or disease risk in previous studies.

Genotyping was performed without knowing the subjects' case or control status. Genotyping of the three potentially functional polymorphisms T-365C, T125C, and Ala275Pro (rs416748, rs13306541 and rs3747531) was performed using the Taq-Man allelic discrimination assay on the platform of 7900HT Real-time PCR System (Applied Biosystems, Foster City, CA, USA). Two negative controls were included in

each 384-well reaction plate and the genotyping results were determined by using SDS 2.3 Allelic Discrimination Software (Applied Biosystems). Moreover, to confirm the genotyping results, about 10% of the samples were randomly selected and retested by direct DNA sequencing on a 3730xl DNA analyzer (Applied Biosystems) and the accordance rate reached 100%.

Statistical analysis

The Hardy-Weinberg equilibrium between SNPs was evaluated using the χ^2 goodness-of-fit test among the control subjects. Two-sided χ^2 tests were used to evaluate differences in the distributions of demographic characteristics, selected variables, and genotypes between the cases and controls. Logistic regression analyses were employed to estimate crude and adjusted odds ratios (ORs) and 95% confidence intervals (95% CIs) for the association between genetic variants and EH risk in an additive model. $P \leq 0.05$ was considered statistically significant. All statistical analyses were performed with SPSS 13.0 (SPSS Inc., Chicago, IL, USA).

Results

Demographic information

Characteristics of the 617 EH cases and the 620 controls are shown in Table 1. No significant difference was observed in age and sex between the cases and controls ($P = 0.725$ and 0.974 , respectively). Compared with the control subjects, patients with EH had higher levels of body mass index (BMI), SBP, DBP, Glucose, TC, TG, and LDL-C, but lower HDL-C.

Association between *MSR1* polymorphisms and the risk of EH

The observed genotype frequencies for rs416748, rs13306541 and rs3747531 were all consistent with the Hardy-Weinberg equilibrium in controls ($P = 0.629$, 0.203 and 0.288 , respectively). As shown in Table 2, logistic regression analysis revealed that individuals with variant alleles of rs13306541 and rs3747531 were significantly associated with altered risk of EH (adjusted per-allele OR = 1.63, 95% CI = 1.27-

2.09, $P < 0.001$ for rs1330654; adjusted per-allele OR = 1.29, 95% CI = 1.09- 1.52, $P = 0.003$ for rs3747531, respectively). However, no significant association was observed with rs416748 and risk of EH.

Stratified analyses of the polymorphisms and EH

Stratification analyses were conducted to evaluate the potential association of genetic variants of three SNPs with the risk of EH in subgroup populations. As shown in Table 3, in the subgroup of less than 57 years old, variant allele of rs13306541 (G) were both significantly associated with systolic blood pressure and diastolic blood pressure ($P = 0.027$ for SBP and $P = 0.037$ for DBP, respectively). Also, this G allele was significantly associated with systolic blood pressure ($P = 0.016$) in the subgroup of females. However, there was no obvious evidence of significant association between rs416748, rs3747531 and EH risk among all subgroups.

Dose response of rs13306541 and rs3747531 on EH risk

Furthermore, as shown in Table 4 we combined these two loci on an allele manner to detect the dose response and found that the risk of EH significantly increased with the number of risk allele increasing. Individuals with 2 - 4 risk alleles had a 1.43-fold (95%CI: 1.22-1.67) increased risk of EH compared with those having none of the risk alleles.

Discussion

Essential hypertension is a multifactorial complex disease where both genetic and environmental factors interact to produce the specific blood pressure level or a given individual. The genetic contribution to blood pressure (BP) variation ranges from 30 to 50% [12]. Candidate genes that determine BP variation include those whose products have a direct role in BP regulation. We demonstrated for the first time that genetic variants of *MSR1* are significantly associated with essential hypertension in Chinese population.

We evaluated the associations of three genetic variants of *MSR1* with EH susceptibility in an independent case-control study with 617 EH cases and 620 controls in a Chinese population. Each of these three genetic variants could have an important impact on *MSR1* function. For example, the SNPs in the promoter region of rs416748 and rs13306541 could affect transcription of the *MSR1* gene. The missense change of rs3747531 could affect *MSR1* binding with its ligands, because it changes a conserved residue in the first Gly-X-Y repeat of the collagenous domain of the protein, which is critical for ligand binding ^[9-10]. We found that rs13306541 and rs3747531 but not rs416748 was significantly associated with increased risk of EH in the study cohort. The effect of the variant allele of rs13306541 (G) appeared to be stronger in females, and in the subgroup of less than 57 years old.

Logistic regression analysis supported the hypothesis that rs13306541 and rs3747531 may contribute to EH susceptibility in our population. Both SNPs showed the potential to predict the risk of EH in future in combination with traditional risk factors of EH in Chinese population. They may be used to build the risk prediction model to serve as the genetic test for high risk population of EH. The SNP rs13306541 located in the promoter region that is responsible for transcription of the *MSR1* gene. Thus, it may influence the expression level of this gene. Nakayama et al. have found that the *MSR1* gene expression level in peripheral blood mononuclear cells (PBMCs) is increased in patients with acute coronary syndrome (ACS), indicating that the *MSR1* gene expression level in cells also provides a predictive marker for a re-attack of a cardiovascular event ^[13]. Qian LL et al. showed that SR-A is critical for vascular remodeling by regulating macrophage polarization. Therefore, SR-A may be a useful therapeutic target for the intervention of hypertensive vascular remodeling ^[14]. It is plausible that individuals may differ in the macrophage polarization, depending on the *MSR1* genotype. These findings imply a significant pathophysiological effect in cells for the *MSR1* gene in the development of EH in humans.

Furthermore, in this independent case-control study, we failed to observe

significant associations of rs416748 with the risk of EH. The small sample size may account for the negative results. The possibility may be that the association between these variants and EH risk is very modest and our analysis would not have enough statistical power to detect it. Another explanation is that this association is not real in EH. Larger sample size and well-organized studies are warranted to further clarify the associations of the SNP with EH.

In our study, we used candidate gene association studies on assumptions about biological relevant genes. Despite some successes, single gene association studies remain problematic. Small scale studies are easy to yield a false positive association. There is evidence of publication bias in the literature that might enrich false positives [15, 16]. With improved genotyping technologies, genome-wide association studies (GWASs) have become an important approach in genetic studies. GWASs are large scale association mapping using SNPs, making no assumptions of the genomic location or function of the causal variant and are an unbiased and fairly comprehensive option that can be attempted even in the absence of convincing evidence regarding function or location of the causal genes [17]. Recent GWASs and meta-analysis in EH identified several new susceptibility loci [18-21]. Therefore, large scale association studies are warranted to further validate our results.

One of the strengths in the current study is that, based on an independent Chinese study, we firstly confirmed the *MSRI* gene as a susceptibility gene for EH in Chinese population. However, the limitations of this study need to be addressed. First of all, the relatively small sample size may underpower the results of our study. Secondly, although great attention was paid in the study design and analysis, selection bias in this study might have affected our results. Thirdly, all our data were obtained at the time of diagnosis, thus prospectively followed-up clinical outcome including severe cardiac events may be required to analyze the association between the genetic variants of *MSRI* and EH prognosis. Lastly, our study was performed only in a Chinese population. Data should be extrapolated to other regions and ethnic groups cautiously.

In conclusion, this study provided strong evidence in an independent population

that genetic variants of *MSR1*, defined by rs13306541 and rs3747531, respectively, were susceptible SNPs for EH in Chinese population. The functional significance of the variants remains to be further investigate, which may help in elucidating the genetic mechanism of EH.

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Table 1 Baseline characteristics of the study population

Variable	Case (n = 617)	Control (n = 620)	P-value
Age	57.36 ± 7.98	56.37 ± 7.55	0.725
Sex (male), n (%)	324 (52.51)	325 (52.42)	0.974
BMI (kg/m ²)	25.51 ± 2.14	20.79 ± 0.95	< 0.001
SBP (mmHg)	154.59 ± 20.14	133.04 ± 17.66	< 0.001
DBP (mmHg)	93.82 ± 9.97	82.90 ± 9.41	< 0.001
Glucose (mmol/l)	5.73 ± 0.68	5.49 ± 0.70	< 0.001
TC (mmol/l)	5.22 ± 0.97	4.69 ± 0.69	< 0.001
TG (mmol/l)	1.92 ± 1.86	1.02 ± 0.51	< 0.001
HDL-C (mmol/l)	1.29 ± 0.33	1.43 ± 0.30	< 0.001
LDL-C (mmol/l)	3.15 ± 0.72	2.74 ± 0.50	< 0.001

BMI: body mass index, SBP: systolic blood pressure, DBP: diastolic blood pressure, TC: total cholesterol, TG: triglyceride, HDL-C: high-density lipoprotein cholesterol, LDL-C: low-density lipoprotein cholesterol.

Table 2 Distribution of genotypes of the SNPs and their associations with EH risk

SNP	Genotype	Controls		Cases	
		N(%)	N(%)	Adjusted OR (95% CI) ^a	P-value ^a
rs416748	GG	184 (29.68)	171 (27.71)	1.00	
	AG	302 (48.71)	307 (49.76)	1.09 (0.84-1.41)	0.540
	AA	134 (21.61)	139 (22.53)	1.06 (0.90-1.24)	0.495
	AG/AA	436 (70.32)	446 (72.29)	1.09 (0.85-1.40)	0.491
	Per allele			1.06 (0.91-1.24)	0.473
rs13306541	AA	513 (82.74)	471 (76.34)	1.00	
	AG	99 (15.97)	116 (18.80)	1.29 (0.96-1.73)	0.094
	GG	8 (1.29)	30 (4.86)	2.04 (1.37-3.02)	<0.001
	AG/GG	107 (17.26)	146 (23.66)	1.50 (1.13-1.99)	0.004
	Per allele			1.63 (1.27-2.09)	<0.001
rs3747531	GG	276 (44.52)	257 (41.65)	1.00	
	CG	284 (45.81)	247 (40.03)	0.94 (0.73-1.19)	0.583
	CC	60 (9.67)	113 (18.32)	1.42 (1.19-1.70)	<0.001
	CG/CC	344 (55.48)	360 (58.35)	1.12 (0.90-1.41)	0.311
	Per allele			1.29 (1.09-1.52)	0.003

a: Adjusted for age and sex.

Table 3 Stratified analyses of SNPs and EH

Model	G1974			G1975			G1976		
	Group	SR-mid	RR-mid	Group	SR-mid	RR-mid	Group	SR-mid	RR-mid
Age 57	ALL	0.73-0.93	0.74-0.93	G1	0.70-0.93	0.73-0.93	G1	0.70-0.93	0.74-0.93
	AG-00	0.72-0.93	0.74-0.93	G1-00	0.72-0.93	0.73-0.93	AG-01	0.70-0.93	0.74-0.93
	P-value	0.07	0.07	P-value	0.56	0.79	P-value	0.74	0.68
Age 57	ALL	0.73-0.93	0.74-0.93	G1	0.70-0.93	0.73-0.93	G1	0.70-0.93	0.74-0.93
	AG-00	0.72-0.93	0.74-0.93	G1-00	0.72-0.93	0.73-0.93	AG-01	0.70-0.93	0.74-0.93
	P-value	0.07	0.07	P-value	0.07	0.00	P-value	0.65	0.09
Age	ALL	0.73-0.93	0.74-0.93	G1	0.70-0.93	0.73-0.93	G1	0.70-0.93	0.74-0.93
	AG-00	0.72-0.93	0.74-0.93	G1-00	0.72-0.93	0.73-0.93	AG-01	0.70-0.93	0.74-0.93
	P-value	0.57	0.02	P-value	0.02	0.01	P-value	0.03	0.05
Female	ALL	0.73-0.93	0.74-0.93	G1	0.70-0.93	0.73-0.93	G1	0.70-0.93	0.74-0.93
	AG-00	0.72-0.93	0.74-0.93	G1-00	0.72-0.93	0.73-0.93	AG-01	0.70-0.93	0.74-0.93
	P-value	0.06	0.00	P-value	0.02	0.02	P-value	0.01	0.06

Table 4 Dose response of rs13306541 and rs3747531 on EH Risk

Risk allele number ^a	Case (n = 617) n (%)	Control (n = 620) n (%)	Adjusted OR (95% CI) ^b	P-value
0	165 (26.8)	209 (33.7)	1.00	
1	279 (45.2)	303 (48.9)	1.16 (0.89-1.51)	0.263
2-4	173 (28.0)	108 (17.4)	1.43 (1.22-1.67)	< 0.001

a: rs13306541- G and rs3747531- C alleles were assumed as risk alleles; b: Adjusted for age and sex.