Introduction

Hypertension is a major global public-health problem. The World Health Organization (WHO) 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. In 2007, the Wellcome Trust Case Control Consortium (WTCCC) reported the first genome-wide association results for hypertension (3). Over the past few years, the genome-wide association study (GWAS) has helped identify many loci in or near genes that generally were not expected to be associated with BP or essential hypertension (EH).

Previous studies have showed that endothelial dysfunction is involved in the occurrence and progression of many cardiovascular pathological conditions, including EH (4-6). 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 (7). Furthermore, the polymorphism of OLR-1 has been found associated with susceptibility in Chinese essential hypertensive population (8). However, another important receptor for oxidized LDL, the class A scavenger receptor (SR-A), has not been researched for its connection with EH. 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 (MSR1) that is located on chromosome 8p22. The association of MSR1 polymorphism with several cardiovascular conditions, e.g., atherosclerosis (AS), acute myocardial infarction (AMI) or coronary artery disease (CAD) has been investigated (9-11). However, its relationship with EH remains unknown. MSR1 has not been mentioned in the GWASs in EH, probably because of the number of people enrolled in the various GWASs was not enough, thereby limiting the power to detect additional markers. In present study, we explored the role of MSR1 SNPs in a Chinese essential hypertensive population. We are planning a study of independent cases and controls. Prior data indicated that the probability of exposure among controls is 0.33. If the true odds ratio (OR) for disease in exposed subjects relative to unexposed subjects is 1.4 (12), we will need to study 598 case patients and 598 control patients to be able to reject the null hypothesis that this OR equals 1 with probability (power) 0.8. The Type I error probability associated with this test of this null hypothesis is 0.05. We will use an uncorrected chi-squared statistic to evaluate this null hypothesis.

**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 EH who were recruited from the Second People’s Hospital of Wuxi. Hypertension was defined as systolic blood pressure (SBP) >140 mmHg, or diastolic blood pressure (DBP) >90 mmHg in supine position, after 20 min of rest on two separate days (13). 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 SBP <140 mmHg and a DBP <90 mmHg) 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: (I) located in the 5’-flanking regions, 5’-UTR, 3’-UTR, and coding regions with amino acid changes; (II) were shown to be of biological significance according to the literature review; (III) 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 $\chi^2$ goodness-of-fit test among the control subjects. Two-sided $\chi^2$ 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 (CIs) for the association between genetic variants and EH risk in an additive model. P≤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 SBP and DBP (P=0.027 for SBP and P=0.037 for DBP, respectively). Also, this G allele was significantly associated with SBP (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 2.03-fold (95% CI: 1.48-2.78) increased risk of EH compared with those having none of the risk alleles (P for trend <0.001).

Discussion

EH is a multifactorial complex disease where both genetic and environmental factors interact to produce the specific BP level or a given individual. The genetic contribution to
BP variation ranges from 30% to 50% (14). 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 EH 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 (10,11). We found that 13306541 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 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 (15). Qian 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 (16). 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 HE.

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 (17,18). With improved genotyping technologies, 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 (19). Recent GWASs and meta-analysis in EH identified several new susceptibility loci (20-22). 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 MSR1 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

### Table 4 Dose response of rs13306541 and rs3747531 on EH Risk

<table>
<thead>
<tr>
<th>Risk allele number</th>
<th>Case (n=617), n (%)</th>
<th>Control (n=620), n (%)</th>
<th>Adjusted OR (95% CI)</th>
<th>P for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>165 (26.7)</td>
<td>209 (33.7)</td>
<td>1.00</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>1</td>
<td>279 (45.2)</td>
<td>303 (48.9)</td>
<td>1.17 (0.90-1.51)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>157 (25.5)</td>
<td>108 (17.4)</td>
<td>1.84 (1.34-2.53)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>8 (1.3)</td>
<td>0 (0)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>8 (1.3)</td>
<td>0 (0)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>2, 3, 4</td>
<td>173 (28.1)</td>
<td>108 (17.4)</td>
<td>2.03 (1.48-2.78)</td>
<td></td>
</tr>
</tbody>
</table>

*a*, rs13306541- G and rs3747531- C alleles were assumed as risk alleles; *b*, Adjusted for age and sex. EH, essential hypertension.
the association between the genetic variants of MSR1 and EH prognosis. Lastly, our study was performed only in a Chinese population. Data should be extrapolated to other regions and ethnic groups cautiously.

Although the alleles that are associated with a modest increase in risk are constantly being found, their ability to be discriminatory and predictive markers is low. Genetic variants may improve disease prediction beyond traditional risk factors when they are involved in unknown pathways or in pathways with unmeasurable intermediate factors (23). GWASs have thus far failed to unlock the genetics of hypertension, but it is possible that this methodology could identify rare variants associated with an increased risk for EH, thereby leading to new insights for developing better methods for prevention or treatment of hypertension (24). Although a common noncoding SNP might only have a small effect, the underlying gene/protein/mechanism could very well become an important drug target. In the future, novel designs, such as a GWAS of BP traits that respond to drug therapy, may play a larger role in the diagnosis and treatment of hypertension (25).

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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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

References

3. Wellcome Trust Case Control Consortium. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature 2007;447:661-78.