Associations SELE Gene Haplotype Variant and Hypertension in Mongolian and Han Populations

Li Qin¹, Ping Zhao², Zhiyue Liu¹ and Peiye Chang³

Abstract

Genetic variation is thought to contribute to the etiology of hypertension, and E-selectin is a candidate essential hypertension-associated gene.

Objective In this study, we attempted to test the hypothesis that subtle haplotype variants of SELE genes may be sources of essential hypertension in Mongolian and Han populations.

Materials A total of 429 unrelated Mongolian herdsmen and 416 Han farmers were enrolled, including 212 Mongolian essential hypertension (EH) patients, 217 Mongolian normotensives (controls), 200 Han EH patients and 216 Han normotensives (controls).

Methods All nine tag single-nucleotide polymorphisms (SNPs) within the SELE gene were retrieved from HapMap and the genotyping was performed using a polymerase chain reaction (PCR)/ligase detection reaction assay.

Results The distributions of the A-allele frequency of rs3917458 and the C-allele frequency of rs2179172 differed significantly between the hypertensive subjects and controls in the Han population. The frequency of haplotype GGC was significantly higher in the EH group than in the controls in the Mongolian population. In the Han population, a significant difference was observed in the haplotype frequency of TCC between the patients and controls, whereas haplotype ACA was detected significantly less often in the EH subjects than in the controls.

Conclusion Meanwhile, the haplotype TCC in the Han hypertensive patients and the haplotype GGC in the Mongolian patients had independent effects in increasing the risk for EH and maybe used as risk factors for predicting high blood pressure. However, the haplotype ACA had an independent effect in decreasing the risk of hypertension and may be protective in normotensive subjects in the Han population. Therefore, multiple SNPs in combination in SELE may confer a risk of hypertension.

Key words: essential hypertension (EH), Mongolian population, SELE, haplotype polymorphism

(DOI: 10.2169/internalmedicine.54.2797)

Introduction

Essential hypertension (EH), which is characterized by sustained elevated blood pressure (BP) with no identifiable cause, is a public health burden in the worldwide (1) and a complex polygenic disease determined by both genetic and environmental factors (2). A body of evidence suggests that the SELE gene plays an important role in the development and progression of hypertension. E-selectin (SELE, CD62E, ELAM-1), an 11-kD cell surface glycoprotein, is an adhesion molecule of the selection family (3). Its corresponding gene SELE is located on human chromosome 1. The adhesion molecule, E-selectin, SELE, which mediates the initial attachment of leukocytes to vascular endothelial cells, is a specific product of activated endothelial cells (4). Soluble forms are also released from the activated endothelium into the circulation (5, 6) and increased serum levels of sE-selectin have been reported in patients with essential hypertension (7, 8).

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Received for publication March 4, 2014; Accepted for publication June 29, 2014
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Single-nucleotide polymorphisms (SNPs) are a valuable resource for investigating the genetic basis of disease (9). The SELE gene has been investigated worldwide in the search for the inheritable determinants of blood pressure phenotypes in humans, as reports have shown a positive association between polymorphisms of the SELE gene and hypertension (10). Several mutations/polymorphisms have been identified in SELE, which is located on the 1q12-qter. Among them, the L/F554 and S/R128 mutations/polymorphisms have been found to be related to atherosclerosis (11). Moreover, recent studies in a German population showed possible associations between the S128R and L554F variations of the E-selectin gene and severe atherosclerosis, hypertension and cerebrovascular diseases (12, 13). However, the low frequency haplotypes of E-selectin polymorphisms G2692A and C1901T is currently considered to be protective for coronary artery disease (14). Additionally, the association between SELE SNPs and the E-selectin level remains controversial (15-17). Studies to establish an association between essential hypertension and SELE gene polymorphisms have been undertaken in different populations. Recently, Wang et al. (18) found that C602A and T1559C polymorphisms of the SELE gene are associated with essential hypertension in the Chinese population, while T1559C is closely related to hypertension in men.

However, there are few studies on the interactions between haplotypes in the SELE gene and hypertension in the Mongolian and Han populations. The Inner Mongolia Autonomous Region is located in northwestern China and includes the Xilin Gol League, a desert with an arid and cold climate. The prevalence of hypertension is higher the Mongolian population than in China. Therefore, the study of haplotypes in Mongolian and Han populations is of great significance.

The purpose of this study was thus to investigate the associations between haplotype variants of the SELE gene and essential hypertension in the Mongolian and Han populations.

Materials and Methods

Study population

The study population (20-70 years of age) was recruited from the Duolun and Dongwuqi provinces of the Xilin Gol League in Inner Mongolia. Subjects with a history of secondary hypertension, stroke, coronary heart disease, diabetes, kidney failure, thyroid gland disease or excessive drinking were excluded from this study. A total of 429 unrelated Mongolian herdsmen and 416 Han farmers were enrolled, including 212 Mongolian EH patients, 217 Mongolian normotensives (controls), 200 Han EH patients and 216 Han normotensives (controls). Each subject was from a family that had lived in Inner Mongolia for at least three generations without a history of mixed marriage. All individuals provided their written informed consent for the collection of samples and the subsequent analyses. Hypertension was defined as a systolic blood pressure (SBP) of at least 140 mmHg, diastolic blood pressure (DBP) of at least 90 mmHg and/or treatment with antihypertensive medications. The normotensive group was selected based on the following criteria: an SBP of less than 140 mmHg and DBP of less than 90 mmHg with no previous diagnosis of EH.

Phenotype measurements

The subjects were seated in a quiet room and prevented from smoking, exercising or drinking alcohol, tea or coffee for at least one hour before the physical examination. During the clinical examination, demographic information was collected using an interview. Weight, height and body mass index (BMI) were measured according to the standard methods, as follows: Body weight and height were measured with the subjects wearing only light indoor clothing without shoes. BMI was calculated by dividing the weight (kg) by the height squared (m$^2$). BP was measured three times, with a 2-minute interval between each measurement. SBP was recorded to the nearest 2 mmHg at the appearance of the first Korotkoff sound (phase I), and DBP was recorded to the nearest 2 mmHg at the disappearance of the fifth Korotkoff sound (phase V). The SBP and DBP values were calculated as the means of three consecutive physician-obtained measurements. Blood samples were collected after an overnight fast, and the total plasma cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) levels were measured within eight hours at a local hospital.

Selection of single nucleotide polymorphisms (SNPs)

Tagging SNPs were selected from the Chinese HapMap database (http://www.hapmap.org) based on a pairwise r$^2$ of ≥0.5 and a minor allele frequency (MAF) of ≥0.05. In this study, we chose nine tagSNPs of SELE. The details of the nine tagSNPs in SELE are shown in Table 1.

Genotyping

Genomic DNA was extracted from leukocytes in peripheral blood samples using a commercial blood DNA extraction kit (TIANamp Blood DNA kit; TIANGEN BIOTECH, Beijing, China) and stored at -20°C. All genotyping was performed using a polymerase chain reaction (PCR)/ligase detection reaction assay, and primers were synthesized by Shanghai HAYU Biological Engineering Ltd. Each set of ligation detection reaction probes comprised one common probe and two discriminating probes for the two types. The target DNA sequences were amplified using the multiplex PCR method. PCR was carried out for each subject at a final volume of 20 μL containing 1X PCR buffer, 3.0 mM MgCl$_2$, 2.0 mM deoxynucleotide triphosphate, 2 μL of primers, 0.2 μL of Qiagen HotStarTag Polymerase (QIAGEN, Shenzhen, China), 4 μL of 1X Q-solution and 50 ng of genomic DNA. Thermal cycling was performed for rs5356,
rs5359, rs1534904, rs3917458, rs3917419 and rs2179172 using the Gene Amp PCR system 9600 (Norwalk, USA) with initial denaturation for 2 minutes at 95°C, followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing at 50°C for 1 minute and 30 seconds and extension at 65°C for 1 second with final extension at 65°C for 1 minute.

The ligation reaction for each subject was carried out in a final volume of 10 μL, containing 1X NEB Taq DNA ligase buffer, 2 pmol of each probe mix, 0.05 μL of Taq DNA ligase (BIOWING, Jiangsu, China) and 4 μL of the multiplex PCR product. A total of 40 cycles for the ligase detection reaction were performed at 95°C for 2 minutes, followed by 94°C for 15 seconds and 50°C for 2 seconds. The fluorescent products of the ligase detection reaction were differentiated using the PRISM 3730 (ABI).

Statistical analysis. The Statistical Program for Social Sci-
Table 2. Demographics of the Study Participants

<table>
<thead>
<tr>
<th></th>
<th>Mongolian</th>
<th>Han</th>
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<tbody>
<tr>
<td></td>
<td>p</td>
<td></td>
</tr>
<tr>
<td>n</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender(men/women)</td>
<td>139/73</td>
<td>113/87</td>
</tr>
<tr>
<td>Age, years</td>
<td>53.65 ± 12.29</td>
<td>56.21 ± 13.62</td>
</tr>
<tr>
<td>SBP(mmHg)</td>
<td>155.47 ± 13.94</td>
<td>154.74 ± 15.92</td>
</tr>
<tr>
<td>DBP(mmHg)</td>
<td>92.34 ± 10.60</td>
<td>91.33 ± 10.32</td>
</tr>
<tr>
<td>BMI(kg/m²)</td>
<td>27.78 ± 3.97</td>
<td>26.37 ± 3.27</td>
</tr>
<tr>
<td>TC(mM)</td>
<td>5.00 ± 1.05</td>
<td>4.99 ± 1.02</td>
</tr>
<tr>
<td>TG(mM)</td>
<td>2.02 ± 1.32</td>
<td>1.94 ± 1.12</td>
</tr>
<tr>
<td>HDL(mM)</td>
<td>1.25 ± 0.33</td>
<td>1.24 ± 0.32</td>
</tr>
<tr>
<td>LDL(mM)</td>
<td>3.21 ± 0.73</td>
<td>3.12 ± 0.79</td>
</tr>
</tbody>
</table>

Data are reported as mean±standard deviation. BMI: body mass index, SBP: systolic blood pressure, DBP: diastolic blood pressure, HDL-C: high-density lipoprotein-cholesterol, TG: triglycerides, TC: total cholesterol, LDL-C: low-density lipoprotein-cholesterol

* <p < 0.05, **<p < 0.01

**Clinical characteristics of the EH and control subjects.**

The characteristics of the hypertensive and normotensive individuals in the Mongolian and Han populations are shown in Table 2. Significant differences in age, SBP, DBP, BMI, TG, HDL-C and LDL-C were observed between the EH patients and controls in both the Mongolian and Han populations (p<0.01). The Man and TC levels were markedly higher in the EH patients than in the controls among the Mongolian population.

**Single-locus association study of tagSNPs and EH.** The genotype frequencies of all nine tagSNPs of SELE satisfied the Hardy-Weinberg equilibrium for both the EH subjects and controls in the Mongolian and Han populations (p>0.05). The genotype and allele frequency distributions are shown in Table 1. As shown in Table 1, the distribution of allele frequencies of rs2179172 and rs3917458 differed significantly between the EH patients and controls in the Han population (p<0.05). However, there were no significant differences in the genotype or allele distributions for all nine SNPs between the EH patients and controls in the Mongolian population.

**Haplotypes of tagSNPs.** Linkage disequilibrium plots for the SELE gene in the study population are shown in Figs. 1 and 2. The LD (linkage disequilibrium) among the tagSNPs was measured according to the Lewontin standardized disequilibrium coefficient D' in both groups separately (20). For adjacent SNPs in strong LD (D’>0.8), we
chose rs3917458, rs3917419 and rs2179172 in the Han population and rs1534904, rs932307 and rs3917436 in the Mongolian population to structure haplotypes for the subsequent analyses. The frequency of haplotype GGC was significantly higher in the EH group (7.4%) than in the control group (3.7%) in the Mongolian population, as shown in Table 3. In the Han population, a significant difference was observed in the haplotype frequency of TCC between the patients and controls, whereas haplotype ACA occurred significantly less often in the EH patients (2.5%) than in the controls (6%), as shown in Table 4. Individuals who possessed the ACA haplotype had a significantly lower risk of EH in the Han population, whereas the presence of haplotype GGC was significantly associated with a higher risk of EH in the Mongolian population.

### Discussion

Hypertension is a multifactorial disorder that likely results from the effects of multiple genetic variants (21) interacting with environmental factors (22). Chang et al. (23) investigated multiple genes for susceptibility to essential hypertension on chromosome 1q and documented that the 1q23-32 regions harbor multiple EH-susceptible genes that affect BP, providing evidence of an independent association between three genes in this region and BP; SELE was one of the three genes. Meanwhile, Wang et al. (24) investigated multiple genes with respect to susceptibility to EH and found that C602A and T1559C polymorphisms of E-selectin may be independent risk factors for essential hypertension in the Chinese population. In addition, there is a significant interaction between E-selectin and gender. Furthermore, Faruque et al. (10) assessed the association between SELE polymorphisms and both hypertension and blood pressure in African Americans and found two SNPs (rs3917420 and rs5361) in the SELE gene associated with SBP. Moreover, Wang et al. (25) explored the association between two common variants in the E-selectin gene (rs5361A/C and rs5355C/T) and EH in the Uygur, Kazakh and Han populations in the Xinjiang area. These results indicated that the common variant rs5361 is strongly associated with EH in Han individuals and weakly associated in Uygur individuals. Therefore, the CC genotype of rs5361 may be an independent risk factor for EH among Uygur, Kazakh and Han individuals living in the Xinjiang area. However, analyses of single-locus polymorphisms have limitations in correlation studies, and haplotype-based analyses are thought to be much more powerful than marker-by-marker analyses in genes with multiple susceptibility alleles (26). Haplotype blocks may capture epistatic interactions between SNPs (27-29), drastically reducing the number of tests required (controlling type I error), although this method can increase the rate of inevitable type II errors. The benefits of haplotype analyses therefore depend on the extent of LD between the variants in the block and thus the length of the haplotype block and distance between the variants (30). Few studies have reported associations between haplotypes and hypertension, especially in the Mongolian population. The prevalence of hypertension is higher in Mongolia than in China, and the Mongolian population has distinct characteristics of a high inci-

### Table 3. SELE Haplotype Frequency Distribution in the Mongolian Population

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>HT, N (%)</th>
<th>NT, N (%)</th>
<th>x2</th>
<th>OR</th>
<th>95%CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAC</td>
<td>234.93 (0.557)</td>
<td>233.96 (0.542)</td>
<td>0.02</td>
<td>1.06</td>
<td>0.81-1.39</td>
<td>0.655</td>
</tr>
<tr>
<td>GAT</td>
<td>1.07 (0.003)</td>
<td>0.96 (0.002)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>GGC</td>
<td>31.07 (0.074)</td>
<td>16.04 (0.037)</td>
<td>5.46</td>
<td>2.06</td>
<td>1.11-3.83</td>
<td>0.02*</td>
</tr>
<tr>
<td>GGT</td>
<td>61.93 (0.147)</td>
<td>78.04 (0.181)</td>
<td>1.79</td>
<td>0.78</td>
<td>0.54-1.12</td>
<td>0.181</td>
</tr>
<tr>
<td>TAT</td>
<td>0.00 (0.000)</td>
<td>0.08 (0.000)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TGT</td>
<td>93.00 (0.220)</td>
<td>102.92 (0.238)</td>
<td>0.38</td>
<td>0.90</td>
<td>0.66-1.24</td>
<td>0.535</td>
</tr>
</tbody>
</table>

Globe x2 = 7.04
Fisher p = 0.07

HT: hypertensives, NT: normotensives, OR: odds ratio, CI: confidence interval, $\chi^2$: Pearson’s chi-square, *p<0.05.

### Table 4. SELE Haplotype Frequency Distribution in the Han Population

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>HT, N (%)</th>
<th>NT, N (%)</th>
<th>x2</th>
<th>OR</th>
<th>95%CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACA</td>
<td>10.00 (0.025)</td>
<td>24.99 (0.060)</td>
<td>5.75</td>
<td>0.41</td>
<td>0.20-0.87</td>
<td>0.017*</td>
</tr>
<tr>
<td>ATA</td>
<td>0.00 (0.000)</td>
<td>0.01 (0.000)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TCA</td>
<td>236.01 (0.599)</td>
<td>263.02 (0.626)</td>
<td>0.64</td>
<td>0.89</td>
<td>0.67-1.18</td>
<td>0.426</td>
</tr>
<tr>
<td>TCC</td>
<td>75.99 (0.193)</td>
<td>56.99 (0.136)</td>
<td>4.86</td>
<td>1.52</td>
<td>1.05-2.21</td>
<td>0.028*</td>
</tr>
<tr>
<td>TTA</td>
<td>71.99 (0.183)</td>
<td>74.98 (0.179)</td>
<td>0.02</td>
<td>1.03</td>
<td>0.72-1.47</td>
<td>0.877</td>
</tr>
<tr>
<td>TTC</td>
<td>0.01 (0.000)</td>
<td>0.01 (0.000)</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Globe x2 = 9.84
Fisher p = 0.02

HT: hypertensives, NT: normotensives, OR: odds ratio, CI: confidence interval, $\chi^2$: Pearson’s chi-square, *p<0.05.
dence of minority groups. Hence, Mongolians may have different genetic and environmental backgrounds. Therefore, the study of haplotypes, rather than single SNP associations, has been proposed in Mongolian and Han population and is of great significance. Our study included a haplotype analysis to assess the potential influence of the SELE gene on essential hypertension. As tagSNPs are sufficient to capture most of the haplotype structure of the gene (31), analyses based on both SNPs and haplotypes were performed. For adjacent SNPs in strong LD (D’>0.8), we chose rs3917458, rs3917419 and rs2179172 in the Han population and rs1534904, rs932307 and rs3917436 in the Mongolian population in order to structure the haplotypes. Consequently, the frequency of haplotype GGC was significantly higher in the EH group than in the control group in the Mongolian population. In the Han population, a significant difference was observed in the haplotype frequency of TCC in both the patients and controls, whereas haplotype ACA occurred significantly less often in the EH patients than in the controls. Therefore, haplotype ACA had an independent effect on decreasing the risk of hypertension and may have a protective effect in normotensive subjects in the Han population. However, the haplotype TCC in the Han hypertensive patients and the haplotype GGC in the Mongolian patients had independent effects in increasing the risk of EH and may be used as risk factors for predicting high blood pressure.

Although we found associations between SELE gene haplotype variants and hypertension in Mongolian and Han populations in this study, several potential limitations must be noted. First, we did not measure the SELE levels in this study. Therefore, it is difficult to interpret the observed relationship and its biological significance. Second, our study results are restricted to ethnic groups in the Mongolian and Han populations. Third, the lack of significant differences in the genotype and allele distributions for all nine SNPs of the SELE gene between the EH patients and controls in the Mongolian population may be due to the small number of subjects. Finally, the risk of the development of hypertension in the two ethnicities may be different due to true etiologic heterogeneity, which may be driven by differences in gene–gene or gene-environment interactions. Consequently, comprehensive studies of genomic segments in the human genome in large samples are required to fully elaborate the contributions to the physiology of BP and EH.

In conclusion, the present study suggests that the presence of multiple variants in combination in SELE, rather than a single SNP, may alter the risk of hypertension. However, confounding factors and biases remain, and further multicenter, large-scale, multi-ethnic studies are warranted to validate the correlations between haplotype polymorphisms of the SELE gene and EH.

The authors state that they have no Conflict of Interest (COI).

Acknowledgement
This study was supported by the Inner Mongolia Natural Science Foundation Outstanding Youth Program (No. 2012JQ04). We appreciate the support and assistance provided by the Duolun Center for Disease Prevention and Control and the Department of Epidemiology of Inner Mongolia University.

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