Symposium

Linkage Analysis of Twelve Candidate Gene Loci Regulating Water and Sodium Metabolism and Membrane Ion Transport in Essential Hypertension


To investigate the relationship between 12 candidate genes responsible for water regulation, sodium metabolism and membrane ion transport and essential hypertension (EH) in the Chinese. Linkage analysis of EH was performed in 95 Chinese nuclear families including 477 subjects using a technique of fluorescence-based gene scanning with 12 microsatellite markers. Markers were selected on the chromosomal regions covering 12 candidate genes responsible for regulating water and sodium metabolism and membrane ion transport. These candidate genes included sodium hydrogen exchanger 3, sodium hydrogen exchanger 5, chloride bicarbonate exchanger 3, sodium calcium exchanger 1, mineralocorticoid receptor, plasma membrane calcium ATPase 2, ATPase, Na/K transporting alpha, ß-adducin, SA gene, kidney epithelial sodium channel- ß, vasopressin receptor 1A, and 11 ß-hydroxysteroid dehydrogenase type 2 genes. Two-point non-parametric linkage analysis (NPL), maximum LOD score analysis and transmission/disequilibrium test (TDT) were performed using the GENEHUNTER software package. The NPL analysis and LOD score suggested a significant linkage at D12S398 (Z = 2.08, p < 0.05 and LOD score = 1.26, p < 0.01, respectively). TDT indicated a significant disequilibrium of transmission at the locus (Z² = 9.00, p < 0.005). No significant linkages were found at the other loci tested (p > 0.05 or LOD < -1). In conclusion, D12S398, a marker near the vasopressin receptor 1A gene (V1AR), showed a positive linkage with EH based on the results of three statistical methods (NPL, LOD score, and TDT). This region warrants further exploration.


Key Words: hypertension, genetics, water and sodium metabolism, ion transport

Introduction

Essential hypertension is a multifactorial disorder with both genetic and environmental determinants. Multiple genes and gene-to-gene interactions may be involved in the etiology of this disease. Recently, many studies have been conducted on potential genes related to hypertension in order to determine the molecular genetic mechanisms and potential therapeutic targets for this disease. These researches have provided us with useful information, but the genes related to essential hypertension remain to be clarified.
Abnormal responses to excess salt intake or aberrant ion membrane transport have been found in a subset of patients with essential hypertension (1, 2). Genes responsible for the regulation of sodium metabolism, blood volume, and intracellular pH, such as genes related to the epithelial sodium channel, α-adducin and sodium hydrogen exchangers, have been examined in association studies as candidate hypertensive genes. In this study, linkage analysis of 12 genetic loci at or near the candidate genes regulating water and sodium metabolism and membrane ion transport was performed in 95 Chinese families in order to clarify linkage regions associated with essential hypertension.

### Subjects and Methods

#### Subjects

The subjects in this study consisted of 477 individuals from 95 nuclear families. Each family member was determined to have at least two affected siblings. Overall, there were 397 affected and 80 unaffected individuals. Twenty-nine (30.5%) of the 95 families had 2 affected siblings, 30 (31.6%) had 3, 18 (18.9%) had 4, 10 (10.5%) had 5, 7 (7.4%) had 6, and 1 (1.1%) had 8. All participants were Han Chinese currently residing in the Shanghai area.

The hypertensive probands were identified in the Outpatient Department of Hypertension of Ruijin Hospital, Shanghai or selected from a screening survey in the area of Shanghai on the basis of the following criteria: 1) systolic blood pressure ≥150 mmHg and/or diastolic blood pressure ≥95 mmHg; 2) no clinical or biochemical signs of secondary hypertension; 3) an onset of hypertension after 20 or before 60 years of age; and 4) at least one affected sibling pair. If a hypertensive proband met these criteria, he/she and all his/her siblings and parents were examined by trained physicians and epidemiologists of the Shanghai Institute of Hypertension. Normotensive siblings younger than 40 years of age were excluded. Blood pressure was measured using a mercury sphygmomanometer with subjects in the sedentary position after 10 min of rest by experienced and certified examiners. Afterwards, a detailed questionnaire was filled out, a physical examination was made, and a 10 ml blood sample was drawn from all subjects for the purpose of DNA extraction and biochemical assays. Informed consent was obtained from each participant.

#### Extraction of Genome DNA and Biochemical Determination

Blood was sampled after the subjects had fasted for 12 h. Genomic DNA was extracted from peripheral white blood cells of all participants using the standard method. Fasting blood glucose (FG), serum total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), creatinine (Cr), blood urea nitrogen (BUN), and uric acid (UA) were measured.

### Selection of Candidate Genes and Genetic Markers

On 11 autosomes, 12 polymorphic microsatellite markers close to the corresponding candidate genes (less than 7.3 cM) were chosen using one of three genomic databases (ncbi.nlm.nih.gov/, www.chlc.org/ and www.marshmed.org/). These candidate genes were involved in the regulation of water and sodium metabolism, membrane ion transport (Table 1). These microsatellite primers labeled with fluorescent dyes were purchased from Research Genetics, Inc. (Huntsville, USA).

### Genotyping

Multiplex PCR reactions were carried out in 96-well plates using a 5-1 cocktail that included 10 mmol/l Tris-HCL, pH
The microsatellite alleles were analyzed by Genotyper™ ware (Applied Biosystem Inc.) was used for collecting data. 200 mol/l dNTPs, 0.15 U Taq polymerase, 33 ng Taq Start™

nitrogen; Cr, creatinine; UA, blood uric acid.

LDL-C, low density lipoprotein cholesterol; BUN, blood uric pressure; FG, fasting blood glucose; TC, serum total cholesterol; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; FG, fasting blood glucose; TC, serum total cholesterol; HDL-C, high density lipoprotein cholesterol; TG, triglyceride; LDL-C, low density lipoprotein cholesterol; BUN, blood uric nitrogen; Cr, creatinine; UA, blood uric acid.

Table 2. Clinical Characteristics and Biochemistry Data of the Study Population

<table>
<thead>
<tr>
<th></th>
<th>Members unaffected</th>
<th>Members affected</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>80</td>
<td>397</td>
</tr>
<tr>
<td>Sex (M/F) a</td>
<td>30/50</td>
<td>164/233</td>
</tr>
<tr>
<td>Age (years)</td>
<td>48.50 □ 10.2</td>
<td>53.00 □ 11.12**</td>
</tr>
<tr>
<td>Age of onset (years)</td>
<td>□</td>
<td>38.41 □ 10.14</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.71 □ 2.80</td>
<td>24.76 □ 3.29**</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>122.38 □ 9.80</td>
<td>165.03 □ 25.49**</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>79.00 □ 8.90</td>
<td>102.84 □ 11.21**</td>
</tr>
<tr>
<td>FG (mmol/l)</td>
<td>4.89 □ 0.91</td>
<td>5.38 □ 1.79**</td>
</tr>
<tr>
<td>TC (mmol/l)</td>
<td>4.50 □ 0.94</td>
<td>4.92 □ 1.06**</td>
</tr>
<tr>
<td>HDL-C (mmol/l)</td>
<td>1.45 □ 0.34</td>
<td>1.36 □ 0.35</td>
</tr>
<tr>
<td>TG (mmol/l)</td>
<td>1.37 □ 0.43</td>
<td>1.73 □ 0.89**</td>
</tr>
<tr>
<td>LDL-C (mmol/l)</td>
<td>2.79 □ 1.06</td>
<td>3.21 □ 1.08**</td>
</tr>
<tr>
<td>BUN (mmol)</td>
<td>4.35 □ 1.45</td>
<td>4.34 □ 1.45</td>
</tr>
<tr>
<td>Cr (µmol/l)</td>
<td>65.67 □ 14.78</td>
<td>72.04 □ 17.45**</td>
</tr>
<tr>
<td>UA (µmol/l)</td>
<td>255.63 □ 41.26</td>
<td>282.48 □ 55.61**</td>
</tr>
</tbody>
</table>

8.3, 50 mmol/l KCl, 0.1 mg/ml gelatin, 3.0 mmol/l MgCl₂, 200 mol/l dNTPs, 0.15 U Taq polymerase, 33 ng Taq Start™ antibody, 48 mmol/l of each primer and 9.0 ng genomic DNA. PCR reactions were carried out in a Perkin-Elmer Norwalk, USA). The amplifications took 2 min at 94 °C, followed by 34 cycles, each having 20 s at 94 °C, 20 s at 56 °C and 20 s at 72 °C, except that in the first 14 cycles, the annealing temperatures decreased from 63 °C to 56 °C by 0.5 °C per cycle and annealing time from 60 s to 20 s by 3 s per cycle. At the end of the amplifications, the reaction tubes were subjected to 72 °C for 5 min in order to complete all extensions.

PCR products were mixed with loading buffer, denatured and then loaded on a 24 cm, 6% denaturing polyacrylamide gel. After a 2-h electrophoresis, the DNA segments were separated on a 373A automatic DNA sequencer (Applied Biosystem Inc., Foster City, USA). The Genescan™ software (Applied Biosystem Inc.) was used for collecting data. The microsatellite alleles were analyzed by Genotyper™ software (Applied Biosystem Inc.). All genotypes were verified for Mendelian segregation among pedigree members.

Statistic Analysis

Since the mode of inheritance of hypertension is not yet well established, non-parametric linkage (NPL) analysis was carried out using GENEHUNTER software (http://waldo.wi.mit.edu/ftp/distribution/software/genehunter/gh2) to assess the evidence for linkage (3). Briefly, GENEHUNTER performs multipoint analysis which compute a Z score statistic comparing the observed identical-by-descent (IBD) sharing among all affected members to that expected under the null hypothesis of no linkage. On the null hypothesis, the Z score is normally distributed with mean zero and variance one. Marker allele frequencies were calculated using unrelated individuals in this study. An LOD score was also estimated for this set of data. We considered two alleles, D and d, at the disease locus with frequencies 0.075 and 0.925, respectively. The penetrances of the genotypes DD, Dd and dd were assumed to be 0.725, 0.1 and 0.1, respectively. The prevalence of hypertension calculated using these values was consistent with that obtained from our observations in the area of Shanghai (11–15%). We also performed a TDT using the GENEHUNTER program to compare the frequencies of the allele transmitted from heterozygous parents to the affected children with those of untransmitted alleles. The TDT is a test of association in the presence of linkage (4). Probability values less than 0.05 or LOD scores equal to or greater than 1 were considered to indicate statistical significance.

Results

Clinical and Biochemical Data of Affected and Unaffected Groups

Gender ratio was not significantly different between the affected and unaffected groups (p > 0.05). But systolic blood pressure, diastolic blood pressure, body mass index, and the levels of fasting blood glucose serum total cholesterol, triglyceride, low density lipoprotein cholesterol, creatinine and blood uric acid were higher in the affected group than in the unaffected one (p < 0.01). No significant differences in high-density lipoprotein cholesterol or blood uric nitrogen were found between the two groups (Table 2).

Linkage Analysis

The GENEHUNTER program was used to perform linkage analyses on the 95 families. Analyses of the NPL, LOD score and TDT were used for detecting significant regions. Among the 12 microsatellite markers tested, D12S398, a marker 7.3 cM away from vasopressin receptor 1A gene, was significantly linked with essential hypertension. In contrast, no significant positive linkage was found at the other microsatellite markers. The GENEHUNTER program was used to perform linkage analyses on the 95 families. Analyses of the NPL, LOD score and TDT were used for detecting significant regions. Among the 12 microsatellite markers tested, D12S398, a marker 7.3 cM away from vasopressin receptor 1A gene, was significantly linked with essential hypertension. In contrast, no significant positive linkage was found at the other genetic loci. Detailed results of the analyses are given in Table 3.

Discussion

Blood pressure is elevated in response to excess salt intake, sodium retention and plasma volume expansion, and is a result of an abnormal intercellular ion transport and intracellular pH, which are considered surrogate phenotypes of certain
patients in the subgroup of essential hypertension. Moreover, genetic factors might contribute to these phenotypes. Recently, investigators have detected linkages or association of the genetic factors might contribute to these phenotypes. Recent-

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Table 3. Results of LOD Score, NPL and TDT Analyses in the Study

<table>
<thead>
<tr>
<th>Loci</th>
<th>LOD</th>
<th>NPL</th>
<th>Z</th>
<th>Alleles</th>
<th>No. of allele</th>
<th>Trans/untrans</th>
<th>$\chi^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>DSS392</td>
<td>-2.1</td>
<td>1.04</td>
<td></td>
<td>13</td>
<td>10</td>
<td>8 / 16</td>
<td>2.67</td>
</tr>
<tr>
<td>D16S512</td>
<td>-3.0</td>
<td>0.07</td>
<td></td>
<td>9</td>
<td>4</td>
<td>37 / 28</td>
<td>1.25</td>
</tr>
<tr>
<td>D2S163</td>
<td>-5.0</td>
<td>0.57</td>
<td></td>
<td>9</td>
<td>4</td>
<td>33 / 25</td>
<td>1.10</td>
</tr>
<tr>
<td>D2S119</td>
<td>-4.8</td>
<td>0.38</td>
<td></td>
<td>9</td>
<td>3</td>
<td>16 / 8</td>
<td>0.18</td>
</tr>
<tr>
<td>D4S1604</td>
<td>-5.8</td>
<td>0.93</td>
<td></td>
<td>6</td>
<td>4</td>
<td>42 / 24</td>
<td>4.91*</td>
</tr>
<tr>
<td>D3S1263</td>
<td>-5.1</td>
<td>0.58</td>
<td></td>
<td>17</td>
<td>8</td>
<td>9 / 19</td>
<td>3.57</td>
</tr>
<tr>
<td>D19S420</td>
<td>-4.9</td>
<td>0.47</td>
<td></td>
<td>8</td>
<td>4</td>
<td>9 / 13</td>
<td>0.73</td>
</tr>
<tr>
<td>D4S432</td>
<td>-4.2</td>
<td>0.34</td>
<td></td>
<td>10</td>
<td>3</td>
<td>36 / 25</td>
<td>1.98</td>
</tr>
<tr>
<td>D16S412</td>
<td>-2.8</td>
<td>0.57</td>
<td></td>
<td>7</td>
<td>3</td>
<td>26 / 41</td>
<td>3.36</td>
</tr>
<tr>
<td>D12S398</td>
<td>1.26**</td>
<td>2.08*</td>
<td></td>
<td>4</td>
<td>2</td>
<td>0 / 9</td>
<td>9.0***</td>
</tr>
<tr>
<td>D16S503</td>
<td>-6.5</td>
<td>1.58</td>
<td></td>
<td>9</td>
<td>6</td>
<td>5 / 10</td>
<td>1.7</td>
</tr>
</tbody>
</table>

*p < 0.05, ** p < 0.01, *** p < 0.005.

encoding $\alpha$-adducin has been considered to be involved in cellular sodium metabolism. Linkage and association analyses have confirmed the relationship between this gene and blood pressure regulation in Italian population (9, 10). However, in agreement with our results, most studies have failed to demonstrate a linkage between $\alpha$-adducin gene loci and hypertension, or have showed a lack of association between the Gly460Trp variant of $\alpha$-adducin and hypertension in Chinese (11, 12), Scottish (13), Anglo-Australian (14) and African American (15) populations. Inconsistency between populations has also been demonstrated for other hypertensive candidate genes, such as the SA gene, which was positively associated with hypertension in Japanese hypertensive patients (6), but negatively associated with hypertension in Caucasian subjects (16).

There are a number of possible explanations for these discrepancies. Differences in the ethnicities of subjects may have had an important influence on the outcome of the studies. Furthermore, the results of genetic studies on $\alpha$-adducin polymorphism and hypertension in Japanese populations have been inconsistent (17, 18). The discrepancies in genetic analyses in different nations and regions may reflect important differences in the genetic factors. It is worth noting that the frequency of the 460Trp variant in Chinese (45.9% to 48.2%) (12) is much higher than that in Caucasians (13% to 23%) (19). Regional differences in environmental factors, such as differences in salt intake, might be another important factor.

The study design and criteria for subject recruitment are another important consideration. In the case of randomly selected patients with essential hypertension, the multiple and minor gene effects are obscured by the heterogeneity of the study population. A more homogeneous sub population defined by intermediate phenotype might be helpful to disclose the underlying genetic predisposition to essential hypertension. Recently, an association between $\alpha$-adducin polymorphism and hypertension in familial combined hyperlipidemia.
was reported (20).

No linkage was found at the other loci typed in the present study, which might indicate that the polymorphisms of the candidate genes at or near these loci did not play a key role in the etiology of essential hypertension. However, for the above-mentioned reasons, the linkage between these loci and essential hypertension could not be excluded in other races or populations.

In conclusion, D12S368, a marker near the vasopressin receptor 1A gene, showed a positive linkage with essential hypertension based on the results of analyses using LOD scores and NPL and TDT values. On the other hand, there was no evidence of a significant linkage of the other loci tested with essential hypertension. Further studies will be needed to verify the present results identify other genetic links to hypertension.

References