Association of SLC6A9 Gene Variants with Human Essential Hypertension

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Aim: We previously identified a quantitative trait locus (QTL) on rat chromosome 5 that appeared to be primarily controlled by the sympathetic nervous system. Because sympathetic overactivity is related to hypertension, solute carrier family 6, member 9 (SLC6A9) is a candidate gene for the connection of this QTL with blood pressure regulation. In the present study, we therefore explored the role of SLC6A9 genetic variations in human essential hypertension (EH).

Methods: We evaluated three single nucleotide polymorphisms (SNPs) (rs2286245, rs3791124 and rs2486001) in 758 essential hypertension patients and 726 controls. Polymorphism-related genotypes were determined with TaqMan assays.

Results: The allelic frequency of rs2286245 (C versus T, \( p=0.032 \)) showed significant differences between EH and normotensive controls (NT) groups. The genotypic distribution of rs3791124 in its dominant model (AA + GA versus GG, \( p=0.027 \)) also showed significant differences between EH and NT groups. The genotype and allele distributions of rs2486001 did not exhibit any significant differences.

Conclusion: We found an association between SLC6A9 gene polymorphisms and essential hypertension in a Japanese population, suggesting that SLC6A9 is a susceptibility locus for essential hypertension.


Key words: Essential hypertension, SLC6A9, Japanese population, Sympathetic nervous system

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Introduction

Hypertension affects 25% of most adult populations in industrialized countries⁴⁻⁵ and is a major risk factor for stroke and coronary heart disease⁵⁻⁷. The largest group of hypertensive patients has essential
hypertension (EH), a polygenic disorder that results from the inheritance of a number of susceptibility genes. There are most likely several causal genes, which together account for 30% to 50% of the blood pressure variation found among individuals. EH subjects happen to have inherited an aggregate of genes related to hypertension and/or to have been exposed to exogenous factors that predispose them to hypertension.

Using experimental crosses derived from genetically hypertensive and control rat strains, quantitative trait loci (QTL) influencing blood pressure have been mapped to several rat chromosomes. We previously established a new approach to estimate the contribution of blood pressure regulatory systems, using a pharmacogenetic approach, and systematically analyzed the cosegregation of genetic loci for acute cardiovascular responses to drugs that influence the renin-angiotensin system, sympathetic nervous system, or nitric oxide system in F2 populations derived from cross-breeding Prague hypertensive-hypertriglyceridemic rats with normotensive Lewis rats. In this analysis, we identified a QTL on rat chromosome 5 that correlated with basal mean arterial blood pressure. The correlation of this locus to blood pressure disappeared after pentolinium administration, thereby suggesting modulation by the sympathetic nervous system. By comparative mapping, 142 annotated genes were identified in the syntenic locus of this rat QTL on human chromosome 1. Similar to most loci mapped, this locus included a large segment of the genome, and these segments clearly included several potential candidate genes.

Termination of synaptic activity is thought to occur through removal of the neurotransmitter from the synaptic cleft by ion-coupled, high-affinity neurotransmitter transport proteins located in neuronal and glial plasma membranes. Neurotransmitter transporters are membrane-bound proteins that actively transport the released neurotransmitters back into presynaptic neurons and surrounding glia, thereby terminating the activity of monoamine and amino acid neurotransmitters, and helping to replenish presynaptic pools of neurotransmitters.

Glycine has two functions in the central nervous system. Firstly, it is an inhibitory neurotransmitter acting on strychnine-sensitive glycine receptors, which are located mainly in the brainstem and spinal cord. Secondly, in a broader action throughout the CNS, it regulates glutamatergic neurotransmission by acting as an obligatory coagonist of glutamate at the N-methyl-D-asparate (NMDA) receptor. The actions of glycine are terminated by diffusion and/or uptake. Uptake of glycine is achieved by glycine transporters. Glycine transport is mediated by two sodium-dependent carriers, solute carrier family 6, member 9 (SLC6A9) and solute carrier family 6, member 5 (SLC6A5), which have distinct tissue distributions. With the development of potent and specific antagonists of SLC6A9, the role of SLC6A9 in maintaining subsaturating levels of glycine at the glutamatergic synapse was established.

Therefore, the SLC6A9 gene seems to be an attractive candidate gene for sympathetic nervous system-driven blood pressure regulation in the QTL that was earlier identified on rat chromosome 5. In the present study, our aims were to investigate the association between human SLC6A9 and hypertension by analyzing single-nucleotide polymorphisms (SNPs) in the human SLC6A9 gene.

Method

Case-Control Study: Collaborative Study with the Hypertension Section of the Japanese Millennium Project

The study population consisted of 758 essential hypertension (EH) patients and 726 normotensive (NT) healthy control subjects who were recruited through a subgroup collaboration with the hypertension section of the Japanese Millennium Project. Six medical institutes took part in the collaborative study and collected data on hypertensive cases and controls. Hypertensive patients were defined as having SBP 140 mmHg or DBP 90 mmHg, or were receiving chronic antihypertensive medication. To increase the statistical power of the present study, hypertensive subjects additionally had to meet the following criteria: age 60 years old or onset of hypertension at 50 years of age, a family history of hypertension, and not obese (body mass index 26 kg/m²). NT criteria were as follows: SBP/DBP 130/85 mmHg, without a family history of hypertension, and not obese. Both groups were recruited throughout Japan, and informed consent was obtained from each individual as per the protocol approved by each institution’s human studies committee.

Genotyping

Based on allelic frequency information from the web site of the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nih.gov), 3 SNPs in the human SLC6A9 gene with minor allele frequencies higher than 15% were selected. SNPs with relatively high minor allele frequencies have demonstrated utility as genetic markers for genetic case-control studies. Both SNPs were confirmed using the
NCBI web site with accession numbers rs2286245, rs3791124 and rs2486001. We examined the association between EH and these 3 SNPs (Table 1).

Genotypes were determined using Assay-on-Demand kits (Applied Biosystems) together with TaqMan PCR. TaqMan Universal Master Mix (PE Biosystems) was used for PCR in a 25 μL reaction volume containing 50 ng DNA, 700 nM primer, and 100 nM probe final concentrations. Thermal cycling conditions consisted of 95°C for 10 minutes and then 40 cycles 92°C for 15 s and 60°C for 1 minute in a GeneAmp 9700 system. Fluorescence levels of PCR products were measured using an ABI PRISM 7700 Sequence Detector (Applied Biosystems), which resulted in the clear identification of three genotypes for the two alleles.

Statistical Analysis

Data are shown as the mean and SD. All statistical analyses were conducted using StatView 5.0 (SAS Inc.) and Dr. SPSS (SPSS Inc.). Hardy-Weinberg equilibrium was assessed by two analyses. The overall distributions of the genotypes or alleles were analyzed by two analyses using 2×3 or 2×2 contingency tables between EH patients and NT controls.

Results

We previously identified a QTL on rat chromosome 5 between D5Rat147 and D5Rat108 that appeared to affect blood pressure regulation. SLC6A9 was located in the middle of the region. This rat locus was syntenic with a region on human chromosome 1. Mapping data for the human and rat locus were taken from NCBI (Fig. 1).

Basic characteristics of the patient and control groups are given in Table 2. EH patients had a significantly higher body mass index, as well as systolic and diastolic blood pressure; 499 of 758 patients with essential hypertension were treated with oral antihypertensive agents.

We performed a case-control study of the rs2286245, rs3791124 and rs2486001 SNPs using 758 EH patients and 726 NT controls (Table 3). The observed and expected genotypic frequencies of each SNP in NT subjects were in good agreement with the predicted Hardy-Weinberg equilibrium values.

The allelic frequency of rs2286245 (C versus T, \(p = 0.032\)) showed significant differences between EH and NT groups. The genotypic distribution of rs3791124 in its dominant model (AA + GA versus GG, \(p = 0.027\)) also showed significant differences between EH and NT groups. The odds ratio of rs3791124 and rs2286245 for hypertension was estimated to be 1.32 (95% CI: 1.06 to 1.64; \(p = 0.01\)) and 1.26 (95% CI: 0.99 to 1.62; \(p = 0.06\)) after age and BMI adjustment in the dominant model.

Discussion

We performed a genetic case-control study in a Japanese population, and found that SNPs in the SLC6A9 gene were associated with EH. To our knowledge, this is the first study that relates SLC6A9 gene polymorphism to hypertension in humans.

We have established a new approach to estimate the contribution of blood pressure regulatory systems, using a pharmacogenetic approach[12]. In this analysis, the disappearance of a QTL for mean arterial pressure after blockade of the sympathetic nervous system by pentolinium reveals the contribution of the sympathetic nervous system to baseline mean arterial pressure. Using this approach, we determined that the baseline mean arterial pressure of the hypertensive rat strain was controlled by the QTL on rat chromosome 5 through the sympathetic nervous system.

Dense gene maps have been established for the rat by the Rat Genome Database and NCBI. The human genome project has enabled construction of a human genome map that is currently available from NCBI. These maps can be used to project the results of quantitative genetic analysis of rat chromosomes onto the human genome. We have previously reported a strategy to extrapolate data from rat quantitative trait genetics onto the human genome, using a comparative mapping approach[20]. In the current study, we applied our comparative approach to our previously identified QTL for baseline mean arterial pressure on rat chromosome 5, to which contribution of the sympathetic nervous system was suggested, and selected

### Table 1. Location of SNPs analyzed in the present case-control study

<table>
<thead>
<tr>
<th>dbSNP rs#</th>
<th>Cluster ID</th>
<th>Contig position</th>
<th>Region</th>
<th>Function</th>
<th>dbSNP allele</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs2486001</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>A/G</td>
</tr>
<tr>
<td>rs3791124</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>A/G</td>
</tr>
<tr>
<td>rs2286245</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C/T</td>
</tr>
</tbody>
</table>
SLC6A9 as a possible candidate gene for blood pressure regulation.

Although sympathetic nervous activation in essential hypertension has been well documented, with analysis of the regional sympathetic nervous system function demonstrating activation of sympathetic nervous outflows to the heart, kidneys and skeletal muscle vasculature\textsuperscript{23}, the exact pathophysiology of sympathetic nervous dysfunction remains to be delineated. Termination of synaptic activity is thought to occur through neurotransmitter removal from the synaptic cleft by ion-coupled, high-affinity neurotransmitter transporter proteins. SLC6A9 have been suggested to play an important regulatory role at the glycine receptor containing synapses by clearing glycine from the synaptic cleft, and at synapses containing NMDA receptors by maintaining the extracellular glycine level below saturating concentrations at the glycine site on NMDA receptors\textsuperscript{24-27}. As there is increasing evidence that essential hypertension, at least in its early stages, is accompanied by sympathetic hyperactivation, the contribution of the central nervous system to essential hypertension has been reported. Concerning this evidence, SLC6A9 seems to be an attractive candidate gene for essential hypertension, and this gene plays an important role in maintaining sympathetic activity in the central nervous system.

Recently, the association of SLC6A9 polymor-

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**Table 2. Clinical characteristics of NT and EH**

<table>
<thead>
<tr>
<th></th>
<th>EH</th>
<th>NT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>758</td>
<td>726</td>
</tr>
<tr>
<td>Male (%)</td>
<td>564 (74.4)</td>
<td>550 (75.8)</td>
</tr>
<tr>
<td>Age</td>
<td>59.0 ± 11.0*</td>
<td>62.8 ± 9.4</td>
</tr>
<tr>
<td>Body mass index (kg/m(^2))</td>
<td>23.6 ± 3.0*</td>
<td>22.7 ± 2.9</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>163.5 ± 24.6*</td>
<td>115.9 ± 12.0</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>100.3 ± 15.7*</td>
<td>72.0 ± 7.6</td>
</tr>
<tr>
<td>Antihypertensive medication, n (%)</td>
<td>499 (65.8)</td>
<td></td>
</tr>
</tbody>
</table>

*: p < 0.05 by Mann-Whitney’s U-test.

NT: normotensive control, EH: essential hypertension
phism with schizophrenia\textsuperscript{28, 29} or methamphetamine-use disorder\textsuperscript{30} has been reported. There is a rapidly growing body of literature describing inhibitors of SLC6A9\textsuperscript{20, 31}; however, the effect of these inhibitors on blood pressure has not been reported. The elevation of blood pressure in response to psychoemotional stimuli is well known by clinicians, and the SLC6A9 gene may affect blood pressure by altering the response to psychoemotional stimuli.

In summary, we examined polymorphisms of the SLC6A9 gene in a case-control study of the Japanese population. This finding needs further confirmation in a variety of ethnic groups, and functional studies are required to elucidate the mechanisms underlying the association of SLC6A9 polymorphisms with essential hypertension.

### Acknowledgements

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### References

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### Table 3. Genotype distribution in NTs and patients with EH

<table>
<thead>
<tr>
<th>Genotype</th>
<th>rs2286245</th>
<th>rs3791124</th>
<th>rs2486001</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NT (0.028)</td>
<td>EH (0.016)</td>
<td>NT (0.0631)</td>
</tr>
<tr>
<td>TT</td>
<td>20</td>
<td>12</td>
<td>45</td>
</tr>
<tr>
<td>CT</td>
<td>168</td>
<td>153</td>
<td>270</td>
</tr>
<tr>
<td>TT</td>
<td>523</td>
<td>577</td>
<td>398</td>
</tr>
<tr>
<td>p</td>
<td>0.096</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dominant</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT + CT</td>
<td>188</td>
<td>165</td>
<td>315</td>
</tr>
<tr>
<td>CC</td>
<td>523</td>
<td>577</td>
<td>398</td>
</tr>
<tr>
<td>OR</td>
<td>0.796</td>
<td>0.262-1.012</td>
<td>0.4417</td>
</tr>
<tr>
<td>95% CI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p</td>
<td>0.062</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recessive</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT + CT</td>
<td>188</td>
<td>165</td>
<td>315</td>
</tr>
<tr>
<td>CC + CT</td>
<td>691</td>
<td>730</td>
<td>668</td>
</tr>
<tr>
<td>OR</td>
<td>0.568</td>
<td>0.276-1.171</td>
<td>0.9369</td>
</tr>
<tr>
<td>95% CI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p</td>
<td>0.125</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allele</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>208</td>
<td>177</td>
<td>360</td>
</tr>
<tr>
<td>C</td>
<td>1,214</td>
<td>1,307</td>
<td>1,066</td>
</tr>
<tr>
<td>OR</td>
<td>0.79</td>
<td>0.637-0.980</td>
<td>0.2525</td>
</tr>
<tr>
<td>95% CI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p</td>
<td>0.032</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NT: normotensive control, EH: essential hypertension
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