Original Article

Epidemiological Evidence of an Association between SLC6A2 Gene Polymorphism and Hypertension

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Selective blockade of the norepinephrine transporter with reboxetine has been reported to induce a slight but significant increase in blood pressure. This study was designed to examine the relation of genetic variants of the norepinephrine transporter gene (solute carrier family 6, member 2; SLC6A2) with hypertension in a Japanese population. We genotyped five genetic variants of SLC6A2, three in the promoter region and two in the intronic sequence, in 1,950 subjects recruited from the Suita study. One of the variants, an A>G polymorphism in the promoter region (Promoter 3 polymorphism), was found to be associated with hypertension. Multiple logistic analysis indicated that sex (p = 0.0223), age (p < 0.0001), body mass index (p < 0.0001), alcohol consumption (p = 0.0002), and the Promoter 3 genotype (AA = 1, AG + GG = 2) (p = 0.0090) were predictive of hypertensive status. The odds ratio of the AG + GG genotypes for hypertension was 1.35 (95% confidence interval: 1.08–1.69) over the AA genotype. SLC6A2 may be one of the genes that contribute to hypertension in Japanese. To our knowledge, this is the first report to detect associations between SLC6A2 genetic variants and blood pressure. (Hypertens Res 2003; 26: 685–689)

Key Words: genetics, epidemiology, norepinephrine, hypertension

Introduction

Interactions between genetic and environmental factors are thought to play important roles in the pathogenesis of hypertension. The use of association studies in large epidemiological cohorts with a large number of single-nucleotide polymorphisms throughout the entire genome or throughout a single gene is a new strategy for identifying genes that contribute to blood pressure regulation (1–3). In the present study, we applied this strategy to the norepinephrine transporter gene (solute carrier family 6, member 2; SLC6A2) to examine whether its genetic variants influence blood pressure.

The norepinephrine transporter plays important roles in cardiovascular homeostasis. At most synapses, chemical signaling is terminated by a rapid reaccumulation of neurotransmitter into presynaptic terminals. The reuptake of noradrenaline occurs via a specific sodium- and chloride-dependent transporter system known as the norepinephrine transporter system. Genetic dysfunction of SLC6A2 has been reported to cause idiopathic orthostatic intolerance, which is characterized by the absence of orthostatic hypotension but a rapid orthostatic increase in heart rate (4).

Interestingly, in some studies, patients with orthostatic intolerance have been reported to exhibit greater supine blood pressure values than age- and sex-matched control subjects (5). Selective blockade of the norepinephrine transporter by
Hypertension has also been reported (7). Thus, SLC6A2 is a good candidate gene for human essential hypertension.

In the present study, we thoroughly searched for polymorphisms of SLC6A2 and performed an association study using a large epidemiological cohort. To our knowledge, this is the first report to investigate associations between SLC6A2 genetic variants and blood pressure.

**Methods**

Subjects

The selection criteria and design of the Suita study have been described previously (2). The present study was approved by the Ethics Committee of the National Cardiovascular Center and by the Committee on Genetic Analysis and Genetic Therapy of the National Cardiovascular Center. Written informed consent for genetic analysis was obtained from about 3,700 subjects, and the genotype of SLC6A2 was determined in 1,950 consecutive subjects. Subjects were categorized as hypertensives when they had a systolic blood pressure of \( 90 \text{ mmHg} \) or a diastolic blood pressure of \( 60 \text{ mmHg} \). Subjects who were taking hypertensive medication were also categorized as hypertensives.

DNA Studies

DNA was isolated from peripheral leukocytes according to standard procedures. Genomic DNA from 24 subjects was used as a template for sequence analyses. The promoter (up to \(-1.5\text{kb}\)) and coding regions (exons 1–14) were sequenced. Polymorphisms were determined by the TaqMan system. The results were analyzed using an ABI PRISM 7700 Sequence Detection System (PE Biosystems, Foster City, USA) using allelic discrimination software supplied by the manufacturer.

**Statistical Analyses**

Values are expressed as the mean \( \pm \) SEM. All statistical analyses were performed with the JMP statistical package (SAS Institute Inc., Cary, USA). Multiple regression and multiple logistic analyses were performed with other covariates. The effects of polymorphisms on blood pressure and heart rate were assessed in subjects who were not receiving cardiovascular medications, since hypertensive subjects with excellent blood pressure control by medication may have normal blood pressure values. We excluded subjects who were receiving antihypertensive treatment, subjects who had had cerebrovascular accidents, subjects with demonstrated ischemic heart disease, and subjects with atrial fibrillation. Residuals of blood pressure values were calculated by adjusting for sex, age, ethanol consumption, and body mass index (BMI) (residuals of blood pressure values were the observed values minus the values predicted based on the sex, age, ethanol consumption, and BMI). Residuals of heart rate were calculated by adjusting for sex. Data was analyzed using a contingency table analysis and one-way analysis of variance (ANOVA) with Bonferroni correction for multiple comparisons. The sample power was calculated using SPSS software (SPSS Inc., Chicago, USA).

**Results**

Detection of Genetic Variants

We sequenced the promoter region (up to \(-1.5\text{kb}\)) and coding regions (exons 1–14) and found 9 polymorphisms in SLC6A2 (Table 1). Promoter 1, 4, and 5 polymorphisms were in complete disequilibrium among the 24 subjects sequenced, and the Promoter 1 polymorphism was selected for the association study. Intron 8, exon 9, and intron 13 polymorphisms were in tight linkage disequilibrium (\( 45/48 = 93.75\% \) could be explained by the two haplotypes) among the 24 subjects sequenced, and the Intron 8 polymorphism was selected for the association study. Promoter 2, Promoter 3, and intron 5 were not in linkage disequilibrium with the others and were selected for the association study. The primers and probes for genotype determination are summarized in Table 2. No missense mutation was found in the exonic regions.

Association Study

An association study between the polymorphisms and blood pressure status revealed that only the Promoter 3 polymorphism had significant effects on the frequency of hypertension (Table 3). Table 4 shows characteristics of the study population according to the Promoter 3 genotype. The frequencies of the AG and GG genotypes in the Promoter 3 polymorphism were higher among hypertensives. When blood pressure values were adjusted for sex, age, BMI, and

<table>
<thead>
<tr>
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<tbody>
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<td>ggacattgtgta[c/t]ttggtccctct</td>
</tr>
<tr>
<td>Promoter 2 C &gt; G</td>
<td>ccactgcccccc[c/g]atccctaccc</td>
</tr>
<tr>
<td>Promoter 3 A &gt; G</td>
<td>tgcggaagacaa[a/g]gcgggtgtgca</td>
</tr>
<tr>
<td>Promoter 4 C &gt; T</td>
<td>tcatctcctca[c/g]tgaagtgtgttat</td>
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<tr>
<td>Promoter 5 A &gt; G</td>
<td>gggggacatca[a/g]tttaaacaactg</td>
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<td>Intron 5 A &gt; G</td>
<td>cggtcagtgctc[a/g]gtgacaccaag</td>
</tr>
<tr>
<td>Intron 8 C &gt; A</td>
<td>ataggtctcttg[c/a]tgttcttcag</td>
</tr>
<tr>
<td>Exon 9 G &gt; A</td>
<td>ggcgtgcatcac[a/g]gggctcgccagat</td>
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<tr>
<td>Intron 13 T &gt; C</td>
<td>ttccctgtgtgt[c/a]actgecccaagc</td>
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Table 1. Genetic Variants in SLC6A2

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ethanol consumption, all of which are reported to affect blood pressure levels, residuals of systolic blood pressure values tended to be higher in subjects with the AG and GG genotypes than in those with the AA genotype.

Multiple logistic analysis indicated that sex (p < 0.0223), age (p < 0.0001), BMI (p < 0.0001), alcohol consumption (p = 0.0002), and the Promoter 3 genotype (AA = 1, AG + GG = 2) (p = 0.0090, p = 0.045 after Bonferroni’s correction for multiple tests) were predictors of hypertensive status. The odds ratio of the AG + GG genotypes for hypertension was 1.35 (95% confidence interval: 1.08–1.69) over the AA genotype. When the Promoter 3 genotype was analyzed using an A-dominant model (AA + AG = 1, GG = 2), the genotype could not predict hypertensive status (p = 0.55).

Sample Power Calculation

The sample power in the present study was 0.79 for the distribution, sample size, and α value (0.05, two-tailed).

Discussion

At most synapses, chemical signaling is terminated by a rapid reaccumulation of neurotransmitters into presynaptic terminals. The reuptake of noradrenaline occurs via a specific Na⁺- and Cl⁻-dependent transport system which is the target for tricyclic antidepressants such as desipramine and imipramine. Pacholczyk et al. isolated a cDNA encoding a human noradrenaline transporter (SLC6A2) (8). The cDNA sequence predicted a protein of 617 amino acids, with 12–13 highly hydrophobic regions compatible with membrane-spanning domains. They also suggested that analysis of the
structure and function of this transporter may aid structure-based drug design for the treatment of human depression and lead to a determination of whether transporter abnormalities underlie affective disorders.

Genetic dysfunction of SLC6A2 has been reported to cause idiopathic orthostatic intolerance, which is a syndrome characterized by lightheadedness, fatigue, altered mentation, and syncope, and is associated with postural tachycardia and plasma norepinephrine concentrations that are disproportionately high in relation to sympathetic outflow (4).

Interestingly, in some studies, patients with orthostatic intolerance exhibited greater supine blood pressure values than age- and sex-matched control subjects (5). The selective inhibition of norepinephrine reuptake by reboxetine has been reported to induce a slight but significant increase in blood pressure (6). The results of previous reports that involved measurement of the whole body plasma kinetics of tritiated norepinephrine suggest that neuronal norepinephrine reuptake may be impaired in essential hypertension (9). More direct evidence for the impaired neuronal reuptake of norepinephrine in essential hypertension has also been reported by a study using a phenotypically relevant radiotracer method (7). Thus, SLC6A2 is a good candidate gene for human essential hypertension, and the present study showed that one of the promoter variants of SLC6A2 was associated with hypertensive status.

In this study, we analyzed five polymorphisms. The association between the Promoter 3 genotype and hypertension remained significant after multiple regression analysis with Bonferroni’s correction for multiple comparisons, and thus the Promoter 3 genotype was considered useful for the prediction of hypertensive status.

The sample power in this study was 0.79. This means that 79% of studies would be expected to yield a significant effect, rejecting the null hypothesis that the odds are 1.0, and suggesting that this study has adequate statistical power.

The standards of association studies have improved over the years, and it is widely anticipated that such studies will contribute to the understanding of complex traits. To date,
however, only a few association studies have been replicated (10). Therefore, replication in other populations will be necessary to confirm our present observations.

SLC6A2 is located at chromosome 16q12.2. CAPNS2 (calpain small subunit 2) and FLJ31547 (unknown function) have been reported to exist adjacent to SLC6A2. Moreover, the Bardet-Biedl syndrome 2 gene has also been reported to exist at this locus (11). The distance for tight linkage disequilibrium may vary according to the chromosomal region and race, and may be beyond 50 kb (12). In this sense, it may be necessary to sequence and genotype a wider range of this chromosomal region to identify the genetic variations that truly influence blood pressure.

Acknowledgements

We would like to express our profound gratitude to Dr. Soichiro Kitamura, President of the National Cardiovascular Center, for his support of our research. We would also like to thank Dr. Otosaburo Hishikawa, Dr. Katsuyuki Kawanishi, and Mr. Shigeru Kobayashi for their continuous support of our population survey in Suita city. We also thank the members of the Satsuki-Junyukai, and Ms. Akemi Fukumoto for her excellent technical assistance.

References