Original Article

Association of the GNAS1 Gene Variant with Hypertension Is Dependent on Alcohol Consumption

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The β-adrenoceptor (β-AR)–stimulatory guanine nucleotide-binding (Gs) protein system has been shown to play important roles in the cardiovascular system. The gene encoding the β-subunit of Gs proteins (GNAS1) is a candidate genetic determinant for hypertension. Because alcohol consumption is known to affect blood pressure partly through the β-AR–Gs protein system, we examined the possible interaction between GNAS1 T393C polymorphism and drinking status in the association with hypertension in the present study. As a result, a non-significant but reasonable trend supporting the presence of an interaction was shown (p = 0.076). In line with this trend, the T393C polymorphism significantly interacted with drinking status in the association with systolic blood pressure (p = 0.028). Moreover, supporting the presence of an interaction, T allele carriers consistently had a higher probability of hypertension, higher systolic blood pressure, and higher diastolic blood pressure than CC homozygotes in non-drinkers and light drinkers. In contrast, CC homozygotes consistently had a higher probability of hypertension, higher systolic blood pressure, and higher diastolic blood pressure than T allele carriers in moderate to heavy drinkers. The present study also showed a significant interaction between the T393C polymorphism and drinking status in the association with pulse pressure (p = 0.026), reflected by a significant association between the T393C polymorphism and pulse pressure in moderate to heavy drinkers (p = 0.026). These findings may be helpful in conducting further molecular and biological studies on the relationship among the effects of alcohol, the β-AR–Gs protein system, and hypertension. (Hypertens Res 2003; 26: 439–444)

Key Words: guanine nucleotide-binding proteins, β-adrenoceptor, hypertension, polymorphism, alcohol

Introduction

Heterotrimeric guanine nucleotide-binding proteins (G proteins) couple seven transmembrane receptors to adenylyl cyclase. Each G protein is composed of three distinct subunits (α, β, and γ). Based on amino acid similarities of the α-subunits, G proteins are classified into four major classes (Gs, Gi/o, Gq/11, and G12/13) (1–3). Ubiquitously expressed Gs proteins mediate signal transduction across cell membranes. Stimulation of the Gs subfamily activates adenylyl cyclase, resulting in accumulation of the second messenger, cAMP (1–3).

The β-adrenoceptor (β-AR)–Gs protein system has been
shown to play important roles in the cardiovascular system. To date, three distinct β-AR subtypes have been identified (β1-AR, β2-AR, and β3-AR) (4–6). Signals of all three β-AR subtypes are transmitted by coupling to Gs proteins. However, in the cardiovascular system, the α-subunit of Gs proteins couples to β1-AR and β2-AR (7). The gene encoding the α-subunit of Gs proteins (GNAS1), comprising 13 exons, maps to 20q13.2–q13.3 (8).

Recently, based on several lines of biological evidence suggesting an association of the α-subunits of G proteins with hypertension (9–11), an initial study examined the association between a common silent polymorphism (T393C) in GNAS1 and hypertension (12). This study showed that the T393C polymorphism was significantly associated with hypertension. Subsequently, we also studied this association in a large Japanese population (13), resulting in replication of the results of the initial study. Additionally, in the same population, we showed that the T393C polymorphism significantly interacted with cigarette smoking in the pathogenesis of hypertension (13). Because alcohol consumption, like cigarette smoking, is known to affect blood pressure at least partly through the β-AR–Gs protein system (14–16), we speculated that the T393C polymorphism could also interact with alcohol consumption in the pathogenesis of hypertension. Because information on alcohol consumption was not available at the time of this earlier study, we were unable to examine the possible interaction between the T393C polymorphism and drinking status. However, because, more recently, such information was available in subjects included in the population, we were able to examine the possible interaction in the present study.

### Methods

#### Subjects

According to the criteria described below, 699 hypertensive subjects and 1,609 normotensive subjects were selected from among the employees of a company in the Ehime region of Japan (Table 1) (13). All subjects were Japanese. They had participated in medical check-ups 1 to 11 times (mean 6.2 times per person), and the mean values of variables in their personal health records were used in the analyses. All subjects provided their informed consent to participate, and the ethics committee of Ehime University approved the study.

#### Diagnostic Categories

Each subject was assigned to one of the blood pressure diagnostic categories defined by the following criteria. Hypertensive subjects had a previous diagnosis of hypertension and were being treated with antihypertensive medications, or their systolic/diastolic blood pressure (SBP/DBP) was ≥140/90 mmHg. Normotensive subjects had never been treated with medication for hypertension, and their SBP/DBP was <140/90 mmHg. Heavy smokers were defined as subjects smoking 20 or more cigarettes per day. Drinkers (moderate to heavy) were defined as subjects drinking 25 g of ethanol or more per day.

#### DNA Analysis

The polymerase chain reaction (PCR) was used to detect the GNAS1 T393C polymorphism (12). The sense oligonucleotide primer was 5'-CTCTAACTGACATCGGTCAATTTGTTCT-3' and the antisense primer was 5'-ACTAAGGCAACAAGTGGGCCT-3'. The PCR mixture contained 10 ng genomic DNA, 10 pmol of each primer, 250 µmol/l dNTP, 1.5 mmol/l MgCl₂, 50 mmol/l KCl, 10 mmol/l Tris-HCl, pH 8.4, and 1 U Taq DNA polymerase (Takara Shuzo Co., Ltd., Kyoto, Japan) in a final volume of 25 µl. After initial denaturation at 94°C for 5 min, the DNA was amplified by 35 PCR cycles: of denaturation at 94°C for 45 s, annealing at 55°C for 45 s, and extension at

### Table 1. Characteristics of Participants According to Hypertensive Status

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normotensive (n = 1,609)</th>
<th>Hypertensive (n = 699)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (male %)</td>
<td>86.1</td>
<td>89.0</td>
<td>NS</td>
</tr>
<tr>
<td>Age (years)</td>
<td>51.2 (8.1)</td>
<td>55.1 (6.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>22.8 (2.7)</td>
<td>24.4 (3.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>123.7 (9.7)</td>
<td>150.9 (10.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>72.0 (6.2)</td>
<td>87.6 (6.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Pulse pressure (mmHg)</td>
<td>51.7 (5.1)</td>
<td>63.2 (6.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>196.5 (31.2)</td>
<td>203.4 (32.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>60.4 (13.4)</td>
<td>60.5 (13.7)</td>
<td>NS</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>130.0 (74.0)</td>
<td>157.2 (84.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Smoking (heavy smoker %)</td>
<td>28.7</td>
<td>24.7</td>
<td>NS</td>
</tr>
<tr>
<td>Alcohol (moderate to heavy drinker %)</td>
<td>29.1</td>
<td>37.9</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are mean (SD). HDL, high density lipoprotein. NS, not significant. Blood pressure readings prior to the start of antihypertensive treatment were not available for 141 hypertensive subjects whose values were measured under treatment.

The PCR mixture contained 10 ng genomic DNA, 10 pmol of each primer, 250 µmol/l dNTP, 1.5 mmol/l MgCl₂, 50 mmol/l KCl, 10 mmol/l Tris-HCl, pH 8.4, and 1 U Taq DNA polymerase (Takara Shuzo Co., Ltd., Kyoto, Japan) in a final volume of 25 µl. After initial denaturation at 94°C for 5 min, the DNA was amplified by 35 PCR cycles: of denaturation at 94°C for 45 s, annealing at 55°C for 45 s, and extension at
72°C for 2 min, followed by final extension at 72°C for 7 min. The amplified PCR products were digested with 3 U of the restriction enzyme, FokI. The digested samples were separated by electrophoresis through an agarose gel and visualized under ultraviolet light after ethidium bromide staining. A thymine at nucleotide position 393 was shown by a fragment of 345 base pairs (bp), whereas a cytosine at nucleotide position 393 was shown by two fragments of 263 bp and 82 bp. The person who assessed the genotype was blinded to the clinical data of the subjects from whom the samples originated (13).

Statistical Methods
Analysis of variance was used to assess differences in means and variances of continuous variables. Logistic regression models were used to assess whether the GNAS1 T393C polymorphism made a statistically significant contribution to prediction of blood pressure, with consideration of interactions between the polymorphism and drinking status. P values less than 0.05 were considered statistically significant. Statistical analysis was performed with SPSS statistical software.

Results

Association of GNAS1 T393C Polymorphism with Hypertension
A total of 2,308 Japanese individuals from the Ehime region were categorized as hypertensive or normotensive and genotyped for the T393C polymorphism (Table 2). The frequencies in both hypertensive and normotensive subjects were in Hardy-Weinberg equilibrium. Logistic regression analysis showed a significant difference in the frequencies of the alleles ($p = 0.036$) and genotypes ($p = 0.046$ for TT + TC vs. CC) between the hypertensive and normotensive subjects, as shown also in our previous study (13) (Table 2).

Interaction of GNAS1 T393C Polymorphism with Alcohol Consumption in the Association with Hypertension
In the present study, we analyzed the possible interaction of the GNAS1 T393C polymorphism with drinking status in the association with hypertension in a logistic regression model.
T393C polymorphism was associated with hypertension in alcohol consumption (Table 3). This analysis revealed that the polymorphism and hypertension according to stratified alcohol consumption groups also supported this difference. Although a significant association was shown only in non-drinkers and light drinkers, subjects with the TT and TC genotypes consistently had a higher probability of hypertension in moderate to heavy drinkers (p = 0.84, OR = 1.04, 95% CI = 0.71–1.52) (Table 3).

More quantitatively, we further analyzed the interaction between the T393C polymorphism and drinking status in the association with blood pressure in general linear regression models. This analysis showed significant interactions between the T393C polymorphism and drinking status in the association with SBP (p = 0.028) and with pulse pressure (p = 0.026). The analysis also showed a non-significant trend supporting the presence of an interaction between the T393C polymorphism and drinking status in the association with DBP (p = 0.059).

Given these interactions and trends, we next analyzed the association between the T393C polymorphism and blood pressure according to stratified alcohol consumption in general linear regression models (Table 4). This analysis showed a significant association between the T393C polymorphism and pulse pressure in moderate to heavy drinkers. However, except for this association, the analysis failed to show any significant association between the T393C polymorphism and blood pressure in stratified alcohol consumption groups.

### Discussion

We previously showed a significant interaction between the \( GNAS1 \) T393C polymorphism and cigarette smoking status in the association with hypertension in a Japanese population (13). Prompted by the presence of this interaction, in the present study, we assessed the interaction between the \( GNAS1 \) T393C polymorphism and alcohol consumption in the association with hypertension in the same population. As a result, a non-significant trend supporting the presence of an interaction was shown. In line with this trend, our results also showed a significant interaction between the T393C polymorphism and drinking status in the association with SBP, and a non-significant trend supporting the presence of an interaction between the T393C polymorphism and drinking status in the association with DBP. These results suggest that the apparent effect of the T393C polymorphism differed depending on alcohol consumption.

Association analyses of the T393C polymorphism with blood pressure in stratified alcohol consumption groups also supported this difference. Although a significant association was shown only in non-drinkers and light drinkers, subjects with the TT and TC genotypes consistently had a higher probability of hypertension, higher SBP, and higher DBP than subjects with the CC genotype in this group (Tables 3 and 4). In contrast, subjects with the CC genotype consistently had a higher probability of hypertension, higher SBP, and higher DBP than subjects with the TT and TC genotypes in moderate to heavy drinkers (Tables 3 and 4).

The present study also showed a significant interaction between the \( GNAS1 \) T393C polymorphism and alcohol consumption in the association with pulse pressure, reflected by a significant association between the T393C polymorphism and pulse pressure in moderate to heavy drinkers. A wide pulse pressure resulting largely from excessive large artery stiffness is associated with systolic hypertension (17). Consistent with this established association, the present study showed a non-significant but strong trend supporting an association between the T393C polymorphism and SBP in moderate to heavy drinkers (Table 4).

### Table 4. Association of \( GNAS1 \) Genotype with Blood Pressure According to Drinking Status

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Systolic blood pressure</th>
<th>Diastolic blood pressure</th>
<th>Pulse pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-drinkers and light drinkers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT + TC (1,264)</td>
<td>130.4 (16.2)</td>
<td>75.7 (9.7)</td>
<td>54.7 (7.7)</td>
</tr>
<tr>
<td>CC (311)</td>
<td>129.1 (15.2)</td>
<td>74.7 (9.3)</td>
<td>54.4 (7.5)</td>
</tr>
<tr>
<td>p value</td>
<td>0.18</td>
<td>0.092</td>
<td>0.51</td>
</tr>
<tr>
<td>Moderate to heavy drinkers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT + TC (592)</td>
<td>135.3 (14.5)</td>
<td>79.2 (8.5)</td>
<td>56.0 (7.6)</td>
</tr>
<tr>
<td>CC (141)</td>
<td>137.8 (16.3)</td>
<td>80.2 (9.0)</td>
<td>57.6 (8.2)</td>
</tr>
<tr>
<td>p value</td>
<td>0.074</td>
<td>0.23</td>
<td>0.026</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT + TC (1,856)</td>
<td>132.0 (15.8)</td>
<td>76.8 (9.5)</td>
<td>55.1 (7.7)</td>
</tr>
<tr>
<td>CC (452)</td>
<td>131.8 (16.0)</td>
<td>76.4 (9.5)</td>
<td>55.4 (7.9)</td>
</tr>
<tr>
<td>p value</td>
<td>0.82</td>
<td>0.39</td>
<td>0.50</td>
</tr>
</tbody>
</table>

Data are mean (SD). The abbreviations are the same as Table 2.
Taking these results together, the present study suggested an interaction between the GNAS1 T393C polymorphism and alcohol consumption in the association with hypertension and with pulse pressure. Because alcohol is known to affect blood pressure through the β-AR–Gs protein system (14–16), an interaction between the T393C polymorphism and alcohol consumption in the association with hypertension seems reasonable. This interaction could be reflected by the interaction between the T393C polymorphism and alcohol consumption in the association with pulse pressure, because hypertension promotes atherosclerosis (18, 19), which results in large-vessel stiffening and increased wave reflection, and thereby amplifies pulse pressure (20). However, the precise mechanism of these interactions remains elusive. Previous studies have provided evidence that the T allele of the T393C polymorphism is associated with poor responsivity to β-blockade (12) and that the T393C polymorphism interacts with cigarette smoking status in the pathogenesis of hypertension (13). Based on this evidence, we previously speculated that the TT and TC genotypes or genotypes in linkage disequilibrium with them might produce a constant amount of the α-subunit of Gs proteins independent of activation of the sympathetic nervous system (13). In contrast, the CC genotype or genotype in linkage disequilibrium with it might produce a controlled amount of α-subunit of Gs proteins. Indeed, subjects with the CC genotype tended to be more strongly affected by alcohol consumption than subjects with the TT and TC genotypes in the association with hypertension (Tables 3 and 4). Thus, the above explanation appears also to be applicable to the interaction between the T393C polymorphism and alcohol consumption in the association with hypertension. Alternatively, depending on the genotypes, alcohol could influence glucose metabolism, which in turn could influence blood pressure (21). Indeed, GNAS1 gene knockout mice have been shown to exhibit a significant increase in insulin sensitivity (22). Data on the parameters of glucose metabolism, however, were not available in our population, preventing assessment of the association between the T393C polymorphism and glucose metabolism. Another possible explanation for the interaction between the T393C polymorphism and alcohol consumption in the association with hypertension and with pulse pressure might be that this interaction might reflect the interaction between the T393C polymorphism and cigarette smoking status. Indeed, in our population, alcohol consumption was associated with cigarette smoking status (data not shown). Moreover, the T393C polymorphism interacted significantly with cigarette smoking status in the association with hypertension (p = 0.00050) and with pulse pressure (p = 0.00067). However, considering that both cigarette smoking and alcohol consumption could affect blood pressure through the β-AR–Gs protein system, the final explanation may be less plausible than the former two.

The present study has additional limitations. Information on the history of alcohol consumption and the actual amount of alcohol drunk by subjects was not available in our population, preventing quantitative assessment of alcohol consumption. In this regard, analysis of the aldehyde dehydrogenase 2 gene may be helpful to some extent (23). Moreover, the present study did not assess gene–gene interaction, which is a candidate factor for modifying the evaluation of an association. In this context, interaction analyses of the GNAS1 gene with other genes involved in the β-AR–Gs protein system may be helpful to improve understanding of the relation between the β-AR–Gs protein system and hypertension.

References

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