Association of GNAS1 Gene Variant With Hypertension Depending on Smoking Status

Michiko Abe, Jun Nakura, Miyuki Yamamoto, Ji Jing Jin, Zhihong Wu, Yasuharu Tabara, Yoshikuni Yamamoto, Michiya Igase, Katsuhiko Kohara, Tetsuro Miki

Abstract—The β-adrenoceptor (β-AR) G protein system has been shown to have important roles in the cardiovascular system. The gene encoding the α-subunit of G proteins (GNAS1) is a candidate genetic determinant for hypertension. We studied the GNAS1 T393C polymorphism in >2000 Japanese individuals. χ² test showed a marginally significant difference in the frequencies of the alleles (P=0.036) and genotypes (P=0.094) between hypertensives and normotensives. Because hypertension is considered to be a complex disorder resulting from interactions between genetic and environmental factors, we further analyzed the T393C polymorphism, with consideration of interactions between the polymorphism and confounding factors in regression models. These analyses showed a significant interaction between the polymorphism and cigarette smoking in the pathogenesis of hypertension (P=0.0005). The interaction was reflected in a significant association of the polymorphism with hypertension in nonheavy smokers (P=0.0028; odds ratio, 1.52; 95% confidence interval, 1.16 to 2.00). A significant interaction between the polymorphism and aging in the pathogenesis of hypertension was also shown in nonheavy smokers. These findings may be helpful in conducting further molecular and biological studies on the relationship among cigarette smoking, the β-AR-G protein system, and hypertension. (Hypertension. 2002;40:261-265.)

Key Words: G proteins ▪ hypertension, essential ▪ genetics ▪ polymorphism ▪ smoking ▪ age

The β-adrenoceptor (β-AR) G protein system has been shown to have important roles in the cardiovascular system. To date, 3 distinct β-AR subtypes have been identified (β₁-AR, β₂-AR, and β₃-AR).¹⁻³ Signals of all 3 β-AR subtypes are transmitted by coupling to G proteins, resulting in activation of adenyl cyclase and accumulation of the second messenger, cAMP.¹⁻³ G proteins mediate signal transduction across cell membranes. The unique α-subunit of heterotrimeric G proteins, containing the guanine nucleotide-binding site and having intrinsic GTPase activity, confers functional specificity to the corresponding G protein. In the cardiovascular system, the α-subunit of G proteins couples β₁-AR and β₂-AR.⁴ The gene encoding the α-subunit of G proteins (GNAS1), comprising 13 exons, maps to 20q13.2-q13.3.⁵ Many studies have revealed an association between the GNAS1 gene and hypertension.⁶⁻⁶ In contrast, another study assessed the association between the GNAS1 gene and hypertension,⁹ and found a common silent polymorphism (T393C) involving a change of codon 131 from ATT (Ile) to ATC (Ile). The polymorphism was characterized by the presence (+) or absence (−) of a restriction site for FokI. The study analyzed the T393C polymorphism in 268 hypertensive and 231 normotensive whites and found a marginal association of the polymorphism with hypertension. However, association studies are often irreproducible, and the association status is occasionally different in ethnically different populations. Therefore, we analyzed the polymorphism in >2000 Japanese individuals to replicate the association between the GNAS1 gene and hypertension.

Materials and Methods

Subjects
According to the criteria described below, 699 hypertensives and 1609 normotensives were selected from a population composed of 2308 subjects working in a company in the Ehime region of Japan (Table 1). All subjects were Japanese. They had participated in medical check-ups (from spring 1995 to autumn 2001) 1 to 11 times (mean, 6.2 times per person), and the mean values of variables in their personal health records were used in analyses. All subjects gave informed consent, and the ethics committee of Ehime University approved the study.

Diagnostic Categories
Each subject was assigned to one of the blood pressure (BP) 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 BP was ≥140/90 mm Hg. Normotensive subjects had never been treated with medication for hypertension, and their systolic/diastolic
DNA Analysis
The polymerase chain reaction (PCR) was used to detect the GNAS1 T393C polymorphism. The sense oligonucleotide primer was 5'-CTC CTA ACT GAC ATG GTG CAA-3', and the antisense primer was 5'-TAA GCC CAC ACA AGT CGG GGT-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) in a final volume of 25 μL. After initial denaturation at 94°C for 5 minutes, the DNA was amplified by 35 PCR cycles: denaturation at 94°C for 45 seconds, annealing at 55°C for 45 seconds, and extension at 72°C for 2 minutes, followed by final extension at 72°C for 7 minutes. 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 is shown by 2 fragments of 263 bp and 82 bp.

Statistical Methods
ANOVA 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 hypertension, with consideration of interactions between the polymorphism and confounding factors in regression models. In our population, confounding factors were associated with one another (data not shown). Each interaction was therefore assessed separately; that is, the interaction between the T393C polymorphism and each confounding factor was entered with the polymorphism and the corresponding confounding factor in each logistic regression model, and was analyzed in the association with hypertension. Statistical analysis was performed with SPSS statistical software.

Results
Association of GNAS1 T393C Polymorphism With Hypertension
A total of 2308 Japanese individuals from the Ehime region were categorized as hypertensive or normotensive and were genotyped for the T393C polymorphism (Tables 1 and 2). The frequencies of the genotypes (P=0.0005) and the alleles (P=0.0001) in Japanese were significantly different from those in whites. The frequencies in both hypertensives and normotensives were in Hardy-Weinberg equilibrium (P=0.32 and P=0.84, respectively). χ2 test showed a marginally significant difference in the frequencies of the alleles (P=0.036) and genotypes (P=0.094) between the hypertensives and normotensives (Table 2).

Interaction of GNAS1 T393C Polymorphism With Smoking Status in Association With Hypertension
We next analyzed possible interactions of the polymorphism with confounding factors in the association with hypertension in logistic regression models, because the development of hypertension is considered to be attributable, at least in part, to gene-environmental interactions. In the analysis of interactions of the polymorphism with gender, age, body mass index, plasma total cholesterol, HDL cholesterol, and triglyceride levels, this polymorphism and the corresponding interactions were eliminated from the corresponding models assuming additive, dominant, and recessive modes of inheritance. In contrast, in the analysis of the possible interaction between the polymorphism and smoking status, both smoking status and the T393C polymorphism were eliminated from the corresponding model, and the interaction of the polymorphism with smoking status was included in the model assuming a dominant effect of the T allele (P=0.0005). The
interaction was significant, even after adjustment for gender and age (\(P=0.0034\)) and for all confounding factors (\(P=0.025\)).

Given the significant interaction, we next analyzed the association between the T393C polymorphism and hypertension in nonheavy smokers and heavy smokers separately. These analyses revealed that the T393C polymorphism was associated with hypertension in nonheavy smokers (\(P=0.0028\); odds ratio [OR], 1.52; 95% confidence interval [CI], 1.16 to 2.00), whereas the polymorphism was not associated with hypertension in heavy smokers (\(P=0.29\); OR, 0.72; 95% CI, 0.52 to 1.22) (Table 3 and Figure 1). In addition, analyses of the association between smoking status and hypertension in stratified genotypes revealed that cigarette smoking was associated with hypertension in subjects with the TT and TC genotypes (\(P=0.0051\); OR, 0.72; 95% CI, 0.58 to 0.91), whereas cigarette smoking was not associated with hypertension in subjects with the CC genotype (\(P=0.16\); OR, 1.39; 95% CI, 0.88 to 2.20) (Table 4 and Figure 1). The insignificant association in subjects with the CC genotype could be lack of statistical power.

**Interaction of GNAS1 T393C Polymorphism With Age in the Association With Hypertension in Nonheavy Smokers**

We next analyzed the interaction of the T393C polymorphism with age in the association with hypertension in logistic regression models, because a previous report showed that the T393C polymorphism was associated with hypertension in a 59-year age group, but not in 40- to 49-year and 50- to 59-year age groups. This analysis showed the presence of a significant interaction between the polymorphism and age in the association with hypertension in nonheavy smokers (\(P=1.5\times10^{-5}\)). The interaction was significant, even after adjustment for gender and age (\(P=0.0054\)) and for all confounding factors (\(P=0.022\)). In contrast, the interaction was not significant in heavy smokers (\(P=0.48\)).

Given the significant interaction, we further analyzed the association between age and hypertension in subjects with the TT and TC genotypes and in those with the CC genotype separately in nonheavy smokers. Both in subjects with the TT and TC genotypes and in those with the CC genotype, age was significantly associated with hypertension (\(P=4.5\times10^{-18}\) and \(P=0.0014\), respectively). The regression between age and the probability of having hypertension in subjects with the TT and TC genotypes was represented by the equation \(y=\exp(0.076x-4.79)/[1+\exp(0.076x-4.79)]\). The equation was \(y=\exp(0.063x-4.48)/[1+\exp(0.063x-4.48)]\) in subjects with CC genotype (Figure 2). Thus, subjects with TT and TC genotypes showed a steeper slope than did those with CC genotype (Figure 2). The \(P\) values for the association between the polymorphism and hypertension were 0.023 (OR, 1.43; 95% CI, 1.05 to 1.94) and 0.044 (OR, 2.08; 95% CI, 1.39 to 3.18) for all confounding factors.

**TABLE 3.** GNAS1 Genotype Frequency in Hypertensives and Normotensives According to Smoking Status

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Normotensive (n=699)</th>
<th>Hypertensive (n=699)</th>
<th>(P)</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonheavy smokers</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT+TC</td>
<td>899 (77%)</td>
<td>445 (85%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>249 (23%)</td>
<td>81 (15%)</td>
<td>0.0028</td>
<td>1.52</td>
<td>1.16–2.00</td>
</tr>
<tr>
<td>Heavy smokers</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT+TC</td>
<td>377 (82%)</td>
<td>135 (78%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>38 (22%)</td>
<td>84 (18%)</td>
<td>0.29</td>
<td>0.79</td>
<td>0.52–1.22</td>
</tr>
</tbody>
</table>

**TABLE 4.** Smoking Status in Hypertensives and Normotensives According to GNAS1 Genotype

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Normotensive (n=699)</th>
<th>Hypertensive (n=699)</th>
<th>(P)</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonheavy smokers</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT+TC</td>
<td>899 (70%)</td>
<td>445 (77%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>377 (30%)</td>
<td>135 (23%)</td>
<td>0.0051</td>
<td>0.72</td>
<td>0.58–0.91</td>
</tr>
<tr>
<td>Heavy smokers</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT+TC</td>
<td>249 (75%)</td>
<td>81 (68%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>84 (25%)</td>
<td>38 (32%)</td>
<td>0.16</td>
<td>1.39</td>
<td>0.88–2.20</td>
</tr>
</tbody>
</table>

**Figure 1.** Genotypic variations in relationship between smoking status and probability of having hypertension. Simple line indicates TT and TC genotypes; dotted line, CC genotype. There was a statistical significance in the difference of the slope steepness between subjects with TT and TC genotypes and those with CC genotype (\(P=0.0005\)).

**Figure 2.** Genotypic variations in positive relationship between aging and probability of having hypertension in non-smokers. Simple line indicates TT and TC genotypes; dotted line, CC genotype. There was a statistical significance in the difference of the slope steepness between subjects with TT and TC genotypes and those with CC genotype (\(P=1.5\times10^{-5}\)).
CI, 1.02 to 4.24) in ≤59-year and >59-year age groups, respectively.

Discussion

Hypertension is a common complex phenotype and has been intensively studied to identify susceptibility loci in humans. Nonetheless, there is no genotypic polymorphism consistently associated with hypertension in humans, thus far. Large-scale linkage studies of hypertension have detected several regions showing suggestive linkage but no region showing highly significant linkage according to a proposed criterion, and the suggested regions are inconsistent, indicating that the development of hypertension may not be attributable to a few strong genetic factors. Instead, many weak genetic factors may contribute to the development of hypertension directly or indirectly through interactions with genetic or environmental factors. Indeed, the results of our genome-wide linkage disequilibrium mapping of hypertension using tri- and tetranucleotide repeats suggested the presence of many hypertension-related loci with weak effects in the human genome (J.J. Jin, unpublished data, 2002). The T393C polymorphism in GNAS1 or a variant in linkage disequilibrium with it also appears to have a weak effect on the development of hypertension, because the OR was only 1.52, even in nonheavy smokers.

Although the development of hypertension is considered to be attributable at least partly to gene-gene and gene-environmental interactions, fewer interaction analyses have been conducted compared with simple association analyses. This may be attributable to the inconsistency of the associations of several candidate genes with hypertension, because, in theory, associations between polymorphisms and complex phenotypes might be masked in the presence of gene-environmental interactions, even when analyzed in subjects with matched confounding factors. In fact, although the D allele of the G1057D variant in IRS-2 was positively associated with type 2 diabetes in obese subjects and the D allele was negatively associated with type 2 diabetes in nonobese subjects, the G1057D variant was not associated with type 2 diabetes overall. Furthermore, in the case of the T393C polymorphism in GNAS1, our results indicate that the association between the polymorphism and hypertension might be missed when analyzed in heavy smokers and/or in a younger population.

Our findings suggest that the TT and TC genotypes of the T393C polymorphism have a risk-increasing effect on the development of hypertension in nonheavy smokers, and heavy smoking significantly decreases the effect. In contrast, the CC genotype has a relatively protective effect on the development of hypertension in nonheavy smokers, and heavy smoking does not modify the effect significantly. Considering that subjects with the TT and TC genotypes are predominant over those with the CC genotype both in whites and in Japanese, our findings are in line with the epidemiologic evidence that continuous smokers have lower BP than do nonsmokers, although the evidence remains controversial. The protective effect of smoking may be attributable to the phenomenon that smokers have lower body mass index than nonsmokers. However, our results do not support this hypothesis, because the T393C polymorphism appears to interact with smoking status, but not with body mass index, in the association with hypertension.

Because smoking is known to affect BP through the β-AR-G protein system, an interaction between the T393C polymorphism and smoking status in the association with hypertension may be reasonable. However, the precise mechanism of the interaction remains elusive. Given the evidence that the T allele of the T393C polymorphism is associated with poor responsiveness to β-blockade, and because the present study suggested that the polymorphism interacts with smoking status in the pathogenesis of hypertension, a simple explanation may be that the TT and TC genotypes or genotypes in linkage disequilibrium with them might produce excessive α-subunit of G proteins beyond control, leading to increased BP, and at the same time, smoking might decrease BP by an as-yet-unknown mechanism. In contrast, the CC genotype, or genotype in linkage disequilibrium with it, might produce a controlled amount of α-subunit of G proteins. If this is the case, subjects with the TT and TC genotypes may show poor responsiveness to a β-agonist, and subjects with the CC genotypes may show good responsiveness to a β-agonist. This explanation is in line with the phenomenon that BP is harder to control in hypertensive smokers and with the suggestion that following prolonged activation of the sympathetic nervous system (corresponding to continuous heavy smoking), the β1- and β2-AR-mediated responses are diminished, again considering that subjects with the TT and TC genotypes are predominant. The explanation is also in line with our finding that subjects with the CC genotype of the T393C polymorphism have a smaller increment of the probability of hypertension with aging, because a decrease in β-AR-G, protein coupling is observed with aging. According to the explanation, subjects with the TT and TC genotypes should be less affected by β-AR-G, protein uncoupling with aging. Alternatively, depending on the genotypes, smoking could influence glucose metabolism and could, in turn, influence BP. However, because the etiology of hypertension and the effects of smoking and aging are all complicated, the above explanation remains completely speculative.

The present study has several limitations. First, parameters of glucose metabolism were not available in our population. Second, information on the smoking history and on the number of cigarettes smoked by smokers was not available in our population. Third, BP readings before the start of antihypertensive medication were not available for medicated hypertensive subjects. These limitations prevented quantitative assessment of glucose metabolism, smoking, and BP. Finally, because association studies are often irreproducible and the association and interaction shown in the present study are weak, the results of this study need to be assessed in large populations. Despite these limitations, if our findings are confirmed, the findings could be helpful in conducting further molecular and biological studies on the relationship among cigarette smoking, the β-AR-G, protein system, and hypertension.

In conclusion, the present study revealed a significant interaction between the GNAS1 T393C polymorphism and...
cigarette smoking in the pathogenesis of hypertension in a large Japanese population. The interaction was reflected in a significant association of the polymorphism with hypertension in nonheavy smokers. The present study also revealed a significant interaction between the polymorphism and aging in the pathogenesis of hypertension in nonheavy smokers.

**Perspectives**

To establish an association between a polymorphism and a common disorder, replication of an initial report in large populations is indispensable. In this context, based on an initial report showing an association between the GNAS1 T393C polymorphism and hypertension in whites, we assessed the association in a large Japanese population, resulting in replicating the association. Given the replication, we further analyzed the T393C polymorphism, with consideration of interactions between the polymorphism and confounding factors, showing that the polymorphism interacts significantly with smoking in the pathogenesis of hypertension. The interaction was reflected in a significant association of the polymorphism with hypertension in nonheavy smokers. A significant interaction between the polymorphism and aging in the pathogenesis of hypertension was also shown in nonheavy smokers. Because this is the first report showing these interactions, further replication in large populations is required to establish the interactions. Moreover, haplotype studies and examinations of variants in linkage disequilibrium with the polymorphism are also needed to more exactly assess the relation between GNAS1 and hypertension. Thus, although the results of this study remain to be confirmed, this study may have a broad implication, the importance of interaction analyses. In theory, associations between polymorphisms and complex phenotypes might be masked in the presence of gene-environmental interactions, even when analyzed in subjects with matched confounding factors. Indeed, in the case of the GNAS1 T393C polymorphism, the association between the polymorphism and hypertension might be missed when analyzed in heavy smokers and/or in younger populations.

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