Original Article

Association of a GNAS1 Gene Variant with Hypertension and Diabetes Mellitus

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Previous studies have shown that the T allele of the GNAS1 T393C polymorphism is associated with poor responsiveness to β -blockade and that the T393C polymorphism interacts with cigarette smoking and alcohol consumption in the pathogenesis of hypertension. Thus, the T393C polymorphism is likely to interact with β -adrenoceptor (β -AR) stimulation in the pathogenesis of hypertension. Although this interaction might be caused by a direct effect of Gs proteins on the cardiovascular system, it could also result from an indirect effect of Gs proteins mediated by glucose metabolism. Moreover, association studies are often irreproducible. We therefore examined the possible interaction between the T393C polymorphism and γ -glutamyl transpeptidase (GGT), which is an established biomarker of alcohol consumption, in the association with glucose metabolism as well as with hypertension in a Japanese population. Genotyping for GNAS1 was performed by using the polymerase chain reaction-restriction fragment length polymorphism method in all 821 samples. The present study showed a significant interaction between the T393C polymorphism and GGT in the association with hypertension ($p = 0.033$). This interaction was even more significant after adjustment for all confounding factors ($p = 0.0025$). In contrast, analysis of the possible interaction of the T393C polymorphism with GGT in the association with diabetes mellitus or fasting plasma glucose failed to show a significant result. These results did not support the hypothesis that the interaction between the T393C polymorphism and GGT in the association with hypertension could be caused by an indirect effect of Gs proteins mediated by glucose metabolism. (*Hypertens Res* 2004; 27: 919–924)

Key Words: G proteins, glucose, hypertension, polymorphism, sympathetic nervous system

Introduction

Hypertension is considered to be a complex trait to which genetic, environmental, and demographic factors contribute interactively (1). The β -adrenoceptor (β -AR)-stimulatory guanine nucleotide-binding protein (Gs) system has been shown to play important roles in the cardiovascular system. Recently, based on several lines of biological evidence suggesting an association of the α -subunit of Gs proteins with

hypertension (2–4), an initial study examined the association between a common silent polymorphism (T393C) in GNAS1 and hypertension (5). This study showed that the T393C polymorphism was significantly associated with hypertension and with poor responsiveness to β -blockade. Subsequently, we also studied the association in a large Japanese population, resulting in replication of the association between the T393C polymorphism and hypertension (6). Additionally, in the same population, we showed that the T393C polymorphism interacted with cigarette smoking and with al-

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Table 1. Characteristics of Participants According to Hypertension Status

Variable	Normotensive (n = 554)	Hypertensive (n = 267)	p value
Sex (male %)	78.3	89.0	<0.001
Age (years)	52.8 ± 8.6	57.4 ± 8.4	<0.001
BMI (kg/m ²)	22.6 ± 2.9	23.9 ± 2.9	<0.001
SBP (mmHg)	115.1 ± 11.6	143.9 ± 16.6	<0.001
DBP (mmHg)	72.2 ± 8.9	88.9 ± 9.9	<0.001
T-Cho (mg/dl)	197.7 ± 31.2	202.9 ± 37.1	NS
HDL-Cho (mg/dl)	54.2 ± 14.6	52.1 ± 14.1	NS
TG (mg/dl)	116.3 ± 80.9	151.6 ± 129.2	<0.001
FPG (mg/dl)	101.2 ± 17.0	105.7 ± 18.5	<0.001
AST (U/I)	23.3 ± 26.0	25.1 ± 14.3	NS
ALT (U/I)	20.9 ± 32.8	21.4 ± 14.9	NS
GGT (U/I)	31.3 ± 34.8	43.3 ± 45.4	NS

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; T-Cho, plasma total cholesterol; HDL-Cho, plasma high density lipoprotein cholesterol; TG, plasma triglyceride; FPG, fasting plasma glucose; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, γ -glutamyl transpeptidase. Data are mean $\pm$ SD. Blood pressure readings prior to the start of antihypertensive medication were not available for 113 hypertensive subjects whose values were measured under treatment.

cohol consumption in the pathogenesis of hypertension (6, 7). Taken together, these results indicate that the T393C polymorphism is likely to interact with β -AR stimulation in the pathogenesis of hypertension. Although this interaction could be caused by a direct effect of Gs proteins on the cardiovascular system, it could also result from an indirect effect of Gs proteins mediated by glucose metabolism. However, we were unable to assess these two possibilities in the population previously analyzed due to a lack of information on glucose metabolism. In this regard, information on glucose metabolism as well as a well-established biomarker of alcohol consumption, γ -glutamyl transpeptidase (GGT) (8–11), was available in another population. Moreover, association studies are often irreproducible. We therefore examined the possible interaction between the T393C polymorphism and GGT in the association with glucose metabolism as well as with hypertension.

Methods

Subjects

According to the criteria described below, 267 hypertensive subjects and 554 normotensive subjects were selected from a population in the Hyogo region of Japan (Table 1). All subjects were Japanese. They had participated in a medical check-up, and the values of variables in their personal health

records were used in the analyses. All subjects provided 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 medication, or their systolic/diastolic blood pressure (SBP/DBP) was $\geq 140/90$ mmHg. Normotensive subjects had never been treated with medication for hypertension, and their SBP/DBP was $< 140/90$ mmHg. Diabetic subjects were diagnosed according to the WHO98 definition of type 2 diabetes (12). Subjects were considered to have diabetes mellitus if their fasting plasma glucose (FPG) concentration was ≥ 126 mg/dl.

DNA Analysis

Polymerase chain reaction (PCR) was used to detect the GNAS1 T393C polymorphism (5). The sense oligonucleotide primer was 5'-CTCCTAACTGACATGGTGCAA-3' and the antisense primer was 5'-TAAGGCCACACAAGTCGGGGT-3'. The amplified PCR products were digested with 3 U of the restriction enzyme, FokI. A thymine at nucleotide position 393 was shown by a fragment of 345 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.

Statistical Methods

Analysis of variance was used to assess differences in the 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 or diabetes mellitus, with consideration of the interactions between the T393C polymorphism and GGT. Sex, age, body mass index, plasma total cholesterol, high-density lipoprotein-cholesterol, and triglyceride levels were considered to be confounding factors (Table 1). Because the distributions of plasma triglyceride and GGT values were skewed, their logarithmically transformed values were used in the analyses. General linear regression models were used to assess whether the T393C polymorphism made a statistically significant contribution to prediction of blood pressure or of FPG, with consideration of interactions between the polymorphism and GGT. *p* values less than 0.05 were considered statistically significant. Statistical analysis was performed with SPSS statistical software.

Table 2. GNAS1 Genotype and Allele Frequencies in Hypertensive and Normotensive Subjects

Genotype	Genotype frequency		<i>p</i> value [*]	OR [*]	95% CI [*]
	Normotensive (<i>n</i> = 554)	Hypertensive (<i>n</i> = 267)			
GNAS1 genotypes					
TT (%)	187 (33.8)	87 (32.6)	0.954 [*]	1.01 [*]	0.70–1.45 [*]
TC (%)	254 (45.8)	126 (47.2)			
CC (%)	113 (20.4)	54 (20.2)			
GNAS1 alleles					
T (%)	628 (56.7)	300 (56.2)	0.849	0.98	0.79–1.21
C (%)	480 (43.3)	234 (43.8)			

* *p* value, OR and 95% CI are for TT + TC vs. CC. OR, odds ratio; CI, confidence interval.

Fig. 1. Genotypic variations in the positive relationship between GGT and the probability of having hypertension. The solid line indicates the TT and TC genotypes; the dotted line indicates the CC genotype. The regression between GGT and the probability of having hypertension in T carriers was represented by the equation, $Y = \exp(0.006X - 0.946) / [1 + \exp(0.006X - 0.946)]$. The equation was $Y = \exp(0.020X - 1.450) / [1 + \exp(0.020X - 1.450)]$ in CC homozygotes. CC homozygotes show a steeper slope than T carriers ($p = 0.033$; after adjustment for all confounding factors, $p = 0.0025$).

Results

Association of GNAS1 T393C Polymorphism with Hypertension

A total of 821 Japanese individuals from the Hyogo 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. Statistical analysis failed to show a significant difference in the frequencies of the alleles ($p = 0.954$) and genotypes ($p = 0.849$ for TT + TC vs. CC) between the hypertensive and normotensive subjects (Table

2). Moreover, there was no significant difference in GGT and FPG between the genotypes (TT + TC vs. CC) (data not shown).

Interaction of GNAS1 T393C Polymorphism with GGT in Association with Hypertension and Diabetes Mellitus

We next analyzed the possible interaction of the GNAS1 T393C polymorphism with GGT in the association with hypertension in a logistic regression model. This analysis showed a significant interaction ($p = 0.033$) (Fig. 1). This interaction was even more significant after adjustment for all confounding factors ($p = 0.0025$). Because, all nine of the subjects with a GGT level above 150 U/l of GGT had the T allele(s), and despite the fact that the T393C polymorphism was not associated with GGT ($p = 0.686$), we also examined this interaction using GGT stratified by quartiles (13, 22, and 42 U/l). The results showed that the *p* value for the interaction was 0.034. The *p* value was 0.0013 after adjustment for all confounding factors. In contrast, analysis of the possible interaction of the T393C polymorphism with GGT in the association with diabetes mellitus failed to show a significant relation ($p = 0.492$).

We further analyzed the interaction between the T393C polymorphism and GGT in the association with blood pressure in general linear regression models (Table 3 and Fig. 2). This analysis showed a marginally significant interaction between the T393C polymorphism and GGT in the association with DBP ($p = 0.049$). This interaction was more significant after adjustment for all confounding factors ($p = 0.0027$). The analysis also showed a non-significant trend supporting the presence of an interaction between the T393C polymorphism and GGT in the association with SBP ($p = 0.198$). However, this interaction was significant after adjustment for all confounding factors ($p = 0.011$). Analyses of these interactions using GGT stratified by quartiles showed that the *p* values for these interactions were 0.039 and 0.183 for DBP and SBP, respectively. The *p* values were 0.0011 for DBP and 0.0041 for SBP after adjustment for all confounding factors. In contrast, analysis of the possible interaction of the

Table 3. General Linear Model for Regression of GGT in Association with Blood Pressure and FPG According to Genotype

Phenotype	Genotype (n)	Coefficient	Constant	p value for regression	Determination coefficient	p value for interaction
SBP (mmHg)	TT + TC (654)	0.05	123.0	0.037	0.027	0.198
	CC (167)	0.10	119.6	0.006	0.077	
DBP (mmHg)	TT + TC (654)	0.03	76.5	0.003	0.034	0.049
	CC (167)	0.09	74.1	0.003	0.122	
FPG (mg/dl)	TT + TC (654)	5.59	85.0	<0.001	0.065	0.998
	CC (167)	5.58	84.0	<0.001	0.071	

GGT, γ -glutamyl transpeptidase; FPG, fasting plasma glucose; SBP, systolic blood pressure; DBP, diastolic blood pressure.

Fig. 2. Genotype-specific regression slopes of blood pressure on GGT. The solid line indicates the TT and TC genotypes; the dotted line indicates the CC genotype. a: The regression between GGT and SBP in T carriers was represented by the equation, $Y = 0.101X + 119.6$. The equation was $Y = 0.049X + 123.0$ in CC homozygotes. CC homozygotes showed a steeper slope than T carriers ($p = 0.198$; after adjustment for all confounding factors, $p = 0.011$). b: The regression between GGT and DBP in T carriers was represented by the equation, $Y = 0.090X + 74.1$. The equation was $Y = 0.034X + 76.5$ in CC homozygotes. CC homozygotes showed a steeper slope than T carriers ($p = 0.049$; after adjustment for all confounding factors, $p = 0.0027$).

T393C polymorphism with GGT in the association with FPG failed to show a significant result ($p = 0.998$) (Table 3).

Discussion

The present study showed a significant interaction of the GNAS1 T393C polymorphism with GGT in the association with hypertension in a Japanese population. In subjects with lower GGT, the CC genotype appeared to have a protective effect against the development of hypertension, whereas the TT and TC genotypes appeared to have a risk-increasing effect (Figs. 1 and 2). However, CC homozygotes were more sensitive to an increment of GGT than T carriers in the association with hypertension. Consequently, in subjects with higher GGT, the TT and TC genotypes appeared to have a protective effect against the development of hypertension, whereas the CC genotype appeared to have a risk-increasing effect. These relations and directions were very similar to

those in the interaction of the T393C polymorphism with cigarette smoking and alcohol consumption in the association with hypertension (6, 7). Given that both cigarette smoking and alcohol consumption could affect blood pressure through the β -AR-Gs protein system (13–16), and because GGT is a well-established biomarker of alcohol consumption (8–11), the significant interactions of the T393C polymorphism with cigarette smoking, alcohol consumption, and GGT in the association with hypertension may each indicate the presence of an interaction between the T393C polymorphism and β -AR stimulation in the pathogenesis of hypertension from different angles. Indeed, the T393C polymorphism has been shown to be associated with blood pressure response to β -blockers in a Caucasian population (5). However, more accurate assessment will require additional information on drinking history.

The present study failed to show a significant association between the T393C polymorphism and hypertension. How-

ever, given the above interactions, this failure may not be surprising, because an association between a polymorphism and hypertension could be masked in the presence of gene-environmental interactions even when analyzed in subjects with matched confounding factors (6). In this context, the significant association shown in the population previously analyzed was largely dependent on the fact that hypertensive subjects had a significantly higher frequency of the TT and TC genotypes than normotensive subjects both in non-heavy smokers and in non-drinkers or light drinkers. In the present population, a possibly lower β -AR stimulation might have resulted in failure to show a significant association between the T393C polymorphism and hypertension, although the extent of β -AR stimulation is difficult to estimate.

The precise mechanism of the interaction between the T393C polymorphism and β -AR stimulation in the pathogenesis of hypertension is elusive and remains to be investigated. Previous studies have shown that the T allele of the T393C polymorphism is associated with poor responsiveness to β -blockade (5) and that the T393C polymorphism interacts with cigarette smoking and alcohol consumption in the pathogenesis of hypertension (6, 7). 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 α -subunit of Gs proteins independent of activation of the sympathetic nervous system (6). In contrast, the CC genotype or genotype in linkage disequilibrium with it might produce a controlled amount of α -subunit of Gs proteins. In this context, it is noteworthy that CC homozygotes tended to be more strongly affected by an increment of GGT than T carriers in the association with hypertension (Figs. 1 and 2). Thus, the above explanation appears also to be applicable to the interaction between the T393C polymorphism and GGT in the association with hypertension.

Another point of view in regard to the interaction between the T393C polymorphism and β -AR stimulation in the association with hypertension is that, depending on the genotype, β -AR stimulation could influence glucose metabolism, which in turn could influence blood pressure (6). In order to assess this possibility, we examined the possible interaction between the T393C polymorphism and GGT in the association with diabetes mellitus, and failed to show a significant interaction. Analysis of the possible interaction between the T393C polymorphism and GGT in the association with FPG also failed to show a significant interaction. These results appeared to contribute evidence against the hypothesis that the interaction between the T393C polymorphism and GGT in the association with hypertension could be caused by an indirect effect of Gs proteins mediated by glucose metabolism. Further extending this, the interaction between the T393C polymorphism and β -AR stimulation in the association with hypertension might not result from an indirect effect of Gs proteins mediated by glucose metabolism. However, because data on insulin levels, HbA1c, and oral glucose tolerance test

were not available in the present population, a further study including such data should be performed.

Taking advantage of the fact that GGT is a good biomarker of alcohol consumption, the present study provided an additional piece of evidence supporting the presence of an interaction between the GNAS1 T393C polymorphism and β -AR stimulation in the pathogenesis of hypertension. Moreover, by analyzing the possible interaction between the T393C polymorphism and GGT in the association with diabetes mellitus and with FPG, the present study provided evidence against the possibility that the interaction may be caused by an indirect effect of Gs proteins mediated by glucose metabolism. However, 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. Furthermore, in order to establish an association, it is also important that associated alleles affect the gene product in a physiologically meaningful way. In this context, thus far, there is no evidence showing that the T393C polymorphism affects the gene product in a physiologically meaningful way. Therefore, it is necessary to investigate the possible biological change of the gene product by the T393C polymorphism or another variant in linkage disequilibrium with it.

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