Original Article

Interaction between Serotonin 2A Receptor and Endothelin-1 Variants in Association with Hypertension in Japanese

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Serotonin has been implicated in the pathogenesis of hypertension because of its ability to induce vasoconstriction *via* stimulation of serotonin 2 (5-HT2) receptors. Recently, an association between the T102C functional polymorphism of the serotonin 2A (5-HT2A) receptor gene and hypertension in the UK has been reported. Another association study, however, failed to replicate this association in a Chinese population. We therefore investigated the possible association between the 5-HT2A T102C polymorphism and hypertension in two large Japanese populations (n=2,968 total). We also investigated the possible interaction between the 5-HT2A T102C polymorphism and the G/T (Lys198Asn) polymorphism of the endothelin-1 (ET-1) gene, based on robust biological evidence for the existence of an interaction between the serotonin and endothelin systems. The results showed that there was no significant difference in the frequencies of the alleles and genotypes between the hypertensive and normotensive subjects. However, a significant interaction between the 5-HT2A T102C and ET-1 G/T polymorphisms in their association with hypertension (p=0.0040) and with diastolic blood pressure (p=0.0013) was revealed. A marginally significant interaction in the association with systolic blood pressure was also shown (p=0.045). The associations of the 5-HT2A T102C polymorphism with hypertension and diastolic blood pressure in ET-1 T allele carriers were significant (p=0.0056 and 0.021, respectively). The association of the 5-HT2A T102C polymorphism with systolic blood pressure in ET-1 T allele carriers was marginally significant (p=0.054). Thus, the present study suggests that the 5-HT2A T102C and ET-1 G/T polymorphisms are interactively associated with hypertension. (*Hypertens Res* 2006; 29: 227–232)

***Key Words*: serotonin receptor, endothelin, hypertension, genetics, polymorphism**

Introduction

Serotonin (5-hydroxytryptamine; 5-HT) is a naturally occurring vasoactive monoamine and is widely distributed in the human organism (*1*). Serotonin executes diverse cardiophysiological actions, which are mediated by different subtypes of serotonin receptors. Currently, serotonin receptors are divided into seven groups (5-HT1–7). Among these groups, 5-HT2 receptors mediate the vasoconstrictive actions of serotonin, and these are further categorized into three subtypes (A, B, and C). Among these three subtypes, the 5-HT2A

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Table 1. Characteristics of Participants According to Hypertension Status

Variable	Population 1		Population 2	
	Normotensive (n=1,364)	Hypertensive (n=852)	Normotensive (n=502)	Hypertensive (n=250)
Sex (male %)	84.8	89.4*	77.1	78.2
Age (years)	49.5±9.0	53.8±6.6*	52.4±8.8	57.2±8.3*
Body mass index (kg/m^2)	22.6±2.8	24.2±3.2*	22.5±2.8	23.8±2.7*
Systolic blood pressure (mmHg)	122.7±10.7	148.7±12.7*	112.4±10.6	143.4±17.0*
Diastolic blood pressure (mmHg)	72.3±7.3	87.3±8.4*	72.1±8.9	89.4±9.3*
Total cholesterol (mg/dl)	195.4±32.1	203.2±31.6*	198.5±30.9	201.8±36.3
High density lipoprotein cholesterol (mg/dl)	60.8±13.3	60.8±13.2	54.1±14.7	52.1±14.8
Triglyceride (mg/dl)	127.0±75.9	155.3±85.4*	107.9±76.3	137.3±126.8*

Data are mean±SD. *$p<0.05$ *vs*. normotensives. Blood pressure readings before the start of antihypertensive medication were not available for 705 hypertensive subjects whose values were measured under treatment.

receptor is the primary receptor mediating vasoconstriction under conditions of normal blood pressure (*2*). Thus, the 5-HT2A receptor may play an important role in the regulation of blood pressure.

The 5-HT2A receptor gene is located on chromosome 13. Given the biological evidence for a relation between the 5-HT2A receptor and blood pressure, it is important to evaluate how variations in the 5-HT2A receptor gene are associated with blood pressure as genetic factors. In this context, a functional polymorphism (T102C) of the 5-HT2A receptor gene has been investigated in relation to hypertension. An initial study showed that increased frequency of the 102C allele was significantly associated with hypertension in female UK residents (*3*). Another association study, however, failed to show a significant association between the 5-HT2A T102C polymorphism and hypertension in a Chinese population (*4*).

Generally, inconsistent associations could result from various factors, including racial difference, insufficient statistical power, and interactions of polymorphisms with other genetic and environmental factors (*5*). In this context, it may be of significance that the serotonin system has been shown to biologically interact with the endothelin system (*6–14*). This interaction could modify the association between the 5-HT2A T102C polymorphism and hypertension. However, whether genetic interactions between polymorphisms corresponding to the biological interaction significantly influence blood pressure in the general population remains to be assessed. We therefore analyzed the association between the 5-HT2A T102C polymorphism and hypertension in two large Japanese populations, with consideration of the interaction between the 5-HT2A T102C polymorphism and the G/T (Lys198Asn) polymorphism in exon 5 of the endothelin-1 (ET-1) gene, because the ET-1 system (*15*), especially the ET-1 G/T polymorphism (*16*), has been shown to be involved in the development of hypertension.

Methods

Subjects

The clinical characteristics of the subjects included in the study are shown in Table 1. Population 1 (n=2,216) originated from the Ehime region of Japan, and population 2 (n=752) from the Hyogo region of Japan (*17*). All subjects were Japanese urban residents. Subjects in population 1 participated in medical check-ups 1–11 times (average 6.2 times per person), and the mean values of variables in their personal health records were used in the analyses. Subjects in population 2 also underwent a medical check-up, and the values of variables in their personal health records were used in the analyses. All subjects provided informed consent for participation in the molecular-genetic studies. 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 >140/90 mmHg. Normotensive subjects had never been treated with medication for hypertension, and their SBP/DBP was <140/90 mmHg. Blood pressure was measured in the sitting position with the use of a standard sphygmomanometer during medical check-ups.

DNA Analysis

The TaqMan chemical method, which is an established and frequently used method (*18–21*), was used to detect the 5-HT2A T102C polymorphism. The forward primer was 5′-AAATGATGACACCAGGCTCTACAGT-3′, the reverse

Table 2. 5-HT2A Genotype and Allele Frequencies in Hypertensive and Normotensive Subjects

Genotype and allele	Population 1			Population 2			Populations 1 and 2		
	Normo-tensive	Hyper-tensive	*p* value	Normo-tensive	Hyper-tensive	*p* value	Normo-tensive	Hyper-tensive	*p* value
5-HT2A genotypes (*n* (%))									
CC	344 (25.2)	230 (27.0)		123 (24.5)	68 (27.2)		467 (25.0)	298 (27.0)	
CT	645 (47.3)	409 (48.0)		254 (50.6)	108 (43.2)		899 (48.2)	517 (46.9)	
TT	375 (27.5)	213 (25.0)	0.38	125 (24.9)	74 (29.6)	0.15	500 (26.8)	287 (26.0)	0.48
5-HT2A alleles (*n* (%))									
C	1,333 (48.9)	869 (51.0)		500 (49.8)	244 (48.8)		1,833 (49.1)	1,113 (50.5)	
T	1,395 (51.1)	835 (49.0)	0.17	504 (50.2)	256 (51.2)	0.71	1,899 (50.9)	1,091 (49.5)	0.30

5-HT2A, serotonin 2A.

primer was 5′-TGTCCAGTTAAATGCATCAGAAGTG-3′, the T-allele specific probe was 5′-FAM-AACTCTGGAGAA GCT-MGB-3′, and the C-allele specific probe was 5′-VIC-AACTCCGGAGAAGC-MGB-3′. The person who assessed the genotype was blinded to the clinical data of the subjects from whom the samples originated. The ET-1 G/T polymorphism was previously determined in our populations (*17*).

Statistical Methods

Comparisons of categorical variables were performed using the χ^2 test. Analysis of variance was used to assess differences in the means and variances of continuous variables. Because of a skewed distribution of data, logarithmically transformed plasma triglyceride values (TG) were used in the analysis. Logistic regression models were used to assess whether the 5-HT2A T102C polymorphism made a statistically significant contribution to the prediction of hypertension, with consideration of the interaction between the 5-HT2A T102C and ET-1 G/T polymorphisms. General linear models were used to assess whether the 5-HT2A T102C polymorphism made a statistically significant contribution to the prediction of blood pressure, with consideration of the interaction between the 5-HT2A T102C and ET-1 G/T polymorphisms. *p* values less than 0.05 were considered statistically significant. Statistical analysis was performed with SPSS statistical software.

Results

Association of the 5-HT2A T102C Polymorphism with Hypertension

Table 1 presents the clinical characteristics of the participants in populations 1 and 2. In population 1, the relative frequencies of the CC, CT and TT genotypes were 25.9%, 47.6% and 26.5%, respectively. In population 2, the relative frequencies of the CC, CT and TT genotypes were 25.4%, 48.1% and 26.5%, respectively. In population 1, the allele frequencies were 49.7% and 50.3% for the C and T alleles, respectively. In population 2, the allele frequencies were 49.5% and 50.5% for the C and T alleles, respectively. These results are consistent with the Hardy-Weinberg equilibrium. There was no significant difference in the frequencies of the alleles ($p=0.17$) and genotypes ($p=0.38$) between the hypertensive and normotensive subjects in population 1 (Table 2). There was no significant difference in the frequencies of the alleles ($p=0.71$) and genotypes ($p=0.15$) between the hypertensive and normotensive subjects in population 2. Finally, there was no significant difference in the frequencies of the alleles ($p=0.30$) and genotypes ($p=0.48$) between the hypertensive and normotensive subjects in the combined group of populations 1 and 2.

Interaction between the 5-HT2A T102C and ET-1 G/T Polymorphisms in Association with Hypertension

We next analyzed the interaction between the 5-HT2A T102C and ET-1 G/T polymorphisms in their association with hypertension. This analysis showed a significant interaction in population 1 ($p=0.012$, odds ratio [OR]=0.74, 95% confidence interval [95% CI]=0.58–0.94) and failed to show a significant interaction in population 2 ($p=0.14$, OR=0.73, 95% CI=0.48–1.11). Finally, analysis combining populations 1 and 2 yielded a lower *p* value of 0.0040 (OR=0.74, 95% CI=0.60–0.91) for the interaction between the 5-HT2A T102C and ET-1 G/T polymorphisms in their association with hypertension. The interaction was also significant after adjustment for sex and age ($p=0.0022$), and for sex, age, body mass index (BMI), plasma total cholesterol, high density lipoprotein (HDL)–cholesterol, and TG ($p=0.050$). Table 3 shows the opposite directions of the association of the 5-HT2A T102C polymorphism with hypertension between the ET-1 genotypes. The association of the 5-HT2A T102C polymorphism with hypertension in ET-1 T allele carriers was significant ($p=0.0056$). The association of the ET-1 G/T polymorphism with hypertension also showed opposite directions between the 5-HT2A genotypes (Table 4). The association of the ET-1 G/T polymorphism with hypertension in 5-HT2A CC homozygotes was significant ($p=0.028$). The

Table 3. 5-HT2A Genotype Frequency in Hypertensives and Normotensives According to ET-1 Genotype

ET-1 genotype	5-HT2A genotype	Genotype frequency (*n* (%))		*p* value	OR	95% CI
		Normotensive (*n*=1,866)	Hypertensive (*n*=1,102)			
GG	CC	256 (26.4)	139 (24.7)			
	CT	460 (47.4)	258 (45.8)			
	TT	255 (26.3)	166 (29.5)	0.20	1.10	0.95–1.26
GT+TT	CC	211 (23.6)	159 (29.5)			
	CT	439 (49.1)	259 (48.1)			
	TT	245 (27.4)	121 (22.4)	0.0056	0.81	0.70–0.94

ET-1, endothelin-1; 5-HT2A, serotonin 2A; OR, odds ratio; CI, confidence interval.

Table 4. ET-1 Genotype Frequency in Hypertensives and Normotensives According to 5-HT2A Genotype

5-HT2A genotype	ET-1 genotype	Genotype frequency (*n* (%))		*p* value	OR	95% CI
		Normotensive (*n*=1,866)	Hypertensive (*n*=1,102)			
CC	GG	256 (54.8)	139 (46.6)			
	GT+TT	211 (45.2)	159 (53.4)	0.028	1.39	1.04–1.86
CT	GG	460 (51.2)	258 (49.9)			
	GT+TT	439 (48.8)	259 (50.1)	0.65	1.05	0.85–1.31
TT	GG	255 (51.0)	166 (57.8)			
	GT+TT	245 (49.0)	121 (42.2)	0.064	0.76	0.57–1.02

ET-1, endothelin-1; 5-HT2A, serotonin 2A; OR, odds ratio; CI, confidence interval.

association of the ET-1 G/T polymorphism with hypertension in 5-HT2A TT homozygotes was also marginally significant (p=0.064).

Interaction between the 5-HT2A T102C and ET-1 G/T Polymorphisms in Association with Blood Pressure

Given the marginally significant interaction between the 5-HT2A T102C and ET-1 G/T polymorphisms in their association with hypertension, we next analyzed the possible interactions between these polymorphisms in their association with blood pressure in the combined group of populations 1 and 2. This analysis showed a marginally significant interaction between the 5-HT2A T102C and ET-1 G/T polymorphisms in their association with SBP (p=0.045). This interaction was also marginally significant after adjustment for sex and age (p=0.045), and for sex, age, BMI, plasma total cholesterol, HDL-cholesterol, and TG (p=0.058). Moreover, there was a significant interaction between the 5-HT2A T102C and ET-1 G/T polymorphisms in their association with DBP (p=0.0013). This interaction was also significant after adjustment for sex and age (p=0.0018), and for sex, age, BMI, plasma total cholesterol, HDL-cholesterol, and TG (p=0.0023). Table 5 shows the opposite directions of the association of the 5-HT2A T102C polymorphism with blood pressure between the ET-1 genotypes. The association of the 5-HT2A T102C polymorphism with SBP in ET-1 T allele carriers was marginally significant (p=0.054), and the association of the 5-HT2A T102C polymorphism with DBP in ET-1 T allele carriers was significant (p=0.021). Table 6 again shows the opposite directions of the association of the ET-1 G/T polymorphism with blood pressure between the 5-HT2A genotypes. The association of the ET-1 G/T polymorphism with DBP in 5-HT2A CC homozygotes was significant (p=0.0013).

Discussion

Given the biological evidence for a relation of the 5-HT2A receptor to blood pressure, a functional polymorphism (T102C) of the 5-HT2A receptor gene has been investigated in relation to hypertension. An initial study showed that increased frequency of the 102C allele was significantly associated with hypertension in female UK residents (*3*). A subsequent study failed to show a significant association between the 5-HT2A T102C polymorphism and hypertension in a Chinese population (*4*). Consistent with the results of the latter study, the present study failed to show a significant association, although increased frequency of the 102C allele was non-significantly associated with hypertension in the combined group of populations 1 and 2, in line with the results of the former study.

This failure could be attributable to racial difference. How-

Table 5. Blood Pressure for 5-HT2A Genotype According to ET-1 Genotype

BP	ET-1 genotype	5-HT2A genotype			*p* value	
		CC	CT	TT	For regression	For interaction
SBP (mmHg)	GG	129.9±18.2	129.3±19.1	130.4±17.7	0.61	
	GT+TT	132.0±18.0	130.3±18.1	128.8±17.9	0.054	0.045
DBP (mmHg)	GG	77.4±10.6	77.7±11.7	78.5±10.6	0.33	
	GT+TT	79.9±11.2	77.6±10.9	77.4±10.7	0.021	0.0013

Data are mean±SD. 5-HT2A, serotonin 2A; ET-1, endothelin-1; BP, blood pressure; SBP, systolic BP; DBP, diastolic BP.

Table 6. Blood Pressure for ET-1 Genotype According to 5-HT2A Genotype

BP	5-HT2A genotype	ET-1 genotype		*p* value	
		GG	GT+TT	For regression	For interaction
SBP (mmHg)	CC	129.9±18.2	132.0±18.0	0.11	
	CT	129.3±19.1	130.3±18.1	0.29	
	TT	130.4±17.7	128.8±17.9	0.21	0.045
DBP (mmHg)	CC	77.4±10.6	79.9±11.2	0.0019	
	CT	77.7±11.7	77.6±10.9	0.98	
	TT	78.5±10.6	77.4±10.7	0.14	0.0013

Data are mean±SD. ET-1, endothelin-1; 5-HT2A, serotonin 2A; BP, blood pressure; SBP, systolic BP; DBP, diastolic BP.

ever, genetic effects are usually consistent across human populations (*22*). Therefore, the failure might be rather attributable to gene–environmental and/or gene–gene interactions, because such interactions could modify or mask associations. In this respect, the present study revealed a statistically significant interaction between the 5-HT2A T102C and ET-1 G/T polymorphisms in their association with hypertension. Consequently, increased frequency of the 5-HT2A 102C allele is significantly associated with hypertension in ET-1 T allele carriers, consistent with the initial study (*3*). However, it should be noted that this interaction was significant in population 1 and in the combined group of populations 1 and 2, but not in population 2, despite the fact that the OR for the interaction were very similar between the two populations. This implies that studies with modest sample sizes can fail to detect interactions, and a combination of samples will be required to achieve adequate statistical power.

Moreover, the present study showed a significant interaction between the 5-HT2A T102C and ET-1 G/T polymorphisms in their association with blood pressure. In particular, the interaction between the 5-HT2A T102C and ET-1 G/T polymorphisms in their association with DBP was significant in both populations 1 and 2 (data not shown), constituting strong evidence in favor of the existence of this interaction.

A genetic interaction between the 5-HT2A T102C and ET-1 G/T polymorphisms is also supported by persistent biological evidence for the existence of interactions between the serotonin and endothelin systems. For example, subthreshold concentrations of ET-1 amplify the vasoconstrictor effect of serotonin in human arteries (*6*, *7*, *9*, *10*) and in the guinea pig trachea (*11*). Preincubation of a platelet suspension with ET-1 has been shown to inhibit the serotonin-mediated platelet response (*8*). ET-1 has been shown to inhibit serotonergic amplification of epinephrine-induced aggregation of platelets (*8*). Pre-treatment of rabbit platelets with ET-1 has been shown to enhance serotonin-promoted protein tyrosine phosphorylation (*12*). In the rabbit platelet membrane, ET-1 has been shown to enhance serotonin binding and inhibit its internalization (*12*). On the other hand, serotonin also potentiates ET-1–induced vascular smooth muscle cell proliferation (*13*).

The T102C polymorphism is located in the coding sequence in exon 1 of the 5-HT2A receptor gene and does not change any amino acid, and thus it is a silent polymorphism in that both nucleotides result in a codon that encodes Ser at amino acid position 34 (*23*). Nevertheless, the T102C polymorphism results in a differential gene expression (*24*). This functionality of the 5-HT2A T102C polymorphism also increases the plausibility of a genetic interaction between the 5-HT2A T102C and ET-1 G/T polymorphisms.

In conclusion, the present study revealed a significant interaction between the 5-HT2A T102C and ET-1 G/T polymorphisms in the pathogenesis of hypertension in two large Japanese populations. This interaction was supported by several lines of molecular biological evidence. Nevertheless, association studies are often irreproducible, warranting further studies in large populations to investigate the interactions between the serotonin and endothelin systems, with consideration of various gene–environment and gene–gene interactions.

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