*Original Article*

# Association of Dopamine β-Hydroxylase Polymorphism with Hypertension through Interaction with Fasting Plasma Glucose in Japanese

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**Dopamine-β-hydroxylase (DBH) catalyzes the conversion of dopamine to norepinephrine and is released from sympathetic neurons into the circulation. Several lines of evidence, including the finding of elevated plasma DBH activity in essential hypertension, suggest an important role of DBH in hypertension. Recently, a novel polymorphism (−1021C/T) in the 5′ flanking region of the DBH gene has been shown to account for 35–52% of the variation in plasma DBH activity. We therefore investigated the possible association between the DBH −1021C/T polymorphism and hypertension in a large Japanese population. Moreover, because the development of hypertension is considered to be due at least partly to gene-environmental interactions, we also investigated the possible interactions between the DBH −1021C/T polymorphism and environmental factors. Consequently, we found a significant interaction between the DBH −1021C/T polymorphism and fasting plasma glucose (FPG) in the association with hypertension. CC homozygotes showed a steeper increase in probability of hypertension with FPG than T allele carriers. We also found a marginally significant trend suggesting the presence of an interaction between the DBH −1021C/T polymorphism and FPG in the association with blood pressure. Consistent with the presence of the interaction, we found that a 19 bp sequence containing the DBH −1021C/T polymorphism includes two palindromic non-canonical E boxes separated by 5 bps, and closely resembles the glucose response element of the L-type pyruvate kinase gene. These findings could be helpful in conducting further molecular and biological studies on the relationship among glucose metabolism, the sympathetic nervous system, and hypertension. (*Hypertens Res* 2005; 28: 215–221)**

***Key Words*: dopamine-β-hydroxylase, essential hypertension, genetics, polymorphism, glucose**

## Introduction

Hypertension is considered to be a complex trait to which genetic, environmental, and demographic factors contribute interactively (*1*–*5*). Dopamine-β-hydroxylase (DBH) catalyzes the conversion of dopamine to norepinephrine and is released from sympathetic neurons into the circulation. Because the sympathetic nervous system is intimately involved in both the origin and the perpetuation of a hypertensive state (*6*, *7*), DBH may play an important role in the pathogenesis of essential hypertension. Indeed, neonates with DBH deficiency show episodic hypotension (*8*). DBH activity, derived largely from sympathetic nerves, can be measured

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This study was supported by a Grant-in-Aid for Scientific Research on Priority Area C, "Medical Genome Science," from the Ministry of Education, Culture, Sports, Science and Technology of Japan and a Grant-in-Aid for Research on the Human Genome, Tissue Engineering, and Food Biotechnology from the Ministry of Health, Labour, and Welfare of Japan.

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Received November 29, 2004; Accepted in revised form December 17, 2004.

**Table 1. Characteristics of Participants According to Hypertension Status**

| Variable | Normotensive ($n$=547) | Hypertensive ($n$=275) |
|---|---|---|
| Sex (male %) | 78.8 | 89.1 |
| Age (years) | 52.7±8.6 | 57.3±8.5 |
| Body mass index (kg/m$^2$) | 22.6±2.8 | 23.8±2.9 |
| SBP (mmHg) | 112.6±10.7 | 143.2±17.4 |
| DBP (mmHg) | 72.0±9.1 | 89.1±9.9 |
| Total cholesterol (mg/dl) | 198.0±30.6 | 202.4±37.2 |
| HDL cholesterol (mg/dl) | 54.2±14.5 | 51.9±14.0 |
| Triglyceride (mg/dl) | 116.7±81.7 | 150.9±127.7 |
| Fasting plasma glucose (mg/dl) | 101.2±17.3 | 106.0±19.2 |

Data are mean±SD. Blood pressure readings before the start of antihypertensive medication were not available for 118 hypertensive subjects whose values were measured under treatment. SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high density lipoprotein.

in human plasma (*9*, *10*), and elevated plasma DBH activity has also been shown in essential hypertension (*11*, *12*), although the conclusions have not been completely consistent (*13*). Moreover, DBH inhibitors have been shown to produce a dose-dependent decrease in mean arterial blood pressure (*14*, *15*).

The DBH gene, approximately 23 kb in length, is composed of 12 exons (*16*). Recently, a novel polymorphism (−1021C/T) in the 5′ flanking region of the DBH gene has been shown to account for 35–52% of the variation in plasma DBH activity in several ethnically different populations, including Japanese (*17*). The strong association of the DBH −1021C/T polymorphism with plasma DBH activity has also been replicated in a native Western European population (*18*). Thus, considering several lines of evidence for the relation between DBH and blood pressure, the DBH −1021C/T polymorphism appears to be an attractive candidate variable contributing to hypertension. Nevertheless, there have been few reports investigating the possible association between the DBH gene and hypertension. We therefore investigated the possible association between the DBH −1021C/T polymorphism and hypertension. Moreover, because the development of hypertension is considered to be due at least partly to gene-environmental interactions, we also investigated the possible interactions between the DBH −1021C/T polymorphism and environmental factors.

## Methods

### Subjects

According to the criteria described below, 275 hypertensive subjects and 547 normotensive subjects were selected from a population in the Hyogo region of Japan (Table 1) (*19*). All subjects were Japanese urban residents. They had participated in a medical check-up, and the mean values of variables in their personal health records were used in the analyses. All subjects gave their informed consent. 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.

Subjects were considered to have impaired fasting glycemia (IFG) if their fasting plasma glucose (FPG) concentration was ≥110 mg/dl. Subjects were considered to have diabetes mellitus (DM) if their FPG was ≥126 mg/dl.

### DNA Analysis

The TaqMan chemical method, which is an established and frequently used method (*20*–*23*), was used to detect the DBH −1021C/T polymorphism. The forward primer was 5′-GGATCAAGCAGAATGTCCTGAAG-3′, the reverse primer was 5′-GGCACCTCTCCCTCCTGTC-3′, the T-allele specific probe was 5′-Fam-CTCTCCCACAAGTAGA-MGB-3′, and the C-allele specific probe was 5′-Vic-CTC CCGCAAGTAGA-MGB-3′. The person who assessed the genotype was blinded to the clinical data of the subjects from whom the samples originated.

### Statistical Methods

Statistical analysis was performed with SPSS statistical software. Comparisons of categorical variables were performed using the $\chi^2$ test. Analysis of variance was used to assess differences in means and variances of continuous variables. Logarithmically transformed plasma triglyceride (TG) and FPG values were used in the analysis. Logistic regression models were used to assess whether the DBH −1021C/T polymorphism made a statistically significant contribution to prediction of hypertension, with consideration of interactions between the polymorphism and confounding factors. General linear regression models were used to assess whether the DBH −1021C/T polymorphism made a statistically significant contribution to prediction of blood pressure, with consideration of interactions between the polymorphism and confounding factors. $p$ values less than 0.05 were considered statistically significant.

**Table 2. DBH Genotype and Allele Frequencies in Hypertensive and Normotensive Subjects**

| Genotype and allele | Genotype frequency | | *p* value | OR | 95% CI |
|---|---|---|---|---|---|
| | Normotensive | Hypertensive | | | |
| DBH genotypes | | | | | |
| CC (%) | 378 (69.1) | 184 (66.9) | | | |
| CT (%) | 153 (28.0) | 86 (31.3) | | | |
| TT (%) | 16 (2.9) | 5 (1.8) | 0.52* | 0.90* | 0.66–1.23* |
| DBH alleles | | | | | |
| C (%) | 907 (83.1) | 454 (82.5) | | | |
| T (%) | 185 (16.9) | 96 (17.5) | 0.78 | 0.96 | 0.73–1.26 |

**p* value, OR and 95% CI are for CC *vs.* CT+TT. DBH, dopamine-β-hydroxylase; OR, odds ratio; CI, confidence interval.

**Table 3. Logistic Regression Model of FPG in the Association with Hypertension According to DBH Genotype**

| Genotype | Coefficient | Constant | *p* value for regression | OR | 95% CI | *p* value for interaction |
|---|---|---|---|---|---|---|
| CC | 3.12 | −15.14 | $5.4\times10^{-6}$ | 22.59 | 5.90–86.55 | |
| CT+TT | 0.20 | −1.53 | 0.82 | 1.22 | 0.22–6.78 | 0.0086 |

DBH, dopamine-β-hydroxylase; FPG, fasting plasma glucose; OR, odds ratio; CI, confidence interval.

## Results

### Association of DBH −1021C/T Polymorphism with Hypertension

A total of 822 Japanese individuals from the Hyogo region were categorized as hypertensive or normotensive and genotyped for the DBH −1021C/T polymorphism (Tables 1 and 2). The relative frequencies of the CC, CT and TT genotypes were 68%, 29% and 3%, respectively. The allele frequencies were 83% and 17% for the C and T alleles, respectively. These results are consistent with the Hardy-Weinberg equilibrium ($p>0.25$). Because of the relatively small number of subjects with the TT genotype, we analyzed differences between subjects with the CC genotype and those with the CT and TT genotypes. Statistical analysis failed to show a significant difference in the frequencies of the alleles ($p=0.52$) and genotypes ($p=0.78$ for CC *vs.* CT+TT) between the hypertensive and normotensive subjects (Table 2).

### Interaction of DBH −1021C/T Polymorphism with FBS in the Association with Hypertension

We next analyzed possible interactions of the DBH −1021C/T polymorphism with confounding factors in the association with hypertension in logistic regression models, because the development of hypertension is attributable at least partly to gene-environmental interactions. The DBH −1021C/T polymorphism did not interact with sex, age, body mass index (BMI), plasma total cholesterol, high density lipoprotein (HDL)-cholesterol, or TG. In contrast, the DBH −1021C/T polymorphism significantly interacted with FPG ($p=0.0086$) (Table 3). The interaction was significant even after adjustment for sex and age ($p=0.014$), and for sex, age, BMI, plasma total cholesterol, HDL-cholesterol, and TG ($p=0.031$). Subjects with the CC genotype showed a steeper increase in probability of hypertension with FPG than those with the CT and TT genotypes (Fig. 1).

Because the distribution of logarithmically transformed FPG was still slightly skewed, we also examined this interaction using stratification of FPG by quartiles (first quartile <94 mg/dl, second quartile 94 to 99 mg/dl, third quartile 100 to 106 mg/dl, and fourth quartile >106 mg/dl). Consequently, the *p* value for the interaction was 0.014. The *p* value was 0.019 after adjustment for sex and age, and 0.037 after adjustment for sex, age, BMI, plasma total cholesterol, HDL-cholesterol, and TG. Moreover, stratified analyses showed that subjects with the CT and TT genotypes had a significantly higher probability of hypertension than those with the CC genotype in the first quartile (FPG <94 mg/dl) ($p=0.0056$; OR=2.58, 95% CI=1.32–5.05, where OR indicates odds ratio and 95% CI indicates 95% confidence interval).

### Interaction of DBH −1021C/T Polymorphism with FBS in the Association with Blood Pressure

We next analyzed possible interactions of the DBH −1021C/T polymorphism with FPG in the association with blood pressure in general linear models. Analysis only of subjects not on current antihypertensive treatment showed that the DBH −1021C/T polymorphism significantly interacted with FPG ($p=0.045$) in the association with DBP (Table 4). The *p* value was 0.056 after adjustment for sex and age, and 0.055 after

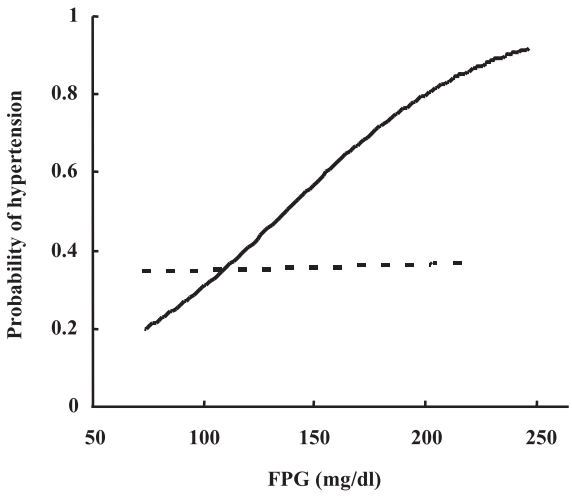


**Fig. 1.** *Genotype-specific regression slopes of hypertension on FPG. The simple line indicates the CC genotype; the dotted line indicates the CT and TT genotypes. The regression between FPG and the probability of having hypertension in subjects with the CC genotype was represented by the equation:* $y = exp(0.02241x - 3.028)/\{1 + exp(0.02241x - 3.028)\}$. *The equation was:* $y = exp(0.00064x - 0.685)/\{1 + exp(0.00064x - 0.685)\}$; *in subjects with the CT and TT genotypes. Subjects with the CC genotype showed a steeper slope than those with the CT and TT genotypes (*$p = 0.0086$*).*

adjustment for sex, age, BMI, plasma total cholesterol, HDL-cholesterol, and TG. Subjects with the CC genotype showed a steeper increase in blood pressure levels with FPG than those with the CT and TT genotypes (Fig. 2b). A similar trend of interaction was shown in the association with SBP ($p = 0.057$) (Table 4 and Fig. 2a). The $p$ value was 0.092 after adjustment for sex and age, and 0.087 after adjustment for sex, age, BMI, plasma total cholesterol, HDL-cholesterol, and TG.

Analyses of the interaction using stratification of FPG by quartiles (first quartile <94 mg/dl, second quartile 94 to 98 mg/dl, third quartile 99 to 106 mg/dl, and fourth quartile >106 mg/dl) showed that the $p$ value for the interaction was 0.089 for SBP and 0.025 for DBP. The $p$ value was 0.091 for SBP and 0.033 for DBP after adjustment for sex and age. The $p$ value was 0.10 for SBP and 0.035 for DBP after adjustment for sex, age, BMI, plasma total cholesterol, HDL-cholesterol, and TG.

## Discussion

The present study provided evidence for the interaction between the DBH −1021C/T polymorphism and FPG in the association with hypertension in a large Japanese population. There was also a marginally significant trend suggesting the presence of an interaction between the DBH −1021C/T polymorphism and FPG in the association with blood pressure. This lack of significance was possibly due to the unstable nature of blood pressure (*19*). In addition, the inclusion or exclusion of subjects who were receiving antihypertensive treatment influenced the distribution of blood pressure, and blood pressure readings before the start of antihypertensive medication were not available for 118 hypertensive subjects in our population.

In theory, the DBH −1021C/T polymorphism might be associated with hypertension, because this polymorphism is associated with plasma DBH activity (*17*, *18*) and plasma DBH activity is associated with hypertension (*11*, *12*). However, in practice, the present study failed to show a significant association between the DBH −1021C/T polymorphism and hypertension. This failure was possibly due to the interaction between the DBH −1021C/T polymorphism and FPG in the association with hypertension. However, evidence for this possibility is insufficient, because data on plasma DBH activity were not available in our population. In addition, the previous reports showing that the DBH −1021C/T polymorphism is associated with plasma DBH activity did not analyze the interaction between the DBH −1021C/T polymorphism and FPG in the association with plasma DBH activity (*17*, *18*).

Supporting the interaction between the DBH gene and FPG, there is biological evidence showing that glucose and other sugars induce an increase of DBH (*24*). Indeed, rats with experimental diabetes have increased plasma DBH activity (*25*). Thus, the most important physiological influence on plasma DBH activity is considered to be the plasma glucose level (*26*). In addition, DBH-containing neurons in the hindbrain that innervate the hypothalamus have been implicated in the feeding response to glucose deprivation (*27*). In humans, the difference in sympathetic response to glucose ingestion related to family history of hypertension suggests the existence of genetic factors influencing the sympathetic response to glucose ingestion (*28*). The DBH gene may be one such genetic factor.

The precise mechanism of the interaction between the DBH −1021C/T polymorphism and FPG in the association with hypertension remains elusive; a simple explanation may be that the CC genotype or a genotype in linkage disequilibrium with it might produce a controlled amount of DBH in association with the plasma glucose level, leading to increased blood pressure. In contrast, the CT and TT genotypes or genotypes in linkage disequilibrium with them might produce a constant amount of DBH irrespective of the plasma glucose level, leading to relatively stable blood pressure. This explanation may be in line with the observation in a previous study that all 19 chimpanzees were homozygous for the C allele (*29*).

Alternatively, depending on the genotype, glucose level could influence plasma insulin level, which in turn could influence blood pressure. However, the previous observation that insulin administration lowered plasma glucose level, but not plasma DBH activity, challenges this possibility (*24*). Moreover, in humans, activation of the sympathetic nervous

**Table 4. General Linear Model for Regression of FPG in the Association with Blood Pressure According to DBH Genotype**

| BP | Genotype (*n*) | Coefficient | Constant | *p* value for regression | Determination coefficient | *p* value for interaction |
|---|---|---|---|---|---|---|
| SBP | CC (562) | 12.1 | 23.5 | 0.00016 | 0.035 | |
| | CT+TT (260) | 2.9 | 106.7 | 0.75 | 0.00056 | 0.057 |
| DBP | CC (562) | 11.8 | 22.1 | 0.0034 | 0.021 | |
| | CT+TT (260) | −3.1 | 91.0 | 0.65 | 0.0011 | 0.045 |

FPG, fasting plasma glucose; DBH, dopamine-β-hydroxylase; BP, blood pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure.

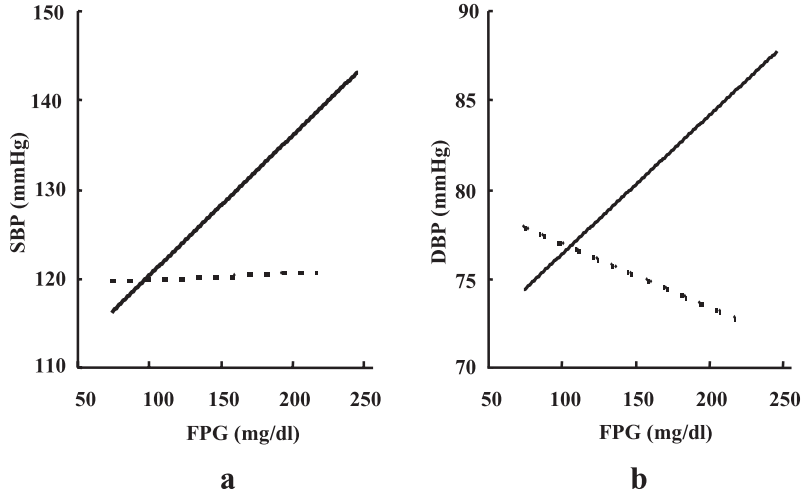


**Fig. 2.** *Genotypic variations in the relationship between FPG and blood pressure. a: The simple line indicates the CC genotype; the dotted line indicates the CT and TT genotypes. The regression between FPG and SBP in subjects with the CC genotype was represented by the equation:* $y = 0.1558x + 104.71$. *The equation was:* $y = 0.0071x + 119.15$; *in subjects with the CT and TT genotypes. Subjects with the CC genotype showed a steeper slope than those with the CT and TT genotypes (*$p = 0.057$). *b: The simple line indicates the CC genotype; the dotted line indicates the CT and TT genotypes. The regression between FPG and DBP in subjects with the CC genotype was represented by the equation:* $y = 0.16x - 4.53$. *The equation was:* $y = 0.22x - 6.10$; *in subjects with the CT and TT genotypes. Subjects with the CC genotype showed a steeper slope than those with the CT and TT genotypes (*$p = 0.045$).

system is related to plasma glucose level but not hyperinsulinemia or insulin hypersecretion in essential hypertension (*30*). However, because the etiology of hypertension, the effects of glucose, and the regulation of the sympathetic nervous system are all complicated, the above explanation remains completely speculative. Epidemiological studies in large populations with information on plasma DBH activity and plasma insulin level as well as biological studies could test this hypothesis.

With respect to the possible functionality of the DBH −1021C/T polymorphism, transient-transfection assays of the reporter gene construct in human neuroblastoma cell lines designed to assess whether this polymorphism directly alters transcriptional activation of the DBH gene have been negative to date (*31*, *32*). In this context, we found that a 19 bp sequence containing the DBH −1021C/T polymorphism (CCCTCAGTCTACTTGYGGG, where Y indicates the C/T polymorphism) includes two palindromic non-canonical E boxes separated by 5 bps, and closely resembles the glucose response element of the L-type pyruvate kinase gene (*33*). The DBH −1021C/T polymorphism resides in a critical 6-bp area. This suggests that the DBH −1021C/T polymorphism may alter the responsiveness to glucose, consistent with the interaction between the polymorphism and FPG, although direct molecular evidence is lacking.

In conclusion, the present study revealed a significant interaction between the DBH −1021C/T polymorphism and FPG in the pathogenesis of hypertension in a large Japanese population. This interaction was partly supported by other epidemiological and molecular biological evidence. Despite several limitations of this study, if our findings are confirmed, they could be helpful in conducting further molecular and biological studies on the relationship among glucose metabolism, the sympathetic nervous system, and hypertension.

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