Table 2. Biochemical characteristics in study subjects

	n	FPG (mg/mL)	FIRI (μU/mL)	LDL-C (mg/dL)	HDL-C (mg/dL)	TG (mg/dL)
NC subjects	15	87.5 ± 9.2	4.22 ± 1.01	120 ± 16	45 ± 14	132 ± 43
Hypertension						
FH(+) group	33	99.4 ± 14.8	8.10 ± 3.03	123 ± 34	43 ± 14	128 ± 55
FH(−) group	39	92.7 ± 8.8	5.26 ± 1.84	137 ± 34	47 ± 13	135 ± 64
P value						
NC *v* FH(+)		.0059	<.0001	NS	NS	NS
NC *v* FH(−)		NS	NS	NS	NS	NS
FH(+) *v* FH(−)		NS	<.0001	NS	NS	NS

FIRI = fasting immunoreactive insulin; FPG = fasting plasma glucose; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; TG = triglyceride; other abbreviations as in Table 1.
Data are presented as mean value ± SD.

not significant). Furthermore, FPG was significantly related to LVM ($r = 0.542$, $P < .0001$) or RWT ($r = 0.574$, $P < .0001$), respectively. The SV/PP ratio showed a weak but significant negative correlation with RWT ($r = -0.362$, $P = .0097$) in hypertensive patients.

Table 4 shows the results of the multivariate analysis. The HOMA value was the strongest contributor to the resultant model, with smaller and approximately equal contributions from BMI and male sex and lesser contributions from genetic predisposition of hypertension and systolic BP; age did not bear a significant relation to LVM. Independent determinants of RWT were the HOMA value and age.

Discussion

Because LV hypertrophy is strongly and independently associated with cardiovascular morbidity and mortality,[1,2] it would be helpful to be able to identify hypertensive patients who are likely to develop LV hypertrophy. We found that hypertensive patients with genetic predisposition to hypertension and insulin resistance had LV hypertrophy, including significant increases in LVM, indexed LVM, and RWT. In a multivariate analysis, HOMA value, male sex, BMI, SBP, and genetic predisposition to hypertension were independently associated with LVM. The HOMA value and age were also independently associated with RWT. Thus, we suggest that a genetic predisposition to hypertension and the HOMA value appear to have additive impacts on LV hypertrophy in patients with essential hypertension and normal glucose tolerance. This relation is independent of known and postulated determinants of LVM such as male sex, overweight, and high BP.

There is growing evidence that LV hypertrophy is influenced by genetic factors.[6–8,23] On the other hand, a number of previous studies have evaluated the relations between LV hypertrophy and insulin resistance.[9–11,13–15] Although genetic predisposition to hypertension and insulin resistance share several physiopathologic abnormalities and are frequently associated, to our knowledge, no study has looked at whether genetic factors and insulin resistance are related to the progression of LV remodeling in essential hypertension. We observed a significant relationship between LVM and genetic predisposition to hypertension. Our findings agree with previous studies[6–8,23] in which genetic factors accounted for a small but discernible proportion of the overall variance in LVM.

It is widely acknowledged that peripheral hyperinsulinemia in patients with hypertension is a marker of insulin resistance.[9,24] Diminished insulin sensitivity with regard to glucose use causes a substantial increase in insulin production in an attempt to maintain normal glucose use, making it possible that cardiovascular trophic effects and other actions of insulin could be exaggerated. The HOMA value was calculated to obtain a better quantitative estimate of insulin resistance.[25] In the present study, we showed an independent association between echocardiographically determined LVM, indexed LVM and RWT, and HOMA value in patients with hypertension, thereby confirming previous positive reports. Interestingly, the strength of association with the HOMA value, as reflected by the magnitude of standardized coefficient, increased in RWT compared with LVM. These findings agree with a previous study[13] in which several components of insulin resistance syndrome were found to be related to thick LV walls and concentric remodeling but less to LVM in elderly men.

Verdecchia et al[10] reported that insulin and insulin growth factor–1 (IGF-1) were powerful independent determinants of LVM in nondiabetic patients with hypertension. The direct effect of insulin on cardiac myocyte growth could be mediated, at least in part, by IGF-1 receptors.[26] Unfortunately, we could not determine IGF-1 binding protein in the present study. However, because fasting insulin levels were positively correlated with LVM and RWT, our data suggest that insulin is a powerful determinant of cardiac myocyte in individuals with untreated essential hypertension and normal glucose tolerance. In addition, hypertensive patients with glucose intolerance have more severe LV hypertrophy and LV diastolic dysfunction than those with normal glucose tolerance.[11,14,27]

Table 3. Echocardiographic characteristics in study subjects

	n	LVM (g)	LVM/height (g/m)	LVM/BSA (g/m^2)	LVM/height$^{2.7}$ ($g/m^{2.7}$)	RWT	Doppler SV (mL)	SV/PP ratio (mL/mm Hg)
NC subjects	15	135 ± 20	79 ± 11	87 ± 11	35 ± 5	0.32 ± 0.03	88.3 ± 13.2	1.63 ± 0.28
Hypertension								
FH(+) group	33	207 ± 55	128 ± 30	126 ± 28	57 ± 12	0.42 ± 0.09	82.7 ± 9.3	1.11 ± 0.25
FH(−) group	39	167 ± 34	106 ± 20	106 ± 19	50 ± 10	0.39 ± 0.07	85.6 ± 12.8	1.15 ± 0.24
P value								
NC *v* FH(+)		<.0001	<.0001	<.0001	<.0001	.0039	NS	<.0001
NC *v* FH(−)		NS	.0316	NS	.0075	NS	NS	<.0001
FH(+) *v* FH(−)		.0007	.0015	.0017	.0151	NS	NS	NS

BSA = body surface area; FH = familial history; LVM = left ventricular mass; NC = normotensive control; NS = not significant; SV = stroke volume; RWT = relative wall thickness; other abbreviations as in Table 1.

Data are presented as the mean value ± SD.

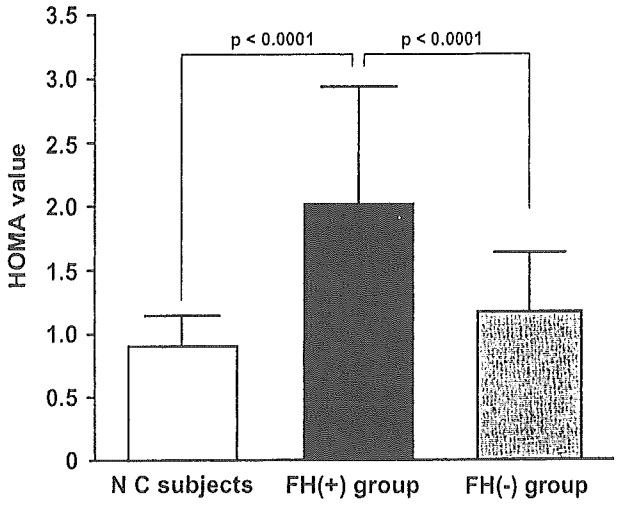

FIG. 1 Comparison of homeostasis model assessment (HOMA) values in normotensive control (NC) subjects and hypertensive patients with family history [FH(+)] and with weak family history [FH(−)]. **Column height** represents mean; **bars** indicate 95% confidence intervals.

In a recent investigation, high HOMA value was related to LVM in women alone, but this relation was largely accounted for by obesity.[15] In the present study, high HOMA values were related to LVM in male hypertensive subjects but not in female hypertensive subjects. Furthermore, this relationship was not accounted for by overweight. The absence of an association between HOMA value and LVM in female hypertensive subjects in our study might be due to the small sample size. Alternatively, it could indicate that insulin affects LV geometry differently in men and women in these Japanese hypertensive patients. When demographic variables (age, BMI, and sex distribution), SBP, genetic predisposition to hypertension, and HOMA value were considered together, the strongest

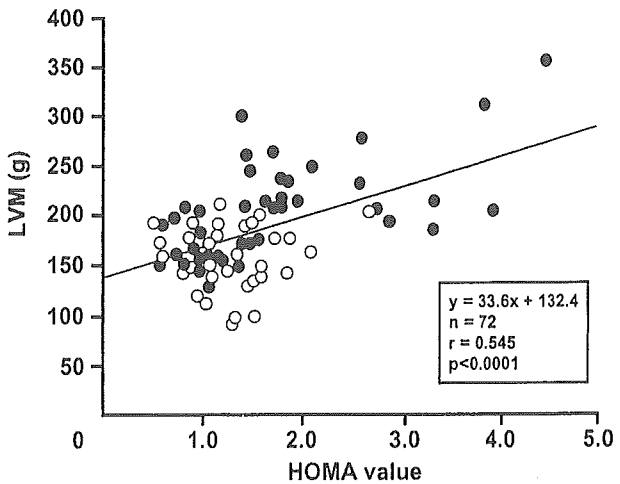

FIG. 2 Relationship between the homeostasis model assessment (HOMA) value and echocardiographically determined left ventricular mass (LVM) in female patients (**open circles**) and male patients (**closed circles**) with essential hypertension. A statistically significant positive relation was found between the HOMA value and LVM.

Table 4. Multivariate analysis of factors relevant to left ventricular mass (LVM) and relative wall thickness (RWT) in patients with hypertension

	LVM		RWT	
Variable	**Standardized coefficient**	***P* value**	**Standardized coefficient**	***P* value**
Age	−0.065	.3028	0.241	.0207
Sex	−0.284	.0032	−0.149	.1498
Body mass index	0.258	.0061	0.084	.4054
Systolic blood pressure	0.217	.0245	0.195	.0631
Genetic predisposition to hypertension	0.214	.0441	−0.138	.2416
HOMA value	0.341	.0011	0.549	<.0001
	Adjusted R^2 = 0.489, P < .0001		Adjusted R^2 = 0.333, P < .0001	

HOMA = homeostasis model assessment.

determinant of LVM was the HOMA value (positive). The contribution of male sex to the predictive model for LVM approximately equaled that of BMI. Genetic predisposition to hypertension and SBP added weakly to the multivariate model, but age did not.

In the present study, coronary risk factors such as BP, pulse pressure, heart rate, lipid profile, and vascular compliance of the large arteries as indicated by the SV/PP ratio were not significantly related to genetic predisposition to hypertension. In contrast, the SV/PP ratio was significantly related to the HOMA value in patients with essential hypertension. The independent impact of arterial status on outcome was recently found in two follow-up studies of patients with hypertension[28] or with both diabetes and glucose intolerance.[29] Together with the present results regarding hypertension and insulin resistance, a strong relationship between LV hypertrophy and cardiovascular morbidity and mortality appears to be mediated through insulin resistance.

In conclusion, there is increasing evidence of a link between insulin and cardiovascular risk,[30] although the independent role of insulin is still undetermined. The present study suggests that genetic predisposition to hypertension and the HOMA value appear to have additive impacts on LV hypertrophy. This relation is independent of well-known and postulated determinants of LVM such as male sex, overweight, and high BP.

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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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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 χ^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	5.4×10^{-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

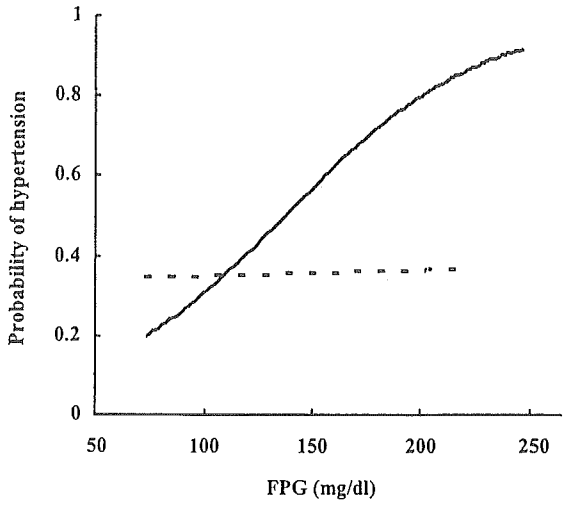

Fig. 1. *Genotype-specific regression slopes of hypertension on FPG. The simple line indicates the CC genotype; the dotted line indicates the CT and TT genotypes. The regression between FPG and the probability of having hypertension in subjects with the CC genotype was represented by the equation:* $y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

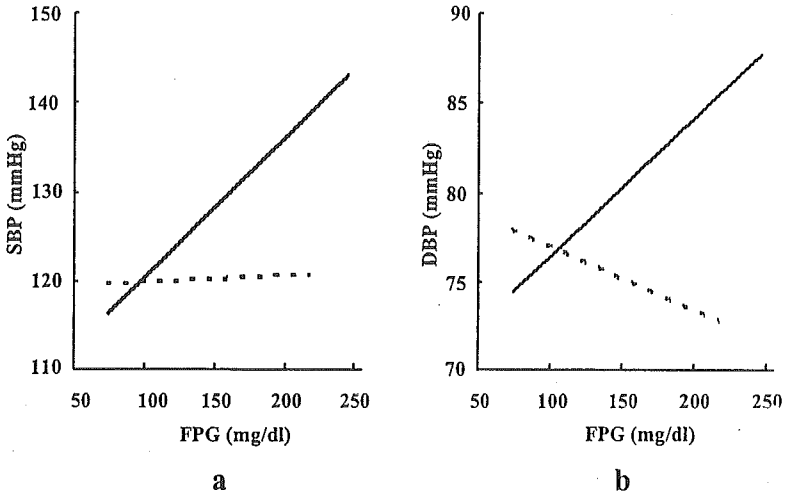

Fig. 2. *Genotypic variations in the relationship between FPG and blood pressure. a: The simple line indicates the CC genotype; the dotted line indicates the CT and TT genotypes. The regression between FPG and SBP in subjects with the CC genotype was represented by the equation:* $y = 0.1558x + 104.71$. *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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Original Article

Combined Analysis of Polymorphisms in Angiotensinogen and Adducin Genes and Their Effects on Hypertension in a Japanese Sample: The Shigaraki Study

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We examined the interactions between lifestyle and polymorphisms of salt-sensitive genes and their effects on hypertension in a general Japanese sample (The Shigaraki Study). The study group consisted of 2,902 subjects who underwent a medical examination in 1999 in Shigaraki, a suburban area in Shiga. Among 1,647 subjects not receiving antihypertensive medication, in a combined analysis of angiotensinogen (AGT) and adducin (ADD1) polymorphisms, double homozygosity of 235Thr or 460Trp was not found to be associated with hypertension. A multiple logistic regression analysis showed that age (odds ratio [OR]: 1.07, 95% confidence interval [95% CI]: 1.06–1.08), body mass index (BMI) (OR: 1.18, 95% CI: 1.13–1.23), alcohol consumption (OR: 1.39, 95% CI: 1.16–1.66), family history of hypertension (OR: 1.57, 95% CI: 1.18–2.07), and combined AGT M235T Thr/Thr and ADD1 Trp/Trp polymorphisms (OR: 1.37, 95% CI: 1.03–1.82) were associated with hypertension. However, there was no interaction between eating salty food and combined AGT and ADD1 polymorphisms. Furthermore, eating salty food was not associated with hypertension in a multivariate analysis. Therefore, a combination of the AGT and ADD1 polymorphisms appears to be associated with hypertension. However, a simple questionnaire regarding salt intake was not sufficient to confirm the relationship between salt intake and hypertension and/or salt-sensitive genes. (*Hypertens Res* 2005; 28: 645–650)

Key Words: **angiotensinogen M235T polymorphism, adducin Gly460Trp polymorphism, hypertension, lifestyle**

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Introduction

The pathophysiological mechanisms related to salt-sensitive essential hypertension are not completely understood. Excess salt intake is an important environmental risk factor for the predisposition to essential hypertension. Therefore, polymorphisms that might increase the formation of angiotensin II (such as the angiotensinogen [AGT] polymorphism) are relevant in the context of sodium sensitivity. The AGT M235T (the substitution of threonine [Thr] for methionine [Met] at codon 235) polymorphism is associated with an increased risk of hypertension (*1*, *2*) and has also been evaluated in relation to salt sensitivity, with controversial results (*3*, *4*). The Gly460Trp genotype of adducin (ADD1) (the substitution of tryptophan [Trp] for glycine [Gly] at codon 460) is also associated with erythrocyte sodium transport, increases in tubular sodium reabsorption, and risk for hypertension (*5–7*). One epidemiologic study showed that the ADD1 Trp/Trp genotype was associated with higher systolic blood pressure (sBP) among men with a high sodium intake (*8*).

The purpose of this study was to elucidate the relationship between AGT, ADD1, both genotypes combined, and hypertension in a general Japanese sample. Moreover, we examined the effects of salt intake and polymorphisms of salt-sensitive genes on hypertension, and we conducted a statistical analysis of the interactions between these factors after adjusting for other lifestyle factors.

Methods

Study Population

The Shigaraki Study was based on a medical examination undertaken in 1999 at Shigaraki, a farming community near Kyoto, in western Japan (*9–11*). A total of 2,902 subjects underwent the examination, of whom 2,395 were enrolled in this genetic study after receiving a full explanation and providing informed consent. Of these subjects, 748 were excluded for the following reasons: undetermined genotype, $n=41$; already taking antihypertensive agents, $n=431$; a serum GOT or GPT level of over 100 IU/l, $n=13$; and/or a history of transient ischemic attack, stroke, angina pectoris, myocardial infarction, or diabetes mellitus, $n=263$. Subjects were between the ages of 30 and 79. This study was approved by the Institutional Review Board of Shiga University of Medical Science (Nos. 11–15, 1999).

Blood Pressure (BP) and Biochemical Examinations

sBP and diastolic blood pressure (dBP) were measured twice using a standard sphygmomanometer on the right arm while the subject was seated after having rested for at least 5 min. Korotokov's first and fourth points were regarded as the sBP and dBP, respectively, and the BP was measured by a well-trained nurse. The mean of the 2 measurements from each subject was used for the data analysis. In this study, participants were considered hypertensive if they had the following BP values: sBP≥140 mmHg or dBP≥90 mmHg. The non-fasting blood glucose level was measured by the hexokinase method. Participants were considered diabetic if they had a blood glucose level of 200 mg/dl or more, or if they were already being treated for diabetes. The body mass index (BMI) was calculated as weight (kg) divided by the square of the height (m).

Assessment of Lifestyle Factors

The patient history regarding daily alcohol intake and number of cigarettes per day was assessed by face-to-face interview (*9*, *10*). The frequency of consumption during a typical week and the alcohol intake on each occasion were determined and used to calculate the alcohol intake per week, which was then divided by 7 to obtain the average intake per day. Subjects were asked to estimate their alcohol intake based on the "gou," a traditional Japanese drinking unit corresponding to 23 g of ethanol. Drinkers were defined as those consuming more than 0.3 gou a week. The participants who reported that they preferred salty foods in a simple questionnaire were defined as those "eating salty food."

Genetic Analysis

DNA was isolated from peripheral leukocytes and the AGT and ADD1 genotypes were determined as previously reported (*12*, *13*). Both genotypes, determined by the polymerase chain reaction (PCR)–restriction fragment length polymorphism (RFLP) method for a total of 75 random samples consisting of 25 PCR products for each genotype, were confirmed by direct sequencing. Briefly, after fractionation of the PCR-RFLP products on 1% agarose gels (Nippon Gene, Tokyo, Japan), the desired DNA bands were excised, and the DNA was purified using a QIAquick Gel Extraction Kit (QIAGEN, Valencia, USA), amplified with the above 5′ primer, and analyzed with an ABI PRISM 310 Genetic Analyzer (Perkin-Elmer, Wellesley, USA).

Statistical Analysis

The Statistical Package for Social Science (SPSS ver. 11.0J; SPSS Japan, Tokyo, Japan) was used for the statistical analysis. Student's or Welch's *t*-test and the Wilcoxon rank sum test (for alcohol consumption) were used for comparisons of means between two categories. For comparisons of means among three or more categories, a one-way analysis of variance or the Kruskal-Wallis test (for alcohol consumption) was used according to the distributions. The χ^2 test was used to compare proportions. Age-adjusted prevalence was calculated directly. A multiple logistic regression analysis was

Table 1. Characteristics of Study Population by AGT M235T Polymorphism in Men and Women, Shigaraki Study in 1999

Risk characteristics	Men (638)				Women (1,009)			
	Met/Met	Met/Thr	Thr/Thr	*p*-value	Met/Met	Met/Thr	Thr/Thr	*p*-value
N (1,647)	29	168	441		40	303	666	
Age (years)	55.9±14.9	56.1±16.3	56.1±15.3	0.997	53.8±16.7	52.7±15.8	53.5±15.2	0.739
BMI (kg/m^2)	22.4±3.7	22.1±3.0	22.6±2.8	0.208	21.9±3.0	22.2±3.0	22.1±3.0	0.890
sBP (mmHg)	128.0±16.9	130.6±16.4	130.2±17.5	0.760	125.0±17.3	123.2±18.5	125.2±19.9	0.324
dBP (mmHg)	77.0±11.3	78.5±12.2	78.3±11.6	0.819	76.0±10.2	73.3±11.1	74.6±11.8	0.194
Alcohol consumption (gou/day)	1.38	0.80	0.80	0.251	0.06	0.07	0.07	0.145
Eating salty food (%)	31.0	25.6	19.5	0.119	15.0	6.9	7.5	0.191

N: number of subjects. Values are means±SD. AGT, angiotensinogen; BMI, body mass index; sBP, systolic blood pressure; dBP, diastolic blood pressure. Alcohol consumption: 1 gou=23 g of ethanol.

Table 2. Characteristics of Study Population by ADD1 Gly460Trp Polymorphism in Men and Women, Shigaraki Study in 1999

Risk characteristics	Men (638)				Women (1,009)			
	Gly/Gly	Gly/Trp	Trp/Trp	*p*-value	Gly/Gly	Gly/Trp	Trp/Trp	*p*-value
N (1,647)	123	305	210		201	497	311	
Age (years)	55.4±15.9	55.3±15.7	57.6±15.1	0.240	54.7±16.6	53.3±15.0	52.2±15.5	0.187
BMI (kg/m^2)	22.3±2.5	22.5±3.0	22.5±3.0	0.673	21.6±2.8	22.3±3.0	22.1±3.0	0.031
sBP (mmHg)	130.6±17.0	129.6±17.0	130.8±17.6	0.726	124.7±20.2	124.8±19.6	124.3±18.6	0.951
dBP (mmHg)	78.3±11.8	78.0±11.6	78.6±12.0	0.836	72.8±12.1	75.0±11.6	74.0±11.1	0.061
Alcohol consumption (gou/day)	0.77	0.87	0.79	0.390	0.06	0.08	0.07	0.352
Eating salty food (%)	24.4	22.3	19.0	0.482	8.0	8.7	5.8	0.322

N: number of subjects. Values are means±SD. BMI, body mass index; ADD1, adducin; sBP, systolic blood pressure; dBP, diastolic blood pressure. Alcohol consumption: 1 gou=23 g of ethanol.

used to clarify the contribution of each independent variable to hypertension. In this analysis, hypertension was regarded as a dependent variable, and each genotype and other factors were regarded as independent variables. The significance of the interaction of eating salty food with AGT and ADD1 genotypes was also tested using an interaction term in this model. All confidence intervals were estimated at the 95% level.

Results

Table 1 shows the characteristics of the study population according to the AGT M235T polymorphism. The frequencies of AGT genotypes Met/Met, Met/Thr, and Thr/Thr were 4.2%, 28.6%, and 67.2%, respectively. No significant differences were observed among the Met/Met, Met/Thr, and Thr/Thr groups with respect to age, BMI, sBP, dBP, alcohol consumption, and the habit of eating relatively more salty food, in comparison to the reported salt intake of other subjects. Table 2 shows the characteristics of the study population according to the ADD1 Gly460Trp polymorphism. The frequencies of ADD1 genotypes Gly/Gly, Gly/Trp, and Trp/Trp were 19.7%, 48.7%, and 31.6%, respectively. Results similar to those given above were obtained. In all, no significant differences were observed among the Gly/Gly, Gly/Trp, and Trp/Trp groups in terms of hypertension.

Table 3 shows the characteristics of the combined AGT and ADD1 polymorphism analysis, AGT M235T Thr/Thr and ADD1 Trp/Trp *vs.* other polymorphisms. There was a significant association between the combined genotypes AGT Thr/Thr and ADD1 Trp/Trp in men and hypertension ($p=0.035$). However, the statistical significance disappeared when we adjusted for age, although the magnitude of the percentage remained almost the same. After adjustments for age, BMI, alcohol consumption, eating salty food, family history of hypertension, and number of cigarettes per day were made, the multivariate prevalence odds ratio and 95% confidence interval (CI) of combined AGT and ADD1 polymorphisms for hypertension were, respectively, 1.33 and 0.88–2.02 for men, and 1.41 and 0.95–2.01 for women. The combined AGT and ADD1 polymorphisms were positively associated with hypertension in both men and women, with an odds ratio of almost the same magnitude; however, the association did not reach a level of statistical significance.

Table 4 shows the multivariate adjusted odds ratios of combined AGT and ADD1 polymorphisms for hypertension when the data for men and women were combined. Multiple

Table 3. Characteristics of Study Population by Combined AGT and ADD1 Polymorphisms in Men and Women, Shigaraki Study in 1999 (AGT M235T Thr/Thr and ADD1 Trp/Trp *vs.* Others)

Risk characteristics	Men (638)			Women (1,009)		
	Others	Thr/Thr and Trp/Trp	*p*-value	Others	Thr/Thr and Trp/Trp	*p*-value
N (1,647)	488	150		800	209	
Age (years)	55.3±15.8	58.6±14.6	0.026	53.4±15.5	52.7±15.4	0.549
BMI (kg/m^2)	22.4±2.9	22.8±3.0	0.189	22.1±3.0	22.1±2.9	0.971
sBP (mmHg)	129.6±16.9	132.0±18.0	0.140	124.3±19.3	125.8±19.7	0.335
dBP (mmHg)	77.9±11.7	79.6±11.9	0.125	74.2±11.6	74.7±11.5	0.598
Alcohol consumption (gou/day)	0.83	0.81	0.723	0.07	0.07	0.144
Family history of hypertension (%)	24.4	18.0	0.104	29.9	30.6	0.834
Eating salty food (%)	22.7	18.0	0.217	8.1	5.7	0.248
Hypertension (%)	28.9	38.0	0.035	23.4	27.3	0.241
Hypertension (%)†	28.9	35.9	0.127	23.4	28.1	0.125

Others: AGT M235T polymorphism, Met/Met and Met/Thr; and ADD1 Gly460Trp polymorphism, Gly/Gly and Gly/Trp. Other abbreviations are listed in Tables 1 and 2. †Age-adjusted prevalence was calculated by the direct method using the "others" group as the standard population.

Table 4. Multivariate Adjusted Relative Odds Ratios and 95% Confidence Intervals (CIs) of Combined Genetic AGT M235T Thr/Thr (TT) and ADD1 Trp/Trp (TT) Polymorphisms for Hypertension (*N*=1,647)

Risk characteristics	Odds ratio (95% CI)	*p*-values
AGT T/T and ADD1 T/T (both TT=1, others=0)	1.37 (1.03–1.82)	0.031
Age (years)	1.07 (1.06–1.08)	<0.001
BMI (kg/m^2)	1.18 (1.13–1.23)	<0.001
Alcohol consumption (gou/day)	1.39 (1.16–1.66)	<0.001
Eating salty food (yes=1, no=0)	1.25 (0.88–1.77)	0.218
Family history of hypertension	1.57 (1.18–2.07)	0.002
Smoking (number of cigarettes/day)	1.00 (0.99–1.01)	0.481
Sex (men=0, women=1)	1.09 (0.81–1.47)	0.481

Abbreviations are listed in Tables 1 and 2.

logistic regression analysis adjusting for age, BMI, alcohol consumption, eating salty food, family history of hypertension, number of cigarettes per day and sex showed that age, BMI, alcohol consumption, family history of hypertension, and combined AGT and ADD1 polymorphisms were associated with hypertension. However, there was no correlation between eating salty food and hypertension. In addition, there was no interaction between eating salty food and the AGT M235T Thr/Thr plus ADD1 Trp/Trp polymorphism (p=0.829).

Discussion

A number of genes have been tested for an association with hypertension, with controversial results. Salt sensitivity is possibly genetically determined. Salt-sensitive individuals tend to more frequently have a familial history of hypertension than do salt-resistant subjects, and there is a familial resemblance in the response of BP to sodium restriction (*14*, *15*). Such findings suggest the existence of genetic determinants that influence the sensitivity of BP to salt. Hunt and co-workers speculated that the AGT genotype affects BP in response to sodium and the development of hypertension. A greater reduction in BP following a reduction in sodium has been reported in subjects with the Thr/Thr genotype than in those with the Met/Met genotype (*16*). Similarly, Beeks and co-workers reported that the 460Trp variant of the ADD1 polymorphism is probably associated with a salt-sensitive form of hypertension (*17*). However, studies of African Americans, who are believed to have a higher prevalence of salt-sensitive hypertension, have not revealed any association between the ADD1 polymorphism and hypertension (*18*, *19*). These discrepancies may be difficult to reconcile. One possible explanation is that essential hypertension is a complex syndrome determined by both genetic and environmental factors. It is possible that the polymorphism of a single gene

exerts only a small effect on the development of hypertension, and this may be masked by differences in genetic phenotypes or environmental factors such as BMI, salt intake, and alcohol consumption (*9–11*). In the Ohasama study (*13*), the Gly460Trp polymorphism of ADD1 was associated with ambulatory BP and home BP, but not casual BP. Casual BP usually does not reflect basal BP, being influenced by physical or psychological stress and environmental factors. However, in the present study, we found an association between the AGT M235T Thr/Thr plus ADD1 Trp/Trp polymorphism and hypertension after adjustment for possible confounding lifestyle factors, which indicates the importance of clarifying the combined effects of certain candidate genes on hypertension. Here, we suggest that a combined genetic analysis for demonstrating the presence of both AGT and ADD1 polymorphisms is a good marker for hypertension, as defined by the casual BP. Therefore, we concluded that the accumulation of genetic risk factors increases the frequency of hypertension, irrespective of exposure to environmental risk factors for hypertension.

There were several limitations to the present study. First, we did not examine other candidate genes that might be associated with hypertension (*20, 21*). Second, the simple questionnaire regarding salt intake used in the present study did not reflect the actual salt intake of each participant. Instead, it might have been more suitable for the purposes of the present study to use a 24-h urinary sodium excretion test, or some other formula to estimate 24-h urinary sodium excretion based on spot urine samples (*22*).

In conclusion, as regards heredity, double homozygosity of 235Thr or 460Trp might be associated with essential hypertension. However, in the present study, no interaction between these genotypes and salt intake could be determined based on the results of a simple questionnaire. In addition, further investigation will need to be carried out using a large-scale sample.

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Original Article

Orthostatic Systolic Hypotension and the Reflection Pressure Wave

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Orthostatic hypotension (OH) is a potent predictor of cardiovascular frailty. Although OH is determined by changes in brachial blood pressure (BP), it has been reported that there are significant differences between central BP and peripheral BP. The prevalence of OH has been reported to be higher in subjects with isolated systolic hypertension. Since an early returning of the reflection pressure wave due to advanced arterial stiffness is one of the underlying mechanisms of systolic hypertension, a significant association between alterations of the reflection pressure wave and OH has been hypothesized. To explore this hypothesis, the orthostatic changes in carotid BP and arterial waveform were evaluated. The study subjects were 155 community residents (69±7 years old). Carotid and brachial BP were measured simultaneously in the supine position and 1 min after standing using a cuff-oscillometric and tonometric method. The carotid augmentation index (AIx) was obtained from the pressure waveform. The orthostatic decline of BP was more prominent in the carotid artery than the brachial artery. Nine subjects were diagnosed with orthostatic systolic hypotension (OSH) from brachial BP, while 21 subjects were diagnosed from carotid BP ($p<0.001$). The orthostatic change in carotid systolic BP was significantly associated with that in carotid AIx ($r=0.361$, $p<0.001$). The decline of the reflection component of carotid pulse pressure (−4.0±8.4 mmHg) was more prominent than that of the incident component (−1.2±9.9 mmHg, $p=0.002$). These results indicate that evaluation of brachial BP may not represent the orthostatic changes in central BP. Alteration of the reflection pressure wave could be one of the underlying mechanisms of OSH in the central artery. (*Hypertens Res* 2005; 28: 537–543)

Key Words: **orthostatic hypotension, reflection pressure wave, augmentation index**

Introduction

Orthostatic hypotension (OH) is a commonly observed phenomenon in the general elderly population (*1–9*). OH is defined as a more than a 20 mmHg reduction in systolic blood pressure (SBP) and/or more than 10 mmHg reduction in diastolic blood pressure (DBP) during a period of 3 min after standing (*10*). Although OH is known to be one of the underlying causes of falls (*1*, *2*), it has also been shown to be an independent risk factor for cognitive dysfunction (*3*, *4*), cardiovascular disease, including stroke (*5*, *6*) and silent cerebral infarction (*7*), and mortality (*8*).

A growing body of evidence shows that the regulation of central blood pressure (BP), such as that in the aorta or carotid artery, is significantly different from that of peripheral BP, such as in the brachial artery (*11–14*). A change in brachial BP does not accurately reflect that in central BP in several conditions, such as smoking (*12*), caffeine intake (*13*) and alcohol intake (*14*). Recently, it has also been reported that squatting is associated with a larger change in aortic BP than in brachial BP (*15*). Based on these observations, it is conceivable that the orthostatic change in brachial BP does not accurately reflect that in central BP.

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In central arteries, including the carotid artery, the pressure waveform consists of two components, the forward or incident component, and the backward or reflection component (*11*, *16*). Early return of the backward reflected pressure wave due to advanced atherosclerosis would result in augmentation of central SBP, which is one of the underlying mechanisms of systolic hypertension (*17*). Since the prevalence of OH has been reported to be higher in subjects with isolated systolic hypertension (*18*), OH may be associated with an orthostatic alteration of the reflection component of central BP.

Based on this background, we hypothesized that 1) the orthostatic change in brachial BP does not accurately reflect that in carotid BP, and 2) standing is associated with an alteration of the reflection of the pressure wave, which precipitates OH in the central arteries. To evaluate these hypotheses, the orthostatic change in carotid BP was measured using a tonometric method in community-dwelling elderly subjects free from any cardiovascular complications and medications. The arterial pressure waveform in the carotid artery was evaluated before and after standing, to examine which component of the pressure waveform is more affected in response to standing.

Methods

Study Subjects

The study subjects were participants in the cardiovascular examination of the Shimanami Health Promoting Program study (J-SHIPP) to investigate factors relating to cardiovascular disease, dementia, and death (*19*). One hundred and fifty-five subjects, aged 50 years or older with no known history or symptoms of cardiovascular disease such as stroke, transient ischemic attack, myocardial infarction, angina, congestive heart failure, or peripheral vascular disease, were enrolled in this study (mean age 68.6±6.8 years; 37 males and 118 females). They were also free from any medications. Informed consent to the procedure was obtained from each subject. All procedures were approved by the ethical committee of Ehime University School of Medicine.

Measurement of Carotid and Brachial Arterial Pressure

Brachial SBP and carotid SBP were measured using a volume-plethysmographic apparatus (form PWV/ABI; Colin Co., Ltd., Komaki, Japan). The subjects were examined in the supine position, with electrocardiogram electrodes placed on both wrists, a microphone for detecting heart sounds placed on the left sternal edge, and a brachial cuff wrapped around a plethysmographic sensor that determines the volume pulse waveform and an oscillometric pressure sensor that measures BP. Pulse volume waveforms for the brachium were recorded for 10 s using a semiconductor pressure sensor with automatic gain analysis and quality adjustment. The components over 5 Hz were stored using a pass-filter, and the wave characteristic points were automatically determined (*20*).

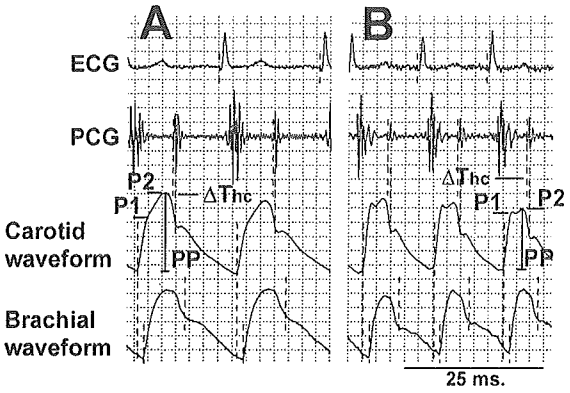

Fig. 1. *Representative tracings of orthostatic changes in brachial and carotid arterial waveforms. Two cardiograms and two waveforms, one for the carotid and one for the brachial arterial waveform, were recorded simultaneously. A shows the waveforms observed in the supine position, and B those after standing up. The dotted line indicates the characteristic points of each waveform and cardiogram. Carotid pressure waveform was analyzed according to the definition of Murgo* et al. *(16), and the augmentation index (%) was obtained using the equation: (P2–P1)×100/PP. In this example, the augmentation index of the carotid arterial waveform was reduced from 29% to 1%. PCG, phonocardiogram. The vertical scale is relative.*

The common carotid arterial pressure waveform was also simultaneously recorded noninvasively using a multi-element tonometric sensor (Fig. 1). The multi-element tonometry sensor consists of 15 sensitive micro-sensors within a width of 19 mm, and it automatically selects the most appropriate waveform from 15 detected pulse waveforms. The tonometry sensor for the carotid artery was fixed on the neck by means of a clip. The length of the clip-arm, angle and hold-down pressure of the sensor head were adjusted for each subject for optimal pressure wave detection. The accuracy and reproducibility of the apparatus has been reported elsewhere (*21*).

The carotid pressure wave was recorded by calibrating the brachial pressure wave, assuming that the mean BP (MBP) was the same at both sites (*22*). For this purpose, the MBP of the carotid pressure wave was computed and set equal to the brachial MBP in the corresponding heart beat. The carotid pressure amplitude was then computed from the DBP and the position of the MBP in the carotid pressure wave. Carotid SBP was calculated proportionally at the peak of the carotid arterial waveform. Invasive validation and reproducibility of measurements have been published in detail previously (*22*). The brachial SBP and carotid SBP were averaged for a series of waves over a 10-s period. Orthostatic systolic hypotension (OSH) was defined as a more than 20 mmHg decline in SBP after standing (*10*).

Table 1. Orthostatic Change in Blood Pressure in Total Population

	Supine	Standing	*p*
Brachial SBP (mmHg)	129±19	131±20	0.048
Carotid SBP (mmHg)	124±22	123±23	0.588
Carotid PP (mmHg)	46±15	41±17	<0.001
Incident component (mmHg)	31±10	30±12	0.143
Reflection component (mmHg)	16±10	12±9	<0.001
MBP (mmHg)	102±15	105±15	<0.001
DBP (mmHg)	78±11	82±11	<0.001
Heat rate (beats/min)	69±11	80±12	<0.001
Augmentation index (%)	31±16	25±18	<0.001

Values are mean±SD. SBP, systolic blood pressure; PP, pulse pressure; DBP, diastolic blood pressure; MBP, mean blood pressure.

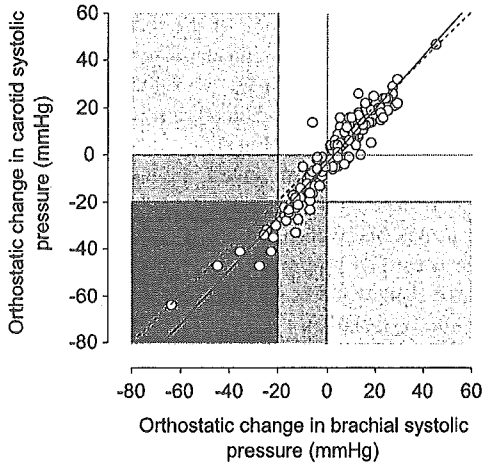

Fig. 2. *Correlation between orthostatic changes in carotid blood pressure and brachial blood pressure. The solid line is the regression line, and the dotted line is the identity line between the two variables.*

Carotid Augmentation Index (AIx)

Carotid BP at the inflection and peak of the waveform, and the time intervals of the first and second systolic peaks were obtained. The AIx was calculated as the ratio of the augmented pressure to the pulse pressure (PP) (*11*, *16*) (Fig. 1). The carotid PP was divided into two components, the backward reflection component of PP (carotid PP × AIx) and the incident component of PP (carotid PP – reflection component). The time interval between the dicrotic notch of the carotid waveform and the second heart sound of the phonocardiogram was defined as the time interval between the carotid artery and heart (ΔT_{hc}). Figure 1 shows actual tracings of the carotid and brachial pressure waveform, and the determination of AIx.

Orthostatic Changes in BP

After obtaining basal parameters in the supine position for more than 10 min, the subject was asked to stand. A brachial cuff placed around the right brachial arm was kept at the level of the heart. One minute after standing, measurements of all parameters were repeated while the subject was in the standing position. The carotid pressure wave was re-calibrated by the brachial pressure wave. Orthostatic changes in brachial SBP (Δbrachial SBP), carotid SBP (Δcarotid SBP) and AIx (ΔAIx) were obtained (Fig. 1). The percentage of orthostatic changes in the two components of carotid PP was also obtained. The percentage of orthostatic change in the reflection component of carotid PP was defined as –100% in the case that carotid AIx changed from positive to negative after standing.

Statistical Analysis

All values are expressed as the mean±SD unless otherwise specified. All analyses were performed using the SPSS software package (SPSS Inc., Chicago, USA). A probability value of less than 0.05 was considered statistically significant.

Results

Changes in brachial and carotid BP and heart rate (HR) in response to standing are summarized in Table 1. The MBP and DBP, as well as HR, were significantly increased after standing up. No significant changes were observed in carotid SBP. However, in a separate analysis, the reflection components of carotid PP showed a significant reduction, resulting in a decreased carotid PP.

Figure 2 depicts the relationship between Δbrachial SBP and Δcarotid SBP. Among all subjects, 9 (5.8%) were diagnosed as having OSH based on the change in brachial SBP, while 21 (13.5%) were diagnosed as having OSH when evaluated from the carotid SBP. The prevalence was significantly different between the two measurements (χ^2=61.0, p<0.001). In the 9 OSH patients determined by brachial BP,