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H. 知的財産権の出願・登録状況

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厚生労働科学研究補助金（長寿科学総合研究事業）
分担研究報告書

インスリン抵抗性感受性遺伝子多型の探索

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研究要旨
メタボリックシンドロームの病態基盤であるインスリン抵抗性をターゲットとし、既知の高血圧等感受性遺伝子多型との相関を検討した。アンジオテンシン変換酵素 I/D 多型、アンジオテンシノーゲン M235T 多型、メチレンテトラヒドロ葉酸還元酵素 C677T 多型、Gタンパク質β３サブユニット C825T 多型、Gタンパク質αサブユニット T393C 多型について HOMA 指数、アディポネクチン、高感度 CRP との関連を検討したが、いずれも有意な相関は認められなかった。これより、これら遺伝子多型はインスリン抵抗性の遺伝的背景とはならないことが示された。

A．研究目的

メタボリックシンドロームとは、内臓脂肪蓄積、糖尿病、高脂血症、高血圧症などの動脈硬化危険因子が重複した病態をいい、将来の心血管系疾患のリスク因子となることが知られている。メタボリックシンドロームには幾つかの基準が存在するが、米国コレステロール教育プログラムⅢでは、肥満、高血糖、高中性脂肪、低 HDL コレステロール、高血圧の集積と定義している。メタボリックシンドロームは、内臓脂肪型肥満、インスリン抵抗性を基礎病態とする心血管系疾患の高リスク状態と言い換えることができる。

インスリン抵抗性と密接に関連して、脂肪細胞由来ホルモンの異常も注目されている。脂肪組織は単にエネルギー（脂肪）を蓄積する臓器であるだけでなく、種々の生理活性物質（アディポサイトカイン）を分泌し、全身の代謝調節や恒常性保持を行っている。しかし、肥満すなわち脂肪細胞の肥大化が起こると、アディポサイトカイン産生異常（インスリン感受性因子の減少とインスリン抵抗性因子の増加）が生じ、これがメタボリックシンドロームの中心的役割を果たしているものとも考えられる。

その一方で、遺伝的な背景もメタボリックシンドロームの発症に関与していると考えられている。従来、高血圧や糖尿病をターゲットとして多くの感受性遺伝子解析研究が行われてきたが、一定した見解は得られていない。その原因として、高血圧などの生活習慣病は多因子疾患であり、疾患発症・進展には遺伝因子以外にも多くのファクターが関与していることから、個々の影響力が弱い遺伝因子と疾患とを直接的に検討することが困難であることが挙げられる。そのため、共通の病態基盤であるインスリン抵抗性やアディポサイトカインを中間形質とした感受性遺伝子解析が必要とされてきた。しかし、これまでに大機母集団を対象として、これら中間形質の遺伝的背景を検討した例は見当たらない。

そこで本研究では、大規模一般地域住民を対象とし、従来から高血圧や糖尿病などの感受性遺伝子として考えられてきた候補遺伝子多型との関連について検討を行った。

B．研究方法

対象は、愛媛県下でコホート設定しているＡ町住民のうち、老人保健法に基づく一般住民検診を受診し、かつ本研究の趣旨に同意の得られた約 3000 例とした。対象者の一般臨床検査所見は健診時の検査値を利用した。加えて、インスリンや高感度 CRP、アディポネクチンなどのインスリン抵抗性マーカーを、末梢血を用いて独自に測定した。測定したインスリン値は、空腹時血糖値とから HOMA 指数を算出して解析に用いた。

対象者の DNA は、末梢血より定法に則って抽出した。抽出した DNA は、DOP-PCR 法により増幅してから分析に供した。解析した遺伝子多型を表１に示した。いずれも従来の研究から、高血圧や動脈硬化の候補遺伝子として報告されている遺伝子多型である。遺伝子多型の解析は、TaqMan プロ

ーブ法で行った。解析に用いたプライマー/プローブの配列を表２に示した。なお、アンジオテンシン変換酵素（ACE）の挿入/欠失（I/D）多型については、PCR-RFLP 方で解析した。

（倫理面への配慮）
愛媛大学医学部ヒトゲノム・遺伝子解析研究倫理委員会において、研究課題名「生活習慣病と動脈硬化性疾患の発症要因の探索」、「生活習慣病における個別化医療を目指した研究」「遺伝子多型解析による個別化医療を目指した研究」として承認を得ている。対象者には、事前に十分な主旨説明の上、書面にて同意を得た。個人情報は、所定の手続きに則って匿名化し管理している。これらはヒトゲノム・遺伝子解析研究に関する倫理指針を遵守したものであり、本研究における倫理面への配慮は十分であると判断した。

表１　解析した遺伝子多型

遺伝子	シンボル	多型	rs 番号
アンジオテンシン変換酵素	ACE	I/D	
アンジオテンシノーゲン	AGT	M235T	rs699
メチレンテトラヒドロ葉酸還元酵素	MTHFR	C677T	rs2274976
Ｇタンパク質β３サブユニット	GNB3	C825T	rs543
Ｇタンパク質αサブユニット	GNAS	T393C	rs7121

表２　解析に用いたプライマー/プローブの配列

遺伝子	プローブ	プライマー
アンジオテンシノーゲン	FAM-CTCCCTGACGGGAG-MGB	5'-GGCTGTGACAGGATGGAAGACT-3'
	VIC-CTCCCTGATGGGAG-MGB	5'-AAGTGGACGTAGGTGTTGAAAGC-3'
メチレンテトラヒドロ葉酸還元酵素	FAM-TGCGGGAGTCGAT-MGB	5'-GACCTGAAGCACTTGAAGGAGAAG-3'
	VIC-CGGGAGCCGATTT-MGB	5'-AAAGAAAAGCTGCGTGATGATG-3'
Ｇタンパク質β３サブユニット	FAM-CACACTCAGGATGTAGT-MGB	5'-CCTGACCGCTTTGCTAAATCA-3'
	VIC-CACACTCAGAATGTAGT-MGB	5'-AAGTCAAAGTCAGGCACGTTCA-3'
Ｇタンパク質αサブユニット	FAM-CTCCCTGACGGGAG-MGB	5'-GGCTGTGACAGGATGGAAGACT-3'
	VIC-CTCCCTGATGGGAG-MGB	5'-AAGTGGACGTAGGTGTTGAAAGC-3'

C．研究結果

ACE 遺伝子 D 多型は、アディポネクチン濃度と有意水準５％で相関したが（表３）、多重比較を補正するとこの相関は有意とはいえなかった。同様に、検討した遺伝子多型はいずれのインスリン抵抗性マーカーとも有意に相関しなかった。（表４～７）。

D．考察

ACE 遺伝子多型の欠失型は、以前より高血圧や糖尿病などのリスク因子となることが多くの成績から報告されてきた。反面、相関を否定する報告も同数程度あり、結果の解釈に一定した見解は得られていない。その一因として、メタボリックシンドロームや生活習慣病が多因子疾患であることが挙げられる。そこで本研究では、より遺伝因子の影響を受けやすいインスリン抵抗性を中間形質として、種々の血中マーカーと従来の疾患感受性候補遺伝子多型との関連を検討した。その結果、いずれの遺伝子多型もインスリン抵抗性の危険因子とならない可能性が示された。

今後、独立した集団で本成績を再検証するとともに、環境因子との相互作用を十分に加味した検討を行うことで、本研究成果の一般化を進めていく必要がある。また、今回検討した遺伝子以外の候補遺伝子についても同様に検討を進めていく必要があると思われる。

表３　ACE 遺伝子多型との相関

	遺伝子多型	N	平均	標準偏差	p
血糖値（mg/dl）	II	890	98.0	21.7	0.681
	ID	967	98.8	21.3	
	DD	292	98.0	20.7	
HbA1C	II	208	5.87	1.27	0.999
	ID	240	5.87	1.27	
	DD	72	5.88	1.34	
HOMA 指数	II	863	1.68	1.51	0.732
	ID	950	1.72	1.90	
	DD	285	1.63	1.33	
アディポネクチン（ug/ml）	II	872	5.98	4.26	0.016
	ID	953	5.92	4.06	
	DD	289	6.73	5.12	
高感度ＣＲＰ（mg/dl）	II	823	0.11	0.18	0.051
	ID	905	0.09	0.16	
	DD	272	0.10	0.17	

表４　AGT 遺伝子多型との相関

	遺伝子多型	N	平均	標準偏差	p
血糖値（mg/dl）	MM	69	96.7	21.6	0.776
	MT	610	98.1	22.5	
	TT	1392	98.4	20.9	
HbA1C	MM	16	5.70	1.14	0.842
	MT	139	5.90	1.37	
	TT	341	5.88	1.27	
HOMA 指数	MM	68	1.57	1.32	0.632
	MT	600	1.64	1.56	
	TT	1362	1.70	1.66	
アディポネクチン（ug/ml）	MM	68	6.19	4.13	0.186
	MT	604	5.79	3.96	
	TT	1373	6.17	4.47	
高感度ＣＲＰ（mg/dl）	MM	65	0.11	0.18	0.970
	MT	572	0.10	0.17	
	TT	1297	0.10	0.17	

表５　MTHFR 遺伝子多型との相関

	遺伝子多型	N	平均	標準偏差	p
血糖値（mg/dl）	CC	832	97.8	20.0	0.154
	CT	933	97.9	19.6	
	TT	330	100.3	27.5	
HbA1C	CC	203	5.78	1.15	0.230
	CT	217	5.88	1.18	
	TT	85	6.06	1.66	
HOMA 指数	CC	817	1.71	1.65	0.939
	CT	913	1.68	1.75	
	TT	320	1.70	1.57	
アディポネクチン（ug/ml）	CC	821	6.15	4.33	0.725
	CT	921	6.00	4.23	
	TT	324	5.99	4.52	
高感度ＣＲＰ（mg/dl）	CC	787	0.11	0.18	0.190
	CT	862	0.10	0.15	
	TT	306	0.10	0.17	

表６　GNB3 遺伝子多型との相関

	遺伝子多型	N	平均	標準偏差	p
血糖値（mg/dl）	CC	517	98.3	20.6	0.952
	CT	1078	98.1	21.1	
	TT	521	98.4	21.8	
HbA1C	CC	141	5.82	1.19	0.889
	CT	246	5.87	1.33	
	TT	123	5.88	1.22	
HOMA 指数	CC	506	1.75	1.82	0.648
	CT	1059	1.66	1.57	
	TT	506	1.70	1.77	
アディポネクチン（ug/ml）	CC	510	5.82	4.38	0.310
	CT	1068	6.17	4.27	
	TT	509	6.08	4.33	
高感度ＣＲＰ（mg/dl）	CC	482	0.11	0.19	0.592
	CT	1006	0.10	0.16	
	TT	486	0.10	0.17	

表７　GNB3 遺伝子多型との相関

	遺伝子多型	N	平均	標準偏差	p
血糖値 (mg/dl)	TT	670	97.3	18.2	0.102
	TC	1036	99.3	23.2	
	CC	412	97.3	21.0	
HbA1C	TT	168	5.77	0.98	0.475
	TC	260	5.92	1.42	
	CC	85	5.90	1.32	
HOMA 指数	TT	650	1.71	2.02	0.562
	TC	1019	1.72	1.54	
	CC	405	1.62	1.41	
アディポネクチン (ug/ml)	TT	656	6.07	4.31	0.916
	TC	1026	6.03	4.35	
	CC	408	6.14	4.18	
高感度ＣＲＰ (mg/dl)	TT	624	0.11	0.18	0.347
	TC	970	0.10	0.16	
	CC	384	0.10	0.17	

Ｅ．結論

アンジオテンシン変換酵素 I/D 多型、アンジオテンシノーゲン M235T 多型、メチレンテトラヒドロ葉酸還元酵素 C677T 多型、Ｇタンパク質β３サブユニット C825T 多型、Ｇタンパク質αサブユニット T393C 多型は、HOMA 指数、アディポネクチン、高感度 CRP を指標としたインスリン抵抗性の遺伝的背景とはならないことが示された。

Ｆ．健康危険情報

なし

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H. 知的財産権の出願・登録状況

なし

別添６

研究成果の刊行に関する一覧表

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研究成果の刊行物・別刷

An Association of 5,10-Methylenetetrahydrofolate Reductase (*MTHFR*) Gene Polymorphism and Ischemic Stroke

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Plasma homocysteine (Hcy) concentration has been shown to be influenced by a mutation in the gene coding methylenetetrahydrofolate reductase (*MTHFR*). Although plasma Hcy has been shown to be related to atherosclerotic disorders, the association between *MTHFR* gene polymorphism and ischemic stroke remains controversial. In the present study we investigated the association between *MTHFR* gene polymorphism and risk factor–dependent augmentation for ischemic stroke in subjects with several risk factors for atherosclerosis, with special emphasis on the risk factor–gene interaction. The diagnosis of cerebral infarction in each patient was confirmed by computed tomography (CT) findings of the brain. *MTHFR* C677T polymorphism was genotyped with a conventional method. In 97 stroke patients (48 cases of atherothrombotic infarction, 38 cases of lacunar infarction, 9 cases of cardiac embolism, 2 others) and 241 age-and sex-matched healthy control subjects, the frequencies of the T allele were 0.44 and 0.39, respectively. In patients with CT-proven atherothrombotic infarction, the T allele frequency was 0.54 ($P = .033$ vs controls). The adjusted odds ratio in subjects with TT genotype for atherothrombotic infarction was 3.87 (95% confidence interval = 1.27–11.8). A general linear model analysis showed that an interaction between the HDL-C and *MTHFR* genotype was significantly associated with atherothrombotic infarction ($F = 5.695$, $P = .018$). These findings indicate that the T allele of the *MTHFR* gene is significantly associated with atherothrombotic infarction. Furthermore, the analysis of risk factor–gene interaction could be a useful tool for deriving specific predictive information about ischemic stroke in an elderly Japanese population. **Key Words:** Risk factor—gene—interaction—homocysteine—polymorphism.

Homocysteine (Hcy) is a sulfur-containing amino acid generated as an intermediate product in methionine metabolism. Hyperhomocysteinemia has been substantiated as a risk factor for occlusive vascular disease in patients with cerebral, coronary, or peripheral arterial diseases.[1-4] Meta-analyses have revealed a consistent association between the plasma level of Hcy and atherosclerotic disorders.[2] Recent studies have suggested that the risk of ischemic stroke is increased in subjects with even slightly elevated Hcy concentrations, previously considered to be within the normal range.[3] Hcy can be transsulfurated to form cysteine or remethylated to form methionine. The latter reaction uses 5-methyltetrahydrofolate as a carbon donor; 5-methyltetrahydrofolate is synthesized from 5,10-methylenetetrahydrofolate through the action of the methylenetetrahydrofolate reductase gene (*MTHFR*), which locates in endothelium or smooth muscle cell. Mutations in the gene coding for both of these enzymes leads to group of disorders in which marked elevation of circulating Hcy was observed.

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There have been numerous genetic association studies of the *MTHFR* C677T variant, particularly in the homozygous state, which have shown both the presence[5-8] and the absence[5,9-16] of significant associations of such end points as coronary heart disease, myocardial infarction, and cerebrovascular disease. Although there are several subtypes in ischemic stroke, atherothrombotic infarction has been reported to be associated with Hcy.[11-13] Accordingly, the subtype of ischemic stroke enrolled in positive studies is thought to be mainly the atherothrombotic type. Recently, we and others observed that risk factor–gene interaction could have an influence on hypertension or carotid atherosclerosis.[16-20] It is also conceivable that the conflicts between *MTHFR* and atherosclerosis may suggest an interaction between of risk factor and gene. Furthermore, the studies of *MTHFR* polymorphism lack statistical power, and a *MTHFR* genotype may even modulate cardiovascular disease risk independently of Hcy.[2] We hypothesized that risk factor-dependent evaluation of the effect of *MTHFR* on a specific subtype of ischemic stroke could provide new information on the genetic predisposition to ischemic stroke. To address this hypothesis, we investigated the association between *MTHFR* polymorphism and subtype of ischemic stroke in elderly subjects with several risk factors for atherosclerosis, with special emphasis on the risk factor–gene interaction.

Materials and Methods

Subjects

The acute ischemic stroke patients defined with following criterion were consecutively enrolled from inpatients in the Internal Medicine Department of Nomura Municipal Hospital between August 1999 and December 2000. Patients with cerebral hemorrhage were not included. A total of 97 patients were enrolled in the study. A total of 241 control subjects with no clinical history of cerebrovascular disease or present neurologic abnormalities were randomly recruited at the time of annual health examination in Nomura-cho, in which Seiyo Municipal Nomura Hospital is a community hospital. Informed consent for the procedure was obtained from each patient. All procedures were approved by Ethics Committee of Ehime University School of Medicine.

Definition of Acute Cerebral Infarction

Clinical syndrome of ischemic stroke was defined as a rapidly developing clinical symptoms and/or signs of focal and at times global loss of brain function, with symptoms or leading to earlier death and with no apparent cause other than that of vascular origin. Acute ischemic stroke was defined as an acute onset of stroke with normal computed tomography (CT) brain findings followed by sequential change in CT lesions. A recent infarct in the clinically relevant area of the brain with CT scan evidence was also included. Patients were divided according to clinical diagnosis and CT findings into 4 groups: those with atherothrombotic infarction, those with lacunar infarction, those with cardiac embolism, and those unclassifiable.[21]

Evaluation of Risk Factors

Information on demographic characteristics and risk factors was collected from clinical files on all cases. Subjects with hypertension were defined as those who had previously been treated for hypertension. Subjects were classified as diabetic when they were being treated for diabetes mellitus. A smoker was defined as a subject with a pack-years index ≥10 with pack-years defined as packs of cigarettes per day multiplied by years smoked. In the blood biochemistry analyses, total cholesterol (T-C), triglycerides (TG), and High-density lipoprotein cholesterol (HDL-C) were measured after fasting within 24 hours after admission. Low-density lipoprotein cholesterol (LDL-C) level was calculated by the Friedewald formula. The use of antilipidemic drugs was assessed.

MTHFR *Genotype Analysis*

Genomic DNA was extracted from peripheral blood lymphocytes using standard procedures.[22] The DNA sample were subjected to amplification by polymerase chain reaction (PCR), and the restriction enzyme HinfI was used to identify those with the mutation, as described by Frosst et al.[23] The PCR reaction generated a fragment of 198 bp that contained codon 677. The point substitution of T for C at codon 677 created a HinfI recognition sequence with resulting 175- and 23-bp fragments. Alanine-coding alleles therefore produced a 198-bp fragment that was easily distinguished from the 175-bp fragment generated by valine alleles. Electrophoresis in a 4% agarose gel followed by ethidium bromide staining and UV illumination allowed detection of mutation alleles.

Statistical Analysis

Statistical analysis was performed using SPSS 10.0J (SPSS, Chicago, IL). The prevalence among the ischemic stroke and control subjects was compared using the χ^2 test. The differences among groups were analyzed using the Mann-Whitney *U*-test. The relation between ischemic stroke and risk factors, including genotype, were examined by logistic multiple regression analysis. A general linear model was used to evaluate the significant contribution of risk factor–gene interactions to ischemic stroke. A *P* value <.05 was considered significant.

Table 1. *Baseline characteristics of control subjects and ischemic stroke patients*

Characteristics	Control (n = 241)	Ischemic stroke (n = 97)	*P*
Age	76 ± 7.1	78 ± 8.3	.081
≥ 75 years, n (%)	152 (63.1)	65 (67.0)	.532
Gender, male, n (%)	118 (49.0)	57 (58.8)	.118
Smokers, n (%)	60 (24.9)	42 (43.3)	.001
History of hypertension, n (%)	101 (41.9)	60 (61.9)	.001
History of diabetes mellitus, n (%)	41 (17.0)	26 (26.8)	.050
Antilipidemic drug use, n (%)	15 (6.2)	2 (2.1)	.168
Total cholesterol (mg/dL)	205.7 ± 31.9	181.1 ± 37.8	<.001
HDL cholesterol (mg/dL)	61.0 ± 14.8	47.7 ± 15.5	<.001
LDL cholesterol (mg/dL)	122.4 ± 28.4	114.2 ± 32.0	.011
Triglyceride (mg/dL)	111.3 ± 65.8	95.9 ± 45.7	.023

HDL, high-density lipoprotein; *LDL*, low-density lipoprotein.

Results

Background of Subjects

The baseline characteristics of the patients and control subjects are given in Table 1. The patients had a significantly higher prevalence of smokers and were more likely to have a history of hypertension (well-known major risk factors for stroke) than the control subjects. The patients had not only significantly lower mean HDL-C levels than controls, but also significantly lower mean T-C, LDL-C, and TG levels. There were no intergroup differences in age, gender, history of diabetes mellitus, or prevalence of antilipidemic drug use.

Distribution of MTHFR *Genotypes in Control Subjects and Ischemic Stroke Patients*

The distribution of the *MTHFR* genotypes in both control subjects and the patient groups is given in Table 2. The genotype distributions of both groups were in agreement with Hardy-Weinberg equilibrium, and the distribution of *MTHFR* genotypes was consistent with a published report in Japanese subjects.[6] The allele frequency of the T mutation was not significantly higher in the ischemic stroke patients compared with the control subjects. The association of the mutation with ischemic stroke was further studied in terms of the subtypes of stroke as well as CT findings of the lesions, and we found 48 cases of atherothrombotic infarction, 38 cases of lacunar infarction, 9 cases of cardiac embolism, and 2 others. The χ^2 test demonstrated a significant difference ($P = .033$) between the atherothrombotic infarction patients and control subjects in the distribution of the *MTHFR* genotype, suggesting a codominant effect of the T allele on the risk of atherothrombotic infarction.

Odds Ratio of Subtypes of Ischemic Stroke Associated With MTHFR *Genotypes*

In ischemic stroke patients with CT-proven atherothrombotic infarction, the T-allele frequency was 0.54 (Table 2). The unadjusted odds ratio (OR) and 95% confidence interval (CI) for atherothrombotic infarction are summarized in Table 3. Subjects with TT genotype as well as T carriers had a significant risk for atherothrombotic infarction even after adjustment with other known risk factors (Table 3).

Table 2. *Distribution of MTHFR genotypes in control subjects and ischemic stroke patients*

	n	MTHFR genotype: CC, n (%)	CT, n (%)	TT, n (%)	C/T frequency	*P**
Control	241	91 (37.8)	110 (45.6)	40 (16.6)	0.61/0.39	—
Ischemic stroke	97	33 (34.0)	43 (44.3)	21 (21.6)	0.56/0.44	.530
Atherothrombotic infarction	48	10 (20.8)	24 (50.0)	14 (29.2)	0.46/0.54	.033
Lacunar infarction	38	17 (44.7)	15 (39.5)	6 (15.8)	0.65/0.35	.703
Cardiac embolism	9	6 (66.7)	2 (22.2)	1 (11.1)	0.78/0.22	.213
Other	2		2 (100)			

*χ^2 statistic test versus control group.

	Atherothrombotic Infarction	*P*-value
	Unadjusted OR (95% CI)	
CT vs CC	1.98 (0.90-4.37)	.094
CT + TT vs CC	2.31 (1.10-4.85)	.031
TT vs CC	3.19 (1.31-7.78)	.011
	Adjusted OR (95% CI)	
CT vs CC	2.30 (0.95-5.56)	.066
CT + TT vs CC	2.53 (1.10-5.82)	.030
TT vs CC	3.87 (1.27-11.8)	.017

CI, confidence interval.

Adjusted for age, gender, smoking habits, history of hypertension, history of diabetes mellitus, antilipidemic drug use, HDL cholesterol, and LDL cholesterol.

Risk of Atherothrombotic Infarction in Subjects With MTHFR *T Carriers (CT + TT) According to Age, Gender, and Other Vascular Risk Factors*

We examined the possible synergistic effect between *MTHFR* T carriers and conventionally known risk factors in both control and atherothrombotic infarction subjects. Table 4 presents the risk of atherothrombotic infarction in subjects with *MTHFR* T carriers stratified according to age, gender, and presence/absence of other vascular risk factors. In this subgroup, significant risks of atherothrombotic infarction were observed in subjects with *MTHFR* T carriers of age ≥ 75 years, female sex, nonsmoker, no history of diabetes mellitus, HDL-C < 40 mg/dL, and LDL-C < 130 mg/dL. A possible interaction between *MTHFR* T carriers and specific risk factors in atherothrombotic infarction was suggested.

MTHFR *Genotypes and Conventional Risk Factors for Atherothrombotic Infarction*

To find possible gene-risk factor interactions for atherothrombotic infarction, multiple regression analysis for atherothrombotic infarction was performed with risk factors in subjects with a specific genotype of *MTHFR* gene polymorphism (Table 5). It was shown that smoker and HDL-C were significantly associated with atherothrombotic infarction in subjects with *MTHFR* CC genotype, and HDL-C in *MTHFR* T carriers. Analysis of covariance failed to demonstrate significant differences between 2 regression lines in smoker and atherothrombotic infarction ($F[1, 285] = 0.698$, $P = .404$).

*Risk Factors–*MTHFR *Gene Interaction*

To further investigate whether the interaction between *MTHFR* genotype and the conventional risk factors could have any influence on atherothrombotic infarction, a general linear model for presence of atherothrombotic infarction was analyzed with the following parameters: gender, age, smoking, history of hypertension and diabetes mellitus, HDL-C, LDL-C, and *MTHFR* gene polymorphism, including interactions between risk factor and *MTHFR* genotype (Table 6). This analysis revealed that interaction between HDL-C and *MTHFR* gene polymorphism ($F[1,275] = 5.695$, $P = .018$) was significantly associated with atherothrombotic infarction.

Discussion

The present study examined the association between *MTHFR* gene polymorphism and etiologic subtypes of ischemic stroke in patients with risk factors, and revealed that the T allele of the *MTHFR* gene was significantly associated with CT-proven atherothrombotic infarction in a Japanese patient population. A multivariate analysis demonstrated that this association was independent of other risk factors, including age, gender, smoking, history of hypertension, and diabetes mellitus, HDL-C, and LDL-C. This analysis also revealed the influence of an HDL-C–*MTHFR* gene interaction on atherothrombotic infarction.

The association between plasma Hcy concentration and atherosclerosis has been the subject of a number of clinical studies.[24,25] The British Regional Heart Study Cohort investigators reported that the mean Hcy level in 107 stroke patients was 13.7 μmol/L (95% CI = 12.7–14.8), a significantly higher level than the mean 11.9 μmol/L (95% CI = 11.3–12.6) in 118 control subjects, and that the OR for stoke increased with increasing Hcy level.[25] Eikelboom et al[13] also reported that higher Hcy level was a strong and independent risk factor for ischemic stroke (adjusted OR = 2.7, 95% CI = 1.4–5.1 for a 5-μmol/L increase in fasting plasma Hcy from 10 to 15 μmol/L).

Although there are several etiologic subtypes in ischemic stroke, an association between atherothrombotic infarction and plasma Hcy levels has been reported.[7,12] The underlying pathophysiology of lacunar infarction is less well understood but appears to involve cerebral

Table 4. *Odds ratios for atherothrombotic infarction in subjects with MTHFR T carriers subcategorized with known risk factor for ischemic stroke.*

Characteristics	Total (n = 289)	Control (n = 241)	Atherothrombotic infarction (n = 48) OR (95% CI)	P
Age				
<75 years	68/108	55/89	13/19 1.34 (0.47-3.86)	.794
≥75 years	120/181	95/152	25/29 3.75 (1.24-11.3)	.017
Gender				
Male	96/148	74/118	22/30 1.64 (0.67-3.99)	.392
Female	92/141	76/123	16/18 4.95 (1.09-22.5)	.032
Smoker				
Yes	55/83	39/60	16/23 1.23 (0.44-3.46)	.798
No	133/206	111/181	22/25 4.63 (1.34-16.0)	.008
History of hypertension				
Yes	86/131	63/101	23/30 1.98 (0.78-5.06)	.190
No	102/158	87/140	15/18 3.05 (0.84-11.0)	.115
History of diabetes mellitus				
Yes	33/56	22/41	11/15 2.38 (0.65-8.70)	.231
No	155/233	128/200	27/33 2.51 (1.00-6.42)	.048
HDL cholesterol				
<40 mg/dL	25/38	8/17	17/21 4.78 (1.13-20.3)	.042
≥40 mg/dL	163/251	142/224	21/27 2.02 (0.78-5.21)	.199
LDL cholesterol				
< 130 mg/dL	119/187	89/150	30/37 2.94 (1.21-7.13)	.014
≥ 130 mg/dL	69/102	61/91	8/11 1.31 (0.32-5.30)	1.000

OR, odds ratio; *CI*, confidence interval.

to subcortical vascular encephalopathy.[27] Li et al[7] found in a case-control study of 1823 stroke patients (807 with cerebral thrombosis, 513 with lacunar infarction, and 503 with intracerebral hemorrhage) and 1832 controls that total plasma Hcy levels were significantly higher in cases than in controls (median, 14.7 vs 12.8 μmol/L; $P < .001$) and were associated with an increased risk of 1.87-fold (95% CI = 1.58–2.22) for overall stroke, 1.72-fold (95% CI = 1.39–2.12) for cerebral thrombosis, 1.89-fold (95% CI = 1.50–2.40) for lacunar infarction, and 1.94-fold (95% CI = 1.48–2.55) for intracerebral hemorrhage. Based on these observations, it has been accepted that Hcy is an independent risk factor for stroke, regardless of the type of ischemic stroke.

Because it has been reported that thermolabile *MTHFR*, which is thought to be associated with *MTHFR* T allele and reduced enzyme activity, accounts for 25% to 30% of elevated Hcy levels in patients with premature vascular disease,[28] it has been strongly suggested that *MTHFR* is one of the candidate genes for ischemic stroke. Li et al[7] reported that the TT genotype of *MTHFR* was associated with an increased risk for overall stroke (OR = 1.27; 95% CI = 1.04–1.56) and thrombotic stroke (OR = 1.37; 95% CI = 1.06–1.78). In the present study, our observation of an association between *MTHFR* polymorphism and atherothrombotic infarction are consistent with these results. But the nonsignificant relationship between the *MTHFR* polymorphism and ischemic stroke observed so far does not contradict the Hcy theory. One reason for this may be marked differences in nutritional status of the various subjects, because individuals with *MTHFR* TT genotype develop elevated Hcy only under conditions of impaired folate status.[29] In most studies, including our study, folate and Hcy concentrations were not measured,[26] and in several studies demonstrating no association between *MTHFR* and risk, the authors reported that their subjects were probably well nourished.[30] In contrast, in our study of ischemic stroke subjects who had a high prevalence of low T-C and low TG, the *MTHFR* TT genotype was a significant predictor of atherothrombotic infarction. Moreover, an association between *MTHFR* polymorphism and silent lacunar infarction was recently reported in a large Japanese general population.[8] The underlying pathophysiology of ischemic stroke due to microangiopathy is less well understood, but appears to involve microatheroma formation as well as lipohyalinosis.[31] However, if the putative deleterious effects of hyperhomocysteinemia are mediated primary via a proatherogenic effect, then it is plausible that Hcy is not as strong a risk factor for lacunar stroke caused by microangiopathy as it is for atherothrombotic infarction. Although we did not find a positive association, the sampling of cases and controls might be too small to reach a conclusion.[8]

This finding may indicate the difference in genetic background between atherothrombotic infarction and lacunar infarction. It is also conceivable that different risk factor–gene interactions could take place in the etiology of ischemic stroke subtypes. Because ischemic stroke is a multifactorial disorder including genetic predisposition in addition to these risk factors, risk factor–gene interaction could decrease or enhance the absolute risk in individual subjects in various ways. The risk factor–gene interactions may also account for the contradictory results of previous studies of *MTHFR* polymorphism and ischemic stroke. In the present study, we found that the presence of atherothrombotic infarction on HDL-C differed significantly between *MTHFR* genotypes.

Unfortunately, we have no clear explanation of how the interaction between HDL-C and *MTHFR* affects atherothrombotic infarction. However, Hcy auto-oxidation has been shown to support the oxidation of LDL through generation of the superoxide anion radical.[32,33] It has also been reported that Hcy and oxidized LDL have distinct effects on endothelial cell thrombogenicity.[34] It has also been reported that VLDL and LDL demonstrate a high binding capacity for Hcy.[35] These findings may indicate synergistic interaction between *MTHFR* and lipid metabolism in the development of atherosclerosis.

Table 5. *Multivariate linear regression analysis for atherothrombotic infarction with conventional risk factors and the MTHFR genotype*

Characteristics	CC genotype (n = 101)	CT + TT genotype (n = 188)	Total (n = 289)
Age	−0.100 (.302)	−0.014 (.836)	−0.031 (.573)
Gender	−0.052 (.654)	−0.056 (.508)	−0.049 (.467)
Smoker	0.275 (.018)	0.052 (.536)	0.128 (.079)
History of hypertension	0.177 (.061)	0.116 (.083)	0.132 (.034)
History of diabetes mellitus	0.179 (.060)	0.037 (.585)	0.099 (.066)
Antilipidemic drug use	0.123 (.195)	−0.096 (.145)	−0.029 (.584)
HDL cholesterol	−0.223 (.023)	−0.413 (<.001)	−0.352 (<.001)
LDL cholesterol	0.056 (.576)	−0.131 (.071)	−0.093 (.100)
MTHFR genotype	···	—	0.138 (.010)
r^2	0.215 (.003)	0.252 (<.001)	0.225 (<.001)

Values are the standard regression coefficients (*P* values). r^2, multiple coefficient of determination. An additive model (CC = 0, CT + TT = 1) was used for MTHFR genotype.

Table 6. *General linear model for atherothrombotic infarction*

Characteristics	F	P
MTHFR T carrier	4.477	.035
Gender, male	0.311	.578
Age	0.382	.537
Smoker	4.092	.044
History of hypertension	5.463	.020
History of diabetes mellitus	2.591	.109
HDL cholesterol	39.49	<.001
LDL cholesterol	2.272	.133
MTHFR T carrier*		
Smoker	2.012	.157
HDL cholesterol	5.695	.018

An additive model (CC = 0, CT + TT = 1) was used for MTHFR genotype.

The net effect of each interaction was estimated using a general linear model including MTHFR T carriers, each conventional risk factor, and *the interaction between MTHFR T carriers and the factor.

We need to be aware of the limitations in interpreting the present results. We can not deny that the relatively small number of patients with atherothrombotic infarction limits the credibility of the results and that subjects might have a subclinical ischemic stroke (silent lacunar stroke), because the majority of the control group was elderly (age 70 years or older). Silent lacunar infraction could likely reduce the difference between cases and controls and thereby bias the results of the study toward the null, especially negative association between *MTHFR* and lacunar infarction. These points need to be addressed in a large population with more precise phenotyping including control subjects.

In summary, we have reported a significant association between *MTHFR* gene polymorphism and atherothrombotic infarction in subjects with risk factors for atherosclerosis. Furthermore, an interaction between HDL-C and *MTHFR* was observed. These findings further support the idea that risk factor–gene interaction could allow us to determine specific predictive information about the development of atherosclerosis.

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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: 1.67, 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 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.

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/m²)	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/m²)	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.

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 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 ($p=0.829$).

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/m²)	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/m²)	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.

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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Radial Augmentation Index: A Useful and Easily Obtainable Parameter for Vascular Aging

Katsuhiko Kohara, Yasuharu Tabara, Akira Oshiumi, Yoshinori Miyawaki, Tatsuya Kobayashi, and Tetsuro Miki

Background: It has been shown that the systolic augmentation index (AI) in the central arteries, including the aorta and carotid arteries, changes with age. The AI can also be obtained from the peripheral arteries. The possible usefulness of AI obtained from the radial artery as an index for vascular aging was investigated.

Methods: Radial arterial waveforms were obtained from 632 subjects with no cardiovascular disease, using radial tonometry. Radial AI was calculated as follows: (Second peak systolic blood pressure [SBP2] − diastolic blood pressure [DBP]) / (first peak SBP − DBP) ×100 (%).

Results: Radial AI was significantly higher in women than in men (81.1% ± 16.1% compared with 69.5% ± 16.3%, $P < .001$). Radial AI was positively related to age in healthy men and women ($r = 0.619$, $P < .001$, and $r = 0.644$, $P < .001$, respectively). When comparing subjects in their 30s to those in their 70s, radial AI increased 1.56 times (from 53.2% to 83.0%) in men and 1.49 times (from 64.6% to 96.4%) in women. Multiple regression analysis showed that age is a potent predictor of radial AI in addition to gender, DBP and pulse rate.

Conclusions: These findings indicate that simple and easily-obtainable radial AI is age-dependent and could be a useful index of vascular aging. Am J Hypertens 2005; 18:11S–14S © 2005 American Journal of Hypertension, Ltd.

Key Words: Augmentation index, wave reflection, arterial stiffness, vascular aging.

Recently, several parameters have been introduced to assess vascular aging.[1–4] One of the parameters is systolic augmentation index (AI), which is obtained from the central arterial waveform as the ratio of augmentation pressure to the total pulse pressure.[1–3] Although many factors have been shown to affect AI, it has been shown that central AI is closely related to several risk factors for atherosclerosis[5–8] and for future cardiovascular events.[9,10] A close relationship between age and central AI has also been reported.[11] Because AI can also be obtained from radial arterial waveform,[1–3,12] radial AI itself could provide information on vascular aging. In the present study, we investigated the relationship of radial AI to age in healthy subjects free of cardiovascular disease.

Methods

Subjects

Subjects were selected among volunteers who agreed with the main purpose of this study and participated in either local resident or employee health examinations. A total of 632 subjects with no history of cardiovascular disease, diabetes, or hyperlipidemia were identified and included in the study. Anthropometric parameters, blood pressure (BP), and radial AI were measured in all participants. Written informed consent for the procedure was obtained from each subject, and the study was approved by the ethical committee of Ehime University School of Medicine.

Brachial BP and Pulse Wave Analysis

Blood pressure and radial AI were measured on the right upper arm using an oscillometric method after 5 min of rest in the sitting position (HEM-907; Omron Healthcare Co., Ltd., Kyoto, Japan). Immediately after measuring BP via the upper arm, the left radial arterial waveform was obtained using the tonometric method. Radial AI was calculated as follows: (Second peak systolic BP [SBP2] − diastolic BP [DBP]) / (first peak SBP − DBP) ×100 (%), which was automatically calculated using a fourth-order differential equation for radial arterial waveform (HEM-9010AI; Omron Healthcare Co., Ltd., Kyoto, Japan) (Fig. 1). The HEM-9010AI device is programmed to determine automatically the pressure against the radial artery to obtain the optimal radial arterial waveform. The SBP2 was also calculated by calibrating with brachial BP as an index of the absolute values of reflection pressure wave.

To investigate the association between radial AI and aortic AI, we also measured aortic AI using the transfer function developed for the SphygmoCor apparatus (AtCor Medical, NSW, Australia) in 154 subjects. Aortic AI was calculated as follows: (SBP2 − SBP1) / pulse pressure (PP) × 100 (%) (Fig. 1).

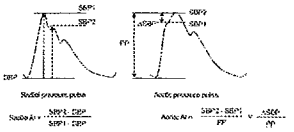

FIG. 1 An actual tracing of a radial arterial waveform. Radial augmentation index (AI) = (SBP2 − DBP)/(SBP1 − DBP) (%). DBP = diastolic pressure; PP = pulse pressure; SBP1 = first systolic blood pressure component; SBP2 = second systolic blood pressure component. Aortic AI is defined as (SBP2 − SBP1)/PP (%). ASBP = SBP2 −SBP1.

Data Analysis

All values are expressed as mean ± SD, if not specified. The difference between men and women was evaluated by analysis of variance. Multiple regression analysis for AI was performed with the following parameters: age, gender, brachial SBP, DBP, pulse rate, body height, body weight, and body mass index (BMI). All statistical analyses were performed using the SPSS statistical software package (SPSS Inc., Chicago, IL). A probability value of $P < .05$ was considered to be statistically significant.

Results

Table 1 summarizes characteristics of the study participants divided by sex. The mean age was 47.0 ± 15.4 years (range 20 to 82 years). The radial AI in men was significantly lower than that in women (69.5 ± 16.3 compared with 81.1 ± 16.1, $P < .001$). Figure 2 illustrates the

Table 1. Clinical characteristics of the study participants

	Male (n = 348)	Female (n = 289)	P value
AI	69.5 ± 16.3	81.1 ± 16.1	<.001
SBP	125.9 ± 16.1	117.0 ± 18.0	<.001
DBP	75.9 ± 12.6	69.6 ± 11.9	<.001
Pulse rate	70.6 ± 11.0	71.5 ± 9.5	NS
Height	169.0 ± 6.0	155.4 ± 6.4	<.001
Weight	65.8 ± 9.5	52.6 ± 7.7	<.001
BMI	23.0 ± 2.9	21.8 ± 3.0	<.001

AI = augmentation index; BMI = body mass index; DBP = diastolic blood pressure; SBP = systolic blood pressure.
Values are mean ± SD.

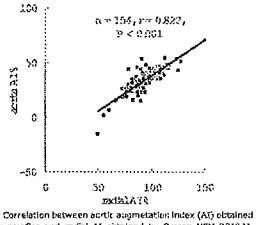

FIG. 2 Correlation between aortic augmentation index (AI) obtained by SphygmoCor and radial AI obtained by Omron HEM-9010AI. There was a highly significant correlation between radial AI and aortic AI.

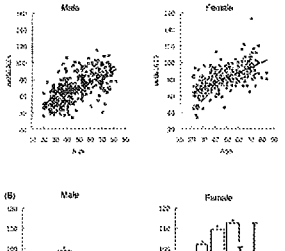

FIG. 3 Correlation between radial augmentation index (AI) and age (**A**) in men ($r = 0.619$, $P < .001$) and women ($r = 0.644$, $P < .001$). Age-related changes in radial AI in men and women are shown for each decade (**B**). Values are mean ± SD. *$P < .01$ between age groups.

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Table 2. Multiple regression analysis for radial augmentation index

	β	t	*P* value
Age (y)	0.420	13.513	<.001
Sex (female)	0.218	5.452	<.001
SBP (mm Hg)	−0.082	−1.779	.076
DBP (mm Hg)	0.375	8.608	<.001
Pulse rate (beats/min)	−0.410	−16.276	<.001
Height (cm)	−0.194	−1.100	.272
Weight (kg)	−0.041	−0.148	.882
BMI (kg/m²)	−0.080	−0.387	.699

Abbreviations as in Table 1.

correlation between radial AI obtained by HEM-9010AI and aortic AI determined by the SphygmoCor device. Radial AI showed a highly significant correlation with aortic AI ($r = 0.822$, $P < .001$).

Radial AI and Age

Figure 3 summarizes the relationship between radial AI and age. There was a significant positive correlation between age and radial AI for both men and women (men: $r = 0.619$, $P < .001$; women: $r = 0.644$, $P < .001$).

Age-related changes in radial AI, expressed as mean value in each decade of life, are also shown in Fig. 3. For men, there were significant age-dependent increases in radial AI from the 20s to the 50s. In women, an age-dependent increase in radial AI was observed from the 20s to the 60s.

Multiple regression analysis further revealed that radial AI was significantly associated with age in addition to pulse rate and DBP. However, body height, weight, BMI, and SBP were not associated with radial AI (Table 2).

Discussion

In the present study, we showed an age-related increase in radial AI in healthy men and women with no cardiovascular disease between 20 and 82 years of age. In a separate study, we evaluated the association between radial AI obtained from HEM-9010AI and aortic AI obtained by SphygmoCor. There was a highly significant and close association between AI obtained by two methods ($r = 0.822$, $P < .001$). This finding indicates the validity of radial AI semi-automatically obtained with HEM-9010AI, which detects the radial artery and determines the appropriate pressure of tonometry to obtain the most proper pressure waveform. The finding that radial AI significantly and closely associated with aortic AI obtained via transmission function is in accordance with the study by Millasseau et al.[12]

Several parameters have been shown to influence the AI, including body height,[13] heart rate,[14] postural change,[15] and gender.[16,17] Accordingly, it is generally necessary to take into account these confounding factors when assessing AI as an index for atherosclerosis. The difference in radial AI between men and women is consistent with previous findings.[13,16,17] Radial AI is higher in women than in men, and it has been suggested that the difference in body height is one of the underlying mechanisms.[13] However, the difference persists even after adjusting for body height. The smaller diameter of the radial artery and higher pulse wave velocity (PWV) in women has also been reported to account for the higher AI in women.[17]

The AI is determined by PWV, distance to the reflection point, and reflection coefficient.[1–3] The PWV increases with the age-related increase in arterial stiffness.[4] The reflection coefficient is influenced by area ratio (that is, the ratio of branches to parent vessel cross-sectional areas) and age. Atherosclerosis has been shown to be associated with a lower area ratio.[11] Body height, a simple estimate of the distance to the reflection point, diminishes somewhat with aging. These findings indicate that all factors defining AI are subject to age-dependent alterations.

In summary, the present study shows that radial AI could be an index of vascular aging. Because central AI reflects the difference between aortic BP and peripheral BP,[1–3] radial AI could also be used as an estimate of central BP. Recent guidelines also point out the potential usefulness in estimating central BP.[18] These findings further support the clinical usefulness of radial AI, a simple and easily obtainable parameter for vascular aging.

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Resistin SNP-420 determines its monocyte mRNA and serum levels inducing type 2 diabetes

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Abstract

Resistin, secreted from adipocytes, causes insulin resistance in rodents. Its roles and main source in humans remain unknown. The G/G genotype of resistin single nucleotide polymorphism, SNP-420, induces type 2 diabetes mellitus (T2DM) by increasing promoter activity. We elucidated factors correlated with serum resistin and effects of SNP-420 on monocyte resistin mRNA. In 198 T2DM and 157 controls, fasting serum resistin was higher in T2DM. Multiple regression analysis revealed that SNP-420 genotype was the strongest determinant of serum resistin. In T2DM, 1-year duration of T2DM and 1% HbA1c was also correlated with 0.19 and 0.54 ng/ml serum resistin, respectively. Logistic regression analysis revealed that serum resistin was an independent factor for T2DM. In 23 healthy volunteers, monocyte resistin mRNA was positively correlated with its simultaneous serum levels and was higher in G/G genotype. Thus, SNP-420 determines monocyte mRNA and serum levels of resistin, which could induce T2DM.

Keywords: Resistin; Insulin resistance; SNP; Polymorphism; Promoter; mRNA; Monocyte; Adipocyte; Type 2 diabetes; Serum level

Type 2 diabetes mellitus (T2DM) is characterized by insulin resistance in insulin target tissues, namely, adipose tissue, skeletal muscle, and liver [1]. T2DM is thought to be polygenic disease, the major genetic factors of which remain to be identified [2]. Single nucleotide polymorphisms (SNPs) in intensively analyzed genes such as peroxisome proliferator-activated receptor γ (PPARγ), calpain-10, and adiponectin have been reported to be associated with T2DM [3–5]. Most recently, we found that the resistin gene promoter SNP-420 is associated with T2DM [6].

Resistin (resistance to insulin) is secreted from adipocytes and antagonizes insulin both in vitro and in vivo [7–14]. Serum levels of resistin are increased in obese diabetic mice and are reduced by PPARγ ligands, suggesting that levels of this molecule are correlated with whole body insulin resistance [7]. Transgenic mice that overexpress *resistin* in the liver have high serum resistin levels and are insulin-resistant [11]. *Resistin* (−/−) mice show lower fasting blood glucose levels, which are increased by resistin injection [13]. A reduction in resistin gene expression by antisense oligonucleotides improves and enhancement by adenovirus encoding resistin worsens insulin resistance [15,16]. These findings suggest that higher serum resistin levels cause insulin resistance and diabetes in rodents whereas some other studies did not agree with this role of resistin [17].

A link between *resistin* and human T2DM has not been clarified [18–36]. Most recently, we found that the G/G genotype of resistin promoter SNP-420 is associated with T2DM susceptibility [6]. A meta-analysis involving three previous reports confirms this association. Sp1 and Sp3 transcription factors specifically bind to the DNA element including −420G and enhance promoter activity. Serum resistin levels are highest in this genotype. Therefore, the specific recognition of −420G by Sp1/3 increases resistin gene promoter activity, which induces insulin resistance and human T2DM through enhanced serum resistin levels.

The issue of whether serum resistin levels are altered in human T2DM or obesity remains controversial [26–31]. Lee et al. [28] showed that serum resistin levels are not associated with T2DM or obesity. McTernan et al. [29] and Youn et al. [31] reported that serum resistin levels are elevated in T2DM, but are not associated with obesity. Fujinami et al. [30] and Azuma et al. [27] found that serum resistin levels are increased in T2DM, and in obesity, respectively. In addition to the SNP-420 genotype, other factors affecting serum resistin levels remain to be elucidated.

In humans, resistin is rarely expressed in adipose tissues and is highly expressed in monocytes or macrophages [32–34], in contrast to its dominant expression in adipose tissues in mice [7]. Since macrophages infiltrating into adipose tissues could account for insulin resistance in obese mice [35–37], the pathophysiological relevance of resistin in human monocytes or macrophages merits further investigation. Whereas Smith et al. [38] showed that resistin mRNA levels are higher in adipose tissues of obese human subjects with the SNP-420 G/G genotype, the association of its levels in monocytes with the genotype should be pursued.

In view of this, we examined which clinical parameters are associated with serum resistin levels in addition to the SNP-420 genotype. Effects of SNP-420 on resistin mRNA levels in monocytes and its relation to serum resistin levels were also assessed in healthy volunteers.

Materials and methods

Subjects. We randomly selected 198 T2DM and 157 control subjects whose fasting serum and DNA were available. All these subjects were unrelated and were native Japanese. In this study, 93 T2DM subjects were recruited from the previous study [6]. Diabetes mellitus was diagnosed based on the American Diabetes Association criteria, as reported in 1998 [39]. These 157 control subjects were chosen based on HbA1c levels of less than 5.6%, fasting plasma glucose levels of less than 110 mg/dl, no history of diabetes, and no evidence of diabetes within first degree relatives. The clinical characteristics of these subjects are given in Table 1.

To assess resistin mRNA in monocytes, another 23 healthy volunteers [11 males and 12 females, means ± SD (range), age: 34.7 ± 5.2 (21–46) years; BMI: 20.6 ± 1.5 (18.1–23.9)] were employed. They had normal glucose tolerance, as assessed by 75 g oral glucose tolerance test with no evidence of diabetes within the first degree relatives. Their routine blood tests were within normal ranges.

All subjects were informed of the purpose of the study and their consent was obtained. The study was approved by the Ethics Committee of the Ehime University Hospital and Ehime Prefectural Hospital.

SNP typing. PCR direct sequencing was performed as described previously [6,20,40]. To type SNP-420, sequences of minus strands were checked using the preR primer and the other strand was also sequenced, when required. Taqman analysis was also employed for typing SNP-420. The probes used were VIC 5′-CATGAAGACGG AGGCC-3′ for −420C and FAM 5′-ATGAAGAGGGAGGCC-3′ for −420G. Forward and reverse primers are 5′-CCACCTCCTGACCAG TCTCT-3′ and 5′-AGCCTTCCCACTCCAACAG-3′, respectively.

Measurement of serum resistin levels. Serum resistin was measured using a human resistin ELISA kit (Linco Research), following the manufacturer's protocol [6]. We confirmed that linearity was maintained below 0.16 ng/ml, as described earlier [6].

Measurement of resistin mRNA levels in monocytes. Peripheral mononuclear cells were collected from whole blood samples using Lymphoprep Tube (AxisShield PoC AS, Oslo, Norway), and monocytes were isolated using an anti-CD14 antibody of the MACS system (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany) by following manufacturers' protocols. Total RNA was isolated from cell homogenates using an RNeasy Mini Kit (Qiagen Sciences, MD). Human resistin and internal control human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA levels were quantified using the two-step TaqMan RT-PCR method using an ABI PRISM 7700 following the manufacturer's protocol (Perkin-Elmer Applied Biosystems, Foster City, CA). The primers and probes used were available as Assay on Demand (Perkin-Elmer Applied Biosystems). Five hundred nanograms of total RNA was incubated in 50 μl reaction mixture for the cDNA synthesis. Three microliters of the synthesized cDNA was then incubated in 25 μl of the RT-PCR mixture including 0.625 μl of either resistin or GAPDH probe. The comparative cycle of the threshold (C_T) method was used for quantitating this mRNA following the manufacturer's protocol (Perkin-Elmer). The resistin mRNA level was corrected by the GAPDH mRNA level. The corrected resistin mRNA level of undifferentiated THP-1 human monocyte cells was used as a control (defined as 1). Duplicate wells for each subject were

Table 1
Clinical characteristics of control and T2DM subjects

	Control	Type 2 diabetes
n (M/F)	157(73/80)	198(106/92)
Age (years)	62.8 ± 12.2	60.3 ± 10.8
Age of onset (years)	—	48.7 ± 11.7
Duration of diabetes (years)	—	11.2 ± 9.0
Height (cm)	155.8 ± 9.3	158.5 ± 8.6
BW (kg)	58.8 ± 12.0	60.2 ± 12.0
max BW (kg)	63.6 ± 12.1	70.1 ± 13.3
ΔBW(kg)	4.4 ± 4.5	9.5 ± 7.4
Age of max BW (years)	45.6 ± 17.9	44.6 ± 13.3
BMI (kg/m²)	24.1 ± 3.4	23.9 ± 3.9
max BMI (kg/m²)	25.9 ± 3.2	27.7 ± 4.2
ΔBMI (kg/m²)	1.8 ± 1.8	3.7 ± 2.8
HbA1c (%)	5.1 ± 0.3	8.4 ± 1.8

Means ± SD are shown. BW, body weight; max BW, maximum body weight; ΔBW = max BW − BW; BMI, body mass index; ΔBMI = max BMI − BMI.

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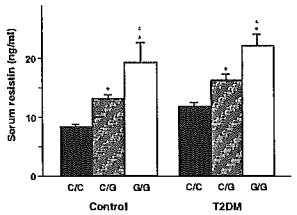

Fig. 1. Serum resistin levels of each genotype in T2DM and control subjects. Serum resistin levels were measured using a human resistin ELISA kit (Linco Research) as described in Materials and methods. Fasting serum samples from 198 T2DM and 157 control subjects were analyzed. The data represent means ± SE for each genotype in either control or T2DM subjects. *Significant difference compared to C/C, **significant difference compared to C/C or C/G. In controls, ANOVA: $F = 35.0$, $P < 0.0001$, Scheffe's test $P < 0.001$ between each pair. In T2DM, ANOVA: $F = 14.3$, $P < 0.0001$; Scheffe's test $P = 0.0053$ (C/C vs C/G), $P < 0.0001$ (C/C vs G/G), and $P = 0.016$ (C/G vs G/G). When all genotypes were combined, serum resistin levels were significantly higher in T2DM than controls (means ± SE, control vs T2DM; 11.2 ± 0.5 vs 15.1 ± 0.7 ng/ml, Student's t test; $P < 0.0001$). Fasting serum resistin levels were also increased as the number of G alleles increased when T2DM and controls were combined (C/C 10.2 ± 0.4; C/G 15.0 ± 0.7; and G/G 21.1 ± 1.7 ng/ml, ANOVA: $F = 38.3$, $P < 0.0001$; Scheffe's test: $P < 0.0001$ between each pair).

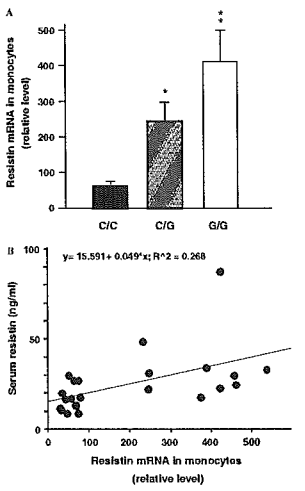

Fig. 2. Resistin mRNA levels in monocytes in healthy volunteers. Resistin mRNA levels in monocytes of 23 healthy volunteers were quantitated using the two-step TaqMan RT-PCR method as described in Materials and methods. The level of human resistin mRNA was normalized by that of human GAPDH mRNA in each sample for meaningful comparisons, and the relative amounts of resistin mRNA were determined by calculating from the threshold cycles. Resistin mRNA levels corrected by GAPDH mRNA levels in undifferentiated THP-1 cells are defined as 1. (A) Resistin mRNA in healthy volunteers with each genotype. The data represent means ± SE using duplicate wells for each subject. ANOVA and Scheffe's test were used for statistical analysis. C/C ($n = 9$), C/G ($n = 11$), and G/G ($n = 3$). ANOVA: $F = 8.87$, $P = 0.0018$; Scheffe's test: *$P < 0.05$ and **$P < 0.005$ compared to C/C genotype. (B) Correlation between resistin mRNA in monocytes and its simultaneous serum resistin levels. Fusing serum resistin levels at the time of monocyte isolation were measured as described in Materials and methods. Simple regression analysis was used for statistical analysis. Fasting serum resistin level = 15.59 + 0.049 × resistin mRNA in monocytes ($R^2 = 0.268$). $R = 0.518$, $P = 0.011$.

analyzed. The resistin mRNA level in total RNA from human primary cultured adipocytes (Zen-Bio, NC) was quantitated as described above, but using three replicate wells, to compare resistin mRNA levels between human monocytes and adipocytes.

Statistical analysis. To examine the effect of the −420(C/G genotype on serum resistin levels, a single regression analysis involving the genotype, gender, age, age of onset, duration of T2DM, BMI, maximum body mass index in life (max BMI), or HbA1c as an independent variable was performed. A multiple regression analysis was then performed using only the significant factors of these variables. In these regression analyses, the genotypes for −420C/C, −420C/G, and −420G/G were denoted by two dummy variables (c1, c2) = (0,0), (1,0), and (0,1), respectively. To estimate the effects of serum resistin levels on T2DM, a multiple logistic regression analysis adjusted simultaneously for potentially confounding variables was performed. The variables considered in this model were age, gender, max BMI, and serum resistin. In the logistic regression analysis, the Wald test was used to assess statistical significance. Analysis of variance (ANOVA) followed by Sheffe's test is used in Figs. 1 and 2A. Student's t test is also used in Fig. 1 where indicated. Simple regression analysis is used in Fig. 2B.

Results

Serum resistin levels were higher in T2DM

We first compared serum resistin levels between 198 cases (SNP-420 genotype – n; C/C – 87, C/G – 87, and G/G – 24) and 157 controls (C/C – 80, C/G – 64, and G/G – 13) (Fig. 1). Serum resistin levels were significantly higher in T2DM than in controls (means ± SE, control vs T2DM; 11.2 ± 0.5 vs 15.1 ± 0.7 ng/ml, Student's t test, $P < 0.0001$). Fasting serum resistin levels increased with increasing number of G alleles in controls, T2DM, and both (both combined; C/C 10.2 ± 0.4; C/G 15.0 ± 0.7; and G/G 21.1 ± 1.7 ng/ml, ANOVA; $F = 38.3$, $P < 0.0001$, Scheffe's test; $P < 0.0001$ between each pair, see Fig. 1 legend for the other results).

SNP-420 genotype primarily determined serum resistin levels also increased with longer duration of T2DM and higher HbA1c

To examine which factors affect fasting serum resistin levels, we then analyzed 198 T2DM subjects (Table 2). A single regression analysis involving the genotype (C/G or G/G vs C/C), age, gender, age of onset, duration of T2DM, BMI, max BMI, or HbA1c as an independent variable revealed that only the genotype, duration of T2DM, and HbA1c were significantly associated with serum resistin levels.

A multiple regression analysis involving these three independent variables showed that serum resistin levels were ~4.4 ng/ml higher in C/G, and ~10.6 ng/ml higher in G/G than in C/C (Table 3). An increase in 1-year duration of T2DM and 1% of HbA1c was correlated with an increase in serum resistin at levels of 0.19 and 0.54 ng/ml, respectively.

A single regression analysis also revealed that serum resistin levels were determined by the genotype in 157 control subjects, whereas age, gender, BMI, max BMI, or HbA1c had no effects (data not shown). Neither BMI nor max BMI was associated with serum resistin levels, even when adjusted for genotype, age, gender, and HbA1c, either in the cases or the controls (data not shown). Therefore, serum resistin levels were strongly correlated with the SNP-420 genotype in both T2DM and controls. The duration of T2DM and HbA1c was positively correlated with these levels only in T2DM.

Table 2
Simple regression analysis involving fasting serum resistin level as a dependent variable in T2DM subjects

Variables	Parameter estimate	Standard error	P
C/G	4.36	1.33	0.0012
G/G	10.22	2.02	<0.0001
Gender (female)	0.93	1.33	0.488
Age	0.07	0.06	0.353
Age of onset	−0.09	0.06	0.145
Duration	0.24	0.08	0.002
BMI	−0.04	0.17	0.798
max BMI	0.14	0.16	0.373
HbA1c	0.86	0.38	0.023

Each of genotype of SNP-420, gender, age, age of onset of T2DM, duration of T2DM, BMI, max BMI, and HbA1c was involved in the analysis as an independent variable. Statistical analyses were performed as described in Materials and methods.

Table 3
Regression analysis for serum resistin in T2DM or T2DM as dependent variables

Variables	Estimate	Standard error	P
Serum resistin in T2DM			
Intercept	5.31	3.20	
C/G	4.42	1.36	0.0013
G/G	10.57	2.14	<0.0001
Duration of diabetes	0.19	0.07	0.0090
HbA1c	0.54	0.37	0.1466
T2DM (logistic regression)			
Intercept	−2.38	1.22	
Serum resistin	0.07	0.02	<0.0001
Age	−0.02	0.01	0.0725
Gender (female)	−0.29	0.24	0.2136
max BMI	0.13	0.03	0.0003

Each of serum resistin in T2DM, and T2DM was involved in the analysis as a dependent variable. The independent variables in each analysis are shown below each intercept. Statistical analyses were performed as described in Materials and methods.

Serum resistin level was an independent factor for T2DM

To determine whether serum resistin is associated with T2DM, a logistic regression analysis involving serum resistin level, age, gender, and max BMI was employed. Serum resistin level was found to be an independent determinant for T2DM (Table 3). Therefore, serum resistin levels, primarily determined by the SNP-420 genotype, could induce T2DM.

Resistin mRNA level in monocytes was higher in the G/G genotype and positively correlated with serum resistin levels

To determine whether the resistin SNP-420 genotype is associated with resistin gene expression in human monocytes, we analyzed its mRNA levels using RT-PCR (Fig. 2). To assess isolated effects of the SNP-420 genotype, 23 healthy volunteers were employed. Resistin mRNA was significantly higher in the C/G or G/G genotype than in the C/C genotype. Consistent with the data on serum resistin levels (Fig. 1), resistin mRNA in monocytes appears to be highest in the G/G genotype (means ± SE, C/C 62.6 ± 4.0; C/G 243.8 ± 54.0; and G/G 412.8 ± 87.5), although the difference did not quite reach the levels of significance when compared between G/G and C/G ($P = 0.07$) (Fig. 2A).

Finally, when these volunteers were analyzed together, resistin mRNA levels were positively correlated with serum resistin levels ($R = 0.518$, $P = 0.011$) (Fig. 2B). We also found that resistin mRNA level was more than ~100-fold higher in human monocytes than in human primary cultured adipocytes (resistin mRNA in human primary cultured adipocytes, means ± SE of three replicate wells; 0.61 ± 0.06). Therefore, the SNP-420 genotype determines resistin mRNA in monocytes and serum levels, which could induce T2DM.

Discussion

We report here that the resistin promoter SNP-420 genotype was associated with its monocyte mRNA and serum levels, and that T2DM subjects had higher serum resistin levels than controls. A logistic regression analysis revealed that serum resistin level was an independent factor for T2DM. Therefore, the SNP-420 determines monocyte mRNA and serum levels of resistin, which could induce T2DM.

We found that the SNP-420 genotype was a major determinant of serum resistin levels. Serum resistin levels were highest in the G/G genotype, followed by the C/G and C/C genotypes. This order was also confirmed in a report on Korean subjects [26]. Haplotypes including this SNP-420 showed a similar tendency in Japanese subjects [41]. We also found that resistin mRNA levels in monocytes were higher in healthy volunteers with the G/G genotype. Smith et al. [38] showed that obese human subjects with the G/G genotype also have higher resistin mRNA levels in their abdominal subcutaneous fat.

We found that resistin mRNA in monocytes was positively correlated with serum resistin levels. We also found that resistin mRNA was more than ~100-fold higher in monocytes than in primary cultured adipocytes in humans. Whereas it is dominantly expressed in adipose tissues of mice, resistin is most highly expressed in macrophages in humans [32–34]. Therefore, monocytes are promising candidates for the main source of serum resistin in humans, although other regulatory factors or secretory tissues could also affect serum resistin levels.

The association of resistin mRNA in adipose tissue with serum resistin or insulin resistance has been reported by other investigators. Heilbronn et al. [42] reported that serum resistin is positively correlated with resistin mRNA in the subcutaneous adipose tissue of obese subjects. The fat content in the liver and HOMA-IR has been also reported to be positively correlated with resistin mRNA in subcutaneous adipose tissues of obese subjects [38]. A total of four independent reports have shown that the activity of the mutant resistin promoter including −420G is higher than that of the wild type promoter including −420C [6,26,38,41]. Therefore, G of SNP-420 enhances resistin gene promoter activity, which could increase resistin mRNA levels in adipose tissues as well as monocytes, leading to whole body insulin resistance.

We have shown that serum resistin levels were associated with T2DM. The serum levels increased with the number of G alleles in both T2DM and control subjects. The duration of T2DM and HbA1c was also positively correlated with serum resistin in T2DM. Serum resistin levels have been reported to be increased or unchanged in human T2DM or obesity [14,26–31]. The discrepancy between previous reports may be resolved by considering the SNP-420 genotype as well as the duration of T2DM and HbA1c. It should be noted that serum resistin probably exists as a hexamer (major form) or trimer (a more biologically active form) in mice, which may also affect the assay results [43].

In summary, we elucidated factors correlated with serum resistin levels and effects of SNP-420 on resistin mRNA in monocytes. Fasting serum resistin was significantly higher in T2DM and its independent determinant. Resistin monocyte mRNA levels were positively correlated with their simultaneous serum levels. Therefore, the SNP-420 determines the monocyte mRNA and serum levels of resistin, which could induce T2DM. It is not presently clear how resistin induces insulin resistance in human subjects and whether adipocytes or macrophages are the main sources of serum resistin. Further experiments will be required to clarify these points.

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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 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.

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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)	72.6±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.

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′-GCTCACCTCTCCCTCCTGTC-3′, the T-allele specific probe was 5′-Fam-CTCTCCCACAAGTAGA-MGB-3′, and the C-allele specific probe was 5′-Vic-CTC CCTCAAGTAGA-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*	0.80*	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^{-5}	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 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.

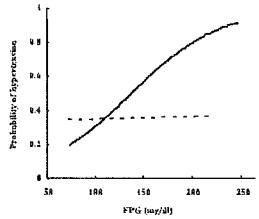

Fig. 1. *Genotype-specific regression slopes of hypertension on FPG. The single line indicates the CC genotype; the dotted line indicates the CT and TT genotypes. The regression between FPG and the probability of having hypertension in subjects with the CC genotype was represented by the equation: y = exp(0.0241x − 3.638)/{1 + exp(0.0241x − 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).*

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.

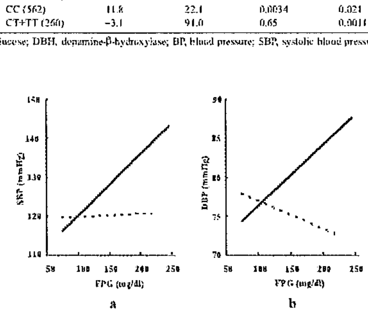

Fig. 2. *Genotypic variations in the relationship between FPG and blood pressure. a: The simple line indicates the CC genotype; the dotted line indicates the CT and TT genotypes. The regression between FPG and SBP in subjects with the CC genotype was represented by the equation, y = 0.1558x + 104.71.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