Original Article

Association of Sixty-One Non-Synonymous Polymorphisms in Forty-One Hypertension Candidate Genes with Blood Pressure Variation and Hypertension

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We previously selected a group of hypertension candidate genes by a key word search using the OMIM database of NCBI and validated 525 coding single nucleotide polymorphisms (SNPs) in 179 hypertension candidate genes by DNA sequencing in a Japanese population. In the present study, we examined the association between 61 non-synonymous SNPs and blood pressure variations and hypertension. We used DNA samples taken from 1,880 subjects in the Suita study, a population-based study using randomly selected subjects. Analyses of covariance adjusting for age, body mass index, hyperlipidemia, diabetes, smoking, drinking, and antihypertensive medication revealed that 17 polymorphisms in 16 genes (*APOB*, *CAST*, *CLCNKB*, *CTNS*, *GHR*, *GYS1*, *HF1*, *IKBKAP*, *KCNJ11*, *LIPC*, *LPL*, *P2RY2*, *PON2*, *SLC4A1*, *TRH*, *VWF*) were significantly associated with blood pressure variations. Multivariate logistic regression analysis with adjustment for the same factors revealed that 11 polymorphisms in 11 genes (*CAST*, *CTLA4*, *F5*, *GC*, *GHR*, *LIPC*, *PLA2G7*, *SLC4A1*, *SLCI8A1*, *TRH*, *VWF*) showed significant associations with hypertension. Five polymorphisms in five genes, *CAST* (calpastatin), *LIPC* (hepatic lipase), *SLC4A1* (band 3 anion transporter), *TRH* (thyrotropin-releasing hormone), and *VWF* (von Willebrand factor), were significantly associated with both blood pressure variation and hypertension. Thus, our study suggests that these five genes were susceptibility genes for essential hypertension in this Japanese population. (*Hypertens Res* 2006; 29: 611–619)

***Key Words*: genetic variants, hypertension, calpastatin, lipase, von Willebrand factor**

Introduction

Hypertension is one of the major risk factors for cardiovascular disease morbidity and mortality (*1–4*). In order to reduce events related to cardiovascular disease, control of hypertension is very important (*5*, *6*). The clinical phenotypes of hypertension are known to be affected by both lifestyle and genetic factors (*1*). Although studies of Mendelian inheritance in hypertension are limited, the causative genes have recently been identified in cases with glucocorticoid-remediable aldosteronism, Liddle syndrome, and pseudohypoaldo-

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steronism (*7–10*). Essential hypertension, however, is a multifactorial disease caused by the interaction of environmental factors with specific genotypes of multiple genes.

To delineate the genetic factors underlying hypertension, numerous association analyses have been performed. In these studies, hypertensives and matched controls with normal blood pressure are genotyped for a marker such as a single nucleotide polymorphism (SNP) thought to be etiologically important, and then allele or genotype frequencies in cases and controls are compared. In this study design, cases and controls must be representative and must be matched as closely as possible, except for blood pressure. To achieve these criteria, a subject group from the general population is widely used (*11–13*).

The National Cardiovascular Center conducts the Suita Study for the purpose of identifying the most common risk factors or characteristics that contribute to cardiovascular disease, including hypertension, in the Japanese population. This study is based on a random sampling of 15,200 Japanese residents of Suita, a City near Osaka and part of the second-largest urban area of Japan. The residents, between 30 and 79 years of age, were arbitrarily selected from the city population registry and were stratified by sex and decennial boundaries. By February 1997, 53% of the selected subjects had paid an initial visit to the National Cardiovascular Center. Since then, participants have visited the National Cardiovascular Center every 2 years for regular health checkups.

SNPs have received much attention as a means of identifying the genotypes of multiple genes for common diseases, such as myocardial infarction, asthma, and hypertension. In particular, SNPs concomitant with a missense mutation (non-synonymous SNPs) can potentially alter the protein function and gene expression level. In the translated protein, the amino acid changes caused by the missense mutation have the potential to affect protein function. Therefore, non-synonymous SNPs are the primary targets when searching for DNA variations that are causative for hypertension (*14–16*).

We previously selected a group of hypertension candidate genes by a key word search using the OMIM database of NCBI and retrieved SNPs from the public database (*17*). We verified 525 coding SNPs in 179 hypertension candidate genes by DNA sequencing of samples from 32 Japanese individuals and successfully identified a total of 143 SNPs in 93 candidate genes, including 104 missense mutations in 65 genes. Some of the missense mutations including the C677T polymorphism in *MTHFR* (*18*) and the T268M substitution in angiotensinogen, *AGT* (*19*), have previously been examined for their association with hypertension in our population, but the others remain to be assessed. This study was undertaken to examine the association of these missense mutations with blood pressure variation or hypertension in a general population.

Methods

Subjects of the Population Study

The subjects of the Suita study consisted of 15,200 men and women (30–79 years of age), who were randomly selected from the municipal population registry and stratified by gender and age in 10-year intervals. They were all invited, by letter, to receive medical and behavioral examinations every 2 years at the Division of Preventive Cardiology, National Cardiovascular Center, Japan. DNA from the leukocytes was collected between April 2002 and February 2003 from participants who gave written informed consent for genetic analyses. A total of 1,880 samples were collected during this period. The study protocol was approved by the Ethical Review Committee of the National Cardiovascular Center. Routine blood examinations that included measurements of total serum cholesterol, high-density lipoprotein (HDL)–cholesterol, triglycerides, and glucose levels were performed. A physician or nurse interviewed each patient with regard to smoking and alcohol drinking habits and personal history of common diseases.

Blood pressures were measured after at least 10 min of rest in a sitting position. Systolic and diastolic blood pressure (SBP/DBP) values were taken as the mean of 2 measurements recorded by well-trained doctors using a mercury sphygmomanometer. Hypertension was defined as a mean SBP of $\geq$140 mmHg, a mean DBP of $\geq$90 mmHg, or current use of antihypertensive medication (*20*, *21*). Diabetes was defined as fasting plasma glucose levels $\geq$7.0 mmol/l (126 mg/dl), non-fasting plasma glucose levels $\geq$11.1 mmol/l (200 mg/dl), HbA1c $\geq$6.5%, or current use of antidiabetic medication. Hyperlipidemia was defined as total cholesterol levels $\geq$5.68 mmol/l (220 mg/dl) or current use of antihyperlipidemia medication. Body mass index (BMI) was calculated as weight (in kg) divided by height (in m) squared.

Genotyping of Polymorphisms

Non-synonymous SNPs with a minor allele frequency of greater than 3% described in our previous study (*17*) were genotyped by the TaqMan-polymerase chain reaction (PCR) system (*22*, *23*). However, some of these SNPs could not be genotyped in case of the nearest-neighbor sequence. Six SNPs (rs16027, rs362331, rs362272, rs1805020, rs1805021, and rs1982073) that were previously assigned as non-synonymous SNPs were here mapped in intron by the current version of dbSNP database (http://www.ncbi.nlm.nih.gov/SNP), build 122. Thus, these SNPs were excluded from the present analyses, leaving a total of 61 non-synonymous SNPs that were genotyped in this study.

Statistical Analysis

Analysis of variance was used to compare mean values between groups, and if overall significance was demonstrated, the intergroup difference was assessed by means of a general linear model. Frequencies were compared by χ^2 analysis.

Analyses of covariance for SBP and DBP in each sex of genotypes were performed with consideration of potentially confounding risk variables, including age, BMI, present illness (hyperlipidemia and diabetes mellitus), lifestyle (smoking and drinking), and antihypertensive medication. For multivariate risk predictors, the adjusted odds ratios were given with the 95% confidence intervals. The association between genotype and risk of hypertension was expressed in terms of odds ratios adjusted for possible confounding effects including age, BMI, present illness (hyperlipidemia and diabetes mellitus), and lifestyle (smoking and drinking). SAS statistical software (release 8.2; SAS Institute Inc., Cary, USA) was used for statistical analyses.

Results

Basic Characteristics of Subjects in the Suita Study

The characteristics of the 1,880 participants (866 men and 1,014 women) are summarized in Table 1. Age, SBP, DBP, BMI, percentage of current smokers and drinkers, and prevalence of hypertension and diabetes mellitus were significantly higher in men than in women. Total cholesterol, HDL-cholesterol, and percentage of hyperlipidemia were significantly higher in women than in men.

Table 1. Basic Characteristics of Subjects in Suita, a Japanese Urban Population, 2002

	Women (n=1,014)	Men (n=866)
Age (years)	63.3±11.0	66.3±11.1*
SBP (mmHg)	128.0±19.7	131.8±19.4*
DBP (mmHg)	76.6±9.8	79.7±10.7*
Body mass index (kg/m^2)	22.3±3.2	23.3±3.0*
Total cholesterol (mg/dl)	215.7±30.6*	197.9±30.6
HDL-cholesterol (mg/dl)	64.3±15.5*	55.0±14.3
Current smokers (%)	6.3	29.9†
Current drinkers (%)	29.5	67.1†
Present illness (%)		
Hypertension	38.1	47.3†
Hyperlipidemia	54.5†	27.8
Diabetes mellitus	4.3	11.1†

Values are mean±SD or percentage. Hypertension indicates SBP ≥140 mmHg and/or DBP ≥90 mmHg or antihypertensive medication; hyperlipidemia, total cholesterol ≥220 mg/dl or antihyperlipidemia medication; diabetes, fasting plasma glucose ≥126 mg/dl or non-fasting plasma glucose ≥200 mg/dl or HbA1c ≥6.5% or antidiabetic medication. *$p<0.05$ between women and men by Student's t-test. †$p<0.05$ between women and men by χ^2 test. SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high-density lipoprotein.

Susceptible Missense Mutations Related to Blood Pressure Variation and Hypertension

We genotyped 61 non-synonymous SNPs by the TaqMan-PCR system in 1,880 individuals; 796 of whom were hypertensives and 1,084 of whom were normotensives. Non-synonymous SNPs genotyped in this study in conjunction with the allele frequencies are listed in Table 2.

Analysis of covariance adjusting for age, BMI, hyperlipidemia, diabetes mellitus, smoking, drinking, and antihypertensive medication revealed that 17 polymorphisms in 16 genes (*APOB*, *CAST*, *CLCNKB*, *CTNS*, *GHR*, *GYS1*, *HF1*, *IKBKAP*, *KCNJ11*, *LIPC*, *LPL*, *P2RY2*, *PON2*, *SLC4A1*, *TRH*, *VWF*) were significantly associated with blood pressure variation in either a dominant or a recessive genetic model (Table 3). Among them, four SNPs (*GYS1*: glycogen synthase; *LIPC*: heptic lipase; *TRH*: thyrotropin-releasing hormone; *VWF*: von Willebrand factor) were associated with blood pressure in men or women on the basis of a probability value <0.01 in either a dominant or recessive genetic model.

Multivariate logistic regression analysis with adjustment for the same factors revealed that 11 polymorphisms in 11 genes (*CAST*, *CTLA4*, *F5*, *GC*, *GHR*, *LIPC*, *PLA2G7*, *SLC4A1*, *SLCI8A1*, *TRH*, *VWF*) showed significant association with hypertension (Table 4). Among them, two SNP, rs754615 in calpastatin (*CAST*) and rs9016 in a group-specific component (*GC*) were associated with hypertension in women on the basis of a probability value <0.01. When the controls were defined as SBP ≤120 mmHg, DBP ≤80 mmHg, or non-medication, and the hypertensives were defined as SBP ≥160 mmHg, DBP ≥100 mmHg, or current use of antihypertensive medication, 5 out of 11 SNPs showed positive association with hypertension after adjustment for the confounding factors described above as follows. Rs754615 of *CAST* was associated with hypertension in women (GG+GC *vs*. CC, odds ratio: 0.17, 95% confidence interval: 0.03–0.88, $p=0.035$). Rs9016 of *GC* was associated with hypertension in women (CC *vs*. CT+TT, odds ratio: 0.19, 95% confidence interval: 0.06–0.56, $p=0.003$). Rs1390938 of *SLC18A1* was associated with hypertension in women (TT+TC *vs*. CC, odds ratio: 0.60, 95% confidence interval: 0.38–0.92, $p=0.020$). Rs5036 of *SLC4A1* was associated with hypertension in men (AA *vs*. AG+GG, odds ratio: 0.57, 95% confidence interval: 0.34–0.96, $p=0.035$). Rs1063856 of *VWF* was associated with hypertension in men (AA *vs*. AG+GG, odds ratio: 0.51, 95% confidence interval: 0.28–0.92, $p=0.026$).

Association analysis using two different statistical calculations showed that five genes, *CAST* (calpastatin), *LIPC*

Table 2. List of Non-Synonymous SNPs Genotyped in this Study

Gene symbol	Reference SNP (dbSNP)	Allele 1/2	Amino acid change	Allele 1 Homo	Hetero	Allele 2 Homo	Allele frequency	
							Allele 1	Allele 2
ABCC8	rs757110	G/T	Ala1369Ser	296	841	729	0.384	0.616
ADRB2	rs1042713	G/A	Gly16Ala	473	902	461	0.503	0.497
APOA4	rs5104	A/G	Asn147Ser	776	882	220	0.648	0.352
APOB	rs1367117	C/T	Thr98Ile	1,581	267	20	0.918	0.082
	rs679899	C/T	Ala618Val	32	405	1,439	0.125	0.875
APOC4	rs1132899	T/C	Leu36Pro	182	808	885	0.313	0.687
	rs5167	G/T	Arg96Leu	432	960	484	0.486	0.514
CALCA	rs5241	C/A	Ser76Arg	1,777	99	0	0.974	0.026
CAST	rs754615	G/C	Cys408Ser	1,405	439	35	0.865	0.135
CCR2	rs1799864	G/A	Val64Ile	936	779	163	0.706	0.294
CDKN1A	rs1801270	C/A	Ser31Arg	523	947	406	0.531	0.469
CFTR	rs213950	G/A	Val470Met	722	878	280	0.618	0.382
CLCNKB	rs2015352	G/T	Arg27Leu	133	738	996	0.269	0.731
CPT2	rs1799821	G/A	Val368Ile	9	198	1,672	0.057	0.943
	rs1799822	A/G	Met647Val	1,670	199	9	0.942	0.058
CSF1	rs1058885	T/C	Leu408Pro	279	894	688	0.390	0.610
CTLA4	rs231775	G/A	Ala17Thr	722	877	281	0.617	0.383
CTNS	rs161400	T/C	Ile260Thr	1,662	211	7	0.940	0.060
CYP21A2	rs6474	G/A	Arg103Lys	857	799	222	0.669	0.331
F5	rs6020	G/A	Arg513Lys	230	854	795	0.350	0.650
F7	rs6046	G/A	Arg413Gln	1,647	224	8	0.936	0.064
GC	rs7041	T/G	Asp432Glu	1,064	679	137	0.747	0.253
	rs4588	A/C	Lys436Thr	148	746	979	0.278	0.722
	rs9016	C/T	Arg445Cys	1,786	90	2	0.975	0.025
GHR	rs6182	G/T	Cys440Phe	1,588	273	18	0.918	0.082
	rs6180	C/A	Leu544Ile	593	904	381	0.556	0.444
	rs6184	C/A	Pro579Thr	1,577	294	0	0.921	0.079
GIPR	rs1800437	G/C	Glu354Gln	1,147	634	96	0.780	0.220
GYS1	rs5447	A/G	Met416Val	1,512	342	23	0.897	0.103
HF1	rs800292	G/A	Val62Ile	657	915	304	0.594	0.406
	rs1061170	C/T	His402Tyr	6	222	1,643	0.063	0.937
	rs1065489	G/T	Glu936Asp	525	951	401	0.533	0.467
IKBKAP	rs1538660	C/T	Pro1158Leu	792	874	210	0.655	0.345
KCNJ11	rs5219	A/G	Lys23Glu	253	834	788	0.357	0.643
LIPA	rs1051339	G/A	Gly23Arg	1,650	219	11	0.936	0.064
LIPC	rs6078	G/A	Val95Met	1,083	691	105	0.760	0.240
	rs6083	A/G	Asn215Ser	14	284	1,574	0.083	0.917
LPL	rs328	C/G	Ser474Stop	1,412	435	33	0.867	0.133
NOTCH3	rs1044009	C/T	Ala2223Val	299	883	696	0.394	0.606
P2RY2	rs1626154	T/C	Cys334Arg	12	259	1,600	0.076	0.924
PCSK1	rs6234+	C/G	Gln665Glu	1,121	665	92	0.774	0.226
	rs6235+	G/C	Ser690Thr	1,122	666	92	0.774	0.226
PLA2G7	rs1805017	G/A	Arg92His	1,175	612	91	0.789	0.211
	rs1805018	T/C	Ile198Thr	1,179	620	79	0.793	0.207
	rs1051931	T/C	Val379Ala	24	358	1,498	0.108	0.892
PON1	rs854560	T/A	Leu55Met	1,525	294	10	0.914	0.086
	rs662	A/G	Gln192Arg	214	852	767	0.349	0.651
PON2	rs11545941	C/G	Ala148Gly	1,175	627	74	0.793	0.207
SELE	rs5368	C/T	His468Tyr	1,125	676	78	0.779	0.221
	rs5355	C/T	Leu575Phe	1,695	178	4	0.950	0.050

Table 2. (Continued)

Gene symbol	Reference SNP (dbSNP)	Allele 1/2	Amino acid change	Allele 1 Homo	Hetero	Allele 2 Homo	Allele frequency	
							Allele 1	Allele 2
SLC18A1	rs1390938	T/C	Ile136Thr	128	703	1,044	0.256	0.744
SLC2A2	rs1800572	G/A	Val101Ile	1,769	109	1	0.970	0.030
SLC4A1	rs5035	A/C	Asp38Ala	1,715	163	2	0.956	0.044
	rs5036	A/G	Lys56Glu	1,317	524	37	0.841	0.159
	rs2285644	C/T	Pro854Leu	1,697	176	7	0.949	0.051
TRH	rs5658	G/C	Val8Leu	210	812	856	0.328	0.672
VWF	rs1800377	G/A	Val471Ile	1,329	504	44	0.842	0.158
	rs1800378	A/G	His484Arg	238	855	785	0.354	0.646
	rs1063856	A/G	Thr789Ala	1,626	236	17	0.928	0.072
	rs216321	A/G	Gln852Arg	63	576	1,240	0.187	0.813
WRN	rs1346044	T/C	Cys1367Arg	1,608	263	8	0.926	0.074

+: SNPs in linkage disequilibrium. Present rs numbers of SNPs are obtained from dbSNP database (http://www.ncbi.nlm.nih.gov/SNP/), build 122. SNP, single nucleotide polymorphism.

(hepatic lipase), *SLC4A1* (band 3 anion transporter), *TRH*, and *VWF*, were significantly associated with both blood pressure variation and hypertension. The blood pressure variations by genotypes of these genes were 4.4 mmHg, 3.5 mmHg, 1.6 mmHg, 4.5 mmHg, and 5.5 mmHg, respectively.

Discussion

In this study, we performed an association of a large number of non-synonymous SNPs previously identified in Japan with blood pressures variation and hypertension in a general population. The results showed that 16 and 11 genes showed an association with blood pressure variation and hypertension, respectively, and five genes (*CAST*, *LIPC*, *SLC4A1*, *TRH*, *VWF*) showed an association with both blood pressure variation and hypertension.

Some of the SNPs showed relatively large blood pressure variation (>5 mmHg; Table 3). For example, the mean blood pressure variations contributed by the genotypes of *APOB* (apolipoprotein B), *CTNS* (cystinosin), *GHR* (growth hormone receptor), and *VWF* were 12.2 mmHg, 9.9 mmHg, 8.1 mmHg, and 5.5 mmHg, respectively. These SNPs have a minor allele frequency of below 0.1, suggesting that the blood pressure variation of these genes may be overestimated. *CAST*, *KCNJ11* (potassium channel, inwardly rectifying, subfamily J, member 11), *LPL* (lipoprotein lipase), *TRH*, and *VWF*, in which the minor allele frequencies were over 0.1, showed a moderate blood pressure change of between 4–5 mmHg by the genotypes.

CAST (5q14–q22) encodes an intracellular protease inhibitor, calpastatin, that regulates a calcium-dependent cysteine proteinase, calpain, ubiquitously present in a variety of tissues and cells (*24*). Calpain activity is tightly regulated with intracellular calcium concentration, and the calpain-calpastatin system governs the non-lysozomal intracellular degradation of proteins. Calpastatin consists of an N-terminal domain L and four repetitive calpain-inhibition domains (domains 1–4). The missense mutation we reported here is the Cys-to-Ser substitution at position 408 that is present in domain 2. In Milan hypertensive rats, calpastatin activity was decreased compared to that in Milan normotensive rats (*25*). Patients with essential hypertension showed lower calpastatin activity in red cells than normotensive subjects (*26*). These reports suggest a possible link between *CAST* and hypertension.

LIPC, located on chromosome 15q21, encodes hepatic lipase. It is a key enzyme in lipoprotein metabolism together with lecithin cholesterol acyl transferase. Hepatic lipase is synthesized by the liver and resides in the hepatic endothelial cell lining (*27*). Genetic polymorphisms in the promoter region of *LIPC* have been associated with high plasma HDL-cholesterol concentrations (*28*). In the current study, the Val149Met polymorphism in *LIPC* was associated with HDL cholesterol ($p=0.04$; data not shown). Here, we showed an association of Val49Met substitution with blood pressure variation and hypertension. The mechanisms by which this substitution affects the blood pressure variations are not clear.

SLC4A1 encodes a plasma membrane anion exchanger, termed band 3, abundantly present at the erythrocyte membrane. It performs electroneutral exchange of Cl^- for HCO_3^- across the membrane. It is also present in renal tubular cells, defects of which cause distal renal tubular acidosis characterized by defective urinary acidification by the distal nephron (*29*). We showed that the Lys-to-Glu substitution at position 56 in *SLC4A1* is associated with hypertension. This substitution has previously been reported as band 3 Memphis (*30*). This variant did not show functional difference towards the specific band 3 inhibitor, stilbenedisulfonates, although the detailed analysis has not been done (*31*). If the mutation affects the anion transport in a low amount, it might influence the cation transport. Long-term exposure to the variant may

Table 3. Association of Blood Pressure Variation with Genotypes

Gene	SNP amino acid change	Allele1/2 (allele freq.)	Sex	BP	Genotype group	BP mean±SEM (mmHg)	*p**	Variation of mean BP (mmHg)
APOB	rs1367117	C/T	Men	SBP	CC+TC	132.0±0.6		
	T98I	(0.918/0.082)			TT	119.7±5.8	0.035	12.2
CAST	rs754615	G/C	Women	DBP	GG+GC	76.5±0.3		
	C408S	(0.865/0.135)			CC	80.9±2.2	0.042	4.4
CLCNKB	rs2015352	G/T	Women	DBP	GG	74.3±1.1		
	R27L	(0.269/0.731)			GT+TT	76.8±0.3	0.034	2.5
CTNS	rs161400	T/C	Men	DBP	TT+TC	79.8±0.3		
	I260T	(0.940/0.060)			CC	69.8±4.4	0.026	9.9
GHR	rs6182	G/T	Men	DBP	CC+CT	79.8±0.3		
	C440F	(0.918/0.082)			TT	71.7±4.0	0.046	8.1
GYS1	rs5447	A/G	Men	DBP	AA	80.2±0.4		
	M416V	(0.897/0.103)			AG+GG	77.8±0.8	0.006	2.4
HF1	rs800292	G/A	Men	DBP	GG+GA	79.4±0.4		
	V62I	(0.594/0.406)			AA	81.2±0.8	0.047	1.8
IKBKAP	rs1538660	C/T	Women	SBP	CC+CT	128.5±0.6		
	P1158L	(0.655/0.345)			TT	125.2±1.6	0.046	3.3
KCNJ11	rs5219	A/G	Men	SBP	AA	128.1±1.6		
	K23E	(0.357/0.643)			AG+GG	132.3±0.6	0.015	4.2
LIPC	rs6078	G/A	Men	SBP	GG	133.4±0.8		
	V95M	(0.760/0.240)			GA+AA	129.9±0.9	0.004	3.5
LPL	rs328	C/G	Women	DBP	CC+CG	76.5±0.3		
	S474X	(0.867/0.133)			GG	81.2±2.1	0.029	4.7
P2RY2	rs1626154	T/C	Women	DBP	TT+TC	75.0±0.8		
	C334R	(0.076/0.924)			CC	76.9±0.3	0.025	1.8
PON2	rs11545941	C/G	Women	DBP	CC	77.0±0.4		
	A148G	(0.793/0.207)			CG	76.0±0.5	0.032	2.5
					GG	74.6±1.5		
SLC4A1	rs5036	A/G	Men	DBP	AA	79.3±0.4		
	K56E	(0.841/0.159)			AG+GG	80.8±0.7	0.040	1.6
TRH	rs5658	G/C	Women	SBP	GG+GC	127.6±0.6		
	V8L	(0.328/0.672)			CC	132.1±1.5	0.006	4.5
VWF	rs1800377	G/A	Men	SBP	GG	132.8±0.7		
	V471I	(0.842/0.158)			GA+AA	129.5±1.1	0.009	3.4
VWF	rs1063856	A/G	Women	DBP	AA+AG	76.5±0.3		
	T789A	(0.928/0.072)			GG	82.0±2.7	0.045	5.5

*Analyses of covariate analysis, adjusted for age, body mass index (BMI), present illness (hyperlipidemia and diabetes mellitus), antihypertensive medication, and lifestyle (smoking and drinking). SNP, single nucleotide polymorphism; BP, blood pressure; SBP, systolic blood presssure; DBP, diastolic blood pressure.

result in a slight but significant dysfunction of anion exchange, thereby leading to hypertension.

TRH encodes the thyrotropin-releasing hormone (TRH), which is a tripeptide functioning as a regulator of the biosynthesis of thyroid-stimulating hormone. TRH also plays an important role in central cardiovascular regulation. Overexpression of the TRH precursor has been shown to induce hypertension in normal rats, which was reversed by TRH antisense treatment (*32*). This treatment also reduced the central TRH hyperactivity in spontaneously hypertensive rats and normalized blood pressure. TRH decreased leptin and mediated the leptin-induced pressor effect (*33*). The polymorphisms in the promoter region of the TRH receptor that belongs to the G protein–coupled seven-transmembrane domain receptor superfamily have been associated with essential hypertension (*34*, *35*). The Leu-to-Val substitution at position 8 in the thyrotropin-releasing hormone precursor is present in the signal sequence that is cleaved off during the formation of TRH. Thus, there would be a possible link between the Leu8Val substitution in TRH and hypertension

Table 4. Allele Frequency and Odds Ratio of Presence of Hypertension by Genotypes of Polymorphisms

Gene	SNP amino acid change	Allele1/2 (allele freq.)	Sex	Genotype group	Odds ratios (95% CI)	*p**
CAST	rs754615	G/C	Women	GG+GC	1	
	C408S	(0.865/0.135)		CC	0.25 (0.09–0.68)	0.007
CTLA4	rs231775	G/A	Men	GG+GA	1	
	A17T	(0.617/0.383)		AA	1.50 (1.00–2.24)	0.050
F5	rs6020	A/G	Women	AA+AG	1	
	K513R	(0.650/0.350)		GG	0.58 (0.39–0.88)	0.010
GC	rs9016	C/T	Women	CC	1	
	R445C	(0.975/0.025)		CT+TT	0.31 (0.15–0.66)	0.002
GHR	rs6180	C/A	Women	CC+CA	1	
	L544I	(0.556/0.444)		AA	0.70 (0.50–1.00)	0.048
LIPC	rs6078	G/A	Men	GG	1	
	V95M	(0.760/0.240)		GA+AA	1.42 (1.07–1.90)	0.016
PLA2G7	rs1805018	T/C	Women	TT+TC	1	
	I198T	(0.793/0.207)		CC	2.30 (1.14–4.64)	0.020
SLC18A1	rs1390938	T/C	Women	TT+TC	1	
	I136T	(0.256/0.744)		CC	0.73 (0.55–0.98)	0.033
SLC4A1	rs5036	A/G	Men	AA	1	
	K56E	(0.841/0.159)		AG+GG	0.70 (0.51–0.97)	0.031
TRH	rs5658	G/C	Women	GG+GC	1	
	V8L	(0.328/0.672)		CC	0.63 (0.41–0.98)	0.041
VWF	rs1063856	A/G	Women	AA	1	
	T789A	(0.928/0.072)		AG+GG	0.65 (0.43–0.97)	0.034

*Conditional logistic analysis, adjusted for age, body mass index (BMI), present illness (hyperlipidemia and diabetes mellitus), and lifestyle (smoking and drinking). SNP, single nucleotide polymorphism; CI, confidence intervals.

due to the insufficient production of TRH.

VWF encodes von Willebrand factor, which is synthesized and stored in the endothelium and is an essential plasma protein for platelet plug formation at the site of vessel injuries. It is widely regarded as a marker of endothelial cell damage/dysfunction. Elevated levels of plasma VWF are related to adverse cardiovascular outcomes (*36*). Hypertensive patients with target organ damage are at high risk of adverse cardiovascular events, particularly myocardial infarction and stroke (*37*), and there is a relationship between target organ damage and endothelial damage/dysfunction in hypertension. Although the functional significance of the Val471Ile mutant remains to be determined, the mutant likely has adverse effects on the vasculature.

We would point out that SNPs positively associated with blood pressure/hypertension may be merely markers, and true DNA variation may be present in the other sites in linkage disequilibrium. It has been well established that the human chromosome is divided into discrete blocks of sequences called haplotype blocks, which are separated by hot spots of recombination (*38*). In haplotype blocks, a small number of common haplotypes are present. The size of the haplotype blocks occasionally extends to more than 100 kb (*39*). Therefore, the variation that actually confers the susceptibility to disease may be present in adjacent genes in the same haplotype blocks.

Given the relatively small number of tests performed in the present study, the association of individual SNPs with hypertension or blood pressure variation can be considered marginally significant at best. All the *p*-values were greater than 0.004 (Tables 3 and 4), but the significance vanished after correction by the Bonferroni method. However, these SNPs in the hypertension candidate genes are non-synonymous, which could potentially affect the protein function. In addition, these SNPs had a positive association with both blood pressure variation and hypertension. Taking these results together, we can regard these five genes as candidate genes for hypertension. Many reports of association study failed to be confirmed. Thus, the association between the SNPs identified in the present study and blood pressure/hypertension will need to be confirmed by another set of studies.

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