Table 5. Accumulated Clinical Profiles of Subjects with Missense Mutations in *HSD11B2* in the General Population

	L14F	R74H	R147H
Number	14	20	8
Age (years old)	67.7±12.3	64.8±13.3	61.5±12.0
Sex (M/F)	7/7	9/11	5/3
Body mass index (kg/m^2)	23.4±4.0	22.4±2.9	23.9±1.7
Systolic blood pressure (mmHg)	125.4±23.0	128.7±23.4	124.9±19.9
Diastolic blood pressure (mmHg)	75.4±11.0	78.2±10.0	75.8±12.9
Total cholesterol (mg/dl)	213.9±34.0	213.8±37.0	199.3±36.4
HDL-cholesterol (mg/dl)	57.9±12.1	63.1±16.9	52.1±18.5
Triglyceride (mg/dl)	93.8±49.3	120.1±93.4	140.7±90.1
Creatinine (mg/dl)	0.8±0.2	0.7±0.2	0.8±0.2
Over proteinuria (yes/no)	1/13	0/20	0/8
FBS (mg/dl)	100.4±20.9	94.5±10.3	99.6±22.3
HbA1c (%)	5.7±0.8	5.4±0.7	5.6±0.9
Current smoker (yes/no)	2/12	4/16	1/7
Current drinker (yes/no)	5/9	9/11	4/4
Hypertension (yes/no)	6/8	8/12	3/5
Hyperlipidemia (yes/no)	10/4	11/9	6/2
Diabetes mellitus (yes/no)	6/8	2/18	2/6
Antihypertensive treatment (yes/no)	4/10	2/18	2/6

Values were expressed as mean±SD. Hypertension: systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥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. M, male; F, female; HDL, high-density lipoprotein; FBS, fasting blood sugar.

Table 6. Number of Subjects with Missense/Frameshift Mutations in the Hypertensive and the General Populations

Mutations	Hypertensive population (n=953)	General population	
		Hypertensive subjects (n=1,480)	Normotensive subjects (n=2,175)
L14F	5	6	8
R74H	1	8	12
R147H	3	3	5
T156I	1	n.d.	n.d.
4884Gdel	1	n.d.	n.d.
R335H	1	0	0
Total	12	17	25

n.d., not determined.

that the heterozygous carriers with the defective allele of the *HSD11B2* gene showed essential hypertension (*16*). It is evident that this frameshift mutation results in the dysfunction of HSD11B2. The allele frequency of this mutation was very low (0.052%, 1 allele/1,906 alleles) in the Japanese hypertensive population. However, it is worth noting that this defective allele might be prevalent in other ethnic populations, because the frequency of some genetic mutations varies with ethnicity. Recently, rare genetic mutations collectively contributing to a quantitative trait variation, such as plasma levels of HDL-cholesterol, have been reported (*33*). We have performed large-scale sequence analyses of five hypertension candidate genes, *WNK4*, *SCNN1B*, *SCNN1G*, *NR3C2* and *RGS2*, to evaluate this hypothesis and found that a low but significant percentage of the hypertensive subjects had missense/frameshift mutations (*24–26*, *34*). Collectively, these rare mutations may make an at least partial contribution to hypertension.

The deduced NAD-binding sites reside in the conserved region from T82 to A111 (*2*), and the deduced catalytic site resides in the conserved region from Y232 to K236 (*35*). So far, more than ten genetic defects in patients with AME, most of whom had a severe deficiency of enzymatic activity confirmed by the expression analysis, have been reported and none of them overlap with the five missense mutations identified in the present study. Therefore, the effects on the HSD11B2 enzymatic activity of the mutations are not clear. In the future, an *in vitro* expression study should be performed to evaluate the activity of mutants and the ratios of urinary cortisol to cortisone metabolites in carriers of the mutations.

In the Caucasian population, a mutation at E178 that is synonymous with 553G>A which can be distinguished by *Alu* I restriction enzyme digestion, has been identified with a prevalence of 8.6% in the control subjects (*21*, *23*). This polymorphism was associated with end-stage renal disease but not with essential hypertension. We did not identify this polymor-

A

```
                    *
h-HSD    1 MERWPWPSGGAWLLVAARALLQLLRSDLRLGRPLLAALALLAALD   45
m-HSD    1 MERWPWPSGGAWLLVAARALLQLLRSDLRLGRPLLAALALLAALD   45
r-HSD    1 MERWPWPSGGAWLLVAARALIQLLRADLRLGRPLLAALALLAALD   45

                                          *
h-HSD   46 WLCQRLLPPPAALAVLAAAGWIALSRLARPQRLPVATRAVLITGC   90
m-HSD   46 WLCLRLMPPPAALVVLAGAGWIALSRLARPPRLPVATRAVLITGC   90
r-HSD   46 WLCQSLLPPSAALAVLAAAGWIALSRLARPQRLPVATRAVLITGC   90

                      *         *
h-HSD  136 QMDLTKPGDISRVLEFTKAHTTSTGLWGLVNNAGHNEVVADAELS  180
m-HSD  136 QMDLTKAEDISRVLEITKAHTASTGLWGLVNNAGLNIVVADVGLS  180
r-HSD  136 QMDLTKPADISRALEFTKAHTTSTGLWGLVNNAGHNDVVADVELS  180

                                   *
h-HSD  316 SDLTPVVDAITDALLAARPRRRYYPGQGLGLMYFIHYYLPEGLRR  360
m-HSD  316 PDLSPVVDAIIDALLAAQPRSRYYPGRGLGLMYFIHHYLPEGLRR  360
r-HSD  316 PDLSPVVDAITDALLAARPRPRYYPGRGLGLMYFIHYYLPEGLRR  360
```

B

Wild type

```
                    *
          ACTGTGGGGAGCCCAGCGGGGGACATGCCA
216       T  V  G  S  P  A  G  D  M  P       225
          TTCAAGACAGAGTCAGTGAGAAACGTGGGT
265       F  K  T  E  S     V  R  N  V  G    274
```

4884Gdel allele

```
          ACTGTGGGAGCCCAGCGGGGGACATGCCAT
216       T  V  G  A  Q  R  G  T  C  H       225
          TCAAGACAGAGTCAGTGAGAAACGTGGGTC
265       S  R  Q  S  Q     *  *  *
```

Fig. 2. *Partial amino acid sequence surrounding the mutations in* HSD11B2. *A: Alignment of partial amino acid sequences of HSD11B2 from two species and human HSD11B2. HSD11B2 sequences are from* Homo sapiens *(h),* Mus musculus *(m), and rabbit (r). Numbers indicate the position of amino acid sequence. The asterisks indicate the positions at which missense mutations occur (L14F, R74H, R147H, T156I, R335H) B: Nucleotide and amino acid sequences of wild-type allele and 4884Gdel allele. Numbers indicate the amino acid residues. An asterisk indicates the base deleted in the 4884Gdel allele, which causes a frameshift mutation from S218. This results in a 51-amino-acid extension that is is terminated by a stop codon (indicated by three asterisks).*

phism in our Japanese population.

In the Caucasian population, an intensive genetic study on the *HSD11B2* gene using 587 subjects, including 260 patients with end-stage renal disease, has been conducted, in which one missense mutation, L148V, and three synonymous mutations, T156, E178, and D388, were identified by the combination of single strand conformational polymorphism analysis and DNA sequencing (*36*). The results showed that allele frequencies did not differ significantly between control subjects and end-stage renal disease patients or between patients with hypertension and patients with end-stage renal disease. We did not identify these mutations in our Japanese population. Our results support their findings that the mutations in the *HSD11B2* gene do not affect hypertension.

In summary, we suggest that rare mutations in *HSD11B2*, L14F, R74H, R147H, T156I, R335H, and 4884Gdel may not collectively contribute to the pathogenesis of hypertension, although it was not clear whether abnormalities of electrolytes, renin activity, or aldosterone concentration were present, since our hypertensive patients with these missense/frameshift mutations were taking antihypertensive drugs. Further functional analyses of *HSD11B2* mutants are necessary to clarify the functional defects caused by these genetic variations in Japanese.

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生活習慣病　高血圧

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かわの　ゆうへい

- 高血圧は普遍的な疾患であり，有病率は加齢とともに大きく増加する．
- 高血圧の発症や進展には，遺伝因子と環境要因がともに関与している．
- 高血圧の早期発見と適切な管理は，心血管疾患の予防にきわめて重要である．
- 全成人は定期的な血圧測定を受けるべきで，小児についても血圧測定が望まれる．
- 高血圧の早期発見には検診の充実が重要であるが，それで十分とはいえない．
- 高血圧と仮面高血圧の早期発見には，家庭血圧計による家族全員の血圧測定が望まれる．

Key Words　高血圧，検診，家庭血圧，遺伝子，生活習慣，仮面高血圧

はじめに

高血圧は普遍的な疾患であるが，脳卒中や心筋梗塞など，多くの心血管疾患の主要な危険因子であることは，よく知られている．降圧治療により高血圧患者の心血管予後および生命予後が改善することも，多くの臨床試験により証明されている．したがって，高血圧の予防や早期発見および適切な管理はきわめて重要であり，それにより心血管疾患が減少すれば，利得は非常に大きいと考えられる[1]．高血圧の診断は血圧値によってなされるので，早期発見に血圧測定が必要なことは当然であるが，その対象や時期については，年齢や他の危険因子などを考慮すべきであろう．また，仮面高血圧が注目されている今，高血圧の診断は検診などでの随時血圧測定でよいのであろうか．本稿では，これらの事柄をふまえながら，高血圧の早期発見について述べていきたい．

□ 高血圧の診断と疫学

高血圧の診断基準は，収縮期血圧 140 mmHg 以上あるいは拡張期血圧 90 mmHg 以上であり，国際的な合意が得られている[2~4]．ただし，臨床的に高血圧と診断するには，1 度だけの血圧測定では不十分で，繰り返しの測定を要する．

表 1 に，日本高血圧学会のガイドライン（JSH 2004）における成人の血圧値の分類を示す[4]．高血圧は軽症，中等症，重症に分かれ，また収縮期血圧 140 mmHg 以上で拡張期血圧 90 mmHg 未満は収縮期高血圧となる．正常血圧も至適，正常，正常高値に分かれる．正常高値血圧は 130-139/85-89 mmHg で，正常や至適群に比べて心血管リスクが高く，高血圧に進展しやすいことから，高血圧の早期発見の点からも注意すべき状態と考えられる．米国のガイドライン（JNC 7）は，より低い血圧値を含む 120-139/80-89 mmHg を前高血圧（Prehypertension）と呼び，注意を喚起している[2]．

表 1　成人における血圧値の分類

分類	収縮期血圧		拡張期血圧
至適血圧	＜120	かつ	＜80
正常血圧	＜130	かつ	＜85
正常高値血圧	130～139	または	85～89
軽症高血圧	140～159	または	90～99
中等症高血圧	160～179	または	100～109
重症高血圧	≧180	または	≧110
収縮期高血圧	≧140	かつ	＜90

（高血圧治療ガイドライン 2004[4]より引用）

高血圧は普遍的な疾患であり，わが国における患者数は約 3500 万人といわれている．また，血圧は加齢とともに上昇し，わが国では高齢者の 60% 以上は高血圧である．

図 1 に，2000 年に行われたわが国の第 5 次循環

* 国立循環器病センター　高血圧腎臓内科

0913-7963/06/¥500/論文/JCLS

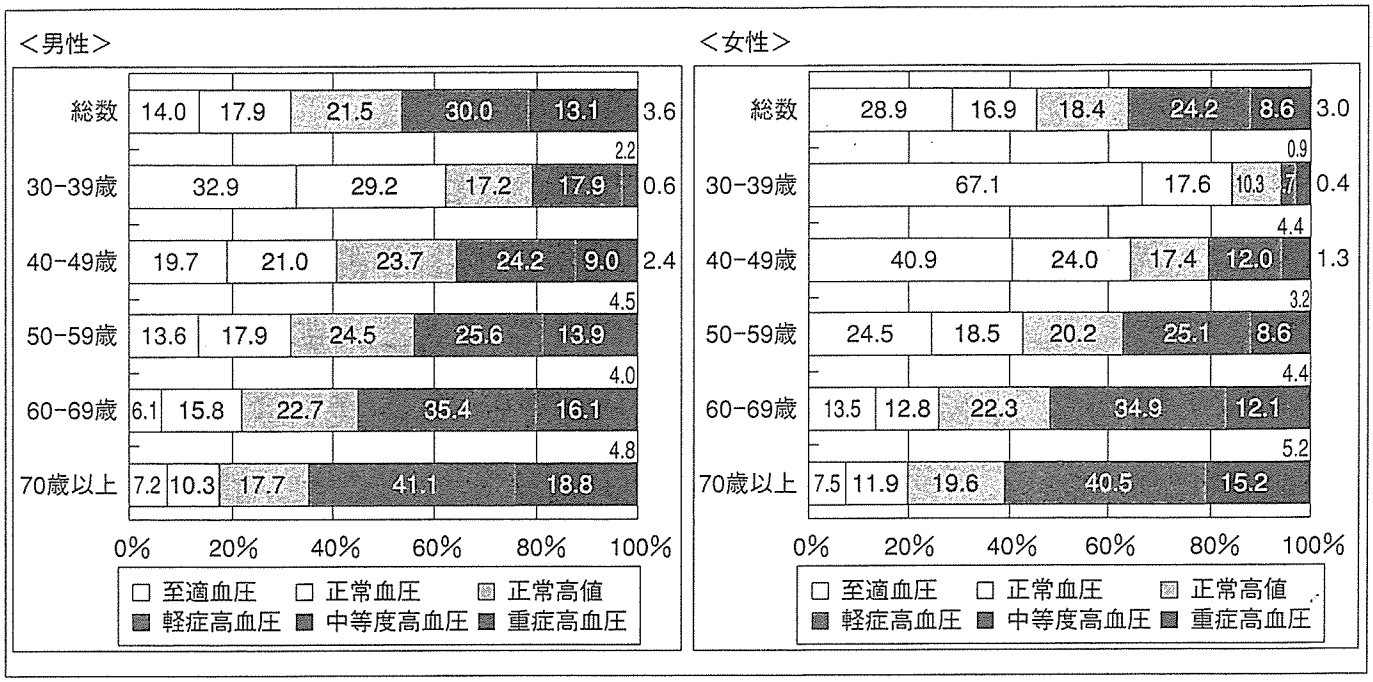

図1　厚生労働省第5次循環器疾患基礎調査（2000年）における性・年齢階級別の血圧区分（2回の平均値による）
（厚生労働省：第5次循環器疾患基礎調査（平成12年）[5]より）

器疾患基礎調査における，性・年齢階級別の血圧区分を示す[5]．2回の測定の平均値が高血圧であった者の頻度は，30歳以上の男性では47%と高く，女性では36%であった．また，その頻度は，男性では30歳代22%，40歳代36%，50歳代44%，60歳代55%，70歳以上65%で，女性はそれぞれ5%，18%，37%，51%，61%であった．この数字は降圧治療中の者の血圧を含んでおり，実際の高血圧者の頻度はさらに高いと考えられる．

このように高血圧の頻度は高く，また年齢とともに上昇する．高血圧の早期発見のためには，全成人における定期的な血圧測定がきわめて重要と考えられる．

□ 遺伝因子と環境要因

高血圧者の大部分を占める本態性高血圧の成因はいまだ特定されてはいないが，遺伝因子と環境要因がともに関与していることは疑いない（図2）．各個人におけるこれらの要因を考慮することは，高血圧の早期発見の面からも重要と考えられる．

遺伝因子が高血圧の発症に役割を持つことは，多くの研究で示されている．特に両親が高血圧の場合には，子供が高血圧になる可能性が高く，定期的な血圧測定が望まれる．

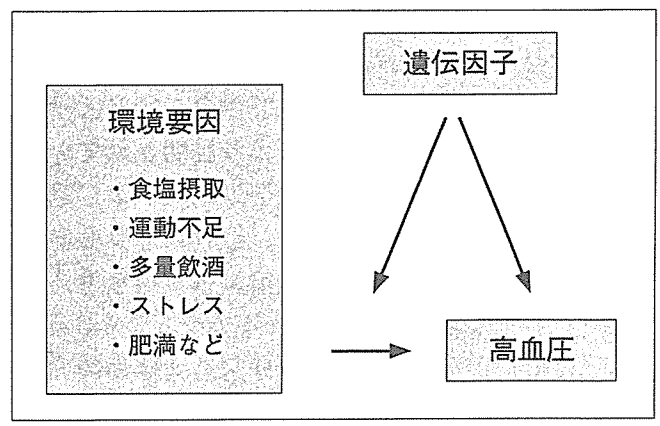

図2　高血圧の成因における遺伝と環境の影響

高血圧の遺伝子研究は急速に進行しており，われわれもミレニアム・ゲノム・プロジェクトにおいて，多くの血圧に関係する遺伝子やSNPを同定した[6,7]．これまでの知見では，一部のまれな二次性高血圧は特定の遺伝子変異によるが，本態性高血圧については，関連する遺伝子は多いものの，大部分の成因を説明できる単一の遺伝子変化はないようである．しかし，遺伝子が高血圧に関与することは明らかであり，生活習慣と高血圧との関係にも遺伝子が影響すると考えられる（図2）．遺伝子診断による高血圧の予知はまだ研究段階であるが，将来は実用化が期待される．

環境要因もまた，高血圧の発症や進展に大きな

表2　未治療の外来血圧正常者における仮面高血圧の頻度

	報告年	雑誌	CBPM 正常血圧基準	非医療環境下血圧 血圧測定法	非医療環境下血圧 高血圧基準	頻度 (%)	平均年齢 (歳)	男性 (%)
Sega	2001	*Circulation*	<140	HBPM	≧132	10.1*	25～74†	51.0
			<90		≧83	11.4*		
Hozawa	2002	*Hypertens Res*	<140/90	HBPM	≧135/85	11.0	40以上†	32.0
Hond	2002	*Blood Press Monit*	<140/90	HBPM	≧135/85	46.7*	52.8	46.8
Selenta	2000	*Arch Fam Med*	<140	daytime ABPM	≧135	23.0	17～68†	48.3
	2000		<90		≧85	24.0		
Hond	2003	*Blood Press Monit*	<140/90	daytime ABPM	≧135/85	33.3*	52.8	48.8
Bjorklund	2003	*Circulation*	<140/90	daytime ABPM	≧135/85	30.4*	70.0	100
Liu	1999	*Ann Intern Med*	<140/90	daytime ABPM	>134/90	20.7*	30～66†	46.4
Enstrom	1991	*J Hypertens*	<90	24 h ABPM/ at screening	≧85/≧90	14.0	40～64†	100
Imai	1996	*Hypertens Res*	≦140/90	24 h ABPM	≧133/78	13.6*	20～79†	31.4
Sega	2001	*Circulation*	<140	24 h ABPM	≧125	11.6*	25～74†	51-58
			<90		≧79	11.3*		
Hozawa	2002	*Hynertens Res*	<140/90	24 h ABPM	≧135/85	9.0	40以上†	32.0

（小原　拓，他：血圧 11：783-787，2004[14]より引用）

役割を有している．そのほとんどは生活習慣に関するもので，高血圧の危険因子として食塩の過剰摂取，肥満，運動不足，多量飲酒，ミネラルの摂取不足，ストレスなどがあげられる[8]（図2）．また，これらの生活習慣の修正は，高血圧の管理において広く推奨されている[1~4,9]．

生活習慣による血圧変化は，それぞれの項目や個人によって異なるが，食塩摂取は1 g/日あたり1 mmHgほど，体重増加は1 kgあたり1 mmHgほど収縮期血圧を上昇させる[8,9]．したがって，体重が大きく増加した場合などは高血圧を発症するリスクが高く，適切に血圧を測定することが望まれる．

□ 定期検診と家庭血圧測定

高血圧の有病率は高く，年齢とともに増加するので，早期発見には全成人における定期的な血圧測定が重要と考えられる．高血圧はSilent killerといわれるように自覚症状に乏しく，血圧値が診断基準になることから，血圧測定は絶対条件である．男性では30歳以上，女性では40歳以上になると高血圧の頻度が高くなるので，定期検診などの機会に必ず血圧測定を受けるべきであろう．特に正常高値血圧の者は高血圧を発症する可能性が高く，注意深い経過観察を要する．

小児では，高血圧の診断基準は成人とは異なり，その頻度や血圧の平均値はより低い．しかし，小児においても高血圧はまれではなく，また肥満者の増加にともない血圧値が高くなっていることが示されている[10]．血圧が高い者はその後も高いというtracking現象があるので，小児においても入学時などでの検診時に血圧が測定されることが望ましい．

検診や外来で測定される随時血圧は，各個人の通常の血圧を表しているとは限らない．随時血圧は高いが24時間血圧や家庭血圧は正常な白衣高血圧や，その逆の仮面高血圧を呈する者が少なくない[11]．白衣高血圧は，持続性高血圧より臓器障害や予後は良好であり，随時血圧のみで管理されれば過剰な治療を受けることになろう．仮面高血圧は，臓器障害や予後が持続性高血圧と同等であり，注意すべき病態であることが示されている[12,13]．

白衣高血圧や仮面高血圧の頻度は，診断基準などにより異なるが，前者は検診で高血圧とされる者の約20%，後者は正常血圧とされる者の10～20%にのぼる[14]（表2）．わが国における数は，それぞれ約700万人と推計される．このことは，全国民が検診を受けても約1,400万人は誤った管理を受ける可能性を示しており，重要な問題と考えられる．家庭血圧測定のさらなる普及と，それに基づいた管理システムの確立が望まれる．

おわりに

高血圧の早期発見の重要性と，関連する事柄について述べた．わが国では検診システムがかなり整っているが，すべての成人が定期的に血圧測定を受けているわけではない．したがって，高血圧であっても診断されていない者は少なくないと考えられる．高血圧の早期発見には検診のさらなる普及が望まれるが，それだけでは不十分なことは白衣高血圧や仮面高血圧の病態や予後に示されている．家庭血圧計はかなり普及しているが，これが常備され家族全員の血圧が測定できれば，より効果的な高血圧の早期発見と適切な管理が可能となるであろう．

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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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Masked Hypertension and Target Organ Damage in Treated Hypertensive Patients

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Background: Recent studies have shown that an elevated ambulatory or home blood pressure (BP) in the absence of office BP—a phenomenon called masked hypertension—is associated with poor cardiovascular prognosis. However, it remains to be elucidated how masked hypertension modifies target organ damage in treated hypertensive patients.

Methods: A total of 332 outpatients with chronically treated essential hypertension were enrolled in the present study. Patients were classified into four groups according to office (<140/90 or ≥140/90 mm Hg) and daytime ambulatory (<135/85 or ≥135/85 mm Hg) BP levels; ie, controlled hypertension (low office and ambulatory BP), white-coat hypertension (high office but low ambulatory BP), masked hypertension (low office but high ambulatory BP), and sustained hypertension (high office and ambulatory BP). Left ventricular mass index, carotid maximal intima-media thickness, and urinary albumin levels were determined in all subjects.

Results: Of the patients, 51 (15%), 65 (20%), 74 (22%), and 142 (43%) were identified as having controlled hypertension, white-coat hypertension, masked hypertension, and sustained hypertension, respectively. Left ventricular mass index, maximal intima-media thickness, and urinary albumin level in masked hypertension were significantly higher than in controlled hypertension and white-coat hypertension, and were similar to those in sustained hypertension. Multivariate regression analyses revealed that the presence of masked hypertension was one of the independent determinants of left ventricular hypertrophy, carotid atherosclerosis, and albuminuria.

Conclusions: Our findings indicate that masked hypertension is associated with advanced target organ damage in treated hypertensive patients, comparable to that in cases of sustained hypertension. Am J Hypertens 2006; 19:880–886 © 2006 American Journal of Hypertension, Ltd.

Key Words: Blood pressure, ambulatory, cardiac hypertrophy, atherosclerosis, albuminuria.

Several population-based studies and prospective clinical studies have shown that ambulatory blood pressure (BP) is a significant predictor for cardiovascular morbidity and mortality even after adjustment for conventional BP.[1–3] In fact, left ventricular (LV) hypertrophy and other end-organ damage are more closely associated with average BP levels assessed by 24-h ambulatory monitoring than isolated BP readings taken in the office.[4,5] There is often a discrepancy between office and ambulatory BP, and many studies have evaluated the association between white-coat hypertension, a normal ambulatory but elevated office BP, and cardiovascular risk.[6,7] On the other hand, the converse of white-coat hypertension called "reverse white-coat hypertension," "white-coat normotension," or "isolated ambulatory hypertension," ie, a high ambulatory but normal office BP, has received little attention. This phenomenon is also called "masked hypertension" on the grounds that the hypertension is not detected by routine methods in the clinic.[8] Recent studies indicated that an elevated ambulatory or home BP despite a normal or well-controlled office BP is associated with poor cardiovascular prognosis in both untreated and treated hypertensive patients.[9–11] The present study was conducted to verify

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the possible association between masked hypertension and target organ abnormalities such as LV hypertrophy, carotid arteriosclerosis, and albuminuria in treated hypertensive patients.

Methods

Subjects

A total of 332 outpatients with treated essential hypertension (163 men and 169 women; mean age, 66 ± 10 years) were enrolled in the present study. Patients with secondary hypertension, stroke, ischemic heart disease including myocardial infarction, congestive heart failure, chronic glomerulonephritis, nephrotic syndrome, renal failure (serum creatinine ≥160 μmol/L), or poorly controlled (fasting plasma glucose ≥10.0 mmol/L or hemoglobin A_{1c} ≥8.0%) or insulin-treated diabetes mellitus were excluded from this study. Diabetes mellitus was diagnosed according to the American Diabetes Association criteria, such as a fasting plasma glucose of ≥7.0 mmol/L and/or a plasma glucose level at 2 h after a 75-g oral glucose load of ≥11.1 mmol/L, or when medication was taken for treatment of hyperglycemia. A diagnosis of hyperlipidemia required a serum total cholesterol level of ≥5.69 mmol/L and/or a serum triglyceride level of ≥1.69 mmol/L or the use of lipid-lowering drugs.

All patients had taken antihypertensive drugs for at least 1 year (average, 13 years). Of the patients, 237 patients (71%) were treated with Ca channel blockers, 111 (33%) with angiotensin II receptor blockers, 56 (17%) with angiotensin converting enzyme inhibitors, 106 (32%) with β-blockers, 66 (20%) with diuretics, and 34 (10%) with other classes of agents. Combination drug treatments were used in 188 subjects (57%). All subjects gave their informed consent to participate in the present study. All procedures of the present study were carried out in accordance with institutional and national ethical guidelines for human studies.

Measurement of BP

In each visit, office BP was measured twice by a physician in a hospital outpatient clinic with the patient in a sitting position after ≥20 min of rest, using an appropriate-sized arm cuff and mercury sphygmomanometer. The first and fifth Korotkoff sounds were used to identify systolic and diastolic values, respectively. Office BP was determined by averaging six measurements taken on three separate occasions during a 3-month period.

In the same study period, all subjects underwent 24-h ambulatory BP monitoring. BP and heart rate were measured every 30 min during the day and night by the oscillometric method using an automatic monitoring device (TM-2421, A&D Co., Tokyo, Japan).[12] Accuracy and performance of this device have been previously demonstrated.[13] The patients were instructed to continue with their normal daily activities during measurements and to note their activity and location in a diary. According to the diary, daytime and night-time were determined as the waking and sleeping periods of the patient, respectively, and mean values of 24-h, daytime, and night-time BP (systolic and diastolic) were calculated. We also analyzed short-term BP variability and circadian BP variation. Short-term BP variability was calculated as the standard deviation (SD) of daytime and night-time ambulatory BP obtained every 30 min. Circadian BP variation was defined as a nocturnal dipping in BP and calculated as 100 × (daytime BP − night-time BP) / daytime BP.

In the present study, all subjects were classified into four groups based on the levels of office and daytime ambulatory BP, as follows: 1) controlled hypertension (ie, office BP <140/90 mm Hg and daytime ambulatory BP <135/85 mm Hg); 2) white-coat hypertension (ie, isolated uncontrolled office hypertension, office BP ≥140/90 mm Hg, and ambulatory BP <135/85 mm Hg); 3) masked hypertension (ie, isolated uncontrolled ambulatory hypertension, office BP <140/90 mm Hg and ambulatory BP ≥135/85 mm Hg); and 4) sustained hypertension (ie, uncontrolled hypertension, office BP ≥140/90 mm Hg and ambulatory BP ≥135/85 mm Hg).

Echocardiography

A comprehensive two-dimensional echocardiography was performed using a cardiac ultrasound unit (Sonos 5500, Philips Medical Systems, Andover, MA) as previously described.[14] Echocardiographic parameters were measured by the consensus of two experienced investigators who were blinded to the clinical data including office and ambulatory BP of the subjects. Measurements included interventricular septal thickness (IVSTd), posterior wall thickness (PWTd), LV diameter at end-diastole (LVDd), and LV diameter at end-systole (LVDs). The LV mass was estimated using the formula validated by Devereux and Reichek[15]: LV mass (g) = $1.04 \times \{(IVSTd + PWTd + LVDd)^3 - LVDd^3\} - 13.6$. The LV mass was normalized for body surface area and expressed as the LV mass index. The intra- and interobserver coefficients of variation of LV mass index were 6.7% and 9.8%, respectively.

Carotid Ultrasonography

Ultrasound examinations of both carotid arteries were performed using a high resolution Duplex scanner (model SSA-390A, Toshiba, Tokyo, Japan) with the probe at a frequency of 7.5 MHz for the B-scan, as previously described.[16] All measurements were performed by two trained sonographers who were unaware of the subjects' clinical data. The carotid arteries were carefully examined with regard to wall changes from different longitudinal and transverse views. The common carotid artery, the carotid bulb, and the internal and external carotid arteries were studied in all subjects. Each ultrasound image was taken at the end-diastolic phase. We assessed carotid intima-media thickness (IMT) and plaques by measuring generally used

parameters such as conventional IMT and maximal IMT.[16] Conventional IMT was defined as an average of six IMT approximately 15 mm proximal to the carotid bulb in the right and left common carotid arteries avoiding discrete plaques. Maximal IMT was defined as the maximal thickness of intima-media including plaques. Maximal IMT was assessed from the region branching off from the brachiocephalic artery (right) or aorta (left) to the bifurcation of the common carotid artery. The intra- and inter-observer coefficients of variation of maximal IMT were 4.2% and 7.9%, respectively.

Biochemical Measurements

Blood samples were obtained in the morning after an overnight fast. Total cholesterol, triglycerides, fasting plasma glucose, hemoglobin A_{1c}, fasting insulin, and serum creatinine levels were determined by standard laboratory measurements. The homeostasis model assessment (HOMA) index, a parameter of insulin resistance, was calculated as fasting plasma glucose × fasting insulin/22.5. Creatinine clearance was calculated from the Cockcroft-Gault formula.[17] The urinary albumin (U-Alb) level was measured as the ratio of albumin to creatinine excretion in the urine and expressed as $\log_{10}$ mg/g Cr.

Statistical Analysis

Statistical analysis was performed using StatView Version 5 Software (Abacus Concepts, Berkeley, CA). Values are expressed as the mean ± SD. The significance of differences among the four groups with controlled, white-coat, masked, and sustained hypertension was evaluated by unpaired ANOVA with subsequent Fisher's multiple comparison test. A stepwise multiple regression analysis was performed to identify independent determinants of target organ damage (LV mass index, maximal IMT, and U-Alb levels). A value of $P < .05$ was accepted as statistically significant.

Results

General characteristics of the four subject groups classified according to certain levels of office BP (<140/90 or ≥140/90 mm Hg) and daytime ambulatory BP (<135/85 or ≥135/85 mm Hg) are summarized in Table 1. Of the patients, 51 (15%), 65 (20%), 74 (22%), and 142 (43%) were identified as having controlled hypertension, white-coat hypertension, masked hypertension, and sustained hypertension, respectively. Age was youngest and the proportion of men was highest in subjects with masked hypertension. The rate of habitual drinkers was significantly

Table 1. Clinical characteristics and antihypertensive treatment of study patients

Characteristic	Controlled hypertension (n = 51)	White-coat hypertension (n = 65)	Masked hypertension (n = 74)	Sustained hypertension (n = 142)
Age (y)	67 ± 8	67 ± 7	63 ± 11*†	67 ± 10‡
Sex (male/female)	24/27	23/42	48/26*†	68/74‡
Body mass index (kg/m^2)	24 ± 3	24 ± 3	25 ± 4	24 ± 3
Duration of hypertension (y)	19 ± 11	20 ± 11	17 ± 10	18 ± 11
Diabetes mellitus (%)	14	23	20	21
Hyperlipidemia (%)	59	72	70	67
Current smoking (%)	16	17	20	18
Habitual drinking (%)	51	46	66†	52
Total cholesterol (mmol/L)	5.1 ± 0.6	5.3 ± 0.8	5.3 ± 0.8	5.3 ± 0.7
Triglycerides (mmol/L)	1.4 ± 0.6	1.3 ± 0.8	1.6 ± 0.9†	1.5 ± 0.9
Fasting plasma glucose (mmol/L)	5.7 ± 1.6	5.7 ± 1.2	5.7 ± 1.0	5.8 ± 1.0
Hemoglobin A_{1c} (%)	5.5 ± 0.7	5.7 ± 0.9	5.5 ± 0.6	5.7 ± 0.8
Fasting insulin (mU/L)	6.0 ± 2.6	6.7 ± 4.3	7.4 ± 4.5	7.7 ± 11.7
HOMA index	1.5 ± 0.9	1.8 ± 1.5	1.9 ± 1.2	2.0 ± 3.1
Creatinine clearance (mL/min)	81 ± 26	81 ± 23	89 ± 36	80 ± 26
Antihypertensive treatment				
Period of medication (y)	13 ± 9	14 ± 10	11 ± 9†	13 ± 9
Ca channel blockers (%)	57	74*	76*	73*
AII receptor blockers (%)	41	28	38	31
ACE inhibitors (%)	18	12	15	20
β-Blockers (%)	31	45	31	27†
Diuretics (%)	20	23	27	15‡
Others (%)	16	5	8	12
Combination treatment (%)	55	62	68	49‡
Total number of classes	1.8 ± 1.0	1.9 ± 0.8	1.9 ± 0.8	1.8 ± 1.0

ACE = angiotensin-converting enzyme; AII = angiotensin II; HOMA = homeostasis model assessment.
Values are mean ± SD or percentage.
* $P < .05$ *v* controlled hypertension; † $P < .05$ *v* white-coat hypertension; ‡ $P < .05$ *v* masked hypertension.