Association of gene polymorphisms with blood pressure and the prevalence of hypertension in community-dwelling Japanese individuals

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Abstract. Hypertension is a complex multifactorial disorder that is thought to result from an interaction between genetic background and environmental factors. Although various loci and genes have been implicated in predisposition to hypertension by genetic linkage analyses and candidate gene association studies, the genes that confer susceptibility to this condition remain to be identified definitively. We examined the relations of nine candidate gene polymorphisms to blood pressure (BP) and the prevalence of hypertension in a population-based study. The 2238 subjects (1110 women, 1128 men) were aged 40 to 79 years and were randomly recruited for a population-based prospective cohort study of aging and age-related diseases in Japan. BP was measured with subjects having rested in a sitting position for at least 15 min. Genotypes for the 160C→T (Arg54Trp) polymorphism of *QPCT*, the C→T (Pro198Leu) polymorphism of *GPX1*, the 137,346T→C polymorphism of *FYN*, the -344C→T polymorphism of *CYP11B2*, and the A→G (Ser49Gly) polymorphism of *ADRB1* were determined with a fluorescence-based allele-specific DNA primer assay system; those for the A→G polymorphism of *CNR2*, the I/D (22,375delAC) polymorphism of *CAV1*, and the -1213T→C polymorphism of *ESR2* by melting curve analysis, and that for the $(GT)_n$ polymorphism of *COL1A2* were determined by DNA fragment analysis. The polymorphism of *FYN* was associated with systolic and diastolic BP in women. In men, polymorphisms of *CNR2*, *QPCT*, *GPX1*, *COL1A2*, *CYP11B2*, and *ESR2* were associated with systolic and diastolic BP, those of *CAV1* and *FYN* with systolic BP, and that of *ADRB1* with diastolic BP. The polymorphisms of *QPCT* and *CYP11B2* were also associated with the prevalence of hypertension in men. These results suggest that polymorphisms of *QPCT* and *CYP11B2* are determinants of BP and the development of hypertension in Japanese men.

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Introduction

Hypertension is a complex multifactorial disorder that is thought to result from an interaction between an individual's genetic background and various environmental factors (1). Given that hypertension is a major risk factor for coronary heart disease, stroke, and chronic renal failure, personalized prevention of hypertension is an important public health goal. One approach to personalized prevention of, and selection of the most appropriate treatment for, hypertension is to identify genes that confer susceptibility to this condition. Although genetic linkage analyses (2-5) and candidate gene association studies (6-9) have implicated various loci and genes in the predisposition to hypertension, the genes that confer genetic susceptibility to this condition remain to be identified definitively. In addition, because of ethnic divergence of gene polymorphisms as well as of environmental factors and lifestyle, it is important to examine polymorphisms related to hypertension in each ethnic group.

We have been attempting to identify genes significantly associated with blood pressure (BP) in Japanese women or men with a population-based approach. In the present study, we selected nine candidate genes that might be expected to contribute to the regulation of BP (Table I) and examined the relations of polymorphisms of these genes to BP, even though there was no apparent biological link among these genes. Our aim was to identify a single polymorphism significantly associated with BP for each gene. Among several polymorphisms previously identified, we selected those that might be expected to affect gene function. We thus examined the relations of these polymorphisms to BP and the prevalence of hypertension in community-dwelling Japanese women and men.

Materials and methods

Study population. The National Institute for Longevity Sciences, the Longitudinal Study of Aging, is a population-

Table I. The nine gene polymorphisms examined in the present study.

Locus	Gene	Symbol	Polymorphism	dbSNP
1p36.11	Cannabinoid receptor 2	*CNR2*	A→G	rs2501431
2p22.2	Glutaminyl-peptide cyclotransferase	*QPCT*	160C→T (Arg54Trp)	rs2255991
3p21.3	Glutathione peroxidase	*GPX1*	C→T (Pro198Leu)	rs1050450
6q21	FYN oncogene related to SRC, FGR, YES	*FYN*	137,346T→C	rs706895
7q22.1	Collagen, type I, α-2	*COL1A2*	$(GT)_n$	ND
7q31.1	Caveolin 1	*CAV1*	I/D (22,375delAC)	rs3840634
8q21-q22	Cytochrome P450, subfamily Y XIB, polypeptide 2	*CYP11B2*	-344C→T	rs1799998
10q24-q26	ß-1-adrenergic receptor	*ADRB1*	A→G (Ser49Gly)	rs1801252
14q23.2	Estrogen receptor 2	*ESR2*	-1213T→C	ND

ND, not detected in dbSNP.

based prospective cohort study of aging and age-related diseases (10). The subjects were unrelated individuals stratified by both age and sex, and were randomly selected from resident registrations in the city of Obu and town of Higashiura in central Japan (11-13). The lifestyle of residents of this area is typical of that of individuals in most regions of Japan. The numbers of men and women recruited were similar and the age at baseline was 40-79 years, with similar numbers of participants in each decade (40, 50, 60 and 70s). The subjects are being followed up every 2 years. All participants were subjected at a special center to a detailed examination, which included not only medical evaluation but also assessment of exercise physiology, body composition, nutrition, and psychology. Individuals with coronary heart disease, valvular heart disease, cardiomyopathies, or renal or endocrinologic diseases that cause secondary hypertension were excluded from the present study. We thus examined the relations of gene polymorphisms to BP or the prevalence of hypertension in 2238 individuals (1110 women, 1128 men). Individuals whose genotypes were not successfully determined were excluded from the analysis. The study protocol complies with the Declaration of Helsinki and was approved by the Committee on Ethics of Human Research of the National Institute for Longevity Sciences. Written informed consent was obtained from each subject.

Measurement of BP. BP was measured with an automatic sphygmomanometer (BP-203RV-II; Colin, Tokyo, Japan) in subjects having rested in a sitting position for at least 15 min. BP in each subject was confirmed with the measurement made by a physician with a mercury manometer according to the guidelines of the American Heart Association (14). Normal BP was defined as both a systolic BP of <140 mmHg and a diastolic BP of <90 mmHg. Hypertension was defined as a systolic BP of ≥140 mmHg or a diastolic BP of ≥90 mmHg (or both), or the use of antihypertensive medication.

Determination of genotype. Genotypes for polymorphisms of *QPCT*, *GPX1*, *FYN*, *CYP11B2*, and *ADRB1* were determined with a fluorescence-based allele-specific DNA primer assay system (Toyobo Gene Analysis, Tsuruga, Japan) (15). Primers and other conditions for genotyping are shown in Table II. The polymorphic region of each gene was amplified by the polymerase chain reaction (PCR) with allele-specific sense primers labeled at the 5' end with either fluorescein isothiocyanate or Texas red and with an antisense primer labeled at the 5' end with biotin. The reaction mixture (25 μl) contained 20 ng of DNA, 5 pmol of each primer, 0.2 mmol/l of each deoxynucleoside triphosphate, 2.5-4 mmol/l $MgCl_2$, and 1 U of rTaq DNA polymerase (Toyobo, Osaka, Japan) in polymerase buffer. The amplification protocol comprised initial denaturation at 95˚C for 5 min; 35 cycles of denaturation at 95˚C for 30 sec, annealing at 60-70˚C for 30 sec, and extension at 72˚C for 30 sec; and a final extension at 72˚C for 2 min. The amplified DNA was incubated with streptavidin-conjugated magnetic beads in the wells of a 96-well plate at room temperature, and the plate was then placed on a magnetic stand. The supernatants from each well were transferred to the wells of a 96-well plate containing 0.01 mol/l NaOH and were measured for fluorescence with a microplate reader (Fluoroscan Ascent; Dainippon Pharmaceutical, Osaka, Japan) at excitation and emission wavelengths of 485 and 538 nm, respectively, for fluorescein isothiocyanate and of 584 and 612 nm, respectively, for Texas red.

Genotypes for polymorphisms of *CNR2*, *CAV1*, and *ESR2* were determined by melting curve analysis (intercalater-mediated fluorescence resonance energy transfer probe method). The polymorphic region of each gene was amplified by PCR (Table II) in a reaction mixture (25 μl) containing 20 ng of DNA, 5 pmol of each primer, 0.2 mmol/l of each deoxynucleoside triphosphate, 2 mmol/l $MgCl_2$, and 1.25 U of rTaq DNA polymerase in polymerase buffer. The amplification protocol comprised initial denaturation at 95˚C for 5 min; 45 cycles of denaturation at 95˚C for 30 sec, annealing at 65˚C for 30 sec, and extension at 72˚C for 30 sec; and a final extension at 72˚C for 2 min. A mixture (2 μl) of 10 pmol of probe and SYBR-Green was added to the PCR products, which were then transferred to a PRISM 7700 instrument (Applied Biosystems, Foster City, CA) for measurement of melting temperature. The program for analytic melting comprised incubation at 95˚C for 30 sec, 40˚C for 1 min, and temperatures increasing to 80˚C over 10 min. The fluorescence signals were detected at excitation and emission wavelengths of 485 and 612 nm, respectively.

Table II. Primers, probes, and other PCR conditions for genotyping.

Gene	Polymorphism	Sense primer with FITC	Sense primer with Texas red
QPCT	160C→T (Arg54Trp)	TTTGAATTCATCGGCTCTxCG	TTTGAATTCATCGGCTCTxTG
GPX1	C→T (Pro198Leu)	GCGCCCTAGGCACAGCTxAG	GCGCCCTAGGCACAGCTxGG
FYN	137,346T→C	GGAGTAATTGACAAGGCTCAxCG	AGGAGTAATTGACAAGGCTCAxTG
CYP11B2	-344C→T	TATTAAAAGAATCCAAGGxCC	GTCTATTAAAAGAATCCAAGGxTC
ADRB1	A→G (Ser49Gly)	GAGACAGCGGCTCGGGGxCT	GACAGCGGCTCGGGGxTT

	Antisense primer with biotin	Annealing (˚C)	Cycles	Mg^{2+} (mM)	Taq/KOD
QPCT	GGTATCGCTCTATCAGCAATGG	62.5	35	2.5	Taq
GPX1	GTGTGCCCCTACGCAGGTACA	65.0	35	2.5	Taq
FYN	CCTTTCCTCATGCCCCCTAAT	67.5	35	4.0	Taq
CYP11B2	GGACTTTATCTTATCGTGAGATGA	60.0	35	3.0	Taq
ADRB1	GCCGCCCGCCTCGTTG	70.0	35	3.5	Taq

Gene	Polymorphism	Sense primer	Antisense primer
CNR2	A→G	GGGCAGGTAGGAGACTAGTGCTGAGAG	CTCACCCGTGGAAGGGCACTG
CAV1	I/D (22,375delAC)	AAAGGTGATGGATCATTTCCCATTATACAC	TGGGCAATGGTCATCCATGACTG
ESR2	-1213T→C	GAACAGGAGCCAGGGGCACAG	CCTGAAGACAAGTACCTTGCAGCTGAG

	Probe	Annealing (˚C)	Cycles	Mg^{2+} (mM)	Taq/KOD
CNR2	CACATGATGCCCAGGGTC	65.0	45	2.0	Taq
CAV1	CAAAATGTGTGTCCATTTCAGG	65.0	45	2.0	Taq
ESR2	AACAGTAAAATTCTGCCTGGG	65.0	45	2.0	Taq

Gene	Polymorphism	Sense primer with FAM	Antisense primer
COL1A2	$(GT)_n$	CAGCACGGTGTCTACCACTGC	ATTACTCCTTAGTATCCACAGTATGTATAC

	Annealing (˚C)	Cycles	Mg^{2+} (mM)	Taq/KOD
COL1A2	60.0	35	1.2	KOD

FITC, fluorescein isothiocyanate; FAM, 6-carboxyfluorescein. Oligonucleotide sequences are 5'→3'.

The GT repeats [$(GT)_n$] in the first intron of *COL1A2* were amplified by PCR with a sense primer labeled at the 5' end with 6-carboxyfluorescein and with an antisense primer (Table II). The reaction mixture (25 μl) contained 20 ng of DNA, 5 pmol of each primer, 0.2 mmol/l of each deoxynucleoside triphosphate, 1.2 mmol/l $MgSO_4$, and 0.4 U of KOD plus DNA polymerase (Toyobo) in polymerase buffer. The amplification protocol comprised initial denaturation at 94˚C for 5 min; 35 cycles of denaturation at 94˚C for 15 sec, annealing at 60˚C for 30 sec, and extension at 68˚C for 30 sec; and a final extension at 68˚C for 2 min. The size of the $(GT)_n$-containing PCR products was determined with a PRISM 3100 DNA sequencer and with GeneScan and Genotyper software (Applied Biosystems).

Statistical analysis. Age and BP were compared among three groups by one-way analysis of variance and the Tukey-Kramer *post hoc* test, and between two groups by the unpaired Student's t-test. BP values were analyzed in individuals who were not taking antihypertensive drugs. The prevalence of hypertension was compared between two groups (2x2) or among three groups (3x2) by the Chi-square test in all individuals. Allele frequencies were estimated by the gene-counting method, and the Chi-square test was used to identify significant departure from Hardy-Weinberg equilibrium. A P value of <0.05 was considered statistically significant.

Results

Relation of the A→G polymorphism of CNR2 to BP. For men, the distribution of genotypes for the A→G polymorphism of *CNR2* was in Hardy-Weinberg equilibrium and individuals in the combined group of *AG* and *GG* genotypes were younger than those with the *AA* genotype (Table III). Systolic and diastolic BP were significantly higher in men with the *GG* genotype than in those with the *AA* genotype or with the *AG* genotype or in those in the combined group of *AA* and *AG*

Table III. Blood pressure (BP) for male subjects according to *CNR2* genotype.

Characteristics	*AA*	*AG*	*GG*	*AA + AG*	*AG + GG*
Number of subjects (n=874)[a]	295	425	154	720	579
Age (years)	60.2±0.6	58.5±0.5	59.2±0.8	59.2±0.4	58.7±0.4[b]
Systolic BP (mmHg)	120.5±1.0[c]	120.3±0.8[d]	125.0±1.4	120.4 ±0.6[e]	121.6±0.7
Diastolic BP (mmHg)	74.8±0.6[f]	74.9±0.5[g]	78.0±0.9	74.9±0.4[h]	75.8±0.4
Number of all subjects (n=1122)	387	549	186	936	735
Hypertension (%)	34.4	33.5	37.6	33.9	34.6

[a]Subjects not taking antihypertensive medication. Data for age and BP are means ± SE. Data for the combined group of *AA* and *AG* genotypes (*AA + AG*) were compared with those for individuals with the *GG* genotype (recessive genetic model). Data for the combined group of *AG* and *GG* genotypes (*AG + GG*) were compared with those for individuals with the *AA* genotype (dominant genetic model). [b]P=0.0313 versus AA; [c]P=0.0230, [d]P=0.0101, [e]P=0.0025, [f]P=0.0071, [g]P=0.0065, [h]P=0.0010 versus *GG*.

Table IV. Blood pressure (BP) for male subjects according to *QPCT* genotype.

Characteristics	*CC*	*CT*	*TT*	*CC + CT*	*CT + TT*
Number of subjects (n=878)[a]	387	379	112	766	491
Age (years)	58.8±0.5	59.8±0.5	58.4±0.9	59.3±0.4	59.5±0.4
Systolic BP (mmHg)	119.5±0.9	122.4±0.9	121.7±1.7	121.1±0.6	122.3±0.8[b]
Diastolic BP (mmHg)	74.6±0.5	76.3±0.5	75.1±1.0	75.5±0.4	76.1±0.5[c]
Number of all subjects (n=1128)	487	501	140	988	641
Hypertension (%)	29.2[e]	39.5[e]	35.0[e]	34.4	38.5[d]

[a]Subjects not taking antihypertensive medication. Data for age and BP are means ± SE. Data for the combined group of *CC* and *CT* genotypes (*CC + CT*) were compared with those for individuals with the *TT* genotype (recessive genetic model). Data for the combined group of *CT* and *TT* genotypes (*CT + TT*) were compared with those for individuals with the *CC* genotype (dominant genetic model). [b]P=0.0233, [c]P=0.0434, [d]P=0.0010 versus CC. [e]P=0.0027 (*CC* versus *CT* versus *TT*, 3x2 Chi-square test).

genotypes. The differences in systolic and diastolic BP between individuals with the *GG* genotype and those in the combined group of *AA* and *AG* genotypes (expressed as a percentage of the larger value) were 3.7 and 4.0%, respectively. The prevalence of hypertension did not differ among *CNR2* genotypes for men. For women, neither systolic or diastolic BP nor the prevalence of hypertension differed among *CNR2* genotypes (data not shown).

Relation of the 160C→T (Arg54Trp) polymorphism of QPCT to BP. The distribution of genotypes for the160C→T polymorphism of *QPCT* was in Hardy-Weinberg equilibrium and age did not differ among genotypes for men (Table IV). Systolic and diastolic BP were significantly greater for men in the combined group of *CT* and *TT* genotypes than for those with the *CC* genotype; the differences in systolic and diastolic BP between these groups were 2.3 and 2.0%, respectively. The prevalence of hypertension also differed significantly among genotypes (*CC* versus *CT* versus *TT*), being greater for men in the combined group of *CT* and *TT* genotypes than for those with the *CC* genotype. The odds ratio of the *T* allele compared with the *C* allele for predisposition to hypertension was 1.3. There were no differences in systolic or diastolic BP or in the prevalence of hypertension among *QPCT* genotypes in women (data not shown).

Relation of the C→T (Pro198Leu) polymorphism of GPX1 to BP. Among men, the distribution of genotypes for the C→T (Pro198Leu) polymorphism of *GPX1* was in Hardy-Weinberg equilibrium and age did not differ among genotypes (Table V). Systolic and diastolic BP were significantly higher for men with the *CT* genotype or for those in the combined group of *CT* and *TT* genotypes than for those with the *CC* genotype. The differences in systolic and diastolic BP between individuals in the combined group of *CT* and *TT* genotypes and those with the *CC* genotype were 3.6 and 3.7%, respectively. The prevalence of hypertension did not differ among *GPX1* genotypes for men. Among women, no difference in systolic or diastolic BP or in the prevalence of hypertension was detected among *GPX1* genotypes (data not shown).

Relation of the 137,346T→C polymorphism of FYN to BP. For women, the distribution of genotypes for the 137,346T→C polymorphism of *FYN* was in Hardy-Weinberg equilibrium and age did not differ among genotypes (Table VI). Systolic and diastolic BP were significantly higher in women with the *TC* genotype or in those in the combined group of *TC* and *CC* genotypes than in those with the *TT* genotype. The differences in systolic and diastolic BP between individuals in the combined group of *TC* and *CC* genotypes and those

Table V. Blood pressure (BP) for male subjects according to *GPX1* genotype.

Characteristics	*CC*	*CT*	*TT*	*CC + CT*	*CT + TT*
Number of subjects (n=879)[a]	750	126	3	876	129
Age (years)	59.3±0.4	58.7±0.9	59.0±6.3	59.2±0.4	58.7±0.9
Systolic BP (mmHg)	120.5±0.6	125.0±1.5[b]	122.0±9.9	121.2±0.6	125.0±1.5[c]
Diastolic BP (mmHg)	75.0±0.4	78.0±0.9[d]	71.7±6.1	75.4±0.4	77.9±0.9[e]
Number of all subjects (n=1128)	971	154	3	1125	157
Hypertension (%)	34.0	37.7	0	34.5	36.9

[a]Subjects not taking antihypertensive medication. Data for age and BP are means ± SE. Data for the combined group of *CC* and *CT* genotypes (*CC + CT*) were compared with those for individuals with the *TT* genotype (recessive genetic model). Data for the combined group of *CT* and *TT* genotypes (*CT + TT*) were compared with those for individuals with the *CC* genotype (dominant genetic model). [b]P=0.0188, [c]P=0.0072, [d]P=0.0078, [e]P=0.0040 versus *CC*.

Table VI. Blood pressure (BP) for female subjects according to *FYN* genotype.

Characteristics	*TT*	*TC*	*CC*	*TT + TC*	*TC + CC*
Number of subjects (n=883)[a]	338	423	122	761	545
Age (years)	59.0±0.5	59.6±0.5	58.1±0.9	59.3±0.4	59.3±0.3
Systolic BP (mmHg)	117.9±1.0	121.6±0.9[b]	119.6±1.7	119.9±0.7	121.1±0.8[c]
Diastolic BP (mmHg)	71.5±0.6	73.8±0.5[d]	72.4±1.0	72.8±0.4	73.5±0.5[e]
Number of all subjects (n=1109)	426	532	151	958	683
Hypertension (%)	32.4	37.3	31.8	35.1	36.1

[a]Subjects not taking antihypertensive medication. Data for age and BP are means ± SE. Data for the combined group of *TT* and *TC* genotypes (*TT + TC*) were compared with those for individuals with the *CC* genotype (recessive genetic model). Data for the combined group of *TC* and *CC* genotypes (*TC + CC*) were compared with those for individuals with the *TT* genotype (dominant genetic model). [b]P=0.0217, [c]P=0.0138, [d]P=0.0132, [e]P=0.0104 versus *TT*.

Table VII. Blood pressure (BP) for male subjects according to *FYN* genotype.

Characteristics	*TT*	*TC*	*CC*	*TT + TC*	*TC + CC*
Number of subjects (n=875)[a]	339	409	127	748	536
Age (years)	59.0±0.5	59.4±0.5	59.1±0.9	59.2±0.4	59.4±0.4
Systolic BP (mmHg)	119.5±1.0	122.3±0.9	121.3±1.6	121.2±0.6	122.1±0.7[b]
Diastolic BP (mmHg)	74.7±0.6	75.8±0.5	76.1±0.9	75.3±0.4	75.9±0.5
Number of all subjects (n=1122)	441	527	154	968	681
Hypertension (%)	32.4	36.6	31.8	34.7	35.5

[a]Subjects not taking antihypertensive medication. Data for age and BP are means ± SE. Data for the combined group of *TT* and *TC* genotypes (*TT + TC*) were compared with those for individuals with the *CC* genotype (recessive genetic model). Data for the combined group of *TC* and *CC* genotypes (*TC + CC*) were compared with those for individuals with the *TT* genotype (dominant genetic model). [b]P=0.0344 versus *TT*.

with the *TT* genotype were 2.6 and 2.7%, respectively. The prevalence of hypertension did not differ among *FYN* genotypes for women.

For men, the distribution of *FYN* genotypes was in Hardy-Weinberg equilibrium and age did not differ among genotypes (Table VII). Systolic BP was significantly higher for men in the combined group of *TC* and *CC* genotypes than for those with the *TT* genotype; the difference in systolic BP between these groups was 2.1%. The prevalence of hypertension did not differ among *FYN* genotypes for men.

Relation of the $(GT)_n$ polymorphism of COL1A2 to BP. Given that the mean and median numbers of GT repeats for *COL1A2* in the study subjects were 14.2 and 12, respectively, we designated $(GT)_{n\leq12}$ and $(GT)_{n\geq13}$ as short (*S*) and long (*L*) alleles, respectively. The distribution of the *SS*, *SL*, and *LL*

Table VIII. Blood pressure (BP) for male subjects according to *COL1A2* genotype.

Characteristics	*SS*	*SL*	*LL*	*SS + SL*	*SL + LL*
Number of subjects (n=854)[a]	252	415	187	667	602
Age (years)	59.7±0.6	59.4±0.5	58.7±0.7	59.5±0.4	59.2±0.4
Systolic BP (mmHg)	123.1±1.1	120.4±0.9	120.6±1.3	121.4±0.7	120.5±0.7[b]
Diastolic BP (mmHg)	76.8±0.7	74.7±0.5[c]	75.4±0.8	75.5±0.4	7 4.9±0.4[d]
Number of all subjects (n=1095)	308	543	244	851	787
Hypertension (%)	33.3	34.6	36.1	34.2	35.1

[a]Subjects not taking antihypertensive medication. Data for age and BP are means ± SE. *S*, short repeat allele [$(GT)_n \leq 12$]; *L*, long repeat allele [$(GT)_n \geq 13$]. Data for the combined group of *SS* and *SL* genotypes (*SS + SL*) were compared with those for individuals with the *LL* genotype (recessive genetic model). Data for the combined group of *SL* and *LL* genotypes (*SL + LL*) were compared with those for individuals with the *SS* genotype (dominant genetic model). [b]P=0.0460, [c]P=0.0341, [d]P=0.0178 versus *SS*.

Table IX. Blood pressure (BP) for male subjects according to *CAV1* genotype.

Characteristics	*II*	*ID*	*DD*	*II + ID*	*ID + DD*
Number of subjects (n=879)[a]	796	82	1	878	83
Age (years)	59.1±0.3	60.6±1.1	48.0	59.2±0.4	60.4±1.1
Systolic BP (mmHg)	120.8±0.6	125.0±1.9	126.0	121.2±0.6	125.1±1.9[b]
Diastolic BP (mmHg)	75.2±0.4	77.4±1.2	75.0	75.4±0.4	77.3±1.2
Number of all subjects (n=1128)	1028	99	1	1127	100
Hypertension (%)	34.2	36.4	0	34.4	36.0

[a]Subjects not taking antihypertensive medication. Data for age and BP are means ± SE. Data for the combined group of *II* and *ID* genotypes (*II + ID*) were compared with those for individuals with the *DD* genotype (recessive genetic model). Data for the combined group of *ID* and *DD* genotypes (*ID + DD*) were compared with those for individuals with the *II* genotype (dominant genetic model). [b]P=0.0325 versus *II*.

genotypes of *COL1A2* was in Hardy-Weinberg equilibrium and age did not differ among genotypes for men (Table VIII). Systolic BP was significantly higher in men with the *SS* genotype than in those in the combined group of *SL* and *LL* genotypes, whereas diastolic BP was significantly higher in men with the *SS* genotype than in those with the *SL* genotype or in those in the combined group of *SL* and *LL* genotypes. The differences in systolic and diastolic BP between individuals with the *SS* genotype and those in the combined group of *SL* and *LL* genotypes were 2.1 and 2.5%, respectively. The prevalence of hypertension did not differ among *COL1A2* genotypes for men. There were no differences in systolic or diastolic BP or in the prevalence of hypertension among *COL1A2* genotypes in women (data not shown).

Relation of the I/D (22,375delAC) polymorphism of CAV1 to BP. For men, the distribution of genotypes for the 22,375 I/D polymorphism of *CAV1* was in Hardy-Weinberg equilibrium and age did not differ among genotypes (Table IX). Systolic BP was significantly higher for men in the combined group of *ID* and *DD* genotypes than for those with the *II* genotype; the difference in systolic BP between these groups was 3.4%. The prevalence of hypertension did not differ among *CAV1* genotypes for men. For women, neither systolic or diastolic BP nor the prevalence of hypertension differed among *CAV1* genotypes (data not shown).

Relation of the -344C→T polymorphism of CYP11B2 to BP. The distribution of genotypes for the -344C→T polymorphism of *CYP11B2* was in Hardy-Weinberg equilibrium and age did not differ among genotypes for men (Table X). Systolic and diastolic BP were significantly higher in men with the *CT* genotype or with the *TT* genotype or in those in the combined group of *CT* and *TT* genotypes than in those with the CC genotype. The difference in systolic or diastolic BP between individuals in the combined group of *CT* and *TT* genotypes and those with the *CC* genotype was 4.9%. The prevalence of hypertension also differed significantly among genotypes (*CC* versus *CT* versus *TT*), being greater for men in the combined group of *CT* and *TT* genotypes than for those with the *CC* genotype. The odds ratio of the *T* allele compared with the *C* allele for predisposition to hypertension was 1.2. Although there were no differences in systolic or diastolic BP among *CYP11B2* genotypes in women (data not shown), the prevalence of hypertension differed among genotypes [*CC* (26.6%) versus *CT* (33.9%) versus *TT* (38.3%), P=0.0333], being greater for women in the combined group of *CT* and *TT* genotypes (36.0%) than for those with the *CC* genotype (26.6%, P=0.0272) as well as greater for women with the *TT* genotype (38.3%) than for those in the combined group of *CC* and *CT* genotypes (32.3%, P=0.0387). The odds ratio of the *T* allele compared with the *C* allele for predisposition to hypertension was 1.3.

Table X. Blood pressure (BP) for male subjects according to *CYP11B2* genotype.

Characteristics	*CC*	*CT*	*TT*	*CC + CT*	*CT + TT*
Number of subjects (n=876)[a]	109	418	349	527	767
Age (years)	59.5±1.0	59.1±0.5	59.2±0.5	59.2±0.4	59.1±0.4
Systolic BP (mmHg)	115.9±1.7	122.6±0.8[b]	121.1±0.9[c]	121.2±0.8	121.9±0.6[d]
Diastolic BP (mmHg)	72.2±1.0	76.2±0.5[e]	75.5±0.6[f]	75.3±0.5	75.9±0.4[g]
Number of all subjects (n=1125)	130	541	454	671	995
Hypertension (%)	23.9[i]	35.3[i]	36.6[i]	33.1	35.9[h]

[a]Subjects not taking antihypertensive medication. Data for age and BP are means ± SE. Data for the combined group of *CC* and *CT* genotypes (*CC + CT*) were compared with those for individuals with the *TT* genotype (recessive genetic model). Data for the combined group of *CT* and *TT* genotypes (*CT + TT*) were compared with those for individuals with the *CC* genotype (dominant genetic model). [b]P=0.0010, [c]P=0.0171, [d]P=0.0007, [e]P=0.0014, [f]P=0.0115, [g]P=0.0007, [h]P=0.0053 versus *CC*. [i]P=0.0188 (*CC* versus *CT* versus *TT*, 3 x 2 Chi-square test).

Table XI. Blood pressure (BP) for male subjects according to *ADRB1* genotype.

Characteristics	*AA*	*AG*	*GG*	*AA + AG*	*AG + GG*
Number of subjects (n=876)[a]	627	233	16	860	249
Age (years)	59.1±0.4	59.7±0.6	59.2±2.4	59.3±0.4	59.7±0.6
Systolic BP (mmHg)	120.6±0.7	122.6±1.1	125.9±4.3	121.1±0.6	122.8±1.1
Diastolic BP (mmHg)	75.0±0.4	76.4±0.7	79.5±2.7	75.4±0.4	76.6±0.7[b]
Number of all subjects (n=1125)	804	301	20	1105	321
Hypertension (%)	33.7	35.9	45.0	34.3	36.5

[a]Subjects not taking antihypertensive medication. Data for age and BP are means ± SE. Data for the combined group of *AA* and *AG* genotypes (*AA + AG*) were compared with those for individuals with the *GG* genotype (recessive genetic model). Data for the combined group of *AG* and *GG* genotypes (*AG + GG*) were compared with those for individuals with the *AA* genotype (dominant genetic model). [b]P=0.0430 versus *AA*.

Relation of the A→G (Ser49Gly) polymorphism of ADRB1 to BP. For men, the distribution of genotypes for the A→G polymorphism of *ADRB1* was in Hardy-Weinberg equilibrium and age did not differ among genotypes (Table XI). Diastolic BP was significantly higher for men in the combined group of *AG* and *GG* genotypes than for those with the *AA* genotype, the difference in diastolic BP between these groups being 2.1%. The prevalence of hypertension did not differ among *ADRB1* genotypes for men. Although systolic and diastolic BP did not differ among *ADRB1* genotypes for women (data not shown), the prevalence of hypertension differed significantly among genotypes [*AA* (32.6%) versus *AG* (41.4%) versus *GG* (29.6%), P=0.0270], being greater for women in the combined group of *AG* and *GG* genotypes (40.3%) than for those with the *AA* genotype (32.6%, P=0.0156). The odds ratio of the *G* allele compared with the T allele for predisposition to hypertension was 1.3.

Relation of the -1213T→C polymorphism of ESR2 to BP. In men, the distribution of genotypes for the -1213T→C polymorphism of *ESR2* was not in Hardy-Weinberg equilibrium and age did not differ among genotypes (Table XII). Systolic and diastolic BP were significantly higher in men with the *TC* genotype or in those in the combined group of *TC* and *CC* genotypes than in those with the *TT* genotype. The differences in systolic and diastolic BP between individuals in the combined group of *TC* and *CC* genotypes and those with the *TT* genotype were 3.3 and 4.2%, respectively. The prevalence of hypertension did not differ among *ESR2* genotypes for men. For women, there was no difference in systolic or diastolic BP or in the prevalence of hypertension among *ESR2* genotypes (data not shown).

Discussion

The regulation of blood pressure involves the integration of a variety of biological systems that control the structure and tone of the vasculature as well as the volume and composition of body fluid. It also involves the adaptation of these systems to constantly changing physiological needs (16). We have now examined the relations of nine candidate gene polymorphisms to BP and the prevalence of hypertension in community-dwelling Japanese women and men. Our results show that the polymorphism of *FYN* was associated with systolic and diastolic BP in women as well as with systolic BP in men; the polymorphisms of *CNR2*, *QPCT*, *GPX1*, *COL1A2*, *CYP11B2*, and *ESR2* with systolic and diastolic BP in men; and those of *CAV1* and *ADRB1* with systolic and diastolic BP, respectively, in men. The polymorphisms of *QPCT* and *CYP11B2* were also associated with the prevalence

Table XII. Blood pressure (BP) for male subjects according to *ESR2* genotype.

Characteristics	*TT*	*TC*	*CC*	*TT + TC*	*TC + CC*
Number of subjects (n=879)[a]	773	97	9	870	106
Age (years)	59.4±0.3	57.4±1.0	61.7±3.3	59.2±0.4	57.7±0.9
Systolic BP (mmHg)	120.7±0.6	125.5±1.7[b]	117.2±5.7	121.2±0.6	124.8±1.7[c]
Diastolic BP (mmHg)	75.0±0.4	79.0±1.1[d]	70.9±3.5	75.5±0.4	78.3±1.0[e]
Number of all subjects (n=1128)	991	126	11	1117	137
Hypertension (%)	33.5	42.1	27.3	34.5	40.9

[a]Subjects not taking antihypertensive medication. Data for age and BP are means ± SE. Data for the combined group of *TT* and *TC* genotypes (*TT + TC*) were compared with those for individuals with the *CC* genotype (recessive genetic model). Data for the combined group of *TC* and *CC* genotypes (*TC + CC*) were compared with those for individuals with the *TT* genotype (dominant genetic model). [b]P=0.0257, [c]P=0.0212, [d]P=0.0014, [e]P=0.0027 versus *TT*.

of hypertension in men. These observations thus suggest that polymorphisms of *QPCT* and *CYP11B2* are determinants of BP and the development of hypertension in Japanese men.

Given that selection bias can influence the results of genetic association studies, it is important that study populations be genetically and ethnically homogeneous. Our study subjects were recruited randomly from individuals residing in the city of Obu and town of Higashiura in central Japan, where the population is thought to share the same ethnic ancestry and to possess a homogeneous genetic background. We also showed that, with the exception of *ESR2*, the genotype distributions of the examined polymorphisms were in Hardy-Weinberg equilibrium in the study population. We thus appeared to avoid admixture and selection bias.

We detected associations of all nine polymorphisms with BP in men, whereas only the *FYN* polymorphism was associated with BP in women. The reason for this sex difference remains unclear. It might, however, be attributable, at least in part, to the difference in the plasma concentration of estrogen between men and women, given that estrogen exerts various favorable effects on vasomotor function, including stimulation of the production of nitric oxide and prostaglandin I_2 as well as inhibition of the release of endothelin-1 by vascular endothelial cells (17).

The formation of amino-terminal pyroglutamate from its glutaminyl precursor is an important posttranslational or cotranslational event in the processing of numerous bioactive neuropeptides, hormones, and cytokines during their maturation in the secretory pathway. These regulatory peptides require the amino-terminal pyroglutamate to develop the correct conformation for binding to their receptors or to protect their amino termini from exopeptidase-mediated degradation (18,19). The glutaminyl cyclases are acyltransferases that catalyze this posttranslational modification (20,21). They are abundant in mammalian neuroendocrine tissues, such as the hypothalamus and pituitary gland (21,22), and are highly conserved from yeast to humans. In humans, the glutaminyl-peptide cyclotransferase (glutaminyl cyclase) gene (*QPCT*) is located at chromosomal position 2p22.2. Ezura *et al* (23) examined the relations of 13 polymorphisms in this region to bone mineral density (BMD) in adult women and detected associations between the genotypes of six polymorphisms and BMD for the radius. The 160C→T (Arg54Trp) polymorphism of *QPCT* showed the most pronounced association, with the *T* allele being associated with low BMD. These results indicate that genetic variation in *QPCT* is an important determinant of BMD in adult women and may therefore contribute to susceptibility to osteoporosis. We have now shown that the 160C→T (Arg54Trp) polymorphism of *QPCT* was associated both with systolic and diastolic BP and with the prevalence of hypertension in Japanese men, with the *T* allele being associated with high BP. The effect of this polymorphism on gene expression or protein function has not been determined. This is the first demonstration of an association of this polymorphism of *QPCT* with BP or the prevalence of hypertension, but the underlying molecular mechanism of the association remains to be elucidated.

The cytochrome P450, subfamily Y XIB, polypeptide 2 (aldosterone synthase) gene (*CYP11B2*) encodes an enzyme that participates in the terminal steps of aldosterone synthesis in the zona glomerulosa cells of human adrenal glands, and its expression is regulated by angiotensin II and potassium (24). The candidacy of this gene in the present study is based on its pathogenic role in the syndrome of glucocorticoid-remediable aldosteronism (25). Several common polymorphisms of *CYP11B2* have been described (26-28). The -344C→T polymorphism, which is located in a putative binding site for a steroidogenic transcription factor, has been associated with hypertension (29-31) and with other hypertensive intermediate phenotypes such as plasma aldosterone level (32), urinary aldosterone excretion rate (30), and the aldosterone/renin ratio (27,28). Although some studies have not confirmed these relations (33,34), this locus may be important in the regulation of BP and the development of hypertension (35). We have now shown that the -344C→T polymorphism of *CYP11B2* was associated with both systolic and diastolic BP and the prevalence of hypertension in Japanese men, with the *T* allele being associated with high BP. Our results are thus consistent with previous observations (29-31).

Given the multiple comparisons of genotypes with BP or the prevalence of hypertension in the present study, it is not possible to completely exclude potential statistical errors such as false positives. It is also possible that one or more of the polymorphisms associated with BP or the prevalence of hypertension in our study are in linkage disequilibrium with

other polymorphisms of the same genes or of nearby genes that are actually responsible for the development of high BP. Furthermore, the relevance of the identified polymorphisms to gene transcription or to protein structure or function was not determined in the present study.

In conclusion, our results implicate *QPCT* and *CYP11B2* in the regulation of BP and the development of hypertension in Japanese men. Determination of genotypes for these polymorphisms may prove informative for assessment of the genetic risk for hypertension and may contribute to the personalized prevention of this condition. Given that multiple variants, each having a small effect, will likely ultimately be found to be responsible for a large fraction of the genetic component of essential hypertension, identification of additional hypertension susceptibility genes will allow more accurate assessment of the genetic component of this condition.

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