Association of the genetic variants of *APOA5* and *PRKCH* with hypertension in community-dwelling Japanese individuals

YOSHIJI YAMADA[1], FUJIKO ANDO[2] and HIROSHI SHIMOKATA[2]

[1]Department of Human Functional Genomics, Life Science Research Center, Mie University, Tsu, Mie;
[2]Department of Epidemiology, National Institute for Longevity Sciences, Obu, Aichi, Japan

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Abstract. Hypertension is a complex multifactorial disorder that is thought to result from the interaction between genetic background and environmental factors. Although various loci and genes have been implicated in the 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 have now examined the relation of five candidate gene polymorphisms to blood pressure (BP) and the prevalence of hypertension in an 8-year population-based longitudinal cohort study. The 2267 subjects (1128 women, 1139 men) were aged 40-79 years and were randomly recruited to a population-based prospective cohort study of aging and age-related diseases in Japan. BP was measured after subjects had rested in a sitting position for at least 15 min. Genotypes for the -765G→C polymorphism of *PTGS2* and the 67G→A (Ala23Thr) polymorphism of *CCL11* were determined using a fluorescence-based allele-specific DNA primer assay system, and those of the 1444T→C (3'-UTR) polymorphism of *CRP*, the -1131T→C polymorphism of *APOA5* and the 1425G→A (Val374Ile) polymorphism of *PRKCH* using melting curve analysis. Longitudinal analysis of the relation between systolic or diastolic BP and the five polymorphisms with a mixed-effect model revealed that the polymorphism of *CRP* was significantly related to systolic BP in all subjects, that of *APOA5* to systolic BP in men, and that of *PRKCH* to diastolic BP in women. Longitudinal analysis of the relation between the prevalence of hypertension and the five polymorphisms with a generalized estimating equation revealed that the *CRP*, *APOA5* and *CCL11* polymorphisms were significantly related to the prevalence of hypertension in men, the *PTGS2* polymorphism to its prevalence in all subjects, and the *PRKCH* polymorphism to its prevalence in all subjects and in women. The *APOA5* and *PRKCH* polymorphisms were thus associated with both BP and the prevalence of hypertension in men and women, respectively. These results suggest that the *APOA5* and *PRKCH* polymorphisms are determinants of BP and the development of hypertension in Japanese men and women, respectively.

Correspondence to: Dr Yoshiji Yamada, Department of Human Functional Genomics, Life Science Research Center, Mie University, 1577 Kurima-machiya, Tsu, Mie 514-8507, Japan
E-mail: yamada@gene.mie-u.ac.jp

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Introduction

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

We have been attempting to identify the genes associated with blood pressure (BP) and the prevalence of hypertension in Japanese women and men recruited to a population-based prospective cohort study with a candidate gene approach. In the present study, we selected five candidate genes that might be expected to contribute to the regulation of BP (Table I). Although there is no apparent biological link between these genes, we examined the relation of their polymorphisms to systolic and diastolic BP and to the prevalence of hypertension. Among the various polymorphisms previously identified, we selected those that might be expected to affect gene function. We thus examined the relation of these polymorphisms to systolic and diastolic BP and to the prevalence of hypertension in community-dwelling Japanese women and men.

Materials and methods

Study population. The National Institute for Longevity Sciences-Longitudinal Study of Aging (NILS-LSA) is a population-based prospective cohort study of aging and age-related diseases, the details of which have been described previously (10-13). We examined the relation of gene polymorphisms to

Table I. The five gene polymorphisms examined in the study.

Locus	Gene	Symbol	Polymorphism	dbSNP
1q21-q23	C-reactive protein, pentraxin-related	*CRP*	1444T→C (3'-UTR)	rs1130864
1q25.2-q25.3	Prostaglandin-endoperoxide synthase 2	*PTGS2*	-765G→C	rs20417
11q23	Apolipoprotein A-V	*APOA5*	-1131T→C	rs662799
14q22-q23	Protein kinase C, η	*PRKCH*	1425G→A (Val374Ile)	rs2230500
17q21.1-q21.2	Chemokine (C-C motif) ligand 11	*CCL11*	67G→A (Ala23Thr)	rs3744508

BP and the prevalence of hypertension in 2267 individuals (1128 women, 1139 men) recruited to the NILS-LSA. Individuals whose genotypes were not successfully determined were excluded from the analysis. The study protocol complied with the Declaration of Helsinki and was approved by the Committee on Ethics of Human Research of the National Center for Geriatrics and Gerontology. 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 who had been resting in a sitting position for at least 15 min. The BP of each subject was confirmed by measurement performed with a mercury manometer by a physician 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 as the taking of antihypertensive medication.

Genotyping of polymorphisms. Genotypes for the *PTGS2* and *CCL11* polymorphisms 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 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 (for *PTGS2*) or 6 (for *CCL11*) 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 65˚C (for *PTGS2*) or 67.5˚C (for *CCL11*) for 30 sec, and extension at 72˚C for 30 sec, followed by 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 respective excitation and emission wavelengths of 485 and 538 nm for fluorescein isothiocyanate, and 584 and 612 nm for Texas red.

Genotypes for the *CRP*, *APOA5* and *PRKCH* polymorphisms were determined by melting curve analysis (intercalater-mediated fluorescence resonance energy transfer probe method) (Table II). The polymorphic region of each gene was amplified by PCR in a reaction mixture (25 μl) containing 20 ng DNA, 5 pmol of each primer, 0.2 mmol/l of each deoxynucleoside triphosphate, 3 (for *CRP*) or 2 (for *APOA5* and *PRKCH*) 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 (for *CRP* and *PRKCH*) or 40 (for *APOA5*) cycles of denaturation at 95˚C for 30 sec, annealing at 65˚C for 30 sec, and extension at 72˚C for 30 sec, followed by a final extension at 72˚C for 2 min. A mixture (2 μl) of 10 pmol of probe labeled at the 5' end with Texas red and 1/400 diluted SYBR-Green I 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, then temperatures increasing to 80˚C over 10 min. The fluorescence signals were detected at excitation and emission wavelengths of 485 and 612 nm, respectively.

Statistical analysis. Age, body mass index and BP were compared in subjects with hypertension to the controls using the unpaired Student's t-test, and the prevalence of smoking was compared in the two groups by the χ^2 test. BP values were analyzed in individuals who were not taking antihypertensive drugs. We examined the effects of the genetic variants of *CRP*, *PTGS2*, *APOA5*, *PRKCH* and *CCL11* on systolic and diastolic BP and on the prevalence of hypertension based on an 8-year longitudinal cohort study. The data for the examination of each subject in the first wave (November 1997 to April 2000), in the second wave (April 2000 to May 2002), in the third wave (May 2002 to May 2004) and in the fourth wave (June 2004 to July 2006) were pooled and analyzed. Systolic and diastolic BP and the prevalence of hypertension were evaluated in terms of all subjects, women, or men. Longitudinal changes in BP were analyzed with a mixed-effect model (16), a type of statistical analysis commonly used for repeated measurements. Responses for points close in time are usually more highly correlated with each other than responses for points far apart in time. Special methods of analysis are therefore usually needed to accommodate the correlation structure of the repeated measurements. This autoregressive structure was controlled in the mixed-effect model. Systolic and diastolic BP were thus compared in the two groups (dominant or recessive model) by a mixed-effect model with adjustment for age and sex in all

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

Gene	Polymorphism	Sense primer with FITC	Sense primer with Texas red	Antisense primer with biotin	Annealing (°C)	Cycles	Mg^{2+} (mmol/l)
PTGS2	-765G→C	GAGGAGAATTTACCTTTCCXGC	GTATTATGAGGAGAATTTACCTTTCCXCC	GTTCTCCGTACCTTCACCCCCT	65.0	35	2.5
CCL11	67G→A	GGGGCTTACCTGGCCCAXTG	GGGGCTTACCTGGCCCAXCG	CCTCCAACATGAAGGTCTCCGCAG	67.5	35	6.0
Gene	Polymorphism	Sense primer	Antisense primer	Probe with Texas red	Annealing (°C)	Cycles	Mg^{2+} (mmol/l)
CRP	1444T→C	GAGCTCGTTAACTATGCTGGGA	TTATCTCCAAGATCTGTCCAACTTG	GCTGGGAAACGGTCCAAA	65.0	45	3.0
APOA5	-1131T→C	GGGACTCTGAGCCCCAGGAACTG	CGAGTGGAGTTCAGCTTTTCCTCATG	GAGCGAAAGTGAGATTTGCC	65.0	40	2.0
PRKCH	1425G→A	CCTCCTTTTGCTTTGCCATAGGTG	TCAGCACCTTCACAGCATAGAGGTCTC	TGCTTGCAAGAGTAAAAGAAACA	65.0	45	2.0

FITC, fluorescein isothiocyanate. Oligonucleotide sequences are 5'→3'.

subjects or for age in women or in men. Longitudinal changes in the prevalence of hypertension were compared in the two groups by a generalized estimating equation (17) with adjustment for age and sex in all subjects, or for age in women or in men. Age-related changes in systolic or diastolic BP or in the prevalence of hypertension were estimated by quadratic curve controlling for the observation year during which the subjects attended at least one follow-up examination. Allele frequencies were estimated by the gene-counting method, and the χ^2 test was used to identify significant departure from Hardy-Weinberg equilibrium. A P-value of <0.05 was considered statistically significant. Statistical analysis was performed with SAS software release 9.13 (SAS Institute, Cary, NC).

Results

The baseline characteristics (first wave) of the 2267 study subjects are shown in Table III. Age, body mass index and systolic and diastolic BP were greater in subjects with hypertension than in the controls in the case of both the men and women. The prevalence of smoking was greater in the controls than in the hypertensive subjects in the case of the men.

The relation of the five polymorphisms to systolic and diastolic BP was analyzed with a mixed-effect model in all subjects, in women, and in men (Table IV). The 1444T→C (3'-UTR) polymorphism of *CRP* was significantly related to systolic BP in all subjects, with the *CC* genotype reflecting a higher BP. The -1131T→C polymorphism of *APOA5* was significantly related to systolic BP in men, with the *CC* genotype being associated with a lower BP. The 1425G→A (Val374Ile) polymorphism of *PRKCH* was significantly related to diastolic BP in women, with the *A* allele being associated with a higher BP.

The relation of the five polymorphisms to the prevalence of hypertension was analyzed with a generalized estimating equation for all subjects, for women, and for men (Table V). The 1444T→C (3'-UTR) polymorphism of *CRP*, the -1131T→C polymorphism of *APOA5*, and the 67G→A (Ala23Thr) polymorphism of *CCL11* were associated with the prevalence of hypertension among men in a recessive model. The *CC* genotype of *CRP* and the *AA* genotype of *CCL11* were risk factors for hypertension, whereas the *CC* genotype of *APOA5* was protective against this condition. The -765G→C polymorphism of *PTGS2* was associated with the prevalence of hypertension in all subjects, with the variant *C* allele protecting against this condition. The 1425G→A (Val374Ile) polymorphism of *PRKCH* was associated with the prevalence of hypertension among all subjects and women, with the variant *A* allele representing a risk factor for this condition. The genotype distributions of the five polymorphisms in male and female controls were all in Hardy-Weinberg equilibrium.

Given that the -1131T→C polymorphism of *APOA5* and the 1425G→A (Val374Ile) polymorphism of *PRKCH* were associated with both BP and the prevalence of hypertension in men and women, respectively, the relation between systolic or diastolic BP and age was analyzed longitudinally, according to genotypes for *APOA5* in men and those for *PRKCH* in women, with a mixed-effect model (Fig. 1) and the relation between the prevalence of hypertension and age was analyzed longitudinally with a generalized estimating equation (Fig. 2).

Table III. Baseline characteristics (first wave, n=2267) of male and female subjects with hypertension and the controls.

Characteristic	Men			Women		
	Hypertension	Control	P-value	Hypertension	Control	P-value
Number of subjects (n=2267)	358	781		377	751	
Age (years)	63.2±0.6	57.4±0.4	<0.0001	64.5±0.5	56.6±0.4	<0.0001
Body mass index (kg/m^2)	23.6±0.1	22.6±0.1	<0.0001	23.9±0.2	22.4±0.1	<0.0001
Smoking [n (%)]	100 (27.9)	333 (42.6)	<0.0001	19 (5.0)	63 (8.4)	0.0673
Number of subjects (n=1847)[a]	156	781		159	751	
Systolic BP (mmHg)	145.2±1.0	115.9±0.5	<0.0001	148.4±1.0	113.6±0.5	<0.0001
Diastolic BP (mmHg)	89.3±0.7	72.2±0.3	<0.0001	86.6±0.7	69.5±0.3	<0.0001

Data for age, body mass index, and BP are represented as the means ± SE. [a]Subjects not taking antihypertensive medication.

Systolic and diastolic BP and the prevalence of hypertension was lower in men with the *CC* genotype of *APOA5* than in the combined group, 40-80 years of age, of those with the *TT* or *TC* genotypes. Systolic and diastolic BP and the prevalence of hypertension was higher in the combined group of women of 40-80 years of age with the *GA* or *AA* genotypes of *PRKCH* than in those with the *GG* genotype

Discussion

The regulation of BP 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 (18). We have now examined the relation of five candidate gene polymorphisms to systolic and diastolic BP, and the prevalence of hypertension in community-dwelling Japanese women and men. Our results show that the -1131T→C polymorphism of *APOA5* and the 1425G→A (Val374Ile) polymorphism of *PRKCH* are associated with both BP and the prevalence of hypertension in men and women, respectively. These observations suggest that *APOA5* and *PRKCH* are, respectively, susceptibility loci for the development of hypertension in Japanese men and women.

APOA5 is located approximately 27 kb upstream of the well-characterized *APOA1-APOC3-APOA4* gene cluster at chromosome 11q23 (19). The -1131T→C polymorphism in the promoter region of human *APOA5* was found to be associated with plasma triglyceride levels in populations of various ethnicities, with the *C* allele being a risk factor for increased triglyceride concentrations (20-23). This polymorphism was also associated with high-density lipoprotein (HDL)-cholesterol levels, in addition to triglyceride levels in both Asian and Caucasian populations, with individuals with the *C* allele exhibiting reduced HDL-cholesterol concentrations (21-23). A peroxisome proliferator response element (PPRE) has been identified at a position 328 bp downstream of the -1131T→C polymorphism in the promoter region of *APOA5* (24,25). The expression of *APOA5* was also found to be increased by fibrates acting through peroxisome proliferator-activated receptor α and the PPRE. These observations suggest that the -1131T→C polymorphism of *APOA5* might influence gene expression and thereby affect the circulating concentrations of triglycerides and HDL-cholesterol. We have now shown that the -1131T→C polymorphism of *APOA5* was significantly related to systolic BP and the prevalence of hypertension in men, with the variant *CC* genotype being associated with a lower BP and protecting against hypertension. The molecular mechanism responsible for this association remains to be elucidated.

Protein kinase C (PKC) is a serine-threonine kinase that regulates a wide variety of important cellular functions, including proliferation, differentiation, and apoptosis. The η isoform of PKC (PRKCH) is regulated by diacylglycerol and phospholipids, but is insensitive to Ca^{2+} (26,27). Although its specific substrates remain to be identified, PRKCH has been implicated in the cellular response to oxidative stress. The overexpression of PRKCH in human monocytic cells resulted in the induction of inducible nitric oxide synthase and nitric oxide production in response to the exposure of the cells to endotoxin (28). Evidence also suggests that PRKCH promotes cell growth through the suppression of cyclin E expression (29) and caspase-3 activity (30), as well as through the activation of the Akt signaling pathway (31). The 1425G→A (Val374Ile) polymorphism of *PRKCH* was shown to be associated with the incidence of cerebral infarction in a Japanese population (32), with the *A* allele being a risk factor for this condition. This polymorphism is located within the ATP binding site of PRKCH (27). The Val374Ile substitution enhances the autophosphorylation and kinase activity of PRKCH induced by cell stimuli, thereby promoting signaling by this enzyme (32). We have now shown that the 1425G→A (Val374Ile) polymorphism of *PRKCH* was significantly related to diastolic BP and the prevalence of hypertension in women, with the variant *A* allele being associated with a higher BP and a risk factor for hypertension. This association might be attributable to an effect of this polymorphism on vascular inflammation, although the underlying molecular mechanism remains to be elucidated.

Given the multiple comparisons of genotypes with BP or the prevalence of hypertension in the present study, it is not possible to exclude completely potential statistical errors,

Table IV. Relation of five polymorphisms to systolic and diastolic BP (mmHg) analyzed with a mixed-effect model (first wave to fourth wave).[a]

Gene	Polymorphism			Dominant model		P-value	Recessive model		P-value
CRP	1444T→C (3'-UTR)			*TT*	*TC + CC*		*TT + TC*	*CC*	
		All subjects	No. of samples	4820	687		5484	23	
			Systolic BP	119.9±0.4	120.3±1.0	0.7343	119.9±0.4	132.5±5.3	**0.0183**
			Diastolic BP	73.8±0.2	73.9±0.6	0.9460	73.8±0.2	79.7±3.2	0.0663
		Women	No. of samples	2337	348		2676	9	
			Systolic BP	118.5±0.6	119.4±1.5	0.5466	118.5±0.5	131.9±7.7	0.0822
			Diastolic BP	72.1±0.3	72.9±0.9	0.3478	72.1±0.3	77.1±4.5	0.2761
		Men	No. of samples	2483	339		2808	14	
			Systolic BP	121.3±0.5	121.1±1.5	0.9055	121.2±0.5	131.8±7.4	0.1530
			Diastolic BP	75.5±0.3	74.7±0.9	0.3872	75.4±0.3	81.4±4.5	0.1791
PTGS2	-765G→C			*GG*	*GC + CC*		*GG + GC*	*CC*	
		All subjects	No. of samples	5129	374		5501	2	
			Systolic BP	120.1±0.4	118.1±1.4	0.1705	120.0±0.4	112.9±16.6	0.6710
			Diastolic BP	73.9±0.2	73.1±0.8	0.3556	73.9±0.2	71.9±10.0	0.8415
		Women	No. of samples	2473	207		2680	0	
			Systolic BP	118.9±0.6	116.2±1.9	0.1842	118.7±0.5		ND
			Diastolic BP	72.3±0.3	71.4±1.1	0.4337	72.2±0.3		ND
		Men	No. of samples	2656	167		2821	2	
			Systolic BP	121.3±0.5	120.0±2.1	0.5366	121.3±0.5	116.7±16.2	0.7785
			Diastolic BP	75.5±0.3	74.7±1.3	0.5655	75.4±0.3	75.0±9.8	0.9668
APOA5	-1131T→C			*TT*	*TC + CC*		*TT + TC*	*CC*	
		All subjects	No. of samples	2384	3118		4809	693	
			Systolic BP	119.3±0.6	120.5±0.5	0.1156	120.0±0.4	119.6±1.0	0.6845
			Diastolic BP	73.4±0.3	74.2±0.3	0.0639	73.9±0.2	73.6±0.6	0.6452
		Women	No. of samples	1135	1549		2321	363	
			Systolic BP	117.6±0.8	119.4±0.7	0.0935	118.3±0.6	120.9±1.5	0.1062
			Diastolic BP	71.6±0.5	72.6±0.4	0.1075	72.1±0.3	72.9±0.9	0.3822
		Men	No. of samples	1249	1569		2488	330	
			Systolic BP	120.9±0.7	121.5±0.7	0.5211	121.6±0.5	118.4±1.4	**0.0332**
			Diastolic BP	75.1±0.5	75.7±0.4	0.2601	75.6±0.3	74.3±0.9	0.1684
PRKCH	1425G→A (Val374Ile)			*GG*	*GA + AA*		*GG + GA*	*AA*	
		All subjects	No. of samples	3528	1983		5260	251	
			Systolic BP	119.8±0.5	120.3±0.6	0.5487	119.9±0.4	121.7±1.7	0.2971
			Diastolic BP	73.6±0.3	74.2±0.4	0.2100	73.8±0.2	75.6±1.0	0.0664
		Women	No. of samples	1729	959		2567	121	
			Systolic BP	118.0±0.7	119.8±0.9	0.0941	118.6±0.5	119.4±2.4	0.7455
			Diastolic BP	71.7±0.4	73.1±.5	**0.0311**	72.1±0.3	73.4±1.4	0.3804
		Men	No. of samples	1799	1024		2693	130	
			Systolic BP	121.6±0.6	120.6±0.8	0.3124	121.1±0.5	124.0±2.3	0.2167
			Diastolic BP	75.6±0.4	75.2±0.5	0.5941	75.3±0.3	77.9±1.4	0.0715
CCL11	67G→A (Ala23Thr)			*GG*	*GA + AA*		*GG + GA*	*AA*	
		All subjects	No. of samples	4196	1286		5400	82	
			Systolic BP	120.1±0.4	119.4±0.8	0.4401	119.9±0.4	121.6±2.9	0.5699
			Diastolic BP	73.8±0.3	73.8±0.5	0.8746	73.8±0.2	74.1±1.8	0.8873
		Women	No. of samples	2055	613		2630	38	
			Systolic BP	118.5±0.6	118.6±1.1	0.9520	118.6±0.5	115.6±4.4	0.4959
			Diastolic BP	72.1±0.4	72.3±0.7	0.7400	72.2±0.3	69.8±2.6	0.3583
		Men	No. of samples	2141	673		2770	44	
			Systolic BP	121.5±0.6	120.3±1.0	0.2676	121.1±0.5	126.4±3.8	0.1715
			Diastolic BP	75.5±0.3	75.2±0.6	0.6233	75.4±0.3	77.7±2.3	0.3310

[a]Systolic or diastolic BP was compared between two groups (dominant or recessive model) for each polymorphism, with adjustment for age and sex in all subjects or for age in women and in men. P-values <0.05 are shown in bold. ND, not determined.

Table V. Relation of five polymorphisms to the prevalence of hypertension analyzed with a generalized estimating equation (first wave to fourth wave).[a]

Gene	Polymorphism			Hypertension (%)	Control (%)	P-value (dominant)	P-value (recessive)
CRP	1444T→C (3'-UTR)						
		All subjects	*TT*	2051 (87.4)	4182 (87.5)	0.6198	0.0690
			TC	274 (11.7)	580 (12.1)		
			CC	21 (0.9)	15 (0.3)		
		Women	*TT*	927 (84.4)	2057 (87.3)	0.1111	0.6673
			TC	164 (14.9)	293 (12.4)		
			CC	8 (0.7)	6 (0.3)		
		Men	*TT*	1124 (90.1)	2125 (87.8)	0.3698	**0.0360**
			TC	110 (8.8)	287 (11.9)		
			CC	13 (1.0)	9 (0.4)		
PTGS2	-765G→C						
		All subjects	*GG*	2235 (95.23)	4442 (93.10)	**0.0363**	ND
			GC	111 (4.73)	327 (6.85)		
			CC	1 (0.04)	2 (0.04)		
		Women	*GG*	1045 (95.0)	2161 (92.0)	0.0640	ND
			GC	55 (5.0)	188 (8.0)		
			CC	0 (0)	0 (0)		
		Men	*GG*	1190 (95.4)	2281 (94.2)	0.2882	ND
			GC	56 (4.5)	139 (5.7)		
			CC	1 (0.1)	2 (0.1)		
APOA5	-1131T→C						
		All subjects	*TT*	1002 (42.6)	2094 (43.9)	0.4507	0.5061
			TC	1051 (44.7)	2078 (43.6)		
			CC	298 (12.7)	598 (12.5)		
		Women	*TT*	434 (39.3)	1016 (43.2)	0.0712	0.1970
			TC	494 (44.8)	1030 (43.8)		
			CC	176 (15.9)	307 (13.1)		
		Men	*TT*	568 (45.6)	1078 (44.6)	0.5598	**0.0287**
			TC	557 (44.7)	1048 (43.4)		
			CC	122 (9.8)	291 (12.0)		
PRKCH	1425G→A (Val374Ile)						
		All subjects	*GG*	1440 (61.3)	3072 (64.3)	**0.0324**	0.8568
			GA	813 (34.6)	1492 (31.2)		
			AA	98 (4.2)	215 (4.5)		
		Women	*GG*	667 (60.4)	1546 (65.6)	**0.0178**	0.5752
			GA	390 (35.3)	708 (30.0)		
			AA	47 (4.3)	103 (4.4)		
		Men	*GG*	773 (62.0)	1526 (63.0)	0.4975	0.8233
			GA	423 (33.9)	784 (32.4)		
			AA	51 (4.1)	112 (4.6)		
CCL11	67G→A (Ala23Thr)						
		All subjects	*GG*	1839 (79.0)	3640 (76.5)	0.2896	0.4509
			GA	446 (19.1)	1047 (22.0)		
			AA	44 (1.9)	71 (1.5)		
		Women	*GG*	846 (77.5)	1809 (77.3)	0.7159	0.1258
			GA	237 (21.7)	495 (21.2)		
			AA	8 (0.7)	36 (1.5)		
		Men	*GG*	993 (80.2)	1831 (75.7)	0.0734	**0.0256**
			GA	209 (16.9)	552 (22.8)		
			AA	36 (2.9)	35 (1.5)		

[a]The prevalence of hypertension was compared between two groups (dominant or recessive model) for each polymorphism, with adjustment for age and sex in all subjects or for age in women and in men. P-values <0.05 are shown in bold. ND, not determined.

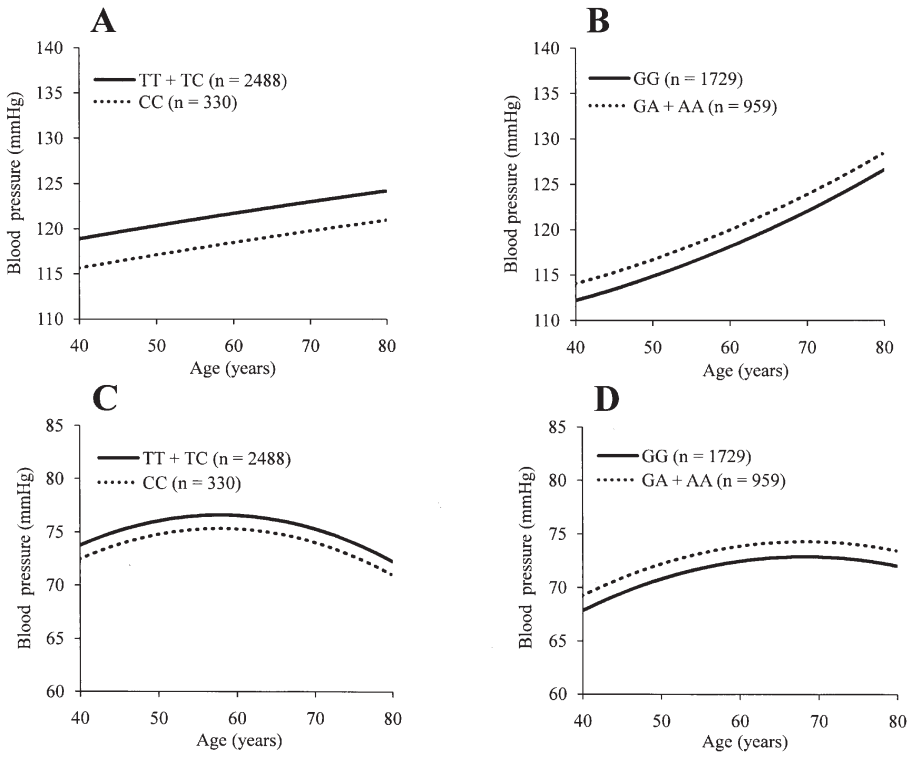

Figure 1. Longitudinal analysis of relations between systolic (A and B) or diastolic (C and D) BP and age according to the genotype for *APOA5* (*TT + TC* versus *CC*) in men (A and C) or to the genotype for *PRKCH* (*GG* versus *GA + AA*) in women (B and D) with a mixed-effect model.

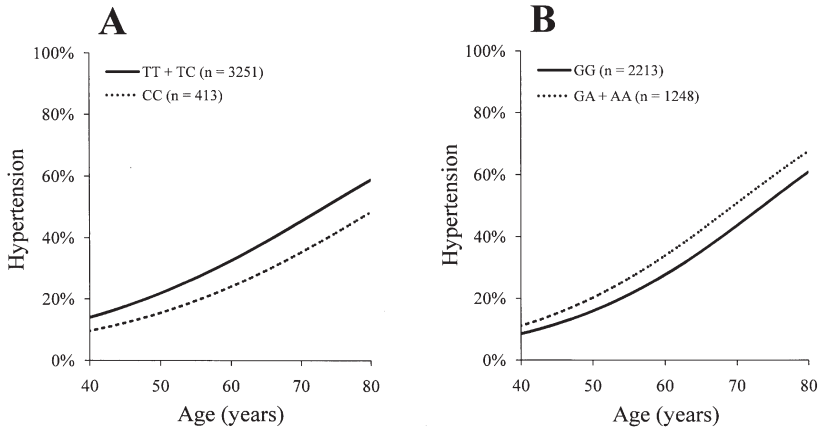

Figure 2. Longitudinal analysis of relations between the prevalence of hypertension and age according to the genotype for *APOA5* (*TT + TC* versus *CC*) in men (A) or to the genotype for *PRKCH* (*GG* versus *GA + AA*) in women (B) with a generalized estimating equation.

such as false positives. It is also possible that one or more of the polymorphisms associated with the BP or the prevalence of hypertension in our study is in linkage disequilibrium with other polymorphisms of the same genes or of nearby genes that are actually responsible for the development of hypertension. 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 the -1131T→C polymorphism of *APOA5* and the 1425G→A (Val374Ile) polymorphism of *PRKCH* in the regulation of BP and the development of hypertension in Japanese men and women, respectively. The determination of genotypes for these polymorphisms may prove to be informative to the assessment of the genetic risk for hypertension. 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 for a more accurate assessment of the genetic risk for this condition.

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