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ORIGINAL ARTICLE

Association of polymorphisms of *SORBS1*, *GCK* and *WISP1* with hypertension in community-dwelling Japanese individuals

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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 have now examined the relationships of 22 candidate gene polymorphisms with the prevalence of hypertension and with blood pressure (BP) in a 6-year population-based longitudinal cohort study and observed significant relationships of three polymorphisms of *SORBS1*, *GCK* and *WISP1* with hypertension. The 2233 subjects (1106 women, 1127 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 with subjects having rested in the sitting position for at least 15 min. Genotypes for the 682A→G (Thr228Ala) polymorphism of *SORBS1*, the −30G→A polymorphism of *GCK* and the 2364A→G polymorphism of *WISP1* were determined by melting curve analysis. Longitudinal analysis with a generalized estimating equation revealed that the polymorphisms of *SORBS1* and *GCK* and that of *WISP1* were significantly associated with the prevalence of hypertension in women and men, respectively. Longitudinal analysis with a mixed-effect model revealed that the polymorphism of *SORBS1* was significantly related to diastolic BP in women and that those of *GCK* and *WISP1* were significantly related to both systolic and diastolic BP in women and men, respectively. These results suggest that *SORBS1* and *GCK* are susceptibility loci for hypertension in Japanese women and that *WISP1* is such a locus in 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 kidney disease, personalized prevention of hypertension is an important public health goal. An 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] genome-wide mapping with microsatellite markers,[6] genome-wide association studies[7] and candidate gene association studies[8–12] have implicated various loci and genes in predisposition to hypertension, the genes that confer susceptibility to this condition remain to be identified definitively. In addition, ethnic divergence of lifestyle and environmental factors as well as of genetic background necessitates examination of polymorphisms related to hypertension in each ethnic group.

With the use of a candidate gene approach, we have been attempting to identify genes associated with blood pressure (BP) and the prevalence of hypertension in Japanese women and men recruited to a population-based prospective cohort study. In this study, we have selected 22 candidate gene polymorphisms that might be expected to contribute to the regulation of BP and the development of hypertension (Table 1) and have examined the relationships of these polymorphisms with systolic and diastolic BP and the prevalence of hypertension, even though there is no apparent biological link among these genes. Our aim was to identify one to two genetic markers significantly associated with hypertension for each gene. Among the 22 polymorphisms examined in the study, the 682A→G (Thr228Ala) polymorphism of *SORBS1*, the −30G→A polymorphism of *GCK* and the 2364A→G polymorphism of *WISP1* were significantly associated with hypertension in community-dwelling Japanese individuals. Given that among the various polymorphisms identified earlier, these polymorphisms were shown to be related to phenotypes,[13–15] they might be expected to affect gene function. We now show the relationships of

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Table 1 The 22 gene polymorphisms examined in this study

Locus	*Gene*	*Symbol*	*Polymorphism*	*dbSNP*
1p31	Leptin receptor	*LEPR*	G→A (Arg109Lys)	rs1137100
1q22	Farnesyl diphosphate synthase	*FDPS*	A→C	rs2297480
1q32	Complement factor H	*CFH*	T→C (Tyr402His)	rs1061170
3q27	Alpha-2-HS-glycoprotein	*AHSG*	C→A (Thr270Thr)	rs1071592
6p21.3	Advanced glycosylation end product-specific receptor	*AGER*	G→A (Gly82Ser)	rs2070600
6q27	Thrombospondin 2	*THBS2*	3949T→G (3′-UTR)	rs8089
7p15.3–p15.1	Glucokinase	*GCK*	−30G→A	rs1799884
8q24.1–q24.3	WNT1-inducible signaling pathway protein 1	*WISP1*	2364A→G	rs2929970
10q23.3–q24.1	Sorbin and SH3 domain-containing 1	*SORBS1*	682A→G (Thr228Ala)	rs2281939
11p15.1	ATP-binding cassette, subfamily C, member 8	*ABCC8*	−3C→T	rs1799854
11p15.1	ATP-binding cassette, subfamily C, member 8	*ABCC8*	T→G (Ser1369Ala)	rs757110
11q13.1	Actinin, alpha 3	*ACTN3*	C→T (Arg577Stop)	rs1815739
11q13.4	Low-density lipoprotein receptor-related protein 5	*LRP5*	4037T→C (Val1330Ala)	rs3736228
12q13.11	Vitamin D receptor	*VDR*	23005G→A	rs11568820
13q34	Coagulation factor VII	*F7*	11496G→A (Arg353Gln)	rs6046
14q24.2–q24.3	Deiodinase, iodothyronine, type II	*DIO2*	C→T	rs12885300
15q24.1	Cytochrome *P*450, family 1, subfamily A, polypeptide 2	*CYP1A2*	−163A→C	rs762551
16q12.2	Solute carrier family 6, member 2	*SLC6A2*	1287A→G (Thr429Thr)	rs5569
17p11.1	A kinase anchor protein 10	*AKAP10*	2073A→G (Ile646Val)	rs203462
17q11.2–q12	Chemokine (C-C motif) ligand 2	*CCL2*	−2518C→T	rs1024611
20q13.1–q13.2	Protein tyrosine phosphatase, non-receptor type 1	*PTPN1*	C→T	rs718049
22q11.23	Glutathione *S*-transferase theta 1	*GSTT1*	I/D (3′-UTR)	Not detected

Table 2 Primers, probes and other PCR conditions for genotyping

Gene	*Polymorphism*	*Sense primer*	*Antisense primer*	*Probe with Texas red*	Mg^{2+}*(mmol/l)*
SORBS1	682A→G (Thr228Ala)	ATTTCATTCCCCCTCATCTGCAGAG	ACCAGCGGAGGTGGTGGTGAG	ATGGTGGCGCCTGGCTAAT	2.0
GCK	−30G→A	CTCCTGGTCACCATGACAACCACAG	TGCTCCAGCCAGGTGTGGAGTG	CCTCTCAGGAGCACAGTAAGC	2.0
WISP1	2364A→G	CAAATGGCCAGTTTTCTGGTAGGAAG	TTCAACCTCTTCAGCTTTAAACCTTTATTAAGTC	GGAGGTTTACCGGTTGTTTAGA	3.0

Oligonucleotide sequences are 5′→3′.

the polymorphisms of *SORBS1*, *GCK* and *WISP1* with BP and the prevalence of hypertension in community-dwelling Japanese individuals.

METHODS

Study population

The National Institute for Longevity Sciences—Longitudinal Study of Aging is a population-based prospective cohort study of aging and age-related diseases, the details of which have been described earlier.[16–19] We examined the relationships of genetic variants with BP and the prevalence of hypertension in 2233 individuals (1106 women, 1127 men) recruited to the National Institute for Longevity Sciences—Longitudinal Study of Aging. 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 Center for Geriatrics and Gerontology. Written informed consent was obtained from each subject.

Measurement of BP

Blood pressure was measured with an automatic sphygmomanometer (BP-203RV-II; Colin, Tokyo, Japan) in subjects who had rested in the sitting position for at least 15 min. BP in each subject was confirmed by measurement with a mercury manometer performed by a physician according to the guidelines of the American Heart Association.[20] Hypertension was defined as a systolic BP of $\geqslant$140 mm Hg, a diastolic BP of $\geqslant$90 mm Hg or the taking of antihypertensive medication. Normal BP was defined as both a systolic BP of $<$140 mm Hg and a diastolic BP of $<$90 mm Hg without the taking of antihypertensive medication.

Genotyping of polymorphisms

Genotypes for polymorphisms of *SORBS1*, *GCK* and *WISP1* were determined by melting curve analysis (intercalater-mediated fluorescence resonance energy transfer probe method) (Table 2). The polymorphic region of each gene was amplified by PCR in a reaction mixture (25 μl) containing 20 ng of DNA, 5 pmol of each primer, 0.2 mmol l^{-1} of each deoxynucleoside triphosphate, 2 mmol l^{-1} (for *SORBS1* and *GCK*) or 3 mmol l^{-1} (for *WISP1*) $MgCl_2$ and 1.25 U of rTaq DNA polymerase (Toyobo, Osaka, Japan) in polymerase buffer. The amplification protocol comprised initial denaturation at 95°C for 5 min; 45 cycles of denaturation at 95°C for 30 s, annealing at 65°C for 30 s and extension at 72°C for 30 s; and 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 a 1:400 dilution of SYBR Green I was added to the PCR products, which was then transferred to a Prism 7700 instrument (Applied Biosystems, Foster City, CA, USA) for measurement of melting temperature. The program for analytical melting comprised incubation at 95°C for 30 s, 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.

Statistical analysis

Age, body mass index, and systolic and diastolic BP were compared between subjects with hypertension and controls by the unpaired Student's *t*-test, and

the prevalence of smoking was compared between 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 genetic variants of *SORBS1*, *GCK* and *WISP1* on the prevalence of hypertension and on systolic and diastolic BP based on a 6-year longitudinal cohort study. The data for examination of each subject in the first wave (November 1997 to April 2000), second wave (April 2000 to May 2002), third wave (May 2002 to May 2004) and fourth wave (June 2004 to July 2006) were pooled and analyzed. Systolic and diastolic BP and the prevalence of hypertension were evaluated for both sexes combined as well as for women and men separately. Longitudinal changes in the prevalence of hypertension were compared between two groups (dominant or recessive genetic model) by a generalized estimating equation,[21] with adjustment for age and sex in all subjects or for age in women or men. Longitudinal changes in systolic and diastolic BP in individuals not taking antihypertensive medication were compared between two groups (dominant or recessive model) in a mixed-effect model,[22] with adjustment for age and sex in all subjects or for age in women or men. Age-related changes in the prevalence of hypertension or in systolic or diastolic BP were estimated with quadratic curves 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, USA).

RESULTS

The baseline characteristics (first wave) of the 2233 study subjects are shown in Table 3. Age, body mass index, as well as systolic and diastolic BP were greater in subjects with hypertension than in controls for both women and men, whereas the prevalence of smoking was greater in controls than in hypertensive subjects.

The relationships of the three polymorphisms with the prevalence of hypertension were analyzed with a generalized estimating equation and adjustment for age and sex in all subjects or for age in women or men examined separately (Table 4). The 682A→G (Thr228Ala) polymorphism of *SORBS1* and the −30G→A polymorphism of *GCK* were significantly associated with the prevalence of hypertension among women in a dominant model. The variant *G* allele of the *SORBS1* polymorphism was a risk factor for hypertension, whereas the variant *A* allele of the *GCK* polymorphism was protective against this condition. The 2364A→G polymorphism of *WISP1* was significantly associated with the prevalence of hypertension among men in a dominant model, with the variant *G* allele representing a risk factor for this condition. The genotype distributions of the three polymorphisms in control subjects were all in Hardy–Weinberg equilibrium. The relationships between the prevalence of hypertension and age analyzed longitudinally with a generalized estimating equation are shown in Figure 1. The prevalence of hypertension was greater in the combined group of all subjects with the *AG* or *GG* genotype of *SORBS1* than in those with the *AA* genotype from 40 to 80 years of age (Figure 1a), was lower in the combined group of women with the *GA* or *AA* genotype of *GCK* than in those with the *GG* genotype (Figure 1b) and was greater in the combined group of men with the *AG* or *GG* genotype of *WISP1* than in those with the *AA* genotype (Figure 1c).

The relationships of the three polymorphisms with systolic or diastolic BP in individuals not taking antihypertensive medication were analyzed with a mixed-effect model, with adjustment for age and sex in all subjects or for age in women or men examined separately (Table 5). The 682A→G (Thr228Ala) polymorphism of *SORBS1* was related to diastolic BP for women in a recessive model, with the variant *G* allele being associated with increased BP. The −30G→A polymorphism of *GCK* was related to systolic and diastolic BP for women in a dominant model, with the variant *A* allele being associated with a reduced BP. The 2364A→G polymorphism of *WISP1* was related to systolic and diastolic BP for men in a dominant model, with the variant *G* allele being associated with a higher BP. The relationships between systolic or diastolic BP and age analyzed longitudinally according to genotype for *WISP1* in men with a mixed-effect model are shown in Figure 2. Systolic (Figure 2a) and diastolic (Figure 2b) BPs were greater in the combined group of men with the *AG* or *GG* genotype of *WISP1* than in those with the *AA* genotype from 40 to 80 years of age.

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.[23] We have now examined the relationships of three candidate gene polymorphisms with the prevalence of hypertension and with systolic and diastolic BP in community-dwelling Japanese women and men. Our results show that the 682A→G (Thr228Ala) polymorphism of *SORBS1* and the −30G→A polymorphism of *GCK* were related to both the prevalence of hypertension and BP in women and that the 2364A→G polymorphism of *WISP1* was related to both these parameters in men. These observations thus suggest that *SORBS1* and *GCK* are susceptibility loci for hypertension in Japanese women and that *WISP1* is such a locus in men.

Table 3 Baseline characteristics (first wave) of women and men with hypertension and corresponding controls

	Women			*Men*		
Characteristic	*Hypertension*	*Control*	P-*value*	*Hypertension*	*Control*	P-*value*
Number of subjects (*n*=2233)	370	736		356	771	
Age (years)	64.4 ± 0.5	56.6 ± 0.4	<0.0001	63.2 ± 0.6	57.3 ± 0.4	<0.0001
Body mass index (kg m^{-2})	24.0 ± 0.2	22.4 ± 0.1	<0.0001	23.7 ± 0.1	22.6 ± 0.1	<0.0001
Smoking (*n* (%))	18 (4.9)	61 (8.3)	0.0375	99 (27.8)	330 (42.8)	<0.0001
Systolic BP (mm Hg)	143.1 ± 0.8	113.6 ± 0.5	<0.0001	141.0 ± 0.8	115.9 ± 0.5	<0.0001
Diastolic BP (mm Hg)	84.0 ± 0.5	69.5 ± 0.3	<0.0001	85.9 ± 0.5	72.3 ± 0.3	<0.0001
Number of subjects (*n*=1781)[a]	144	736		130	771	
Systolic BP (mm Hg)	150.4 ± 1.0	113.6 ± 0.5	<0.0001	150.3 ± 1.1	115.9 ± 0.4	<0.0001
Diastolic BP (mm Hg)	88.1 ± 0.7	69.5 ± 0.3	<0.0001	92.5 ± 0.7	72.3 ± 0.3	<0.0001

Data for age, body mass index and blood pressure (BP) are means ± s.e.
[a]Subjects not taking antihypertensive medication.

Table 4 Relations of three polymorphisms to the prevalence of hypertension analyzed with a generalized estimating equation (first wave to fourth wave)

Gene symbol	*Polymorphism*	*Subjects*	*Genotype*	*Hypertension*	*Control*	P-*value (dominant)*	P-*value (recessive)*
SORBS1	682A→G (Thr228Ala)						
		Total	*AA*	1792 (76.5)	3818 (80.1)	**0.0039**	0.1749
			AG	512 (21.9)	896 (18.8)		
			GG	39 (1.7)	55 (1.2)		
		Women	*AA*	847 (77.0)	1891 (80.6)	**0.0249**	0.3641
			AG	227 (20.6)	417 (17.8)		
			GG	26 (2.4)	39 (1.7)		
		Men	*AA*	945 (76.0)	1927 (79.6)	0.0628	0.2649
			AG	285 (22.9)	479 (19.8)		
			GG	13 (1.1)	16 (0.7)		
GCK	−30G→A						
		Total	*GG*	1639 (69.7)	3006 (62.9)	**0.0068**	0.5414
			GA	638 (27.1)	1597 (33.4)		
			AA	74 (3.1)	175 (3.7)		
		Women	*GG*	798 (72.3)	1463 (62.1)	**0.0025**	0.2016
			GA	279 (25.3)	798 (33.9)		
			AA	27 (2.5)	95 (4.0)		
		Men	*GG*	841 (67.4)	1543 (63.7)	0.3861	0.7655
			GA	359 (28.8)	799 (33.0)		
			AA	47 (3.8)	80 (3.3)		
WISP1	2364A→G						
		Total	*AA*	895 (38.2)	1919 (40.2)	**0.0184**	0.4578
			AG	1133 (48.3)	2219 (46.5)		
			GG	318 (13.6)	635 (13.3)		
		Women	*AA*	458 (41.7)	929 (39.5)	0.5437	0.2908
			AG	526 (47.9)	1108 (47.1)		
			GG	115 (10.5)	315 (13.4)		
		Men	*AA*	437 (35.0)	990 (40.9)	**0.0074**	0.0597
			AG	607 (48.7)	1111 (45.9)		
			GG	203 (16.3)	320 (13.2)		

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 or men examined separately.
Values in parentheses are percentages. *P*-values of <0.05 are shown in bold.

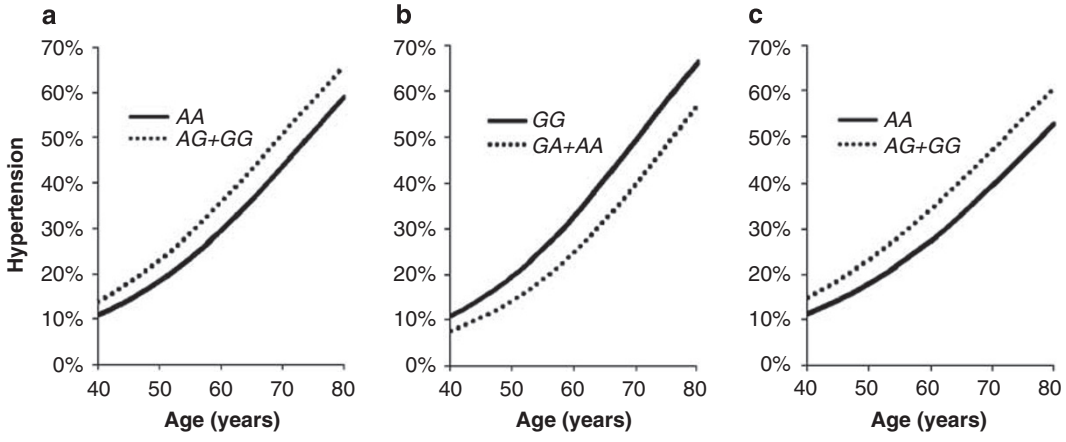

Figure 1 Longitudinal analysis of relationships between the prevalence of hypertension and age according to genotype for *SORBS1* (*AA vs. AG+GG*) in women and men combined (**a**), for *GCK* (*GG vs. GA+AA*) in women (**b**) or for *WISP1* (*AA vs. AG+GG*) in men (**c**), with a generalized estimating equation and adjustment for either age and sex (**a**) or age (**b** and **c**).

Sorbin and SH3 domain-containing 1 (SORBS1) is a human homolog of c-Cbl-associated protein (CAP),[24] which is an important signaling molecule in insulin stimulation of glucose uptake in mouse adipocytes.[24–27] Phosphorylation of c-Cbl results in the dissociation of the c-Cbl–CAP complex from the insulin receptor and its translocation to a lipid raft domain of the plasma membrane. Subsequent

Table 5 Relations of three polymorphisms to systolic and diastolic blood pressure (BP, mm Hg) in individuals not taking antihypertensive medication as analyzed with a mixed-effect model (first wave to fourth wave)

Gene symbol	*Polymorphism*			*Dominant model*		P-*value*	*Recessive model*		P
SORBS1	682A→G (Thr228Ala)			*AA*	*AG+GG*		*AA+AG*	*GG*	
		Total	Sample no.	4407	1093		5432	68	
			Systolic BP	119.9±0.4	120.3±0.8	0.6179	119.9±0.4	123.7±3.1	0.2355
			Diastolic BP	73.8±0.2	73.9±0.5	0.8963	73.8±0.2	76.6±1.9	0.1379
		Women	Sample no.	2157	520		2627	50	
			Systolic BP	118.6±0.6	118.7±1.2	0.8973	118.5±0.5	124.7±3.7	0.0939
			Diastolic BP	72.2±0.4	72.1±0.7	0.8528	72.1±0.3	76.4±2.2	**0.0471**
		Men	Sample no.	2250	573		2805	18	
			Systolic BP	121.1±0.6	121.7±1.1	0.6246	121.3±0.5	117.1±6.2	0.5007
			Diastolic BP	75.4±0.3	75.6±0.7	0.7841	75.5±0.3	73.3±3.7	0.5700
GCK	−30G→A			*GG*	*GA+AA*		*GG+GA*	*AA*	
		Total	Sample no.	3490	2020		5310	200	
			Systolic BP	120.3±0.5	119.4±0.6	0.2339	120.0±0.4	120.4±1.9	0.8164
			Diastolic BP	74.1±0.3	73.4±0.4	0.1399	73.8±0.2	74.5±1.1	0.5721
		Women	Sample no.	1683	1004		2580	107	
			Systolic BP	119.5±0.7	117.1±0.9	**0.0262**	118.6±0.5	118.9±2.7	0.9182
			Diastolic BP	72.8±0.4	71.2±0.5	**0.0120**	72.2±0.3	72.7±1.6	0.7506
		Men	Sample no.	1807	1016		2730	93	
			Systolic BP	121.0±0.6	121.7±0.8	0.5117	121.2±0.5	122.0±2.6	0.7819
			Diastolic BP	75.3±0.4	75.7±0.5	0.6084	75.4±0.3	76.3±1.6	0.5897
WISP1	2364A→G			*AA*	*AG+GG*		*AA+AG*	*GG*	
		Total	Sample no.	2195	3309		4766	738	
			Systolic BP	119.1±0.6	120.5±0.5	0.0573	119.9±0.4	120.5±1.0	0.5698
			Diastolic BP	73.3±0.3	74.2±0.3	0.0522	73.8±0.2	74.4±0.6	0.3371
		Women	Sample no.	1074	1608		2330	352	
			Systolic BP	118.6±0.8	118.6±0.7	0.9498	118.6±0.6	118.9±1.5	0.8248
			Diastolic BP	72.2±0.5	72.2±0.4	0.9953	72.2±0.3	72.4±0.9	0.8283
		Men	Sample no.	1121	1701		2436	386	
			Systolic BP	119.6±0.8	122.3±0.6	**0.0073**	121.2±0.5	121.9±1.3	0.6141
			Diastolic BP	74.4±0.5	76.1±0.4	**0.0057**	75.3±0.3	76.2±0.8	0.2939

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 or men examined separately.
P-values of <0.05 are shown in bold.

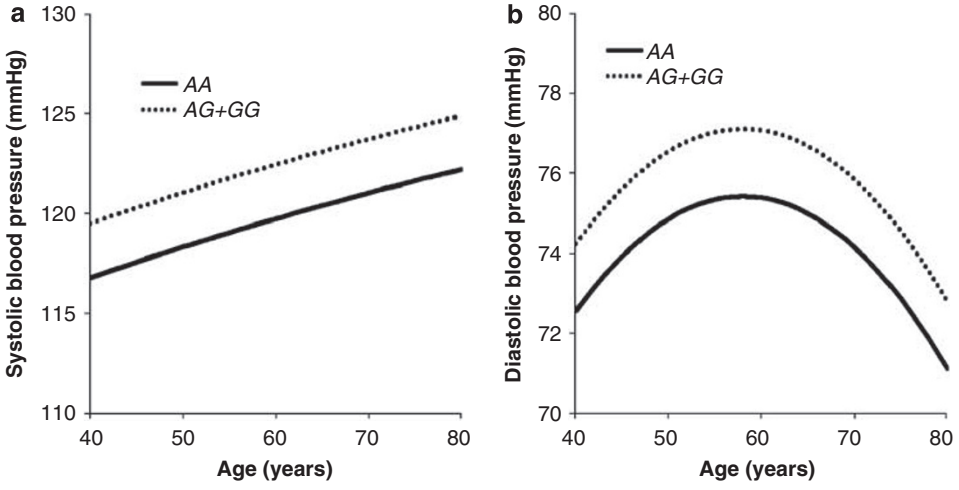

Figure 2 Longitudinal analysis of relationships between systolic (**a**) or diastolic (**b**) BP and age according to genotype for *WISP1* (*AA vs. AG+GG*) in men with a mixed-effect model and adjustment for age.

interactions of the c-Cbl–CAP complex with proteins such as flotillin, Crkα, C3G and TC10 eventually leads to the translocation of vesicles containing the glucose transporter SLC2A4 (GLUT4) from the cytoplasm to the plasma membrane. The central role of the c-Cbl–CAP complex in the regulation of insulin-stimulated glucose uptake suggests that genetic variation of *SORBS1* may be related to insulin

resistance. Fourteen single-nucleotide polymorphisms have been identified in human *SORBS1*, among which, only 682A→G (Thr228Ala), which corresponds to a predicted phosphorylation site for mitogen-activated protein kinase, was found to be related to obesity and type 2 diabetes mellitus in Chinese adults, with the *G* allele being protective against these conditions.[13] In contrast, the *GG* (Ala/Ala) genotype of the 682A→G (Thr228Ala) polymorphism of *SORBS1* was associated with lacunar infarction in the Japanese population.[28] The 682A→G (Thr228Ala) polymorphism (rs2281939) is located in exon 7 of *SORBS1*. A linkage disequilibrium (LD) block (standardized LD coefficient (r^2)$\geqslant$0.3) containing this polymorphism includes ~5 kb from exon 7 to the 3′ region of the gene (International HapMap Project, http://www.hapmap.org/index.html.ja). We have now shown that this polymorphism of *SORBS1* was related to both the prevalence of hypertension and diastolic BP in Japanese women, with the *G* (Ala) allele representing a risk factor for hypertension. The risk allele for hypertension is thus consistent with that for lacunar infarction in the earlier study.[28]

Glucokinase is expressed in pancreatic β cells and hepatocytes, with its expression being controlled by two tissue-specific gene promoters.[29] Pancreatic glucokinase serves as the sensor for glucose in the regulation of insulin secretion.[30] Mutations of *GCK* account for 10–50% of cases of maturity-onset diabetes of the young.[30] The −30G→A polymorphism of *GCK* was shown to be associated with reduced β-cell function and impaired glucose tolerance in Japanese population.[14,31] The −30G→A polymorphism (rs1799884) is located in the β-cell-specific promoter region of *GCK*. An LD block containing this polymorphism includes ~7.5 kb from 5′ region of the gene to intron 2. The *A* allele of this polymorphism was also found to increase the risk for coronary heart disease and was consistently related to this condition after adjustment for type 2 diabetes mellitus in a white population.[32] The risk for coronary heart disease conferred by the *A* allele was even greater in individuals with type 2 diabetes mellitus than in non-diabetic individuals. We have shown earlier that the −30G→A polymorphism of *GCK* was associated with hypertension, with the *A* allele being protective against this condition, in a cross-sectional case–control study.[11] We have now shown that this polymorphism was related to both the prevalence of hypertension and systolic and diastolic BP in Japanese women, with the variant *A* allele protecting against hypertension, consistent with our earlier study.[11] The mechanisms responsible for the association of the *A* variant with an increased risk of coronary heart disease,[32] with reduced β-cell function and impaired glucose tolerance,[14,31] and with a reduced risk of hypertension (present study) remain to be elucidated. The −258G→A polymorphism located in the liver-specific promoter of *GCK* was also shown to be associated with hypertension in a Taiwanese population.[33]

Wingless-type MMTV (mouse mammary tumor virus) integration site family member 1 (WNT1) belongs to a family of cysteine-rich, glycosylated signaling proteins that mediate diverse developmental processes, such as control of cell proliferation, adhesion, polarity and fate. WNT1-inducible signaling pathway protein 1 (WISP1) is induced by WNT1 and belongs to the CCN family, which includes CTGF (connective tissue growth factor), Cyr61 (cysteine-rich 61) and Nov (nephroblastoma-overexpressed gene).[34] WISP1 is a target of the Wnt-β–catenin pathway, with its expression being regulated by β-catenin.[35,36] WISP1 activity and availability are modulated by its interaction with decorin and biglycan, two extracellular matrix-associated proteoglycans that are abundant in bone and cartilage.[37] The 2364A→G polymorphism was shown to be related to spinal osteoarthritis in postmenopausal Japanese women, with the *G* allele protecting against this condition.[15] The 2364A→G polymorphism (rs2929970) is located in the 3′-untranslated region of *WISP1*. An LD block containing this polymorphism includes ~5.5 kb from intron 3 to 3′ region of the gene. We have now shown that this polymorphism was related to both the prevalence of hypertension and systolic and diastolic BP in Japanese men, with the variant *G* allele representing a risk factor for hypertension, although the underlying mechanism of the association remains to be elucidated.

Our study has several limitations. (i) Given the multiple comparisons of genotypes with BP or the prevalence of hypertension in this study, it is not possible to exclude completely potential statistical errors such as false positives. (ii) It is possible that one or more of the polymorphisms related to BP or the prevalence of hypertension in our study are in LD with other polymorphisms of the same or nearby genes that are actually responsible for the development of hypertension. (iii) The functional relevance of the identified polymorphisms to gene transcription or to protein structure or function was not determined in this study. (iv) Given the lack of replication, validation of our findings will require their replication with independent subject panels.

In conclusion, our results suggest that the 682A→G (Thr228Ala) polymorphism of *SORBS1* and the −30G→A polymorphism of *GCK* may play a role in the development of hypertension in Japanese women and that the 2364A→G polymorphism of *WISP1* may play such a role in Japanese men. Determination of genotypes for these polymorphisms may prove informative for 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 a more accurate assessment of the genetic risk for this condition.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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