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ORIGINAL ARTICLE

Accumulation of common polymorphisms is associated with development of hypertension: a 12-year follow-up from the Ohasama study

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Hypertension is a complex multi-factorial and polygenic disorder. Nevertheless, most studies have focused on single-gene effects. Furthermore, a majority of these studies have been cross-sectional and diagnosed hypertension using conventional blood pressure (BP) measurements, which are known to be subject to biases, including the so-called white-coat effect. Thus, we performed a longitudinal association study to clarify the effects of polymorphism accumulation on the development of hypertension that is defined on the basis of self-measured BP at home (home BP). In 403 Japanese aged 40–79 years with home normotension (home BP $<$135/85 mm Hg, and not treated with antihypertensive medication at baseline), we examined the associations of 51 single-nucleotide polymorphisms (SNPs) classically nominated or reported to be associated with hypertension in the Japanese Millennium Genome Project for Hypertension with a 12-year risk of progression to home hypertension (home BP $\geqslant$135/85 mm Hg, or start of antihypertensive medication). Out of 51 SNPs, four significantly and independently predicted the risk of progression of home hypertension, even after adjustment for possible confounding factors, including baseline home BP value. These were rs3767489 near the regulator of G-protein signaling 2 (RGS2), rs4961 in adducin 1 (ADD1), rs2236957 in the calcium channel, voltage-dependent, α-2/δ-subunit 2 (CACNA2D2) and rs769214 in catalase (CAT). Accumulation of these SNPs significantly improved the predictive values for the development of home hypertension. In conclusion, this longitudinal study, which was based on home BP measurement, showed that accumulation of common polymorphisms reliably predicted the risk of future hypertension in the Japanese general population.
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Keywords: blood pressure; development of hypertension; general population; genetics; single-nucleotide polymorphism

INTRODUCTION

Hypertension is a complex multi-factorial and polygenic disorder that results from an interaction between an individual's genetic background and various environmental factors.[1] This disorder is a major risk factor for cardiovascular events such as stroke and myocardial infarction.

In previous studies, numerous genes have been reported to be associated with hypertension,[2] although most of these studies have focused on single-gene effects. However, the combined effects of two or more genes should be considered to accurately predict the prevalence and incidence of complex phenotypes such as hypertension.

Most of the studies on the gene polymorphisms have been performed based on conventional blood pressure (BP).[3,4] Conventional BP measurements, however, are known to be subject to biases, such as observer biases, regression dilution bias and the so-called white-coat effect.[5] In contrast, self-measured BP at home (home BP) allows multiple BP measurements outside hospital, is free of these biases, provides more reproducible information and has more predictive power than conventional BP measurement.[5,6]

Recently, in a case–control study of the Millennium Genome Project for Hypertension in Japan, 38 single-nucleotide polymorphisms (SNPs) were reported to be associated with hypertension.[4] However,

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the study was cross-sectional and the measurement was based on conventional BP.

The present study was undertaken to determine the effects of polymorphism accumulation on the development of hypertension in the Japanese general population, based on home BP.

METHODS

Design

This study is a part of a longitudinal observational study of subjects participating in a BP measurement project in Ohasama, Japan. The socioeconomic and demographic characteristics of this region and full details of the project have been described elsewhere.[7] The study protocol was approved by the institutional review board of Tohoku University School of Medicine and by the Department of Health of the Ohasama Town Government. All study subjects provided written informed consent.

Definition of hypertension

On the basis of several guidelines,[8–10] subjects with home systolic BP $\geqslant$135 mm Hg and/or home diastolic BP $\geqslant$85 mm Hg were classified as having high home BP, whereas others were classified as having normal home BP. Development of home hypertension (hypertension based on home BP measurements) was defined as either progression to high home BP or the start of antihypertensive medication.[11] ΔBP was defined as follow-up home BP—baseline home BP.

Subjects

Between 1988 and 1994, we contacted 2716 subjects aged $\geqslant$40 years living in three districts of Ohasama town. Subjects who were not at home during the normal working hours of the study nurses (n=575) and those hospitalized (n=121) or incapacitated (n=31) were ineligible. Of the remaining 1989 residents, 1957 (98.4%) participated in baseline examinations of home BP measurements. We excluded 44 subjects because home BP values were based on averages of $<$3 readings (3 days). To examine the risk of development of home hypertension, 630 individuals who were 80 years or over, had been treated with antihypertensive medication or had home systolic/diastolic BP values of $\geqslant$135/85 mm Hg were further excluded from the present analysis. Of the remaining 1283 subjects, 577 (45.0%) gave their written informed consent and provided blood samples for DNA extraction.

Selection of SNPs and genotyping

Genomic DNA was extracted from peripheral blood, using a QIAamp DNA blood kit (QIAGEN GmbH, Hilden, Germany). We analyzed 53 susceptible SNPs for hypertension; 38 SNPs reported by the Japanese Millennium Genome Project for Hypertension[4] and 15 classical candidate SNPs [2,12–17] reported to be associated with hypertension in the Japanese population, and to have sufficient frequency in minor alleles to conduct meaningful analysis between genotype and hypertension.[18,19] All SNPs were analyzed by TaqMan probe assay (Applied Biosystems, Foster City, CA, USA) using commercially available primers and probe sets purchased from the Assay-on-Demand system or custom-made oligonucleotides (Supplementary Tables 1 and 2). In all, 51 SNPs were successfully genotyped (genotyping of CALCR (rs1042138) and CYP17 (rs6162) was unsuccessful). Fluorescence levels of PCR products were measured using an ABI PRISM 7900HT sequence detector (Applied Biosystems). Details of SNPs from the Millennium Genome Project for Hypertension in Japan and those classically nominated are listed in Supplementary Tables 1 and 2, respectively.

Therefore, we examined the association between genetic variants of these 51 SNPs and the development of hypertension using home BP.

Home BP measurement

Home BP was measured with the HEM401C at baseline and with the HEM747ICN at follow-up. Both are semiautomatic devices produced by Omron Life Science, Kyoto, Japan, and are based on the cuff-oscillometric method, which generates a digital display of both systolic and diastolic BP. The devices satisfy the criteria of the Association for the Advancement of Medical Instrumentation.[20]

Public health nurses calibrated the devices and instructed the subjects on how to measure BP. Under the same conditions as in the guidelines for the Japanese Society of Hypertension (JSH),[8] all subjects were asked to measure BP at home once in the morning within 1 h after waking, after micturition, sitting after 1–2 min of rest, before drug ingestion and before breakfast, and to record the results over a 4-week period. Home BP measurements were conducted among subjects who collected their own BP data for at least 3 days during the 4-week study period. This criterion was based on our previous observation regarding the average BP values obtained over a given study period.[7] Home BP was defined as the mean of all measurements obtained in each individual. The mean number of home baseline and follow-up BP measurements was 22.7 (s.d. 8.4) and 24.2 (s.d. 5.0), respectively.

Data collection and analysis

Information on smoking status, history of diabetes mellitus, hypercholesterolemia or cardiovascular disease and use of antihypertensive medication was obtained from questionnaires sent to the subjects at the time of home BP measurements and from the medical charts of the Ohasama Hospital, which included the results of laboratory investigations performed at the time of annual health checkups. Subjects using lipid-lowering drugs or those with serum cholesterol levels of 5.68 mmol l^{-1} were considered to have hypercholesterolemia. Subjects with a fasting glucose level of 7.0 mmol l^{-1} or a nonfasting glucose level of 11.1 mmol l^{-1} or those using insulin or oral antihyperglycemic drugs were defined as having diabetes mellitus.

The association between genotypes and development of hypertension was examined by multiple logistic regression analysis, after adjusting for baseline home BP values, age, sex, obesity (body mass index (BMI) $\geqslant$25), smoking status (current or former *vs.* never) and a history of diabetes mellitus, hypercholesterolemia or cardiovascular disease. We examined the associations of each SNP with incidence of hypertension using four different models (minor allele dominant, minor allele recessive, minor allele additive and minor allele frequency models). For each SNP, we selected one of the four models with the highest likelihood of developing hypertension in the logistic regression model.

Variables were compared using analysis of variance (ANOVA), analysis of covariance (ANCOVA) and χ^2-test, as appropriate. Statistical analysis was performed with SAS software, version 9.1 (SAS Institute, Cary, NC, USA). Parametric data are shown as mean (s.d.). Values of $P<0.05$ were considered statistically significant.

RESULTS

Follow-up

Among the 577 normotensive subjects at the time of the baseline survey, 23 died or moved from town before the follow-up measurement. Of the remaining 554 subjects, 403 (72.7%) took part in the follow-up home BP measurements. Those who took part in the follow-up measurements were significantly younger, although baseline home BP levels did not differ (Supplementary Table 3). The mean duration of the period between the baseline and follow-up home BP measurements was 12.2 (2.0) years. At the time of follow-up measurements, 150 subjects (37.2%) developed home hypertension.

Baseline characteristics

The baseline characteristics of the 403 subjects are shown in Table 1. Age, BMI, obesity, systolic BP and diastolic BP among those who developed hypertension were significantly higher when compared with those who maintained normotension.

SNPs significantly associated with development of hypertension

Of the 51 SNPs examined, four significantly and independently predicted the development of hypertension on multiple logistic regression analysis adjusted for confounding factors: rs3767489 near the regulator of G-protein signaling 2 (RGS2), rs4961 in adducin 1

Table 1 Baseline characteristics

	All subjects	*Sustained normotension*	*Developed hypertension*	P-*value*
Number of subjects	403	253	150	
Percentage of men (%)	29.0	27.3	32.0	0.3
Age (years)	55.8 (7.0)	55.0 (6.7)	57.1 (7.3)	0.003
Body mass index ($kg\,m^{-2}$)	23.5 (3.0)	23.1 (2.9)	24.1 (3.0)	0.001
Obesity (%)	30.5	25.7	38.7	0.006
Current or former smoker (%)	18.9	17.0	22.0	0.2
Previous history of diabetes mellitus (%)	12.7	11.9	14.0	0.5
Hypercholesterolemia (%)	30.5	32.4	27.3	0.3
Cardiovascular disease (%)	2.7	2.4	3.3	0.6
Systolic home BP (mm Hg)	116.0 (9.0)	113.7 (9.1)	119.8 (7.5)	<.0001
Diastolic home BP (mm Hg)	70.2 (7.4)	69.0 (7.6)	72.3 (6.6)	<.0001

Data are given as means (s.d.) or percentage of subjects. Obesity was defined as body mass index (BMI) $\geqslant 25$ ($kg\,m^{-2}$). Statistical significance between subjects who sustained normotension and subjects who developed hypertension was compared using the *t*-test for continuous variables and the χ^2-test for categorical variables.

Table 2 Multivariate logistic regression analysis of SNPs associated with incidence of hypertension

	Gene symbol	*Odds ratio*	*95% CI*	P-*value*	*Model*	*Number of subjects successfully genotyped*
1	RGS2	1.8	1.1–2.9	0.01	AA (*vs.* GA+GG)	397
2	ADD1	1.9	1.1–3.1	0.02	AA (*vs.* AC+CC)	384
3	CACNA2D2	1.7	1.1–2.8	0.03	AA (*vs.* GA+GG)	394
4	CAT	1.6	1.0–2.5	0.04	TC+TT (*vs.* CC)	397

Abbreviations: ADD1, α-adducin1; CACNA2D2, calcium channel, voltage-dependent, α-2/δ-subunit2; CAT, catalase; RGS2, regulator of G-protein signaling 2; SNP, single-nucleotide polymorphism. The four SNPs significantly associated with incidence of hypertension from multivariable logistic regression analysis are shown. Analysis was performed with adjustment for baseline BP, age, sex, obesity (body mass index (BMI) $\geqslant 25$), smoking status and a history of diabetes mellitus, hypercholesterolemia or cardiovascular disease.

(ADD1), rs2236957 in the calcium channel, voltage-dependent, α-2/δ-subunit 2 (CACNA2D2) and rs769214 in catalase (CAT) (Table 2). The minor allele dominant model showed the highest likelihood of developing hypertension for RGS2, CACNA2D2 and CAT, whereas the minor allele recessive model showed the highest likelihood for ADD1 (Supplementary Tables 4 and 5). Details of the associations between other SNPs and hypertension are also shown in Supplementary Tables 4 and 5.

The frequency of the RGS2, ADD1, CACNA2D2 and CAT genotypes are shown in Table 3. All of these satisfied Hardy–Weinberg's equilibrium (all $P>0.1$). The allelic frequencies of these SNPs were similar to the frequencies reported in a database of Japanese Single-Nucleotide Polymorphisms (JSNP),[21] except for the frequency of the rs769214 in CAT, which has not yet been reported.

Although there were no differences in baseline home BP values by genotype, the follow-up home BP values were higher for AA in RGS2, AA in ADD1, AA in CACNA2D2 and TT+TC in CAT (Table 3). The development of hypertension was higher with these genotypes than with other genotypes (Table 3).

Cumulative effect of four risk-associated SNPs on the development of hypertension

We defined AA in RGS2, AA in ADD1, AA in CACNA2D2 and TT or TC in CAT as risk-associated SNPs, and analyzed the association between the number of risk-associated SNPs, defined as the sum of RGS2 (AA=1; GG, GA=0), ADD1 (AA=1; CC, AC=0), CACNA2D2 (AA=1; GG, GA=0) and CAT (TT, TC=1; CC=0), the change in home BP values and the development of hypertension (Table 4).

There was a significant association between the number of risk-associated SNPs and ΔBP values (P=0.02/0.001). Development of hypertension significantly increased as the number of risk-associated SNPs increased (P=0.02; Table 4). The odds ratios for development of hypertension in subjects with 1, 2, 3 and 4 of these risk-associated SNPs were 1.6-, 2.6-, 4.7- and 16.9-fold higher than those in subjects with no risk-associated SNPs, respectively (P=0.2, 0.01, 0.001 and 0.005, respectively; Figure 1).

DISCUSSION

Our longitudinal study in a general Japanese population based on home BP revealed that the SNP near RGS2, and SNPs in ADD1, CACNA2D2 and CAT, significantly predicted the risk of progression to hypertension, independent of possible confounding factors including age, obesity and baseline BP levels. The combination of AA in RGS2, AA in ADD1, AA in CACNA2D2 and TT+TC in CAT accurately predicted the risk of progression to hypertension; 75% of subjects with all four SNPs progressed to hypertension.

The functional roles of these SNPs are not known, although they apparently have a role in regulating BP. RGS proteins negatively regulate G protein-coupled receptor (GPCR) signaling by accelerating the inactivation of Gα proteins through stimulation of their GTPase-activating protein (GAP) activity.[3] RGS2 mediates the action of most physiological vasoconstrictors, including norepinephrine, angiotensin II, endothelin-1 and thrombin.[3] Several studies have reported the relationship between genetic variations in human RGS2 and hypertension,[3,22] which is consistent with the present results. However, the SNP that we analyzed was located more than 10-kb upstream from the SNP directly coding RGS2. Further studies are therefore necessary to analyze the role of RGS2 polymorphisms themselves on the risk of hypertension.

ADD1 is involved in cell signal transduction, regulation of actin cytoskeleton and ion transport across the cell membrane.[23] The Gly460Trp polymorphism was found in human α-adducin, and the

Table 3 Change in home BP values and incidence of hypertension according to four significant SNPs

Genotype	*AA*	*GA*	*GG*	P-*value**	*GG+GA*	P-*value*†
RGS2						
n (%)	140 (35)	192 (48)	65 (16)		257	
BP (mm Hg)						
Baseline	116/70 (9/7)	116/70 (9/8)	117/71 (8/6)	0.5/0.8	116/70 (9/7)	0.7/0.7
Follow-up	132/75 (17/9)	127/74 (15/9)	129/74 (14/9)	0.02/0.2	127/74 (15/9)	0.008/0.1
ΔBP (mm Hg)[a]	16/5 (15/9)	11/3(14/9)	12/4 (12/7)	0.02/0.1	12/4 (13/8)	0.006/0.05
Treatment at follow-up (%)[b]	11	11	6	0.5	10	0.8
Hypertension (%)[c]	46	32	34	0.04	33	0.01
Genotype	*AA*	*AC*	*CC*	P-*value**	*CC+AC*	P-*value*†
ADD1						
n (%)	97 (25)	186 (48)	101 (26)		287	
BP (mm Hg)						
Baseline	115/70 (9/8)	116/70 (9/7)	117/71 (9/7)	0.5/0.4	116/70 (9/7)	0.5/0.9
Follow-up	130/76 (16/9)	128/74 (16/9)	129/74 (15/9)	0.6/0.08	129/74 (16/9)	0.4/0.03
ΔBP (mm Hg)	15/6 (13/8)	13/4 (14/8)	13/3 (14/8)	0.4/0.04	13/4 (14/8)	0.2/0.02
Treatment at follow-up (%)	14	9	9	0.3	9	0.1
Hypertension (%)	45	33	36	0.1	34	0.047
Genotype	*AA*	*GA*	*GG*	P-*value**	*GG+GA*	P-*value*†
CACNA2D2						
n (%)	112 (28)	184 (46)	98 (25)		282	
BP (mm Hg)						
Baseline	116/71 (9/8)	116/70 (9/7)	117/70 (10/8)	0.5/0.8	116/70 (9/7)	0.7/0.7
Follow-up	130/75 (11/9)	128/74 (15/9)	130/74 (16/7)	0.02/0.2	127/74 (15/9)	0.008/0.1
ΔBP (mm Hg)	14/5 (15/8)	12/4 (13/9)	13/4 (14/8)	0.4/0.6	12/4 (13/9)	0.3/0.3
Treatment at follow-up (%)	17	7	10	0.01	8	0.007
Hypertension (%)	46	32	37	0.07	34	0.03
Genotype	*CC*	*TC*	*TT*	P-*value**	*TT+TC*	P-*value*†
CAT						
n (%)	159 (40)	189 (48)	46 (12)		238	
BP (mm Hg)						
Baseline	116/71 (8/7)	116/70 (9/8)	116/71 (9/7)	0.5/0.4	116/70 (9/7)	0.5/0.9
Follow-up	127/73 (15/8)	130/75 (16/9)	129/74 (15/9)	0.6/0.08	129/74 (16/9)	0.4/0.03
ΔBP (mm Hg)	11/2 (13/8)	14/5 (14/8)	13/4 (15/8)	0.1/0.01	14/5 (14/8)	0.06/0.006
Treatment at follow-up (%)	7	12	16	0.1	13	0.07
Hypertension (%)	32	41	39	0.2	41	0.06

Abbreviations: ADD1, α-adducin1; BP, blood pressure; CACNA2D2, calcium channel, voltage-dependent, α-2/δ-subunit2; CAT, catalase; RGS2, regulator of G-protein signaling 2; SNP, single-nucleotide polymorphism.
Data are given as means (s.d.) or percentage of subjects. Statistical significance was determined by *t*-test, ANOVA or χ^2-test.
**P*-value of ANOVA or χ^2 test among three groups.
†*P*-value of *t*-test or χ^2 test in two groups; GG+GA *vs.* AA (RGS2, CACNA2D2), CC+AC *vs.* AA (ADD1), TT+TC *vs.* CC (CAT).
[a]ΔBP is follow-up home BP−baseline home BP.
[b]Treatment at follow-up is the use of antihypertensive treatments at the time of follow-up measurement (%).
[c]Hypertension is the incidence of hypertension based on home BP.

460Trp allele was associated with primary hypertension and faster proximal tubular resorption through the activation of Na,K-ATPase.[23] There have been no studies on the association between CACNA2D2 and hypertension, whereas CACNA1C polymorphisms are reportedly associated with the efficacy of calcium channel blockers in the treatment of hypertension.[24] The function of the L-type Ca2+ channel is characterized by its main subunit, α1C (CACNA1C) (Cav1.2), as well as the auxiliary subunits α2δ (CACNA2D) and β (CACNB). The main subunitα1C (CACNA1C) (Cav1.2) mRNA is predominantly expressed in the ventricle and CACNA2D2 mRNA is abundantly expressed in the atrium.[25] CAT is an important antioxidant enzyme that detoxifies H_2O_2 into oxygen and water, and thus limits the deleterious effects of reactive oxygen species (ROS).[15] CAT regulates plasma levels of ROS and together with nitric oxide (NO), influences angiotensin-converting enzyme (ACE) activation, LDL oxidation, adhesion molecule expression, platelet aggregation, endothelial cell apoptosis and vascular smooth cell growth.

Most previous studies only considered single-gene effects, although hypertension is a complex multi-factorial and polygenic disorder. Staessen *et al.*[26] reported that a combination of ACE, ADD and aldosterone synthase polymorphisms, which were identified among SNPs in the rennin–angiotensin–aldosterone system, contribute to the

Table 4 Changes in home BP values and incidence of hypertension according to the number of risk-associated SNPs

	Number of risk-associated SNPs					
	0	*1*	*2*	*3*	*4*	P-*value*
n	57	134	133	41	8	—
Age (years)	56 (7)	55 (7)	57 (7)	55 (8)	58 (8)	0.1
BMI (kg m^{-2})	23 (3)	24 (3)	24 (3)	24 (3)	24 (4)	0.7
Obesity (%)	30	34	32	24	38	0.8
BP (mm Hg)						
Baseline	116/71 (9/6)	115/70 (9/7)	116/70 (9/7)	116/70 (9/8)	115/69 (11/12)	0.9/0.8
Follow-up	126/72 (13/7)	128/73 (16/9)	129/75 (17/9)	133/77 (15/9)	138/78 (15/10)	0.07/0.02
ΔBP (mm Hg)[a]	10/2 (12/8)	12/4 (15/9)	13/4 (14/8)	18/7 (15/8)	24/9 (16/11)	0.02/0.009
Treatment at follow-up[b](%)	2	8	15	15	25	0.02
Hypertension (%)[c]	25	31	43	51	75	0.002

Abbreviations: BMI, body mass index; BP, blood pressure.
Data are given as means (s.d.) or percentage of subjects. Statistical significance was determined using ANOVA or χ^2-test. The number of the risk-associated SNPs was calculated by the sum of RGS2 (AA=1; GG, GA=0), ADD1 (AA=1; CC, AC=0), CACNA2D2 (AA=1; GG, GA=0) and CAT (TT, TC=1; CC=0).[3]Obesity: BMI $\geqslant$25 kg m^{-2}.
[a]ΔBP is follow-up home BP−baseline home BP.
[b]Treatment at follow-up is the use of antihypertensive treatment at the time of follow-up measurement (%).
[c]Hypertension is the incidence of hypertension based on home BP.

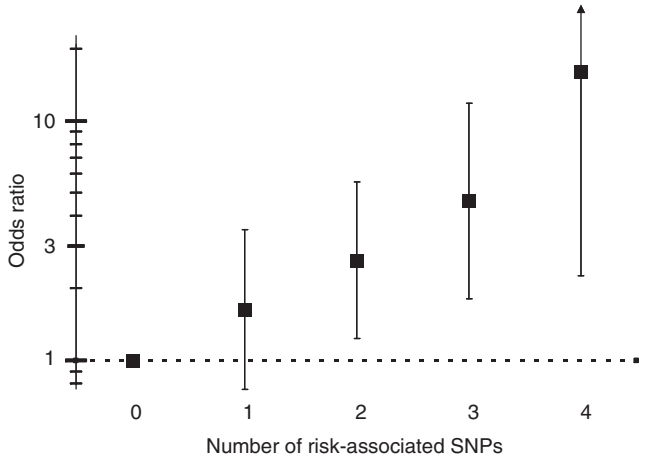

Figure 1 Cumulative effects of risk-associated SNPs on the risk of developing hypertension. Odds ratios and 95% confidence intervals for the risk of development of hypertension among the five groups who were defined according to the number of risk-associated SNPs, and adjusted for baseline BP, age, sex, obesity, smoking status and previous history of diabetes mellitus, hypercholesterolemia or cardiovascular disease.

incidence of hypertension based on casual BP. Recently, Yamada *et al.*[27] reported a combination of three SNPs, which were identified from candidate SNPs in online databases, associated with hypertension in a case–control study. Our longitudinal observation revealed that accumulation of four risk-associated SNPs, which were selected from classical candidate SNPs and candidates from the Millennium Genome Project for Hypertension in Japan, was associated with risk of progression to hypertension diagnosed by home BP.

In the present study, the effects of SNPs on BP were analyzed based on home measurements. Home BP makes it possible to obtain multiple measurements of BP over a long observation period under well-controlled conditions,[5] and it has stronger predictive power for mortality and morbidity than casual BP,[6] indicating that these BP values provide better phenotypes for BP. Therefore, our results were more reliable when compared with previous studies using casual BP. In this study, we only show home BP data, as fewer subjects had casual BP (n=331) data during the follow-up period when compared with subjects who measured home BP (n=403). Comparison between these groups would have raised the limited statistical power.

Our study should be interpreted within the context of its potential limitations. We could not adjust for multiple comparisons. It is possible that the four significant SNPs selected in the present study were merely a reflection of type 1 error, although this is less likely because three of these four SNPs were previously reported to be independently associated with hypertension. Herbert *et al.*[28] used a two-stage testing strategy and used two other cohorts to bypass the multiple comparisons, but we did not have another cohort to verify our results. The second limitation is the limited statistical power derived from the small sample size, which might have caused false-negative associations in some SNPs. Although gender differences in genetic influence on hypertension have also been reported,[29] we may have overlooked such differences among certain subgroups, as we could not perform stratified analysis because of limited statistical power.

Third, we followed up BP changes in normotensive subjects aged $\geqslant$40 years without antihypertensive treatment. It is currently difficult to observe the natural history of hypertension for a long-term period because antihypertensive medication is often administrated to prevent cardiovascular disease. In such cases, the true effect of genetic factors on natural BP change may be masked by the effects of antihypertensive medication. Thus, we excluded hypertensive patients at the start of follow-up in this study. Therefore, we may have overlooked the effects of important candidate genes affecting BP at ages below 40 years, because there are many differences in symptoms and etiologies between hypertension in younger and elderly subjects, and different genetic factor(s) might be associated with hypertension in different generations. Furthermore, as the prevalence of hypertension becomes higher in older individuals, probably more than half of elderly subjects (>65 years old) would be excluded because they already had hypertension, and only very healthy elderly subjects with regard to BP would be selected in the study group. Thus, we may have missed the effects of important candidate genes affecting BP in elderly subjects.

Finally, the possibility of selection bias needs to be considered when generalizing the present findings, because only 45% of those eligible to participate in the study agreed to take part. However, the potential selection bias seems to be minimal, as the home BP values among the study participants were similar to those of nonparticipants. Marked differences also exist among the environmental and genetic factors associated with hypertension between Japan and the United States or Europe. Therefore, another study, including a larger sample size, different ethnic groups and younger subjects should help to clarify the role of these polymorphisms.

In conclusion, this study showed that an accumulation of common polymorphisms accurately predicted future risk of developing hypertension. General applicability of the present findings, as well as the responsible mechanisms, should be examined in further studies.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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