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Ⅳ. 研究成果の刊行物・別刷

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Detection of Midpregnancy Fall in Blood Pressure by Out-of-Office Monitoring

Hirohito Metoki, Takayoshi Ohkubo, Yurie Sato, Maiko Kawaguchi, Misato Nishimura, Yumiko Watanabe and Yutaka Imai

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Letter to the Editor

Letters to the Editor will be published, if suitable, as space permits. They should not exceed 1000 words (typed double-spaced) in length and may be subject to editing or abridgment.

Detection of Midpregnancy Fall in Blood Pressure by Out-of-Office Monitoring

To the Editor:

Silva et al[1] have demonstrated the absence of a midpregnancy fall in diastolic blood pressure (BP) in women with a low educational level. Their previous articles also showed that maternal socioeconomic status is associated with a risk of gestational hypertension and preeclampsia.[2] However, they did not demonstrate a midpregnancy fall in systolic BP in all of the educational subgroups. This might be attributable to few measurement points being obtained during pregnancy or to some other confounding factors.

Ambulatory BP measurement is one way to resolve inferior results from isolated BP measurements. Hermida et al[3] measured ambulatory BP in 403 pregnant women for 48 consecutive hours every 4 weeks from the first obstetric visit until delivery. They found that BP steadily decreased up to 20 weeks of pregnancy and increased up to the day of delivery. Conversely, in women with gestational hypertension and preeclampsia, BP remained stable until the 22nd week of gestation and then linearly increased for the remainder of the pregnancy.

The American Heart Association, American Society of Hypertension, and Preventive Cardiovascular Nurses Association scientific statements indicate that home BP monitoring might overcome many of the limitations of traditional office BP measurements, and it is less expensive and easier to perform than ambulatory BP monitoring.[4] Home BP measurements are theoretically ideal for monitoring changes in BP during pregnancy, because home measurement is the optimal way to record multiple readings taken at the same time of day over prolonged periods.[4] We recently conducted a prospective observational study (Babies and Their Parents' Longitudinal Observation in Suzuki Memorial Hospital on Intrauterine Period Study), in which we averaged 100 BP measurement points during pregnancy in 101 normotensive pregnant women.[5] We found that BP during pregnancy is associated with both seasonal effects and gestational age. We found that the gestational week when BP reached the nadir differed according to the season in which delivery was predicted.

As Silva et al[1] commented in their Discussion, the absence of a midpregnancy fall in BP is reportedly associated with preeclampsia, according to a population study, and may be a sign of latent endothelial dysfunction. However, the amplitude of the fall in midpregnancy BP seems smaller than that of circadian, daily, and seasonal BP variations. Home BP measurement would be a good tool with which to detect such small changes in BP during early pregnancy.

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Disclosures

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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 ⩾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) ≥ 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) ≥ 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).[31]Obesity: BMI ⩾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.

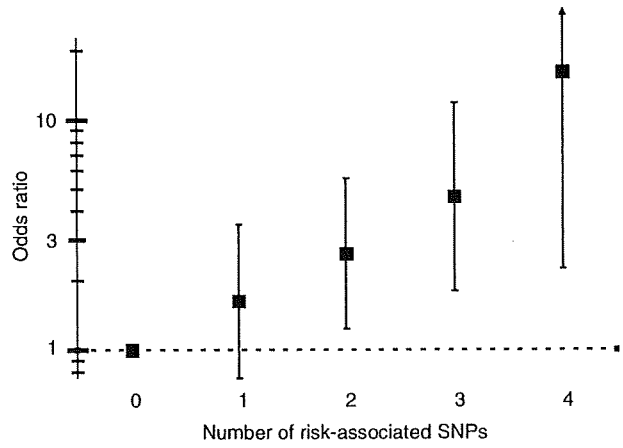

Figure 1 Cumulative effects of risk-associated SNPs on the risk of developing hypertension. Odds ratios and 95% confidence intervals for the risk of development of hypertension among the five groups who were defined according to the number of risk-associated SNPs, and adjusted for baseline BP, age, sex, 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 ⩾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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Parental longevity and offspring's home blood pressure: the Ohasama study

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Objective Longevity is clustered in particular families. Some studies using conventional blood pressure (BP) reported an association between parental longevity and offspring's BP. No study has used self-measurement of BP at home (home BP). We examined the association between parental longevity and home BP values of adult Japanese offspring.

Method Home and conventional BPs were measured in 1961 residents aged 40 years and over in the general population of Ohasama, Japan. Information about the ages of offspring's parents (age at death or current age) was obtained from a standardized questionnaire.

Results The mean ± SD values of systolic/diastolic home BP in offspring whose mothers died at less than 69 years of age, at 69–84 years of age, and in offspring whose mothers were alive at age 84 years were 127.4 ± 13.2/76.2 ± 9.1, 124.8 ± 15.0/74.4 ± 10.0, and 123.4 ± 15.2/74.4 ± 10.3 mmHg ($P = 0.0002/0.009$), respectively. Corresponding values in offspring whose fathers died at less than 66 years of age, at 66–80 years of age, and in offspring whose fathers were alive at age 80 years were 125.7 ± 15.2/75.6 ± 10.6, 124.7 ± 14.1/75.0 ± 9.2 and 122.4 ± 14.6/73.6 ± 9.5 mmHg ($P = 0.001/0.003$), respectively. Multivariate analysis demonstrated associations that were only weakly observed for conventional BP values (conventional BP: $P = 0.3/0.4$ for maternal and $P = 0.3/0.3$ for paternal longevity; home BP: $P = 0.05/0.2$ for maternal and $P = 0.0004/0.007$ for paternal longevity).

Conclusion Parental premature death was significantly associated with higher home BP levels in adult offspring, suggesting that parental longevity might be a useful additional marker for screening adult offspring at higher risk of hypertension. *J Hypertens* 28:272–277 © 2010 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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Keywords: general population, home blood pressure, hypertension, offspring, parental longevity

Abbreviations: ANCOVA, analysis of covariance; ANOVA, analysis of variance; BMI, body mass index; BP, blood pressure

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Introduction

Longevity is clustered in particular families [1]. This phenomenon may be caused by genetic and environmental factors, but these factors are not well known. Hypertension, which is also caused by genetic and environmental factors [2,3], is a major risk factor for cardiovascular events such as stroke and myocardial infarction [4,5]. Some studies have focused on associations between hypertension and noncardiovascular mortality [6].

Although some studies in Western countries have reported an association between parental longevity and offspring's conventional blood pressure (BP) [7], no study has investigated the association using self-measurement of BP (home BP). Conventional BP measurements are known to have biases, such as observer biases, regression dilution biases, and the so-called white-coat effect. In contrast, home BP allows multiple BP measurements outside the hospital, is free of these biases, provides more reproducible information, and has more predictive power than conventional BP measurements [8–12]. The Japanese population is known to have the longest longevity in the world, but no studies have investigated this association [13].

In this Japanese study, the association between parental longevity and home BP values of adult offspring was examined.

Methods

Design

The present study is based on a longitudinal observation of individuals who had been participating in a BP measurement project in Ohasama, Iwate Prefecture, Japan, since 1987. Ohasama, a rural community, had a total population

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of 7496 in 1992. The socioeconomic and demographic characteristics of this region and the details of this project have been previously described [5]. The study protocol was approved by the Institutional Review Board of Tohoku University School of Medicine and by the Department of Health of Ohasama Town Government.

Participants

In Japan, annual health check-ups were available for farmers, the self-employed, pensioners, and dependents aged at least 40 years. Among the residents of Ohasama, 3076 were eligible for annual health check-ups in 1992 [14]. Home and conventional BPs were measured in 1961 residents aged 40 years and over, representing 64% of the total eligible population.

Classification of longevity status

Information on the ages of offspring's parents (at death or current age) was obtained from a standardized questionnaire. The cut-off points of parental longevity status were determined such that the number of offspring in each tertile was the same (Table 1). Paternal and maternal longevity classes were analyzed separately. Offspring whose mothers died at less than 69 years of age were classified into the premature death group, whose mothers were alive at age 69 but died by 84 years of age were classified into the intermediate group, and whose mothers were alive at age 84 were classified into the longevity group. Similarly, offspring whose fathers died at less than 66 years of age were classified into the premature death group, whose fathers were alive at age 66 but died by 80 years of age were classified into the intermediate group, and whose fathers were alive at age 80 were classified into the longevity group. Offspring whose mothers were still alive and were less than 84 years old (618 mothers) or offspring whose fathers were still alive and were less than 80 years old (478 fathers) were excluded from corresponding analyses because these offspring could be classified into both intermediate and longevity groups.

Conventional blood pressure measurement

Two consecutive measurements of BP were taken by a nurse or technician at local medical centers, using a semiautomatic device (USM-700F; UEDA Electronic Works Co. Ltd, Tokyo, Japan) with the participants seated and at rest for at least 2 min. The conventional BP was defined as the average of the two readings.

Table 1 Classification of parental longevity status

	Premature death	Intermediate	Longevity	Other	Total
Mother's age (years)	<69	69 to 84	≥84		
Number of offspring	438	466	439	618	1961
Father's age (years)	<66	66 to 80	≥80		
Number of offspring	514	484	485	478	1961

We classified the offspring into three groups according to the ages of their parents (at death or current age). Maternal and paternal longevity classes were analyzed separately. Offspring whose mothers were still alive and were less than 84 years old (618 mothers) or whose fathers were still alive and were less than 80 years old (478 fathers) were excluded from corresponding analyses.

Home blood pressure measurement

Home BP was measured with the HEM401C, a semiautomatic device based on the cuff-oscillometric method that generates a digital display of both systolic and diastolic BP (Omron Healthcare, Kyoto, Japan). The devices used met the criteria of the Association for the Advancement of Medical Instrumentation [15].

Public health nurses calibrated the devices and instructed the participants on how to measure BP. All participants 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. This protocol was the same as the guidelines of the Japanese Society of Hypertension [11]. Participants were asked to record the results over a 4-week period.

Home BP measurements were collected from participants who measured their own BP data on at least 3 days during the 4-week study period. The home BP was defined as the mean of all measurements obtained in each individual.

Definition of hypertension

On the basis of several guidelines [11,12,16–18], participants with home systolic BP at least 135 mmHg and/or home diastolic BP at least 85 mmHg or taking antihypertensive medication were classified as having home hypertension, whereas those with conventional systolic BP at least 140 mmHg and/or conventional diastolic BP at least 90 mmHg or taking antihypertensive medication were classified as having conventional hypertension.

Data collection and analysis

Information on smoking status, parental hypertension, history of diabetes mellitus, hypercholesterolemia, and/or cardiovascular disease, as well as use of antihypertensive medication, was obtained from questionnaires and from the medical charts of the Ohasama Hospital, which included the results of laboratory investigations performed during annual health check-ups. Participants using lipid-lowering drugs or those with serum cholesterol levels of 5.68 mmol/l were considered to have hypercholesterolemia. Participants with a fasting glucose level of 7.0 mmol/l or a nonfasting glucose level of 11.1 mmol/l, or those using insulin or oral hypoglycemic drugs, were defined as having diabetes mellitus. Body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2).

Variables were compared using the chi-squared test, analysis of variance (ANOVA), and analysis of covariance (ANCOVA) adjusted for sex, age, BMI, smoking status, parental hypertension, and history of diabetes mellitus, hypercholesterolemia and/or cardiovascular disease, as

appropriate. Statistical analysis was performed using SAS software, Version 9.1 (SAS Institute Inc., Cary, North Carolina, USA). Parametric data are shown as means ± SD SD or means [95% confidence interval (CI)]. Values of $P < 0.05$ were considered statistically significant.

Results

Characteristics of offspring by parental longevity status

The offspring's characteristics by parental longevity status are shown in Table 2. The percentages of offspring classified into the maternal premature death, intermediate, and longevity groups were 32.6% ($n=438$), 34.7% ($n=466$), and 32.7% ($n=439$), respectively (Table 2). The corresponding percentages for fathers were 34.7% ($n=514$), 32.6% ($n=484$), and 32.7% ($n=485$), respectively (Table 2).

Maternal longevity was significantly associated with offspring's younger age, a lower percentage receiving antihypertensive medication, and lower prevalence of home and conventional hypertension (Table 2).

Height and weight were slightly but significantly associated with paternal longevity. Although the prevalence of home hypertension was higher in the paternal premature death group, it did not reach statistical significance ($P=0.1$) (Table 2).

Parental longevity and offspring's blood pressure

The mean ± SD values of systolic/diastolic BP according to parental longevity status are shown in Table 3. Parental longevity was significantly associated with offspring's home BP ($P=0.0002/0.009$ for maternal and $P=0.001/0.003$ for paternal longevity, respectively). Such associations were only weakly observed for conventional BP values ($P=0.01/0.1$ for maternal and $P=0.3/0.1$ for paternal longevity, respectively). We found similar significant relationships using home BP values defined as the average of the first two readings ($P=0.002/0.01$ for maternal and $P=0.002/0.008$ for paternal longevity, respectively). Multivariate analyses adjusted for possible confounding factors did not modify most of these significant associations (Table 3). The adjusted mean values and their 95% CIs in each group were 126.4/75.6 (125.2–127.5/74.8–76.4), 124.5/74.5 (123.4–125.6/73.7–75.3) and 124.7/74.9 (123.6–125.9/74.1–75.7) for maternal ($P=0.05/0.2$); and 125.8/75.5 (124.7–126.8/74.8–76.2), 124.4/74.9 (123.3–125.5/74.1–75.6), 122.7/73.8 (121.6–123.8/73.0–74.6) for paternal ($P=0.0004/0.007$), respectively.

Similar relationships were observed for those not on antihypertensive medications (Table 3). Separate analyses according to sex of offspring showed consistent results (data not shown).

Combination of paternal and maternal longevity and offspring's blood pressure

The combination of maternal longevity and paternal longevity was strongly associated with offspring's home BP levels. Offspring whose mothers died at less than 69 years of age and whose fathers died at less than 66 years of age had a significantly higher level of home systolic BP

Table 2 Characteristics of offspring according to maternal and paternal longevity status

	Maternal longevity status				Paternal longevity status			
	Premature death	Intermediate	Longevity	P	Premature death	Intermediate	Longevity	P
Number of offspring (n)	438	466	439		514	484	485	
Age (years)	61.6 ± 9.0	61.8 ± 8.9	60.0 ± 7.9	0.003	59.5 ± 9.5	60.0 ± 9.5	59.9 ± 8.9	0.6
Men (%)	36.8	33.7	32.8	0.4	36.6	33.5	33.6	0.5
Height (cm)	152.4 ± 8.3	151.9 ± 8.2	152.6 ± 8.3	0.5	153.5 ± 8.7	152.6 ± 8.1	152.1 ± 8.2	0.02
Weight (kg)	54.7 ± 8.8	54.9 ± 8.7	54.8 ± 8.8	0.9	56.0 ± 9.1	55.0 ± 8.6	54.4 ± 8.7	0.01
BMI (kg/m^2)	23.5 ± 3.2	23.7 ± 2.9	23.5 ± 3.1	0.5	23.7 ± 3.2	23.6 ± 3.0	23.5 ± 3.0	0.4
Ever smoker (%)	16.4	17.4	15.0	0.6	16.5	15.7	16.7	0.9
Ever drinker (%)	24.9	23.2	24.8	0.8	27.0	24.2	24.1	0.5
Antihypertensive medication (%)	43.4	35.2	27.8	<0.0001	32.7	35.5	30.9	0.3
History of parental hypertension (%)	23.7	23.4	20.3	0.4	21.6	24.0	22.1	0.6
Previous history of hypercholesterolemia (%)	32.7	33.5	30.8	0.7	31.5	32.0	30.5	0.9
Diabetes mellitus (%)	11.4	10.5	10.7	0.9	11.3	10.5	9.9	0.8
Cardiovascular disease (%)	8.0	5.8	4.8	0.1	7.2	5.0	5.8	0.3
Antihypertensive medication (%)	43.4	35.2	27.8	<0.0001	32.7	35.5	30.9	0.3
Hypertension								
Conventional BP (%)	55.0	48.1	39.0	<0.0001	47.3	45.5	41.2	0.1
Home BP (%)	52.5	45.3	37.1	<0.0001	45.5	42.2	39.2	0.1
Number of offspring with antihypertensive medication (n)	190	164	122		168	172	150	
Uncontrolled hypertension								
Conventional BP (%)	42.6	38.4	44.3	0.6	39.9	43.0	42.7	0.8
Home BP (%)	45.3	49.4	55.7	0.2	51.2	52.3	42.0	0.1

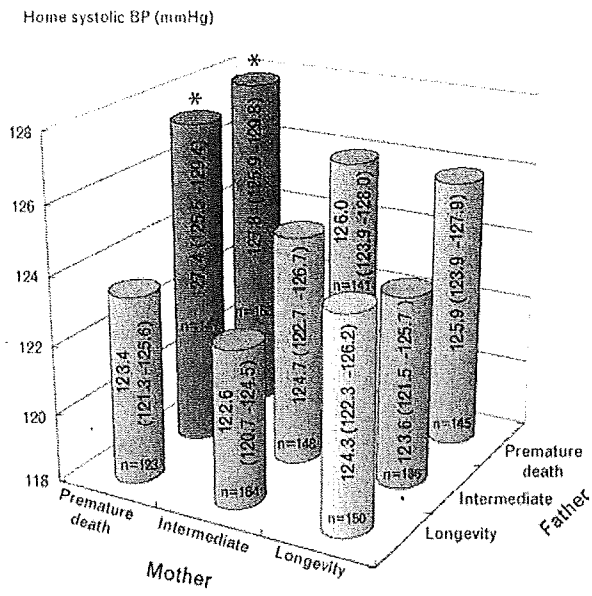
Data are given as mean ± SD or percentage of offspring. Statistical significance among three groups was compared using the ANOVA for continuous variables and the chi-squared test for categorical variables. Definitions of hypertension: home BP, systolic BP ≥ 135 mmHg and/or diastolic BP ≥ 85 mmHg or taking antihypertensive medication; conventional BP, systolic BP ≥ 140 mmHg and/or diastolic BP ≥ 90 mmHg or taking antihypertensive medication. Definitions of uncontrolled hypertension: home BP, systolic BP ≥ 135 mmHg and/or diastolic BP ≥ 85 mmHg; conventional BP, systolic BP ≥ 140 mmHg and/or diastolic BP ≥ 90 mmHg. BMI, body mass index; BP, blood pressure.

Table 3 Parental longevity and offspring's BP

	All offspring						Untreated offspring					
	Maternal longevity status						Maternal longevity status					
	Premature death	Intermediate	Longevity	*P*	*P**	*P***	Premature death	Intermediate	Longevity	*P*†	*P*††	*P*†††
Number of offspring	438	466	439				248	302	317			
Conventional												
Systolic BP (mmHg)	132.8 ± 16.0	131.4 ± 15.8	129.6 ± 15.9	0.01	0.3	0.3	129.2 ± 14.4	128.5 ± 15.0	126.5 ± 15.0	0.07	0.2	0.2
Diastolic BP (mmHg)	74.7 ± 11.4	73.9 ± 10.6	73.2 ± 10.7	0.1	0.4	0.4	73.4 ± 9.7	72.6 ± 10.4	72.0 ± 10.2	0.2	0.4	0.4
Home												
Systolic BP (mmHg)	127.4 ± 13.2	124.8 ± 15.0	123.4 ± 15.2	0.0002	0.05	0.05	122.7 ± 11.5	120.4 ± 13.9	119.0 ± 13.2	0.004	0.02	0.02
Diastolic BP (mmHg)	76.2 ± 9.1	74.4 ± 10.0	74.4 ± 10.3	0.009	0.2	0.2	73.7 ± 7.9	72.2 ± 9.1	72.1 ± 9.4	0.06	0.2	0.2
Pulse pressure (mmHg)	51.3 ± 9.1	50.3 ± 9.7	49.0 ± 9.2	0.002	0.2	0.2	49.0 ± 8.1	48.2 ± 8.8	46.9 ± 7.7	0.01	0.04	0.04
Heart rate (b.p.m.)	66.4 ± 8.2	67.6 ± 7.8	66.8 ± 7.3	0.08	0.07	0.07	66.8 ± 7.7	68.2 ± 7.0	67.4 ± 7.0	0.09	0.1	0.1
	Paternal longevity status						Paternal longevity status					
	Premature death	Intermediate	Longevity	*P*	*P**	*P***	Premature death	Intermediate	Longevity	*P*†	*P*††	*P*†††
Number of offspring	514	484	485				346	312	335			
Conventional												
Systolic BP (mmHg)	131.3 ± 15.9	130.7 ± 15.9	129.7 ± 16.1	0.3	0.3	0.3	128.4 ± 15.0	126.9 ± 14.6	126.4 ± 15.1	0.2	0.1	0.1
Diastolic BP (mmHg)	74.3 ± 11.4	74.0 ± 10.4	73.0 ± 10.9	0.1	0.3	0.3	73.2 ± 11.2	72.8 ± 9.4	71.4 ± 10.3	0.06	0.08	0.07
Home												
Systolic BP (mmHg)	125.7 ± 15.2	124.7 ± 14.1	122.4 ± 14.6	0.001	0.0004	0.0003	121.5 ± 13.8	119.5 ± 12.0	118.3 ± 12.6	0.005	0.002	0.001
Diastolic BP (mmHg)	75.6 ± 10.6	75.0 ± 9.2	73.6 ± 9.5	0.003	0.007	0.007	73.4 ± 10.0	72.5 ± 8.3	71.6 ± 8.6	0.03	0.04	0.03
Pulse pressure (mmHg)	50.1 ± 9.4	49.7 ± 9.1	48.9 ± 9.7	0.1	0.03	0.03	48.0 ± 8.3	47.1 ± 7.5	46.7 ± 8.4	0.08	0.01	0.01
Heart rate (b.p.m.)	67.2 ± 8.3	66.8 ± 7.7	67.4 ± 7.2	0.5	0.6	0.7	67.8 ± 7.6	67.4 ± 7.2	67.8 ± 7.0	0.7	0.5	0.5

Data are given as means ± SD. *P* shows *P* values of ANOVA among three groups. *P** shows *P* values adjusted for sex, age, BMI, smoking status, a history of diabetes mellitus, hypercholesterolemia, or cardiovascular disease. *P*** shows *P* value adjusted for sex, age, BMI, smoking status, a history of diabetes mellitus, hypercholesterolemia, or cardiovascular disease, and parental hypertension. *P*†, *P*†† and *P*††† shows *P* values of ANOVA/ANCOVA among three groups in untreated offspring. BP, blood pressure.

Fig. 1



Combination of maternal and paternal longevity and offspring's home BP. Home systolic BP among nine groups defined according to maternal and paternal longevity. Gray bars and * show significant associations compared with both parental longevity group adjusted for sex, age, BMI, smoking status, a history of diabetes mellitus, hypercholesterolemia, and/or cardiovascular disease. Data are given as adjusted mean values and their 95% confidence intervals. BP, blood pressure.

than offspring whose mothers were alive by age 84 and whose fathers were alive by age 80 ($128.9 \pm 12.7/77.2 \pm 9.6$ mmHg vs. $122.5 \pm 14.1/74.3 \pm 9.5$ mmHg, $P = 0.0001/0.009$); no significant associations were observed for conventional BP ($133.1 \pm 14.5/75.6 \pm 11.9$ mmHg vs. $129.5 \pm 15.8/73.8 \pm 10.8$ mmHg, $P = 0.05/0.2$). Similar relationships were observed using home BP values defined as the average of the first two readings ($P = 0.0007/0.04$). These associations were significant after adjustment for possible confounding factors (Fig. 1).

Parental longevity and history of parental hypertension

When maternal longevity and history of maternal hypertension were entered into the same model simultaneously, only maternal longevity was significantly associated with offspring's systolic BP ($P = 0.04$ for maternal longevity, $P = 0.1$ for history of maternal hypertension). Paternal longevity and paternal hypertension were independently and significantly related with offspring's systolic BP ($P = 0.0004$ for paternal longevity, $P = 0.01$ for history of paternal hypertension) when paternal longevity and history of paternal hypertension were entered into the same model.

Discussion

We found significant associations between parental longevity and offspring's BP using home BP measurement. Hypertension was more frequent, and home systolic and diastolic BPs were higher in the parental premature death group than in the parental longevity group. Parental longevity was more strongly associated with offspring's home BP than with offspring's conventional BP.

To our knowledge, no previous studies have examined the association between parental longevity and offspring's BP using home BP. Home BP makes it possible to obtain multiple measurements of BP over a long observation period under well controlled conditions [8], and it has stronger predictive power for mortality and morbidity than conventional BP [9–11], indicating that these BP values provide a better phenotype for BP. In the present study, the effects of parental longevity on offspring's BP were analyzed on the basis of both home BP and conventional BP measurements, and we found that associations between parental longevity and offspring's BP were more marked for home BP than for conventional BP. Furthermore, home BP values were significantly associated with parental longevity, even with home BP values defined as the average of the first two readings. We previously reported that the predictive value of home BP increased progressively with the number of measurements, but that home BP had a stronger predictive power than conventional BP, even for a lower number of measurements [19]. Measurement conditions might be important, as well as the number of measurements.

Previous studies reported the relationships of BP with age at death of parents and longevity. Hammond *et al.* [20] reported that a history of high BP was more frequent in offspring with the shortest-lived parents (a group defined by both parents having died at <70 years of age) than in other groups. Another study showed that the prevalence of hypertension was lower in the offspring of centenarians [21]. In the PRIME study, systolic and diastolic BPs were lower in offspring whose fathers and mothers were alive at 80 years of age [22]. A recent study reported that paternal longevity but not maternal longevity was associated with offspring's BP [7]. These studies used the same cut-off points for the age at death of fathers and mothers. Our results using home BP further demonstrated that both paternal and maternal longevities contribute equally to offspring's BP.

In this study, parental longevity was associated with offspring's home BP equal to or greater than the association with parental hypertension. Previous studies showed the association between parental hypertension and offspring's BP [23,24]. A self-reported family history of hypertension is sometimes known to be inaccurate. In the Framingham Offspring study, a negative offspring report of parental high BP had a negative predictive value of only 53%, whereas a positive offspring report of parental high BP had a positive predictive value of 83% [25]. In our study, parental longevity was also more

closely associated with offspring's BP than with parental hypertension. Since parental age is easy to remember, it is possible that the ages of parents (at death or current age) appear to be a more accurate predictor than a family history of hypertension.

Our study should be interpreted within the context of its potential limitations. Our analyses were based on all-cause mortality of parents because the questionnaire did not require that the primary causes of parental deaths be specified. Unlike the previous study, associations between parental longevity and offspring's conventional BP were not significant in this study. Some differences in the characteristics of offspring may have influenced the findings. Offspring in our present study were about 10 years older, and standard deviations were also larger than those in the previous study. Regardless of potential limitations, home BP detected significant differences in a dispersed population.

In conclusion, parental premature death was significantly associated with higher home BP levels in adult offspring, suggesting that parental longevity might be useful additional information in screening adult offspring who may be at higher risk for hypertension.

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There are no conflicts of interest.

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Cellular mRNA expressions of anti-oxidant factors in the blood of preeclamptic women

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Objective To assess the alterations of mRNA expressions associated with oxidative stress in the cellular component of blood from pregnant women with pre-eclampsia.

Methods Peripheral blood samples were obtained from pregnant women with and without pre-eclampsia. Cellular RNA was subjected to a reverse transcription polymerase chain reaction (PCR) assay in order to examine the mRNA distribution among women with pre-eclampsia ($n = 24$) and control subjects ($n = 24$) during 35–41 weeks of gestation. The data were analyzed by non-parametric statistics.

Results Significant differences between the pre-eclampsia subjects and the controls were observed in the gene expressions associated with oxidative stress. Lower values in the pre-eclampsia group were found for heme oxygenase (HO)-1, HO-2, catalase and superoxide dismutase (SOD). The HO-1, HO-2 and the catalase levels significantly correlated with proteinuria, and the HO-2 level with systolic blood pressure.

Conclusion Significantly lower concentrations of HO-1, HO-2, SOD and catalase are found in the cellular component of blood from pre-eclamptic patients. The values correlate with the severity of pre-eclampsia. These findings indicate that enhanced oxidative stress and a decrease in the number of anti-oxidant enzymes may be associated with pre-eclampsia. Copyright © 2009 John Wiley & Sons, Ltd.

KEY WORDS: cellular RNA in maternal blood; pre-eclampsia; anti-oxidant enzymes; heme oxygenase-1; fetal cells; nucleic acids and proteins; RNA

INTRODUCTION

Pre-eclampsia (PE) affects 3–8% of pregnancies and it has a significant impact on the health of both the mother and fetus (Sibai *et al.*, 2005). Endothelial dysfunction and failure of cytotrophoblast invasion feature prominently in the pathogenesis of PE. In early gestation, extravillous trophoblasts invade the decidua of the uterus and remodel the endothelial cells of the spiral arteries, thereby leading to an increase in the blood supply to the placenta. In fact, oxygen tension has been reported to increase steeply from <20 mmHg at 8 weeks of gestation to >50 mmHg at 12 weeks of gestation (Jauniaux *et al.*, 2000). Furthermore, the increase in oxygen tension causes increased oxidative stress, thereby leading to the up-regulation of anti-oxidant enzymes in the normal placenta.

However, in cases that subsequently develop PE, the remodeling of endothelial cells of spiral arteries by the extravillous trophoblasts is incomplete; as a consequence, the vascular resistance is not low enough to deliver an adequate blood supply to the fetoplacental unit, thus resulting in a relatively low oxygen tension of the intervillous space. The alterations of oxygen tension may therefore be a cause of the increased oxidative stress of the placenta. Although oxidative stress may be prevented by increased levels of endogenous anti-oxidants in the placenta, excess oxidative stress may lead to the development of PE. Several anti-oxidant systems have been identified within the placenta, including heme oxygenase (HO)-1, HO-2, copper/zinc superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). These anti-oxidant systems may play an important role in the development of PE.

In 2000, Poon *et al.* described circulation of cell-free mRNA derived from the placenta in the plasma of pregnant women, enabling the development of several promising approaches for non-invasive evaluation of placental function (Poon *et al.*, 2000). We have reported that human chorionic gonadotrophin (hCG) and human placental lactogen (hPL) gene expressions in the maternal plasma reflected placental gene expressions (Okazaki *et al.*, 2006). Furthermore, we showed that vascular endothelial growth factor (VEGF), VEGF receptor 1 [fms-like tyrosine kinase 1 (FLT-1)] and endoglin (Eng) mRNAs were detected in the plasma of pregnant women in the third trimester, and that these levels were higher in pregnancies complicated by PE, in comparison to normal pregnancies (Purwosunu *et al.*, 2008). Moreover, VEGF, FLT-1 and Eng mRNA concentrations correlated with the severity of PE. These alterations

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were also observed in plasminogen activator inhibitor-1, tissue-type plasminogen activator, corticotrophin-releasing hormone, selectin P and placenta-specific 1 mRNA (Purwosunu *et al.*, 2007a,b). Therefore, evaluation of placental mRNA levels in maternal plasma may allow for the indirect monitoring of the placental function.

That the expression of placenta-specific genes, such as hPL and βhCG, is detectable in the cellular component of maternal blood (Okazaki *et al.*, 2006) suggests that some trophoblasts circulate in the blood of normal pregnant women. Furthermore, the mRNA concentrations of hPL and βhCG correlate with the protein assay. We have reported that the cellular mRNA concentration of hPL during the third trimester was 16.7 times greater, and that of βhCG during the first trimester was 8.5 times greater, than those of the plasma component of maternal blood (Okazaki *et al.*, 2006). These results suggest that analysis of the cellular component of maternal blood may be ideal for evaluating the placental function. We reported the up-regulated mRNA expression of pregnancy-specific β1-glycoprotein 1 (PSBG) and trophoblast glycoprotein (TPBG) in the cellular component of blood from patients affected with PE, and a direct correlation between the PSBG expression levels and the clinical severity of PE (Okazaki *et al.*, 2007). As oxidative stress may play an important role in the pathogenesis of PE, in this study we assessed the mRNA expression levels of anti-oxidant enzymes in the cellular component of blood from women with PE.

MATERIALS AND METHODS

Subjects

We included pre-eclamptic and normal pregnant women who visited the Department of Obstetrics and Gynecology, University of Indonesia at Cipto Mangunkusumo National Hospital. Subjects were recruited between December 2005 and February 2006. A total of 48 singleton pregnancies were included, with 24 in each group of PE and control. All women provided their written informed consent to participate in the study, and the protocol was approved by the Research Ethics Committees of both University of Indonesia and Showa University. We defined mild and severe PE, as well as hemolysis, elevated liver enzymes, and low platelets (HELLP) syndrome, as described elsewhere (Purwosunu *et al.*, 2007b). The severities of hypertension and proteinuria were defined by highest level of hypertension during hospital stay and urinary protein excretion in a 24-hour urine specimen, respectively. The control group included pregnant women with no pre-existing medical diseases or prenatal complications.

Processing of blood samples

The blood samples (2.5 mL) were collected in PAXgene blood RNA tubes (PreAnalytiX, Hombrechtikon, Switzerland) and kept at room temperature for 3 h, and then were stored at −20 °C until they were transported to Japan. A molecular analysis was performed in the Department of Obstetrics and Gynecology at Showa University School of Medicine, Tokyo, Japan. RNA extraction and reverse transcription polymerase chain reaction (RT-PCR) were performed according to protocols described elsewhere (Okazaki *et al.*, 2006). In brief, the blood samples were centrifuged twice at 4000 g for 10 min at room temperature; the entire supernatant and any mRNA present in the residual plasma were removed. The pellet was then washed, resuspended and incubated in optimized buffer solution containing proteinase K to digest protein. A second round of centrifugation was performed to remove any residual cell debris, and the resulting supernatant was then transferred to a fresh microcentrifuge tube. We added 100% ethanol to the supernatant to adjust the binding conditions, and the resultant lysate was then applied to a PAXgene spin column (PreAnalytiX); thus resulting in the selective binding of RNA to the silica-gel membrane of the spin column. After the column was washed three times, pure RNA was then eluted in 80 µL of RNase-free water.

Real-time quantitative RT-PCR

The mRNA was reverse transcripted using an Omniscript RT Kit (Qiagen). Real-time quantitative PCR was then performed using a QuantiTect Probe PCR Kit (Qiagen), according to the manufacturer's instructions. The cDNA products were amplified by real-time quantitative PCR according to the manufacturer's instructions (QuantiTect Probe PCR kit, Qiagen) using a 2-µL aliquot of cDNA and the kit's components in a reaction volume of 20 µL. TaqMan PCR analyses for HO-1, HO-2, SOD, GPx and CAT were performed using predeveloped and commercially available primers and probe sets (Cat# Hs00157965_m1 for HO-1, Cat# Hs00157965_m1 for HO-2, Cat# Hs00166575_m1 for SOD, Cat#Hs00829989_gH for GPx and Cat# Hs00156308_m1 for CAT: Applied Biosystems, Foster City, CA). As an initial step, we verified that each PCR assay was specific to mRNA and not to genomic DNA. The amplification data were collected and analyzed with an ABI Prism 7900T Sequence Detector (Applied Biosystems). Each sample was analyzed in duplicate, and multiple negative water blanks were included in every analysis. The following thermal profile was used: 15-min denaturation at 95 °C and 15-s annealing at 94 °C, followed by 1-min extension at 60 °C. The quantification of the gene expression was performed with investigators blinded to sample background. The calibration curves for HO-1, HO-2, SOD, GPx and CAT mRNA quantification were made using placental mRNA obtained from normal pregnancies undergoing elective cesarean delivery, with calculations performed as previously described (Farina *et al.*, 2006) and they were expressed as relative concentrations (RCs).