must await large scale, randomized trials of the effects of BP variability lowering therapy on major causes of morbidity and mortality.

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Serum Magnesium, Ambulatory Blood Pressure, and Carotid Artery Alteration: The Ohasama Study

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BACKGROUND

To investigate the associations of 24-h ambulatory blood pressure (ABP) and serum magnesium level (sMg) with risk of carotid artery alteration in a general population.

METHODS

sMg and ABP, monitored every 30 min, were measured in 728 subjects (mean age, 67 years) from the Japanese general population. The extent of carotid artery alteration was evaluated according to mean common carotid intima-media thickness (IMT) and the presence of focal carotid plaque. To determine the association of sMg and carotid artery alteration, analysis of covariance (ANCOVA) (for adjusted mean IMT) or multiple logistic regression analysis (for odds ratio (OR) for the presence of carotid plaques) was used.

RESULTS

Lower sMg was significantly associated with mean IMT ($P = 0.004$) and risk of ≥2 carotid plaques ($P = 0.03$) after adjusting for possible confounding factors, including 24-h ABP (systolic), creatinine clearance (Ccr) (estimated using the Cockcroft–Gault equation), and serum minerals (sodium, potassium, calcium, and inorganic phosphorus). Even when 24-h ABP values were within normal range (<130/80 mm Hg), lower sMg levels (<2.2 mg/dl) were significantly associated with mean IMT ($P = 0.007$) and risk of ≥2 carotid plaques (OR, 2.14; 95% confidence interval, 1.18–3.85; $P = 0.01$).

CONCLUSIONS

Both 24-h ABP and lower sMg were closely and independently associated with risk of carotid artery alteration. Further investigations are needed to examine the relationship between sMg levels and the incidence of cardiovascular disease.

Keywords: *ambulatory blood pressure; blood pressure; carotid plaque; general population; hypertension; intima-media thickness; serum magnesium*

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Magnesium (Mg) plays a physiological role in many functions, including anti-inflammatory and antiplatelet activities.[1,2] Previous studies have demonstrated that serum Mg levels (sMg) are inversely associated with both intima-media thickness (IMT)[3] and incidence of hypertension.[4] Another study showed an association between sMg and age-, sex-, and race-adjusted incidence of ischemic stroke, but the association did not remain significant after adjusting for a history of hypertension and diabetes mellitus.[5] The usefulness of sMg as a marker of cardiovascular disease thus remains controversial. On the other hand, a systematic review recommended higher intake of dietary Mg for the prevention of hypertension, cardiovascular disease, and metabolic syndrome.[6] Daily Mg intake reportedly correlates with sMg among Japanese people,[7] suggesting the importance of investigating the contribution of sMg to the prevention of cardiovascular disease in Japanese communities.

High-resolution B-mode ultrasonography of the carotid artery provides a valid, noninvasive, and reproducible means of identifying plaque and quantifying IMT, both of which are widely regarded as markers of the generalized atherosclerosis and arteriosclerosis that predicts cardiovascular disease independent of conventional risk factors.[8,9] Carotid IMT and plaque more accurately predict the risk of future myocardial infarction and stroke than traditional risk factors.[10,11]

Ambulatory blood pressure (ABP) offers higher predictive powers of future cardiovascular disease than casual blood pressure (CBP).[12] Furthermore, we have reported that ABP values are more closely associated with mean IMT and carotid plaques than CBP.[13]

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We conducted a cross-sectional study to determine the association between sMg and carotid artery alterations in a general population. We also evaluated the association between carotid artery alteration and the risk conferred by combined sMg and ABP levels.

METHODS

Design. This investigation was part of the Ohasama study. The socioeconomic and demographic characteristics of this region and full details of the project have been described previously.[12–15] The study protocol was approved by the institutional review board of Tohoku University School of Medicine (Sendai, Japan), and by the Department of Health of the Ohasama Town Government.

Study population. In 1998, the total population of Ohasama was 7,202. Of these inhabitants, 3,077 were ≥55 years old. Individuals who were not home during normal working hours of the study nurses (n = 492), and those hospitalized, mentally ill, or bedridden (n = 185) were excluded from the study.[15] Of the remaining 2,400 eligible individuals, 728 (mean age, 66.7 years; 68.4% women) gave written informed consent, completed a carotid ultrasound examination, and underwent measurement of ABP values and various biochemical values, including sMg.

Carotid ultrasonography. We used a real-time, B-mode ultrasound imaging unit (Sonolayer SSA-250A; Toshiba, Tokyo, Japan) with a 7.5-MHz annular array probe, providing an axial resolution of 0.25 mm. A trained physician examined ultrasonograms using a standardized protocol described elsewhere.[15] All subjects were examined in a sitting position. Observers measured the IMT on the near and far walls of both common carotid arteries, ~1 cm proximal to the carotid bulb on the longitudinal view. Mean IMT was defined as the mean of the maximum wall thickness for the near and far walls of both the left and right common carotid arteries. The common carotid artery, carotid bifurcation, internal carotid artery, and external carotid artery were examined bilaterally for the presence of plaque (absence, 1, or 2 or more), defined as a focal lesion relative to adjacent segments, with protrusion into the lumen composed of either calcified deposits alone or the presence of a combination of calcified and noncalcified material.[11] If plaque lay at an IMT measurement point, the IMT was scanned and evaluated from other angles. When plaque involved the entire circumference of the artery at a measurement point, IMT was measured at three of the four locations described above, and the values were averaged. As a result, mean IMT did not include values obtained at sites of plaque lesions.

The reproducibility of IMT measurements was assessed in 29 subjects, with 6 observers measuring IMT of 4 to 6 subjects twice. The intraobserver coefficient of variation and correlation coefficient for mean IMT between the first and second observations was 6.3% and r = 0.91 (P < 0.0001), respectively (mean ± s.d.) of the difference, 0.035 ± 0.035 mm). Interobserver coefficient of variation and correlation coefficient between two observers was 11.9% and r = 0.62 (P < 0.0003), respectively (mean ± s.d. of the difference, 0.085 ± 0.062 mm). These data show that the reproducibility of IMT measurements was comparable to that seen in other studies.[8,10,11,16]

BP measurements. ABP was monitored using an ABPM-630 (Nippon Colin, Komaki, Japan), a fully automatic device that uses the cuff-oscillometric method and is preset to measure BP every 30 min. The device was attached and detached the following morning by a well-trained public health nurse who visited the subject on a weekday morning. Each subject was instructed to record their daily activities in a diary. According to the diary, "daytime" and "nighttime" were determined as periods of being awake and asleep, respectively. Artifactual measurements during recordings were defined according to the described criteria[17] and automatically omitted from analysis: systolic BP <60 mm Hg and mean BP <40 mm Hg; mean BP >200 mm Hg and/or systolic BP >250 mm Hg, with no similar preceding or subsequent respective value; pulse pressure ≤10 mm Hg; and abrupt increase or decrease in pulse pressure, systolic BP, mean BP, and/or heart rate of ≥50% from the value immediately before or after respective readings. On average, 2.8 readings were excluded per subject. CBP was measured twice consecutively in the sitting position, after a rest interval of ≥2 min, by a physician using a mercury sphygmomanometer or an automatic device (HEM907; Omron Healthcare, Kyoto, Japan) at the time of carotid ultrasonography. The average of the two readings was defined as CBP. Both devices for ABP[17] and CBP[18] have been validated and meet the criteria of the Association for the Advancement of Medical Instrumentation.[19]

Thresholds of ABP levels were based on international database on ABP monitoring in relation to cardiovascular outcomes,[20] and ABP hypertension was defined as ≥130/80 mm Hg for 24-h, ≥140/85 mm Hg for daytime, and ≥120/70 mm Hg for nighttime. CBP hypertension was defined as ≥140/90 mm Hg.

Physical and biochemical examinations. sMg was measured using a xylidyl blue colorimetric method. Hypercholesterolemia was defined as total cholesterol ≥220 mg/dl, use of medication for hypercholesterolemia, and/or a history of hypercholesterolemia. Diabetes mellitus was defined as a nonfasting glucose level ≥200 mg/dl, hemoglobin A_{1c} level ≥6.5%, use of medication for diabetes, and/or a history of diabetes mellitus. Body mass index (BMI) was defined as weight in kg divided by the square of height in meters. Serum creatinine was obtained using the Jaffe method (n = 502) or an enzymatic method (n = 226). Creatinine clearance (Ccr) was calculated using the Cockcroft–Gault formula-adjusted body surface area as calculated by the DuBois formula.[21,22] Serum creatinine is reportedly lower when measured by the enzymatic method than when measured by the Jaffe method.[23,24] Therefore, based on evidence for Japanese subjects,[24] we evaluated serum creatinine as +0.2 mg/dl if

Table 1 | Characteristics of study subjects among quartiles of sMg

	sMg quartile				
	I	II	III	IV	*P*
sMg levels, mg/dl	<2.1	2.1–2.2	2.2–2.3	≥2.3	
N	182	137	140	269	
Age, years	67 ± 6	67 ± 6	67 ± 6	66 ± 6	0.2
Men, %	34	36	32	28	0.4
BMI, kg/m^2	24 ± 3	24 ± 3	24 ± 3	24 ± 3	0.5
Smoker, %	20	22	23	15	0.2
Drinker, %	31	31	29	32	0.9
Hypercholesterolemia, %	30	37	40	49	0.0008
Diabetes, %	15	15	13	14	0.9
History of CVD, %	17	15	17	15	0.9
Treatment[a], %	37	42	40	41	0.7
Serum Mg, mg/dl	1.9 ± 0.1	2.1 ± 0.0	2.2 ± 0.0	2.4 ± 0.1	—
Serum Na, mEq/l	141.8 ± 2.0	142.2 ± 1.9	142.3 ± 1.7	142.7 ± 1.9	0.07
Serum K, mEq/l	4.4 ± 0.5	4.5 ± 0.7	4.6 ± 0.6	4.8 ± 0.7	<0.0001
Serum Ca, mg/dl	9.1 ± 0.3	9.2 ± 0.4	9.3 ± 0.4	9.3 ± 0.4	<0.0001
Serum IP, mg/dl	3.4 ± 0.6	3.3 ± 0.7	3.2 ± 0.6	3.1 ± 0.6	0.002
Ccr, ml/min/1.73 m^2	73.4 ± 24.5	74.3 ± 29	71.2 ± 19.4	72.8 ± 36.1	0.8
Total cholesterol, mg/dl	195.4 ± 33.4	197.0 ± 31.0	199.3 ± 30.6	207.0 ± 32.8	0.0007
HDL cholesterol, mg/dl	54.7 ± 14.6	57.7 ± 14.8	55.8 ± 14.5	58.9 ± 15.0	0.02
LDL cholesterol, mg/dl	114.0 ± 28.9	114.2 ± 28.2	117.6 ± 26.5	122.2 ± 30.2	0.01
Triglyceride, mg/dl	140.5 ± 102.1	124.3 ± 57.5	131.6 ± 69.9	128.7 ± 70.8	0.3
24-h ABP, mm Hg					
Systolic	125.6 ± 13.5	127.2 ± 11.6	126.6 ± 12.9	124.5 ± 12	0.2
Diastolic	73 ± 7.4	74.2 ± 7.3	73.8 ± 8.3	73.7 ± 7.1	0.5
Daytime ABP, mm Hg					
Systolic	131.5 ± 14.8	133 ± 12.6	132.5 ± 13.9	130.3 ± 12.9	0.2
Diastolic	77.3 ± 8.5	78.5 ± 7.7	78.1 ± 9.1	77.7 ± 7.8	0.6
Nighttime ABP, mm Hg					
Systolic	114.2 ± 14.4	116.3 ± 13.2	115.7 ± 14.1	113.5 ± 13.5	0.2
Diastolic	64.6 ± 7.6	66.2 ± 8.5	65.7 ± 8.3	65.9 ± 7.7	0.3
CBP, mm Hg					
Systolic	140 ± 21.7	144.2 ± 19.9	148.6 ± 24.6	147.5 ± 21.6	0.0009
Diastolic	77 ± 10	78.3 ± 10.3	80.1 ± 11.6	80.2 ± 11.4	0.01
Mean IMT, mm	0.75 ± 0.13	0.75 ± 0.14	0.72 ± 0.14	0.71 ± 0.13	0.001
Plaques					
≥1, %	42	41	35	36	0.4
≥2, %	23	22	17	12	0.009

Values are mean ± s.d. or %; *N*, number of subjects; LDL (n = 723) was calculated by the Friedewald equation.
ABP, ambulatory blood pressure; BMI, body mass index; Ca, calcium; CBP, casual blood pressure; Ccr, creatinine clearance; CVD, cardiovascular disease; HDL, high-density lipoprotein; IMT, intima-media thickness; IP, inorganic phosphorus; K, potassium; LDL, low-density lipoprotein; Na, sodium; sMg, serum magnesium level.
[a]Treatment means use of antihypertensive medications.

measured by an enzymatic method, to calculate estimated Ccr. Ccr (ml/min/1.73 m^2) was calculated as ((140 − age) × weight (kg) × 1.73/BSA (m^2) (× 0.85: women))/(72 × serum creatinine (mg/dl)), where BSA (m^2) = weight (kg)$^{0.425}$ × height (m)$^{0.725}$ × 0.007184.

Statistical analysis. We examined associations of the quartiles of sMg and population characteristics by analysis of variance or the χ^2-test. Analysis of covariance (ANCOVA) or multiple logistic regression analysis was used to determine the association of sMg and carotid artery alteration (continuous mean

Table 2 | Adjusted mean IMT and ORs and 95% CIs for plaques associated with quartiles of sMg ($n = 782$)

	sMg quartile				
	I	II	III	IV	Trend *P*
ANCOVA	mm (95% CI)	mm (95% CI)	mm (95% CI)	mm (95% CI)	
Mean IMT	0.75 (0.73–0.77)	0.75 (0.73–0.77)	0.72 (0.70–0.74)	0.71 (0.70–0.73)	0.004
Multiple logistic regression analysis	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (reference)	
Plaques ≥2	1.83 (1.03–3.26)	1.88 (1.03–3.43)	1.39 (0.75–2.59)	1	0.03

Mean IMT and ORs for the presence of plaques were adjusted for age, sex, BMI, smoking, drinking, antihypertensive medications, 24-h ABP (systolic), cardiovascular diseases, hypercholesterolemia, diabetes mellitus, Ccr, sNa, sK, sCa, and sIP.
ABP, ambulatory blood pressure; ANCOVA, analysis of covariance; BMI, body mass index; Ccr, creatinine clearance; CI, confidence interval; IMT, intima-media thickness; OR, odds ratio; sCa, serum calcium; sIP, serum inorganic phosphorus; sK, serum potassium; sMg, serum magnesium level; sNa, serum sodium.

IMT and presence of plaque), adjusted for age, sex, BMI, smoking, drinking, use of antihypertensive medications, 24-h ABP (systolic), cardiovascular disease, hypercholesterolemia, diabetes mellitus, Ccr, and serum minerals (sodium, potassium, calcium, and inorganic phosphorus). *P* for trends from multiple logistic regression analysis was evaluated using odds ratio (OR) for a one-category increase in the quartile of sMg. We examined the relationship between carotid artery alteration and the combination of sMg and ABP levels using ANCOVA or multiple logistic regression analysis. The Bonferroni method was used on mean IMT in the ANCOVA. Interactions were evaluated between sMg and 24-h ABP (hypertension vs. no hypertension), sex (male vs. female), age (<65 years vs. ≥65 years), and use of antihypertensive medication (treated vs. untreated) on carotid artery alteration.

All statistical analyses were conducted using SAS version 9.1 software (SAS Institute, Cary, NC). Values are expressed as means ± s.d. Two-tailed values of $P < 0.05$ were considered statistically significant.

RESULTS

Subject characteristics

sMg values ranged from 1.7 to 2.8 mg/dl. BMI (mean ± s.d.) was 23.8 ± 3.0 kg/m^2. A total of 292 subjects (40.1%) were taking antihypertensive medications. In 292 treated subjects, detailed information on antihypertensive medications was collected from 287, and 10 users of diuretics were identified. One hundred and forty (19.2%) were smokers, 227 (31.2%) were drinkers, 294 (40.4%) had hypercholesterolemia, 105 (14.4%) had diabetes mellitus, and 115 (15.8%) had a history of cardiovascular disease. Mean IMT was 0.73 ± 0.13 mm, and 277 subjects (38.1%) had plaques. Mean values of 24-h, daytime, and nighttime ABP were 125.7 ± 12.5/73.6 ± 7.5, 131.5 ± 13.6/77.8 ± 8.2, and 114.6 ± 13.8/65.6 ± 7.9 mm Hg, respectively. CBP was 145.2 ± 22.1/79.0 ± 11.0 mm Hg. Of the 728 study subjects, 275 (37.8%), 229 (31.5%), and 287 (39.4%) were classified as having 24-h, daytime, and nighttime ABP hypertension, respectively. A total of 441 subjects (60.6%) showed CBP hypertension.

sMg was not significantly associated with systolic or diastolic ABP values. Quartiles of sMg were positively and significantly associated with CBP (systolic, $P = 0.0009$; diastolic, $P = 0.01$), and negatively associated with mean IMT ($P = 0.001$) and ≥2 plaques ($P = 0.009$), but not with ≥1 plaques ($P = 0.4$; **Table 1**).

Multivariate analyses by ANCOVA were performed to examine whether the positive association between sMg and CBP was independent of confounding factors. The positive association between the quartiles of sMg and systolic CBP was weak but still significant ($P = 0.02$), whereas the association with diastolic CBP was not significant ($P = 0.2$).

Table 3 | Adjusted mean IMT associated with the combination of sMg level and ABP level

BP criteria		sMg	*N*	Mean IMT, mm (95% CI)	*P**
24-h	<130/80	Higher	254	0.69 (0.68–0.71)	(Ref.)
		Lower	199	0.73 (0.72–0.75)	0.007
	≥130/80	Higher	155	0.74 (0.72–0.76)	0.003
		Lower	120	0.77 (0.75–0.80)	<0.0001
Daytime	<140/85	Higher	281	0.70 (0.68–0.71)	(Ref.)
		Lower	218	0.74 (0.72–0.75)	0.005
	≥140/85	Higher	128	0.74 (0.72–0.76)	0.02
		Lower	101	0.77 (0.75–0.80)	<0.0001
Nighttime	<120/70	Higher	253	0.70 (0.68–0.71)	(Ref.)
		Lower	188	0.74 (0.72–0.76)	0.004
	≥120/70	Higher	156	0.74 (0.72–0.76)	0.005
		Lower	131	0.76 (0.74–0.79)	<0.0001
Casual	<140/90	Higher	138	0.70 (0.68–0.72)	(Ref.)
		Lower	149	0.74 (0.72–0.76)	0.1
	≥140/90	Higher	271	0.72 (0.70–0.73)	1.0
		Lower	170	0.75 (0.74–0.77)	0.003

Values for mean IMT were adjusted for age, sex, BMI, smoking, drinking, antihypertensive medications, cardiovascular diseases, hypercholesterolemia, diabetes mellitus, Ccr, sNa, sK, sCa, and sIP; Higher sMg was defined as ≥2.2 mg/dl (median).
ABP, ambulatory blood pressure; BMI, body mass index; BP, blood pressure; Ccr, creatinine clearance; CI, confidence interval; IMT, intima-media thickness; OR, odds ratio; sCa, serum calcium; sIP, serum inorganic phosphorus; sK, serum potassium; sMg, serum magnesium level; sNa, serum sodium.
**P* vs. higher sMg and lower BP group by Bonferroni method in analysis of covariance.

sMg and carotid artery alteration

Lower sMg was significantly associated with higher mean IMT (adjusted mean IMT, mm (95% confidence interval)

Table 4 | Adjusted ORs and 95% CI for plaques ≥2 associated with the combination of sMg level and ABP level

BP criteria		sMg	*N*	OR (95% CI)	*P**
24-h	<130/80	Higher	254	1	(Ref.)
		Lower	199	2.14 (1.18–3.85)	0.01
	≥130/80	Higher	155	1.93 (1.02–3.64)	0.04
		Lower	120	2.36 (1.22–4.56)	0.01
Daytime	<140/85	Higher	281	1	(Ref.)
		Lower	218	1.99 (1.16–3.42)	0.01
	≥140/85	Higher	128	1.66 (0.87–3.18)	0.1
		Lower	101	1.93 (1.00–3.74)	0.05
Nighttime	<120/70	Higher	253	1	(Ref.)
		Lower	188	2.07 (1.15–3.75)	0.02
	≥120/70	Higher	156	1.73 (0.92–3.25)	0.09
		Lower	131	2.16 (1.14–4.10)	0.02
Casual	<140/90	Higher	138	1	(Ref.)
		Lower	149	2.56 (1.18–5.54)	0.02
	≥140/90	Higher	271	1.96 (0.95–4.03)	0.07
		Lower	170	2.69 (1.28–5.65)	0.009

ORs for presence of plaques were adjusted for age, sex, BMI, smoking, drinking, antihypertensive medications, cardiovascular diseases, hypercholesterolemia, diabetes mellitus, Ccr, sNa, sK, sCa, and sIP; Higher sMg was defined ≥2.2 mg/dl (median).
ABP, ambulatory blood pressure; BMI, body mass index; BP, blood pressure; Ccr, creatinine clearance; CI, confidence interval; IMT, intima-media thickness; OR, odds ratio; sCa, serum calcium; sIP, serum inorganic phosphorus; sK, serum potassium; sMg, serum magnesium level; sNa, serum sodium.
*P vs. higher sMg and lower BP group.

among quartiles of sMg = 0.75 (0.73–0.77), 0.75 (0.73–0.77), 0.72 (0.70–0.74), and 0.71 (0.70–0.73); $P = 0.004$) and OR for ≥2 plaques (OR (95% confidence interval) among quartiles of sMg = 1.83 (1.03–3.26), 1.88 (1.03–3.43), 1.39 (0.75–2.59), and 1 (reference); P for trend = 0.03) after adjustment for possible confounding factors. sMg was not associated with OR for ≥1 plaques (data not shown) (**Table 2**). Interactions of sex (male vs. female), age (≤65 vs. ≥65 years), and use of antihypertensive medications (treated vs. untreated) with these results were not observed; interaction P (mean IMT and ≥2 plaques, respectively) = 0.09 and 0.3 for sex, 0.97 and 0.3 for age, and 0.3 and 0.2 for use of antihypertensive medication.

Association of 24-h ABP and sMg with risk of carotid artery alteration

sMg was dichotomized by the median value (2.2 mg/dl) because almost the same values for the adjusted mean IMT or OR for ≥2 carotid plaques were observed between the first and second, and the third and fourth quartiles of sMg (**Table 2**). Lower sMg (≤2.2 mg/dl) levels and higher ABP levels (≥130/80 mm Hg for 24-h, ≥140/85 mm Hg for daytime, and ≥120/70 mm Hg for nighttime)[20] were independently associated with adjusted mean IMT (**Figure 1a** and **Table 3**) and OR for ≥2 carotid plaques (**Figure 1b** and **Table 4**), whereas not with OR for ≥1 carotid plaques (data not shown). Even when ABP was within normal range, lower sMg was significantly associated with adjusted mean IMT and OR for ≥2 carotid plaques. No significant interactions were identified between sMg and 24-h ABP on mean IMT ($P = 0.96$) and OR for ≥2 carotid plaques ($P = 0.4$).

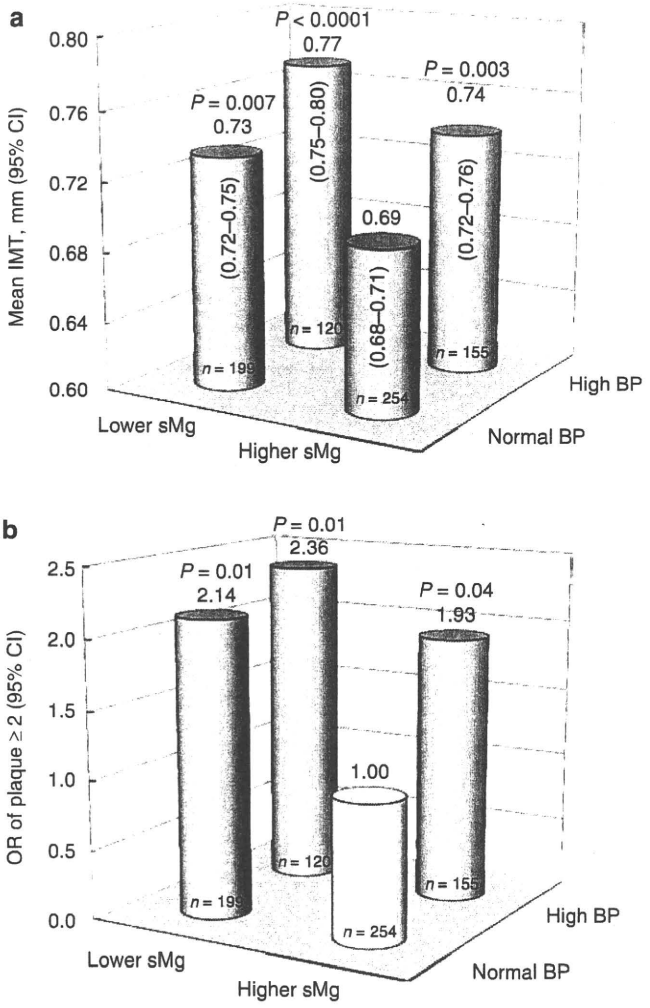

Figure 1 | Association with IMT and a combination of sMg level and 24-h ABP level. (**a**) Adjusted mean IMT. (**b**) Adjusted ORs and 95% CI for ≥2 plaques. All models were adjusted for age, sex, BMI, smoking, drinking, antihypertensive medications, cardiovascular diseases, hypercholesterolemia, diabetes mellitus, Ccr, sNa, sK, sCa, and sIP. Higher sMg was defined as ≥2.2 mg/dl (median); normal BP was defined as <130/80 mm Hg; *P* vs. higher sMg and normal BP group by Bonferroni method in the analysis of covariance or by multiple logistic regression analysis. ABP, ambulatory blood pressure; BMI, body mass index; BP, blood pressure; Ccr, creatinine clearance; CI, confidence interval; IMT, intima-media thickness; OR, odds ratio; sCa, serum calcium; sIP, serum inorganic phosphorus; sK, serum potassium; sMg, serum magnesium level; sNa, serum sodium.

sMg, 24-h ABP, and carotid artery alteration in untreated subjects

To exclude the influence of antihypertensive medications, subanalyses in untreated subjects ($n = 436$) were performed. Similar associations as seen in the main analyses were observed (data not shown).

DISCUSSION

The present cross-sectional study showed that lower sMg levels were significantly associated with risks of carotid artery

alteration, including mean IMT and ≥2 plaques after adjusting for possible confounding factors in a Japanese general population. A previous study reported that sMg was inversely associated with age-adjusted carotid wall thickness for rural Americans.[3] Moreover, to the best of our knowledge, the present study is the first to demonstrate that, irrespective of ABP levels, lower sMg was significantly and independently associated with carotid artery alteration. These results suggest that, in general populations, sMg can play a crucial role as a marker of atherosclerosis and arteriosclerosis.

Clinical investigations have revealed that in patients with severe complications such as after coronary artery bypass graft[25] and advanced atherosclerosis (symptomatic peripheral artery disease),[26] low sMg levels indicate an increased risk of major cardiac (Q-wave infarction and all-cause mortality) and neurological (ischemic stroke and carotid revascularization) events. Furthermore, in patients with type 1 diabetes mellitus from childhood to adolescence, low sMg levels have been associated with early atherosclerosis, including higher mean IMT levels.[27] These results show that Mg depletion is more closely linked to the pathogenesis of atherosclerosis in subjects with cardiovascular complications.

In the present study, sMg was positively and significantly associated with CBP levels even after adjusting for confounding factors. To examine the generalizability of our study findings, we compared our findings with those from studies of other ethnicities. In the Atherosclerosis Risk in Communities (ARIC) study, in which almost all subjects were Caucasian and African American (45–64 years old), sMg was inversely and significantly associated with systolic CBP.[3] On the other hand, in a Canadian population (19–62 years old), no association between sMg and CBP was observed.[28] Thus, it is difficult to determine whether sMg was independently associated with CBP, or whether unknown confounding factors contributed to this relationship. Therefore, in this study, we focused on the association of sMg with ABP, which has stronger predictive power for cardiovascular risks than CBP.[12,29–31]

The mechanisms by which low sMg contributes to carotid artery alteration are hypothesized to be as follows. Low extracellular Mg levels have been shown to accelerate atherosclerosis by promoting the elevation of inflammatory cytokines, lipid oxidation, and the inhibition of endothelial cell growth.[32,33] In rodents, ionized Mg has been reported as an essential cofactor in enzymatic functions for DNA repair.[34]

Similar results indicating that lower sMg levels were associated with higher mean IMT were observed in both of the ARIC study[3] and our study. Subjects in the ARIC study were mostly Caucasian or African American, and all subjects in our study were Japanese. Therefore, it is suggested that the association between lower sMg levels and higher IMT could be, in part, generalized from the results of these two epidemiological studies. On the other hand, there are no other investigations that examine the association between sMg levels and carotid plaques. Therefore, further investigations on other ethnic and cultural populations are needed to confirm the generalizability of our study findings to other populations.

The validity of the relationships between the quartiles of sMg and demographic characteristics such as diabetes, BMI, and lipid profiles is uncertain (**Table 1**). Findings in which sMg levels were not associated with BMI ($P = 0.5$) and the presence of diabetes ($P = 0.9$) should be examined in Asian populations. The association between lower sMg levels and a higher prevalence of diabetes was significant in both the ARIC study[35] and a epidemiological study including African American and Hispanic subjects,[36] whereas a significant association between lower sMg and higher BMI was observed only in the ARIC study.[35] The associations with lipid profiles (total, high-density lipoprotein, low-density lipoprotein) of the present study were similar with results of the ARIC study[35] and a cross-sectional study including Canadian subjects.[28] Therefore, the positive associations between sMg levels and lipid profiles might be, in part, generalized from these results.

The present study has several limitations. First, the influence of antihypertensive medications should be considered, particularly diuretics, which may increase urinary Mg excretion. In the present study, only 10 subjects used diuretics, and the use of antihypertensive medications did not interact with the association between sMg and carotid artery alteration (P for interaction ≥0.2). Furthermore, in subanalyses for untreated subjects ($n = 436$), similar significant associations were observed. Thus, it is suggested that lower sMg levels might contribute to carotid artery alteration without confounding by antihypertensive medications such as diuretics. Second, the possibility of selection bias should be considered when generalizing the present findings, as only 30% of those eligible to participate in the study agreed to do so. However, this may not have caused much selection bias, as the IMT values obtained in our study did not differ from those obtained from other large population studies in which IMT was measured in areas free of plaque.[11,16]

In conclusion, in this older and mostly female Japanese population, it was demonstrated that higher ABP levels and lower sMg were closely and independently associated with risk of carotid artery alteration, including mean IMT and carotid plaque, suggesting that a low sMg level represents an independent risk factor or predictor of carotid artery alteration. Further investigations in subjects with different demographic backgrounds are needed to examine the relationship between sMg levels and the incidence of cardiovascular disease.

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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 mmoll^{-1} were considered to have hypercholesterolemia. Subjects with a fasting glucose level of 7.0 mmoll^{-1} or a nonfasting glucose level of 11.1 mmoll^{-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).[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.

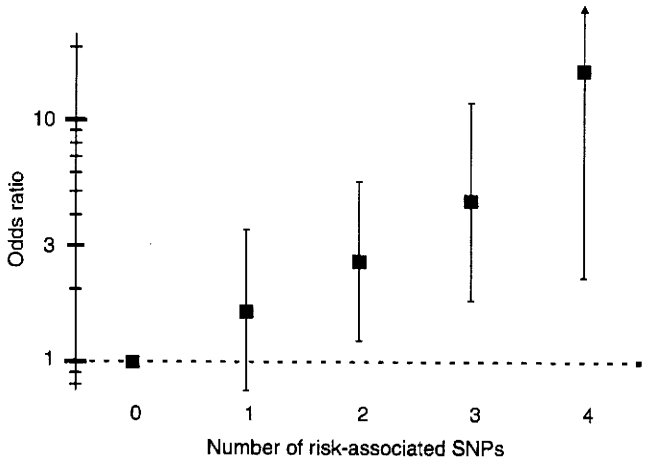

Figure 1 Cumulative effects of risk-associated SNPs on the risk of developing hypertension. Odds ratios and 95% confidence intervals for the risk of development of hypertension among the five groups who were defined according to the number of risk-associated SNPs, and adjusted for baseline BP, age, sex, 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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ORIGINAL ARTICLE

Relationship of dysregulation of glucose metabolism with white-coat hypertension: the Ohasama study

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Characteristics of glucose metabolism in subjects with white-coat hypertension (WCHT) have not been fully investigated. The purpose of this study was to determine the relationship between glucose metabolism and WCHT on the basis of blood pressure (BP) at home (HBP) in the general population. Participants were from Ohasama, a rural Japanese community, and included 466 residents (mean age, 61.0 years) who had no history of diabetes mellitus. HBP and oral glucose tolerance test values were measured. Participants were classified into four groups on the basis of their HBP and casual-screening BP (CBP) values: normotension (NT) (HBP < 135/85 mm Hg, CBP < 140/90 mm Hg); WCHT (HBP < 135/85 mm Hg, CBP ⩾ 140/90 mm Hg); masked hypertension (HBP ⩾ 135/85 mm Hg, CBP < 140/90 mm Hg); or sustained hypertension (SHT) (HBP ⩾ 135/85 mm Hg, CBP ⩾ 140/90 mm Hg). The relationships between glucose metabolism and BP among the four groups were examined using multivariate analysis adjusted for possible confounding factors. Factors in relation to glucose metabolism, such as fasting glucose level, 2-h postchallenge glucose level and homeostasis model assessment-insulin resistance index, were significantly higher in subjects with WCHT and SHT than in those with NT (all $P<0.03$). When men and women were analyzed separately, these relationships were more pronounced in women. Our results suggest that dysregulation of glucose metabolism in WCHT might contribute to the increase in the long-term cardiovascular risk among the general population.
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Keywords: general population; glucose metabolism; home blood pressure; oral glucose tolerance test; white-coat hypertension

INTRODUCTION

Self-measurement of blood pressure (BP) at home (HBP) has been recognized as a useful tool for accurate diagnosis and treatment of hypertension. Previous reports have indicated that HBP is correlated with target-organ damage, and predict the prognosis of hypertension better than casual-screening BP (CBP).[1,2]

The measurement of BP outside medical settings has identified a subgroup of individuals with white-coat hypertension (WCHT)[3] who have persistently elevated CBP but normal HBP or ambulatory BP (ABP) levels, and a subgroup of individuals with masked hypertension (MHT)[4] who have normal CBP but elevated HBP or ambulatory BP levels. The clinical significance of WCHT in relation to cardiovascular disease risk is controversial.[5,6] Similarly, there is little conclusive evidence about the association between WCHT and metabolic abnormalities.[7]

Oral glucose tolerance test (OGTT) is widely used for diagnosing diabetes mellitus (DM). Fasting glucose level is insufficient to diagnose DM; however, measuring glucose level 2-h after an oral glucose load has strong predictive power for cardiovascular disease.[8–10] Although several studies have shown the association between OGTT and CBP,[11,12] the association between OGTT and HBP remains unclear. Moreover, the relationship of WCHT and MHT with glucose metabolism is undetermined. Therefore, the aim of this study was to determine the relationship between glucose metabolism and WCHT, as well as MHT on the basis of HBP in the general population.

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METHODS

Study population

This investigation is a part of a longitudinal observational study of HBP measurements among Ohasama residents that started in 1987. The socio-economic and demographic characteristics of this region and full details of the project have been described elsewhere.[13] Between 2000 and 2008, we contacted all 4809 individuals aged ⩾35 years in four districts of Ohasama town. Those who were not at home during the normal working hours of the study nurses (n=1298) and those hospitalized (n=192) or incapacitated (n=120) were not eligible. Of the remaining 3199 residents, 2181 (68%) gave written, informed consent to participate in the HBP measurement program. Of those, 700 individuals (19%) voluntarily participated in the OGTT. We excluded those treated with antidiabetic (n=11) and antihypertensive agents (n=223) from this analysis. The total number of participants statistically analyzed was thus 466. The study protocol was approved by the Institutional Review Board of Tohoku University School of Medicine, Sendai, Japan, and by the Department of Health of the Ohasama Town Government.

BP measurement

HBP was measured using the semi-automatic HEM-747IC-N or HEM701C (Omron Healthcare, Kyoto, Japan), a device based on the cuff-oscillometric method that generates a digital display of both systolic and diastolic BP values.[14] Physicians and public health nurses instructed the participants on how to use the device and record HBP results. The participants then measured their own BP once in the morning, in the sitting position within 1 h after awaking and after 2 min of rest and recorded such measurements for 4 weeks. Although many participants measured their HBP values twice or more per occasion, we used the first value from each measurement in our analysis to exclude individual selection bias.[15] HBP was defined as the mean of all measurements. The mean number of total HBP measurements was 24. CBP measurements were taken after at least 2 min of rest, twice consecutively, using an automatic device (HEM-907, Omron Healthcare) before OGTT. The average of two consecutive readings from each individual was used as CBP. The HBP and CBP measuring devices used in this study have been validated[14,16,17] and meet the criteria established by the Association for the Advancement of Medical Instrumentation.[18]

OGTT and other information

OGTT was carried out using a 75-g glucose-equivalent carbohydrate load (Trelan G; Ajinomoto Pharma, Tokyo, Japan) after the fasting blood samples were collected. Blood samples were drawn at 60 min (1 h) and 120 min (2 h), and glucose levels and insulin were measured. Information on the use of antihypertensive, hyperlipidemic and diabetic medications at baseline was obtained from interviews conducted at the time of OGTT, from records of annual health checkups and from records of Ohasama Hospital. Serum adiponectin was measured using a latex particle-enhanced turbidimetric immunoassay (SRL, Tokyo, Japan).

Classification of groups

Participants were classified into four groups (normotension (NT), WCHT, MHT and sustained hypertension (SHT)) on the basis of their HBP and CBP levels: NT, with systolic CBP < 140 mm Hg and diastolic CBP < 90 mm Hg, and systolic HBP < 135 mm Hg and diastolic HBP < 85 mm Hg; WCHT, with systolic CBP ⩾ 140 mm Hg or diastolic CBP ⩾ 90 mm Hg or both, and systolic HBP < 135 mm Hg and diastolic HBP < 85 mm Hg; MHT, with systolic CBP < 140 mm Hg and diastolic CBP < 90 mm Hg, and systolic HBP ⩾ 135 mm Hg or diastolic HBP ⩾ 85 mm Hg or both; and SHT, with systolic CBP ⩾ 140 mm Hg or diastolic CBP ⩾ 90 mm Hg or both, and systolic HBP ⩾ 135 mm Hg or diastolic HBP ⩾ 85 mm Hg or both (Figure 1). Cutoff values were derived from several guidelines.[19–21]

On the basis of OGTT, subjects were classified as having DM, impaired glucose intolerance, impaired fasting glucose or normal glucose tolerance according to the World Health Organization classification[22] (Figure 2).

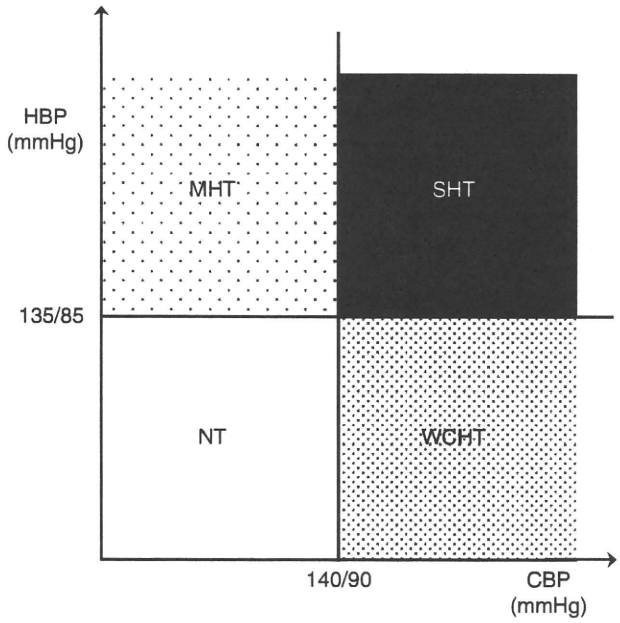

Figure 1 Distribution of subjects classified into four groups on the basis of HBP and CBP levels. CBP, casual-screening blood pressure; HBP, home blood pressure; MHT, masked hypertension; NT, normotension; SHT, sustained hypertension; WCHT, white-coat hypertension.

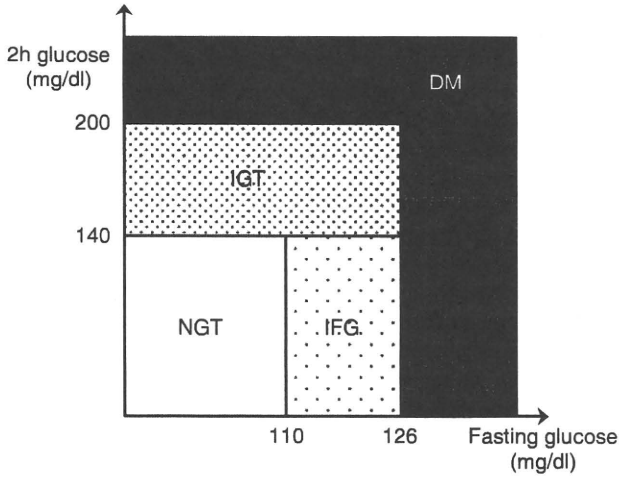

Figure 2 Distribution of subjects classified into four groups on the basis of fasting glucose and 2-h glucose level, which were determined by OGTT. DM, diabetes mellitus; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; NGT, normal glucose tolerance.

Data analysis

Dyslipidemia was defined in accordance with criteria of the Japanese metabolic syndrome[23] as low high-density lipoprotein-cholesterol (<40 mg per 100 ml (1.03 mmol l^{-1})), high triglyceride (⩾150 mg per 100 ml (1.68 mmol l^{-1})) and/or the use of antilipidemic treatment. Area under the blood concentration time curve was calculated using fasting plasma glucose, 1-h glucose and 2-h glucose by quadrature by parts (area under the blood concentration time curve=(fasting plasma glucose+1-h glucose)×0.5+(1-h glucose+2-h glucose)×0.5). The homeostasis model assessment-insulin resistance index (HOMA-IR) was calculated using the following formula: HOMA-IR=fasting glucose (mg per 100 ml)×fasting insulin (μUnits per ml)/405. Insulin sensitivity was determined by the Matsuda DeFronzo index based on the following formula: 10 000/sqrt (fasting glucose (mg per 100 ml)×fasting insulin (μUnits per ml)) (mean glucose (mg per 100 ml)×mean insulin (μUnits per ml)).[24]

All data are expressed as means ± s.d. Variables were compared using Fisher's exact test, ANOVA (analysis of variance) or ANCOVA (analysis of covariance), followed by Tukey's multiple comparison test. Associations between indices for glucose metabolism and BPs as continuous variables were examined with multiple regression analysis adjusted by age, body mass index, dyslipidemia, history of cardiovascular disease, drinking habit and smoking habit. Statistical significance was established at $P<0.05$. All statistical calculations were carried out using the SAS system (version 9.1, SAS Institute, Cary, NC, USA).

RESULTS

The characteristics of the study participants are given in Table 1. The mean age was 61.0 ± 9.6 years and the proportion of men and women was 29:71. Mean systolic/diastolic CBP and HBP values were 131.3 ± 18.3/76.1 ± 11.2 mm Hg and 122.3 ± 15.0/74.5 ± 9.0 mm Hg, respectively. Of the 466 subjects, 268 were classified as having NT, 49 were classified as having MHT, 90 were classified as having WCHT and the remaining 59 were classified as having SHT. Both CBP and HBP values in the NT group were significantly lower those in than the other groups. Subjects in the NT group tended to be younger than those in the other categories of BP classification.

The relationships between glucose metabolism and each BP group were analyzed using ANCOVA (Table 2). Among subjects with WCHT and SHT, significantly higher glucose levels and HOMA-IR values and significantly lower Matsuda DeFronzo index values were observed when compared with NT (all $P<0.03$). Among those with MHT, there were no indices for glucose metabolism, which showed significant

Table 1 Characteristics of study participants

	All subjects	*Subjects with NT*	*Subjects with MHT*	*Subjects with WCHT*	*Subjects with SHT*	*ANOVA P-value*
Number of subjects (*n*)	466	268	49	90	59	—
Gender (women, %)	71.0	77.2	49.0	76.7	52.5	<0.0001
Age (years)	61.0 ± 9.6	59.8 ± 9.7	63.4 ± 9.7	62.4 ± 8.7	62.4 ± 10.0	0.02
Body mass index ($kg\,m^{-2}$)	23.3 ± 3.2	22.7 ± 3.0	24.0 ± 3.6*	23.5 ± 2.8	24.9 ± 3.6*,‡	<0.0001
Height (cm)	55.6 ± 10.3	54.1 ± 9.2	59.6 ± 13.9	54.6 ± 8.7†	60.2 ± 11.6	0.02
Weight (kg)	154.2 ± 8.5	154.1 ± 8.1	156.8 ± 10.4*	152.3 ± 7.9†	155.2 ± 8.8*,‡	<0.0001
Systolic HBP (mm Hg)	122.3 ± 15.0	114.4 ± 10.7	139.5 ± 8.9*	122.5 ± 8.5*,†	143.4 ± 10.3*,‡	<0.0001
Diastolic HBP (mm Hg)	74.5 ± 9.0	70.3 ± 6.9	84.6 ± 6.8*	74.3 ± 5.7*,†	85.4 ± 7.1*,‡	<0.0001
Home heart rate (b.p.m.)	65.2 ± 7.7	64.6 ± 7.1	66.9 ± 8.1	66.1 ± 7.6	64.9 ± 9.5	0.2
Systolic CBP (mm Hg)	131.3 ± 18.3	120.8 ± 12.2	127.1 ± 9.2*	149.4 ± 9.1*,†	154.5 ± 14.4*,†,‡	<0.0001
Diastolic CBP (mm Hg)	76.1 ± 11.2	70.6 ± 8.5	75.5 ± 7.8*	85.2 ± 9.3*,†	87.7 ± 9.5*,†	<0.0001
HDL (mg per 100 ml)	62.3 ± 15.4	63.0 ± 16.0	59.2 ± 14.8	62.5 ± 14.2	61.6 ± 14.8	0.4
Triglyceride (mg per 100 ml)	100.3 ± 62.5	89.8 ± 52.7	115.6 ± 63.5*	109.1 ± 74.4	122.1 ± 73.5*	0.0002
Drinking habit (%)	41.9	40.7	59.2	26.7	55.9	0.0002
Smoking habit (%)	13.7	12.3	30.6	7.8	15.3	0.004
Dyslipidemia (%)	19.1	15.3	28.6	18.9	28.8	0.03
[a]IFG(%)	5.4	2.2	6.1	11.1	10.2	0.002
[a]IGT(%)	20.0	17.2	18.4	24.4	27.1	0.2
[a]Diabetes mellitus (%)	6.9	3.7	6.1	8.9	18.6	0.001
Past history of CVD (%)	2.8	1.9	8.2	1.1	5.1	0.04

Abbreviations: ANOVA, analysis of variance; CBP, casual-screening blood pressure; CVD, cardiovascular disease; HBP, home blood pressure; HDL, high-density lipoprotein; IFG, impaired fasting glucose; IGT, impaired glucose intolerance; MHT, masked hypertension; NT, normotension; SHT, sustained hypertension; WCHT, white-coat hypertension.
*$P<0.05$ compared with NT.
†$P<0.05$ compared with MHT.
‡$P<0.05$ compared with WCHT.
[a]IFG, IGT and diabetes mellitus were defined by the oral glucose tolerance test.

Table 2 Variables in relation to glucose metabolism

	All subjects	*Subjects with NT*	*Subjects with MHT*	*Subjects with WCHT*	*Subjects with SHT*	*ANCOVA P-value*
Number of subjects (*n*)	466	268	49	90	59	—
Fasting plasma glucose (mg per 100 ml)	95.1 ± 10.9	93.0 ± 9.7	95.1 ± 10.3	98.7 ± 12.3*	99.1 ± 11.9*	0.0003
One-hour glucose (mg per 100 ml)	157.1 ± 52.6	148.0 ± 51.0	156.2 ± 44.1	171.4 ± 49.1*	177.7 ± 61.4*	0.001
Two-hour glucose (mg per 100 ml)	126.7 ± 43.1	119.7 ± 37.6	121.6 ± 39.3	136.8 ± 50.1*	146.9 ± 49.4*,†	0.0007
Glucose AUC_{0-120} (mg per 100 ml h)	268.0 ± 71.5	254.3 ± 67.1	264.6 ± 60.3	289.2 ± 71.0*	300.7 ± 82.6*,†	0.0002
Δ60 glucose (mg per 100 ml h)	62.0 ± 48.0	55.0 ± 47.2	61.0 ± 39.3	72.7 ± 44.6*	78.6 ± 56.6*	0.01
Δ120 glucose (mg per 100 ml h)	31.6 ± 39.3	26.7 ± 34.5	26.5 ± 36.8	38.1 ± 45.1	47.9 ± 46.7*,†	0.008
HOMA	1.32 ± 0.86	1.20 ± 0.71	1.33 ± 1.09	1.47 ± 0.81*	1.66 ± 1.15*	0.03
MDI	8.89 ± 4.54	9.68 ± 4.87	9.53 ± 4.78	7.25 ± 3.13*,†	7.26 ± 3.54*,†	0.0009

Abbreviations: ANCOVA, analysis of covariance; AUC_{0-120}, area under the blood concentration time curve; HOMA, homeostasis model assessment; MDI, Matsuda DeFronzo index; MHT, masked hypertension; NT, normotension; SHT, sustained hypertension; WCHT, white-coat hypertension.
Adjusted for sex, age, body mass index, dyslipidemia, history of cardiovascular disease, drinking habit and smoking habit.
Δ60 glucose=1-h glucose—fasting plasma glucose; Δ120 glucose=2-h glucose—fasting plasma glucose.
*$P<0.05$ compared with NT.
†$P<0.05$ compared with MHT.

Table 3 Variables in relation to glucose metabolism according to sex

	All subjects	*Subjects with NT*	*Subjects with MHT*	*Subjects with WCHT*	*Subjects with SHT*	*ANCOVA P-value*
Men						
Number of subjects (*n*)	135	61	25	21	28	
Age (years)	61.2±9.1	61.0±9.4	59.2±8.7	63.8±6.2	61.5±10.6	0.4
Fasting plasma glucose (mg per 100 ml)	97.4±10.9	96.1±10.4	96.2±12.4	98.5±8.4	100.5±11.9	0.6
One-hour glucose (mg per 100 ml)	168.5±58.5	164.7±61.5	164.5±48.5	165.2±52.3	182.6±64.9	0.7
Two-hour glucose (mg per 100 ml)	129.0±47.4	124.9±46.8	130.2±44.4	124.6±39.4	140.1±56.4	0.7
Glucose AUC_{0-120} (mg per 100 ml h)	281.7±78.2	275.2±80.8	277.7±70.0	276.8±65.1	302.9±88.0	0.7
Δ60 glucose (mg per 100 ml h)	71.1±54.2	68.7±57.9	68.2±42.3	66.7±49.2	82.0±59.9	0.8
Δ120 glucose (mg per 100 ml h)	31.6±43.6	28.9±42.2	34.0±40.8	26.1±39.4	39.6±52.1	0.8
HOMA	1.31±0.96	1.15±0.62	1.33±1.15	1.29±0.81	1.64±1.37	0.3
MDI	9.76±5.29	11.0±6.11	10.1±5.05	8.51±3.83	7.75±3.62*	0.03
Women						
Number of subjects (*n*)	331	207	24	69	31	
Age (years)	61.0±9.8	59.5±9.8	67.7±8.8*	62.0±9.3	63.3±9.5	0.0003
Fasting plasma glucose (mg per 100 ml)	94.2±10.7	92.1±9.3	94.0±7.6	98.7±13.3*	97.7±12.0*	0.0005
One-hour glucose (mg per 100 ml)	152.5±49.3	143.0±46.5	147.5±38.1	173.3±48.3*	173.2±58.7*	0.0006
Two-hour glucose (mg per 100 ml)	125.7±41.3	118.2±34.4	112.7±31.6	140.5±52.6*,†	153.1±42.1*,†	<0.0001
Glucose AUC_{0-120} (mg per 100 ml h)	262.4±67.9	248.2±61.4	250.8±45.8	292.9±72.7*	298.7±78.8*	<0.0001
Δ60 glucose (mg per 100 ml h)	58.3±44.8	50.9±42.9	53.5±35.2	74.6±43.4*	75.5±54.2*	0.005
Δ120 glucose (mg per 100 ml h)	31.5±37.5	26.1±32.0	18.7±30.9	41.8±46.3*,†	55.4±40.6*,†	0.0002
HOMA	1.33±0.81	1.21±0.74	1.33±1.04	1.52±0.82*	1.67±0.92*	0.2
MDI	8.53±4.16	9.29±4.38	8.98±4.52	6.86±2.80*	6.83±3.46*	0.3

Abbreviations: ANCOVA, analysis of covariance; AUC_{0-120}, area under the blood concentration time curve; HOMA, homeostasis model assessment; MDI, Matsuda DeFronzo index; MHT, masked hypertension; NT, normotension; SHT, sustained hypertension; WCHT, white-coat hypertension.
Adjusted for age, body mass index, dyslipidemia, history of cardiovascular disease, drinking habit and smoking habit.
Δ60 glucose=1-h glucose—fasting plasma glucose; Δ120 glucose=2-h glucose—fasting plasma glucose.
* $P<0.05$ compared with NT.
† $P<0.05$ compared with MHT.

differences from those in subjects with NT. Similarly, no significant difference was observed between subjects with WCHT and those with SHT. Further analysis in subjects in which serum adiponectin levels were measured ($n=167$) showed that significantly lower adiponectin levels were observed in subjects with WCHT (10.5 ± 6.0) when compared with those with NT (14.7 ± 6.7) ($P=0.044$). Similar trends were observed only in women (data not shown).

The results in which men and women were analyzed separately are shown in Table 3. There were no significant differences in fasting glucose levels, 2-h glucose levels, HOMA-IR and the Matsuda DeFronzo index between MHT and NT regardless of sex. For women with WCHT and SHT, glucose levels were significantly higher than those with NT. Meanwhile, no significant differences of glucose levels among BP groups were observed in men. HOMA-IR in women was significantly higher in individuals with WCHT and SHT (1.5 ± 0.8 and 1.7 ± 0.9, respectively) than in those with NT (1.2 ± 0.7), whereas HOMA-IR in men did not differ among the four BP groups. Similar results were observed with regard to the Matsuda DeFronzo index, although the Matsuda DeFronzo index in men was significantly higher in individuals with SHT (7.7 ± 3.6) than in those with NT (11.0 ± 6.1). However, there was no significant interaction between BP groups and sex in relation to glucose levels, HOMA-IR and the Matsuda DeFronzo index (all $P>0.2$).

The results of multiple regression analysis indicated that CBP values were significantly associated with several indices for glucose metabolism even adjusted by confounding factors. When systolic HBP and systolic CBP values were simultaneously included in the model (Table 4), systolic CBP, but not systolic HBP, was significantly associated with indices for glucose metabolism, especially with fasting plasma glucose ($P=0.14$ for systolic HBP, $P<0.0001$ for systolic CBP).

DISCUSSION

In this study, glucose levels in subjects with WCHT and SHT were significantly higher than those with NT. In a previous study, young subjects with WCHT tended to have metabolism dysregulation.[25] In a population-based study, individuals with WCHT showed impaired insulin sensitivity compared with normotensive subjects in their late middle age.[26] Sympathetic nervous system activity has been associated with the development of WCHT and with insulin resistance.[27,28] Furthermore, CBP values were reported to be positively correlated with HOMA-IR.[29] Central sympathetic hyperactivity has also reported to exist in WCHT in the clinical setting.[27] Although we have not investigated sympathetic nerve activities in this study, the strong relationship between CBP and glucose metabolisms would support the existence of sympathetic nervous system hyperactivity in individuals with WCHT and SHT.

In this study, significant correlations between glucose dysregulation and WCHT were not observed in men. Sympathetic nerve activity would differ between men and women with WCHT. However, to our knowledge, there was no previous study about the sympathetic nerve system for difference between men and women with WCHT. Thus we cannot explain this difference between men and women from the viewpoint of the sympathetic nerve system. Other factors would contribute to hyperglycemia in individuals with WCHT, whereas the result might be just by chance because of a small number of participants. The difference between men and women should be investigated on the basis of a large population.

Table 4 Independent relations between indices for glucose metabolism and BP as determined by multiple regression analysis

	HBP		*CBP*		*Sex*		*Age*		*BMI*		*Dyslipidemia*		*Drinking habit*		*Smoking habit*		
	β	*s.e.*	*β*	*s.e.*	*β*	*s.e.*	*β*	*s.e.*	*β*	*s.e.*	*β*	*s.e.*	*β*	*s.e.*	*β*	*s.e.*	R^2
Fasting plasma glucose (mg per 100 ml)	−0.060	0.040	0.168	0.031†	2.180	1.364	0.116	0.06*	0.385	0.164*	1.406	1.274	−1.150	1.157*	−0.170	1.651	0.1227
One-hour glucose (mg per 100 ml)	0.094	0.198	0.416	0.152*	12.630	6.696	0.830	0.27†	0.880	0.806	8.043	6.254	4.134	5.682	−6.550	8.105	0.0962
Two-hour glucose (mg per 100 ml)	0.191	0.163	0.346	0.125*	4.129	5.513	0.348	0.22	1.533	0.664*	6.960	5.149	4.184	4.678	5.757	6.673	0.0899
Glucose AUC $_{0-120}$ (mg per 100 ml h)	0.160	0.267	0.673	0.205†	15.790	9.029	1.062	0.37†	1.839	1.087	12.230	8.432	5.650	7.661	−3.760	10.930	0.1112
Δ60 glucose (mg per 100 ml h)	0.153	0.183	0.248	0.140	10.460	6.187	0.714	0.25†	0.495	0.745	6.637	5.779	5.286	5.250	−6.380	7.489	0.0749
Δ120 glucose (mg per 100 ml h)	0.251	0.150	0.178	0.116	1.949	5.093	0.232	0.21	1.149	0.613	5.554	4.756	5.336	4.321	5.929	6.164	0.0640
HOMA	0.002	0.003	0.004	0.002	−0.030	0.103	−0.010	0†	0.090	0.012†	0.226	0.096*	0.146	0.087	−0.050	0.125	0.1949
MDI	−0.010	0.016	−0.040	0.012†	0.332	0.529	0.019	0.02	−0.470	0.064‡	−1.440	0.494†	−1.570	0.449†	−1.330	0.640*	0.2459
[a]Adiponectin (μg ml^{-1})	−0.030	0.040	0.026	0.028	−4.730	1.239†	0.221	0.05‡	−0.740	0.146‡	−1.000	1.151	−1.030	1.025	−0.300	1.528	0.3758

Abbreviations: AUC_{0-120}, area under the blood concentration time curve; BMI, body mass index; BP, blood pressure; CBP, casual-screening blood pressure; HBP, home blood pressure; HOMA, homeostasis model assessment; MDI, Matsuda DeFronzo index.
*$P<0.05$, †$P<0.005$, ‡$P<0.0001$.
Δ60 glucose=1-h glucose—fasting plasma glucose; Δ120 glucose=2-h glucose—fasting plasma glucose.
[a]The number of subjects who have the data of adiponectin is 167.

The association between WCHT and the risk of cardiovascular disease is inconsistent. Although many reports have shown that the risk of cardiovascular disease in subjects with WCHT was comparable with NT,[6,30] our previous report indicated that WCHT is correlated with high risk for development of SHT and suggested that WCHT would carry a poor cardiovascular prognosis on a long-term basis.[31] The cumulative hazard for stroke in the WCHT group was equal to that of the ambulatory hypertensive group according to the results of a meta-analysis of prospective studies, including the Ohasama study.[32] Thus, dysregulation of glucose metabolism might be associated with WCHT, which is a risk factor for cardiovascular disease in the long term. Diabetic nephropathy and diabetic retinopathy were more progressive in diabetic individuals with WCHT than in those with NT.[33] It would be useful for individuals with WCHT to undergo an OGTT to detect dysregulation of glucose metabolism in the early stages. Furthermore, early detection and prevention for progression from WCHT to SHT should be monitored by consecutive measurements of HBP.

Significantly low adiponectin levels were observed in subjects with WCHT compared with those with NT. The observations also support the involvement of insulin resistance in glucose dysmetabolism. The role of adipocytokine might explain sex differences for glucose metabolism as this tendency was observed especially in women; however, the number of subjects was very small especially when men and women were analyzed separately. The association between adipocytokine or sex difference and glucose metabolism should be investigated with a large number of participants.

No significant difference in indices for glucose metabolism was observed between subjects with WCHT and those with SHT. However, the tendency of low adiponectin levels was observed in subjects with WCHT compared with those with SHT. Although there were small (although not statistically significant) differences in indices for glucose metabolism between WCHT and SHT, significantly low weight and body mass index were observed in subjects with WCHT when compared with those with SHT. Despite this, the level of adiponectin in subjects with WCHT was lower and the level of glucose metabolism dysregulation was comparable when compared with those with SHT. Thus, we believe that WCHT is not comparable with SHT and might not be a safe condition.

There was no specific tendency for glucose metabolism in MHT in this study. In previous studies, fasting glucose levels were reported to be significantly higher in the MHT group than in the NT group, and those in the MHT group were similar to the SHT group.[7,34] These results were inconsistent with our findings that glucose metabolism of subjects with MHT was comparable with those with NT. The most likely explanation is that individuals treated with antihypertensive medication were excluded from this study. Several previous studies in relation to the prognosis of MHT consisted of subjects treated with antihypertensive medication[7,35] or included subjects both with and without antihypertensive medication.[36] Although the high risk of cerebrovascular and cardiovascular disease in subjects with MHT has been established by these previous studies, the risk for individuals with MHT without antihypertensive medication would be a separate concern. Exclusion of subjects taking antihypertensive medication in the current study resulted in an insufficient number of subjects in each BP category and might lead to insufficient statistical power to draw a conclusion. Thus, further research including individuals who use antihypertensive medication would be necessary to clarify the association between MHT and dysregulation of glucose metabolism.

It should also be noted that subjects who were previously diagnosed with DM and those treated with antidiabetic agents did not participate

in this study. Several studies including our previous study have shown that many subjects with MHT had a history of DM[37] or were prescribed antidiabetic treatment.[38,39] In this study, subjects with MHT might have been excessively excluded, and thus glucose levels of the MHT group might be underestimated and create a weak association between MHT and glucose levels. The possibility of selection bias should be considered when generalizing the report findings. Furthermore, the number of subjects with MHT (n=49) in this study was relatively small, which resulted in an insufficient statistical power; the association between MHT and the dysregulation of glucose metabolism remain a matter for debate. It is also well known that patients with MHT have a greater frequency of target-organ damage[34,37] and have a greater risk of cardiovascular disease.[36] Thus, it is important to promote further research with a large number of subjects, including those with DM to confirm the association between dysregulation of glucose metabolism and MHT.

According to the multiple regression model, CBP would be more useful for predicting dysregulation of glucose metabolism or insulin resistance than HBP. In the previous study, it was established that HBP value has a stronger predictive power for target-organ damage, morbidity and mortality than has the CBP value.[1,2] Glucose metabolism is also treated as a risk factor for cardiovascular diseases.[9,10] It seems reasonable to suppose that HBP and glucose metabolism would affect to cardiovascular diseases independently. Further follow-up studies are required to investigate long-term prognosis in terms of comparing BP information and glucose metabolism.

There were several limitations in this study. OGTT data were obtained at only one measurement in one occasion. If we carry out OGTT twice or more, the classification based on OGTT might be changed. We excluded patients with DM or with a history of DM. The study participants might not be the same as the entire population of Ohasama, and study participants' potential awareness of health concerns would be higher than the other residents in the general population. Thus, the possibility of selection bias needs to be considered when generalizing the present findings. Furthermore, this study included a comparably small number of men without data of participants' detailed lifestyle, although sex-specific associations were observed. Women have reported to have a greater tendency to be influenced by the white-coat effect than men,[40,41] and decreased glucose tolerance related to poor lifestyle choices was more common in women than in men.[42] Therefore, further prospective studies based on a sufficient number of subjects with detailed information are required to overcome these limitations.

In conclusion, strong associations between dysregulation of glucose metabolism and WCHT were observed in this study. Our findings suggest that dysregulation of glucose metabolism might contribute to the increase in the long-term risk of poor prognosis for subjects with WCHT. It is useful for individuals with WCHT to undergo OGTT to detect early stages of dysregulation of glucose metabolism. Consecutive measurements of HBP would also be important to detect and to prevent progression from WCHT to SHT.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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