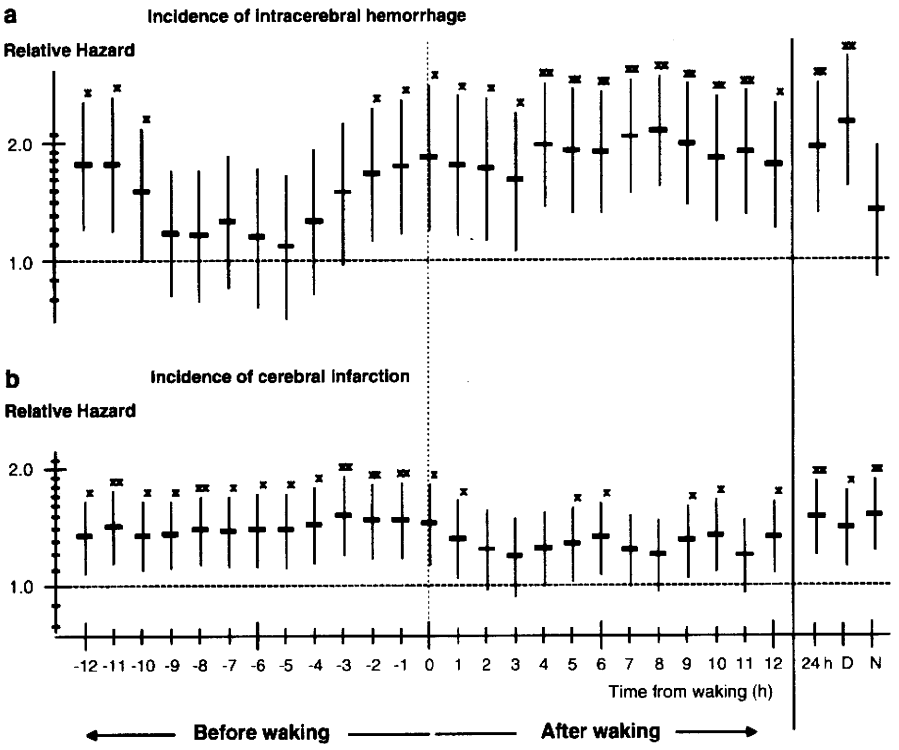

Figure 3 Relative hazard for incidence of stroke subtypes per 1-s.d. elevation of SBP values. Relative hazards and 95% confidence intervals for incidence of (**a**) hemorrhage stroke and (**b**) cerebral infarction per 1-s.d. elevation of SBPs over a mean follow-up of 10.2 years in Ohasama, Japan. Left panel: Numbers indicate 2-h moving averages of SBP over 24-h period. Right panel: 24 h, D and N on the right slide panel indicate 24-h, daytime, and nighttime mean SBP values, respectively. Each analysis was adjusted for age, gender, smoking status, antihypertensive medication, history of heart disease, hypercholesterolemia and diabetes mellitus. $*P<0.05$; $**P<0.002$ (Bonferroni's adjustment).

CONCLUSION

The results of the Ohasama study show that ambulatory BP values are uniquely associated with cardiovascular diseases and their prognosis. Ambulatory BP values provide not only static, but also dynamic information about BP that is applicable to the effective management of hypertension and cardiovascular diseases.

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Association of environmental tobacco smoke exposure with elevated home blood pressure in Japanese women: the Ohasama study

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Objective **Only a few of numerous epidemiological studies have demonstrated a positive association between environmental tobacco smoke (ETS) exposure and blood pressure (BP), despite experimental studies showing such a positive association. The association between home blood pressure (HBP) and ETS exposure was investigated in the general population.**

Methods **Five hundred and seventy-nine nonsmoking Japanese women were enrolled. The participants were classified into four categories according to their responses to a self-administered questionnaire: unexposed women (non-ETS), women exposed at home [ETS(home)], at the workplace/other places [ETS(work/other)] and at home and at the workplace/other places [ETS(both)]. Variables were compared using analysis of covariance adjusted for age, marital status, body mass index, diabetes mellitus, stroke, heart disease, hyperlipidemia, alcohol intake, salt intake and activity levels.**

Results **In participants without antihypertensive medication, systolic morning HBP in ETS(both) was 4 mmHg higher than that in non-ETS (116.8 ± 1.01 vs. 113.1 ± 1.08 mmHg, $P = 0.02$) and systolic morning HBP in ETS(home) and systolic evening HBP in ETS(both) were 3 mmHg higher than those in non-ETS (116.2 ± 1.07 vs. 113.1 ± 1.08 mmHg, $P = 0.04$; and 115.3 ± 1.02 vs. 111.9 ± 1.09 mmHg, $P = 0.03$, respectively). In participants with antihypertensive medication, ETS exposure status was not significantly associated with increased HBP levels.**

Conclusions **A positive association between HBP levels and ETS exposure was confirmed. HBP measurement is recommended in population-based studies investigating the effects of ETS exposure. ETS exposure may increase BP, thereby synergistically contributing to unfavorable cardiovascular outcomes along with other deleterious effects of ETS.** ***J Hypertens*** **28:1814–1820 © 2010 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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Abbreviations: ANCOVA, analysis of covariance; ANOVA, analysis of variance; BMI, body mass index; BP, blood pressure; CBP, casual clinic blood pressure; ETS, environmental tobacco smoke; ETS(both), participants exposed to ETS both at home and at the workplace and/or other places; ETS(everyday), participants exposed to ETS everyday; ETS(home), participants exposed to ETS at home; ETS(occasionally), participants exposed to ETS less frequently than everyday; ETS(work/other), participants exposed to ETS at the workplace and/or other places; HBP, home blood pressure; non-ETS, participants not exposed to ETS

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Introduction

Exposure to environmental tobacco smoke (ETS) is a well known risk factor for morbidity and mortality from cardiovascular diseases such as coronary heart disease [1] and stroke [2–9]. Numerous studies have investigated the pathophysiological changes caused by ETS exposure, and one of the findings of these studies is that ETS causes endothelial dysfunction, such as impaired endothelium-dependent vasodilatation [10–14] and decreased nitric oxide production [15]. Some experimental studies have also shown that blood pressure (BP) is elevated for a short time period [16] or for 24 h after brief ETS exposure [15].

These pathophysiological and hemodynamic findings imply that ETS exposure increases BP in the general population. To the best of our knowledge, however, only a few of the numerous epidemiological studies investigating this relationship have shown a positive association between chronic ETS exposure and BP [17,18]. One possible reason for these inconsistent findings is that

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most results are based on measurement of casual clinic blood pressure (CBP), which is less sensitive in detecting true changes in BP compared to home blood pressure (HBP) measurement [19,20]. HBP is measured by individuals themselves at home with a validated device over a long observation period, providing more reproducible and reliable values with less random error, without observer bias and without the white-coat effect [19,20]. Because of these advantages, HBP values have better predictive power for morbidity and mortality from cardiovascular diseases than CBP values [19–22], and HBP monitoring is now widely recommended in guidelines [19,21] and in a scientific statement [20].

To test the hypothesis that HBP measurements detect differences in BP between individuals exposed and those not exposed to ETS in the general population, the association between HBP values and ETS exposure was examined in a population-based, cross-sectional study.

Methods

Study population

The study was conducted as a part of the Ohasama study, a Japanese community-based, BP measurement project [23,24]. The total population of Ohasama was 7202 in 1998. Of this total population, 4964 were 35 years old or older. Of those, 1410 working outside of the town were considered ineligible and excluded from the study because they were not in the town during normal working hours. Of the remaining 3554 individuals, 213 were also excluded from the study because they were hospitalized, mentally ill, or bedridden. A total of 3341 participants were thus eligible for the study. A questionnaire was sent to each participant, and 1895 of the eligible participants gave their informed consent and responded to the questionnaire. Of those, 585 were excluded from the analysis because they were ex-smokers or current active smokers. Thus, the number of lifelong nonsmokers was 1310. Another 505 individuals with incomplete answers to the questions regarding demographic factors including ETS exposure were also excluded. Of the remaining 805 individuals, 754 who measured their HBP in the morning on at least three occasions (3 days) during the 4-week study period were included. This criterion was based on our previous observation that the average BP on the first three occasions was not significantly different from the mean for the entire study period [23]. Men ($n = 175$) were also excluded from the analysis because their number was small. Therefore, the study included 579 women [54.9% of the total number of lifelong nonsmoking women ($n = 1054$)].

Table 1 compares the characteristics of the included study participants with lifelong nonsmoking women who participated in the study but were ultimately excluded from the analysis due to incomplete data on ETS exposure (nonparticipants). The participants were characterized by a lower mean age, by lower percentages of participants taking antihypertensive medication and having a history of stroke and by higher percentages of participants being married and current drinkers.

Table 1 Characteristics of participants and nonparticipants in lifelong nonsmoking women ($n = 998$)

	Participants	Nonparticipants[a]	*P* value
N	579	419	
Mean age (years)	59.2 ± 13.1	64.1 ± 11.2	<0.0001
Marital status (married %)	71.0	61.3	0.0014
BMI (kg/m^2)	23.7 ± 3.3	23.7 ± 3.4	NS
Antihypertensive medication (%)	18.1	25.1	0.0081
History			
Diabetes mellitus (%)	8.6	9.3	NS
Stroke (%)	1.0	2.9	0.0323
Heart disease (%)	6.0	5.0	NS
Hyperlipidemia (%)	12.1	14.8	NS
Alcohol intake (current drinker %)	24.5	15.0	0.0003
Salt intake (≥12.28 g/day %)	50.1	43.9	NS
Time spent walking (≥1 h/day %)	79.8	81.1	NS

BMI, body mass index; ETS, environmental tobacco smoke. Student's *t*-test for continuous variables and χ^2-test for categorical variables. Continuous variables are expressed as mean ± SD. NS = $P > 0.05$. [a]Lifelong nonsmoking female participants who participated in the study but were ultimately excluded from the analysis due to incomplete data on ETS exposure.

Blood pressure and pulse rate measurement

The procedures used for HBP, pulse rate and CBP measurements, as well as the measuring devices, have been described elsewhere [23,25,26]. Briefly, physicians and public health nurses conducted health education classes to inform the participants about the HBP and pulse rate recording method, to teach them how to measure their own HBP and pulse rate, and to validate their ability to perform these tasks consistently. The women were then asked to measure their HBP and pulse rate every morning and evening and to record the results for 4 weeks. Measurements of morning HBP and pulse rate were made within 1 h of waking, before breakfast or taking any drugs, with the women seated and having rested for at least 2 min [27]. Measurements of evening HBP and pulse rate were obtained in a homologous way just before going to bed. The HBP and pulse rate of an individual were defined as the mean of all measurements obtained from that person. The mean ± SD numbers of morning HBP, morning pulse rate, evening HBP and evening pulse rate measurements were 22.6 ± 6.5 ($n = 579$), 22.4 ± 6.6 ($n = 567$), 22.8 ± 6.5 ($n = 577$) and 22.7 ± 6.6 ($n = 566$), respectively.

Two consecutive measurements of CBP were taken by a nurse or technician after the participant had been seated at rest for at least 2 min [23]. CBP was defined as the average of the two readings.

Blood pressure and pulse rate measuring device

HBP and pulse rate were measured with the HEM 701C (Omron Healthcare Co. Ltd, Kyoto, Japan), an automatic device based on the cuff-oscillometric method that

generates a digital display of systolic BP, diastolic BP and pulse rate. CBP was measured with a USM-700F (UEDA Electronic Works Co. Ltd, Tokyo, Japan), a fully automatic device based on the Korotkoff sound technique (a microphone method). The circumference of the arm was less than 34 cm in most cases, so a standard arm cuff was used for both BP measurements. All devices used in this study had been validated [25,26] and satisfied the criteria of the Association for the Advancement of Medical Instrumentation [28].

Definition of environmental tobacco smoke exposure

Environmental tobacco smoke exposure status was evaluated by the following two questions: 'How often are you exposed to smoke from cigarette smoking by other family members or guests at home?' and 'How often are you exposed to smoke from cigarette smoking by other persons at the workplace and/or other places?'. The women who responded 'hardly exposed' to both questions were categorized as those not exposed to ETS (non-ETS), whereas those who responded 'everyday', '3 or 4 days a week', '1 or 2 days a week' or 'occasionally' were categorized as those exposed to ETS. The exposed women were further classified into three categories according to their location of ETS exposure: those exposed to ETS at home [ETS(home)], those exposed to ETS at the workplace and/or other places [ETS(work/other)] and those exposed to ETS both at home and at the workplace and/or other places [ETS(both)]. For an additional analysis based on frequency of ETS exposure, the women who responded 'everyday' to either question were categorized as those exposed to ETS everyday [ETS(everyday)], whereas the remaining women who responded '3 or 4 days a week', '1 or 2 days a week' and 'occasionally' to either question were categorized as those exposed to ETS less frequently than everyday [ETS(occasionally)].

Data analysis

Information on smoking status, ETS exposure status, marital status, history of diabetes mellitus, history of stroke, history of heart disease, history of hyperlipidemia, alcohol intake, salt intake and activity levels (time spent walking per day) was obtained from the questionnaire. A standardized methodology was used to calculate dietary salt (NaCl) intake from a Japanese version of the food-frequency questionnaire. The reproducibility and validity of this version were previously reported in detail [29,30]. Information on age and use of antihypertensive medication was obtained from another questionnaire sent to each household at the time of the HBP measurements. Body mass index (BMI) information was obtained from medical records kept at Ohasama Hospital and from annual health check-up records.

The participants were stratified according to use of antihypertensive medication to avoid possible mitigation of pressor effect of ETS, because relatively small differences in BP between the participants exposed and those not exposed to ETS were expected to be detected from previous findings [17,18]. Variables were compared using the t-test, analysis of variance (ANOVA), χ^2-test, a logistic regression analysis adjusted for age (years) or analysis of covariance (ANCOVA) adjusted for age (years), marital status (married or single/divorced/widowed), BMI (kg/m^2), history of diabetes mellitus, history of stroke, history of heart disease, history of hyperlipidemia, alcohol intake (current drinker or not current drinker), salt intake (less than the median of 12.28 g/day or greater than or equal to the median) and time spent walking (less than 1 h/day or greater than or equal to 1 h/day), as appropriate. The level of statistical significance was set at $P < 0.05$. Data are presented as percentages or means ± SD (for the t-test and ANOVA) or means ± SE (for ANCOVA). All analyses were performed with SAS software version 9.1 (SAS Institute Inc., Cary, North Carolina, USA).

Results

Home blood pressure and pulse rate of the participants without antihypertensive medication

The characteristics of the study participants are presented in Table 2. Mean age, marital status and percentages of current drinkers were significantly different among the categories of ETS exposure status. This might have been due to the marked differences in age, because working women are usually younger than retirement age and their spouses may be comparatively younger and healthier. Younger women may also have more social opportunities to consume alcohol. A logistic regression analysis was performed to determine whether these factors are significantly different among the categories of ETS exposure status after adjusting for age. The results showed that marital status was not significantly different ($P = 0.40$), whereas percentages of current drinkers remained significantly different among the categories of ETS exposure status ($P = 0.01$).

Table 3 shows HBP and pulse rate levels by location of ETS exposure. The systolic morning HBP value in ETS(both) was approximately 4 mmHg higher than that in non-ETS ($P = 0.02$), and the systolic morning HBP value in ETS(home) and the systolic evening HBP value in ETS(both) were approximately 3 mmHg higher than those in non-ETS ($P = 0.04$ and $P = 0.03$, respectively). There was also a tendency for systolic morning HBP and systolic evening HBP values of all categories exposed to ETS to be higher than those in non-ETS. Systolic morning HBP and systolic evening HBP levels were not significantly different among the categories exposed to ETS, and diastolic HBP and pulse rate levels were not significantly associated with any ETS exposure status. There were no significant interactions between age and ETS exposure status on any HBP and pulse rate levels (all P for interaction >0.2).

Table 2 Characteristics of the participants without antihypertensive medication by ETS location ($n=474$)

	non-ETS	ETS(work/other)	ETS(home)	ETS(both)	*P* value
N	143	47	129	155	
Mean age (years)	64.0 ± 10.7	47.7 ± 9.4	58.3 ± 12.8	52.3 ± 10.7	<0.0001
Marital status (married %)	64.3	85.1	72.9	83.2	0.0007
BMI (kg/m^2)	23.2 ± 3.2	23.4 ± 2.4	23.6 ± 3.5	23.5 ± 3.2	NS
History					
Diabetes mellitus (%)	6.3	8.5	8.5	7.1	NS
Stroke (%)	0.7	0.0	0.8	0.0	NS
Heart disease (%)	6.3	4.3	4.7	2.6	NS
Hyperlipidemia (%)	14.0	4.3	12.4	6.5	NS
Alcohol intake (current drinker %)	12.6	36.2	24.0	40.0	<0.0001
Salt intake (≥12.28 g/day%)	50.3	44.7	47.3	55.5	NS
Time spent walking (≥1 h/day %)	81.1	70.2	82.9	83.2	NS

BMI, body mass index; ETS, exposure to environmental tobacco smoke. Analysis of variance for continuous variables and χ^2-test for categorical variables. Continuous variables are expressed as mean ± SD. NS = $P > 0.05$.

Because percentages of current drinkers were significantly different among the categories of ETS exposure status after adjusting for age, subgroup analysis was performed in noncurrent drinkers. The results showed a similar tendency presented in Table 3 (data not presented).

Table 4 presents the results of the additional analysis based on frequency of ETS exposure. There was a similar tendency for systolic morning HBP and systolic evening HBP values of all categories exposed to ETS, including the values in ETS(occasionally), to be higher than those in non-ETS, as presented in Table 3. The results showed significant differences between the systolic morning HBP value in ETS(everyday) and that in non-ETS and between the systolic evening HBP value in ETS(everyday) and that in non-ETS ($P = 0.02$ and $P = 0.03$, respectively).

Home blood pressure and pulse rate of the participants with antihypertensive medication

Home blood pressure and pulse rate levels by location and frequency of ETS exposure ($n = 105$) showed no significant differences in systolic HBP values between any ETS exposure group and the non-ETS group ($P > 0.2$ and $P > 0.5$, respectively). No other HBP and pulse rate levels were significantly associated with any ETS exposure status (data not presented).

Casual clinic blood pressure and pulse rate of the participants without antihypertensive medication

Table 5 shows mean CBP levels by location of ETS exposure. CBP values were available from 296 (62.4%) study participants without antihypertensive medication. The systolic and diastolic CBP values in ETS(home) were significantly higher than those in non-ETS ($P = 0.02$ and $P = 0.04$, respectively). No other significant differences in CBP values were seen between any ETS exposure group and the non-ETS group ($P > 0.6$).

Discussion

The present results confirm that there is a relationship between increased HBP levels and ETS exposure in Japanese women without antihypertensive medication. HBP measurements detect approximately a 3–4 mmHg difference in BP between the ETS(home) and the ETS-(both) groups and the non-ETS group, whereas CBP measurements detect significant differences only between the ETS(home) group and the non-ETS group. Thus, HBP measurement is a more sensitive measurement for detecting small BP changes.

In the present study, systolic morning HBP values in ETS(home) and in ETS(both) and systolic evening HBP value in ETS(both) were significantly higher than those in non-ETS, whereas diastolic HBP and pulse rate levels were not significantly associated with any ETS exposure status. These findings are consistent with those of Heiss *et al.* [15] and Mahmud and Feely [16], who investigated the relationship between ETS exposure and BP levels in experimental studies. Makris *et al.* [17] investigated the association between ambulatory BP values and ETS exposure in 254 clinically normotensive nonsmokers who were self-referred to their outpatient

Table 3 HBP and PR of the participants without antihypertensive medication by ETS location

	non-ETS	ETS(work/other)	ETS(home)	ETS(both)
Systolic morning HBP (mmHg)	113.1 ± 1.08	114.7 ± 1.85	116.2 ± 1.07[a]	116.8 ± 1.01[a]
Diastolic morning HBP (mmHg)	71.0 ± 0.73	71.4 ± 1.24	71.6 ± 0.72	72.0 ± 0.68
Morning PR (beats/min)	66.2 ± 0.62	66.9 ± 1.06	66.9 ± 0.63	66.9 ± 0.59
Systolic evening HBP (mmHg)	111.9 ± 1.09	114.2 ± 1.86	114.3 ± 1.08	115.3 ± 1.02[a]
Diastolic evening HBP (mmHg)	69.0 ± 0.74	70.3 ± 1.26	69.4 ± 0.73	70.6 ± 0.69
Evening PR (beats/min)	68.7 ± 0.60	68.4 ± 1.02	68.7 ± 0.60	69.4 ± 0.57

BMI, body mass index; ETS, exposure to environmental tobacco smoke; HBP, home blood pressure; PR, pulse rate. Analysis of covariance. Data were adjusted for age, marital status (married or single/divorced/widowed), BMI, history of diabetes mellitus, history of stroke, history of heart disease, history of hyperlipidemia, alcohol intake (current drinker or not current drinker), salt intake (≥12.28 g/day or <12.28 g/day) and time spent walking (≥1 h/day or <1 h/day). Data are expressed as mean ± SE.
[a] $P < 0.05$ compared to non-ETS.

Table 4 HBP and PR of the participants without antihypertensive medication by ETS frequency

	Non-ETS	ETS(occasionally)	ETS(everyday)
N	143	155	176
Systolic morning HBP (mmHg)	113.0 ± 1.08	115.9 ± 0.98	116.7 ± 0.95[a]
Diastolic morning HBP (mmHg)	71.1 ± 0.72	72.0 ± 0.66	71.5 ± 0.64
Morning PR (beats/min)	66.2 ± 0.62	66.5 ± 0.57	67.2 ± 0.55
Systolic evening HBP (mmHg)	111.9 ± 1.08	114.2 ± 0.99	115.2 ± 0.96[a]
Diastolic evening HBP (mmHg)	69.1 ± 0.74	70.2 ± 0.67	69.9 ± 0.65
Evening PR (beats/min)	68.7 ± 0.60	68.6 ± 0.55	69.3 ± 0.53

BMI, body mass index; ETS, exposure to environmental tobacco smoke; HBP, home blood pressure; PR, pulse rate. Analysis of covariance. Data were adjusted for age, marital status (married or single/divorced/widowed), BMI, history of diabetes mellitus, history of stroke, history of heart disease, history of hyperlipidemia, alcohol intake (current drinker or not current drinker), salt intake (≥12.28 g/day or <12.28 g/day) and time spent walking (≥1 h/day or <1 h/day). Data are expressed as mean ± SE.
[a] $P < 0.05$ compared to non-ETS.

hypertension clinic. Their results show that 24-h and daytime systolic BP, heart rate and daytime diastolic BP values are significantly higher in those with at least 1 h daily ETS exposure, compared with those with less exposure and those without ETS exposure. Although the study population and categories of ETS exposure status are different, the present results are consistent with their findings in that out-of-clinic BP measurements detect a difference in BP between individuals exposed and those not exposed to ETS.

Not only were the systolic HBP values of the ETS(home), the ETS(both) and the ETS(everyday) groups significantly higher than those in non-ETS, but systolic morning HBP and systolic evening HBP values of all categories exposed to ETS, including the ETS(work/other) and the ETS(occasionally) groups, tended to be higher than those in non-ETS in the present study. These findings indicate that ETS exposure may elevate systolic HBP regardless of location and frequency of exposure, which is consistent with the previous findings that even a small amount of ETS exposure causes detrimental effects at the clinical level [31,32]. Since systolic HBP is a strong predictive factor for morbidity and mortality from cardiovascular diseases [33,34], the present results may also reflect that a pressor effect, as well as other deleterious effects, of ETS exposure contribute to increased morbidity and mortality from cardiovascular diseases [1–9] in the general population.

Considering the fact that the pathophysiological and hemodynamic effects of ETS exposure last for 24 h after 30 min of ETS exposure at the experimental level [15], that the systolic HBP values of all categories exposed to ETS were consistently higher than those of the non-ETS group, and that the present results were obtained from multiple HBP measurements for a mean of 3 weeks, the present results may reflect a nonlinear persistent pressor effect caused by ETS exposure in the general population. Although there is a possibility that the present results may reflect a much shorter duration of pressor effects of ETS just after exposure, especially in the morning when many smokers tend to smoke just after waking, the present results are important from a prognostic hemodynamic standpoint. Since HBP measurement detects small BP changes, it may reflect persistent effects of ETS exposure and is more feasible to monitor a large population regularly, a further study using HBP measurement is necessary to clarify the chronic deleterious hemodynamic effects of ETS exposure at the population level, with more detailed data on ETS exposure status. HBP measurement may also be useful for future studies investigating the hemodynamic effects of other air pollutants, such as ambient particulate matter [35].

Differences in HBP between women exposed and those not exposed to ETS were not observed in women with antihypertensive medication. This might be because the relatively small pressor effect of ETS exposure was mitigated by the large BP-lowering effects of antihypertensive drugs. It is necessary to consider a pressor effect of ETS exposure at least when interpreting HBP data from normotensive or prehypertensive patients in clinical practice. The present results obviously raise concerns over public health. Achievement of smoke-free environments is thus also important from a hemodynamic standpoint.

Several limitations of the present study need to be discussed. First, as more detailed data on time, duration

Table 5 CBP of the participants without antihypertensive medication by ETS location ($n = 296$)

	non-ETS	ETS(work/other)	ETS(home)	ETS(both)
N	102	21	96	77
Systolic CBP (mmHg)	126.6 ± 1.46	125.4 ± 3.24	131.5 ± 1.46[a]	126.5 ± 1.68
Diastolic CBP (mmHg)	71.6 ± 0.89	70.8 ± 1.98	74.2 ± 0.89[a]	72.4 ± 1.03

BMI, body mass index; CBP, casual clinic blood pressure; ETS, exposure to environmental tobacco smoke. Analysis of covariance. Data were adjusted for age, marital status (married or single/divorced/widowed), BMI, history of diabetes mellitus, history of stroke, history of heart disease, history of hyperlipidemia, alcohol intake (current drinker or not current drinker), salt intake (>12.28 g/day or <12.28 g/day) and time spent walking (≥1 h/day or <1 h/day). Data are expressed as mean ± SE. [a] $P < 0.05$ compared to non-ETS.

and quantity of ETS exposure were unavailable in our study population, the dose–response relationship between HBP levels and ETS exposure is unknown. A further study using HBP measurement is necessary with more detailed data on ETS exposure status. Second, although age distribution of the categories of ETS exposure status was uneven, age did not significantly interact with ETS exposure status on the present results. Third, as the study was cross-sectional, the results do not show a causal relationship between ETS exposure and BP elevation or development of hypertension. A longitudinal study is necessary to investigate this causal relationship in the Ohasama study, as well as in other populations. Fourth, the study excluded men due to the small number of lifelong nonsmoking men. It remains to be investigated whether a positive association between ETS exposure and BP is present in men. Lastly, since a biological marker of ETS exposure, such as cotinine concentration, was not measured, there may be misclassification of ETS exposure status. However, ETS exposure status in a self-administered questionnaire is shown to be generally accurate in a large-scale cohort study in a Japanese population, with a slightly higher rate of passive smokers falsely reporting themselves to be nonpassive smokers compared to Western studies [36]. Therefore, we believe that the present results are acceptable, but they may underestimate the true magnitude of the hemodynamic effects of ETS exposure due to these misclassifications.

In conclusion, this is the first population-based study demonstrating a significant association between increased HBP and ETS exposure. HBP measurement is recommended to investigate the effects of ETS exposure in the general population. ETS exposure may increase BP levels, which may synergistically contribute to unfavorable cardiovascular outcomes, along with the other deleterious effects of ETS.

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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

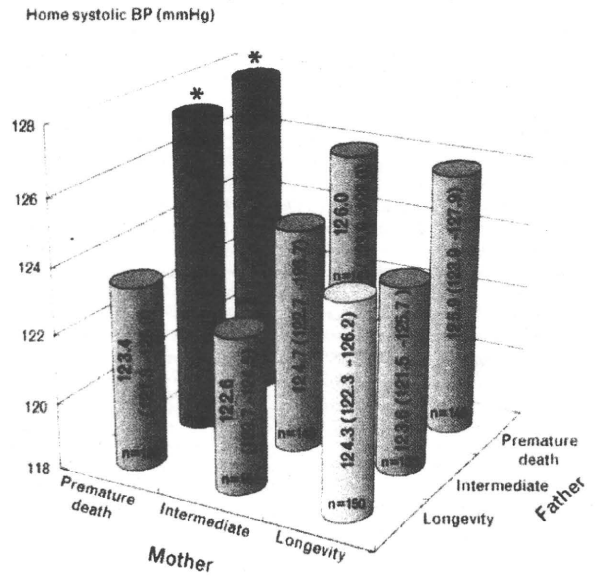
Data are given as mean ± SD or percentage of offspring. Statistical significance among three groups was compared using the ANOVA for continuous variables and the chi-squared test for categorical variables. Definitions of hypertension: home BP, systolic BP > 135 mmHg and/or diastolic BP > 85 mmHg or taking 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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Parthenogenetic chimaerism/mosaicism with a Silver-Russell syndrome-like phenotype

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ABSTRACT

Introduction We report a 34-year-old Japanese female with a Silver-Russell syndrome (SRS)-like phenotype and a mosaic Turner syndrome karyotype (45,X/46,XX).

Methods/Results Molecular studies including methylation analysis of 17 differentially methylated regions (DMRs) on the autosomes and the *XIST*-DMR on the X chromosome and genome-wide microsatellite analysis for 96 autosomal loci and 30 X chromosomal loci revealed that the 46,XX cell lineage was accompanied by maternal uniparental isodisomy for all chromosomes (upid(AC)mat), whereas the 45,X cell lineage was associated with biparentally derived autosomes and a maternally derived X chromosome. The frequency of the 46,XX upid(AC)mat cells was calculated as 84% in leukocytes, 56% in salivary cells, and 18% in buccal epithelial cells.

Discussion The results imply that a parthenogenetic activation took place around the time of fertilisation of a sperm missing a sex chromosome, resulting in the generation of the upid(AC)mat 46,XX cell lineage by endoreplication of one blastomere containing a female pronucleus and the 45,X cell lineage by union of male and female pronuclei. It is likely that the extent of overall (epi)genetic aberrations exceeded the threshold level for the development of SRS phenotype, but not for the occurrence of other imprinting disorders or recessive Mendelian disorders.

Although a mammal with maternal uniparental disomy for all chromosomes (upd(AC)mat) is incompatible with life because of genomic imprinting,[1] a mammal with a upd(AC)mat cell lineage could be viable in the presence of a co-existing normal cell lineage. In the human, Strain *et al*[2] have reported 46,XX peripheral blood cells with maternal uniparental isodisomy for all chromosomes (upid(AC)mat) in a 1.2-year-old phenotypically male patient with aggressive behaviour, hemifacial hypoplasia and normal birth weight. Because of the 46,XX disorders of sex development, detailed molecular studies were performed, revealing the presence of a normal 46,XY cell lineage in a vast majority of skin fibroblasts and a upid(AC)mat 46,XX cell lineage in nearly all blood cells. In addition, although the data are insufficient to draw a definitive conclusion, Horike *et al*[3] have also identified 46,XX peripheral blood cells with possible upd(AC)mat in a phenotypically male patient through methylation analyses for plural differentially methylated regions (DMRs) in 11 patients with Silver–Russell syndrome (SRS)-like phenotype. This patient was found to have a normal 46,XY cell lineage and a triploid 69,XXY cell lineage in skin fibroblasts.

However, such patients with a upd(AC)mat cell lineage remain extremely rare, and there is no report describing a human with such a cell lineage in the absence of a normal cell lineage. Here, we report a female patient with a upid(AC)mat 46,XX cell lineage and a non-upd 45,X cell lineage who was identified through genetic screenings of 103 patients with SRS-like phenotype.

MATERIALS AND METHODS

Case report

This Japanese female patient was conceived naturally and born at 40 weeks of gestation by a normal vaginal delivery. At birth, her length was 44.0 cm (−3.1 SD), her weight 2.1 kg (−2.9 SD) and her occipitofrontal head circumference (OFC) 30.5 cm (−2.3 SD). The parents and the younger brother were clinically normal (the father died from a traffic accident).

At 2 years of age, she was referred to us because of growth failure. Her height was 77.7 cm (−2.5 SD), her weight 8.45 kg (−2.6 SD) and her OFC 43.5 cm (−2.5 SD). Physical examination revealed several SRS-like somatic features such as triangular face, right hemihypoplasia and bilateral fifth finger clinodactyly. She also had developmental retardation, with a developmental quotient of 56. Endocrine studies for short stature were normal as were radiological studies. Cytogenetic analysis using lymphocytes indicated a low-grade mosaic Turner syndrome (TS) karyotype, 45,X[3]/46,XX[47]. Thus, a screening of TS phenotype[4] was performed, detecting horseshoe kidney but no body surface features or cardiovascular lesion. Chromosome analysis was repeated at 6 and 32 years of age using lymphocytes, revealing a 45,X[8]/46,XX[92] karyotype and a 45,X[12]/46,XX[88] karyotype, respectively. On the last examination at 34 years of age, her height was 125.0 cm (−6.2 SD), her weight 57.5 kg (−2.0 SD) and her OFC 51.2 cm (−2.8 SD). She was engaged in a simple work and was able to get on her daily life for herself.

Sample preparation

This study was approved by the Institutional Review Board Committees at National Center for Child health and Development. After obtaining written informed consent, genomic DNA was extracted from leukocytes of the patient, the mother and the brother and from salivary cells, which comprise ~40% of buccal epithelial cells and ~60% of leukocytes,[5] of the patient. Lymphocyte metaphase spreads and leukocyte RNA were also

obtained from the patient. Leukocytes of healthy adults and patients with imprinting disorders were utilised for controls.

Primers and probes

The primers utilised in this study are summarised in supplementary methods and supplementary tables 1–3.

DMR analyses

We first performed bio-combined bisulfite restriction analysis (COBRA)[6] and bisulfite sequencing of the *H19*-DMR (A) on chromosome 11p15.5 by the previously described methods[7] and methylation-sensitive PCR analysis of the *MEST*-DMR (A) on chromosome 7q32.2 by the previously described methods[8] with minor modifications (the methylated and unmethylated allele-specific primers were designed to yield PCR products of different sizes, and the PCR products were visualised on the 2100 Bioanalyzer (Agilent, Santa Clara, California, USA)). This was because hypomethylation (epimutation) of the normally methylated *H19*-DMR of paternal origin and maternal uniparental disomy 7 are known to account for 35–65% and 5–10% of SRS patients, respectively.[9 10] In addition, fluorescence in situ hybridisation (FISH) analysis was performed with a ~84-kb RP5-998N23 probe containing the *H19*-DMR (BACPAC Resources Center, Oakland, California, USA). We also examined multiple other DMRs by bio-COBRA. The ratio of methylated clones (the methylation index) was calculated using peak heights of digested and undigested fragments on the 2100 Bioanalyzer using 2100 expert software.

Genome-wide microsatellite analysis

Microsatellite analysis was performed for 96 autosomal loci and 30 X chromosomal loci. The segment encompassing each locus was PCR-amplified, and the PCR product size was determined on the ABI PRISM 310 autosequencer using GeneScan software (Applied Biosystems, Foster City, California, USA).

PCR analysis for Y chromosomal loci

Standard PCR was performed for six Y chromosomal loci. The PCR products were electrophoresed using the 2100 Bioanalyzer.

Expression analysis

Quantitative real-time reverse transcriptase PCR analysis was performed for three paternally expressed genes (*IGF2*, *SNRPN* and *ZAC1*) and four maternally expressed genes (*H19*, *MEG3*, *PHLDA2* and *CDKN1C*) that are known to be variably (usually weakly) expressed in leukocytes (UniGene, http://www.ncbi.nlm.nih.gov/sites/entrez?db=unigene), using an ABI Prism 7000 Sequence Detection System (Applied Biosystems). *TBP* and *GAPDH* were utilised as internal controls.

RESULTS

DMR analyses

In leukocytes, the bio-COBRA indicated severely hypomethylated *H19*-DMR, and bisulfite sequencing combined with *rs2251375* SNP typing for 30 clones revealed maternal origin of 29 hypomethylated clones and non-maternal (paternal) origin of a single methylated clone in this patient (figure 1A). Thus, the marked hypomethylation of the *H19*-DMR was caused by predominance of maternally derived clones rather than hypomethylation of the *H19*-DMR of paternal origin. FISH analysis for 100 lymphocyte metaphase spreads excluded an apparent deletion of the paternally derived *H19*-DMR or duplication of the maternally derived *H19*-DMR (Supplementary figure 1).

Methylation-sensitive PCR amplification for the *MEST*-DMR delineated a major peak for the methylated allele and a minor peak for the unmethylated allele (figure 1B). This also indicated the predominance of maternally derived clones and the co-existence of a minor portion of paternally derived clones. Furthermore, autosomal DMRs invariably exhibited markedly abnormal methylation patterns consistent with predominance of maternally inherited DMRs, whereas the methylation index of the *XIST*-DMR on the X chromosome remained within the female reference range (figure 1C). The abnormal methylation patterns were less obvious in salivary cells (thus, in buccal epithelial cells) than in leukocytes, except for the methylation index for the *XIST*-DMR that mildly exceeded the female reference range (figure 1A–C).

Microsatellite analysis

Major peaks consistent with maternal uniparental isodisomy and minor peaks of non-maternal (paternal) origin were identified for at least one locus on each autosome, with the minor peaks of non-maternal origin being more obvious in salivary cells than in leukocytes (figure 1D and supplementary table 4). Furthermore, the frequency of the upid(AC)mat cells was calculated as 84% in leukocytes, 56% in salivary cells and 18% in epithelial buccal cells, using the area under curves for the maternally and the non-maternally inherited peaks (supplementary note). Such minor peaks of non-maternal origin were not detected for all the 30 X chromosomal loci examined.

PCR analysis for Y chromosomal loci

PCR amplification failed to detect any trace of Y chromosome-specific bands in leukocytes and salivary cells (Supplementary figure 2).

Expression analysis

Expression analysis using control leukocytes indicated that, of the seven examined genes, *SNRPN* expression alone was strong enough to allow for a precise assessment (Supplementary figure 3). *SNRPN* expression was extremely low in this patient (figure 1E).

DISCUSSION

These results imply that this patient had a upid(AC)mat 46,XX cell lineage and a non-upd 45,X cell lineage. Indeed, methylation patterns of the *XIST*-DMR is explained by assuming that the two X chromosomes in the upid(AC)mat cells undergo random X-inactivation and that 45,X cells with the methylated *XIST*-DMR on a single active X chromosome[11] are relatively prevalent in buccal epithelial cells. Furthermore, lack of non-maternally derived minor peaks for microsatellite loci on the X chromosome is explained by assuming that the two X chromosomes in the upid(AC)mat cells and the single X chromosome in the 45,X cells are derived from a common X chromosome of maternal origin, with no paternally derived sex chromosome. It is likely, therefore, that a parthenogenetic activation took place around the time of fertilisation of a sperm missing a sex chromosome, resulting in the generation of the 46,XX cell lineage with upid (AC)mat by endoreplication (the replication of DNA without the subsequent completion of mitosis) of one blastomere containing a female pronucleus and the 45,X cell lineage with biparentally derived autosomes and a maternally derived X chromosome by union of male and female pronuclei (figure 2), although it is also possible that a paternally derived sex chromosome was present in the sperm but was lost from the normal

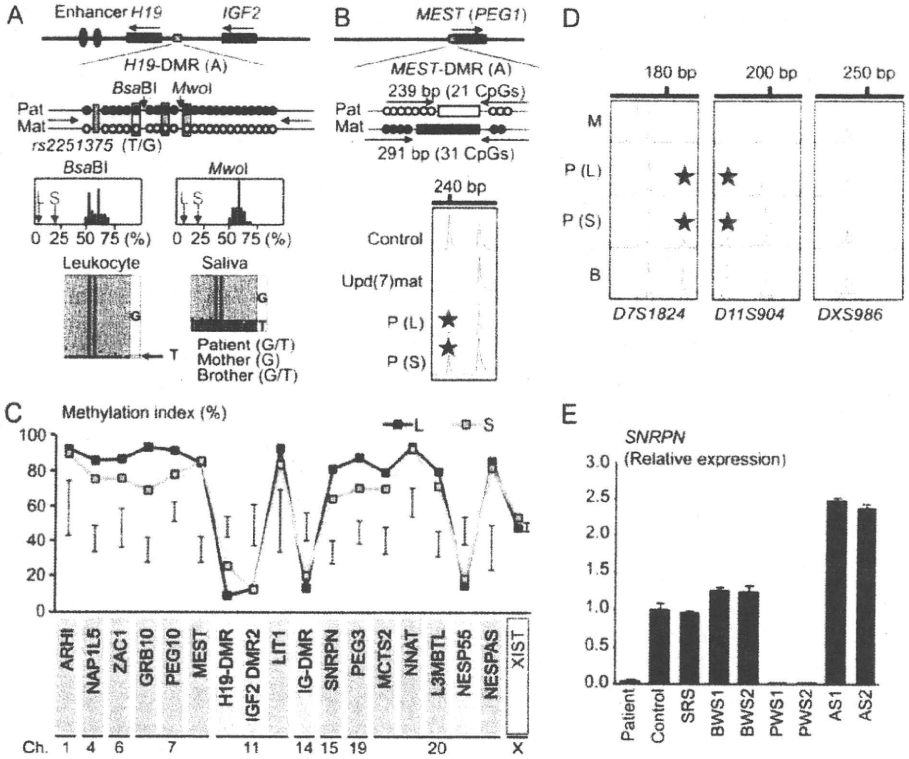

Figure 1 Representative molecular results. Pat, paternally derived allele; Mat, maternally derived allele; P, patient; M, mother; B, brother; L, leukocytes; and S, salivary cells. Filled and open circles in A and B represent methylated and unmethylated cytosine residues at the CpG dinucleotides, respectively. A. Methylation patterns of the *H19*-DMR (A) harbouring 23 CpG dinucleotides and the T/G SNP (*rs2251375*) (a grey box). The PCR products are digested with *Bsa*BI when the cytosine at the sixth CpG dinucleotide (highlighted in yellow) is methylated and with *Mwo*I when the two cytosines at the ninth and the 11th CpG dinucleotides (highlighted in orange) are methylated. For the bio-COBRA data, the black histograms represent the distribution of methylation indices (%) in 50 control participants, and L and S denote the methylation indices for leukocytes and salivary cells of this patient, respectively. For the bisulfite sequencing data, each line indicates a single clone. B. Methylated and unmethylated allele-specific PCR analysis for the *MEST*-DMR (A). In a control participant, the PCR products for methylated and unmethylated alleles are delineated, and the unequal amplification is consistent with a short product being more easily amplified than a long product. In a previously reported patient with upd(7)mat,[8] the methylated allele only is amplified. In this patient, major peaks for the methylated allele and minor peaks for the unmethylated allele (red asterisks) are detected. C. Methylation patterns for the 18 DMRs examined. The DMRs highlighted in blue and pink are methylated after paternal and maternal transmissions, respectively. The black vertical bars indicate the reference data (maximum–minimum) in 20 normal control participants, using leukocyte genomic DNA (for the *XIST*-DMR, 16 female data are shown). D. Representative microsatellite analysis. Minor peaks (red asterisks) have been identified for *D7S1824* and *D11S904* but not for *DXS986* of the patient. Since the peaks for *D7S1824* and *D11S904* are absent in the mother and clearly present in the brother, they are assessed to be of paternal origin. E. Relative expression level (mean ± SD) of *SNRPN* on chromosome 15. The data have been normalised against *TBP*. SRS, an SRS patient with an epimutation (hypomethylation) of the *H19*-DMR; BWS1, a BWS patient with an epimutation (hypermethylation) of the *H19*-DMR; BWS2, a BWS patient with upd(11)pat; PWS1, a PWS patient with upd(15)mat; PWS2, a PWS patient with an epimutation (hypermethylation) of the *SNRPN*-DMR; AS1, an Angelman syndrome (AS) patient with upd(15)pat; and AS2, an AS patient with an epimutation (hypomethylation) of the *SNRPN*-DMR.

cell lineage at the very early developmental stage. Hence, in a strict sense, this patient is neither a chimera resulting from the fusion of two different zygotes nor a mosaic caused by a mitotic error of a single zygote. In this regard, a triploid cell stage is assumed in the generation of a upid(AC)mat cell lineage, and such triploid cells may have been detected in skin fibroblasts of the patient reported by Horike *et al*.[5]

The upid(AC)mat cells accounted for the majority of leukocytes even in adulthood of this patient, despite global negative selective pressure.[12 15] This phenomenon, though intriguing, would not be unexpected in human studies because leukocytes are usually utilised for genetic analyses. Rather, if the upid(AC)mat cells were barely present in leukocytes, they would not have been detected. It is likely, therefore, that upid(AC)mat cells have occupied a relatively large portion of the definitive haematopoietic tissues primarily as a stochastic event. Furthermore, parthenogenetic chimera mouse studies have revealed that parthenogenetic cells are found at a relatively high frequency in some tissues/organs including blood and are barely identified in other tissues/organs such as skeletal muscle and liver.[15] Such a possible tissue-specific selection in favour of the preservation of parthenogenetic cells in the definitive haematopoietic tissues may also be relevant to the predominance of the upid(AC)mat cells in leukocytes. In addition, a reduced growth potential of 45,X cells[14] may also have contributed to the skewed ratio of the two cell lineages.

Clinical features of this patient would be determined by several factors. They include: (1) the ratio of two cell lineages in various tissues/organs, (2) the number of imprinted regions or DMRs relevant to the development of specific imprinting disorders (eg, plural regions/DMRs on chromosomes 7 and 11 for SRS[9 10] and a single region/DMR on chromosome 15 for Prader–Willi syndrome (PWS)),[15] (3) the degree of clinical effects of dysregulated imprinted regions/DMRs (an (epi)dominant effect has been

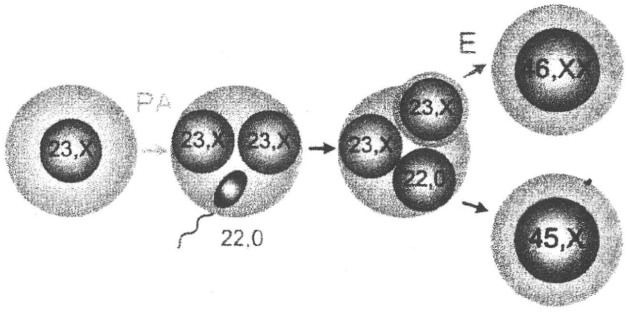

Figure 2 Schematic representation of the generation of the upid(AC)mat 46,XX cell lineage and the non-upd 45,X cell lineage. Polar bodies are not shown. PA, parthenogenetic activation; and E, endoreplication of one blastomere containing a female pronucleus.

assumed for the 11p15.5 imprinted regions including the *IGF2–H19* domain on the basis of SRS or Beckwith–Wiedemann syndrome (BWS) phenotype in patients with multilocus hypomethylation[16] and BWS-like phenotype in patients with a upid (AC)pat cell lineage,[17] a mirror image of a upid(AC)mat cell lineage). (4) expression levels of imprinted genes in upid(AC)mat cells (although *SNRPN* expression of this patient was consistent with upid(AC)mat cells being predominant in leukocytes. complicated expression patterns have been identified for several imprinted genes in androgenetic and parthenogenetic fetal mice. probably because of perturbed *cis*- and *trans*-acting regulatory mechanisms)[18] and (5) unmasking of possible maternally inherited recessive mutation(s) in upid(AC)mat cells.[19] Collectively, it appears that the extent of overall (epi)genetic aberrations exceeded the threshold level for the development of SRS phenotype and horseshoe kidney characteristic of TS[4] but remained below the threshold level for the occurrence of other imprinting disorders or recessive Mendelian disorders.

In summary, we identified a upid(AC)mat 46,XX cell lineage in a woman with an SRS-like phenotype and a 45,X cell lineage accompanied by autosomal haploid sets of biparental origin. This report will facilitate further identification of patients with a upid(AC)mat cell lineage and better clarification of the clinical phenotypes in such patients.

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Competing interests None.

Patient consent Obtained.

Ethics approval This study was conducted with the approval of the Institutional Review Board Committees at National Center for Child health and Development.

Contributors Drs Kazuki Yamazawa (first author) and Kazuhiko Nakabayashi (second author) contributed equally to this work.

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ELSEVIER

SHORT REPORT

High cardiovascular risk factors among obese children in an urban area of Japan☆

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Summary The association between degree of obesity and cardiovascular and related metabolic risk factors were examined in 355 Japanese obese school children from 11 to 12 years old. The parameters evaluated were blood pressure, serum lipids, fasting blood glucose, and serum ALT and AST. ALT, AST and triglycerides were more commonly evaluated in obese boys than in obese girls, while HDL-cholesterol was more commonly lowered in obese girls. Hypercholesterolemia was 2-fold, and abnormal liver functions were 3-fold more common in severely obese than in moderate obese children. Thus, cardiovascular and related metabolic risk factors are present in obesity in school-aged children, particularly in boys.

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