REFERENCES

1. MacKay A, Berg C, Atrash H. Pregnancy-related mortality from preeclampsia and eclampsia. Obstet Gynecol 2001;97:533-8.

2. Ng EK, Tsui NB, Lau TK, et al. mRNA of placental origin is readily detectable in maternal plasma. Proc Natl Acad Sci U S A 2003; 100:4748-53.

3. Poon LL, Leung TN, Lau TK, Lo YM. Presence of fetal RNA in maternal plasma. Clin Chem 2000;46:1832-4.

4. Okazaki S, Sekizawa A, Purwosunu Y, Iwasaki M, Farina A, Okai T. Measurement of mRNA of trophoblast-specific genes in cellular and plasma components of maternal blood. J Med Genet 2006;43:e47.

5. Ng EK, Leung TN, Tsui NB, et al. The concentration of circulating corticotropin-releasing hormone mRNA in maternal plasma is increased in preeclampsia. Clin Chem 2003; 49:727-31.

6. Farina A, Chan CW, Chiu RW, et al. Circulating corticotropin-releasing hormone mRNA in maternal plasma: relationship with gestational age and severity of preeclampsia. Clin Chem 2004;50:1851-4.

7. Sekizawa A. [Detection of the damage of placental villous trophoblasts and the possible pathogenesis of preeclampsia]. Acta Obst Gynaec Jpn 2003;55:830-7.

8. Purwosunu Y, Sekizawa A, Farina A, et al. Evaluation of physiological alterations of the placenta through analysis of cell-free messenger ribonucleic acid concentrations of angiogenic factors. Am J Obstet Gynecol 2008;198: 124.e1-7.

9. Purwosunu Y, Sekizawa A, Farina A, et al. Cell-free mRNA concentrations of CRH, PLAC1, and selectin-P are increased in the plasma of pregnant women with preeclampsia. Prenat Diagn 2007;27:772-7.

10. Purwosunu Y, Sekizawa A, Koide K, et al. Cell-free mRNA concentrations of plasminogen activator inhibitor-1 and tissue-type plasminogen activator are increased in the plasma of pregnant women with preeclampsia. Clin Chem 2007;53:399-404.

11. American College of Obstetricians and Gynecologists. ACOG practice bulletin no.: 33. Diagnosis and management of preeclampsia and eclampsia. Int J Gynaecol Obstet 2002;77: 67-75.

12. Fujito N, Samura O, Miharu N, Tanigawa M, Hyodo M, Kudo Y. Increased plasma mRnas of placenta-specific 1 (PLAC1) and glial cells-missing 1 (GCM1) in mothers with pre-eclampsia. Hiroshima J Med Sci 2006;55:9-15.

13. Redman CW, Sargent IL. Latest advances in understanding preeclampsia. Science 2005;308:1592-4.

14. Sibai B, Dekker G, Kupferminc M. Pre-eclampsia. Lancet 2005;365:785-99.

15. Conde-Agudelo A, Villar J, Lindheimer M. World Health Organization systematic review of screening tests for preeclampsia. Obstet Gynecol 2004;104:1367-91.

16. Farag K, Hassan I, Ledger WL. Prediction of preeclampsia: can it be achieved? Obstet Gynecol Surv 2004;59:464-82; quiz 485.

17. Espinoza J, Romero R, Nien J, et al. Identification of patients at risk for early onset and/or severe preeclampsia with the use of uterine artery Doppler velocimetry and placental growth factor. Am J Obstet Gynecol 2007;196: 326.e1-13.

18. Levine RJ, Lam C, Qian C, et al. Soluble endoglin and other circulating antiangiogenic factors in preeclampsia. N Engl J Med 2006;355:992-1005.

19. Levine RJ, Maynard SE, Qian C, et al. Circulating angiogenic factors and the risk of preeclampsia. N Engl J Med 2004;350:672-83.

20. Venkatesha S, Toporsian M, Lam C, et al. Soluble endoglin contributes to the pathogenesis of preeclampsia. Nat Med 2006;12:642-9.

OBSTETRICS

Performance of messenger RNAs circulating in maternal blood in the prediction of preeclampsia at 10-14 weeks

Antonio Farina, MD, PhD; Cinzia Zucchini, PhD; Akihiko Sekizawa, MD; Yuditiya Purwosunu, MD; Paola de Sanctis, PhD; Giuseppe Santarsiero, MD; Nicola Rizzo, MD; Danila Morano, MD; Takashi Okai, MD

OBJECTIVE: The purpose of this study was to determine whether the combined distribution of a panel of cellular messenger RNA markers can detect preeclampsia long before onset.

STUDY DESIGN: We compared blood at 10-14 weeks from 11 women who ultimately experienced preeclampsia with 88 matched control subjects. After multiples of the median conversion of all the markers, logistic regression was used to calculate the risk of the development of preeclampsia.

RESULTS: Higher multiples of the median values than expected were found for endoglin, fms-related tyrosine kinase 1, and transforming growth factor–β1. Lower multiples of the median values were found for placental growth factor and placental protein 13. Endoglin fms-related tyrosine kinase 1 and transforming growth factor–β1 had the best discriminant power. Messenger RNA species provided independent contributions to the prediction of preeclampsia. In fact, 11 women with preeclampsia scored a median risk of 50% of experiencing preeclampsia. Control subjects scored a median risk of preeclampsia of 0.18%. The detection rate at a 5% false positive rate was 72.3%.

CONCLUSION: The messenger RNA dosage in maternal blood would be a useful method for the calculation of the risk of the development of preeclampsia.

Key words: logistic regression, mRNA, maternal blood, preeclampsia, screening

Cite this article as: Farina A, Zucchini C, Sekizawa A, et al. Performance of messenger RNAs circulating in maternal blood in the prediction of preeclampsia at 10-14 weeks. Am J Obstet Gynecol 2010;203:●●●●.

Hypertensive disorders in pregnancy, including preeclampsia and pregnancy-induced hypertension, are associated with significant morbidity and mortality rates for the mother and the neonate.[1] Preeclampsia is a multisystem disorder that is unique to pregnancy and causes significant morbidity and death worldwide. It affects 2-5% of pregnancies[2]; the clinical features are well recognized and characteristically manifest in the second to third trimester, whereas the underlying disease and major pathogenic changes already occur at earlier stages of pregnancy because of failure of the trophoblast cells to invade, with an increase in the apoptotic and necrotic index. Because several known risk factors are not highly predictive or modifiable, attempts to prevent preeclampsia are confined mostly to those patients who are at increased risk because of their medical history. Given the morbidity that is associated with preeclampsia, an enormous variety of biomolecules have been studied to detect those that show evidence of alteration in the maternal circulation during early pregnancy, before the manifestation of clinical symptoms.[3] The identification of reliable screening markers thus would permit major improvements in obstetric care through better targeting of antepartum surveillance. A great number of possible predictive markers have been tested, which include placental factors,[4] abnormal fibrolytic activity,[5] markers of endothelial dysfunction,[6] markers of oxidative stress,[7] and markers of abnormal trophoblast invasion.[8,9] An aberrant quantitative expression of some circulating placental-specific messenger RNAs (mRNAs) in the maternal blood that was found in preeclampsia cases compared with control subjects seems to be a promising tool for the early detection of the disease.[10] Even if there is no clear evidence that mRNAs offer some advantages over protein markers, molecular analysis has the advantage of enrolling a great number of markers at the same time and can also provide some useful information about the pathophysiologic condition of the disease. The disturbances in the expression of these molecules have led to the proposal that they may be used as early predictive markers of preeclampsia and/or intrauterine growth restriction before the onset of

From the Department of Histology, Embryology, and Applied Biology, Division of Prenatal Medicine, University of Bologna, Bologna, Italy (Drs Farina, Zucchini, de Sanctis, Santarsiero, Rizzo, and Morano); the Department of Obstetrics and Gynecology, Showa University School of Medicine, Tokyo, Japan (Drs Farina, Sekizawa, Purwosunu, and Okai); and the Department of Obstetrics and Gynecology, University of Indonesia, Cipto Mangunkusumo National Hospital, Jakarta, Indonesia (Dr Purwosunu).

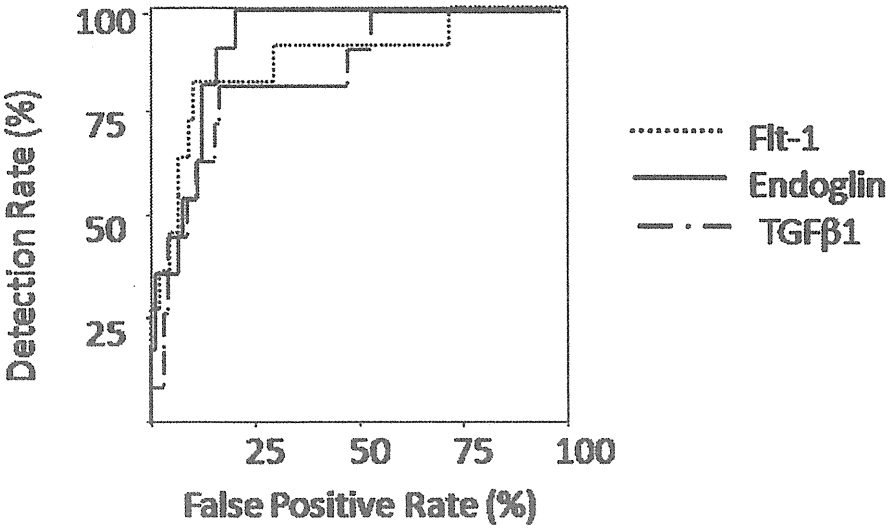
Received Jan. 29, 2010; revised May 26, 2010; accepted July 27, 2010.

Reprints: Antonio Farina, MD, PhD, Division of Prenatal Medicine, University of Bologna, 40126 Bologna, Italy. antonio.farina@unibo.it.

Supported in part by a PRIN project 2007 and Ricerca Fondamentale Orientata (A.F.) and by Grants-in-Aid for Scientific Research from the Ministry of Education, Science, Sport and Culture of Japan (Grant no. 09158522) and from the Ministry of Health, Labor and Welfare of Japan.

0002-9378/$36.00 • © 2010 Mosby, Inc. All rights reserved. • doi: 10.1016/j.ajog.2010.07.043

FIGURE 1
Receiver operating characteristic curve for the messenger RNA species that show discriminant power between cases of preeclampsia and control subjects



The X-axis shows 1-specificity or false positive rate; Y-axis shows the detection rate.

FLT1, fms-related tyrosine kinase 1; *TGFβ1*, transforming growth factor beta 1.

Farina. Prediction of preeclampsia by means of circulating placental mRNA at 10-14 weeks of gestation. Am J Obstet Gynecol 2010.

clinical symptoms. However, because it seems that no single test is highly sensitive or predictive, several markers might plausibly be used at the same time.

In this study, we dosed the concentration of a panel of cellular mRNAs that was comprised of fms-related tyrosine kinase 1 (FLT1), endoglin, placental growth factor (PlGF), transforming growth factor–β1 (TGFβ1), and placental protein 13 (PP13) that were circulating in maternal blood of women with preeclampsia who had been matched with appropriate control subjects, with a view to the calculation of a new posterior risk of preeclampsia for each subject in the data series.

Materials and Methods

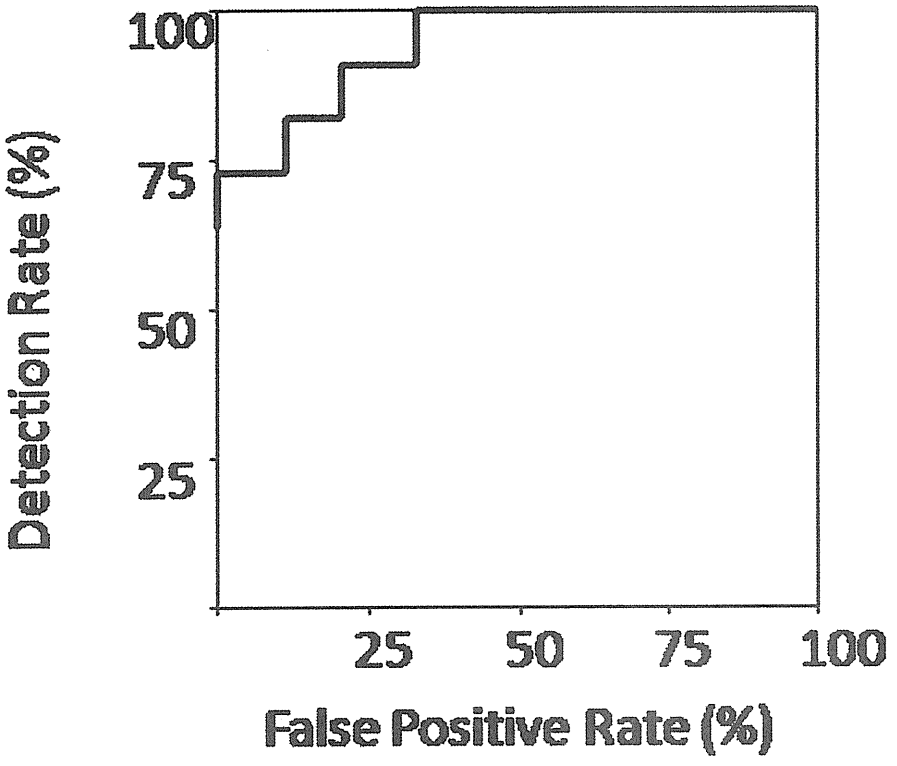
The study population consisted of 99 women who attended the Division of Prenatal Medicine, University of Bologna, Bologna, Italy. Gestational age was calculated by ultrasound measurements at 11-14 weeks' gestation. A second ultrasound examination was performed at 22-24 weeks' gestation for measurement of fetal growth and examination for fetal defects. Those cases in which no major fetal defects were detected were included in the study. Eleven women with preeclampsia but who were asymptomatic at the time of blood drawing were matched with 88 control subjects (1:8 match for fetal sex and gestational age expressed in weeks + days). All women were informed and agreed to participate in the study, which was approved by the local Hospital Ethics Committee.

Preeclampsia was defined as gestational hypertension (systolic pressure of >140 mm Hg or diastolic blood pressure of >90 mm Hg on at least 2 occasions after 20 weeks' gestation) with proteinuria (>0.3 g/d). *Severe preeclampsia* was defined by the presence of 1 or both of the following events: (1) severe gestational hypertension (systolic pressure of >160 mm Hg or diastolic pressure of >110 mm Hg on at least 2 occasions after 20 weeks' gestation) and (2) severe proteinuria (≥5g protein in a 24-hour urine specimen or ≥3 g on 2 random urine samples that were collected at least 4 hours apart). *Intrauterine growth restriction* was defined as the estimated fetal weight being 2.0 standard deviations below the mean expected weight for the gestational age, as determined by ultrasound evaluation.

Blood samples were taken at the time of first examination (11-14 weeks' gestation) from subjects who had been scheduled for chorionic villous sampling or amniocentesis. The blood samples (2.5 mL) were collected in PAXgene blood RNA tubes (PreAnalytic, Hombrechtikon, Switzerland), kept at room temperature for 3 hours, and then stored at –20°C until being transported to Japan. Molecular analysis was performed in the Department of Obstetrics and Gynecology at Showa University School of Medicine, Tokyo, Japan. RNA extraction and real-time polymerase chain reaction (PCR) were performed according to protocols described elsewhere.[11] In brief, cellular component samples were centrifuged twice at 4000 g for 10 minutes at room temperature to remove the entire supernatant and any mRNA that was present in the residual plasma. The pellet was then washed, resuspended, and incubated in optimized buffer solution that contained proteinase K to digest protein. A second round of centrifugation was performed to remove any residual cell debris, and the resulting supernatant was transferred to a fresh microcentrifuge tube. We added 100% ethanol to the supernatant to adjust the binding conditions; the resultant lysate was then applied to a PAXgene spin column (PreAnalytiX; PreAnalytic), which resulted in selective binding of RNA to the silica-gel membrane of the spin column. After the column was washed 3 times, pure RNA was eluted in 80 μL of RNase-free water.

Reverse transcription of the mRNA was performed using an Omniscript RT Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. Complementary DNA products were amplified by real-time quantitative PCR according to the manufacturer's instructions (QuantiTect Probe PCR kit; Qiagen) with a 2-μL aliquot of complementary DNA and the kit's components in a reaction volume of 20 μL. QuantiTect PCR analyses for vascular endothelial growth factor, FLT1, endoglin, PlGF, and TGFβ1 were performed with predeveloped and com-

FIGURE 2
Receiver operating characteristic curve shows the combined discriminant power of Flt-1, endoglin, TGFβ1, and parity



The X-axis shows 1-specificity or false positive rate; Y-axis shows the detection rate.

FLT1, fms-related tyrosine kinase 1; *TGFβ1*, transforming growth factor beta 1.

Farina. Prediction of preeclampsia by means of circulating placental mRNA at 10-14 weeks of gestation. Am J Obstet Gynecol 2010.

mercially available primers and probe sets (catalog no. Hs00900054_m1 for vascular endothelial growth factor; catalog no. Hs01052936_m1 for FLT1; catalog no. Hs00923997_g1 for endoglin; catalog no. Hs00182176_m1 for PlGF; and catalog no. Hs0000171257_m1 for TGFβ1; Applied Biosystems, Foster City, CA). The following thermal cycling protocol was used for PCR: initial denaturation at 95°C for 15 minutes, 40 cycles of denaturation at 94°C for 15 seconds, and annealing at 60°C for 1 minute. As an initial step, we verified that each PCR assay was specific to mRNA and not to genomic DNA. Amplification data were collected and analyzed with an ABI Prism 7900T Sequence Detector (Applied Biosystems). Each sample was analyzed in duplicate, and multiple negative water blanks were included in every analysis. Quantification of gene expression was performed with investigators blinded to the outcome of pregnancy. Amounts of mRNA samples were expressed in term of copies per milliliter. To quantify mRNA concentrations, we prepared plasmid DNA for calibration curves as previously described.[11]

Data analysis was performed by nonparametric statistics because of the small sample size. A 1:8 match (1 case with 8 control subjects) for gestational age expressed in days, and fetal sex was determined. Such a match would guarantee a proper comparison, even in the presence of a relatively low number of cases. The median mRNA concentration of each available marker (FLT1, endoglin, PlGF, TGFβ, and PP13) as a function of increasing gestational age was measured initially and calculated by weighted log10–linear regression. All data were expressed as multiples of the median. We used a logistic regression to calculate the posterior risk of preeclampsia in both control subjects and affected cases, taking the panel of available mRNA values that were expressed in multiples of the median and parity as predictors of the disease. The detection and false-positive rates were calculated for each available marker with the use of a univariable receiver operating characteristic (ROC) curve. Multivariable analysis was performed with logistic regression to calculate the risk for each patient for classification as a control or preeclampsia case. The logistic output was adjusted for the incidence of preeclampsia in the general population (2%) by the calculation of the sampling fraction as described by Collett.[12] Again, quantitative data were transformed into an ordinal scale (in 4 categories <25th percentile, 25-50th percentile, 50-75th percentile, and >75th percentile).

To obtain a more robust risk estimation, a multivariable ROC curve for the calculation of multivariable detection rate was built with the use of the calculated risk for preeclampsia by logistic regression analysis as the test variable, for each patient in the series.

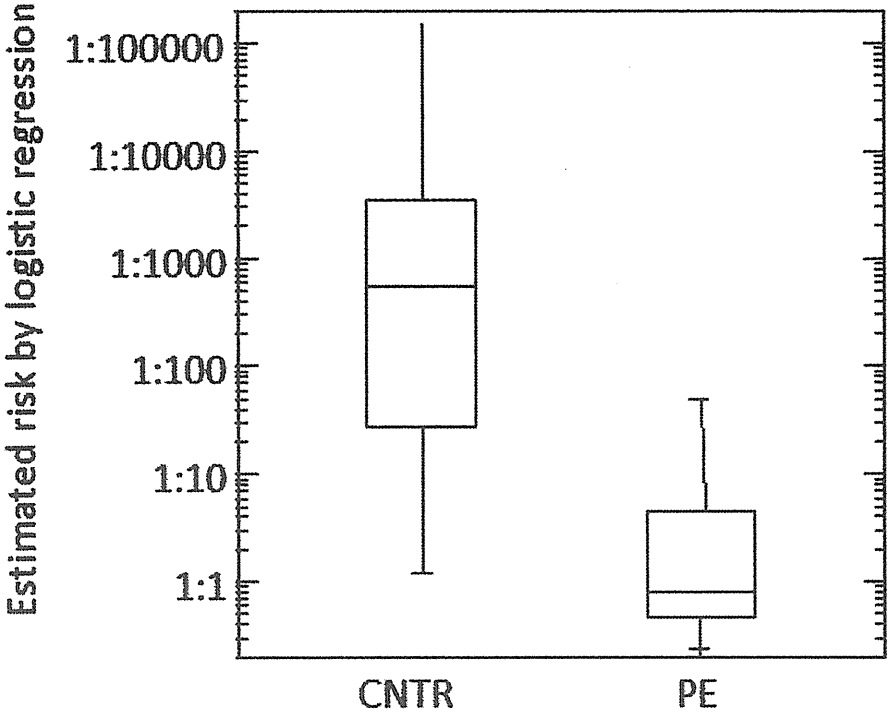
RESULTS

The findings of this study demonstrate that at 11-14 weeks of gestation, women who subsequently experience preeclampsia have an increased maternal concentration of mRNA for FLT1, endoglin, and TGFβ1 and reduced levels of mRNA for PP13 and PlGF. These findings are compatible with previous studies that reported reduced levels of PP13 and PlGF and increased FLT1, endoglin, and TGFβ1 in early second-trimester pregnancy in women destined to experience preeclampsia.[10,13,14]

Tables 1 and 2 show the demographic characteristics of the patients and the log multiples of the median of the mRNA species that were considered in the study, stratified according to the 2 subgroups that were generated. The median gestational age at the time of blood test was 12 + 3 weeks (range, 11–14 weeks) and 12 + 3 weeks (range, 11–14 weeks) for control subjects and cases, respectively.

ROC curve analysis showed that FLT1 has the highest detection rate at a 5%

FIGURE 3
Box and whiskers plot shows the risk distribution for preeclampsia in cases and controls



CNTR, controls; *PE*, preeclampsia.

Farina. Prediction of preeclampsia by means of circulating placental mRNA at 10-14 weeks of gestation. Am J Obstet Gynecol 2010.

false-positive rate, followed by endoglin and TGFβ1 (Figure 1). Table 3 shows the output of the ROC curves for those mRNA species, with a significant detection rate for preeclampsia. As shown, only FLT1, endoglin, and TGFβ1 were included in the analysis.

After adjustment for the incidence of the disease (2%), we found that the median risk of preeclampsia was reduced by 10 times in control subjects and 20 times higher in our cases. Figure 2 expresses the risk (in a box and whiskers plot) for both cases and control subjects. The detection rate of the combined marker panel at 5% false positive was 72.3%, as reported in Figure 3. Table 4 shows the actual risk of preeclampsia for each of the preeclamptic cases found in our series along with the available clinical information.

TABLE 1
Demographic and clinical characteristics of pregnant women who provided CVS

Variable	Controls, n = 88	Preeclampsia, n = 11	*P* value[a]
Gestational age at the time of blood test, d	84 (72-96)	84 (71-96)	ns
Maternal age, y	36 (35–38)	36 (35–38)	ns
Percentage of primiparae	52.3	81.8	ns
Week at delivery	39 (36–41)	36 (34–41)	ns
Neonatal weight, g	3150 (2560–4180)	2930 (2000–3510)	ns

Data are expressed as a median (min-max) or percentage.
CVS, chorionic villous samples; *ns*, not significant.

[a] Mann-Whitney U-test or Fisher's exact test.

Farina. Prediction of preeclampsia by means of circulating placental mRNA at 10-14 weeks of gestation. Am J Obstet Gynecol 2010.

COMMENT

The onset of preeclampsia is associated with maternal and neonatal morbidity and death. Medical treatment is often started too late when there is the presence of hypertension and proteinuria, which are probably end-stage manifestations of this multisystem disorder. Currently, Doppler ultrasound analysis of the uteroplacental circulation is the most widely used test to detect poor placental perfusion and represents a routine antenatal screening tool to detect women who are at a higher risk of preeclampsia. Many studies are available in the literature; unfortunately, they are difficult to compare because of differences in the Doppler sampling techniques, definitions of abnormal flow velocity waveform, populations, gestational age at examination, and the criteria for a diagnosis of preeclampsia. A review of 43 studies that included >42,000 women and evaluated uterine artery Doppler images reports that the positive predictive value of an abnormal Doppler image is low in both high- and low-risk populations.[9,15,16] In low-risk women, for example, the risk seems to increase from 2.5% to 8-15%, although a negative test reduces the probability of disease to 1.5-2%. In high-risk women, a rise from 14% to 29-32% would be quoted for those women who are screened as positive, and a reduction as low as 6-9% is expected for those women who are screened negative. Thus, Doppler imaging seems more effective in the assignment of a higher risk ratio (compared with baseline) in low-risk patients than in high-risk patients. Again, the predictive ability of Doppler imaging differs significantly according to gestation at delivery. In addition to first trimester Doppler images of the uterine arteries with maternal factor and/or biochemical markers for calculating a posterior risk of preeclampsia,

TABLE 2
Log MoM values in cases and controls

mRNA	PE cases, n = 11	Controls, n = 88	*P* value[a]
FLT1	0.34 (−0.16 to 0.74)	0 (−0.92 to 0.45)	< .001
Endoglin	0.32 (0.25 to 0.51)	0 (−1.05 to 0.84)	< .001
PlGF	−0.35 (−0.57 to 0.31)	0 (−1.39 to 0.97)	.772
TGFβ1	0.28 (−0.04 to 0.61)	0 (−1.19 to 0.49)	< .001
PP13	−0.15 (−1.99 to 0.29)	0 (−1.95 to 0.42)	< .001

Data are expressed in median and SD.
FLT1, fms-related tyrosine kinase 1; *MoM*, multiple of median; *mRNA*, messenger RNA; *PE*, preeclampsia; *PlGF*, placental growth factor; *PP13*, placental protein 3; *TGFβ1*, transforming growth factor β1.
[a] Mann-Whitney U test
Farina. Prediction of preeclampsia by means of circulating placental mRNA at 10-14 weeks of gestation. Am J Obstet Gynecol 2010.

TABLE 3
Univariable and multivariable ROC curves analysis

Variable	Area	SE	*P* value	95% CI	DR% at 5% FPR
FLT1	0.872	0.064	< .001	0.747–0.997	45.5
ENG	0.884	0.034	< .001	0.817–0.952	36.4
TGFβ1	0.835	0.058	< .001	0.720–0.949	27.3
All + Parity	0.946	0.024	< .001	0.899–0.994	72.3

CI, confidence interval; *DR*, detection rate; *ENG*, endoglin; *FLT1*, fms-related tyrosine kinase 1; *FPR*, false positive rate; *MoM*, multiple of median; *ROC*, receiver operating characteristic; *TGFβ1*, transforming growth factor β1.
Farina. Prediction of preeclampsia by means of circulating placental mRNA at 10-14 weeks of gestation. Am J Obstet Gynecol 2010.

more recent papers have used a statistical algorithm that is quite similar to that used in the present study.[17-20]

FLT1 and endoglin recently have been reported as playing important roles in the pathophysiologic condition of preeclampsia.[21] Protein concentrations of FLT1 and endoglin in plasma are increased before the onset of preeclampsia and correlate with disease severity.[21-23] These reports have indicated that FLT1 and endoglin from the placenta induce severe maternal endothelial dysfunction, which is concordant with the present finding that FLT1 and endoglin are likely to offer the best predictors among genes that are derived from the placenta. Although the precise relationship of endoglin to FLT1 is unknown, it appears that both endoglin and FLT1 contribute to the pathogenesis of the maternal syndrome through separate mechanisms. Several lines of evidence support this hypothesis.[21-23] Endoglin level is elevated in the sera of preeclamptic women 2-3 months before the onset of clinical signs of preeclampsia, correlates with disease severity, and falls off after delivery. Endoglin is a coreceptor for TGFβ1 and is highly expressed on cell membranes of vascular endothelium and syncytiotrophoblasts.[23] Endoglin inhibits TGFβ1 signaling in endothelial cells and blocks TGFβ1-mediated activation of endothe-

TABLE 4
Estimated risk for preeclampsia cases asymptomatic at the time of blood test

Risk	Case ID	Gestational age at the time of PE onset	IUGR	Type of PE	Parity	MoM FLT1	MoM Endoglin	MoM TGFβ1
1:2	IB	25+3	Yes	Severe	0000	2.61	2.58	4.12
1:2	CV	30+2	Yes	Severe	0000	1.97	2.09	2.21
1:2	AB	31+5	No	Mild	0000	3.43	1.95	2.18
1:2	FD	30+3	No	Severe	1001	5.47	2.17	1.91
1:2	ER	37+5	No	Mild	0010	4.29	3.23	2.62
1:2	EB	36+0	No	Mild	2002	2.08	2.04	1.66
1:3	JC	35+2	Yes	Severe	0010	2.48	3.22	2.13
1:3	GD	37+3	Yes	Severe	0000	2.18	2.02	1.61
1:7	JP	35+2	No	Mild	0010	2.19	2.43	0.91
1:21	SE	32+6	No	Mild	0020	0.69	1.91	1.82
1:51	CB	33+5	No	Mild	0000	1.36	1.78	1.04

FLT1, fms-related tyrosine kinase 1; *IUGR*, intrauterine growth restriction; *TGFβ1*, transforming growth factor β1.
Farina. Prediction of preeclampsia by means of circulating placental mRNA at 10-14 weeks of gestation. Am J Obstet Gynecol 2010.

lial nitric oxide synthase and vasodilation, which suggests that dysregulated TGFβ1 signaling may be involved in the pathogenesis of preeclampsia. TGFβ1 has also been associated with the prediction of preeclampsia, but with discordant results, being reported both higher or lower in preeclampsia cases.[24,25]

In previous studies on chorionic villous sampling, we proved for the first time that the pathophysiologic alterations that are involved in preeclampsia that have been identified as mRNA species aberrations start from the trophoblasts long before the clinical onset.[14]

Only 2 previous studies have reported so far the detection rate of mRNA for FLT1 and endoglin at 15-20 weeks' gestation.[13,26] In maternal blood, the detection rate with a 5% false-positive rate was a bit higher than in cellular components (PAX method) for FLT1 (58% vs 43%) but were quite similar for endoglin (43% vs 47%). Even if there is no evidence to demonstrate which source of samples is better for the screening of preeclampsia, we have chosen mononuclear fraction because, based on a previous result, it seems a bit better than whole blood in the detection of preeclamptic patients. The plasma method, instead, so far has been reported more extensively for the diagnosis and screening of fetal genetic diseases because it is more specific in the detection of fetal- and or placental-specific mRNAs sequences. Again, the plasma method needs a more difficult and sophisticated approach, which is not properly available in our laboratories.

Several other mRNA species (eg, plasminogen activator inhibitor-1 selectin P tissue plasminogen activator vascular endothelial growth factor placenta-specific protein 1 heme oxygenase 1 PlGF) have been investigated at 15-20 weeks' gestation, but the detection rate of all of them was <30%. All of them, however, contributed to discriminating preeclampsia cases from control subjects.

In this study, we moved the screening from 15-20 to 11-14 weeks' gestation by using FLT1, endoglin, TGFβ1, and parity. This is a novel result that allows a direct and clear comparison with the risk estimation at the first trimester that has been described by other groups by means of Doppler imaging, biochemical markers, and maternal history.[17-20] The combination of factors that we described yielded a good detection rate and a risk estimation that is realistic. In fact, the risk modification (10-fold reduction and 20-fold increase in preeclampsia risk in control subjects and in cases, respectively) is to be expected in a screening protocol. Again, the enrollment of a population with intrauterine growth restriction, without preeclampsia and/or gestational hypertension, would better define the detection rate and the false-positive rate of the mRNA markers and would also make the risk estimation even more realistic.

In conclusion, we have demonstrated that a panel of mRNA levels in the cellular components of blood from first-trimester pregnant women can be used to predict preeclampsia. Among the genes that were analyzed, antiangiogenic genes (such as FLT1 and endoglin) were the best markers to predict the subsequent onset of preeclampsia. These findings indicate that these factors play a crucial role in the pathogenesis of preeclampsia and that the alteration has started and can be assessed by the first trimester. ■

REFERENCES

1. Walker JJ. Pre-eclampsia. Lancet 2000;356:1260-5.
2. De Groot CJ, Bloemenkamp KW, Duvekot EJ, et al. Preeclampsia and genetic risk factors for thrombosis: a case-control study. Am J Obstet Gynecol 1999;181:975-80.
3. Myatt L, Miodovnik M. Prediction of preeclampsia. Semin Perinatol 1999;23:45-57.
4. Lambert-Messerlian GM, Silver HM, Petraglia F, et al. Second-trimester levels of maternal serum human chorionic gonadotropin and inhibin a as predictors of preeclampsia in the third trimester of pregnancy. J Soc Gynecol Investig 2000;7:170-4.
5. Halligan A, Bonnar J, Sheppard B, Darling M, Walshe J. Haemostatic, fibrinolytic and endothelial variables in normal pregnancies and pre-eclampsia. BJOG 1994;101:488-92.
6. Ballegeer V, Spitz B, Kieckens L, Moreau H, Van Assche A, Collen D. Predictive value of increased plasma levels of fibronectin in gestational hypertension. Am J Obstet Gynecol 1989;161:432-6.
7. Cotter AM, Molloy AM, Scott JM, Daly SF. Elevated plasma homocysteine in early pregnancy: a risk factor for the development of severe preeclampsia. Am J Obstet Gynecol 2001;185:781-5.
8. Clausen T, Djurovic S, Brosstad FR, Berg K, Henriksen T. Altered circulating levels of adhesion molecules at 18 weeks' gestation among women with eventual preeclampsia: indicators of disturbed placentation in absence of evidence of endothelial dysfunction? Am J Obstet Gynecol 2000;182:321-5.
9. Chien PF, Arnott N, Gordon A, Owen P, Khan KS. How useful is uterine artery Doppler flow velocimetry in the prediction of pre-eclampsia, intrauterine growth retardation and perinatal death? An overview. BJOG 2000;107:196-208.
10. Purwosunu Y, Sekizawa A, Okazaki S, et al. Prediction of preeclampsia by analysis of cell-free messenger RNA in maternal plasma. Am J Obstet Gynecol 2009;200:386.e1-7.
11. Okazaki S, Sekizawa A, Purwosunu Y, Iwasaki M, Farina A, Okai T. Measurement of mRNA of trophoblast-specific genes in cellular and plasma components of maternal blood. J Med Genet 2006;43:e47
12. Collett D. Modelling binary data. New York, NY: Chapman & Hall; 1999.
13. Shimizu H, Sekizawa A, Purwosunu Y, et al. PP13 mRNA expression in the cellular component of maternal blood as a marker for pre-eclampsia. Prenat Diagn 2009;29:1231-6.
14. Farina A, Sekizawa A, De Sanctis P, et al. Gene expression in chorionic villous samples at 11 weeks' gestation from women destined to develop preeclampsia. Prenat Diagn 2008;28:956-61.
15. Papageorghiou AT, Yu CK, Nicolaides KH. The role of uterine artery Doppler in predicting adverse pregnancy outcome. Best Pract Res Clin Obstet Gynaecol 2004;18:383-96.
16. Conde-Agudelo A, Villar J, Lindheimer M. World Health Organization systematic review of screening tests for preeclampsia. Obstet Gynecol 2004;104:1367-91.
17. Akolekar R, Etchegaray A, Zhou Y, Maiz N, Nicolaides KH. Maternal serum activin at 11-13 weeks of gestation in hypertensive disorders of pregnancy. Fetal Diagn Ther 2009;25:320-7.
18. Poon LC, Staboulidou I, Maiz N, Plasencia W, Nicolaides KH. Hypertensive disorders in pregnancy: screening by uterine artery Doppler at 11-13 weeks. Ultrasound Obstet Gynecol 2009;34:142-8.
19. Poon LC, Kametas NA, Maiz N, Akolekar R, Nicolaides KH. First-trimester prediction of hypertensive disorders in pregnancy. Hypertension 2009;53:812-8.
20. Onwudiwe N, Yu CK, Poon LC, Spiliopoulos I, Nicolaides KH. Prediction of pre-eclampsia by a combination of maternal history, uterine artery Doppler and mean arterial pressure. Ultrasound Obstet Gynecol 2008;32:877-83.
21. Levine RJ, Lam C, Qian C, et al. Soluble endoglin and other circulating antiangiogenic factors in preeclampsia. N Engl J Med 2006;355:992-1005.
22. Levine RJ, Maynard SE, Qian C, et al. Circulating angiogenic factors and the risk of preeclampsia. N Engl J Med 2004;350:672-83.
23. Venkatesha S, Toporsian M, Lam C, et al. Soluble endoglin contributes to the patho-

genesis of preeclampsia. Nat Med 2006;12: 642-9.
24. Enquobahrie DA, Williams MA, Qiu C, Woelk GB, Mahomed K. Maternal plasma transforming growth factor-beta1 concentrations in preeclamptic and normotensive pregnant Zimbabwean women. J Matern Fetal Neonatal Med 2005;17:343-8.
25. Lim JH, Kim SY, Park SY, Yang JH, Kim MY, Ryu HM. Effective prediction of preeclampsia by a combined ratio of angiogenesis-related factors. Obstet Gynecol 2008;111:1403-9.
26. Sekizawa A, Purwosunu Y, Farina A, et al. Prediction of preeclampsia by an analysis of placenta-derived cellular mRNA in the blood of pregnant women at 15-20 weeks of gestation. BJOG 2010;117:557-64.

Parental longevity and offspring's home blood pressure: the Ohasama study

Yumiko Watanabe[a,e], Hirohito Metoki[a,b,f], Takayoshi Ohkubo[c,f], Takuo Hirose[a,f], Masahiro Kikuya[a], Kei Asayama[f], Ryusuke Inoue[d], Azusa Hara[a,f], Taku Obara[a,f], Haruhisa Hoshi[g], Kazuhito Totsune[a,f] and Yutaka Imai[a,f]

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.**

Journal of Hypertension 2010, 28:272–277

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

[a]Department of Clinical Pharmacology and Therapeutics, [b]Department of Medical Genetics, [c]Department of Planning for Drug Development and Clinical Evaluation, [d]Department of Medical Informatics, Tohoku University Graduate School of Pharmaceutical Sciences and Medicine, [e]Tohoku University Institute for International Advanced Research and Education, [f]Tohoku University 21st Century COE Program 'Comprehensive Research and Education Center for Planning of Drug Development and Clinical Evaluation', Sendai and [g]Ohasama Hospital, Hanamaki, Japan

Correspondence to Takayoshi Ohkubo, MD, PhD, Department of Planning for Drug Development and Clinical Evaluation, Clinical Pharmacology and Therapeutics, Tohoku University Hospital, 1-1 Seiryo-cho, Aoba-ku, Sendai, Miyagi 980-8574, Japan
Tel: +81 22 795 4528; fax: +81 22 795 4532;
e-mail: tohkubo@mail.tains.tohoku.ac.jp

Received 22 May 2009 Revised 31 August 2009
Accepted 7 September 2009

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

0263-6352 © 2010 Wolters Kluwer Health | Lippincott Williams & Wilkins DOI:10.1097/HJH.0b013e328332fa78

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

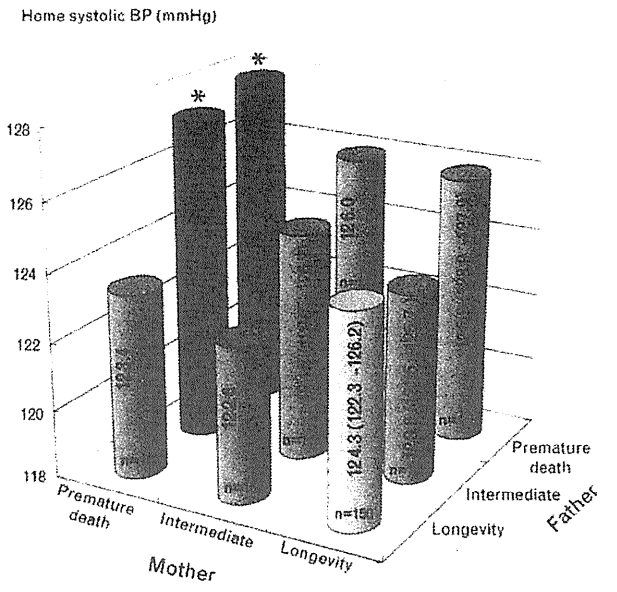
Data are given as mean ± SD or percentage of offspring. Statistical significance among three groups was compared using the ANOVA for continuous variables and the chi-squared test for categorical variables. Definitions of hypertension: home BP, systolic BP ≥ 135 mmHg and/or diastolic BP ≥ 85 mmHg or taking 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.

Acknowledgements

The work was supported by Grants for Scientific Research (15790293, 16590433, 17790381, 17790381, 18390192, 18590587, 19590929 and 19790423) from the Ministry of Education, Culture, Sports, Science, and Technology, Japan; a Grant-in-Aid (H17-Kenkou-007, H18-Junkankitou [Seishuu]-Ippan-012, and H20-Junkankitou [Seishuu]-Ippan-009, 013) from the Ministry of Health, Labor and Welfare, Health and Labor Sciences Research Grants, Japan; a Grant-in-Aid for Japan Society for the Promotion of Science (JSPS) fellows (16.54041, 18.54042, 19.7152, 20.7198, 20.7477 and 20.54043), Tokyo, Japan; Health Science Research Grants and Medical Technology Evaluation Research Grants from the Ministry of Health, Labor and Welfare, Japan; Japan Atherosclerosis Prevention Fund; the Uehara Memorial Foundation; the Takeda Medical Research Foundation; National Cardiovascular Research Grants; and Biomedical Innovation Grants.

There are no conflicts of interest.

References

1 Perls TT, Wilmoth J, Levenson R, Drinkwater M, Cohen M, Bogan H, *et al.* Life-long sustained mortality advantage of siblings of centenarians. *Proc Natl Acad Sci U S A* 2002; **99**:8442–8447.
2 Lifton RP, Gharavi AG, Geller DS. Molecular mechanisms of human hypertension. *Cell* 2001; **104**:545–556.
3 Staessen JA, Wang J, Bianchi G, Birkenhager WH. Essential hypertension. *Lancet* 2003; **361**:1629–1641.
4 Lewington S, Clarke R, Qizilbash N, Peto R, Collins R. Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies. *Lancet* 2002; **360**:1903–1913.
5 Imai Y, Satoh H, Nagai K, Sakuma M, Sakuma H, Minami N, *et al.* Characteristics of a community-based distribution of home blood pressure in Ohasama in northern Japan. *J Hypertens* 1993; **11**:1441–1449.
6 Grossman E, Messerli FH, Boyko V, Goldbourt U. Is there an association between hypertension and cancer mortality? *Am J Med* 2002; **112**:479–486.
7 Zureik M, Galan P, Bertrais S, Courbon D, Czernichow S, Blacher J, *et al.* Parental longevity and 7-year changes in blood pressures in adult offspring. *Hypertension* 2005; **46**:287–294.
8 Sakuma M, Imai Y, Nagai K, Watanabe N, Sakuma H, Minami N, *et al.* Reproducibility of home blood pressure measurements over a 1-year period. *Am J Hypertens* 1997; **10 (7 Pt 1)**:798–803.
9 Ohkubo T, Imai Y, Tsuji I, Nagai K, Kato J, Kikuchi N, *et al.* Home blood pressure measurement has a stronger predictive power for mortality than does screening blood pressure measurement: a population-based observation in Ohasama, Japan. *J Hypertens* 1998; **16**:971–975.
10 Ohkubo T. Prognostic significance of variability in ambulatory and home blood pressure from the Ohasama study. *J Epidemiol* 2007; **17**:109–113.
11 Imai Y, Otsuka K, Kawano Y, Shimada K, Hayashi H, Tochikubo O, *et al.* Japanese society of hypertension (JSH) guidelines for self-monitoring of blood pressure at home. *Hypertens Res* 2003; **26**:771–782.
12 Parati G, Stergiou GS, Asmar R, Bilo G, de Leeuw P, Imai Y, *et al.* European Society of Hypertension guidelines for blood pressure monitoring at home: a summary report of the Second International Consensus Conference on Home Blood Pressure Monitoring. *J Hypertens* 2008; **26**:1505–1526.
13 Statistics and Information Department, Minister's Secretariat, Ministry of Health, Labour and Welfare. The 20th life tables. Tokyo: Health and Welfare Statistics Association; 2007.
14 Nakayama M, Metoki H, Terawaki H, Ohkubo T, Kikuya M, Sato T, *et al.* Kidney dysfunction as a risk factor for first symptomatic stroke events in a general Japanese population: the Ohasama study. *Nephrol Dial Transplant* 2007; **22**:1910–1915.
15 Imai Y, Abe K, Sasaki S, Minami N, Munakata M, Sakuma H, *et al.* Clinical evaluation of semiautomatic and automatic devices for home blood pressure measurement: comparison between cuff-oscillometric and microphone methods. *J Hypertens* 1989; **7**:983–990.
16 Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL Jr, *et al.* Seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. *Hypertension* 2003; **42**:1206–1252.
17 Mancia G, De Backer G, Dominiczak A, Cifkova R, Fagard R, Germano G, *et al.* 2007 Guidelines for the Management of Arterial Hypertension: The Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). *J Hypertens* 2007; **25**:1105–1187.
18 Ogihara T, Kikuchi K, Matsuoka H, Fujita T, Higaki J, Horiuchi M, *et al.* The Japanese Society of Hypertension Guidelines for the Management of Hypertension (JSH 2009). *Hypertens Res* 2009; **32**:3–107.
19 Ohkubo T, Asayama K, Kikuya M, Metoki H, Hoshi H, Hashimoto J, *et al.* How many times should blood pressure be measured at home for better prediction of stroke risk? Ten-year follow-up results from the Ohasama study. *J Hypertens* 2004; **22**:1099–1104.
20 Hammond EC, Garfinkel L, Seidman H. Longevity of parents and grandparents in relation to coronary heart disease and associated variables. *Circulation* 1971; **43**:31–44.
21 Terry DF, Wilcox M, McCormick MA, Lawler E, Perls TT. Cardiovascular advantages among the offspring of centenarians. *J Gerontol* 2003; **58**:M425–M431.
22 Yarnell J, Yu S, Patterson C, Cambien F, Arveiler D, Amouyel P, *et al.* Family history, longevity, and risk of coronary heart disease: the PRIME Study. *Int J Epidemiol* 2003; **32**:71–77.
23 Hunt SC, Williams RR, Barlow GK. A comparison of positive family history definitions for defining risk of future disease. *J Chronic Dis* 1986; **39**:809–821.
24 Wang NY, Young JH, Meoni LA, Ford DE, Erlinger TP, Klag MJ. Blood pressure change and risk of hypertension associated with parental hypertension: the Johns Hopkins Precursors study. *Arch Internal Med* 2008; **168**:643–648.
25 Murabito JM, Nam BH, D'Agostino RB Sr, Lloyd-Jones DM, O'Donnell CJ, Wilson PW. Accuracy of offspring reports of parental cardiovascular disease history: the Framingham Offspring Study. *Ann Internal Med* 2004; **140**:434–440.

Hypertension Research (2010) 33, 652–656

www.nature.com/hr

REVIEW SERIES

Diurnal blood pressure variation and cardiovascular prognosis in a community-based study of Ohasama, Japan

Diurnal variations in blood pressure: clinical implications and pathogenesis

Hirohito Metoki[1,2], Takayoshi Ohkubo[3] and Yutaka Imai[1]

The introduction of 24-h ambulatory blood pressure (BP) monitoring has enabled BP evaluations at specific times of the day. Associations between diurnal BP variation and cardiovascular prognosis have been investigated in the Ohasama study, which is an epidemiological survey of hypertension using ambulatory and home BP monitoring that has been ongoing since 1985 in the general population of Ohasama, a town located in northern Japan. A diminished nocturnal decline in systolic BP was associated with a greater common carotid intima-media thickness as well as a higher risk of cardiovascular morbidity and mortality, especially the risk for cerebral infarction. The consumption of large amounts of alcohol was associated with a higher morning pressor surge. A large nocturnal decline in BP and a large morning pressor surge were both associated with a risk of cerebral hemorrhage. Ambulatory BP monitoring provides not only static, but also dynamic information about BP that should be considered to ensure effective management of hypertension and cardiovascular diseases.
Hypertension Research (2010) **33**, 652–656; doi:10.1038/hr.2010.70; published online 30 April 2010

Keywords: ambulatory blood pressure monitoring; morning pressor surge; nocturnal decline in blood pressure

INTRODUCTION

High blood pressure (BP) is associated with target-organ damage and a poor cardiovascular prognosis. The introduction of 24-h ambulatory BP monitoring has enabled BP to be evaluated at specific times of day. A single BP value obtained using an ambulatory device on rising in the morning is a better discriminator of future cardiovascular events than the mean of three measurements taken under standardized conditions in a hospital or clinic.[1] Several recently proposed indices of circadian BP variation might be relevant to the diagnosis and management of hypertension with special reference to target-organ damage and prognosis. The present review describes diurnal BP variation and cardiovascular prognosis from the results of the Ohasama study, which is an epidemiological survey of hypertension based on ambulatory BP monitoring that was started in 1985 among the general population of Ohasama, a town located in northern Japan.

STUDY POPULATION

Ohasama had a population of 9400 in 1985. We have obtained ambulatory BP data over the past 20 years by monitoring over 3000 inhabitants aged $\geqslant$20 years, as well as outcomes and information about risk factors and predictors. To prospectively investigate the association between BP levels and subsequent risk of outcomes, we excluded individuals aged $<$40 years at the time of ambulatory BP monitoring because death or stroke occurrence was less frequent among younger persons. Thus, several indexes of BP obtained by ambulatory monitoring were prospectively analyzed among 1542 inhabitants of Ohasama aged $\geqslant$40 years.

NOCTURNAL DECLINE IN BP

BP generally increases on awakening in the morning and falls while asleep during the nighttime. This circadian variation in BP is regulated by the autonomic nervous and endocrine systems, and modified by several factors such as physical and mental activities as well as environmental stressors. Nocturnal BP usually falls 10–20% from the diurnal value and is referred to as nocturnal dipping. However, nocturnal dipping is attenuated or disappears under several pathophysiological conditions and persons with this phenomenon are referred to as 'non-dippers.' Those with higher nocturnal BP than the diurnal value are referred to as 'inverted dippers' or 'risers.' A person with a large nocturnal decline in BP is defined as an 'extreme

[1]Department of Clinical Pharmacology and Therapeutics, Tohoku University Graduate School of Pharmaceutical Sciences and Medicine, Sendai, Japan; [2]Department of Medical Genetics, Tohoku University Graduate School of Pharmaceutical Sciences and Medicine, Sendai, Japan and [3]Department of Planning for Drug Development and Clinical Evaluation, Tohoku University Graduate School of Pharmaceutical Sciences and Medicine, Sendai, Japan
Correspondence: Dr Y Imai, Department of Clinical Pharmacology and Therapeutics, Tohoku University Hospital, 1-1 Seiryo-cho, Aoba-ku, Sendai, Miyagi 980-8574, Japan.
E-mail: rinsyo@mail.pharm.tohoku.ac.jp
Received 7 February 2010; revised 10 March 2010; accepted 28 March 2010; published online 30 April 2010

dipper.' However, nocturnal BP remains normal or high among hypertensive patients. Therefore, 'extreme dippers' among hypertensive patients are equivalent to 'diurnal risers.'[2] Such disordered circadian BP variations are associated with a poor cardiovascular prognosis[3–6] and are regarded as targets for antihypertensive therapy.

The decline in nocturnal BP was calculated in the Ohasama study as follows: nocturnal decline in BP (%)=(daytime BP−nighttime BP)×100/daytime BP. We classified the subtypes of nocturnal decline in BP as follows: extreme dipper (20% nocturnal decline in BP from diurnal value), dipper (10–19% nocturnal decline in BP), non-dipper (0–9% nocturnal decline in BP) and riser (0% nocturnal decline in BP or nocturnal elevation).

NOCTURNAL DECLINE IN BP AND CARDIOVASCULAR MORTALITY

We reported the association between ambulatory BP and cardiovascular prognosis in 1997. That report describes that ambulatory BP predicted mortality more effectively than casual screening of BP during a mean follow-up period of 5.1 years.[7] During the same observation period, the mortality risk was highest among risers, followed by non-dippers. Mortality rates did not differ between extreme dippers and dippers. This relationship in both treated and untreated individuals was more remarkable for cardiovascular, than for non-cardiovascular mortality, and was not changed after adjustment for 24-h, daytime and nighttime BP levels.[8] Follow-up for a mean of 9.2 years showed that a diminished nocturnal decline in BP was associated with a risk for cardiovascular mortality, which was independent of the overall BP load during a 24-h period.[9]

NOCTURNAL DECLINE IN BP AND RISK OF STROKE

Analysis of data over a mean follow-up period of 10.4 years revealed no consistent association between dipping profile and the risk of total stroke. The data did not fit a linear model; the relative hazard per 1 s.d. increase of nocturnal decline in BP was 1.1 (95% CI, 0.8–1.4, P=0.7). The risk for cerebral infarction was significantly higher among individuals with a diminished nocturnal decline (risers and non-dippers) than among those with a decline of $\geqslant$10% (dippers and extreme dippers). The relative hazard among individuals with a diminished nocturnal decline was 1.6 (95% CI, 1.0–2.5, P=0.04). Extreme dippers had 2.7-fold higher risk (95% CI, 1.1–6.4, P=0.02) of cerebral hemorrhage than those with a nocturnal decline of <20% (dippers, non-dippers and risers).

MORNING BP SURGE

BP that abruptly increases around awakening in the morning is called the 'morning pressor surge.'[10,11] The association between morning BP surge and cardiovascular disease has received focus because cardiovascular events occur more frequently in the morning[12–14] and a mean follow-up of 20 months has revealed that elderly patients with a large morning pressor surge have a threefold higher risk of stroke.[15]

The amplitude of the morning pressor surge in the Ohasama study was defined based on earlier findings[11] as follows: morning pressor surge in systolic blood pressure (SBP)=2-h mean SBP after waking−2-h mean SBP before waking.

The morning pressor surge can also be calculated by a method that generates the 'sleep-trough' morning pressor surge,[15] which is calculated as follows: sleep-trough morning pressor surge in SBP=2-h mean SBP after waking−lowest SBP defined as mean BP of three readings centered on the lowest nighttime reading.

An association between morning pressor surge and the incidence of total stroke (n=128) and of cerebral infarction (n=86) was not identified during a 10.4-year follow-up. However, the risk for cerebral hemorrhage (n=27) was significantly high in the fifth quintile group with a morning pressor surge amplitude of 25 mm Hg (RH, 4.0; 95% CI, 1.1–14.6, P=0.04), when the second quintile of the morning pressor surge (amplitude 3–11 mm Hg) was set as the reference category.[16] The predictive value of the sleep-trough morning surge was similar to that of the sleeping-to-waking morning surge in the same population.[16]

The International Database of Ambulatory Blood Pressure in relation to Cardiovascular Outcome has recently been established.[17,18] A morning surge in BP exceeding the 90th percentile in this database was a significant and independent predictor of mortality and cardiovascular events even after correcting the night-to-day BP ratio, the 24-h BP level and other covariables.[19] Moreover, consistent with our earlier findings,[16] Asians with a morning surge in the top decile were at a significantly higher risk for hemorrhagic stroke (n=51; HR [95% CI], 2.28 [1.09–4.26], P=0.03), but not for ischemic stroke (n=127; HR, 1.41 [0.67–2.98], P=0.37), than those with a lower morning surge.

BP AT A SPECIFIC TIME OF DAY

Although the predictive value of BP increases with increasing numbers of measurements,[20,21] BP values obtained at different times of the day (nighttime, morning and daytime) have not been compared with values obtained by the same number of measurements. A simple calculated mean of the BP values recorded every 30 min during the nighttime (8 h) generates 16 values; similarly, a simple mean of the daytime (16 h) BP values recorded every 30 min yields 28 values. Therefore, if the predictive power of BP obtained during the daytime was more powerful than taken during the nighttime, it could reflect the larger number of measurements taken during the daytime.

The means of four BP readings obtained every 30 min for two consecutive hours in a day (moving averages) during the Ohasama study were defined as '2h-BP' (see Figure 1).[22] When readings were omitted because of missed and/or artifactual measurements, calculations were based on the remaining readings (minimum of one) obtained during the 2-h period. The 2h-BP allows a comparison of

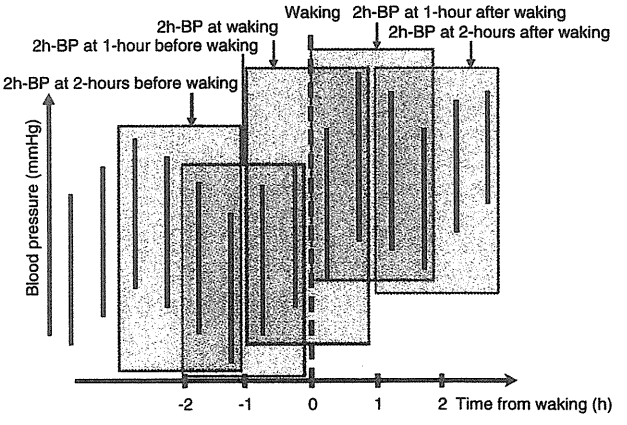


Figure 1 Definition of moving average of 2-h SBP/2-h DBP. Means of four SBP/DBP readings obtained over two consecutive daytime hours (moving averages) are defined as 2h-SBP/2h-DBP. Horizontal line indicates time (hours) from waking. Vertical line shows BP (mm Hg). Reproduced from our earlier article22 with permission from the Lippincott Williams & Wilkins.

the predictive value of BP taken at different times using the same number of BP measurements.

BP AT A SPECIFIC TIME OF DAY AND CARDIOVASCULAR MORTALITY

When nighttime and daytime SBP values were simultaneously included in the same Cox model, only nighttime BP significantly predicted cardiovascular mortality risk from the 10.8-year follow-up data. We concluded that the relationship between ambulatory SBP and cardiovascular mortality is not U- or J-shaped, and that the prognostic value of BP during the nighttime is better than that during the daytime.[23] We applied 2h-BP to evaluate the relevance of BP at a particular time of day to the risk of stroke mortality. Total cerebrovascular and cardiovascular mortality risk was significantly associated with elevated 2h-BP recorded during the night and early morning. Hemorrhagic stroke mortality was significantly associated with elevated daytime 2h-BP. The mortality of cerebral infarction and heart disease was significantly associated with elevated nighttime 2h-BP.[22]

BP AT A SPECIFIC TIME OF DAY AND RISK OF STROKE

The risk of stroke incidence in the Ohasama study was more closely associated with daytime, than with nighttime BP over a mean follow-up of 6.4 years.[24]

Using 2h-BP to evaluate the relevance of BP taken at a specific time of day to determine the risk of stroke morbidity showed that risk for total stroke incidence was significantly associated with systolic 2h-BP values (2h-SBPs) throughout the day (Figure 2). Risk for incidence of intracerebral hemorrhage was significantly associated with elevated daytime 2h-SBPs, but less so with nighttime 2h-SBPs (Figure 3a). Risk for the incidence of cerebral infarction was significantly associated with nighttime 2h-SBPs, but less so with daytime 2h-SBPs (Figure 3b).

DIURNAL BP VARIATION AND TARGET-ORGAN DAMAGE

Cross-sectional analyses regarding target-organ damage were performed during the Ohasama study. Nighttime BP was most closely associated with carotid artery alterations among values for daytime, nighttime and casual BP. Although a morning pressor surge was not associated with carotid artery alterations, a diminished nocturnal decline in SBP was associated with common carotid intima-media thickness after adjustment for confounding factors.[25] Daytime and nighttime BP values were both associated with silent cerebrovascular lesions, whereas casual BP in the same population was not.[26]

FACTORS ASSOCIATED WITH DIURNAL BP VARIATION

Alcohol consumption and diurnal BP variation

We found, using 2h-BP, that BP rapidly increased before awakening and that morning BP was higher among Ohasama inhabitants who consumed alcohol. The morning pressor surge was significantly higher among those who consumed large amounts of alcohol than in those who consumed none, whereas alcohol consumption status was not significantly associated with the magnitude of the nocturnal decline in BP.[27]

Genetic polymorphisms and diurnal BP variation

Daytime SBP and diastolic blood pressure (DBP) values were higher in individuals with the C allele in the angiotensin II type 1 receptor gene A/C^{1166} polymorphism (130.5 ± 14.0/77.4 ± 8.3 mm Hg) than in those with the AA genotype (127.7 ± 13.6/75.8 ± 8.3 mm Hg, P=0.03/0.04), although the difference was not statistically significant after adjusting for age, gender, body mass index and smoking status.[28] Nighttime BP values were significantly lower among individuals with the MM genotype in the angiotensinogen M235T polymorphism than in those with the T allele (105.2 ± 13.0/60.1 ± 6.9 *vs.* 110.6 ± 13.3/63.6 ± 7.8 mm Hg, P=0.04/0.02). The nocturnal decline in BP was significantly larger among those with the MM genotype than with the T allele (17.4/19.8 *vs.* 13.5/16.2 mm Hg, P=0.004/0.01).[29] The nocturnal decline in BP was significantly greater among individuals with a homozygous CC aldosterone synthase gene (CYP11B2) C-334T polymorphism than in others (15.4%/17.7% *vs.* 13.3%/16.1%, P=0.007/0.03), although 24-h ambulatory BP levels did not significantly differ among the genotypes.[30]

We recently focused on the (pro)renin receptor gene. Although casual BP was not associated, 24-h, daytime and nighttime SBP and DBP values were significantly higher among male carriers of the IVS5+169T rather than the C allele of the (pro)renin receptor gene. BP values did not significantly differ among the three genotypes of female IVS5+169C>T carriers.[31]

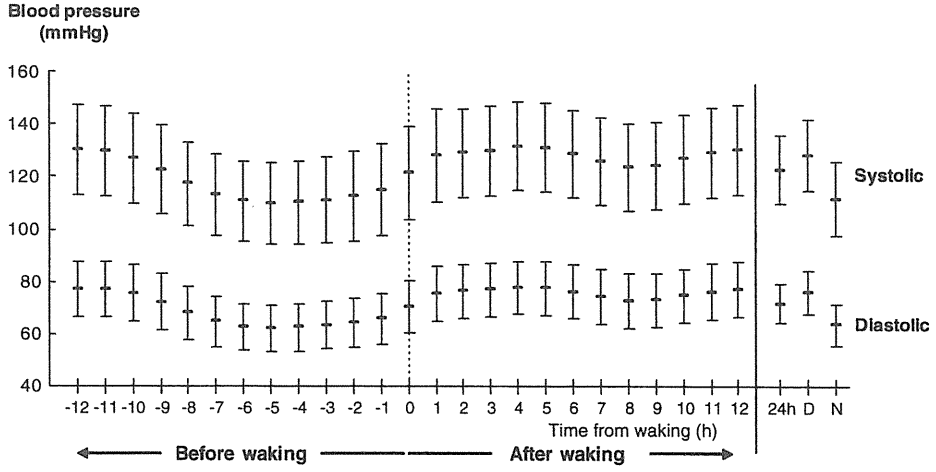


Figure 2 Circadian BP variation of SBP and DBP using 2-h SBP/2-h DBP. Left panel: 2-h moving averages of SBP and DBP over 24-h period based on time (hours) from waking. Right panel: 24-h, daytime and nighttime mean BP values are shown as 24 h, D and N, respectively.

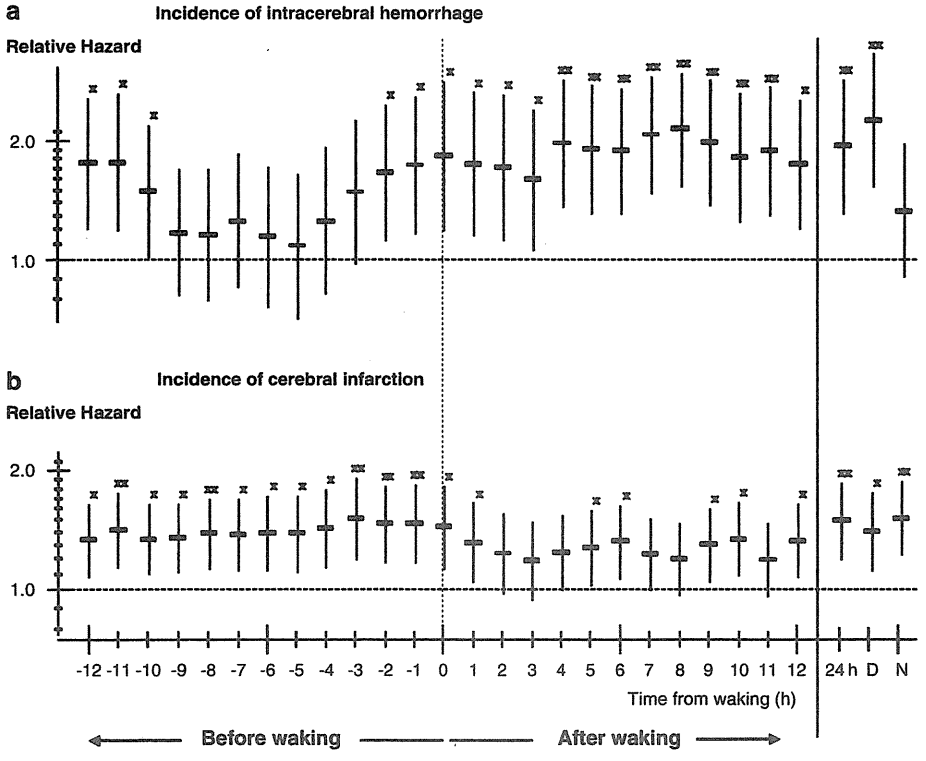


Figure 3 Relative hazard for incidence of stroke subtypes per 1-s.d. elevation of SBP values. Relative hazards and 95% confidence intervals for incidence of (**a**) hemorrhage stroke and (**b**) cerebral infarction per 1-s.d. elevation of SBPs over a mean follow-up of 10.2 years in Ohasama, Japan. Left 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.

ACKNOWLEDGEMENTS

We are grateful to all collaborating mentors, colleagues, foundations and individuals.

1 Gosse P, Cipriano C, Bemurat L, Mas D, Lemetayer P, N'Tela G, Clementy J. Prognostic significance of blood pressure measured on rising. *J Hum Hypertens* 2001; **15**: 413–417.
2 Nishiyama A, Imai Y, Ohkubo T, Tsuji I, Nagai K, Kikuchi N, Kato J, Sekino M, Aihara A, Kikuya M, Satoh H, Hisamichi S. Determinants of circadian blood pressure variation: a community-based study in Ohasama. *Tohoku J Exp Med* 1997; **183**: 1–20.
3 Staessen JA, Thijs L, Fagard R, O'Brien ET, Clement D, de Leeuw PW, Mancia G, Nachev C, Palatini P, Parati G, Tuomilehto J, Webster J. Predicting cardiovascular risk using conventional vs ambulatory blood pressure in older patients with systolic hypertension. systolic hypertension in Europe trial investigators. *JAMA* 1999; **282**: 539–546.
4 Shimada K, Kawamoto A, Matsubayashi K, Ozawa T. Silent cerebrovascular disease in the elderly. Correlation with ambulatory pressure. *Hypertension* 1990; **16**: 692–699.
5 Verdecchia P, Porcellati C, Schillaci G, Borgioni C, Ciucci A, Battistelli M, Guerrieri M, Gatteschi C, Zampi I, Santucci A, Santucci C, Reboldi G. Ambulatory blood pressure. An independent predictor of prognosis in essential hypertension. *Hypertension* 1994; **24**: 793–801.
6 Kario K, Pickering TG, Matsuo T, Hoshide S, Schwartz JE, Shimada K. Stroke prognosis and abnormal nocturnal blood pressure falls in older hypertensives. *Hypertension* 2001; **38**: 852–857.
7 Ohkubo T, Imai Y, Tsuji I, Nagai K, Watanabe N, Minami N, Itoh O, Bando T, Sakuma M, Fukao A, Satoh H, Hisamichi S, Abe K. Prediction of mortality by ambulatory blood pressure monitoring versus screening blood pressure measurements: a pilot study in Ohasama. *J Hypertens* 1997; **15**: 357–364.
8 Ohkubo T, Imai Y, Tsuji I, Nagai K, Watanabe N, Minami N, Kato J, Kikuchi N, Nishiyama A, Aihara A, Sekino M, Satoh H, Hisamichi S. Relation between nocturnal decline in blood pressure and mortality. The Ohasama study. *Am J Hypertens* 1997; **10**: 1201–1207.
9 Ohkubo T, Hozawa A, Yamaguchi J, Kikuya M, Ohmori K, Michimata M, Matsubara M, Hashimoto J, Hoshi H, Araki T, Tsuji I, Satoh H, Hisamichi S, Imai Y. Prognostic significance of the nocturnal decline in blood pressure in individuals with and without high 24-h blood pressure: the Ohasama study. *J Hypertens* 2002; **20**: 2183–2189.
10 DeQuattro V, Lee DD, Allen J, Sirgo M, Plachetka J. Labetalol blunts morning pressor surge in systolic hypertension. *Hypertension* 1988; **11**: I198–I201.
11 Gosse P, Lasserre R, Minifie C, Lemetayer P, Clementy J. Blood pressure surge on rising. *J Hypertens* 2004; **22**: 1113–1118.
12 Muller JE, Stone PH, Turi ZG, Rutherford JD, Czeisler CA, Parker C, Poole WK, Passamani E, Roberts R, Robertson T, Sobel BE, Willerson JT, Braunwald E. Circadian variation in the frequency of onset of acute myocardial infarction. *N Engl J Med* 1985; **313**: 1315–1322.
13 Muller JE, Ludmer PL, Willich SN, Tofler GH, Aylmer G, Klangos I, Stone PH. Circadian variation in the frequency of sudden cardiac death. *Circulation* 1987; **75**: 131–138.
14 Tsementzis SA, Gill JS, Hitchcock ER, Gill SK, Beevers DG. Diurnal variation of and activity during the onset of stroke. *Neurosurgery* 1985; **17**: 901–904.
15 Kario K, Pickering TG, Umeda Y, Hoshide S, Hoshide Y, Morinari M, Murata M, Kuroda T, Schwartz JE, Shimada K. Morning surge in blood pressure as a predictor of silent and clinical cerebrovascular disease in elderly hypertensives: a prospective study. *Circulation* 2003; **107**: 1401–1406.
16 Metoki H, Ohkubo T, Kikuya M, Asayama K, Obara T, Hashimoto J, Totsune K, Hoshi H, Satoh H, Imai Y. Prognostic significance for stroke of a morning pressor surge and a nocturnal blood pressure decline: the Ohasama study. *Hypertension* 2006; **47**: 149–154.

17 Kikuya M, Hansen TW, Thijs L, Bjorklund-Bodegard K, Kuznetsova T, Ohkubo T, Richart T, Torp-Pedersen C, Lind L, Ibsen H, Imai Y, Staessen JA. Diagnostic thresholds for ambulatory blood pressure monitoring based on 10-year cardiovascular risk. *Circulation* 2007; **115**: 2145–2152.
18 Boggia J, Li Y, Thijs L, Hansen TW, Kikuya M, Bjorklund-Bodegard K, Richart T, Ohkubo T, Kuznetsova T, Torp-Pedersen C, Lind L, Ibsen H, Imai Y, Wang J, Sandoya E, O'Brien E, Staessen JA. Prognostic accuracy of day versus night ambulatory blood pressure: a cohort study. *Lancet* 2007; **370**: 1219–1229.
19 Li Y, Thijs L, Hansen TW, Kikuya M, Boggia J, Richart T, Metoki H, Ohkubo T, Trop-Pedersen C, Kuznetsova T, Stolarz-Skizypek K, Tikhonoff V, Malyutina S, Casiglia E, Nikitin Y, Sandoya E, Kawecka-Jaszcz K, Ibsen H, Imai Y, Wang J, Staessen J. Prognostic value of the morning blood pressure surge in 5645 subjects from 8 populations. *Hypertension* 2010; **55**: 1040–1048.
20 Fagard RH, Staessen JA, Thijs L. Prediction of cardiac structure and function by repeated clinic and ambulatory blood pressure. *Hypertension* 1997; **29**: 22–29.
21 Ohkubo T, Asayama K, Kikuya M, Metoki H, Hoshi H, Hashimoto J, Totsune K, Satoh H, Imai Y. How many times should blood pressure be measured at home for better prediction of stroke risk? Ten-year follow-up results from the Ohasama study. *J Hypertens* 2004; **22**: 1099–1104.
22 Metoki H, Ohkubo T, Kikuya M, Asayama K, Obara T, Hara A, Hirose T, Hashimoto J, Totsune K, Hoshi H, Satoh H, Imai Y. Prognostic significance of night-time, early morning, and daytime blood pressures on the risk of cerebrovascular and cardiovascular mortality: the Ohasama Study. *J Hypertens* 2006; **24**: 1841–1848.
23 Kikuya M, Ohkubo T, Asayama K, Metoki H, Obara T, Saito S, Hashimoto J, Totsune K, Hoshi H, Satoh H, Imai Y. Ambulatory blood pressure and 10-year risk of cardiovascular and noncardiovascular mortality: the Ohasama study. *Hypertension* 2005; **45**: 240–245.
24 Ohkubo T, Hozawa A, Nagai K, Kikuya M, Tsuji I, Ito S, Satoh H, Hisamichi S, Imai Y. Prediction of stroke by ambulatory blood pressure monitoring versus screening blood pressure measurements in a general population: the Ohasama study. *J Hypertens* 2000; **18**: 847–854.
25 Shintani Y, Kikuya M, Hara A, Ohkubo T, Metoki H, Asayama K, Inoue R, Obara T, Aono Y, Hashimoto T, Hashimoto J, Totsune K, Hoshi H, Satoh H, Imai Y. Ambulatory blood pressure, blood pressure variability and the prevalence of carotid artery alteration: the Ohasama study. *J Hypertens* 2007; **25**: 1704–1710.
26 Aono Y, Ohkubo T, Kikuya M, Hara A, Kondo T, Obara T, Metoki H, Inoue R, Asayama K, Shintani Y, Hashimoto J, Totsune K, Hoshi H, Satoh H, Izumi S, Imai Y. Plasma fibrinogen, ambulatory blood pressure, and silent cerebrovascular lesions: the Ohasama study. *Arterioscler Throm Vasc Biol* 2007; **27**: 963–968.
27 Nakashita M, Ohkubo T, Hara A, Metoki H, Kikuya M, Hirose T, Tsubota-Utsugi M, Asayama K, Inoue R, Kanno A, Obara T, Hoshi H, Totsune K, Satoh H, Imai Y. Influence of alcohol intake on circadian blood pressure variation in Japanese men: the Ohasama study. *Am J Hypertens* 2009; **22**: 1171–1176.
28 Kikuya M, Sugimoto K, Katsuya T, Suzuki M, Sato T, Funahashi J, Katoh R, Kazama I, Michimata M, Araki T, Hozawa A, Tsuji I, Ogihara T, Yanagisawa T, Imai Y, Matsubara M. A/C1166 gene polymorphism of the angiotensin II type 1 receptor (AT1) and ambulatory blood pressure: the Ohasama Study. *Hypertens Res* 2003; **26**: 141–145.
29 Fujiwara T, Katsuya T, Matsubara M, Mikami T, Ishikawa K, Kikuya M, Ohkubo T, Hozawa A, Michimata M, Suzuki M, Metoki H, Asayama K, Araki T, Tsuji I, Higaki J, Satoh H, Hisamichi S, Ogihara T, Imai Y. T+31C polymorphism of angiotensinogen gene and nocturnal blood pressure decline: the Ohasama study. *Am J Hypertens* 2002; **15**: 628–632.
30 Matsubara M, Kikuya M, Ohkubo T, Metoki H, Omori F, Fujiwara T, Suzuki M, Michimata M, Hozawa A, Katsuya T, Higaki J, Tsuji I, Araki T, Ogihara T, Satoh H, Hisamichi S, Nagai K, Kitaoka H, Imai Y. Aldosterone synthase gene (CYP11B2) C-334 T polymorphism, ambulatory blood pressure and nocturnal decline in blood pressure in the general Japanese population: the Ohasama Study. *J Hypertens* 2001; **19**: 2179–2184.
31 Hirose T, Hashimoto M, Totsune K, Metoki H, Asayama K, Kikuya M, Sugimoto K, Katsuya T, Ohkubo T, Hashimoto J, Rakugi H, Takahashi K, Imai Y. Association of (pro)renin receptor gene polymorphism with blood pressure in Japanese men: the Ohasama study. *Am J Hypertens* 2009; **22**: 294–299.

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

Mami Seki[a], Ryusuke Inoue[b], Takayoshi Ohkubo[c], Masahiro Kikuya[a], Azusa Hara[a], Hirohito Metoki[a], Takuo Hirose[c], Megumi Tsubota-Utsugi[d], Kei Asayama[c], Atsuhiro Kanno[a], Taku Obara[a,e], Haruhisa Hoshi[f], Kazuhito Totsune[a], Hiroshi Satoh[g] and Yutaka Imai[a]

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.**

Journal of Hypertension 2010, 28:1814–1820

Keywords: blood pressure, blood pressure monitoring ambulatory, cardiovascular diseases, home blood pressure monitoring, particulate matter, passive smoking

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

[a]Tohoku University Graduate School of Pharmaceutical Sciences and Medicine, the Department of Clinical Pharmacology and Therapeutics, [b]Tohoku University Graduate School of Medicine, the Department of Medical Informatics, [c]Tohoku University Graduate School of Pharmaceutical Science, the Department of Planning for Drug Development and Clinical Evaluation, Sendai, [d]Nutritional Epidemiology Program, National Institute of Health and Nutrition, Tokyo, [e]Department of Pharmaceutical Sciences, Tohoku University Hospital, Sendai, [f]Ohasama Hospital, Iwate and [g]Tohoku University Graduate School of Medicine, the Department of Environmental Health Sciences, Sendai, Japan

Correspondence to Takayoshi Ohkubo, MD, PhD, Department of Planning for Drug Development and Clinical Evaluation, Tohoku University Graduate School of Pharmaceutical Science, 6-3 Aramaki-aza-aoba, Aoba-ku, Sendai 980-8578, Japan
Tel: +81 22 795 4528; fax: +81 22 795 4532;
e-mail: tohkubo@mail.tains.tohoku.ac.jp

Received 27 July 2009 Revised 2 March 2010
Accepted 22 March 2010

See editorial comment on page 1806

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

0263-6352 © 2010 Wolters Kluwer Health | Lippincott Williams & Wilkins DOI:10.1097/HJH.0b013e32833a3911