Table 1 Patient characteristics

Variable	Mean ± SD
n	2287
Age (years)	56 ± 16
Sex (%male)	56
CVD (%)	4.8
Height (cm)	162 ± 9
Body weight (kg)	61 ± 12
Systolic BP (mmHg)	128 ± 19
Diastolic BP (mmHg)	81 ± 13
Total cholesterol (mg/dl)	209 ± 36
LDL-cholesterol (mg/dl)	124 ± 33
HDL-cholesterol (mg/dl)	59 ± 16
Triglyceride (mg/dl)	127 ± 93
Plasma glucose (mg/dl)	103 ± 25
cfPWV (cm/s)	1256 ± 388
baPWV (cm/s)	1484 ± 342
eGFR (ml/min per 1.73 m^2)	79 ± 20

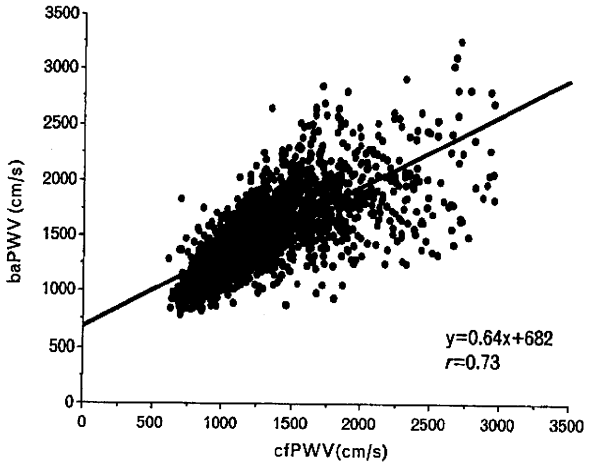
BP, blood pressure; CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate; PWV, pulse wave velocity.

American populations. Both cfPWV and baPWV were significantly and positively associated with age ($r = 0.56$ and 0.64; Fig. 2 and Table 2), systolic blood pressure (SBP) ($r = 0.49$ and 0.61), and the Framingham risk score ($r = 0.48$ and 0.63; Fig. 3). Stepwise multiple regression analyses indicated that the two primary determinants of both cfPWV and baPWV were age and SBP, explaining 43 and 60% of variances associated with cfPWV and baPWV, respectively. Figure 4 shows the results of the cross-sectional analyses involving the ROC of PWV to predict the presence of both stroke and CAD in a cohort of 814 patients (36 strokes and 40 CAD). The areas under the ROC curve for cfPWV and baPWV were comparable in stroke (0.62 and 0.63) and CAD (0.60 and 0.60).

Discussion

Pulse wave velocity is an established index of arterial stiffness and its first clinical use can be traced to Bramwell and the Nobel laureate, A.V. Hill [5]. In the present study, we performed comparative analyses of cfPWV and baPWV in a large number of patients who participated in the multicenter study. First, we demonstrated that baPWV was significantly and positively associated with cfPWV in the pooled population. Second, both PWV measures exhibit similar associations with various risk factors for CAD. Third, the areas under the ROC curve to predict the presence of CAD and stroke were comparable for cfPWV and baPWV. Collectively, these results indicate that cfPWV and baPWV are indices of arterial stiffness that are similarly related to CHD risk factors and predict clinical events to similar extents.

Fig. 1



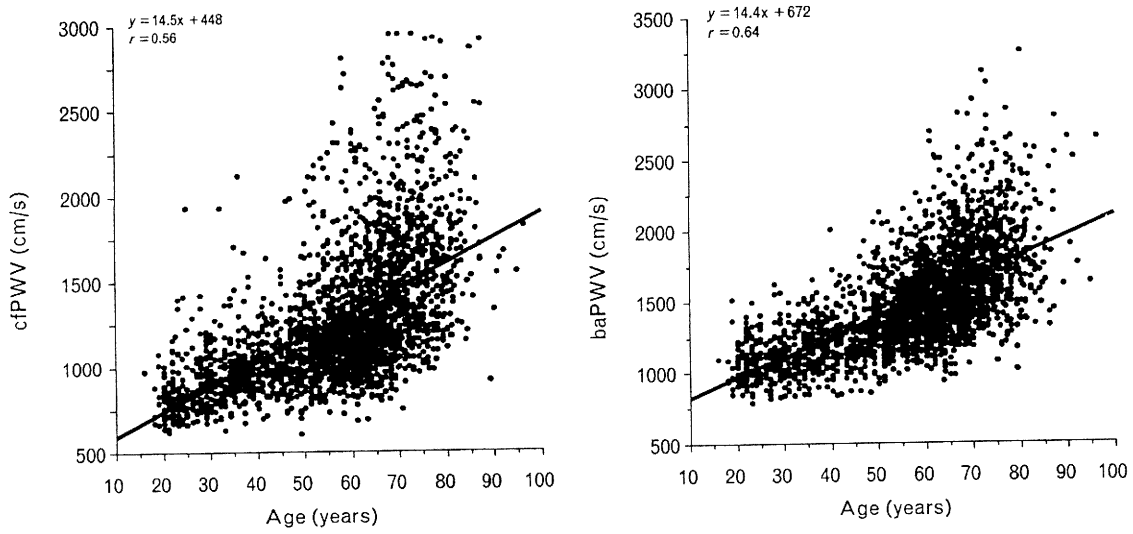
Association between carotid-femoral pulse wave velocity (cfPWV) and brachial-ankle pulse wave velocity (baPWV).

There was a strong positive association between cfPWV and baPWV, suggesting that both measures of PWV are indices of 'central' (or cardiothoracic) artery stiffness. These results are consistent with previous small-scale studies showing that baPWV is more closely associated with the index of central artery stiffness [8,13,19]. We have also previously reported that changes in central artery stiffness induced by a lifestyle modification are closely associated with the corresponding changes in baPWV [8]. Thus, in contrast to the prevailing notion, baPWV appears to reflect central arterial stiffness rather than peripheral artery stiffness. However, the regression line between cfPWV and baPWV deviated from the line of identity. On average, baPWV was approximately 20% higher than cfPWV. This finding indicates that some (albeit small) portions of baPWV may be determined by 'peripheral' (or muscular) arterial stiffness as suggested by a previous study [8].

The comparative assessment and analyses of different indices of arterial stiffness, particularly the comparisons with cfPWV, are becoming increasingly important given the recent European guidelines for the management of arterial hypertension proposing that a cfPWV value of more than 1200 cm/s be used as an index of subclinical organ damage [9]. The regression line obtained in the present study reveals that a baPWV value of 1450 cm/s is equivalent to the threshold value of 1200 cm/s proposed by the European Society of Hypertension and the European Society of Cardiology [9]. Such setting of a threshold PWV value may become a necessity if arterial stiffness measures were to be fully integrated into routine clinical settings.

Carotid-femoral pulse wave velocity has been shown to be accurate, reliable, and relatively simple to use and has been strongly linked with cardiovascular disease [1,2,14]. However, this methodology has not been widely incorporated in the routine clinical settings. The use of pressure transducers or Doppler probes on target arteries may be perceived as somewhat difficult to clinical staff. Additionally, some patients may feel uncomfortable exposing the inguinal area during the acquisition of

Fig. 2



Relation between age and pulse wave velocity (PWV) measures.

femoral pressure waveforms. These trends were particularly evident in generally demure Japanese population and required a development of a novel arterial stiffness index. baPWV has a procedural advantage of being very simple to use, only requiring the wrapping of blood pressure cuffs on four extremities. As a result, it has become a very popular modality to assess arterial stiffness in Japan [13], and it has been incorporated in thousands of local (i.e. nonresearch-oriented) clinics and hospitals.

Brachial-ankle pulse wave velocity has been criticized that the pulse wave does not travel directly from the brachial arteries to the post-tibial arteries in the same arterial tree and that the nomenclature of PWV is inappropriate. However, the same argument can be made for the well established cfPWV. cfPWV measures the velocity of pulse wave from carotid to femoral arteries, and these two arteries are not connected directly in the same arterial tree. Another issue pertaining to cfPWV is that there has been no consensus in terms of how the arterial path length should be measured. In large epidemiological studies from France that yielded the most clinically significant findings on cfPWV [1,20,21], the straight distance between the carotid and femoral arteries was applied. On the contrary, different investigations, including the Framingham Heart Study, employed the subtraction of the carotid artery length from the carotid to femoral straight distance in order to account for the pulse traveling in the opposite direction [22]. Rajzer *et al.* [23] recently compared the values of aortic PWV obtained with different arterial path length measurement: the carotid to femoral straight distance vs. the subtraction of the carotid artery length from the suprasternal notch to femoral straight distance. They reported that PWV measured with the former method was 25% higher compared with that using the latter method. We recently measured the aortic path lengths directly by the three-dimensional transverse magnetic resonance image arterial tracing in 256 apparently healthy adults and found that PWV calculated with the straight distance between carotid and femoral sites 26% overestimated the actual arterial path length [24]. Thus, it should be acknowledged that both cfPWV and baPWV have inherent problems with regard to the measurement of arterial path length.

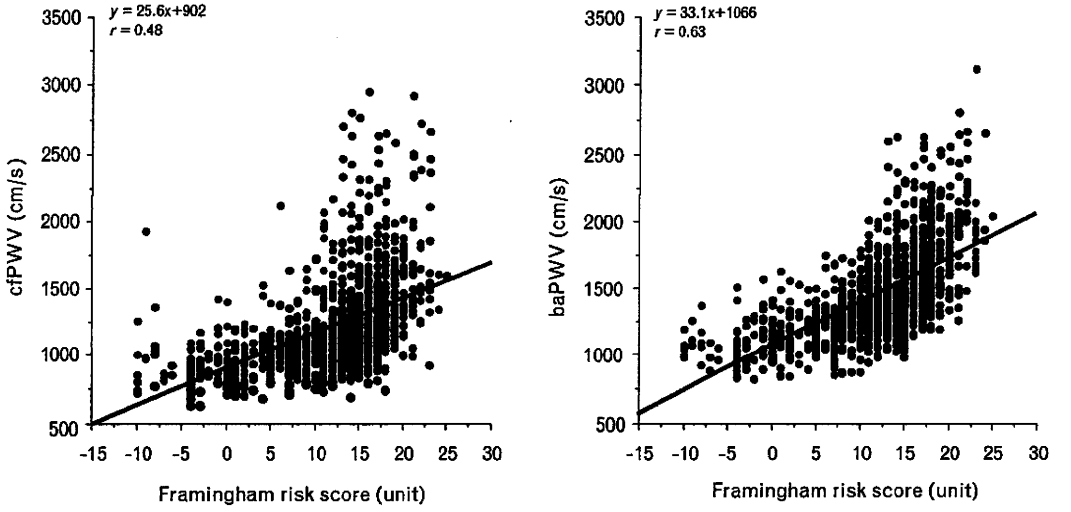
Table 2 Associations between pulse wave velocity (PWV) and risk factors for coronary heart disease

Variable	cfPWV	baPWV
Age	0.56	0.64
Systolic BP	0.49	0.61
Diastolic BP	0.13	0.23
Mean BP	0.48	0.58
Pulse pressure	0.50	0.56
FRS	0.48	0.63
eGFR	−0.32	−0.25

BP, blood pressure; eGFR, estimated glomerular filtration rate; FRS, Framingham risk score; PWV, pulse wave velocity. Values are Pearson correlation coefficients. All are significant at $P < 0.001$.

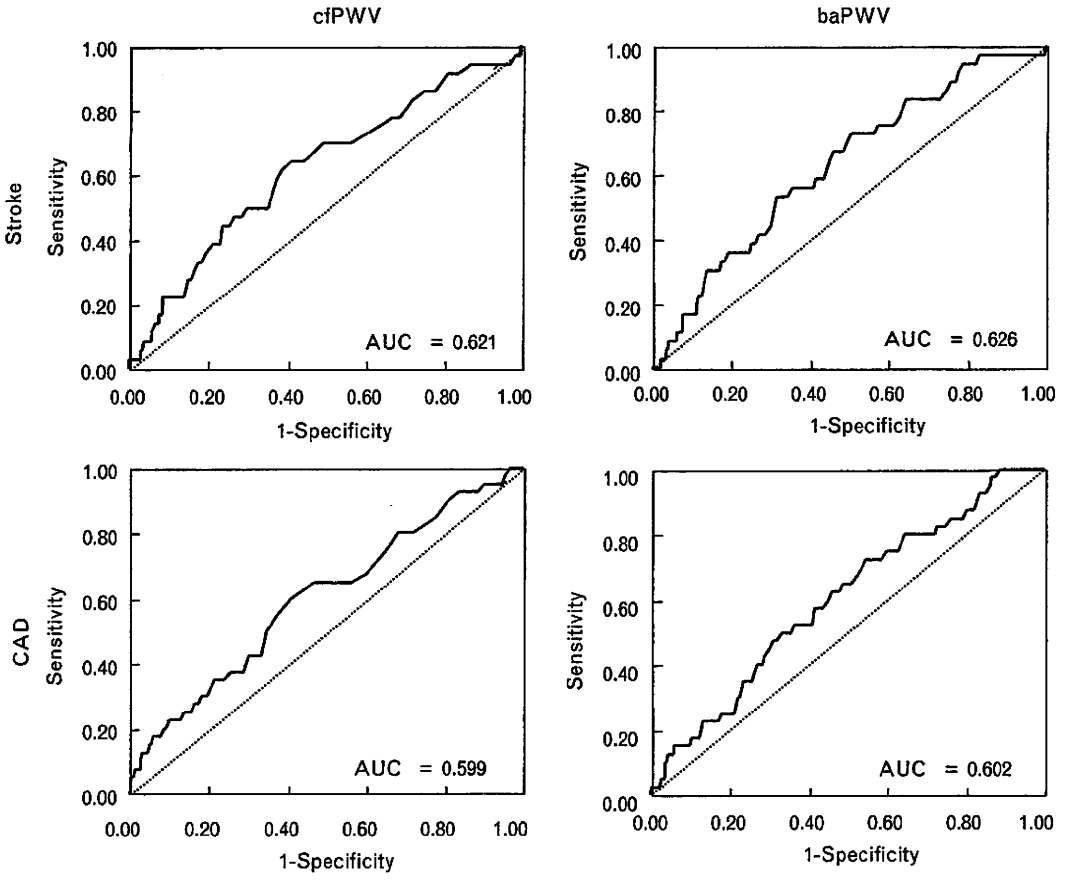
Both cfPWV and baPWV were significantly and similarly associated with various risk factors for CAD in the present study. Multiple regression analyses revealed that the two primary determinants of cfPWV and baPWV were the same (age and SBP). Interestingly, the strength of associations was somewhat greater for baPWV. These results are consistent with a recent epidemiological study [14] showing that both cfPWV and baPWV were significantly associated with the presence and severity of coronary calcification among overweight postmenopausal women. Interestingly, baPWV displayed stronger associations with the presence of coronary calcium than cfPWV

Fig. 3



Relation between the Framingham risk scores and pulse wave velocity (PWV) measures.

Fig. 4



Receiver operating characteristic (ROC) curves for incidences of stroke and coronary artery disease (CAD). AUC, area under the curve.

[14]. A similar finding that baPWV is more strongly related to left ventricular mass than cfPWV has also been reported [15]. Although baPWV is predominantly a measure of central artery stiffness [8], it also displays a modest correlation with peripheral artery stiffness (e.g. leg PWV) [8]. Thus, baPWV may be affected by more peripheral or systemic disease processes.

The areas under the ROC curve were also similar for baPWV and cfPWV. The results from this cross-sectional analysis indicate that both cfPWV and baPWV have similar abilities to associate with the presence of CAD and stroke. However, the values depicting the areas under the ROC curve were somewhat lower than what have been reported in the literature. This may be related to a lower cardiovascular risk in the Japanese population. Future prospective longitudinal studies are warranted to properly address this issue.

Carotid-femoral pulse wave velocity values reported in the present study appear high compared with some of the previously published studies [3,11] but are consistent with other studies [1,25]. The divergent cfPWV values are attributed to a different method used to measure the arterial path length (20% differences in mean values). In the latter studies, the arterial path length is the distance between the carotid and femoral recording sites, whereas the distance between carotid and femoral recording sites minus the distance from the carotid location to the supersternal notch is used in the former studies. Although this choice of methodology would produce approximately 20% differences in cfPWV values [24], it is still a matter of debate which arterial path length should be measured for the calculation of cfPWV. However, Sugawara *et al.* [24] have recently demonstrated that subtraction of the distance from the carotid location to the supersternal notch is the closest to the actual aortic length directly measured by the three-dimensional MRI. In baPWV, arterial path length is automatically estimated from one's height.

In summary, the newer automated measure of PWV (baPWV) was strongly associated with the gold standard measure of PWV (cfPWV). Additionally, both cfPWV and baPWV exhibit similar association with established risk factors for CAD and provide similar areas under the ROC curve for both stroke and CAD. Given the simplicity of the technique, baPWV is a promising new technique that is ideal for large-scale population studies and for incorporation into routine clinical settings. However, a more thorough analysis against a conventional technique is desirable.

References

1 Blacher J, Asmar R, Djane S, London GM, Safar ME. Aortic pulse wave velocity as a marker of cardiovascular risk in hypertensive patients. *Hypertension* 1999; **33**:1111–1117.

2 Blacher J, Pannier B, Guerin AP, Marchais SJ, Safar ME, London GM. Carotid arterial stiffness as a predictor of cardiovascular and all-cause mortality in end-stage renal disease. *Hypertension* 1998; **32**:570–574.

3 Mattace-Raso FU, van der Cammen TJ, Hofman A, van Popele NM, Bos ML, Schalekamp MA, *et al.* Arterial stiffness and risk of coronary heart disease and stroke: the Rotterdam Study. *Circulation* 2006; **113**:657–663.

4 Hodes RJ, Lakatta EG, McNeil CT. Another modifiable risk factor for cardiovascular disease? Some evidence points to arterial stiffness. *J Am Geriatr Soc* 1995; **43**:581–582.

5 Bramwell JC, Hill AV. The velocity of the pulse wave in man. *Proc Royal Soc London* 1922; **93**:298–306.

6 Kelly R, Hayward C, Avolio A, O'Rourke M. Noninvasive determination of age-related changes in the human arterial pulse. *Circulation* 1989; **80**:1652–1659.

7 Laurent S, Cockcroft J, Van Bortel L, Boutouyrie P, Giannattasio C, Hayoz D, *et al.* Expert consensus document on arterial stiffness: methodological issues and clinical applications. *Eur Heart J* 2006; **27**:2588–2605.

8 Sugawara J, Hayashi K, Yokoi T, Cortez-Cooper MY, DeVan AE, Anton MA, Tanaka H. Brachial-ankle pulse wave velocity: an index of central arterial stiffness? *J Hum Hypertens* 2005; **19**:401–406.

9 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.

10 Avolio AP, Clyde KM, Beard TC, Cooke HM, Ho KKL, O'Rourke MF. Improved arterial distensibility in normotensive subjects on a low salt diet. *Arterioscler* 1986; **6**:166–169.

11 Mitchell GF, Parise H, Benjamin EJ, Larson MG, Keyes MJ, Vita JA, *et al.* Changes in arterial stiffness and wave reflection with advancing age in healthy men and women: the Framingham Heart Study. *Hypertension* 2004; **43**:1239–1245.

12 Cortez-Cooper MY, Supak JA, Tanaka H. A new device for automatic measurements of arterial stiffness and ankle-brachial index. *Am J Cardiol* 2003; **91**:1519–1522; A1519.

13 Yamashina A, Tomiyama H, Takeda K, Tsuda H, Arai T, Hirose K, *et al.* Validity, reproducibility, and clinical significance of noninvasive brachial-ankle pulse wave velocity measurement. *Hypertension Res* 2002; **25**:359–364.

14 Venkitachalam L, Mackey RH, Sutton-Tyrrell K, Patel AS, Boraz MA, Simkin-Silverman LR, Kuller LH. Elevated pulse wave velocity increases the odds of coronary calcification in overweight postmenopausal women. *Am J Hypertens* 2007; **20**:469–475.

15 Yu WC, Chuang SY, Lin YP, Chen CH. Brachial-ankle vs. carotid-femoral pulse wave velocity as a determinant of cardiovascular structure and function. *J Hum Hypertens* 2008; **22**:24–31.

16 Tanaka H, DeSouza CA, Seals DR. Absence of age-related increase in central arterial stiffness in physically active women. *Arterioscler Thromb Vasc Biol* 1998; **18**:127–132.

17 Sugawara J, Komine H, Hayashi K, Yoshizawa M, Yokoi T, Maeda S, Tanaka H. Agreement between carotid and radial augmentation index: does medication status affect the relation? *Artery Res* 2008; **2**:74–76.

18 Imai E, Horio M, Nitta K, Yamagata K, Iseki K, Hara S, *et al.* Estimation of glomerular filtration rate by the MDRD study equation modified for Japanese patients with chronic kidney disease. *Clin Exp Nephrol* 2007; **11**:41–50.

19 Munakata M, Ito N, Nunokawa T, Yoshinaga K. Utility of automated brachial ankle pulse wave velocity measurements in hypertensive patients. *Am J Hypertens* 2003; **16**:653–657.

20 Laurent S, Boutouyrie P, Asmar R, Gautier I, Laloux B, Guize L, *et al.* Aortic stiffness is an independent predictor of all-cause and cardiovascular mortality in hypertensive patients. *Hypertension* 2001; **37**:1236–1241.

21 Meaume S, Rudnichi A, Lynch A, Bussy C, Sebban C, Benetos A, Safar ME. Aortic pulse wave velocity as a marker of cardiovascular disease in subjects over 70 years old. *J Hypertens* 2001; **19**:871–877.

22 Mitchell GF, Guo CY, Benjamin EJ, Larson MG, Keyes MJ, Vita JA, *et al.* Cross-sectional correlates of increased aortic stiffness in the community: the Framingham Heart Study. *Circulation* 2007; **115**:2628–2636.

23 Rajzer MW, Wojciechowska W, Klocek M, Palka I, Brzozowska-Kiszka M, Kawecka-Jaszcz K. Comparison of aortic pulse wave velocity measured by three techniques: Complior, SphygmoCor and Arteriograph. *J Hypertens* 2008; **26**:2001–2007.

24 Sugawara J, Hayashi K, Yokoi T, Tanaka H. Age-associated elongation of the ascending aorta in adults. *J Am College Cardiol: Cardiovasc Imaging* 2008; **1**:739–748.

25 Laurent S, Katsahian S, Fassot C, Tropeano AI, Gautier I, Laloux B, Boutouyrie P. Aortic stiffness is an independent predictor of fatal stroke in essential hypertension. *Stroke* 2003; **34**:1203–1206.

Relationship Between Blood Pressure Category and Incidence of Stroke and Myocardial Infarction in an Urban Japanese Population With and Without Chronic Kidney Disease

The Suita Study

Yoshihiro Kokubo, MD, PhD; Satoko Nakamura, MD, PhD; Tomonori Okamura, MD, PhD; Yasunao Yoshimasa, MD, PhD; Hisashi Makino, MD, PhD; Makoto Watanabe, MD, PhD; Aya Higashiyama, MD; Kei Kamide, MD, PhD; Katsuyuki Kawanishi, MD, PhD; Akira Okayama, MD, PhD; Yuhei Kawano, MD, PhD

Background and Purpose—Chronic kidney disease (CKD) is increasingly recognized as an independent risk factor for stroke and myocardial infarction (MI). Few studies, however, have examined the relationship between blood pressure (BP) category and these diseases in subjects with and without CKD.

Methods—We studied 5494 Japanese individuals (ages 30 to 79, without stroke or MI at baseline) who completed a baseline survey and received follow-up through December 2005. The glomerular filtration rate (GFR) was estimated using the Modification of Diet in Renal Disease study equation modified by the Japanese coefficient. CKD was defined as an estimated GFR <60 mL/min/1.73m^2. BP categories were defined by the European Society of Hypertension and European Society of Cardiology 2007 criteria.

Results—In 64 395 person-years of follow-up, we documented 346 incidences of cardiovascular diseases (CVD; 213 strokes and 133 MI events). Compared with the GFR (≥90 mL/min/1.73m^2) group, the hazard ratios (95% confidential intervals) for stroke were 1.9 (1.3 to 3.0) in the GFR 50 to 59 mL/min/1.73m^2 group and 2.2 (1.2 to 4.1) in the GFR <50 mL/min/1.73m^2 group. Results for cerebral infarction were similar. Compared with the optimal BP subjects without CKD, the normal BP, high-normal BP, and hypertensive subjects without CKD showed increased risks of CVD and stroke; however the impact of each BP category on CVD (*P* for interaction: 0.04 in men, 0.49 in women) and stroke (0.03 in men, 0.90 in women) was more evident in men with CKD.

Conclusions—CKD may increase the association of BP and CVD in a Japanese urban population. (***Stroke.* 2009;40:2674-2679.**)

Key Words: chronic kidney disease ■ blood pressure category ■ stroke ■ myocardial infarction ■ epidemiology ■ prospective studies ■ general population

Recently, chronic kidney disease (CKD) has become a major public health problem and a risk factor for all-causes mortality, stroke, and myocardial infarction (MI).[1] In end-stage renal disease, the cardiovascular disease (CVD) mortality rate is more than 10 times as high as that in the general population.[2] In asymptomatic general populations or outpatients, a severely or moderately decreased glomerular filtration rate (GFR) has been shown by most but not all studies to be an independent risk factor for stroke and MI.[1] However, in low-risk or general populations, the relationship between levels of kidney function and clinical outcomes has not been as clear. Some studies have demonstrated no association between CKD and CVD,[3,4] whereas others have shown CKD as an independent risk factor for CVD.[5–8] These inconsistencies may be attributable to differences between the selected study populations as well as the severity of the CKD.

The frequency of hypertension is relatively higher in Japanese than in Western countries.[9] Hypertension is one of the major risk factors for both CVD and CKD. Recently, a larger prospective study has indicated that CKD increased the association between blood pressure (BP) categories and CVD, although the relevant data were gathered from 10 rural areas with different methods

Received February 19, 2009; final revision received March 25, 2009; accepted April 14, 2009.

From the Department of Preventive Cardiology (Y. Kokubo, T.O., M.W., A.H., A.O.), the Division of Hypertension and Nephrology (S.N., K. Kamide, Y. Kawano), and the Department of Atherosclerosis and Diabetes (Y.Y., H.M.), National Cardiovascular Center, Osaka, Japan; the Department of Geriatric Medicine (K. Kamide), Osaka University Graduate School of Medicine, Osaka, Japan; the Suita Medical Association (K. Kawanishi), Osaka, Japan; and the Japan Anti-Tuberculosis Association (A.O.), Tokyo, Japan.

Correspondence to Yoshihiro Kokubo, MD, PhD, FAHA, Department of Preventive Cardiology, National Cardiovascular Center, 5-7-1, Fujishiro-dai, Suita, Osaka, 565-8565 Japan. E-mail ykokubo@hsp.ncvc.go.jp

Stroke is available at http://stroke.ahajournals.org

DOI: 10.1161/STROKEAHA.109.550707

for the measurement of creatinine.[10] A few studies in general population have demonstrated a stronger association between BP and CVD in subjects with CKD.[5,10] We examined the association between BP category and incidence of stroke and MI subjects with and without CKD in a Japanese urban population.

Methods

Study Subjects

Suita city is located adjacent to Osaka city, which is the second largest metropolitan area in Japan. The Suita Study,[11–13] an epidemiological study of cerebrovascular and cardiovascular diseases, was based on a random sampling of 12 200 Japanese urban residents. As a baseline, participants (aged 30 to 79 years) were randomly selected from the municipality population registry and stratified into groups by sex and age in 10-year increments in 1989. Of these, 6485 people underwent regular health checkups between September 1989 and March 1994.

Cohort members in the study population were excluded from these analyses if they had a past or present history of CVD at baseline (n=208), were missing data (n=170), attended health checkups after April 1994 (n=79), or failed to complete the follow-up health surveys or questionnaires after the baseline examination (n=534). After applying these exclusions, a total of 5494 participants aged 30 to 79 years old were selected. Informed consent was obtained from all participants. This study was approved by the Institutional Review Board of the National Cardiovascular Center.

Measurement of Blood Pressure and Covariates

Well-trained physicians measured BP 3 times using a mercury column sphygmomanometer, an appropriate-size cuff, and a standard protocol. Before the initial BP reading was obtained, participants were seated at rest for at least 5 minutes. First, systolic blood pressure (SBP) was measured for the purpose of obtaining approximate SBP levels. SBP and diastolic blood pressures (DBP) were taken as the average of the second and third measurements, which were recorded more than 1 minute apart.

At the time of the baseline examination, subjects were classified into 1 of the 5 BP categories based on the European Society of Hypertension and European Society of Cardiology (ESH-ESC) 2007 criteria[14]: optimal (SBP <120 mm Hg and DBP <80 mm Hg), normal (SBP 120 to 129 mm Hg or DBP 80 to 84 mm Hg), high-normal BP (SBP 130 to 139 mm Hg or DBP 85 to 89 mm Hg), and hypertensive (SBP ≥140 mm Hg or DBP ≥90 mm Hg). Antihypertensive drug users were classified according to their BP levels at the baseline survey. If the SBP and DBP readings for a subject were in different categories, the subjects were categorized into the higher of the two BP categories.

At the baseline examination, we performed routine blood tests that included serum total cholesterol, HDL cholesterol, and glucose levels. Physicians or nurses administered questionnaires covering personal habits and present illness. Body mass index (BMI) was calculated as weight (kilograms) divided by height (meters) squared. Hypercholesterolemia was defined as total cholesterol levels ≥5.7 mmol/L or current use of antihyperlipidemic medications. Diabetes was defined as a fasting plasma glucose level ≥7.0 mmol/L, a nonfasting plasma glucose level ≥11.0 mmol/L, or current use of antidiabetic medications.

Definition of CKD

Serum creatinine (Cre) was measured by noncompensated kinetic Jaffé methods. The glomerular filtration rate (GFR) of each participant was calculated from the Cre value and the age, using the MDRD equation modified by the Japanese coefficient (0.881), as follows[15]:

$$\text{GFR (ml/min/1.73 m}^2) = 0.881 \times 186 \times \text{age}^{-0.203} \times \text{Cre}^{-1.154} \text{ (for men)}$$

$$\text{and GFR (ml/min/1.73 m}^2) = 0.881 \times 186 \times \text{age}^{-0.203} \times \text{Cre}^{-1.154} \times 0.742 \text{ (for women).}$$

CKD was defined as an estimated GFR <60 mL/min/1.73m^2.

Confirmation of Stroke and MI and End Point Determination

The confirmation of stroke and MI in the Suita Study has been described elsewhere.[11–13] In brief, the 5 hospitals in this area, where acute stroke and MI patients were admitted, were all capable of performing computed tomographic scans or MRI. Medical records were reviewed by registered hospital physicians or research physicians who were blinded to the baseline data. Strokes were defined according to the U.S. National Survey of Stroke criteria.[16] For each stroke subtype (ie, cerebral infarction [thrombotic or embolic infarction], intracerebral hemorrhage, and subarachnoid hemorrhage), a definite diagnosis was established based on examination of computed tomographic scans, magnetic resonance images, or autopsies. Definite and probable MIs were defined according to the criteria set out by the MONICA project.[17] Sudden deaths of unknown origin were deaths that occurred within 24 hours from the onset of symptoms, and were also classified as MI. In this study CVD was defined as stroke or MI.

To detect MI and stroke occurrences, each participant's health status was checked at clinical visits to the National Cardiovascular Center every 2 years. Yearly questionnaires by mail or telephone were also completed for all participants. In addition, to complete our surveillance for fatal strokes and MIs, we conducted a systematic search for death certificates. All the data (health check-ups, questionnaires, and death certificates) were checked against medical records to confirm the incidence of CVD. We identified possible strokes or MIs using data from (1) the health examination and questionnaires from the stroke and MI registries without informed consent for medical records survey; and (2) death certificates bearing a diagnosis of probable stroke or MI without registration of CVD incidence.

The end points of the current follow-up study were (1) date of the first MI or stroke event (2); date of death (3); date of leaving Suita; and (4) December 31, 2005 (censored).

Statistical Analysis

Analyses of variances and χ^2 tests were used to compare mean values and frequencies. The Cox proportional-hazard ratios (HRs) were fitted to the GFR categories and CKD after adjusting for sex and age in 5-year increments as stratified variables and other potential confounding factors at the baseline survey: namely, present illness of hypertension, hypercholesterolemia and diabetes, smoking status (never, quit, and current smoker), and drinking status (never, quit, and current drinker). The Cox proportional HRs were fitted to the combination of the BP categories and CKD (positive or negative) after adjusting for sex and age in 5-year increments as stratified variables and other potential confounding factors including an interactive term for CKD and BP categories. The fit of the proportional hazards model was evaluated by examining discrete regression models and by permitting the proportionality assumption to vary with time, and assessments of nonlinearity involving associations with blood pressure and GFR categories were made. The probability values for the model of interaction between CVD incidence and log (person year) were 0.38 in men and 0.81 in women. Proportionality was also checked by log-log survival plot.

To express the impact of CKD on CVD occurrence in the participants, we estimated the population attributable fraction (PAF, %). PAF was estimated as follows:

$$Pe \times (HR-1)/HR,$$

in which Pe is the proportion of incident cases in CKD, and HR is the multiple-adjusted hazard ratio.[18] All statistical analyses were conducted using the SAS statistical package software (release version 8.2, SAS Institute Inc).

Results

Figure 1 shows that the frequency of CKD increases with age in both men and women. At the baseline survey, both men and women with CKD (8.9% for men and 11.3% for women)

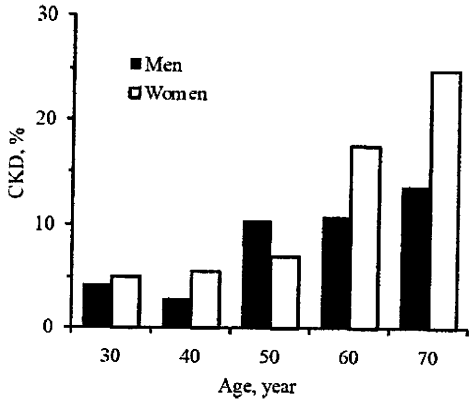


Figure 1. Frequencies of CKD according to sex and age.

were older, had higher prevalence of hypertension and hypercholesterolemia, and had a lower frequency of current drinking than those without CKD (Table 1).

During an average 11.7-year follow-up period, we documented 213 strokes and 133 MIs. In men and women combined, compared with subjects for GFR ≥90 mL/min/1.73m^2 the multivariable HRs (95% confidence intervals; CIs) for CVD incidence were 1.75 (1.22 to 2.50) in GFR=50 to 59 mL/min/1.73m^2 and 2.48 (1.56 to 3.94) in <50 mL/min/1.73m^2 (Table 2). In addition, the risks of CVD for each GFR category in men and women separately were similar to the risks for all participants. The multivariable HR (95% CIs) of CVD incidence for CKD was 1.70 (1.30 to 2.23) in all subjects (data not shown).

In Table 3, the multivariable HRs (95% CIs) for strokes were 1.94 (1.26 to 2.98) in the GFR=50 to 59 mL/min/1.73m^2 and 2.19 (1.18 to 4.06) in the GFR <50 mL/min/1.73m^2 compared with subjects for GFR ≥90 mL/min/1.73m^2 Results for cerebral infarction were similar to strokes. Age-adjusted HRs (95% CIs) for intracerebral hemorrhage were 1.93 (0.77 to 4.85) in the GFR=50 to 59 mL/min/1.73m^2 and 2.52 (0.72 to 8.80) in the GFR <50 mL/min/1.73m^2 (supplemental Table I, available online at http://stroke.ahajournals.org).

In Figure 2, compared with the optimal BP subjects without CKD, the normal BP, high-normal BP, and hypertensive subjects without CKD showed increased risks of CVD, whereas the impact of each BP category on CVD was more evident in subjects with CKD (probability values for interaction between CKD and BP category were 0.04 in men, 0.49 in women, and 0.06 in all subjects). Results of stroke were similar (probability values for the interaction were 0.03 in men and 0.90 in women, data not shown). Supplemental Table II shows the hazard ratios for the association between 10 mm Hg of SBP and the risk of CVD in subjects with or without CKD.

Using the HRs, we estimated the population attributable fraction of CVD to exposure for CKD at baseline by sex. We found that 8.3% in men and 17.6% in men with CVD incidences could be described as excessive incidence attributable to CKD.

Discussion

In this cohort study of a general urban Japanese population, CKD was a risk factor for CVD and its subtypes. A stronger association between BP and the incidence of CVD was

Table 1. Baseline Characteristics of Study Subjects According to Chronic Kidney Disease

	Men			Women		
Variables	CKD (−)	CKD (+)	*P* Value	CKD (−)	CKD (+)	*P* Value
No. of subjects	2341	229		2593	331	
Age at baseline, y	55±13	61±12	<0.001	53±13	62±12	<0.001
Body mass index, kg/m^2	22±3	23±3	<0.001	22±3	22±3	0.332
Blood pressure category, %			0.005			<0.001
Optimal	31.7	24.0		43.9	27.2	
Normal	19.2	14.4		16.6	15.4	
High-normal blood pressure	16.2	20.5		14.0	14.8	
Hypertension	32.9	41.1		25.5	42.6	
Present illness, %*						
Hypercholesterolemia	28.1	35.8	0.014	40.7	54.7	<0.001
Diabetes	6.1	6.6	0.791	3.2	5.4	0.036
Smoking status, %			0.007			0.713
Current	51	42		12	12	
Quit	30	40		4	4	
Never	19	18		84	83	
Drinking status, %			0.024			0.017
Current	76	68		34	26	
Quit	3	6		2	3	
Never	21	26		65	71	

*Hypercholesterolemia; antilipidemic drug use or total cholesterol ≥5.7 mmol/L (220 mg/dl), diabetes; antihyperglycemic drug use or fasting blood sugar ≥7.0 mmol/L (126 mg/dl).

Plus–minus values are means±SD.

Table 2. Age and Multivariable Adjusted Hazard Ratios (95% CIs) for Incidence of Cardiovascular Disease† According to Category of Glomerular Filtration Rate by Sex

	Glomerular Filtration Rate, ml/min/1.73m^2				
Variables	≥90	60 to 89	50 to 59	<50	*P* for Trend
Men and Women					
Cases, n	94	176	51	25	
Person-years	28 736	29 336	4764	1558	
Age-adjusted	1	1.22 (0.94–1.58)	1.71 (1.20–2.42)	2.49 (1.59–3.90)	<0.001
Multivariable adjusted*	1	1.21 (0.93–1.58)	1.75 (1.22–2.50)	2.48 (1.56–3.94)	<0.001
Men					
Cases, n	50	124	24	11	
Person-years	12 092	14 835	1928	522	
Age-adjusted	1	1.20 (0.85–1.70)	1.63 (1.00–2.68)	2.17 (1.11–4.23)	0.008
Multivariable adjusted*	1	1.21 (0.85–1.70)	1.78 (1.08–2.94)	2.38 (1.21–4.68)	0.004
Women					
Cases, n	44	52	27	14	
Person-years	16 644	14 502	2836	1036	
Age-adjusted	1	1.22 (0.81–1.83)	1.79 (1.09–2.92)	2.81 (1.53–5.18)	<0.001
Multivariable adjusted*	1	1.21 (0.80–1.84)	1.76 (1.05–2.93)	2.31 (1.20–4.43)	0.002

*Multivariable adjusted for age, BMI, smoking, drinking, and present illness (hypertension, diabetes, and hypercholesterolemia).
†Cardiovascular disease includes both stroke and MI.

observed in the presence of CKD. Furthermore, we found that 8% in men and 18% in women of CVD incidence may be derived from CKD cases.

Go et al reported that both severe and moderate renal diseases were risk factors for CVD incidence.[6] A pooled analysis of community-based studies demonstrated that CKD is an independent risk factor for the composite of all-cause mortality in blacks and whites and CVD incidence in blacks.[5] In contrast, NHANES I did not provide relationships between mortality and moderately higher serum creatinine levels.[4] The Framingham Heart Study and Offspring cohorts have shown no significant association between the presence of kidney disease and CVD incidence.[3]

The results of our study are essentially compatible with previous cohort studies in Japan. The Hisayama study demonstrated that CKD was a risk factor for incidence of coronary heart disease in men and ischemic stroke in women.[8] The Ohasama study indicated that decreased kidney function increased the risk of first symptomatic stroke events.[19] This study used creatinine clearance rather than estimated GFR. Irie et al showed that subjects with GFR <60 had a higher risk of CVD mortality[7] but did not examine the risk of GFR 50 to 59 mL/min/1.73m^2 The NIPPON DATA 90 indicated that CKD was an independent risk factor for cardiovascular death in a community-dwelling Japanese population.[20] The end point of these studies was also mortality. Ninomiya et al

Table 3. Age-Sex and Multivariable Adjusted Hazard Ratios (95% CIs) for Incidence of All Strokes, Cerebral Infarction, and Myocardial infarction According to Category of Glomerular Filtration Rate

	Glomerular Filtration Rate, ml/min/1.73m^2				
Variables	≥90	60 to 89	50 to 59	<50	*P* for Trend
Person-years	28 258	28 690	4528	1446	
All strokes					
Cases, n	65	99	36	13	
Age and sex adjusted	1	1.02 (0.73–1.41)	1.78 (1.17–2.70)	1.93 (1.05–3.54)	0.004
Multivariable adjusted*	1	1.04 (0.74–1.45)	1.94 (1.26–2.98)	2.19 (1.18–4.06)	<0.001
Cerebral infarction					
Cases, n	42	66	24	9	
Age and sex adjusted	1	0.99 (0.66–1.49)	1.72 (1.03–4.19)	2.01 (0.97–4.19)	0.020
Multivariable adjusted*	1	0.98 (0.65–1.49)	1.81 (1.07–3.07)	2.26 (1.07–4.78)	0.008
Myocardial infarction					
Cases, n	29	77	15	12	
Age and sex adjusted	1	1.68 (1.08–2.61)	1.64 (0.87–3.09)	4.26 (2.14–8.45)	<0.001
Multivariable adjusted*	1	1.60 (1.03–2.49)	1.51 (0.80–2.88)	3.56 (1.73–7.30)	0.002

*Multivariable adjusted for age, sex, BMI, smoking, drinking, and present illness (hypertension, diabetes, and hypercholesterolemia).

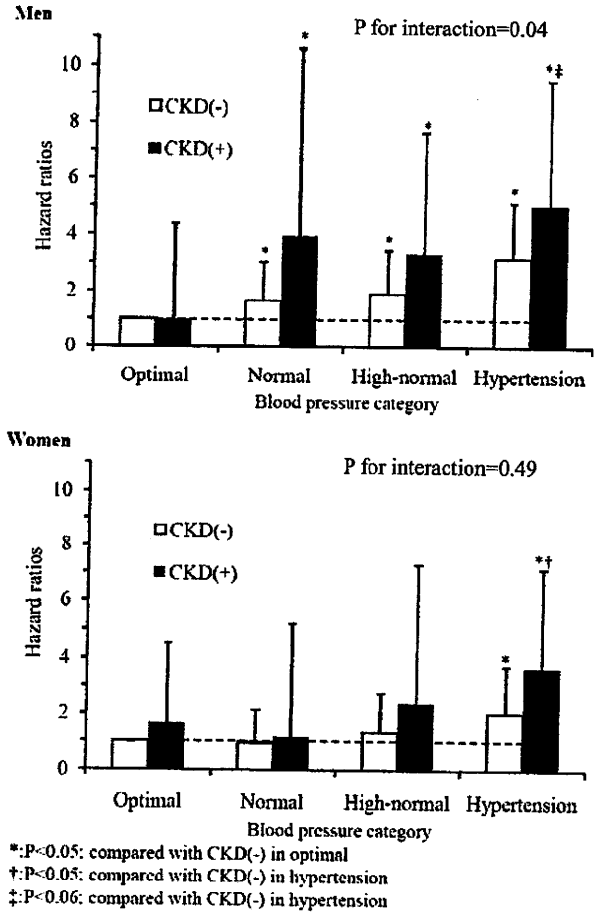


Figure 2. The combination of CKD and BP categories on multivariable hazard ratios for CVD. Data for men and women are presented separately. Multivariable analyses are adjusted age in 5-year increments as stratified variables and other potential confounding factors of hypercholesterolemia, diabetes, and smoking and drinking status.

has recently reported that CKD was risk factors for CVD and stroke in women and that CKD increased the association between BP category and CVD in all subjects from 10 combined different cohort studies using different methods of creatinine measurement.[10] All of our samples were measured using the same analyzer at one laboratory.

Compared with the previous studies, our study has several methodological strengths. First, we could perform subanalysis by age and CVD subtype, because we evaluated a large cohort of participants. Second, each participant's health status was checked during a clinical visit at the National Cardiovascular Center every 2 years. In addition, each year, a health questionnaire was given to each participant via mail or telephone. We could evaluate the registry of CVD incidence with the data obtained from clinical visits, annual questionnaires, or death certificates. Finally, our cohort population was selected at random from an urban population, in contrast to most other cohort studies in Japan, which have relied on rural populations.[7,8,19]

There may be some reasons why CKD is more positively associated with CVD in blacks or Japanese than in whites. Blacks and Japanese are more likely to have hypertension at an earlier age.[9,21] Therefore, the period of hypertension exposure tends to be longer in blacks and Japanese than in whites. The GFR estimation has been adjusted by a factor suitable for Japanese populations.[15]

Reduced kidney function is associated with increased levels of inflammatory factors,[22,23] abnormal apolipoprotein levels,[22] elevated plasma homocysteine,[22] enhanced coagulability,[23] anemia, left ventricular hypertrophy, increased arterial calcification, endothelial dysfunction, and arterial stiffness.[2,24] How these and other factors interact to increase the risk of adverse outcomes remains unclear but is the focus of ongoing investigations.[24]

Subjects with GFR levels of 50 to 59 mL/min/1.73m^2 were observed to be at risk for stroke. It is desirable to prevent CVD in subjects with both high-risk (<50 mL/min/1.73m^2) and less severe kidney disease (50 to 59 mL/min/1.73m^2), although an accelerated decline in GFR occurred for the subjects whose initial GFR <50 mL/min/1.73m^2.[25]

Hypertension is a strong risk factor for early decline in kidney function; hypertensive patients (BP ≥160/95 mm Hg) have a 5-fold greater decline in GFR (2.7 mL/min/1.73m^2/yr) compared with patients with BP <140/90 mm Hg.[26] Furthermore, in this study, the association between BP and the incidence of CVD were evident by CKD. The risk of CVD was higher in CKD subjects with normal and high-normal BP than in non-CKD subjects in the same BP categories. Using the combination of BP and CKD, it could be possible to screen more efficiently for higher risk of stroke and MI. This is compatible with the CKD clinical guidelines, which state that the preferable BP for subjects with CKD is 130/80 mm Hg.[27] For the prevention of CVD incidence for all hypertensive subjects in health check-ups, it might be desirable to measure serum creatinine levels and to intervene in lifestyle modification such as reducing salt intake, more frequent exercise, or quit smoking.

Our study has several limitations. The primary limitation is dilution bias,[28] in that the current study was based on single-day measurement of creatinine levels. The creatinine levels might have been misclassified, despite the fact that measurements of creatinine levels on a single day have been found to be accurate in other epidemiological studies. Second, we did not perform a creatinine clearance test or 2 measurements of serum creatinine at least 3 months apart. Although our definition of CKD is based on a single assessment of serum creatinine, the equation provides an accurate estimated GFR value.[15] Third, even with the moderate sample size (n=5494) and 12-year duration, the numbers of end points were limited, especially when the data were stratified by 2 variables, such as sex and glomerular filtration rates. A study with more participants with the same protocol is required to validate to the association between BP category and CVD by CKD.

In conclusion, CKD was associated with an increased risk for stroke and MI in a general urban Japanese population. Furthermore, the association between BP and CVD may be evident by CKD. To prevent the incidence of stroke and MI, it is necessary for subjects with CKD to control their BP by lifestyle modification and proper clinical treatment.

Acknowledgments

We express our deepest gratitude to Dr Yasushi Kotani, all members of the Suita City Health Center, and the Suita Medical Association. We also thank all researchers and the staff of the Division of Preventive Cardiology for performing medical examinations and the follow-up. We also thank *Satsuki-Junyukai*, the volunteers for the administration of the Suita study.

Sources of Funding

This study was supported by Grant-in-Aid from the Ministry of Health, Labor, and Welfare of Japan (H20-SeiShu-013, H19-SeiShu-017, and H18-SeiShu-012), and by a Research Grant for Cardiovascular Disease from the Ministry of Health, Labor, and Welfare (19S-6, 18S-1).

Disclosures

None.

References

1. Sarnak MJ, Levey AS, Schoolwerth AC, Coresh J, Culleton B, Hamm LL, McCullough PA, Kasiske BL, Kelepouris E, Klag MJ, Parfrey P, Pfeffer M, Raij L, Spinosa DJ, Wilson PW. Kidney disease as a risk factor for development of cardiovascular disease. *Hypertension*. 2003;42:1050–1065.
2. USRDS: The United States renal data system. *Am J Kidney Dis*. 2003;42:1–230.
3. Culleton BF, Larson MG, Wilson PW, Evans JC, Parfrey PS, Levy D. Cardiovascular disease and mortality in a community-based cohort with mild renal insufficiency. *Kidney Int*. 1999;56:2214–2219.
4. Garg AX, Clark WF, Haynes RB, House AA. Moderate renal insufficiency and the risk of cardiovascular mortality: Results from the NHANES I. *Kidney Int*. 2002;61:1486–1494.
5. Weiner DE, Tighiouart H, Amin MG, Stark PC, MacLeod B, Griffith JL, Salem DN, Levey AS, Sarnak MJ. Chronic kidney disease as a risk factor for cardiovascular disease and all-cause mortality: A pooled analysis of community-based studies. *J Am Soc Nephrol*. 2004;15:1307–1315.
6. Go AS, Chertow GM, Fan D, McCulloch CE, Hsu CY. Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. *N Engl J Med*. 2004;351:1296–1305.
7. Irie F, Iso H, Sairenchi T, Fukasawa N, Yamagishi K, Ikehara S, Kanashiki M, Saito Y, Ota H, Nose T. The relationships of proteinuria, serum creatinine, glomerular filtration rate with cardiovascular disease mortality in Japanese general population. *Kidney Int*. 2006;69:1264–1271.
8. Ninomiya T, Kiyohara Y, Kubo M, Tanizaki Y, Doi Y, Okubo K, Wakugawa Y, Hata J, Oishi Y, Shikata K, Yonemoto K, Hirakata H, Iida M. Chronic kidney disease and cardiovascular disease in a general Japanese population: The Hisayama Study. *Kidney Int*. 2005;68:228–236.
9. Yamori Y, Nara Y, Mizushima S, Mano M, Sawamura M, Kihara M, Horie R. International cooperative study on the relationship between dietary factors and blood pressure: A report from the Cardiovascular Diseases and Alimentary Comparison Study. *J Cardiovasc Pharmacol*. 1990;16 Suppl8:S43–S47.
10. Ninomiya T, Kiyohara Y, Tokuda Y, Doi Y, Arima H, Harada A, Ohashi Y, Ueshima H. Impact of kidney disease and blood pressure on the development of cardiovascular disease: An overview from the Japan Arteriosclerosis Longitudinal Study. *Circulation*. 2008;118:2694–2701.
11. Kokubo Y, Kamide K, Okamura T, Watanabe M, Higashiyama A, Kawanishi K, Okayama A, Kawano Y. Impact of high-normal blood pressure on the risk of cardiovascular disease in a Japanese urban cohort. The Suita Study. *Hypertension*. 2008;52:652–659.
12. Kokubo Y, Okamura T, Yoshimasa Y, Miyamoto Y, Kawanishi K, Kotani Y, Okayama A, Tomoike H. Impact of metabolic syndrome components on incidence of cardiovascular disease in a general urban Japanese population: The Suita Study. *Hypertens Res*. 2008;31:2027–2035.
13. Okamura T, Kokubo Y, Watanabe M, Higashiyama A, Miyamoto Y, Yoshimasa Y, Okayama A. Low-density lipoprotein cholesterol and non-high-density lipoprotein cholesterol and the incidence of cardiovascular disease in an urban Japanese cohort study: The suita study. *Atherosclerosis*. 2009;203:587–592.
14. Mancia G, De Backer G, Dominiczak A, Cifkova R, Fagard R, Germano G, Grassi G, Heagerty AM, Kjeldsen SE, Laurent S, Narkiewicz K, Ruilope L, Rynkiewicz A, Schmieder RE, Boudier HA, Zanchetti A. 2007 Guidelines for the management of arterial hypertension. *J Hypertens*. 2007;25:1105–1187.
15. Imai E, Horio M, Nitta K, Yamagata K, Iseki K, Hara S, Ura N, Kiyohara Y, Hirakata H, Watanabe T, Moriyama T, Ando Y, Inaguma D, Narita I, Iso H, Wakai K, Yasuda Y, Tsukamoto Y, Ito S, Makino H, Hishida A, Matsuo S. Estimation of glomerular filtration rate by the MDRD study equation modified for Japanese patients with chronic kidney disease. *Clin Exp Nephrol*. 2007;11:41–50.
16. Walker AE, Robins M, Weinfeld FD. The National Survey of Stroke. Clinical findings. *Stroke*. 1981;12:I13–44.
17. Tunstall-Pedoe H, Kuulasmaa K, Amouyel P, Arveiler D, Rajakangas AM, Pajak A. Myocardial infarction and coronary deaths in the World Health Organization MONICA project. Registration procedures, event rates, and case-fatality rates in 38 populations from 21 countries in four continents. *Circulation*. 1994;90:583–612.
18. Rockhill B, Newman B, Weinberg C. Use and misuse of population attributable fractions. *Am J Public Health*. 1998;88:15–19.
19. Nakayama M, Metoki H, Terawaki H, Ohkubo T, Kikuya M, Sato T, Nakayama K, Asayama K, Inoue R, Hashimoto J, Totsune K, Hoshi H, Ito S, Imai Y. 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.
20. Nakamura K, Okamura T, Hayakawa T, Kadowaki T, Kita Y, Ohnishi H, Saitoh S, Sakata K, Okayama A, Ueshima H. Chronic kidney disease is a risk factor for cardiovascular death in a community-based population in Japan: Nippon data90. *Circ J*. 2006;70:954–959.
21. Rahman M, Douglas JG, Wright JT Jr. Pathophysiology and treatment implications of hypertension in the African-American population. *Endocrinol Metab Clin North Am*. 1997;26:125–144.
22. Muntner P, Hamm LL, Kusek JW, Chen J, Whelton PK, He J. The prevalence of nontraditional risk factors for coronary heart disease in patients with chronic kidney disease. *Ann Intern Med*. 2004;140:9–17.
23. Shlipak MG, Fried LF, Crump C, Bleyer AJ, Manolio TA, Tracy RP, Furberg CD, Psaty BM. Elevations of inflammatory and procoagulant biomarkers in elderly persons with renal insufficiency. *Circulation*. 2003;107:87–92.
24. Feldman HI, Appel LJ, Chertow GM, Cifelli D, Cizman B, Daugirdas J, Fink JC, Franklin-Becker ED, Go AS, Hamm LL, He J, Hostetter T, Hsu CY, Jamerson K, Joffe M, Kusek JW, Landis JR, Lash JP, Miller ER, Mohler ER III, Muntner P, Ojo AO, Rahman M, Townsend RR, Wright JT. The Chronic Renal Insufficiency Cohort Study: Design and methods. *J Am Soc Nephrol*. 2003;14:S148–S153.
25. Imai E, Horio M, Yamagata K, Iseki K, Hara S, Ura N, Kiyohara Y, Makino H, Hishida A, Matsuo S. Slower decline of glomerular filtration rate in the Japanese general population: A longitudinal 10-year follow-up study. *Hypertens Res*. 2008;31:433–441.
26. Vupputuri S, Batuman V, Muntner P, Bazzano LA, Lefante JJ, Whelton PK, He J. Effect of blood pressure on early decline in kidney function among hypertensive men. *Hypertension*. 2003;42:1144–1149.
27. Bakris GL, Williams M, Dworkin L, Elliott WJ, Epstein M, Toto R, Tuttle K, Douglas J, Hsueh W, Sowers J. Preserving renal function in adults with hypertension and diabetes. *Am J Kidney Dis*. 2000;36:646–661.
28. MacMahon S, Peto R, Cutler J, Collins R, Sorlie P, Neaton J, Abbott R, Godwin J, Dyer A, Stamler J. Blood pressure, stroke, and coronary heart disease. Part 1, Prolonged differences in blood pressure: Prospective observational studies corrected for the regression dilution bias. *Lancet*. 1990;335:765–774.

連載

知って得するワンポイントアドバイス

抗血栓薬の不応症（レジスタンス）

国立循環器病研究センター脳血管内科 医長

長束 一行

Kazuyuki NAGATSUKA

抗血栓薬の不応症（レジスタンス）として，早くから注目されたのはアスピリン抵抗性である．アスピリンは多くの血栓症発症予防のために用いられてきたが，全体でみると血栓症を約23%予防するとされているが[1]，逆の方向から考えるとアスピリンを服用していても年間約6%の血栓症が1年間に生じるということになる．血小板機能に対するアスピリンの作用に個人差がみられるという研究が数多く発表されるようになり，血小板機能からみたアスピリン抵抗性をもつ群に血栓症の再発が多いとのデータも発表されたことにより，一気にアスピリン抵抗性が注目されることとなった．

血小板機能からみたアスピリン抵抗性については，検査法によりその頻度が大きく異なり，数%から45%とかなりのばらつきがある．どの検査が最も臨床的なアスピリン抵抗性，すなわちアスピリンによる再発予防効果が弱い群をみつけるのに適しているかは，まだ明らかではない．血小板機能検査には血小板凝集能，血小板トロンボキサン産生能，尿中トロンボキサン代謝産物排泄量，ずり応力下での血小板凝集能，VerifyNowという抗血小板薬の抵抗性を評価する専門機器などさまざまである．血小板凝集能は古くからある血小板機能検査であるが，凝集を起こさせるための惹起物質に何を選ぶのかが問題となる．アスピリンはアラキドン酸凝集，コラーゲン凝集を主に抑制するが，ADP凝集は一次凝集しか抑制しない．アラキドン酸凝集はアスピリンが主に抗血小板剤作用を発揮する，アラキドン酸カスケードそのものによる凝集をみるために，高感度にアスピリンの作用を反映するが，その凝集反応は通常凝集するかしないかに大きく分かれるために，微妙な差はわかりにくい．一方，コラーゲン凝集はアスピリン投与による抑制効果はばらつきが多くなるし，試薬の濃度にも依存する．

血小板のトロンボキサン産生能はアラキドン酸カスケードそのものを評価可能であるが，逆にそこだけしか評価できない．尿中トロンボキサン代謝産物はサイクロオキシゲナーゼ2の機能も反映するといわれている．

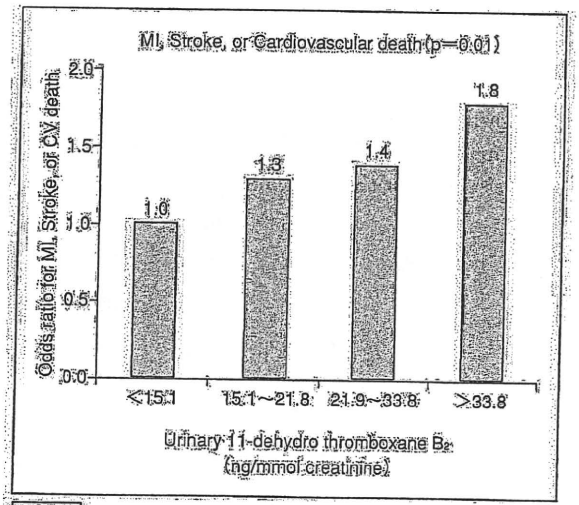


図1 尿中トロンボキサン代謝産物量と血栓症発症リスク

尿中トロンボキサン代謝産物量が多い群では血栓症のリスクが高くなっている．

（文献2より引用改変）

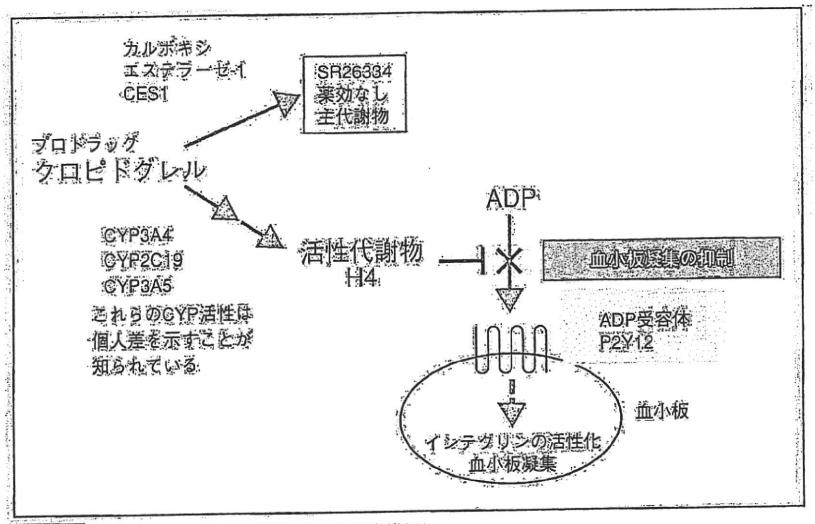


図2 クロピドグレルの代謝と作用機序

クロピドグレルはプロドラッグで，CYP2C19などの代謝酵素で代謝され，活性化物質へと変化し，抗血小板機能を発揮する．

アスピリン抵抗性に関する代表的な論文を紹介すると，Eikelboomら[9]は，尿中トロンボキサン代謝産物量を測定し，4分位に分けて3年間血栓症をイベントとしてフォローしたところ，尿中トロンボキサン代謝産物量が多いほど血栓症の発生が多かったと報告している（図1）．

アスピリン抵抗性の原因については，さまざまな説がある．投与量，代謝産物の影響，併用薬の影響，遺伝子多型，服薬コンプライアンスなどさまざまなものが推測されているが，いまだ明らかにされていない．Ohmoriら[10]は，コラーゲン凝集をマーカーとして4分位に分け，81mgのアスピリンを服用している脳梗塞，心筋梗塞の既往のある136例をフォローしたところ，特に1年以内の血栓症の発症率は最もコラーゲン凝集が抑制されていない群で明らかに高かったと報告している．現在，国立循環器病研究センター研究所病因部の宮田敏行が主任研究者となり，「アスピリンレジスタンスの実態ならびにその遺伝子背景に関する研究（ProGEAR study）」が進行中であるが，この研究ではアラキドン酸およびコラーゲン凝集，血小板トロンボキサン産生能，尿中トロンボキサン代謝産物量，遺伝子解析に加え，血中アセチルサリチル酸濃度血中濃度を抜き打ちで計測することにより，服薬コンプライアンスも評価している．592例が登録され，2010年2月末に2年間の追跡がすべての症例で終了し，間もなく解析結果が報告できる．

アスピリン以外の抗血小板剤ではクロピドグレル抵抗性が最近注目されているが，こちらはメカニズムがかなり解明されている．クロピドグレルはプロドラッグで，代謝産物が抗血小板機能を有している．その代謝に関わるCYP2C19には遺伝子多型が存在し，遺伝子多型をもつ症例では代謝産物の産生量が減り，同じ投与量では抗血小板作用が弱くなる[11]（図2，3）．遺伝子多型をもつ頻度は欧米では30%前後とされているが，日本人では60～70%ともっと遺伝子多型をもつ頻度が非常に高いことがわかっている．また，プロトンポンプ阻害薬を併用すると抗血小板作用が弱まることも最近欧米で報告され，話題となっている[11]．

本当にクロピドグレルは日本人では効いていないことが多いのであろうか．クロピドグレルと同じ系統のチエノピリジン系抗血小板薬のチクロピジンは，欧米では500mgの投与量であったが，日本では合併症が多いということで，いつの間にか200mgの投与量が標準となっているが，日本人でのエビデンスを集めたわけではない．このことからクロピドグレルは，当初は欧米と同じ75mgという投与量は多いのではないかと危惧されていたが，製薬会社が行ったチクロピジン200mgとクロピドグレル75mg投与の二重盲検試験で

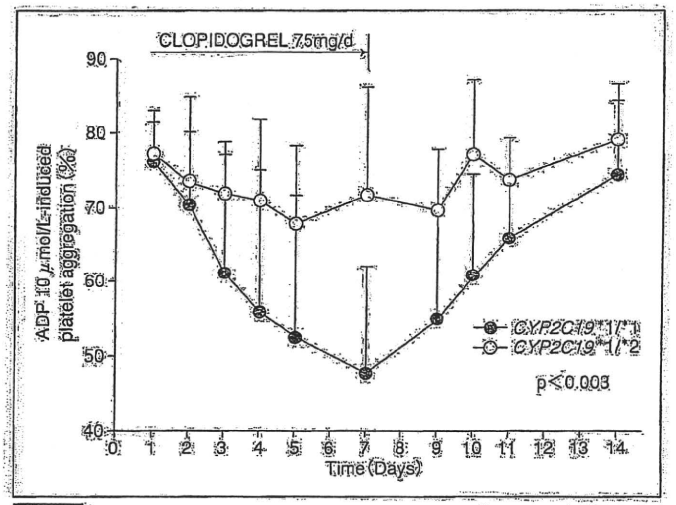


図3 健常者にクロピドグレルを投与した場合のADP凝集抑制効果

CYP2C19の遺伝子多型をもたない群（○）は大きく凝集能が抑制されるが，遺伝子多型をもつ群（●）では凝集能の抑制効果が弱い．

（文献5）より引用改変）

は出血合併症に差がなかったと報告されている．今後日本人におけるクロピドグレルと遺伝子多型，抗血小板作用，イベント抑制率については，独自にしかも公的資金で調査する必要があり，現在，循環器病研究委託費「複雑化する脳・心血管疾患病態における適切な抗血栓治療の開拓」による研究の一環として，多施設前向き共同試験（Cognac study）を開始している．

文献

1) Collaborative meta-analysis of randomised trials of antiplatelet therapy for prevention of death, myocardial infarction, and stroke in high risk patients. BMJ 324: 71-86, 2002

2) Eikelboom JW, Hirsh J, Weitz JI, et al: Aspirin-resistant thromboxane biosynthesis and the risk of myocardial infarction, stroke, or cardiovascular death in patients at high risk for cardiovascular events. Circulation 105: 1650-1655, 2002

3) Ohmori T, Yatomi Y, Nonaka T, et al: Aspirin resistance detected with aggregometry cannot be explained by cyclooxygenase activity: Involvement of other signaling pathway(s) in cardiovascular events of aspirin-treated patients. J Thromb Haemost 4: 1271-1278, 2006

4) Simon T, Verstuyft C, Mary-Krause M, et al: Genetic determinants of response to clopidogrel and cardiovascular events. N Engl J Med 360: 363-375, 2009

5) Hulot JS, Bura A, Villard E, et al: Cytochrome p450 2c19 loss-of-function polymorphism is a major determinant of clopidogrel responsiveness in healthy subjects. Blood 108: 2244-2247, 2006

6) Ho PM, Maddox TM, Wang L, et al: Risk of adverse outcomes associated with concomitant use of clopidogrel and proton pump inhibitors following acute coronary syndrome. JAMA 301: 937-944, 2009

心臓　第41巻　第2号(2009年2月15日発行)別刷

血栓症とその予防薬に対する遺伝子のかかわり

宮田敏行

日本心臓財団

心血管系血栓症―その病態解明と治療への応用―

血栓症とその予防薬に対する遺伝子のかかわり

Genetic involvement in thrombosis diseases and treatments in Japanese

宮田敏行

● はじめに

多因子疾患である静脈血栓塞栓症(venous thromboembolism；VTE)は，その遺伝的背景として凝固制御因子であるアンチトロンビン，プロテインC，プロテインSの先天性欠損症が広く知られている．白人種では，比較的頻度の高い凝固V因子Leiden変異とプロトロンビンG20210A変異もVTEの遺伝的背景として知られている．VTEの環境因子として，手術，外傷，癌，脊椎損傷，長期臥床，経口避妊薬，妊娠，産褥期が知られている．このように，VTEの発症の危険因子が明らかになっているにもかかわらず，米国ではVTEの発症が増加している．これは医師や国民のVTEへの関心の低さに原因があると考えられ，2008年3月号のArterioscler Thromb Vasc Biol誌は，VTEに関する8つの総説を掲載しVTEへ喚起を呼びかけた．また，2007年にBlood誌のPerspectiveにもVTEが取り上げられた．

VTEの発症は加齢で増加する．米国ミネソタ州の1966～1990年の統計によると，VTE発症者数は55歳から増加し，80歳では約年に1回/100人の発症となり，80歳以上では45歳以下と比べて約1,000倍上昇するという．今後，本邦でもVTE発症は診断法の普及とともに増加すると考えられるので，VTE発症に関与する因子を，日本人を対象に調査することは重要であると考えられる．こういった調査を大規模な一般住民を対象に行うと，VTEの背景因子が明らかにできると考えられる．

国立循環器病センター循環器予防検診部は，循環器疾患の背景因子を解明することを目的として，地域一般住民を対象としたコホート研究を進めている．本稿では一般住民およびVTE発症患者を対象とした研究を紹介する．また，ファルマコゲノミクス研究として進めているワルファリンとアスピリンにかかわる遺伝子研究も紹介したい．

● 一般住民を対象にしたVTEに関する研究

当センター予防検診部は，地域一般住民を対象としたコホート研究を1989年から開始している．これは住民台帳から性別と10歳ごとで無作為に抽出した30歳代から70歳代の14,200人を対象にしている．最近では2年に1度，のべ4,000人程度の方の参加がある．**図1**に血液凝固のカスケード反応を示す．血液の流動性は凝固因子と制御因子のバランスで成り立っており，過凝固因子状態もしくは低制御因子状態は，VTEのリスクとなる．われわれはこれまで**図1**の＊印で示す因子を含めて，計13種の血漿因子の活性もしくは抗原量を測定してきた．なかでも，アンチトロンビン，プロテインC，プロテインSの先天性欠損症はVTEのリスクであるため，一般住民を対象に活性を測定し，欠損症の頻度を求めることは重要である．

われわれはアンチトロンビン，プロテインC，プロテインS，プラスミノーゲンの活性について一般住民を対象に測定した．その結果を**図2**に示す[1]．プラスミノーゲンは活性値が60%程度に小さなピークが見られるが，これはプラスミノーゲンAla620Thr変異保有者である．プロテインCとプロテインSの活性はアンチトロンビンより広い活性分布を示した．次いで，これら4因子の活性を男女に分けて10歳代ごとで示す(**図3**)[1]．男性では4つのいずれの因子の活性も加齢により低下し，これが高齢者の血栓傾向を一部説明するかもしれない．一方，女性は30歳代と40歳代のプロテインCとプロテインS活性が低いことがわかる．これらの活性値から，一般住民におけるそれぞれの因子の欠損症の頻度を求めることができる．すなわち，プロテインCは0.13%(4,517人中6人)，プロテインSは1.12%および1.60%(男性：1,252人

国立循環器病センター研究所病因部
National Cardiovascular Center Research Institute
Toshiyuki Miyata

● Key words；静脈血栓塞栓症，血栓性素因，プロテインS，ワルファリン，アスピリン

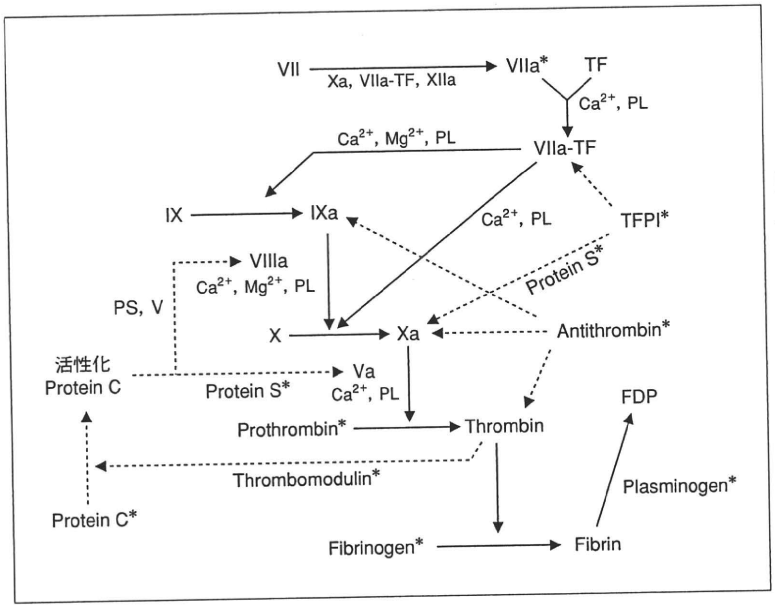


図 1
血液凝固カスケード
血液凝固は凝固Ⅶa因子が組織因子(tissue factor；TF)に結合することで開始される．この複合体によりⅨやⅩが活性化され大量のトロンビンが生成する(実線)．一方，凝固を抑制する因子として，プロテアーゼインヒビターであるアンチトロンビンと組織因子経路インヒビター(TFPI)，および内皮細胞上でプロテインC抗凝固反応を担うプロテインCとプロテインSがある．これらの抑制反応は点線で示した．一般住人を対象に活性を測定した因子は＊印で示した．ここに示した因子以外に，フォンビルブランド因子(vWf)とPAI-1も一般住民を対象に測定を終了している．

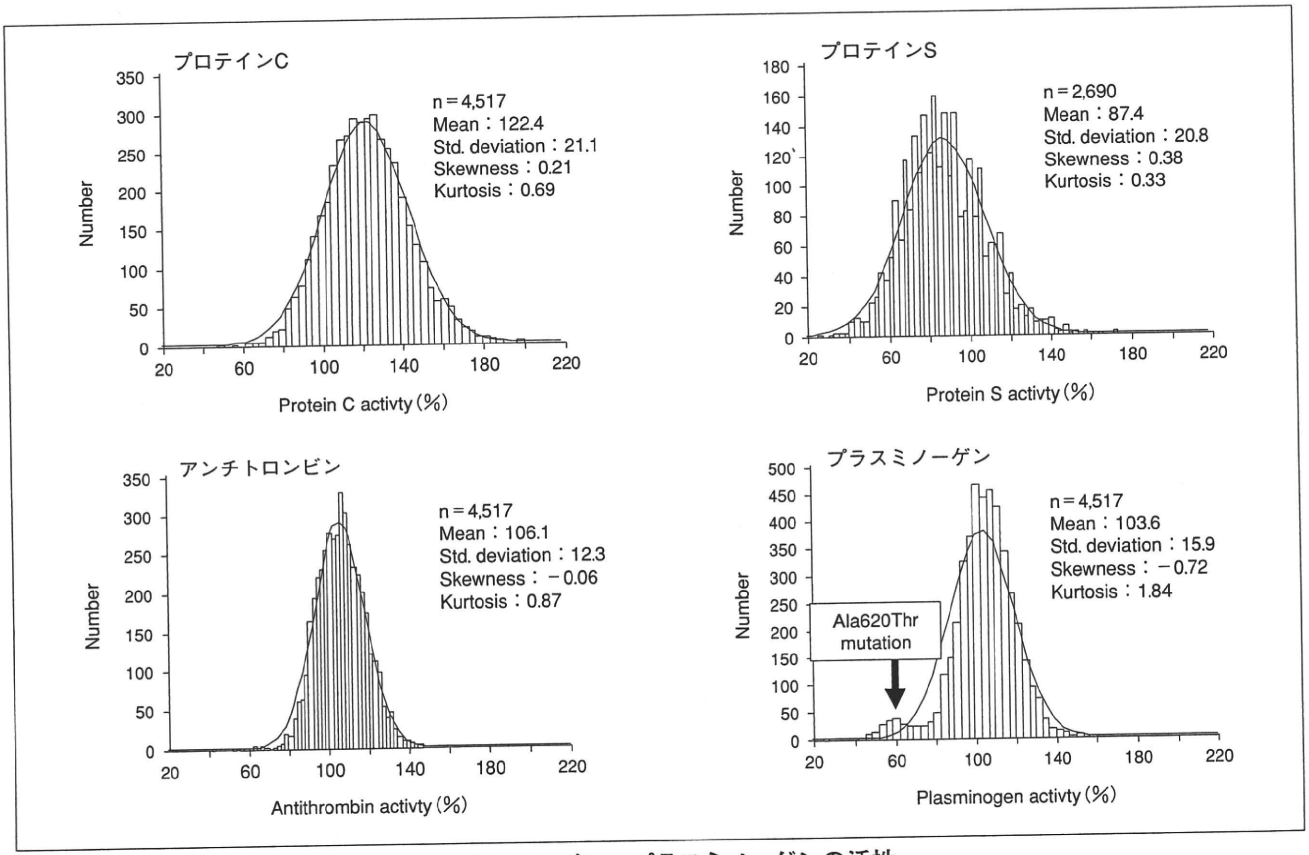


図 2　プロテインC，プロテインS，アンチトロンビン，プラスミノーゲンの活性
測定対象は一般住民4,517人．プロテインSのみ2,690人で測定した．プロテインCとプロテインSは広い活性幅を示す．プラスミノーゲンは活性60%程度を示すピークが存在した．これらの多くはAla620Thr変異(いわゆるplasminogen Tochigi変異)を有する．

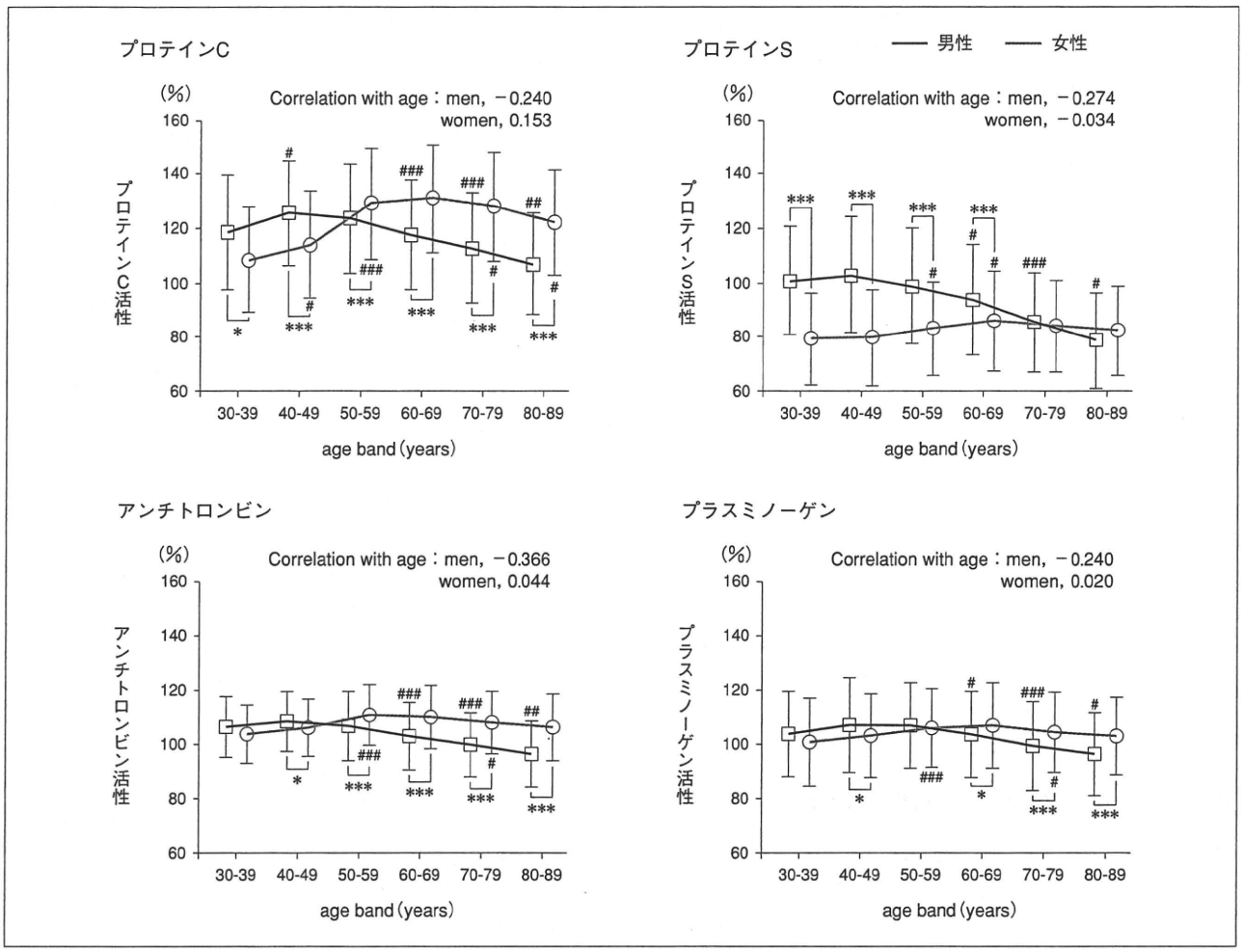


図3 プロテインC，プロテインS，アンチトロンビン，プラスミノーゲン活性の性差と年齢差
活性と男女別に10歳ごとに示した．プロテインCとプロテインSの活性に大きな性差が見られる．特に，30歳代と40歳代の女性のプロテインS活性は同年代の男性より約20%低い．

中14人，女性：1,438人中23人)，アンチトロンビンは0.15%(4,517人中7人)，プラスミノーゲンは4.29%(4,517人中ヘテロ192人，ホモ2人)であった．こうして求めた一般住民での頻度と，国立循環器病センターで収集したVTE患者(108人)の活性値から測定した欠損症の頻度を比較した．その結果，プロテインC欠損症およびアンチトロンビン欠損症はオッズ比が52および38であり，VTEの強い危険因子であることが判明した[1]．一方，プラスミノーゲン欠損症のオッズ比は0.65であるVTEの危険因子ではなかった[1]．これらの結果は，欧米での報告によく一致した．プロテインS活性はVTE患者で測定していなかったので，欠損症がリスクになるかどうかはわれわれの結果では明らかではないが，欧米の結果ではプロテインS欠損症もVTEのリスクであることが明らかとなっている．以上の結果は，活性値を測定し，低い活性値を示す人を欠損症と考えて，欠損症の頻度を一般住民群とVTE群で比較したものである．

表1に日本人と欧米人の欠損症の頻度を，一般住民とVTE患者で示した[1]．日本人に広く見られるプラスミノーゲン欠損症は，幸いなことに，VTE危険因子ではなかった．VTEの危険因子であるアンチトロンビンとプロテインCの欠損症の頻度は，日本人と欧米人で差は見られない．プロテインS欠損症は日本人に多く見られるVTEの危険因子であった．

一般住民を対象に欠損症の頻度を求めることができたので，これを用いて日本人の欠損症の人数を推定できる．

表1　日本人と欧米人での血栓性因子欠損症の頻度の比較

		一般住民	VTE患者
プラスミノーゲン	日本人	4.29	2.8
欠損症(%)	欧米人	0.3～0.5	n.d.
アンチトロンビン	日本人	0.15	5.6
欠損症(%)	欧米人	0.17	1.1
プロテインC	日本人	0.13	6.5
欠損症(%)	欧米人	0.15～0.33	3.2
プロテインS	日本人	1.12	n.d.
欠損症(%)	欧米人	0.03～0.13	1.3～2.2

日本人のプラスミノーゲン欠損症のほとんどはA620T変異を持ち，この変異は白人種には存在しない．

その推定では，プロテインS欠損症は約140万人，プロテインC欠損症は約16万人，アンチトロンビン欠損症は約19万人となる．欠損症によるVTE発症の浸透率は明らかとなっていないものの，それほど高いものではないと想像される．したがって，活性低値保有者が必ずしもVTEを発症するものではないが，こういった人は，VTEの発症を避ける生活様式をとることが薦められよう．

● VTE患者を対象にした遺伝子の研究

厚生労働省科学研究費補助金難治性疾患克服研究事業，血液凝固異常症に関する調査研究班では「特発性血栓症サブグループ」を組織し，VTE患者の遺伝的背景に関する研究を進めてきた．本サブグループは6施設(大阪大学；川崎富夫，京都府立医科大学；辻肇，名古屋大学；小嶋哲人，自治医科大学；坂田洋一，慶應義塾大学；村田満，国立循環器病センター；宮田敏行)で構成された．本サブグループでVTE患者約170人を登録し，一般住民を対象としてVTEの遺伝的背景を調査した．

まず，候補となる次の5つの変異を検討した[2]．ADAMTS13 P475S変異は，血栓性血小板減少性紫斑病(thrombotic thrombocytopenic purpura；TTP)の原因遺伝子である．P475S変異はわれわれが同定したミスセンス変異で，これによりプロテアーゼ活性は低下する(しかし，TTPの発症を起こすまでにはいたらない)．プラスミノーゲンAla620Thr変異はすでに述べたように活性低下を示す変異である．プロテインS K196E変異も活性低下を示す．凝固XII因子－4C＞T変異は変異により本来のATG配列より上流に新しいATGができるので，ここから翻訳が始まり本来のATGからの翻訳量が減少するため血中XII因子量が低下する．PAI-1 4G/5GはプロモーターI領域の変異で転写因子の結合に影響を与え，PAI-1 mRNA量が変化する．

これら5多型をタイピングした結果，プロテインS K196E変異の変異Eアレル頻度が，VTE群で有意に高く，変異EアレルがVTEのリスクであることが判明した(オッズ比；5.6，95%信頼区域；2.90-9.46)(**表2**)[2]．ほかの4多型はプラスミノーゲンA620T変異を含めて一般住民群とVTE群で頻度に差が見られず，VTEと関連を示さなかった．プロテインS K196E変異は九州大学濱崎教授が行った研究でもVTEと関連を示した(オッズ比；3.7)．このように，2つの独立した研究から本変異とVTEの関連が明らかになった．われわれの研究から，一般住民3,651人中にヘテロ接合体が66人，ホモ接合体はいなかったので，アレル頻度は0.9%と計算された．これは，以前名古屋大学が算出した0.8%，今回九州大学が求めた0.8%とよく一致した．これより，一般住民の約55人に1人がヘテロ接合体と算出された．この頻度から，12,000人に1人がホモ接合体と計算された．日本人総人口を1億2,000万人とすると，1万人がホモ接合体であると推定された．

プロテインS K196E変異は1993年に名古屋大学と三重大学がVTE患者に同定したミスセンス変異であり，血中に本変異を有する異常分子が存在する．本変異体は活性化プロテインCのコファクター活性を示さないことが発現実験で明らかとなっている．われわれは本変異のヘテロ接合体保有者の血中プロテインS活性を調べるため，一般住民1,862人の活性を測定し，遺伝型との関連を調べた[3]．このうち34人がヘテロ接合体であり，プロテインS活性は40%から110%まで広い範囲を示した(**図4**)．一方，野生型プロテインSをもつ1,828人の活性も40%から170%までの広い活性分布を示した．このように血中のプロテインS活性だけではK196E変異を識別できないことが判明した．しかし，遺伝型に分けて活性を比較すると，変異Eアレルのヘテロ接合体者は平均16%の有意な活性低下を示すことが明らかとなった[3]．

プロテインS K196E変異はプロテインS活性を低下させることから，ほかの血栓性疾患の素因になる可能性があり，この点が今後の課題である．また，本変異は日本人だけでなく東アジア人に共通に見られる変異と考えられるので，韓国人や中国人を対象にした研究も進められるべきであろう．

図3に一般住民を対象にしたプロテインS活性の性・

表2 日本人VTE患者を対象とした5遺伝子多型の症例対照研究

		ADAMTS13 P475S	PLG A620T	PS K196E	FXII −4C>T	PAI-1 4G/5G
VTE	Major homo	139	152	146	63	61
	Hetero	20	9	13	75	69
	Minor homo	1	0	2	23	30
	Total	160	161	161	161	160
	MAF	0.069	0.028	0.053	0.376	0.403
General population (Suita study)	Major homo	3,290	3,501	3,585	1,513	1,468
	Hetero	332	149	66	1,651	1,686
	Minor homo	17	0	0	486	497
	Total	3,639	3,650	3,651	3,650	3,651
	MAF	0.050	0.020	0.009	0.359	0.367
	χ^2	2.179	0.987	75.464	0.372	3.402
	P	0.336	0.320	<0.0001	0.830	0.183
	Major allele	Pro	Ala	Lys	T	4G
	Minor allele	Ser	Thr	Glu	C	5G

MAF：minor allele frequency

（文献2より改変引用）

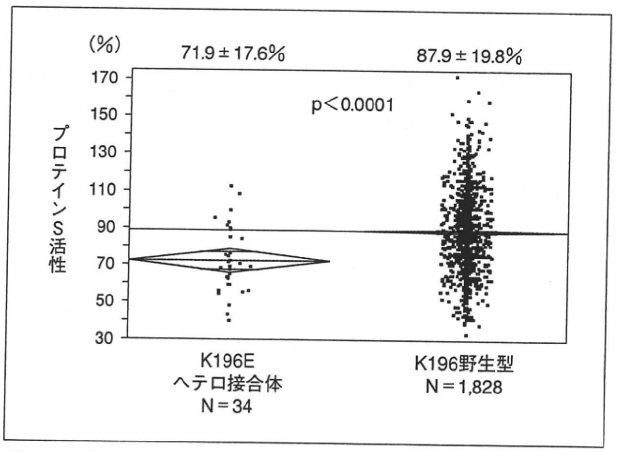


図4 プロテインS K196E変異保有者の血中プロテインS活性

一般住民1,862人の血中プロテインS活性を測定し，K196E変異の遺伝型に分けて活性を比較した．ヘテロ接合体(34人)のプロテインS活性(71.9±17.6%，平均値±SD)は野生型(1,828人)のプロテインS活性(87.9±19.8%)より有意に低い(p<0.0001)．

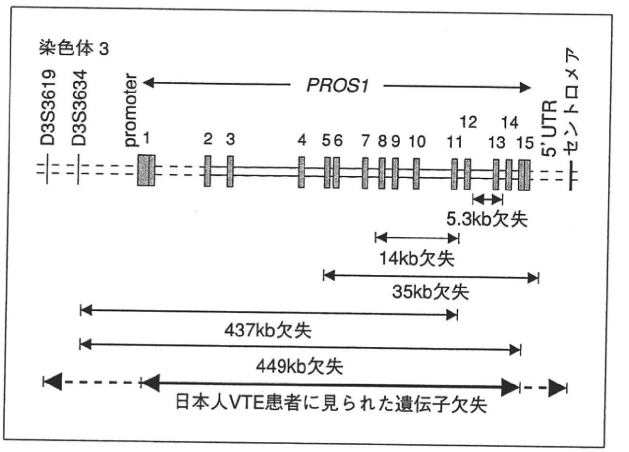


図5 プロテインS遺伝子に同定された遺伝子欠損

日本人VTE患者に認められた欠損はプロテインS遺伝子の全域が欠損していた．

年齢差を示したが，プロテインS活性は多くの後天性因子の影響を受ける．特に妊娠時のプロテインS活性は欠損症に匹敵する程度にまで低下することが知られており，注意が必要である．**図5**にプロテインSの抗凝固能についてまとめた．プロテインS活性の抗凝固能は活性化プロテインCのコファクター，およびTFPIのXa因子阻害の促進で説明される．プロテインSは血中では補体制御因子であるC4BPと複合体を形成し，複合体のプロテインSは抗凝固能を持たず，遊離型だけが抗凝固能を示す．C4BPはα鎖だけで構成される分子とβ鎖を含む分子がある．プロテインSはβ鎖にのみ結合する．α鎖は

表3　173人のVTE患者に見られたアミノ酸配列が変化する変異を有する患者数

遺伝子名	患者数
プロテインS	24
プロテインC	12
アンチトロンビン	14
プロテインS＋プロテインC	5
合計	55

炎症で増加するがβ鎖は増加しない．したがって，炎症時でも遊離プロテインS量は大きな変動はなく，プロテインSによる抗凝固能も大きく変化するものではない．

プロテインS欠損症とみなされる程度にまで活性が低下した患者のプロテインS遺伝子の解析を行っても，その半数にしか原因変異は同定されず，残りの症例には変異を同定できないことが世界的な問題になっている．これは，重症血友病患者の遺伝子解析を思い起こさせる．この場合，第Ⅷ因子遺伝子のAlu配列特異的逆位(inversion)で説明された．しかし，プロテインS遺伝子近傍にはAlu配列は存在しない．そこで，プロテインS遺伝子の欠失を想定し，VTE患者163人のプロテインS遺伝子欠損の解析を行った[4]．その結果，プロテインS活性21%を示す1人の患者に遺伝子全域の欠失を認めた．163人中に1人では高頻度に認められることにはならないが，プロテインS活性が50%以下で，かつミスセンス変異が同定されない患者とすると6人に絞られ，そのうちの1人が遺伝子欠失を保有していたことになる．プロテインSの遺伝子欠失はVTE患者に広く認められるものではないが，活性低下症例には存在することが明らかとなった．

● VTE患者にみられる稀な変異

VTEの遺伝的背景として，アンチトロンビン，プロテインC，プロテインSの遺伝子変異が知られているが，どれくらいの頻度でどういった変異が存在するのかは，日本人を対象に行った研究はなかった．そこで，サブグループで収集したVTE患者173人を対象に3つの遺伝子の蛋白質コード全領域のDNAシークエンスを行った．その結果を**表3**に示す．VTE患者54人にミスセンス変異などのアミノ酸変化を伴う遺伝子異常を同定した．プロテインS遺伝子欠損患者1人を加えると，総計55人，約32%の患者に，39種のアミノ酸の変化を伴う変異を同定した．なかでもプロテインS K196Eは前述のように最も多くの患者に見られ，次いでプロテインCのK193del変異とV339M変異が4人ずつの患者に同定されたので，これらの変異は日本人のVTEのリスクとして重要であることが明らかとなった．V339M変異保有者のプロテインCアミド活性は1人(77%)を除き，低い活性を示したが，K193del変異保有者はいずれもアミド活性は90%以上を示した．本変異保有者は九州大学も同定しており，その抗凝固活動は低値を示していることから，本変異は抗凝固活性には影響を与えるがアミド活性には影響しないものと考えられる．素因スクリーニングとしてプロテインCアミド活性の測定の際に注意が必要である．K196残基はプロテインC軽鎖のC末端から6残基目に位置するので，この残基がアミド活性に影響しないことを説明できよう．

表4　2つの遺伝子に変異を有する5人の患者

プロテインS	プロテインC	VTE発症年齢	家族歴
K196E	K193del	57	情報なし
K196E	R221W	40	no
K196E	R27W	39	yes
K196E	V339M	25	yes
K196E	V339M	55	no

表5　変異保有者と非保有者のVTE発症年齢の比較

	変異保有者	変異非保有者
人数	55*	118
発症年齢 平均±SD	44.7±16.5	52.6±16.1

*5人はプロテインSとプロテインCに変異を持つ．2人はプロテインS K196E変異を持つ．1人はプロテインS K196E，R101Cの複合ヘテロ体である．
p=0.0031

VTE患者の5人は，2つの遺伝子に変異を保有していた．この5人の患者はいずれもプロテインS K196E変異を保有していた(**表4**)．このことから，プロテインS K196E変異はほかの変異と重複することによりVTE発症のリスクを上げていると考えられた．

VTE患者55人にアミノ酸変化を伴う遺伝子異常を同定したので(**表3**)，次にこれら遺伝子変異保有者のVTE発症年齢を変異非保有者と比較した(**表5**)．その結果，変異保有者のVTEは非保有者より有意に若年で発症することが判明し，遺伝子変異のVTE発症への寄与が明らかになった．