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ORIGINAL ARTICLE

Association between arterial stiffness and platelet activation

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Increased arterial stiffness is strongly associated with atherosclerosis, while platelet activation is an important trigger of thrombotic events in patients with atherosclerosis. However, little is known about the effect of arterial stiffness on platelet activation. We therefore investigated the association between arterial stiffness and platelet activation in 38 normal volunteers (20 men and 18 women) aged 23–77 years (mean = 49 ± 15 years). Arterial stiffness was assessed by measuring brachial–ankle pulse wave velocity (ba-PWV) and heart–brachial PWV (hb-PWV). Flow cytometric analyses were performed to evaluate platelet activation by measuring surface expression of P-selectin and platelet–neutrophil complexes (PNC) before and after activation by ADP. We also calculated the difference between basal and stimulated states of P-selectin and PNC to assess platelet activation reserve. PWVs were significantly correlated with age and BP ($r = 0.60–0.81$). For platelet activation and activation reserve, correlations with age were less strong but remained significant ($r = 0.36–0.61$), with the exception of P-selectin (not significant, NS), and correlations with SBP were similar ($r = 0.35–0.53$). A significant correlation was found between PWVs and platelet activation ($r = 0.43–0.74$). Multiple regression analysis demonstrated significant correlations between platelet activation and reserve and PWVs (coefficient = 2.17–6.59), when both age and BP were adjusted for simultaneously. In conclusion, platelet activation was associated with arterial stiffness, suggesting that arterial stiffness may play an important role in thrombotic events.

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Introduction

Platelet activation and aggregation are important triggers of thrombotic events in patients with atherosclerosis. In such patients, platelets are activated at the site of atheroma[1] due to increased shear stress in the narrowed vessels.[2,3] Increased platelet activation is observed in patients with coronary risk factors and cardiovascular events.[4–12]

Increased arterial stiffness, measured with pulse wave velocity (PWV), has been shown to be associated with atherosclerosis and risk factors of atherosclerotic cardiovascular disease,[13–21] and is an independent predictor of cardiovascular events,[22,23] Therefore, although platelets are likely to be activated in patients with atherosclerotic disease who exhibit increased arterial stiffness, little is known about the relation of arterial stiffness itself to platelet activation.

Recently, platelet activation has been widely evaluated by measuring soluble P-selectin; a platelet surface molecule also termed CD62P.[4,6–8,11] Although the measurement of soluble P-selectin is simple and useful, it is an indirect method of evaluating platelet activation. On the other hand, platelet activation can be detected directly by measuring surface antigen CD62P using flow cytometry.[2,3,5,9,10,12] Furthermore, detection of platelet–neutrophil complexes (PNC), which are formed as a result of interaction with CD62P provides an additional means to detect platelet activation.[24]

The purpose of this study was to investigate the association between arterial stiffness and platelet activation by measuring PWV, P-selectin, and PNC in subjects without atherosclerotic disease.

Materials and methods

Subjects

We studied 38 healthy nonsmoking volunteers (20 men and 18 women), aged 23–77 years

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(mean = 49 ± 15 years) with no evidence of heart disease on physical examination, standard 12-lead electrocardiography, chest radiography, echocardiography, or blood chemistry analysis. Subjects had no self-reported past history or current evidence of cardiovascular disease, hypertension, hypercholesterolaemia, diabetes mellitus or renal disease. Basic characteristics of subjects are shown in Table 1. None of the subjects had frequent ectopic beats or atrial fibrillation and none had taken any medication for at least 10 days. Informed consent was obtained before performing the study and the study protocol was approved by the Local Ethics Committee of Kochi Medical School.

Evaluation of arterial stiffness

Arterial stiffness was evaluated by PWV, measured using volume-plethysmographic apparatus (Colin, Komaki, Japan).[18–21] Data were acquired with subjects lying supine in a quiet and temperature-controlled room at 11 AM, at least 3 h after breakfast. Surface electrodes were attached to both wrists for ECG measurement, a microphone was positioned at the left sternal edge to detect heart sounds, and cuffs incorporating plethysmographic and oscillometric sensors were fastened around both the brachial regions and ankles to measure pulse wave forms and blood pressure. Brachial–ankle PWV (ba-PWV) and heart–brachial PWV (hb-PWV) were measured as follows. The time interval between the wave foot of the brachial waveform and that of the ankle waveform was defined as the time interval between the brachial region and ankle, while the time interval between the heart and the right brachial artery was defined as the time interval between the second heart sound and the right brachial waveform. The distance between these sampling points was calculated automatically according to the height of the subject. PWVs were calculated by dividing each distance by the respective time interval. Right brachial blood pressure (systolic and diastolic) and pulse rate were concurrently measured.

Table 1 Clinical characteristics of subjects

Parameters	*All subjects (n = 38)*
Age (years)	49 ± 15
Gender, male/female	20/18
Systolic blood pressure (mmHg)	125 ± 16
Diastolic blood pressure (mmHg)	77 ± 10
Pulse rate (bpm)	66 ± 10
Blood sugar (mg/dl)	98.5 ± 18.5
Total cholesterol (mg/dl)	192.6 ± 20.7
Blood urea nitrogen (mg/dl)	14.0 ± 18.5
Creatinine (mg/dl)	0.69 ± 0.15
PNC (%)	9.5 ± 4.9
PNC(ADP) (%)	20.2 ± 9.9
Δ-PNC	10.7 ± 6.9
P-selectin (%)	13.1 ± 1.7
P-selectin(ADP) (%)	36.6 ± 9.2
Δ-P-selectin	23.6 ± 9.1
hb-PWV (m/s)	5.3 ± 0.9
ba-PWV (m/s)	13.8 ± 3.0

Values are expressed as mean ± s.d.
PNC = platelet neutrophil complexes; ADP = adenosine diphosphate; Δ-PNC = PNC (ADP)−PNC; Δ-P-selectin = P-selectin (ADP)−P-selectin; hb-PWV = heart–brachial pulse wave velocity; ba-PWV = brachial–ankle pulse wave velocity.

Measurement of platelet activation

Sample preparation and measurement of platelet P-selectin (CD62P) and PNC levels were performed according to the method described by Peters *et al.*[24] To minimize platelet activation during blood collection, blood was drawn via a 21 G butterfly needle without the use of a tourniquet. After discarding the first 2 ml of blood, a further 2 ml was collected and immediately added to 200 μl of sodium citrate (3.13%). All antibodies were sourced as follows: Fluorescein isothiocyanate (FITC) labelled IgG1 anti-CD62P from Dainippon Pharmaceutical, Osaka, Japan, phycoerythin (PE) labelled IgG2a anti-CD42b and FITC labelled IgG1 anti-CD11b from Beckman Coulter, Fullerton, CA, USA. As negative controls, FITC-labelled IgG1 (Beckman Coulter, Fullerton, CA, USA) and double-stained (FITC/PE) IgG1 and IgG2a (Dako, High Wycombe, Bucks, UK) irrelevant antibodies were included.

Sample preparation for the measurement of platelet CD62P level: In all, 5 μl of blood was added to a round-bottomed polystyrene tube containing 50 μl of platelet buffer (10 mmol/l HEPES, 145 mmol/l NaCl, 5 mmol/l KCl, 1 mmol/l $MgSO_4$; pH 7.4), and 5 μl of anti-CD62P or control IgG1 antibody. Following gentle suspension, samples were incubated in the dark at room temperature for 20 min without stirring. Then 250 μl of fixative was added and the tubes were incubated for an additional 10 min. The samples were then diluted with 500 μl of buffer and analysed. Flow cytometric analysis was performed within 1 h of fixation.

Sample preparation for the measurement of PNC level: In all, 50 μl of blood was added to a round-bottomed polystyrene tube containing 5 μl of anti-CD42b, and 5 μl of anti-CD11b or isotype control antibodies. Following gentle mixing, samples were incubated in the dark at room temperature for 10 min without stirring. Then 500 μl of fixative was added and the tubes were incubated for additional 10 min. Flow cytometric analysis was performed within 1 h of preparation.

Flow cytometric analysis

Blood samples were analysed in a COULTER EPICS XL Profile Flow Cytometer, Miami, FL, USA, using either single or double fluorochromes. The peak emission intensity of FITC fluorescence was

detected at 515 nm and that of phycoerythin fluorescence at 580 nm.

Measurement of platelet CD62P level: After forward and side scatter measurements were made with gain setting in logarithmic mode, platelet-sized events were counted. CD62P-positive platelets were defined as those with a fluorescence intensity exceeding that of 98% of the platelets staining with control antibody.

Measurement of PNC level: After forward and side scatter measurements were made with gain setting in linear mode, neutrophil-sized events were selected. Results were defined as positive when the fluorescence intensity exceeded that of 98% of the isotype-matched (IgG1 and IgG2a) control antibodies staining. Events positive for both CD11b and CD42b were considered to represent PNCs and were expressed as percentages of events with positive CD11b staining.

Evaluation of platelet activation reserve: We evaluated platelet activation reserve, that is, the ability of the platelets to be activated, in a separate experiment. Platelets were activated with 5 μl of adenosine diphosphate (ADP). We also calculated the difference between basal and stimulated states of P-selectin expression (Δ-P-selectin) and PNC level (Δ-PNC) to determine activation reserve.

Statistical analysis

Data are presented as mean±s.d. Univariate linear correlation analysis and multiple regression analysis were used for statistical evaluation. The variables significantly associated with platelet activation on univariate analysis were included in a multiple regression analysis in order to adjust PWV for each variable. Gender differences were evaluated with ANOVA. *P*-values <0.05 were considered to represent statistical significance.

Results

Both ba-PWV and hb-PWV exhibited significant positive correlations with age, systolic, and diastolic blood pressure ($r=0.60$–0.81, $P<0.05$ or <0.01), and pulse rate ($r=0.44$, $P<0.05$, $r=0.65$, <0.01, respectively) (Table 2). For platelet activation and activation reserve, correlations with age were less strong but remained significant ($r=0.36$–0.61, $P<0.05$ or <0.01) with the exception of Δ-P-selectin (not significant, NS), and correlations with systolic and diastolic blood pressure were similar ($r=0.35$–0.53, $P<0.05$ or <0.01) with the exception of P-selectin (NS) (Table 3). However, platelet activation and activation reserve exhibited no significant correlation with pulse rate, blood glucose, total cholesterol, blood urea nitrogen or creatinine. No significant gender-related differences were observed in any of these correlations (Tables 2 and 3).

Table 2 Correlation between PWV and clinical indices

	hb-PWV	*ba-PWV*
Age	0.74**	0.80**
Systolic blood pressure	0.61**	0.81**
Diastolic blood pressure	0.60**	0.74**
Pulse rate	0.44*	0.65**
Blood sugar	−0.05	−0.17
Total cholesterol	−0.03	−0.30
Blood urea nitrogen	−0.32	0.32
Creatinine	0.04	−0.14
Gender		
Male	5.5±1.0	14.1±3.0
Female	5.2±0.8	13.6±3.1

PNC = platelet neutrophil complexes; ADP = adenosine diphosphate; Δ-PNC = PNC (ADP)−PNC; Δ-P-selectin = P-selectin (ADP)−P-selectin; hb-PWV = heart–brachial pulse wave velocity; ba-PWV = brachial–ankle pulse wave velocity.
For parameters from age to creatinine, values are correlation coefficients.
*$P<0.05$.
**$P<0.01$.
For gender, values are mean±s.d., with differences evaluated with ANOVA.

PWVs exhibited significant positive correlations ($r=0.43$–0.74, $P<0.05$ or <0.01) to all indices of platelet activation and reserve (Table 4, Figure 1). When age or blood pressures were adjusted for on multivariate analysis, some indices of platelet activation and reserve were significantly related to PWVs ($r=0.34$–7.67, $P<0.05$ or <0.01). When both age and blood pressures were simultaneously adjusted for, significant correlations remained between platelet activation and reserve and PWVs ($r=2.17$–6.59, $P<0.05$ or <0.01) (Table 4). In other words, although the relationship between PWVs and the indices of platelet activation was strongly affected by age and blood pressure, a significant association remained when these factors were adjusted for.

Discussion

The main finding of this study was that platelet activation and activation reserve were associated with arterial stiffness when analyses were adjusted for age and blood pressure. This suggests that increased arterial stiffness might play an important role in thrombotic events.

Patients with hypertension, cerebrovascular disease, coronary heart disease, diabetes mellitus, and renal failure are recognized to have less arterial compliance than normal subjects.[13–15,17–19] Increased PWV has also been reported to be an independent predictor of cardiovascular events in patients with hypertension or renal failure, and in elderly subjects.[22,23] The association between increased arterial stiffness and high incidence of cardiovascular events may be explained by the existence of atherosclerosis. Hirai *et al*[25] have demonstrated strong associations between abdominal aortic and

Table 3 Correlation between platelet activation and clinical indices

	PNC	*PNC (ADP)*	*Δ-PNC*	*P-selectin*	*P-selectin (ADP)*	*Δ-P-selectin*
Age	0.51**	0.61**	0.52**	0.36*	0.38*	0.32
Systolic blood pressure	0.41*	0.53**	0.48**	0.41*	0.43*	0.35*
Diastolic blood pressure	0.43*	0.49**	0.40*	0.25	0.40*	0.36*
Pulse rate	0.28	0.25	0.16	0.04	0.15	0.15
Blood sugar	0.09	−0.18	−0.31	−0.17	0.13	0.16
Total cholesterol	−0.14	−0.07	0.001	−0.10	−0.13	−0.11
Blood urea nitrogen	−0.01	0.12	0.18	−0.05	0.05	0.06
Creatinine	0.05	−0.13	−0.22	0.04	−0.17	−0.18
Gender						
Male	10.3±5.9	19.7±8.7	9.4±6.9	13.1±1.8	35.5±9.3	22.4±9.0
Female	8.8±3.8	20.7±11.4	11.9±6.8	13.0±1.7	37.7±9.2	24.7±9.3

PNC = platelet neutrophil complexes; ADP = adenosine diphosphate; Δ-PNC = PNC (ADP)−PNC; Δ-P-selectin = P-selectin (ADP)−P-selectin; hb-PWV = heart–brachial pulse wave velocity; ba-PWV = brachial–ankle pulse wave velocity.
For parameters from age to creatinine, values are correlation coefficients.
*$P<0.05$.
**$P<0.01$.
For gender, values are mean±s.d., with differences evaluated with ANOVA.

Table 4 Relation between platelet activations and PWV

	PNC	*PNC (ADP)*	*Δ-PNC*	*P-selectin*	*P-selectin (ADP)*	*Δ-P-selectin*
Not adjusted						
hb-PWV	0.62**	0.74**	0.63**	0.45**	0.57**	0.50**
ba-PWV	0.59**	0.71**	0.61**	0.47**	0.51**	0.43*
Adjusted for age						
hb-PWV	2.86**	6.95**	4.09*	0.75	6.55**	5.80*
ba-PWV	0.79	2.01**	1.22*	0.28	1.75*	1.47
Adjusted for systolic blood pressure						
hb-PWV	3.20**	7.23**	4.04**	0.59	5.09*	4.50*
ba-PWV	1.21**	2.64**	1.44*	0.23	1.48	1.25
Adjusted for diastolic blood pressure						
hb-PWV	3.08**	7.67**	4.59**	0.87*	5.32**	4.46*
ba-PWV	0.97**	2.50**	1.54**	0.34*	1.45*	1.10
Adjusted for age and systolic blood pressure						
hb-PWV	2.80*	6.43**	3.63*	0.58	5.93*	5.35*
ba-PWV	1.08	2.32*	1.24	0.24	1.72	1.48
Adjusted for age and diastolic blood pressure						
hb-PWV	2.63*	6.59**	3.97**	0.78	6.06*	5.28*
ba-PWV	0.76	2.17*	1.40	0.40	1.66	1.26

PNC = platelet neutrophil complexes; ADP = adenosine diphosphate; Δ-PNC = PNC (ADP)−PNC; Δ-P-selectin = P-selectin (ADP)−P-selectin; hb-PWV = heart–brachial pulse wave velocity; ba-PWV = brachial–ankle pulse wave velocity.
'Not adjusted' — values are correlation coefficients between PWVs and indices of platelet activation before adjustment.
*$P<0.05$.
**$P<0.01$.
Other values are regression coefficients between PWVs and indices of platelet activation adjusted for age and/or blood pressures as indicated.
*$P<0.05$.
**$P<0.01$.

carotid arterial stiffness and the degree of coronary artery disease. Popele *et al*[26] recently reported that aortic stiffness as measured by PWV is strongly associated with common carotid intima-media thickness, carotid arterial plaques, and the presence of peripheral arterial disease. Moreover, some population-based studies have demonstrated higher blood pressure, increased age, and male gender to be associated with increased PWV.[16,20,21] Pulse pressure may also relate to arterial stiffness and cardiovascular events, with higher pulse pressure reflecting elevated systolic pressure and reduced diastolic pressure due to increased arterial stiffness. In the present study, significant relationships were observed between PWVs and age, blood pressure, and pulse rate, in accordance with previous studies.

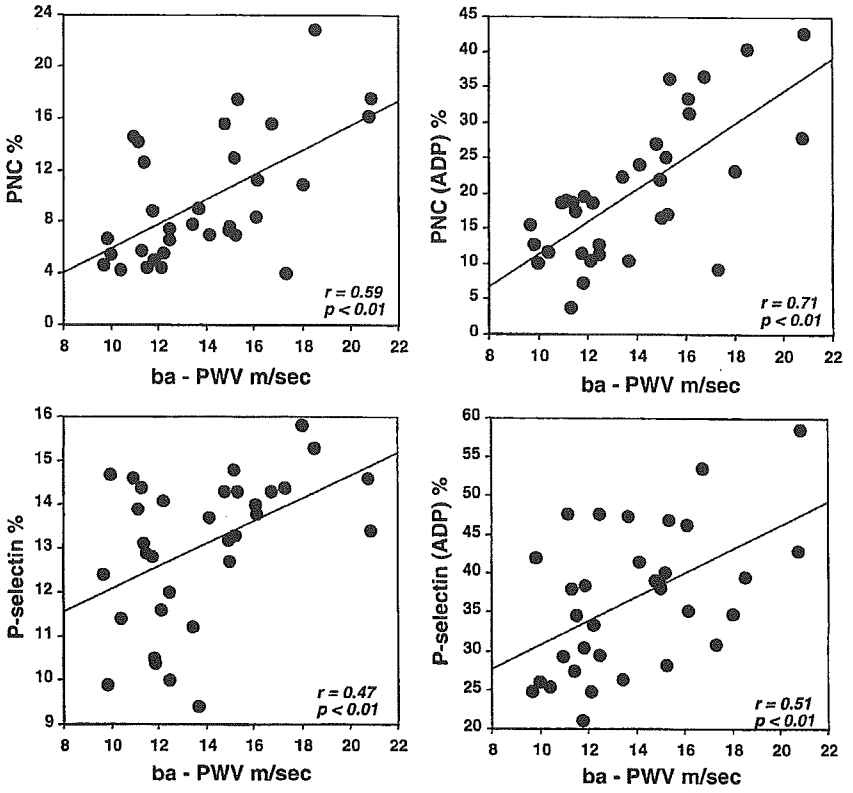

Figure 1 Correlation between ba-PWV and PNC (upper two panels). PNC = platelet neutrophil complexes; ADP = adenosine diphosphate; Δ-PNC = PNC (ADP)−PNC; Δ-P-selectin = P-selectin (ADP)−P-selectin; hb-PWV = heart–brachial pulse wave velocity; ba-PWV = brachial–ankle pulse wave velocity.

P-selectin is a component of α-granules that is expressed on the platelet surface membrane and released into the plasma upon platelet activation. Although the bulk of circulating soluble P-selectin appears to be platelet derived,[27] the substance is also found in the Weibel–Palade bodies of endothelial cells.[28] Direct measurement of platelet membrane P-selectin is therefore a more sensitive method of assessing platelet activation. In the present study, we evaluated platelet activation by measuring membrane activation markers using flow cytometry with activation-dependent monoclonal antibodies. PNC levels were also measured using the same method. P-selectin levels in our normal subjects aged 49±15 years were 13.1±1.7%; this was higher than that in quoted by other studies, possibly due to the differences in monoclonal antibodies or in sample manipulation.

P-selectin expressed on activated platelets causes formation of PNC. Moreover, platelets and platelet-derived P-selectin play an important role in thrombus growth at the site of atherosclerosis.[2] *In vivo* and *in vitro* studies have shown that shear stress and exposure to atherogenic stimuli, such as oxidization by low-density lipoprotein or cigarette smoking, induce rapid P-selectin-dependent aggregation and accumulation of leukocytes and platelets.[4,5,11] Activated platelets accumulating in thrombi at the site of ruptured atherosclerotic plaques will express CD62P. In clinical studies, P-selectin has been shown to be a marker of platelet activation related to adverse cardiovascular events such as hypertension, coronary artery disease, cerebrovascular disease, and peripheral arterial disease,[6,7,10–12] and also to be a predictor of cardiovascular events.[8,12] PNC, forming as a result of the interaction of platelet P-selectin and neutrophils also promotes platelet activation.[24] This is the first study to demonstrate that P-selectin and PNC were significantly correlated with arterial stiffness evaluated by PWV in normal subjects. In an analysis of four randomized trials, Hebert *et al*[29] showed that aspirin therapy was beneficial in the primary prevention of vascular disease. Higher levels of other membrane markers such as von Willebrand factor receptor are observed in activated platelets, which are affected by aspirin or ticlopidine.[30] Therefore, our results indicate that, in the normal population, antiplatelet agents may play a role in preventing cardiovascular events through factors other than P-selectin.

Although the exact mechanism accounting for the relationship between platelet activation and arterial stiffness is unknown, it is possible to make

the following speculations. When arterial stiffness is raised, shear stress might play an important role in platelet activation. Using cone-plate viscometry,[3] Goto *et al* showed that platelet activation (measured by P-selectin surface expression, von Willebrand factor-mediated platelet aggregation and translocation of GP Ibα) was induced by high shear rate of $10\,800\,s^{-1}$. Higher arterial stiffness increases blood flow velocity and produces a steep systolic pressure waveform,[31] and it is possible that the resulting increased shear stress could promote platelet activation. Another possible mechanism is that endothelial dysfunction may interact with arterial stiffness and platelet hyperactivity. Kobayashi *et al*[32] showed significant correlation between endothelial dysfunction measured by flow-mediated dilatation and ba-PWV. Platelets are also activated by endothelial dysfunction. On the other hand, activated platelets themselves may cause arterial stiffness via vascular smooth muscle cell growth factors and extracellular matrix modulator released from platelets, that is, PDGF.[33] However, this response also occurs at the site of endothelial injury. Further study is therefore required to clarify whether arterial stiffness causes platelet activation or alternatively whether platelet activation might result in arterial stiffness.

Limitations

Despite the small sample size, it is possible that the broad age range (23–77 years) of our subjects caused outliers in PWV and platelet activation. However, significant correlations were found when age and blood pressure were adjusted for, suggesting that the influence of age did not entirely explain the correlation between PWV and platelet activation. In the present study, ba-PWV was 14.1 ± 3.0 m/s in men and 13.6 ± 3.1 m/s in women; values higher than those reported by Yamashina *et al*.[20] Furthermore, it is not known whether such a relationship between arterial stiffness and platelet activation is found in patients with conditions such as hypertension, diabetes mellitus, coronary heart disease, and stroke. Further studies should be therefore performed in such patients, using larger sample sizes.

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ORIGINAL ARTICLE

Trunk deformity is associated with a reduction in outdoor activities of daily living and life satisfaction in community-dwelling older people

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Abstract We have evaluated the association between trunk deformities of the sagittal plane and functional impairment of daily living in community-dwelling elderly subjects. The analysis involved a detailed assessment of indoor and outdoor activities of daily living, satisfaction with life, and mental status. The participants in this study were 236 community-dwelling older adults, aged 65 years and older, living in Kahoku district of Kochi in Japan. The participants were classified based on their posture, which was assessed using photographs of the subjects, and interviewed to assess their basic activities of daily living (BADL), instrumental ADL (IADL), and cognitive well-being in the cross-sectional study. The statistical analysis was performed using the Mann-Whitney *U*-test. The lumbar kyphosis group received significantly lower BADL and IADL scores than the normal group. The trunk deformity group which were defined as kyphosis, flat back, and lumbar lordosis groups exhibited decreases in activities that included going out, shopping, depositing and withdrawing money, and visiting friends in the hospital. These activities require going outdoors; thus, this study showed that the trunk deformity group had limitations in outdoor activities. There was no significant difference between the geriatric depression score (GDS) and the pattern of posture. The abnormal trunk deformity groups tended to score lower than the normal group with regard to subjective healthiness and life satisfaction measures, including subjective health condition, everyday feeling, satisfaction with human relationships, satisfaction with economic condition, and satisfaction with present life.

Keywords Activities of daily living · Kyphosis · Life satisfaction · Trunk deformity

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Introduction

Several studies have reported on the relationship between trunk deformity and lumbago [1,2]. It is predictable that patients with abnormal posture would be at increased risk for falling, as their balance is perturbed by the posture abnormality [3,4]. Loss of distal lumbar lordosis is the main cause of sagittal imbalance in individuals who do not maintain sagittal alignment [5]. This abnormal posture could lead to the limitation of daily activities.

There have been several evaluations of posture and functional activities to date [6]; however, very few involve elderly subjects. Ettinger et al. [7] reported that kyphotic women did not have greater back pain, disability caused by back problems, or poorer health than non-kyphotic women. Another study showed a poor correlation between quality of life and abnormal findings on radiography or densitometry [8].

Vertebral body compression fractures have been shown to be associated with the severity of kyphosis [9]. Ryan et al. [10] reported that there was a significant association between scores of osteoporosis severity and limitations in functional activity. Vertebral compression fractures associated with osteoporosis can be self-limiting, causing considerable pain and disability [8].

Vertebral compression fractures are associated with significant impairments in physical, functional, and psychosocial performance in the elderly [11,12,13]. It is crucial to improve the mental status of the elderly. However, there have been few reports regarding the

association between trunk deformity and psychogenic activity in elderly patients.

In this study, we have evaluated the daily activities and mental condition of community-dwelling elderly subjects with regard to the severity of trunk deformity in the sagittal plane.

Materials and methods

Subjects

Participants who applied in 1999 included 236 community-dwelling older adults, aged 65 years and older, living in Kahoku district in Kochi prefecture, who had been enrolled in one of several studies involving annual medical check-ups (beginning in 1994). The population of Kahoku rural area is 5800 people, 50% of whom are engaged in agricultural work.

Study participants were observed from July to August 1999, and then classified based on their posture, which was assessed using photographs taken by researchers. In total, there were 145 females (mean; 79.0 years) and 91 males (mean; 80.3 years) with a mean age of 80 years (range, 65–94 years), and a mean height of 149.1 cm. Functional status of the lumbar spine [14] and knee [15] were measured using the assessment of the Japanese Orthopaedic Association (JOA). In this study, JOA scores for assessing treatment of low back pain was calculated without incorporating urinary bladder function. Comorbidities were hypertension (31.6%), cardiac arrhythmia (6.1%), diabetes mellitus (5.7%), cerebrovascular disorder (4.2%), coronary artery disease (3.2%), senile dementia (2.1%), and Parkinson's disease (0.4%).

Methods

Interviews and examinations, conducted at the community plaza of Kahoku, consisted of a questionnaire covering physical health, functional status, and mental status. The analysis of trunk deformity was examined by photography. The presence or absence of disease was based on the subjects' self-report of a doctor's diagnosis. Informed consent was obtained from all participants.

Postural analysis

In order to protect the participants' privacy when undergoing the community health check-ups, we constructed a device with which to assess trunk posture without requiring that the subjects disrobe. Participants wore clothes typical of the summer season, and the device was equipped with a band that, when twisted, would reveal the alignment of the body. Each participant had reflective surface markers attached at various locations, including C7, T6 (xyphoid process level), L4 (Jacob line level), the left greater trochanter, the left lateral chondyle of the femur, and the left lateral malleous, as shown in Fig. 1. For the photograph, participants were positioned carefully and asked to remain relaxed while standing up straight. Posture in the sagittal plane was classified into one of the following five types: I normal, II thoracic

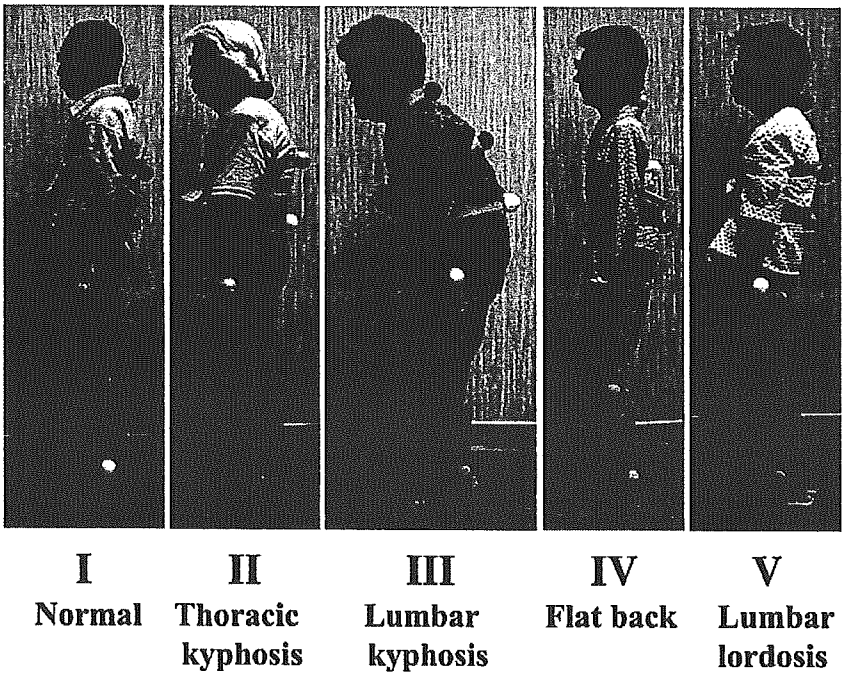

Fig. 1 Classification of trunk deformity from photographs

kyphosis, III lumbar kyphosis, IV flat back, and V lumbar lordosis, as described by Ando et al. [16,17,18]. Three orthopaedic doctors independently determined the classification, and we adopted the classification given by at least two doctors. In cases in which none of the doctors' classifications agreed, we discussed the case and jointly decided on the classification. The classification system is shown in Fig. 1, which includes photographs of patients representing each of the posture groups.

ADL analysis

The subjects were asked questions regarding basic activities of daily living (BADL) (walking, ascending and descending stairs, feeding, dressing, using the toilet, bathing, grooming, and taking medicine) and instrumental ADL (IADL) (using public transportation, shopping for groceries, preparing meals, paying bills, depositing and withdrawing money, writing, reading newspapers, reading magazines or books, taking an interest in news of health, visiting friends, giving advice to family or friends, visiting friends in the hospital, and talking to young people) [19]. We assessed the ADL score using a 4-point scale, based on the help required for each activity: 3 completely independent; 2 some help needed; 1 much help needed; and 0 completely dependent.

Mental state analysis

Geriatric Depression Scale

The Geriatric Depression Scale (GDS) [20,21], a measure of depressive symptomatology assessed on scale of 0–30, was administrated. We assessed the short form of 15 items, it is interpreted that a score >5 points is suggestive of depression, a score >10 points is almost always depression.

Visual analog scale (VAS)

Each year, we conducted an assessment of subjective quality of life (QOL), especially subjective healthiness and life satisfaction, using a validated self-reported visual analogue scale (VAS) [22]. The components of questions were subjective health condition, everyday feeling, satisfaction with human relationship to others, satisfaction with human relationship to family, satisfaction with economic condition, satisfaction with present life, and subjective happiness. The VAS questionnaire ended with a summing-up graph in the form of a 100 mm bar, graded with the subjectively worst condition on the left and the best one on the right. The subject was asked to place a mark on the 100 mm bar based on his or her condition. We defined the distance (mm) from the left to the marked position as the VAS score (0–100), with high scores indicating a high QOL [23].

Statistical analysis

For the classification of posture, Cohen's kappa coefficients were used to test statistical reliability. To determine inter-observer reliability, each reviewer's responses were compared with those of the other reviewers.

Data concerning ADL, GDS and life satisfaction were expressed by mean, SD, and SEM. The differences among the pattern of trunk deformities were evaluated using Kruskal-Wallis test, between with (II–V) and without trunk deformities (I) were evaluated using Mann-Whitney test. Differences were considered significant at $P<0.05$.

Results

The classification of trunk deformity resulted in five groups: I normal group (109 subjects; 46.2%), II thoracic kyphosis group (47 subjects; 19.9%), III lumbar kyphosis group (41 subjects; 17.4%), IV flat back group (28 subjects; 11.9%), and V lumbar lordosis group (11 subjects; 4.7%). There was a mean inter-observer kappa coefficient of 0.47 for both observation times, with a mean inter-observer agreement of 60.2%. We calculated a mean intra-observer kappa coefficient of 0.55 for the two observation times, with a mean inter-observer agreement of 68.3%. Table 1 shows the baseline characteristics in each group. There was no significant difference in age, sex, and overall health status such as comorbidities among the groups.

The mean BADL score of abnormal trunk posture (II–V) was 23.1; that of the normal (I) group was 23.6. The lumbar kyphosis group had significantly lower BADL scores than the normal group ($P=0.017$) (Table 2). With regard to BADL, walking was more likely to be limited in the abnormal trunk posture group (II–V) than in normal participants (I) ($P=0.02$).

The mean IADL score of abnormal trunk posture (II–V) was 10.3, that of the normal (I) group was 11.2. There was no significant difference in IADL among these groups ($P=0.1$) (Table 3). However, the abnormal posture groups (II–V) had lower IADL scores that differed significantly from the normal group (I) ($P=0.047$) (Table 3).

The achieved ratio of transportation of IADLs was associated with trunk deformity ($P=0.04$) (Table 4). The group with trunk deformity group had significant disturbances in certain IADLs, including transportation, shopping for groceries, depositing and withdrawing money, and visiting friends in the hospital (Table 4). Subjects with lumbar lordosis did not exhibit significant differences from the normal group, because of the small size of this group.

There was no significant difference between GDS and the pattern of trunk deformity ($P=0.70$) (Table 5). Measures of subjective healthiness and life satisfaction (Table 6), assessed using a validated, self-reported, visual analogue scale (VAS), were not significantly dif-

Table 1 Baseline characteristics of participants. All data are expressed as mean (95% confidence interval). *I* normal, *II* thoracic kyphosis, *III* lumbar kyphosis, *IV* flat back, and *V* lumbar lordosis

	I	II	III	IV	V	Total
Number	109	47	41	28	11	236
Age	78.4 (68.9, 87.8)	81.3 (71.3, 91.4)	80.8 (70.0, 91.8)	80.2 (70.9, 89.5)	80.6 (71.8, 89.5)	80
Gender (Female, Male)	55, 54	30, 17	34, 7	18, 10	7, 4	144, 92
Height	152.0 (133.5, 170.6)	145.7 (128.8, 162.7)	142.8 (126.3, 159.2)	150.8 (134.8, 166.9)	151.6 (133.4, 169.8)	149.1
Weight	54.3 (33.7, 74.9)	47.3 (33.1, 61.5)	47.4 (29.3, 65.5)	48.1 (31.0, 65.3)	53.0 (28.5, 77.5)	50.9
JOA score (lumbar)	25.6 (18.2, 33)	25.2 (18, 32.4)	24.1 (14.7, 33.5)	24.4 (15.4, 33.4)	27.5 (23.5, 31.5)	25.2
JOA score (knee)	92.0 (67.03, 116.9)	88.9 (59.18, 118.65)	88.0 (58.53, 117.45)	89.6 (66.81, 112.45)	93.3 (63.11, 123.49)	90.5
Coexisting illness						
Hypertension	34	15	17	6	2	74
Cardiac arrhythmia	5	4	4	1	0	14
Diabetes mellitus	5	2	2	3	1	13
Cerebrovascular disorder	3	2	2	1	0	10
Coronary artery disease	4	1	2	0	0	7
Senile dementia	1	2	0	2	0	5
Parkinson disease	0	0	1	0	0	1

Table 2 Total BADL score (points) by the classification of trunk deformity in comparison with the normal trunk group (I). *I* normal, *II* thoracic kyphosis, *III* lumbar kyphosis, *IV* flat back, and *V* lumbar lordosis

	Mean	SD	95% confidence intervals
I	23.6	0.9	21.8; 25.4
II	23.3	1.56	20.18; 26.42
III	22.7	3.81	15.08; 30.32
IV	23.3	1.76	19.78; 26.82
V	23.7	0.65	22.4; 25.0
II–V	23.1	2.51	18.1; 28.12

Table 3 Total IADL score (points) by the classification of trunk deformity in comparison with the normal trunk group (I). *I* normal, *II* thoracic kyphosis, *III* lumbar kyphosis, *IV* flat back, and *V* lumbar lordosis

	Mean	SD	95% confidence intervals
I	11.2	3.13	4.94; 17.46
II	10.6	3.09	4.42; 16.78
III	9.8	4.09	1.62; 17.98
IV	10	4.42	1.16; 18.84
V	12	1.41	9.18; 14.82
II–V	10.3	3.68	2.94; 17.66

ferent from normal in participants with trunk deformity ($P=0.08$). However, the abnormal trunk deformity group tended to have lower scores with regard to subjective health condition ($P=0.03$), everyday feeling ($P=0.007$), satisfaction with human relationships to family ($P=0.035$), satisfaction with economic condition ($P=0.03$), and satisfaction with present life ($P=0.051$) than those of the normal group.

Discussion

Trunk posture in the elderly, especially kyphosis, is known to be associated with vertebral compression fractures. Measurement of kyphosis may be useful in assessing the severity of spinal osteoporosis [9]. The high prevalence of back pain demonstrates the importance of pain management in the treatment of osteoporosis [24]. The number of recent vertebral fractures was also a significant predictor of poor performance in functional reach and walking speed tests [25]. Women with multiple vertebral deformities had significantly greater impairment of ADL function than women without such deformities [26].

Lyles et al. [12] showed that patients with vertebral compression fractures had reduced levels of functional performance, pain with activity, and difficulty in activities in comparison with patients that did not have fractures. Kyphosis is associated with qualitatively and quantitatively diminished function, especially with regard to the performance of mobility tasks [27]. Our results also showed that the walking activity of the

Table 4 The accomplished ratio (%) of IADL items compared between subjects with and without trunk deformity. Kruskal-Wallis test: among five groups, Mann-Whitney test: between with (II–V) and without trunk deformities (I). *I* normal, *II* thoracic kyphosis, *III* lumbar kyphosis, *IV* flat back, and *V* lumbar lordosis

Classification	I	II	III	IV	V	*P*-value	II–V	*P*-value
Going out using public transportation	93.1(%)	82.2(%)	75(%)	76.9(%)	77.8(%)	0.04	78.4(%)	0.003
Shopping for groceries	100	95.6	94.6	96	100	0.24	95.8	0.037
Preparing meals	98.1	93.5	91.9	92.3	100	0.37	93.3	0.09
Paying bills	99	93.3	94.6	92.3	100	0.27	94.1	0.052
Depositing and withdrawing money	98	91.3	91.9	88.5	100	0.17	91.7	0.035
Writing paper	93.2	83	86.5	80	100	0.13	85	0.053
Reading newspaper	82.4	75.6	67.6	76.9	90.9	0.32	74.8	0.17
Reading magazine or book	81	72.1	72.2	72	81.8	0.66	73	0.17
Taking an interest in news of health	97	90.7	86.5	91.7	100	0.17	90.4	0.052
Visiting friends	79	78.6	73	80	81.8	0.94	77.4	0.78
Giving advice to family or friend	85.1	75	73	80	100	0.19	77.6	0.16
Visiting friend in the hospital	98	89.1	91.9	88.5	100	0.12	90.8	0.023
Talking to young people	92.2	87	86.5	80.8	90.9	0.52	85.8	0.14

Table 5 Geriatric depression scale (GDS) by the classification of trunk deformity. *I* normal, *II* thoracic kyphosis, *III* lumbar kyphosis, *IV* flat back, and *V* lumbar lordosis

	Mean	SD	95% confidence intervals
I	5.8	3.65	−1.5; 13.1
II	5.9	3.79	−1.68; 13.48
III	6.6	3.76	−0.92; 14.12
IV	6.4	3.14	0.12; 12.68
V	5.4	3.78	−2.16; 12.96
II–V	6.2	3.62	−1.04; 13.44

abnormal trunk deformity group was more limited than that of normal participants. In contrast, kyphosis is associated with decreased bone mineral density (BMD) and loss of height, but does not cause substantial chronic back pain, disability, or poor health in older women [7]. However, previous studies have not assessed patterns of trunk deformity in the context of detailed assessments of functional impairment of daily living in the elderly.

In this study, we classified trunk deformity into five groups: I normal; II thoracic kyphosis; III lumbar kyphosis; IV flat back; and V lumbar lordosis. Previously, we reported that standing trunk posture was closely associated not only with distance and time parameters of gait, but also with functional performance measures such as functional reach and timed up and go tests in elderly subjects dwelling in a rural community [17]. In the present study, we evaluated if trunk deformity is associated with the results of a detailed assessment of indoor and outdoor activities of daily living, satisfaction with life, and mental status.

This study demonstrated that the lumbar kyphosis group had decreased activities of daily living, manifested primarily in the basic ADL of walking. Of the IADL, the trunk deformity group exhibited decreased activities of daily life such as going out, shopping, depositing and withdrawing money, and visiting friends in the hospital. These activities require going out of doors. This means the abnormal trunk deformity group experienced limited outdoor activities. The lumbar kyphosis group had greater interest in their own health, possibly because they have plenty of time to think about their own health at home, as their outdoor activities are limited.

So far, few reports have discussed the association between trunk deformity and mental status. However, vertebral deformity was shown to be associated with psychological morbidity in elderly Chinese women [28].

With regard to subjective healthiness and life satisfaction, there was no significant difference among the trunk deformity groups; however, the abnormal posture group tended to score lower than the normal group on measures of their own subjective health condition, everyday feeling, satisfaction with human relationships,

Table 6 Satisfaction-with-life score by the trunk deformity. Mann-Whitney test: between with (II–V) and without trunk deformities (I). *I* normal, *II* thoracic kyphosis, *III* lumbar kyphosis, *IV* flat back, and *V* lumbar lordosis

	Mean; SD		
	Normal (I)	Abnormal (II–V)	*P*-value
Subjective health condition	64.9; 17.85	59.3; 18.75	0.033
Everyday feeling	68.5; 18.56	61.7; 19.52	0.007
Satisfaction with human relationship to others	80.8; 16.31	77.6; 19.17	0.29
Satisfaction with human relationship to family	82.1; 14.55	77.5; 16.61	0.035
Satisfaction with economic condition	62.1; 20.91	55.6; 22.72	0.03
Satisfaction with present life	68.3; 20.55	62.5; 23.2	0.051
Subjective happiness	67.1; 20.09	64.0; 21.37	0.32
Total	457.8; 181.07	428.7; 155.95	0.08

satisfaction with economic condition, and satisfaction with present life. Therefore, the trunk deformity group experienced less subjective healthiness and satisfaction with life. This result may be explained by the limitation of outdoor activities that can lead to a limited social life and difficulty in enjoying a healthy and active life in the community.

Schreiner et al. reported that Geriatric Depression Scale was accurate and reliable in dementia among Japanese subjects [29]. Although the subjective impression of mental status in the trunk deformity group was not as favorable as that of normal group, the GDS in the trunk deformity group did not reveal significant difference in that of normal group. In another study, a significant association was found between BMD of the hip and depressive symptoms after adjustment for osteoporosis risk factors [30]. They suggested the relationship between low BMD and depression was associated with endogenous steroid. We should evaluate the GDS of the larger number of participants by the classification of trunk posture and detailed background of participants to clarify the relation of depression and spinal deformity.

In this study, we did not take X-rays of thoracic and lumbar lesions in the participants, and therefore cannot speculate on the association between spinal posture and vertebral fracture. Further examinations of the radiography of spine and bone densities, such as dual energy X-ray absorptiometry (DXA) or quantitative ultrasound, are necessary to assess the association between spinal posture and vertebral osteoporosis in the community-dwelling elderly. Further studies are also needed to evaluate the cause of the limitation in outdoor activities experienced by study participants with lumbar kyphosis, and to clarify and assess the relationship between mental status and trunk deformity through long-term follow-up.

In conclusion, patients in this study with trunk deformities exhibited decreases in activities that require going outdoors.The abnormal trunk deformity groups also tended to score lower than the normal group with regard to subjective healthiness and life satisfaction measures.

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第46回日本老年医学会学術集会記録
〈シンポジウム1：高齢者総合的機能評価ガイドライン，健康増進と介護予防〉
2. 老年症候群の評価と介護予防：生活改善に機能評価を生かす

2) 栄養と生活機能

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松林 公蔵[3] 小澤 利男[4] 土居 義典[1]

Key words：後期高齢者，生活機能，アルブミン，魚摂取，イコサペンタエン酸（EPA）
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はじめに

近年，生活習慣病と糖尿病，高脂血症との関連から，栄養過多や肥満を問題として取り上げられることが多いが，高齢者では反対に低栄養が問題となることが少なくなく，生活機能障害や予後を左右することが多い．栄養と生活機能障害との関連について，特に地域在住の後期高齢者を対象とした本邦の報告は少なく，栄養管理の指針は十分とはいえない．そこで，今回，地域在住高齢者の生活機能評価の経年的変化から，生活機能維持に関連する栄養学的マーカーについて検討した．

地域在住高齢者のこれまでの知見

1) 香北町健康長寿計画

高知大学老年病科と高知県香北町では，官学共同事業として，「健やかに老いるために」をテーマにこれまでさまざまな取り組みを展開してきた．地域高齢者に対する老年医学的総合機能評価（Comprehensive Geriatric Assessment：CGA）を地域在住高齢者に適用し，その結果に基づいた介入を試みた．内容は大きく3つに分かれる．すなわち，ADL等の調査を目的とした65歳以上の住民アンケート，後期高齢者を中心とした機能検診，これらの結果に基づく運動教室や文化教室などの介入事業である．

2) 1991年参加集団の動向

松林ら[1]は，1991年アンケート参加集団1,488例の解析を行い，10年後の生死やADL低下に関連する因子について解析し，歩行や階段昇降，摂食，入浴，更衣，整容などの基本的日常活動度低下が生存に，また，ADL低下すなわち要介護の出現に関連する危険因子として，年齢，性（女性），視聴覚などの情報関連機能低下，脳卒中などが関連することを明らかにした．同じように，今回私たちは，1991年に機能検診を受診した後期高齢者330例を追跡したが，機能検診連続参加者は4年後には半減以下，7年後には72例に，10年後には39例にまで減少した．その原因は死亡が年々増加したこともあるが，機能検診には参加できず，アンケート調査のみに参加する高齢者も増加した．図1に示すように検診連続参加者の基本的ADLは，全体の加齢に伴うと考えられるADL低下と比べて，明らかに良好に保たれており，逆にアンケートのみの参加者は全体の動向と同様に低下していた．

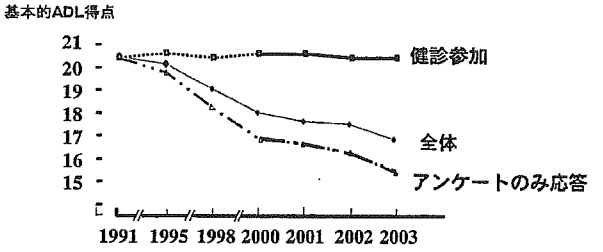

図1 1991年時長寿健診後期高齢者の基本的ADLの変化
歩行，階段昇降，摂食，入浴，排泄，更衣，整容の7項目（3段階：21点満点）
330例（男154，女176）平均年齢 80±4歳

ADLばかりでなく，認知機能検査のMMSや歩行機能を総合的に判定するアップアンドゴーテストも機能検診連続参加者には低下がほとんどみられず，機能が維持

Nutritional factors and functional assessment
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2) Kiyohito Okumiya：総合地球環境学研究所研究所
3) Kozo Matsubayashi：京都大学東南アジアセンター
4) Toshio Ozawa：東京都老人医療センター

表1　1991年時長寿健診後期高齢者：10年後（2001年）自立に関連する要因

	調整オッズ比	p	95% 信頼区間
年齢	0.87	0.026	0.764 ～ 0.983
MMS	1.16	0.040	1.007 ～ 1.342
Up&Go	0.83	0.003	0.737 ～ 0.940
アルブミン	4.32	0.055	0.969 ～ 19.22
総コレステロール	0.99	0.286	0.982 ～ 1.005

（性，血圧，高血圧歴で補正）

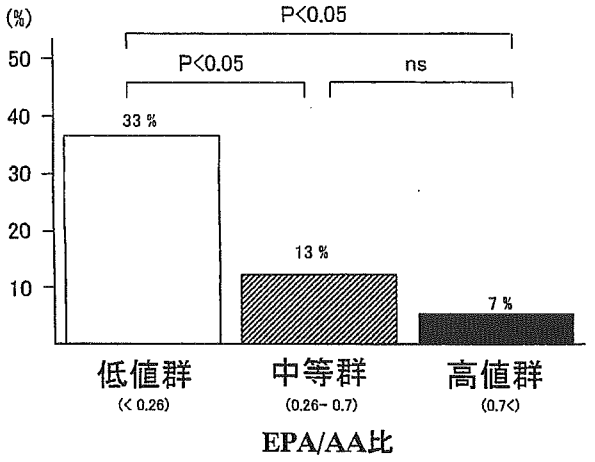

図2　追跡2年後の基本的ADL低下の頻度

されている高齢者のみが連続して機能健診に参加している実態が明らかになった．

(1) アルブミン

高齢者の栄養状態を最もよく反映すると言われるアルブミン[2)3)]は，連続健診参加者では，男女ともに高齢になるほどむしろ増加し，一般に言われるように加齢とともには減少しなかった．これは，他の研究でもみられるように，アルブミン低下がみられる対象は順次死亡したり，ADLの低下が見られたりして，機能検診受診をしなくなるため（選択的脱落）に生じると考えられる．

(2) 血清総コレステロール

女性は男性より高いが，2002年までは低下せず，逆に男性では2002年以降増加する．男女ともに加齢とは関係なく，血清総コレステロール値はほぼ一定に保たれていた．この結果にも選択的脱落が生じたためと考えられる．

(3) 自立に関連する要因

1991年時の検診参加者を対象として，4年後，7年後，10年後（表1）の自立に関連する要因について検討した．後期高齢者では，血清学的なアルブミンやコレステロール値は自立に関連する有意な要因とはならず，むしろ，MMSやアップアンドゴーテスト等の機能検査の方がより強く，将来の高齢者の自立を予測しうることが明らかとなった．

これまで多くの研究で，血清アルブミン値や総コレステロール値が生命・機能予後の推定に有用であることが示されている[2)3)]．しかし，先に述べたように，私たちの後期高齢者を対象とした集会型の健診では，生活機能が維持されている高齢者のみが参加する傾向が強く，機能的予後推定に血清アルブミン，コレステロールの測定が大きな役割を果していなかった．

栄養に関するアンケート型問診と生活機能との関連

長寿健診は，基本健診をベースとして，さらに機能健診を合わせて実施されている．基本健診の健康診査受診票の中での問診項目には，食事に関する問診項目があり，その中で近年報告されている魚の摂取頻度に焦点を当て，生活機能との関連について検討した．近年，魚の摂取頻度が高いほど，動脈硬化性疾患の発生が減少すると報告されている[4)~6)]．しかし，動脈硬化の進展した高齢者において，魚の摂取の習慣と動脈硬化性疾患発症や，生活機能障害との関連を検討した報告は少ない．

そこで，後期高齢者の魚摂取の実態を知るために，その魚摂取頻度と，血清イコサペンタエン酸（EPA）とアラキドン酸（AA）を測定し，その比（EPA/AA）によって，魚摂取と肉類摂取のバランスの指標とした（EPA/AAが高値ほど魚摂取が多い）．対象は65歳以上の地域在住高齢者（平均年齢78±5歳），生活機能障害として，開始時および2年後に歩行，階段，食事，着替え，排泄，入浴，洗面・整髪の7項目を21点満点とする基本的・日常生活活動度（BADL）得点および認知機能検査としてMini-Mental State Examination（MMS：30点満点）を追跡開始時と2年後に施行した．

(1) 血清EPA/AA比の分布によって，3つのグルー

プに，すなわち，低値群（～0.25），中等度群（0.26～0.70），高値群（0.71～）に分類した．欧米人の平均は0.04，日本人の平均は0.4～0.6と報告されている．実際の魚摂取頻度と血清EPA/AAの相関は，魚摂取の頻度が高いほどEPA/AAが高く，食事摂取の質をよく反映していた．

(2) 血清EPA/AA比の再現性について，追跡開始時と1年後の血清EPA/AAの相関について検討したが，比較的良好な相関が認められ（n＝217，r＝0.582，P＜0.001），食生活の内容が年単位ではそれほど大きく変わらないことが推測された．

(3) EPA/AA比の低値，中等度，高値群の間で，年齢，性，血圧，総コレステロール（TC），HDLコレステロール（HDL），LDLコレステロール（LDL），リポプロテイン(a)(Lp(a))，高感度CRP（hsCRP）に有意な差はなかった．

(4) 動脈硬化の一つの指標である平均脈波速度（PWV）は，低値群，中等度群の順で高値群で有意に高く（低値群：1,896，中等度群：1,787，高値群：1,724cm/s，p＜0.05），魚摂取の低い群での動脈硬化の進展が推測された．

(5) 認知機能（MMS）との関連では，高値群，中等度群とは同様であったが，低値群で有意に他の2群と比べて，認知機能が低下していた（低値群：19.2±4.9，中等度群：20.6±1.2，高値群：20.7±1.1，p＜0.05）．

(6) 基本的ADLとの関連では，低値群で，他の2群に比べて，高値で基本的ADLが保たれていた（低値群：26.2±4.3，中等度群：27.5±2.5，高値群：27.4±2.2，p＜0.05）．

(7) 自立喪失（要介護）高齢者の割合：2年後の基本的ADLが20点未満になった高齢者を自立喪失（要介護）と定義した．その割合は，魚摂取頻度低値群で高く約3割にのぼり，逆に高値群では5%と有意に低かった（図2）．

おわりに

高齢者にとって栄養の問題は重要である．血清アルブミン値や血清蛋白の値が低いことが高齢者の虚弱性と関連があることが報告されている[3]．今回，我々が示したように，すべての高齢者健診の現場で，これらの指標が有用な栄養学的マーカーとは限らない．後期高齢者の集会型の健診には「選択的脱落」が生じ，十分なスクリーニング機能が発揮されない可能性もある．

今回の我々の検討では，魚食の多い日本人においても，EPAを多く含む魚油を摂取する食習慣のあるグループでは動脈硬化の進展が遅く，認知機能低下や要介護状態を回避できる可能性が示され，今後，栄養指導等の地域介入を勧める一つの根拠となりえる．

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Lifelong Left Ventricular Remodeling of Hypertrophic Cardiomyopathy Caused by a Founder Frameshift Deletion Mutation in the Cardiac Myosin-Binding Protein C Gene Among Japanese

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OBJECTIVES We studied the longitudinal evolution of hypertrophic cardiomyopathy (HCM) caused by a founder frameshift mutation in the cardiac myosin-binding protein C (MyBPC) gene.

BACKGROUND Mutations in the MyBPC gene have been associated with delayed expression of HCM and a good prognosis. Few studies, however, demonstrated the phenotype-genotype correlations in the longitudinal study.

METHODS We studied long-term evolution of clinical features of 15 unrelated families who were found to have an identical frameshift mutation in the MyBPC gene: a one-base deletion of a thymidine at nucleotide 11645 (V592fs/8).

RESULTS Thirty-nine individuals in 15 families were genotype-positive. Thirty of the 39 individuals with the mutation were phenotype-positive. The disease penetrance was 100% in subjects ≥50 years and 65% in those <50 years. "End-stage" HCM (ejection fraction <50%) was observed in 7 (18%) of the 39 genotype-positive individuals (7 [23%] of the 30 phenotype-positive patients); 6 of them were 60 years or older. Seven patients were hospitalized for treatment of repeated congestive heart failure, and four patients died or had implantable cardioverter-defibrillator discharge (13%; incidence, 1.4%/year) during a mean follow-up period of 9.2 ± 5.5 years.

CONCLUSIONS Elderly patients with a V592fs/8 mutation in the MyBPC gene may evolve into the "end-stage" HCM, characterized by left ventricular systolic dysfunction, cavity dilation, and irreversible heart failure. The clinical course in patients with this mutation is not benign in the long run, with progressive left ventricular remodeling with advancing age. (J Am Coll Cardiol 2005;46:1737–43) © 2005 by the American College of Cardiology Foundation

Hypertrophic cardiomyopathy (HCM) is a primary myocardial disorder with heterogeneous morphologic, functional, and clinical features (1–4). Recent molecular genetic studies have revealed that HCM is caused by mutations in 10 genes that encode sarcomeric contractile proteins (5–9).

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Cardiac myosin-binding protein C (MyBPC) is one of these sarcomeric proteins, and mutations in the MyBPC gene have been reported to be associated with delayed expression of hypertrophy and a relatively good prognosis (10–14). On the other hand, a recent report showed that patients with mutations in the MyBPC gene did not differ significantly from patients with thick-filament HCM or thin-filament HCM with respect to age at diagnosis or severity of phenotype (15).

Few studies, however, have demonstrated longitudinal evolution of phenotype in relation to genotype, although the HCM phenotype itself is recognized to be a slowly progressive disorder that manifests remarkable evolution of clinical features throughout life (16).

We analyzed the MyBPC gene in probands from families with HCM and had the opportunity to study 15 unrelated families living in Kochi prefecture, Japan, who were found to have an identical frameshift mutation in the MyBPC gene: a one-base deletion of a thymidine at nucleotide 11645 (V592fs/8) (17). The results of clinical and genetic investigations in these 15 families during a long period of time are presented herein.

METHODS

Subjects. The subjects were 94 probands with familial or sporadic HCM. Twenty-two subjects were familial HCM,

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Abbreviations and Acronyms

AF	= atrial fibrillation
ECG	= electrocardiogram/electrocardiographic
EF	= ejection fraction
HCM	= hypertrophic cardiomyopathy
ICD	= implantable cardioverter-defibrillator
LVEDD	= left ventricular end-diastolic diameter
LVH	= left ventricular hypertrophy
MLVWT	= maximum left ventricular wall thickness
MyBPC	= cardiac myosin-binding protein C

whereas the other 72 subjects were not confirmed to have relatives with HCM. All probands were evaluated at the Kochi Medical School Hospital for confirmation of diagnosis, risk assessment, and symptom management between 1982 and 2004. The diagnosis of HCM was based on echocardiographic demonstration of an unexplained left ventricular hypertrophy (LVH) (i.e., maximum left ventricular wall thickness [MLVWT] ≥15 mm). Relatives of probands were contacted by probands themselves and visited our clinic of their own free will. After the identification of a V592fs/8 mutation, pedigree analysis, including both clinical evaluation and genotyping, was performed. Informed consent was obtained from all subjects or their parents in accordance with the guidelines of the Ethics Committee on Medical Research of Kochi Medical School.

Clinical evaluation. The evaluation of probands and relatives included medical history, clinical examination, 12-lead electrocardiography, M-mode, two-dimensional and Doppler echocardiography, and ambulatory 24-h Holter electrocardiographic (ECG) analysis. The severity and distribution of LVH were assessed in the parasternal short-axis plane at mitral valve and papillary muscle levels (18,19). Maximum left ventricular wall thickness was defined as the greatest thickness in any single segment. Left ventricular end-diastolic diameter (LVEDD) and end-systolic diameter were measured from M-mode and two-dimensional images obtained from parasternal long-axis views. Ejection fraction (EF) was determined from apical two- and four-chamber views because the left ventricle is of heterogeneous shape and the septum itself is usually hypokinetic in HCM. Left ventricular outflow tract gradient was calculated from continuous-wave Doppler using the simplified Bernoulli equation.

Disease penetrance was determined by the following criteria for relatives: 1) MLVWT ≥13 mm; 2) presence of major abnormalities on the ECG (i.e., Q-wave ≥0.04 s in duration or one-fourth of the ensuing R-wave in depth in at least two leads, significant ST-T changes, and Romhilt-Estes score >4); or 3) a combination of criteria 1 and 2.

Data regarding survival and clinical status of patients were collected during serial clinic visits. Evaluation of the phenotype was completed before determination of the genotype. Three modes of HCM-related death were defined: 1) sudden and unexpected death (including resuscitated cardiac arrest), in which the collapse occurred in the absence or <1 h from the onset of symptoms in patients who previously experienced a relatively stable or uneventful course; 2) heart failure-related death, which was in the context of progressive cardiac decompensation ≥1 year before death, particularly if complicated by pulmonary edema or evolution to the end-stage phase (including patients with heart transplantation); and 3) stroke-related death, which occurred in patients who died as a result of embolic stroke.

Genetic analysis. Peripheral blood samples were taken at the time of clinical evaluation, and they were frozen and stored at −20°C. We extracted DNA using a DNA purification kit from QIAGEN Inc. (no.51104; Hilden, Germany). In vitro amplification of genomic DNA was performed using polymerase chain reaction. Oligonucleotide primers were used to amplify exon 18 of the MyBPC gene. Information on primer sequences and polymerase chain reaction conditions is available upon request. Sequencing was performed using a BigDye Terminator Cycle Sequencing Kit from Applied Biosystems Inc. (no.4336774; Foster City, California). The sequences were analyzed on an ABI PRISM 3100-Avant Genetic Analyzer in accordance with the manual of the manufacturer.

In patients in whom the mutation was identified, confirmation was obtained by reanalysis with direct sequencing from a second blood sample. The presence of a V592fs/8 mutation, which abolishes a *Bsm*FI restriction site, was confirmed by digestion of genomic DNA with this enzyme.

To investigate if families carrying the identical mutation were related, haplotype analysis was performed using microsatellite markers defining the MyBPC gene locus. Markers MyBPC3-CA, D11S4109, D11S1784, and D11S1326, flanking the MyBPC gene, were used. To describe haplotype results, the length (base pair) of allele was put in parentheses after each marker.

RESULTS

Genetic results. A V592fs/8 mutation, a frameshift mutation that causes truncation of cardiac MyBPC protein, was identified in 15 of 94 probands. Relatives of 15 probands were studied further, totaling 64 members, including 15 probands, of the various families (Figs. 1A to 1G). Of the 64 individuals, 39 had a V592fs/8 mutation in the MyBPC gene. This mutation was thought to be disease-causing based on presence of the mutation in all affected individuals and absence of the sequence variation in at least 200 chromosomes from healthy individuals.

Haplotype analysis with highly polymorphic markers was performed in these families to investigate whether a V592fs/8 mutation was likely to have arisen from a common ancestor (founder effect). We found that a unique haplotype, MyBPC3-CA(282)-D11S4109(151)-D11S1784(138)-D11S1326(249), was linked to the V592fs/8 mutation in all 15 families, indicating that a common founder of the mutation was likely in these families.