European Journal of Neurology 2007, 14: 1091–1097 doi:10.1111/j.1468-1331.2007.01904.x

NAD(P)H oxidase $p22^{phox}$ C242T polymorphism and ischemic stroke in Japan: the Fukuoka Stroke Registry and the Hisayama study

J. Kuroda[a,b], T. Kitazono[a], T. Ago[a,b], T. Ninomiya[a], H. Ooboshi[a], M. Kamouchi[a], Y. Kumai[a], N. Hagiwara[a], S. Yoshimura[a], K. Tamaki[c], K. Kusuda[b], K. Fujii[d], T. Nagao[e], Y. Okada[f], K. Toyoda[f], H. Nakane[g], H. Sugimori[g], Y. Yamashita[b], Y. Wakugawa[a], K. Asano[a], Y. Tanizaki[a], Y. Kiyohara[a], S. Ibayashi[a] and M. Iida[a]

[a]*Department of Medicine and Clinical Science, Graduate School of Medical Sciences, Kyushu University, Maidashi, Higashi-ku, Fukuoka, Japan;* [b]*Department of Medicine, Seiai Rehabilitation Hospital and Fukuoka Institute of Neurogenetics and Stroke, Minamiohri, Onojo City, Fukuoka, Japan;* [c]*Hakujyuji Hospital, Ishimaru, Nishi-ku, Fukuoka, Japan;* [d]*Fukuoka Red Cross Hospital, Ohkusu, Minami-ku, Fukuoka, Japan;* [e]*Imazu Red Cross Hospital, Imazu, Nishi-ku, Fukuoka, Japan;* [f]*National Hospital Organization Kyushu Medical Center, Jigyohama, Chuo-ku, Fukuoka, Japan; and* [g]*National Hospital Organization Fukuoka Higashi Medical Center, Chidori, Koga City, Fukuoka, Japan*

Keywords:
brain infarction, genetics, NAD(P)H oxidase, polymorphism, superoxide

Received 30 March 2007
Accepted 16 June 2007

The C242T polymorphism of $p22^{phox}$, a component of NAD(P)H oxidase, may have an impact on cardiovascular diseases; however, the association between this polymorphism and brain infarction is not fully understood. Here, we investigate the relationship between the C242T polymorphism and brain infarction in Japan. We recruited 1055 patients with brain infarction and 1055 control subjects. A chi-squared test revealed that the T-allele frequency was lower in patients with cardioembolic infarction (5.6%) than in control subjects (11.0%, $P < 0.001$); however, allele frequencies in patients with lacunar and atherothrombotic infarction (11.2%) were not significantly different from those in control subjects (11.0%). A multivariate-adjusted conditional logistic regression analysis also revealed no association between CT + TT genotype, and lacunar and atherothrombotic infarction (odds ratio = 0.97, 95% confidence interval: 0.72–1.32). To investigate the functional effects of the C242T polymorphism, we examined superoxide production in COS-7 cells cotransfected with Nox4 and $p22^{phox}$ of each genotype. The superoxide-producing activity in those cells expressing $p22^{phox}$ with the T allele was not significantly different from that in cells expressing $p22^{phox}$ with the C allele. The present results suggest that the $p22^{phox}$ C242T polymorphism may have a protective effect against cardioembolic infarction, but is not related to lacunar and atherothrombotic infarction in Japan.

Introduction

Reactive oxygen species (ROS) appear to play a major role in the development of arteriosclerosis of cerebral arteries [1] and thereby contribute to ischemic cerebrovascular diseases. Recent evidence has suggested that a major source of ROS in the vascular wall is NAD(P)H oxidase, which produces superoxide in conjunction with oxidation of NADPH or NADH [2–6]. $p22^{phox}$, an essential membrane-associated factor of NAD(P)H oxidase, forms a heterodimer with another membrane-integrated protein, $gp91^{phox}$ or its homologue, and plays a crucial role in the activation and stabilization of NAD(P)H oxidase [7].

Correspondence: Takanari Kitazono, MD, PhD, Department of Medicine and Clinical Science, Graduate School of Medical Sciences, Kyushu University, 3–1–1, Maidashi, Higashi-ku, Fukuoka 812–8582, Japan (tel.: +81 92 642 5256; fax: +81 92 642 5271; e-mail: kitazono@intmed2.med.kyushu-u.ac.jp).

Several allelic polymorphisms in the $p22^{phox}$ gene have been reported [8]. Amongst them, the C242T polymorphism results in a substitution of histidine with tyrosine at residue 72, which may change molecular structure of NAD(P)H oxidase and modulate its activity. The association of this polymorphism with cardiovascular diseases has been investigated with quite conflicting results. Inoue *et al.* [9] first reported that the T allele of the $p22^{phox}$ gene has a protective effect against coronary risk. By contrast, other reports have suggested that the T allele of the $p22^{phox}$ gene is a risk factor for coronary artery disease (CAD) [10,11]. Moreover, no association between the C242T polymorphism with CAD was also reported [12–15].

In terms of ischemic stroke, two different results regarding the $p22^{phox}$ C242T polymorphism have been reported. Ito *et al.* [16] have indicated that the T allele of the $p22^{phox}$ gene is significantly associated with atherothrombotic brain infarction. However, Shimo-Nakanishi *et al.* [17] showed no association between this polymorphism and thrombotic brain infarction. Thus,

the precise relationship between the C242T polymorphism and brain infarction remains unsolved. The first objective of this study is to settle the question of whether the C242T polymorphism of $p22^{phox}$ is associated with brain infarction.

Amongst seven isoforms of $gp91^{phox}$, termed the Nox family, Nox4 was initially identified in the kidney and was pointed out to possibly have a role in oxygen sensing [18]. We have shown recently that Nox4 is the major catalytic subunit of NAD(P)H oxidase in vascular endothelial cells [4,6], and is expressed abundantly in the basilar arterial smooth muscle and endothelial cells [5]. It is also reported that Nox4 has an important role in the development of vascular lesions such as restenotic lesions after carotid injury [19]. Because Nox4, as well as other Nox family members, is considered to form heterodimer with $p22^{phox}$ [7], it is possible that genetic variation in $p22^{phox}$ may affect the activity of Nox4.

The second objective of the present study is to investigate the effect of the polymorphism on the superoxide-producing activity of 'vascular' NAD(P)H oxidase, Nox4. For this purpose, we examined the superoxide-producing activity in COS-7 cells cotransfected with cDNAs for Nox4 and $p22^{phox}$ of each genotype.

Subjects and methods

Study population

The Fukuoka Stroke Registry (FSR) is a hospital-based registration of stroke patients. Stroke specialists from the 11 medical centers in southern Japan have participated in the FSR. In the present study, case subjects were recruited from consecutive outpatients with brain infarction who visited seven medical centers in the FSR, namely, Kyushu University Hospital, National Hospital Organization Kyushu Medical Center, National Hospital Organization Fukuoka Higashi Medical Center, Fukuoka Red Cross Hospital, Hakujyuji Hospital, Imazu Red Cross Hospital and Seiai Rehabilitation Hospital, from April 1 to July 31, 2004.

Control subjects were selected from participants of the Hisayama study. The Hisayama study, an epidemiological study of cardiovascular diseases, was established in 1961 in Hisayama, a suburban community adjacent to Fukuoka City, Japan. In 2002, a screening survey for the present study was performed in Hisayama. Briefly, a total of 3328 participants aged 40 years or over (78% of the total population of this group) consented to participate in the examination and underwent a comprehensive assessment. We have been performing a population-based cohort study for 45 years, in which most residents of Hisayama have participated, and study team physicians have been collecting detailed clinical information through periodic medical examination. Control subjects were selected randomly from participants without cardiovascular diseases including stroke and myocardial infarction, and were properly matched to case subjects for sex and age (± 5 years) using these clinical data.

This study was approved by the local ethics committees of Kyushu University and each hospital. Informed consent was obtained from all individuals that participated and genomic DNA was extracted from whole blood.

Determination of brain infarction and its subtype

Stroke was defined as a sudden onset of non-convulsive and focal neurological deficit persisting for more than 24 h and was classified as either brain infarction, brain hemorrhage, subarachnoid hemorrhage or undetermined type of stroke by means of brain imaging including computed tomography and/or magnetic resonance imaging. Brain infarction was further classified into four subtypes, namely, lacunar infarction, atherothrombotic infarction, cardioembolic infarction, and unclassified type of brain infarction, on the basis of the Classification of Cerebrovascular Disease III proposed by the National Institute of Neurological Disorders and Stroke [20], as well as on the basis of the diagnosis criteria of the Trial of Org 10172 in Acute Stroke Treatment study [21] and the Cerebral Embolism Task Force for brain infarction subtypes [22]. The subclassification of brain infarction was also made by reference to detailed clinical features and brain imaging including computed tomography, magnetic resonance imaging, cerebral angiography, echocardiography, and carotid duplex imaging.

Risk factors

Hypertension was defined as systolic blood pressure ≥140 mmHg and/or diastolic pressure ≥90 mmHg or as current treatment with antihypertensive drugs. Diabetes mellitus was determined by either a 75 g oral glucose tolerance test according to the diagnostic criteria of the World Health Organization in 1998, casual blood glucose levels (> 11.1 mmol/l), or a medical history of diabetes. Hyperlipidemia was defined as either a cholesterol level > 220 mg/dl, low-density lipoprotein-cholesterol level > 140 mg/dl, high-density lipoprotein-cholesterol level < 40 mg/dl, or current treatment with a cholesterol-lowering drug. Atrial fibrillation was diagnosed based on electrocardiographic findings. Case subjects who reported smoking at least one cigarette per

day and those who reported consuming alcohol at least once a month at the time of onset of stroke were defined as smokers and drinkers, respectively. Control subjects were also asked about their smoking and drinking habits at the time of collecting blood and were categorized as either current users or not.

Determination of p22^{phox} C242T polymorphism

Genotyping of p22^{phox} C242T polymorphism was achieved by a rapid-cycle polymerase chain reaction and melting curve analysis using fluorescent probes on the LightCycler™ instrument (Roche, Mannheim, Germany) according to the method described previously [23]. Sequences of primers and probes using this study were as follows: 5′-GTTTGTGGGAGGAAAGAGG-3′ (forward primer) and 5′-CTCACAGGAGATGCAGGAC-3′ (reverse primer); GGACAGAAGCACATGACCG-fluorescein (sensor probe) and LC Red 640-CGTGGTGAAGCTGTTCGGGCCCTT-phosphate (anchor probe).

Transfection of cDNAs encoding Nox4 and p22^{phox} of each genotype into COS-7 cells

The cDNAs for Nox4 and p22^{phox} of each genotype were ligated to the mammalian expression vector pcDNA3.0 or pcDNA3.1/Zeo(+) (Invitrogen, Carlsbad, CA, USA). Monkey kidney COS-7 cells were transfected with cDNAs for Nox4 and p22^{phox} simultaneously, using Lipofectamine Plus reagent (Invitrogen).

Preparation of membrane fraction

The membrane fraction of the cells transfected with cDNAs for Nox4 and p22^{phox} was prepared as described previously [18]. Transfected cells were cultured for 48 h and harvested with trypsin/ethylenediaminetetraacetic acid (EDTA) for 5 min at room temperature. Harvested cells were lysed by sonication in the presence of proteinase inhibitors. The sonicates were centrifuged for 10 min at 10 000 × g. The resultant supernatant was further centrifuged for 60 min at 100 000 × g. The pellet was used as the membrane fraction.

Assay for superoxide production using chemiluminescence

Superoxide production was determined as superoxide dismutase (SOD)-inhibitable chemiluminescence detected with 5 μM lucigenin or an enhancer-containing luminol-based detection system (DIOGENES; National Diagnostics, Atlanta, GA, USA) as described previously [4,18]. The membrane fraction of transfected COS-7 cells (5 μg protein) was suspended in 200 μl of an assay buffer composed of 100 mM potassium phosphate (pH 7.0), 10 μM flavin adenine dinucleotide (FAD), 1 mM NaN_3, and 1 mM ethyleneglycol bis (2-aminoethylether) tetraacetic acid (EGTA). After preincubation with 5 μM lucigenin or enhanced luminol-based substrate (200 μl), NADH or NADPH was added to a final concentration of 500 μM. The chemiluminescence was continuously monitored using a luminometer (MiniLumat LB9506; EG&G Berthold, Bad Wildbad, Germany). The reaction was terminated by the addition of SOD (100 μg/ml).

Detection of superoxide anion by dihydroethidium

An oxidative fluorescent dye, dihydroethidium, was used to evaluate superoxide anion production as described previously [4,24]. Transfected COS-7 cells on 35-mm glass-bottom dishes (MatTek, Ashland, MA, USA) were treated with 10 μM dihydroethidium for 5 min at room temperature. Ethidium fluorescence (excitation at 490 nm, emission at 610 nm) was examined by fluorescence microscopy (DM IRB, Leica, Wetzlar, Germany).

Statistical analysis

The SAS (Cary, NC, USA) software package was used to perform all statistical analyses. The Hardy-Weinberg equilibrium for the p22^{phox} genotype distribution was assessed by the chi-squared test. We analyzed the genotype and allele frequencies in patients and control groups by the chi-squared test. The age- and sex- or multivariate-adjusted odds ratios (ORs) and 95% confidence intervals (CIs) were estimated with the use of conditional logistic regression analysis. In the present study, we recruited 1055 patients and the same number of control subjects. To estimate the differences in the frequencies of the CT + TT genotype between case and control subjects, the sample size was sufficient to detect ORs of 1.5-fold or larger with 80% statistical power at the 5% level of significance, assuming that the frequency of the T allele was 0.1 and that the penetrance of the CC genotype for brain infarction was 0.01 (the required sample size was at least 528 in each group for a total of 1056). Thus, the number of patients and control subjects in our study provided enough power to analyze precise statistics. One-way factorial ANOVA followed by a Scheffe multiple comparison test was used to compare the NAD(P)H-dependent superoxide production of COS-7 membranes. $P < 0.05$ was considered statistically significant in all analyses.

Table 1 Baseline characteristics of the study population

	Case	Control	*P*-value
Number	1055	1055	
Age, years	70 ± 10	70 ± 10	NS
Female, %	37.3	37.3	NS
Hypertension, %	78.8	56.1	< 0.0001
Diabetes, %	30.3	22.5	< 0.0001
Hyperlipidemia, %	48.9	43.4	< 0.05
Atrial fibrillation, %	15.3	2.3	< 0.0001
Smoking, %	43.9	22.3	< 0.0001
Drinking, %	48.3	46.5	NS
Subtypes of brain infarction, *n* (%)			
Lacunar infarction	445 (42.2)		
Atherothrombotic infarction	358 (33.9)		
Cardioembolic infarction	134 (12.7)		
Unclassified infarction	118 (11.2)		

Statistical analysis was performed using the chi-squared test; NS, not significant.

Results

Characteristics of the study population

A total of 2110 subjects (1055 case and 1055 control subjects) were studied. Baseline characteristics of both study groups are shown in Table 1. The prevalence of hypertension, diabetes, hyperlipidemia, and atrial fibrillation was higher in case subjects than in control subjects. A smoking habit was also significantly more frequent in case subjects than in control subjects. The distribution of stroke subtype was 42.2% with lacunar infarction, 33.9% with atherothrombotic infarction, 12.7% with cardioembolic infarction, and 11.2% with an unclassified type of infarction.

Distribution and allele frequency of $p22^{phox}$ C242T polymorphism

The genotype distribution was in accordance with Hardy-Weinberg expectations. The T-allele frequency of $p22^{phox}$ was 10.4% in stroke patients and 11.0% in control subjects. In terms of stroke subtype, this allele frequency was 11.2% in patients with lacunar infarction, 11.2% in patients with atherothrombotic infarction, and 5.6% in patients with cardioembolic infarction (Table 2). Comparison by the chi-squared test revealed that the T-allele frequency of the C242T polymorphism was significantly lower in patients with cardioembolic infarction than in control subjects; however, the frequencies of the T allele and CT + TT genotypes in the patients with lacunar infarction and atherothrombotic infarction were similar to those in the control subjects. To evaluate the influence of risk factors, we performed the conditional logistic regression analyses matched for age and sex. As shown in Table 3, we found no association between the C242T polymorphism and either lacunar or atherothrombotic infarction after adjustment for age and sex. These relationships remained substantially unchanged even after adjustments for hypertension, diabetes, hyperlipidemia, and atrial fibrillation. Because the frequency of the TT genotype in subjects with cardioembolic infarction was rather small for conditional logistic regression analysis using multiple confounding factors, a significant negative association between CT + TT genotype of $p22^{phox}$ and cardioembolic infarction was found when adjusted for age and sex. These results indicate that the C242T polymorphism of the $p22^{phox}$ gene is not involved in lacunar and atherothrombotic infarction; however, the T allele of C242T polymorphism may be negatively associated with cardioembolic infarction.

Functional effects of the $p22^{phox}$ C242T polymorphism

To investigate the effects of the polymorphism on the superoxide-producing activity, we selected Nox4 as a catalytic subunit partner of $p22^{phox}$ for the following two reasons. First, Nox4 is predominantly expressed in vascular endothelial cells and is considered to play an

Table 2 Genotype distribution and allele frequencies for C242T polymorphism of the $p22^{phox}$ gene in each subtype of brain infarction: an overview

	Genotypes *n* (%)		
Clinical subtypes	CC	CT + TT	T allele (%)
Case subjects			
All types of brain infarction (*n* = 1055)	851 (80.7)	189 + 15 (19.3)	10.4
Lacunar (*n* = 445)	350 (78.7)	90 + 5 (21.3)	11.2
Atherothrombotic (*n* = 358)	286 (79.9)	64 + 8 (20.1)	11.2
Cardioembolic (*n* = 134)	120 (89.6)	13 + 1 (10.4)[a]	5.6[b]
Unclassified (*n* = 118)	95 (80.5)	22 + 1 (19.5)	10.2
Control subjects (*n* = 1055)	840 (79.6)	198 + 17 (20.4)	11.0

Statistical analysis was performed using the chi-squared test; [a]$P < 0.01$; [b]$P < 0.001$ versus control subjects.

Table 3 Age- and sex- or multivariate-adjusted odds ratios (ORs) and 95% confidence intervals (CIs) of genotypes of the C242T polymorphism of p22^{phox} for brain infarction and its subtype

	Number		Age and sex adjusted		Multivariate adjusted[a]	
Genotypes	Case	Control	OR	95% CI	OR	95% CI
All types of brain infarction						
CC	851	840	1.00	Reference	1.00	Reference
CT	189	198	0.94	0.76–1.17	0.91	0.69–1.21
TT	15	17	0.87	0.44–1.75	0.97	0.41–2.29
CC	851	840	1.00	Reference	1.00	Reference
CT + TT	204	215	0.94	0.76–1.16	0.92	0.70–1.20
Lacunar and atherothrombotic infarction						
CC	636	647	1.00	Reference	1.00	Reference
CT	154	147	1.07	0.83–1.37	0.95	0.70–1.29
TT	13	9	1.46	0.62–3.42	1.37	0.50–3.74
CC	636	647	1.00	Reference	1.00	Reference
CT + TT	167	156	1.09	0.85–1.39	0.97	0.72–1.32
Lacunar infarction						
CC	350	355	1.00	Reference	1.00	Reference
CT	90	82	1.11	0.80–1.53	1.00	0.67–1.50
TT	5	8	0.64	0.21–1.96	0.62	0.18–2.20
CC	350	355	1.00	Reference	1.00	Reference
CT + TT	95	90	1.07	0.78–1.47	0.97	0.66–1.43
Atherothrombotic infarction						
CC	286	292	1.00	Reference	1.00	Reference
CT	64	65	1.00	0.76–1.17	0.75	0.45–1.27
TT	8	1	8.00	1.00–63.96	13.71	1.07–175.30
CC	286	292	1.00	Reference	1.00	Reference
CT + TT	72	66	1.12	0.77–1.63	0.86	0.52–1.42
Cardioembolic infarction						
CC	120	102	1.00	Reference		
CT	13	28	0.40	0.20–0.82[b]		
TT	1	4	0.20	0.02–1.87		
CC	120	102	1.00	Reference		
CT + TT	14	32	0.38	0.19–0.76[c]		

Statistical analysis was performed using the conditional logistic regression analysis; [a]Adjusted for age, sex, hypertension, diabetes, hyperlipidemia, atrial fibrillation, smoking, and drinking; [b]$P < 0.05$; and [c]$P < 0.01$.

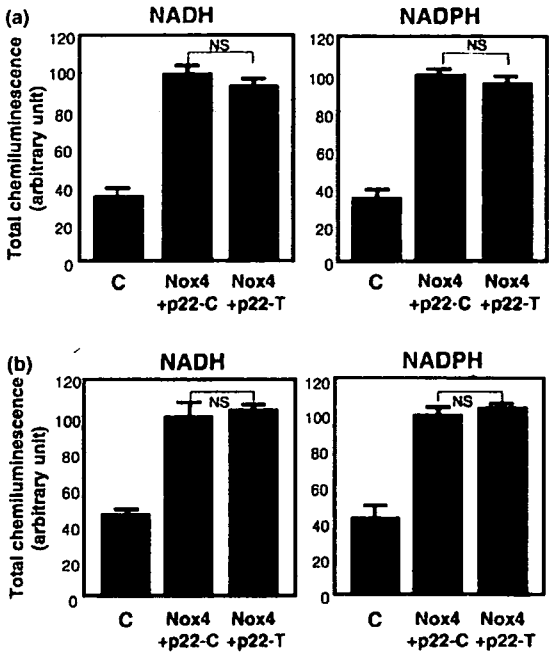

Figure 1 NAD(P)H-dependent superoxide production by the membrane fraction of COS-7 cells cotransfected with Nox4 and p22^{phox} of each genotype. Chemiluminescence changes caused by the addition of NADH or NADPH were continuously monitored with use of 5 μM lucigenin (a) or an enhanced luminal-based substrate, DIOGENES (b). The total chemiluminescence change in the membrane fraction of COS-7 cells expressing p22^{phox} with the C allele was set to 100. Each graph represents the means ± SD of the superoxide-producing activity obtained from four independent experiments. C; control, p22-C; p22^{phox} with the C allele, p22-T; p22^{phox} with the T allele, NS; not significant.

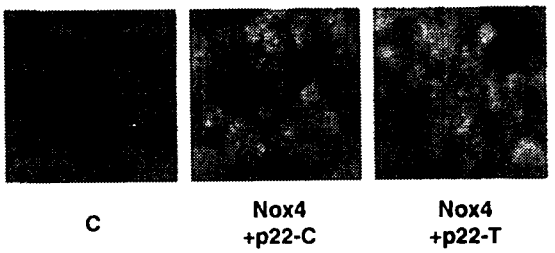

Figure 2 Effects of the C242T polymorphism of the p22^{phox} gene on superoxide production in living cells. Superoxide production in living COS-7 cells cotransfected with Nox4 and p22^{phox} of each genotype was determined by fluorescence microscopy using dihydroethidium. C; control, p22-C; p22^{phox} with the C allele, p22-T; p22^{phox} with the T allele.

important role in the development of arteriosclerosis. Secondly, Nox4 appears to produce superoxide independent of any regulatory cytosolic factors unlike Nox1 and Nox2, allowing us to simplify the experimental protocol.

As shown in Fig. 1, the membrane fraction produced superoxide following the addition of NADH as well as NADPH, in accordance with a previous finding that both NADH and NADPH can act as electron donors for superoxide production by Nox4 [18]. The superoxide production by the membrane fraction of COS-7 cells expressing p22^{phox} with the C allele was almost identical to that of cells expressing p22^{phox} with the T allele (Fig. 1), indicating that the polymorphism has no effect on superoxide-producing activity. We also examined superoxide production using a fluorescent dye, dihydroethidium, and found that the intensity of ethidium fluorescence was almost identical in the both types of transfected cells (Fig. 2).

Discussion

The major new finding of the present study is that the genotype and allele frequencies of the $p22^{phox}$ C242T polymorphism in the patients with lacunar and atherothrombotic brain infarction are similar to those in control subjects in the Japanese population, indicating a lack of association between brain infarction of lacunar and atherothrombotic subtypes and the C242T polymorphism. Because the T-allele frequency in patients with cardioembolic infarction was smaller than that in control subjects, the CT + TT genotype of the C242T polymorphism of $p22^{phox}$ may have a protective effect against cardioembolic brain infarction.

There have been two conflicting reports regarding the association between the C242T polymorphism of $p22^{phox}$ and the risk of stroke in the Japanese population. Ito *et al.* [16] investigated the association of this polymorphism with ischemic stroke in 301 stroke patients and reported that the CT + TT genotype was more frequent in ischemic stroke patients than in the control group (21.7% and 13.3%, respectively; $P = 0.01$). Thus, the T allele may be a risk factor for ischemic stroke. By contrast, Shimo-Nakanishi *et al.* [17] investigated $p22^{phox}$ genotypes in 120 Japanese patients with lacunar or atherothrombotic brain infarction and found no association between the polymorphism and these subtypes of brain infarction (CT + TT genotype frequencies in patients and control subjects were 15.0% and 13.0%, respectively). Utilizing clinical information, we performed conditional logistic regression analysis after adjustment for risk factors and found that the C242T polymorphism of $p22^{phox}$ was not related to lacunar or atherothrombotic infarction.

Amongst clinical subtypes of brain infarction, patients with cardioembolic infarction tend to have a lower frequency of the T allele than other stroke types and control subjects. The frequency of the T allele of $p22^{phox}$ was significantly lower in subjects having atrial fibrillation than those without atrial fibrillation (T-allele frequencies in subjects with and without atrial fibrillation were 6.0% and 11.1%, respectively; $P < 0.03$). Thus, reduced T-allele frequency in patients with cardioembolic brain infarction may be due to a low frequency of the T allele in subjects having atrial fibrillation. It is possible that the C242T polymorphism is associated with the occurrence of atrial fibrillation. Further studies are required to clarify the association between the polymorphism and atrial fibrillation.

The functional effects of the C242T polymorphism of the $p22^{phox}$ gene on the activity of NAD(P)H oxidase are also not well understood. Phagocytes such as neutrophils and macrophage express $gp91^{phox}$, also termed Nox2, which plays an important role in host defense and inflammation. Shimo-Nakanishi *et al.* [17] have shown that the superoxide-producing activity in neutrophils with the T allele is higher than that in neutrophils with the C allele, and the activity is also higher in promyelocytic HL-60 cells transfected with $p22^{phox}$ harboring the T allele than cells transfected with $p22^{phox}$ harboring the C allele. By contrast, Wyche *et al.*[25] reported that the superoxide-producing activity of neutrophils from individuals with the TT genotype is significantly lower than that of neutrophils from individuals with CC and CT genotypes.

Recent evidence has suggested that vascular superoxide production, which plays an important role in the pathogenesis of brain infarction based on arteriosclerosis, is more complicated. We have shown recently that Nox4 is expressed most abundantly amongst Nox family members in vascular endothelial cells [4]. Moreover, Nox4 is present and may be functionally active in the cerebral arterial wall [5]. Guzik *et al.* [26] reported that the presence of the T allele was associated with reduced vascular NAD(P)H oxidase activity in the saphenous vein, suggesting that the C242T polymorphism of $p22^{phox}$ could also modulate vascular superoxide-producing activity, such as that of Nox4. Thus, it is important to investigate the effect of the polymorphism on superoxide-producing activity of the Nox4-$p22^{phox}$ heterodimer. In the present study, we found that the C242T polymorphism of $p22^{phox}$ is not associated with superoxide production by the Nox4-$p22^{phox}$ heterodimer using monkey kidney COS-7 cells co-transfected with Nox4 and $p22^{phox}$ of each genotype. The finding may support our interpretation that there is no association between the polymorphism and lacunar and atherothrombotic infarction. On the other hand, the C242T polymorphism of $p22^{phox}$ may have a protective role in the occurrence of atrial fibrillation. It is possible that NAD(P)H oxidases have some other function in addition to superoxide production which are affected by this polymorphism. As an example of the function other than superoxide production, it has been reported that NAD(P)H oxidase of HL-60 (a human myeloid cell line) may have the activity of proton channel [27]. Thus, the polymorphism may affect the activity of unknown functions of NAD(P)H oxidases or $p22^{phox}$ itself.

In conclusion, the C242T polymorphism of the $p22^{phox}$ gene may not be involved in lacunar and atherothrombotic infarction in the Japanese population and may not affect the superoxide-producing activity of NAD(P)H oxidase in vascular walls. On the other hand, the presence of the T allele may have a protective role in the occurrence of atrial fibrillation and cardioembolic infarction.

Acknowledgements

We thank the members of the FSR study group contributing to this study including Drs. Tsuyoshi Omae, Kazuki Ota, Tsuyoshi Imamura, Kazuhiro Kishikawa, Tomoko Kagiyama, Fumiko Sadanaga, Junichi Takada, Shigeru Fujimoto, Yoko Wakugawa, Hideaki Kurushima, Juro Jinnouchi, Takeshi Uwatoko, Eiichi Ishikawa, Yuko Hirai, Seiji Goto, Noriko Makihara, and Yoko Yokoyama. We are also grateful to Ms. Rie Tominaga (Seiai Rehabilitation Hospital) for her technical assistance.

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医学と薬学
57巻2号・2007年2月
57(2) : 169-171, 2007

メタボリックシンドロームにおける高尿酸血症の意義

札幌医科大学医学部第2内科
東浦　勝浩・斎藤　重幸・竹内　宏
高木　覚・浦　信行・島本　和明

I. 目　的

肥満と高尿酸血症（以下，HU）の関連については多くの報告があるが，HUがメタボリックシンドローム（以下，MetS）といかに関連するのかについては明らかではない。そこで本稿では端野・壮瞥町の住民健診受診者を対象に，地域一般住民男性におけるMetSの頻度と危険因子集積におけるHUの意義についての検討結果を報告する。

II. 方　法

対象は2002年に北海道端野町，壮瞥町の住民健診を受診した，薬剤治療者を除く男性588名（平均年齢63±13歳）。安静座位にて血圧を測定し，早朝空腹時に採血を施行して血糖，血清尿酸値，脂質パラメーター，血清インスリン値を測定した。HOMA-IR（homeostasis model assessment for insulin resistance）は空腹時血糖×血清インスリン濃度/405にて計算した。また，腹囲径は立位，軽呼気時，臍周囲にて計測した。全米コレステロール教育プログラム（NCEP-ATP-III）のMetSの診断基準[1]を一部改変，つまり肥満（腹囲≧85 cm），血圧高値（≧130/85 mmHg），高中性脂肪血症（≧150 mg/dl），低HDL血症（<40 mg/dl），空腹時血糖高値（≧110 mg/dl）の中で，三つ以上を有する者をMetSとして，非MetSの2群に分けて検討した。また，血清尿酸値>7.0 mg/dlをHUと定義した。なお，本検討は全対象から筆式にてインフォームドコンセントを得て施行した。

III. 結　果

対象の平均body mass index（BMI）は23.6±3.0 kg/m^2，平均腹囲径は84.8±8.6 cmであった。住民健診受診者全体の15.9%にHUを認め，また25.3%がMetSの診断基準を満たした。血清尿酸値はインスリン抵抗性の指標であるHOMA-IRと有意に正に相関した。MetSの中でHUを有するのは約20%であり，血清尿酸値はMetSで非MetSと比して有意に高かった（図1）。MetSの各危険因子と血清尿酸値の関係においては，腹部肥満，高中性脂肪血症，低HDL血症，血圧高値を有する群で，有さ

Significance of hyperuricemia for metabolic syndrome
Katsuhiro Higashiura, Shigeyuki Saitoh, Hiroshi Takeuchi, Satoru Takagi, Nobuyuki Ura, Kazuaki Shimamoto　*Second Department of Internal Medicine, Sapporo Medical University*
Key words: metabolic syndrome, hyperuricemia, insulin resistance

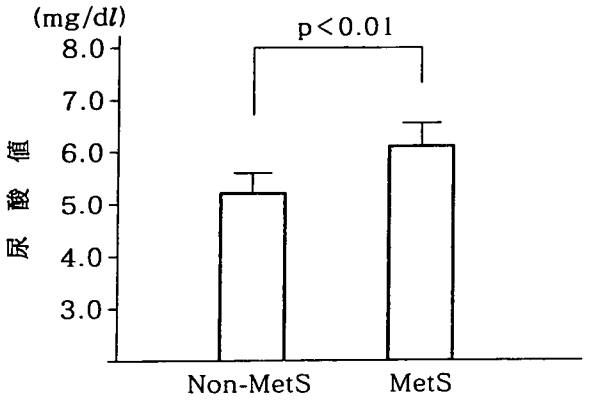

図 1　メタボリックシンドローム (MetS) の有無と血清尿酸値

表 1　重回帰分析（目的変数：危険因子の集積）

	β	t	p
AGE	0.23	5.04	<0.01
BMI	0.38	7.47	<0.01
HOMA-IR	0.27	5.44	<0.01
UA	0.10	2.19	<0.05

調整済み R^2値：0.33

危険因子の集積を従属変数とした重回帰分析では，年齢，BMI，HOMA-IR とともに尿酸(UA)も有意な説明因子として採択された。

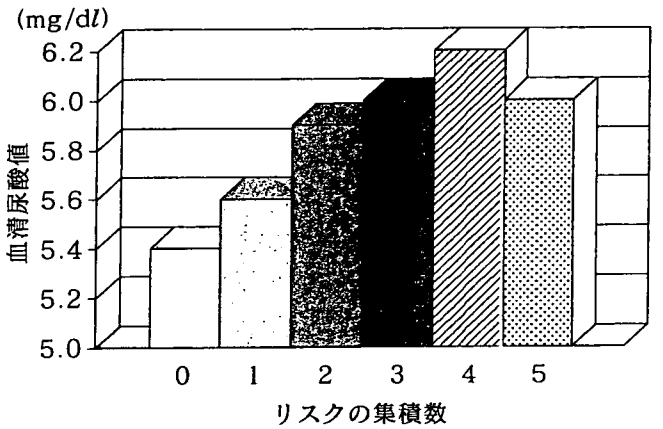

図 2　リスクの集積と血清尿酸値

ない群に比べ血清尿酸値は有意に高かった。

また，各危険因子の集積個数が多いほど血清尿酸値は段階的に上昇（図 2）し，高尿酸血症を有する群では有さない群に比べて危険因子の集積個数が有意に多かった。バリマックス因子分析を施行すると，HU は肥満，高中性脂肪血症，低 HDL 血症とともにインスリン抵抗性に関連する因子として選択された。一方で，危険因子の集積を目的変数として重回帰分析を施行すると，年齢，BMI，HOMA-IR に加えて，血清尿酸値も危険因子集積に有意に関連する因子であることが明らかになった（表 1）。

IV. 考　察

今回の地域住民健診の男性における検討により，HU はインスリン抵抗性を背景因子として MetS に関連していることが明らかとなった。MetS における HU の機序については腹部内臓脂肪の増加に伴う肝臓での中性脂肪合成亢進と連動した尿酸の *de novo* 合成増大[2]と，インスリン抵抗性/高インスリン血症に伴う腎臓での，Na の再吸収亢進に伴う尿酸の排泄低下[3]が関連していると考えられており，今回の検討結果はこれらの仮説に矛盾しないと考えられた。

一方で，血清尿酸値は BMI，HOMA-IR 等で補正した後も危険因子集積の有意な因子として選択されており，HU はインスリン抵抗性を介さない機序で危険因子集積に関わっている可能性が示唆された。この点については HU がレニン-アンジオテンシン系の賦活化に関わるとの報告[4]や炎症性マーカー[5]との関連についての指摘もあり，これらの機序を介して HU が MetS 発症に関わる可能性も考慮されるが，詳細については今後の検討課題と考える。

近年，高血圧[6]あるいは糖尿病患者[7]等のハイリスクグループにおいて，HU が心血管疾患の独立した危険因子であるとの報告が散見されている。これらのこともインスリン抵抗性に関連して生じた HU が，他の機転を介して危険因子集積に関わる可能性を示した本検討の結果を支持するものと考えられる。

ま と め

インスリン抵抗性は MetS の背景因子であると同時に HU の発症因子であり，HU 自体も MetS の病態形成に関わっている可能性が示唆

された。

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Original Article

Relationship between Visceral Fat and Cardiovascular Disease Risk Factors: The Tanno and Sobetsu Study

Yu CHIBA[1)], Shigeyuki SAITOH[1)], Satoru TAKAGI[1)], Hirofumi OHNISHI[1)], Nobuo KATOH[1)], Junichi OHATA[1)], Motoya NAKAGAWA[1)], and Kazuaki SHIMAMOTO[1)]

We assessed the amount of visceral fat using ultrasonography (US) and studied its relationship to cardiovascular disease risk factors, particularly blood pressure. The subjects in the first study were 45 male and 61 female outpatients. We measured the visceral fat area (VFA) of each subject using abdominal CT and waist circumference (WC), and visceral fat distance (VFD) using US. The subjects in the second study were 353 male and 457 female inhabitants of a rural community, for whom VFD and WC were measured. We divided subjects into tertiles based on VFD and WC, and studied the relationship between each group and individual risk factors. In an analysis of outpatient subjects, the correlation coefficient between VFA and VFD was satisfactory: r=0.660 for men and r=0.643 for women. In the analysis of the rural subjects, the high VFD group had a significantly higher odds ratio than the low VFD group in high blood pressure (HBP) and hypertriglyceridemia (HTG) for men and in HBP, HTG and low high-density lipoprotein cholesterolemia (LHDL) for women. Moreover, adjusting VFD for body mass index revealed that, in comparison to WC, VFD was significantly related to risk factors. VFD was used as an independent variable in multiple regression analysis with blood pressure level as a dependent variable; no significant association between WC and blood pressure was obtained. Visceral fat assessment by US may be useful for epidemiological study and for clinics with no abdominal CT equipment for identifying high-risk individuals, such as those with metabolic syndrome. (*Hypertens Res* 2007; 30: 229–236)

***Key Words*: ultrasonography, visceral obesity, cardiovascular disease risk factors, waist circumference, hypertension**

Introduction

Obesity is often complicated by arteriosclerotic diseases such as hypertension, ischemic heart disease and cerebrovascular disease as well as by their risk factors (*1*, *2*). Since the late 1980s, these complications have been explained by the concept of a multiple risk factor syndrome such as syndrome X (*3*), the deadly quartet (*4*), and visceral fat syndrome (*5*). More recently, the term metabolic syndrome (MS) has been adopted by the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATPIII) (*6*). Visceral obesity, in which fat markedly accumulates in the peritoneal mesentery and around the greater omentum, is thought to be a fundamental pathology for MS in particular. The incidence of cardiovascular disease is high even in non-obese individuals with a body mass index (BMI) within the normal range who have an accumulation of visceral fat (*7*), and accurate assessment of both body fat distribution and visceral fat accumulation is critical for assessing the risk of arteriosclerotic disease.

Previous studies have shown that waist-to-hip ratio, waist-to-height ratio, waist circumference (WC), and visceral fat

From the [1)]Second Department of Internal Medicine, Sapporo Medical University School of Medicine, Sapporo, Japan.
Address for Reprints: Hirofumi Ohnishi, M.D., Ph.D., Second Department of Internal Medicine, Sapporo Medical University School of Medicine, S-1, W-16, Chuo-ku, Sapporo 060–8543, Japan. E-mail: hohnishi@sapmed.ac.jp
Received June 28, 2006; Accepted in revised form November 16, 2006.

assessed by abdominal CT are relatively good indicators of the risk of cardiovascular disease (*8–13*). Abdominal CT enables quantification of the visceral fat area (VFA) and therefore serves as the gold standard for visceral fat assessment. On the other hand, WC measurement is recommended as a simpler and easier screening method (*14*). However, abdominal CT has drawbacks, including exposure to radiation, lack of ease and simplicity, and high cost. WC includes subcutaneous fat, and WC measurement therefore has drawbacks such as an inability to account for an individual's height and a low level of reproducibility in the case of marked obesity.

Simple methods for assessing visceral fat accumulation using ultrasonography (US) have been studied in recent years (*15–20*). In addition, previous studies have indicated a relationship between hypertension and visceral fat assessed by abdominal CT and WC, but US was not used in any of those studies (*21–24*). Thus, in the present study, we assessed the usefulness of visceral fat assessment by US in outpatients. Then, based on the results of a cross-sectional study, we assessed the relationships between abdominal obesity determined by US and cardiovascular disease risk factors, particularly blood pressure levels.

Methods

Study 1

The subjects were 45 men and 61 women outpatients (mean ages: 55.4±19.4 years for men and 67.5±10.8 years for women). Individuals with cardiovascular disease, renal disease or a severe debilitating disease were excluded from participation. Height, body weight, WC, VFA and total fat area (TFA) were determined by abdominal CT, and visceral fat distance (VFD) was determined by US. The subcutaneous fat area (SFA) was calculated by subtracting VFA from TFA.

Informed consent was obtained from each outpatient, who completed a form consenting to testing. Height, body weight and visceral fat levels were measured on the same day, and BMI was calculated. Correlations between VFA, SFA, VFD, BMI and WC were investigated.

Measurement of Visceral Fat Levels

CT equipment from Toshiba Medical Systems (Tokyo, Japan) was used for abdominal CT. Imaging was done at the end of expiration at the umbilical level. Tracing in cross-sectional images was done using a trackball; the total cross-sectional area was determined by automatic calculation of portions with a CT number of −200 to 1,000 Hounsfield units (HU) using the method of Grauer *et al.* (*25*). In addition, portions with a CT number of −200 to −10 HU were separated as adipose tissue and their areas were automatically calculated.

WC was measured with non-stretchable measuring tape while subjects bared the circumference of the abdomen. The umbilical circumference was measured in increments of 0.1 cm during expiration while standing (*14*).

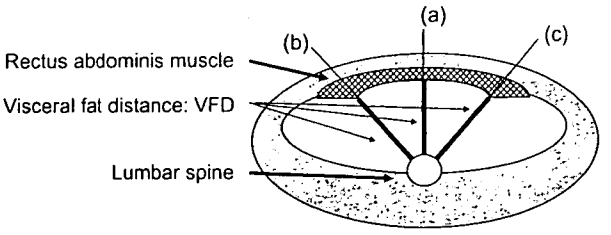

Fig. 1. *VFD was measured between the peritoneum and the lumbar spine, and which was taken as the average value. VFD = (a + b + c)/3. Each subject assumed a supine position, and at the end of expiration the distance from the peritoneum to the front of the vertebral body was measured perpendicularly three times with a 3.5 MHz linear probe while making the slightest contact possible, and the average value was used as the VFD.*

VFD was measured using VF-750XT portable ultrasonography equipment (Fukuda Electrical, Tokyo, Japan) by the method of Stolk *et al.* (*18, 19*). That is, each subject assumed a supine position, and at the end of expiration the distance from the peritoneum to the front of the vertebral body was measured perpendicularly three times with a 3.5 MHz linear probe while making the least possible amount of contact, and the average value was used as the VFD (Fig. 1). All measurements were performed by the same investigator.

Study 2

The subjects were 353 men and 457 women (mean ages: 62.8±12.2 years for men and 57.8±12.6 years for women) out of 1,455 individuals who underwent screening for local residents of a rural community; individuals being treated for hypertension, diabetes or hyperlipidemia were excluded. The study was approved by the Ethics Committee of Sapporo Medical University, and written informed consent was obtained from each subject.

For all subjects, height and body weight were measured after fasting for 8 h or longer since their last meal, blood pressure levels were measured and blood samples were taken. The blood samples were used to measure high-density lipoprotein (HDL)–cholesterol levels (HDL-c), triglyceride levels (TG), fasting plasma glucose levels (FPG) and serum insulin levels. Afterwards, WC and VFD were measured. Height and body weight were measured at intervals of 0.1 cm and 0.1 kg, respectively, with subjects lightly dressed and shoes removed. Blood pressure was measured twice consecutively on the upper arm using an automated sphygmomanometer (HEM-907, Omron Instruments, Tokyo, Japan) with subjects in a seated resting position, and the average was used for systolic blood pressure (SBP) and diastolic blood pressure (DBP).

Table 1. Characteristics of the Subjects for Study 1

	Men (n=45)	Women (n=61)	p-value
Age (years)	55.4±19.4	67.5±10.8	<0.001
Body weight (kg)	67.1±11.8	56.4±8.8	<0.001
BMI (kg/m^2)	24.2±3.2	24.7±3.9	0.462
Lean: BMI<22	11/45 (24%)	14/61 (23%)	
Overweight: 22≤BMI<25	17/45 (38%)	23/61 (38%)	
Obese: 25≤BMI	17/45 (38%)	24/61 (39%)	
WC (cm)	84.9±8.8	85.6±10.1	0.787
VFD (cm)	5.2±1.2	4.9±1.43	0.459
SFA (cm^2)	147.0±63.8	221.2±132.4	<0.001
VFA (cm^2)	137.0±62.6	128.9±51.8	0.606

All values are mean±SD. BMI, body mass index; WC, waist circumference; VFD, visceral fat distance; SFA, subcutaneous fat area; VFA, visceral fat area.

Table 2. Correlation between Adipose Tissue Measured by CT and Other Anthropometric Parameters

	Adipose tissue measured by CT	
	SFA	VFA
Men (n=45)		
BMI	0.763*	0.565*
WC	0.861*	0.646*
VFD	0.237	0.660*
Women (n=61)		
BMI	0.591*	0.571*
WC	0.595*	0.499*
VFD	0.289**	0.643*

Values are Pearson's correlation coefficients. *p<0.001, **p<0.05. SFA, subcutaneous fat area; VFA, visceral fat area; BMI, body mass index; WC, waist circumference; VFD, visceral fat distance.

Measurement Methods

HDL-c was measured by the enzymatic method (homogenous), TG was measured by the enzymatic colorimetric method (free glycerol elimination), FPG was measured by the GOD immobilized oxygen electrode maximum reaction acceleration method, and serum insulin level was measured by the enzyme immunoassay method. In addition, homeostasis model assessment index (HOMA-IR) was calculated on the basis of FPG and serum insulin levels (*26*).

Diagnostic Criteria for Cardiovascular Disease Risk Factors

Diagnostic criteria for cardiovascular disease risk factors followed the NCEP ATPIII criteria for MS (*6*). High blood pressure (HBP) was defined as SBP ≥130 mmHg and/or DBP ≥85 mmHg or higher, hypertriglyceridemia (HTG) was defined as TG ≥150 mg/dl, low HDL cholesterolemia (LHDL) was defined as HDL-c <40 mg/dl for men and <50 mg/dl for women, and high fasting plasma glucose (HFPG) was defined as FPG ≥110 mg/dl.

Statistical Analysis

Statistical analysis was done using Windows SPSS version 11.5J. Numerical values are shown as means (mean)±SD. The correlation between two variables was evaluated using Pearson's correlation coefficient. Comparison between two groups was done with an unpaired t-test. For logistic regression analysis, subjects were divided into tertiles based on VFD and WC, adjusted for age (model 1) and then adjusted for age and BMI (model 2); with the low VFD and low WC groups as a reference, odds ratios (OR) and individual cardiovascular disease risk factors were examined. Comparison of three groups was done by multiple comparisons after one-way ANOVA. For multiple regression analysis, blood pressure level served as a dependent variable, and the relationships between cardiovascular disease risk factors with VFD and WC were studied. In all instances, the level of significance was p<0.05.

Results

Study 1

Table 1 shows characteristics of the 45 male and 61 female outpatient subjects whose visceral fat levels were measured by abdominal CT. No significant difference between the male and female subjects was found in BMI, WC, VFD or VFA. SFA was significantly larger for women than for men.

The correlations between SFA and VFA determined by abdominal CT and BMI, VFD and WC are shown in Table 2. The correlation coefficients between VFA and VFD were r=0.660 (p<0.001) for men and r=0.643 (p<0.001) for women. In addition, VFA had a stronger correlation to VFD than to BMI or WC. Moreover, BMI and WC had stronger

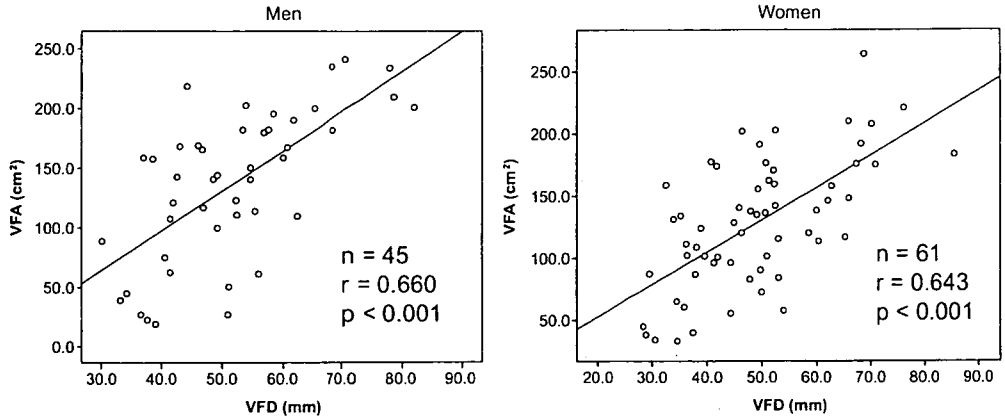

Fig. 2. *Scattergrams of relationship between VFD and VFA for men and women. VFD, visceral fat distance assessed by ultrasonography; VFA, visceral fat area assessed by CT. There were significant positive correlations between VFD and VFA in both men and women.*

Table 3. Characteristics of the Study Subjects of Residents of a Rural Community

	Men (n=353)	Women (n=457)	p-value
Age (years)	62.8±12.2	57.8±12.6	<0.001
Body weight (kg)	63.9±10.1	53.7±7.6	<0.001
BMI (kg/m²)	23.7±3.2	23.0±3.2	0.002
Lean: BMI<22	107/353 (30%)	177/457 (39%)	
Overweight: 22≤BMI<25	143/353 (41%)	171/457 (37%)	
Obese: 25≤BMI	103/353 (29%)	109/457 (24%)	
WC (cm)	84.7±9.1	82.6±9.9	0.002
VFD (cm)	5.5±1.7	4.7±1.3	<0.001
SBP (mmHg)	131.9±20.1	127.0±21.2	0.001
DBP (mmHg)	75.5±11.6	71.9±10.6	<0.001
HDL-c (mg/dl)	51.3±11.7	59.3±14.5	<0.001
TG (mg/dl)	115.1±75.2	88.3±49.2	<0.001
FPG (mg/dl)	96.8±15.7	94.4±17.7	0.041
Serum insulin levels (μU/ml)	4.5±4.7	4.4±2.9	n.s.
HOMA-IR	1.13±1.38	1.04±0.72	n.s.

All values are mean±SD. BMI, body mass index; WC, waist circumference; VFD, visceral fat distance; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL-c, high-density lipoprotein-cholesterol; TG, triglyceride; FPG, fasting plasma glucose; HOMA-IR, homeostasis model assessment index; n.s., not significant.

correlations to SFA than to VFA (Table 2).

Figure 2 shows scattergrams of the relationships between VFD and VFA for men and women. There were significant positive correlations between VFD and VFA in both sexes.

Study 2

Table 3 shows the characteristics of the subjects in Study 2. Average VFDs were 5.5±1.7 cm for men and 4.7±1.3 cm for women, and average WCs were 84.7±9.1 cm for men and 82.6±9.9 cm for women.

The subjects were divided into tertiles based on VFD and WC; OR for cardiovascular disease risk factors with individual low-tertile groups as a reference are shown in Table 4. Adjusted only for age (model 1), OR increased significantly for the male VFD group in comparison to that for the low VFD group in HBP (OR: 3.45 [95% CI: 1.83–5.77]; p<0.001) and HTG (OR: 3.74 [1.72–8.12]; p<0.05), and it increased significantly for the female group in HBP (OR: 2.31 [1.37–3.92]; p<0.05), HTG (OR: 13.3 [3.02–58.5]; p<0.05) and LHDL (OR: 4.62 [2.47–8.62]; p<0.001). Similarly, OR increased significantly for the male WC group in comparison to that for the low WC group in HBP (OR: 2.00 [1.15–3.45]; p<0.05), HTG (OR: 3.09 [1.41–6.75]; p<0.05) and LHDL (OR: 8.82 [1.98–39.3]; p<0.05), and it increased significantly for the female group in HBP (OR: 1.95 [1.18–3.23];

Table 4. Odds Ratios and 95% CIs of CAD Risk Factors by Tertile of VFD and WC

	HBP	HTG	HFPG	LHDL
Men (n=353)				
Model 1				
VFD				
Lower tertile	1.00	1.00	1.00	1.00
Middle tertile	1.79 (1.04–3.09)*	2.31 (1.04–5.16)*	1.04 (0.4–2.44)	1.95 (0.83–4.59)
Upper tertile	3.45 (1.83–5.77)†	3.74 (1.72–8.12)*	0.80 (0.32–2.00)	2.02 (0.85–4.77)
WC				
Lower tertile	1.00	1.00	1.00	1.00
Middle tertile	2.10 (1.22–3.59)*	3.41 (1.56–7.44)*	0.79 (0.32–1.99)	16.4 (3.79–71.1)†
Upper tertile	2.00 (1.15–3.45)*	3.09 (1.41–6.75)*	1.26 (0.54–2.96)	8.82 (1.98–39.3)*
Model 2				
VFD				
Lower tertile	1.00	1.00	1.00	1.00
Middle tertile	1.67 (0.95–2.95)	2.21 (0.97–5.04)	0.88 (0.36–2.13)	1.71 (0.71–4.14)
Upper tertile	2.75 (1.37–5.50)*	3.35 (1.35–8.32)*	0.52 (0.17–1.62)	1.44 (0.52–4.04)
WC				
Lower tertile	1.00	1.00	1.00	1.00
Middle tertile	1.60 (0.86–2.96)	3.09 (1.31–7.31)*	0.71 (0.25–1.96)	17.6 (3.77–82.2)†
Upper tertile	1.15 (0.51–2.59)	2.54 (0.87–7.41)	1.00 (0.29–3.46)	10.1 (1.75–58.1)*
Women (n=457)				
Model 1				
VFD				
Lower tertile	1.00	1.00	1.00	1.00
Middle tertile	1.76 (1.04–2.98)*	6.28 (1.38–28.6)*	0.52 (0.16–1.72)	2.32 (1.23–4.38)*
Upper tertile	2.31 (1.37–3.92)*	13.3 (3.02–58.5)*	1.82 (0.71–4.69)	4.62 (2.47–8.62)†
WC				
Lower tertile	1.00	1.00	1.00	1.00
Middle tertile	1.05 (0.63–1.76)	3.79 (1.21–11.8)*	1.10 (0.43–2.82)	2.72 (1.52–4.86)*
Upper tertile	1.95 (1.18–3.23)*	5.79 (1.93–17.4)*	0.93 (0.37–2.34)	2.46 (1.36–4.43)*
Model 2				
VFD				
Lower tertile	1.00	1.00	1.00	1.00
Middle tertile	1.27 (0.73–2.22)	4.59 (0.99–21.3)	0.56 (0.16–1.92)	1.91 (0.99–3.70)
Upper tertile	1.06 (0.55–2.04)	6.36 (1.30–31.3)*	2.16 (0.67–6.92)	2.94 (1.40–6.17)*
WC				
Lower tertile	1.00	1.00	1.00	1.00
Middle tertile	0.65 (0.37–1.15)	2.37 (0.73–7.73)	0.90 (0.32–2.47)	1.78 (0.95–3.33)
Upper tertile	0.74 (0.37–1.45)	2.06 (0.56–7.57)	0.60 (0.17–2.05)	0.97 (0.45–2.09)

Model 1: adjusted for age; Model 2: adjusted for age and BMI. Significantly different from the Lower tertile: *p<0.05, †p<0.001. CI, confidence interval; CAD, cardiovascular disease; HBP, high blood pressure; HTG, hypertriglyceridemia; HFPG, high fasting plasma glucose; LHDL, low high-density lipoprotein cholesterolemia; VFD, visceral fat distance; WC, waist circumference.

p<0.05), HTG (OR: 5.79 [1.93–17.4]; p<0.05) and LHDL (OR: 2.46 [1.36–4.43]; p<0.05).

When additionally adjusted for BMI (model 2), OR increased significantly for the male VFD group in comparison to that for the low VFD group in HBP (OR: 2.75 [1.37–5.50]; p<0.05) and HTG (OR: 3.35 [1.35–8.32]; p<0.05). However, no significant association was found between WC and HBP or between WC and HTG. In addition, OR increased significantly for the female high VFD group in comparison to that for the low VFD group in HTG (OR: 6.36 [1.30–31.3]; p<0.05) and LHDL (OR: 2.94 [1.40–6.17]; p<0.05). However, no significant association was found between WC and any of the factors.

Table 5 shows the results of multiple regression analysis with SBP and DBP as dependent variables. For men, VFD was selected as a significant independent variable for both SBP and DBP. However, there was no significant association between WC and SBP or between WC and DBP.

Table 5. Results of Multiple-Regression Analysis Related to SBP and DBP

	Independent	Dependent			
		SBP		DBP	
		β	p-value	β	p-value
Men (n=353)	VFD	2.093	0.015	1.049	0.047
	WC	0.287	0.226	0.163	0.265
Women (n=457)	VFD	1.422	0.118	0.739	0.154
	WC	0.110	0.425	−0.057	0.466

Dependent variables: systolic blood pressure (SBP) or diastolic blood pressure (DBP). Independent variables: visceral fat distance (VFD) or waist circumference (WC) and additionally adjusted for age, triglyceride (TG), high-density lipoprotein-cholesterol (HDL-c), fasting plasma glucose (FPG), body mass index (BMI). β: standardized regression coefficient.

Although the data are not shown, when VFD was divided into tertiles, HOMA-IR increased significantly in the higher tertiles. Moreover, in multiple regression analysis using HOMA-IR as a dependent variable and using age, SBP, TG and VFD as independent variables, VFD was found to be a significant independent variable of HOMA-IR for both men and women.

Discussion

The significance of visceral obesity has been noted in recent years, and the accumulation of visceral fat must be accurately assessed. However, abdominal CT is not a simple technique, and WC also has the drawback of leading to an assessment that includes subcutaneous fat. In contrast, US involves no radiation exposure, the technique can be quickly learned, it is typically completed in less than 5 min, and it has been reported to have a good level of reproducibility (*15–20*). In the present study we therefore investigated whether US can be used as an easy screening method for the accurate estimation of the accumulation of visceral fat in Japanese as well.

When the correlations between VFA, SFA, BMI, VFD and WC were examined, VFD was found to have a stronger positive correlation with VFA than with SFA for both men and women. Additionally, BMI and WC each had a stronger positive correlation with SFA than with VFA. This is because measurements of BMI and WC are assessment methods that include elements of subcutaneous fat. The present study indicated that VFD measurement is a simple method for assessing visceral fat that does not include elements of subcutaneous fat and that VFD measurement is a useful means of assessing visceral fat in a large number of subjects.

The relationships between visceral fat and cardiovascular disease risk factors were then assessed in a study using US performed on inhabitants of a rural community who were not being treated for hypertension, diabetes or hyperlipidemia. The data presented in Table 4, obtained after adjustment for age and BMI (model 2), showed that VFD was significantly correlated with HBP, HTG and LHDL in men and with HTG and LHDL in women. On the other hand, WC was correlated with LHDL in men but showed only weak correlations with risk factors in women.

What eliminated the relationship between WC and cardiovascular disease risk factors in women subjects in particular was the effect of subcutaneous fat. Subcutaneous fat has less of an effect on arteriosclerosis than visceral fat and instead has antiarteriosclerotic action (*27*). In general, visceral obesity, a condition in which visceral fat readily accumulates, affects men more than women; women are affected by female sex hormones and exhibit body types that feature subcutaneous obesity (*28*, *29*). Thus, in assessment by BMI and WC, the effects of subcutaneous fat are more intensely reflected in women than in men. This fact is supported by the stronger correlation of BMI and WC to SFA than to VFA in the study of outpatient cases (Study 1).

We could not find a significant association between FPG and a rise in VFD or WC for either men or women. The reasons are threefold. First, individuals on medication for type 2 diabetes were excluded in this study and, second, the study was conducted in a homogenous population with a relatively low FPG. Third, we could not find participants with impaired glucose tolerance (IGT) because we did not conduct oral glucose tolerance test (OGTT) in this study. Thus, there was a small number of participants with high FPG and there was no significant relationship between FPG and VFD for either men or women.

The results of multiple regression analysis showed that VFD was an independent explanatory variable of blood pressure in men. No significant relationship was found between WC and blood pressure in men or women. VFD may be a good indicator of blood pressure in men. Moreover, VFD may also be a useful index for the management of blood pressure in men with metabolic syndrome.

In a state of visceral fat accumulation, it is thought that free fatty acid produced by the decomposition of TG flows into the liver and induces insulin resistance. Moreover, substances that induce insulin resistance such as tumor necrosis factor (TNF)-α are produced from visceral fat. Studies have indicated the possibility that elevation of blood pressure is induced in a state of insulin resistance by various mechanisms *via* adipocytokines (*30*). It has also been reported that compensatory hyperinsulinemia, which occurs in a state of insulin

resistance, plays a role in blood pressure elevation *via* renal mechanisms (*31*).

In multiple regression analysis, no relationship was found between VFD and blood pressure in women. Possible reasons for this are the influence of an autocorrelation due to the addition of BMI to the adjusted items and both the small mean value and the low distribution of VFD in female subjects.

WC measurement is a very useful screening method for assessing visceral fat because it is simple and cheap. It does, however, have drawbacks, such as an inability to assess tall individuals differently than short ones and a low level of reproducibility in the case of marked obesity, since WC includes subcutaneous fat. Therefore, the Japanese criteria of MS recommend assessing real visceral fat accumulation by CT when we find individuals with WC ≥85 cm in men and ≥90 cm in women. Although abdominal CT is the gold standard for visceral fat assessment, it entails exposure to radiation, lack of ease and simplicity, and high cost. General practitioners may have a good deal of opportunity to assess individuals with MS, but very few physicians have CT equipment in their clinics. Assessment by US is a simpler technique than abdominal CT and allows general practitioners to assess visceral fat accumulation in their clinics. When we find high-risk individuals with an accumulation of risk factors and without abdominal obesity (WC <85 cm in men, WC <90 cm in women), it is important to confirm their fat distribution by other methods than WC. In such cases, the US method may be useful simply assessing the accumulation of visceral fat.

One limitation of the present study is that all of the subjects were Japanese; thus the results may not apply to Westerners or individuals of certain ethnic groups. The female body type in particular differs between Westerners and Japanese. Nevertheless, diagnostic criteria for WC that take into account ethnicity have been incorporated in the International Diabetes Federation (IDF)'s diagnostic criteria for MS. While there are differences in extent, the relationship between visceral fat accumulation and cardiovascular disease risk factors is universal (*32*, *33*).

No statistical analysis was performed to evaluate the differences in the measured parameters between premenopausal and postmenopausal women in our study group. In general, postmenopausal women tend toward obesity more than premenopausal women, and their blood pressure levels and visceral fat levels are known to increase (*34*, *35*). A study taking this into account is needed in the future. Additionally, the present study involved cross-sectional studies, and additional prospective studies on the relationship between abdominal obesity and elevated blood pressure are needed.

In conclusion, US is a simpler technique than abdominal CT, and its usefulness in visceral fat assessment was demonstrated in the screening of residents of a rural community. VFD is thought to be a good index for assessing not only visceral fat accumulation but also cardiovascular risk factors. Moreover, in men VFD showed a significant correlation with blood pressure. Visceral fat assessment by US may be useful for epidemiological studies and for clinics with no abdominal CT equipment to identify high-risk individuals such as those with metabolic syndrome.

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Original Article

Visceral Obesity in Japanese Patients with Metabolic Syndrome: Reappraisal of Diagnostic Criteria by CT Scan

Mariko EGUCHI[1], Kazufumi TSUCHIHASHI[1], Shigeyuki SAITOH[1], Yoshihiro ODAWARA[2], Tohru HIRANO[2], Tomoaki NAKATA[1], Tetsuji MIURA[1], Nobuyuki URA[1], Masato HAREYAMA[2], and Kazuaki SHIMAMOTO[1]

To reappraise the cutoff level of abdominal circumference (AC) for diagnosis of visceral obesity in Japanese, we examined the association of visceral fat deposition with other constituents of metabolic syndrome and atherosclerotic cardiovascular disease (ASCD). CT was used for determination of visceral-fat area (VFA), subcutaneous-fat area (SFA) and AC on CT (AC_{CT}) in 420 Japanese patients with (n=180) or without ASCD (n=240). VFA cutoff levels were calculated by receiver operating characteristic (ROC) analysis. AC_{CT} correlated with VFA (r=0.828), SFA (r=0.795), and AC measured with an anthropometric tape (AC_M, r=0.96). The VFA cutoff levels yielding the maximum sensitivity and specificity to predict two or more components of metabolic syndrome were 92 cm^2 in males and 63 cm^2 in females, which correspond to AC_M values of 83 cm and 78 cm, respectively. The male AC_M cutoff level was similar to the AC in current Japanese criteria (85 cm), but the female AC_M cutoff level was considerably smaller than the criteria, and this change in cutoff level increased the prevalence of metabolic syndrome in females three-fold. The cutoff levels of VFA for predicting presence of ASCD were 98 cm^2 in males and 75 cm^2 in females, corresponding to AC_M values of 84 cm and 80 cm, respectively. The present results obtained by CT support the validity of the current Japanese criteria for visceral obesity in males but not in females. AC_M of 78 cm appears to be a cutoff level suitable for diagnosing visceral obesity in Japanese females, though further confirmation is needed. (*Hypertens Res* 2007; 30: 315–323)

***Key Words*: metabolic syndrome, coronary arterial disease, visceral obesity, aging**

Introduction

Clustering of major risk factors (hypertension, diabetes mellitus, and hyper-lipidemia) has been shown to have synergistic effects on the development of atherosclerotic cardiovascular disease (ASCD) (*1*, *2*). The contribution of clustered minor risk factors for ASCD has also received attention recently, and attractive clinical concepts (*3–6*) emerged in the 1980s: metabolic syndrome X, insulin resistance syndrome, visceral fat syndrome, and multiple risk factor syndrome. Currently, the cluster of minor metabolic factors for ASCD is referred to as metabolic syndrome, and consists of visceral obesity, glucose intolerance or insulin resistance, dyslipidemia, and raised blood pressure. However, several definitions of metabolic syndrome, which differ in their required combinations of risk factors and cutoff levels for each factor, have been proposed (*7–9*).

One of the marked differences among the current diagnostic criteria of metabolic syndrome is the method used to assess visceral obesity and its requirement for diagnosis. In the definition of metabolic syndrome by the National Choles-

From the [1]Second Department of Internal Medicine and [2]Department of Radiology, Sapporo Medical University School of Medicine, Sapporo, Japan.
Address for Reprints: Mariko Eguchi, M.D., Ph.D., Second Department of Internal Medicine, Sapporo Medical University School of Medicine, S-1, W-16, Chuo-ku, Sapporo 060–0061, Japan. E-mail: eguchi@sapmed.ac.jp
Received August 3, 2006; Accepted in revised form December 12, 2006.

Table 1. Clinical Backgrounds in Studied Subjects

	All (n=420)	Male (n=235)	Female (n=185)
Age (years old)	62±15	63±14	61±17
Gender [male/female]	235/185		
Risk factors (n (%))			
Hypertension	275 (66)	163 (69)	112 (61)
Diabetes mellitis	141 (34)	84 (36)	57 (31)
Hyperlipidemia	297 (71)	167 (71)	130 (70)
Hyperuricemia	132 (32)	93 (40)	39 (21)*
Smoking	174 (42)	143 (63)	31 (17)*
Family history	65 (16)	34 (15)	31 (17)
Weight (kg)	60±14	65±14	53±11*
BMI (kg/m^2)	23±4	23±4	22±4*
Systolic blood pressure (mmHg)	134±21	135±20	133±20
Diastolic blood pressure (mmHg)	77±13	77±13	76±13
Major disease (n (%))			
Coronary heart disease	122 (29)	88 (37)	34 (18)
Cardiomyopathy	33 (8)	19 (8)	14 (8)
Valvular disease	40 (10)	15 (6)	25 (14)
Aortic disease	41 (10)	27 (11)	14 (8)
Arrhythmia	61 (15)	38 (16)	23 (12)
Renal disease	56 (13)	27 (11)	29 (16)
Stroke	12 (3)	7 (3)	5 (3)
Others	54 (17)	14 (6)	40 (22)
Medication (n (%))			
Antihypertensive agents	241 (57)	149 (63)	92 (50)*
Antihyperlipidemia agents	112 (26)	49 (21)	63 (34)*
Antidiabetic agents	81 (19)	45 (19)	36 (20)

All the variables are expressed as mean±1 SD. *$p<0.05$ *vs.* male group, respectively.

terol Education Program Adult Treatment Panel III (NCEP ATP III) (*7*), visceral obesity is not a requisite. However, visceral obesity needs to be present in metabolic syndrome as defined by the International Diabetes Federation (IDF 2005) (*8*) and the Examination Committee of Criteria for Metabolic Syndrome in Japan (Japanese criteria) (*9*). In these definitions, visceral obesity is assessed by abdominal (waist) circumference, but its cutoff level is not the same: abdominal circumferences (ACs) are ≥102 cm in males and ≥88 cm in females in the NCEP ATP III criteria, ≥85–94 cm in males and ≥80–90 cm in females, depending on ethnic groups, in the IDF criteria, and ≥85 cm in males or ≥90 cm in females in the Japanese criteria. These differences in diagnostic criteria of visceral obesity derive from different rationales in each subject population.

In the present study, we used multi-detector-row CT (MDCT) to reappraise visceral obesity criteria for the diagnosis of metabolic syndrome and screening of ASCD in Japanese subjects. Since visceral fat, but not subcutaneous fat, is primarily responsible for the production of cytokines relevant to the development of metabolic syndrome (*10*, *11*), the amounts of visceral and subcutaneous fat were separately determined by MDCT together with AC. The relationships between the amount of visceral fat and metabolic syndrome or ASCD were analyzed by use of receiver operating characteristic (ROC) curves, and the results suggest that the current Japanese criterion of visceral obesity in males (AC=85 cm) is valid but that the criterion for females needs to be modified possibly to AC of 78 cm.

Methods

Study Subjects

We enrolled 420 consecutive patients who underwent MDCT at Sapporo Medical University Hospital between January 2001 and December 2003 (Table 1). Informed consent for use of their data in scientific research was obtained from all study subjects. Data from each subject were saved in anonymous formats and securely stored in a computer. Information on coronary risk factors, including data on the blood pressure category, serum triglyceride and high-density lipoprotein (HDL) cholesterol levels and presence/absence of ASCD, was obtained by physical and laboratory examinations. Unless otherwise stated, metabolic syndrome was diagnosed in accordance with the current Japanese criteria (*10*), which