全に対する有用な治療選択としてLVASと心臓移植は受け入れられるようになってきた．最近では，体外設置型東洋紡製左室脱血方式による3年11ヵ月の補助後に心臓移植がおこなわれ，退院した症例も経験されるようになった．しかし，体外設置型LVAS装着患者のQOL (quality of life) の改善が課題であり，小型駆動装置の臨床への導入が必要であり，さらに在宅治療の検討も今後の課題である．また，体格の小さな患者に装着できる埋込み型LVASが必要であり，小型化が可能な無拍動流型LVASの開発が積極的に進められており，欧米では数種類の臨床治験がおこなわれており，わが国で開発された2種のLVASがわが国内で用いられるようになることが望まれる．

また，LVASによる長期生存が可能となるにしたがい，心臓移植の代替治療選択としてLVASによるdestination therapyの検討がおこなわれ，米国では心臓移植の対象とならない重症心不全患者についての検討により，薬物療法よりHeartmate-VEの有用性が示され，destination therapyとして認可された[5] (図❷)．わが国においても検討を開始する時期にきていると考える．

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NAKATANI Takeshi

国立循環器病センター臓器移植部長
なかたに・たけし
1951年，大阪府生まれ．
1969年，府立三国ヶ丘高校卒業．
1976年，神戸大学医学部卒業．
大阪大学医学部第一外科入局．
1982年，国立循環器病センター研究所人工臓器部研究員．
1986年，米国テキサス心臓研究所リサーチフェロー．
1999年，国立循環器病センター研究所生体工学部研究室長（心臓血管外科併任）．
国立循環器病センター研究所実験治療開発部長．
2000年，国立循環器病センター臓器移植部長．
専門・研究分野：心臓血管外科，人工臓器（補助人工心臓，補助循環），心臓移植・組織・細胞移植，再生治療．
趣味：音楽鑑賞．
E-mail：tnakatan@hsp.ncvc.go.jp

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Sex Differences in Early Mortality of Patients Undergoing Primary Stenting for Acute Myocardial Infarction

Masami Kosuge, MD; Kazuo Kimura, MD; Sunao Kojima, MD*; Tomohiro Sakamoto, MD*; Masaharu Ishihara, MD**; Yujiro Asada, MD†; Chuwa Tei, MD††; Shunichi Miyazaki, MD‡; Masahiro Sonoda, MD‡‡; Kazufumi Tsuchihashi, MD§; Masakazu Yamagishi, MD‡; Yoshihiko Ikeda, MD§§; Mutsunori Shirai, MD***; Hisatoyo Hiraoka, MD†††; Takeshi Inoue, MD‡‡‡; Fumio Saito, MD§§§; Hisao Ogawa, MD*; on behalf of the Japanese Acute Coronary Syndrome Study (JACSS) Investigators

Background Limited information exists regarding the impact of gender on in-hospital outcome after primary stenting for acute myocardial infarction (AMI).

Methods and Results A total of 2,981 patients (790 women and 2,191 men) participated in the study who were admitted within 24 h after symptom onset and underwent emergency primary stenting for AMI. Compared with men, women were significantly older; had higher incidences of hypertension, diabetes mellitus, hyperlipidemia, Killip class ≥2, and cardiogenic shock; had a higher blood glucose level and a lower serum creatinine level on admission. Other baseline characteristics, including the incidences of ST-segment elevation AMI, anterior infarction, 3-vessel disease, initial or final Thrombolysis in Myocardial Infarction (TIMI) flow grade did not significantly differ between the sexes. The in-hospital mortality rate was significantly higher in women than in men (9.4% vs 5.2%, p<0.001). On multivariate analysis, age, Killip class, blood glucose level, serum creatinine level, and final TIMI grade were independent predictors of in-hospital death, but female gender was not (odds ratio 1.01, p=0.69).

Conclusions Our findings suggest that in patients undergoing primary stenting for AMI, women have higher in-hospital mortality than men, but female gender itself is not independently associated with increased in-hospital mortality after adjustment for baseline differences. (*Circ J* 2006; **70:** 217–221)

Key Words: Myocardial infarction; Stents; Survial; Women

A number of studies have addressed sex-related differences in outcomes in patients with acute myocardial infarction (AMI). A few studies have reported similar or lower mortality rates after AMI in women than in men[1–4] but most have concluded that mortality is higher in women irrespective of reperfusion modality.[5–12] The reasons for poorer outcomes in women remain unclear. Increased mortality in women might be partially explained by their higher age at presentation and higher risk profiles. Some[5–8] but not all[9–12] studies have shown that female gender itself is independently associated with increased mortality after adjustment for baseline differences. One potential explanation for the persistence of increased mortality after risk adjustment is that women frequently receive less aggressive treatment for AMI than men.[7,13,14] Primary balloon angioplasty, compared with thrombolytic therapy, has been shown to improve outcomes for women, but mortality remains high.[2,10] Limited information exists regarding the impact of sex on outcomes after contemporary interventional techniques, such as stent implantation, for AMI. We therefore analyzed a database from a large, retrospective, multicenter observational study of patients with AMI who underwent emergency primary stenting to assess the outcomes of women compared with those of men.

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*Division of Cardiology, Yokohama City University Medical Center, Yokohama, *Department of Cardiovascular Medicine, Kumamoto University School of Medicine, Kumamoto, **Department of Cardiology, Hiroshima City Hospital, Hiroshima, †First Department of Pathology, Miyazaki University Medical School, Miyazaki, ††The First Department of Internal Medicine, Kagoshima University Faculty of Medicine, Kagoshima, ‡Division of Cardiology, Department of Internal Medicine, National Cardiovascular Center, Suita, ‡‡The Second Department of Cardiology, National Hospital Kyusyu Cardiovascular Center, Kagoshima, §Second Department of Internal Medicine, Sapporo Medical University School of Medicine, Sapporo, §§Department of Pathology, National Cardiovascular Center, Suita, ***Department of Microbiology, School of Medicine, Yamaguchi University, Ube, †††Department of Internal Medicine and Molecular Science, Graduate School of Medicine, Osaka University, Suita, ‡‡‡Division of Cardiology, Oita National Hospital, Oita and §§§Department of Cardiology, Nihon University Surugadai Hospital, Tokyo, Japan*

Mailing address: Kazuo Kimura, MD, The Division of Cardiology, Yokohama City University Medical Center, 4-57 Urafune-cho, Minami-ku, Yokohama 232-0024, Japan. E-mail: c-kimura@urahp.yokohama-cu.ac.jp

Methods

Patients

The patients included in the current study were selected from those enrolled into the Japan Acute Coronary Syndrome Study,[15] a retrospective, observational multicenter trial. Between January 2001 and December 2003, patients who were admitted to 35 participating hospitals in Japan within 48 h after the onset of AMI were studied. A diagnosis of AMI required at least 2 of the following characteristics: typical chest pain persisting for 30 min or longer,

Table 1 Baseline Characteristics

	Women (n=790)	Men (n=2,191)	p value
Age (years)	73±10	65±11	<0.001
Body surface area (m^2)	1.47±0.14	1.71±0.16	<0.001
Time from symptom onset to admission (h)	4.9±5.0	4.5±5.0	0.09
Killip ≥2 on admission	173 (22%)	331 (15%)	<0.001
Cardiogenic shock on admission	68 (9%)	142 (7%)	0.045
Previous infarction	78 (10%)	245 (11%)	0.31
Previous angina	308 (39%)	799 (37%)	0.21
Diabetes mellitus	273 (35%)	672 (31%)	0.044
Hyperlipidemia	293 (37%)	716 (33%)	0.025
Hypertension	506 (64%)	1,136 (52%)	<0.001
Smoking	131 (17%)	1,248 (57%)	<0.001
Blood glucose level on admission (mmol/L)	10.7±5.1	9.8±4.2	<0.001
Serum creatinine on admission (mg/dl)	0.8±0.7	1.0±0.9	<0.001
ST-segment elevation	688 (87%)	1,930 (88%)	0.46
Peak creatine kinase (IU/L)	2,829±2,676	3,418±3,387	<0.001

Data are presented as mean values±SD or percentages of patients.

Table 2 Angiographic Findings

	Women (n=790)	Men (n=2,191)	p value
Number of diseased vessels			0.97
1	445 (57%)	1,246 (57%)	
2	240 (30%)	657 (30%)	
3	105 (13%)	288 (13%)	
Infarct-related artery			0.29
LAD	384 (49%)	1,024 (47%)	
RCA	283 (36%)	840 (38%)	
LCX	107 (13%)	287 (13%)	
LMT	16 (2%)	33 (2%)	
Bypass graft	0	7 (0.3%)	
TIMI flow grade 0 at initial CAG	512 (65%)	1,456 (67%)	0.40
Final TIMI flow grade ≥2	758 (96%)	2,090 (95%)	0.51
Final TIMI flow grade 3	714 (90%)	1,967 (87%)	0.63

Data are presented percentages of patients.
LAD, left anterior descending artery; RCA, right coronary artery; LCX, left circumflex coronary artery; LMT, left main trunk; TIMI, Thrombolysis in Myocardial Infarction.

ischemic electrocardiographic changes, and a peak creatine kinase level equivalent to more than twice the upper limit of normal. A total of 4,432 patients fulfilled the following criteria: 1 admission within 24 h after the onset of AMI; 2 coronary angiography performed immediately after admission; and 3 availability of a detailed clinical history. Reperfusion therapy was performed in 3,635 patients (82%). Of these 3,635 patients, we studied 2,981 (82%) who underwent stenting of the infarct-related artery. The study protocol was reviewed and approved by the ethical committee of each participating hospital.

Definitions

Diabetes mellitus was defined as a fasting glucose concentration of ≥7.0 mmol/L, a blood glucose concentration of ≥11.0 mmol/L on a 75-g, 2-h oral glucose tolerance test, or the use of anti-diabetic treatment. Hypertension was defined as a history of a systolic blood pressure of ≥140 mmHg, a diastolic pressure of ≥90 mmHg, or the use of anti-hypertensive treatment. Hyperlipidemia was defined as a fasting total cholesterol concentration of ≥220 mg/dl, a fasting triglyceride concentration of ≥150 mg/dl, or the use of anti-hyperlipidemic treatment. Preinfarction angina was defined as the presence of typical chest pain occurring at rest or during exercise and persisting for less than 30 min, within 24 h before the onset of AMI.

Coronary Angiography and Coronary Intervention

Coronary angiography was performed immediately after admission. The perfusion status of the infarct-related artery was assessed according to the Thrombolysis in Myocardial Infarction (TIMI) study classification[16] The recanalization method was left to the physicians' discretion. Stenting was done in all patients in whom the procedure was feasible. Final TIMI flow grade was assessed on the basis of the final angiograms obtained at admission.

Statistical Analysis

Data are expressed as means±SD. Patients with and those without preinfarction angina were compared by using unpaired t-tests. Differences in prevalence were assessed by chi-square tests. A probability value of <0.05 was considered to indicate a statistically significant difference. Multiple logistic regression analysis was used to examine determinants of in-hospital mortality. Analyzed variables included age, sex, hypertension, diabetes mellitus, prior infarction, preinfarction angina, body surface area, time to admission, Killip class, infarct location, blood glucose level and serum creatinine level on admission, occlusion status at the culprit lesion, number of diseased vessels, and final TIMI flow grade. Odds ratios and 95% confidence intervals were calculated. Data were analyzed with the use of SPSS software (Release 10, SPSS, Chicago, IL, USA).

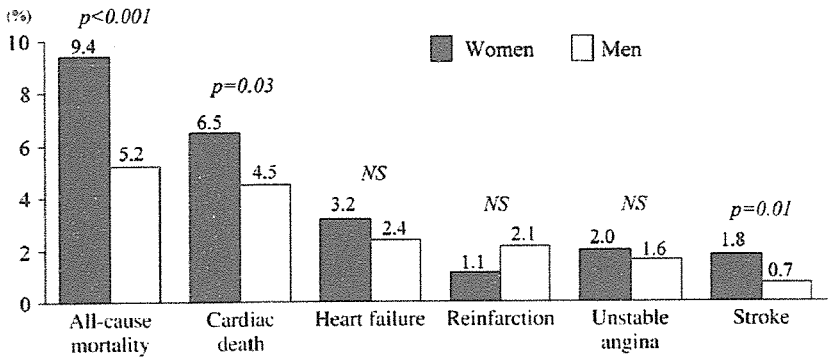

Fig 1. Overall, in-hospital mortality was significantly higher in women (black bars) than in men (white bars). The rates of cardiac death and stroke were also significantly higher in women than in men.

Results

Baseline Characteristics

Of the 2,981 subjects, 2,191 (73%) were men and 790 (27%) were women. The baseline characteristics of the subjects are presented according to sex in Table 1. Women were more likely to be elderly and had a lower body surface area; higher incidences of hyperlipidemia, diabetes mellitus, hypertension, Killip class ≥2, and cardiogenic shock; and a lower frequency of smoking. There was a trend toward a longer time from symptom onset to admission in women, but the difference with men did not reach statistical difference. The frequency of previous infarction, preinfarction angina, and ST-segment elevation did not differ between the sexes. On admission the blood glucose level was significantly higher and the peak creatine kinase level and serum creatinine level were significantly lower in women than in men.

Angiographic Findings

Angiographic findings of the patients are shown in Table 2. There were no significant differences between women and men in the number or distribution of diseased coronary vessels, including 3-vessel disease and left main coronary disease. The initial and final TIMI flow grades did not differ between the sexes.

In-Hospital Outcomes

Clinical outcomes in hospital are shown in Fig 1. In-hospital mortality was significantly higher in women than men. Of the patients who died in the hospital (74 women and 115 men), 51 women and 98 men died of cardiac causes. Women had a higher rate of stroke. Multivariate analysis showed that age, Killip class, blood glucose level or serum creatinine level on admission, and final TIMI grade were independent predictors of in-hospital death, whereas female gender was not (Table 3). Multivariate analysis according to sex showed that age, Killip class, blood glucose level or serum creatinine level on admission,

Table 3 Multivariate Analysis of Factors Associated With In-Hospital Mortality in All Patients

Variable	*Odds ratio (95%CI)*	*p value*
Age	*1.08 (1.04–1.12)*	*<0.001*
Female	*1.01 (0.47–2.04)*	*0.69*
Hypertension	*1.09 (0.42–1.29)*	*0.29*
Diabetes mellitus	*1.04 (0.54–2.02)*	*0.40*
Prior infarction	*1.81 (0.92–3.56)*	*0.09*
Absence of preinfarction angina	*1.55 (0.87–2.76)*	*0.14*
Body surface area	*0.68 (0.43–1.06)*	*0.09*
Time to admission	*1.03 (0.98–1.09)*	*0.23*
Killip class ≥2	*6.43 (3.67–11.3)*	*<0.001*
Anterior infarction	*1.56 (0.89–2.71)*	*0.11*
Blood glucose level on admission	*1.01 (1.01–1.30)*	*0.049*
Serum creatinine level on admission	*1.45 (1.24–1.69)*	*<0.001*
TIMI flow grade 0 at initial CAG	*1.21 (0.68–2.16)*	*0.51*
Multivessel disease	*1.31 (0.74–2.33)*	*0.30*
Final TIMI flow grade	*0.60 (0.41–0.85)*	*0.005*

CI, confidence interval; CAG, coronary angiography; TIMI, Thrombolysis in Myocardial Infarction.

Table 4 Multivariate Analysis of Factors Associated With In-Hospital Mortality According to Sex

Variable	*Women*		*Men*	
	Odds ratio (95%CI)	*p*	*Odds ratio (95%CI)*	*p*
Age	*1.11 (1.03–1.19)*	*0.004*	*1.07 (1.03–1.11)*	*0.001*
Hypertension	*1.09 (0.39–3.03)*	*0.86*	*0.62 (0.31–1.26)*	*0.19*
Diabetes mellitus	*1.03 (0.30–2.91)*	*0.91*	*1.10 (0.47–2.58)*	*0.82*
Prior infarction	*0.95 (0.22–4.19)*	*0.95*	*2.33 (1.06–5.16)*	*0.036*
Absence of preinfarction angina	*1.51 (0.24–1.83)*	*0.42*	*1.52 (0.74–3.17)*	*0.26*
Body surface area	*0.80 (0.06–13.3)*	*0.59*	*0.19 (0.01–2.50)*	*0.21*
Time to admission	*1.08 (1.01–1.17)*	*0.037*	*0.99 (0.91–1.07)*	*0.76*
Killip class ≥2	*5.90 (2.24–15.6)*	*<0.001*	*6.60 (3.24–13.4)*	*<0.001*
Anterior infarction	*1.74 (0.64–4.76)*	*0.28*	*1.68 (0.83–3.38)*	*0.15*
Blood glucose level on admission	*1.17 (1.01–20.6)*	*0.038*	*1.20 (1.05–23.7)*	*0.046*
Serum creatinine level on admission	*1.59 (1.15–11.3)*	*0.005*	*1.42 (1.17–1.71)*	*<0.001*
TIMI flow grade 0 at initial CAG	*0.95 (0.36–2.53)*	*0.91*	*1.36 (0.64–2.86)*	*0.42*
Multivessel disease	*1.05 (0.41–2.65)*	*0.94*	*1.51 (0.72–3.20)*	*0.28*
Final TIMI flow grade	*0.46 (0.23–0.90)*	*0.024*	*0.62 (0.40–0.97)*	*0.037*

CI, confidence interval; CAG, coronary angiography; TIMI, Thrombolysis in Myocardial Infarction.

and final TIMI grade were independent predictors of in-hospital death in both women and men (Table 4).

Discussion

The present study showed that in patients undergoing primary stenting for AMI, women had a higher rate of in-hospital mortality than men did. However, multivariate analysis showed that female gender itself was not an independent predictor of in-hospital mortality.

Similar to previous studies[1–11] women were older than men by 8 years on average and had a lower body surface area, a higher Killip class, and higher incidences of hypertension, diabetes mellitus, and hyperlipidemia. On coronary angiography, the extent of underlying coronary atherosclerosis, evaluated on the basis of the number of diseased vessels, did not differ between women and men, consistent with the findings of previous investigations[2,5,10,12] The current study also found that baseline and post-procedural TIMI flow grades were similar in women and men. Several studies have similarly shown that the TIMI flow grade before and after primary angioplasty for AMI did not differ between women and men[2,10,17] In contrast, Lansky et al found that baseline and post-procedural TIMI grade 3 flows were better in women than in men[11]

Mortality was higher in women than in men, consistent with the results of most previous studies[5–12] This poorer outcome in women was most likely related to the facts that women were older than men, had higher incidences of coronary risk factors such as hyperlipidemia, diabetes mellitus, and hypertension, as well as higher incidences of Killip class ≥2, and cardiogenic shock. These and other factors such as a higher blood glucose level on admission might have negatively affected outcome. Several studies have shown that woman have poorer outcomes than men;[9–11,14] however, after adjustment for other baseline characteristics, female sex itself was not an independent risk factor for increased mortality. Those studies therefore concluded that a higher age or more adverse risk profiles in women contributed to the poorer outcomes. However, several studies reported that female gender itself was an independent predictor of increased mortality, even after adjusting for other baseline characteristics[5–8] These inconsistent results suggest that methodological, as well as biologic factors, must be considered when interpreting the impact of gender on survival after AMI.

Women have been reported to be significantly less likely to receive thrombolytic therapy, percutaneous transluminal coronary angioplasty (PTCA), and coronary artery bypass grafting[6,7,13] The less frequent use of reperfusion therapy in women might be related to their higher mortality. Furthermore, randomized studies have shown that percutaneous coronary interventions are more effective reperfusion strategies than intravenous thrombolysis[18] In the GUSTO II-B PTCA substudy, Tamis-Holland et al suggested that women derived a larger absolute benefit from primary PTCA than from thrombolytic therapy as compared with men[10] Stone et al reported that primary PTCA reduced the risk of intracranial bleeding and improved survival in women enrolled in the PAMI trial[2] In the present study, all patients underwent primary stenting, thereby eliminating possible differences in mortality caused by treatment bias.

Recent studies have investigated the influence of sex on outcome in patients receiving primary coronary intervention for AMI, but whether female gender itself is an independent predictor of increased mortality after AMI remains controversial. Vakili et al reported that after correcting for age and baseline risk differences, women undergoing primary coronary intervention for a first AMI have a higher in-hospital mortality rate than men[5] Lansky et al have shown that female gender is not an independent determinant of death at 1 year and attributed the higher mortality rate in women after interventional treatment for AMI to differences in body size and clinical risk factors[11] Mehilli et al showed that women with AMI who received percutaneous coronary intervention have outcomes similar to those of men, despite more adverse risk profiles[3] Differences in study design, entry criteria, and length of follow-up make it difficult to compare results in different studies. The present study included high-risk patients who are usually excluded from clinical trials, such as those who are elderly and have shock, as well as a high proportion of patients with diabetes mellitus (31%). In addition, we included the blood glucose level and serum creatinine level on admission in our risk analysis. These factors have been shown to be associated with an increased risk of death after AMI[8,19–22] but were not always included in risk analysis in previous studies assessing the impact of sex on outcomes after AMI.

Study Limitations

Several important limitations need to be considered when interpreting our results. First, this was a retrospective, observational, non-randomized study. However, our database was relatively large and included patients treated at hospitals of various sizes and settings, making it more representative of current practice patterns than previous single-site databases or randomized trials. Second, our data did not include information on treatment with aspirin, statin, β-blockers, angiotensin converting enzyme inhibitors and angiotensin II receptor antagonists or on the door-to-balloon time, all factors shown to influence mortality from AMI. Furthermore, angiographic data such as the number of stenosed lesions, lesion length, and reference diameter were not obtained. However, in the current study, body surface area, a surrogate for coronary vessel size, was lower in women than in men. Another limitation was that cardiac and non-cardiac causes of death were not fully examined. Further investigations are necessary to determine why women with AMI are more likely to have poorer outcomes compared with men. In addition, prospective studies are needed to verify the effects of gender on clinical outcomes after primary stenting for AMI.

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Circadian Variation of Endothelial Function in Idiopathic Dilated Cardiomyopathy

Takeshi Maruo, MD[b], Satoshi Nakatani, MD, PhD[a,*], Hideaki Kanzaki, MD[a], Hiroyuki Kakuchi, MD[a], Masakazu Yamagishi, MD, PhD[a], Masafumi Kitakaze, MD, PhD[a], Tohru Ohe, MD, PhD[b], and Kunio Miyatake, MD, PhD[a]

This study measured flow-mediated dilation (FMD) of the brachial artery 3 times a day (6:30 A.M., 11:30 A.M., and 9 P.M.) in 7 normal subjects and 14 patients with idiopathic dilated cardiomyopathy (7 in New York Heart Association [NYHA] functional class I or II and 7 in NYHA functional class III or IV). FMD in normal subjects and patients in NYHA class I or II showed a circadian variation, being lowest in the morning and highest at night. Compared with them, FMD in patients in NYHA class III or IV was lower and almost constant during the day, showing loss of significant circadian variation in endothelial function in patients with congestive heart failure. **(Am J Cardiol 2006;97:699–702)**

Cardiovascular events (stroke, sudden death, and acute myocardial infarction) frequently occur early in the morning.[1–3] The increased incidence of events in the morning can be due to various factors, such as sympathetic activation, hemodynamic changes, neurohumoral factors, and increases in coagulation. Recent studies have suggested that circadian variation, with the attenuation of endothelial function in the morning, might be 1 of the causes and may contribute to the risk for cardiovascular events.[4–8] However, little is known about circadian variation in endothelial function in congestive heart failure. Thus, in the present study, we investigated changes in endothelial function throughout the day in patients with idiopathic dilated cardiomyopathy (IDC) and congestive heart failure.

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We studied 7 normal subjects and 14 patients with IDC in stable condition (Table 1). Seven patients with IDC were in New York Heart Association (NYHA) functional class I or II, and the other 7 patients were in NYHA functional class III or IV. All patients were in sinus rhythm. The diagnosis of IDC was done by cardiac catheterization and right ventricular biopsy.[9] We excluded patients with a history or evidence of ischemic heart disease, hypercholesterolemia, diabetes, habitual drinking, smoking, and chronic renal failure because these alone have influence on endothelial function. All 14 patients received stable doses of heart failure medications, including digitalis, furosemide, angiotensin-converting enzyme inhibitors, β blockers, amiodarone, and angiotensin II receptor blockers. Between the 2 IDC groups, there was no significant difference in the use of cardiovascular drugs.

Endothelial function was noninvasively assessed by flow-mediated dilation (FMD) of the brachial artery in response to reactive hyperemia.[10] To measure FMD, we used a high-resolution ultrasound system equipped with a 10-MHz linear array transducer (Aloka SSD-5500, Aloka, Co., Tokyo, Japan). All images were recorded for subsequent quantitative analysis. Within a 24-hour period, FMD measurement was repeated 3 times, at 6:30 A.M., 11:30 A.M., and 9 P.M.[8] Subjects were asked to rest in a supine position in a temperature-controlled laboratory (22°C to 25°C) for ≥15 minutes. We scanned the brachial artery of the dominant arm in a longitudinal section 5 cm proximal to the antecubital fossa. After recording the baseline brachial artery image, a blood pressure cuff around the forearm was inflated to 200 mm Hg for 5 minutes and released. After cuff deflation, brachial artery images were continuously recorded for ≥90 seconds. FMD was defined as the maximal percentage increase in a vessel diameter during reactive hyperemia. After FMD measurements, nitroglycerin-induced vasodilation was evaluated to assess endothelium-independent dilation. The maximal percentage increase in vessel diameter before and after the sublingual administration of glycerin trinitrate 0.3 mg was determined.[10] This was measured at 6:30 A.M. and 9 P.M. The luminal diameters were measured between the media-adventitia interfaces at the onset of the R wave. The measurements were done by a single cardiologist who was blinded to the clinical information of the study participants. The intraobserver variability (coefficient of variation) for repeated measurements of diameter at baseline and at reactive hyperemia were <3%, in agreement with a previous report.[11]

Other measurements included left ventricular end-diastolic and end-systolic diameters and the ejection fraction, calculated by Teichholz's method. As a sympathovagal tone index, we determined the coefficient of variation of heart rate variability from 5-minute electrocardiograms at night,

[a]Department of Cardiology, National Cardiovascular Center, Osaka; and [b]Department of Cardiovascular Medicine, Okayama University Graduate School of Medicine, Okayama, Japan. Manuscript received June 11, 2005; revised manuscript received and accepted September 9, 2005.

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* Corresponding author: Tel: 81-6-6833-5012; fax: 81-6-6872-7486.
E-mail address: nakatas@hsp.ncvc.go.jp (S. Nakatani).

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Table 1
Patient characteristics

Variable	Normal Subjects (n = 7)	Patients with IDC	
		NYHA Functional Class I or II (n = 7)	NYHA Functional Class III or IV (n = 7)
Age (yrs)	36 ± 2†	46 ± 5	54 ± 4
Men/women	6/0	5/2	6/1
Systolic blood pressure (mm Hg)	120 ± 4*†	104 ± 12	107 ± 14
Diastolic blood pressure (mm Hg)	73 ± 10	62 ± 7	69 ± 12
Heart rate (beats/min)	59 ± 3†	65 ± 3†	83 ± 4
Coefficient of variation of heart rate variability (%)	4.9 ± 0.6†	4.1 ± 0.7†	1.5 ± 0.2
Left ventricular end-diastolic diameter (mm)	45 ± 1*†	71 ± 4	71 ± 5
Ejection fraction (%)	56 ± 4*†	20 ± 9	23 ± 9
Total cholesterol (mg/dl)		171 ± 22	175 ± 19
Atrial natriuretic peptide (pg/ml)		42 ± 12†	219 ± 61
Brain natriuretic peptide (pg/ml)		141 ± 49†	720 ± 322

Data are presented as mean ± SD.
* p <0.05 versus NYHA functional class I to II; †p <0.05 versus NYHA functional class III to IV.

avoiding the morning surge of the sympathetic nervous system. Serum levels of brain natriuretic peptide and atrial natriuretic peptide in the morning were measured in all patients with IDC with highly sensitive and specific immunoradiometric assay kits (Shionogi Co., Ltd., Osaka, Japan).

Data are expressed as mean ± SEM. Circadian variation in FMD was calculated by the formula (maximal FMD − minimal FMD)/minimal FMD × 100 (%). Comparisons of FMD among the different times were made by 1-way analysis of variance for repeated measures, followed by Scheffé's test. Comparisons between 2 groups were analyzed by the Mann-Whitney U statistic test, and those among >2 groups were analyzed by the Tukey-Kramer test. A p value <0.05 was considered statistically significant.

Patients' characteristics are listed in Table 1. In patients in NYHA class III or IV, heart rate was higher, and the coefficient of variation of heart rate variability was smaller compared with normal subjects and patients in NHYA class I or II. Atrial and brain natriuretic peptides in patients in NYHA class III or IV were greater than in patients in NYHA class I or II.

In normal subjects and patients in NYHA class I or II, FMD at 6:30 A.M. was markedly decreased compared with that at 9 P.M. and showed significant circadian variation (78 ± 12% for normal subjects, 126 ± 22% for patients in NYHA class I or II), whereas FMD in patients in NYHA class III or IV was low and relatively constant through the whole day (28 ± 7%, p <0.05 vs normal subjects and patients in NYHA class I or II) (Figure 1).

There were no significant differences in FMD at 6:30 A.M. (normal subjects, 5.3 ± 0.6%; patients in NYHA class I or II, 4.1 ± 0.5%; patients in NYHA class III or IV, 4.4 ± 0.6%; p = NS) among the 3 groups. In contrast, FMD in patients in NYHA class III or IV (4.3 ± 0.6%) was lower at 9 P.M. compared with normal subjects (8.0 ± 1.0%, p = 0.010) and patients in NYHA class I or II (7.7 ± 0.9%, p = 0.025). The coefficient of variation of heart rate variability showed a positive correlation with FMD at 9 P.M. (r = 0.60, p = 0.013; Figure 2). Nitroglycerin-induced vasodilation showed no significant differences between 2 different times in each group (21.3 ± 1.5% vs 19.3 ± 1.9% for normal subjects, 17.9 ± 2.6% vs 14.7 ± 2.9% for patients in

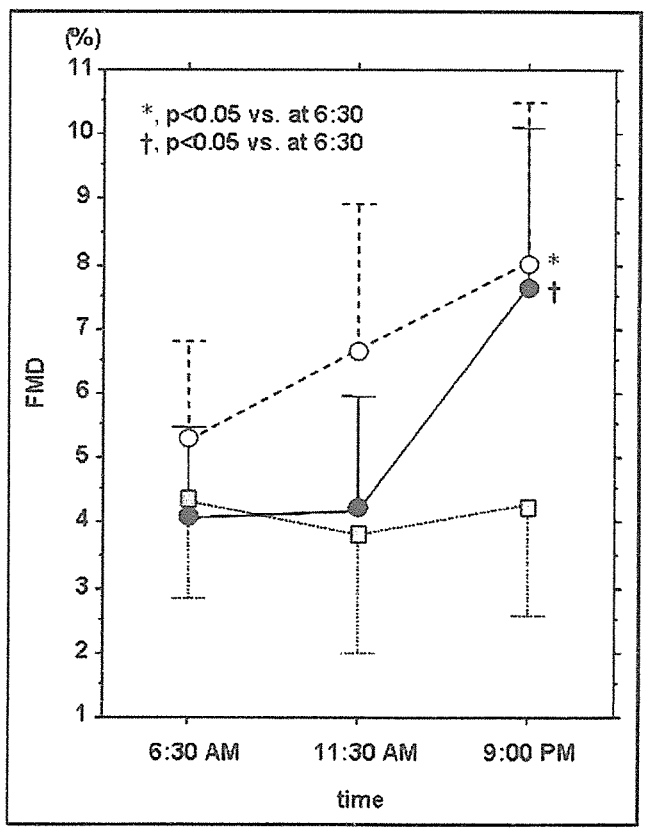

Figure 1. Circadian variation in FMD. In normal subjects *(open circles)* and patients in NYHA class I or II *(filled circles)*, FMD at 6:30 A.M. was markedly decreased compared with that at 9 P.M. and showed the presence of circadian variation (normal subjects, 5.3 ± 0.6% vs 8.0 ± 1.0%, p = 0.021; patients in NYHA class I or II, 4.1 ± 0.5% vs 7.7 ± 0.9%, p = 0.007), whereas FMD in patients in NYHA class III or IV *(squares)* was low and relatively constant throughout the day (4.4 ± 0.6% at 6:30 A.M., 3.8 ± 0.7% at 11:30 A.M., and 4.3 ± 0.6% at 9 P.M., p = NS).

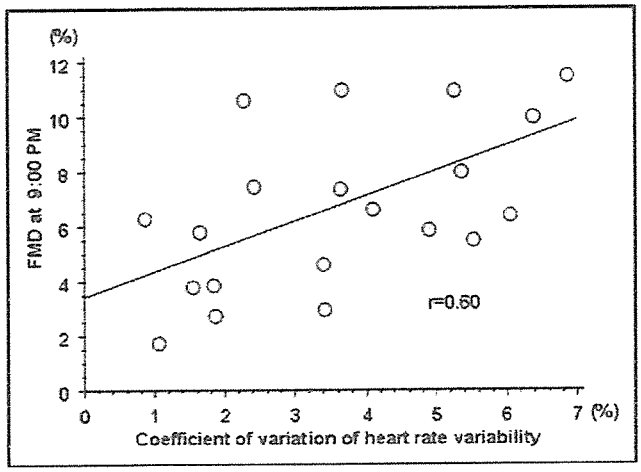

Figure 2. Correlation between FMD at 9 P.M. and the coefficient of variation of heart rate variability. The coefficient of variation showed a positive correlation with FMD at 9 P.M. ($r = 0.60$, $p = 0.013$).

NYHA class I or II, and 20.2 ± 3.9% vs 18.7 ± 1.3% for patients in NYHA class III or IV; all p = NS).

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This is the first study of circadian variation in endothelial function in patients with IDC. We found that FMD in normal subjects and patients in NYHA class I or II was lowest in the morning and highest at night, whereas FMD in patients with severe congestive heart failure was depressed throughout the day. Therefore, circadian variation in endothelial function changed according to the severity of congestive heart failure. Moreover, we suggest that decreased FMD at night is related to the sympathetic nervous accentuation in severe heart failure.

FMD of peripheral arteries has been widely used as a noninvasive and reliable index of endothelial function and has been shown to decrease in patients with congestive heart failure.[12] Several reports have shown that endothelial function has a circadian variation, with morning attenuation in normal subjects.[4,5] This circadian variation in endothelial function may be caused by the interplay of endothelin-1, nitric oxide, and vascular function[5] and might play an important role in the occurrence of acute cardiovascular events at this time. We found that circadian variation, with the morning attenuation of FMD, was maintained in patients with mild congestive heart failure, as in normal subjects.

Endothelial dysfunction may occur early in the course of the disease in congestive heart failure. Thus, the assessment of endothelial function should have important clinical implications for the evaluation of disease status and the prognoses of these patients.[13,14] Depressed FMD indicates the failure of appropriate vascular dilation and may increase left ventricular afterload, decrease organ perfusion, and lead to disease progresson.[13,14] The loss of circadian variation should indicate the loss of the potential protective role of endothelium and contribute to the increased incidence of cardiovascular events in patients with IDC.

The sympathetic nervous system influences endothelial function by increasing constrictors such as α- and β-adrenergic stimulation.[15] In patients with stable or variant angina, endothelial function was impaired in the morning,[6,16] whereas that in patients with acute coronary syndrome showed no diurnal variation.[17] We speculate that endothelial function may be impaired throughout the day, with continuous sympathetic stimulation in patients with severe congestive heart failure. The significant correlation between FMD and heart rate variability could support this speculation.

One of the limitations of our study is that we did not discontinue medications, because of severely reduced left ventricular function and for ethical reasons. Thus, we could not completely eliminate the effects of drugs (e.g., angiotensin-converting enzyme inhibitors and β blockers) on endothelial function.[18,19] Because there was no significant difference in medications between patients with mild and those with severe congestive heart failure, the present result would be little affected by medication. Second, we examined a small number of patients. Despite the small number, the difference in FMD among the 3 groups was very prominent and consistent in each patient. Therefore, we believe that the results would not be altered substantially with more patients, although they should be confirmed in a larger number of patients.

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Determinants of rapid progression of aortic root dilatation and complications in Marfan syndrome☆

Aleksandar M. Lazarevic[a], Satoshi Nakatani[a,*], Yutaka Okita[b], Jelena Marinkovic[c], Yutaka Takeda[a], Keiji Hirooka[a], Hiroshi Matsuo[a], Soichiro Kitamura[b], Masakazu Yamagishi[a], Kunio Miyatake[a]

[a]*Department of Cardiology, National Cardiovascular Center, Suita, Osaka, Japan*
[b]*Department of Cardiothoracic Surgery, National Cardiovascular Center, Suita, Osaka, Japan*
[c]*Cardiovascular Research Center, Dedinje Cardiovascular Institute, Belgrade University Medical School, Belgrade, Serbia and Montenegro*

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Abstract

Background: Progressive aortic dilatation has prognostic significance in the Marfan syndrome.
Methods: To identify which patients were at high risk of rapid progression, we echocardiographically studied 43 patients (age 22 ± 14 years) with the mean follow-up period of 5.2 ± 3.2 years. Aortic diameters, left ventricular (LV) size, fractional shortening, and the severity of aortic and mitral regurgitation were assessed. Transmitral peak early and atrial flow velocities, their ratio and the deceleration time of peak early velocity were also obtained.
Results: Mean annual increases of aortic diameters were 0.4 ± 0.3 mm at the annulus, 1.5 ± 1.3 mm at the sinuses of Valsalva, 0.7 ± 0.6 mm at the supraaortic ridge and 0.4 ± 0.4 mm at the proximal ascending aorta. Patients were divided into 2 groups according to the aortic growth rate at the sinuses of Valsalva level: rapid (R, >3% per year, 15 patients) or slow (S, ≤3% per year, 28 patients) progression groups. Measured variables did not show significant differences between the 2 groups except older age, higher blood pressure and more severe aortic regurgitation in group R. Multiple regression analysis identified prolonged deceleration time as the most important variable predicting aortic complications. Aortic dissection occurred more frequently in group R (7 patients, 47%) than in group S (0%, P<0.001).
Conclusions: Marfan patients at older age, with higher blood pressure, and with significant aortic regurgitation were at high risk of progression of aortic dilatation, with the most remarkable increase at the sinuses of Valsalva. Prolonged deceleration time may relate to an increased risk for aortic complications.

Keywords: Marfan syndrome; Echocardiography; Aortic dissection; LV function

1. Introduction

One of the most serious clinical manifestations of the Marfan syndrome is progressive aortic root dilatation which may lead to aortic dissection, rupture or regurgitation that are responsible for decreased life expectancy in these patients [1–4]. Although aortic root dilatation is observed in 60–80% of Marfan patients [5–7], aortic growth rate among individuals may vary [8] and factors predicting the rate of change of the aortic diameter are still not completely

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* Corresponding author. Department of Cardiology National Cardiovascular Center 5-7-1 Fujishiro-dai, Suita Osaka 565-8565, Japan. Tel.: +81 6 6833 5012; fax: +81 6 6872 7486.
E-mail address: nakatas@hsp.ncvc.go.jp (S. Nakatani).

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clarified. It would be clinically important to define which patients are at high risk of rapid progression.

Several factors have been reported to relate to aortic complications in the Marfan syndrome including aortic size, systolic blood pressure and aortic growth rate [8]. However, determinants of aortic growth rate remains elusive. Because the abnormality in extracellular connective tissue matrix in the Marfan syndrome [9] involves not only aorta but also heart muscle [10], left ventricular (LV) systolic and diastolic function may have some influence on aortic growth rate and complications. In the present study, we investigated determinants of rapid progression of aortic root dilatation and aortic complications with a special interest of the effect of LV function on them. Further, we clarified characteristics of patients who might have poor prognosis.

2. Materials and methods

2.1. Study population

We studied retrospectively 114 consecutive patients with the Marfan syndrome or relatives of patients, who underwent clinical and echocardiographic examinations. Inclusion criteria were: (1) positive strict, internationally established diagnostic criteria [11,12] for the Marfan syndrome, (2) echocardiographic follow-up ≥ 1 year (2-dimensional echocardiography and Doppler color flow mapping), and (3) no proximal aortic surgery before the initial examination. The final study group included 43 patients (age 22 ± 14 years [range 1–59 years], 23 males), with the mean follow-up period of 5.2 ± 3.2 years (range 1–10 years).

2.2. Echocardiography

Two-dimensional, M-mode and Doppler echocardiograms were obtained with a commercially available cardiac ultrasound system, using a 2.5-MHz transducer and stored on videotape and/or strip chart for later analysis. Aortic root measurements were made in 2-dimensional parasternal long-axis view at end-diastole at 4 levels: aortic annulus, sinuses of Valsalva, supraaortic ridge and proximal ascending aorta 1–2 cm above the supraaortic ridge according to the method of Roman et al. (Fig. 1) [13]. All measurements were made using the leading edge technique on up to 5 cycles and averaged. The echocardiographic evidence of aortic root dilatation was determined using Roman's nomograms based on age and body surface area [13]. Dimensions of the sinuses of Valsalva and supraaortic ridge were used to determine an aortic ratio as follows:

$$\text{Aortic ratio} = \frac{\text{measured diameter}}{\text{expected diameter}}$$

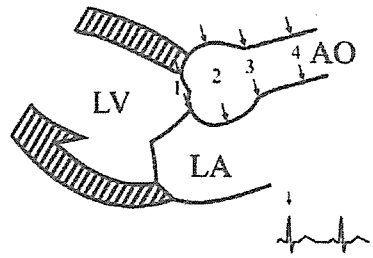

Fig. 1. Schematic illustration of the long-axis two-dimensional echocardiogram. Aortic root measurements were obtained at 4 levels, including; 1) the annulus, 2) sinuses of Valsalva, 3) supraaortic ridge and 4) proximal ascending aorta. AO=aorta; LA=left atrium; LV=left ventricle.

where the expected diameter was obtained using the regression equation derived from Roman's nomograms [13]. The mean annual rate of aortic root dilatation was determined by dividing the absolute change in aortic diameter between the final and initial echocardiograms by the duration of follow-up in years. We also assessed the annual change in the aortic ratio by dividing the change in aortic ratio between the final and initial echocardiograms by the time interval between them. The severity of aortic regurgitation and mitral regurgitation was graded semi-quantitatively (1–4) using color Doppler jet area criteria [14–16]. Echocardiographic evidence of mitral valve prolapse was evaluated using established echocardiographic criteria [17]. LV end-diastolic and end-systolic diameters, fractional shortening, and diastolic interventricular septum and LV posterior wall thicknesses were determined according to the recommendations of the American Society of Echocardiography [18]. Standard pulsed wave Doppler flow velocities measurements were obtained from the apical long-axis view, with the sample volume placed at the level of the mitral valve leaflet tips. The following Doppler indexes were measured: peak early (E) and atrial (A) transmitral filling velocities, E deceleration time and E/A ratio. A single investigator (A.M.L) blinded to the clinical data performed the off-line analysis.

2.3. Statistical analysis

Results are reported as mean value $\pm$ SD. Comparison of data between patients groups was performed by use of a two-tailed, unpaired Student's t test or Mann–Whitney U test. Mean annual increases at the aortic root levels were compared using analysis of variance. The χ^2 test (with continuity correction when applicable) was used to compare frequencies among the patients groups. Multivariate and univariate linear regression analysis was used to evaluate which variables such as blood pressure, heart rate, LV diameter indexes, fractional shortening, aortic regurgitation and LV diastolic filling indexes correlated best with the progression of aortic root dilatation. Independence of relation was tested using logistic regression. Significance was established at $P<0.05$.

3. Results

3.1. Changes in aortic diameters and grouping of patients

Because of suboptimal images, measurements at the supraaortic ridge level and those at the ascending aorta level were not available in 5 and 11 patients, respectively. Aortic diameters increased with time (Fig. 2). Mean annual increases were 0.4 ± 0.3 mm at the level of annulus, 1.5 ± 1.3 mm at the level of the sinuses of Valsalva, 0.7 ± 0.6 mm at the supraaortic ridge and 0.4 ± 0.4 mm at the proximal ascending aorta. The most rapid change was seen at the sinuses of Valsalva diameter ($F=17$, $P<0.001$ vs. 3 other aortic diameters). The change in aortic ratio was also the highest at the level of the sinuses of Valsalva ($3 \pm 4\%$ per year). Because the annual changes in aortic root diameters were most prominent at the aortic sinus level, patients were divided into 2 groups according to the mean annual change in aortic ratio at the sinuses of Valsalva: rapid (R, >3% per year, 15 patients) or slow (S, ≤3% per year, 28 patients) progression groups. During the follow-up period, 7 patients in group R had aortic dissection requiring aortic surgery and 2 patients died. One patient died 2 months after aortic root and valve replacement and another patient died 3 years after Bentall's operation from colon cancer. Aortic dissection [2 proximal (involving the ascending aorta) and 5 distal (all dissections that originate after the left subclavian artery)] occurred more frequently in group R (7 patients, 47%) than in group S (0%, $\chi^2=12.37$, $P<0.001$). Only 1 patient from group S underwent prophylactic aortic root and valve replacement due to ascending aortic aneurysm and severe aortic regurgitation.

3.2. Characteristics of the patients

Demographic and clinical data for all 43 patients at the time of the initial examination are described in Table 1. Patients in group R were older than those in group S ($P<0.01$). There were no significant differences in sex, heart rate and body surface area, but systolic ($P<0.02$) and diastolic ($P<0.05$) blood pressures were higher in group R.

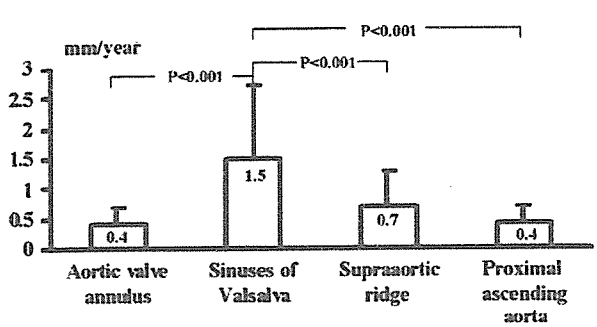

Fig. 2. Mean annual increases of aortic root diameter at 4 levels in all study patients.

Table 1
Demographic and clinical data in all study patients and in the slow (S) and rapid (R) progression group at entry

	Total ($n=43$)	Group S ($n=28$)	Group R ($n=15$)
Age (years)	22 ± 14	18 ± 12	30 ± 15*
Male sex	23 (53%)	14 (50%)	9 (60%)
Body surface area (m^2)	1.56 ± 0.37	1.51 ± 0.4	1.67 ± 0.2
Heart rate (beats/min)	73 ± 8	72 ± 7	75 ± 9
Blood pressure (mm Hg)			
Systolic	117 ± 15	112 ± 13	124 ± 16†
Diastolic	67 ± 9	65 ± 8	70 ± 9‡
Pulse pressure	50 ± 12	48 ± 11	54 ± 13
Mitral valve prolapse	24 (56%)	17 (61%)	7 (47%)
Aortic root dilatation	40 (93%)	26 (93%)	14 (93%)
Duration of follow-up (years)	5.2 ± 3.2	6.3 ± 3.1	3.3 ± 2.5§

*$P<0.01$; †$P<0.02$; ‡$P<0.05$; §$P<0.005$ compared with group S. Data presented are mean value ± SD or number (%) of patients.

Pulse pressure was comparable between the 2 groups. Aortic root dilatation was similarly seen in group R and in group S. The presence of mitral valve prolapse was comparable in both groups. Duration of follow-up was shorter in group R ($P<0.005$). There were no differences in the incidence of patients using β-adrenergic blocking drugs between the 2 groups.

3.3. Echocardiographic data (Table 2)

At the initial examination mean aortic sinus diameter ($P=0.001$) and aortic ratio ($P=0.01$) were larger in group R. LV diameters and fractional shortening were comparable between the 2 groups, but LV wall thickness was larger in group R ($P=0.01$ for interventricular septum and $P=0.02$ for posterior wall). Aortic regurgitation was more common ($P=0.001$) and more severe in group R ($P=0.02$). The prevalence ($P=0.349$) and severity of mitral regurgitation ($P=0.13$) was comparable in both groups (Table 2).

Echocardiographic data at the most recent follow-up or before surgery showed no significant differences in LV diameters, fractional shortening and severity of mitral regurgitation between groups R and S. However, aortic sinus diameter ($P<0.001$) and aortic ratio ($P<0.001$) were larger and aortic regurgitation was more severe in group R ($P<0.001$).

Pulsed Doppler indexes were obtained in 27 out of 43 patients (12 patients in group R and in 15 patients in group S) (Table 3). At the time of the initial pulsed Doppler examination, there were no differences in age (30 ± 14 vs. 27 ± 12 years), blood pressures (125 ± 28 vs. 117 ± 11 mm Hg for systolic pressure and 69 ± 9 vs. 68 ± 8 mm Hg for diastolic pressure), LV dimensions (29 ± 4 vs. 30 ± 4 mm/m^2 for end-diastolic diameter index and 18 ± 4 vs. 19 ± 3 mm/m^2 for end-systolic diameter index), fractional shortening ($37 \pm 5\%$ vs. $36 \pm 3\%$), LV wall thickness (8.4 ± 1.4 vs. 9.2 ± 1.6 mm for interventricular septum and 8.1 ± 1.2 vs. 8.8 ± 1.5 for posterior wall) and the severity of aortic

Table 2
Echocardiographic variables

	Total ($n=43$)		Group S ($n=28$)		Group R ($n=15$)	
	Initial	Follow-up	Initial	Follow-up	Initial	Follow-up
Aortic sinus diameter (mm)	36.8 ± 8.7	43.2 ± 11.5	33.7 ± 7.2	39 ± 7	42.7 ± 8.4*	51 ± 14†
Aortic sinus ratio	1.36 ± 0.23	1.49 ± 0.32	1.30 ± 0.20	1.37 ± 0.2	1.48 ± 0.24‡	1.72 ± 0.4†
LV EDD (mm)	48 ± 7	52 ± 8	47 ± 8	51 ± 8	50 ± 5	54 ± 9
LV EDDI (mm/m^2)	32 ± 8	32 ± 6	33 ± 9	31 ± 6	31 ± 6	33 ± 7
LV ESD (mm)	31 ± 5	34 ± 7	30 ± 6	33 ± 6	32 ± 4	35 ± 8
LV ESDI (mm/m^2)	21 ± 5	21 ± 5	21 ± 6	20 ± 4	20 ± 5	22 ± 6
FS (%)	36 ± 4	34 ± 5	36 ± 4	34 ± 5	36 ± 5	34 ± 5
IVS (mm)	8.1 ± 1.8	8.6 ± 1.6	7.6 ± 1.6	8.3 ± 1.5	9.0 ± 1.7‡	9.2 ± 1.7
PW (mm)	7.7 ± 1.7	8.3 ± 1.5	7.3 ± 1.5	7.9 ± 1.4	8.5 ± 1.8§	8.9 ± 1.6‖
Aortic regurgitation						
Prevalence	16 (37%)	22 (51%)	5 (18%)	8 (29%)	11 (73%)*	14 (93%)†
Severity	0.5 ± 0.8	0.9 ± 1.2	0.3 ± 0.8	0.4 ± 0.9	0.9 ± 0.7§	1.9 ± 1.1†
Mitral regurgitation						
Prevalence	12 (28%)	19 (44%)	6 (21%)	11 (39%)	6 (40%)	8 (53%)
Severity	0.4 ± 0.7	0.8 ± 1.1	0.2 ± 0.5	0.6 ± 1.0	0.7 ± 1.0	1.0 ± 1.2

*$P=0.001$; †$P<0.001$; ‡$P=0.01$; §$P=0.02$; ‖ $P=0.04$ compared with group S. Data presented are mean value ± SD or number (%) of patients. EDD=end-diastolic diameter; EDDI=end-diastolic diameter index, ESD=end-systolic diameter; FS=fractional shortening; IVS=diastolic interventricular septum thickness; PW=diastolic LV posterior wall thickness.

regurgitation (0.7 ± 0.5 vs. 0.5 ± 1.0) and mitral regurgitation (0.7 ± 1.0 vs. 0.2 ± 0.5) between the 2 groups. However, patients in group R showed longer deceleration time ($P=0.005$), lower E ($P=0.015$) and lower E/A ($P=0.002$) compared to those in group S. A wave velocity did not differ significantly between the 2 groups. Follow-up data were available in 17 patients. At the most recent follow-up, deceleration time was longer in group R ($P=0.03$). Other Doppler indexes were comparable between the 2 groups.

3.4. Possible predictors of rapid progression of aortic root dilatation

To identify possible predictors of rapid aortic root dilatation, we performed multiple regression analyses. Aortic root dilatation at the sinuses of Valsalva level and that at the supraaortic ridge level were determined by diastolic pressure (t value=2.091, $P=0.043$ for the sinus level, t value=2.348, $P=0.024$ for the supraaortic ridge level). Changes of aortic ratios at the sinus and supraaortic ridge levels were influenced by diastolic pressure and aortic regurgitation (t value=2.340, $P=0.024$ and t value=2.163, $P=0.037$ for the aortic sinus, and t value=3.374, $P=0.002$ and t value=3.438, $P=0.002$ for the supraaortic ridge). Fig. 3 shows t values of each parameter to predict aortic growth rate with overall multiple correlation coefficient.

Multivariate logistic regression analysis was used to identify the best predictor of rapid aortic dilatation (group R patients). In this analysis, the parameters which differed significantly between groups R and S at initial evaluation were included. They were aortic sinus diameter, aortic sinus ratio, systolic and diastolic blood pressures, degree of aortic regurgitation, deceleration time, E and E/A. The only independent predictor of rapid dilatation was E/A ratio ($P=0.0223$). We also assessed the independent predictors of aortic complications using the logistic regression with the same parameters. The only independent predictor of aortic complications was deceleration time ($P=0.0295$).

4. Discussion

4.1. Features of the patients with rapid progression of the aortic root dilatation

The present study showed that the Marfan patients at older age, with higher blood pressure, and with significant aortic regurgitation were at high risk of rapid progression of

Table 3
Doppler left ventricular diastolic filling variables

	Total		Group S		Group R	
	Initial ($n=27$)	Follow-up ($n=17$)	Initial ($n=15$)	Follow-up ($n=8$)	Initial ($n=12$)	Follow-up ($n=9$)
DT	184 ± 26	196 ± 40	172 ± 15	175 ± 22	199 ± 30*	216 ± 43†
E	62 ± 15	57 ± 14	68 ± 13	63 ± 13	54 ± 14‡	51 ± 14
A	44 ± 13	44 ± 9	40 ± 14	42 ± 10	48 ± 12	46 ± 8
E/A	1.55 ± 0.58	1.35 ± 0.45	1.84 ± 0.57	1.55 ± 0.36	1.19 ± 0.35§	1.18 ± 0.46

*$P=0.005$; †$P=0.03$; ‡$P=0.015$; §$P=0.002$ compared with group S. Data presented are mean value ± SD or number of patients. A=atrial diastolic filling velocity; DT=deceleration time of the early diastolic filling velocity; E=early diastolic filling velocity.

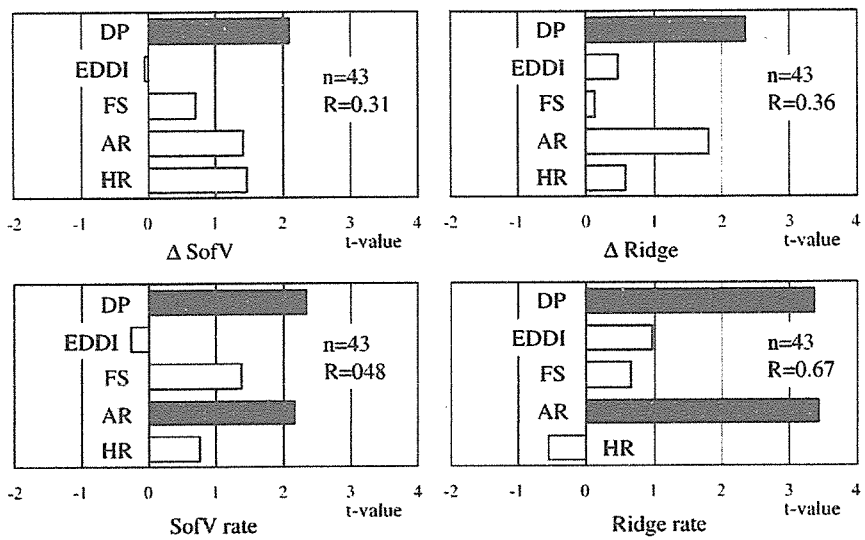

Fig. 3. Impact of patients' characteristics on annual change of aortic root diameter and on change of the aortic ratio; t values of each parameter are shown in bar graphs. Black bars indicate $P<0.05$ and white bars indicate $P>0.05$. R shows multiple correlation coefficient. AR=severity of aortic regurgitation; DP=diastolic blood pressure; EDDI=end-diastolic diameter index; FS=left ventricular fractional shortening; HR=heart rate; Δ ridge=change in supraaortic ridge diameter per year; ridge rate=change in supraaortic ridge ratio; Δ SofV=change in aortic sinuses of Valsalva diameter per year; SofV rate=change in aortic sinus ratio.

aortic root dilatation and subsequent aortic complications. Despite significant initial differences between groups in the prevalence and severity of aortic regurgitation, we found that LV size and systolic function were comparable between groups with rapid and slow progression. However, interestingly, abnormal LV diastolic filling pattern, indicating impaired LV relaxation, was more common in patients with rapid progression. Note that in this analysis, factors influencing Doppler filling parameters such as age, blood pressure, LV size, wall thickness, and aortic and mitral regurgitation grades were all comparable between patients with slow and rapid progression. Although there was a relatively small number of patients who had pulsed Doppler recordings, the features associated with impaired diastolic LV relaxation could predict more rapid aortic root dilatation and development of complications in select Marfan patients. Further, our findings could have a logical explanation when considering the potential abnormalities of the collagen matrix that may involve the myocardium in the Marfan syndrome [10]. The abnormalities of the extracellular connective tissue matrix in patients with the Marfan syndrome could involve heart muscle as well as aorta and heart valves, leading to ventricular diastolic dysfunction [10]. It has been speculated that defective microfibrils and elastic fibers in the cardiac cytoskeleton weaken the elastic restoring forces in the Marfan syndrome, and therefore LV relaxation is impaired [10].

4.2. Determinants of rapid aortic root dilatation

Since the Marfan patients with aortic root dilatation may have poor prognosis, it would be clinically important to predict which patients would progressively develop aortic root dilatation. Therefore, we performed multivariate analysis considering clinical and echocardiographic parameters as independent variables and changes in aortic root diameters as the dependent variables. We found that aortic regurgitation grade, diastolic blood pressure and LV size had significant influence on the rate of aortic root dilatation. This finding is in accord with the previously published study regarding the poorer prognosis (decreased long-term survival) in patients with diastolic murmur or cardiomegaly on initial physical examination [4]. The presence of aortic regurgitation or dilated LV, or both should be a sign to proceed in an aggressive manner to further treatment.

4.3. Clinical implications

Our study confirms the importance of age, high blood pressure, larger initial aortic ratio, and presence and severity of aortic regurgitation, abnormal Doppler diastolic filling pattern at baseline as markers of high risk of rapid progression of aortic dilatation and subsequent aortic complications. This underscores the need for more frequent control and aggressive treatment in the Marfan patients with these features.

Diastolic dysfunction has been known to precede systolic dysfunction in some forms of heart failure. We found that even in patients with rapid progression of aortic dilatation (which could represent more severe disease expression) systolic function was preserved. It appears that in the Marfan syndrome, diastolic dysfunction precedes systolic dysfunction. It may be possible that our findings, indicating impaired relaxation, may show an overture to rapid progression of aortic dilatation and the risk for aortic complications in select Marfan patients.

4.4. Study limitations

In this retrospective study, some patients had suboptimal echocardiographic recordings for measurements of aortic diameters (at supraaortic ridge and ascending aorta level) and these measurements were not included in the analysis. Furthermore, although all study patients had color Doppler recordings, only 63% of all patients had pulsed Doppler recordings. Despite of the small number of patients, the difference in LV filling pattern between the 2 groups was very prominent and consistent.

Although transmitral flow velocity pattern provides a measure of diastolic filling, almost all of the indexes derived from the pattern are load dependent [19]. Again, note that in this study LV size, wall thickness, blood pressure, and the severity of aortic regurgitation and mitral regurgitation were comparable between the 2 patient groups in which pulsed Doppler examination was performed. Therefore, effects of loading conditions would be minor.

Since only 2 patients, both from the rapid progression group, received β-blocker therapy, effect of therapy could not be addressed in this study. To clarify the relationship between LV performance and progression of aortic root dilatation, a prospective study should be conducted with a larger number of patients.

4.5. Conclusions

Marfan patients at older age, with higher blood pressure, and with significant aortic regurgitation were at high risk of progression of aortic dilatation, with the most remarkable increase at the sinuses of Valsalva. LV systolic function appeared not to relate to the progression of aortic root dilatation. Prolonged deceleration time may relate to an increased risk for aortic complications.

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Altered expression balance of matrix metalloproteinases and their inhibitors in human carotid plaque disruption: Results of quantitative tissue analysis using real-time RT-PCR method

Takeo Higashikata[a], Masakazu Yamagishi[a,*], Toshio Higashi[b], Izumi Nagata[b], Koji Iihara[b], Susumu Miyamoto[b], Hatsue Ishibashi-Ueda[c], Noritoshi Nagaya[d], Takashi Iwase[d], Hitonobu Tomoike[a], Aiji Sakamoto[e]

[a] *Division of Cardiovascular Medicine and Bioscience, National Cardiovascular Center and Research Institute, 5-7-1 Fujishiro-dai, Suita, Osaka 565-8565, Japan*

[b] *Division of Neurosurgery, National Cardiovascular Center, Suita, Osaka, Japan*

[c] *Division of Pathology, National Cardiovascular Center, Suita, Osaka, Japan*

[d] *Division of Regenerative Medicine and Tissue Engineering, National Cardiovascular Center and Research Institute, Suita, Osaka, Japan*

[e] *Division of Biotechnology and Bioscience, National Cardiovascular Center and Research Institute, Suita, Osaka, Japan*

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Abstract

Background: The balance between degradation and synthesis of extracellular matrix determines its content in atherosclerotic tissue. To examine the role of expression balance of matrix metalloproteinases (MMPs) to their inhibitors, tissue inhibitors of metalloproteinases (TIMPs) and tissue factor pathway inhibitor-2 (TFPI-2) in the development and disruption of atherosclerotic plaque, these gene expressions in human carotid plaque were quantitatively determined by real-time reverse transcription (RT)-polymerase chain reaction (PCR) method.

Methods: Total RNA for cDNA synthesis was extracted from tissues in 24 patients with carotid endarterectomy. The amounts of cDNAs for MMP-1, -2, -3 and -9, TFPI-2 and TIMP-1, -2 and -3 were determined by real-time RT-PCR method, and normalized with glutaraldehyde 3-dehydrogenase.

Results: In plaques, the expression MMP-1 (1.53 ± 0.25, mean ± S.E.M.), MMP-3 (1.99 ± 0.59) and MMP-9 (2.00 ± 0.51) was augmented compared to those in the adjacent control regions (0.60 ± 0.16, 0.46 ± 0.18 and 0.58 ± 0.21, respectively, $p < 0.05$). The expression of TFPI-2 was lower in plaques (0.32 ± 0.08) than in controls (0.94 ± 0.23, $p < 0.01$). Although the expression of TIMP-1 was higher in plaques (1.28 ± 0.23) than in controls (0.81 ± 0.10, $p < 0.05$), the indices of MMP-1/TIMP-1, MMP-3/TIMP-3 and MMP-9/TIMP-1 were still significantly higher in plaques. Interestingly, MMP-9 and the resulting MMP-9/TIMP-1 balance in plaques with disruption were significantly higher (3.36 ± 1.52 and 1.66 ± 0.12, $n = 11$) than those in non-disrupted plaques (1.11 ± 0.52 and 0.76 ± 0.12, $n = 13$, $p < 0.05$).

Conclusion: With the decreased expression of TFPI-2, upregulation of MMPs in atherosclerotic plaque was disproportional to that of TIMPs, suggesting that imbalanced degradation and synthesis of extracellular matrix persists in advanced lesions, particularly in plaques with disruption.

Keywords: Atherosclerosis; Extracellular matrix; Matrix metalloproteinases; Tissue inhibitor of metalloproteinases; Tissue factor pathway inhibitor-2; Plaque disruption

1. Introduction

Disruption of atherosclerotic plaque during its development can expose the thrombogenic core to luminal blood flow, frequently resulting in ischemic cardiac events and stroke

* Corresponding author. Tel.: +81 6 6833 5012; fax: +81 6 6833 9865.
E-mail address: myamagi@hsp.ncvc.go.jp (M. Yamagishi).

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[1,2]. During this process, structural changes in extracellular matrix (ECM) were shown to play a crucial role in plaque development and disruption [3]. The structural integrity of plaques seems to depend on a balance between synthesis and degradation of the ECM which is mainly regulated by proteinases such as matrix metalloproteinases (MMPs) including interstitial collagenase or MMP-1, gelatinase A or MMP-2, stromelysin 1 or MMP-3, gelatinase B or MMP-9 [4,5].

The activities of MMPs are controlled on multiple levels: transcription and translation of their inactive precursors (zymogens), post-translational activation of zymogens by proteolysis and interactions with tissue inhibitors of metalloproteinases (TIMPs) [6] and/or tissue factor pathway inhibitor-2 (TFPI-2) [7]. Indeed, TIMPs-1, -2, -3 and TFPI-2 are expressed in atherosclerotic lesions [7–9], and these inhibitors bind to and inactivate most of the MMPs [7,10]. Thus, the expression balance of MMP to TIMP and TFPI-2 is considered to regulate the net degeneration of ECM, thus contributing to maintaining plaque stability [7,11,12]. However, few systematic data exist regarding quantitative evaluation of the expression of MMPs and their inhibitors in human atherosclerotic plaques, probably because of technical difficulties in simultaneous determination of multiple gene expression in small tissue samples obtained in clinical settings. In the present study, we used real-time reverse transcription (RT)-polymerase chain reaction (PCR) and analyzed gene expression levels of MMPs, TIMPs and TFPI-2 in human carotid plaque and an adjacent control region. We also compared expression and function of MMPs between histologically disrupted and non-disrupted plaques.

2. Subjects and methods

2.1. Subjects

The protocol of this study was approved by the institutional committee for ethical review. Written informed consent was obtained from all 24 patients who underwent carotid endarterectomy for severe stenosis of the extracranial carotid artery (all male with mean age of 68 ± 2 years). All patients presented clinical symptoms of cerebral ischemic attack related to carotid stenosis. Seven patients had a history of recent ischemic attack within 1 month prior to endarterectomy. The prevalence of risk factors for atherosclerosis was as follows: hypertension (systolic pressure >160 mmHg) in 20, hyperlipidemia (total cholesterol >220 mg/dl) in 22, smoking in 15 and diabetes mellitus (fasting blood glucose >110 mg/dl) in 10 patients. High sensitive (hs) CRP level (normal range <3 mg/l) just before surgery was 2.45 ± 0.43 mg/l (Table 1).

2.2. Tissue sampling

Samples of the plaque region were obtained immediately after endarterectomy. Endarterectomy was extended in a caudal direction to include a sample of minimally affected common carotid artery proximal to the plaque but in continuity with the plaque to act as a paired control. Under these conditions, the stenotic segment and adjacent areas were dissected undisruptedly as a single specimen, preserving circumferential integrity as much as possible. Also special care was taken not to damage luminal surface and plaque interior. After removing a part of the tissue for histological examination, all samples were immediately frozen in liquid nitrogen and stored at −80 °C until extraction of mRNA.

Procedures for RNA preparation and cDNA synthesis were already described elsewhere in detail [13]. Briefly, the samples were homogenized in 1.0 ml ISOGEN™ reagent (Nippon Gene, Tokyo, Japan), thoroughly mixed with 0.2 ml chloroform and centrifuged at 15,000 × *g* for 15 min at 4 °C. The aqueous supernatant was transferred into a micro test tube, mixed with 0.6 ml isopropanol and centrifuged at 15,000 × *g* for 15 min at 4 °C. The precipitated total RNA was rinsed with 70% ethanol, air-dried and then resuspended in RNase-free water. Then, all the total RNA was treated with DNase Free™ reagent (Ambion, Austin, TX) for 60 min, and then reverse-transcribed with Superscript II™ (Invitrogen, Carlsbad, CA) at 37 °C for 60 min using random primers (TaKaRa, Tokyo, Japan). The integrity of each cDNA mixture was checked by amplification of glutaraldehyde 3-phosphate dehydrogenase (GAPDH) with *Ex Taq* (TaKaRa), using the primer set 5′-ACCACAGTCCATGCCATCAC-3′/5′-TCCACCACCCTGTTGCTGTA-3′.

Table 1
Patient Characteristics

	All patients (n = 24)	With disruption (n = 11)	Without disruption (n = 13)	p-Value
Age	68 ± 2	66 ± 3	69 ± 2	NS
Male sex	24	11	13	NS
Hypertension	20	8	12	NS
Diabetes	10	5	5	NS
HbA1c (%)	6.5 ± 0.4	7.0 ± 0.8	6.2 ± 0.4	NS
Hyperlipidemia	22	9	13	NS
LDL (mg/dl)	132 ± 6	140 ± 10	128 ± 7	NS
Smoking	15	5	10	NS
hs-CRP (mg/l)	2.45 ± 0.43	2.68 ± 0.49	2.12 ± 0.81	NS

2.3. Primers and probes for real-time RT-PCR

Using Primer Express™ software (Applied Biosystems, Foster, CA), primers were designed for each of the genes for MMP-1, -2, -3 and -9, TFPI-2 and TIMP-1, -2 and -3, and the TaqMan probe inherent to each primer set was prepared, which was an oligonucleotide labeled with a reporter dye (FAM) at the 5′-end and a quencher dye (TAMRA) at the 3′-end. The sequences of the primers and TaqMan probes of MMPs-1, -2, -3, -9, TIMPs-1, -2 and -9 were reported elsewhere [13], and those for TFPI-2 were SENSE = CGATGCTTGCTGGAGGATAGA; ANTISENSE = ACACTGGTCGTCCACACTCACT; Taqman probe = 5′-FAM-AAGTTCCCAAAGTTTGCCGGCTGC-TAMRA-3′; TFPI-2 SENSE = CGATGCTTGCTGGAGGATAGA; ANTISENSE = ACACTGGTCGTCCACACTCACT; Taqman probe = 5′-FAM-AAGTTCCCAAAGTTTGCCGGCTGC-TAMRA-3′.

Real-time RT-PCR was performed using an ABI PRISM 7700 Sequence Detection System (Applied Biosystems). The reaction solution was assembled in a volume of 25 μl, which comprised TaqMan Universal PCR Master Mix (Applied Biosystems), forward and reverse primers (final concentration 300 nM each), TaqMan probe (final concentration 200 nM) and cDNA mixture (about 2.5 ng). Throughout this study, the cDNA mixture from a particular sample was used to generate the working standard for quantitation of the cDNA of interest, which plots the relationship between the dilution of the standard cDNAs and the corresponding C_t value (the number of cycles necessary to obtain a threshold fluorescent signal) [13]. The initial quantity of the cDNA of interest in a certain cDNA mixture was calculated from the working standard and then normalized to that of GAPDH determined with TaqMan Assay Reagent Endogenous Control™ (Applied Biosystems). The normalized value for each target cDNA reflects the expression level of the corresponding gene in a test sample relative to the standard tissue. The accuracy of the present real-time RT-PCR for determining mRNA expression in human vascular tissue was already confirmed by comparing the results with those determined by conventional RT-PCR method [13].

2.4. Expression and function of MMP

To determine expression and function of MMP in its protein level, carotid tissue samples from 10 patients, in whom enough amounts of proteins could be extracted, were examined by Western blotting and gel zymography. The extracted protein was separated by SDS-PAGE and blotted onto a Hybond-ECL nitrocellulose membrane (Amersham) with the use of primary (40 μg/ml) and secondary (1:2000, Amersham) antibodies. As for zymography, proteins with gelatinolytic activity were identified by use of substrate gels prepared by incorporation of gelatin (1 mg/ml; Wako) into a SDS-PAGE. After elecrophoresis, gels were washed in 2.5% Triton X-100 for 30 min to remove SDS. The gel was equilibrated for 30 min at room temperature with gentle agitation then incubated for overnight at 37 °C in 50 mM Tris/HCl, pH 7.5, containing 0.2 M NaCl, 5 mM $CaCl_2$ and 0.02% Brij 35. Gels were then fixed and stained with 0.25% Coomassie Brilliant Blue R-250 (Wako). The product of the optical net density of the band was compared with a positive control (HT-1080 human fibrosarcoma cells for Western blotting and human MMP-2 and human MMP-9, 1.5 ng, CC073; CHEMICON for zymography) to obtain a ratio comparable between gels.

2.5. Histology and immunohistochemistry

A part of the plaque was placed in tissue fixative (Histochoice, Hedwin, Baltimore). After overnight fixation, the samples were paraffin embedded and sectioned at 4-μm intervals. Tissue sections were deparaffinized with xylene followed by immersion in graded alcohol. They were washed three times for 5 min each in phosphate-buffered saline (PBS) and blocked with bovine serum albumin for 60 min. Specimens were then incubated with primary antibodies against CD-68, MMPs, TIMPs and TFPI-2 (Fuji Chemical, Tokyo, Japan) overnight at 4 °C. After they were washed in PBS, specimens were incubated with biotinylated rabbit anti-mouse IgG for 60 min at room temperature. Specimens were then washed with PBS, stained with horseradish peroxidase-conjugated streptavidin, and finally incubated with substrate solution for 1–15 min. The tissue sections were also stained with hematoxylin–eosin and elastica van Gieson for evaluation of plaque composition and fibrous cap disruption, as described by Carr et al. [2]. Plaque was defined as atheromatous if the area of lipid core was ≥30% of the whole plaque area and as fibrous plaque if <30% in terms of its vulnerability [14].

2.6. Data analysis

The mean and standard error of triplicate data are presented. Statistical analysis was performed by paired *t*-test using Stat View 5.0 software on a Macintosh computer and by Wilcoxon matched-pair signed-rank test if appropriate. A p-value <0.05 was considered significant.

3. Results

3.1. Patient and plaque characteristics

Atheromatous plaque was observed in 15 samples and fibrous plaque in 9 samples. Disruption of the fibrous cap was observed in 11 samples with atheromatous plaque and was not observed in 13 samples, which consisted of 4 atheromatous and 9 fibrous plaques. Although levels of HbA1c and LDL-cholesterol in patients with plaque disruption tended to be higher than those in patients without disruption, there was no statistically significant difference in their clinical back-

Table 2
MMP, TFPI-2 and TIMP levels

Mrna	Control	Plaque	p-Value
MMP-1	0.60 ± 0.16	1.53 ± 0.25	<0.01
MMP-2	0.80 ± 0.11	0.88 ± 0.14	NS
MMP-3	0.46 ± 0.18	1.99 ± 0.59	<0.05
MMP-9	0.58 ± 0.21	2.00 ± 0.51	<0.01
TFPI-2	0.94 ± 0.23	0.32 ± 0.08	<0.01
TIMP-1	0.81 ± 0.10	1.28 ± 0.23	<0.05
TIMP-2	1.12 ± 0.15	0.95 ± 0.17	NS
TIMP-3	0.47 ± 0.16	0.67 ± 0.17	NS

ground. Also there was no difference in hs-CRP level between two patient groups, although mean value in all patients was higher than normal value (Table 1).

3.2. Expression levels of MMPs, TIMPs and TFPI-2

From removed samples with a wet weight of 11.69 ± 2.64 mg, 0.49 ± 0.22 μg total RNA was extracted for analysis. Amplification of GAPDH was equivalent among all the cDNAs synthesized. Each primer set for PCR exponentially amplified its target cDNA according to the cycle number. Normalized values for MMP, TIMP and TFPI-2 gene expression in plaque and adjacent control tissue (controls) are summarized in Table 2. In the plaques, the gene expression levels of MMP-1 (1.53 ± 0.25), MMP-3 (1.99 ± 0.59) and MMP-9 (2.00 ± 0.51) were significantly higher than those in the controls (0.60 ± 0.16, 0.46 ± 0.18 and 0.58 ± 0.21, respectively, $p < 0.05$). However, no difference was found in the expression level of MMP-2 gene between the plaques and controls (0.88 ± 0.14 versus 0.80 ± 0.11). It was quite interesting that TFPI-2 gene expression was significantly higher in the controls (0.94 ± 0.23) than that in plaques (0.32 ± 0.08, $p < 0.01$).

As for TIMP genes, the only TIMP-1 gene was significantly upregulated in plaques in comparison with that in the controls (1.28 ± 0.23 versus 0.81 ± 0.10, $p < 0.05$) (Table 2). Among the combination of the ratios of the four MMPs to the three TIMPs examined in this study, the expression ratios of MMP-1 to TIMP-1, MMP-3 to TIMP-3 and MMP-9 to TIMP-1 were significantly higher in plaques than in the controls (2.98 ± 0.77 versus 0.99 ± 0.43, 2.18 ± 0.53 versus 0.63 ± 0.22 and 1.80 ± 0.14 versus 0.83 ± 0.09, respectively, $p < 0.05$) (Fig. 1). Of interest, in plaques with disruption of fibrous cap, MMP-9 expression (3.36 ± 1.52) and the ratio of MMP-9 to TIMP-1 (1.66 ± 0.12) were significantly higher than those in plaques without disruption (1.11 ± 0.52 and 0.76 ± 0.12, respectively), although TFPI-2 gene expression was not different between these groups (0.27 ± 0.08 versus 0.40 ± 0.18).

MMP-9 protein was expressed both in disrupted and non-disrupted plaques, but was not expressed or only slightly expressed in controls. Under these conditions, net expression of MMP-9 was significantly higher in disrupted (2.61 ± 0.17,

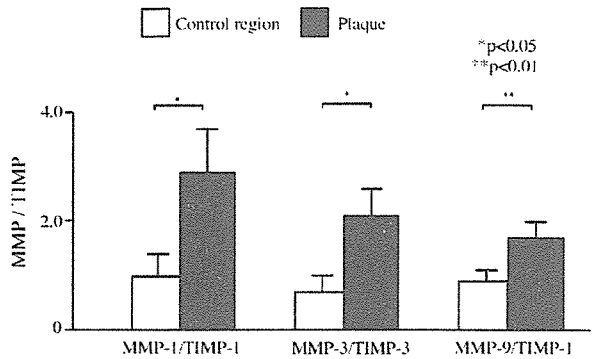

Fig. 1. Imbalanced expression of matrix metalloproteinase (MMP) to tissue inhibitor of matrix metalloproteinase (TIMP) genes in carotid plaque. Vertical axis represented the ratio of MMP/TIMP. Open columns represent values from control regions and closed columns values from plaques.

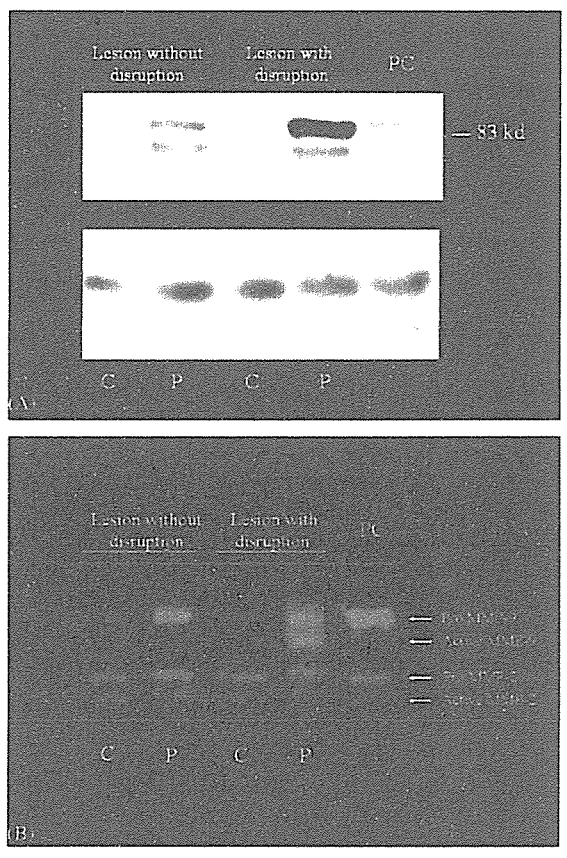

Fig. 2. Expression and function of MMP-9 in protein level. (A) Western blotting for matrix metalloproteinase MMP-9 (upper) and an internal marker protein, endothelin receptor (ETR) (lower), in non-ruptured lesion, ruptured carotid lesions and positive control (PC). MMP-9 was clearly expressed in the plaque (P) and PC, whereas in the control region (C), little expression of MMP-9 was observed. Note that both pro* and active** form of MMP-9 appears to be highly expressed in the ruptured plaque, in comparison with the non-ruptured plaque, although ETR protein is equally expressed. (B) By zymography, increased size and staining of both pro- and active forms of MMP-9 particularly in ruptured plaques, although MMP-2 activity was not different in each lane as observed in mRNA analysis.