13. Watanabe N, Taniguchi T, Taketoh H, et al. Elevated remnant-like lipoprotein particles in impaired glucose tolerance and type 2 diabetic patients. Diabetes Care 1999;22:152–6.
14. The American Diabetes Association. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 1997;19:1183–97.
15. Klein R, Klein BE, Moss SE. Relation of glycemic control to diabetic microvascular complications in diabetes mellitus. Ann Intern Med 1996;24:90–6.
16. Ito H, Harano Y, Suzuki M, et al. Risk factor analyses for macrovascular complication in non-obese NIDDM patients: Multiclinical Study for Diabetic Macroangiopathy (MSDM). Diabetes 1996;45: S19–23.
17. Reaven GM, Chen YD, Jeppesen J, Maheux P, Krauss RM. Insulin resistance and hyperinsulinemia in individuals with small, dense low density lipoprotein particles. J Clin Invest 1993;92:141–6.
18. Doi H, Kugiyama K, Oka H, et al. Remnant lipoproteins induce proatherothrombogenic molecules in endothelial cells through a redox-sensitive mechanism. Circulation 2000;102:670–6.
19. Griendling KK, Alexander RW. Endothelial control of the cardiovascular system: recent advances. FASEB J 1996;10:283–92.
20. Cybulsky MI, Gimbrone MA Jr. Endothelial expression of a mononuclear leukocyte adhesion molecule during atherogenesis. Science 1991;25:788–91.
21. Saniabadi AR, Umemura K, Shimoyama M, Adachi M, Nakano M, Nakashima M. Aggregation of human blood platelets by remnant like lipoprotein particles of plasma chylomicrons and very low density lipoproteins. Thromb Haemost 1997;77:996–1001.
22. Cohn JS, Marcoux C, Davignon J. Detection, quantification, and characterization of potentially atherogenic triglyceride-rich remnant lipoproteins. Arterioscler Thromb Vasc Biol 1999;19:2474–86.

Journal of the American College of Cardiology
© 2004 by the American College of Cardiology Foundation
Published by Elsevier Inc.

Vol. 43, No. 7, 2004
ISSN 0735-1097/04/$30.00
doi:10.1016/j.jacc.2003.09.063

Atherosclerosis

Echolucent Carotid Plaques Predict Future Coronary Events in Patients With Coronary Artery Disease

Osamu Honda, MD,* Seigo Sugiyama, MD, PHD,* Kiyotaka Kugiyama, MD, PHD,†
Hironobu Fukushima, MD,* Shinichi Nakamura, MD,* Shunichi Koide, MD,* Sunao Kojima, MD,*
Nobutaka Hirai, MD,* Hiroaki Kawano, MD, PHD,* Hirofumi Soejima, MD, PHD,*
Tomohiro Sakamoto, MD, PHD,* Michihiro Yoshimura, MD, PHD,* Hisao Ogawa, MD, PHD*†
Kumamoto and Yamanashi, Japan

OBJECTIVES The purpose of this study was to examine whether echolucent carotid plaques predict future coronary events in patients with clinically stable coronary artery disease (CAD).

BACKGROUND Although rupture of coronary plaques is considered a major cause of acute coronary syndromes (ACS), the clinical estimation of coronary vulnerability still remains inconclusive. Ultrasound evaluation of carotid plaques with integrated backscatter (IBS) analysis can indicate the consistency/structure of the plaques. Lipid-rich lesions known as "unstable plaques" appear as echolucent plaques with low IBS values using this technique.

METHODS We investigated the echogenicity of carotid plaques using ultrasound with IBS in 286 consecutive CAD patients (71 with ACS and 215 with stable CAD). Coronary plaque complexity was also determined angiographically in stable CAD patients followed up for 30 months or until the occurrence of coronary events.

RESULTS The calibrated IBS values of carotid plaques in ACS patients were significantly lower than those in stable CAD patients ($p < 0.01$). Echolucent carotid plaques accurately predicted the existence of complex coronary plaques (predictive power of 83%). Kaplan-Meier analysis demonstrated a significantly higher probability of coronary events developing in patients with echolucent carotid plaques than in patients without this type of plaque ($p < 0.001$). The presence of echolucent carotid plaques in stable CAD patients predicted future coronary events independent of other risk factors (odds ratio 7.0, 95% confidence interval 2.3 to 21.4; $p < 0.001$).

CONCLUSIONS Echolucent carotid plaques with low IBS values predicted coronary plaque complexity and the development of future coronary complications in patients with stable CAD. Qualitative evaluation of carotid plaques using ultrasound with IBS is a clinically useful procedure for risk assessment of CAD patients. (J Am Coll Cardiol 2004;43:1177–84) © 2004 by the American College of Cardiology Foundation

Acute coronary syndromes (ACS) are almost invariably associated with ruptured coronary plaques, commonly referred to as "unstable plaques" (1–4). Evaluation of not only the luminal stenoses but also the instability of atherosclerotic plaques is important in determining the extent of clinical disease activity and the risk of subsequent vascular complications. Coronary plaques can be examined by either intravascular ultrasound (5) or angioscopy (6), with both techniques identifying coronary plaque composition and the condition of the plaque surface. However, these techniques are invasive and, therefore, may not be practical for routine use in the management and risk assessment of patients with coronary artery disease (CAD) (7). Recent studies support the concept that plaque instability is not merely a local vascular incident but rather that plaque instability exists simultaneously at multiple sites in the systemic vascular bed (8,9). Thus, it is possible that coronary plaque vulnerability may be assessed by evaluating plaque characteristics such as stability, composition, and surface condition in other vessels such as the carotid arteries.

See page 1185

Non-invasive carotid artery ultrasound is an established, validated method for visualizing and quantifying atherosclerotic lesions (10–12). The intima-media thickness (IMT) of carotid arteries is associated with both coronary risk factors (13) and cardiovascular complications (14). Carotid plaque echogenicity has also been reported to be associated with stroke and other cerebrovascular events (15,16). Recent

From the *Department of Cardiovascular Medicine, Graduate School of Medical Sciences, Kumamoto University, Kumamoto, Japan; †Second Department of Internal Medicine, Graduate School of Yamanashi University, Yamanashi, Japan. This study was supported in part by Grants-in-Aid C(2)-14570679 (Ministry of Education, Culture, Sports, Science, and Technology, Tokyo) and 14C-4 (Ministry of Health, Labor, and Welfare, Tokyo); the Smoking Research Foundation, Tokyo; The Naito Foundation; Mochida Memorial Foundation for Medical and Pharmaceutical Research; Suzuken Memorial Foundation; and the Research Foundation for Community Medicine, "Research Meeting on Hypertension and Arteriosclerosis 2002," Tokyo, Japan. Drs. Honda and Sugiyama contributed equally to this work.

Manuscript received April 23, 2003; revised manuscript received July 31, 2003, accepted September 29, 2003.

Abbreviations and Acronyms

ACS	= acute coronary syndromes
AMI	= acute myocardial infarction
CAD	= coronary artery disease
(c)IBS	= (calibrated) integrated backscatter
IMT	= intima-media thickness
HDL	= high-density lipoprotein
hs-CRP	= high-sensitivity C-reactive protein
LDL	= low-density lipoprotein
OMI	= old myocardial infarction
PCI	= percutaneous coronary intervention
ROI	= region of interest
UAP	= unstable angina pectoris

studies have assessed the composition of carotid plaques (17–19) by using ultrasound with integrated backscatter (IBS) analysis and showed histologically that echolucent plaques were lipid- and macrophage-rich (20) and therefore unstable (17–19). Currently, identifying a high-risk CAD population that is vulnerable to having coronary plaques is clinically difficult. In the present study, we measured the echogenicity of carotid plaques using IBS and investigated the association between carotid plaque echogenicity and angiographic coronary plaque complexity. We also examined whether echolucent carotid plaque with a low value of calibrated IBS (cIBS) predicted future coronary events in patients with clinically stable CAD.

METHODS

Study patients. This study enrolled 286 consecutive patients with CAD: 71 patients with ACS (39 with acute myocardial infarction [AMI] and 32 with unstable angina pectoris [UAP]) and 215 patients with clinically stable CAD. All patients underwent elective or diagnostic catheterization at the Kumamoto University Hospital. A diagnosis of AMI was made if the patient had typical chest pain with ST-segment elevation on an electrocardiogram and an increase in the plasma level of creatine kinase-MB isoenzyme (CK-MB) to greater than twice the upper limit of the normal range. A diagnosis of UAP (Braunwald's class IIB or IIIB [21]) was made if the patient displayed characteristic symptoms at rest associated with transient ischemic ST-segment shifts and normal plasma levels of CK-MB and cardiac troponin T. Stable CAD was defined as a patient with no episodes of angina at rest but with angiographically documented organic stenosis >50% in at least one of the major coronary arteries. The exclusion criteria for the study included severe valvular heart disease, cardiomyopathy, trauma within the previous month, end-stage renal failure, cardiomyopathy, and malignant, infectious, chronic inflammatory, and autoimmune diseases. This study protocol was conducted in accordance with guidelines approved by the ethics committee at our institution.

Ultrasound evaluation. A carotid ultrasound examination was performed in the ultrasound laboratory using an 11.0-MHz, linear-array transducer (SONOS-5500, Philips, Andover, Massachusetts) within one week after an acute coronary event in ACS patients and within a few days after admission but before coronary angiography in stable CAD patients. Patients with ACS who were in an unstable or severe condition one week after an acute coronary event, including patients receiving respiratory or circulatory support, were excluded from the study. Two operators performed all of the carotid scans without any information on the clinical characteristics of the patients. Each common, internal, and external carotid artery was imaged in the anterior oblique, lateral, and posterior oblique planes to identify atherosclerotic lesions. On a longitudinal image of each carotid artery, IMT was defined as the distance from the leading edge of the lumen-intima interface to the leading edge of the media-adventitia interface. Atherosclerotic plaque was defined as a lesion with a focal IMT of 1.1 mm or more, with a localized protrusion of the vessel wall into the lumen (22). Maximum IMT (IMT_{max}) was defined as the greatest axial thickness in the carotid arteries. In this study, we arbitrarily defined the cut-off point for increased IMT_{max} as 2.73 mm, which represented the 75th percentile of the distribution of IMT_{max} in ACS patients.

Measurement of IBS. We measured the IBS values of all carotid atherosclerotic plaques, as described previously by Takiuchi et al. (19). For each plaque, conventional high-resolution, B-mode images were obtained, followed by the acquisition of 60 IBS images. Atherosclerotic plaques were analyzed using the manual definition mode to outline the region of interest (ROI), as shown in Figure 1. Instrument imaging adjustments, such as transmit power, focus, time-gain compensation, and gain setting, including the depth gain compensation curve, were all set at fixed values, with the system control remaining unchanged for the measurement of all plaques. The average power of the IBS signal within the ROI was measured and displayed in decibels for a total of 60 frames. In the case of heterogeneous plaques, we excluded excessively high echoic areas with acoustic shadowing, which indicated calcification from the ROI setting.

We adopted the adventitia as the reference object and then expressed the relative IBS value of the intima-media complex as the difference in IBS values between the intima-media and adventitia (cIBS = intima-media IBS value − adventitia IBS value). In each patient, we selected plaque with the most echolucent cIBS value among all of the carotid plaques. The IBS values in the adventitia were within the dynamic range of the ultrasound machine, with no significant difference observed between patients with and those without echolucent carotid plaques (IBS: 53.1 ± 4.8 dB vs. 52.3 ± 5.0 dB, respectively; p = ns). In our study, the inter-observer variability for the repeated measurements of plaque IBS values was 0.7 ± 0.4 dB, whereas the intra-observer variability for these repeated measurements was 0.6 ± 0.4 dB. We arbitrarily defined the cut-off point for echolucent plaque as −13.4 dB, which was the 75th percentile of the cIBS values in the ACS patient group.

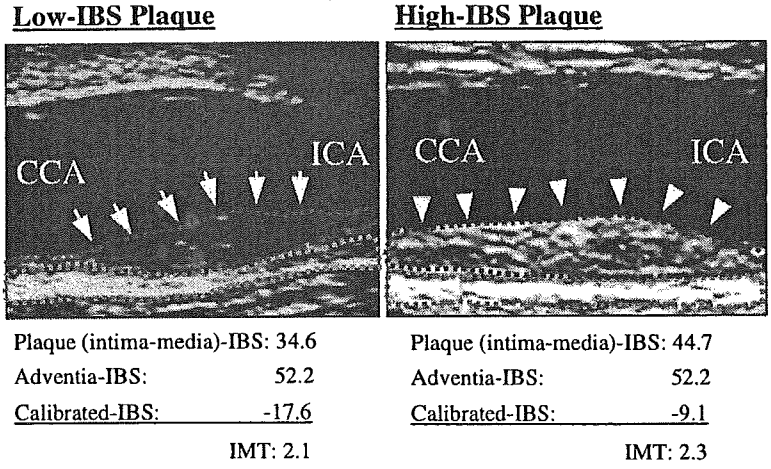

Figure 1. Representative ultrasound images of atherosclerotic carotid plaques with low and high calibrated integrated backscatter (IBS) values. The **red dotted line** indicates region of interest in the plaque (intima-media complex), and the **blue dotted line** indicates region of interest in the adventitia using a manual outline definition mode. **(Left)** Low IBS plaque is identified by **arrows**. The calibrated IBS value and maximum intima-medial thickness (IMT) of this plaque are −17.6 dB and 2.1 mm, respectively. **(Right)** High IBS plaque is identified by **arrowheads**. The calibrated IBS value and maximum IMT of this plaque are −9.1 dB and 2.3 mm, respectively. CCA = common carotid artery; ICA = internal carotid artery.

Determination of serum C-reactive protein. High-sensitivity C-reactive protein (hs-CRP) in fasting serum samples was assayed by rate nephelometry (Dade Behring, Marburg, GMBH). In stable CAD patients, 90% had hs-CRP levels below 0.3 mg/dl and 99% had levels below 1.0 mg/dl.

Angiographic analysis. Measurements of coronary stenosis and the definition of coronary complex plaques in patients with stable CAD were performed independently by two cardiologists who had no knowledge of the patients' clinical characteristics. Coronary lesions were considered complex if they caused at least 50% stenosis and had complex morphologic features such as eccentric lesions with a narrow neck, overhanging edges, irregular borders, or plaque ulceration (9,23,24).

Follow-up study. After the angiographic data were obtained, the 215 patients with stable CAD were followed up every month at the hospital or at a home visit for a period of up to 30 months or until the occurrence of one of the following clinical coronary events: sudden hospitalization or coronary revascularization (e.g., percutaneous coronary intervention [PCI], coronary artery bypass graft surgery) due to recurrent or refractory angina pectoris, UAP, non-fatal AMI, or cardiac death. Patients with ACS were excluded from the follow-up analysis because they had a coronary event ratio significantly higher than that of stable CAD patients. All stable CAD patients received the standardized medical therapy outlined in Table 1. The cause of death was determined from an examination of hospital records. We found 23% of patients with stable CAD had PCI-mediated restenosis at follow-up coronary angiography. Revascularization therapy based only on angiographic data, including PCI-mediated restenosis, was not counted as a coronary event. The need for and timing of revascularization was decided by the attending physician and interventional cardiologists, independent of this prospective study. The attending physician and interventional cardiologist were blinded as to whether the patients had echolucent carotid plaques.

Statistical analysis. All descriptive data are expressed as the mean value ± SD. The Mann-Whitney *U* test was used to evaluate differences in plaque cIBS, IMT_{max}, and CRP between the two study groups. The frequencies for gender, smoking, hypertension, diabetes mellitus, medication therapy, invasive therapy, multi-vessel CAD, left main trunk lesion, left ventricular dysfunction, and history of old myocardial infarction (OMI) were compared between the two groups by using chi-square analysis. The mean values of continuous variables with a normal distribution, including age, body mass index, total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, and triglycerides, were compared between the two groups by using the unpaired *t* test. Survival analysis was carried out using the Kaplan-Meier method (log-rank test), according to the presence or absence of echolucent carotid plaques (cIBS value <-13.4 dB). The predictive value for coronary events during the follow-up period was assessed by Cox proportional hazards analysis. The continuous covariates in this analysis were age, body mass index, total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, hs-CRP, IMT_{max}, and plaque cIBS, and the categorical covariates were male gender, smoking, diabetes mellitus (25), hypertension (>140/90 mm Hg or taking any antihypertensive medications), history of OMI, and multi-vessel CAD.

The data were analyzed initially using a univariate model to determine the risk factors that had a significant association with future coronary events. Multivariate analysis was then applied using only the covariates that significantly predicted coronary events in the univariate analysis. In the logistic regression analysis, continuous covariates were expressed as

Table 1. Baseline Clinical Characteristics of Patients With Stable Coronary Artery Disease With or Without Echolucent Carotid Plaque

	Echolucent Carotid Plaque		
	With (n = 112)	Without (n = 103)	p Values
Age (yrs)	69.4 ± 7.1	67.6 ± 8.8	0.24
Gender (male/female)	94/18	65/38	< 0.001
Smoking	28 (25%)	32 (31%)	0.32
Hypertension	74 (66%)	58 (56%)	0.12
Diabetes mellitus	47 (42%)	32 (31%)	0.10
BMI (kg/m^2)	23.6 ± 2.7	24.1 ± 3.7	0.17
Total cholesterol (mg/dl)	191 ± 37.2	194 ± 40.0	0.58
HDL cholesterol (mg/dl)	46 ± 11.4	53 ± 18.1	0.004
LDL cholesterol (mg/dl)	126 ± 31.9	120 ± 34.3	0.13
Triglycerides (mg/dl)	140 ± 71.6	137 ± 90.8	0.33
C-reactive protein (mg/dl)	0.21 ± 0.2	0.11 ± 0.1	< 0.001
IMT_{max} (mm)	2.68 ± 1.0	1.69 ± 1.0	< 0.001
Medications			
Beta-blocker	26 (23%)	15 (15%)	0.11
Calcium antagonist	93 (83%)	75 (73%)	0.07
ACE inhibitor or ARB	60 (54%)	44 (43%)	0.11
Nitrate	51 (46%)	34 (33%)	0.06
Aspirin	106 (95%)	90 (87%)	0.06
HMG-CoA reductase inhibitor	46 (41%)	35 (34%)	0.28
Fibrate	6 (5%)	7 (7%)	0.66
Invasive therapy with POBA/stenting/ CABG [n]	30 (27%) [4/11/15]	13 (13%) [3/4/6]	0.01
Multi-vessel CAD	84 (75%)	37 (36%)	< 0.001
Left main trunk lesion	16 (14%)	5 (5%)	0.02
LV dysfunction (EF <40%) (%)	8 (8%)	6 (6%)	0.63
History of old myocardial infarction	44 (39%)	22 (21%)	0.004

Data are presented as the mean value ± SD or number (%) of patients.
ACE = angiotensin-converting enzyme inhibitor; ARB = angiotensin II receptor blocker; BMI = body mass index; CABG = coronary artery bypass graft surgery; CAD = coronary artery disease; EF = ejection fraction; HDL = high-density lipoprotein; HMG-CoA = 3-hydroxy-3-methylglutaryl coenzyme A; IMT_{max} = maximum intima-media thickness; LDL = low-density lipoprotein; LV = left ventricular; POBA = plain old balloon angioplasty.

categorized covariates (old age >75 years, high total cholesterol >240 mg/dl, low HDL cholesterol <40 mg/dl, high LDL cholesterol >160 mg/dl, hypertriglyceridemia >150 mg/dl, body mass index >25 kg/m^2, and high hs-CRP >0.3 mg/dl), according to the cut-off points of the American Heart Association (26–29). Statistical significance was defined as $p < 0.05$. To evaluate the probability of predicting angiographic evidence of coronary plaques, a test of the sensitivity (SE) and specificity (SP) of the carotid plaques' echolucency, as compared with either smooth or complex coronary plaques, was carried out using the following equations. Sensitivity = number of patients with complex coronary plaques among patients with echolucent carotid plaques/number of tested patients with complex coronary plaques. Specificity = number of patients without complex coronary plaques among patients without echolucent carotid plaques/number of patients without complex coronary plaques. The pretest likelihood (PL) was defined as the probability that complex coronary plaques existed in the patient to be tested (PL = number of patients with complex coronary plaques/total number of stable CAD patients tested in present study) (30). The predictive power (PP = PL × SE/PL × SE + [1 − PL][1 − SP]) and the likelihood ratio (LR = SE/[1 − SP]) were calculated according to Bayes' theorem (30). All analyses were carried out using StatView version 5.0 (Tokyo, Japan).

RESULTS

Carotid plaque evaluation in CAD patients. Of the stable CAD patients, 79.2% had multiple carotid plaques, with 93.8% of the patients with echolucent plaques possessing multiple echolucent plaques in their carotid arteries. The cIBS values of all plaques examined in the study ranged from 4.1 to −28.9 dB. The standard variation of the cIBS value of multiple carotid plaques in each patient was 3.6 ± 1.8 dB. Figure 1 shows representative echolucent carotid plaques with a low cIBS value (−17.6 dB, IMT of 2.1 mm) and a high cIBS value (−9.1 dB, IMT of 2.3 mm). The median (inter-quartile range) cIBS values of carotid plaques in patients with ACS were significantly lower than those in stable CAD patients: −17.7 (−20.2 to −13.4) versus −13.7 (−18.3 to −9.8) dB ($p = 0.007$) (Fig. 2). However, patients with either ACS or stable CAD showed no significant difference in IMT_{max} (2.2 ± 0.8 mm vs. 2.2 ± 1.0 mm; $p = 0.91$) or in the frequency of traditional coronary risk factors (data not shown). Thus, the carotid plaques in

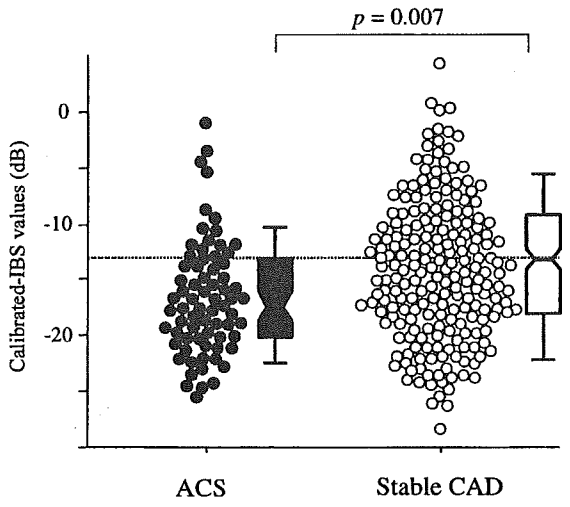

Figure 2. Comparison of calibrated integrated backscatter (IBS) values between patients with stable coronary artery disease (CAD) and patients with acute coronary syndrome (ACS). The **dotted line** indicates the cut-off level for echolucent plaque (−13.4 dB, which represents the 75th percentile of the distribution in patients with ACS). **Box and whisker plot:** lines within boxes represent median values; upper and lower lines of boxes represent 25th and 75th percentiles, respectively; and **upper and lower bars outside of boxes** represent 90th and 10th percentiles, respectively.

patients with ACS were more echolucent than those in stable CAD patients, despite similar values of IMT_{max}.

Baseline clinical characteristics and echolucent carotid plaques in stable CAD patients. The baseline clinical characteristics of the patients with stable CAD with or without echolucent carotid plaques are shown in Table 1. The stable CAD patient group with echolucent carotid plaques had significantly higher levels of hs-CRP, lower levels of HDL cholesterol, and increased IMT_{max} and were predominantly male. Multivariate logistic regression analysis showed a significant and independent association between increased levels of hs-CRP and the presence of echolucent carotid plaques (odds ratio [OR] 3.5, 95% confidence interval [CI] 1.4 to 8.5; $p < 0.01$).

Ultrasound analysis of carotid plaques and angiographic morphology of coronary plaques in stable CAD patients. Complex coronary plaques were found in 116 (54%) of 215 stable CAD patients, with 43 (20%) of these 215 patients having multiple complex plaques in their coronary arteries. The reproducibility of assessment in coronary plaque complexity was 93%. The significant risk factors for the presence of complex coronary plaques were carotid echolucency, high hs-CRP levels, increased IMT_{max}, low levels of HDL cholesterol, and male gender. On multivariate logistic regression analysis, using these parameters as covariates, carotid plaque echolucency was shown to be the strongest independent predictor of complex coronary plaques (OR 11.5, 95% CI 5.5 to 23.7; $p < 0.001$). Table 2 shows that patients with echolucent carotid plaques frequently had complex coronary plaques ($p < 0.001$, chi-square test). The pretest likelihood of complex coronary plaques was 54%, and we found that echolucent carotid plaques predicted the existence of complex coronary plaques with a sensitivity of 80%, a specificity of 81%, and a predictive power of 83%. The likelihood ratio for estimating the existence of complex coronary plaques was 4.2.

Table 2. Relationship Between Echolucency of Carotid Plaques and Angiographic Complex Coronary Plaques

	Coronary Artery Complexity		
	Complex Plaque	Smooth Plaque	Total
With echolucent carotid plaque	93	19	112
Without echolucent carotid plaque	23	80	103
Total	116	99	215

Chi-square value = 78.1, $p < 0.001$. Sensitivity = 80%; specificity = 81%; predictive power = 83%; likelihood ratio = 4.2.

Prognostic value of echolucent carotid plaques in stable CAD patients. All patients completed the follow-up period. Patients with stable CAD were followed from one to 30 months (mean 14). Patients with echolucent carotid plaques (n = 112) had 29 coronary events during the follow-up period, whereas patients without echolucent plaques (n = 103) had only four coronary events ($p < 0.001$) (Table 3). All but 1 of the 29 coronary events in the total echolucent group (i.e., 97%) were recorded in patients diagnosed with stable CAD with multiple echolucent carotid plaques. There were no significant differences in the dosages of administered medications between the patients with and those without coronary events (data not shown). Kaplan-Meier analysis in patients with stable CAD demonstrated that the presence of echolucent carotid plaques was associated with a significantly higher probability of developing coronary events ($p < 0.001$) (Fig. 3). Univariate Cox proportional hazards model analysis of the coronary risk factors and carotid ultrasound data showed that carotid

Table 3. Summary of Coronary Events During the Follow-Up Period in Patients With Stable Coronary Artery Disease

	Echolucent Carotid Plaque		
	With (n = 112)	Without (n = 103)	p Values
Total coronary events	29	4	< 0.001
Cardiac death	4	0	
ACS	19	2	
Non-fatal acute myocardial infarction	2	1	
Unstable angina	17	1	
ACS treated with			
PCI	11	2	
CABG	3	0	
Medical alone	5	0	
Recurrent angina (sudden hospitalization)	6	2	
Invasive therapy	4	2	
Medical alone	2	0	

ACS = acute coronary syndromes; CABG = coronary artery bypass graft surgery; PCI = percutaneous coronary intervention.

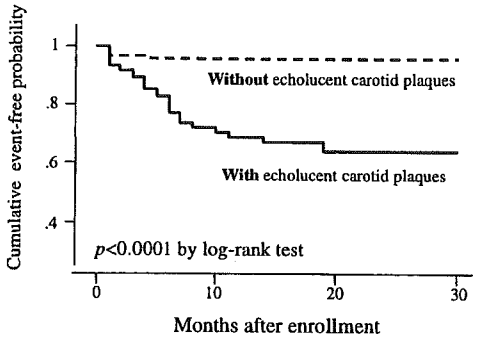

Figure 3. Kaplan-Meier curves comparing the probability of developing a coronary event during a follow-up period of 30 months in 215 patients with stable coronary artery disease, grouped according to the presence or absence of echolucent carotid plaques. The **solid line** indicates patients with echolucent carotid plaques (n = 112), and the **dotted line** indicates patients without echolucent carotid plaques (n = 103).

plaque echolucency, IMT_{max}, multi-vessel CAD, and a history of OMI were all significant predictors of clinical coronary events ($p < 0.01$) (Table 4). Multivariate Cox proportional hazards model analysis of these four risk factors demonstrated that the presence of echolucent carotid plaques was the most significant and independent predictor of future coronary complications in patients with stable CAD (OR 7.0, 95% CI 2.3 to 21.4; $p < 0.001$) (Table 5). The absence of echolucent carotid plaques provided a significantly higher negative predictive value for future coronary complications (OR 0.1, 95% CI 0.03 to 0.27; $p < 0.001$).

DISCUSSION

This study demonstrates, for the first time, that echolucent carotid plaques with low IBS values are strongly associated with the angiographic appearance of complex coronary plaques and that echolucent carotid plaque was a significant and independent predictor of future coronary events in stable CAD patients. Furthermore, the echolucent carotid plaque is a stronger indicator of future coronary events than increased IMT in carotid arteries. Thus, objective evaluation of carotid plaque quality, utilizing an ultrasound technique with IBS, is clinically and practically informative for risk stratification of patients with CAD.

Table 4. Univariate Cox Proportional Hazards Analysis of Risk Factors for a Coronary Event in Patients With Stable Coronary Artery Disease

	Odds Ratio	95% CI	p Values
Age (per yr)	1.01	0.97–1.06	0.61
Gender (male)	1.78	0.73–4.31	0.20
Smoking	1.47	0.72–3.00	0.29
Hypertension	0.88	0.43–1.73	0.66
Diabetes mellitus	1.71	0.86–3.38	0.12
BMI (per kg/m^2)	0.93	0.83–1.03	0.17
Total cholesterol (per mg/dl)	0.99	0.99–1.00	0.19
HDL cholesterol (per mg/dl)	0.98	0.96–1.01	0.20
LDL cholesterol (per mg/dl)	1.00	0.98–1.01	0.35
Triglycerides (per mg/dl)	1.00	0.99–1.00	0.79
hs-CRP (per mg/dl)	1.48	0.83–2.60	0.19
History of OMI	3.74	1.86–7.53	0.0002
Multi-vessel CAD	3.99	1.65–9.68	0.002
IMT_{max} (per 1.0 mm)	1.49	1.14–1.95	0.003
Echolucency of carotid plaque (per c-IBS −1.0 dB)	1.21	1.14–1.29	< 0.0001

CI = confidence interval; c-IBS = calibrated integrated backscatter; hs-CRP = high-sensitivity C-reactive protein; OMI = old myocardial infarction; other abbreviations as in Table 1.

Table 5. Multivariate Cox Proportional Hazards Analysis of Risk Factors for a Coronary Event in Patients With Stable Coronary Artery Disease

	Odds Ratio	95% CI	p Values
Presence of echolucent carotid plaque (c-IBS <−13.4 dB)	7.0	2.3–21.4	< 0.001
History of OMI	2.3	1.1–5.0	0.033
Increased IMT_{max} (>2.73 mm)	1.5	0.7–3.0	0.25
Multi-vessel CAD	1.3	0.5–3.6	0.61

Abbreviations as in Tables 1 and 4.

It is important to evaluate plaque vulnerability as well as the degree of coronary stenosis when assessing disease activity and severity in atherosclerosis. Recent technologic advances have provided methods such as intravascular ultrasound (5) and angioscopy (6), which allow the direct examination of coronary plaques and provide information on coronary plaque composition and surface conditions. However, these techniques are invasive and are not practical on a routine basis for the management and risk assessment of CAD patients (7). Angiographically, the presence of coronary plaques with complex morphologic features correlates with pathologic plaque rupture, whereas superficial thrombus is recognized as the hallmark of ACS (9,23,24,31). It has been shown that such lesions are associated with rapid progression of coronary stenosis and general clinical instability in patients with stable CAD (31). Recent observations support the concept that plaque instability is not merely a local vascular incident, but that plaque destabilization occurs at multiple sites in the entire systemic vascular bed (8,9). Thus, it may be possible that the presence of unstable carotid plaques might reflect, in part, the activation of the systemic vascular tree, and that unstable coronary plaques may exist throughout the entire vasculature.

High-resolution carotid ultrasound detects lipids, thrombi, and hemorrhages in carotid plaques as echolucent structures (10–12) The echolucency of carotid plaque signifies a high lipid content and a higher risk for future ischemic cerebrovascular events (15,16). We recorded 11 cerebrovascular events, including ischemic stroke and transient ischemic attacks, in the present study, with 10 occurring in the echolucent group and only one in the echogenic group. This different prevalence was statistically significant ($p < 0.01$), indicating that carotid plaque echolucency by IBS assessment reflects carotid plaque vulnerability. Takiuchi et al. (19) reported on the usefulness of ultrasound IBS determination of atherosclerotic plaque tissue composition

and found that echolucent plaques with a low IBS value appeared as lipid-rich unstable plaques. Similarly, Kawasaki et al. (32) demonstrated that IBS analysis was useful for evaluating plaque tissue components in echolucent plaque with low IBS values, indicating a lipid-rich condition in the carotid arteries. Recently, an investigation of plaque echolucency by IBS analysis was used clinically for investigating various vasculatures, including coronary arteries (32,33). The present study demonstrated that the IBS value of carotid plaques in patients with ACS was significantly lower than that in patients with stable CAD, despite comparable values of IMT. This suggested that carotid atherosclerotic plaques are more vulnerable in ACS patients than in stable CAD patients, and furthermore, that the echolucency of carotid plaques is a significant indicator of angiographically unstable coronary plaques and future coronary events in stable CAD patients. Non-invasive, easily repeatable, and inexpensive methods that detect instability of coronary lesions are needed for the management of high-risk CAD patients. In this regard, qualitative ultrasound evaluation of carotid plaque according to echogenicity provides clinically important information on coronary plaque vulnerability and future clinical outcome.

It is now widely accepted that inflammation has an important role in the genesis of plaque vulnerability (1–4). Pathologic investigations have shown that unstable plaques are characterized by active inflammation of the fibrous cap at the time of plaque disruption (3,4). In this study, the plasma levels of hs-CRP were significantly higher in patients with echolucent carotid plaques than in patients without such lesions. The higher levels of hs-CRP were found to be independently associated with the presence of echolucent carotid plaques. Clinical reports indicate that elevated levels of systemic inflammatory markers predict future cardiovascular complications (29). Our results suggest that echolucent plaques, in combination with elevated hs-CRP levels, indicate the presence of both ultrasonographically unstable and biochemically activated plaques.

Because the most echolucent plaque may, by itself, determine the risk for rupture, it is important to evaluate whether vulnerable plaques are present in the vascular tree rather than focusing on the number of plaques or the average vulnerability of several plaques. To identify high-risk patients with high sensitivity, we selected a value in each patient for the most echolucent plaque as the "targeted plaque" among all carotid plaques. We consider that monitoring of these targeted carotid plaques using IBS may provide us with a new clinical and practical strategy to treat patients with a high probability of coronary complex plaques, thereby preventing future cardiovascular complications in these patients.

It is well established that subjective assessment, gray-scale analysis, and IBS analysis are useful methods for evaluating atherosclerotic plaque echogenicity. Previously, Gronholdt et al. (34) reported that subjective assessment of carotid echolucency correlated with gray-scale analysis of plaque echogenicity. In our study, subjective evaluation of carotid plaque echolucency was found to correlate with objective cIBS-assisted evaluation ($p < 0.001$, chi-square test). The cIBS values were significantly higher in patients with subjectively echogenic plaques than in patients with subjectively echolucent plaques (-9.2 ± 3.6 vs. -18.8 ± 3.8, $p < 0.001$). Recently, Rossi et al. (35) reported that cIBS values in the carotid intima-media complex were significantly related to age in the normal healthy population, but not in hypertensive patients. We found no significant difference in carotid plaque cIBS values between young and old (age >75 years) CAD patients (cIBS median: −13.5 vs. −14.1 dB, $p = 0.56$).

This study was limited by the relatively small number of patients studied. We enrolled only stable CAD patients with mild stenotic lesions (>50% stenosis), as most acute coronary events appear to occur with low-grade or mild coronary stenosis. Low utilization of invasive therapy, instead of a high multi-vessel ratio, may frequently result in cases with mild stenotic coronary lesions and enrollment of patients with OMI or patients treated previously with invasive procedures such as PCI or coronary artery bypass graft surgery. A longitudinal, prospective study utilizing carotid ultrasound evaluation with IBS in a large number of patients with homogeneous risk is required in order to assess the precise prognostic value of echolucent carotid plaques in determining future cardiovascular events.

Conclusions. Echolucent carotid plaques with low IBS values predict the presence of complex coronary plaques and the development of future coronary complications in stable CAD patients. Non-invasive and qualitative carotid plaque evaluation employing the ultrasound technique with IBS is clinically useful for the assessment of coronary plaque vulnerability and is informative in the risk stratification of CAD patients.

Reprint requests and correspondence: Dr. Seigo Sugiyama, 1-1-1 Honjo, Kumamoto City, Kumamoto, Japan 860-8556. E-mail: ssugiyam@kumamoto-u.ac.jp.

REFERENCES

1. Fuster V, Badimon L, Badimon JJ, Chesebro JH. The pathogenesis of coronary artery disease and the acute coronary syndromes (1). N Engl J Med 1992;326:242–50.
2. Libby P. Molecular bases of the acute coronary syndromes. Circulation 1995;91:2844–50.
3. Davies MJ. Stability and instability: two faces of coronary atherosclerosis. The Paul Dudley White Lecture 1995. Circulation 1996;94: 2013–20.
4. Falk E, Shah PK, Fuster V. Coronary plaque disruption. Circulation 1995;92:657–71.
5. Nissen SE, Gurley JC, Grines CL, et al. Intravascular ultrasound assessment of lumen size and wall morphology in normal subjects and patients with coronary artery disease. Circulation 1991;84:1087–99.
6. Uchida Y, Nakamura F, Tomaru T, et al. Prediction of acute coronary syndromes by percutaneous coronary angioscopy in patients with stable angina. Am Heart J 1995;130:195–203.
7. Kern MJ, Meier B. Evaluation of the culprit plaque and the physiological significance of coronary atherosclerotic narrowings. Circulation 2001;103:3142–9.

8. Rothwell PM, Villagra R, Gibson R, Donders RC, Warlow CP. Evidence of a chronic systemic cause of instability of atherosclerotic plaques. Lancet 2000;355:19–24.
9. Goldstein JA, Demetriou D, Grines CL, Pica M, Shoukfeh M, O'Neill WW. Multiple complex coronary plaques in patients with acute myocardial infarction. N Engl J Med 2000;343:915–22.
10. Bots ML, Hoes AW, Koudstaal PJ, Hofman A, Grobbee DE. Common carotid intima-media thickness and risk of stroke and myocardial infarction: the Rotterdam Study. Circulation 1997;96: 1432–7.
11. Gronholdt ML. Ultrasound and lipoproteins as predictors of lipid-rich, rupture-prone plaques in the carotid artery. Arterioscler Thromb Vasc Biol 1999;19:2–13.
12. Sabetai MM, Tegos TJ, Nicolaides AN, Dhanjil S, Pare GJ, Stevens JM. Reproducibility of computer-quantified carotid plaque echogenicity: can we overcome the subjectivity? Stroke 2000;31:2189–96.
13. Bots ML, Breslau PJ, Briet E, et al. Cardiovascular determinants of carotid artery disease: the Rotterdam Elderly Study. Hypertension 1992;19:717–20.
14. Nichols WW, Pepine CJ, O'Rourke MF. Carotid-artery intima and media thickness as a risk factor for myocardial infarction and stroke. N Engl J Med 1999;340:1762–3.
15. Polak JF, Shemanski L, O'Leary DH, et al. Hypoechoic plaque at US of the carotid artery: an independent risk factor for incident stroke in adults aged 65 years or older. The Cardiovascular Health Study. Radiology 1998;208:649–54.
16. Gronholdt ML, Nordestgaard BG, Schroeder TV, Vorstrup S, Sillesen H. Ultrasonic echolucent carotid plaques predict future strokes. Circulation 2001;104:68–73.
17. Barzilai B, Saffitz JE, Miller JG, Sobel BE. Quantitative ultrasonic characterization of the nature of atherosclerotic plaques in human aorta. Circ Res 1987;60:459–63.
18. Shepard RK, Miller JG, Wickline SA. Quantification of atherosclerotic plaque composition in cholesterol-fed rabbits with 50-MHz acoustic microscopy. Arterioscler Thromb 1992;12:1227–34.
19. Takiuchi S, Rakugi H, Honda K, et al. Quantitative ultrasonic tissue characterization can identify high-risk atherosclerotic alteration in human carotid arteries. Circulation 2000;102:766–70.
20. Gronholdt ML, Nordestgaard BG, Bentzon J, et al. Macrophages are associated with lipid-rich carotid artery plaques, echolucency on B-mode imaging, and elevated plasma lipid levels. J Vasc Surg 2002;35:137–45.
21. Braunwald E. Unstable angina: a classification. Circulation 1989;80: 410–4.
22. Handa N, Matsumoto M, Maeda H, et al. Ultrasonic evaluation of early carotid atherosclerosis. Stroke 1990;21:1567–72.
23. Ambrose JA, Winters SL, Stern A, et al. Angiographic morphology and the pathogenesis of unstable angina pectoris. J Am Coll Cardiol 1985;5:609–16.
24. Rehr R, Disciascio G, Vetrovec G, Cowley M. Angiographic morphology of coronary artery stenoses in prolonged rest angina: evidence of intracoronary thrombosis. J Am Coll Cardiol 1989;14:1429–37.
25. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 1997;20:1183–97.
26. Grundy SM, Balady GJ, Criqui MH, et al. Primary prevention of coronary heart disease: guidance from Framingham. A statement for healthcare professionals from the American Heart Association Task Force on Risk Reduction. Circulation 1998;97:1876–87.
27. Pearson TA, Blair SN, Daniels SR, et al. The AHA guidelines for primary prevention of cardiovascular disease and stroke: 2002 update. Consensus panel guide to comprehensive risk reduction for adult patients without coronary or other atherosclerotic vascular diseases. American Heart Association Science Advisory and Coordinating Committee. Circulation 2002;106:388–91.
28. Williams MA, Fleg JL, Ades PA, et al. Secondary prevention of coronary heart disease in the elderly (with emphasis on patients ≥75 years of age): an American Heart Association scientific statement from the Council on Clinical Cardiology, Subcommittee on Exercise, Cardiac Rehabilitation, and Prevention. Circulation 2002;105:1735–43.
29. Pearson TA, Mensah GA, Alexander RW, et al. Markers of inflammation and cardiovascular disease: application to clinical and public health practice. A statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. Circulation 2003;107:499–511.
30. Diamond GA, Forrester JS. Analysis of probability as an aid in the clinical diagnosis of coronary-artery disease. N Engl J Med 1979;300: 1350–8.
31. Yokoya K, Takatsu H, Suzuki T, et al. Process of progression of coronary artery lesions from mild or moderate stenosis to moderate or severe stenosis: a study based on four serial coronary arteriograms per year. Circulation 1999;100:903–9.
32. Kawasaki M, Takatsu H, Noda T, et al. Noninvasive quantitative tissue characterization and two-dimensional color-coded map of human atherosclerotic lesions using ultrasound integrated backscatter: comparison between histology and integrated backscatter images. J Am Coll Cardiol 2001;38:486–92.
33. Kawasaki M, Takatsu H, Noda T, et al. In vivo quantitative tissue characterization of human coronary arterial plaques by use of integrated backscatter intravascular ultrasound and comparison with angioscopic findings. Circulation 2002;105:2487–92.
34. Gronholdt ML, Nordestgaard BG, Wiebe BM, Wilhjelm JE, Sillesen H. Echo-lucency of computerized ultrasound images of carotid atherosclerotic plaques are associated with increased levels of triglyceride-rich lipoproteins as well as increased plaque lipid content. Circulation 1998;97:34–40.
35. Rossi M, Cupisti A, Perrone L, Santoro G. Carotid ultrasound backscatter analysis in hypertensive and in healthy subjects. Ultrasound Med Biol 2002;28:1123–8.

Polymorphism in Glutamate-Cysteine Ligase Modifier Subunit Gene Is Associated With Impairment of Nitric Oxide–Mediated Coronary Vasomotor Function

Shin-ichi Nakamura, MD, PhD; Seigo Sugiyama, MD, PhD; Daisuke Fujioka, MD; Ken-ichi Kawabata, MD, PhD; Hisao Ogawa, MD, PhD; Kiyotaka Kugiyama, MD, PhD

Background—The minor −588T allele of polymorphism −588C/T of a modifier subunit gene in glutamate-cysteine ligase (GCLM), a rate-limiting enzyme for glutathione (GSH) synthesis, was associated with lower plasma GSH levels and was a risk factor for myocardial infarction.

Methods and Results—We examined effects of the −588C/T polymorphism on coronary arterial diameter and blood flow responses to intracoronary infusion of acetylcholine in 157 consecutive subjects who had normal coronary angiograms. In multivariate linear regression analysis with covariates including traditional risk factors, the minor −588T allele had an independent association with impaired dilation or enhanced constriction of epicardial coronary arteries in response to acetylcholine, and it was independently associated with blunted increase in coronary flow response to acetylcholine. In a subgroup of 59 consecutive subjects, constrictor responses of epicardial coronary diameter to intracoronary infusion of N^G-monomethyl-L-arginine, reflecting the presence of coronary nitric oxide (NO) bioactivity, had an inverse and independent association with the −588T allele in multivariate analysis.

Conclusions—The −588T polymorphism of the GCLM gene causes a decrease in endothelial NO bioactivity, leading to impairment of endothelium-dependent vasomotor function in large and resistance coronary arteries. The GCL–GSH–NO axis may play a role in the defense system against coronary artery disease. (***Circulation.*** **2003;108:1425-1427.)**

Key Words: antioxidants ■ genetics ■ nitric oxide ■ acetylcholine

Exposure to oxidants may initiate an adaptive intracellular antioxidant response, such as induction of antioxidant genes. Glutathione (GSH), tripeptide thiols, is a major and naturally occurring antioxidant, and it has a predominant role in the regulation of intracellular redox state and protects cells from oxidative injury.[1] Previous in vitro studies demonstrated that GSH depletion inhibits nitric oxide (NO) production in endothelial cells.[2] Furthermore, we and others have shown that supplementation of GSH or its precursor improved endothelial vasomotor dysfunction in patients with coronary risk factors in which oxidative stress has a pathogenic role.[3,4]

Glutamate-cysteine ligase (GCL) is a rate-limiting enzyme for GSH synthesis.[1,5–7] GCL is a heterodimer composed of a catalytic subunit (GCLC) and a modifier subunit (GCLM). GCLM has a physiologically important regulatory function. Recently, we found polymorphism −588C/T in the 5′-flanking region of the GCLM gene.[8] The minor −588T allele of the polymorphism suppresses oxidant-induced upregulation of GCLM gene expression and is associated with lower plasma GSH levels, and this polymorphism is a genetic risk factor for myocardial infarction (MI).[8] However, mechanisms by which this polymorphism is linked to the genesis of MI remain undefined. The present study thus examined whether this polymorphism may be implicated in coronary endothelial vasomotor dysfunction, which plays an important role in the pathogenesis of coronary artery disease.

Methods

Study Subjects

This study enrolled 157 consecutive subjects who had quantitative coronary angiography with intracoronary injection of acetylcholine (ACh) at Kumamoto University Hospital. All of the subjects were ethnic Japanese and underwent diagnostic cardiac catheterization for evaluation of atypical chest pain. All of the 157 subjects had angiographically documented normal coronary arteries (<10% stenosis), normal ventriculography, and no coronary spasm during intracoronary infusion of ACh. No subject had previous MI, congestive heart failure, cardiomyopathy, valvular heart disease, left ventricular hypertrophy, or other serious diseases. These study subjects included most of those examined in our previous study.[9] All of the subjects gave written, informed consent for this study and genetic analysis. The study protocol followed the national guidelines for

Received June 4, 2003; revision received July 29, 2003; accepted July 30, 2003.

From the 2nd Department of Internal Medicine (S.-i.N., D.F., K.-i.K., K.K.), Interdisciplinary Graduate School of Medicine and Engineering, University of Yamanashi, Yamanashi, Japan; and the Department of Cardiovascular Medicine (S.-i.N., S.S., H.O.), Graduate School of Medical Sciences, Kumamoto University, Japan.

Correspondence to Kiyotaka Kugiyama, MD, PhD, 2nd Department of Internal Medicine, Interdisciplinary Graduate School of Medicine and Engineering, University of Yamanashi, 1110 Shimokato, Nakakoma-gun, Yamanashi, 409-3898 Japan. E-mail kugiyama@yamanashi.ac.jp

Circulation is available at http://www.circulationaha.org

DOI: 10.1161/01.CIR.0000091255.63645.98

genetic analysis in Japan and was approved by the ethics committee at Kumamoto University Hospital.

Study Protocol

After baseline angiography, incremental doses of ACh (10, 50, and 100 μg/min) were infused directly into the left coronary artery through the Judkins catheter for 2 minutes, as described previously.[3,9] Fifteen minutes after the ACh infusion, incremental doses of N^G-monomethyl-L-arginine (L-NMMA; 25 and 50 μmol/min, each for 4 minutes), an inhibitor of NO synthase, were infused into the left coronary artery through the Judkins catheter in a subgroup of 59 consecutive subjects. Subsequently, L-NMMA (50 μmol/min) was infused for an additional 5 minutes into the left coronary artery, and at the last minute of the L-NMMA infusion, 50 μg/min of ACh was simultaneously injected into the left coronary artery in the same manner as before the L-NMMA infusion.[9] Finally, intracoronary injection of isosorbide dinitrate (1 mg) was given. Hemodynamic measurements and coronary angiography were repeated before and at each of the infusions.

Quantitative Coronary Angiography and Measurement of Coronary Blood Flow

A quantitative coronary angiographic study was performed in all of the 157 subjects in the same manner, as described.[3,9] The lumen diameter at the center of the left anterior descending coronary artery (LAD) was measured quantitatively by two observers who were blinded to the clinical data of the study subjects.

Blood flow velocity in the proximal segment of the LAD was measured in a subgroup of 99 consecutive subjects using a 0.014-inch wire equipped with a Doppler crystal at its tip, as described previously.[3,9] Responses of coronary artery diameter and blood flow to the infusion of the drugs were expressed as percentage changes from baseline coronary diameter and blood flow, respectively, which were measured just before each infusion.

Genotyping

Genomic DNA was extracted from peripheral blood lymphocytes. Genotyping was performed by a polymerase chain reaction–based method of restriction fragment length polymorphism with forward (5′-CTCAAGGGCAAAGACTCA-3′) and reverse (5′-CCGCCTGGTGAGGTAGACAC-3′) primers, as described previously.[8]

Statistical Analysis

Data are expressed as mean (±SEM) unless otherwise indicated. For comparison of coronary responses between subjects with and without the −588T allele, 2-way analysis of variance for repeated measures was used with Bonferroni's multiple-comparison tests. Analyses for the associations were performed using the multivariate linear regression technique, with coronary arterial responses as the dependent variable. Of the independent variables, age, body mass index (kg/m^2), and total cholesterol levels (mg/dL) were treated as continuous variables. Other independent variables, including male sex, smoking (>10 cigarettes per day for 1 year), hypertension (defined as blood pressure ≥140/90 mm Hg or as taking an antihypertensive medication), diabetes mellitus (defined according to the American Diabetes Association report or as taking an antidiabetic medication), and the −588T polymorphism, were treated as categorical variables. Statistical significance was defined as $P<0.05$. Statistical analysis was performed with StatView 5.0 (SAS Institute).

Results

Genotypes of Study Subjects

The −588TT, CT, and CC genotypes were present in 1 (0.6%), 34 (22%), and 122 (78%) of 157 subjects studied, respectively. The frequencies of traditional coronary risk factors were comparable between subjects with and without the −588T allele (data not shown).

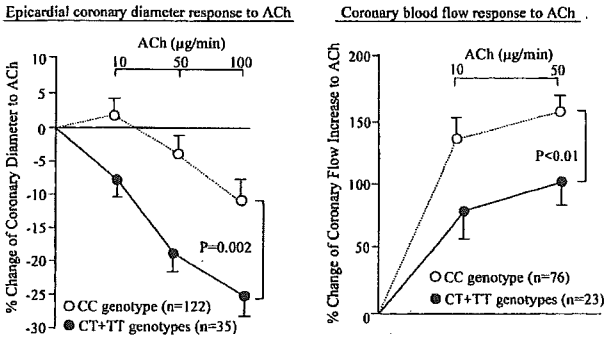

Figure 1. Left, Percent changes (mean±SEM) from baseline in epicardial diameter of the LAD in response to intracoronary infusion of ACh. Right, Percent changes (mean±SEM) from baseline in coronary blood flow response to ACh infusion.

Responses of Epicardial Coronary Diameter and Coronary Blood Flow to ACh

Subjects with CT and TT genotypes had an impaired dilation or enhanced constriction of epicardial coronary arteries and a blunted increase in coronary blood flow in response to ACh infusion, as compared with those with the CC genotype, as shown in Figure 1. The dilator response of epicardial coronary arteries to nitrate was comparable between subjects with and without the −588T allele (26±4% in subjects with CC genotype versus 25±4% in those with CT and TT genotypes; P=NS). In multivariate linear regression analysis, the −588T allele was independently associated with constrictor response of epicardial coronary diameter and with impairment of coronary flow increase in response to ACh at all of doses among the covariates including traditional risk factors (diameter response to 50 μg/min of ACh: standardized regression coefficient=−0.34; $P<0.001$; flow response to 10 μg/min of ACh: standardized regression coefficient=−0.39; $P<0.01$). Among the covariates with a significant association, the association rank-ordered as follows: (1) for diameter response to 50 μg/min of ACh: age > −588T polymorphism > smoking; and (2) for flow response to 10 μg/min of ACh: −588T polymorphism > diabetes > total cholesterol levels.

Responses of Epicardial Arterial Diameter to L-NMMA

Subjects with CT and TT genotypes had less constrictor response to intracoronary infusion of L-NMMA than did those with CC genotype, as shown in Figure 2. In multivariate linear regression analysis, the −588T allele was independently associated with decrease in diameter response to L-NMMA at both doses among the covariates (response to 50 μmol/min of L-NMMA: standardized regression coefficient=0.42; $P<0.01$). The simultaneous infusion of L-NMMA with ACh augmented the constrictor response of epicardial coronary arteries to ACh (50 μg/min) in subjects with CC genotype, whereas the simultaneous L-NMMA infusion had minimum effect on the constrictor response to ACh in those with CT and TT genotypes, as shown in Figure 2.

Discussion

This study indicated that the −588T polymorphism of the GCLM gene had a significant and independent association

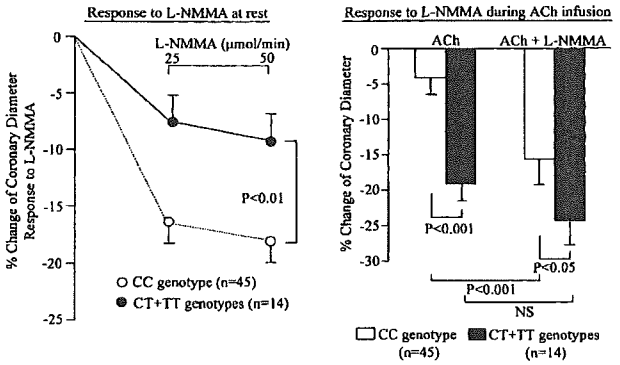

Figure 2. Left, Percent changes (mean±SEM) from baseline in epicardial diameter of the LAD in response to intracoronary infusion of L-NMMA alone. Right, Percent changes (mean±SEM) from baseline in the epicardial coronary diameter in response to ACh alone (50 μg/min) and combined infusion of ACh (50 μg/min) and L-NMMA (50 μmol/min).

with abnormal vasomotor reactivity to ACh in large and resistance coronary arteries. The epicardial coronary diameter response to nitrates was not significantly different between subjects with and without the T allele. Thus, the −588T polymorphism of GCLM gene has an important and causative role in impairment of endothelium-dependent vasomotor function in large and resistance coronary arteries. The present study further showed that the constrictor response of epicardial coronary arteries to L-NMMA at basal conditions, reflecting basal coronary NO bioactivity, was independently decreased with the presence of the −588T polymorphism. Furthermore, the combined infusion of L-NMMA did not significantly augment the constrictor response of epicardial diameter to ACh in subjects with the T allele. Thus, these results suggest that the −588T polymorphism of the GCLM gene may cause a decrease in the basal and stimulated release of endothelial NO, leading to impairment of endothelium-dependent dilation and enhancement of constriction in response to ACh in coronary arteries.

It has been shown that GSH has a central role in protection of endothelial cells from oxygen free radicals and preservation of endothelium-derived NO in arteries exposed to oxidative stress.[1–7] When cells are exposed to oxidative stress, GSH synthesis is increased through upregulation of GCL gene expression, providing a protective/adaptive mechanism against oxidative stress.[1,5–7] However, we have previously shown that the −588T polymorphism suppresses the increase in GCLM gene expression in response to oxidative stress and that this polymorphism is associated with low plasma GSH concentration.[8] Therefore, the −588T polymorphism may weaken the intracellular production of GSH in response to oxidative stress, leading to an increase in the susceptibility to impairment of NO-mediated endothelial vasomotor function. In addition, endothelium-derived NO has various antiatherothrombogenetic properties. Thus, a decrease in NO bioactivity due to the blunted GSH induction may partly play a role in the genesis of coronary artery disease in patients with the −588T polymorphism of the GCLM gene. The associations in the present study were, however, marginal and based on a small population size. Although it is necessary to replicate our findings in a larger number of patients, the −588T polymorphism of GCLM gene could have a causative role in oxidant-induced vascular dysfunction.

The present data are consistent with our previous report showing that polymorphism in the GCLC gene is also associated with coronary vasomotor dysfunction and MI.[10] Thus, the previous and present data support our hypothesis that the GCL–GSH–NO axis may act as a defense system against oxidant-induced cardiovascular complications.

Acknowledgments

This study was supported by grants-in-aid for (B) (2)-15390244, Priority Areas (C) "Medical Genome Science 15012222" from the Ministry of Education, Culture, Sports, Science, and Technology; H15-Chozy-012 Health and Labor Sciences Research Grants for Comprehensive Research on Aging and Health; the Smoking Research Foundation; and Arteriosclerosis Prevention Fund, Tokyo, Japan.

References

1. Meister A, Anderson ME. Glutathione. *Annu Rev Biochem.* 1983;52:711–760.
2. Murphy ME, Piper HM, Watanabe H, et al. Nitric oxide production by cultured aortic endothelial cells in response to thiol depletion and replenishment. *J Biol Chem.* 1991;266:19378–19383.
3. Kugiyama K, Ohgushi M, Motoyama T, et al. Intracoronary infusion of reduced glutathione improves endothelial vasomotor response to acetylcholine in human coronary circulation. *Circulation.* 1998;97:2299–2301.
4. Vita JA, Frei B, Holbrook M, et al. L-2-Oxothiazolidine-4-carboxylic acid reverses endothelial dysfunction in patients with coronary artery disease. *J Clin Invest.* 1998;101:1408–1414.
5. Rahman I, MacNee W. Lung glutathione and oxidative stress: implications in cigarette smoke–induced airway disease. *Am J Pathol.* 1999;277:L1067–L1088.
6. Cho S, Hazama M, Urata Y, et al. Protective role of glutathione synthesis in response to oxidized low density lipoprotein in human vascular endothelial cells. *Free Radic Biol Med.* 1999;26:589–602.
7. Moinova HR, Mulcahy RT. An electrophile responsive element (EpRE) regulates γ-naphthoflavone induction of the human γ-glutamylcysteine synthetase regulatory subunit gene. *J Biol Chem.* 1998;273:14683–14689.
8. Nakamura S, Kugiyama K, Sugiyama S, et al. Polymorphism in the 5′-flanking region of human glutamate-cysteine ligase modifier subunit gene is associated with myocardial infarction. *Circulation.* 2002;105:2968–2973.
9. Kugiyama K, Doi H, Motoyama T, et al. Association of remnant lipoprotein levels with impairment of endothelium-dependent vasomotor function in human coronary arteries. *Circulation.* 1998;97:2519–2526.
10. Koide S, Kugiyama K, Sugiyama S, et al. Association of polymorphism in glutamate-cysteine ligase catalytic subunit gene with coronary vasomotor dysfunction and myocardial infarction. *J Am Coll Cardiol.* 2003;41:539–545.