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 $N^\alpha$-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...
Study Protocol
After baseline angiography, incremental doses of ACh (10, 50, and 100 \(\mu g/min\)) were infused directly into the left coronary artery through the Judkins catheter for 2 minutes, as described previously.\(^9\) Fifteen minutes after the ACh infusion, incremental doses of \(N^\circ\)-monomethyl-L-arginine (L-NMMA; 25 and 50 \(\mumol/min\), 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 \(\mumol/min\)) was infused for an additional 5 minutes into the left coronary artery, and at the last minute of the L-NMMA infusion, 50 \(\mug/min\) 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'-CTCAAGGGCAAAAGCTCA-3') and reverse (5'-CCGCTTGGTGGTAGCAGAC-3') primers, as described previously.\(^8\)

Statistical Analysis
Data are expressed as mean (\(\pm\)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 (2 by 3) 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 \(\geqslant 140/90\) 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).

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 \(\pm\)4% in subjects with CC genotype versus 25 \(\pm\)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 \(\mug/min\) of ACh: standardized regression coefficient\(=-0.34\); \(P<0.001\); flow response to 10 \(\mug/min\) 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 \(\mug/min\) of ACh: age > \(588T\) polymorphism > smoking; and (2) for flow response to 10 \(\mug/min\) 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 \(\mumol/min\) 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 \(\mug/min\)) 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
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 The present data suggest that the −588T polymorphism in the 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**

Polymorphism in Glutamate-Cysteine Ligase Modifier Subunit Gene Is Associated With Impairment of Nitric Oxide–Mediated Coronary Vasomotor Function
Shin-ichi Nakamura, Seigo Sugiyama, Daisuke Fujioka, Ken-ichi Kawabata, Hisao Ogawa and Kiyotaka Kugiyama

_Circulation._ 2003;108:1425-1427; originally published online September 15, 2003;
doi: 10.1161/01.CIR.0000091255.63645.98
_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2003 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/108/12/1425

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation_ is online at:
http://circ.ahajournals.org//subscriptions/