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Association of Polymorphism in Glutamate-Cysteine Ligase Catalytic Subunit Gene With Coronary Vasomotor Dysfunction and Myocardial Infarction

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OBJECTIVES The purpose of this study was to test the hypothesis that polymorphisms in the promoter region of the glutamate-cysteine ligase catalytic subunit *(GCLC)* gene may be associated with coronary endothelial vasomotor dysfunction and myocardial infarction (MI).

BACKGROUND Glutamate-cysteine ligase is a rate-limiting enzyme for synthesis of glutathione (GSH) that plays a crucial role in the intracellular antioxidant defense systems. Oxidants transcriptionally upregulate the *GCLC* gene for GSH synthesis, providing a protective mechanism against oxidant-induced endothelial dysfunction or activation, which plays a pathogenetic role in cardiovascular diseases.

METHODS The association of the possible polymorphisms with coronary arterial diameter responses to acetylcholine was determined in 62 male subjects. The frequency of polymorphisms was compared between 255 male patients with MI and 179 male control subjects.

RESULTS We found a polymorphism (−129C/T) in which the T allele showed lower promoter activity (50% to 60% of the activity of the C allele) in response to H_2O_2 in human endothelial cells. Endothelium-dependent dilation of coronary arteries was impaired in subjects with the −129T allele (n = 31), as compared with the age-matched subjects without the −129T allele (n = 31). The T allele was highly frequent in patients with MI as compared with control subjects, and it was a significant risk factor for MI, independent of traditional coronary risk factors (odds ratio [OR] 1.81, 95% confidence interval [CI] 1.08 to 3.03; p = 0.03).

CONCLUSIONS The −129T polymorphism of the *GCLC* gene may suppress the *GCLC* gene induction response to an oxidant, and it is implicated in coronary endothelial vasomotor dysfunction and MI. (J Am Coll Cardiol 2003;41:539–45) © 2003 by the American College of Cardiology Foundation

BASIC SCIENCE IN CONTEXT

There are many factors that determine a person's risk for the development of atherosclerosis, including dyslipidemia, diabetes, hypertension, inflammation, and oxidative stress. Recently, considerable attention has been given to the prospect that natural genetic variability in genes, known as single nucleotide polymorphisms (SNPs, pronounced "snips"), may identify genetic risk factors for coronary artery disease that are related to known risk factors. The SNPs are common, small genetic variations that can occur within a person's deoxyribonucleic acid sequence.

The current study identifies a novel SNP in the promoter region of the glutamate-cysteine ligase catalytic subunit gene (GCLC), a gene that has an important role in the synthesis of other genes that prevent damage from oxidative stress. In addition to identifying the SNP, the study shows that the SNP is more frequently present in patients with myocardial infarction, and that it is associated with increased coronary artery endothelial dysfunction. Importantly, the report also demonstrates that the SNP in the promoter region of the gene has a functional effect on the activity of the promoter. All of these combined increase the likelihood that the SNP described in this report is a risk factor for myocardial infarction.

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Endothelial dysfunction or activation is known to be an early event in atherosclerotic development and importantly contributes to the pathogenesis of coronary artery disease (1–3). Coronary risk factors alter endothelial functions, partially through oxidative stress as a common feature of the risk factors (1–7). We have shown that oxidative stress plays a crucial role in the pathogenesis of endothelial vasomotor dysfunction in patients with coronary risk factors, including hypercholesterolemia (1), smoking (4), diabetes (5), and hypertriglyceridemia (6,7).

There are various antioxidant defense systems against oxidative stress in mammalian cells. Exposure to oxidants may initiate an adaptive intracellular antioxidant response (i.e., induction of antioxidant genes such as manganese superoxide dismutase) (8), thioredoxin reductase (9), metal-

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Abbreviations and Acronyms

ACh	= acetylcholine
bp	= basepair
DNA	= deoxyribonucleic acid
GCL	= glutamate-cysteine ligase
GCLC	= glutamate-cysteine ligase-catalytic subunit
GCLR	= glutamate-cysteine ligase-regulatory subunit
GSH	= reduced glutathione
HUVECs	= human umbilical vein endothelial cells
MI	= myocardial infarction
PBS	= phosphate-buffered saline
PCR	= polymerase chain reaction

lothionein (10), and glutathione (GSH) (11,12), protecting cells from subsequent exposure to oxidant stresses. Glutathione, a triple peptide present in virtually all cells, has a predominant role in the regulation of the intracellular redox state and protects cells from oxidative injury (12). Indeed, GSH depletion has been demonstrated to suppress endothelial nitric oxide production (13). Furthermore, we and others have shown that GSH supplementation improved an endothelial vasomotor abnormality in the coronary arteries of patients with coronary risk factors or coronary spastic angina in which oxidative stress has a pathogenic role (14–16). Glutathione is synthesized within the cells by the action of glutamate-cysteine ligase (GCL) (12,17). Glutamate-cysteine ligase is a heterodimer composed of a heavy catalytic subunit *(GCLC)* and a light regulatory subunit *(GCLR)* (12,17). The *GCLC* gene has catalytic activity and works in the physiologic concentrations of glutamine under *GCLR* (12,17). It has been shown that GSH production is paralleled with *GCLC* gene expression, which is regulated primarily at the transcription level (11,17–19). The *GCLC* gene is shown to have oxidative stress-responsive elements in the promoter/enhancer region (11,17–19). Several *cis*-acting deoxyribonucleic acid (DNA) elements contribute to the transcriptional upregulation of the *GCLC* gene in response to oxidative stress, providing a protective mechanism against oxidative stress (11,17,18).

A weakness in the defense system of vascular cells against oxidative stress, as well as an increase in oxidative stress, might potentially contribute to endothelial vasomotor dysfunction. A genetic weakness in the antioxidant defense system might have an influence on the disease susceptibility of vascular cells to coronary risk factors, as there is a considerable variation in the vasomotor response of coronary arteries among patients with the same risk factor profiles. Thus, this study examined the hypothesis that the functional variations in the promoter region of the *GCLC* gene may be present, and that they may be implicated in the endothelial vasomotor dysfunction of coronary arteries. Furthermore, we examined the possible association of gene variants with myocardial infarction (MI) in which oxidative stress-induced endothelial dysfunction may play a pathogenetic role (20).

Table 1. Clinical Characteristics of Control Subjects and Patients With MI for Analysis of the Association Between *GCLC* Gene Polymorphism and MI

	Control Subjects (n = 179)	Patients With MI (n = 255)	p Value
Age (yrs)	59 ± 13	67 ± 11	< 0.001
Hypertension	70 (39.1%)	138 (54.1%)	< 0.001
Diabetes mellitus	41 (22.9%)	87 (34.1%)	< 0.01
Total cholesterol (mg/dl)	187 ± 33	195 ± 40	< 0.05
Cigarette smoking	119 (66.5%)	146 (57.3%)	0.91
Body mass index (kg/m^2)	23.9 ± 3.4	23.7 ± 3.3	0.62

Data are presented as the mean value ± SD or number (%) of patients.
GCLC = glutamate-cysteine ligase-catalytic subunit; MI = myocardial infarction.

METHODS

Study subjects enrolled for analysis of the association of *GCLC* gene polymorphism with coronary vasomotor function. This study initially enrolled 179 consecutive male subjects (mean age 59 years [range 23 to 81 years]) who had quantitative coronary angiography with an intracoronary injection of acetylcholine (ACh) (4) at Kumamoto University Hospital. All of them underwent diagnostic cardiac catheterization for evaluation of atypical chest pain or ST-segment depression on rest or exercise electrocardiograms, without chest pain. It is well known that the coronary endothelial vasomotor response to ACh is considerably affected by coronary risk factors, especially age (21). To reduce this influence, we compared coronary vasomotor functions between all subjects with the minor allele at the polymorphic sites and age-matched subjects without the minor allele from the initially enrolled 179 subjects. Age matching was performed by a computer-based method to exclude a selection bias. All of the 179 subjects also served as control subjects for the analysis of polymorphisms with MI, and their characteristics are shown in Table 1. All of the 179 subjects had angiographically normal coronary arteries (<10% narrowing after nitrate administration) and no coronary spasm during intracoronary infusion of ACh (4). No subject had a previous MI, congestive heart failure, cardiomyopathy, valvular heart disease, left ventricular hypertrophy, or other serious diseases. All medications were withdrawn at least three days before the study. None of the study subjects had taken pharmacologic doses of antioxidants for at least one month before the study. Written, informed consent for this study and genetic analysis was obtained from all subjects before the study. The study protocol was in agreement with the national guidelines for the genetic analysis in Japan and was approved by the ethics committee at Kumamoto University Hospital.

Quantitative coronary angiography. A quantitative coronary angiographic study was performed in all of the 179 subjects in the same manner, as described in previous reports (4,6,14,15). In brief, after baseline angiography, ACh (50 μg/min) was infused directly into the left coronary artery through the Judkins catheter for 2 min, and then angiography was performed. After an additional 15 min, an

intracoronary injection of isosorbide dinitrate (1 mg) was given. Two minutes after that, coronary angiography was performed in multiple projections in all study subjects. The trunk of the left anterior descending coronary artery was divided into proximal and distal segments of equal length. The lumen diameter at the center of each segment was measured quantitatively with the use of a computer-assisted coronary angiographic analysis system (Cardio 500, Kontron Instruments, München, Germany) by two observers (H. F. and O. H.) who were blinded to the clinical data of the study subjects. Coronary artery diameter responses to infusion of ACh and nitrate were expressed as percent changes from baseline coronary diameters.

Patients and control subjects for analysis of the association of *GCLC* gene polymorphism with MI. This study also included 255 consecutive male patients with MI who underwent coronary angiography at Kumamoto University Hospital. These patients were examined for a possible association of *GCLC* gene polymorphisms with MI. The clinical characteristics of these patients with MI are shown in Table 1. Criteria for MI included chest symptoms, characteristic electrocardiographic changes, and elevation of creatine kinase to more than twice the upper limit of normal. The findings of coronary angiography and left ventriculography supported the diagnosis of MI. Written, informed consent for the genetic analysis was obtained from all patients before the study. The study protocol was also approved by the ethics committee at Kumamoto University Hospital.

All of the 179 consecutive men who were initially enrolled for analysis of the association of *GCLC* gene polymorphism with coronary vasomotor function served as control subjects for analysis of the association of *GCLC* gene polymorphism with MI.

Identification of polymorphisms in the promoter region. Genomic DNA was extracted from peripheral blood lymphocytes by using the phenol chloroform protocol. The promoter region of the *GCLC* gene was amplified by polymerase chain reaction (PCR) from genomic DNA obtained from 12 patients with MI and 12 control subjects, using 11 sets of primer pairs covering the region from −3753 to +520, on the basis of the published sequence of the *GCLC* promoter sequence (18). The PCR products were sequenced by using the ABI PRISM dye terminator cycle-sequencing kit (Perkin-Elmer, Norwalk, Connecticut) on an ABI Genetic Analyzer 373S (PE Biosystem, Foster City, California). Sequences were analyzed and compared among the patients and control subjects to detect polymorphisms.

Genotyping. We identified two polymorphisms that were not linked: −3506A/G and −129C/T. Genotypes of each polymorphism were determined by the PCR-based restriction fragment length polymorphism method by two investigators (S. S. and S. K.) who had no knowledge of the angiographic and clinical data of the control subjects and patients. The −129C/T polymorphism creates another novel site for the *Tsp*45I restriction enzyme in the presence of the T allele. A set of primers was designed to amplify a 613-base pair (bp) fragment of the *GCLC* promoter by PCR (forward: 5′-TCGTCCCAAGTCTCACAGTC-3′; reverse: 5′-CGCCCTCCCCGCTGCTCCTC-3′ (Hokkaido System Science, Sapporo, Japan), encompassing the −129C/T polymorphic site and an additional site for *Tsp*45I as a control. Subjects with the CC genotype were identified by the presence of 500- and 113-bp bands; those with the TT genotype were identified by the presence of 302-, 198-, and 113-bp bands; and those with the CT genotype were identified by the presence of all four bands, as shown in Figure 1.

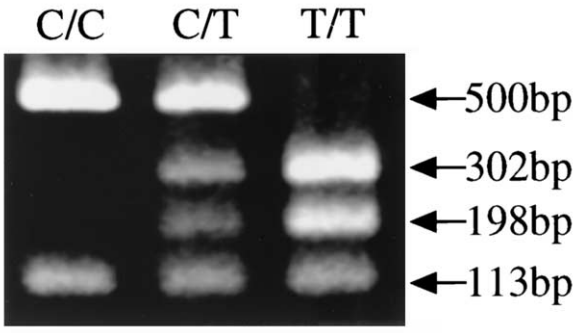

Figure 1. Ethidium bromide gel showing three genotypes from a single nucleotide polymorphism (C or T) in the promoter region 129 bases upstream of the *GCLC* gene. The 613-bp polymerase chain reaction amplification fragment contains an invariant *Tsp*45I restriction site, yielding a constant 113-bp fragment seen in **all lanes**. Individuals homozygous for the C allele have no additional *Tsp*45I restriction sites and consequently show only two bands: at 500-bp and the invariant band at 113-bp. The −129T allele creates an additional *Tsp*45I site such that homozygous T-allele individuals have three bands at 302-, 198-, and 113-bp. Heterozygous individuals show all four bands.

Similarly, the −3506A/G polymorphism creates another novel site for the *Nla*III restriction enzyme in the presence of the G allele. A set of primers was designed to amplify an 874-bp fragment of the *GCLC* promoter by PCR (forward: 5′-AAGTCCCAGGAAGAATCACA-3′; reverse: 5′-CGCTCTCCAGGAACCCATCT-3′ (Hokkaido System Science), encompassing the −3506A/G polymorphic site and an additional site for *Nla*III as a control. Subjects with the AA genotype were identified by the presence of 722-, 104-, and 48-bp bands; those with the GG genotype were identified by the presence of 513-, 209-, 104-, and 48-bp bands; and those with the AG genotype were identified by the presence of all five bands.

Cell culture. Primary cultures of human umbilical vein endothelial cells (HUVECs) were obtained as previously described (22). The HUVECs at second passage were used in this study.

Construction of *GCLC* reporter vectors containing the −129C/Tpolymorphic site. Recombinant expression vectors were created by cloning restriction fragments isolated from the 5′-flanking sequences of the *GCLC* gene into pGL3-Basic (Promega, Madison, Wisconsin) for determination of promoter activity. Two DNA fragments covering

Table 2. Genotype Frequencies of *GCLC* −129C/T Polymorphism in Control Subjects and Patients With MI

	Control Subjects (n = 179)	Patients With MI (n = 255)	OR (95% CI)	p Value
−129C/T				
GCLC/TT	1/179 (0.5%)	8/255 (3.1%)	—	—
GCLC/CT	30/179 (16.8%)	64/255 (25.1%)	—	—
GCLC/CC	148/179 (82.7%)	183/255 (71.8%)	—	—
−129T allele vs. C allele	—	—	1.90 (1.23–2.92)	< 0.005
−129TT and CT vs. CC	—	—	1.88 (1.17–3.02)	< 0.01
−129TT vs. CT and CC	—	—	5.77 (0.72–46.53)	0.059*

*Fisher's exact probability test. Data are presented as the number (%) of patients.
CI = confidence interval; *GCLC* = glutamate-cysteine ligase-catalytic subunit; MI = myocardial infarction; OR = odds ratio.

the region from −1393 to +493 were amplified by PCR with genomic DNA (from −129C wild-type homozygote or −129T variant homozygote) and sequenced. The fragments were isolated by *Xho* I/*Hind* III restriction digestion and cloned into the *Xho* I/*Hind* III sites of pGL3-Basic, creating the recombinant plasmid pGL3-$GCLC_{wildtype;\ -129C}$ and pGL3-$GCLC_{variant;\ -129T}$, respectively.

Luciferase reporter gene assay. The DNA was introduced into the cells by the liposome-mediated transfection method. Briefly, subconfluent cultures of HUVECs in a six-well tissue culture plate were transfected using LIPOFECTIN Reagent (Life Technologies, Inc., Rockville, Maryland). The transfection used 1 μg of pGL3 luciferase reporter vector with the *GCLC* promoters and 0.025 μg of PRL-TK control vector (Promega), with the herpes simplex virus thymidine kinase promoter to provide constitutive expression of Renilla luciferase expression in 2 ml of serum-free medium per one well. Forty-eight hours after transfection, the transfected cells were treated for 18 h with 100 μmol/l of H_2O_2 or phosphate-buffered saline (PBS) as a time control. The treated cells were harvested using passive lysis buffer (Promega). Luciferase activity was measured using a dual-luciferase assay system (Promega) and luminometer. Luciferase levels were expressed in arbitrary units after normalization to Renilla luciferase levels.

Nuclear extracts preparation and electrophoretic mobility shift assay. Confluent HUVECs were treated with 100 μmol/l of H_2O_2 or PBS in serum-free medium for 1 to 18 h. After the treatment, nuclear extraction from the cells and electrophoretic mobility shift assay were performed as described previously (22). The sequences of the probes containing the −129C/T polymorphic site were as follows: $GCLC_{wildtype;\ -129C}$: 5′-GCTCCCCTCAACTG*C*GACCCAATCACCCTT-3′; $GCLC_{variant;\ -129T}$: 5′-GCTCCCCTCAACTG*T*GACCCAATCACCCTT-3′ (Hokkaido System Science).

Statistical analysis. Mean values of continuous variables with normal distribution and frequencies between groups were compared by the unpaired *t* test and chi-square analysis or the Fisher exact test, respectively. To evaluate the −129T polymorphism as an independent risk factor differing between patients with MI and control subjects, multiple logistic regression analysis was performed using the following factors as categorical co-variates: age (≥70 years), smoking history (defined as smoking ≥10 cigarettes per day for ≥10 years), hypertension (>140/90 mm Hg or current treatment with antihypertensive medication), diabetes mellitus (according to the American Diabetes Association report [23]), hypercholesterolemia (>220 mg/dl), body mass index (>26 kg/m^2), and the −129T polymorphism (TT and CT genotypes). Statistical significance was defined as $p < 0.05$. Analyses were performed partly using StatView version 5.0 (SAS Institute, Cary, North Carolina).

RESULTS

Identification of *GCLC* gene polymorphisms and association with MI. Two novel polymorphisms (−129C/T and −3506A/G) were identified in the 5′-flanking region of the *GCLC* gene. The two polymorphisms were not linked. Figure 1 shows representative agarose gels loaded with PCR products encompassing the −129C/T polymorphic site after digestion with *Tsp*45I.

The −129TT, CT, and CC genotypes were present in 8 (3.1%), 64 (25.1%), and 183 (71.8%) of the 255 consecutive male patients with MI, respectively, and they were present in 1 (0.5%), 30 (16.8%), and 148 (82.7%) of the 179 male control subjects, respectively. The genotype distribution in either patients with MI or control subjects was consistent with the population being in Hardy-Weinberg equilibrium. In analyses of the additive and dominant effects of the −129T polymorphism, the frequencies were significantly higher in patients with MI than in control subjects ($p < 0.01$), as shown in Table 2. The −129T polymorphism (TT and CT genotypes) was a significant risk for MI, independent of the traditional coronary risk factors in multivariate logistic regression analysis (odds ratio [OR] 1.81, 95% confidence interval [CI] 1.08 to 3.03; $p = 0.03$).

The frequency of the −3506A/G polymorphism was comparable between patients with MI and control subjects (data not shown).

Effects of *GCLC* gene polymorphisms on coronary vasomotor responses. The frequencies of coronary risk factors were comparable between subjects with and without the −129T allele, as shown in Table 3. Intracoronary ACh infusion dilated 13 proximal and 15 distal coronary seg-

Table 3. Clinical Characteristics of Study Subjects for Analysis of the Association Between −129C/T Polymorphism and Coronary Vasomotor Function

	−129CC Genotype (n = 31)	−129CT or TT Genotype (n = 31)	p Value
Age (yrs)	61 ± 10	61 ± 10	NS
Diabetes mellitus	11 (35.5%)	7 (22.6%)	NS
Cigarette smoking	25 (80.6%)	24 (77.4%)	NS
Hypertension	11 (35.5%)	10 (32.3%)	NS
Total cholesterol (mg/dl)	182 ± 27	195 ± 29	NS
Body mass index (kg/m^2)	23.7 ± 2.4	24.1 ± 3.3	NS

Data are presented as the mean value ± SD or number (%) of patients.
MI = myocardial infarction; NS = not significant.

ments and constricted the remaining segments (18 proximal and 16 distal segments), resulting in a dilator response of both coronary segments, as a whole, in the −129CC subjects (Fig. 2). In contrast, the ACh infusion dilated six proximal and five distal coronary segments and constricted the remaining segments (25 proximal and 26 distal segments), resulting in a constrictor response of both coronary segments, as a whole, in the −129CT and TT subjects (Fig. 2). Nitrate dilated coronary arteries with a comparable magnitude between subjects with and without the T allele (percent diameter change from baseline in proximal segment: 24 ± 1% in the −129CC subjects vs. 24 ± 2% in the −129CT and TT subjects, p = NS; distal segment: 24 ± 2% in the −129CC subjects vs. 25 ± 2% in the −129CT and TT subjects, p = NS). The −3506A/G polymorphism did not affect the coronary vasomotor response to either ACh or nitrate (data not shown).

Promoter activities of the *GCLC* gene. We examined the effects of only the −129C/T polymorphism on the promoter activities because this polymorphism, but not the −3506A/G, was associated with coronary vasomotor dysfunction and MI. The luciferase activity in cells transfected with the construct containing the −129T allele was significantly lower than that in cells with the −129C allele in the control condition (PBS), as shown in Figure 3. The luciferase levels were induced in either cells with the −129T or −129C allele when cells were treated with H_2O_2, but the induced levels were significantly lower in cells with the −129T allele than in those with the −129C allele, as shown in Figure 3.

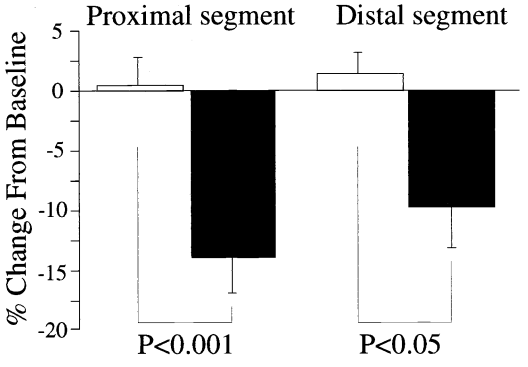

Figure 2. Percent changes (mean ± SEM) in lumen diameter from baseline in response to acetylcholine in the proximal and distal segments of the left anterior descending coronary arteries in subjects with the −129C/T or T/T genotype **(solid bars)** (n = 31) and in age-matched subjects with the −129C/C genotype **(open bars)** (n = 31).

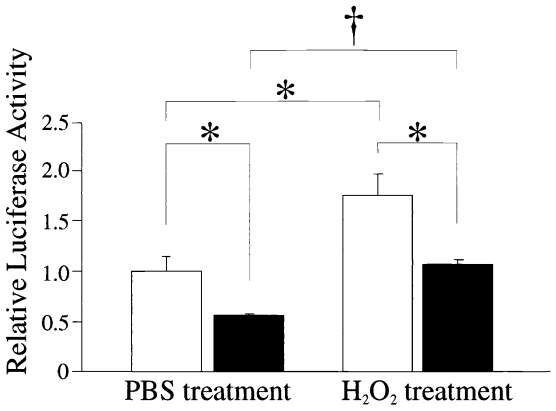

Figure 3. Effects of the −129C/T polymorphism on *GCLC* promoter activity in human umbilical vein endothelial cells. The transfected cells were incubated for 18 h with H_2O_2 (100 μmol/l) or phosphate-buffered saline (PBS) (as a time control). Promoter activity is expressed as relative luciferase activity normalized to Renilla activity. Data are presented as the mean value ± SEM from eight independent experiments. *$p < 0.05$, †$p < 0.01$. **Open bars** = −129C; **solid bars** = −129T.

Electrophoretic mobility shift assay. A nuclear protein complex with the sequence of *GCLC* gene promoter containing the −129C allele was observed in the control condition (PBS), as shown in Figure 4. The nuclear protein complex with the −129C probe was induced earlier when cells were treated with H_2O_2. However, the specific complex with the sequence containing the −129T allele was very weakly observed in either the control condition (PBS) or with H_2O_2 treatment, as shown in Figure 4.

DISCUSSION

Association of −129C/T polymorphism of the *GCLC* gene with coronary vasomotor dysfunction. The present study identified two polymorphisms in the promoter region of the *GCLC* gene: −129C/T and −3506A/G. The association study showed that subjects with −129C/T and T/T genotypes are highly associated with abnormal vasomotor reactivity in epicardial coronary arteries, as reflected by enhanced constriction or impaired dilation in response to ACh, whereas the −3506A/G polymorphism was not associated. The epicardial coronary dilator response to nitrate, an endothelium-independent dilator, was not significantly different between the −129C/C genotype and C/T and T/T genotypes. Thus, endothelial vasomotor function of coronary arteries is impaired in subjects with the −129T allele.

Glutathione is synthesized from its constituent amino acids in two sequentials by GCL and GSH synthetase (12). Glutamylcysteine, synthesized by GCL, is rapidly converted to GSH by GSH synthetase. Glutamate-cysteine ligase is the rate-limiting enzyme in GSH synthesis, whereas GSH

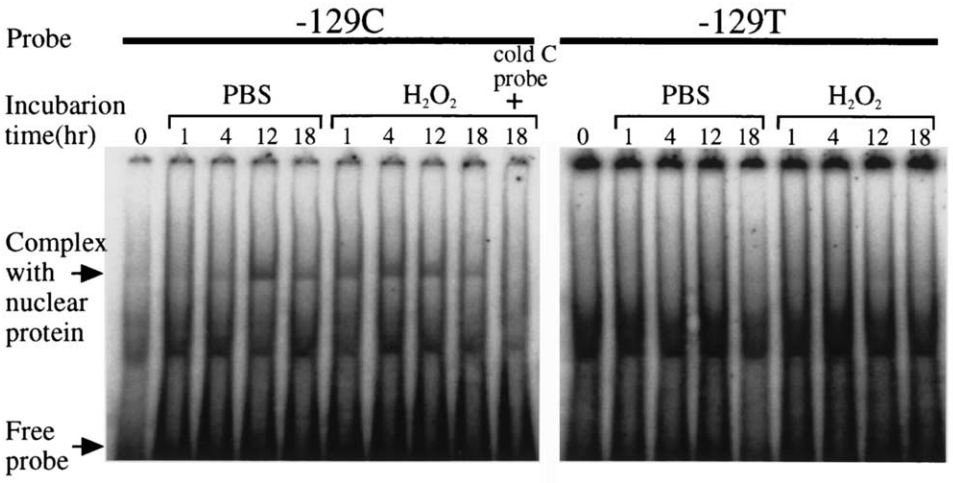

Figure 4. Electrophoretic mobility shift assay: allele-specific binding of nuclear protein to the −129C/T polymorphic site. Nuclear extracts were obtained from human umbilical vein endothelial cells after treatment for the indicated time with H_2O_2 (100 μmol/l) or phosphate-buffered saline (PBS) (as a time control). **Left panel** = interaction with the −129C probe; **right panel** = interaction with the −129T probe.

synthetase had apparently no regulatory role (12,17). When cells are challenged with sublethal oxidative stress or GSH depletion, *GCLC* gene expression was upregulated through activation of oxidative stress-responsive elements in the promoter regions (11,17–19). This leads to GSH synthesis and provides a protective/adaptive mechanism against oxidative stress (11,12). In this context, the present study demonstrated that the −129T allele had lower promoter activity either in the control condition or after H_2O_2 treatment. Thus, the −129T polymorphism may suppress the increase in *GCLC* gene expression in response to oxidative stress, and it may possibly weaken the intracellular production of GSH in response to oxidative stress, leading to an increase in the susceptibility to oxidant-induced endothelial injury, which is thought to occur as a part of the pathogenesis of coronary vasomotor abnormality. Furthermore, the present gel-shift assay showed that the exposure of human endothelial cells to H_2O_2 increased the binding of one nuclear protein factor to the oligonucleotide probe around the −129 position with the C allele, whereas the same band was very weakly observed with the T allele probe. Thus, the nuclear protein bound more strongly to the sequence with the C allele may be an activator of transcriptional activity. Although there is no putative enhancer element that contains the −129 position on the computer-based data research, there are several binding sites for nuclear proteins (i.e., CCAAT binding protein, near the −129C/T polymorphic site). Thus, it is possible that the −129T allele might modify the binding of nuclear proteins to unidentified *cis*-elements around the −129 position, leading to suppression of *GCLC* gene expression.

Association of −129C/Tpolymorphism of GCLC gene with MI. Oxidative stress upregulates endothelial expression of pro-atherothrombogenic molecules and causes endothelial vasomotor dysfunction in coronary arteries, leading to coronary events (1–7). Also, GSH suppresses the induction of these molecules and improves abnormal endothelial vasomotor function (7,14,15). The present study also showed that the −129T allele is highly frequent in patients with MI. Although this association was only marginal and based on a small population size, the present results indicate that the −129T allele may increase the susceptibility of oxidative stress-induced endothelial dysfunction or activation, leading to atherothrombotic events in patients with MI.

Previous reports. Evidence suggests that low levels of serum GSH are a risk of coronary artery disease (24). Administration of buthionine sulfoximine, an inhibitor of GCL activity, for several weeks severely suppressed both basal and inducible GSH production to <20% of normal GSH levels in animal models, resulting in cell damage in the kidney, lung, and brain (25). It has been previously reported that a genetic defect in *GCLC* is associated with a severe decrease in the intracellular GSH content and causes hemolytic anemia and neurologic and psychiatric disorders (26,27). However, there was no description regarding cardiovascular disorders in these previous reports, probably because intracellular GSH levels were undetectable or extremely low, leading to severe cell damage in major organs, such as the liver, kidney, or brain, where GSH has a crucial role in their functions rather than cardiovascular systems. Therefore, the animals treated with buthionine sulfoximine and the patients with a *GCLC* complete defect may not serve as a model of MI, which develops over an extremely long-term process and occurs in older age in subjects with the present polymorphism in *GCLC* gene.

Study limitations. This study is limited by the relatively small number of control subjects and patients studied. It is hard to demonstrate direct evidence that intracellular GSH levels in coronary vascular cells are decreased in subjects with the −129T allele. Furthermore, the mechanisms by which the −129T polymorphism in the *GCLC* gene is

linked to pathogenesis of coronary endothelial vasomotor dysfunction and MI remain undefined. We cannot exclude the possibility that this polymorphism is a marker for other functional gene variants. Also, a case-control study has a cross-sectional nature, and it may have an inherent selection bias of cases and controls. A longitudinal study with a large number of study patients with homogeneous risk is required to assess the precise role of this gene variant in the pathogenesis of cardiovascular diseases.

Conclusions. The −129T polymorphism of the *GCLC* gene may suppress *GCLC* gene induction, and it is implicated in coronary endothelial vasomotor dysfunction and MI.

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