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# Association of Gene Polymorphisms With Coronary Artery Disease in Low- or High-Risk Subjects Defined by Conventional Risk Factors

Akihiro Hirashiki, MD,* Yoshiji Yamada, MD, PHD,† Yosuke Murase, MD,* Yoriyasu Suzuki, MD,* Hiroki Kataoka, MD,* Yasutsugu Morimoto, MD,* Toru Tajika, MD,* Toyoaki Murohara, MD, PHD,‡ Mitsuhiro Yokota, MD, PHD, FACC§

*Okazaki, Kakamigahara, and Nagoya, Japan*

**OBJECTIVES** The aim of the study was to identify genes that confer susceptibility to coronary artery disease (CAD) in low- or high-risk men or women separately and thereby to assess the genetic risk of CAD in such individuals.

**BACKGROUND** The prevention of CAD would be facilitated by the identification of genes that confer susceptibility to this condition independently in low- or high-risk individuals, as defined by conventional risk factors.

**METHODS** The study population comprised 1,661 unrelated Japanese individuals, including 1,011 patients with CAD and 650 control subjects. Among all study subjects, 601 individuals (high-risk subjects) had hypertension, diabetes mellitus, and hypercholesterolemia, and 1,060 individuals (low-risk subjects) had none of these risk factors for CAD. The genotypes for 37 polymorphisms of 31 candidate genes were determined by a fluorescence- or colorimetry-based allele-specific DNA primer-probe assay system.

**RESULTS** Multivariate logistic regression analysis, with adjustment for age, body mass index, and the prevalence of smoking and hyperuricemia, revealed that the −219G→T polymorphism of the apolipoprotein E gene in low-risk men, the −1171/5A→6A polymorphism of the stromelysin-1 gene in low-risk women, the 1019C→T polymorphism of the connexin 37 gene in high-risk men, and the 3932T→C polymorphism of the apolipoprotein E gene in high-risk women were significantly associated with CAD. A stepwise forward selection procedure revealed that the effects of these polymorphisms on CAD were statistically independent of age or conventional risk factors.

**CONCLUSIONS** Genotyping of these polymorphisms may prove informative for assessment of the genetic risk of CAD in low- or high-risk men or women. (J Am Coll Cardiol 2003;42:1429–37)
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Coronary artery disease (CAD) is a multifactorial disorder that is thought to result from an interaction between genetic background and environmental factors such as diet, smoking, and physical activity. It is usually associated with conventional risk factors, including hypertension, diabetes mellitus, and hypercholesterolemia (1). However, in some individuals, CAD is not associated with such risk factors, suggesting that other genetic factors contribute to a predisposition to coronary atherosclerosis and its thrombotic complications (2). In general, individuals with hypertension, diabetes mellitus, and hypercholesterolemia and those with none of these factors are considered at high and low risk, respectively, for development of CAD. It is thus important to identify genes that confer susceptibility to CAD in these high- and low-risk individuals independently.

Genetic epidemiologic studies have suggested that certain genetic variants, including polymorphisms in the genes encoding platelet glycoprotein IIIa (3), methylenetetrahydrofolate reductase (4), and plasminogen-activator inhibitor-1 (5), are associated with an increased prevalence of CAD in high- or low-risk subjects. However, the genes that contribute to genetic susceptibility to CAD in individuals with three major conventional risk factors—hypertension, diabetes mellitus, and hypercholesterolemia—or in those with none of these factors remain to be identified. In addition, because of ethnic divergence of gene polymorphisms, it is important to examine polymorphisms related to CAD in low- or high-risk individuals of each ethnic group.

In a previous association study of 112 polymorphisms in 71 genes of 445 individuals with myocardial infarction (MI) and 464 control subjects, we identified 19 and 18 polymor-

From the *Division of Cardiology, Okazaki City Hospital, Okazaki; †Department of Gene Therapy, Gifu International Institute of Biotechnology, Kakamigahara; and the Departments of ‡Cardiology and §Clinical Pathophysiology, Nagoya University Graduate School of Medicine, Nagoya, Japan. This work was supported in part by a grant-in-aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (Tokyo; to Dr. Yokota); a grant from Mitsui Life Social Welfare Foundation (Tokyo; to Dr. Yokota); a grant-in-aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (Tokyo; to Dr. Yamada); a grant from the Japan Cardiovascular Research Foundation (Osaka; to Dr. Yamada); and a grant from the Takeda Science Foundation (Osaka; to Dr. Yamada).

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**Abbreviations and Acronyms**

BMI = body mass index
BP = blood pressure
CAD = coronary artery disease
$HbA_{1c}$ = glycosylated hemoglobin
LDL = low-density lipoprotein
MI = myocardial infarction
SNP = single nucleotide polymorphism

phisms that are possibly related to MI in Japanese men and women, respectively (6). We have performed an association study for 37 polymorphisms of 31 candidate genes (including the polymorphisms previously related to MI) and CAD in the absence or presence of hypertension, diabetes mellitus, and hypercholesterolemia. Our aim was to identify genes that confer susceptibility to CAD in low- or high-risk men or women independently and thereby to assess the genetic risk of CAD in such individuals separately.

## METHODS

**Study population.** The study population comprised 1,661 unrelated Japanese individuals (1,060 men and 601 women) who either visited outpatient clinics of or were admitted to one of the participating hospitals (Appendix) between July 1994 and December 2001, either because they were experiencing various symptoms or for a medical checkup and who were found to have either hypertension (systolic blood pressure [BP] ≥140 mm Hg or diastolic BP ≥90 mm Hg, or both), diabetes mellitus (fasting blood glucose ≥6.93 mmol/l or glycosylated hemoglobin [$HbA_{1c}$] ≥6.5%, or both), and hypercholesterolemia (serum total cholesterol ≥5.72 mmol/l) or none of these major risk factors for CAD. A total of 1,011 subjects (696 men and 315 women) had CAD; all of these individuals underwent coronary angiography and left ventriculography. The diagnosis of CAD was defined as >50% stenosis in any major coronary artery, as revealed by coronary angiography. Among the 1,011 CAD subjects, 744 (535 men and 209 women) had MI. The 650 control subjects (364 men and 286 women) exhibited normal electrocardiograms at rest and no signs of myocardial ischemia during exercise stress testing; these examinations were performed in control subjects as part of a medical checkup in the absence of cardiovascular symptoms. Among all 1,661 study subjects, the 601 individuals (364 men and 237 women) with hypertension, diabetes mellitus, and hypercholesterolemia were classified as high risk, and the 1,060 individuals (696 men and 364 women) with none of these conditions were classified as low risk. Individuals with valvular heart disease, congenital malformations of the heart or vessels, or metabolic or endocrinologic diseases, as well as those taking drugs that cause secondary hypertension, diabetes mellitus, or hypercholesterolemia, were excluded from the study. The study protocol was approved by the Committees on the Ethics of Human Research of Nagoya University Graduate School of Medicine, Gifu International Institute of Biotechnology, Okazaki City Hospital, Kosei Hospital, and Nagoya Daini Red Cross Hospital, and written, informed consent was obtained from each participant.

**Selection of candidate gene polymorphisms for CAD.** We previously performed a screening association study with 112 polymorphisms of 71 genes in 451 men (219 patients with MI and 232 controls) and 458 women (226 patients with MI and 232 controls) (6). From this screening study, we identified 19 and 18 polymorphisms possibly related to MI ($p < 0.1$) in men and women, respectively (four polymorphisms were related to MI in both men and women). In addition to these 33 polymorphisms of 27 genes, for the present study we selected four polymorphisms of four genes (angiotensin I-converting enzyme, angiotensin II receptor type 1, glycoprotein IIIa, and methylenetetrahydrofolate reductase genes) that have been associated with cardiovascular disease. Most of the 37 polymorphisms of these 31 genes are located in the promoter region, exons, or splice donor or acceptor sites in introns and might be expected to affect the function of the encoded protein or its expression (Table 1). We therefore examined the possible association of these 37 polymorphisms with CAD.

**Genotyping of polymorphisms.** Venous blood (7 ml) was collected from each subject into tubes containing 50 mmol/l EDTA (disodium salt), and genomic DNA was isolated with a kit (Qiagen, Chatsworth, California). The genotypes of polymorphisms were determined by a fluorescence- or colorimetry-based allele-specific DNA primer-probe assay system, as previously described (6) (Table 2).

**Statistical analysis.** Quantitative clinical data were compared between patients with CAD and control subjects by the unpaired Student *t* test. Qualitative data were compared by the chi-square test. Allele frequencies were estimated by the gene counting method, and the chi-square test was used to identify significant departures from the Hardy-Weinberg equilibrium. We performed multivariate logistic regression analysis to adjust risk factors, with CAD as a dependent variable and age, gender, body mass index (BMI), smoking status (0 = nonsmoker; 1 = smoker), hyperuricemia (0 = no history; 1 = positive history), and the genotype of each polymorphism as independent variables. Each genotype was assessed according to dominant, recessive, and additive genetic models, and the p value, odds ratio, and 95% confidence interval were calculated (JMP version 5; SAS Institute, Cary, North Carolina). We also performed a stepwise forward selection procedure to examine the effects of genotypes as well as other characteristics on CAD. Unless indicated otherwise, a p value <0.05 was considered statistically significant.

## RESULTS

We first examined the relation of 37 gene polymorphisms to CAD in the total study population of 1,661 subjects, whose characteristics are shown in Table 3. Age and the percentage

**Table 1.** Gene Polymorphisms Examined for Association With Coronary Artery Disease

| Gene | Symbol | Polymorphism | Function of Protein |
|---|---|---|---|
| Angiotensin I-converting enzyme | *ACE* | Insertion/deletion in intron 16 | Conversion of angiotensin I to angiotensin II |
| Angiotensin II receptor type 1 | *AGTR1* | −535C→T | Type 1 cell surface receptor for angiotensin II |
| Angiotensinogen | *AGT* | −6G→A | Precursor of angiotensin I |
| Apolipoprotein C-III | *APOC3* | −482C→T | Major component of triglyceride-rich lipoprotein and HDL |
| Apolipoprotein C-III | *APOC3* | 1100C→T | |
| Apolipoprotein E | *APOE* | −219G→T | Component of chylomicrons and very-low-density lipoprotein remnants and ligand for the LDL receptor and LDL receptor-like protein |
| Apolipoprotein E | *APOE* | 3932T→C (Cys112Arg) | |
| Apolipoprotein E | *APOE* | 4070C→T (Arg158Cys) | |
| ATP-binding cassette transporter-1 | *TAP1* | 1051G→A (Arg219Lys) | Cholesterol efflux pump in cellular lipid removal pathway |
| CD14 | *CD14* | −260C→T | Myeloid cell surface receptor for lipopolysaccharide |
| CC chemokine receptor-2 | *CCR2* | 190G→A (Val64Ile) | Receptor for monocyte chemoattractant protein-1 |
| Connexin-37 | *GJA4* | 1019C→T (Pro319Ser) | Gap junction component in endothelium and other tissues |
| Endothelial nitric oxide synthase | *NOS3* | −786T→C | Production of nitric oxide from L-arginine |
| Endothelin-1 | *EDN1* | 5665G→T (Lys198Asn) | Vasoconstrictor produced by vascular endothelial cells |
| E-selectin | *SELE* | 561A→C (Ser128Arg) | Adhesion of leukocytes to vascular endothelial cells |
| Fatty acid-binding protein-2 | *FABP2* | 2445G→A (Ala54Thr) | Uptake, metabolism, and transport of long-chain fatty acids |
| G protein $\beta 3$ subunit | *GNB3* | 825C→T (splice variant) | Signal transduction by pertussis toxin–sensitive G proteins |
| Glycoprotein IIIa | *ITGB3* | 1565T→C (Leu33Pro) | Platelet receptor for fibrinogen and von Willebrand factor |
| Glycoprotein Ia | *ITGA2* | 1648A→G (Lys505Glu) | Collagen receptor on platelets and other cell types |
| Glycoprotein Ib-alpha | *GPIBB* | 1018C→T (Thr145Met) | Platelet surface receptor for von Willebrand factor |
| Insulin receptor substrate-1 | *IRS1* | 3494G→A (Gly972Arg) | Substrate of insulin receptor tyrosine kinase |
| Interleukin-6 | *IL6* | −634C→G | Promotion and regulation of acute inflammatory response |
| Interleukin-10 | *IL10* | −819T→C | Anti-inflammatory cytokine that inhibits cytotoxic activity of and cytokine synthesis by macrophages |
| Interleukin-10 | *IL10* | −592A→C | |
| 5,10-methylenetetrahydrofolate reductase | *MTHFR* | 677C→T (Ala222Val) | Catalysis of the methylation of homocysteine |
| NADH/NADPH oxidase p22 phox | *p22-PHOX* | 242C→T (His72Tyr) | Production of superoxide in vascular cells |
| Paraoxonase-1 | *PON1* | 584G→A (Gln192Arg) | Confers antioxidant properties on HDL |
| Plasminogen activator inhibitor-1 | *PAI1* | −668/4G→5G | Inhibition of the conversion of plasminogen to plasmin |
| Platelet-activating factor acetylhydrolase | *PLA2G7* | 994G→T (Val279Phe) | Hydrolysis of platelet-activating factor and related lipids |
| Stromelysin-1 | *MMP3* | −1171/5A→6A | Matrix metalloproteinase with a broad substrate specificity |
| Thrombomodulin | *THBD* | 2136C→T (Ala455Val) | Vascular endothelial cell surface receptor for thrombin |
| Thrombopoietin | *THPO* | 5713A→G | Stimulation of platelet production from megakaryocytes |
| Thrombospondin-4 | *THBS4* | 1186G→C (Ala387Pro) | Cell adhesion, angiogenesis, and ligand for CD36 |
| Transforming growth factor-beta$_1$ | *TGFB1* | 869T→C (Leu10Pro) | Regulates cell proliferation, differentiation, and function |
| Tumor necrosis factor-alpha | *TNFA* | −863C→A | Pro-inflammatory cytokine with effects on lipid metabolism, blood coagulation, insulin resistance, and vascular endothelial function |
| Tumor necrosis factor-alpha | *TNFA* | −850C→T | |
| Tumor necrosis factor-alpha | *TNFA* | −238G→A | |

ATP = adenosine triphosphate; HDL and LDL = high- and low-density lipoprotein, respectively.

**Table 2.** Primers, Probes, and Other Conditions for Genotyping

| Gene | SNP | Labels | Primers (5′→3′) | Cycles | Probes (5′→3′) | Formamide |
|---|---|---|---|---|---|---|
| Apolipoprotein C-III | −482C→T | | CGGAGCCACTGATGCXCG | | AGCCACTGATGCXCGGTCT | |
| | | | CGGAGCCACTGATGCXTG | 35 | AGCCACTGATGCXTGGTCT | 30% |
| | | Biotin | TGTTTGGAGTAAAGGCACAGAA | | | |
| Apolipoprotein E | −219G→T | FITC | GAATGGAGGAGGGTGTCTXGA | | | |
| | | TxR | AGAATGGAGGAGGGTGTCTXTA | 35 | | |
| | | Biotin | CCAGGAAGGGAGGACACCTC | | | |
| Apolipoprotein E | 3932T→C | FITC | GGACATGGAGGACGTXCG | | | |
| | | TxR | CGGACATGGAGGACGTXTG | 40 | | |
| | | Biotin | CGCGGTACTGCACCAGGC | | | |
| CC chemokine receptor 2 | 190G→A | FITC | GCAGTTTATTAAGATGAGGXCG | | | |
| | | TxR | TTGCAGTTTATTAAGATGAGGXTG | 40 | | |
| | | Biotin | GGTGCTCCCTGTCATAAATTTGA | | | |
| Connexin 37 | 1019C→T | TxR | CTCAGAATGGCCAAAAXCC | | | |
| | | FITC | CCTCAGAATGGCCAAAAXTC | 35 | | |
| | | Biotin | GCAGAGCTGCTGGGACGA | | | |
| Endothelial nitric oxide synthase | −786T→C | TxR | ATCAAGCTCTTCCCTGGXCG | | | |
| | | FITC | ATCAAGCTCTTCCCTGGXTG | 35 | | |
| | | Biotin | TCAGCAGAGAGACTAGGGCTGA | | | |
| E-selectin | 561A→C | | ACATTCACCGTGGCCAXTG | | CACCGTGGCCAXTGCAGGAT | |
| | | | CATTCACCGTGGCCAXGG | 35 | CACCGTGGCCAXGGCAGGAT | 45% |
| | | Biotin | AGCTGCCTGTACCAATACATCC | | | |
| Fatty acid-binding protein 2 | 2445G→A | | TCACAGTCAAAGAATCAAGXGC | | GAATCAAGXGCTTTTCGAAACATT | |
| | | | ATTCACAGTCAAAGAATCAAGXAC | 40 | GAATCAAGXACTTTTCGAAACATT | 37.5% |
| | | Biotin | CAAAAACAACTTCAATGTTTCGA | | | |
| Glycoprotein Ibα | 1018C→T | FITC | CCCAGGGCTCCTGXCG | | | |
| | | TxR | CCCCAGGGCTCCTGXTG | 40 | | |
| | | Biotin | TGAGCTTCTCCAGCTTGGGTG | | | |
| Insulin receptor substrate-1 | 3494G→A | | GGGCCCTGCACCTCCXGG | | CACCTCCXGGGGCTGCTAG | |
| | | | GGGCCCTGCACCTCCXAG | 40 | CACCTCCXAGGGCTGCTAG | 35% |
| | | Biotin | GGGTAGGCCTGCAAATGCTA | | | |
| Interleukin-10 | −819T→C | | TACCCTTGTACAGGTGATGTAXTA | | GTACAGGTGATGTAXTATCTCTGTG | |
| | | | TACCCTTGTACAGGTGATGTAXCA | 35 | GTACAGGTGATGTAXCATCTCTGTG | 40% |
| | | Biotin | ATAGTGAGCAAACTGAGGCACA | | | |
| Interleukin-10 | −592A→C | FITC | CAGAGACTGGCTTCCTACAXGA | | | |
| | | TxR | CCAGAGACTGGCTTCCTACAXTA | 35 | | |
| | | Biotin | GCCTGGAACACATCCTGTGA | | | |
| NADH/NADPH oxidase p22 phox | 242C→T | FITC | ACCACGGCGGTCATGXGC | | | |
| | | TxR | ACCACGGCGGTCATGXAC | 40 | | |
| | | Biotin | GCAGCAAAGGAGTCCCGAGT | | | |
| Platelet-activating factor acetylhydrolase | 994G→T | FITC | TTCTTTTGGTGGAGCAACXGT | | | |
| | | TxR | ATTCTTTTGGTGGAGCAACXTT | 40 | | |
| | | Biotin | TCTTACCTGAATCTCTGATCTTCA | | | |
| Stromelysin-1 | −1171/5A→6A | FITC | TTTGATGGGGGGAAAAXAC | | | |
| | | TxR | TTGATGGGGGGAAAAXCC | 40 | | |
| | | Biotin | CCTCATATCAATGTGGCCAA | | | |
| Thrombomodulin | 2136C→T | FITC | CCCGACTCGGCCCTTXCC | | | |
| | | TxR | CCCGACTCGGCCCTTXTC | 40 | | |
| | | Biotin | GTCACAGTCGGTGCCAATGT | | | |

**Table 3.** Characteristics of All Study Subjects

| Characteristic | Controls (n = 650) | Subjects With CAD (n = 1,011) |
|---|---|---|
| Age (yrs) | 55.9 ± 11.5 | 62.8 ± 9.8* |
| Men/women (%) | 56.0/44.0 | 68.8/31.2* |
| Body mass index (kg/m$^2$) | 23.2 ± 3.0 | 23.4 ± 3.0 |
| Smoking (%) | 40.2 | 40.9 |
| Hypertension (%) | 34.2 | 37.5 |
| Diabetes mellitus (%) | 34.2 | 37.5 |
| Hypercholesterolemia (%) | 34.2 | 37.5 |
| Hyperuricemia (%) | 8.6 | 11.6 |

*$p < 0.0001$ versus corresponding controls. Data for age and body mass index are presented as the mean value ± SD.
CAD = coronary artery disease.

of men were greater among subjects with CAD than among controls. Multivariate logistic regression analysis with adjustment for age, gender, BMI, and the prevalence of smoking and hyperuricemia revealed that 10 single nucleotide polymorphisms (SNPs) were related to CAD in the total study population on the basis of a p value <0.05 in a dominant, recessive, or additive genetic model (Table 4).

We next divided the study population into men and women as well as low- and high-risk individuals. The characteristics of the 1,060 male subjects are shown in Table 5. For low-risk men, age was higher and the prevalence of smoking was lower in subjects with CAD than in controls. For high-risk men, age and the prevalence of hyperuricemia were higher in subjects with CAD than in controls. There were no differences in systolic or diastolic BP, fasting blood glucose, $HbA_{1c}$ in blood, or the serum concentration of total cholesterol between CAD patients and controls for either low- or high-risk men. The characteristics of the 601 female subjects are shown in Table 6. For low-risk women, age and BMI were higher and the prevalence of hyperuricemia was lower in subjects with CAD than in controls. For high-risk women, the prevalence of hyperuricemia was higher in subjects with CAD than in controls. There were no differences in systolic or diastolic BP, fasting blood glucose, $HbA_{1c}$ in blood, or the serum concentration of total cholesterol between CAD patients and controls for either low- or high-risk women.

Multivariate logistic regression analysis with adjustment for age, BMI, and the prevalence of smoking and hyperuricemia revealed that three and eight SNPs were related to CAD in low- and high-risk men, respectively (Table 7), and that four and three SNPs were related to CAD in low- and high-risk women, respectively (Table 8), on the basis of a p value <0.05 in a dominant, recessive, or additive genetic model. However, because of the multiple comparisons of genotypes, we considered a p value <0.005 to be significant for such associations. On the basis of this criterion, the −219G→T SNP of the apolipoprotein E gene (*APOE*) was significantly associated with CAD in low-risk men, and the 1019C→T SNP of the connexin 37 gene (*GJA4*) was associated with CAD in high-risk men (Table 7). Also, the −1171/5A→6A SNP of the stromelysin-1 gene (*MMP3*) was significantly associated with CAD in low-risk women, and the 3932T→C SNP of *APOE* was associated with CAD in high-risk women (Table 8). Each SNP significantly associated with CAD in each subgroup was not related to CAD in the other subgroups. The −219G→T SNP of *APOE* in low-risk men (p = 0.0035; 370 subjects with MI) and the 1019C→T SNP of *GJA4* in high-risk men (p = 0.0243; 165 subjects with MI) were also associated with MI, but the p values for this association were larger than those for CAD. The −1171/5A→6A SNP of *MMP3* in low-risk women (128 subjects with MI) and the 3932T→C SNP of *APOE* in high-risk women (81 subjects with MI) were not associated with MI.

Finally, we performed a stepwise forward selection procedure to examine the effects of genotypes for *APOE* (−219G→T and 3932T→C), *GJA4*, and *MMP3*, as well as other CAD characteristics (Table 9). Age, *APOE* (−219G→T) genotype, and smoking, in descending order of statistical significance, affected the prevalence of CAD in low-risk men, and the *GJA4* genotype, age, and hyperuricemia influenced the prevalence of CAD in high-risk men. Age, the *MMP3* genotype, and hyperuricemia, in descending order of statistical significance, affected the prevalence of CAD in low-risk women, whereas the *APOE* (3932T→C)

**Table 4.** Multivariate Logistic Regression Analysis of Polymorphisms Associated With Coronary Artery Disease in the Total Study Population

| | | Dominant | | Recessive | | Additive | |
|---|---|---|---|---|---|---|---|
| Gene | Polymorphism | p Value | OR (95% CI) | p Value | OR (95% CI) | p Value | OR (95% CI) |
| *MMP3* | −1171/5A→6A | 0.0352 | 3.0 (1.0–7.1) | 0.2410 | | **0.0221** | 3.3 (1.1–7.8) |
| *GJA4* | 1019C→T (Pro319Ser) | **0.0241** | 1.3 (1.1–1.6) | 0.0417 | 1.8 (1.0–3.2) | 0.0332 | 1.9 (1.1–3.4) |
| *APOE* | 3932T→C (Cys112Arg) | **0.0352** | 2.0 (1.0–3.5) | 0.5487 | | 0.0434 | 2.2 (1.1–3.7) |
| *FABP2* | 2445G→A (Ala54Thr) | 0.6459 | | **0.0362** | 2.1 (1.2–3.7) | 0.0371 | 2.0 (1.1–3.7) |
| *p22-PHOX* | 242C→T (His72Tyr) | 0.0455 | 0.6 (0.5–0.9) | 0.4549 | | **0.0363** | 0.6 (0.4–0.8) |
| *SELE* | 561A→C (Ser128Arg) | 0.0564 | | 0.8485 | | **0.0364** | 0.3 (0.2–0.8) |
| *IRS1* | 3494G→A (Gly972Arg) | 0.0434 | 1.8 (1.1–3.0) | 0.7058 | | **0.0371** | 2.0 (1.2–3.3) |
| *APOE* | −219G→T | 0.5971 | | **0.0401** | 1.5 (1.1–2.0) | 0.2139 | |
| *APOC3* | −482C→T | 0.9782 | | **0.0431** | 1.3 (1.0–1.7) | 0.1350 | |
| *NOS3* | −786T→C | 0.0515 | | 0.9576 | | **0.0484** | 1.5 (1.0–2.2) |

The lowest p value among dominant, recessive, and additive models for each polymorphism is shown in **boldface**.
CI = confidence interval; OR = odds ratio.

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**Table 5.** Characteristics of the 1,060 Male Study Subjects With a Low- or High-Risk of Coronary Artery Disease

| Characteristic | Low-Risk Men (n = 696) | | High-Risk Men (n = 364) | |
|---|---|---|---|---|
| | Controls (n = 248) | Subjects With CAD (n = 448) | Controls (n = 116) | Subjects With CAD (n = 248) |
| Age (yrs) | 52.6 ± 10.9 | 61.8 ± 9.7* | 57.4 ± 9.6 | 60.7 ± 8.8† |
| Body mass index (kg/m$^2$) | 23.1 ± 2.7 | 22.9 ± 2.7 | 24.6 ± 2.8 | 24.5 ± 2.7 |
| Smoking (%) | 62.1 | 46.0* | 65.5 | 66.1 |
| Hyperuricemia (%) | 9.3 | 8.0 | 12.1 | 21.8‡ |
| Systolic blood pressure (mm Hg) | 118.8 ± 10.3 | 120.4 ± 11.1 | 159.0 ± 22.1 | 158.5 ± 19.2 |
| Diastolic blood pressure (mm Hg) | 71.4 ± 8.9 | 70.7 ± 8.6 | 95.2 ± 14.6 | 94.6 ± 14.1 |
| Fasting blood glucose (mmol/l) | 5.45 ± 0.59 | 5.41 ± 0.65 | 9.19 ± 2.45 | 9.30 ± 2.68 |
| Glycosylated hemoglobin (%) | 4.9 ± 0.2 | 5.2 ± 0.4 | 7.5 ± 1.2 | 7.8 ± 1.6 |
| Serum total cholesterol (mmol/l) | 4.70 ± 0.57 | 4.66 ± 0.61 | 6.40 ± 0.60 | 6.36 ± 0.63 |

*$p < 0.0001$, †$p < 0.005$, and ‡$p < 0.05$ versus corresponding controls. Data for age and body mass index are presented as the mean value ± SD.
CAD = coronary artery disease.

genotype and age influenced the prevalence of CAD in high-risk women.

## DISCUSSION

Coronary atherosclerosis results from excessive inflammatory and fibroproliferative responses to various forms of insult to the endothelium and smooth muscle of the artery wall, with the participation of large numbers of growth factors, cytokines, and vasoregulatory molecules (7). The genes shown to be significantly associated with CAD in the present study play important roles in lipid metabolism (*APOE*), gap-junctional communication between vascular endothelial cells (*GJA4*), and vascular matrix metabolism (*MMP3*).

Given that interactions between genetic and environmental factors may be important in the etiology of CAD, we examined the effects of genotypes, as well as age, BMI, and the prevalence of smoking and hyperuricemia, on the prevalence of CAD in low- or high-risk men or women. An examination of possible interactions among gene polymorphisms was not a purpose of the present study. A stepwise forward selection procedure revealed that genotypes for *APOE* (−219G→T or 3932T→C), *GJA4*, or *MMP3* significantly influenced the prevalence of CAD in low- or high-risk men or women, and that the effects of these genetic factors were statistically independent of age, smoking, or hyperuricemia, as well as hypertension, diabetes mellitus, and hypercholesterolemia. Our present results indicate that smoking is an important environmental factor for CAD in low-risk men, consistent with the notion that the cessation of smoking is important in the prevention of CAD in these individuals.

Among the total of four SNPs of three genes significantly associated with CAD in the present study, −219G→T of *APOE* was associated with CAD in low-risk men, and −1171/5A→6A of *MMP3* was associated with CAD in low-risk women. Apolipoprotein E is a structural component of both chylomicrons and very-low-density lipoprotein remnants, and it is responsible for the binding and uptake of these particles by the low-density lipoprotein (LDL) receptor and LDL receptor-like protein (8,9). The −219G→T SNP of *APOE* was previously associated with MI for men in France and Northern Ireland, with the T allele representing a risk factor for MI (10). Consistent with its location in the promoter region of *APOE*, the −219G→T SNP was shown to be associated with the plasma concentration of apolipoprotein E, with the T allele conferring a reduced apolipoprotein E concentration (10). The deleterious influence of the T allele on MI therefore cannot be explained by its effect on the circulating level of apolipoprotein E. We have

**Table 6.** Characteristics of the 601 Female Study Subjects With a Low- or High-Risk for Coronary Artery Disease

| Characteristic | Low-Risk Women (n = 364) | | High-Risk Women (n = 237) | |
|---|---|---|---|---|
| | Controls (n = 180) | Subjects With CAD (n = 184) | Controls (n = 106) | Subjects With CAD (n = 131) |
| Age (yrs) | 55.2 ± 12.4 | 66.4 ± 11.1* | 63.4 ± 9.6 | 65.5 ± 8.4 |
| Body mass index (kg/m$^2$) | 21.7 ± 2.7 | 22.3 ± 2.9† | 24.6 ± 3.2 | 24.6 ± 3.7 |
| Smoking (%) | 10.6 | 13.0 | 11.3 | 15.3 |
| Hyperuricemia (%) | 5.0 | 1.1† | 9.4 | 19.1† |
| Systolic blood pressure (mm Hg) | 119.6 ± 13.4 | 120.1 ± 10.7 | 169.5 ± 23.1 | 170.6 ± 23.4 |
| Diastolic blood pressure (mm Hg) | 69.6 ± 9.3 | 70.0 ± 10.3 | 96.1 ± 15.5 | 95.1 ± 16.2 |
| Fasting blood glucose (mmol/l) | 5.24 ± 0.57 | 5.33 ± 0.68 | 9.41 ± 2.69 | 9.32 ± 2.53 |
| Glycosylated hemoglobin (%) | 5.1 ± 0.5 | 5.2 ± 0.4 | 7.8 ± 1.2 | 7.6 ± 1.4 |
| Serum total cholesterol (mmol/l) | 4.76 ± 0.64 | 4.77 ± 0.68 | 6.44 ± 0.69 | 6.41 ± 0.59 |

*$p < 0.0001$ and †$p < 0.05$ versus corresponding controls. Data for age and body mass index are presented as the mean value ± SD.
CAD = coronary artery disease.

**Table 7.** Multivariate Logistic Regression Analysis of Polymorphisms Associated With Coronary Artery Disease in Low- or High-Risk Men

| | | Dominant | | Recessive | | Additive | |
|---|---|---|---|---|---|---|---|
| **Gene** | **Polymorphism** | **p Value** | **OR (95% CI)** | **p Value** | **OR (95% CI)** | **p Value** | **OR (95% CI)** |
| Low-risk men | | | | | | | |
| *APOE* | −219G→T | 0.2336 | | **0.0002** | 2.0 (1.4–2.8) | 0.0336 | 2.0 (1.0–3.7) |
| *CCR2* | 190G→A (Val64Ile) | 0.0809 | | 0.0080 | 2.8 (1.4–6.3) | **0.0051** | 3.0 (1.4–6.9) |
| *IL10* | −819T→C | 0.8048 | | **0.0309** | 0.5 (0.3–0.9) | 0.0547 | |
| High-risk men | | | | | | | |
| *GJA4* | 1019C→T (Pro319Ser) | 0.1475 | | 0.0017 | 7.3 (2.3–28.2) | **0.0015** | 7.5 (2.3–29.4) |
| *IL10* | −592A→C | 0.0517 | | 0.0169 | 2.7 (1.3–6.7) | **0.0098** | 3.1 (1.4–7.6) |
| *IL10* | −819T→C | 0.0688 | | 0.0155 | 2.8 (1.3–6.9) | **0.0102** | 3.1 (1.4–7.8) |
| *CCR2* | 190G→A (Val64Ile) | 0.2051 | | 0.0139 | 2.5 (1.2–5.3) | **0.0129** | 2.6 (1.2–5.7) |
| *p22-PHOX* | 242C→T (His72Tyr) | 0.0143 | 0.5 (0.3–0.9) | 0.8458 | | **0.0130** | 0.5 (0.3–0.9) |
| *PLA2G7* | 994G→T (Val279Phe) | **0.0143** | 2.0 (1.2–3.5) | 0.7889 | | 0.0466 | 1.8 (1.0–3.2) |
| *THMD* | 2136C→T (Ala455Val) | 0.3491 | | 0.0336 | 0.4 (0.2–0.9) | **0.0330** | 0.4 (0.2–0.9) |
| *NOS3* | −786T→C | **0.0369** | 1.9 (1.1–3.7) | 0.8611 | | 0.0497 | 1.9 (1.0–3.8) |

The lowest p value among dominant, recessive, and additive models for each polymorphism is shown in **boldface**.
CI = confidence interval; OR = odds ratio.

shown that the T allele of this SNP is a risk factor for CAD in low-risk men, consistent with the previous observation for MI (10).

Stromelysin-1 is a member of the matrix metalloproteinase family, with a broad substrate specificity (11). Thus, it catalyzes the degradation of many of the constituents of the extracellular matrix found in atherosclerotic plaques (11). The −1171/5A→6A SNP of *MMP3* has been associated with promoter activity, with the 6A allele showing reduced gene transcription (12). The 6A allele of the −1171/5A→6A SNP was also previously associated with an increased rate of progression of coronary atherosclerosis in a male population in England (13). Moreover, the 6A/6A genotype was associated with an increased intima-media thickness of the carotid artery both in Finnish men (14) and men and women in New York (15). Consistent with these previous observations (13–15), we have now shown that the 6A allele is a risk factor for CAD in low-risk women.

The 1019C→T SNP of *GJA4* was significantly associated with CAD in high-risk men, whereas the 3932T→C SNP of *APOE* was associated with CAD in high-risk women. Connexin 37 is a gap junction protein in the arterial endothelium, including that of human coronary arteries, and contributes to the growth and regeneration after injury of endothelial cells (16,17). It forms functional intercellular channels with a voltage dependence and unitary conductance properties that are distinct from those of other channels (18). The carboxyl terminal domain of this protein also plays a role in pH regulation (19). The 1019C→T (Pro319Ser) SNP of *GJA4* was previously associated with carotid intimal thickening in Swedish men, with the C allele being overrepresented in individuals with atherosclerotic plaques (20). The C allele of this SNP was also associated with CAD in a Taiwanese population (21). However, the population sizes of both of these previous studies were small. In contrast to their findings, we have shown that the T allele of this polymorphism is a risk factor for CAD in high-risk men, with an odds ratio of 7.5, the highest such value obtained in the present study. The functional impact of the 1019C→T SNP of *GJA4* has not been determined.

In humans, three alleles ($\epsilon$2, $\epsilon$3, $\epsilon$4) of *APOE* have been described. The C allele of the 3932T→C (Cys112Arg) SNP of *APOE*, which is located in the LDL receptor-binding domain of the encoded protein (8), is a major determinant of the $\epsilon$4 allele, which is associated with a reduced binding of triglyceride-rich lipoproteins to the

**Table 8.** Multivariate Logistic Regression Analysis of Polymorphisms Associated With Coronary Artery Disease in Low- or High-Risk Women

| | | Dominant | | Recessive | | Additive | |
|---|---|---|---|---|---|---|---|
| **Gene** | **Polymorphism** | **p Value** | **OR (95% CI)** | **p Value** | **OR (95% CI)** | **p Value** | **OR (95% CI)** |
| Low-risk women | | | | | | | |
| *MMP3* | −1171/5A→6A | **0.0034** | 2.9 (1.4–6.0) | 0.1069 | | 0.0049 | 2.9 (1.4–6.0) |
| *GP1BB* | 1018C→T (Thr145Met) | **0.0123** | 0.4 (0.2–0.8) | 0.5273 | | 0.0154 | 0.4 (0.2–0.8) |
| *IRS1* | 3494G→A (Gly972Arg) | **0.0132** | 4.4 (1.4–15.4) | 1.0000 | | **0.0132** | 4.4 (1.4–15.4) |
| *SELE* | 561A→C (Ser128Arg) | **0.0467** | 0.3 (0.1–0.9) | 1.0000 | | **0.0467** | 0.3 (0.1–0.9) |
| High-risk women | | | | | | | |
| *APOE* | 3932T→C (Cys112Arg) | **0.0049** | 2.4 (1.3–4.6) | 0.4367 | | 0.0068 | 2.4 (1.3–4.6) |
| *SELE* | 561A→C (Ser128Arg) | 0.0493 | 0.4 (0.1–1.0) | 0.8448 | | **0.0297** | 0.3 (0.1–0.9) |
| *FABP2* | 2445G→A (Ala54Thr) | 0.3026 | | 0.0325 | 3.0 (1.2–8.9) | **0.0305** | 3.2 (1.2–10.1) |

The lowest p value among dominant, recessive, and additive models for each polymorphism is shown in **boldface**.
CI = confidence interval; OR = odds ratio.

**Table 9.** Genotypes and Other Characteristics Associated With Coronary Artery Disease in Low- or High-Risk Men or Women, as Determined by a Stepwise Forward Selection Procedure

| Variable | p Value |
|---|---|
| Low-risk men | |
| Age | <0.0001 |
| *APOE* (−219G→T, recessive) | 0.0002 |
| Smoking | 0.0049 |
| High-risk men | |
| *GJA4* (1019C→T, recessive or additive) | 0.0018 |
| Age | 0.0034 |
| Hyperuricemia | 0.0210 |
| Low-risk women | |
| Age | <0.0001 |
| *MMP3* (−1171/5A→6A, dominant) | 0.0029 |
| Hyperuricemia | 0.0067 |
| High-risk women | |
| *APOE* (3932T→C, dominant) | 0.0034 |
| Age | 0.0259 |

LDL receptor and LDL receptor-like protein and thus with an increased serum concentration of cholesterol (22). The ε4 allele has previously been associated with CAD (23,24). Our results indicate that the C allele of the 3932T→C SNP of *APOE* was associated with CAD in high-risk women, consistent with these previous observations (23,24).

The reason for the difference in SNPs associated with CAD between low-risk men and women or between high-risk men and women remains to be elucidated. The gender difference in the association between SNPs and CAD might be attributable, at least in part, to the difference in the serum concentration of estrogen between men and women, given that estrogen exerts various favorable effects on vasomotor function, including stimulation of the production of nitric oxide and prostaglandin $I_2$, as well as inhibition of the release of endothelin-1 by vascular endothelial cells (25). In addition, given that the 37 polymorphisms examined in the present study likely represent only a small proportion of those potentially associated with CAD, it remains possible that further investigations will uncover polymorphisms that are associated with this condition in both men and women.

There are several limitations of the present study: 1) given the complex nature of atherosclerosis, etiologies may vary among study populations, making replication of the results difficult. 2) After classifying our study population into high- and low-risk groups of men and women, the numbers of individuals in each subgroup were relatively small. 3) Although we selected controls from individuals with no history of CAD who exhibited normal electrocardiograms at rest and no signs of myocardial ischemia during exercise stress testing, without performing coronary angiography, we could not exclude the possibility that some of these subjects were affected by CAD. 4) Given that the subjects with CAD in the present study were survivors of CAD, they are likely not representative of all CAD patients. However, the mortality of MI in Japan is approximately one-fifth of that in the U.S. and one-seventh of that in the U.K. Any survivor bias present in our study is thus likely to be small. 5) Given the multiple comparisons of genotypes with CAD in the present study, we adopted a strict criterion of statistical significance ($p < 0.005$ for association). However, it is not possible to completely exclude potential statistical errors such as false-positives. 6) Finally, it is also possible that one or more of the SNPs associated with CAD in our study are in linkage disequilibrium with polymorphisms of other nearby genes that are actually responsible for the development of this condition.

Despite these various limitations, our present results suggest that *APOE* is a susceptibility locus for CAD in low-risk Japanese men and high-risk women. They also suggest that *MMP3* is a susceptibility locus for CAD in low-risk women, and that *GJA4* constitutes such a locus in high-risk men. Genotyping of these SNPs may prove informative for assessment of the genetic risk of CAD in low- or high-risk men or women.

**Reprint requests and correspondence:** Dr. Yoshiji Yamada, FAHA, Department of Gene Therapy, Gifu International Institute of Biotechnology, 1-1 Naka-Fudogaoka, Kakamigahara, Gifu 504-0838, Japan. E-mail: yoyamada@giib.or.jp.

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## APPENDIX

The following physicians and institutions participated in this study: T. Tanaka, H. Kanda, H. Ishihara (Okazaki City Hospital); H. Horibe, M. Watarai, F. Takatsu (Kosei Hospital); T. Okada, H. Hirayama (Nagoya Daini Red Cross Hospital); S. Ichihara, A. Yamada, H. Izawa (Nagoya University Hospital).