

PREDICTION OF THE RISK OF MYOCARDIAL INFARCTION FROM POLYMORPHISMS IN CANDIDATE GENES

YOSHIJI YAMADA, M.D., PH.D., HIDEO IZAWA, M.D., PH.D., SAHOKO ICHIHARA, M.D., PH.D., FUMIMARO TAKATSU, M.D., PH.D., HITOSHI ISHIHARA, M.D., PH.D., HARUO HIRAYAMA, M.D., PH.D., TAKAHIITO SONE, M.D., PH.D., MASASHI TANAKA, M.D., PH.D., AND MITSUHIRO YOKOTA, M.D., PH.D.

ABSTRACT

Background Although epidemiologic studies have suggested that several genetic variants increase the risk of myocardial infarction, large-scale association studies that examine many polymorphisms simultaneously are required to allow reliable prediction of the genetic risk of myocardial infarction.

Methods We used a fluorescence- or colorimetry-based allele-specific DNA-primer-probe assay system to determine the genotypes of 112 polymorphisms of 71 candidate genes in 2819 unrelated Japanese patients with myocardial infarction (2003 men and 816 women) and 2242 unrelated Japanese controls (1306 men and 936 women).

Results In an initial screening of the 112 polymorphisms for an association with myocardial infarction in 909 subjects, 19 polymorphisms were selected in men and 18 in women by means of logistic-regression analysis, after adjustment for age, body-mass index, and the prevalence of smoking, hypertension, diabetes mellitus, hypercholesterolemia, and hyperuricemia. In a large-scale study involving the selected polymorphisms and the remaining 4152 subjects, similar logistic-regression analysis revealed that the risk of myocardial infarction was significantly associated with the C1019T polymorphism in the connexin 37 gene ($P < 0.001$) in men and the 4G-668/5G polymorphism in the plasminogen-activator inhibitor type 1 gene ($P < 0.001$) and the 5A-1171/6A polymorphism in the stromelysin-1 gene ($P < 0.001$) in women.

Conclusions Determination of the genotypes of the connexin 37, plasminogen-activator inhibitor type 1, and stromelysin-1 genes may prove reliable in predicting the genetic risk of myocardial infarction and might thus contribute to the primary prevention of this condition. (N Engl J Med 2002;347:1916-23.)

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MYOCARDIAL infarction is a complex multifactorial and polygenic disorder that is thought to result from an interaction between a person's genetic makeup and various environmental factors.^{1,2} In general, the incidence of myocardial infarction increases additively as a function of the number of conventional risk factors, including hypertension, diabetes mellitus, and hypercholesterolemia.² Although each risk factor itself is partly under genetic control, a family history of myocardial infarction is also an independent predictor, sug-

gesting the existence of additional susceptibility genes for this condition.¹ Furthermore, some patients who have had a myocardial infarction do not have any conventional risk factors, suggesting the contribution of an uncharacterized genetic component. Given that myocardial infarction is a leading cause of death in the Western world and markedly impairs the quality of life by causing heart failure or refractory arrhythmias, prevention of this disease is an important public health goal. One approach to preventing this condition is to identify disease-susceptibility genes. Genetic-linkage studies³ and candidate-gene analyses⁴⁻⁷ have implicated a locus and several candidate genes in the predisposition to myocardial infarction. Although epidemiologic studies have revealed that several genetic variants, including those of angiotensin-converting enzyme,⁴ platelet glycoprotein IIIa,⁵ coagulation factor VII,⁶ and cholesterol-ester transfer protein,⁷ increase the risk of myocardial infarction, the results of these studies remain controversial, with no consensus on their implications. In addition, because of racial and ethnic differences in genetic polymorphisms, it is important to construct a data base of polymorphisms related to myocardial infarction in each racial and ethnic group.

The purpose of the present study was to identify polymorphisms that confer susceptibility to myocardial infarction and thereby to contribute to the primary prevention of this condition.

METHODS

Study Population

The study population comprised 5061 unrelated Japanese subjects (3309 men and 1752 women) who either visited outpatient clinics or were admitted to 1 of the 15 participating hospitals (see the Appendix) between July 1994 and December 2001. The 2819 patients with myocardial infarction (2003 men and 816 women) all underwent coronary angiography and left ventriculography. The diagnosis of myocardial infarction was based on typical electrocardiographic changes and increased serum activities of enzymes such

From the Department of Gene Therapy, Gifu International Institute of Biotechnology, Mitake (Y.Y., M.T.); the Cardiovascular Division, Department of Pathophysiology, Nagoya University Graduate School of Medicine, Nagoya (H. Izawa, S.I., M.Y.); the Division of Cardiology, Kosei Hospital, Anjo (F.T.); the Division of Cardiology, Okazaki City Hospital, Okazaki (H. Ishihara); the Cardiovascular Center, Nagoya Daini Red Cross Hospital, Nagoya (H.H.); and the Department of Cardiology, Ogaki Municipal Hospital, Ogaki (T.S.) — all in Japan. Address reprint requests to Dr. Yokota at the Department of Clinical Laboratory Medicine, Nagoya University Hospital, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan, or at myokota@med.nagoya-u.ac.jp.

as creatine kinase, aspartate aminotransferase, and lactate dehydrogenase. The diagnosis was confirmed by the presence of wall-motion abnormality on left ventriculography and attendant stenosis in any of the major coronary arteries or in the left main trunk, as documented by coronary angiography.

The 2242 control subjects (1306 men and 936 women) were recruited from persons found to have at least one of the conventional risk factors for coronary artery disease, including habitual cigarette smoking (10 or more cigarettes daily), obesity (a body-mass index [the weight in kilograms divided by the square of the height in meters] of at least 26), hypertension (defined by a systolic blood pressure of at least 140 mm Hg, a diastolic blood pressure of at least 90 mm Hg, or both), diabetes mellitus (defined by a blood glucose level of at least 126 mg per deciliter [6.93 mmol per liter] after an overnight fast, a glycosylated hemoglobin value of at least 6.5 percent, or both), hypercholesterolemia (serum total cholesterol level of at least 220 mg per deciliter [5.72 mmol per liter]), or hyperuricemia (serum uric acid level of at least 7.7 mg per deciliter [0.46 mmol per liter] for men and at least 5.5 mg per deciliter [0.33 mmol per liter] for women), but who had no history of coronary artery disease. They had normal electrocardiograms at rest and no signs of myocardial ischemia on exercise stress testing. The study protocol was approved by the committees on the ethics of human research of Nagoya University Graduate School of Medicine and Gifu International Institute of Biotechnology, and written informed consent was obtained from each participant.

Selection of Polymorphisms

With the use of public data bases, including PubMed and Online Mendelian Inheritance in Man, we selected 71 candidate genes that have been characterized and potentially associated with coronary atherosclerosis or vasospasm, hypertension, diabetes mellitus, or hyperlipidemia on the basis of a comprehensive overview of vascular biology, platelet and leukocyte biology, coagulation and fibrinolysis cascades, as well as lipid and glucose metabolism and other metabolic factors. We further selected 112 polymorphisms of these genes — most of which were in the promoter regions, exons, or splice-donor or splice-acceptor sites in introns — that might be expected to cause changes in the function or level of expression of the encoded protein (Table 1). The minus signs before the numbered nucleotide in some polymorphisms, such as C-535T in Table 1, refer to the 5' upstream region relative to the transcription-initiation site of a gene.

Genotyping of Polymorphisms

Venous blood (7 ml) was collected from each subject into tubes containing 50 mmol of EDTA per liter, and genomic DNA was isolated with a kit (Qiagen). Genotypes of the 112 polymorphisms were determined with a fluorescence- or colorimetry-based allele-specific DNA-primer-probe assay system (Toyobo Gene Analysis) (described in detail in Supplementary Appendix 1, available with the full text of this article at <http://www.nejm.org>). Polymorphic regions of each gene were amplified by the polymerase chain reaction (PCR) with two allele-specific sense (or antisense) primers labeled at the 5' end with either fluorescein isothiocyanate or Texas red and an antisense (or sense) primer labeled at the 5' end with biotin. Alternatively, the polymorphic regions were amplified with two allele-specific sense (or antisense) primers and a biotin-labeled antisense (or sense) primer or with a sense primer and a biotin-labeled antisense primer. The reaction mixture (25 μ l) contained 20 ng of DNA, 5 pmol of each primer, 0.2 mmol of each deoxynucleoside triphosphate per liter, 1 to 4 mmol of magnesium chloride per liter, and 1 U of DNA polymerase (rTaq or KODplus, Toyobo) in corresponding DNA polymerase buffer. The amplification protocol comprised an initial period of denaturation at 95°C for 5 minutes, 35 to 45 cycles of denaturation at 95°C for 30 seconds, annealing at 55° to 67.5°C for 30 seconds, extension at 72°C for 30 seconds, and a final period of extension at 72°C for 2 minutes.

To determine the genotype by means of fluorescence, we incubated amplified DNA with streptavidin-conjugated magnetic beads in 96-well plates at room temperature. The plates were placed on a magnetic stand, and the supernatants were then transferred to the wells of the 96-well plate containing 10 mmol of sodium hydroxide per liter and assessed for fluorescence at excitation and emission wavelengths of 485 and 538 nm, respectively, in the case of fluorescein isothiocyanate and of 584 and 612 nm, respectively, in the case of Texas red. To determine the genotype by means of colorimetry, we denatured amplified DNA with 0.3 mol of sodium hydroxide per liter and subjected it to hybridization at 37°C for 30 minutes in hybridization buffer containing 30 to 45 percent formamide with each of two allele-specific capture probes fixed to the bottom of the wells of a 96-well plate. After thorough washing of the wells, alkaline phosphatase-conjugated streptavidin was added to each well and the plate was incubated at 37°C for 15 minutes while being agitated. The wells were again washed, and after the addition of a solution containing 0.8 mmol of 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium (monosodium salt) per liter and 0.4 mmol of 5-bromo-4-chloro-3-indolyl phosphate *p*-toluidine salt per liter, the absorbance of the samples was assessed at a wavelength of 450 nm.

To confirm the accuracy of genotyping with the use of this method, we randomly selected 50 DNA samples and subjected them to PCR and restriction-fragment-length polymorphism analysis or to direct DNA sequencing of the PCR products. In each instance, the genotype determined by the allele-specific DNA-primer-probe assay system was identical to that determined by the confirmatory methods.

Association Study

We first performed a screening study using the 112 polymorphisms of the 71 candidate genes to screen 909 subjects who were randomly selected from the total study population of 5061 subjects. In this screening study, the cutoff *P* value was defined as less than 0.1 for multivariate logistic-regression analysis involving dominant, recessive, or additive genetic models in order to avoid false negative associations. From the screening study, we selected 19 polymorphisms related to myocardial infarction in men and 18 related to myocardial infarction in women. We then performed a large-scale study to assess the association between these polymorphisms and the risk of myocardial infarction in the remaining 4152 study subjects. An association was considered significant at a *P* value of less than 0.001 on multivariate logistic-regression analysis.

Statistical Analysis

Measured variables were compared between patients with myocardial infarction and controls with use of the unpaired Student's *t*-test or Mann-Whitney *U* test. Categorical variables were compared with use of the chi-square test. Allele frequencies were estimated by the gene-counting method, and the chi-square test was used to identify departures from Hardy-Weinberg equilibrium. We performed multivariate logistic-regression analysis to adjust risk factors, with myocardial infarction as a dependent variable and independent variables that included age, body-mass index, smoking status (a value of 0 assigned for a nonsmoker and 1 for a smoker), metabolic variables (a value of 0 assigned for the absence of a history of hypertension, diabetes mellitus, hypercholesterolemia, or hyperuricemia and a value of 1 for the presence of such a history), and the genotype of each polymorphism. Each genotype was assessed with the use of dominant, recessive, and additive genetic models, and the *P* value, odds ratio, and 95 percent confidence interval were calculated.

RESULTS

The characteristics of the 909 subjects in the screening study for the 112 polymorphisms are shown in

TABLE 1. THE 112 POLYMORPHISMS EXAMINED IN THE SCREENING STUDY.*

GENE	POLYMORPHISM	GENE	POLYMORPHISM
Angiotensin-converting enzyme	I/D in intron 16	Interleukin-1 α	C-889T
Angiotensin II receptor type I	C-535T	Interleukin-1 β	C-511T C3953T
Angiotensinogen	G-6A	Interleukin-6	C-634G G-174C
Apolipoprotein A-1	G-75A C83T	Leptin	C-1887A
Apolipoprotein B	I/D in signal peptide	Lipoprotein lipase	G280A (Asp9Asn) A1127G (Asn291Ser)
Apolipoprotein C-III	C-482T C1100T	Low-density lipoprotein receptor-related protein	C766T
Apolipoprotein E	A-491T G-219T T3932C (Cys112Arg) C4070T (Arg158Cys)	Lp(a) lipoprotein	C93T G121A A11764C (Thr12Pro)
ATP-binding cassette transporter	C-477T G1051A (Arg219Lys)	Manganese superoxide dismutase	C47T (Ala16Val) T173C (Ile58Thr)
Atrial natriuretic peptide	G664A (Val7Met)	Matrix Gla protein	G-7A A7158G (Thr83Ala)
Atrial natriuretic peptide clearance receptor	A-55C	Metalloproteinase-1 (collagenase)	G-1607GG
β_2 -adrenergic receptor	A46G (Arg16Gly) C79G (Gln27Glu) C491T (Thr164Ile)	Metalloproteinase-12 (macrophage elastase)	A-82G
β_3 -adrenergic receptor	T190C (Trp64Arg)	Methionine synthase	A2756G (Asp919Gly)
β -fibrinogen	G-854A G-455A C148T G8059A (Arg448Lys)	Methylenetetrahydrofolate reductase	C677T (Ala222Val)
CD14 receptor	C-260T	Monocyte chemoattractant protein 1	G-2518A C242T (His72Tyr)
CC chemokine receptor 2	G190A (Val64Ile)	<i>p22^{phox}</i>	T1128C (Leu7Pro)
Cholesterol-ester transfer protein	A1061G (Ile405Val) A1163G (Asp442Gly) G1200A (Arg451Gln) G1691A (Arg506Gln)	Neuropeptide Y	T-107C A172T (Met55Leu) G584A (Gln192Arg)
Coagulation factor V	G11,496A (Arg353Glu)	Paraoxonase	C14546 (Leu125Val) G4428A (Ser563Asn)
Coagulation factor VII	C46T	Peroxisome-proliferator-activated receptor α	C696G (Leu162Val)
Coagulation factor XII	G163T (Val34Leu)	Peroxisome-proliferator-activated receptor γ 2	C34G (Pro12Ala) C344A (Pro115Gln)
Coagulation factor XIII A subunit	C1019T (Pro319Ser)	Plasminogen-activator inhibitor type 1	4G-668/5G
Connexin 37	T-786C G894T (Glu298Asp)	Platelet-activating factor acetylhydrolase	G994T (Val279Phe)
Endothelial nitric oxide synthase	G5665T (Lys198Asn) G98T A561C (Ser128Arg) C1839T (Leu554Phe)	Prothrombin	G20,210A
Endothelin-1	C5775G (Arg213Gly) G2445A (Ala54Thr)	P-selectin	A76,666C (Thr715Pro)
E-selectin	G84,635A (Val249Ile) C807T G873A A1648G (Lys505Glu) C1018T (Thr145Met)	Scavenger receptor BI	G4A (Gly2Ser) G403A (Val135Ile) T102C
Extracellular superoxide dismutase	T1565C (Leu33Pro)	Serotonin receptor 2A	5A-1171/6A
Fatty-acid-binding protein 2	A97C (Lys121Gln)	Stromelysin-1	G-33A GG-10TA G845A (Ala25Thr)
Fractalkine receptor	C825T (splice variant)	Thrombomodulin	C2136T (Ala455Val)
Glycoprotein Ia	G845A (Cys282Tyr) C-480T G-250A	Thrombopoietin	A5713G
Glycoprotein Ib α	G3494A (Gly972Arg)	Thrombospondin 1	A2210G (Asn700Ser)
Glycoprotein IIIa	G-1082A T-819C A-592C	Thrombospondin 4	G1186C (Ala387Pro)
Glycoprotein PC-1		Tissue-factor-pathway inhibitor	G874A (Val264Met)
G protein β 3 subunit		Transforming growth factor β 1	C-509T T869C (Leu10Pro)
Hemochromatosis-associated protein		Tumor necrosis factor α	C-863A C-850T G-308A G-238A
Hepatic lipase		von Willebrand factor	C-1234T G-1051A

*Minus signs indicate the number of nucleotides upstream from the transcription-initiation site. For nonsynonymous polymorphisms, the resulting amino acid change is shown in parentheses.

Table 2. Among the men, there were no significant differences in age, body-mass index, or the prevalence of conventional risk factors for coronary artery disease, including smoking, hypertension, diabetes mellitus, hypercholesterolemia, and hyperuricemia, between patients with myocardial infarction and controls. For women, age, body-mass index, and the prevalence of hypertension, hypercholesterolemia, and hyperuricemia did not differ significantly between patients with myocardial infarction and controls, but the prevalence of both smoking and diabetes mellitus was higher among patients with myocardial infarction than among controls. On the basis of multivariate logistic-regression analysis with adjustment for age, body-mass index, and the prevalence of smoking, hypertension, diabetes mellitus, hypercholesterolemia, and hyperuricemia, 19 polymorphisms were selected for further study in men and 18 for further study in women (Table 3). Only four of these polymorphisms were observed in both sexes.

The characteristics of all 4152 subjects in the large-scale study are shown in Table 4. For men, age, body-mass index, and the prevalence of smoking did not differ significantly between patients with myocardial infarction and controls; the prevalence of both hypertension and hyperuricemia was lower and that of both diabetes mellitus and hypercholesterolemia was higher among patients than controls. For women, age and the prevalence of both hypertension and hyperuricemia did not differ significantly between patients with myocardial infarction and controls; body-mass index and the prevalence of smoking, diabetes mellitus, and hypercholesterolemia were greater among patients than controls. In the large-scale study of 19 polymorphisms in men and 18 polymorphisms in women, multivariate logistic-regression analysis with adjustment

for age, body-mass index, and the prevalence of smoking, hypertension, diabetes mellitus, hypercholesterolemia, and hyperuricemia revealed that one polymorphism (the replacement of cytosine with thymine at position 1019 [C1019T] in the connexin 37 gene) was associated with a significant risk of myocardial infarction in men and two polymorphisms (the replacement of four guanines with five guanines at position -668 [4G-668/5G] in the plasminogen-activator inhibitor type 1 gene and the replacement of five adenines with six adenines at position -1171 [5A-1171/6A] in the stromelysin-1 gene) were associated with a significant risk of myocardial infarction in women ($P < 0.001$ for all comparisons with the use of either a dominant or a recessive genetic model) (Table 5). The replacement of cytosine with thymine at position 242 in the *p22^{phox}* gene was also potentially associated with a risk of myocardial infarction in men ($P < 0.01$). The genotypic distributions of these polymorphisms are shown in Table 6 and were in Hardy-Weinberg equilibrium.

DISCUSSION

We examined the relation of 112 polymorphisms in 71 candidate genes to the risk of myocardial infarction. Our large-scale study, involving 4152 subjects, revealed that the C1019T polymorphism in the connexin 37 gene was associated with a significant risk of myocardial infarction in men and that the 4G-668/5G polymorphism in the plasminogen-activator inhibitor type 1 gene and the 5A-1171/6A polymorphism in the stromelysin-1 gene were associated with a significant risk of myocardial infarction in women.

Many studies have examined the relations between polymorphisms and coronary artery disease or myocardial infarction. The results of most of these stud-

TABLE 2. CHARACTERISTICS OF THE 909 SUBJECTS INCLUDED IN THE SCREENING STUDY.*

CHARACTERISTIC	MEN (N=451)		WOMEN (N=458)	
	CONTROLS (N=232)	PATIENTS WITH MYOCARDIAL INFARCTION (N=219)	CONTROLS (N=232)	PATIENTS WITH MYOCARDIAL INFARCTION (N=226)
Age (yr)	52.4±3.6	51.8±6.0	62.6±8.8	62.2±8.3
Body-mass index	23.8±2.5	24.2±2.7	23.4±3.2	23.2±2.9
Smoker (%)	60.3	60.7	9.5	16.5†
Hypertension (%)	43.5	42.9	69.8	65.5
Diabetes mellitus (%)	11.2	16.0	15.5	36.7‡
Hypercholesterolemia (%)	45.3	52.5	59.9	66.8
Hyperuricemia (%)	16.4	21.0	10.3	11.9

*Plus-minus values are means ±SD.

†P=0.028 for the comparison with controls.

‡P<0.001 for the comparison with controls.

TABLE 3. POLYMORPHISMS RELATED TO MYOCARDIAL INFARCTION IN THE SCREENING STUDY.

GENE	POLYMORPHISM	GENETIC MODEL	P VALUE
Men			
Platelet-activating factor acetylhydrolase	G994T	Additive	<0.001
<i>p22^{phox}</i>	C242T	Dominant	0.006
Connexin 37	C1019T	Additive	0.007
Thrombospondin 4	G1186C	Dominant	0.013
Angiotensinogen	G-6A	Recessive	0.019
Tumor necrosis factor α	C-863A	Dominant	0.045
Transforming growth factor β 1	T869C	Additive	0.049
G protein β 3 subunit	C825T	Additive	0.051
Apolipoprotein C-III	C-482T	Recessive	0.057
Interleukin-10	T-819C	Recessive	0.061
Thrombomodulin	C2136T	Additive	0.065
Apolipoprotein E	C4070T	Additive	0.074
Glycoprotein Ia	A1648G	Recessive	0.080
Interleukin-10	A-592C	Recessive	0.088
Apolipoprotein E	G-219T	Recessive	0.092
Thrombopoietin	A5713G	Recessive	0.094
Apolipoprotein C-III	C1100T	Recessive	0.095
CC chemokine receptor 2	G190A	Recessive	0.097
Endothelial nitric oxide synthase	T-786C	Dominant	0.098
Women			
Paraoxonase	G584A	Dominant	0.009
Interleukin-6	C-634G	Additive	0.009
Connexin 37	C1019T	Dominant	0.013
ATP-binding cassette transporter 1	G1051A	Additive	0.014
Tumor necrosis factor α	C-850T	Additive	0.015
Endothelin-1	G5665T	Recessive	0.028
Apolipoprotein E	C4070T	Recessive	0.038
Apolipoprotein C-III	C-482T	Recessive	0.044
Apolipoprotein E	T3932C	Dominant	0.047
CD14 receptor	C-260T	Additive	0.050
Tumor necrosis factor α	G-238A	Dominant	0.052
Plasminogen-activator inhibitor type 1	4G-668/5G	Recessive	0.055
Fatty-acid-binding protein 2	G2445A	Additive	0.057
Insulin receptor substrate-1	G3494A	Dominant	0.058
Stromelysin-1	5A-1171/6A	Additive	0.072
Glycoprotein Ib α	C1018T	Additive	0.072
E-selectin	A561C	Dominant	0.074
Endothelial nitric oxide synthase	T-786C	Dominant	0.087

TABLE 4. CHARACTERISTICS OF THE 4152 SUBJECTS INCLUDED IN THE LARGE-SCALE STUDY.*

CHARACTERISTIC	MEN (N=2858)		WOMEN (N=1294)	
	CONTROLS (N=1074)	PATIENTS WITH MYOCARDIAL INFARCTION (N=1784)	CONTROLS (N=704)	PATIENTS WITH MYOCARDIAL INFARCTION (N=590)
Age (yr)	62.0±10.4	62.1±10.1	62.8±12.0	62.5±10.8
Body-mass index	23.6±2.7	23.6±2.9	22.9±3.3	23.4±3.6†
Smoker (%)	55.2	57.8	9.0	14.9‡
Hypertension (%)	57.3	47.0§	58.2	57.9
Diabetes mellitus (%)	16.2	34.7§	14.4	43.0§
Hypercholesterolemia (%)	33.4	42.6§	45.7	54.5‡
Hyperuricemia (%)	17.7	13.2‡	10.2	12.6

*Plus-minus values are means ±SD.

†P<0.01 for the comparison with controls.

‡P<0.005 for the comparison with controls.

§P<0.001 for the comparison with controls.

GENETIC RISK OF MYOCARDIAL INFARCTION

TABLE 5. MULTIVARIATE LOGISTIC-REGRESSION ANALYSIS OF POLYMORPHISMS ASSOCIATED WITH MYOCARDIAL INFARCTION IN THE LARGE-SCALE STUDY OF 4152 SUBJECTS.*

GENE AND POLYMORPHISM	DOMINANT MODEL		RECESSIVE MODEL		ADDITIVE MODEL	
	P VALUE	ODDS RATIO (95% CI)	P VALUE	ODDS RATIO (95% CI)	P VALUE	ODDS RATIO (95% CI)
Men (n=2858)						
Connexin 37, C1019T	<0.001	1.4 (1.1-1.6)†	0.09	—	0.002	1.4 (1.1-1.6)‡
<i>p22^{phox}</i> , C242T	0.007	0.7 (0.6-0.9)§	0.64	—	0.008	0.7 (0.6-0.9)¶
Women (n=1294)						
Plasminogen-activator inhibitor type 1, 4G-668/5G	<0.001	1.6 (1.2-2.1)	0.28	—	<0.001	1.6 (1.2-2.1)**
Stromelysin-1, 5A-1171/6A	<0.001	4.7 (2.0-12.2)††	0.08	—	<0.001	4.9 (2.1-12.7)‡‡

*CI denotes confidence interval.

†The value is for the comparison of the C/T plus T/T genotypes with the C/C genotype.

‡The value is for the comparison of the C/T genotype with the C/C genotype.

§The value is for the comparison of the C/T plus T/T genotypes with the C/C genotype.

¶The value is for the comparison of the C/T genotype with the C/C genotype.

||The value is for the comparison of the 4G/5G plus 5G/5G genotypes with the 4G/4G genotype.

**The value is for the comparison of the 4G/5G genotype with the 4G/4G genotype.

††The value is for the comparison of the 5A/6A plus 6A/6A genotypes with the 5A/5A genotype.

‡‡The value is for the comparison of the 6A/6A genotype with the 5A/5A genotype.

ies, however, remain controversial, with no consensus on their implications, mainly because of the limited size of the study populations, the ethnic diversity of polymorphisms, and complicating environmental factors. The chief cause of myocardial infarction is atherosclerotic coronary artery disease, which contributes to hemodynamically significant narrowing of the arterial lumen, alters the control of vasomotor tone, and increases the likelihood that plaque will be disrupted and thrombi will form. We thus selected 71 candidate genes on the basis of a comprehensive overview of vascular biology, platelet and leukocyte biology, coagulation, and fibrinolysis systems, as well as lipid and glucose metabolism and other metabolic factors. Indeed, genes associated with myocardial infarction may have roles in various aspects of the pathogenesis of this condition, including gap-junctional communication between vascular endothelial cells (connexin 37),⁸⁻¹⁰ the production of reactive oxygen species by vascular smooth-muscle cells (*p22^{phox}*),¹¹⁻¹⁴ fibrinolysis (plasminogen-activator inhibitor type 1),¹⁵⁻¹⁷ and matrix metabolism (stromelysin-1).¹⁸⁻²¹ We examined 112 polymorphisms in 909 subjects, 19 polymorphisms in 2858 men, and 18 polymorphisms in 1294 women, resulting in the determination of 179,402 genotypes and possibly representing the largest such association study of polymorphisms to date.

The C1019T polymorphism of the connexin 37 gene was associated with thickening of the carotid intima in Swedish men, with the C allele being overrep-

TABLE 6. DISTRIBUTIONS OF POLYMORPHISMS ASSOCIATED WITH MYOCARDIAL INFARCTION AMONG THE 4152 SUBJECTS IN THE LARGE-SCALE STUDY.*

GENE AND POLYMORPHISM	PATIENTS WITH MYOCARDIAL INFARCTION	
	CONTROLS	percent
Men (n=2858)		
Connexin 37, C1019T at 1p35.1		
C/C	71.7	66.2
C/T	25.4	29.6
T/T	2.9	4.2
<i>p22^{phox}</i> , C242T at 16q24		
C/C	75.9	80.7
C/T	22.5	17.9
T/T	1.5	1.4
Women (n=1294)		
Plasminogen-activator inhibitor type 1, 4G-668/5G at 7q21.3-q22		
4G/4G	44.8	36.5
4G/5G	44.9	50.9
5G/5G	10.3	12.6
Stromelysin-1, 5A-1171/6A at 11q23		
5A/5A	5.7	1.2
5A/6A	31.3	28.1
6A/6A	63.0	70.8

*Because of rounding, percentages may not total 100.

resented in men with atherosclerotic plaques.⁹ The C allele of this gene was also associated with coronary artery disease in a Taiwanese population.¹⁰ However, both these studies were small, and in contrast to their findings, our results suggest that the T allele of this polymorphism is a risk factor for myocardial infarction in men.

The 4G allele of the plasminogen-activator inhibitor type 1 gene was associated with myocardial infarction in a small group of Swedish men.¹⁵ Large studies of men in the United States¹⁶ and of white women in the Netherlands,¹⁷ however, did not confirm such an association. Furthermore, the 4G/4G genotype was associated with a lower risk of death from cerebrovascular causes than was the 5G/5G genotype in the latter study.¹⁷ Our results suggest that the 5G allele is a risk factor for myocardial infarction in women, a view consistent with the latter observation.

The 6A allele of the stromelysin-1 gene was associated with an increased rate of progression of coronary atherosclerosis in a small population of men in England.¹⁸ The 6A/6A genotype was also associated with an increased intima-media thickness of the carotid artery in Finnish men²⁰ and in men and women in New York.²¹ We found that the 6A allele is a risk factor for myocardial infarction in women, consistent with these previous observations.

In our large-scale study, neither of the polymorphisms that were associated with a significant risk of myocardial infarction in women was associated with a significant risk of this condition in men. The reason for this difference remains unclear. In Japan, the incidence of myocardial infarction is low in women, especially among premenopausal women, probably because such women are protected by their high serum level of estrogen.²² Indeed, most women with myocardial infarction in our study were postmenopausal. The sex-based difference in the association between genetic polymorphisms and the risk of myocardial infarction might thus be attributable, at least in part, to the differences in the levels of estrogen or other hormones between men and women.

Our results indicate that the identification of genotypes of plasminogen-activator inhibitor type 1 and stromelysin-1, especially the latter, given its high odds ratio of 4.7 (for the comparison of the 5A/6A plus 6A/6A genotypes with the 5A/5A genotype), may prove to be a reliable means of predicting the genetic risk of myocardial infarction in women and might thus contribute to the primary prevention of this condition. For men, however, although a polymorphism of the connexin 37 gene was associated with a significant risk of myocardial infarction, the odds ratio was only 1.4 (for the comparison of the C/T plus T/T genotypes with the C/C genotype). Further studies are thus required in men to identify additional polymor-

phisms that are associated with a significant risk of myocardial infarction and that have higher odds ratios.

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APPENDIX

In addition to the authors, the following investigators and Japanese institutions participated in the study: *Kosei Hospital, Anjo* — H. Horibe, M. Watarai, K. Takemoto, and S. Shimizu; *Okazaki City Hospital, Okazaki* — A. Hirashiki, Y. Murase, and Y. Suzuki; *Nagoya Daini Red Cross Hospital, Nagoya* — Y. Yoshida and T. Okada; *Nagoya University Hospital, Nagoya* — R. Ishiki, F. Somura, A. Yamada, and T. Kato; *Ogaki Municipal Hospital, Ogaki* — K. Takagi; *Hamamatsu Medical Center, Hamamatsu* — C. Takakana; *Chita City Hospital, Chita* — M. Maeda and Y. Nishinaka; *Hekinan City Hospital, Hekinan* — T. Fukumitsu; *Nagoya East City Hospital, Nagoya* — H. Kanda; *Nagoya National Hospital, Nagoya* — T. Watanabe; *Showa Hospital, Komae* — S. Ishikawa and E. Saito; *Toyota Memorial Hospital, Toyota* — H. Inagaki and S. Kamihara; *Tokai Central Hospital, Kagami-hara* — S. Ogawa and T. Fujimura; *National Chubu Hospital, Obu* — J. Goto; and *Marine Clinic, Nagoya* — S. Kato.

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