# Association of genetic variants with myocardial infarction in Japanese individuals with or without metabolic syndrome

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**Abstract.** The etiology of metabolic syndrome (MetS) is highly complex, with both genetic and environmental factors being thought to play an important role. Although MetS has been recognized as a risk factor for myocardial infarction (MI), the genetic risk for MI in individuals with or without MetS has remained uncharacterized. We examined a possible association of genetic variants with MI in individuals with or without MetS separately. The study population comprised 4,424 individuals, including 1,918 individuals with MetS (903 subjects with MI and 1,015 controls) and 2,506 individuals without MetS (499 subjects with MI and 2,007 controls). The 150 polymorphisms examined in the present study were selected by genome-wide association studies of MI and ischemic stroke with the use of Affymetrix GeneChip Human Mapping 500K Array Set. Initial screening by the Chi-square test revealed that the C→T polymorphism (rs1794429) of *LRPAP1*, the A→G polymorphism (rs12373237) of *LAMA3* and the A→G polymorphism (rs3782257) of *NCOR2* were significantly (false discovery rate of <0.05) associated with MI for individuals with MetS, and that the C→G polymorphism (rs13051704) of *TFF1* was significantly related to MI for individuals without MetS. Subsequent multivariable logistic analysis with adjustment for covariates revealed that rs1794429 of *LRPAP1* (recessive model; P=0.0218; odds ratio=0.71) and rs3782257 of *NCOR2* (dominant model; P=0.0057; odds ratio=1.94) were significantly associated with MI among individuals with MetS, and that rs13051704 of *TFF1* (additive model; P=0.0100; odds ratio=0.55) was significantly associated with MI among individuals without MetS. The genetic variants that confer susceptibility to MI differ between individuals with or without MetS. Stratification of subjects according to the presence or absence of MetS may thus be important for personalized prevention of MI based on genetic information.

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*Key words:* genetics, polymorphism, myocardial infarction, coronary heart disease, metabolic syndrome

## Introduction

Metabolic syndrome (MetS) is defined by a clustering of abdominal obesity, an increased serum concentration of triglycerides, a decreased serum concentration of high density lipoprotein (HDL)-cholesterol, high blood pressure and an increased fasting plasma glucose level (1). The etiology of MetS is highly complex, with both genetic and environmental factors being thought to play an important role (2,3). Although MetS has been recognized as a risk factor for atherosclerotic diseases, such as coronary heart disease and myocardial infarction (MI) (4-6), the genetic risk for MI in individuals with or without MetS remains uncharacterized. Given that coronary heart disease and MI remain the leading cause of death in Western countries (7) and the second leading cause of death in Japan, disease prevention is an important strategy for reducing the overall burden of these disorders. The identification of genetic markers for disease risk is thus essential both for risk prediction and for potential intervention to reduce the chance of future cardiovascular events.

We herein performed an association study for 150 single nucleotide polymorphisms and MI in 4,424 Japanese individuals, including 1,918 or 2,506 individuals with or without MetS, respectively. The purpose of the present study was to identify genetic variants that confer susceptibility to MI in

Table I. Primers, probes and other conditions for the genotyping of polymorphisms related (P<0.01) to myocardial infarction.

| Gene | Polymorphism | Sense primer (5'->3') | Antisense primer (5'->3') | Probe 1 (5'->3') | Probe 2 (5'->3') | Annealing temp. (˚C) | Cycles |
|---|---|---|---|---|---|---|---|
| *LRPAP1* | C->T (rs1794429) | CCTTTTCGTTCCAAAAGTTCCTTC | AGCATTCCTGAGTCTTTGAGAGG | ATTCTGCAAATCACTTACTTTCC | TATTCTGCAAATCATTTACTTTCC | 60 | 50 |
| *LAMA3* | A->G (rs12373237) | GAATGATAGCGATTCTCCTCCTCA | TATAGCTCAATAAAGTGGTTACCAA | TTATACACATAGGTCAACTTTTTTG | ATTATACACATAGGTCGACTTTTTT | 60 | 50 |
| *NCOR2* | A->G (rs3782257) | TGGTTCCTGGGGACCCTGAGA | GGACATTCCCTAGGAACTGAGG | GTGGAACCTATAGAAATCCGG | GTGGAACCTATAGAGATCCGG | 60 | 50 |
| *TFF1* | C->G (rs13051704) | TGCCACCCTGAGTTACTCTCCA | TGTGCCAGGTGCTGTTCTAGG | CCAATTTACAGGATGGGCAAA | CCAATTTACAGGATCGGCAAAA | 60 | 50 |
| *CLEC16A* | C->T (rs9925481) | GTACCTACTTTGTAGTGTTCATACA | CCAGGCACTGTTTGGAGCACA | AATTAAATGAGCGAATATGTGCAA | AATTAAATGAGTGAATATGTGCAAT | 60 | 50 |
| *SEMA3F* | A->G (rs12632110) | GGTGCTGCACCGTGGATGTGA | CTCCAGATCACTCCTCTACTACA | TGTGAGTCCTTGCACAGTGGT | CATTTCACCACTGCGCAAGGA | 60 | 50 |

individuals with or without MetS and, thereby, to provide a basis for the personalized prevention of this condition in such individuals separately.

## Patients and methods

*Study population*. The study population comprised a total of 4,424 unrelated Japanese individuals (2,712 men, 1,712 women) who visited the outpatient clinics or were admitted to one of the participating hospitals (Gifu Prefectural General Medical Center and Gifu Prefectural Tajimi Hospital in Gifu Prefecture, and Hirosaki University Hospital, Reimeikyo Rehabilitation Hospital and Hirosaki Stroke Center in Aomori Prefecture, Japan) between October 2002 and March 2008, due to various symptoms or for an annual health checkup, or who were recruited to a population-based prospective cohort study of aging and age-related diseases in Nakanojo, Gunma Prefecture, Japan.

The diagnosis of MetS was based on a modified version of the definition proposed by the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity (1). In this modified version, which was also used in the West of Scotland Coronary Prevention Study (8) and the Women's Health Study (9), body mass index (BMI) replaces waist circumference. On the basis of the recent recognition of a need to revise BMI criteria for obesity in Japanese and other Asian populations (10), we set the cut-off point for obesity as a BMI of ≥25 kg/m$^2$. The subjects with MetS thus had three or more of the following five components: i) a BMI of ≥25 kg/m$^2$; ii) a serum triglyceride concentration of ≥1.65 mmol/l (150 mg/dl) or drug treatment for elevated triglycerides; iii) a serum HDL-cholesterol concentration of <1.04 mmol/l (40 mg/dl) for men or <1.30 mmol/l (50 mg/dl) for women, or drug treatment for reduced HDL-cholesterol; iv) a systolic blood pressure of ≥130 mmHg, a diastolic blood pressure of ≥85 mmHg, or drug treatment for hypertension; and v) a fasting plasma glucose concentration of ≥5.50 mmol/l (100 mg/dl) or drug treatment for elevated glucose. Based on these criteria, the 1,918 individuals (1,189 men, 729 women) were selected as individuals with MetS, whereas 2,506 individuals (1,523 men, 983 women) who had none or one of the five components of MetS were selected as individuals without MetS.

The 1,402 subjects with MI (1,108 men, 294 women) underwent coronary angiography and left ventriculography. The diagnosis of MI was based on typical electrocardiographic changes, as well as on increases both in the serum activity of creatine kinase (MB isozyme) and in the serum concentration of troponin T. The diagnosis was confirmed by the presence of a wall-motion abnormality on left ventriculography and by identification of the responsible stenosis in any of the major coronary arteries or in the left main trunk by coronary angiography. The control subjects comprised 3,022 individuals (1,604 men, 1,418 women) who visited the outpatient clinics of the participating hospitals for an annual health checkup or who were community-dwelling individuals recruited to the prospective cohort study. They had no history of coronary heart disease, aortic aneurysm or peripheral arterial occlusive disease,

Table II. Characteristics of the subjects with myocardial infarction (MI) and the controls among individuals with or without metabolic syndrome.

| | With metabolic syndrome | | | Without metabolic syndrome | | |
|---|---|---|---|---|---|---|
| Characteristic | MI | Controls | P-value | MI | Controls | P-value |
| No. of subjects | 903 | 1,015 | | 499 | 2,007 | |
| Age (years) | 64.8±10.1 | 67.9±9.4 | <0.0001 | 67.2±10.4 | 65.4±11.3 | 0.0011 |
| Gender (male/female; %) | 77.5/22.5 | 48.2/51.8 | <0.0001 | 81.8/18.2 | 55.6/44.4 | <0.0001 |
| Body mass index (kg/m$^2$) | 25.0±7.5 | 25.3±3.3 | 0.2498 | 22.2±7.5 | 22.5±2.9 | 0.0508 |
| Current or former smoker (%) | 28.8 | 23.1 | 0.0046 | 25.1 | 21.6 | 0.1035 |
| Hypertension (%) | 80.1 | 63.5 | <0.0001 | 29.5 | 54.7 | <0.0001 |
| Systolic blood pressure (mmHg) | 146±26 | 143±20 | 0.0082 | 135±26 | 134±21 | 0.1593 |
| Diastolic blood pressure (mmHg) | 77±14 | 81±12 | <0.0001 | 70±14 | 77±12 | <0.0001 |
| Diabetes mellitus (%) | 58.7 | 26.5 | <0.0001 | 28.5 | 8.8 | <0.0001 |
| Fasting plasma glucose (mmol/l) | 8.08±3.54 | 7.50±3.42 | 0.0003 | 6.72±3.08 | 5.88±2.02 | <0.0001 |
| Blood glycosylated hemoglobin (%) | 6.8±1.9 | 5.9±1.5 | <0.0001 | 6.1±1.5 | 5.3±1.0 | <0.0001 |
| Hypercholesterolemia (%) | 30.5 | 34.1 | 0.0951 | 24.2 | 27.2 | 0.1883 |
| Serum total cholesterol (mmol/l) | 5.21±1.07 | 5.26±0.95 | 0.3426 | 5.07±0.97 | 5.08±0.89 | 0.8830 |
| Serum HDL-cholesterol (mmol/l) | 1.12±0.34 | 1.27±0.32 | <0.0001 | 1.33±0.33 | 1.60±0.38 | <0.0001 |
| Serum LDL-cholesterol (mmol/l) | 3.20±0.95 | 3.04±0.83 | 0.0002 | 3.18±0.87 | 2.93±0.78 | <0.0001 |
| Serum triglyceride (mmol/l) | 2.04±1.27 | 2.23±1.37 | 0.0017 | 1.24±0.69 | 1.22±0.61 | 0.6616 |
| Serum creatinine ($\mu$mol/l) | 92.0±92.0 | 71.7±53.1 | <0.0001 | 87.6±77.9 | 57.7±21.2 | <0.0001 |

Quantitative data are means ± SD. LDL, low density lipoprotein; HDL, high density lipoprotein.

ischemic or hemorrhagic stroke or other cerebral diseases, or of atherosclerotic, thrombotic, embolic or hemorrhagic disorders.

The study protocol complied with the Declaration of Helsinki and was approved by the Committees on the Ethics of Human Research of Mie University Graduate School of Medicine, Hirosaki University Graduate School of Medicine, Gifu International Institute of Biotechnology, Tokyo Metropolitan Institute of Gerontology and participating hospitals. Written informed consent was obtained from each participant.

*Selection of polymorphisms*. Our aim was to identify genetic variants that confer susceptibility to MI in Japanese individuals with or without MetS in a case-control association study. A total of 150 polymorphisms examined in the present study were selected by genome-wide association studies of MI and ischemic stroke (P-value for allele frequency <1.0x10$^{-7}$) with the use of the Affymetrix GeneChip Human Mapping 500K Array Set (11,12).

*Genotyping of polymorphisms*. Venous blood (7 ml) was collected into tubes containing 50 mmol/l EDTA (disodium salt), and genomic DNA was isolated with a kit (Genomix; Talent, Trieste, Italy). Genotypes of the 150 polymorphisms were determined at G&G Science (Fukushima, Japan) by a method that combines the polymerase chain reaction and sequence-specific oligonucleotide probes with suspension array technology (Luminex, Austin, TX, USA). Primers, probes and other conditions for genotyping of polymorphisms related to MI (P<0.01 by the Chi-square test) are shown in Table I. Detailed genotyping methodology was described previously (13).

*Statistical analysis*. Quantitative data were compared between subjects with MI and the controls by the unpaired Student's t-test. Categorical 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 departure from Hardy-Weinberg equilibrium. In an initial screening, the genotype distributions (3x2) and allele frequencies (2x2) of each polymorphism were compared between subjects with MI and controls by the Chi-square test. Given the multiple comparisons of genotypes, the false discovery rate (FDR) was calculated by the method of Benjamini and Hochberg (14) from the distribution of P-values for allele frequencies of the 150 polymorphisms. Polymorphisms with FDR of <0.05 were further examined by multivariable logistic regression analysis with adjustment for covariates. Multivariable logistic regression analysis was thus performed with MI as a dependent variable and independent variables including age, gender (0, woman; 1, man), BMI, smoking status (0, non-smoker; 1, current or former smoker), serum concentration of creatinine and the prevalence of hypertension, diabetes mellitus and hypercholesterolemia (0, no history of these conditions; 1, positive history), and genotype of each polymorphism; P-value, odds ratio and 95% confidence interval were calculated. Each genotype was assessed according to dominant, recessive and additive genetic models. Additive models included additive 1 (heterozygotes vs. wild-type homozygotes) and additive 2 (variant homozygotes vs. wild-type homozygotes) models, which were analyzed simultaneously with a single statistical model. We also performed a stepwise forward selection procedure to examine the effects of genotypes, as well as

Table III. Polymorphisms related (P-value for allele frequency <0.01) to myocardial infarction (MI) as determined by the Chi-square test.

| Gene | Polymorphism | dbSNP | MI (%) | Controls (%) | P-value (genotype) | P-value (allele) | FDR (allele) |
|---|---|---|---|---|---|---|---|
| With metabolic syndrome | | | | | | | |
| *LRPAP1* | C→T | rs1794429 | | | 0.0096 | 0.0024 | 0.0444 |
| | *CC* | | 10 (1.1) | 4 (0.4) | | | |
| | *CT* | | 136 (15.1) | 115 (11.5) | | | |
| | *TT* | | 753 (83.8) | 887 (88.2) | | | |
| *LAMA3* | A→G | rs12373237 | | | 0.0263 | 0.0074 | 0.0444 |
| | *AA* | | 18 (2.0) | 15 (1.5) | | | |
| | *AG* | | 205 (22.8) | 183 (18.2) | | | |
| | *GG* | | 676 (75.2) | 808 (80.3) | | | |
| *NCOR2* | A→G | rs3782257 | | | 0.0024 | 0.0096 | 0.0444 |
| | *AA* | | 33 (3.7) | 73 (7.3) | | | |
| | *AG* | | 337 (37.5) | 378 (37.5) | | | |
| | *GG* | | 529 (58.8) | 556 (55.2) | | | |
| Without metabolic syndrome | | | | | | | |
| *TFF1* | C→G | rs13051704 | | | 0.0005 | 0.0001 | 0.0124 |
| | *CC* | | 41 (8.3) | 110 (5.6) | | | |
| | *CG* | | 190 (38.3) | 680 (34.5) | | | |
| | *GG* | | 265 (53.4) | 1,184 (60.0) | | | |
| *CLEC16A* | C→T | rs9925481 | | | 0.0047 | 0.0016 | 0.1180 |
| | *CC* | | 395 (79.6) | 1,460 (74.1) | | | |
| | *CT* | | 94 (19.0) | 473 (24.0) | | | |
| | *TT* | | 7 (1.4) | 37 (1.9) | | | |
| *LRPAP1* | C→T | rs1794429 | | | 0.0145 | 0.0056 | 0.2608 |
| | *CC* | | 2 (0.4) | 9 (0.5) | | | |
| | *CT* | | 61 (12.3) | 297 (15.0) | | | |
| | *TT* | | 433 (87.3) | 1,671 (84.5) | | | |
| *SEMA3F* | A→G | rs12632110 | | | 0.0210 | 0.0070 | 0.2608 |
| | *AA* | | 123 (24.8) | 394 (20.0) | | | |
| | *AG* | | 246 (49.6) | 980 (49.7) | | | |
| | *GG* | | 127 (25.6) | 600 (30.4) | | | |

other covariates on MI. The P-levels for inclusion in and exclusion from the model were 0.25 and 0.1, respectively. In the stepwise forward selection procedure, each genotype was examined according to a dominant or recessive model on the basis of statistical significance in the multivariable logistic regression analysis. With the exception of the initial screen by the Chi-square test (FDR <0.05), a P-value of <0.05 was considered statistically significant. Statistical significance was examined by two-sided tests performed with JMP version 5.1 software and JMP Genomics version 3.2 software (SAS Institute, Cary, NC, USA).

## Results

Baseline characteristics of the subjects with MI and the controls in the presence or absence of MetS are shown in Table II. Among individuals with MetS, the frequency of male subjects, the prevalence of smoking, hypertension and diabetes mellitus, as well as systolic blood pressure, fasting plasma glucose level, blood glycosylated hemoglobin content, serum concentrations of low density lipoprotein (LDL)-cholesterol and creatinine were higher, whereas age, diastolic blood pressure and serum concentrations of HDL-cholesterol and triglycerides were lower in subjects with MI than in the controls. Among individuals without MetS, age, the frequency of male subjects and the prevalence of diabetes mellitus, as well as fasting plasma glucose level, blood glycosylated hemoglobin content and serum concentrations of LDL-cholesterol and creatinine were higher, whereas the prevalence of hypertension, diastolic blood pressure and serum concentration of HDL-cholesterol were lower in subjects with MI than in the controls.

An initial screen by the Chi-square test revealed that the C→T polymorphism (rs1794429) of *LRPAP1*, the A→G polymorphism (rs12373237) of *LAMA3* and the A→G polymorphism (rs3782257) of *NCOR2* were significantly (FDR of

Table IV. Multivariable logistic regression analysis of polymorphisms related to myocardial infarction with adjustment for age, gender, body mass index, smoking status, serum creatinine concentration and the prevalence of hypertension, diabetes mellitus and hypercholesterolemia.

| Gene | Polymorphism | Dominant | | Recessive | | Additive 1 | | Additive 2 | |
|---|---|---|---|---|---|---|---|---|---|
| | | P-value | OR (95% CI) | P-value | OR (95% CI) | P-value | OR (95% CI) | P-value | OR (95% CI) |
| With metabolic syndrome | | | | | | | | | |
| *LRPAP1* | C→T | 0.1377 | | **0.0218** | 0.71 (0.53-0.95) | 0.2920 | | 0.1200 | |
| *LAMA3* | A→G | 0.7548 | | 0.1255 | | 0.4855 | | 0.8423 | |
| *NCOR2* | A→G | **0.0057** | 1.94 (1.22-3.15) | 0.4220 | | **0.0079** | 1.94 (1.20-3.20) | **0.0064** | 1.94 (1.22-3.18) |
| Without metabolic syndrome | | | | | | | | | |
| *TFF1* | C→G | **0.0199** | 0.59 (0.38-0.93) | **0.0375** | 0.78 (0.62-0.99) | 0.0801 | | **0.0100** | 0.55 (0.35-0.87) |

OR, odds ratio; CI, confidence interval. P-values of <0.05 are shown in bold.

Table V. Effects of genotypes and other characteristics on myocardial infarction as determined by a stepwise forward selection procedure (P<0.05).

| Characteristics | P-value | $R^2$ |
|---|---|---|
| With metabolic syndrome | | |
| Male gender | <0.0001 | 0.0556 |
| Diabetes mellitus | <0.0001 | 0.0818 |
| Hypertension | <0.0001 | 0.0174 |
| Body mass index | <0.0001 | 0.0067 |
| Age | 0.0001 | 0.0062 |
| *NCOR2* (A→G, dominant) | 0.0051 | 0.0030 |
| *LAPAP1* (C→T, recessive) | 0.0117 | 0.0024 |
| Serum creatinine concentration | 0.0187 | 0.0021 |
| Without metabolic syndrome | | |
| Male gender | <0.0001 | 0.0935 |
| Diabetes mellitus | <0.0001 | 0.0300 |
| Hypertension | <0.0001 | 0.0288 |
| Smoking | <0.0001 | 0.0110 |
| Serum creatinine concentration | <0.0001 | 0.0099 |
| Hypercholesterolemia | 0.0009 | 0.0048 |
| *TFF1* (C→G, dominant) | 0.0230 | 0.0023 |

$R^2$, contribution rate.

<0.05) associated with MI among individuals with MetS; the C→G polymorphism (rs13051704) of *TFF1* was significantly related to MI among individuals without MetS (Table III).

Multivariable logistic regression analysis with adjustment for age, gender, BMI, smoking status, serum concentration of creatinine and the prevalence of hypertension, diabetes mellitus and hypercholesterolemia revealed that rs1794429 of *LRPAP1* (recessive model) and rs3782257 of *NCOR2* (dominant and additive 1 and 2 models) were significantly (P<0.05) associated with MI for individuals with MetS, and that rs13051704 of *TFF1* (dominant, recessive and additive 2 models) was significantly related to MI for individuals without MetS (Table IV).

A stepwise forward selection procedure was performed to examine the effects of genotypes for the polymorphisms related to MI by multivariable logistic regression analysis, as well as of age, gender, BMI, smoking status, serum concentration of creatinine and the prevalence of hypertension, diabetes mellitus and hypercholesterolemia on MI (Table V). For individuals with MetS, the prevalence of diabetes mellitus, male gender, hypertension, BMI, age, *NCOR2* genotype (dominant model), *LRPAP1* genotype (recessive model), serum concentration of creatinine, in descending order of statistical significance, were significant (P<0.05) and independent determinants of MI. For individuals without MetS, male gender, diabetes mellitus, hypertension, smoking, serum concentration of creatinine, hypercholesterolemia, *TFF1* genotype (dominant model), in descending order of statistical significance, were significant and independent determinants of MI.

## Discussion

We examined the possible relations of 150 polymorphisms of 144 candidate genes to the prevalence of MI in Japanese individuals with or without MetS. Our association study with three steps of analysis (Chi-square test, multivariable logistic regression analysis with adjustment for covariates and stepwise forward selection procedure) revealed that the C→T polymorphism (rs1794429) of *LRPAP1* and the A→G polymorphism (rs3782257) of *NCOR2* were significantly associated with MI in individuals with MetS, and that the C→G polymorphism (rs13051704) of *TFF1* was significantly associated with MI in individuals without MetS.

The low-density lipoprotein receptor-related protein associated protein 1 (LRPAP1) works as a chaperone protein, stabilizing the nascent low-density lipoprotein receptor-related protein (LRP) in the endoplasmic reticulum and Golgi complex (15). The LRP is an endocytic receptor for several ligands, such as α2-microglobulin and apolipoprotein E. LRP is involved in the clearance of lipids from the bloodstream and is expressed in atherosclerotic plaque (16,17). A 21-bp insertion/deletion polymorphism in intron 1 of *LRPAP* was shown to be related to the risk for early-onset MI (18). We now showed that the C→T polymorphism in intron 1 (rs1794429) of *LRPAP1* was significantly associated with MI for individuals with MetS, with the minor *C* allele representing a risk factor for MI, but not for those without MetS. These observations suggested that rs1794429 of *LRPAP1* may interact with one or more components of MetS, although the underlying molecular mechanism remains unclear.

The nuclear receptor corepressor 2 (NCOR2), also known as the silencing mediator of retinoid and thyroid hormone receptor (SMRT), mediates the repression of gene transcription by the thyroid hormone and retinoic acid receptors (19,20). NCOR2 recruits the peroxisome proliferator-activated receptor γ (PPARG) and inhibits PPARG-mediated gene transcription (21). Activation of PPARG regulates adipogenesis, improves insulin resistance and represses atherosclerosis. A PPARG agonist pioglitazone was shown to reduce the risk of cardiovascular events and to delay the progression of atherosclerosis in individuals with type 2 diabetes mellitus (22,23). We now showed that the A→G polymorphism in the intron region (rs3782257) of *NCOR2* is significantly associated with MI in individuals with MetS, with the *G* allele representing a risk factor for MI. Previous and our observations suggest that *NCOR2* may be involved in the progression of MetS and the development of MI for individuals with MetS through its inhibitory effect on the expression of PPARG, although the underlying mechanism remains to be elucidated.

Trefoil factor 1 (TFF1), a member of the trefoil family, is characterized as having at least one copy of the trefoil motif, a 40-amino acid domain that contains three conserved disulfides. TFF1 is expressed in mucous cells of the stomach and is thought to protect the mucosa against insults, to stabilize the mucus layer and to accelerate healing of the epithelium (24). *TFF1* is a tumor suppressor gene implicated in gastric cancer (25). The relation of genetic variants of TFF1 to atherosclerotic disease has not been shown. We now showed that the C→G polymorphism in intron 1 (rs13051704) of *TFF1* is significantly associated with MI in individuals without MetS, with the minor *C* allele representing a risk factor for MI, although the mechanism responsible for this association remains unclear.

Our study has several limitations. i) It is possible that one or more of the polymorphisms associated with MI are in linkage disequilibrium with other polymorphisms in the same gene or in other nearby genes that are actually responsible for the development of this condition. ii) The functional relevance of the identified polymorphisms to gene transcription or to protein function was not determined in the present study. iii) Although we adopted the criterion of FDR <0.05 for association to compensate for the multiple comparisons of genotypes with MI, it is not possible to completely exclude potential statistical errors, such as false positives. iv) Given that the results of the present study were not replicated, validation of our findings will require their replication with independent subject panels.

In conclusion, our present results suggest that *LRPAP1* and *NCOR2* may be susceptibility loci for MI for Japanese individuals with MetS and that *TFF1* may be such a locus for individuals without MetS. Given that genetic variants that confer susceptibility to MI differ between individuals with or without MetS, stratification of subjects according to the presence or absence of MetS may thus be important for personalized prevention of MI based on genetic information. Given that our present study may be considered as hypothesis generating, validation of our findings will require their replication with independent subject panels.

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