Association of polymorphisms of *BTN2A1* and *ILF3* with myocardial infarction in Japanese individuals with different lipid profiles

TETSURO YOSHIDA[1], KIMIHIKO KATO[2], MITSUTOSHI OGURI[3], HIDEKI HORIBE[4], TOSHIKI KAWAMIYA[4], KIYOSHI YOKOI[4], TETSUO FUJIMAKI[1], SACHIRO WATANABE[5], KEI SATOH[6], YUKITOSHI AOYAGI[7], MASASHI TANAKA[7], HIROTO YOSHIDA[8], SHOJI SHINKAI[8], YOSHINORI NOZAWA[9] and YOSHIJI YAMADA[10]

[1]Department of Cardiovascular Medicine, Inabe General Hospital, Inabe; [2]Meitoh Hospital, Nagoya and Life Science Research Center, Mie University, Tsu; [3]Department of Cardiology, Japanese Red Cross Nagoya First Hospital, Nagoya; [4]Department of Cardiovascular Medicine, Gifu Prefectural Tajimi Hospital, Tajimi; [5]Department of Cardiology, Gifu Prefectural General Medical Center, Gifu; [6]Department of Vascular Biology, Institute of Brain Science, Hirosaki University Graduate School of Medicine, Hirosaki; [7]Department of Genomics for Longevity and Health and [8]Research Team for Social Participation and Health Promotion, Tokyo Metropolitan Institute of Gerontology, Tokyo; [9]Gifu International Institute of Biotechnology and Tokai Gakuin University, Kakamigahara; [10]Department of Human Functional Genomics, Life Science Research Center, Mie University, Tsu, Japan

Received December 14, 2010; Accepted February 7, 2011

DOI: 10.3892/mmr.2011.441

Abstract. Dyslipidemia is an important risk factor for myocardial infarction (MI). We previously showed that gene polymorphisms associated with MI differed among individuals with different lipid profiles. We also showed that rs6929846 of *BTN2A1* and rs2569512 of *ILF3* were significantly associated with MI in Japanese individuals. In the present study, we examined the relationship between rs6929846 of *BTN2A1* or rs2569512 of *ILF3* and MI in individuals with low or high serum concentrations of triglycerides, high density lipoprotein (HDL) cholesterol, or low density lipoprotein (LDL) cholesterol, respectively. The study population comprised 5513 unrelated Japanese individuals, including 1537 subjects with MI and 3976 controls. Multivariable logistic regression analyses with adjustment for covariates revealed that rs6929846 of *BTN2A1* was significantly associated with MI in individuals with low ($P=3.1x10^{-5}$; odds ratio, OR=1.66) or high ($P=1.1x10^{-6}$; OR=2.09) triglycerides; in individuals with low (P=0.0082; OR=1.75) or high ($P=2.0x10^{-9}$; OR=1.85) HDL cholesterol; and in individuals with low ($P=3.2x10^{-7}$; OR=1.75) or high ($P=2.8x10^{-5}$; OR=2.18) LDL cholesterol. Similar analyses revealed that rs2569512 of *ILF3* was significantly associated with MI in individuals with low (P=0.0066; OR=1.47) or high (P=0.0013; OR=1.88) triglycerides; in individuals with low (P=0.0059; OR=1.96) or high (P=0.0020; OR=1.51) HDL cholesterol; and in individuals with low (P=0.0004, OR=1.62) LDL cholesterol, but not in those with high LDL cholesterol. The results suggest that the relationship between rs6929846 of *BTN2A1* or rs2569512 of *ILF3* and MI is influenced by the serum concentrations of HDL and LDL cholesterol, respectively. Stratification of subjects according to lipid profiles may thus be useful for the personalized prevention of MI based on genetic information.

Correspondence to: Dr Yoshiji Yamada, Department of Human Functional Genomics, Life Science Research Center, Mie University, 1577 Kurima-machiya, Tsu, Mie 514-8507, Japan
E-mail: yamada@gene.mie-u.ac.jp

Key words: genetics, polymorphism, myocardial infarction, triglycerides, high density lipoprotein cholesterol, low density lipoprotein cholesterol

Introduction

Cardiovascular disease is the leading cause of morbidity and mortality in industrialized countries, and will soon be the leading cause of death in developing countries (1). Disease prevention is an important strategy for reducing the overall burden of coronary heart disease (CHD) and myocardial infarction (MI), and the identification of biomarkers for disease risk is important both for risk prediction and for potential intervention to reduce the possibility of future events.

We previously showed that genetic variants that confer susceptibility to MI differ among individuals with different lipid profiles (2,3). We also showed that the C→T polymorphism (rs6929846) of *BTN2A1* and the A→G polymorphism (rs2569512) of *ILF3* were significantly associated with MI in Japanese individuals by a genome-wide association study (4). To further examine whether the association of polymorphisms

with MI is influenced by lipid profiles, we examined the association between rs6929846 of *BTN2A1* or rs2569512 of *ILF3* and MI in 5513 Japanese individuals with low or high serum concentrations of triglycerides, high density lipoprotein (HDL) cholesterol, or low density lipoprotein (LDL) cholesterol, respectively.

Materials and methods

Study population. The study population comprised 5513 unrelated Japanese individuals (3113 males, 2400 females) who either visited the outpatient clinics of or were admitted to participating hospitals (Gifu Prefectural General Medical Center, Gifu; Gifu Prefectural Tajimi Hospital, Tajimi; Hirosaki University Hospital and Hirosaki Stroke Center, Hirosaki; Japanese Red Cross Nagoya First Hospital, Nagoya; and Inabe General Hospital, Inabe, Japan) between October 2002 and March 2009 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, Kusatsu, and Tokyo, Japan. The 1,537 subjects with MI (1224 males, 313 females) all underwent coronary angiography and left ventriculography. The diagnosis of MI was based on typical electrocardiographic changes and on increases in both the serum activity of creatine kinase (MB isozyme) and the serum concentration of troponin T. The diagnosis was confirmed by the presence of a wall motion abnormality by left ventriculography, and by the identification of the responsible stenosis in any of the major coronary arteries or in the left main trunk by coronary angiography. The 3976 control individuals (1889 males, 2087 females) were recruited from individuals who visited outpatient clinics of the participating hospitals for an annual health checkup, or were community-dwelling individuals enrolled in cohort studies. They had no history of MI or CHD, ischemic or hemorrhagic stroke, peripheral arterial occlusive disease, or other atherosclerotic, thrombotic, embolic, or hemorrhagic disorders. Among the subjects, 3601 and 1912 had low (<1.70 mmol/l) or high (≥1.70 mmol/l) serum concentrations of triglycerides, respectively; 884 and 4629 had low (<1.03 mmol/l) or high (≥1.03 mmol/l) serum concentrations of HDL cholesterol, respectively; and 4260 and 1253 had low (<3.63 mmol/l) or high (≥3.63 mmol/l) serum concentrations of LDL cholesterol, respectively. The subjects with MI and the controls either had or did not have other conventional risk factors for CHD, including hypertension (systolic blood pressure of ≥140 mmHg or diastolic blood pressure of ≥90 mmHg, or taking anti-hypertensive medication), diabetes mellitus [fasting plasma glucose level of ≥6.93 mmol/l or blood glycosylated hemoglobin (hemoglobin A1c) content of ≥6.5%, or taking anti-diabetes medication], and CKD {estimated glomerular filtration rate (eGFR) <60 ml min^{-1} 1.73 m^{-2}; eGFR (ml min^{-1} 1.73 m^{-2}) = 194 x [age (years)]$^{-0.287}$ x [serum creatinine (mg/dl)]$^{-1.094}$ [x 0.739 if female]} (5,6).

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.

Genotyping of polymorphisms. Venous blood (7 ml) was collected into tubes containing 50 mmol/l ethylenediaminetetraacetic acid (disodium salt), and genomic DNA was isolated with a kit (Genomix; Talent, Trieste, Italy). Genotypes of single nucleotide polymorphisms (SNPs, rs6929846 and rs2569512) were determined at G&G Science (Fukushima, Japan) by a method that combines polymerase chain reaction (PCR) and sequence-specific oligonucleotide probes with suspension array technology (Luminex, Austin, TX). Primers, probes, and other PCR conditions for the genotyping of the two SNPs (4) and detailed genotyping methodology (7) were as described previously.

Statistical analysis. Quantitative data were compared between subjects with MI and controls by the unpaired Student's t-test. Categorical data were compared by the χ^2 test. The genotype distribution of each SNP was compared between subjects with MI and controls by the χ^2 test. SNPs with a P-value for genotype distribution 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, sex (0, female; 1, male), body mass index (BMI), smoking status (0, non-smoker; 1, smoker), serum concentrations of triglycerides, HDL cholesterol, LDL cholesterol or creatinine, history of hypertension or diabetes mellitus (0, no history; 1, positive history), and the genotype of each SNP; P-values, odds ratios (ORs), and 95% confidence intervals were calculated. Each genotype was assessed according to dominant, recessive, and additive genetic models. Additive models included the additive 1 model (heterozygotes versus wild-type homozygotes) and the additive 2 model (variant homozygotes versus wild-type homozygotes), which were analyzed simultaneously with a single statistical model. A P-value of <0.05 was considered statistically significant. Statistical significance was examined by two-sided tests performed with JMP version 6.0 and JMP Genomics version 3.2 software (SAS Institute, Cary, NC).

Results

Genetic variants related to MI in individuals with low or high serum concentrations of triglycerides. The characteristics of the subjects with low or high serum concentrations of triglycerides are shown in Table I. For individuals with low serum triglycerides, the frequency of male subjects, serum concentrations of LDL cholesterol and creatinine, as well as the prevalence of smoking, hypertension, and diabetes mellitus were greater, whereas age, BMI, eGFR, and the serum concentration of HDL cholesterol were lower, in subjects with MI compared to the controls. For individuals with high serum triglycerides, the frequency of male subjects, serum concentrations of LDL cholesterol and creatinine, and the prevalence of hypertension and diabetes mellitus were greater, whereas age, eGFR, and the serum concentration of HDL cholesterol were lower, in subjects with MI compared to the controls.

Table I. Baseline characteristics of the subjects with myocardial infarction (MI) and controls among individuals with low or high serum concentrations of triglycerides.

	Low serum triglycerides (n=3601)			High serum triglycerides (n=1912)		
Characteristic	MI	Controls	P-value	MI	Controls	P-value
No. of subjects	924	2677		613	1299	
Age (years)	66.2±10.3	67.7±10.5	<0.0001	63.7±10.1	68.3±8.7	<0.0001
Sex (male/female, %)	78.0/22.0	45.6/54.4	<0.0001	82.1/17.9	51.4/48.6	<0.0001
Body mass index (kg/m^2)	23.4±3.3	23.8±3.3	<0.0001	24.7±3.4	24.4±3.1	0.1551
Current or former smoker (%)	31.8	26.6	0.0025	33.8	32.8	0.6738
Hypertension (%)	70.0	49.4	<0.0001	76.0	59.4	<0.0001
Diabetes mellitus (%)	47.4	24.4	<0.0001	52.4	31.0	<0.0001
Serum triglycerides (mmol/l)	1.06±0.37	1.07±0.33	0.5034	2.56±0.80	2.58±1.14	0.3297
Serum HDL cholesterol (mmol/l)	1.24±0.35	1.57±0.39	<0.0001	1.11±0.30	1.31±0.31	<0.0001
Serum LDL cholesterol (mmol/l)	3.21±0.89	2.97±0.76	0.0025	3.20±1.01	3.02±0.84	0.02592
Serum creatinine (μmol/l)	87.5±87.9	68.2±34.8	<0.0001	98.7±112.8	70.7±24.0	<0.0001
eGFR (ml min^{-1} 1.73 m^{-2})	68.2±22.8	71.4±18.4	<0.0001	65.1±22.1	68.4±16.0	0.0060

Quantitative data are the means ± SD. eGFR, estimated glomerular filtration rate.

Table II. Comparisons of genotype distributions of two SNPs between subjects with myocardial infarction (MI) and controls among individuals with low or high serum concentrations of triglycerides.

Gene	SNP	dbSNP	MI no. (%)	Controls no. (%)	P-value (genotype)
Low serum triglycerides					
BTN2A1	C→T	rs6929846			4.6x10^{-11}
	CC		732 (79.2)	2365 (88.3)	
	CT		183 (19.8)	299 (11.2)	
	TT		9 (1.0)	13 (0.5)	
ILF3	A→G	rs2569512			0.0476
	AA		93 (10.0)	347 (13.0)	
	AG		422 (45.7)	1217 (45.4)	
	GG		409 (44.3)	1113 (41.6)	
High serum triglycerides					
BTN2A1	C→T	rs6929846			1.4x10^{-9}
	CC		471 (76.8)	1148 (88.4)	
	CT		135 (22.0)	145 (11.2)	
	TT		7 (1.2)	6 (0.4)	
ILF3	A→G	rs2569512			0.0007
	AA		59 (9.6)	176 (13.6)	
	AG		254 (41.5)	599 (46.1)	
	GG		300 (48.9)	524 (40.3)	

Comparison of genotype distributions by the χ^2 test revealed that rs6929846 of *BTN2A1* and rs2569512 of *ILF3* were significantly (P<0.05) associated with MI in individuals with low or high serum concentrations of triglycerides (Table II). Multivariable logistic regression analysis with adjustment for age, sex, BMI, smoking status, serum

Table III. Multivariable logistic regression analysis of SNPs associated with myocardial infarction among individuals with low or high serum concentrations of triglycerides.

		Dominant		Recessive		Additive 1		Additive 2	
Gene	SNP	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)
Low serum triglycerides									
BTN2A1	C→T	4.3×10^{-5}	1.63 (1.29-2.06)	0.9908		3.1×10^{-5}	1.66 (1.31-2.10)	0.8572	
ILF3	A→G	0.0066	1.47 (1.12-1.95)	0.3706		0.0099	1.47 (1.10-1.83)	0.0099	1.47 (1.10-1.83)
High serum triglycerides									
BTN2A1	C→T	1.1×10^{-6}	2.09 (1.55-2.82)	0.0928		3.8×10^{-6}	2.05 (1.51-2.78)	0.0617	
ILF3	A→G	0.0062	1.66 (1.16-2.41)	0.0050	1.39 (1.10-1.74)	0.0498	1.47 (1.01-2.17)	0.0013	1.88 (1.29-2.78)

OR, odds ratio; CI, confidence interval. Multivariable logistic regression analysis was performed with adjustment for age, sex, body mass index, smoking status, serum concentrations of HDL cholesterol, LDL cholesterol and creatinine, and the prevalence of hypertension and diabetes mellitus.

concentrations of HDL cholesterol, LDL cholesterol and creatinine, and the prevalence of hypertension and diabetes mellitus revealed that rs6929846 of *BTN2A1* (dominant and additive 1 models) and rs2569512 of *ILF3* (dominant and additive 1 and 2 models) were significantly associated with MI in individuals with low serum triglycerides, and that rs6929846 of *BTN2A1* (dominant and additive 1 models) and rs2569512 of *ILF3* (dominant, recessive, and additive 1 and 2 models) were significantly associated with MI in individuals with high serum triglycerides (Table III).

Genetic variants related to MI in individuals with low or high serum concentrations of HDL cholesterol. The characteristics of the subjects with low or high serum concentrations of HDL cholesterol are shown in Table IV. For individuals with low serum HDL cholesterol, the frequency of male subjects, serum concentrations of LDL cholesterol and creatinine, and the prevalence of hypertension and diabetes mellitus were greater, whereas age and serum concentrations of triglycerides and HDL cholesterol were lower, in subjects with MI compared to the controls. For individuals with high serum HDL cholesterol, the frequency of male subjects, BMI, serum concentrations of LDL cholesterol and creatinine, and the prevalence of hypertension and diabetes mellitus were greater, whereas age, eGFR, and serum concentrations of triglycerides and HDL cholesterol were lower, in subjects with MI compared to the controls.

Comparison of genotype distributions by the χ^2 test revealed that rs6929846 of *BTN2A1* and rs2569512 of *ILF3* were significantly associated with MI in individuals with low or high serum concentrations of HDL cholesterol (Table V). Multivariable logistic regression analysis with adjustment for age, sex, BMI, smoking status, serum concentrations of triglycerides, LDL cholesterol and creatinine, and the prevalence of hypertension and diabetes mellitus revealed that rs6929846 of *BTN2A1* (dominant and additive 1 models) and rs256951 of *ILF3* (dominant, recessive, and additive 2 models) were significantly associated with MI in individuals with low serum HDL cholesterol, and that rs6929846 of *BTN2A1* (dominant, recessive, and additive 1 and 2 models) and rs2569512 of *ILF3* (dominant and additive 1 and 2 models) were significantly associated with MI in individuals with high serum HDL cholesterol (Table VI).

Genetic variants related to MI in individuals with low or high serum concentrations of LDL cholesterol. The characteristics of the subjects with low or high serum concentrations of LDL cholesterol are shown in Table VII. For individuals with low serum LDL cholesterol, the frequency of male subjects, BMI, serum concentrations of triglycerides, LDL cholesterol and creatinine, as well as the prevalence of hypertension and diabetes mellitus were greater, whereas age, eGFR, and the serum concentration of HDL cholesterol were lower, in subjects with MI compared to the controls. For individuals with high serum LDL cholesterol, the frequency of male subjects, BMI, serum concentrations of LDL cholesterol and creatinine, and the prevalence of smoking, hypertension and diabetes mellitus were greater, whereas age and the serum concentration of HDL cholesterol were lower, in subjects with MI compared to the controls.

Table IV. Baseline characteristics of the subjects with myocardial infarction (MI) and controls among individuals with low or high serum concentrations of HDL cholesterol.

	Low serum HDL cholesterol (n=884)			High serum HDL cholesterol (n=4629)		
Characteristic	MI	Controls	P-value	MI	Controls	P-value
No. of subjects	516	368		1021	3608	
Age (years)	63.3±10.4	68.1±9.7	<0.0001	66.1±10.1	67.8±10.0	<0.0001
Sex (male/female, %)	89.7/10.3	70.4/29.6	<0.0001	74.5/25.5	45.2/54.8	<0.0001
Body mass index (kg/m^2)	24.5±3.3	24.6±3.1	0.4538	23.7±3.4	23.3±3.3	0.0043
Current or former smoker (%)	40.9	37.8	0.3494	28.4	27.7	0.6532
Hypertension (%)	68.8	56.0	<0.0001	74.2	52.3	<0.0001
Diabetes mellitus (%)	51.9	34.5	<0.0001	48.1	25.8	<0.0001
Serum triglycerides (mmol/l)	1.79±0.97	2.25±1.71	<0.0001	1.19±0.69	1.50±0.87	0.0085
Serum HDL cholesterol (mmol/l)	0.86±0.12	0.90±0.10	<0.0001	1.35±0.29	1.55±0.36	<0.0001
Serum LDL cholesterol (mmol/l)	3.17±1.01	2.85±0.81	<0.0001	3.22±0.91	3.00±0.79	<0.0001
Serum creatinine (μmol/l)	96.9±109.3	78.8±59.0	<0.0001	89.5±92.8	68.0±27.3	<0.0001
eGFR (ml min^{-1} 1.73m^{-2})	66.8±23.0	67.2±17.7	0.7563	67.1±22.3	70.7±17.7	<0.0001

Quantitative data are the means ± SD. eGFR, estimated glomerular filtration rate.

Table V. Comparisons of genotype distributions of two SNPs between subjects with myocardial infarction (MI) and controls among individuals with low or high serum concentrations of HDL cholesterol.

Gene	SNP	dbSNP	MI no. (%)	Controls no. (%)	P-value (genotype)
Low serum HDL cholesterol					
BTN2A1	C→T	rs6929846			0.0050
	CC		406 (78.7)	316 (85.9)	
	CT		107 (20.7)	47 (12.8)	
	TT		3 (0.6)	5 (1.3)	
ILF3	A→G	rs2569512			0.0007
	AA		51 (9.9)	59 (16.0)	
	AG		215 (41.7)	173 (47.0)	
	GG		250 (48.4)	136 (37.0)	
High serum HDL cholesterol					
BTN2A1	C→T	rs6929846			1.5×10^{-17}
	CC		797 (78.0)	3197 (88.6)	
	CT		211 (20.7)	397 (11.0)	
	TT		13 (1.3)	14 (0.4)	
ILF3	A→G	rs2569512			0.0002
	AA		101 (9.9)	464 (12.9)	
	AG		461 (45.2)	1643 (45.5)	
	GG		459 (45.0)	1501 (41.6)	

Comparison of genotype distributions by the χ^2 test revealed that rs6929846 of *BTN2A1* and rs2569512 of *ILF3* were significantly associated with MI in individuals with low serum LDL cholesterol, and that rs6929846 of *BTN2A1*, but

Table VI. Multivariable logistic regression analysis of SNPs associated with myocardial infarction among individuals with low or high serum concentrations of HDL cholesterol.

Gene	SNP	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)
Low serum HDL cholesterol									
BTN2A1	C→T	0.0226	1.60 (1.07-2.40)	0.1003		0.0082	1.75 (1.16-2.67)	0.1272	
ILF3	A→G	0.0234	1.67 (1.07-2.62)	0.0155	1.46 (1.08-1.99)	0.1189		0.0059	1.96 (1.21-3.16)
High serum HDL cholesterol									
BTN2A1	C→T	2.0×10^{-9}	1.85 (1.51-2.26)	0.0453	2.33 (1.01-5.33)	1.1×10^{-8}	1.82 (1.48-2.23)	0.0254	2.57 (1.11-5.87)
ILF3	A→G	0.0026	1.47 (1.15-1.90)	0.0961		0.0081	1.43 (1.10-1.86)	0.0020	1.51 (1.17-1.98)

OR, odds ratio; CI, confidence interval. Multivariable logistic regression analysis was performed with adjustment for age, sex, body mass index, smoking status, serum concentrations of triglycerides, LDL cholesterol and creatinine, and the prevalence of hypertension and diabetes mellitus.

not rs2569512 of *ILF3*, was significantly associated with MI in individuals with high serum LDL cholesterol (Table VIII). Multivariable logistic regression analysis with adjustment for age, sex, BMI, smoking status, serum concentrations of triglycerides, HDL cholesterol and creatinine, and the prevalence of hypertension and diabetes mellitus revealed that rs6929846 of *BTN2A1* (dominant and additive 1 models) and rs2569512 of *ILF3* (dominant, recessive, and additive 1 and 2 models) were significantly associated with MI in individuals with low serum LDL cholesterol, and that rs6929846 of *BTN2A1* (dominant, recessive, and additive 1 and 2 models) was significantly associated with MI in individuals with high serum LDL cholesterol (Table IX).

Discussion

The butyrophilin, subfamily 2, member A1 gene (*BTN2A1*) is a member of the BTN2 subfamily of genes, which encode proteins belonging to the butyrophilin protein family (Entrez Gene, NCBI). We previously showed that the C→T polymorphism (rs6929846) of *BTN2A1* and the A→G polymorphism (rs2569512) of *ILF3* were significantly associated with MI in Japanese individuals by a genome-wide association study (4). The *T* allele of rs6929846 increased the transcription activity of *BTN2A1* and the over-expression of *BTN2A1* decreased the expression of elastin mRNA and increased the mRNA expression of matrix metallopeptidase 3 and interleukin 5 in human cell cultures (4).

The results of the present study show that the association between rs6929846 of *BTN2A1* and MI was influenced by the serum concentration of HDL cholesterol; the association was more apparent in individuals with high HDL cholesterol than in those with low HDL cholesterol, with the *T* allele representing a risk factor for MI. The butyrophilin family was originally identified on the basis of its ability to promote the production of milk fat globulesin (8), and is involved in lipid, fatty acid, and sterol metabolism (Entrez Gene, NCBI). Given that the regulation of the serum concentration of HDL cholesterol may itself have a genetic component (9), interactions between *BTN2A1* and other genes related to HDL cholesterol metabolism or between *BTN2A1* and environmental factors such as diet and exercise may play roles in the development of MI.

Interleukin enhancer binding factor 3, 90 kDa (*ILF3*) is a subunit of the nuclear factor of activated T-cells, a transcription factor required for the expression of interleukin 2 in T-cells (10). Our previous study (4) demonstrated that *ILF3* is abundantly accumulated in the necrotic core of the coronary plaque, suggesting that *ILF3* may play a role in the development of coronary thrombosis, although the functional relevance of rs2569512 located in intron 7 of *ILF3* to the pathogenesis of MI remains to be elucidated.

The results of the present study show that the relationship between the A→G polymorphism (rs2569512) of *ILF3* and MI was affected by the serum concentration of LDL cholesterol. A significant association was observed in individuals with low LDL cholesterol, with the *G* allele representing a risk factor for MI, but not in those with high LDL cholesterol. Given that genetic factors contribute to the regulation of the serum concentration of LDL cholesterol (9), the interactions between *ILF3* and other genes related to LDL cholesterol

Table VII. Baseline characteristics of the subjects with myocardial infarction (MI) and controls among individuals with low or high serum concentrations of LDL cholesterol.

	Low serum LDL cholesterol (n=4260)			High serum LDL cholesterol (n= 253)		
Characteristic	MI	Controls	P-value	MI	Controls	P-value
No. of subjects	1080	3180		457	796	
Age (years)	65.8±10.2	68.2±10.0	<0.0001	63.8±10.5	66.6±9.8	<0.0001
Sex (male/female, %)	82.8/17.2	49.8/50.2	<0.0001	72.2/27.8	38.6/61.4	<0.0001
Body mass index (kg/m^2)	23.8±3.4	23.3±3.3	<0.0001	24.1±3.4	23.8±3.4	0.0474
Current or former smoker (%)	32.6	29.8	0.0840	32.6	24.0	0.0011
Hypertension (%)	73.1	52.9	<0.0001	70.9	51.8	<0.0001
Diabetes mellitus (%)	48.7	27.7	<0.0001	51.0	21.9	<0.0001
Serum triglycerides (mmol/l)	1.69±0.99	1.56±1.06	<0.0001	1.58±0.81	1.58±0.70	0.6649
Serum HDL cholesterol (mmol/l)	1.19±0.35	1.49±0.39	<0.0001	1.19±0.31	1.48±0.37	<0.0001
Serum LDL cholesterol (mmol/l)	2.74±0.57	2.70±0.57	0.0386	4.30±0.70	4.13±0.46	0.0005
Serum creatinine (μmol/l)	94.5±107.4	69.2±29.7	<0.0001	86.2±73.9	68.2±38.8	<0.0001
eGFR (ml min^{-1} 1.73m^{-2})	66.3±22.6	70.3±17.0	<0.0001	68.6±22.2	70.7±20.6	0.1604

Quantitative data are the means ± SD. eGFR, estimated glomerular filtration rate.

Table VIII. Comparisons of genotype distributions of two SNPs between subjects with myocardial infarction (MI) and controls among individuals with low or high serum concentrations of LDL cholesterol.

Gene	SNP	dbSNP	MI no. (%)	Controls no. (%)	P-value (genotype)
Low serum LDL cholesterol					
BTN2A1	C→T	rs6929846			1.1x10^{-14}
	CC		847 (78.4)	2807 (88.3)	
	CT		224 (20.8)	356 (11.2)	
	TT		9 (0.8)	17 (0.5)	
ILF3	A→G	rs2569512			0.0008
	AA		107 (9.9)	424 (13.3)	
	AG		472 (43.7)	1458 (45.9)	
	GG		501 (46.4)	1298 (40.8)	
High serum LDL cholesterol					
BTN2A1	C→T	rs6929846			5.8x10^{-7}
	CC		356 (77.9)	706 (88.7)	
	CT		94 (20.6)	88 (11.1)	
	TT		7 (1.5)	2 (0.2)	
ILF3	A→G	rs2569512			0.3211
	AA		45 (9.9)	99 (12.4)	
	AG		204 (44.6)	358 (45.0)	
	GG		208 (45.5)	339 (42.6)	

metabolism or between *ILF3* and environmental and lifestyle factors may play a role in the pathogenesis of MI. The underlying molecular mechanism of the association of *ILF3* with LDL cholesterol metabolism remains to be elucidated.

In conclusion, the relationship between rs6929846 of *BTN2A1* or rs2569512 of *ILF3* and MI were influenced by the serum concentrations of HDL and LDL cholesterol, respectively, suggesting that interactions between gene

Table IX. Multivariable logistic regression analysis of SNPs associated with myocardial infarction among individuals with low or high serum concentrations of LDL cholesterol.

		Dominant		Recessive		Additive 1		Additive 2	
Gene	SNP	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)
Low serum LDL cholesterol									
BTN2A1	C→T	$5.8x10^{-7}$	1.72 (1.39-2.12)	0.9527		$3.2x10^{-7}$	1.75 (1.41-2.18)	0.8914	
ILF3	A→G	0.00160	1.51 (1.17-1.95)	0.0086	1.24 (1.06-1.46)	0.01360	1.40 (1.08-1.84)	0.0004	1.62 (1.24-2.13)
High serum LDL cholesterol									
BTN2A1	C→T	$2.8x10^{-5}$	2.18 (1.52-3.15)	0.0170	8.99 (1.71-71.77)	0.00020	2.04 (1.41-2.96)	0.0118	10.2 (1.93-81.29)

OR, odds ratio; CI, confidence interval. Multivariable logistic regression analysis was performed with adjustment for age, sex, body mass index, smoking status, serum concentrations of triglycerides, HDL cholesterol and creatinine, and the prevalence of hypertension and diabetes mellitus.

tic variants and lipid profiles may play crucial roles in the development of MI. Stratification of subjects based on lipid profiles may thus be useful in order to achieve personalized prevention of MI with the use of genetic information. Validation of our findings will require their replication with independent subject panels of other ethnic groups.

Acknowledgements

This work was supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (nos. 18209023, 18018021, and 19659149 to Y.Y.) and by a Research Grant from Mie Medical Valley Project (to Y.Y.).

References

1. Murray CJ and Lopez AD: Alternative projections of mortality and disability by cause 1990-2020: global burden of disease study. Lancet 34: 1498-1504, 1997.
2. Yoshida T, Yajima K, Hibino T, Kato K, Matsuo H, Segawa T, Watanabe S, Oguri M, Yokoi K, Nozawa Y, Kimura G and Yamada Y: Association of gene polymorphisms with myocardial infarction in individuals with different lipid profiles. Int J Mol Med 20: 581-590, 2007.
3. Yoshida T, Kato K, Yokoi K, Oguri M, Watanabe S, Metoki N, Yoshida H, Satoh K, Aoyagi Y, Nozawa Y and Yamada Y: Association of genetic variants with myocardial infarction in Japanese individuals with different lipid profiles. Int J Mol Med 25: 607-616, 2010.
4. Yamada Y, Nishida T, Ichihara S, Sawabe M, Fuku N, Nishigaki Y, Aoyagi Y, Tanaka M, Fujiwara Y, Yoshida H, Shinkai S, Satoh K, Kato K, Fujimaki T, Yokoi K, Oguri M, Yoshida T, Watanabe S, Nozawa Y, Hasegawa A, Kojima T, Han BG, Ahn Y, Lee M, Shin DJ, Lee JH and Jang Y: Association of a polymorphism of *BTN2A1* with myocardial infarction in East Asian populations. Atherosclerosis: Dec 15, 2010 (E-pub ahead of print).
5. National Kidney Foundation: K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. Am J Kidney Dis 39 (Suppl 1): S1-S266, 2002.
6. Matsuo S, Imai E, Horio M, Yasuda Y, Tomita K, Nitta K, Yamagata K, Tomino Y, Yokoyama H and Hishida A; Collaborators developing the Japanese equation for estimated GFR: Revised equations for estimated GFR from serum creatinine in Japan. Am J Kidney Dis 53: 982-992, 2009.
7. Itoh Y, Mizuki N, Shimada T, Azuma F, Itakura M, Kashiwase K, Kikkawa E, Kulski JK, Satake M and Inoko H: High-throughput DNA typing of HLA-A, -B, -C, and -DRB1 loci by a PCR-SSOP-Luminex method in the Japanese population. Immunogenetics 57: 717-729, 2005.
8. Ogg SL, Weldon AK, Dobbie L, Smith AJ and Mather IH: Expression of butyrophilin (Btn1a1) in lactating mammary gland is essential for the regulated secretion of milk-lipid droplets. Proc Natl Acad Sci USA 101: 10084-10089, 2004.
9. Kathiresan S, Melander O, Guiducci C, Surti A, Burtt NP, Rieder MJ, Cooper GM, Roos C, Voight BF, Havulinna AS, Wahlstrand B, Hedner T, Corella D, Tai ES, Ordovas JM, Berglund G, Vartiainen E, Jousilahti P, Hedblad B, Taskinen MR, Newton-Cheh C, Salomaa V, Peltonen L, Groop L, Altshuler DM and Orho-Melander M: Six new loci associated with blood low-density lipoprotein cholesterol, high-density lipoprotein cholesterol or triglycerides in humans. Nat Genet 40: 189-197, 2008.
10. Shim J, Lim H, R Yates J and Karin M: Nuclear export of NF90 is required for interleukin-2 mRNA stabilization. Mol Cell 10: 1331-1344, 2002.