

Association of *FURIN* and *ZPR1* polymorphisms with metabolic syndrome

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Received April 3, 2015; Accepted June 8, 2015

DOI: 10.3892/br.2015.484

Abstract. Although genome-wide association studies (GWASs) have identified various genes and loci in predisposition to metabolic syndrome (MetS) or each component of this condition, the genetic basis of MetS in individuals remains to be identified definitively. The aim of the present study was to examine the possible association of MetS in individuals with 29 polymorphisms that were previously identified as susceptibility loci for coronary artery disease or myocardial infarction by meta-analyses of GWASs. The study population comprised 1,822 subjects with MetS and 1,096 controls. Subjects with MetS had ≥ 3 of the 5 components of the diagnostic criteria for MetS, whereas control individuals had 0-1 of the 5 components. The genotypes for the 29 polymorphisms were determined by the multiplex bead-based Luminex assay. Comparisons of allele frequencies by the χ^2 test revealed that rs17514846 (A→C) of the furin (paired basic amino acid-cleaving enzyme) gene (*FURIN*; $P=0.0006$), rs964184 (C→G) of the ZPR1 zinc finger gene (*ZPR1*; $P=0.0078$) and rs599839 (G→A) of the proline/serine-rich coiled-coil 1 gene ($P=0.0486$) were significantly ($P<0.05$) associated with the prevalence of MetS. Multivariable logistic regression analysis with adjustment for age, gender and smoking status revealed that rs17514846 of *FURIN* ($P=0.0016$; odds ratio, 0.76; dominant model) and rs964184 of *ZPR1* ($P=0.0164$; odds ratio, 1.21; dominant model) were significantly associated with MetS. The minor A allele of rs17514846 of *FURIN* was significantly associated with a decrease in the serum concentration of triglycerides

($P=0.0293$) and to an increase in the serum concentration of high-density lipoprotein (HDL) cholesterol ($P=0.0460$). The minor G allele of rs964184 of *ZPR1* was significantly associated with increases in the serum concentration of triglycerides ($P=6.2 \times 10^{-9}$) and fasting plasma glucose level ($P=0.0028$) and to a decrease in the serum concentration of HDL cholesterol ($P=0.0105$). *FURIN* and *ZPR1* may thus be susceptibility loci for MetS.

Introduction

Metabolic syndrome (MetS) is an escalating public health problem worldwide, as this syndrome is an important risk factor for cardiovascular disease and diabetes mellitus (1). 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 blood glucose level (1,2). MetS confers a 5- or 2-fold increase in the risk of type 2 diabetes mellitus or cardiovascular disease, respectively, over the next 5-10 years (1). Individuals with MetS have a 2- to 4-fold increased risk of stroke, a 3- to 4-fold risk of myocardial infarction and a 2-fold risk of mortality (2,3). Therefore, the prevention and early diagnosis and treatment for MetS are important strategies for reducing the overall burden of cardiovascular disease. Given that genetic factors have been shown to contribute to individual susceptibility to MetS (4), the identification of genetic markers for disease risk is essential. Although recent genome-wide association studies (GWASs) have identified various genes in predisposition to MetS (5) or each component of MetS (6-8), the genetic basis of MetS as a composite phenotype remains to be identified definitively. Recent meta-analyses of GWASs also identified various genes and loci that confer susceptibility to coronary artery disease (CAD) or myocardial infarction in Caucasian populations (9,10). As MetS is an important risk factor for CAD, we hypothesized that certain polymorphisms

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Key words: metabolic syndrome, dyslipidemia, diabetes mellitus, genetics, polymorphism

may contribute to the genetic susceptibility to CAD through affecting the susceptibility to MetS.

The aim of the present study was to examine the possible association of MetS in Japanese individuals with 29 single-nucleotide polymorphisms (SNPs) that were previously identified as susceptibility loci for CAD or myocardial infarction in Caucasian populations by the meta-analyses of GWASs.

Subjects and methods

Study population. The study comprised 2,918 Japanese individuals (1,822 subjects with MetS and 1,096 controls) who either visited the outpatient clinics or were admitted to the participating hospitals (Gifu Prefectural Tajimi Hospital, Tajimi; Gifu Prefectural General Medical Center, Gifu; Japanese Red Cross Nagoya First Hospital, Nagoya; Inabe General Hospital, Inabe; Hirosaki University Hospital and Hirosaki Stroke Center, Hirosaki, Japan) between 2002 and 2012.

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 (11) and the Women's Health Study (12), body mass index (BMI) replaces waist circumference. Given that cut-off values of waist circumferences in Japan remain controversial, BMI was used in the present study instead of waist circumference. On the basis of the recognition of BMI criteria for obesity in Japanese and other Asian populations (13), the cut-off point for obesity was set as a BMI of ≥ 25 kg/m². In the preliminary experiments of 1,211 men and 583 women, BMI was significantly correlated with waist circumferences for men ($r=0.79$, $P=2.8 \times 10^{-262}$) and for women ($r=0.72$, $P=9.7 \times 10^{-95}$). A cut-off value of 25 kg/m² for BMI corresponded to 88.3 or 88.4 cm of waist circumference in men or women, respectively. A total of 1,822 subjects with MetS therefore had ≥ 3 of the following 5 components: i) A BMI of ≥ 25 kg/m²; ii) a serum concentration of triglycerides ≥ 1.65 mmol/l (150 mg/dl) or drug treatment for elevated triglycerides; iii) a serum concentration of HDL cholesterol <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 or diastolic blood pressure of ≥ 85 mmHg, or drug treatment for hypertension; and v) a fasting plasma glucose level of ≥ 5.50 mmol/l (100 mg/dl) or drug treatment for elevated glucose. History of obesity, dyslipidemia, hypertension or diabetes mellitus was evaluated from a detailed questionnaire. The controls comprised a total of 1,096 individuals who had 0-1 of the 5 components of diagnostic criteria for MetS.

The study protocol complied with the Declaration of Helsinki and was approved by the Ethics Committees in each participating Hospital. Written informed consent was obtained from all the subjects.

Selection and genotyping of polymorphisms. The SNPs that were shown to be significantly associated with CAD or myocardial infarction in Caucasian populations were searched by the meta-analyses of GWASs (9,10). These SNPs were

Table I. Characteristics of the 2,918 study subjects.

Characteristics	Metabolic syndrome	Controls	P-value
Subjects, no.	1,822	1,096	
Age, years	64.4 \pm 10.3	63.4 \pm 11.7	0.0415
Gender (m/f, %)	67.4/32.6	57.9/42.1	<0.0001
BMI, kg/m ²	25.5 \pm 3.6	21.8 \pm 2.3	<0.0001
Current or former smoker, %	30.9	24.1	<0.0001
Dyslipidemia, %	61.3	36.0	<0.0001
Diabetes mellitus, %	57.7	15.7	<0.0001
Hypertension, %	83.0	43.9	<0.0001
Systolic blood pressure, mmHg	151 \pm 25	129 \pm 23	<0.0001
Diastolic blood pressure, mmHg	81 \pm 16	72 \pm 14	<0.0001
Serum total cholesterol, mmol/l	5.30 \pm 1.15	5.06 \pm 0.92	<0.0001
Serum triglycerides, mmol/l	2.21 \pm 1.46	1.04 \pm 0.45	<0.0001
Serum HDL cholesterol, mmol/l	1.12 \pm 0.29	1.55 \pm 0.40	<0.0001
Serum LDL cholesterol, mmol/l	3.20 \pm 1.00	3.01 \pm 0.83	<0.0001
Fasting plasma glucose, mmol/l	8.15 \pm 3.64	5.42 \pm 2.28	<0.0001
Blood glycosylated hemoglobin, %	7.10 \pm 1.79	6.26 \pm 1.63	<0.0001
Serum creatinine, μ mol/l	94.4 \pm 114.1	79.4 \pm 80.9	<0.0001

Quantitative data are mean \pm standard deviation. Quantitative data are compared between two groups by the Mann-Whitney U test. HDL, high-density lipoprotein; LDL, low-density lipoprotein; BMI, body mass index; m, male; f, female.

examined with the SNP database (dbSNP, National Center for Biotechnology Information, Bethesda, MD, USA) to find SNPs with a minor allele frequency of ≥ 0.015 in a Japanese population. Finally, 29 SNPs (14) were selected and the association with MetS was examined.

Venous blood (7 ml) was collected into tubes containing 50 mmol/l ethylenediaminetetraacetic acid (disodium salt), the peripheral blood leukocytes were isolated and genomic DNA was extracted from these cells with a DNA extraction kit (Genomix; Talent, Trieste, Italy). Genotypes of SNPs were determined at G&G Science (Fukushima, Japan) by a method that combines polymerase chain reaction and sequence-specific oligonucleotide probes with suspension array technology (Luminex Corporation, Austin, TX, USA), as described previously (14-17). Detailed genotyping methodology was also as described previously (18).

Statistical analysis. The χ^2 test was used to compare categorical variables. Quantitative data were compared between

Table II. Comparison of the genotype distributions and allele frequencies of rs17514846 of *FURIN*, rs964184 of *ZPR1* or rs599839 of *PSRC1* by the χ^2 test between subjects with metabolic syndrome and controls.

Gene	SNP	MetS, n (%)	Controls, n (%)	Call rate (%)	P-value, genotype	P-value, allele
<i>FURIN</i>						
rs17514846 (A→C)	CC	1,338 (73.8)	739 (68.5)	99.1	0.0027 ^a	0.0006 ^a
	CA	438 (24.2)	303 (28.1)			
	AA	37 (2.0)	37 (3.4)			
Hardy-Weinberg P		0.8689	0.3902			
<i>ZPR1</i>						
rs964184 (C→G)	CC	932 (51.2)	610 (55.9)	99.8	0.0297 ^a	0.0078 ^a
	CG	738 (40.5)	409 (37.5)			
	GG	151 (8.3)	72 (6.6)			
Hardy-Weinberg P		0.7731	0.7581			
<i>PSRC1</i>						
rs599839 (G→A)	AA	1,573 (86.8)	910 (84.0)	99.2	0.1229	0.0486 ^a
	AG	225 (12.4)	163 (15.1)			
	GG	15 (0.8)	10 (0.9)			
Hardy-Weinberg P		0.0302	0.3726			

^aP<0.05. *FURIN*, furin (paired basic amino acid cleaving enzyme) gene; *ZPR1*, ZPR1 zinc finger gene; *PSRC1*, proline/serine-rich coiled-coil 1 gene; MetS, metabolic syndrome; SNP, single-nucleotide polymorphism; Hardy-Weinberg P, P-value for the Hardy-Weinberg equilibrium.

two groups by the Mann-Whitney U test, as data were not normally distributed (P<0.01 by the Kolmogorov-Smirnov and Lilliefors test). Allele frequencies were estimated by the gene counting method and allele frequencies of each SNP were compared between subjects with MetS and controls by the χ^2 test. Multivariate logistic regression analysis was performed with MetS as a dependent variable and independent variables, including age, gender (0 female; 1 male), BMI, smoking status (0 non-smoker; 1 current or former smoker) and each genotype; and the P-value, odds ratio and 95% confidence interval were calculated. Genotypes of each polymorphism were assessed according to dominant (0 wild-type homozygote; 1 heterozygote and variant homozygote), recessive (0 wild-type homozygote and heterozygote; 1 variant homozygote) and additive genetic models. Additive models comprised additive 1 (heterozygotes versus wild-type homozygotes) and additive 2 (variant homozygotes versus wild-type homozygotes) models, which were analyzed simultaneously with a single statistical model. P<0.05 was considered to indicate a statistically significant difference. Statistical test was performed with JMP version 5.1 and JMP Genomics version 6.0 softwares (SAS Institute Inc., Cary, NC, USA).

Results

Study characteristics. Characteristics of the study subjects are shown in Table I. Age, the frequency of men, BMI, the prevalence of smoking, dyslipidemia, diabetes mellitus, hypertension as well as serum concentrations of creatinine were greater in subjects with MetS compared to the controls.

Identification of the SNPs associated with MetS prevalence. On the basis of comparisons of genotype distributions or allele frequencies by the χ^2 test, 3 SNPs were significantly (P<0.05) associated with the prevalence of MetS (Table II). Genotype distributions and allele frequencies of rs17514846 (A→C) of the furin (paired basic amino acid cleaving enzyme) gene (*FURIN*) and rs964184 (C→G) of the ZPR1 zinc finger gene (*ZPR1*) were significantly associated with the prevalence of MetS. Allele frequencies of rs599839 (G→A) of the proline/serine-rich coiled-coil 1 gene were also significantly associated with MetS. Genotype distributions of 3 SNPs were in Hardy-Weinberg equilibrium (P>0.05) among control individuals. These SNPs were further examined by multivariable logistic regression analysis with adjustment for covariates.

Multivariable logistic regression analysis with adjustment for age, gender and smoking status revealed that rs17514846 of *FURIN* (dominant, recessive and additive 1 and 2 models) was significantly (P<0.05) associated with MetS with the minor A allele being protective against this condition (Table III). Similar analysis revealed that rs964184 of *ZPR1* (dominant and additive 2 models) was significantly associated with MetS with the minor G allele representing a risk factor for this condition.

Finally, the associations of rs17514846 of *FURIN* or rs964184 of *ZPR1* to the components of MetS were examined, including BMI, systolic and diastolic blood pressure, serum concentrations of triglycerides and HDL cholesterol and fasting plasma glucose level in all the subjects (Table IV). The rs17514846 of *FURIN* was significantly (P<0.05) associated with serum concentrations of triglycerides (dominant and recessive models) and HDL cholesterol (dominant model) with the minor A allele being associated with the decreased

Table III. Multivariable logistic regression analysis of the polymorphisms associated with metabolic syndrome.

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)
<i>FURIN</i>								
rs17514846 (A→C)	0.0016 ^a	0.76 (0.65-0.90)	0.0157 ^a	0.56 (0.35-0.90)	0.0089 ^a	0.79 (0.67-0.94)	0.0077 ^a	0.53 (0.33-0.85)
<i>ZPRL</i>								
rs964184 (C→G)	0.0164 ^a	1.21 (1.03-1.40)	0.0706	1.31 (0.98-1.77)	0.0528	1.17 (1.00-1.37)	0.0280 ^a	1.40 (1.04-1.91)
<i>PSRC1</i>								
rs599839 (G→A)	0.0740	0.82 (0.66-1.02)	0.8474	0.92 (0.41-2.14)	0.0734	0.82 (0.66-1.02)	0.7951	0.90 (0.40-2.09)

^aP<0.05. Multivariable logistic regression analysis was performed with adjustment for age, gender and smoking status. *FURIN*, furin (paired basic amino acid cleaving enzyme) gene; *ZPRL*, ZPRL zinc finger gene; *PSRC1*, proline/serine-rich coiled-coil 1 gene; OR, odds ratio; CI, confidence interval.

Table IV. Comparison of each metabolic syndrome component between two groups (dominant or recessive model) of the rs1751486 or rs964184 polymorphisms.

Characteristics		Genotype		P-value		
				Dominant ^a	Recessive ^a	
<i>FURIN</i> (rs1751486)		<i>CC</i>	<i>CA</i>	<i>AA</i>		
Body mass index, kg/m ²	24.2±3.6	24.0±3.7	23.8±4.1	0.0559	0.2975	
Systolic blood pressure, mmHg	144±27	142±27	139±27	0.1149	0.2222	
Diastolic blood pressure, mmHg	78±16	78±15	76±16	0.6812	0.2317	
Serum triglycerides, mmol/l	1.80±1.35	1.73±1.25	1.43±0.75	0.0293 ^b	0.0396 ^b	
Serum HDL cholesterol, mmol/l	1.27±0.39	1.30±0.43	1.31±0.35	0.0460 ^b	0.3238	
Fasting plasma glucose, mmol/l	7.19±3.47	7.05±3.55	6.36±2.15	0.0809	0.1570	
<i>ZPRL</i> (rs964184)		<i>CC</i>	<i>CG</i>	<i>GG</i>		
Body mass index, kg/m ²	24.1±3.5	24.1±3.7	24.4±3.6	0.9126	0.1129	
Systolic blood pressure, mmHg	144±27	144±26	143±27	0.8475	0.7523	
Diastolic blood pressure, mmHg	78±15	79±16	78±15	0.9351	0.4987	
Serum triglycerides, mmol/l	1.65±1.21	1.84±1.38	2.21±1.52	6.2x10 ^{-9b}	1.4x10 ^{-6b}	
Serum HDL cholesterol, mmol/l	1.30±0.40	1.26±0.39	1.24±0.37	0.0105 ^b	0.1043	
Fasting plasma glucose, mmol/l	6.94±3.27	7.25±3.51	7.75±4.31	0.0028 ^b	0.0530	

^aData for each parameter were compared between two groups [dominant (AA vs. AB+BB) or recessive (AA+AB vs. BB) model; A, major allele; B, minor allele] by the Mann-Whitney U test. ^bP<0.05. HDL, high-density lipoprotein; *FURIN*, furin (paired basic amino acid cleaving enzyme) gene; *ZPRL*, ZPRL zinc finger gene.

serum triglycerides and to the increased serum HDL cholesterol. The rs964184 of *ZPRL* was significantly associated with serum concentrations of triglycerides (dominant and recessive models), HDL cholesterol (dominant model) and fasting plasma glucose level (dominant model). The minor G allele of this SNP was associated with the increased serum triglycerides and fasting plasma glucose level and to the decreased serum HDL cholesterol.

Discussion

The present study examined the associations of 29 SNPs identified by the meta-analyses of GWASs for CAD in Caucasian

populations (9,10) to MetS in 2,918 Japanese individuals. The study showed that rs17514846 of *FURIN* and rs964184 of *ZPRL* were significantly associated with the prevalence of MetS, with the minor A allele of rs17514846 being protective against and the minor G allele of rs964184 representing a risk factor for this condition.

FURIN is an enzyme that belongs to the proprotein convertase subtilisin/kexin (PCSK) family, a type 1 membrane-bound protease that processes latent precursor proteins into their biologically active products (19) and it is expressed in numerous tissues, including neuroendocrine organs, liver, gut and brain (Entrez Gene, NCBI). The expression of *FURIN* was enhanced by growth factors, such as

transforming growth factor $\beta 1$ and interleukin 12 (20,21), and elevated FURIN levels were shown to promote the metastatic activity of cancer, atherosclerosis and the pseudomonas infection of cystic fibrosis (22-25). A previous study (26) showed that FURIN may play a pivotal role in the renin-angiotensin system in that this enzyme participates in the rennin receptor processing and in maintaining the sodium-electrolyte balance. Several SNPs of *FURIN* were thus associated with hypertension (27-29). Several PCSKs, including FURIN, are shown to cleave endothelial and lipoprotein lipases, leading to their inactivation (30,31). Endothelial lipase regulates HDL metabolism through its cleavage into free fatty acids and triglycerides (30), whereas lipoprotein lipase plays a critical role in metabolism of triglycerides by hydrolyzing triglyceride-rich lipoprotein to free fatty acids (32). These observations suggest that FURIN plays an important role in lipid metabolism through the cleavage of endothelial and lipoprotein lipases. The present study has shown that rs17514846 of *FURIN* was significantly associated with MetS with the minor A allele correlating to the decreased serum triglycerides and the increased serum HDL cholesterol. The association of rs17514846 of *FURIN* with MetS may be attributable to the effects of this SNP on metabolism of triglycerides and HDL cholesterol, although the underlying molecular mechanism remains to be elucidated.

ZPR1 is a regulatory protein for cell proliferation and signal transduction and may have multiple physiological functions (33,34). Deficiency of ZPR1 is suggested to cause neurodegenerative disorders such as spinal muscular atrophy (35,36). The most relevant transcription factor that binds to the promoter region of *ZPR1* is peroxisome proliferator-activated receptor γ , which plays an important role in insulin sensitivity and obesity (37,38). This promoter region is also bound by hepatocyte nuclear factor 4 α , which activates a variety of genes involved in glucose, fatty acid and cholesterol metabolism (39). The previous GWASs in various ethnic groups showed the association of rs964184 of *ZPR1* with serum concentrations of triglycerides, low-density lipoprotein cholesterol and HDL cholesterol (40-43). Our previous study showed that rs964184 of *ZPR1* was associated with hypertriglyceridemia in Japanese individuals (44). The rs964184 is located near the apolipoprotein A5-A4-C3-A1 (*APOA5-A4-C3-A1*) locus, which is associated with plasma triglycerides in diverse populations (45-49). Expression of *APOA5* is an efficient regulator of plasma triglycerides by enhancing the catabolism of triglyceride-rich lipoprotein (50) and prohibiting the transportation of triglycerides (51). These observations suggest that the association of rs964184 of *ZPR1* with serum triglycerides and HDL cholesterol may be attributable to linkage disequilibrium with functional polymorphisms in *APOA5* that influence lipid metabolism (52). The present study showed that the minor G allele of rs964184 of *ZPR1* was significantly associated with the increased serum triglycerides and decreased serum HDL cholesterol in Japanese individuals, consistent with the previous studies (40-44).

An increase in the serum concentration of triglycerides is an important risk factor for type 2 diabetes mellitus (53). Several studies suggested that polymorphisms of *APOA5* may play an important role in the development of type 2 diabetes mellitus (54,55). Our previous study showed that rs964184 of *ZPR1* was significantly associated with type 2 diabetes

mellitus in Japanese individuals (56). In the present study, the G allele of rs964184 was associated with the increased fasting plasma glucose level. These observations suggest that rs964184 of *ZPR1* may alter the metabolism of triglycerides, HDL cholesterol or glucose through the interaction with *APOA5*, leading to the development of MetS, although the molecular mechanism remains unclear.

There were several limitations in the present study: i) Given that the results were not replicated, validation of the findings is required in other independent subject panels or in other ethnic groups. ii) It is possible that rs17514846 of *FURIN* or rs964184 of *ZPR1* is in linkage disequilibrium with other polymorphisms in the same gene or in other nearby genes that are responsible for the development of MetS. iii) The functional relevance of rs17514846 of *FURIN* or rs964184 of *ZPR1* to the pathogenesis of MetS remains unclear.

In conclusion, the present results suggest that *FURIN* and *ZPR1* may be susceptibility loci for MetS in Japanese individuals. Determination of genotypes for these polymorphisms may prove informative for assessment of the genetic risk for MetS in the Japanese population.

Acknowledgements

The present study was supported by a Collaborative Research grant from the Gifu Prefectural General Medical Center (no. H24-26 to Y.Y.) and a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (no. 24590746 to Y.Y.).

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