

ORIGINAL ARTICLE

Association of variations in the *FTO*, *SCG3* and *MTMR9* genes with metabolic syndrome in a Japanese population

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Metabolic syndrome is defined as a cluster of multiple risk factors, including central obesity, dyslipidemia, hypertension and impaired glucose tolerance, that increase cardiovascular disease morbidity and mortality. Genetic factors are important in the development of metabolic syndrome, as are environmental factors. However, the genetic background of metabolic syndrome is not yet fully clarified. There is evidence that obesity and obesity-related phenotypes are associated with variations in several genes, including *NEGR1*, *SEC16B*, *TMEM18*, *ETV5*, *GNPDA2*, *BDNF*, *MTCH2*, *SH2B1*, *FTO*, *MAF*, *MC4R*, *KCTD15*, *SCG3*, *MTMR9*, *TFAP2B*, *MSRA*, *LYPLAL1*, *GCKR* and *FADS1*. To investigate the relationship between metabolic syndrome and variations in these genes in the Japanese population, we genotyped 33 single-nucleotide polymorphisms (SNPs) in 19 genes from 1096 patients with metabolic syndrome and 581 control individuals who had no risk factors for metabolic syndrome. Four SNPs in the *FTO* gene were significantly related to metabolic syndrome: rs9939609 ($P=0.00013$), rs8050136 ($P=0.00011$), rs1558902 ($P=6.6 \times 10^{-5}$) and rs1421085 ($P=7.4 \times 10^{-5}$). rs3764220 in the *SCG3* gene ($P=0.0010$) and rs2293855 in the *MTMR9* gene ($P=0.0015$) were also significantly associated with metabolic syndrome. SNPs in the *FTO*, *SCG3* and *MTMR9* genes had no SNP \times SNP epistatic effects on metabolic syndrome. Our data suggest that genetic variations in the *FTO*, *SCG3* and *MTMR9* genes independently influence the risk of metabolic syndrome.

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INTRODUCTION

Metabolic syndrome is a common clinical phenotype that is concurrent with metabolic abnormalities, including central obesity, glucose intolerance, dyslipidemia and hypertension.¹ Several different definitions of the syndrome exist² and there are still debates on the adequacy of the concept; however, metabolic syndrome has attracted considerable interest. Although the pathogenesis of metabolic

syndrome is not fully understood, the predominant underlying risk factor is considered to be central obesity due to an atherogenic diet and physical inactivity in the presence of some genetic background.^{2,3} Adipose tissue, especially visceral fat, secretes various adipocytokines. An increase in adipose tissue mass leads to an alteration in the plasma levels of adipocytokines, resulting in the development of dyslipidemia, hypertension and insulin resistance.^{3,4}

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Results from studies using twins and families have suggested that genetic and environmental factors contribute to the clustering of metabolic abnormalities in various ethnic groups.^{5–10} We determined in a previous study that four single-nucleotide polymorphisms (SNPs) (rs2294901, rs6133922, rs6077785 and rs6108572) in the McKusick–Kaufman syndrome (*MKKS*) gene were significantly associated with metabolic syndrome in the Japanese population¹¹ by screening SNPs in 85 obesity-related genes that had been reported as of 2005.¹² We carried out a large-scale case–control association study and found that secretogranin III (*SCG3*)¹³ and myotubularin-related protein 9 (*MTMR9*)¹⁴ conferred susceptibility to the obesity phenotype in the Japanese population. Recent genome-wide association studies revealed the SNPs associated with obesity and fat distribution (waist circumference and waist to hip ratio).^{15–19} We confirmed that some of these SNPs are also associated with obesity and visceral fat area as determined by computed tomography in the Japanese population.^{20–22}

In this study, we investigated the association between metabolic syndrome and 33 SNPs related to obesity and the obesity-related phenotype. We found that SNPs in the fat mass and obesity associated (*FTO*), *SCG3* and *MTMR9* genes are associated with metabolic syndrome.

MATERIALS AND METHODS

Study subjects

The sample size of the group of Japanese subjects with metabolic syndrome was 1096 (male to female ratio, 594:502; age, 53.8 ± 12.5 years). The sample size of the group of Japanese controls was 581 (male to female ratio, 182:399; age, 47.2 ± 14.8 years). Metabolic syndrome was diagnosed as reported previously.^{11,23} In brief, metabolic syndrome is defined by the presence of two or more metabolic abnormalities in addition to obesity (body mass index > 25 kg m⁻²). The metabolic abnormalities were as follows: (1) triglyceride level ≥ 150 mg per 100 ml and/or high-density lipoprotein cholesterol level < 40 mg per 100 ml, or under treatment for this type of dyslipidemia; (2) systolic blood pressure ≥ 130 mmHg and/or diastolic blood pressure blood pressure ≥ 85 mmHg, or under treatment for hypertension; and (3) fasting glucose level ≥ 110 mg per 100 ml, or under treatment for diabetes. The control group consisted of the subjects who were not obese (body mass index < 25 kg m⁻²) and who exhibited none of the metabolic abnormalities described above. Clinical characteristics of subjects are summarized in Table 1. Subjects with metabolic syndrome were recruited from outpatient clinics. Control subjects were selected from non-obese Japanese volunteers who had undergone a medical examination for common disease screening. Written informed consent was obtained from each subject and the protocol was approved by the ethics committee of each institution and by that of Kyoto University.

DNA preparation and SNP genotyping

Using Genomix (Talent Srl, Trieste, Italy), genomic DNA was extracted from blood samples collected from each subject. We constructed Invader probes (Third Wave Technologies, Madison, WI, USA) for rs3101336, rs2568958 and rs2815752 in the neuronal growth regulator 1 (*NEGR1*) gene; rs10913469 in the SEC16 homolog B (*SEC16B*) gene; rs6548238 and rs7561317 in the transmembrane protein 18 (*TMEM18*) gene; rs7647305 in the ets variant 5 (*ETV5*) gene; rs10938397 in the glucosamine-6-phosphate deaminase 2 (*GNPDA2*) gene; rs6265 and rs925946 in the brain-derived neurotrophic factor (*BDNF*) gene; rs10838738 in the mitochondrial carrier homolog 2 (*MTCH2*) gene; rs7498665 in the SH2B adaptor protein 1 (*SH2B1*) gene; rs9939609, rs8050136, rs6499640, rs1121980, rs1558902 and rs1421085 in the *FTO* gene; rs1424233 in the v-maf musculo-aponeurotic fibrosarcoma oncogene homolog (*MAF*) gene; rs17782313, rs12970134, rs489693 and rs17700144 in the melanocortin 4 receptor (*MC4R*) gene; rs29941 and rs11084753 in the potassium channel tetramerization domain-containing 15 (*KCTD15*) gene; rs3764220 in the *SCG3* gene; rs2293855 in the *MTMR9* gene; rs987237 in the transcription factor AP-2β (*TFAP2B*) gene; rs7826222 in the methionine sulfoxide reductase A (*MSRA*) gene; rs2605100 in the lysophospholipase-like-1 (*LYPLAL1*) gene; rs780094 and

Table 1 Clinical characteristics of the subjects

	Case	Control	P-value
Gender (men/women)	594/502	182/399	<0.0001
Age (years)	53.8 ± 12.5	47.2 ± 14.8	<0.0001
Body mass index (kg m ⁻²)	32.0 ± 5.2	21.4 ± 2.1	<0.0001
Plasma glucose (mg per 100 ml)	131.9 ± 45.1	90.9 ± 7.7	<0.0001
Triglycerides (mg per 100 ml)	184.0 ± 145.3	76.0 ± 28.8	<0.0001
High-density lipoprotein cholesterol (mg per 100 ml)	51.3 ± 18.0	67.3 ± 14.0	<0.0001
Systolic blood pressure (mm Hg)	139.9 ± 17.0	111.1 ± 10.2	<0.0001
Diastolic blood pressure (mm Hg)	85.3 ± 12.2	69.4 ± 7.5	<0.0001
<i>Prevalence of metabolic disease</i>			
Dyslipidemia	858 (78%)	0	
Hypertension	974 (89%)	0	
Impaired fasting glucose	770 (70%)	0	
<i>No. of subjects under treatment</i>			
Dyslipidemia	319	0	
Hypertension	466	0	
Diabetes	379	0	

Data are shown as the mean ± s.d. Men/women ratio was analyzed by the χ^2 -test. P-values for quantitative traits were analyzed using the Mann–Whitney U-test.

rs1260326 in the glucokinase regulator (*GCKR*) gene; and rs174547 in the fatty acid desaturase 1 (*FADS1*) gene. The SNPs were genotyped using Invader assays as previously described.²⁴ The success rate of these assays was > 99.0%.

Statistical analysis

For the additive model, we coded genotypes as 0, 1 or 2, depending on the number of copies of the risk alleles. For the dominant model, homozygosity and heterozygosity with the risk allele were coded as 1 and the other was coded as 0. For the recessive model, homozygosity with the risk allele was coded as 1 and others were coded as 0. Additive, dominant and recessive models were chosen for each SNP, according to data from the previous reports.^{13–19} Odds ratios (ORs) and P-values adjusted for age and gender were calculated using multiple logistic regression analysis with genotypes, age and gender as the independent variables. The Hardy–Weinberg equilibrium was assessed using the χ^2 -test.²⁵ Simple comparison of the clinical data between case and control groups was carried out using the Mann–Whitney U-test. To test SNP×SNP epistasis for case–control population-based samples, we used the logistic regression model for each SNP1 and SNP2, and fits the model in the form of $Y = \beta_0 + \beta_1 \times \text{SNP1} + \beta_2 \times \text{SNP2} + \beta_3 \times \text{SNP1} \times \text{SNP2} + \beta_4 \times \text{age} + \beta_5 \times \text{gender}$. Statistical analysis was performed using the software R (<http://www.r-project.org/>). P-values were corrected by Bonferroni adjustment and $P < 0.00152$ (0.05/33) was considered significant.

We examined the power ($\alpha = 0.00152$) of the test for 1096 cases and 581 controls using GDesignPlus (StaGen, Tokyo, Japan). We considered the controls of five different levels of minor allele frequencies (0.1–0.5) and four different levels of the ORs (1.2–1.5). Obtained power was indicated in Supplementary Table 1.

RESULTS

We used measurements of body mass index (> 25 kg m⁻²) instead of waist circumference, as there are still debates on the criteria for waist circumference, especially in Japanese women. The prevalence of metabolic syndrome increased with subject age and has been previously reported to be ~6–7 times higher in men than in women in Japan.²³ Among our subjects, the ratio of men to women and the average age were both significantly higher in the case group than in the control group (Table 1). To adjust for the effects of age and gender, logistic regression analysis was performed. The most significant

Table 2 Genotype and association tests

SNP ID	Nearby gene	Allele1/ Allele2	Risk Allele	Genotype		Model	P-value	OR (95% CI)
				Case	Control			
rs3101336	NEGR1	A/G	G	4/153/938	1/88/492	a	0.55	1.09 (0.82–1.46)
rs2568958	NEGR1	A/G	A	932/154/4	490/88/1	a	0.60	1.08 (0.81–1.44)
rs2815752	NEGR1	A/G	A	938/154/4	492/88/1	a	0.58	1.09 (0.81–1.45)
rs10913469	SEC16B	T/C	C	596/430/69	331/224/26	a	0.054	1.19 (1.00–1.43)
rs6548238	TMEM18	T/C	C	8/169/919	7/101/473	a	0.037	1.32 (1.02–1.71)
rs7561317	TMEM18	G/A	G	917/171/8	472/102/7	a	0.037	1.32 (1.02–1.71)
rs7647305	ETV5	C/T	C	1015/77/0	537/39/1	a	0.57	1.13 (0.75–1.70)
rs10938397	GNPDA2	A/G	G	513/470/111	289/235/55	a	0.37	1.08 (0.92–1.27)
rs6265	BDNF	A/G	G	182/522/388	100/283/196	a	0.29	1.09 (0.93–1.26)
rs925946	BDNF	T/G	T	2/74/1020	0/44/537	a	0.75	0.94 (0.63–1.40)
rs10838738	MTCH2	G/A	G	95/437/564	67/234/277	a	0.023	0.83 (0.71–0.98)
rs7498665	SH2B1	G/A	G	30/280/784	9/133/439	a	0.045	1.25 (1.01–1.55)
rs9939609	FTO	T/A	A	650/379/65	389/170/20	a	0.00013	1.45 (1.20–1.75)
rs8050136	FTO	C/A	A	650/377/65	389/171/19	a	0.00011	1.45 (1.20–1.76)
rs6499640	FTO	A/G	A	33/248/814	9/134/438	a	0.35	1.11 (0.89–1.37)
rs1121980	FTO	A/G	A	74/404/614	25/196/357	a	0.0028	1.32 (1.10–1.58)
rs1558902	FTO	A/T	A	63/381/648	19/170/391	a	6.6×10 ⁻⁵	1.47 (1.22–1.78)
rs1421085	FTO	C/T	C	63/380/648	19/170/390	a	7.4×10 ⁻⁵	1.47 (1.21–1.78)
rs1424233	MAF	C/G	C	644/368/83	329/211/40	a	0.63	1.04 (0.88–1.23)
rs17782313	MC4R	T/C	C	638/392/66	348/200/33	a	0.25	1.11 (0.93–1.32)
rs12970134	MC4R	A/G	A	21/304/762	16/157/404	a	0.68	1.04 (0.85–1.28)
rs489693	MC4R	C/A	A	682/367/45	365/191/24	a	0.43	1.08 (0.89–1.30)
rs17700144	MC4R	A/G	A	2/67/1025	1/31/546	a	0.21	1.32 (0.85–2.05)
rs29941	KCTD15	T/C	C	672/378/45	349/209/22	a	0.62	1.05 (0.87–1.26)
rs11084753	KCTD15	G/A	G	96/472/528	56/243/282	a	0.60	1.04 (0.89–1.23)
rs3764220	SCG3	A/G	A	844/240/11	438/120/20	d	0.0010	3.74 (1.70–8.23)
rs2293855	MTMR9	A/G	A	482/466/146	215/282/83	r	0.0015	1.43 (1.15–1.78)
rs987237	TFAP2B	A/G	G	664/382/48	363/188/27	a	0.48	1.07 (0.89–1.28)
rs7826222	MSRA	G/C	C	423/511/162	232/269/79	a	0.66	1.04 (0.89–1.21)
rs2605100	LYPLAL1	A/G	G	39/315/740	19/159/399	a	0.67	0.96 (0.79–1.17)
rs780094	GCKR	T/C	C	213/510/368	124/287/169	a	0.17	1.11 (0.96–1.28)
rs1260326	GCKR	T/C	T	364/519/211	160/297/124	a	0.072	1.15 (0.99–1.33)
rs174547	FADS1	T/C	C	426/537/133	232/262/87	a	0.48	0.95 (0.81–1.11)

Abbreviations: a, additive model; CI, confidence interval; d, dominant model; OR, odds ratio; r, recessive model; SNP, single-nucleotide polymorphism. The OR for each SNP was adjusted simultaneously for age and gender.

associations were observed for rs1558902 ($P=6.6\times 10^{-5}$, allele-specific OR (95% confidence interval (CI) adjusted for age and gender)=1.47 (1.22–1.78)) and rs1421085 ($P=7.4\times 10^{-5}$, allele-specific OR (95% CI adjusted for age and gender)=1.47 (1.21–1.77)) in the *FTO* gene (Table 2). These two SNPs were previously reported as variations associated with waist circumference^{18,19} and associated with visceral fat area as determined by computed tomography.²² Two other SNPs (rs9939609 and rs8050136) in the *FTO* gene were also significantly associated with metabolic syndrome even when the conservative Bonferroni's correction was applied ($P<0.00152$). Significant associations were also observed between metabolic syndrome and rs3764220 in the *SCG3* gene ($P=0.0010$) and rs2293855 in the *MTMR9* gene ($P=0.00146$). SNPs, rs6548238 and rs7561317 in the *TMEM18* gene, rs7498665 in the *SH2B1* gene and rs1121980 in the *FTO* gene, were marginally associated with metabolic syndrome ($P<0.05$). SNP rs10838738 in the *MTCH2* gene was also marginally associated with metabolic syndrome, although presence of the G-allele, which is a risk allele for obesity,^{15,16} was associated with a reduced risk of metabolic syndrome. Other SNPs did not show any significant associations with metabolic syndrome. The lack of significant association of these SNPs is most likely due to the relatively lower power of this study

Table 3 Results for SNP pairs for epistasis in case-control analysis

SNP1	SNP2	OR (95% CI)	Epistasis P-value
rs2293855	rs3764220	0.07 (0.01–0.70)	0.023
rs2293855	rs1421085	0.80 (0.55–1.18)	0.27
rs2293855	rs1558902	0.81 (0.55–1.19)	0.29
rs2293855	rs8050136	0.84 (0.57–1.24)	0.38
rs2293855	rs9939609	0.82 (0.56–1.21)	0.32
rs3764220	rs1421085	1.60 (0.44–5.82)	0.48
rs3764220	rs1558902	1.60 (0.44–5.84)	0.47
rs3764220	rs8050136	1.58 (0.43–5.74)	0.49
rs3764220	rs9939609	1.57 (0.43–5.71)	0.49

Abbreviations: CI, confidence interval; OR, odds ratio; SNP, single-nucleotide polymorphism.

(Supplementary Table 1). All SNPs were in Hardy–Weinberg equilibrium ($P>0.05$), with the exception of rs6499640 ($P=0.0099$) and rs1424233 ($P=0.0034$) in the case group and rs3764220 in the control subjects ($P=0.0018$).

As six SNPs were significantly associated with metabolic syndrome, we tested SNP×SNP epistasis. Four SNPs in the *FTO* gene were in a

Table 4 Genotype and association tests of dyslipidemia, hypertension and impaired fasting glucose

Control Genotype	Dyslipidemia (n=858)			Hypertension (n=974)			Impaired fasting glucose (n=770)		
	Genotype	P-value	OR (95% CI)	Genotype	P-value	OR (95% CI)	Genotype	P-value	OR (95% CI)
rs9939609 389/170/20	511/297/48	0.00051	1.42 (1.17–1.73)	575/342/57	9.5×10 ⁻⁵	1.47 (1.21–1.79)	458/266/44	0.00024	1.46 (1.19–1.79)
rs8050136 389/171/19	511/295/48	0.00045	1.43 (1.17–1.74)	575/339/57	8.9×10 ⁻⁵	1.48 (1.22–1.80)	458/265/44	0.00020	1.47 (1.20–1.80)
rs1558902 19/170/391	47/298/510	0.00024	1.45 (1.19–1.77)	55/344/573	4.8×10 ⁻⁵	1.50 (1.23–1.83)	43/268/456	0.00011	1.49 (1.22–1.83)
rs1421085 19/170/390	47/297/509	0.00026	1.45 (1.19–1.77)	55/343/573	5.4×10 ⁻⁵	1.50 (1.23–1.82)	43/267/456	0.00012	1.49 (1.22–1.83)
rs3764220 438/120/20	658/190/9	0.0042	3.37 (1.47–7.76)	752/210/11	0.0028	3.34 (1.52–7.38)	592/170/8	0.0026	3.81 (1.60–9.10)
rs2293855 215/282/83	366/372/118	0.0098	1.35 (1.08–1.70)	430/415/127	0.00098	1.46 (1.17–1.83)	351/315/104	0.00039	1.53 (1.21–1.93)

Abbreviations: CI, confidence interval; OR, odds ratio.

The OR for each single-nucleotide polymorphism was adjusted simultaneously for age and gender.

linkage disequilibrium ($r^2 > 0.97$), thus, pair of these SNPs were not analyzed. Any pairs of SNPs did not show significant epistatic effect on metabolic syndrome (Table 3). We performed the multiple logistic regression analysis with three genotypes (rs1558902, rs3764220 and rs2293855), age and gender as the independent variables and found that effects of these SNPs in the three genes on metabolic syndrome were additive: rs1558902 (additive model), $P=4.1 \times 10^{-5}$, OR (95% CI)=1.50 (1.23–1.81); rs3764220 (dominant model), $P=0.00018$, OR (95% CI)=4.77 (2.11–10.79); rs2293855 (recessive model), $P=0.00090$, OR (95% CI)=1.46 (1.17–1.82). Similar results were obtained using rs9939609, rs8050136 and rs1421085, instead of rs1558902.

Next, we examined the effects of significant SNPs on each metabolic disorder (dyslipidemia, hypertension and impaired fasting glucose). SNPs (rs9939609, rs8050136, rs1558902 and rs1421085) in the *FTO* gene were significantly associated with dyslipidemia, hypertension and impaired fasting glucose (Table 4). rs2293855 in the *MTMR9* gene was significantly associated with hypertension and impaired fasting glucose, and was marginally associated with dyslipidemia. rs3764220 in the *SCG3* gene was marginally associated with all three metabolic disorders. There were no obvious differences among the effects (OR) on each metabolic disorder.

DISCUSSION

The SNPs that were most significantly associated with metabolic syndrome (rs1558902 and rs1421085) exist in the *FTO* gene. Visceral fat accumulation is the most predominant factor for the development of metabolic syndrome. SNPs (rs1558902 and rs1421085) were reported to be associated with waist circumference and visceral fat area as measured by computed tomography.^{18,19,22} SNPs (rs9939609, rs8050136, rs1558902 and rs1421085) were also associated with obesity and type 2 diabetes.^{15–17} We have previously reported that rs3764220 in the *SCG3* gene was associated with obesity and subcutaneous fat area as measured by computed tomography¹³ and that rs2293855 in the *MTMR9* gene was associated with obesity and hypertension.¹⁴ Therefore, it is likely that rs9939609, rs8050136, rs1558902 and rs1421085 in the *FTO*, rs3764220 in the *SCG3* and rs2293855 in the *MTMR9* genes would be susceptible for metabolic syndrome. Epistasis, or gene–gene interaction, has recently received much attention in human genetics.²⁶ In this study, effect of these SNPs on metabolic syndrome was independent, and epistatic effect was not observed.

In the simulation study, the power of this test was 0.317 in the following condition; the sizes of the case and the control groups are 1096 and 581, respectively, OR is 1.4, risk allele frequency is 0.2 and the model of inheritance is additive. Therefore, further studies would

be necessary to elucidate the association between SNPs and metabolic syndrome.

SNPs (rs780094 and rs1260326) in the *GCKR* gene were previously reported to be associated with metabolic disorders;^{27–29} however, the present study did not show the association with metabolic syndrome. The C-allele of rs780094 was reported to be associated with increased fasting plasma glucose and lower triglyceride levels. The risk allele of rs780094 had an antagonistic affect on plasma glucose and triglycerides levels. SNP rs174547 in the *FADS1* gene, which is in almost complete linkage disequilibrium with rs174550, is associated with dyslipidemia and type 2 diabetes.^{28,30} Similar to rs780094, the effects of the risk allele of rs174547 on type 2 diabetes, hypertension and dyslipidemia are not the same. Thus, rs780094 and rs174550 are not likely to be important in the development of metabolic syndrome.

SNPs (rs6548238 and rs7561317) in the *TMEM18* gene were marginally associated with metabolic syndrome. We have reported that these SNPs are associated with obesity.²¹ Takeuchi *et al.* reported that rs48454344, which is in almost complete linkage disequilibrium with rs6548238 and rs7561317,²¹ was associated with body mass index and type 2 diabetes.³¹ Therefore, rs6548238 and rs7561317 in the *TMEM18* gene might be susceptible for the development of metabolic syndrome.

The G-allele of rs10838738 in the *MTCH2* gene, which is a risk allele for obesity,¹⁶ reduced the risk of metabolic syndrome. Takeuchi *et al.*³¹ reported that the G-allele is a risk factor for obesity and is protective for type 2 diabetes. Although further investigations would be necessary, there is a possibility that there are some variations that are specifically associated with components of metabolic syndrome, apart from obesity.

Our approach effectively indicated that SNPs in the *FTO*, *SCG3* and *MTMR9* genes are associated with metabolic syndrome in the Japanese population, in addition to the previously reported SNPs in the *MKKS* gene. SNPs in the *FTO*, *SCG3* and *MTMR9* genes had no SNP×SNP epistatic effects on metabolic syndrome. As metabolic syndrome is a complex concept and the criteria are still controversial, further investigations are necessary to elucidate the roles of SNPs in the *MKKS*, *FTO*, *SCG3* and *MTMR9* genes in the development of metabolic syndrome.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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