ORIGINAL ARTICLE

Screening of 336 single-nucleotide polymorphisms in 85 obesity-related genes revealed McKusick–Kaufman syndrome gene variants are associated with metabolic syndrome

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Genetic factors are important in the development of metabolic syndrome. However, the genetic background of metabolic syndrome remains unclear. We screened polymorphisms in 85 obesity-related genes to determine which may be associated with metabolic syndrome. A total of 336 single-nucleotide polymorphisms (SNPs) in 85 genes selected from the JSNP database were genotyped. We conducted case–control association analyses using patients with metabolic syndrome ($n=1080$) and control individuals ($n=528$) who had no risk of the metabolic syndrome. Three SNPs in the McKusick–Kaufman syndrome (*MKKS*) gene were significantly related to metabolic syndrome by case–control association study; rs1545 (odds ratio (OR) adjusted for age and gender, 1.45; 95% confidence interval (CI), 1.21–1.74; $P=0.000043$ (additive model)); rs1547 (OR, 1.45; 95% CI, 1.21–1.74; $P=0.000041$); and rs2294901 (OR, 1.46; 95% CI, 1.22–1.75; $P=0.000033$). We selected five tag SNPs (rs2294901, rs221667, rs6133922, rs6077785 and rs6108572) in the *MKKS* gene. They were in one linkage disequilibrium (LD) block and rs6133922 ($P=0.00042$), rs6077785 ($P=0.000013$) and rs6108572 ($P=0.000019$) as well as rs2294901 were significantly associated with metabolic syndrome. TGAAA haplotype was protective against the metabolic syndrome ($P=0.0074$), and CCGTT haplotype was susceptible ($P=0.00070$) to the metabolic syndrome. Our data suggest that genetic variations at *MKKS* gene influence the risk of metabolic syndrome.

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INTRODUCTION

Metabolic syndrome is a common clinical phenotype presenting as concurrent metabolic abnormalities, including central obesity, glucose intolerance, dyslipidemia and hypertension.¹ Several different definitions exist² and there are still debates on the adequacy of its concept;

however, metabolic syndrome has attracted considerable interest recently. Although the pathogenesis of metabolic syndrome is not fully understood, the predominant underlying risk factor is considered to be visceral obesity due to an atherogenic diet and physical inactivity in the presence of some genetic background.^{1,3} Adipose tissue,

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especially visceral fat, secretes various adipokines. An increase in adipose tissue mass leads to an alteration in the plasma level of adipokines, resulting in the development of dyslipidemia, hypertension and insulin resistance.^{3,4} Studies using twins and families have suggested that genetic and environmental factors contribute to the clustering of metabolic syndrome in various ethnic groups.^{5–10} In a study of Japanese-American families, significant genetic influences were noted on all metabolic syndrome components, especially dyslipidemia.^{7,10} Several genome-wide linkage studies conducted to identify chromosomal segments that are linked with metabolic syndrome have been reported, but no specific gene or variation has been found.² More than 20 genetic association studies with metabolic syndrome have been reported.^{2,11} The published results at these candidate loci are not consistent and the genetic background of metabolic syndrome remains unclear.

In this study, we screened single-nucleotide polymorphisms (SNPs) in 85 obesity-related genes reported¹¹ for a possible relation to metabolic syndrome. We found that four tag SNPs (rs2294901, rs6133922, rs6077785 and rs6108572) in the McKusick–Kaufman syndrome gene (*MKKS*) were significantly associated with metabolic syndrome.

MATERIALS AND METHODS

Study subjects

The sample size of the first set of Japanese subjects with metabolic syndrome was 729 (case-1; male to female ratio 337:392; age, 54 ± 14 year). The sample size of the first set of Japanese controls was 441 (control-1; male to female ratio 112:329; age, 47 ± 16 year). The sample size of the second set of Japanese subjects with metabolic syndrome was 351 (case-2; male to female ratio 218:133; age, 54 ± 10 year), whereas that of the second set of controls was 87 (control-2; male to female ratio 57:30; age, 51 ± 7 year). Metabolic syndrome was diagnosed principally according to the new definition criteria released by the Japanese Committee for the Diagnostic Criteria of Metabolic Syndrome in April 2005.¹² According to these criteria, metabolic syndrome is defined by the presence of two or more abnormalities in addition to visceral fat obesity (waist circumference: ≥ 85 cm in men, ≥ 90 cm in women). These three abnormalities were as follows: (1) triglycerides ≥ 150 mg per 100 ml and/or high-density lipoprotein cholesterol < 1.03 mmol l⁻¹ (40 mg per 100 ml) or under treatment for this type of dyslipidemia, (2) systolic blood pressure ≥ 130 mm Hg and/or diastolic blood pressure ≥ 85 mm Hg or under treatment for hypertension and (3) fasting glucose ≥ 6.1 mmol l⁻¹ (110 mg per 100 ml) or under treatment for diabetes. We used body mass index (> 25 kg m⁻²) instead of waist circumference, as there are still debates on the criteria for waist

circumference, especially in women. Control was composed of the subjects who were not obese (body mass index < 25 kg m⁻²) and no metabolic abnormalities described above were present. Clinical characteristics of cases and controls are summarized in Table 1.

Subjects with metabolic syndrome were recruited from the outpatient clinics. Control subjects were selected from non-obese Japanese volunteers from the subjects 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 RIKEN.

DNA preparation and SNP genotyping

Genomic DNA was prepared from each blood sample according to the standard methods. The genes reviewed by Rankinen *et al.*¹¹ were used for the analysis of metabolic syndrome. The genes located on the X- and Y-chromosomes were not analyzed in this study. The SNPs within such genes were selected from the IMS-JST (Institute of Medical Science-Japan Science and Technology Agency) SNP database.¹³ A total of 755 SNPs in 122 genes were found in the IMS-JST database. Among the 755 SNPs, we chose for use in this study the 336 SNPs with a minor allele frequency more than 0.2, and the expected allele frequencies were not largely diverged from Hardy–Weinberg equilibrium ($P > 0.001$) (Supplementary Table 1). Invader probes (Third Wave Technologies, Madison, WI, USA) were synthesized for these SNPs, and the SNPs were genotyped in cases and controls by a combination of multiplex PCR and the Invader assay as described earlier.¹⁴

In addition to rs2294901, three SNPs (rs221667, rs6133922 and rs6077785) were selected as tag SNPs using a Haploview 4.0,¹⁵ according to the haplotype map of the human genome (HapMap). In addition, SNP rs6108572 that was reported by Benzinou *et al.*¹⁶ in the *MKKS* gene was also genotyped. Four SNPs (rs221667, rs6133922, rs6077785 and rs6108572) were genotyped using TaqMan probes (C_2230457_10, C_25623400_10 and C_2482540_10; Applied Biosystems, Foster City, CA, USA). TaqMan probe for rs6108572 was designed and synthesized by Applied Biosystems.

Statistical analysis

For each case–control study, χ^2 -test (additive model) was performed according to Sladek *et al.*¹⁷ We coded genotypes as 0, 1 and 2, depending on the number of copies of the risk alleles. Odds ratio (OR) adjusted for age and gender was calculated using multiple logistic regression with genotypes, age and gender as independent variables.

We used the *Q*-value as an approach to the problem of multiple testing.¹⁸ Statistical analysis was performed using the software R (<http://www.r-project.org/>), and the *Q*-value was calculated using *Q-VALUE* (<http://faculty.washington.edu/~jstorey/qvalue/>).

Table 1 Clinical characteristics of the subjects

	Case-1	Control-1	P-value	Case-2	Control-2	P-value
Gender (male/female)	337/392	112/329	<0.0001	218/133	57/30	0.58
Age (year)	54 $\pm$ 14	47 $\pm$ 16	<0.0001	54 $\pm$ 10	51 $\pm$ 7	0.037
Body mass index (kg m ⁻²)	32.7 $\pm$ 5.3	21.2 $\pm$ 2.2	<0.0001	30.5 $\pm$ 4.5	21.9 $\pm$ 2.0	<0.0001
Fasting glucose (mmol l ⁻¹)	7.8 $\pm$ 2.7	5.0 $\pm$ 0.4	<0.0001	6.5 $\pm$ 1.6	5.3 $\pm$ 0.5	<0.0001
Triglycerides (mg per 100 ml)	178 $\pm$ 115	73 $\pm$ 28	<0.0001	188 $\pm$ 187	93 $\pm$ 27	<0.0001
High-density lipoprotein cholesterol (mmol l ⁻¹)	1.33 $\pm$ 0.51	1.76 $\pm$ 0.36	<0.0001	1.35 $\pm$ 0.42	1.61 $\pm$ 0.31	<0.0001
Systolic blood pressure (mm Hg)	140 $\pm$ 17	111 $\pm$ 10	<0.0001	141 $\pm$ 18	114 $\pm$ 10	<0.0001
Diastolic blood pressure (mm Hg)	84 $\pm$ 12	69 $\pm$ 7	<0.0001	87 $\pm$ 13	71 $\pm$ 8	<0.0001
<i>No. of subjects under treatment</i>						
Dyslipidemia	143	0		88	0	
Hypertension	222	0		119	0	
Diabetes	225	0		91	0	

Data are shown mean $\pm$ s.d. Male/female ratio was analyzed by χ^2 -test. *P*-values for quantitative traits were analyzed using Student's *t*-test.

Table 2 Genotype frequencies and association tests of significant SNPs and replication study

Set	Rs number	Gene	Allele		Case			Control			MAF (%)		P-value (additive model)	Odds ratio (95% CI)	Q-value
			1	2	11	12	22	11	12	22	Case	Control			
Case-1 vs control-1	rs2294901	MKKS	T	C	370	300	59	274	141	21	0.29	0.21	4.5×10^{-5}	1.49 (1.21–1.83)	0.0056
	rs1547	MKKS	T	C	59	303	367	22	142	273	0.29	0.21	5.6×10^{-5}	1.48 (1.20–1.83)	0.0056
	rs1545	MKKS	T	G	59	300	370	21	143	274	0.29	0.21	5.9×10^{-5}	1.48 (1.20–1.82)	0.0056
Case-2 vs control-2	rs2294901	MKKS	T	C	199	120	32	54	30	3	0.26	0.21	0.15	1.34 (0.90–1.99)	—
	rs1547	MKKS	T	C	33	121	197	3	31	53	0.27	0.21	0.16	1.32 (0.89–1.95)	—
	rs1545	MKKS	T	G	32	120	199	3	30	55	0.26	0.21	0.15	1.34 (0.90–1.99)	—
Combined	rs2294901	MKKS	T	C	569	420	91	328	171	24	0.28	0.21	3.3×10^{-5}	1.46 (1.22–1.75)	—
	rs1547	MKKS	T	C	92	424	564	25	173	326	0.28	0.21	4.1×10^{-5}	1.45 (1.21–1.74)	—
	rs1545	MKKS	T	G	91	420	569	24	173	329	0.28	0.21	4.3×10^{-5}	1.45 (1.21–1.74)	—

Abbreviations: CI, confidence interval; MAF, minor allele frequency; SNP, single-nucleotide polymorphism. The odds ratio for each SNP was adjusted simultaneously for age and gender using additive model.

The Hardy–Weinberg equilibrium was assessed using the χ^2 -test.¹⁹ We used the linkage disequilibrium (LD) measures D' and r^2 , calculated as reported earlier.²⁰ Haplotype phasing was estimated using the EM algorithm.²¹

RESULTS

The cutoff points for the visceral fat obesity (waist circumference: 85 cm in men, 90 cm in women) are based on the cutoff point for the visceral fat area (100 cm²) determined by computerized tomography scan.^{12,22} Neither body mass index nor waist circumference could estimate the visceral fat mass as precisely as computerized tomography scan. Therefore, we performed the case–control association study using case-1 and control-1 subjects who had no risk of the metabolic syndrome. We found that three SNPs (rs1545, rs1547 and rs2294901) in the *MKKS* gene were significantly related to metabolic syndrome even when the conservative Bonferroni's correction was applied (Table 2). The log-quantile-quantile P -value plot indicates that P -values of these three SNPs were significantly lower than the expected P -values (Figure 1). Q -values of these SNPs were also significantly small (Table 2), suggesting that *MKKS* gene would be related to the metabolic syndrome. To confirm the associations of these SNPs, we performed case–control association using the second set of subjects. Although the minor allele frequency of case-2 were higher than control-2, it was not significant because of the small number of control-2 (Table 2). When the case-2 was compared with control-1, significant associations were observed ($P=0.017$ in rs1547, $P=0.022$ in rs1545 and $P=0.019$ in rs2294901).

We examined the power of the test for this association study. The test was χ^2 -test (additive model) with the significance level $0.05/336=1.49 \times 10^{-4}$ (the Bonferroni's correction). Prevalence of metabolic syndrome was estimated as 12.1 and 1.7% among male and female groups, respectively.¹² Thus, we assumed that the penetrances of associated SNPs were different among males and females. We also assumed two conditions: one was that the effect of increasing one risk allele for disease was equal among males and females; the other was that allele frequencies of the SNPs were the same between male and female groups. Under these assumptions, we examined the power of the test for 729 cases (case-1) and 441 controls (control-1) using Bayes' theorem. We considered the cases of four different levels of minor allele frequencies (0.1–0.4) and eight different levels of the effects (1.1–1.8), and performed 100 000 times simulation studies for each condition. Obtained power was indicated in Supplementary Table 2. We also give obtained powers in the 1080 cases and 528 controls namely combined two case and control groups (Supplementary Table 3).

Figure 1 The log-quantile-quantile P -value plot for the analysis of case-1 vs control-1. The horizontal line, $y=-\log_{10}(0.05/336)$, indicates the threshold of significance using Bonferroni correction. The three single-nucleotide polymorphisms (SNPs) reached the threshold of significance. Black circles represent significant SNPs.

According to the Hapmap database, 38 SNPs (minor allele frequency $>5\%$) exist in the *MKKS* gene and they are in one LD block (Supplementary Figure 1). To investigate whether *MKKS* gene would be associated with the metabolic syndrome, we selected four tag SNPs (rs2294901, rs221667, rs6133922 and rs6077785). These SNPs captured 38 of 38 (100%) alleles at $r^2 > 0.95$. As Benzinou *et al.*¹⁶ reported that rs6108572 was associated with metabolic disorder in French obese children and there was no allele frequency data in the Japanese, we also genotyped rs6108572 in our sample. The four SNPs (rs2294901, rs6133922, rs6077785 and rs6108572) showed strong association with metabolic syndrome, whereas rs221667 showed weak association (Table 3). Minor alleles of four SNPs (rs2294901, rs6133922, rs6077785 and rs6108572) and major allele of rs221667 were risk allele for the metabolic syndrome. All SNPs were in Hardy–Weinberg equilibrium ($P > 0.10$).

Table 3 Genotype frequencies and association tests of tag SNPs in the *MKKS* gene

rs number	Chromosomal location	Allele		Case			Control			MAF (%)		P-value (additive model)	Odds ratio (95% CI)
		1	2	11	12	22	11	12	22	Case	Control		
rs2294901	10333503	T	C	569	420	91	328	171	24	0.28	0.21	3.3×10^{-5}	1.46 (1.22–1.75)
rs221667	10341145	C	G	689	342	46	301	193	30	0.20	0.24	0.010	1.25 (1.04–1.50)
rs6133922	10349073	A	G	653	365	58	364	146	16	0.22	0.17	4.2×10^{-4}	1.39 (1.14–1.69)
rs6077785	10359454	A	T	559	419	95	327	175	23	0.28	0.21	1.3×10^{-5}	1.48 (1.23–1.77)
rs6108572	10360759	T	A	83	430	566	20	177	329	0.28	0.21	1.9×10^{-5}	1.48 (1.23–1.79)

Abbreviations: CI, confidence interval; MAF, minor allele frequency; SNP, single-nucleotide polymorphism.

Case-1, case-2, control-1 and control-2 were used for the analysis. The odds ratio for each SNP was adjusted simultaneously for age and gender using additive model.

Table 4 Linkage disequilibrium (LD) coefficients between three single-nucleotide polymorphisms (SNPs) and other tag SNPs of the *MKKS* gene

		r^2						
		rs2294901	rs1545	rs1547	rs221667	rs6133922	rs6077785	rs6108572
D'	rs2294901	—	1.000	1.000	1.000	1.000	0.993	0.800
	rs1545	0.998	—	0.998	1.000	1.000	0.992	0.800
	rs1547	0.984	0.982	—	1.000	1.000	0.977	0.798
	rs221667	0.094	0.094	0.096	—	1.000	1.000	0.893
	rs6133922	0.752	0.752	0.740	0.071	—	0.991	0.745
	rs6077785	0.971	0.969	0.955	0.096	0.729	—	0.818
	rs6108572	0.629	0.628	0.617	0.074	0.426	0.647	—

Case-1, case-2, control-1 and control-2 were used for the analysis.

Table 5 Haplotype structure and frequencies in the *MKKS* gene

Haplotype ID	Haplotype	Frequency		χ^2	P-value	Permutation P-value
		Case	Control			
Haplotype 1	TCAAA	0.484	0.523	4.3	0.038	0.15
Haplotype 2	TGAAA	0.197	0.238	7.2	0.0074	0.029
Haplotype 3	CCGTT	0.180	0.133	11.5	0.00070	0.0026
Haplotype 4	CCATT	0.055	0.040	3.2	0.072	0.31
Haplotype 5	CCGTA	0.043	0.034	1.3	0.26	0.78
Haplotype 6	TCAAT	0.031	0.024	1.2	0.27	0.79

Case-1, case-2, control-1 and control-2 were used for the analysis. Haplotype phasing was estimated using Haploview 4.0. Single-nucleotide polymorphisms that consisted each haplotype were rs2294901, rs221667, rs6133922, rs6077785 and rs6108572. 100 000 permutations were performed.

Linkage disequilibrium analysis of SNPs in the *MKKS* gene showed that they were in one block (Table 4) and rs6108572 was not captured by other tag SNPs. Thus, we added rs6108572 as a tag SNP in the *MKKS* gene. Haplotype analysis showed that TGAAA haplotype was protective against the metabolic syndrome ($P=0.0074$), and CCGTT haplotype was susceptible ($P=0.00070$) to the metabolic syndrome (Table 5).

The prevalence of metabolic syndrome increased with age and was approximately 6–7 times higher in men than in women in Japan.¹² Thus, interactions of age and gender as well as genetic factors play important roles in the development of metabolic syndrome. When the case–control association study was performed in men and women separately, P -values were less than 0.05, except for rs6133922 in men and rs221667 in women (Table 6). When the case and control were divided by age (over 50 years and under 50 years), significant

associations were observed both in younger and elder groups, except for rs221667 in elder group (Table 6). This finding is most likely due to the decrease in the number of each genotype. To investigate the effect of age and gender, logistic regression analysis was performed. The objective variable was the binary variable for the presence or absence of metabolic syndrome. The explanatory variables were age, gender and SNP. The results are shown in Table 7. These tag SNPs (rs2294901, rs221667, rs6133922, rs6077785 and rs6108572) showed a significant association with metabolic syndrome after correction for the confounding factors (age and gender).

DISCUSSION

We found that SNPs in *MKKS* were significantly associated with the metabolic syndrome. Mutations in *MKKS* cause Bardet–Biedl syndrome, which is an autosomal recessive mendelian disorder characterized by progressive obesity, retinal dystrophy, learning disability, polydactyly, renal and cardiac malformations and hypogenitalism.^{23,24} In some cases of Bardet–Biedl syndrome, the patients also had metabolic syndrome,²⁵ and *MKKS*-null mice developed obesity and hypertension in addition to the phenotype resembling that of the Bardet–Biedl syndrome.²⁶ Recently, T variant of rs6108572 was reported to be associated with quantitative components of the metabolic syndrome (dyslipidemia) as well as obesity and in French Caucasian children.¹⁶ T variant of rs6108572 was also associated with obesity in French Caucasian adults, and TT carriers of obese patients increased prevalence of arterial hypertension. Therefore, *MKKS* would be a good candidate gene for the development of metabolic syndrome, as the variant of *MKKS* was indicated to be related to metabolic syndrome in two populations, the Japanese and French Caucasians. SNPs rs1547 and rs1545 are the nonsynonymous SNPs Arg517Cys and Gly532Val, respectively. SNP rs2294901 is

Table 6 The association tests of tag SNPs in the MKKS gene stratified by gender and age

Samples	rs number	Case				Control				P-value	Odds ratio
		11 ^a	12	22	Total	11	12	22	Total		
Male	rs2294901	297	210	48	555	107	49	11	167	0.026	1.38 (1.03–1.83)
	rs221667	365	166	21	552	97	61	10	168	0.036	1.36 (1.02–1.82)
	rs6133922	339	182	31	552	116	45	7	168	0.081	1.32 (0.97–1.79)
	rs6077785	291	210	50	551	107	50	11	168	0.019	1.40 (1.05–1.86)
	rs6108572	45	213	296	554	10	49	108	167	0.017	1.42 (1.06–1.90)
Female	rs2294901	272	210	43	525	221	122	13	356	0.00044	1.53 (1.20–1.96)
	rs221667	324	176	25	525	204	132	20	356	0.19	1.19 (0.93–1.52)
	rs6133922	314	183	27	524	248	101	9	358	0.0021	1.45 (1.11–1.89)
	rs6077785	268	209	45	522	220	125	12	357	0.00024	1.55 (1.21–1.98)
	rs6108572	38	217	270	525	10	128	221	359	0.00038	1.51 (1.17–1.94)
≥ 50 year	rs2294901	383	274	55	712	166	79	12	257	0.0022	1.46 (1.14–1.86)
	rs221667	452	226	32	710	152	88	18	258	0.092	1.22 (0.97–1.55)
	rs6133922	432	244	33	709	179	72	8	259	0.019	1.37 (1.05–1.78)
	rs6077785	375	274	57	706	165	81	12	258	0.0018	1.47 (1.15–1.87)
	rs6108572	52	280	379	711	12	90	156	258	0.029	1.31 (1.03–1.66)
< 50 year	rs2294901	186	146	36	368	162	92	12	266	0.0022	1.57 (1.18–2.10)
	rs221667	237	116	14	367	149	105	12	266	0.044	1.26 (0.93–1.70)
	rs6133922	221	121	25	367	185	74	8	267	0.0065	1.55 (1.14–2.11)
	rs6077785	184	145	38	367	162	94	11	267	0.0010	1.62 (1.22–2.16)
	rs6108572	31	150	187	368	8	87	173	268	0.000092	1.81 (1.34–2.45)

^aAllele1 and allele2 of each single-nucleotide polymorphism (SNP) are indicated in Table 2. Case-1, case-2, control-1 and control-2 were used for the analysis. The odds ratio for each SNP was adjusted for age or gender using additive model.

Table 7 The results of logistic regression analysis of tag single-nucleotide polymorphisms (SNPs) in the MKKS gene

SNP ID	Explanatory variable	Estimate	s.e.	z-value	P-value
rs2294901	Genotype (additive model, 0/1/2)	0.378	0.093	4.066	0.000048
	Age (year)	0.035	0.004	8.461	<0.000001
	Gender (male/female, 1/0)	0.882	0.115	7.640	<0.000001
rs221667	Genotype (additive model, 0/1/2)	0.220	0.094	2.345	0.019
	Age (year)	0.035	0.004	8.524	<0.000001
	Gender (male/female, 1/0)	0.862	0.115	7.496	<0.000001
rs6133922	Genotype (additive model, 0/1/2)	0.327	0.100	3.260	0.0011
	Age (year)	0.035	0.004	8.384	<0.000001
	Gender (male/female, 1/0)	0.877	0.115	7.620	<0.000001
rs6077785	Genotype (additive model, 0/1/2)	0.392	0.093	4.234	0.000023
	Age (year)	0.035	0.004	8.393	<0.000001
	Gender (male/female, 1/0)	0.877	0.115	7.597	<0.000001
rs6108572	Genotype (additive model, 0/1/2)	0.395	0.095	4.168	0.000031
	Age (year)	0.035	0.004	8.476	<0.000001
	Gender (male/female, 1/0)	0.892	0.115	7.724	<0.000001

Case-1, case-2, control-1 and control-2 were used for the analysis.

located in the 3'-flanking region of MKKS. MKKS is a chaperonin-like protein and a novel centrosomal component required for cytokinesis.²⁷ The apical domain region (amino acids 198–370) is important for centrosomal localization. Thus, Arg517Cys (rs1547) and Gly532Val (rs1545) may not affect its function as severely as Bardet-Biedl syndrome; however, these nonsynonymous changes may alter the

function of the MKKS protein and contribute to the development of metabolic syndrome. SNPs rs6108572 and rs6077785 are located in intron 1 and there are several SNPs in almost absolute LD ($r^2=1.0$) with rs6077785 in intron 1. Thus, these SNPs would affect the transcriptional activity of MKKS gene, although further investigation would be necessary.

In summary, we have successfully identified SNPs in the MKKS gene that is related to metabolic syndrome. Our approach would be effective, although further studies to elucidate the mechanisms by which these SNPs affect the development of metabolic syndrome are needed.

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