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ORIGINAL ARTICLE

Variations in the *FTO* gene are associated with severe obesity in the Japanese

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Abstract Variations in the fat-mass and obesity-associated gene (*FTO*) are associated with the obesity phenotype in many Caucasian populations. This association with the obesity phenotype is not clear in the Japanese. To investigate the relationship between the *FTO* gene and obesity in the Japanese, we genotyped single nucleotide polymorphisms (SNPs) in the *FTO* genes from severely obese subjects [$n = 927$, body mass index (BMI) ≥ 30 kg/m^2] and normal-weight control subjects ($n = 1{,}527$, BMI < 25 kg/m^2). A case-control association analysis revealed that 15 SNPs, including rs9939609 and rs1121980, in a linkage disequilibrium (LD) block of approximately 50 kb demonstrated significant associations with obesity; rs1558902 was most significantly associated with obesity. *P* value in additive mode was 0.0000041, and odds ratio (OR) adjusted for age and gender was 1.41 [95% confidential interval (CI) = 1.22–1.62]. Obesity-associated phenotypes, which include the level of plasma glucose, hemoglobin A1c, total cholesterol, triglycerides, high-density lipoprotein (HDL) cholesterol, and blood pressure were not associated with the

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rs1558902 genotype. Thus, the SNPs in the *FTO* gene were found to be associated with obesity, i.e., severe obesity, in the Japanese.

Keywords Fat-mass and obesity-associated gene · Obesity · Japanese population · Association · SNP

Introduction

Obesity is the most common nutritional disorder in developed countries, and it is a major risk factor for hypertension, cardiovascular disease, and type 2 diabetes (Kopelman 2000; Wilson et al. 2003). Genetic and environmental factors contribute to obesity development (Maes et al. 1997; Barsh et al. 2000; Rankinen et al. 2006). Recent progress in single nucleotide polymorphism (SNP) genotyping techniques has enabled genome-wide association studies on common diseases (Herbert et al. 2006; Frayling et al. 2007; Scuteri et al. 2007; The Wellcome Trust Case Control Consortium 2007; Hinney et al. 2007). Using a large-scale case-control association study, we found that secretogranin III (SCG3) (Tanabe et al. 2007) and myotubularin-related protein 9 (MTMR9) (Yanagiya et al. 2007) are involved in susceptibility to the obesity phenotype. Genome-wide association studies have shown that the fat-mass and obesity-associated gene (*FTO*) is also associated with the obesity phenotype (Frayling et al. 2007; Scuteri et al. 2007; Hinney et al. 2007). This association was also found in many Caucasian and Hispanic American populations (Frayling et al. 2007; Scuteri et al. 2007; Dina et al. 2007; Field et al. 2007; Andreasen et al. 2008; Wåhlén et al. 2008; Peeters et al. 2008), whereas it was not found in the Chinese Han population (Li et al. 2008). Among Japanese, body mass index (BMI) was higher in subjects who had the A allele of rs9939609, similar to that observed in Caucasians; however, this finding was not significant (Horikoshi et al. 2007). Another group reported that rs9939609 was associated with BMI in the Japanese (Omori et al. 2008). Thus, the association of SNPs in the *FTO* gene with obesity in the Japanese remains controversial.

To investigate the relationship between the *FTO* gene and obesity in the Japanese, we performed a case-control association study using patients with severe adult obesity (BMI $\geq$ 30 kg/m^2) and normal-weight controls (BMI $<$ 25 kg/m^2); we found that SNPs in intron 1 of the *FTO* gene were associated with severe adult obesity.

Materials and methods

Study subjects

The sample size for severely obese Japanese subjects (BMI $\geq$ 30 kg/m^2) was 927 (male:female ratio 419:508, age 48.7 $\pm$ 14.2 years, BMI 34.2 $\pm$ 5.4 kg/m^2), whereas that for Japanese normal weight controls (BMI $<$ 25 kg/m^2) was 1,527 (male:female ratio 685:842, age 48.1 $\pm$ 16.5 years, BMI 21.7 $\pm$ 2.1 kg/m^2). The severely obese subjects were recruited from among outpatients of medical institutes. Patients with secondary obesity and obesity-related hereditary disorders were not included, and neither were patients with medication-induced obesity. The normal-weight controls were recruited from among subjects who had undergone a medical examination for screening of common diseases. Clinical features of the subjects are illustrated in Table 1. Additionally, 1,604 subjects were recruited (male:female ratio 803:801, age 48.7 $\pm$ 16.9 years, BMI 22.66 $\pm$ 3.16 kg/m^2) from the Japanese general population. Each subject provided written informed consent, and the protocol was approved by the ethics committee of each institution and that of RIKEN.

DNA preparation and SNP genotyping

Genomic DNA was prepared from the blood sample of each subject by using the Genomix (Talent Srl, Trieste, Italy). We searched for dbSNPs with minor allele frequencies (MAF) $>$ 0.10 in the *FTO* gene of Japanese people. We selected 90 SNPs and were able to construct Invader probes (Third Wave Technologies, Madison, WI)

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Table 1 Clinical characterization of obese and control subjects

	Obese	Control	*P* value
Gender (M/F)	419/508	658/842	
Age (year)	49.1 ± 14.2	48.2 ± 16.5	0.049
Body mass index (kg/m^2)	34.50 ± 5.39	21.65 ± 2.08	<0.000001
Glucose (mg/dl)	129.2 ± 49.6	97.7 ± 23.9	<0.000001
HbA1c (%)	6.5 ± 1.8	5.1 ± 0.6	<0.000001
Total cholesterol (mg/dl)	209.9 ± 37.9	201.2 ± 36.4	<0.000001
Triglycerides (mg/dl)	153.2 ± 99.5	104.0 ± 73.2	<0.000001
High-density lipoprotein cholesterol (mg/dl)	53.1 ± 18.9	65.1 ± 15.7	<0.000001
Systolic blood pressure (mmHg)	136.4 ± 18.1	123.4 ± 17.8	<0.000001
Diastolic blood pressure (mmHg)	83.8 ± 12.0	76.0 ± 11.1	<0.000001

P values were analyzed using Mann–Whitney *U* test. Data are mean ± standard deviation

for them (Supplementary Table 1). SNPs were genotyped using Invader assays as described previously (Ohnishi et al. 2001; Takei et al. 2002). Nine SNPs (rs9937053, rs9939973, rs9940128, rs7193144, rs8043757, rs9923233, rs9926289, rs9939609, and rs9930506) reported in a previous genome-wide association study (Scuteri et al. 2007) were genotyped using TaqMan probes (C__29910458_10, C__11776771_10, C__29621384_10, C__29387650_10, C__29387665_10, C__29693738_10, C__30270568_10, C__30090620_10, and C__29819994_10; Applied Biosystems, Foster City, CA, USA).

Statistical analysis

Genotype or allele frequencies were compared between cases and controls in three different modes. In the first mode, i.e., the additive mode, χ^2 test was performed according to Sladek et al. (Sladek et al. 2007). In the second mode, i.e., the minor allele recessive mode, frequencies of the homozygous genotype for the minor allele were compared using a 2 × 2 contingency table. In the third mode, i.e., the minor allele dominant mode, frequencies of the homozygous genotype for the major allele were compared using a 2 × 2 contingency table. A test of independence was performed using Pearson's χ^2 method. *P* values were corrected by Bonferroni adjustment and $P < 0.00017$ [0.05/99 (total SNP number)/3 (number of modes)] was considered significant. The odds ratio (OR) and 95% confidence interval (CI) were calculated by Woolf's method. We coded genotypes as 0, 1, and 2, depending on the number of copies of the risk alleles. OR adjusted for age and gender was calculated using multiple logistic regression with genotypes, age, and gender as independent variables. Hardy–Weinberg equilibrium was assessed using the χ^2 test (Nielsen et al. 1998). Haplotype blocks were determined using Haploview (Barrett et al. 2005). Simple comparison of the clinical data among the different genotypes was performed using one-way analysis of variance (ANOVA). Simple comparison of the clinical data between case and control groups was analyzed using Mann–Whitney *U* test. Difference in BMI between genotypes was analyzed using a multiple linear regression, with BMI as the dependent variable and genotype as the independent variable, and with gender and age as covariates for BMI. Statistical analyses were performed using StatView 5.0 (SAS Institute, Cary, NC, USA). Power was calculated by the Monte Carlo method.

Results

Case-control association studies

We searched for dbSNPs with MAF > 0.10 in the *FTO* gene. By using Invader and TaqMan assay, we successfully genotyped 99 SNPs spanning the *FTO* gene (Supplementary Table 1). Using these SNPs, we performed tests of independence between the phenotype and genotypes of obesity at each SNP by using severely obese subjects (BMI ≥ 30 kg/m^2) and normal weight controls (BMI < 25 kg/m^2). For each SNP, the lowest *P* value among the three different modes was selected as the minimum *P* value. All SNPs, including rs1421084, were in Hardy–Weinberg equilibrium ($P > 0.01$) (Supplementary Table 1).

The power of the test was calculated by Monte Carlo method with different MAFs and different effect sizes. Effect of the risk allele on penetrance was assumed to be multiplicative; i.e., the penetrances for three genotypes were assumed to be a, ar, and ar^2, respectively, where a and r denote the lowest penetrance and genotype relative risk, respectively. Supplementary Table 2 shows the calculated values of the power of the test with different MAFs and different genotype relative risks (r). The lowest penetrance (a) was calculated for each gender by assuming the affection rates of 2.3% for men and 3.4% for women (Yoshiike et al. 2002). Genotype relative risk (r) was assumed to be

the same for both genders. Supplementary Table 2 shows that the test has significant power at relative high risk allele frequency when genotype relative risk is >1.7.

As shown in Fig. 1 and Supplementary Table 1, 15 SNPs demonstrated significant associations with the obesity phenotype; the threshold of significance using Bonfferoni correction was $P < 0.00017$. These SNPs included rs9939609 (Frayling et al. 2007) and rs1121980 (Hinney et al. 2007) that were reported to be significantly associated with the obesity phenotype in the Caucasian population, as determined by genome-wide association studies; rs9930506 (Scuteri et al. 2007) showed marginal association with obesity in the Japanese. Linkage disequilibrium (LD) analysis revealed that these 15 SNPs were in almost complete LD ($D' > 0.98$, $r^2 > 0.80$) and were located within the same LD block of approximately 50 kb (Fig. 1). The most significant association was observed for rs1558902 [additive mode, $P = 0.0000041$ and allele-specific OR (95% CI) adjusted for age and gender was 1.41 (1.22–1.62)]. The minor alleles of rs9939609 (MAF = 0.24) and rs1121980 (MAF = 0.26) were significantly more frequent in the obese group than in the normal-weight control group (additive mode, $P = 0.000012$ and $P = 0.000051$, respectively), and ORs were 1.38 (95% CI = 1.20–1.59) and 1.33 (95% CI = 1.16–1.52), respectively (Table 2, Supplementary Table 1). The MAF of both SNPs in the control group was 0.18; this was consistent with data obtained from the haplotype map of the human genome (HapMap) (Supplementary Table 1). Our data indicated that the SNPs in the *FTO* gene were associated with severe obesity in the Japanese.

Analysis of various quantitative phenotypes with rs1558902

To investigate whether the genotypes of SNP rs1558902 are associated with the phenotypes of metabolic disorders, we compared the following among the different genotypes in the cases, controls, and combined groups: ANOVA results, BMI, levels of fasting plasma glucose, hemoglobin A1c (HbA1c), total cholesterol, triglycerides, HDL cholesterol, and blood pressure. As rs1558902 showed the most significant association with obesity and its call rate was the highest, we analyzed various quantitative phenotypes by using this SNP. The quantitative phenotypes regarding BMI and the levels of fasting plasma glucose, HbA1c, total cholesterol, triglycerides, HDL cholesterol, and blood pressure were not found to be significantly associated with the genotypes at rs1558902 in either the case or control group (Table 3). Although there was no significant difference in BMI values among genotypes in either the control or case group, the direction of the difference (AA > AT > TT) was in accordance with the association between the qualitative obesity phenotype and the genotype shown.

Finally, we examined the BMI distribution of rs1558902 in the Japanese general population and found that rs1558902 genotype was significantly associated with BMI

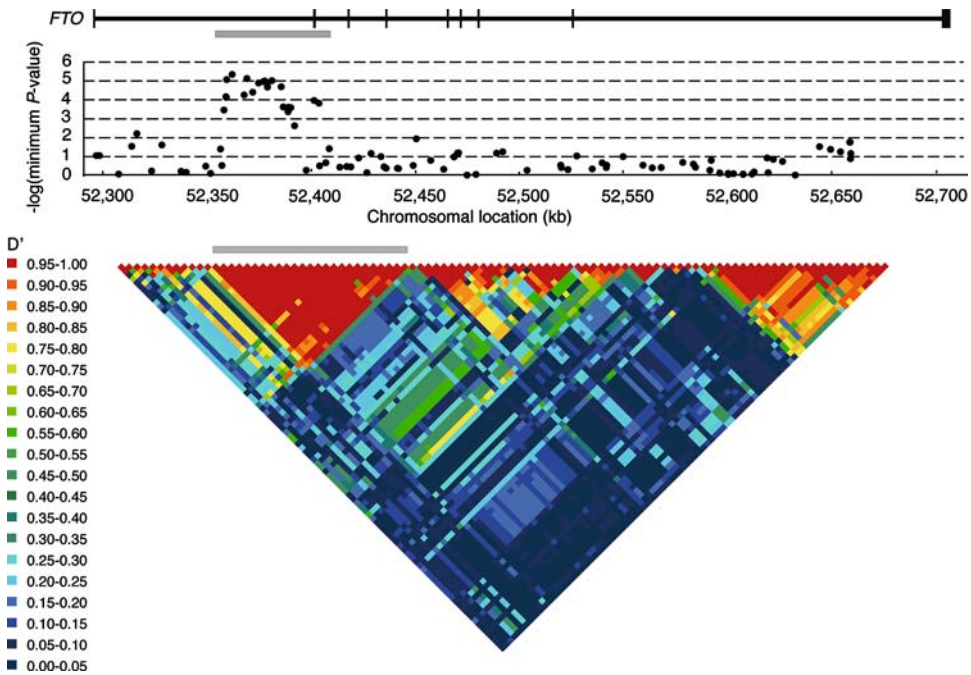

Fig. 1 Linkage disequilibrium (LD) mapping, polymorphisms, and *P* values obtained in the test of independence between the phenotype and genotypes of obesity at various single nucleotide polymorphisms (SNPs) in the fat-mass and obesity-associated gene (*FTO*) gene. *P* values are expressed as negative logarithm of the minimum *P* values obtained in the three models (additive, minor allele dominant, and minor allele recessive modes). LD coefficients (*D'*) between each pair of SNPs were calculated and are displayed as a strand in the LD blocks. Minor allele frequencies of all SNPs used in this analysis are ≥10%. The genomic structure is shown in the *upper*. The *gray bar* marks the LD block associated with obesity

Table 2 Associations of single nucleotide polymorphisms (SNPs) in the fat-mass and obesity-associated gene (*FTO*) gene with obesity existing in the 50-kb linkage disequilibrium (LD) block

dbSNP ID	Allele	Genotype								Additive mode			Recessive mode			Dominant mode		
		Case				Control												
	1/2	11	12	22	Sum	11	12	22	Sum	OR (95% CI)	$\chi 2$	*P* value	$\chi 2$	*P* value	OR (95% CI)	$\chi 2$	*P* value	OR (95% CI)
rs9937053	A/G	59	360	494	913	63	414	773	1250	1.31 (1.13–1.51)	12.3	0.00047	2.0	0.16	1.30 (0.90–1.88)	13.0	0.00031	1.37 (1.16–1.63)
rs9939973	A/G	61	367	496	924	75	504	941	1520	1.32 (1.15–1.51)	15.7	0.000077[a]	3.0	0.081	1.36 (0.96–1.93)	16.1	0.000061[a]	1.40 (1.19–1.66)
rs9940128	A/G	60	366	498	924	75	500	941	1516	1.31 (1.15–1.50)	15.2	0.00010[a]	2.6	0.11	1.33 (0.94–1.89)	15.9	0.000068[a]	1.40 (1.19–1.65)
rs1421085	C/T	49	338	537	924	57	443	1019	1519	1.38 (1.20–1.59)	19.6	0.000011[a]	3.3	0.068	1.44 (0.97–2.12)	20.0	0.0000078[a]	1.47 (1.24–1.74)
rs1558902	A/T	48	341	536	925	52	449	1021	1522	1.41 (1.22 -1.62)	21.2	0.0000041[a]	4.6	0.032	1.55 (1.04–2.31)	20.8	0.0000052[a]	1.48 (1.25–1.75)
rs1121980	A/G	61	367	499	927	73	504	947	1524	1.33 (1.16–1.52)	16.5	0.000051[a]	3.6	0.059	1.40 (0.99–1.99)	16.5	0.000050[a]	1.41 (1.19–1.66)
rs7193144	C/T	49	339	532	920	55	447	1014	1516	1.39 (1.21–1.61)	20.4	0.0000067[a]	4.0	0.044	1.49 (1.01–2.22)	20.3	0.0000067[a]	1.47 (1.24–1.74)
rs8043757	T/A	48	319	541	908	54	436	1027	1517	1.36 (1.18–1.57)	17.4	0.000037[a]	4.2	0.040	1.51 (1.02–2.25)	16.4	0.000052[a]	1.42 (1.20–1.69)
rs8050136	A/C	51	336	538	925	56	450	1018	1524	1.38 (1.20–1.59)	19.4	0.000012[a]	4.7	0.031	1.53 (1.04–2.26)	18.5	0.000017[a]	1.45 (1.22–1.71)
rs3751812	T/G	51	340	534	925	55	458	1013	1526	1.38 (1.20–1.59)	19.6	0.0000098[a]	5.1	0.024	1.56 (1.06–2.31)	18.5	0.000017[a]	1.45 (1.22–1.71)
rs9923233	C/G	51	335	533	919	55	449	1010	1514	1.38 (1.20–1.60)	19.8	0.0000093[a]	5.0	0.025	1.56 (1.06–2.30)	18.7	0.000015[a]	1.45 (1.23–1.72)
rs9926289	A/G	50	323	531	904	56	425	993	1474	1.37 (1.19 -1.58)	18.7	0.000020[a]	3.9	0.047	1.48 (1.00–2.19)	18.1	0.000021[a]	1.45 (1.22–1.72)
rs9939609	A/T	51	334	534	919	56	443	1005	1504	1.38 (1.20–1.59)	19.5	0.000012[a]	4.5	0.034	1.52 (1.03–2.24)	18.7	0.000015[a]	1.45 (1.23–1.72)
rs7185735	G/A	51	340	536	927	55	455	1014	1524	1.38 (1.20–1.59)	19.9	0.0000089[a]	5.0	0.025	1.55 (1.05–2.30)	18.8	0.000014[a]	1.45 (1.23–1.72)
rs9931494	G/C	64	363	494	921	71	504	942	1517	1.35 (1.18–1.55)	18.4	0.000018[a]	5.6	0.018	1.52 (1.07–2.15)	16.9	0.000039[a]	1.42 (1.20–1.67)
rs17817964	T/C	62	361	500	923	68	524	930	1522	1.30 (1.14–1.49)	13.5	0.00022	5.8	0.016	1.54 (1.08–2.19)	11.4	0.00075	1.33 (1.13–1.57)
rs9930506	G/A	67	365	488	920	82	521	913	1516	1.28 (1.12–1.46)	12.8	0.00038	3.5	0.061	1.37 (0.98–1.92)	12.1	0.00051	1.34 (1.14–1.58)
rs9932754	C/T	66	368	491	925	78	525	919	1522	1.29 (1.13–1.48)	13.6	0.00023	4.2	0.040	1.42 (1.01–2.00)	12.6	0.00040	1.35 (1.14–1.59)
rs9922619	T/G	66	368	489	923	78	529	919	1526	1.29 (1.13–1.48)	13.5	0.00024	4.3	0.038	1.43 (1.02–2.01)	12.3	0.00044	1.34 (1.14–1.58)
rs7204609	C/T	134	418	373	925	273	717	529	1519	0.83 (0.73–0.93)	9.68	0.0022	5.0	0.025	0.77 (0.62–0.97)	7.5	0.0063	0.79 (0.67–0.94)
rs12149832	A/G	53	349	525	927	62	480	982	1524	1.33 (1.15–1.53)	15.2	0.000098[a]	3.5	0.061	1.43 (0.98–2.08)	14.8	0.00012[a]	1.39 (1.17–1.64)

The odds ratio (OR) for each SNP was adjusted simultaneously for age and gender using additive model

CI confidence interval, χ^2 chi-square

[a] Significant *P* value ($P < 0.00017$)

Table 3 Comparison of various quantitative phenotypes among different genotypes at single nucleotide polymorphism (SNP) rs1558902 in obese and control subjects

	Obese			Control		
	AA ($n = 48$)	AT ($n = 341$)	TT ($n = 536$)	AA ($n = 52$)	AT ($n = 448$)	TT ($n = 1022$)
Age (year)	49.8 ± 15.3	49.6 ± 14.3	48.8 ± 14.1	46.9 ± 15.4	46.9 ± 16.7	48.8 ± 16.5
P value		0.64			0.098	
BMI (kg/m^2)	35.16 ± 5.70	34.61 ± 5.43	34.39 ± 5.33	21.94 ± 2.23	21.62 ± 2.10	21.65 ± 2.06
P value		0.58			0.56	
Glucose (mg/dl)	142.8 ± 54.8	125.4 ± 43.2	130.8 ± 53.3	101.7 ± 44.1	96.3 ± 18.1	98.2 ± 24.7
P value		0.054			0.34	
HbA1c (%)	6.9 ± 2.1	6.4 ± 1.7	6.5 ± 1.8	5.1 ± 1.2	5.0 ± 0.5	5.1 ± 0.7
P value		0.19			0.15	
Total cholesterol (mg/dl)	215.1 ± 46.7	211.3 ± 38.8	208.6 ± 36.6	195.6 ± 38.8	201.4 ± 37.8	201.4 ± 35.6
P value		0.37			0.53	
Triglycerides (mg/dl)	171.7 ± 119.5	151.3 ± 102.1	153.2 ± 96.0	111.7 ± 70.6	102.0 ± 71.4	104.4 ± 74.2
P value		0.42			0.63	
HDL cholesterol (mg/dl)	53.2 ± 13.8	54.8 ± 24.0	52.0 ± 15.4	62.1 ± 14.2	65.1 ± 15.9	65.3 ± 15.6
P value		0.14			0.53	
SBP (mmHg)	134.2 ± 20.4	137.0 ± 17.8	136.2 ± 18.2	122.7 ± 17.3	123.2 ± 18.8	123.5 ± 17.5
P value		0.61			0.91	
DBP (mmHg)	80.3 ± 11.7	84.1 ± 12.0	83.9 ± 12.0	75.5 ± 11.1	75.2 ± 11.7	76.3 ± 10.9
P value		0.14			0.22	

Data of each quantitative phenotype were compared among different genotypes at the rs1558902 in obese and control subjects. *P* values were analyzed using analysis of variance in each group of obese and control subjects. Data are mean ± standard deviation.

HDL high-density lipoprotein, *SBP* systolic blood pressure, *DBP* diastolic blood pressure.

Table 4 Association of body mass index (BMI) with rs1558902 genotypes in the Japanese general population

	AA	AT	TT	*P* value (additive model)[a]
BMI (kg/m^2) (*n*)	23.17 ± 3.20 (59)	22.79 ± 3.26 (482)	22.57 ± 3.11 (1063)	0.041

[a] The difference in BMI according to genotypes was analyzed using a multiple linear regression, with BMI as the dependent variable and genotype as the independent variable and with gender and age as covariates for BMI. Data are represented as mean ± standard deviation

(Table 4). This result would confirm the association of rs1558902 with obesity.

Discussion

Recent genome-wide association studies have shown that the *FTO* gene is associated with obesity (Frayling et al. 2007; Scuteri et al. 2007; Hinney et al. 2007). The associations between variations in the *FTO* gene and the obesity phenotype have been observed in many Caucasian subjects (Frayling et al. 2007; Scuteri et al. 2007; Dina et al. 2007; Field et al. 2007; Andreasen et al. 2008; Wåhlén et al. 2008; Peeters et al. 2008). However, these associations were controversial with regard to Asian subjects (Horikoshi et al. 2007; Li et al. 2008; Omori et al. 2008). BMI values did not significantly differ among the genotypes in the general population of Chinese and Japanese (Horikoshi et al. 2007; Li et al. 2008). We performed a case-control association study with regard to severe obesity and found that the SNPs in the *FTO* gene were significantly associated with severe obesity. Although the SNPs demonstrated the most significant association in the Japanese, which was different from that in Caucasians, the significantly associated SNPs existed in a similar block as that in Caucasians. Therefore, the *FTO* gene could also contribute to the development of severe obesity in the Japanese.

BMI was modestly different among rs1558902 genotypes in the general population in this study; rs9939609 was not significantly associated with BMI in the general population (AA 23.22 ± 3.14 vs AT 22.79 ± 3.25 vs TT 22.58 ± 3.13, $P = 0.063$). In the Japanese population, rs1558902 may be more tightly associated with BMI than rs9939609. The National Nutrition Survey of Japan reported that the prevalence of subjects with a BMI of

$\geq$30 kg/m^2 is only 2.3% in men and 3.4% in women aged 20 years and older (Yoshiike et al. 2002), and the mean BMI was approximately 23 kg/m^2 for ages 15–84 years (Yoshiike et al. 1998). Inconsistency in the results of effects of variations in the *FTO* gene on BMI between Japanese and Europians may be due to the relatively small mean and variance of BMI in the former than the latter.

The significant SNPs were located in intron 1 of the *FTO* gene. The rs1558902 and other significant SNPs, for example, rs9939609 and rs1121980, would affect transcriptional activity of the *FTO* gene, although further investigation is necessary. The precise mechanism by which the *FTO* gene leads to obesity development is unclear (Gerken et al. 2007; Sanchez-Pulido et al. 2007). However, the *FTO* gene is expressed in the hypothalamus and regulated by fasting and leptin (Frayling et al. 2007; Gerken et al. 2007). Using large-scale case-control association studies, we determined that the *SCG3* (Tanabe et al. 2007) and *MTMR9* (Yanagiya et al. 2007) genes are involved in susceptibility to the obesity phenotype. These two genes are expressed in the hypothalamus. Genetic studies in mice have suggested that mutations in several genes, such as those encoding leptin, proopiomelanocortin, and melanocortin-4 receptor, are implicated in a monogenic form of inherited obesity (Barsh et al. 2000; Rankinen et al. 2006). Such mutations have also been reported in obese humans. As most such genes are expressed in the hypothalamus and have been indicated to play important roles in the regulation of food intake, genes expressed in the hypothalamus are likely to be good candidates for susceptibility to obesity.

In summary, we have identified the genetic variations in the *FTO* gene that may influence the risk of severe obesity in the Japanese.

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