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ORIGINAL ARTICLE

Computed tomography analysis of the association between the *SH2B1* rs7498665 single-nucleotide polymorphism and visceral fat area

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Visceral fat accumulation has an important role in increasing morbidity and mortality rate by increasing the risk of developing several metabolic disorders, such as type 2 diabetes, dyslipidemia and hypertension. New genetic loci that contribute to the development of obesity have been identified by genome-wide association studies in Caucasian populations. We genotyped 1279 Japanese subjects (556 men and 723 women), who underwent computed tomography (CT) for measuring visceral fat area (VFA) and subcutaneous fat area (SFA), for the following single-nucleotide polymorphisms (SNPs): *NEGR1* rs2815752, *SEC16B* rs10913469, *TMEM18* rs6548238, *ETV5* rs7647305, *GNPDA2* rs10938397, *BDNF* rs6265 and rs925946, *MTCH2* rs10838738, *SH2B1* rs7498665, *MAF* rs1424233, and *KCTD15* rs29941 and rs11084753. In the additive model, none of the SNPs were significantly associated with body mass index (BMI). The *SH2B1* rs7498665 risk allele was found to be significantly associated with VFA (P=0.00047) but not with BMI or SFA. When the analysis was performed in men and women separately, no significant associations with VFA were observed (P=0.0099 in men and P=0.022 in women). None of the other SNPs were significantly associated with SFA. Our results suggest that there is a VFA-specific genetic factor and that a polymorphism in the *SH2B1* gene influences the risk of visceral fat accumulation.
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Keywords: computed tomography; Japanese subjects; obesity; *SH2B1*; visceral fat area

INTRODUCTION

Obesity, especially visceral fat obesity, is a risk factor for several metabolic disorders, including type 2 diabetes, dyslipidemia and hypertension.[1] Several studies have indicated that adipose tissue, especially that in the visceral region, secretes various adipocytokines and that an increase in adipose tissue mass leads to alteration in the plasma levels of adipocytokines, resulting in the development of dyslipidemia, hypertension, and insulin resistance.[2,3] Intra-abdominal fat accumulation (central adiposity) is determined in terms of waist circumference; waist-hip ratio; or visceral fat area (VFA), which is measured using computed tomography (CT).[1,4,5] Recently, two genome-wide association studies were conducted to identify the loci

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linked with waist circumference and waist-hip ratio.[6,7] In a previous study, we have reported that the rs1558902 and rs1421085 genotypes of the fat mass and obesity-associated gene (*FTO*) were significantly associated with VFA, as well as with subcutaneous fat area (SFA) and body mass index (BMI).[8]

We performed a large-scale, case–control association study and found that secretogranin III (*SCG3*)[9] and myotubularin-related protein 9 (*MTMR9*)[10] conferred susceptibility to an obese phenotype in the Japanese population. Recent progress in genome-wide association studies has increased the number of known genetic susceptibility loci for obesity.[11–13] Some of the obesity-associated loci identified by the genome-wide association studies were found to be replicated in the Japanese population.[14,15] Some of the obesity-related loci were found to overlap with the waist circumference waist-hip ratio-related loci, for example, the loci within the *FTO* gene and near the melanocortin 4 receptor (*MC4R*) gene.

In this study, we investigated whether the recently reported obesity-related loci were associated with VFA, which is an important factor responsible for increased morbidity and mortality rates.

MATERIALS AND METHODS

Study subjects

In this study, we enrolled 1279 Japanese subjects from outpatient clinics; these patients had agreed to undergo CT testing (in the supine position) to determine the VFA and SFA values at the umbilical level (L4–L5). Both VFA and SFA values were calculated using the FatScan software program (N2system, Osaka, Japan).[16] The patients visited the hospitals to undergo the treatment for obesity and/or metabolic abnormalities such as hypertension, dyslipidemia and type 2 diabetes. Patients with secondary obesity and obesity-related hereditary disorders were excluded from this study. Patients with disease or under treatment that strongly affect the body weight were also excluded. The clinical data were taken at the first visit to the hospital. The clinical characteristics of the subjects are summarized in Table 1. Metabolic syndrome and metabolic abnormalities were diagnosed according to the criteria released by the Japanese Committee for the Diagnostic Criteria of Metabolic Syndrome in April 2005.[4,5] 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 extraction and single-nucleotide polymorphism genotyping

Genomic DNA was extracted from the blood samples collected from each subject by using Genomix (Talent Srl, Trieste, Italy). We selected 12 single-nucleotide polymorphisms (SNPs) identified as susceptibility loci for obesity by genome-wide association studies in Caucasian populations[11–13] and constructed Invader probes (Third Wave Technologies, Madison, WI, USA) for the following SNPs: rs2815752 in the neuronal growth regulator 1 gene (*NEGR1*); rs10913469 in the SEC16 homolog B gene (*SEC16B*); rs6548238 in the transmembrane protein 18 gene (*TMEM18*); rs7647305 in the ets variant 5 gene (*ETV5*); rs10938397 in the glucosamine-6-phosphate deaminase 2 gene (*GNPDA2*); rs6265 and rs925946 in the brain-derived neurotrophic factor gene (*BDNF*); rs10838738 in the mitochondrial carrier homolog 2 gene (*MTCH2*); rs7498665 in the SH2B adaptor protein 1 gene (*SH2B1*); rs1424233 in the v-maf musculo-aponeurotic fibrosarcoma oncogene homolog gene (*MAF*); and rs29941 and rs11084753 in the potassium channel tetramerisation domain-containing 15 gene (*KCTD15*). The SNPs were genotyped using Invader assays as previously described.[17] The success rate of these assays was $>99.0\%$.

Statistical analysis

For the additive model, we coded the 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. Multiple linear regression analyses were carried out to test the independent effect of the risk alleles on BMI, VFA and SFA by taking into account the effects of other variables (that is, age and gender) that were assumed to be independent of the effect of each SNP. The Hardy–Weinberg equilibrium was assessed using the χ^2-test.[18] Statistical analysis was carried out using the software R (http://www.r-project.org/). *P*-values were corrected by Bonferroni's adjustment and $P<0.0042$ (0.05/12) was considered statistically significant.

RESULTS

The clinical characteristics and genotypes of the subjects are shown in Tables 1 and 2, respectively. All the SNPs were in the Hardy–Weinberg equilibrium. The BMI, VFA and SFA values for each SNP genotype are represented in Table 3. Multiple linear regression analyses of the anthropometric parameters with respect to the 12 SNPs analyzed are shown in Table 4. No SNPs were not significantly associated with BMI in this population, although a previous study reported that the *SEC16B* rs10913469 and *TMEM18* rs6548238 SNPs were significantly associated with obesity ($BMI>30\,kg\,m^{-2}$) in the Japanese population.[15]

The *SH2B1* rs7498665 SNP was significantly associated with VFA ($P=0.00047$) even when the conservative Bonferroni's correction was applied ($P<0.0042$). Previous reports indicate that the rs7498665 SNP is associated with waist circumference[19] or visceral fat mass[20] in the dominant model. The VFA values of the rs7498665 genotype (Table 3) suggest that the dominant model would be the best-fitted model. Therefore, we performed multiple regression analyses by using the

Table 1 Clinical characteristics of the subjects

	Men	*Women*	*Total*
n	556	723	1279
Age (years)	49.4 ± 12.2	52.2 ± 11.3	51.0 ± 11.8
BMI ($kg\,m^{-2}$)	30.2 ± 6.1	28.1 ± 5.3	29.0 ± 5.8
VFA (cm^2)	155.3 ± 67.7	99.8 ± 53.6	123.9 ± 66.1
SFA (cm^2)	206.7 ± 108.6	241.6 ± 97.2	226.5 ± 103.7
Waist circumference (cm)	97.5 ± 11.3	91.8 ± 10.3	94.2 ± 11.1
Prevalence of metabolic disease (%)			
Dyslipidemia	293 (53)	244 (34)	537 (42)
Hypertension	379 (68)	452 (63)	831 (65)
Impaired fasting glucose	177 (32)	176 (24)	353 (28)
Metabolic syndrome	248 (45)	162 (22)	410 (32)

Abbreviations: BMI, body mass index; SFA, subcutaneous fat area; VFA, visceral fat area.
Data are shown as mean ± s.d.

Table 2 Genotypic characteristics of the subjects

SNP ID	*Nearby gene*	*Allele 1/2*	*Risk allele*	*Genotype*	*HWE P-value*
rs2815752	*NEGR1*	A/G	A	1113/163/3	0.24
rs10913469	*SEC16B*	T/C	C	690/510/78	0.20
rs6548238	*TMEM18*	T/C	C	6/201/1071	0.29
rs7647305	*ETV5*	T/C	C	201/576/500	0.10
rs10938397	*GNPDA2*	A/G	G	615/537/126	0.58
rs6265	*BDNF*	A/G (Met/Val)	G	207/609/462	0.79
rs925946	*BDNF*	T/G	T	3/100/1175	0.57
rs10838738	*MTCH2*	G/A	G	107/555/616	0.25
rs7498665	*SH2B1*	G/A (Ala/Thr)	G	29/305/945	0.46
rs1424233	*MAF*	A/G	A	726/469/82	0.59
rs29941	*KCTD15*	T/C	C	774/444/60	0.72
rs11084753	*KCTD15*	G/A	G	105/535/638	0.63

Abbreviations: HWE, Hardy–Weinberg equilibrium; SNP, single-nucleotide polymorphism.

Table 3 Mean BMI, VFA and SFA for 12 obesity-risk variants

		Mean ± s.d.								
		BMI ($kg\,m^{-2}$)			VFA (cm^2)			SFA (cm^2)		
SNP ID	Nearby gene	11	12	22	11	12	22	11	12	22
rs2815752	*NEGR1*	29.1 ± 5.9	28.3 ± 5.3	28.5 ± 3.1	124.8 ± 66.7	118.5 ± 62.6	95.2 ± 38.8	226.1 ± 103.1	228.3 ± 108.5	251.0 ± 79.8
rs10913469	*SEC16B*	28.8 ± 5.9	29.2 ± 5.6	29.7 ± 6.5	123.0 ± 66.4	124.9 ± 65.2	124.8 ± 70.4	221.7 ± 103.7	231.6 ± 102.6	234.0 ± 110.5
rs6548238	*TMEM18*	25.9 ± 7.5	29.0 ± 7.2	29.0 ± 5.5	85.9 ± 70.6	123.4 ± 67.1	124.3 ± 65.9	211.0 ± 135.0	222.8 ± 111.3	227.3 ± 102.2
rs7647305	*ETV5*	29.0 ± 5.3	29.1 ± 5.4	29.0 ± 6.3	124.5 ± 66.8	124.5 ± 66.3	123.2 ± 65.8	234.1 ± 99.5	225.6 ± 100.0	224.6 ± 109.6
rs10938397	*GNPDA2*	28.8 ± 5.9	29.1 ± 5.8	29.2 ± 5.3	122.7 ± 68.0	124.5 ± 64.0	127.4 ± 66.3	224.5 ± 103.4	227.9 ± 103.3	229.3 ± 107.5
rs6265	*BDNF*	28.6 ± 5.9	28.7 ± 5.3	29.6 ± 6.3	122.4 ± 68.2	122.9 ± 64.7	126.1 ± 67.1	220.3 ± 92.9	223.5 ± 102.2	233.2 ± 109.9
rs925946	*BDNF*	36.0 ± 10.7	29.5 ± 6.1	28.9 ± 5.7	142.6 ± 11.3	123.3 ± 63.3	124.0 ± 66.4	416.8 ± 155.7	236.3 ± 118.6	225.2 ± 101.9
rs10838738	*MTCH2*	28.7 ± 4.9	29.3 ± 6.4	28.7 ± 5.3	124.5 ± 58.3	125.1 ± 68.5	122.6 ± 65.2	214.1 ± 93.1	233.6 ± 109.6	222.2 ± 99.8
rs7498665	*SH2B1*	29.7 ± 4.8	29.5 ± 6.2	28.8 ± 5.7	134.4 ± 65.3	134.5 ± 70.5	120.2 ± 64.3	231.1 ± 95.9	235.1 ± 98.4	223.6 ± 105.5
rs1424233	*MAF*	29.0 ± 6.0	29.1 ± 5.7	28.4 ± 3.8	123.1 ± 64.7	124.0 ± 65.7	130.6 ± 80.4	222.0 ± 102.6	234.0 ± 109.1	219.7 ± 74.5
rs29941	*KCTD15*	28.8 ± 5.6	29.1 ± 6.0	30.4 ± 6.0	123.9 ± 65.2	122.4 ± 67.3	136.5 ± 68.5	224.8 ± 103.7	228.2 ± 103.5	236.6 ± 106.6
rs11084753	*KCTD15*	29.5 ± 5.7	29.1 ± 5.8	28.9 ± 5.8	128.3 ± 71.9	122.5 ± 64.9	124.5 ± 66.2	233.0 ± 98.6	227.4 ± 102.0	224.7 ± 106.1

Abbreviations: BMI, body mass index; SFA, subcutaneous fat area; SNP, single-nucleotide polymorphism; VFA, visceral fat area.
11, allele1/allele1; 12, allele1/allele2; 22, allele2/allele2. Allele 1 and allele 2 in each SNP is indicated in Table 2.

Table 4 Relationship between obesity loci and adiposity measures

		BMI			VFA			SFA		
SNP ID	Nearby gene	β	s.e.	P-value	β	s.e.	P-value	β	s.e.	P-value
rs2815752	*NEGR1*	0.611	0.448	0.17	7.423	4.847	0.13	−6.293	7.978	0.43
rs10913469	*SEC16B*	0.325	0.255	0.20	2.827	2.753	0.30	4.516	4.532	0.32
rs6548238	*TMEM18*	0.267	0.403	0.51	6.773	4.352	0.12	2.557	7.178	0.72
rs7647305	*ETV5*	0.025	0.221	0.91	1.984	2.386	0.41	2.565	3.929	0.51
rs10938397	*GNPDA2*	0.199	0.236	0.40	0.804	2.547	0.75	4.065	4.190	0.33
rs6265	*BDNF*	0.508	0.223	0.023	1.390	2.410	0.56	6.954	3.968	0.080
rs925946	*BDNF*	0.816	0.545	0.14	0.390	5.897	0.95	18.972	9.685	0.050
rs10838738	*MTCH2*	0.162	0.243	0.51	0.292	2.628	0.91	2.726	4.326	0.53
rs7498665	*SH2B1*	0.536	0.310	0.085	11.717	3.343	0.00047	8.341	5.555	0.13
rs1424233	*MAF*	0.050	0.252	0.84	−2.945	2.722	0.28	−5.311	4.479	0.24
rs29941	*KCTD15*	0.481	0.265	0.070	2.588	2.871	0.37	3.589	4.727	0.45
rs11084753	*KCTD15*	0.332	0.243	0.17	1.562	2.626	0.55	3.242	4.322	0.45

Abbreviations: BMI, body mass index; SFA, subcutaneous fat area; SNP, single-nucleotide polymorphism; VFA, visceral fat area.
Data were derived from a linear regression analysis. BMI, VFA and SFA were adjusted for age and gender.

dominant model and found a significant association between this SNP and VFA (P=0.00022). This association remained significant even after adjusting for age, gender and BMI in the dominant model (P=0.00096). The other SNPs did not show any significant association with VFA. No SNPs, including the *SH2B1* rs7498665, were associated with SFA.

BMI, VFA and SFA are known to be affected by gender; therefore, we compared the anthropometric parameters (BMI, VFA and SFA) among the different genotypes in the men and women (Supplementary Tables 1–3). Association between *SH2B1* rs7498665 SNP and VFA was not significant both in men (P=0.0099) and women (P=0.022). This negative association is most likely due to the decrease in the number of each genotype. The VFA values of the rs7498665 genotype (Supplementary Table 2) suggest that the dominant model would be the best-fitted model both in men and women. By using the dominant model, revealed no significant association between the rs7498665 genotype and VFA in men (P=0.0061) and women (P=0.015).

To confirm the association of the *SH2B1* rs7498665 SNP with VFA, two SNPs (rs4788102 and rs8049439) in linkage disequilibrium of rs7498665 reported by previous study[11] were genotyped (Supplementary Table 4). Both rs4788102 (P=0.00058) and rs8049439 (P=0.0021) SNPs were significantly associated with VFA.

DISCUSSION

In this study, we showed that the *SH2B1* rs7498665 SNP was significantly associated with VFA. Haupt *et al.*[20] used whole-body magnetic resonance imaging to show that this SNP (dominant model) was associated with visceral fat mass. They also reported that the *SH2B1* rs7498665 SNP was not associated with BMI or with non-visceral fat mass. Jamshidi *et al.*[19] reported that the *SH2B1* rs7498665 SNP (dominant model) was associated with waist circumference. Several studies have reported a negative association between the *SH2B1* rs7498665 SNP and abdominal adipose mass (measured using dual energy X-ray absorptiometry)[21] or waist circumference.[22,23]

CT- or magnetic resonance imaging-based analyses are more accurate than waist circumference- and dual energy X-ray absorptiometry-based abdominal fat-mass analysis for evaluating the association between this SNP and visceral fat mass. These data from this study and from the study performed by Haupt *et al.* strongly suggest that the *SH2B1* rs7498665 SNP is associated with visceral fat accumulation.

SH2B1 has four splicing isoforms; that is, α, β, γ and δ, of which SH2-Bβ was originally identified through its association with Janus kinase 2 (JAK2) protein, a cytoplasmic tyrosine kinase that mediates cytokine functions.[24] *SH2B1*-knockout mice have been reported to show severely impaired insulin signaling in the skeletal muscles, liver and adipose tissue, and progressively develop hyperinsulinemia, hyperglycemia and glucose intolerance.[25] *SH2B1*-knockout mice also developed hyperlipidemia, leptin resistance, hyperphagia and obesity.[26] Although data for mesenteric fat have not been reported, both subcutaneous inguinal fat and intra-abdominal (epididymal) fat were found to be increased in *SH2B1*-knockout mice.[26,27] Neuron-specific restoration of *SH2B1* in knockout mice corrected the metabolic disorders, improved leptin regulation of orexigenic neuropeptide expression in the hypothalamus, and protected against high-fat diet-induced leptin resistance and obesity.[27] Ventromedial hypothalamic lesions are reported to induce visceral fat accumulation that does not result in obesity, and to induce hyperglycemia, hyperinsulinemia and hypertriglyceridemia.[28] *SH2B1* was specifically expressed in the brain, including the hypothalamus, in mice with neuron-specific *SH2B1* restoration.[27] Therefore, *SH2B1* expression in hypothalamus (possibly the ventromedial hypothalamic) may have an important role in visceral fat accumulation. As the *SH2B1* rs7498665 SNP is a non-synonymous SNP (G/A, Ala484Thr) and exits in the proline-rich region, the function of the SH2B1 protein might be deteriorated in subjects with the risk G-allele, leading to visceral fat accumulation. The rs4788102 SNP exists in the 5′-flanking region of the *SH2B1* gene, thus, the expression of *SH2B1* may be changed in the subjects with the risk A-allele. It is necessary to investigate whether these SNPs are functional.

In summary, we showed that the *SH2B1* rs7498665 SNP is significantly associated with VFA. This SNP is not associated with BMI or SFA, suggesting that there is a VFA-specific genetic factor. Our results also suggest that the *SH2B1* gene has a role in visceral fat accumulation. However, these results need to be confirmed in other populations.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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