Journal of Human Genetics (2010) 55, 738–742
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www.nature.com/jhg

ORIGINAL ARTICLE

Polymorphisms in *NRXN3*, *TFAP2B*, *MSRA*, *LYPLAL1*, *FTO* and *MC4R* and their effect on visceral fat area in the Japanese population

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The predominant risk factor of metabolic syndrome is intra-abdominal fat accumulation, which is determined by waist circumference and waist–hip ratio measurements and visceral fat area (VFA) that is measured by computed tomography (CT). There is evidence that waist circumference and waist–hip ratio in the Caucasian population are associated with variations in several genes, including neurexin 3 (*NRXN3*), transcription factor AP-2β (*TFAP2B*), methionine sulfoxide reductase A (*MSRA*), lysophospholipase-like-1 (*LYPLAL1*), fat mass and obesity associated (*FTO*) and melanocortin 4 receptor (*MC4R*) genes. To investigate the relationship between VFA and subcutaneous fat area (SFA) and these genes in the recruited Japanese population, we genotyped 8 single-nucleotide polymorphisms (SNPs) in these 6 genes from 1228 subjects. Multiple regression analysis revealed that gender, age, and rs1558902 and rs1421085 genotypes (additive model) in *FTO* were significantly associated with body mass index (BMI; *P*=0.0039 and 0.0039, respectively), SFA (*P*=0.0027 and 0.0023, respectively) and VFA (*P*=0.045 and 0.040, respectively). However, SNPs in other genes, namely, *NRXN3*, *TFAP2B*, *MSRA*, *LYPLAL1* and *MC4R* were not significantly associated with BMI, SFA or VFA. Our data suggest that some SNPs, which were identified in genome-wide studies in the Caucasians, also confer susceptibility to fat distribution in the Japanese subjects.
Journal of Human Genetics (2010) **55**, 738–742; doi:10.1038/jhg.2010.99; published online 12 August 2010

Keywords: fat mass and obesity associated (*FTO*); lysophospholipase-like 1 (*LYPLAL1*); melanocortin 4 receptor (*MC4R*); methionine sulfoxide reductase A (*MSRA*); neurexin 3 (*NRXN3*); subcutaneous fat area; transcription factor AP-2-β (*TFAP2B*); visceral fat area

INTRODUCTION

Metabolic syndrome is a common clinical phenotype that is manifested as concurrent metabolic abnormalities, including central obesity, glucose intolerance, dyslipidemia and hypertension.[1] Because several other definitions also exist,[2] the adequacy of this concept remains debatable. Recently, metabolic syndrome has attracted considerable interest because of increasing the number of patients. 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 addition to certain genetic factors.[1,2] Adipose tissue, especially the visceral fat, secretes various adipocytokines. An increase in the adipose

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Received 14 June 2010; revised and accepted 21 July 2010; published online 12 August 2010

Table 1 Clinical characteristics of the subjects

	Men (n=518)	*Women (n=710)*	*Total (n=1228)*
Age (years)	49.7 ± 11.9	52.1 ± 11.3	51.1 ± 11.6
BMI (kg $^{-2}$)	30.3 ± 6.2	28.2 ± 5.3	29.1 ± 5.8
VFA (cm^2)	160.9 ± 66.8	102.5 ± 54.5	127.1 ± 66.5
SFA (cm^2)	213.0 ± 110.8	246.4 ± 98.6	232.3 ± 105.2

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

tissue mass leads to an alteration in the plasma adipocytokine level, resulting in dyslipidemia, hypertension and insulin resistance.[3,4] Intra-abdominal fat accumulation (central adiposity) is determined in terms of the waist circumference and waist–hip ratio measurements and visceral fat area (VFA) that is measured by computed tomography (CT).[1,5,6] Recently, two genome-wide association studies had been conducted to identify the loci that were linked with waist circumference or waist–hip ratio.[7,8]

In this study, we investigated the association of single-nucleotide polymorphisms (SNPs) in neurexin 3 (*NRXN3*), transcription factor AP-2β (*TFAP2B*), methionine sulfoxide reductase A (*MSRA*), lysophospholipase-like-1 (*LYPLAL1*), fat mass and obesity associated (*FTO*) and melanocortin 4 receptor (*MC4R*) genes with VFA and subcutaneous fat area (SFA) that were determined by CT.

MATERIALS AND METHODS

Study subjects

We recruited 1228 Japanese subjects from outpatient clinics after they agreed to undergo CT examinations (supine position) that were performed to determine the VFA and SFA values at the level of the umbilicus (L4–L5). Both VFA and SFA were calculated using the FatScan software program (N2system, Osaka, Japan).[9] The clinical characteristics of the patients are summarized in Table 1.

All subjects provided their informed written consent, and the protocol was approved by the ethics committee of each institution and by that of RIKEN.

DNA extraction 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 rs1558902 and rs1421085 in *FTO*, rs489693 and rs17700144 in *MC4R*, rs1046997 in *NRXN3*, rs987237 in *TFAP2B*, rs782622 (rs545854) in *MSRA* and rs2605100 in *LYPLAL1*. The SNPs were genotyped using Invader assays as described previously.[10] The success rate of this assays was >99.0%.

Statistical analysis

Differences in the quantitative clinical data between case and control groups were tested by the Mann–Whitney *U*-test. Differences in the quantities of clinical data between the different genotypes were assessed by the Kruskal–Wallis test. We coded genotypes as 0, 1 or 2 depending on the number of copies of the risk alleles. Multiple linear regression analysis was performed to test the independent effect of risk alleles on body mass index (BMI), VFA or SFA by considering the effects of other variables (age and gender), which were assumed to be independent of the effect of the SNPs. The significance of the association between an independent variable and dependent variable was determined using a *t*-test. Hardy–Weinberg equilibrium was assessed using the χ^2-test.[11]

RESULTS

BMI, VFA and SFA are known to be affected by gender, and an association between rs2605100 in *LYPLAL1* and the waist–hip ratio has been reported only in women.[7] Therefore, we first compared the anthropometric parameters (BMI, VFA and SFA) among the different genotypes in the men and women. One SNP—rs10146997 in *NRXN3*—was found to be monomorphic, as reported in the HapMap database. Two SNPs (rs1558902 and rs1421085) in *FTO* were significantly associated with BMI in women (Table 2). The association of rs489693 and rs17700144 in *MC4R* with BMI has been reported in the Caucasian population;[7,8] however, such association was not observed in our study. The SNPs in *TFAP2B*, *MSRA* and *LYPLAL1* were not associated with BMI, which is a finding consistent with that of Lindgren *et al*.[7] No SNPs in the five genes were associated with VFA in either men or women (Table 3). Although rs489693 was marginally associated with VFA in men, the risk allele was different from that reported in the Caucasian population.[7] Two SNPs (rs1558902 and rs1421085) in *FTO* were significantly associated with SFA both in men (P=0.034 and 0.040, respectively) and women (P=0.010 and 0.0083, respectively) (Table 4). SNPs in other genes were not significantly associated with SFA. All SNPs were found to exhibit Hardy–Weinberg equilibrium ($P>0.10$).

Next, we attempted to perform multiple linear regression analysis by using BMI, VFA and SFA as the dependent variables, and with age, gender or genotype as an explanatory variable. We transformed genotypes to 0, 1 or 2 depending on the number of copies of the risk alleles. The A-allele of rs1558902 and the C-allele of rs1421085 in *FTO* were significantly associated with increases in BMI (P=0.0039 and 0.0039, respectively), VFA (P=0.045 and 0.040, respectively) and SFA (P=0.0027 and 0.0023, respectively) even after age and gender were included in the model (Supplementary Tables 1, 2, and 3). Multiple linear regression analysis showed that the SNPs of other genes were not significantly associated with BMI, VFA and SFA.

We also conducted power analysis of linear regression (additive model) with a significance level of 0.05, considering the effect size of the parameters. For rs1558902, the estimated effect sizes per allele (regression coefficients) for VFA and SFA were 5.8 and 14.4 cm^2, respectively (Supplementary Tables 2 and 3). The power of our statistical test was calculated on the basis of these estimated effect sizes and by performing 10 000 simulations. When the allele frequency was assumed to be 0.2, the power was estimated to be 0.32 for VFA and 0.81 for SFA; however, when the allele frequency was assumed to be 0.1, the respective powers were estimated to be 0.20 and 0.56.

DISCUSSION

The most important risk factor for metabolic syndrome is visceral fat obesity. According to the criteria released by the Japanese Committee for the Diagnostic Criteria of Metabolic Syndrome in April 2005,[5] metabolic syndrome is defined by the presence of two or more abnormalities (dyslipidemia, impaired glucose tolerance or diabetes and hypertension), in addition to visceral fat obesity. The cutoff points for visceral fat obesity (waist circumference, 85 cm in men and 90 cm in women) are based on the cutoff point for VFA (100 cm^2) that is determined by CT.[5,6] Visceral fat mass measurement by CT is more precise than that derived from BMI or waist circumference measurements. Furthermore, for predicting metabolic risk-factor clustering, VFA is superior to waist circumference or BMI.[12] Therefore, we examined the association of VFA and SFA with the SNPs related to waist circumference and waist–hip ratio, and identified in a genome-wide study.[7,8] We found that the SNPs in *FTO* were significantly associated with BMI, as we have previously reported.[13] We also found that these SNPs were associated with VFA and SFA; however, the association between these SNPs and VFA was marginal because VFA was not significantly different among the genotypes in men. We did not find any association between other SNPs and BMI, VFA or SFA. These findings may be due to the low power of this study. Therefore, further studies with more subjects should be conducted to conclude that SNPs in genes other than *FTO* are not associated with VFA or SFA.

Table 2 Comparison of BMI among the different genotypes

						BMI ($kg\,m^{-2}$)			
SNP ID	*Closest gene*	*Allele1/allele2*	*Risk allele*	*Gender*	*Genotype*	*11*	*12*	*22*	P-*value*
rs1558902	*FTO*	A/T	A	Men	25/190/302	30.3 ± 5.1	30.9 ± 5.9	29.9 ± 6.4	0.058
				Women	39/239/430	29.6 ± 6.7	28.8 ± 5.6	27.7 ± 5.0	0.012
				Total	64/429/732	29.9 ± 6.1	29.7 ± 5.8	28.6 ± 5.7	0.00021
rs1421085	*FTO*	C/T	C	Men	25/189/304	30.3 ± 5.1	30.9 ± 5.9	30.0 ± 6.4	0.076
				Women	39/238/431	29.6 ± 6.7	28.8 ± 5.6	27.7 ± 5.0	0.0093
				Total	64/427/735	29.9 ± 6.1	29.7 ± 5.8	28.6 ± 5.7	0.00022
rs489693	*MC4R*	C/A	A	Men	308/184/26	30.8 ± 6.9	29.5 ± 4.6	30.2 ± 6.6	0.24
				Women	422/238/50	28.4 ± 5.2	27.8 ± 5.5	28.3 ± 6.2	0.13
				Total	730/422/76	29.4 ± 6.1	28.5 ± 5.2	28.9 ± 6.4	0.045
rs17700144	*MC4R*	A/G	A	Men	0/30/488	—	30.7 ± 5.6	30.3 ± 6.2	0.48
				Women	2/42/662	25.0, 26.2	27.1 ± 3.8	28.3 ± 5.4	0.34
				Total	2/72/1150	25.0, 26.2	28.6 ± 4.9	29.1 ± 5.9	0.62
rs987237	*TFAP2B*	A/G	G	Men	330/168/19	30.2 ± 6.2	30.6 ± 6.1	30.1 ± 6.3	0.56
				Women	434/242/32	28.2 ± 5.5	28.0 ± 5.2	28.8 ± 4.7	0.47
				Total	764/410/51	29.1 ± 5.9	29.1 ± 5.7	29.3 ± 5.4	0.80
rs7826222	*MSRA*	C/G	C	Men	77/235/205	30.7 ± 5.1	30.5 ± 5.9	30.0 ± 6.9	0.22
				Women	117/321/271	28.0 ± 5.3	28.2 ± 4.8	28.2 ± 5.9	0.68
				Total	194/556/476	29.1 ± 5.4	29.2 ± 5.4	29.0 ± 6.4	0.44
rs2605100	*LYPLAL1*	A/G	G	Men	22/145/351	30.5 ± 5.2	30.9 ± 6.6	30.1 ± 6.1	0.23
				Women	14/200/495	26.6 ± 4.7	28.7 ± 6.0	28.0 ± 5.0	0.24
				Total	36/345/846	29.0 ± 5.3	29.6 ± 6.3	28.9 ± 5.6	0.16

Abbreviations: BMI, body mass index; SNP, single-nucleotide polymorphism.
P-values were analyzed using the Kruskal–Wallis test. *P*values for 12 vs 22 at rs17700144 were analyzed using the Mann–Whitney *U*-test. Data are presented as the mean ± s.d.

Table 3 Comparison of VFA among the different genotypes

						VFA (cm^2)			
SNP ID	*Closest gene*	*Allele1/allele2*	*Risk allele*	*Gender*	*Genotype*	*11*	*12*	*22*	P-*value*
rs1558902	*FTO*	A/T	A	Men	25/190/302	163.2 ± 52.1	166.9 ± 67.5	157.1 ± 67.4	0.22
				Women	39/239/430	105.8 ± 69.3	106.2 ± 53.1	100.1 ± 53.7	0.23
				Total	64/429/732	128.2 ± 68.7	133.0 ± 67.0	123.6 ± 66.0	0.039
rs1421085	*FTO*	C/T	C	Men	25/189/304	163.2 ± 52.1	167.2 ± 67.6	156.8 ± 67.2	0.18
				Women	39/238/431	105.8 ± 69.3	106.1 ± 53.2	100.0 ± 53.4	0.25
				Total	64/427/735	128.2 ± 68.7	133.1 ± 67.2	123.5 ± 65.7	0.037
rs489693	*MC4R*	C/A	A	Men	308/184/26	166.7 ± 67.2	154.7 ± 66.2	135.5 ± 56.7	0.047
				Women	422/238/50	105.6 ± 55.1	95.5 ± 51.2	109.1 ± 61.8	0.089
				Total	730/422/76	131.4 ± 67.6	121.3 ± 65.2	118.2 ± 61.0	0.035
rs17700144	*MC4R*	A/G	A	Men	0/30/488	—	161.5 ± 62.4	160.9 ± 67.1	0.87
				Women	2/42/662	68.3, 90.2	104.8 ± 50.8	102.4 ± 54.8	0.60
				Total	2/72/1150	68.3, 90.2	128.4 ± 62.2	127.2 ± 66.9	0.69
rs987237	*TFAP2B*	A/G	G	Men	330/168/19	158.4 ± 65.7	169.0 ± 69.1	132.6 ± 56.7	0.06
				Women	434/242/32	102.7 ± 53.4	101.3 ± 55.7	109.4 ± 61.5	0.71
				Total	764/410/51	126.7 ± 65.2	129.0 ± 69.9	118.0 ± 60.3	0.65
rs7826222	*MSRA*	C/G	C	Men	77/235/205	161.7 ± 65.6	162.9 ± 66.6	158.5 ± 67.7	0.80
				Women	117/321/271	105.4 ± 57.1	103.3 ± 54.8	100.5 ± 53.1	0.74
				Total	194/556/476	127.7 ± 66.5	128.5 ± 66.9	125.4 ± 66.3	0.71
rs2605100	*LYPLAL1*	A/G	G	Men	22/145/351	153.2 ± 65.4	167.9 ± 74.5	158.5 ± 63.4	0.51
				Women	14/200/495	94.1 ± 41.1	101.7 ± 53.0	103.1 ± 55.5	0.92
				Total	36/345/846	130.2 ± 63.6	129.5 ± 70.9	126.1 ± 64.9	0.86

Abbreviations: VFA, visceral fat area; SNP, single-nucleotide polymorphism.
P-values were analyzed using the Kruskal–Wallis test. *P*-values for 12 vs 22 at rs17700144 were analyzed using the Mann–Whitney *U*-test. Data are presented as the mean ± s.d.

SNPs in *FTO* were associated with SFA and VFA. Therefore, some of the SNPs, which were identified by genome-wide association studies in the Caucasian population, were also found to confer susceptibility to body fat distribution in the Japanese subjects. On the other hand, rs10146997 in *NRXN3* was found to be monomorphic in our subjects, and we could not find associations between SNPs in *MC4R*, *TFAP2B*,

Table 4 Comparison of SFA among the different genotypes

						SFA (cm^2)			
SNP ID	*Closest gene*	*Allele1/allele2*	*Risk allele*	*Gender*	*Genotype*	*11*	*12*	*22*	P-*value*
rs1558902	*FTO*	A/T	A	Men	25/190/302	215.9 ± 110.5	223.0 ± 104.4	206.2 ± 114.7	0.034
				Women	39/239/430	279.6 ± 129.9	255.9 ± 96.9	238.5 ± 95.6	0.010
				Total	64/429/732	254.3 ± 125.7	241.3 ± 101.5	225.2 ± 105.1	0.0024
rs1421085	*FTO*	C/T	C	Men	25/189/304	215.9 ± 110.5	223.1 ± 104.6	206.4 ± 114.4	0.040
				Women	39/238/431	279.6 ± 129.9	256.2 ± 96.9	238.4 ± 95.6	0.0083
				Total	64/427/735	254.3 ± 125.7	241.6 ± 101.6	225.1 ± 104.9	0.0021
rs489693	*MC4R*	C/A	A	Men	308/184/26	220.8 ± 114.4	199.7 ± 97.0	214.0 ± 149.6	0.16
				Women	422/238/50	250.0 ± 95.2	242.8 ± 106.6	232.9 ± 86.8	0.29
				Total	730/422/76	237.7 ± 104.6	223.9 ± 104.6	226.4 ± 111.7	0.051
rs17700144	*MC4R*	A/G	A	Men	0/30/488	—	227.6 ± 119.8	212.1 ± 110.3	0.34
				Women	2/42/662	179.3, 180.0	222.4 ± 76.3	248.6 ± 99.9	0.15
				Total	2/72/1150	179.3, 180.0	224.5 ± 96.1	233.1 ± 105.9	0.67
rs987237	*TFAP2B*	A/G	G	Men	330/168/19	207.8 ± 106.8	225.0 ± 119.6	195.0 ± 96.8	0.20
				Women	434/242/32	251.5 ± 102.6	237.8 ± 92.1	246.2 ± 88.7	0.38
				Total	764/410/51	232.6 ± 106.6	232.6 ± 104.3	227.1 ± 94.2	0.96
rs7826222	*MSRA*	C/G	C	Men	77/235/205	230.0 ± 119.7	211.9 ± 109.2	207.9 ± 109.3	0.44
				Women	117/321/271	241.9 ± 93.0	247.1 ± 91.3	247.9 ± 109.0	0.65
				Total	194/556/476	237.1 ± 104.3	232.3 ± 100.7	230.7 ± 110.8	0.52
rs2605100	*LYPLAL1*	A/G	G	Men	22/145/351	217.3 ± 106.5	216.2 ± 112.5	211.4 ± 110.7	0.66
				Women	14/200/495	224.2 ± 94.0	257.5 ± 108.6	242.8 ± 94.2	0.17
				Total	36/345/846	220.0 ± 100.5	240.1 ± 112.0	229.7 ± 102.5	0.21

Abbreviations: SFA, subcutaneous fat area; SNP, single-nucleotide polymorphism.
P-values were analyzed using the Kruskal–Wallis test. *P*-values for 12 vs 22 at rs17700144 were analyzed using the Mann–Whitney *U*-test. Data are presented as the mean ± s.d.

MSRA or *LYPLAL1* and VFA or SFA. Thus, there is a possibility that genetic susceptibility to body fat distribution is likely to differ among various ethnic groups.

rs1558902 and rs1421085 in *FTO* were in linkage disequilibrium with rs9939609, which is associated with obesity and type II diabetes.[14–16] Concentrations of circulating adipocytokines are affected by the accumulation of adipose tissue, especially visceral adipose tissue. Therefore, rs1558902 and rs1421085 probably affect subcutaneous and visceral fat accumulation, leading to the development of type II diabetes by altered adipocytokine secretion. The precise mechanism of how *FTO* affects adipose tissue accumulation is not clear yet; however, there is evidence that *FTO* is involved in the development of obesity. *FTO* is ubiquitously expressed, and a strong expression is observed in the arcuate, paraventricular, dorsomedial and ventromedial nuclei, which are critical energy-regulation sites.[16–18] *FTO* also exists in the nucleus and is reported to be a member of the Fe(II) and 2-oxoglutarate-dependent oxygenase superfamily.[17,19] *FTO*-deficient and dominant-negative mutant *FTO* mice have shown reduced fat mass and increased energy expenditure.[20,21] These reports indicate that FTO has an important role in energy homeostasis by regulating energy expenditure. Although the effects of the SNPs rs1558902 and rs1421085 on gene expression need to be elucidated, variations in *FTO* probably affect subcutaneous and visceral fat accumulation.

In summary, we showed that rs1558902 and rs1421085 in *FTO* may be associated with SFA and VFA in the Japanese population. In our study, rs489693 and rs17700144 in *MC4R*, rs1046997 in *NRXN3*, rs987237 in *TFAP2B*, rs782622 (rs545854) in *MSRA* and rs2605100 in *LYPLAL1* were not associated with SFA or VFA.

ACKNOWLEDGEMENTS

We express our appreciation to Ms Ritsuko Kusano for her contribution to our study. This work was supported by a grant from the Grants-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology of Japan (21591186) and by the Mitsui Life Science Social Welfare Foundation.

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Supplementary Information accompanies the paper on Journal of Human Genetics website (http://www.nature.com/jhg)