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ORIGINAL ARTICLE

Association of gene polymorphism of the fat-mass and obesity-associated gene with insulin resistance in Japanese

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It was reported that gene polymorphisms in the fat-mass and obesity-associated gene (*FTO*) were associated with obesity and diabetes in several genome-wide association studies. A recent report indicated that *FTO*-knockout mice exhibited phenotypes of skinny body shape and normal metabolic profiles. Thus, *FTO* could be important in metabolic disorders. The aim of this study was to clarify the role of single nucleotide polymorphisms (SNPs) in *FTO* in metabolic disorders such as hypertension, obesity, diabetes, dyslipidemia, insulin resistance and metabolic syndrome in the Japanese general population using data from a cohort study in Hokkaido, namely the Tanno–Sobetsu study. Written informed consent for the genetic analysis was obtained from each subject participating in the study. A total of 1514 subjects were genotyped by TaqMan PCR methods for three SNPs, rs9939609, rs1121980 and rs1558902, in *FTO*. Association analyses between the SNPs and metabolic parameters were performed. Although two SNPs, rs9939609 and rs1558902, were not significantly associated with hypertension, obesity, metabolic syndrome or any metabolic parameters, additive and recessive models of rs1121980 were strongly associated with plasma immunoreactive insulin (IRI) level and homeostasis model assessment insulin resistance (HOMA-IR), even after adjusting for confounding factors such as age, gender and body mass index. A haplotype of three SNPs was also significantly associated with IRI and HOMA-IR. One SNP, rs1121980, and a haplotype of three SNPs in *FTO* that contains this SNP, might be important in the progression of insulin resistance in Japanese subjects.
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INTRODUCTION

Metabolic syndrome (MS) consisting of central obesity, high blood pressure, abnormal glucose tolerance or abnormal lipid profiles is considered an independent risk factor for cardiovascular diseases such as ischemic heart disease and stroke.[1,2] In Japan, central obesity based on visceral fat accumulation is an essential diagnosis criterion for MS. One of the main pathways to central obesity is considered to be an imbalance in the secretion of adipocytokines from adipose tissues and subsequent following insulin resistance.[3] Genetic background influences metabolic disorders. Recently, several genome-wide association studies revealed that single nucleotide polymorphisms (SNPs) of the fat-mass and obesity-associated gene (*FTO*) might be predisposing factors for obesity, diabetes and MS.[4–6]

Recently, it was reported that *FTO*-knockout mice showed several characteristic phenotypes, which included skinny body shape and normal metabolic profiles.[7] Thus, *FTO* could be important in fat accumulation and the regulation of glucose or lipid metabolism.

In previous genetic analyses, SNPs rs9939609, rs1121980 and rs1558902 in *FTO* were strongly associated with obesity, defined by body mass index (BMI).[8–11] There remain questions concerning whether these SNPs affect obesity and other metabolic disorders in Japanese subjects, who have a quite different body shape and diet from subjects in Western countries. Furthermore, the allele frequencies of *FTO* SNPs in Japanese subjects are quite different from those in Caucasian subjects (http://hapmap.ncbi.nlm.nih.gov/).

It was reported by Japanese investigators that rs1558902, but not rs9939609, in *FTO* was associated with BMI in a case (severe obesity group: BMI $\geqslant 30\,kg\,m^{-2}$)–control (nonobesity group: BMI $\leqslant 23\,kg\,m^{-2}$) study.[9] However, there are no reports on investigations of the relationship between *FTO* SNPs and metabolic disorders including hypertension, obesity, DM and MS as well as other parameters such as insulin resistance in the Japanese general population. In this study, we investigate the role of *FTO* SNPs in metabolic disorders in a cohort study, namely, the Tanno–Sobetsu study in Hokkaido, northern Japan.

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METHODS

Study subjects

We recruited 1514 subjects (803 in Tanno town and 711 in Sobetsu town) who had undergone medical checkups in these towns in Hokkaido, Japan, in 2002. The detailed epidemiological findings have already been reported.[12–17] Subjects completed a standard questionnaire regarding their medical history, and smoking and drinking habits. We measured the systolic blood pressure (SBP), diastolic blood pressure (DBP), BMI, abdominal circumference, total cholesterol, triglyceride, high-density lipoprotein cholesterol, plasma glucose and immunoreactive insulin (IRI). Blood samples were collected during fasting in the early morning. Obesity was defined as a BMI $>25\,kg\,m^{-2}$. Dyslipidemia was defined as total cholesterol $\geqslant 220$ mg per 100 ml and/or drug treatment for hypercholesterolemia. Diabetes was defined as fasting blood sugar $\geqslant 126$ mg per 100 ml and/or drug treatment for hyperglycemia. The Japanese definition of MS [18] was used as the diagnosis for MS. Briefly, criterion 1 and two of criteria 2–4 needed to be met.

1. visceral fat: (male) abdominal circumference $\geqslant 85$ cm
   (female) abdominal circumference $\geqslant 90$ cm
2. lipid abnormality: treatment for dyslipidemia or
   triglyceride $\geqslant 150$ mg per 100 ml and/or high-density lipoprotein cholesterol <40 mg per 100 ml
3. blood pressure: treatment for hypertension or
   SBP $\geqslant 130$ and/or DBP $\geqslant 85$ mm Hg
4. hyperglycemia: treatment for diabetes or fasting blood sugar $\geqslant 110$ mg per 100 ml.

Homeostasis model assessment insulin resistance (HOMA-IR) was used to determine insulin sensitivity, and was calculated as plasma glucose (mg per 100 ml) $\times$ IRI ($\mu U\,ml^{-1}$)/405.[19] Blood pressure was measured twice after 5 min of rest, with the subjects seated. Hypertension was defined as SBP $\geqslant 140$, DBP $\geqslant 90$ mm Hg or the current use of antihypertensive agents. Three hundred and ninety-five subjects were taking antihypertensive agents, and these subjects were included in the study. Individuals undergoing medical treatment and receiving diet therapy or exercise therapy for diabetes mellitus (n=84) were also included. Precise information on the types of antihypertensive agents or the nature of the treatment for diabetes was not obtained. All participants gave written informed consent to participate in the genetic analyses and in all other procedures associated with the study. The ethics committee of Osaka University approved the study protocol. The final number of subjects participating in the genetic study was 1488.

Genotyping

Genomic DNA was extracted from 200 µl of buffy coat using a QIAamp DNA Blood Kit (Qiagen, Hilden, Germany). We selected three SNPs, rs9939609, rs1121980 and rs1558902, in *FTO*, which were identified as being associated with obesity and/or diabetes in previous reports.[6,9,20,21] These SNPs were genotyped using TaqMan PCR methods with the following probes: C__30090620_10 for rs9939609, C__2031261_10 for rs1121980 and C__8917111_10 for rs1558902 (Applied Biosystems, Foster City, CA, USA).

Statistical analysis

Hardy–Weinberg equilibrium was calculated by a χ^2-test. Linkage disequilibrium was evaluated by SNP Alyze version 2.1 (DYNACOM Co., Ltd, Mohara, Japan). Associations between the polymorphisms and clinical variables were analyzed using one-way analysis of variance and analysis of covariance adjusted for confounding factors. Differences in genotype or allele distribution were examined by χ^2-analysis. All numerical values are expressed as mean ± s.d. Values of $P<0.05$ were considered to indicate statistical significance. To adjust for multiple testing of the three gene polymorphisms by Bonferroni's correction, we arbitrarily adopted $P<0.017$ (=0.05/3) as the level of statistical significance. Haplotype estimation was performed by the expectation-maximization algorithm. All analyses except analysis of covariance were performed with JMP statistical software (version 5; SAS Institute Inc., Cary, NC, USA), and analysis of covariance was performed with SPSSα statistical software (release 11.0.1; SPSS Inc., Chicago, IL, USA).

RESULTS

The total number of study subjects who were successfully genotyped for all three SNPs, rs9939609, rs1121980 and rs1558902 of *FTO*, was 1488. The characteristics of study subjects are shown in Table 1. In this cohort, the average BMI was much lower than that of study subjects in previous studies.[5,9,22,23] The prevalence of obesity defined as BMI $\geqslant 25\,kg\,m^{-2}$ according to Japanese criteria was 33%. DM, hypertension and MS by the Japanese definitions were exhibited by the study subjects at 7.8, 44.4 and 15.9%, respectively.

From the genotyping, the prevalences of each genotype in the three SNPs were determined to be AA/AT/TT=56/475/957 in rs9939609, AA/AG/GG=81/519/885 in rs1121980 and AA/AT/TT=59/468/959 in rs1558902. These allele frequencies are in accordance with Hardy–Weinberg equilibrium (data not shown).

The three SNPs, rs9939609, rs1121980 and rs1558902 in *FTO*, were tested for associations with hypertension, diabetes, dyslipidemia, obesity and MS in all the subjects using χ^2-tests. As shown in Table 2, there were no significant associations with hypertension or metabolic disorders in additive, dominant or recessive models. Table 3 shows blood pressure level and various metabolic parameters compared among genotypes for the three models of three SNPs in *FTO* using analysis of variance.

Additive and dominant models of rs1121980 showed significant differences in levels of IRI (P=0.022, 0.01, respectively) and HOMA-IR (P=0.029, 0.008, respectively), as shown in Table 3. Table 4 shows detailed data of IRI and HOMA-IR values composed among the genotypes in rs1121980. After adjusting for confounding factors including age, gender, BMI, abdominal circumference and presence of DM, there were still significant differences in IRI and HOMA-IR among genotypes in both additive and dominant models (P=0.005, 0.001, respectively) as determined by analysis of covariance.

Because some antihypertensive drugs might affect insulin sensitivity, we investigated the genotype comparison of HOMA-IR in both additive and dominant models for rs1121980 in subjects without hypertension (n=740). Significant differences were again identified: AA (n=38): 1.82 ± 4.04 *vs.* AG(223): 1.09 ± 0.84 *vs.* GG(418): 1.10 ± 0.94 in the additive model (P=0.004) and AA: 1.82 ± 4.04 *vs.* AG+GG(641): 1.10 ± 0.91 in the dominant model (P=0.0009).

Table 1 Characteristics of study subjects

	N=*1488*
Age (year)	62.7 ± 11.63
Gender (*n*, male/female)	M/F=582:906 (M: 39.1%)
BMI ($kg\,m^{-2}$)	23.81 ± 3.24
Abdominal circumference (cm)	83.96 ± 10.20
Systolic blood pressure (mm Hg)	137.43 ± 22.79
Diastolic blood pressure (mm Hg)	76.37 ± 11.67
Total cholesterol (mg per 100 ml)	201.18 ± 31.64
HDL-CHO (mg per 100 ml)	50.59 ± 12.28
LDL-CHO (mg per 100 ml)	130.04 ± 29.62
Triglyceride (mg per 100 ml)	102.72 ± 57.86
FBS (mg per 100 ml)	97.8 ± 24.83
IRI ($\mu U\,ml^{-1}$)	5.203 ± 3.71
HOMA-IR	1.32 ± 1.43
	(mean ± s.d.)

Abbreviations: BMI, body mass index; FBS, fasting blood sugar; HDL-CHO, high-density-lipoprotein cholesterol; IRI, immunoreactive insulin; LDL-CHO, low-density-lipoprotein cholesterol; HOMA-IR, homeostasis model assessment insulin resistance.
HOMA-IR=(FBS×fasting IRI)/405.

Table 2 Relationships between three SNPs in *FTO* and metabolic diseases

	rs9939609			*rs1121980*			*rs1558902*		
	Additive model	*Dominant model AA* vs. *AT+TT*	*Recessive model TT* vs. *AT+AA*	*Additive model*	*Dominant model AA* vs. *AG+GG*	*Recessive model GG* vs. *AG+AA*	*Additive model*	*Dominant model AA* vs. *AT+TT*	*Recessive model TT* vs. *AT+AA*
Dyslipidemia	0.5628	0.729	0.2845	0.5386	0.3025	0.4837	0.7339	0.5975	0.4805
Diabetes	0.4729	0.4982	0.4221	0.5166	0.3312	0.7613	0.4923	0.4362	0.514
Hypertension	0.5142	0.3994	0.3291	0.6366	0.9944	0.3623	0.5557	0.6346	0.2859
Obesity	0.9303	0.9001	0.7057	0.8787	0.9718	0.6202	0.8242	0.9073	0.5357
Treatment history									
Dyslipidemia	0.86	0.8151	0.6768	0.6608	0.5948	0.3888	0.8033	0.9036	0.5535
Diabetes	0.4843	0.2792	0.822	0.403	0.4852	0.3648	0.4943	0.3398	0.677
Hypertension	0.4274	0.2045	0.5304	0.6523	0.3601	0.6956	0.4885	0.3156	0.3666
Metabolic syndrome	0.7067	0.9954	0.4213	0.6112	0.9917	0.3415	0.6153	0.8625	0.3767

Values are indicated as *P*-values.

Table 3 Relationships between three SNPs in *FTO* and metabolic parameters

	rs9939609			*rs1121980*			*rs1558902*		
	Additive model	*Dominant model AA* vs. *AT+TT*	*Recessive model TT* vs. *AT+AA*	*Additive model*	*Dominant model AA* vs. *AG+GG*	*Recessive model GG* vs. *AG+AA*	*Additive model*	*Dominant model AA* vs. *AT+TT*	*Recessive model TT* vs. *AT+AA*
T-CHO (mg per 100 ml)	0.1799	0.6608	0.0641	0.3366	0.351	0.1719	0.3976	0.7541	0.1748
HDL-CHO (mg per 100 ml)	0.4853	0.5142	0.4232	0.8663	0.948	0.6243	0.5043	0.4449	0.5204
LDL-CHO (mg per 100 ml)	0.2946	0.5909	0.1191	0.5649	0.4123	0.3708	0.4825	0.7386	0.2273
TG (mg per 100 ml)	0.872	0.6059	0.8236	0.6308	0.6027	0.3557	0.6878	0.4164	0.9499
FBS (mg per 100 ml)	0.9072	0.7465	0.7072	0.9321	0.7166	0.9876	0.9539	0.8355	0.7836
IRI (μU ml^{-1})	0.3919	0.2543	0.3002	0.022	0.01	0.0876	0.2408	0.311	0.1145
HOMA-IR	0.7414	0.4844	0.6122	0.0294	0.008	0.3577	0.6697	0.5585	0.4156
SBP (mm Hg)	0.7555	0.6449	0.4893	0.3885	0.8893	0.1772	0.6958	0.9078	0.3991
DBP (mm Hg)	0.9621	0.9567	0.7814	0.3831	0.8359	0.2113	0.7915	0.6504	0.7127
BMI	0.6663	0.9483	0.3768	0.4627	0.9628	0.23	0.3762	0.9994	0.1787

Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; FBS, fasting blood sugar; HDL-CHO, high-density-lipoprotein cholesterol; HOMA-IR, homeostasis model assessment insulin resistance; IRI, immunoreactive insulin; LDL-CHO, low-density-lipoprotein cholesterol; SBP, systolic blood pressure; T-CHO, total cholesterol; TG, triglyceride.
Values are indicated as *P*-values.

There was strong linkage disequilibrium among the three SNPs in *FTO*. The r^2 values were 0.811 between rs9939609 and rs1121980, 0.956 between rs9939609 and rs1558902 and 0.821 between rs1121980 and rs1558902. Table 5 shows the results of comparison for metabolic parameters for each haplotype of the three SNPs in additive, dominant and recessive models analyzed by analysis of variance. Low-frequency haplotypes, namely, those with frequencies below 1%, were excluded from analysis. Five haplotypes were analyzed. H2 had a strong association with insulin resistance (Table 5), although it was present at a low frequency (n=21).

Table 4 Detailed data of IRI and HOMA-IR values compared among genotypes in rs1121980

SNP rs1121980	*Genotype group*	*HOMA-IR*	P-*value*	*IRI* (μU ml^{-1})	P-*value*
	GG (828)	1.29 ± 1.42		5.06 ± 3.38	
	AG (482)	1.30 ± 1.02	0.006	5.27 ± 3.67	0.020
	AA (76)	1.74 ± 2.99		6.26 ± 6.34	
	GG	1.29 ± 1.42	0.794	5.05 ± 3.38	0.421
	AG+AA	1.36 ± 1.46		5.41 ± 4.15	
	GG+AG	1.29 ± 1.29	0.002	5.14 ± 3.49	0.013
	AA	1.73 ± 2.99		6.26 ± 6.34	

Abbreviations: HOMA-IR, homeostasis model assessment insulin resistance; IRI, immunoreactive insulin; SNP, single nucleotide polymorphism.
Values are shown as mean ± s.d.
Subjects with data of HOMA-IR and IRI were analyzed in Table 4.

DISCUSSION

This study is the first reported investigation of the association between genetic variations in *FTO* and detailed metabolic parameters in the Japanese general population. We selected three SNPs, rs9939609, rs1121980 and rs1558902 in *FTO*, that had been found to be strongly associated with obesity, defined by BMI, in genome-wide association

Table 5 The results of comparison between haplotypes with three SNPs for metabolic parameters

	rs9939609/rs1121980/rs1558902	*T-CHO*	*HDL-CHO*	*LDL-CHO*	*IRI*	*HOMA-IR*
H1	AA/AA/AA (56) *vs.* others	0.6608	0.5142	0.5909	0.2543	0.4844
H2	AT/AA/AT (21) *vs.* others	0.1853	0.2637	0.3121	0.0006[a]	<0.0001[b]
H3	AT/AG/AT (441) *vs.* others	0.2977	0.5497	0.3463	0.9984	0.4927
H4	TT/AG/TT (67) *vs.* others	0.8063	0.4806	0.4044	0.5983	0.7029
H5	TT/GG/TT (878) *vs.* others	0.1305	0.5129	0.3147	0.1002	0.39

Abbreviations: HDL-CHO, high-density-lipoprotein cholesterol; HOMA-IR, homeostasis model assessment insulin resistance; IRI, immunoreactive insulin; LDL-CHO, low-density-lipoprotein cholesterol; T-CHO, total cholesterol.
Values are indicated as *P*-values.
[a]AT/AA/AT 8.11 ± 9.89 (*N*=19). Others 5.16 ± 3.54 (*N*=1370).
[b]AT/AA/AT 2.72 ± 5.59 (*N*=19). Others 1.30 ± 1.28 (*N*=1370).

studies in various ethnicities.[22,24–29] In Japan, Hotta *et al.*[9] reported that rs1558902 in *FTO* was most significantly associated with obesity in a case–control association study using severely obese Japanese subjects (average BMI $\geqslant 30\,kg\,m^{-2}$).[9] In this study, we investigated the association between various metabolic parameters including hypertension, DM, obesity and MS in subjects participating in the Tanno–Sobetsu cohort study, a study of a Japanese representative rural cohort in Hokkaido. The average BMI of the study subjects was $23.81 \pm 3.24\,kg\,m^{-2}$, which is close to the national average in Japan. In this study, none of the three SNPs was associated with obesity, defined by BMI, higher abdominal circumference or prevalence of MS, defined by Japanese criteria. In addition, none of the three SNPs was associated with hypertension, dyslipidemia or prevalence of DM.

Only one SNP, rs1121980, showed a strong correlation with HOMA-IR, which is an index of insulin resistance, in additive and dominant models. Subjects with AA in rs1121980 had a much higher HOMA-IR and a higher insulin resistance than subjects without the AA genotype (P=0.008). This P-value is considered significant ($P<0.017$) after Bonferroni's correction to adjust for multiple testing of the three SNPs. Subjects with the haplotype H2, which includes AA in rs1121980, had a higher HOMA-IR than other subjects (Table 5). Thus, we conclude that rs1121980 in *FTO* is associated with insulin resistance in the Japanese general population. Because a recent report indicates that a gain of function of *FTO* induces insulin resistance,[7] rs1121980 located in an intron may regulate *FTO* gene function by affecting splicing variation. After adjusting for obesity and the prevalence of DM, rs1121980 is independently associated with insulin resistance. Therefore, rs1121980 may affect insulin resistance, directly and not only indirectly by obesity.

In this study, three SNPs in *FTO* were not associated with obesity. Several reasons for this are considered. One is the difference between Caucasian and Japanese general populations in the severity of obesity. In fact, SNPs in *FTO* were associated with obesity in a study using Japanese subjects with severe obesity (average BMI $\geqslant 30\,kg\,m^{-2}$).[9] Another reason is the differences in allele frequency among *FTO* SNPs. In the cases of rs9939609, allele frequency information obtained from HapMap database (http://hapmap.ncbi.nlm.nih.gov/) shows significant differences between Caucasian (AA/AT/TT=0.117/0.667/0.217) and Japanese populations (0.067/0.200/0.733).

In summary, an SNP located in an intron, rs1121980, and a haplotype of three SNPs in *FTO* that includes this SNP, may be important in the progression of insulin resistance in Japanese subjects. This SNP may be an independent risk factor for future MS, hypertension and DM in Japanese subjects. However, this study has limitations because of its cross-sectional design. Prospective studies investigating the relationship between these SNPs and the development of MS, hypertension and DM over a long time scale are necessary.

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