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Genetic Variations in the *FTO* Gene Are Associated with Type 2 Diabetes and Obesity in South Indians (CURES-79)

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Abstract

Aim: The present study investigated the association of six variants—*rs9940128*, *rs7193144*, and *rs8050136* (in intron 1), *rs918031* and *rs1588413* (in intron 8), and *rs11076023* (3′ untranslated region)—across three regulatory regions of the *fat mass and obesity-associated* (*FTO*) gene with obesity and type 2 diabetes mellitus (T2DM) in a South Indian population.
Methods: Unrelated study subjects ($n = 1,852$; 1,001 normal glucose-tolerant [NGT] controls and 851 cases [T2DM]) were randomly selected from the Chennai Urban Rural Epidemiological Study (CURES). Genotyping was done by the polymerase chain reaction-restriction fragment length polymorphism method, and 20% of samples were sequenced to validate the genotypes obtained. Haplotype analysis was also carried out.
Results: The three polymorphisms *rs9940128 A/G*, *rs1588413 C/T*, and *rs11076023 A/T* of the *FTO* gene were associated with T2DM in our study population. The *rs8050136 C/A* variant was associated with obesity, and its association with T2DM was also mediated through obesity. The *rs1588413 C/T* variant showed an association with obesity in the total study subjects, but when the NGT subjects alone were analyzed, the association with obesity was lost. The haplotype ACCTCT confers a lower risk of T2DM in this South Indian population.
Conclusions: Among South Indians, the *rs9940128 A/G*, *rs11076023 A/T*, and *rs1588413 C/T* variants of the *FTO* gene were associated with T2DM, whereas the *rs8050136 C/A* variant was associated with obesity.

Introduction

Obesity and type 2 diabetes mellitus (T2DM) are complex disorders with strong genetic components, and both disorders have an impact on morbidity and mortality. Recent genome-wide association studies of T2DM discovered a novel *fat mass and obesity-associated* (*FTO*) gene on chromosome 16q12.2 that predisposes to T2DM through effects on obesity as assessed by body mass index (BMI).[1] The *FTO* gene was originally cloned from the *Ft* (*fused toes*) mutant mouse.[2] The gene function is still unknown, but based on the predicted structure, the *FTO* gene encodes for a non-heme and 2-oxoglutarate-dependent oxygenase with nucleic acid demethylase activity.[3]

Two independent genome-wide association studies[1,4] and other studies[5] have replicated *FTO* gene variants and demonstrated their association with BMI and with risk of being overweight in children and adults in cohorts of Europeans, European Americans, and Hispanic Americans. Follow-up studies of relatively smaller cohorts confirmed association of the same region with obesity in German and Belgian children and adults.[6,7] Subsequent replication studies confirmed common variants located in the intron 1 of the *FTO* gene with obesity in Asian[8–13] and African American[14] subjects. However, studies in an Oceanic population,[15] Japanese,[16] and Han Chinese[17] could not replicate these findings.

Asian Indians have a unique phenotype characterized by increased abdominal obesity and visceral fat despite low BMI, hyperinsulinemia,[18] insulin resistance,[19] and dyslipidemia,[20] features that have been referred to as the "Asian Indian phenotype,"[21] which results in increased susceptibility to T2DM.[22–24] Recently, studies on Asian Indians from Pune and Mysore[25] and North Indian Sikhs[26] have reported an association of the *FTO* gene variant *rs9939609 T/A* and *rs7193144 C/T* in intron 1 with T2DM and a weaker association with obesity as defined by BMI. Currently India has 50 million people with diabetes mellitus, and these numbers are projected to increase to 87 million by 2030. Hence studies on genetics of diabetes are urgently needed. This is particularly true because there are genetic differences described in Indians.[27]

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The aim of the present study was to investigate the association of some of the other common variants in the *FTO* gene with T2DM and obesity in a South Indian population.

Research Design and Methods

Subjects and study design

Using a case-control approach, we recruited a total of 1,852 unrelated study subjects, 1,001 controls (normal glucose-tolerant [NGT]) and 851 cases (T2DM) (795 men and 1,057 women, mean age 43 ± 14 years, mean BMI $24.2 \pm 4.6\,kg/m^2$) from the Chennai Urban Rural Epidemiological Study (CURES), an ongoing epidemiological study conducted on a representative population (>20 years) of Chennai, the fourth largest city in India. The methodology of the study has been published elsewhere[28] and is briefly outlined here. In Phase 1 of CURES, 26,001 individuals were recruited based on a systematic random sampling technique. Subjects with self-reported diabetes taking drug treatment for diabetes were classified as "known diabetes subjects." All known diabetes subjects ($n = 1{,}529$) were invited to visit the center for detailed studies. In addition, every 10^{th} individual of the 26,001 individuals without known diabetes was invited to undergo oral glucose tolerance tests using a 75-g oral glucose load (dissolved in 250 mL of water) (Phase 3 of CURES). Those who were confirmed by oral glucose tolerance test to have 2-h plasma glucose value $\geq$11.1 mmol/L (200 mg/dL) based on World Health Organization consulting group criteria were labeled as "newly detected diabetes subjects" and those with 2-h plasma glucose value <7.8 mmol/L (140 mg/dL) as being NGT.[29]

Phenotype measurements

Anthropometric measurements including weight, height, and waist were obtained using standardized techniques. The BMI was calculated as weight (in kg) divided by the square of height (in m). Generalized obesity was defined according to the World Health Organization Asia Pacific Guidelines for Asians as non-obese (BMI $<25\,kg/m^2$) and obese (BMI $\geq 25\,kg/m^2$).[30] Biochemical analyses were done on a Hitachi-912 Auto Analyzer (Hitachi, Mannheim, Germany) using kits supplied by Roche Diagnostics (Mannheim). Fasting plasma glucose (glucose oxidase-peroxidase method), serum cholesterol (cholesterol oxidase-phenol-4-amino-antipyrene peroxidase method), serum triglycerides (glycerol phosphatase oxidase-phenol-4-amino-antipyrene peroxidase method), and high-density lipoprotein cholesterol (direct method; polyethylene glycol-pretreated enzymes) were measured. Low-density lipoprotein cholesterol was calculated using the Friedewald formula.[31] Glycated hemoglobin (HbA1C) was estimated by high-performance liquid chromatography using a Variant™ machine (Bio-Rad, Hercules, CA). Serum insulin concentration was estimated using enzyme-linked immunosorbent assay (Dako, Glostrup, Denmark).

Informed consent was obtained from all study participants, and the study was approved by the Madras Diabetes Research Foundation Institutional Ethics Committee.

Genetic analysis

Genomic DNA was extracted from the whole blood by the phenol-chloroform method of DNA extraction.[32] Six *FTO* single nucleotide polymorphisms (SNPs)—*rs9940128*, *rs7193144*, *rs8050136*, *rs918031*, *rs1588413*, and *rs11076023*—were selected based on the literature review and genotyped by polymerase chain reaction on a GeneAmp® PCR system 9700 thermal cycler (Applied Biosystems, Foster City, CA) using the following primers (all from Sigma, Bangalore, India): forward 5′AGGCCTCAGCTTCCCTGAACTGG3′, reverse 5′TGCCATGGAAAATCTGGCTCATGGT3′; forward 5′TTTATGAAAAATAACTCTTTTCCA3′, reverse 5′CAACC AAAACAACATATTTCGTC3′; forward, 5′TTTGTTTTGGC TTTCTGCAGTCT3′, reverse, 5′CAAAAACCACAGGCTC AGATAAT3′; forward 5′GGAGGGCTGCTGAGAGGGGG3′, reverse 5′CTGCCAAGGGCCCAAGAGGC3′; forward 5′GCT CCCGTCTGCTCTGCCCT3′, reverse 5′GCTGTGGGGAAGG GAGGTGGT3′; and forward 5′TGTTTGCCTGTCTGCACT TGCCT3′, reverse 5′GCCCCACCTGTAGGGCACCTT3′, respectively. Restriction fragment length polymorphism was carried out using *Msp*I (*rs9940128*), *Taq*I (*rs7193144*), *Tsp509*I (*rs8050136*), *Hae*III (*rs918031*), *Rsa*I (*rs1588413*), and *Hinf*I (*rs11076023*) restriction enzymes (New England Biolabs, Inc., Beverly, MA). The resulting products were electrophoresed on a 3% agarose gel. To ensure that the genotyping was of adequate quality, we performed random duplicates in 20% of the samples. The assays were performed by a technician who was masked to the phenotype, and there was 99% concordance in the genotyping. Furthermore, a few variants were confirmed by direct sequencing with an ABI Prism® 310 genetic analyzer (Applied Biosystems).

Statistical analysis

Statistical Package for Social Sciences for Windows version 15.0 (SPSS, Chicago, IL) was used for statistical analysis. The effects of the variants on quantitative and categorical variables were analyzed. Allele frequencies were estimated by gene counting. Agreement with Hardy–Weinberg expectations was tested using a χ^2 goodness-of-fit test. Comparison of the means between the two groups was analyzed by Student's *t* test. The χ^2 test was used to compare the proportions of genotypes or alleles. Analyses for T2DM and NGT are given for an "additive" model in which homozygotes for the major allele (0), heterozygotes (1), and homozygotes for the minor allele (2) were coded. One-way analysis of variance was used to compare groups for continuous variables. Logistic regression analysis was used to identify the risk of the genotype combinations for T2DM and obesity. T2DM or obesity was taken as the dependent variable, and the genotypes were used as the independent variable. As subjects with diabetes were older and had higher BMI, we adjusted for age, sex, and BMI in all the logistic regression analyses. It is possible that some of our controls, who are younger, will develop diabetes because the prevalence of diabetes increases with age. In order to circumvent this problem, age was adjusted for in the logistic analysis. Furthermore, to adjust for the possible confounding effect of age, sex, and BMI, these were included as covariates in the analysis of T2DM. For analyzing the risk of obesity, age, sex, and T2DM were included as covariates.

Linkage disequilibrium (LD) and haplotype frequencies were estimated using Haploview software (www.broad.mit .edu/mpg/haploview/).[33] A *P* value <0.05 was considered statistically significant. Significant *P* values obtained were corrected for multiple testing (Bonferroni correction). Power

was estimated using an online post hoc power computation tool (www.dssresearch.com/toolkit/spcalc/power_p2.asp).

Results

Clinical and biochemical characteristics of the study subjects

The study subjects comprised NGT ($n = 1{,}001$) and T2DM ($n = 851$) subjects. Table 1 represents the baseline clinical characteristics of the study subjects. Compared to NGT subjects, the T2DM subjects were older, and clinical parameters such as BMI (T2DM $25.3 \pm 4.3\,kg/m^2$ vs. NGT $23.4 \pm 4.7\,kg/m^2$, $P < 0.0001$), waist circumference (T2DM 91.0 ± 10.1 cm vs. NGT 83.5 ± 12.1 cm, $P < 0.0001$), fasting plasma glucose (T2DM 9.0 ± 3.9 vs. NGT 4.7 ± 0.4 mmol/L, $P < 0.0001$), HbA1C ($P < 0.0001$), and systolic and diastolic blood pressures ($P < 0.0001$) were significantly higher in cases (T2DM) compared to controls (NGT).

Association of FTO *gene polymorphisms with T2DM*

Table 2 shows the genotype and allele frequencies of the six *FTO* SNPs with T2DM screened in the study population. All the genotype frequencies of the six variants in subjects with T2DM and NGT subjects were in Hardy–Weinberg equilibrium (see Supplementary Table S1; Supplementary Data are available online at www.liebertonline.com/dia). The *rs9940128 A/G* polymorphism of the *FTO* gene showed significant association with T2DM, and the *AG* genotype was significantly higher in T2DM subjects (47.7%) compared to the NGT subjects (39.9%) ($P < 0.0001$). The frequency of the *GG* genotype was also significantly higher in T2DM subjects (16.2%) compared to NGT subjects (9.6%) ($P < 0.0001$). The minor allele frequency of the allele *G* was also significantly higher in T2DM subjects (40.1%) compared to NGT subjects (29.5%) ($P < 0.0001$). Logistic regression analysis was performed for *AG* and *GG* genotypes with *AA* as a reference genotype. We observed that the *AG* genotype confers 1.48 times higher risk for T2DM compared to the *AA* genotype ($P = 0.001$) even after adjusting for age, sex, and BMI. Similarly, the *GG* genotype conferred 2.04 times higher risk for T2DM ($P < 0.0001$), after adjusting for age, sex, and BMI. The *rs1588413 C/T* polymorphism of the *FTO* gene was significantly associated with T2DM. The frequency of the *CT* genotype was higher (46.4%) in T2DM subjects compared to 35.5% in the NGT subjects ($P < 0.0001$). Logistic regression analysis revealed that the *CT* genotype conferred 1.81 times higher risk for T2DM compared to the *CC* genotype ($P < 0.0001$). The frequency of the *TT* genotype was also significantly higher in T2DM subjects (8.9%) compared to NGT subjects (5.8%) ($P = 0.0001$). The minor allele frequency of the allele *T* was also significantly higher in T2DM subjects (32.1%) compared to NGT subjects (23.5%) ($P < 0.0001$). The odds ratio (OR) for the *TT* genotype conferred 1.86 times higher risk of T2DM compared to the *CC* genotype ($P = 0.007$), after adjusting for age, sex, and BMI. The *rs8050136 C/A* polymorphism of the *FTO* gene showed a significant association with T2DM. Of the *CA* carriers, 24.7% were in the T2DM group, compared to 19.3% in the NGT group ($P = 0.004$), and the minor allele frequency of the allele *A* was also significant between the two groups ($P = 0.003$). However, logistic regression analysis revealed that while the unadjusted OR for diabetes for the individuals carrying the *CA* genotype was 1.38 (95% confidence interval, 1.10–1.72; $P = 0.004$), on adjusting for age, sex, and BMI, the significance was lost (OR 1.09; 95% confidence interval, 0.83–1.42; $P = 0.52$). The *rs11076023 A/T* polymorphism of the *FTO* gene showed significant protection against T2DM. The frequency of the *AT* genotype was significantly higher (52.0%) in the NGT subjects compared to 49.8% in T2DM subjects ($P = 0.04$). The logistic regression analysis yielded an OR of 0.70 for T2DM for the *AT* genotype compared to the *AA* genotype ($P = 0.01$). The frequency of the *TT* genotype was also significantly higher (23.0%) in NGT subjects compared to 20.1% in T2DM ($P = 0.02$). A statistically significantly lower OR of 0.64 for T2DM was observed for the *TT* genotype ($P = 0.008$) even

Table 1. Clinical and Biochemical Characteristics of the Study Subjects

Parameter	*NGT subjects* (n = *1,001*)	*T2DM subjects* (n = *851*)	P *value*
n (males/females)	418/583	377/474	—
Age (years)	41 ± 13	51 ± 11	<0.001
BMI (kg/m^2)	23.4 ± 4.7	25.3 ± 4.3	<0.0001
Waist circumference (cm)	83.5 ± 12.1	91.0 ± 10.1	<0.0001
Hip circumference (cm)	94.0 ± 10.0	98.0 ± 10.0	<0.0001
Blood pressure (mm Hg)			
Systolic	115 ± 17	129 ± 21	<0.0001
Diastolic	74 ± 11	78 ± 11	<0.0001
Fasting plasma glucose (mmol/L)	4.7 ± 0.4	9.0 ± 3.9	<0.0001
2-h post-load plasma glucose (mmol/L)	5.5 ± 0.05	14.6 ± 3.6	<0.0001
Fasting serum insulin (μIU/mL)	8.3 ± 5.6	11.3 ± 7.2	<0.0001
Glycated hemoglobin (%)	5.5 ± 0.5	8.7 ± 2.4	<0.0001
Serum cholesterol (mg/dL)	173 ± 35	203 ± 43	<0.0001
Serum triglycerides (mg/dL)	110 ± 67	179 ± 125	<0.0001
HDL cholesterol (mg/dL)	43 ± 10	42 ± 10	0.027
LDL cholesterol (mg/dL)	107 ± 30	125 ± 39	<0.0001

Data are mean ± SD values.

BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NGT, normal-glucose tolerant; T2DM, type 2 diabetes mellitus.

Table 2. Association of *FTO* Gene Polymorphisms with Type 2 Diabetes Mellitus

SNP	*NGT subjects* (n = *1,001*)	*T2DM subjects* (n = *851*)	P *value*	*Unadjusted OR, 95% CI,* P	*Adjusted OR, 95% CI,* P[a]
rs9940128 A/G					
AA	506 (50.5%)	307 (36.1%)	<0.0001 (AA vs. AG)	Reference	Reference
AG	399(39.9%)	406 (47.7%)	0.02 (AG vs. GG)	1.67 (1.37–2.04), <0.0001	1.48 (1.16–1.89), 0.001
GG	96 (9.6%)	138 (16.2%)	<0.0001 (AA vs. GG)	2.36 (1.76–3.18), <0.0001	2.04 (1.42–2.94), <0.0001
MAF (G)	29.5%	40.1%	<0.0001		
rs1588413 C/T					
CC	588 (58.7%)	380(44.7%)	<0.0001 (CC vs. CT)	Reference	Reference
CT	355 (35.5%)	395 (46.4%)	0.44 (CT vs. TT)	1.72 (1.42–2.08), <0.0001	1.81 (1.43–2.31), <0.0001
TT	58 (5.8%)	76(8.9%)	0.0001 (CC vs. TT)	2.02 (1.40–2.92), <0.0001	1.86 (1.18–2.92), 0.007
MAF (T)	23.5%	32.1%	<0.0001		
rs11076023 A/T					
AA	250 (25.0%)	256 (30.1%)	0.04 (AA vs. AT)	Reference	Reference
AT	521 (52.0%)	424 (49.8%)	0.48 (AT vs. TT)	0.79 (0.64–0.98), 0.03	0.70 (0.53–0.92), 0.01
TT	230 (23.0%)	171 (20.1%)	0.02 (AA vs. TT)	0.72 (0.55–0.94), 0.01	0.64 (0.46–0.89), 0.008
MAF (T)	49.0%	45.0%	0.01		
rs8050136 C/A					
CC	797 (79.6%)	627(73.7%)	0.004 (CC vs. CA)	Reference	Reference
CA	193 (19.3%)	210 (24.7%)	0.86 (CA vs. AA)	1.38 (1.10–1.72), 0.004	1.09 (0.83–1.42), 0.52
AA	11 (1.1%)	14 (1.6%)	0.32 (CC vs. AA)	1.61 (0.72–3.58), 0.23	1.34 (0.49–3.62), 0.56
MAF (A)	10.7%	14.0%	0.003		
rs7193144 C/T					
CC	764 (76.3%)	622 (73.1%)	0.12 (CC vs. CT)	Reference	Reference
CT	226 (22.6%)	219 (25.7%)	0.93 (CT vs. TT)	1.19 (0.96–1.47), 0.11	0.95 (0.73–1.23), 0.71
TT	11 (1.1%)	10(1.2%)	0.97 (CC vs. TT)	1.11 (0.47–2.64), 0.80	1.04 (0.36–3.00), 0.93
MAF (T)	12.4%	14.0%	0.15		
rs918031 C/T					
CC	247 (24.7%)	222 (26.1%)	0.64 (CC vs. CT)	Reference	Reference
CT	525 (52.4%)	445 (52.3%)	0.69 (CT vs. TT)	0.94 (0.75–1.17), 0.60	0.96 (0.73–1.26), 0.76
TT	229 (22.9%)	184 (21.6%)	0.44 (CC vs. TT)	0.89 (0.68–1.16), 0.40	0.94 (0.67–1.31), 0.72
MAF (T)	49.1%	47.8%	0.43		

[a]OR adjusted for age, sex, and body mass index.
CI, confidence interval; MAF, minor allele frequency; NGT, normal glucose-tolerant; OR, odds ratio; SNP, single nucleotide polymorphism; T2DM, type 2 diabetes mellitus.

after adjusting for age, sex, and BMI. The minor allele frequency of the *T* allele was significantly higher (49%) in the NGT subjects compared to 45% in the T2DM subjects ($P = 0.01$). The *rs7193144 C/T* and *rs918031 C/T* polymorphisms of the *FTO* gene did not show any association with T2DM.

Association of FTO *gene polymorphisms with obesity*

Table 3 shows the genotype and allele frequencies of the six *FTO* SNPs with obesity. The entire study subjects were stratified based on BMI according to the World Health Organization Asia Pacific Guidelines as non-obese (BMI $\leq$25 kg/m^2) and obese (BMI $\geq$25 kg/m^2). With respect to the *rs1588413 C/T* polymorphism, the genotype and allele frequencies were significantly different between obese and non-obese subjects ($P = 0.01$). Of the *CT* genotype, 43% were in the obese group, compared to 38.7% in the non-obese group ($P = 0.01$). Also, the frequency of the *TT* genotype was 9% in obese subjects, compared to 6% among non-obese subjects ($P = 0.004$). To estimate the effect of the genotypes on the disease, logistic regression analysis was performed. The comparison between the *CC* and *CT* genotypes yielded an unadjusted OR of 1.27, which was statistically significant ($P = 0.01$), but the significance was abolished after adjusting for age, sex, and diabetes. When we compared the *CC* genotype with the *TT* genotype, the OR remained significant, conferring 1.53 times higher risk towards obesity ($P = 0.02$), even after adjusting for age, sex, and diabetes. The minor allele frequency of the *T* allele was also significantly higher in obese subjects (30.5%) compared to non-obese subjects (25.4%) ($P = 0.0007$). In the case of the *rs8050136 C/A* polymorphism, the genotype and allele frequencies were also significantly different between the obese and non-obese subjects ($P < 0.0001$). The frequency of the *CA* genotype was significantly higher (29.3%) among the obese group compared to 16.7% among the non-obese group ($P < 0.0001$). The logistic regression analysis yielded an OR of 2.06 for obesity for the *CA* genotype compared to the *CC* genotype, even after adjusting for age, sex, and diabetes. However, the significance of the *AA* genotype was lost when adjusted for age, sex, and T2DM ($P = 0.07$). The minor allele frequency of allele *A* was also significantly higher in the obese group (16.5%) compared to the non-obese group (9.3%) ($P < 0.0001$). There was no association between any of the other four polymorphisms (*rs9940128 A/G*, *rs7193144 C/T*, *rs918031 C/T*, and *rs11076023 A/T*) with obesity.

Association of FTO *gene polymorphisms with obesity among the NGT subjects*

Because of potential confounding between T2DM and obesity, a separate analysis was done to look at the association with obesity in NGT subjects. Table 4 represents the genotype and allele frequencies of the *FTO* gene polymorphisms, when the NGT subjects were stratified on the basis of BMI as obese and non-obese. In the case of *rs8050136 C/A* polymorphism, the frequency of the *CA* genotype was significantly higher (28.8%) in obese subjects compared to 14.8% in the non-obese subjects ($P < 0.0001$). The minor allele frequency of the *A* allele was also significantly higher in obese NGT subjects (15.9%) compared to 8.3% of non-obese NGT subjects ($P < 0.0001$). However, there was no significant difference between the *CA* and *AA* genotypes or the *CC* and *AA* genotypes. The genotype and allele frequencies of the *rs9940128 A/G*, *rs7193144 C/T*, *rs918031 C/T*, *rs1588413 C/T*, and *rs11076023 A/T* polymorphisms were not significantly different between the obese and non-obese subjects.

LD estimation between FTO *SNPs and haplotype analysis*

The haplotype-based analysis was performed using HAPLOVIEW, wherein six locus haplotypes were constructed, and the haplotypes with frequency >1% in either cases (T2DM) or controls (NGT) were selected. None of these six loci was in strong pairwise LD (r^2 <0.7) (see Supplementary Tables S2 and S3). Four haplotype frequencies were significantly different between cases and controls of the six loci in the *FTO* gene (Table 5). The proportion of GCCTTA haplotype was higher in cases (0.032) compared to controls (0.016) ($P = 0.001$). The frequency of the GCCTTT haplotype was also higher in cases (0.019) compared to controls (0.006) ($P = 0.0002$). The proportion of the ACCTCT haplotype was significantly higher in controls (0.161), compared to the cases (0.095) ($P = 1.9\times10^{-9}$). The ACCCCT haplotype was also higher in controls (0.095) compared to cases (0.062) ($P = 0.0003$). Because 21 tests were performed, corresponding to the 21 haplotypes satisfying the selection criterion, a multiple correction was done using the Bonferroni test. The association of the haplotype ACCTCT remained significant as the *P* value was less than the Bonferroni threshold of 0.05/21, which is 0.002. We also performed power calculation to evaluate whether our sample size had sufficient power to detect the observed difference in the proportion of the haplotype ACCTCT in the two groups (controls and cases) and found that the power was 0.65. The other haplotypes—ACCCCT, GCCTTA, and GCCTTT—could not retain their association after multiple testing, and the power was too low, ranging from 0.24 to 0.04. The true association of the haplotype was tested using permutation analysis in HAPLOVIEW, wherein 10,000 permutations were computed, to obtain the differences in haplotype frequencies between cases and controls. We observed that the difference in the proportion in the haplotype ACCTCT between the cases and controls was truly significant. None of the haplotype frequencies tested was significantly different between the obese and non-obese groups (data not shown).

Discussion

Several genome-wide association studies have shown an association of the *FTO* gene variants with obesity and T2DM in various Caucasian[1,4,5] and Asian[9,12,25] populations. In this study we report on the association of the *FTO* gene variants with T2DM and obesity in a representative population of South Indians. The SNPs screened in the present study are *rs9940128 A/G*, *rs7193144 C/T*, and *rs8050136 C/A* (located in intron 1), *rs918031 C/T* and *rs1588413 C/T* (located in intron 8), and *rs11076023 A/T* (in the 3′ untranslated region). These SNPs were selected because of their positive association with obesity and diabetes in various populations.[1,9,12,25,34]

The *rs9940128 A/G* SNP earlier reported by Scuteri et al.[4] showed a significant association with T2DM in our study. The *GG* genotype conferred a two-fold higher risk of T2DM, even after adjusting for age, sex, and BMI. These findings are

Table 3. Association of *FTO* Gene Polymorphisms with Obesity

SNP	*Non-obese subjects (<25 kg/m²)* (n = *1,093*)	*Obese subjects (≥25 kg/m²)* (n = *754*)	P *value*	*Unadjusted OR, 95% CI,* P	*Adjusted OR, 95% CI,* P[a]
rs1588413 C/T					
CC	604 (55.3%)	362 (48.0%)	0.01 (CC vs. CT)	Reference	Reference
CT	423 (38.7%)	324 (43.0%)	0.13 (CT vs. TT)	1.27 (1.05–1.55), 0.01	1.16 (0.95–1.42), 0.13
TT	66 (6.0%)	68 (9.0%)	0.004 (CC vs. TT)	1.71 (1.19–2.47), 0.003	1.53 (1.05–2.22), 0.02
MAF (T)	25.4%	30.5%	0.0007		
rs8050136 C/A					
CC	900 (82.3%)	519 (68.8%)	<0.0001 (CC vs. CA)	Reference	Reference
CA	182 (16.7%)	221 (29.3%)	0.92 (CA vs. AA)	2.10 (1.68–2.63), <0.0001	2.06 (1.63–2.59), <0.0001
AA	11 (1.0%)	14 (1.9%)	0.07 (CC vs. AA)	2.20 (0.99–4.89), 0.05	2.09 (0.92–4.75), 0.07
MAF (A)	9.3%	16.5%	<0.0001		
rs9940128 A/G					
AA	482 (44.1%)	330 (43.8%)	0.88 (AA vs. AG)	Reference	Reference
AG	480 (43.9%)	322 (42.7%)	0.35 (AG vs. GG)	0.98 (0.80–1.19), 0.84	0.87 (0.71–1.07), 0.21
GG	131 (12.0%)	102 (13.5%)	0.43 (AA vs. GG)	1.13 (0.84–1.52), 0.39	0.98 (0.72–1.33), 0.91
MAF (G)	33.9%	34.9%	0.57		
rs7193144 C/T					
CC	825 (75.5%)	555 (73.6%)	0.47 (CC vs. CT)	Reference	Reference
CT	257 (23.5%)	188 (24.9%)	0.48 (CT vs. TT)	1.08 (0.87–1.35), 0.44	1.08 (0.86–1.35), 0.47
TT	11 (1.0%)	10 (1.5%)	0.36 (CC vs. TT)	1.63 (0.69–3.88), 0.26	1.51 (0.61–3.70), 0.36
MAF (T)	12.7%	13.8%	0.38		
rs918031 C/T					
CC	270 (24.7%)	197 (26.1%)	0.95 (CC vs. CT)	Reference	Reference
CT	572 (52.3%)	396 (52.5%)	0.56 (CT vs. TT)	0.94 (0.75–1.18), 0.64	0.95 (0.76–1.20), 0.71
TT	251 (23.0%)	161 (21.4%)	0.38 (CC vs. TT)	0.87 (0.67–1.15), 0.35	0.90 (0.68–1.19), 0.47
MAF (T)	49.1%	47.6%	0.38		
rs11076023 A/T					
AA	295 (27.0%)	211 (28.0%)	0.65 (AA vs. AT)	Reference	Reference
AT	561 (51.3%)	379 (50.3%)	0.89 (AT vs. TT)	0.94 (0.75–1.17), 0.61	0.95 (0.76–1.20), 0.72
TT	237 (21.7%)	164 (21.8%)	0.86 (AA vs. TT)	0.96 (0.74–1.26), 0.80	1.01 (0.77–1.33), 0.91
MAF (T)	47.3%	46.8%	0.80		

[a]OR adjusted for age, sex, and T2DM.

CI, confidence interval; MAF, minor allele frequency; OR, odds ratio; SNP, single nucleotide polymorphism; T2DM, type 2 diabetes mellitus.

Table 4. Genotype and Allele Frequencies of *FTO* Gene Polymorphisms in Normal Glucose-Tolerant Subjects with Obesity

SNP	*Non-obese subjects (BMI <25 kg/m²)* (n = *676*)	*Obese subjects (BMI ≥25 kg/m²)* (n = *323*)	P *value*
rs8050136 C/A			
CC	570 (84.3%)	225 (69.7%)	<0.0001 (CC vs. CA)
CA	100 (14.8%)	93 (28.8%)	0.89 (CA vs. AA)
AA	6 (0.9%)	5 (1.5%)	0.36 (CC vs. AA)
MAF (A)	8.3%	15.9%	<0.0001
rs9940128 A/G			
AA	339 (50.1%)	166 (51.4%)	0.87 (AA vs. AG)
AG	270 (39.9%)	128 (39.6%)	0.80 (AG vs. GG)
GG	67 (9.9%)	29 (9.0%)	0.69 (AA vs. GG)
MAF (G)	29.8%	28.8%	0.65
rs7193144 C/T			
CC	522 (77.2%)	240 (74.3%)	0.30 (CC vs. CT)
CT	146 (21.6%)	80 (24.8%)	0.81 (CT vs. TT)
TT	8 (1.2%)	3 (0.9%)	0.97 (CC vs. TT)
MAF (T)	12.0%	13.3%	0.44
rs918031 C/T			
CC	165 (24.4%)	81 (25.1%)	0.93 (CC vs. CT)
CT	349 (51.6%)	176 (54.5%)	0.24 (CT vs. TT)
TT	162 (24.0%)	66 (20.4%)	0.40 (CC vs. TT)
MAF (T)	49.8%	47.7%	0.40
rs1588413 C/T			
CC	406 (60.1%)	182 (56.3%)	0.18 (CC vs. CT)
CT	228 (33.7%)	125 (38.7%)	0.31 (CT vs. TT)
TT	42 (6.2%)	16 (5.0%)	0.70 (CC vs. TT)
MAF (T)	23.1%	24.3%	0.58
rs11076023 A/T			
AA	171 (25.3%)	79 (24.5%)	0.98 (AA vs. AT)
AT	353 (52.2%)	166 (51.4%)	0.66 (AT vs. TT)
TT	152 (22.5%)	78 (24.1%)	0.65 (AA vs. TT)
MAF (T)	48.6%	49.8%	0.63

BMI, body mass index; MAF, minor allele frequency; SNP, single nucleotide polymorphism.

Table 5. Haplotype Frequencies in Normal Glucose-Tolerant and Type 2 Diabetes Mellitus Subjects

Haplotype (>1% frequency)	*Frequency*	*NGT subjects* [n (*frequency*)]	*T2DM subjects* [n (*frequency*)]	P *value*	*Corrected* P *value*[a]
ACCTCT	0.131	323 (0.161)	161 (0.095)	1.9×10^{-9}	5×10^{-8}
ACCCCA	0.088	198 (0.099)	127 (0.075)	0.01	0.28
ACCCCT	0.08	190 (0.095)	106 (0.062)	3×10^{-4}	8×10^{-3}
GCCCCA	0.07	138 (0.069)	120 (0.071)	0.82	22.10
ACCCTA	0.067	131 (0.066)	119 (0.070)	0.62	16.68
ACCTCA	0.061	135 (0.067)	92 (0.054)	0.09	2.56
GCCTCT	0.058	98 (0.049)	116 (0.068)	0.01	0.34
GCCCTA	0.047	81 (0.040)	94 (0.055)	0.03	0.91
GCCTCA	0.042	80 (0.040)	77 (0.045)	0.45	12.21
ACCTTT	0.033	66 (0.033)	58 (0.034)	0.91	24.48
ATCTCT	0.03	56 (0.028)	55 (0.032)	0.44	11.96
GCCCCT	0.025	41 (0.020)	53 (0.031)	0.03	0.87
ACCTTA	0.025	53 (0.026)	39 (0.023)	0.50	13.57
GCCTTA	0.023	14 (0.016)	24 (0.032)	1×10^{-3}	0.027
ACCCTT	0.017	31 (0.015)	34 (0.020)	0.31	8.32
ACATCT	0.017	38 (0.019)	25 (0.015)	0.30	8.21
GCACCA	0.012	16 (0.008)	30 (0.017)	0.008	0.22
GCCTTT	0.012	11 (0.006)	32 (0.019)	2×10^{-4}	5×10^{-3}
ATCCCT	0.011	22 (0.011)	19 (0.011)	0.91	24.44
ACACCT	0.011	28 (0.014)	12 (0.007)	0.05	1.48
ACATCA	0.01	32 (0.095)	55 (0.014)	0.04	1.00

[a]Corrected *P* value is that after multiple correction (Bonferroni's test).
NGT, normal glucose-tolerant; T2DM, type 2 diabetes mellitus.

similar to those observed among Chinese and Malay populations.[13] However, no significant association with obesity with this polymorphism was observed in our study, unlike that reported in a Japanese population.[9]

The present study shows an association of *FTO* variant *rs8050136 C/A* with obesity, as reported in Europeans.[1,4,35] Similar to Europeans[5] and Asians living in Hong Kong and Korea,[11] our study shows that the *CA* genotype confers 2.0 times higher risk of developing obesity even after adjusting for age, sex, and diabetes. A recent study among the Chinese population reported an independent association of *rs8050136 C/A* with BMI, after adjusting for age and sex.[36] However, among the Han Chinese[17] there was no association with obesity.

This study also shows that there is no independent association of *rs8050136 C/A* with T2DM as its association with T2DM appears to be linked through obesity because the significance was lost after adjusting for obesity. Similar findings were reported in a U.K. population.[35] In the case of Pima Indians also,[37] the association with T2DM was weakened after adjusting for age, sex, birth year, and BMI, suggesting that the association of this polymorphism with T2DM is largely due to the effect on BMI. In further support of this, studies from a multiethnic group in the United States on *rs8050136 C/A* have shown an association with obesity.[38]

Earlier studies in North Indian Sikhs[26] and Asian Indians from the western part of India[25] have shown strong association of intron 1 variant *rs9939609 T/A* of the *FTO* gene with T2DM independent of BMI. In this study we have chosen six other variants of the *FTO* gene, apart from the previously studied *rs9939609 T/A* variant, to look at the association of these variants with T2DM and obesity.

Various reports have described the significant association of the *FTO* variants with obesity within the well-defined LD blocks in intron 1. The first intron of the *FTO* gene was found to be highly conserved across species. The 47-kb region, bounded by flanking recombination hotspots in intron 1, comprises three variants—*rs9940128*, *rs7193144*, and *rs8050136*—studied in our population. Interestingly, a potential transcription factor, CUTL-like-1, was found to regulate *FTO/FTM* gene expression in mice.[39] Similarly, the variants in intron 1 could play a vital role in disrupting the binding of transcription factors, which are essential in regulation of gene expression.

In the case of the *rs918031 C/T* SNP in intron 8 of the *FTO* gene, no association was found with T2DM or obesity, which is similar to a study in a Chinese population.[12] However, *rs1588413 C/T* located in intron 8 showed significant association with T2DM in our study, but this association was not seen in the Chinese population.[12] The *rs1588413 C/T* SNP was not associated with obesity in our population, unlike the results reported in the Chinese population.[12] This clearly points to ethnic differences in susceptibility to obesity and diabetes between two Asian populations. To our knowledge, this variant has not been screened in any other ethnic population.

The *rs11076023 A/T* SNP located in the 3′ untranslated region of the *FTO* gene was not associated with T2DM but was associated with BMI in the Chinese population.[12] In contrast, we found the *TT* genotype of this SNP conferred 0.64 times lower risk of T2DM in our study subjects. Alteration in the 3′ untranslated region due to the occurrence of SNPs might modulate the stability of mRNA, thereby affecting the normal expression of the gene.

None of these six loci was in very strong pairwise LD ($r^2 < 0.7$). The haplotype analysis showed that the GCCTTA and GCCTTT haplotypes confer increased risk of T2DM, but the association diminished after multiple correction (Bonferroni). In contrast, the ACCTCT haplotype was associated with decreased risk of T2DM, which remained significant even after Bonferroni correction. To our knowledge, this is the first study to report on haplotype analysis of the *FTO* gene variants *rs9940128 A/G*, *rs7193144 C/T*, *rs8050136 C/A*, *rs918031 C/T*, *rs1588413 C/T*, and *rs11076023 A/T* in South Indians.

The complex genetic association of the *FTO* gene shows its potential role in energy homeostasis, which is a key factor that is modulated in complex disorders such as T2DM and obesity. Hence such studies are required to refine the strength and exact nature of the genetic signal and to determine which of the variant within a haplotype cluster could be functionally related to obesity or a related trait.[40]

To circumvent the problem of population stratification, we performed a case-control study at six unlinked marker loci believed to be unrelated to the disease under study but known to have allelic diversity among different populations.[41] The allele frequency difference was not statistically significant at any of the loci, indicating that the findings in this study were unlikely to be an artifact of population substructuring.

In conclusion, the three polymorphisms *rs9940128 A/G*, *rs1588413 C/T*, and *rs11076023 A/T* of the *FTO* gene were associated with T2DM in our study population. The *rs8050136 C/A* variant was associated with obesity, and its association with T2DM was also mediated through obesity. The *rs1588413 C/T* variant showed an association with obesity in the total study subjects, but when the NGT subjects alone were analyzed, the association with obesity was lost. The haplotype ACCTCT confers a lower risk of T2DM in this South Indian population.

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Author Disclosure Statement

No competing financial interests exist.

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