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# Analysis of *FTO* Gene Variants with Measures of Obesity and Glucose Homeostasis in the IRAS Family Study

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## Abstract

Multiple studies have identified *FTO* gene variants associated with measures of adiposity in European-derived populations. The study objective was to determine whether *FTO* variants were associated with adiposity, including visceral and subcutaneous adipose tissue (VAT; SAT), and glucose homeostasis measures in the Insulin Resistance Atherosclerosis Family Study (IRASFS). A total of 27 SNPs in *FTO* intron 1, including SNPs prominent in the literature (rs9939609, rs8050136, rs1121980, rs17817449, rs1421085, and rs3751812), were genotyped in 1,424 Hispanic Americans and 604 African Americans. Multiple SNPs were associated with BMI and SAT (*p-values* ranging from 0.001 to 0.033), and trending or associated with waist circumference (*p-values* ranging from 0.008 to 0.099) in the Hispanic Americans. No association was observed with VAT, illustrating that *FTO* variants are associated with overall fat mass instead of specific fat depots. For the glucose homeostasis measures, variants were associated with fasting insulin but, consistent with other studies, after BMI adjustment, no evidence of association remained. The lack of association of *FTO* SNPs with insulin sensitivity is consistent with the lack of association with VAT, since these traits are strongly correlated. In the African Americans, only rs8050136 and rs9939609 were associated with BMI and WAIST (*p-values* of 0.011 and 0.034), and associated or trending towards association with SAT (*p-values* of 0.038 and 0.058). These results confirm that *FTO* variants are associated with adiposity measures, predisposing individuals to obesity by increasing overall fat mass in Hispanic Americans and to a lesser degree in African Americans.

### Keywords

fat mass and obesity associated (*FTO*) gene; single nucleotide polymorphism; genetic association; adiposity; glucose homeostasis

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Recently, Frayling et al. (2007) found 10 SNPs within the first intron of the *fat mass and obesity associated* (*FTO*) gene to be associated with increased body mass index (BMI) in United Kingdom populations of the Wellcome Trust Case Control Consortium (WTCCC). In other studies focusing on Caucasian European cohorts, association of common variants, characterized by strong linkage disequilibrium in *FTO* intron 1, has been observed with increased fat mass in both children and adults (Dina et al., 2007;Scott et al., 2007;Scuteri et al., 2007). For example, Scuteri et al. were able to replicate the association of rs9930506 with BMI, hip circumference, and weight in Sardinians and Hispanic American families of the GenNet study (2007). In addition, association of *FTO* SNPs with BMI have replicated in several other studies, mainly focusing on the common variants rs9939609 and rs8050136 in Europeans (Andreasen et al., 2008;Hinney et al., 2007;Pascoe et al., 2007;Peeters et al., 2008) and a Japanese population (Omori et al., 2008). However, these results were not replicated in a different Japanese population (Horikoshi et al., 2007) and other ethnicities such as Chinese (Li et al., 2008) and Oceanic populations (Ohashi et al., 2007). Several of these studies have assessed the effects of *FTO* variants on quantitative traits such as insulin sensitivity (Andreasen et al., 2008;Do et al., 2008;Jacobsson et al., 2008;Pascoe et al., 2007), and visceral and subcutaneous fat area (Haupt et al., 2008;Peeters et al., 2008) in Europeans. To date, there have been no studies which have systematically investigated *FTO* with adipose tissue distribution (i.e., visceral adipose tissue, subcutaneous adipose tissue; VAT, SAT) and measures of glucose homeostasis (i.e., insulin sensitivity, acute insulin response, disposition index; $S_{I,}$ AIR, DI) in U.S. minority populations. The Insulin Resistance Atherosclerosis Family Study (IRASFS) is an ideal population for such a study. The goal of this project was to replicate the historic association seen with *FTO* SNPs in US minority populations, to determine whether variants affected differential fat distribution (i.e. placement in the visceral or subcutaneous depot), and investigate if SNPs affected glucose homeostasis measures.

## METHODS

### Recruitment

The study design, recruitment, and phenotyping in the IRASFS have been previously described in detail (Henkin et al., 2003). The IRASFS is a family-based multi-center study whose aim is to determine the genetic components of glucose homeostasis and adiposity in Hispanic Americans and African Americans. Enrollment in the study was based on large family size rather than phenotypes. Individuals were recruited from San Antonio, TX (urban Hispanics), San Luis Valley, CO (rural Hispanics), and Los Angeles, CA (African Americans). This report focused on 90 Hispanic American families ($n$=1,424 subjects) and 42 African American families ($n$=604 subjects). A clinical examination included a frequently sampled intravenous glucose tolerance test (FSIGT), anthropometric measures (height, weight, waist and hip circumference), and abdominal CT scanning.

### Adiposity and Glucose Homeostasis Phenotypes

The IRASFS data set contains detailed quantitative measures of adiposity and glucose homeostasis. Measures of body mass index (BMI, $kg/m^2$) were calculated from the height and weight measures obtained from clinical exams. Waist circumference (WAIST, cm) was measured using a metric tape measure and consisted of the circumference of the body between the $10^{th}$ rib and the iliac crest. Waist:hip ratio (WHR) was calculated by taking the ratio of WAIST over the hip circumference (maximum circumference of the buttocks). A CT scan was also performed in order to make precise measurements of abdominal fat distribution. This procedure uses a standard protocol acquiring two 10-mm-thick axial images from the L2–L3 and L4–L5 vertebral levels after a single scout of the abdomen. The CT scans were sent to the Colorado Health Sciences Center, Department of Radiology for analysis to obtain VAT and SAT ($cm^2$) area measurements. Measures of VAT and SAT from the L2–L3 and L4–L5 level

were highly correlated. Therefore, in this study we have focused on measures made at the L4–L5 level to be consistent with previous IRASFS reports and the literature (Hayashi et al., 2003;Katsuki et al., 2003;Pouliot et al., 1992). The visceral to subcutaneous ratio (VSR) was calculated using the ratio of VAT over SAT.

Measures of glucose homeostasis traits included fasting plasma glucose (GFAST), fasting plasma insulin (FINS), insulin sensitivity ($S_I$), acute insulin response (AIR), and disposition index (DI). Plasma glucose and insulin were assayed using standard procedures. Measures of $S_I$ were obtained using the FSIGT and minimal model analysis (MINMOD) software program (Pacini and Bergman, 1986). AIR was measured eight minutes following glucose infusion as the mean insulin increment in plasma insulin concentration above the basal concentration. DI was calculated as the product of $S_I$ and AIR.

## Genotyping

Genomic DNA was purified using PUREGENE DNA isolation kits (Gentra Inc., Minneapolis, MN, USA). Total genomic DNA was quantified using a fluorometric assay provided by a Hoefer DyNA Quant 200 fluorometer (Hoefer Pharmacia Biotech Inc., San Francisco, CA, USA). The DNA samples were diluted to a final concentration of 5 ng/μL. *FTO* intron 1 variants in the region surrounding the SNPs associated with adiposity measures in the literature (60 kb region) were chosen using YRI HapMap (www.hapmap.org) tagSNPs with an $r^2$ threshold of 0.8 and minor allele frequency greater than 5%. Next, these HapMap tagSNPs were incorporated in the CEU HapMap tagSNPs in order to cover variation in Caucasians. Genotypes for 27 SNPs were ascertained using the iPlex MassARRAY SNP genotyping system (Sequenom Inc., San Diego, CA, USA), which utilizes mass tagging to differentiate between alleles (Buetow et al., 2001).

## Statistical Analysis

*FTO* variants were tested for Mendelian inconsistencies using PedCheck (O'Connell and Weeks, 1998). Genotypes deviating from the pedigree structure were changed to missing. A set of unrelated individuals from the Hispanic (*n*=228) and African American (*n*=58) samples were used to calculate maximum likelihood estimates of allele frequencies, departures from Hardy-Weinberg equilibrium proportions, and LD statistics. Power calculations were performed to approximate the ability of the IRASFS Hispanic and African American cohorts to detect statistically significant differences in phenotypic values when compared to a previous report by Scuteri et al. (2007). These power estimates are approximate and were calculated using the program QUANTO (Gauderman 2002), which does not account for within-family correlation.

Variants were tested for association with each quantitative phenotype using the variance component analysis in SOLAR (Almasy and Blangero, 1998). This analysis includes an overall test of genotypic association (i.e. two degree of freedom), and three models defined by the *a priori* genetic models (i.e. additive, dominant, recessive). Familial correlation was accounted for using a kinship coefficient matrix, where a correlation was calculated for each set of related pairs. This familial correlation was incorporated into a covariance-variance matrix used in the tests of significance. Tests were computed by adjusting measures of adiposity and glucose homeostasis for age, gender, and recruitment center (San Antonio, TX, San Luis Valley, CO, Los Angeles, CA) and additionally for BMI. When necessary, quantitative traits were transformed to approximate the distributional assumptions of the test and to minimize heterogeneity of variance. Effect size was also calculated using the variance component model implemented in SOLAR. β values with corresponding standard errors were calculated for standardized BMI, SAT, and WAIST for each SNP after adjustment for age, gender and center.

The quantitative pedigree disequilibrium test (QPDT) program was also used to analyze single SNPs and two, three, and four marker moving window haplotypes (Dudbridge, 2003). This assesses both single SNP and haplotype association. The variance component measured genotype method, as implemented in the program SOLAR, contrasts genotypic means across individuals while adjusting for the familial relationships. In contrast, the quantitative pedigree disequilibrium test (QPDT) form allelic and haplotypic contrasts within a family; thus, the QPDT also accounts for family structure. The variance component model will be more powerful and the QPDT will be conservative while being more robust to population stratification.

A three-stage approach was used to adjust for multiple comparisons. Specifically, principle component (PC) analyses were performed to estimate the number of independent dimensions for each hypothesis while accounting for linkage disequilibrium and trait correlations. The number of estimated independent dimensions for each hypothesis was used to determine a more precise hypothesis-specific Bonferroni adjustment (Gao et al., 2008). The first stage identified SNPs with historical association in *FTO* intron 1. These SNPs were not adjusted because of the strong *a priori* hypothesis that *FTO* variants are associated with BMI and WAIST (Andreasen et al., 2008;Frayling et al., 2007;Hinney et al., 2007;Pascoe et al., 2007;Peeters et al., 2008;Scuteri et al., 2007). The second step computed a principal analysis on the other SNPs in the region to determine how many independent dimensions exist (i.e., accounting for linkage disequilibrium) and applied a Bonferroni adjustment based on the number of independent dimensions: one for each trait (i.e., BMI and WAIST). Given the correlation between BMI and WAIST, this will be slightly conservative. The third step was designed to estimate the number of independent dimensions in the other phenotypes (i.e., WHR, SAT, VAT, VSR, GFAST, FINS, $S_I$, AIR, DI). This was then used to adjust: 1) the SNPs with historic association with BMI and WAIST and 2) all remaining SNPs.

## RESULTS

This study genotyped 27 SNPs in *FTO* intron 1 in 1,424 Hispanic Americans and 604 African Americans. These SNPs captured 77% of the variation in Yoruban and 85% of the variation in Caucasian data from HapMap at an $r^2$ threshold of 0.8. This intron 1 region has been the focus of most prior studies. The genotyping efficiency was 90–99% for all SNPs and none of the variants deviated from Hardy-Weinberg proportions. One SNP failed quality control (rs11861870) and was excluded from the rest of the analysis. Using the method of Gabriel et al. (2002), these SNPs defined four LD blocks in Hispanics (Supplementary Figure 1). African Americans presented a different pattern of LD with only two blocks (Supplementary Figure 2).

Descriptive statistics of each population are summarized in Table 1. Both ethnicities were similar in age, had a higher proportion of females (59%), and have significant proportions of overweight (33%) or obese (Hispanic: 37%, African American: 43%) individuals. Compared to the descriptive statistics of other studies investigating *FTO* in UK and European Caucasian adults (Frayling et al., 2007) and Finns (Scott et al., 2007), the Hispanic and African Americans of the IRASFS had a higher proportion of females, were younger, and had comparable BMI measures. However, compared to French Canadians of the Quebec Family Study (Do et al., 2008) and other populations of European Caucasians (Dina et al., 2007;Pascoe et al., 2007), Hispanic and African Americans had comparable ages and female proportions, but had larger BMI values.

Scuteri et al., (2007) reported effects sizes for these SNPs in European Americans, Hispanic Americans, and African Americans. These ethnic-specific effect sizes were used to estimate the power of the IRASFS cohorts. The Hispanic Americans had 72% power to detect 0.15

standard deviations for variants with an allele frequency of 20%. African Americans showed significantly less power, with 2% power to detect 0.05 standard deviations assuming an allele frequency of 10%.

## Hispanic Americans

**Adiposity Phenotypes—**The results of the association analysis for *FTO* SNPs and adiposity traits focus on the best-fitting additive model for Hispanic Americans, which are summarized in Table 2. Ten SNPs, six of which are in high $r^2$ (denoted by ‡) and clustering in LD blocks 2 and 3 at the 3' end of intron 1, have significant evidence of association with BMI (rs1108102, rs1421085‡, rs11075986, rs1121980‡, rs17817449‡, rs8050136‡, rs3751812‡, rs9939609‡, rs7199182, rs7204609; with *p-values* ranging from 0.003 to 0.05). Five of these SNPs (rs9939609, rs1421085, rs17817449, rs3751812, 1121980) were previously shown to be associated with BMI in other studies mainly focusing on Caucasian populations (Dina et al., 2007;Frayling et al., 2007;Scuteri et al., 2007). Six SNPs were associated with WAIST, with three in high $r^2$ (rs1421085‡, rs11075986, rs1121980‡, rs3751812‡, rs7199182, rs7204609; with *p-values* ranging from 0.008 to 0.04), while two additional SNPs were trending towards association (rs9939609, rs1108102; with *p-values* of 0.065 and 0.077). Associated SNPs clustered towards the 3' end of the intron, with all but rs7199182 and rs7204609 positioned in the LD block with SNPs prominent in the literature (i.e. rs9939609, rs8050136). Six SNPs grouped together in the second and third LD blocks were also associated with SAT (rs1421085‡, rs1121980‡, rs17817449‡, rs8050136‡, rs3751812‡, rs9939609‡, rs7199182; with *p-values* ranging from 0.001 to 0.028). Overall, the variants assocated with adiposity measures of BMI, WAIST, and SAT clustered in the second and third LD block in the middle and at the 3' end of intron 1 (Table 2, Supplementary Figure 1). Using a principle component analysis to adjust for multiple comparisons, the associations with non-a priori SNPs were no longer significant.

The effects of the most statistically significant SNPs on adiposity phenotypes are depicted in Table 3, while the rest of the SNPs are presented in Supplementary Table 1. The minor allele for these SNPs, including all those prominent in the literature (SNPs starred), were associated with an increased BMI, SAT, and WAIST under the additive model. For example, individuals with one copy of the minor allele for rs9939609 and rs8050136 had a 0.3 $kg/m^2$ increase in BMI, while adding an additional allele increased BMI by another 1.3 and 1.7 $kg/m^2$, for each SNP respectively. Individuals with one copy of the minor allele of the most associated SNP, rs3751812, had a 0.5 $kg/m^2$ increase in BMI, a 13 $cm^2$ increase in SAT, and a 0.4 cm increase in WAIST, while adding an additional minor allele increased BMI by 2.4 $kg/m^2$, SAT by 59 $cm^2$, and WAIST by 5.1 cm.

Adjusting the adiposity quantitative traits for BMI to evaluate the influence of *FTO* gene variants on fat distribution independent of overall body size resulted in the evidence for association with WAIST to disappear. However, three SNPs in moderate to high $r^2$ at the 5' end of intron 1 showed nominal evidence of association with WHR (rs8057044, rs17817449, rs8050136; with *p-values* ranging from 0.018 to 0.042), while five additional SNPs in moderate to high $r^2$ showed trending towards association (rs1421085, rs10852521, rs1121980, rs3751812, rs8044769; with *p-values* ranging from 0.064 to 0.071). All of these SNPs clustered in the same region where previous associations with adiposity measures of BMI, WAIST, and SAT were observed before BMI adjustment (Table 2, Supplementary Figure 1). Adjustment for multiple comparisons removed evidence of association with these SNPs.

**Glucose Homeostasis Phenotypes—**In addition, these SNPs were tested for association with glucose homeostasis phenotypes in Hispanic Americans (Supplementary Table 2). Several variants in moderate to high LD in the middle of intron 1 were associated with different

measures of glucose homeostasis in the initial analysis. Similar to other studies of *FTO*, when BMI is included as a covariate in the analysis to determine if the effects of the *FTO* gene variants are independent of overall fat mass, much of the evidence for association disappeared (Table 4). There was, however, still some evidence for association with DI with rs4784323 modestly associated (*p-value* of 0.03) along with four SNPs trending towards association (rs8047395‡, rs10852521‡, rs9940700, rs13334933; with *p-values* ranging from 0.057 to 0.078). All of these SNPs clustered in the middle of the intron in LD block 2, except for rs9940700 and rs13334933, which were within LD block 1 at the 5' end of the intron. In addition, evidence of association with AIR towards the 5' end of the intron persisted (*p-values* ranging from 0.009 to 0.021), along with one SNP trending towards association (rs10852521 with a *p-value* of 0.056). These variants showed no evidence of association after multiple comparison adjustment.

**Haplotype Analysis—**Haplotype analysis using QPDT gave broadly consistent association results in similar locations for the adiposity and glucose homeostasis traits (data not shown) with one, two, three, and/or four haplotype blocks and numerous SNPs suggesting trends (e.g. *p-value*<0.08). Adiposity traits illustrated evidence of association with BMI, WAIST, WHR, SAT, and VAT (*p-values* ranging from 0.011 to 0.077). After BMI adjustment, there was an increase in the evidence of association with WAIST and WHR (*p-values* ranging from 0.003 to 0.08). Several glucose homeostasis phenotypes, such as FINS, $S_I$, and AIR, also showed evidence of association (data not shown). In contrast to the SOLAR results, when adjusting for BMI, the evidence of association persisted, though modestly, in the QPDT analysis. After accounting for BMI, glucose homeostasis measures had similar evidence of association with FINS, $S_I$, AIR, and DI (*p-values* ranging from 0.012 to 0.08) in the 5' end of intron 1 as previously observed before BMI adjustment.

### African Americans

The association results for the African Americans under the additive model were not as consistent as those observed for the Hispanics (data not shown). However, genotypic association analysis showed two SNPs prominent in the literature to be nominally associated with several adiposity measures (Supplementary Table 3). The variants rs8050136 and rs9939609 were associated with BMI (*p-values* of 0.011 and 0.014), WAIST (*p-values* of 0.017 and 0.034), and rs8050136 was additionally associated with SAT (*p-value* of 0.038) with rs9939609 trending towards association (*p-value* of 0.058). Evidence of association was strongest in the dominant model for BMI (*p-value* of 0.003 for both SNPs), WAIST (*p-values* of 0.006 and 0.011), and SAT (*p-value* of 0.017 for both SNPs) (data not shown). Interestingly, the quantitative trait genotypic association also indicated that rs9939609 was marginally associated with DI (*p-value* of 0.049), along with two additional SNPs in high $r^2$ at the 3' end of the intron (rs7204609, rs7199182; with *p*-values of 0.016 and 0.039) (Supplementary Table 4). Association was strongest in the recessive model for rs9939609 (*p-value* of 0.014), rs7199182 (*p-value* of 0.012) and rs7204609 (*p-value* of 0.005) (data not shown).

## DISCUSSION

Variants in the *FTO* gene have been associated with obesity measures in mainly European-derived populations. The function of *FTO* is not known, but two studies have proved that *FTO* is a 2-oxyglutarate-dependent nucleic acid demethylase (Gerken et al., 2007;Sanchez-Pulido and ndrade-Navarro, 2007). It is unknown how this would play a role in obesity. Expression studies in mice and rats have also illustrated differential expression in centers in the brain that govern energy balance (Fredriksson et al., 2008;Stratigopoulos et al., 2008). Recently, the association of *FTO* with obesity has been extended to additional ethnicities with

mixed success (Al-Attar et al., 2008;Horikoshi et al., 2007;Hotta et al., 2008;Li et al., 2008;Ohashi et al., 2007;Omori et al., 2008). This is the first detailed study of *FTO* SNPs in U.S. minority populations with sophisticated measures of adiposity. In Hispanic and African Americans of the IRASFS, 26 SNPs were analyzed with measures of adiposity and glucose homeostasis. These SNPs included variants prominent in the literature, rs9939609 (Frayling et al., 2007), rs8050136 (Scott et al., 2007;Zeggini et al., 2007), and rs1121980, rs1421085, rs17817449, and rs3751812 (Dina et al., 2007).

## Hispanic Americans

We observed that the LD structure of *FTO* intron 1 in Hispanics, which includes three haplotype blocks, is broadly consistent with the LD structure found in Caucasians. The SNPs prominent in the literature clustered in blocks 2 and 3, spanning the middle and 3' end of intron 1. The minor allele frequencies for these variants were lower in Hispanics (20 to 25%) when compared to previous reports in Caucasians (39 to 49%) (Dina et al., 2007;Frayling et al., 2007;Scott et al., 2007;Scuteri et al., 2007;Zeggini et al., 2007).

**Adiposity Phenotypes—**In Hispanic Americans we observed consistent evidence of association with BMI (*p-values* ranging from 0.001 to 0.033) with the six SNPs prominent in the literature and several additional variants within LD blocks 2 and 3. These associations with BMI are consistent with results found in the original studies of European-derived populations (Dina et al., 2007;Frayling et al., 2007;Scuteri et al., 2007). Additionally, the study by Scuteri et al. found association of rs1421085 and rs3751812 with BMI in the European (*n*=1,496) and Hispanic Americans (*n*=839) of the GenNet study (2007). The Hispanics in the GenNet study have comparable minor allele frequencies for these two SNPs (25% and 24% respectively) to the IRASFS Hispanic American cohort (20% for each SNP). Other studies have also validated the association of rs9939609 in Europeans (Andreasen et al., 2008;Pascoe et al., 2007;Peeters et al., 2008), U.S. Caucasians (Hunt et al., 2008), and Japanese (Omori et al., 2008), rs8050136 in Japanese (Omori et al., 2008) and Germans (Haupt et al., 2008), rs1421085 in Belgian Caucasians (Peeters et al., 2008) and French Canadians (Do et al., 2008), and rs17817449 in French Canadians (Do et al., 2008) with BMI. The magnitude and direction of the effects for BMI (0.102–0.16) are comparable to those which are seen in the literature (0.08–0.19) for European-derived populations (Dina et al., 2007; Frayling et al., 2007) as well as for the European and Hispanic Americans of the GenNet study (Scuteri et al., 2007).

**Association with Direct Measures of Adiposity: Visceral and Subcutaneous Adipose Tissue—**A strength of the IRASFS is the availability of quantitative measures of visceral and subcutaneous fat derived from CT exams. We observed association with SAT (*p-values* ranging from 0.001 to 0.028) with the six SNPs prominent in the literature and additional SNPs in blocks 2 and 3 in LD with these variants. However, there was no evidence of association with VAT in our study. These results indicate that *FTO* variants affect overall fat mass, and are not responsible for visceral placement of fat. Frayling et al. originally found rs9939609 to be strongly associated with measures of skin folds (biceps, triceps, subscapular, suprailiac, and midarm circumference), a surrogate measure of SAT, in Caucasians (2007). The variants rs17817449 and rs1421085 were also associated with 6 measures of skin folds (suprailiac, subscapular, abdominal, medial calf, biceps, and triceps) in French Canadians from the Quebec Family Study (*n*=908) (Do et al., 2008). Few studies have examined the association of abdominal fat depots with *FTO* SNPs. A study in Germans (*n*=1,466) used magnetic resonance (MR) imaging to measure subcutaneous and visceral abdominal fat in a subset of subjects (*n*=298), and found that rs8050136 was associated with subcutaneous fat, but not with visceral fat (Haupt et al., 2008). However, after adjusting the results for age and gender, subcutaneous fat was no longer associated and visceral fat was trending towards association (*p-value* of 0.05) (Haupt et al., 2008). Additionally, Klöting and colleagues illustrated that

*FTO* expression was 3-fold higher in SAT when compared to VAT in Europid men and women undergoing abdominal surgery for cholecystectomy or explorative laparotomy (*n*=55) (2008). They also observed an inverse relationship with BMI and VAT *FTO* gene expression (Klöting et al., 2008). In a study of Belgian Caucasians (*n*=1,367), visceral and subcutaneous fat area were measured using CT scans, and they reported that rs1421085 was associated with subcutaneous fat area under the dominant model (Peeters et al., 2008). However, they did not find any association with visceral fat area. These results are consistent with the results reported here, and suggest that *FTO* is working through a mechanism that preferentially places fat in subcutaneous depots, and not visceral depots, contributing to overall body size in Caucasians and Hispanic Americans.

**Association with Biometric Measures of Adiposity—**Of the six SNPs prominent in the literature, only rs1421085, rs1121980, and rs3751812 were additionally associated with WAIST (*p-values* ranging from 0.008 to 0.04), while rs17817449, rs8050136, and rs9939609 were trending towards association (*p-values* ranging from 0.065 to 0.099). Association with WAIST was originally found in several European populations by Frayling et al. with the variant rs9939609 (2007). The evidence of association has also been replicated in Danish populations (Andreasen et al., 2008), and rs17817449 and rs1421085 were associated in French Canadians (Do et al., 2008). However, rs9939609 failed to replicate in Scandinavians (Wahlen et al., 2008) and rs1421085 was not associated in Belgian Caucasians (Peeters et al., 2008).

Adjusting the adiposity quantitative traits for BMI allows for the evaluation of the influence of *FTO* gene variants on fat distribution independent of overall body size. In this analysis, evidence for association with WAIST became non-significant with adjustment for BMI. However, accounting for BMI effects unmasked some evidence for association of *FTO* SNPs with WHR within the second and third haplotype blocks. Of the six prominent SNPs, rs17817449 and rs8050136 were modestly associated (*p-values* of 0.034 and 0.042), while rs1421085, rs1121980, and rs3751812 were trending towards association (*p-values* ranging from 0.064 to 0.075). These results are consistent with those found in Danish populations, where rs9939609 was not associated after BMI adjustment (Andreasen et al., 2008). Before BMI adjustment no association was observed with *FTO* SNPs and WHR. However, individuals of European ancestry from the RISC study (*n*=1,276) showed evidence of association with WHR and rs9939609 when adjusted for age, sex, and recruitment center (Pascoe et al., 2007). Additionally, rs17817449 and rs1421085 were associated with WHR when adjusted for age and sex in French Canadians (Do et al., 2008). Both of these studies differed from our study since they did not additionally adjust obesity measures for BMI.

**Glucose Homeostasis Phenotypes—**Analysis was also performed to evaluate whether *FTO* variants had an effect on glucose homeostasis traits, and some evidence of association was observed. In a recent evaluation in the IRASFS, the variant rs8050136 was not associated with glucose homeostasis measures (Palmer et al., 2008). However, in the study reported here, other SNPs within the first, second, and third LD block show some evidence of association before multiple comparison adjustment. Adjusting these measures for BMI reduces the evidence of association substantially in the SOLAR results and to a lesser extent in QPDT. The SOLAR results are consistent with most of the results found in other studies of European-derived populations (Andreasen et al., 2008;Do et al., 2008;Freathy et al., 2008;Pascoe et al., 2007). However, the lack of consistency between the two statistical methods indicates that there may be some modest influence on quantitative measures of glucose homeostasis. Other studies have also shown mixed results for association with glucose homeostasis measures. For example, in extremely obese Swedish children (*n*=450), it was observed that rs9939609 was associated with $S_I$ and trending towards association with AIR when adjusted for significant covariates (i.e. age and BMI standard deviation score) (Jacobsson et al., 2008). These measures were calculated using the Bergman minimal model from FSIGT measures (Jacobsson et al.,

2008). After stratifying the population by gender, only the males (*n*=218) showed evidence of association with $S_I$ (Jacobsson et al., 2008). Overall, the results from the present study indicate that association with glucose homeostasis measures in Hispanic Americans seem to be mediated through adiposity effects, since adjusting for BMI generally causes the evidence for association with these measures to become non-significant.

An interesting observation from the glucose homeostasis traits was seen after BMI adjustment, where SNPs within the first and second LD block at the 5' end of intron 1 are trending or associated with DI and AIR (p-values ranging from 0.009 to 0.078). So far, no other studies have evaluated the effect of *FTO* SNPs on disposition index.

## African Americans

Analysis of *FTO* intron 1 SNPs was also completed in the African American cohort of the IRASFS. The LD structure and minor allele frequencies in the African Americans (14 to 49%) were comparable to those found in the YRI from HapMap (5 to 48%). As expected, the LD structure found in African Americans differed from the Hispanic Americans, containing only two haplotype blocks. LD pattern differences between the cohorts could be caused by the low number of unrelated individuals used in the analysis of the African Americans or differences in minor allele frequency between the populations.

**Adiposity Phenotypes—**In the African Americans, genotypic association analysis illustrated that rs8050136 and rs9939609 were associated with BMI (*p-values* of 0.011 and 0.014), WAIST (*p-values* of 0.017 and 0.034), and SAT (*p-values* of 0.038 and 0.058). None of the other SNPs prominent in the literature were associated with these measures of adiposity, which may be due to the differences in genetic structure within the African American population when compared to Caucasians and Hispanic Americans. It could also be due to a smaller sample size (*n*=604), which could reduce power to detect associations. Only two other studies have assessed the role of *FTO* variants on obesity and obesity measures in African Americans. One study compared obese children (*n*=578) to control children (*n*=1,424) and found a positive association with rs3751812 and risk of childhood obesity (Grant et al., 2008). However, Scuteri et al. illustrated that rs1421085 and rs3751812 in African Americans of the GenNet study (*n*=1,101) were not associated with BMI, hip circumference, or weight (2007). This could be due to lower minor allele frequencies or smaller effect sizes of variants in the GenNet African Americans when compared to the Hispanic and European Americans of the GenNet study (Scuteri et al., 2007). These results are consistent with our study, where the lack of association for rs1421085 and rs3751812 in IRASFS may be caused by lower minor allele frequencies in the African Americans (14%) when compared to IRASFS Hispanic Americans (20%) and other Caucasian cohorts (39 to 49%) (Dina et al., 2007;Frayling et al., 2007;Scott et al., 2007;Scuteri et al., 2007;Zeggini et al., 2007).

There has also been mixed success in replication of *FTO* variants with obesity measures in other ethnicities (Al-Attar et al., 2008;Horikoshi et al., 2007;Hotta et al., 2008;Li et al., 2008;Ohashi et al., 2007;Omori et al., 2008), similar to what was found in the African Americans of the IRASFS. This could be due to differences in allele frequencies within these populations when compared to Caucasians. For example, in Chinese Hans (*n*=3,210) investigators could not find evidence of association of rs8050136 and rs9939609 with BMI and WAIST (Li et al., 2008). The minor allele frequency for rs9939609 and rs8050136 (12 and 20%) in this population are much lower when compared to the frequencies observed in Caucasians (45 to 48%) (Li et al., 2008).

**Glucose Homeostasis Phenotypes—**To our knowledge, no other studies have assessed whether *FTO* SNPs affect sophisticated measures of glucose homeostasis (i.e. SI, AIR, DI) in

other ethnicities. Genotypic association in the African Americans of the IRASFS illustrated several SNPs (including rs9939609) at the 3' end of *FTO* intron 1 that were marginally associated with DI (*p-values* ranging from 0.016 to 0.049).

### Limitations

Although we were able to successfully replicate the association of *FTO* variants in intron 1 with adiposity phenotypes in the Hispanic Americans of the IRASFS, we were unable to see consistent association with previously associated SNPs in the African Americans. This limited evidence of association may reflect lack of power in our African American cohort; however, it could also be to differences in allele frequency. It may also be due to small effect size, as seen for the variants rs1421085 and rs3751812 in African Americans of the GenNet study, with a decrease in BMI of only 0.062 and 0.023 standard deviations for these two variants (Scuteri et al., 2007). After performing power calculations based on the effect sizes calculated by Scuteri et al. (2007), we have illustrated that we have 2% power to detect a 0.05 change in standard deviation given 10% allele frequency. Thus, the association results for the IRASFS African Americans should be interpreted with caution and will most likely provide useful information for future meta-analysis efforts.

This study has performed a detailed association analysis with *FTO* intron 1 variants, and therefore necessitates the correction for multiple testing. Since both the variants and adiposity and glucose homeostasis phenotypes are correlated, the Bonferroni correction is too highly conservative. Hypothesis-specific multiple comparisons adjustments were employed in this study based on estimation via principal component analysis of the number of independent dimensions of the hypothesis and subsequent Bonferroni adjustment. The non-a priori associated SNPs did not meet this threshold and were no longer statistically significantly associated with measures of SAT, WHR, and the glucose homeostasis traits. However, the historically associated SNPs seen in mainly European-derived Caucasian populations replicated in the IRASFS Hispanic Americans.

It has been suggested that using family data decreases power to detect true associations (Teng and Risch, 1999; Visscher et al., 2008). However, there are several advantages to using family data including in family quality control measures (i.e. minimizing genotype errors via PedCheck) and performing association analyses robust to population stratification (Visscher et al., 2008; Laird and Lange, 2006; Benyamin et al., 2009).

In summary, the results found in this study are consistent with the literature, supporting that variants within *FTO* predispose individuals to obesity by increasing fat mass in the Hispanic Americans of the IRASFS. Additional studies should be completed to validate the association observed with rs8050136 and rs9939609 and obesity measures in African Americans.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Reference List

Al-Attar SA, Pollex RL, Ban MR, Young TK, Bjerregaard P, Anand SS, Yusuf S, Zinman B, Harris SB, Hanley AJ, Connelly PW, Huff MW, Hegele RA. Association between the FTO rs9939609

polymorphism and the metabolic syndrome in a non-Caucasian multi-ethnic sample. Cardiovasc Diabetol 2008;7:5. [PubMed: 18339204]

Almasy L, Blangero J. Multipoint quantitative-trait linkage analysis in general pedigrees. Am J Hum Genet 1998;62:1198–1211. [PubMed: 9545414]

Andreasen CH, Stender-Petersen KL, Mogensen MS, Torekov SS, Wegner L, Andersen G, Nielsen AL, Albrechtsen A, Borch-Johnsen K, Rasmussen SS, Clausen JO, Sandbaek A, Lauritzen T, Hansen L, Jorgensen T, Pedersen O, Hansen T. Low physical activity accentuates the effect of the FTO rs9939609 polymorphism on body fat accumulation. Diabetes 2008;57:95–101. [PubMed: 17942823]

Benyamin B, Vissher PM, McRae AF. Family-based genome-wide association studies. Pharmacogenomics 2009;10:181–190. [PubMed: 19207019]

Buetow KH, Edmonson M, MacDonald R, Clifford R, Yip P, Kelley J, Little DP, Strausberg R, Koester H, Cantor CR, Braun A. High-throughput development and characterization of a genomewide collection of gene-based single nucleotide polymorphism markers by chip-based matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Proc Natl Acad Sci U S A 2001;98:581–584. [PubMed: 11136232]

Dina C, Meyre D, Gallina S, Durand E, Korner A, Jacobson P, Carlsson LM, Kiess W, Vatin V, Lecoeur C, Delplanque J, Vaillant E, Pattou F, Ruiz J, Weill J, Levy-Marchal C, Horber F, Potoczna N, Hercberg S, Le SC, Bougneres P, Kovacs P, Marre M, Balkau B, Cauchi S, Chevre JC, Froguel P. Variation in FTO contributes to childhood obesity and severe adult obesity. Nat Genet 2007;39:724–726. [PubMed: 17496892]

Do R, Bailey SD, Desbiens K, Belisle A, Montpetit A, Bouchard C, Perusse L, Vohl MC, Engert JC. Genetic variants of FTO influence adiposity, insulin sensitivity, leptin levels, and resting metabolic rate in the Quebec Family Study. Diabetes 2008;57:1147–1150. [PubMed: 18316358]

Dudbridge F. Pedigree disequilibrium tests for multilocus haplotypes. Genet Epidemiol 2003;25:115–121. [PubMed: 12916020]

Frayling TM, Timpson NJ, Weedon MN, Zeggini E, Freathy RM, Lindgren CM, Perry JR, Elliott KS, Lango H, Rayner NW, Shields B, Harries LW, Barrett JC, Ellard S, Groves CJ, Knight B, Patch AM, Ness AR, Ebrahim S, Lawlor DA, Ring SM, Ben-Shlomo Y, Jarvelin MR, Sovio U, Bennett AJ, Melzer D, Ferrucci L, Loos RJ, Barroso I, Wareham NJ, Karpe F, Owen KR, Cardon LR, Walker M, Hitman GA, Palmer CN, Doney AS, Morris AD, Smith GD, Hattersley AT, McCarthy MI. A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity. Science 2007;316:889–894. [PubMed: 17434869]

Freathy RM, Timpson NJ, Lawlor DA, Pouta A, Ben-Shlomo Y, Ruokonen A, Ebrahim S, Shields B, Zeggini E, Weedon MN, Lindgren CM, Lango H, Melzer D, Ferrucci L, Paolisso G, Neville MJ, Karpe F, Palmer CN, Morris AD, Elliott P, Jarvelin MR, Smith GD, McCarthy MI, Hattersley AT, Frayling TM. Common variation in the FTO gene alters diabetes-related metabolic traits to the extent expected given its effect on BMI. Diabetes 2008;57:1419–1426. [PubMed: 18346983]

Fredriksson R, Hagglund M, Olszewski PK, Stephansson O, Jacobsson JA, Olszewska AM, Levine AS, Lindblom J, Schioth HB. The obesity gene, FTO, is of ancient origin, up-regulated during food deprivation and expressed in neurons of feeding-related nuclei of the brain. Endocrinology 2008;149:2062–2071. [PubMed: 18218688]

Gabriel SB. The structure of haplotype blocks in the human genome. Science 2002;296:2225–2229. [PubMed: 12029063]

Gao X, Starmer J, Martin ER. A multiple testing correction method for genetic association studies using correlated single nucleotide polymorphisms. Genet Epidemiol 2008;32:361–369. [PubMed: 18271029]

Gauderman WJ. Sample size requirements for matched case-control studies of gene-environment interactions. Stat Med 2002;21:35–50. [PubMed: 11782049]

Gerken T, Girard CA, Tung YC, Webby CJ, Saudek V, Hewitson KS, Yeo GS, McDonough MA, Cunliffe S, McNeill LA, Galvanovskis J, Rorsman P, Robins P, Prieur X, Coll AP, Ma M, Jovanovic Z, Farooqi IS, Sedgwick B, Barroso I, Lindahl T, Ponting CP, Ashcroft FM, O'Rahilly S, Schofield CJ. The obesity-associated FTO gene encodes a 2-oxoglutarate-dependent nucleic acid demethylase. Science 2007;318:1469–1472. [PubMed: 17991826]

Grant SF, Li M, Bradfield JP, Kim CE, Annaiah K, Santa E, Glessner JT, Casalunovo T, Frackelton EC, Otieno FG, Shaner JL, Smith RM, Imielinski M, Eckert AW, Chiavacci RM, Berkowitz RI,

Hakonarson H. Association analysis of the FTO gene with obesity in children of Caucasian and African ancestry reveals a common tagging SNP. PLoS ONE 2008;3:e1746. [PubMed: 18335027]

Haupt A, Thamer C, Machann J, Kirchhoff K, Stefan N, Tschritter O, Machicao F, Schick F, Haring HU, Fritsche A. Impact of variation in the FTO gene on whole body fat distribution, ectopic fat, and weight loss. Obesity (Silver Spring) 2008;16:1969–1972. [PubMed: 18535549]

Hayashi T, Boyko EJ, Leonetti DL, McNeely MJ, Newell-Morris L, Kahn SE, Fujimoto WY. Visceral adiposity and the risk of impaired glucose tolerance: a prospective study among Japanese Americans. Diabetes Care 2003;26:650–655. [PubMed: 12610016]

Henkin L, Bergman RN, Bowden DW, Ellsworth DL, Haffner SM, Langefeld CD, Mitchell BD, Norris JM, Rewers M, Saad MF, Stamm E, Wagenknecht LE, Rich SS. Genetic epidemiology of insulin resistance and visceral adiposity. The IRAS Family Study design and methods. Ann Epidemiol 2003;13:211–217. [PubMed: 12684185]

Hinney A, Nguyen TT, Scherag A, Friedel S, Bronner G, Muller TD, Grallert H, Illig T, Wichmann HE, Rief W, Schafer H, Hebebrand J. Genome Wide Association (GWA) Study for Early Onset Extreme Obesity Supports the Role of Fat Mass and Obesity Associated Gene (FTO) Variants. PLoS ONE 2007;2:e1361. [PubMed: 18159244]

Horikoshi M, Hara K, Ito C, Shojima N, Nagai R, Ueki K, Froguel P, Kadowaki T. Variations in the HHEX gene are associated with increased risk of type 2 diabetes in the Japanese population. Diabetologia 2007;50:2461–2466. [PubMed: 17928989]

Hotta K, Nakata Y, Matsuo T, Kamohara S, Kotani K, Komatsu R, Itoh N, Mineo I, Wada J, Masuzaki H, Yoneda M, Nakajima A, Miyazaki S, Tokunaga K, Kawamoto M, Funahashi T, Hamaguchi K, Yamada K, Hanafusa T, Oikawa S, Yoshimatsu H, Nakao K, Sakata T, Matsuzawa Y, Tanaka K, Kamatani N, Nakamura Y. Variations in the FTO gene are associated with severe obesity in the Japanese. J Hum Genet 2008;53:546–553. [PubMed: 18379722]

Hunt SC, Stone S, Xin Y, Scherer CA, Magness CL, Iadonato SP, Hopkins PN, Adams TD. Association of the FTO gene with BMI. Obesity (Silver Spring) 2008;16:902–904. [PubMed: 18239580]

Jacobsson JA, Danielsson P, Svensson V, Klovins J, Gyllensten U, Marcus C, Schioth HB, Fredriksson R. Major gender difference in association of FTO gene variant among severely obese children with obesity and obesity related phenotypes. Biochem Biophys Res Commun 2008;368:476–482. [PubMed: 18249188]

Katsuki A, Sumida Y, Urakawa H, Gabazza EC, Murashima S, Maruyama N, Morioka K, Nakatani K, Yano Y, Adachi Y. Increased visceral fat and serum levels of triglyceride are associated with insulin resistance in Japanese metabolically obese, normal weight subjects with normal glucose tolerance. Diabetes Care 2003;26:2341–2344. [PubMed: 12882859]

Kloting N, Schleinitz D, Ruschke K, Berndt J, Fasshauer M, Tonjes A, Schon MR, Kovacs P, Stumvoll M, Bluher M. Inverse relationship between obesity and FTO gene expression in visceral adipose tissue in humans. Diabetologia. 2008

Li H, Wu Y, Loos RJ, Hu FB, Liu Y, Wang J, Yu Z, Lin X. Variants in the fat mass-and obesity-associated (FTO) gene are not associated with obesity in a Chinese Han population. Diabetes 2008;57:264–268. [PubMed: 17959933]

Laird NM, Lange W. Family-based designs in the age of large-scale gene-association studies. Nat Rev Genet 2006;7:385–394. [PubMed: 16619052]

O'Connell JR, Weeks DE. PedCheck: a program for identification of genotype incompatibilities in linkage analysis. Am J Hum Genet 1998;63:259–266. [PubMed: 9634505]

Ohashi J, Naka I, Kimura R, Natsuhara K, Yamauchi T, Furusawa T, Nakazawa M, Ataka Y, Patarapotikul J, Nuchnoi P, Tokunaga K, Ishida T, Inaoka T, Matsumura Y, Ohtsuka R. FTO polymorphisms in oceanic populations. J Hum Genet 2007;52:1031–1035. [PubMed: 17928949]

Omori S, Tanaka Y, Takahashi A, Hirose H, Kashiwagi A, Kaku K, Kawamori R, Nakamura Y, Maeda S. Association of CDKAL1, IGF2BP2, CDKN2A/B, HHEX, SLC30A8, and KCNJ11 with susceptibility to type 2 diabetes in a Japanese population. Diabetes 2008;57:791–795. [PubMed: 18162508]

Pacini G, Bergman RN. MINMOD: a computer program to calculate insulin sensitivity and pancreatic responsivity from the frequently sampled intravenous glucose tolerance test. Comput Methods Programs Biomed 1986;23:113–122. [PubMed: 3640682]

Palmer ND, Goodarzi MO, Langefeld CD, Ziegler J, Norris JM, Haffner SM, Bryer-Ash M, Bergman RN, Wagenknecht LE, Taylor KD, Rotter JI, Bowden DW. Quantitative trait analysis of type 2 diabetes susceptibility loci identified from whole genome association studies in the Insulin Resistance Atherosclerosis Family Study. Diabetes 2008;57:1093–1100. [PubMed: 18252897]

Pascoe L, Tura A, Patel SK, Ibrahim IM, Ferrannini E, Zeggini E, Weedon MN, Mari A, Hattersley AT, McCarthy MI, Frayling TM, Walker M. Common variants of the novel type 2 diabetes genes CDKAL1 and HHEX/IDE are associated with decreased pancreatic beta-cell function. Diabetes 2007;56:3101–3104. [PubMed: 17804762]

Peeters A, Beckers S, Verrijken A, Roevens P, Peeters P, Van GL, Van HW. Variants in the FTO gene are associated with common obesity in the Belgian population. Mol Genet Metab 2008;93:481–484. [PubMed: 18055244]

Pouliot MC, Despres JP, Nadeau A, Moorjani S, Prud'homme D, Lupien PJ, Tremblay A, Bouchard C. Visceral obesity in men. Associations with glucose tolerance, plasma insulin, and lipoprotein levels. Diabetes 1992;41:826–834. [PubMed: 1612197]

Sanchez-Pulido L, ndrade-Navarro MA. The FTO (fat mass and obesity associated) gene codes for a novel member of the non-heme dioxygenase superfamily. BMC Biochem 2007;8:23. [PubMed: 17996046]

Scott LJ, Mohlke KL, Bonnycastle LL, Willer CJ, Li Y, Duren WL, Erdos MR, Stringham HM, Chines PS, Jackson AU, Prokunina-Olsson L, Ding CJ, Swift AJ, Narisu N, Hu T, Pruim R, Xiao R, Li XY, Conneely KN, Riebow NL, Sprau AG, Tong M, White PP, Hetrick KN, Barnhart MW, Bark CW, Goldstein JL, Watkins L, Xiang F, Saramies J, Buchanan TA, Watanabe RM, Valle TT, Kinnunen L, Abecasis GR, Pugh EW, Doheny KF, Bergman RN, Tuomilehto J, Collins FS, Boehnke M. A genome-wide association study of type 2 diabetes in Finns detects multiple susceptibility variants. Science 2007;316:1341–1345. [PubMed: 17463248]

Scuteri A, Sanna S, Chen WM, Uda M, Albai G, Strait J, Najjar S, Nagaraja R, Orru M, Usala G, Dei M, Lai S, Maschio A, Busonero F, Mulas A, Ehret GB, Fink AA, Weder AB, Cooper RS, Galan P, Chakravarti A, Schlessinger D, Cao A, Lakatta E, Abecasis GR. Genome-Wide Association Scan Shows Genetic Variants in the FTO Gene Are Associated with Obesity-Related Traits. PLoS Genet 2007;3:e115. [PubMed: 17658951]

Stratigopoulos G, Padilla SL, Leduc CA, Watson E, Hattersley AT, McCarthy MI, Zeltser LM, Chung WK, Leibel RL. Regulation of Fto/Ftm gene expression in mice and humans. Am J Physiol Regul Integr Comp Physiol 2008;294:R1185–R1196. [PubMed: 18256137]

Teng J, Risch N. The relative power of family-based and case-control designs for linkage disequilibrium studies of complex human diseases. II. Individual genotyping. Genome Res 1999;9:234–241. [PubMed: 10077529]

Visscher PM, Andrew T, Nyholt DR. Genome-wide association studies of quantitative traits with related individuals: little (power) lost but much to be gained. Eur J Hum Genet 2008;16:387–390. [PubMed: 18183040]

Wahlen K, Sjolin E, Hoffstedt J. The common rs9939609 gene variant of the fat mass- and obesity-associated gene FTO is related to fat cell lipolysis. J Lipid Res 2008;49:607–611. [PubMed: 18048838]

Zeggini E, Weedon MN, Lindgren CM, Frayling TM, Elliott KS, Lango H, Timpson NJ, Perry JR, Rayner NW, Freathy RM, Barrett JC, Shields B, Morris AP, Ellard S, Groves CJ, Harries LW, Marchini JL, Owen KR, Knight B, Cardon LR, Walker M, Hitman GA, Morris AD, Doney AS, McCarthy MI, Hattersley AT. Replication of genome-wide association signals in UK samples reveals risk loci for type 2 diabetes. Science 2007;316:1336–1341. [PubMed: 17463249]

**Table 1**

Demographic Summary of IRASFS Hispanic American and African American Participants

| | Hispanic Americans | | | African Americans | | | |
|---|---|---|---|---|---|---|---|
| | **n** | **Mean ± SD** | **Median** | **n** | **Mean ± SD** | **Median** | **P-Value** |
| Subjects | 1,268 | | | 581 | | | |
| Demographics | | | | | | | |
| Age (years) | | 42.8 ± 14.6 | 41.3 | | 42.9 ± 14.0 | 41.5 | |
| Female Gender (%) | | 58.8 % | | | 59.2 % | | |
| Normal Weight (%) (BMI < 25 kg/m$^2$) | | 28.9 % | | | 24.0 % | | |
| Overweight (%) (25 kg/m$^2$ ≤ BMI < 30 kg/m$^2$) | | 33.6 % | | | 33.3 % | | |
| Obese (%) (BMI ≥ 30 kg/m$^2$) | | 37.4 % | | | 42.7 % | | |
| Adiposity | | | | | | | |
| BMI (kg/m$^2$) | | 28.9 ± 6.1 | 28.1 | | 30.0 ± 6.8 | 29.0 | |
| Waist Circumference (WAIST; cm) | | 89.8 ± 14.3 | 89.1 | | 91.7 ± 15.1 | 91.3 | |
| Waist Hip Ratio (WHR) | | 0.86 ± 0.09 | 0.85 | | 0.83 ± 0.09 | 0.83 | |
| SAT (cm$^2$) | | 338.8 ± 154.7 | 313.7 | | 356.5 ± 190.1 | 325.6 | |
| VAT (cm$^2$) | | 113.9 ± 61.2 | 105.9 | | 93.7 ± 58.9 | 83.3 | |
| VSR | | 0.38 ± 0.21 | 0.33 | | 0.30 ± 0.19 | 0.26 | |
| Glucose Homeostasis | | | | | | | |
| $S_I$(×10$^{-5}$ min$^{-1}$/[pmol/l]) | | 2.15 ± 1.86 | 1.70 | | 1.63 ± 1.17 | 1.41 | 1.55×10$^{-8}$ |
| AIR(μU/mL) | | 760.2 ± 649.3 | 587.0 | | 1005.8 ± 826.2 | 771.5 | 3.05×10$^{-10}$ |
| DI($S_I$ × AIR; × 10$^{-5}$ min$^{-1}$) | | 1316.5 ± 1236.0 | 1005.2 | | 1425.7 ± 1269.2 | 1151.5 | 0.1081 |
| Fasting Insulin | | 14.9 ± 11.0 | 12.0 | | 14.4 ± 11.3 | 11.0 | |
| Fasting Glucose (mg/dL) | | 93.4 ± 9.5 | 92.0 | | 94.6 ± 9.7 | 93.0 | |

Abbreviations: IRASFS, Insulin Resistance Atherosclerosis Family Study; BMI, body mass index; WAIST, waist circumference; WHR, waist to hip ratio; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; VSR, visceral to subcutaneous ratio; $S_I$, insulin sensitivity; AIR, acute insulin response; DI, disposition index

**Table 2**

Association P-values of *FTO* SNPs with Adiposity Phenotypes in the IRASFS Hispanic Americans under the Additive Model

| SNP | Alleles[a] | MAF | Adiposity Phenotypes Adjusted for Age, Gender, and Center | | | | | | Adiposity Phenotypes Additionally Adjusted for BMI | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | BMI | WAIST | WHR | SAT | VAT | VSR | WAIST | WHR | VAT | VSR |
| rs7186637 | C/T | 0.156 | 0.65 | 0.81 | 0.58 | 0.91 | 0.31 | 0.30 | 0.12 | 0.49 | *0.088* | 0.28 |
| rs1108102 | T/A | 0.009 | *0.05* | *0.077* | 0.12 | 0.13 | 0.70 | 0.45 | 0.95 | 0.41 | 0.41 | 0.51 |
| rs11643744 | A/G | 0.412 | 0.93 | 0.88 | 0.60 | 0.88 | 0.80 | 0.39 | 0.52 | 0.47 | 0.46 | 0.33 |
| rs9940700 | G/C | 0.16 | 0.82 | 0.66 | 0.33 | 0.77 | 0.27 | 0.41 | 0.11 | 0.28 | *0.094* | 0.40 |
| rs13334933 | A/G | 0.161 | 0.71 | 0.78 | 0.40 | 0.99 | 0.37 | 0.31 | 0.12 | 0.31 | 0.10 | 0.32 |
| rs16952517 | G/A | 0.087 | 0.75 | 0.67 | 0.52 | 0.73 | 0.89 | 0.14 | 0.73 | 0.24 | 0.49 | 0.16 |
| rs6499642 | C/T | 0.035 | 0.79 | 0.60 | 1.00 | 0.55 | 0.86 | 0.60 | 0.38 | 1.00 | 0.94 | 0.71 |
| rs4784323 | G/A | 0.322 | 0.88 | 0.66 | 0.44 | 0.67 | 0.27 | 0.16 | 0.76 | 0.61 | 0.41 | 0.22 |
| rs8047395 | G/A | 0.383 | 0.29 | 0.51 | 0.75 | 0.11 | 0.18 | 0.92 | 0.12 | 0.20 | 0.84 | 0.86 |
| rs1421085[Ж] | T/C | 0.201 | **0.004** | **0.021** | 0.91 | **0.003** | 0.13 | 0.36 | 0.17 | *0.065* | 0.56 | 0.40 |
| rs10852521 | T/C | 0.364 | 0.23 | 0.46 | 0.46 | 0.13 | 0.18 | 0.86 | *0.082* | *0.071* | 0.98 | 0.84 |
| rs12447107 | G/C | 0.036 | 0.34 | 0.28 | 0.81 | 0.43 | 0.84 | 0.48 | 0.93 | 0.71 | 0.35 | 0.47 |
| rs11075986 | C/G | 0.123 | **0.025** | **0.02** | 0.30 | 0.16 | 0.98 | 0.13 | 0.46 | 0.91 | 0.25 | 0.13 |
| rs2058908 | C/T | 0.382 | 0.48 | 0.57 | 0.83 | 0.54 | 0.35 | 0.60 | 0.32 | 0.42 | 0.82 | 0.48 |
| rs16952524 | C/A | 0.002 | 0.99 | 0.89 | 0.78 | 0.59 | 0.93 | 0.58 | 0.94 | 0.72 | 0.84 | 0.62 |
| rs1121980[Ж] | G/A | 0.252 | **0.016** | **0.04** | 0.78 | **0.012** | 0.23 | 0.45 | 0.18 | *0.064* | 0.43 | 0.46 |
| rs8057044 | G/A | 0.313 | *0.091* | 0.29 | 0.29 | 0.11 | 0.69 | 0.27 | **0.036** | **0.018** | 0.14 | 0.34 |
| rs17817449[Ж] | T/G | 0.219 | **0.033** | *0.099* | 0.47 | **0.028** | 0.39 | 0.25 | 0.16 | **0.034** | 0.40 | 0.26 |
| rs8063946 | C/T | 0.128 | 0.43 | 0.42 | 0.55 | 0.85 | 0.39 | 0.22 | 0.58 | 0.68 | 0.21 | 0.20 |
| rs8050136[Ж] | C/A | 0.222 | **0.031** | *0.096* | 0.53 | **0.019** | 0.37 | 0.21 | 0.16 | **0.042** | 0.40 | 0.23 |
| rs3751812[Ж] | G/T | 0.203 | **0.001** | **0.008** | 0.97 | **0.001** | 0.10 | 0.34 | 0.23 | *0.075* | 0.48 | 0.39 |
| rs9939609[Ж] | T/A | 0.228 | **0.023** | *0.065* | 0.85 | **0.015** | 0.27 | 0.31 | 0.25 | 0.11 | 0.45 | 0.32 |
| rs7199182 | A/G | 0.02 | **0.003** | **0.013** | 0.25 | **0.014** | **0.008** | 0.21 | 0.93 | 0.97 | 0.20 | 0.18 |
| rs13337696 | C/T | 0.035 | 0.16 | 0.18 | 0.65 | 0.32 | 0.43 | 0.83 | 0.60 | 0.73 | 0.47 | 0.82 |
| rs7204609 | T/C | 0.077 | **0.008** | **0.039** | 0.77 | 0.15 | 0.96 | 0.20 | 0.45 | 0.40 | *0.078* | 0.27 |
| rs8044769 | T/C | 0.39 | 0.37 | 0.61 | 0.32 | 0.17 | 0.50 | 0.70 | 0.17 | *0.071* | 0.64 | 0.75 |

Numbers in bold indicate P-value ≤ 0.05 and numbers in italic indicate P-value ≤ 0.1

Abbreviations: SNPs, single nucleotide polymorphisms; IRASFS, Insulin Resistance Atherosclerosis Family Study; MAF, minor allele frequency; BMI, body mass index; WAIST, waist circumference; WHR, waist to hip ratio; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; VSR, visceral to subcutaneous ratio

[Ж] SNPs prominent in the literature

[a] Major/Minor Allele

**Table 3**

Genotypic Means of Statistically Significantly Associated *FTO* SNPs with Adiposity Phenotypes in IRASFS Hispanic Americans

| Trait | SNP | Alleles[a] | MAF | Genotypic Means ± Standard Deviation (n) | | | Standardized p-value[b] | Standardized β±SE[b] |
|---|---|---|---|---|---|---|---|---|
| | | | | 1/1 | 1/2 | 2/2 | | |
| BMI (kg/m$^2$) | rs1421085[ϡ] | T/C | 0.201 | 28.6 ± 5.9(753) | 29.2 ± 6.4(416) | 31.0 ± 7.6(64) | 0.005 | 0.142±0.051 |
| | rs1121980[ϡ] | G/A | 0.252 | 28.5 ± 5.6(659) | 29.1 ± 6.4(453) | 29.9 ± 7.4(113) | 0.018 | 0.109±0.046 |
| | rs17817449[ϡ] | T/G | 0.219 | 28.7 ± 5.8(722) | 29.0 ± 6.4(437) | 30.4 ± 7.7(72) | 0.038 | 0.102±0.049 |
| | rs8050136[ϡ] | C/A | 0.222 | 28.7 ± 5.8(728) | 29.0 ± 6.4(439) | 30.3 ± 7.5(76) | 0.035 | 0.102±0.049 |
| | rs3751812[ϡ] | G/T | 0.203 | 28.6 ± 5.9(745) | 29.1 ± 6.3(427) | 31.5 ± 7.6(66) | 0.002 | 0.159±0.050 |
| | rs9939609[ϡ] | T/A | 0.228 | 28.7 ± 5.8(717) | 29.0 ± 6.3(438) | 30.7 ± 7.4(82) | 0.026 | 0.107±0.048 |
| SAT (cm$^2$) | rs1421085[ϡ] | T/C | 0.201 | 331.7 ± 148.2(725) | 345.8 ± 160.1(400) | 381.9 ± 187.4(61) | 0.003 | 0.142±0.048 |
| | rs1121980[ϡ] | G/A | 0.252 | 330.1 ± 146.3(633) | 343.3 ± 156.5(439) | 370.1 ± 182.0(107) | 0.012 | 0.108±0.043 |
| | rs17817449[ϡ] | T/G | 0.219 | 333.2 ± 148.0(695) | 343.0 ± 159.7(421) | 378.1 ± 182.8(68) | 0.028 | 0.101±0.046 |
| | rs8050136[ϡ] | C/A | 0.222 | 332.6 ± 148.2(701) | 343.0 ± 160.1(423) | 377.8 ± 179.6(72) | 0.018 | 0.108±0.046 |
| | rs3751812[ϡ] | G/T | 0.203 | 330.9 ± 147.7(718) | 344.0 ± 158.6(410) | 403.1 ± 189.2(63) | 0.001 | 0.152±0.047 |
| | rs9939609[ϡ] | T/A | 0.228 | 332.6 ± 147.8(690) | 342.4 ± 159.5(422) | 387.9 ± 179.2(78) | 0.015 | 0.110±0.045 |
| WAIST (cm) | rs1421085[ϡ] | T/C | 0.201 | 89.5 ± 13.8(757) | 89.8 ± 14.8(416) | 93.7 ± 16.8(64) | 0.021 | 0.113±0.049 |
| | rs1121980[ϡ] | G/A | 0.252 | 89.5 ± 13.5(663) | 89.7 ± 14.7(453) | 90.9 ± 16.7(113) | 0.040 | 0.090±0.044 |
| | rs17817449[ϡ] | T/G | 0.219 | 89.8 ± 13.7(726) | 89.4 ± 14.7(437) | 92.5 ± 17.4(72) | *0.099* | 0.078±0.047 |
| | rs8050136[ϡ] | C/A | 0.222 | 89.7 ± 13.7(732) | 89.4 ± 14.7(439) | 92.4 ± 17.2(76) | *0.096* | 0.078±0.047 |
| | rs3751812[ϡ] | G/T | 0.203 | 89.4 ± 13.8(749) | 89.8 ± 14.6(427) | 94.9 ± 16.7(66) | 0.008 | 0.128±0.048 |
| | rs9939609[ϡ] | T/A | 0.228 | 89.8 ± 13.8(721) | 89.3 ± 14.5(438) | 93.3 ± 17.1(82) | *0.065* | 0.085±0.046 |

The mean trait values for each genotype (11,12, 22) are listed followed by the number of individuals included in the analysis. Covariates include age, gender, and center. Numbers in bold indicate P-value ≤ 0.05 and numbers in italic indicate P-value ≤ 0.1 under the additive model.

Abbreviations: SNPs, single nucleotide polymorphisms; IRASFS, Insulin Resistance Atherosclerosis Family Study; MAF, minor allele frequency; BMI, body mass index; SAT, subcutaneous adipose tissue; WAIST, waist circumference

[ϡ] SNPs prominent in the literature

[a] Major/Minor Allele

[b] Values are calculated for each SNP using standardized BMI, SAT, and WAIST after adjustment for age, gender, and center

**Table 4**

Association P-values of *FTO* SNPs with Glucose Homeostasis Phenotypes in the IRASFS Hispanic Americans under the Additive Model

| SNP | Alleles[a] | MAF | Glucose Homeostasis Phenotypes Adjusted for Age, Gender, Center, and BMI | | | | |
|---|---|---|---|---|---|---|---|
| | | | GFAST | FINS | $S_I$ | AIR | DI |
| rs7186637 | C/T | 0.156 | 0.45 | 0.97 | 0.74 | 0.20 | *0.095* |
| rs1108102 | T/A | 0.009 | 0.80 | 0.76 | 0.28 | 0.81 | 0.54 |
| rs11643744 | A/G | 0.412 | 0.35 | 0.52 | 0.66 | 0.66 | 0.85 |
| rs9940700 | G/C | 0.16 | 0.33 | 0.80 | 0.75 | 0.10 | *0.073* |
| rs13334933 | A/G | 0.161 | 0.34 | 0.88 | 0.66 | 0.16 | *0.078* |
| rs16952517 | G/A | 0.087 | 0.16 | 0.57 | 0.57 | 0.54 | 0.33 |
| rs6499642 | C/T | 0.035 | 0.18 | 0.48 | 0.42 | 0.18 | 0.22 |
| rs4784323 | G/A | 0.322 | 0.53 | 0.17 | 0.96 | **0.021** | **0.03** |
| rs8047395 | G/A | 0.383 | 0.39 | *0.08* | 0.53 | 0.18 | *0.072* |
| rs1421085[Ж] | T/C | 0.201 | 0.47 | 0.22 | 0.90 | 0.73 | 0.56 |
| rs10852521 | T/C | 0.364 | 0.51 | 0.20 | 0.90 | *0.056* | *0.057* |
| rs12447107 | G/C | 0.036 | 0.71 | 0.43 | 0.70 | 0.50 | 0.60 |
| rs11075986 | C/G | 0.123 | 0.82 | 0.76 | 0.92 | **0.009** | **0.016** |
| rs2058908 | C/T | 0.382 | 0.74 | 0.57 | 0.48 | 0.10 | 0.35 |
| rs16952524 | C/A | 0.002 | 0.46 | 0.38 | 0.30 | 0.66 | 0.64 |
| rs1121980[Ж] | G/A | 0.252 | 0.32 | 0.15 | 0.93 | 0.99 | 0.97 |
| rs8057044 | G/A | 0.313 | 0.43 | 0.32 | 0.95 | 0.32 | 0.31 |
| rs17817449[Ж] | T/G | 0.219 | 0.39 | 0.31 | 0.94 | 0.51 | 0.56 |
| rs8063946 | C/T | 0.128 | 0.43 | 0.58 | 0.61 | 0.12 | *0.091* |
| s8050136[Ж] | C/A | 0.222 | 0.31 | 0.40 | 0.89 | 0.53 | 0.66 |
| rs3751812[Ж] | G/T | 0.203 | 0.66 | 0.32 | 0.77 | 0.65 | 0.45 |
| rs9939609[Ж] | T/A | 0.228 | 0.50 | 0.42 | 0.98 | 0.78 | 0.77 |
| rs7199182 | A/G | 0.02 | 0.26 | 0.88 | 1.00 | 0.37 | 0.29 |
| rs13337696 | C/T | 0.035 | 0.91 | 0.15 | 0.40 | 0.28 | 0.53 |
| rs7204609 | T/C | 0.077 | 0.78 | 0.83 | 0.36 | 0.37 | 0.19 |
| rs8044769 | T/C | 0.39 | 0.44 | 0.42 | 0.88 | 0.13 | 0.16 |

Numbers in bold indicate P-value ≤ 0.05 and numbers in italic indicate P-value ≤ 0.1

Abbreviations: SNPs, single nucleotide polymorphisms; IRASFS, Insulin Resistance Atherosclerosis Family Study; MAF, minor allele frequency; BMI, body mass index; GFAST, fasting glucose; FINS, fasting insulin; SI, insulin sensitivity; AIR, acute insulin response; DI, disposition index

[Ж] SNPs prominent in the literature

[a] Major/Minor Allele