

Association of *FTO* With Obesity-Related Traits in the Cebu Longitudinal Health and Nutrition Survey (CLHNS) Cohort

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OBJECTIVE—The underlying genetic component of obesity-related traits is not well understood, and there is limited evidence to support genetic association shared across multiple studies, populations, and environmental contexts. The present study investigated the association between candidate variants and obesity-related traits in a sample of 1,886 adult Filipino women from the Cebu Longitudinal Health and Nutrition Survey (CLHNS) cohort.

RESEARCH DESIGN AND METHODS—We selected and genotyped 19 single nucleotide polymorphisms in 10 genes (*ADRB2*, *ADRB3*, *FTO*, *GNB3*, *INSIG2*, *LEPR*, *PPARG*, *TNF*, *UCP2*, and *UCP3*) that had been previously reported to be associated with an obesity-related quantitative trait.

RESULTS—We observed evidence for association of the A allele of rs9939609 (*FTO* intron 1) with increased BMI ($P = 0.0072$ before multiple test correction), baseline BMI ($P = 0.0015$), longitudinal BMI based on eight surveys from 1983 to 2005 ($P = 0.00029$), waist circumference ($P = 0.0094$), and weight ($P = 0.021$). The increase in average BMI was ~ 0.4 for each additional A allele. We also observed association of the *ADRB3* Trp64Arg variant with BMI, waist circumference, percent body fat, weight, fat mass, arm fat area, and arm muscle area ($P < 0.05$), although the direction of effect is inconsistent with the majority of previous reports.

CONCLUSIONS—Our study confirms that *FTO* is a common obesity susceptibility gene in Filipinos, with an effect size similar to that seen in samples of European origin. *Diabetes* 57: 1987–1991, 2008

Obesity is a worldwide epidemic, affecting individuals across all age groups, socioeconomic classes, and ethnicities; numerous association studies have attempted to identify genetic variants that influence susceptibility to obesity (1). As of 2005, 22 candidate genes contained a variant reported to be associated ($P < 0.05$) with an obesity-related trait in at least five studies; however, additional reports for these genes have been inconsistent (1).

More recently, genome-wide association (GWA) studies

have identified variants in additional genes. Single nucleotide polymorphism (SNP) rs7566605, near insulin-induced gene 2 (*INSIG2*) and found to be associated with BMI (2), has not been consistently replicated (3–7). Several variants in the fat mass- and obesity-associated (*FTO*) gene identified through two independent GWA studies (8,9) and a third study (10) were associated with BMI and risk of being overweight in children and adults in cohorts of Europeans, European Americans, and Hispanic Americans, but not in African Americans. *FTO* association was also observed for hip circumference, waist circumference, and subcutaneous fat mass assessed using skinfolds (8,9). Two studies observed *FTO* association with percentage of fat mass and dual-energy X-ray absorptiometry-derived fat mass in children (8,10). *FTO* variants have the most consistent replication across multiple populations to date, suggesting that this locus is a likely risk factor for obesity.

In the current study, we examined 19 SNPs previously reported to be associated with obesity-related phenotypes for association with BMI, waist circumference, and percent body fat in 1,886 Filipino women from the Cebu Longitudinal Health and Nutrition Survey (CLHNS). For SNPs with initial evidence of association, we performed analysis with additional obesity phenotypes.

RESEARCH DESIGN AND METHODS

We evaluated 1,886 unrelated healthy Cebu Filipino female participants in the ongoing CLHNS (11), mothers of a 1983–1984 birth cohort. Trained field staff conducted in-home interviews and collected measurements and comprehensive environmental data (www.cpc.unc.edu/projects/cebu). We used data collected from nonpregnant subjects during surveys in 1983–1984 (baseline at 4 months postpartum), 1984–1985 (1 year postpartum), 1985–1986, 1991, 1994, 1998, 2002, and 2005. For 2005 cross-sectional traits, outcome and covariate measures from the 2002 survey were substituted for 16 women who were pregnant or missing data in 2005.

All outcome and covariate measures, except baseline BMI, were taken from the 2005 survey. Triceps and suprailiac skinfold thicknesses (TSF and SiSF) represent the mean of three consecutive Harpenden caliper measurements. Cross-sectional arm muscle area (AMA) and arm fat area (AFA) were calculated using mid-arm circumference and triceps skinfold thickness. Body density was calculated using the Durnin-Womersley sum of skinfold equation based on TSF and SiSF for adult women aged 16–68 years (12), and percent body fat was derived from body density using the Siri equation (13). Fat mass was calculated as the product of percent body fat and weight. Height was calculated as an average of eight measures across surveys from 1983–1984 to 2005. Informed consent was obtained from all individuals, and the study protocol was approved by the University of North Carolina Institutional Review Board for the Protection of Human Subjects.

SNP selection and genotyping methods. We reviewed genes that exhibited nine or more reports of association with an obesity phenotype, as summarized by the 2004 obesity gene map (1). SNPs within these genes with more than three positive reports of association and a minor allele frequency >0.01 in the Han Chinese Beijing HapMap samples were subsequently chosen to be genotyped. Variants in *FTO* and *INSIG2*, identified through GWA studies, were also genotyped (2,8).

Genotyping was performed using TaqMan allelic discrimination (Applied

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TABLE 2
Association of *FTO* and *ADRB3* SNPs with obesity-related traits

	<i>FTO</i> rs9939609 (MAF 0.175)			<i>ADRB3</i> rs4994 (MAF 0.085)		
	TT	TA	AA	Additive P	Dominant P	TC/CC
BMI (kg/m ²)	23.9 (23.6–24.1)	24.5 (24.1–24.8)	24.7 (23.7–25.7)	0.0072	0.0080	23.3 (22.8–23.8)
Waist circumference (cm)	80.1 (79.4–80.7)	81.7 (80.7–82.7)	81.1 (78.5–83.7)	0.0094	0.0040	78.9 (77.7–80.2)
Body fat (%)	36.3 (36.0–36.6)	36.4 (35.9–36.9)	36.8 (35.6–38.0)	0.43	0.47	35.7 (35.1–36.3)
Baseline BMI (kg/m ²)*	20.5 (20.3–20.6)	20.9 (20.7–21.1)	21.0 (20.4–21.6)	0.0015	0.0013	20.5 (20.3–20.8)
Weight (kg)	54.2 (53.5–54.8)	55.3 (54.3–56.2)	55.9 (53.4–58.4)	0.024	0.024	52.6 (51.4–53.9)
Fat mass (kg)	20.1 (19.7–20.5)	20.6 (20.1–21.2)	20.9 (19.5–22.3)	0.055	0.06	19.3 (18.6–20.0)
Suprailiac skinfold thickness (mm)	28.3 (27.6–28.9)	28.8 (27.9–29.7)	28.5 (26.2–30.9)	0.37	0.31	27.4 (26.2–28.5)
Triceps skinfold thickness (mm)	23.5 (23.0–24.0)	23.6 (22.9–24.3)	24.0 (22.2–25.8)	0.64	0.87	22.8 (21.9–23.7)
Arm fat area (mm ²)	9.5 (9.4–9.6)	9.5 (9.4–9.7)	9.6 (9.3–10.0)	0.33	0.45	9.3 (9.1–9.5)
Arm muscle area (mm ²)	58.7 (57.6–59.8)	60.2 (58.6–61.7)	61.5 (57.4–65.6)	0.084	0.11	56.1 (54.1–58.1)
Height (cm)	150.4 (150.1–150.8)	150.2 (149.7–150.6)	150.4 (149.2–151.6)	0.42	0.32	150.0 (149.4–150.6)

Data are untransformed means (95% CI). All data except baseline BMI were collected in the 2005 survey. For women who were pregnant or missing data in 2005, measures from the 2002 survey were substituted. Models were adjusted for age, household assets, natural log of income, number of total past pregnancies as a categorical variable (1–4, 5–10, and >10), and menopausal status; baseline BMI is not adjusted for menopausal status. *Baseline BMI and covariates were collected from postpartum surveys in 1983–1984.

TABLE 1
Characteristics of 1,886 women in the CLHNS

BMI (kg/m ²)	24.3 ± 4.4
Waist circumference (cm)	81.1 ± 10.8
Body fat (%)	36.6 ± 5.4
Baseline BMI (kg/m ²)*	20.6 ± 2.4
Arm fat area (mm ²)	9.6 ± 1.5
Arm muscle area (mm ²)	60.0 ± 17.6
Fat mass (kg)	20.6 ± 6.3
Suprailiac skinfold (mm)	28.8 ± 10.1
Triceps skinfold (mm)	23.8 ± 8.0
Weight (kg)	55.1 ± 10.9
Average height (cm)	150.4 ± 4.9
Age (years)	48.4 ± 6.1
Total number of pregnancies	6.5 ± 3.0
Menopausal status (yes/no)	1162/724

Data are means ± SD unless otherwise indicated. All traits are measured from the 2005 survey except where indicated. For women who were pregnant or missing data in 2005, measures from the 2002 survey were substituted. *Baseline BMI was collected from postpartum surveys in 1983–1984 (see research design and methods).

Biosystems, Foster City, CA). The genotype success rate for all SNPs was >98%, and the discrepancy rate among duplicate samples was 0.1%.

Statistical analysis. Tests for consistency of genotype distributions with expected Hardy-Weinberg equilibrium proportions were calculated using Pearson's χ^2 statistic; only rs3856806 was inconsistent ($P = 0.02$). ANCOVA models were used to test for association between genotype and continuously distributed outcomes. Logistic regression models were used for dichotomous outcomes. We performed a longitudinal analysis incorporating all available BMI measurements for the up to eight measurements spanning 22 years using general linear mixed models.

Models were adjusted for age, household assets, natural log of income, number of total past pregnancies as a categorical variable (1–4, 5–10, and >10), and menopausal status; baseline BMI was not adjusted for menopausal status. Each of these predictors was significantly ($P < 0.05$) associated with BMI in a multivariable model in our sample. Continuously distributed traits were transformed to satisfy the model assumption of normally distributed residuals, conditional on the covariates. The additive mode of inheritance assumption was used unless <15 rare homozygotes existed; the dominant mode of inheritance assumption for the minor allele was used for SNPs rs4994, rs8179183, rs1801282, and rs1800629. The rs9939609 SNP in *FTO* was also analyzed under both additive and dominant models for comparison with previous reports. Because of low linkage disequilibrium ($r^2 < 0.5$) between pairs of SNPs, Bonferroni adjustment was used to account for multiple tests.

We estimated that a SNP must explain at least 0.45% of the total variation in BMI to achieve at least 80% statistical power to detect an association in this sample, assuming a significance threshold of 5% and an additive mode of inheritance. For a SNP with a minor allele frequency (MAF) of 0.03 or 0.50, this effect would correspond with a change in mean BMI of 1.2 or 0.42 kg/m², respectively, for each additional copy of the variant allele. For rs9939609, our power to detect a difference in BMI of 0.8 units between the homozygotes, approximately as observed by Frayling et al. (8), was 57%.

RESULTS

Of 19 SNPs tested for association with 2005 BMI, waist circumference, and percent body fat (Table 1), two SNPs were associated ($P < 0.01$) with at least one trait before correction for multiple tests (supplementary Table 1 [available in an online appendix at <http://dx.doi.org/10.2337/db07-1700>]). The A allele of SNP rs9939609 (*FTO* intron 1) was associated with increased BMI ($P = 0.0072$) and waist circumference ($P = 0.0094$). The TT homozygote (Trp64) of SNP rs4994 (*ADRB3* Trp64Arg) was associated with increased BMI ($P = 0.0011$) and waist circumference ($P = 0.0026$). After Bonferroni correction for multiple tests, only rs4994 in *ADRB3* remained significant ($P < 0.002$); however, only the *FTO* association was consistent in magnitude and direction of effect with previous reports (8–10).

TABLE 3
Association of *FTO* and *ADRB3* SNPs with overweight and obesity status

	<i>FTO</i> rs9939609		<i>ADRB3</i> rs4994	
	Odds ratio (95% CI)	<i>P</i>	Odds ratio (95% CI)	<i>P</i>
2005 overweight and obese (BMI ≥ 25 kg/m ²)	1.30 (1.09–1.55)	0.0034	1.33 (1.07–1.63)	0.0077
2005 obese (BMI ≥ 30 kg/m ²)	1.31 (1.00–1.72)	0.054	1.46 (1.05–2.02)	0.023
1983–1984 overweight and obese (BMI ≥ 25 kg/m ²)	1.50 (1.06–2.12)	0.023	1.27 (1.01–1.61)	0.044

1983–1984 obesity (BMI ≥ 30 kg/m²) is not reported because only 2 people were observed with BMI ≥ 30 kg/m². Models were adjusted for age, household assets, natural log of income, number of total past pregnancies as a categorical variable (1–4, 5–10, and >10), and menopausal status; 1983–1984 model is not adjusted for menopausal status.

To further investigate the rs9939609 and rs4994 SNPs, we analyzed additional obesity-related phenotypes of baseline BMI (measured in 1983–1984), weight, fat mass, SiSF, TSF, AFA, AMA, and height (Table 2). For *FTO* variant rs9939609, evidence of association was observed with baseline BMI ($P = 0.0015$) and weight ($P = 0.021$). Marginal evidence of association ($0.05 < P < 0.10$) was observed for fat mass ($P = 0.055$) and AMA ($P = 0.084$), with direction of estimated effects consistent with those seen for BMI and weight. For the *ADRB3* variant rs4994, association was observed for weight ($P = 0.0011$), fat mass ($P = 0.0036$), AFA ($P = 0.016$), and AMA ($P = 0.0008$), and marginal association was observed for TSF ($P = 0.068$), with the direction of estimated effects consistent with those observed for BMI and weight. Unlike the *FTO* variant, no evidence for association was observed with baseline BMI ($P = 0.55$).

We analyzed risk of being either overweight and obese (BMI ≥ 25 kg/m²) or obese (BMI ≥ 30 kg/m²) (14) both in 1983–1984 and in 2005 (Table 3). Using these criteria, 793 and 178 women had a BMI ≥ 25 kg/m² or BMI ≥ 30 kg/m², respectively, in 2005, and 94 women had a BMI ≥ 25 kg/m² in 1983–1984. The A allele of rs9939609 was associated with increased risk of being overweight in 2005 (odds ratio 1.30; $P = 0.0034$) and in 1983–1984 (1.50; $P = 0.023$). The TT homozygote of rs4994 was associated with increased risk of being overweight in 2005 (1.33; $P = 0.0077$) and 1983–1984 (1.46; $P = 0.023$) and obese in 2005 (1.27; $P = 0.044$).

A longitudinal analysis of BMI included an average of 7.3 (range 3–8) measurements per individual spanning 22 years. The global P value for the test of association with rs9939609 was 0.000029 (additive model, Fig. 1A) and, for the test of association with rs4994, 0.016 (Fig. 1B). The direction of the genotypic least-squares means at each time point was consistent with the cross-sectional analysis. The test of rs9939609 and rs4994 for genotype-by-time interaction showed evidence for an increasing effect of genotype over time ($P = 0.047$ and 0.0065, respectively).

DISCUSSION

We evaluated 19 SNPs in a sample of adult Filipino women from the CLHNS cohort, confirmed the association of the A allele of *FTO* variant rs9939609 with BMI and waist circumference, and observed evidence for an association with the TT homozygote of *ADRB3* rs4994 with BMI, waist circumference, and percent body fat. While only rs4994 reached statistical significance after Bonferroni correction, the direction of effect was not consistent with the majority of previous reports (15,16). The failure to replicate many of the SNP associations that have previously

been reported may reflect environmental and genetic differences between the CLHNS cohort and previously studied populations, limited statistical power, and/or false positive results in the literature.

We also observed evidence for association between the Trp64 allele of rs4994 and increased weight, percent fat mass, AFA, AMA, and longitudinal BMI. However, we did not observe evidence for association with baseline BMI, which was measured at a time when few women were overweight. In contrast to our study, two meta-analyses

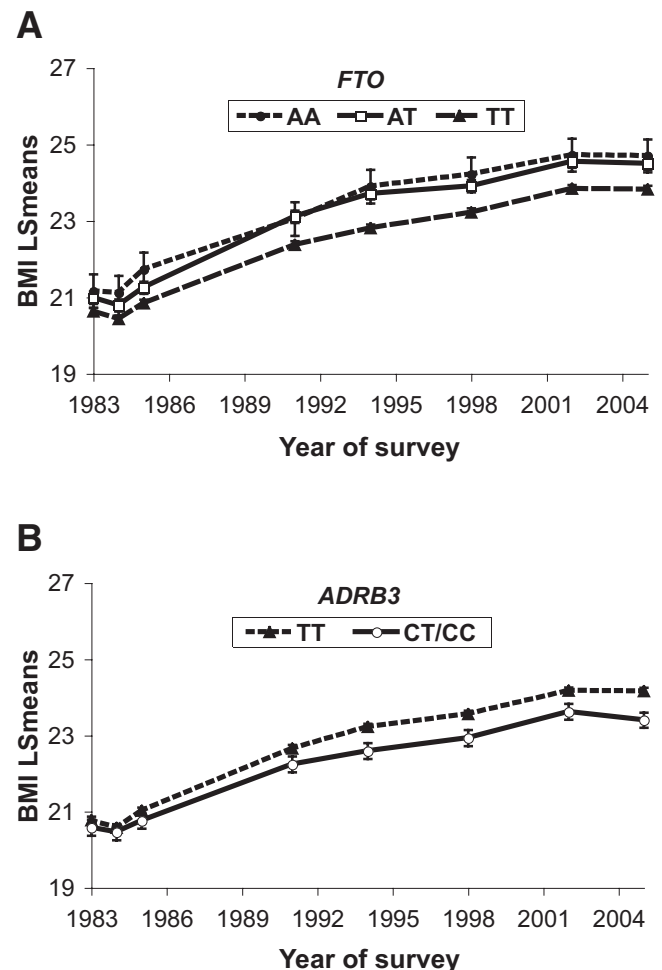


FIG. 1. Longitudinal analysis of BMI using measurements across eight surveys from 1983–1984 to 2005 of *FTO* rs9939609 ($P = 0.000029$) (A) and *ADRB3* rs4994 ($P = 0.016$) (B). BMI is reported as the least-squares means (LSmeans) at each time point. Error bars represent ± 1 SE.

with over 35 subgroups each, one in the Japanese population and one in multiple populations, reported that Arg64 carriers exhibited higher mean BMI than Trp64 homozygotes (15,16). The evidence of opposite alleles associated with increased trait values across studies suggests that these results should be interpreted with caution.

The *FTO* rs9939609 A allele was also associated with several obesity-related traits including longitudinal BMI, reflecting a relatively constant genotype effect over 22 years and strengthening the evidence that this locus influences BMI in this population. We observed evidence for an association with waist circumference but not with skinfold thicknesses, which are surrogate measures for subcutaneous adiposity, consistent with variation in *FTO* influencing central adiposity to a greater extent than subcutaneous fat. These results may be due to our limited power of 47% to detect a change of 1.1 mm between homozygotes, the effect for triceps skinfold previously observed (8).

Recently, two studies reported results that did not replicate association between rs9939609 and obesity in samples of Japanese and Han Chinese. The authors suggested that the findings could be due to relatively low variability in BMI and/or a decreased allele frequency in Asian populations resulting in low power to detect an effect (17,18). The MAF of the rs9939609 variant is 0.18 in the CLHNS sample, less frequent than estimates in European populations (MAF 0.45–0.48) and similar to that in the Han Chinese (MAF 0.11) and Japanese (MAF 0.22) samples (17,18). The discrepancy in results could be due to sampling variability or other differences across studies. Both the Han Chinese and Japanese studies included both men and women, and the mean age was greater, by ~10 and 18 years, respectively, than for the current sample. In addition, the Japanese study was based on a case-control sample for type 2 diabetes.

While the women in this sample were chosen from a single geographic region, we cannot exclude the possibility that our results are influenced by population stratification, as ancestry for this population may include contributions from Polynesia, China and, to a lesser extent, Spain. At the time of this study, sufficient genotype data were not available to fully evaluate such substructure, although we have observed that allele frequencies and linkage disequilibrium patterns in the CLHNS are similar to those for the HapMap Han Chinese samples (19).

In summary, our results corroborate previous reports that a SNP within the first intron of *FTO* is associated with BMI. The *FTO* SNPs have the most consistent prior evidence for association with obesity-related traits reported to date, and our study replicates this evidence, both in direction and approximate magnitude, in a Filipino population, suggesting that *FTO* may be important in many genetic backgrounds.

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