FTO Genotype Is Associated With Exercise Training–induced Changes in Body Composition

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The fat mass (FM) and obesity-associated (FTO) gene is the first obesity-susceptibility gene identified by genome-wide association scans and confirmed in several follow-up studies. Homozygotes for the risk allele (A/A) have 1.67 times greater risk of obesity than those who do not have the allele. However, it is not known whether regular exercise-induced changes in body composition are influenced by the FTO genotype. The purpose of our study was to test whether the FTO genotype is associated with exercise-induced changes in adiposity. Body composition was derived from underwater weighing before and after a 20-week endurance training program in 481 previously sedentary white subjects of the HERITAGE Family Study. FTO single-nucleotide polymorphism (SNP) rs8050136 was genotyped using Illumina GoldenGate assay. In the sedentary state, the A/A homozygotes were significantly heavier and fatter than the heterozygotes and the C/C homozygotes in men ($P = 0.004$) but not in women ($P = 0.331$; gene-by-sex interaction $P = 0.0053$). The FTO genotype was associated with body fat responses to regular exercise ($P < 0.005$; adjusted for age, sex, and baseline value of response trait): carriers of the C allele showed three times greater FM and %body fat losses than the A/A homozygotes. The FTO genotype explained 2% of the variance in adiposity changes. Our data suggest that the FTO obesity-susceptibility genotype influences the body fat responses to regular exercise. Resistance to exercise-induced reduction in total adiposity may represent one mechanism by which the FTO A allele promotes overweight and obesity.

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INTRODUCTION

The prevalence of obesity and its comorbidities has increased during the past three decades and excess body weight is now a major public health problem. The role of genetic factors in the etiology of obesity has been recognized for a long time, but the identification of genes and mutations contributing to body weight gain over time has been slow. The development of microarray-based high-throughput single-nucleotide polymorphism (SNP) genotyping technology has made genome-wide association studies (GWAS) feasible.

A first high-density genome-wide association studies identified the fat mass (FM) and obesity-associated (*FTO*) gene as a strong candidate for obesity-related phenotypes in whites (1–3). Minor alleles in a cluster of SNPs located in the first intron of the gene (e.g., rs9939609 and rs8050136, which are in complete linkage disquilibrium) were associated with ~65% greater risk of obesity in the homozygote state: the homozygotes of the minor allele and heterozygotes had 3–4 and 1–2 kg greater body weight, respectively, than the common allele homozygotes. It has been estimated that the population attributable risk of FTO for obesity is as high as 20%. The initial finding has been replicated in several large cohorts and the association holds both in children and in adults (4). However, the associations show some ethnic differences, being strongest in whites, moderate in Asians, and absent in African Americans.

Given the high prevalence of obesity, a clinically important question with major public health relevance is how the FTO genotype affects prevention of weight gain and treatment of obesity. It has been reported that the association between DNA sequence variation in the FTO locus and obesity is particularly strong in sedentary subjects but not in individuals who are physically active (5,6). These reports suggest that physical activity status may modify the effect of FTO genotype on body weight. However, these data are based on observational studies and there are no data on whether the FTO genotype affects exercise-induced changes in body weight and body composition. The purpose of this study was to test the hypothesis that the obesity-risk allele of the *FTO* gene is associated with

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resistance to lose body fat in response to 20 weeks of exercise in previously sedentary but healthy subjects.

METHODS AND PROCEDURES

Subjects

The overall goal of the HERITAGE Family Study is to investigate the genetic basis of adaptation to exercise training and of concomitant changes in cardiovascular disease and diabetes risk factors. The baseline cohort consists of 503 white subjects from 99 nuclear families and 276 black subjects from 114 family units. Of the white subjects, 481 (233 men, 248 women) completed the 20-week exercise intervention (completed at least 58 of prescribed 60 training sessions), whereas the corresponding numbers in black participants were 259, 88, and 171, respectively. The study design and inclusion criteria have been described previously (7). To be eligible, the individuals were required to be in good health, i.e., free of diabetes, cardiovascular diseases, or other chronic diseases that would prevent their participation in an exercise training program. Subjects were also required to be sedentary, defined as not having engaged in regular physical activity over the previous 6 months. Individuals with resting systolic blood pressure >159 mm Hg and/or diastolic blood pressure >99 mm Hg were excluded. Other exclusion criteria are fully described in a previous publication (7). The prevalence of overweight and obesity were 31.2 and 32.4% in blacks and 30.8 and 19.3 % in whites, respectively. The study protocol had been approved by each of the institutional review boards of the HERITAGE Family Study research consortium. Written informed consent was obtained from each participant.

Exercise program

The exercise intensity of the 20-week program was customized for each participant based on the heart rate (HR)–VO_2 relationship measured at baseline (8). During the first 2 weeks, the subjects exercised at a HR corresponding to 55% of the baseline VO_{2max} for 30 min per session. Duration and intensity of the sessions were gradually increased to 50 min and 75% of the HR associated with baseline VO_{2max} that were then sustained for the last 6 weeks. Frequency of sessions was three times per week, and all exercise was performed on cycle ergometers in the laboratory. HR was monitored during all training sessions by a computerized cycle ergometer system (Universal FitNet System, Cedar Rapids, IA), which adjusted ergometer resistance to maintain the target HR. Trained exercise specialists supervised all exercise sessions.

Body composition

Stature was measured to the nearest 0.1 cm with the subject standing erect on a flat surface, heels, buttocks, and back pressed against the stadiometer, and the head positioned in the Frankfort horizontal plane. Body mass was recorded to the nearest 100 g using a balance scale with subjects clothed only in a light-weight bathing suit. BMI was calculated by dividing body mass (kg) by stature squared (m^2). Body density was assessed by underwater weighing (9). Pulmonary residual volume was measured using the oxygen dilution technique (10) in the Indiana, Minnesota, and Texas clinical centers and by the helium dilution method (11,12) in the Québec clinical center. Body density was converted to percent body fat using the equations of Siri (13) for white men, Lohman (14) for white women, Schutte *et al.* (15) for black men, and Ortiz *et al.* (16) for black women. Reproducibility of the body density, FM, and pulmonary residual volume assessments were very high with intraclass correlations for repeated measures ranging between 0.97 and 1.00 without significant differences between the four clinical centers (17).

Genotype determinations

Genomic DNA was prepared from permanent lymphoblastoid cells by commercial DNA extraction kit (Gentra Systems, Minneapolis, MN). The FTO rs8050136 SNP was genotyped using the Illumina (San Diego, CA) GoldenGate chemistry and Sentrix Array Matrix technology on the BeadStation 500GX. Genotype calling was done with the Illumina BeadStudio software and each call was confirmed manually. For quality control purposes, five CEPH control DNA samples (NA10851, NA10854, NA10857, NA10860, and NA10861; all samples were included in the HapMap Caucasian panel) were genotyped in duplicate. Concordance between the replicates as well as with the rs8050136 genotypes from the HapMap database was 100%.

Statistical analyses

A χ^2-test was used to verify whether the observed genotype frequencies were in Hardy–Weinberg equilibrium. Associations between the FTO SNP and body composition phenotypes were analyzed using a variance components and likelihood ratio test–based procedure in the QTDT software package (available at http://www.sph.umich.edu/csg/abecasis/QTDT). The total association model of the QTDT software utilizes a variance components framework to combine phenotypic means model and the estimates of additive genetic, residual genetic, and residual environmental variances from a variance–covariance matrix into a single likelihood model (18). The evidence of association is evaluated by maximizing the likelihoods under two conditions: the null hypothesis (L_0) restricts the additive genetic effect of the marker locus to zero ($b_a = 0$), whereas the alternative hypothesis does not impose any restrictions on b_a. The quantity of twice the difference of the log likelihoods between the alternative and the null hypotheses ($2[\ln(L_1) - \ln(L_0)]$) is distributed as χ^2 with 1 df (difference in number of parameters estimated). Baseline body composition phenotypes were adjusted for age and sex, whereas body composition phenotype changes with exercise were adjusted for age, sex, and baseline value of the response phenotype.

RESULTS

Baseline characteristics of the subjects are summarized in Table 1. The effects of the exercise program on body composition have been presented in detail elsewhere (19). Briefly, FM and percent body fat decreased significantly with training, whereas fat-free mass increased. Frequencies of the rs8050136 A allele and the A/A genotype were 0.38 and 0.16 in whites

Table 1 Baseline characteristics of the subjects

	Blacks			Whites		
	All	Men	Women	All	Men	Women
N	276	91	185	503	245	258
Age	33.6 (11.7)	34.6 (12.4)	33.2 (11.4)	35.8 (14.6)	36.6 (15.0)	35.0 (14.1)
BMI	27.9 (6.0)	27.3 (5.2)	28.2 (6.3)	25.8 (5.0)	26.7 (4.9)	25.0 (4.9)
Fat mass	25.3 (12.3)	21.0 (11.0)	27.9 (12.4)	20.6 (10.9)	20.2 (10.9)	21.1 (10.8)
Fat %	31.2 (10.4)	23.3 (7.6)	35.9 (8.8)	26.6 (10.1)	22.9 (9.0)	30.1 (9.8)
Fat-free mass	53.1 (11.4)	64.4 (9.3)	46.3 (5.7)	54.2 (11.2)	63.6 (7.8)	45.5 (5.1)

Table 2 Associations between the FTO rs8050136 genotype and BMI and body composition phenotypes in whites of the HERITAGE Family Study

	A/A	A/C	C/C	*P* value (total)	*P* value (TDT)
Baseline, *n*	78	225	200		
BMI, kg/m^2	26.9 (0.6)	25.9 (0.3)	25.4 (4.5)	0.086	0.007
Fat mass, kg,	22.9 (1.2)	20.5 (0.8)	19.9 (0.8)	0.242	0.039
Fat %	28.1 (1.1)	26.5 (0.7)	26.2 (0.7)	0.647	0.094
Fat-free mass, kg	55.5 (1.3)	54.2 (0.8)	53.6 (0.8)	0.026	0.144
Change with exercise, *n*	74	215	192		
BMI, kg/m^2	+0.3 (0.2)	–0.4 (0.1)	–0.2 (0.2)	0.062	0.0035
Fat mass, kg	–0.2 (0.2)	–0.8 (0.1)	–0.8 (0.1)	0.026	0.0065
Fat %	–0.3 (0.2)	–0.9 (0.1)	–1.0 (0.1)	0.026	0.0087
Fat-free mass, kg	+0.5 (0.1)	+0.4 (0.1)	+0.6 (0.1)	0.92	0.225

Table 3 Associations between the FTO rs8050136 genotype and BMI and body composition phenotypes in blacks of the HERITAGE Family Study

	A/A	A/C	C/C	*P* value (total)	*P* value (TDT)
Baseline, *n*	55	141	80		
BMI, kg/m^2	27.3 (0.8)	28.0 (0.5)	28.2 (0.7)	0.417	0.806
Fat mass, kg,	24.5 (1.8)	25.3 (1.1)	25.9 (1.6)	0.411	0.806
Fat %	31.5 (1.4)	30.8 (0.9)	31.6 (1.4)	0.396	0.624
Fat-free mass, kg	50.4 (1.7)	54.4 (1.0)	52.6 (1.4)	0.841	0.699
Change with exercise, *n*	50	132	77		
BMI, kg/m^2	–0.1 (0.1)	–0.2 (0.3)	–0.2 (0.1)	0.671	0.729
Fat mass, kg	–0.8 (0.3)	–0.9 (0.2)	–0.8 (0.3)	0.647	0.156
Fat %	–0.9 (0.3)	–0.8 (0.2)	–0.7 (0.2)	0.442	0.085
Fat-free mass, kg	+0.4 (0.2)	+0.3 (0.1)	+0.3 (0.2)	0.647	0.229

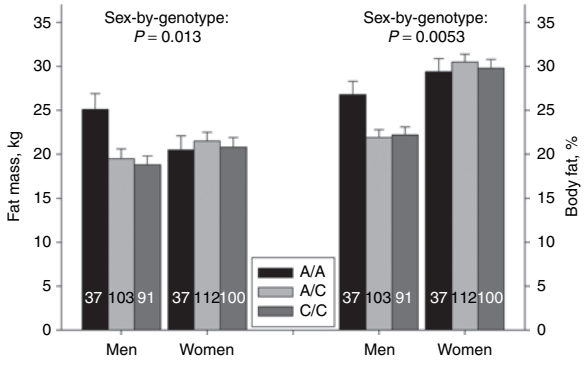

Figure 1 Associations between FTO rs8050136 genotype and baseline fat mass (left) and percent body fat (right) stratified by sex.

and 0.45 and 0.20 in blacks, respectively. Genotype frequencies were in Hardy–Weinberg equilibrium.

In agreement with previous studies, the A/A homozygotes tended to be heavier than the C/C homozygotes, and the transmission disequilibrium test analysis indicated that the A allele was transmitted to heavier offspring more frequently than would be expected by chance in whites (Table 2). We also observed significant sex-by-genotype interactions for fatness: higher level of FM and percent body fat associated with the A/A genotype was evident in males but not in females (Figure 1).

The A/A homozygotes had a blunted adiposity response to the exercise program (Table 2). Heterozygotes and C/C homozygotes showed on average a 4% decrease in FM, whereas the corresponding change in the A/A homozygotes was only –0.1% of baseline FM (Table 2). There were no differences between genotypes in exercise-induced changes in fat-free mass. Also, there was no evidence of sex-by-genotype interactions on body composition changes with the exercise program, i.e., associations between the *FTO* genotype and exercise-induced changes in adiposity did not differ between men and women. In blacks, the FTO genotype was not associated with baseline body composition or with exercise training–induced changes in body composition (Table 3).

DISCUSSION

The association between *FTO* gene and obesity has been confirmed in several studies in white subjects but data on FTO and changes in adiposity in response to regular physical activity have been missing. The main finding of our study is that homozygotes for the obesity-risk allele in the *FTO* gene locus are resistant to exercise-induced changes in adiposity. After 20

weeks of strictly supervised exercise with full compliance, the common allele homozygotes (C/C) lost ~4% of their FM, whereas the change in the A/A homozygotes was only 0.1%.

Previous observational studies have reported evidence of genotype-by-physical activity interactions on obesity, showing that the association between FTO genotype and obesity is strong in sedentary subjects but not among physically active individuals (5,6). These observations could be interpreted to suggest that the obesity-risk allele homozygotes might benefit more from physical activity than the common allele carriers. This hypothesis seems to be in conflict with our findings, but a closer inspection shows that it most likely reflects differences in study designs. First, it is possible that observational studies and exercise intervention trials reflect different aspects of the relationship between physical activity and regulation of body weight and adiposity: observational studies may reflect the role of regular physical activity in the prevention of weight gain, whereas intervention studies test the role of regular exercise in the loss of FM. Second, level of physical activity in cross-sectional, observational studies usually reflects fairly long-term (usually years) activity behavior pattern, whereas the HERITAGE Family Study used a 20-week exercise intervention program in previously sedentary individuals. Third, the amount of activity among the physically active subjects in observational studies is considerably higher than in our study. For example, in Old Order Amish, the average activity level was ~4 h/day (6), whereas the HERITAGE participants exercised for 60 min per session three times per week during the last 6 weeks of the 20-week program. It is possible that weight loss among the FTO risk allele carriers may require higher volume of physical activity over long periods of time. Considering the high frequency of the risk allele, this is an important issue with clinical and public health relevance that needs to be tested in controlled clinical trials.

When FTO was identified as a risk gene for obesity, its function was completely unknown. Recently, it was reported that FTO encodes a 2-oxoglutarate-dependent nucleic acid demethylase, a member of the nonheme dioxygenase superfamily (20,21). It is expressed in several peripheral tissues as well as in brain regions affecting energy balance (20,22). Fasting and feeding modify FTO expression in the arcuate nucleus, although the effects seem to be in opposite directions in mice and rats (22,23). It has been reported that in humans, the FTO obesity-risk allele is associated with lower lipolytic activity in adipose tissue (24). There are no data available at the moment on the effects of exercise on FTO expression and function. Therefore, more studies are needed to understand how FTO may modulate the effects of regular physical activity on body composition. Likewise, little is known about functional properties of the obesity-associated SNPs located in intron 1 of the *FTO* gene. However, recent report indicated that two SNPs are located in binding sites for Cut-like 1 (CUTL1), a potent regulator of gene expression, and one of them, rs8050136, seems to affect CUTL1 binding preference: sequence with the rs8050136 A allele shows greater binding preference by CUTL1 than sequence with the C allele (22).

Our study has several strengths that increase the confidence in our findings. First, the exercise program was designed to provide the same exercise stimulus in terms of frequency, duration, and relative intensity to all subjects. Furthermore, all exercise sessions were performed in laboratory under supervision. These facts together with the full compliance guarantee that interindividual variation in body composition changes cannot be due to variation in exercise dose. Second, the duration of the intervention (20 weeks) was sufficient to produce physiologically meaningful adaptations to regular exercise. Third, all HERITAGE subjects were sedentary and free of chronic diseases at baseline. Therefore, potential confounding due to differences in baseline activity levels, chronic diseases, or medication use was eliminated. Fourth, body composition was measured using underwater weighing technique, the gold standard method at the time of data collection.

Even with these strengths, several questions remain regarding the role of *FTO* genotype in modulation of physical activity–body composition relationship. First, it remains to be tested how the FTO obesity-risk allele carriers respond when an exercise program is implemented together with an energy-restricted diet. Second, future studies should explore whether larger exercise volume could result in greater weight loss also in the FTO risk allele homozygotes. Finally, the *FTO* genotype-by-physical activity interaction in the prevention of unhealthy weight gain should be further addressed. Data from two observational cross-sectional studies suggest that the risk associated with the *FTO* genotype is considerably lower in physically active than in sedentary individuals. We need to understand whether regular physical activity is particularly important in the FTO risk allele carriers to counteract their greater propensity to obesity.

In summary, our data suggest that the obesity-risk allele of the *FTO* gene locus is associated with a blunted body fatness response to regular exercise in previously sedentary but healthy whites. It remains to be tested whether a greater exercise volume either by itself or in combination with a low-calorie diet would induce larger weight losses in carriers of the risk allele.

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DISCLOSURE

The authors declared no conflict of interest.

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