

FTO Gene Variant Is Associated with Obesity: Longitudinal Analyses in Two Cohort Studies and Functional Test

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ABSTRACT

Objective: To examine the longitudinal association of *FTO* variant with obesity, circulating adipokine levels, and *FTO* expression in various materials from human and mouse.

Research Design and Methods: We genotyped rs9939609 in 2,287 men and 3,520 women from two prospective cohorts. Plasma adiponectin and leptin were measured in a subset of diabetic men (n=854) and women (n=987). Expression of *FTO* was tested in adipocytes from db/db mice and mouse macrophages.

Results: We observed a trend toward decreasing associations between rs9939609 and BMI at older age (≥ 65 y) in men, whereas the associations were constant across different age groups in women. In addition, SNP rs9939609 was associated with lower plasma adiponectin [log(e)-means: 1.82 ± 0.04 , 1.73 ± 0.03 , and 1.68 ± 0.05 for TT, TA, and AA genotypes; P for trend=0.02] and leptin (log(e)- means: 3.56 ± 0.04 , 3.63 ± 0.04 , and 3.70 ± 0.06 ; P for trend=0.06] in diabetic women. Adjustment for BMI attenuated the associations. *FTO* gene was universally expressed in human and mice tissues, including adipocytes. In an ancillary study of adipocytes from db/db mice, *FTO* expression was ~50% lower than those from wild-type mice.

Conclusions: The association between *FTO* SNP rs9939609 and obesity risk may decline at older age. The variant affects circulating adiponectin and leptin levels through the changes in BMI. In addition, the expression of *FTO* gene was reduced in adipocytes from db/db mice.

In a recent genome-wide association study (1), Frayling et al. identified a common variant in *FTO* (fat mass and obesity associated) gene (rs9939609) that was related to higher body mass index (BMI) in both children and adults. In addition, adiposity appeared to mediate the association between *FTO* variant and the risk of type 2 diabetes (2; 3). Several other studies have also observed associations between *FTO* variants and obesity related traits in various populations (4-13).

Because most available data are cross-sectional, the longitudinal pattern of the associations between *FTO* variants and adiposity and age-specific genetic effects are not clearly defined. The primary aim of the present study is to address these issues by assessing the genetic effects in two prospective cohorts. Obesity status affects the endocrine function of adipose tissue by altering the secretion of adipokines such as adiponectin and leptin, which have been related to ectopic fat accumulation, insulin sensitivity, and diabetes risk in epidemiological studies (14-16). We therefore examined the associations of *FTO* variant with circulating levels of adiponectin and leptin. In addition to the association analyses, to shed light on its potential functions, we also examined the expression of *FTO* gene in various tissues from humans and mice and investigated the expression in adipocytes from db/db mice and mice macrophages in response to inflammatory stimulants.

MATERIALS AND METHODS

Study population. The Nurses' Health Study (NHS) was established in 1976 when 121,700 female registered nurses aged 30–55 years and residing in 11 large U.S. states completed a mailed questionnaire on their medical history and lifestyle (17). 32,826 women provided blood samples between 1989 and 1990. The Health Professional Follow-up Study (HPFS) is a prospective cohort study of 51,529 U.S.

male health professionals aged 40 to 75 years at study initiation in 1986 (18). Between 1993 and 1999, 18,159 men provided blood samples. Information about health and disease is assessed biennially by self-administered questionnaires in both cohorts.

Diabetes cases were defined as self-reported diabetes confirmed by a validated supplementary questionnaire. For cases occurring before 1998, the diagnosis was made using criteria proposed by the National Diabetes Data Group (NDDG) (19). Medical record review confirmed 98% of the diagnosis. We used the American Diabetes Association diagnostic criteria for diagnosis after the 1998 cycles (20). Subjects for the present study were selected from those who provided blood samples and were free from cardiovascular disease or cancer at baseline. Healthy controls were matched on age and the time of blood drawing with diabetic patients. To reduce potential bias due to population stratification, we included only Caucasians of European ancestry. In total, 1,506 female and 1,076 male diabetic patients, and 2,014 female and 1,211 male non-diabetic controls were included.

Assessment of Adiposity. At baseline (1976 for the NHS and 1986 for the HPFS), participants were asked to report their height and current body weight; the self-reported weight was then updated every two years during the follow-up through 2002 (1976, 1978, 1980, 1982, 1986, 1988, 1990, 1992, 1994, 1996, 1998, 2000, 2002 in the NHS; and 1986, 1988, 1990, 1992, 1994, 1996, 1998, 2000, 2002 in the HPFS) using self-administered questionnaires. To assess the adiposity in early adulthood, the 1980 NHS questionnaire asked about weight at 18 years of age (N=3337) and the 1986 HPFS questionnaire asked about weight at 21 years of age (N=2194). We calculated BMI as weight in kilograms divided by height squared in meters. In 1986-1987, participants in the NHS (N=2333) and HPFS (N=1898)

reported direct measurements of their waists (at the umbilicus) and hips (at the largest circumference) to the nearest quarter of an inch, using a paper tape and detailed measuring directions. The validity of self reported adiposity measures were assessed in a random sample living in the greater Boston area, with high correlation with measured weight ($r \geq 0.96$) and waist ($r = 0.95$) (21; 22). We defined obesity as $BMI \geq 30 \text{ kg/m}^2$.

Assessment of Biomarkers. Blood samples were collected between 1989 and 1990 in NHS and between 1993 and 1999 in HPFS, as previously described (23; 24). Biomarkers were measured in a subset of diabetic men ($n=854$) and women ($n=987$). Plasma adiponectin concentration was measured by competitive radioimmunoassay (Linco Research, St. Charles, MO) with a coefficient of variation of 3.4%. Leptin was assayed by RIA (Linco Research, Inc., St Charles, MO, USA) with intra-assay coefficient of variation of 3.4–8.3%. HbA_{1c} were determined based on turbidimetric immunoinhibition using haemolysed whole blood or packed red cells. The day-to-day variability at HbA_{1c} concentrations of 5.5 and 9.1% was 1.9 and 3.0% respectively.

SNP Selection and Genotype Determination. To date, five SNPs in *FTO* genes have been reported to be associated with obesity traits. These SNPs are in strong to perfect LD. We therefore select one SNP rs9939609, which was found by the first GWA study, as a proxy for other SNPs (pair-wise r^2 with rs9939609: rs17817449, 1.00; rs1421085, 0.97; rs3751812, 1.00, and rs9930506, 0.84; HapMap, CEPH). DNA was extracted from the buffy coat fraction of centrifuged blood using the QIAmp Blood Kit (Qiagen, Chatsworth, CA). The SNP was genotyped using Taqman SNP allelic discrimination by means of an ABI 7900HT (Applied Biosystems, Foster City, CA). The internal quality of genotype data was assessed by typing 10% blinded samples in duplicate;

resulting concordance was $>99\%$. The call rate was higher than 95% and genotype distribution was in Hardy-Weinberg equilibrium (Chi-square test).

Real-time reverse transcription polymerase chain reaction (RT-PCR) and analyses of experimental data. Mouse tissues were collected from wild type ($n=3$; C57BL/6 strain mice obtained from the Jackson laboratory) and db/db mice ($n=3$; 3 months old male), a genetic model of obesity and type 2 diabetes. Mouse 3T3-L1 adipocytes were differentiated in media containing insulin, dexamethasone and isobutylmethylxanthine for 8 days. Human adipocytes were differentiated from pre-adipocytes (obtained from a cell line maintained by Cambrex Corporation) in a similar manner. Mouse macrophages were generated from bone marrow of wild type mice as described (25). Macrophages were cultured in Dulbecco's modification of Eagle's medium (DMEM), 10% fetal bovine serum (FBS) and treated with Interferon γ at a dose of 2 ng/ml overnight. Lipopolysaccharide (10 ng/ml) was then added for an additional 8 hours. RNA was isolated using TRIzol (Invitrogen) and reverse transcribed with the Quantitect RT kit (Qiagen). Human tissues (Brain, heart lung, liver, spleen, intestine, kidney, muscle, leukocytes, preadipocytes, and adipocytes) cDNAs were purchased from Clontech (multiple choice cDNA). SYBR green based real-time PCR reactions were conducted using RealMasterMix (Eppendorf) and detected by the 7300 Real-Time PCR system (Applied Biosystems). The expression of the 36B4 gene, which is a housekeeping gene to serve as a control, was used for normalization to obtain relative expression levels. The slope of efficiency curves for human *FTO* gene is -3.4 and for mouse is -3.6 (-4 means PCR product doubles each cycle). Oligo sequences used were as follows: human *FTO*: 5'-TTAGTTCCACCCACCGAGT-3' and 5'-ACATTCTGCAGAGCCAAGT-3'; mouse

FTO: 5'-ATCACGATGAGAACCTGGTG-3'
and 5'-CCAACATGCCAAGTATCAGG-3';
36B4: 5'-AGATGCAGCAGATCCGCAT-3'
and 5'-GTTCTTGCCCATCAGCACCC-3'.

For gene expression analyses, values were presented as means \pm SEM (n=3) and group means were compared using Student's t-Test. The SAS statistical package was used for the analyses (SAS, Version 8.2 for UNIX). Statistical significance was set at the .05 level, and all tests were 2-tailed.

Statistical analyses. Similar associations between *FTO* SNP rs9939606 and BMI were previously reported in diabetic patients and controls (1) and were also observed in our cohorts. Thus, we pooled diabetics and non-diabetics in the analyses. The geometric means of BMI and waist circumference were compared among the genotypes using general linear models, adjusting for age and diabetes status. In the multivariable analyses, we also adjusted for physical activity (quintiles), smoking (never, past, and current), alcohol intake (nondrinker and drinker [0.1–4.9, 5–10, or >10 g/day]), family history of diabetes (yes/no), and menopausal status [pre- or postmenopausal (never, past, or current hormone use); women only]. Crude associations between genotype and binary outcomes (obesity, diabetes) were tested using Pearson's chi-square statistic, and unconditional logistic regression was used to test for association after adjusting for covariates. General linear models were used to compare the genotype difference in biomarkers (adiponectin and leptin) among diabetic patients, adjusting for age, BMI, physical activity, smoking, alcohol intake, duration of diabetes, HbA(1c), and menopausal status (women only). Plasma adiponectin and leptin levels were logarithmically transformed to achieve a normal distribution. The geometric means of the back-transformed values were presented.

We employed Generalized Estimating Equations (GEE) to analyze the associations between the genotypes and longitudinal BMI values (26; 27). The analysis used the biennially collected repeated measurements of BMI from 1976 to 2002 in the NHS and from 1986 to 2002 in the HPFS. We used exchangeable correlation structure to account for the correlation of the repeated measures. Interaction between genotype and age was tested by creating a product term of the two variables in the model.

RESULTS

Table 1 shows the baseline characteristics of the participants by gender and diabetes status. The frequency of rs9939609 allele A in the study populations was 0.44, similar to the HapMap population frequency of 0.45 in the CEPH (Centre d'Etude du Polymorphisme Humain) and other European populations (1; 4).

Associations with Adiposity at Early and Middle Adulthood. **Table 2** shows the associations of SNP rs9939609 with BMI (N=2236 and 3483 in men and women, respectively) and waist circumference (N=1898 and 2333 in men and women, respectively) in 1986/1987, when both measures were available in the NHS and HPFS, and with BMI at early adulthood (N=2194 and 3337 in men and women, respectively). The SNP was associated with higher BMI and waist circumference in women, under an additive inheritance model. In men, rs9939609 was associated with a higher BMI. SNP rs9939609 was also associated with higher BMI at early adulthood in men and women (**Table 2**). Although the genetic effects in men seemed not to fit well with the additive model, testing for the departure from the additive model was not significant. Because smoking may affect adiposity, we excluded current smokers in a sensitivity analyses but did not find appreciable changes in the associations.

We further assessed the relation between rs9939609 and obesity risk (in 1986/1987). The AA homozygotes were associated with 1.75 (1.26-2.45) and 1.71 (1.36-2.16) times higher obesity risk in men and women, respectively (**Figure 1**). Because few people were obese (2.6% in men 3.4% in women) at early adulthood, we examined the associations between *FTO* genotypes and the risk of overweight (BMI \geq 25kg/m²) instead. In both men and women, the AA homozygotes was associated with 1.57 (1.22-2.03) and 1.84 (1.40-2.40) times higher overweight risk in men and women at early adulthood (**Figure 1**).

Associations with Longitudinal Measurements of Adiposity. rs9939609 was associated with higher BMI during the 26 years of follow-up in women (1976-2002) and was associated with BMI at nearly all time points during 16 years of follow-up in men (1986-2002) except in 1988 and 1998 (**Appendix Figure 1**). Similarly, the AA genotype was associated with greater obesity risk during the follow-up in women and men (except at 1992, 1996, and 2000 in men) (**Appendix Figure 2**).

In longitudinal analyses using GEE, associations were observed between rs9939609 and BMI and the obesity risk in both genders (**Table 3**). Each allele A was associated with 0.25 (standard error, SE=0.11; P=0.03) and 0.59 (SE=0.13; P<0.0001) kg/m² higher BMI, as well as 1.21 (1.05-1.38)- (P=0.007) and 1.19 (1.07-1.31)-fold (P=0.0008) higher risk of obesity in men and women, respectively. We further examined whether the associations between SNP rs9939609 and adiposity were consistent across different age groups (**Figures 2 and 3**). It appears that the association between the SNP and obesity risk declined with older age especially in men, although tests for interaction between genotypes and age were not significant (P=0.20 for men and 0.08 for women).

Case-Control Studies on Type 2 Diabetes. In the crude analyses, the TA and AA genotypes were associated with respectively a 1.17 (1.01-1.36)- and 1.24 (1.02-1.50)-fold higher risk of type 2 diabetes compared with the TT genotype in women (P for trend=0.019) (**Table 4**). The associations between *FTO* genotypes and diabetes risk became non-significant when BMI was adjusted for. However, adjusting for other diabetes risk factors such as age, smoking, alcohol consumption, and physical activity did not alter the association for diabetes. This SNP was not associated with diabetes risk in men.

Associations with Adipokine Concentrations in Patients with Type 2 Diabetes. We further assessed whether *FTO* SNP rs9939609 was associated with adipose-secreted adipokines. We measured leptin and adiponectin in a subset of patients with type 2 diabetes (987 women and 854 men). In women, the *FTO* genotypes were associated with lower plasma adiponectin levels [log(10)-transformed means: 1.82 \pm 0.04, 1.73 \pm 0.03, and 1.68 \pm 0.05 for TT, TA, and AA genotypes; P=0.02], adjusting for age (**Figure 4**). The association remained when smoking, alcohol consumption, physical activity, duration of diabetes, HbA_{1c}, and menopausal status were adjusted for. Further adjustment for BMI attenuated the association. Allele A tended to be associated with higher leptin levels, with borderline significance. The genotype-associated difference in leptin disappeared when BMI was adjusted for. In men, we did not find associations between *FTO* genotypes and adipokine levels.

***FTO* Gene Expression Profile and in Tissues/Cells with High Metabolic Capacities.** To shed light on its potential role in adiposity, we conducted expression profiling of the *FTO* gene in human and mouse tissues and found similar expression patterns between the two species (**Appendix Figure 3**). *FTO* was present at high levels in several metabolically active tissues (from

humans and wild type C56BL/6 mice), including brain, heart, kidney and adipose tissue, with the highest expression in brain from humans and mice. In white adipose tissue isolated from db/db mice, *FTO* expression was reduced by ~50% as compared with lean wild type controls (**Figure 5a**). Interestingly, *FTO* is also expressed by macrophages and was induced by inflammatory stimulants, interferon γ (IFN γ) and lipopolysaccharide (LPS) (**Figure 5b**).

DISCUSSION

In this study, we confirmed that the SNP rs9939609 was associated with higher BMI and obesity risk (1) in men and women from two independent US cohorts. Frayling et al. observed that the *FTO* genotype-associated difference in BMI occurred as early as 7-years old (1). We observed associations between *FTO* SNP and high BMI in early adulthood. The genetic effects were constant across different age in women but tend to decrease at older age in men. The mechanisms underlying this observation are not clear. A longitudinal twin study suggests that the genetic components regulating body weight may vary throughout life (28) and decrease with advanced age (29; 30). Adiposity loss with age may partly explain the reduction in association at senior age. In addition, environmental influences accumulate with age and likely exert stronger influences on later BMI. It appears that the decreasing trend of BMI with advanced age is more pronounced in men than in women, but the test for interaction between gender and *FTO* was not statistically significant.

In women, rs9939609 was associated with greater risk of type 2 diabetes. Adjustment for BMI abolished the association. This finding is consistent with the observations from recent GWA studies (2; 3), supporting the idea that

obesity mediates the effects of *FTO* variant on the development of type 2 diabetes.

Adipose tissue acts as endocrine organ through secreting hormone-like adipokines, which mediate the effects of obesity on various metabolic disorders (31). Adiponectin is the most abundant adipokine in the circulation (32). Adiponectin improves insulin action and metabolism of glucose and lipids (33; 34). Low blood adiponectin levels have been related to increased risk of obesity and diabetes (35; 36). Our data indicated that low adiponectin levels associated with the risk allele A were secondary to the changes in BMI, and may partly mediate the genetic effects on diabetes risk. The association between *FTO* SNP and adiponectin was observed in diabetic patients. Future studies are warranted to replicate the findings in general population.

Little is known about the function of *FTO* gene. Our data comparing both human and mouse genes confirm high expression of *FTO* gene in brain (1). Gerken et al. recently demonstrated *FTO* was highly expressed in arcuate, paraventricular, dorsomedial, and ventromedial nuclei (37). Of note, all these sites are of critical importance in controlling energy balance. These data suggest *FTO* gene may affect the neuroregulation of energy balance. In addition, *FTO* expression was high in heart, kidney, and adipose tissues (particularly brown fat in mice).

The expression of *FTO* was moderately increased in adipocytes compared to pre-adipocytes and was substantially reduced in white adipose tissues of obese, diabetic db/db mice, indicating that it may play a role in adipocyte function but not adipogenesis. The mechanism underlying the reduced expression of *FTO* in adipocytes from the genetic models with direct interruptions of the leptin axis (db/db mice) is not clear. The data suggest *FTO* is likely a part of the pathway mediating the neuroregulation (e.g. leptin) of energy metabolism in adipose tissue, and blocking

leptin signal may inhibit the downstream changes in adipose tissue that induce the expression of *FTO*. Furthermore, *FTO* was found to be expressed in leukocytes and was drastically up-regulated in macrophages stimulated with IFN γ and LPS. Given that metabolic dysregulation is recognized as a state of chronic inflammation (38; 39), it is possible that *FTO* is a molecular link between metabolism and inflammation in the pathogenesis of obesity-related metabolic diseases.

SNP rs9939609 is highly correlated with many other SNPs within a 47kb region encompassing parts of the first two introns as well as exon 2 of *FTO* gene. Sequencing the chromosomes did not result in clear candidate functional variants in *FTO* coding region, minimal splice sites or 3' UTR (1). Further work is warranted to explore the genetic mechanism underpinning the observed associations.

Several limitations need to be acknowledged. Population stratification, mostly arising from either ethnic admixture or genetic substructure within an ethnically homogenous group, may cause spurious associations. However, neither is likely to explain the associations observed in the present study. Our study populations are highly homogeneous by including only European whites. In addition, there is no evidence showing regional difference in rs9939606 across European countries (1).

The participants in our study do not represent random samples of US men and women. However, major cohort studies, such as the Framingham Heart Study, have also not relied on national samples. Clearly, data validity is the chief prerequisite that must be fulfilled before the results can be generalized. In previous analyses, the observed genetic and environmental associations for obesity, type 2 diabetes, coronary heart disease and other

diseases in our cohorts are very similar to those found in other broadly-based US populations (40-44). Nonetheless, the findings in the present study need to be replicated in other ethnic groups.

Adiposity measures were self-reported in our cohorts. However, self-reported information has been reliably validated, with a high correlation with technician-measured variables (21; 22). In addition, previous analyses in NHS and HPFS have demonstrated that self-reported BMI and waist circumference strongly predicted various chronic diseases (40; 45; 46).

We examined only one SNP rs9939609, which was reported by the original GWA study (1). Several other SNPs were associated with obesity in subsequent studies (4; 5), but these SNPs are all in strong to perfect LD with rs9939609. Thus, genotyping of these SNPs would have been redundant.

In conclusion, we confirmed associations between the *FTO* SNP rs9939609 and higher obesity risk. Our data suggest that the genetic association for obesity may decline at older age. In addition, the genetic variant may affect circulating adiponectin levels through the changes in BMI. The expression of *FTO* gene was reduced in adipocytes from db/db mice. Little is known about the function of *FTO* gene, and thus further investigation is warranted to identify the causal genetic variants and potential mechanisms underlying the observed genetic associations

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Table 1. Baseline Characteristics of the Men and Women from the Health Professionals' Follow-up Study (HPFS) and Nurses' Health Study (NHS)

Characteristics	Men (HPFS)		Women (NHS)	
	Diabetic	Non-diabetic	Diabetic	Non-diabetic
N of Participants	1076	1211	1506	2014
Age, years, mean±SD	56±8	55±9	44±7	44±7
Physical Activity,* mean±SD	14.7±19.0	21.5±28.1	3.6±2.8	4.1±2.9
Alcohol Consumption, g/day, mean±SD	11.4±16.8	12.3±15.5	4.1±8.3	6.4±9.8
Smoking, %	11.0	7.0	28.9	21.8
Family History of Diabetes, %	32.3	12.6	52.5	22.7

Cohort baseline, 1976 for the NHS and 1986 for HPFS; physical activity and alcohol

consumption were obtained from the 1980 questionnaire in NHS;

*: In hours/week of physical activity of at least moderate intensity for women and in MET-hours/week for men

Table 2. Body mass index (BMI, in kg/m²) and waist circumference (in cm) according to rs9939609 genotypes

	Mean Measures by Genotypes			TA vs TT	P Values		P Values per allele
	TT	TA	AA		AA vs TA	AA vs TT	
Men, N=2287	34%*	48%*	18%*				
BMI, age 21, N=2194	23.1±3.0	23.1±3.0	23.8±3.2	0.94	0.0001	0.0002	0.0007
BMI, 1986							
All subjects, N=2236	26.2±3.7	26.3±3.6	26.7±4.0	0.45	0.05	0.01	0.02
Non-smoker, N=2042	26.2±3.8	26.3±3.5	26.7±4.0	0.58	0.18	0.09	0.09
Waist circumference, 1987							
All subjects, N=1898	97.7±10.4	98.0±10.0	98.4±10.8	0.44	0.55	0.23	0.12
Non-smoker, N=1740	97.6±10.4	97.9±9.8	98.3±10.8	0.39	0.68	0.28	0.14
Women, N=3520	34%*	46%*	20%*				
BMI, age 18, N=3337	21.7±3.3	21.9±3.5	22.5±3.7	0.06	0.0009	<0.0001	<0.0001
BMI, 1986							
All subjects, N=3483	26.8±5.6	27.4±5.9	28.2±6.1	0.008	0.0099	<0.0001	<0.0001
Non-smoker, N=2624	26.9±5.6	27.5±5.9	28.3±6.2	0.019	0.008	<0.0001	<0.0001
Waist circumference, 1986							
All subjects, N=2333	82.8±13.1	83.9±13.3	85.2±14.3	0.27	0.12	0.02	0.02
Non-smoker, N=2007	82.7±13.0	83.8±13.1	85.4±14.4	0.19	0.10	0.01	0.01

*: Overall percentage of each genotype;

Data are mean ± SD; Except for early adulthood (men, age 21; and women, age 18), analyses were adjusted for age and diabetes status.

Table 3. Generalized Estimating Equations Analyses on the Associations Between rs9939609 and Body Mass Index (BMI) and Obesity in Men (1986 to 2002) and Women (1976 to 2002)

	TT	TA	AA	Per allele, (95% Confidence Interval)	P Values
Men					
BMI	26.5±3.9	26.5±3.8	27.1±4.4	0.25 (0.02-0.47)	0.03
Obesity	1.0	1.13 (0.90-1.41)	1.47 (1.12-1.92)	1.21 (1.05-1.38)	0.007
Women					
BMI	26.9±5.6	27.5±6.0	28.3±6.3	0.59 (0.34-0.84)	<0.0001
Obesity	1.0	1.06 (0.90-1.24)	1.46 (1.20-1.77)	1.19 (1.07-1.31)	0.0008

The effects are presented as mean ± SD, or as odds ratio and 95% confidence intervals.

Table 4. Associations Between rs9939609 Genotypes and The Risk of Type 2 Diabetes in Men and Women

	Diabetic	Non-diabetic	Odds Ratio and 95% CI					
	(%)	(%)	Model 1	P	Model 2	P	Model 3	P
Men, N=2287								
TT	362 (33.6)	406 (33.5)	1.0				1.0	
TA	486 (45.2)	556 (45.9)	0.98 (0.81-1.18)	0.83	0.95 (0.78-1.17)	0.64	0.95 (0.78-1.16)	0.64
AA	228 (21.2)	249 (20.6)	1.03 (0.82-1.29)	0.82	0.94 (0.73-1.21)	0.63	0.99 (0.78-1.26)	0.94
Women, N=3520								
TT	484 (32.1)	726 (36.0)	1.0		1.0		1.0	
TA	739 (49.1)	944 (46.9)	1.17 (1.01-1.36)	0.036	1.08 (0.92-1.27)	0.33	1.21 (1.03-1.43)	0.019
AA	283 (18.8)	344 (17.1)	1.24 (1.02-1.50)	0.034	1.02 (0.83-1.26)	0.84	1.26 (1.03-1.56)	0.029

Model 1, crude analysis; model 2, adjusted for body mass index (BMI); model 3, adjusted for other covariates than BMI, including age, physical activity (quintiles), smoking (never, past, and current), alcohol intake (nondrinker and drinker [0.1–4.9, 5–10, or >10 g/day]), family history of diabetes, and menopausal status [pre- or postmenopausal (never, past, or current hormone use); for women only]

Figure 1

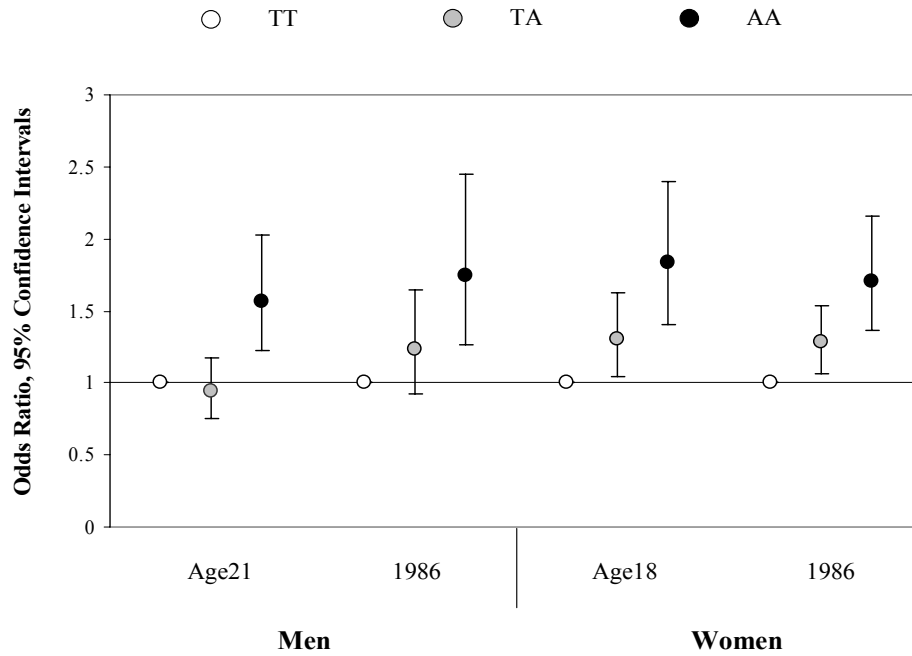


Figure 1. Odds ratios of overweight ($BMI \geq 25 \text{ kg/m}^2$) at early adulthood and obesity ($BMI \geq 30 \text{ kg/m}^2$) in 1986 associated with the TA and AA genotypes of the *FTO* SNP rs9939609 (T>A) compared with the AA genotype in men and women. Risk was adjusted for age and diabetes status. Error bars denote 95 percent confidence intervals.

Figure 2

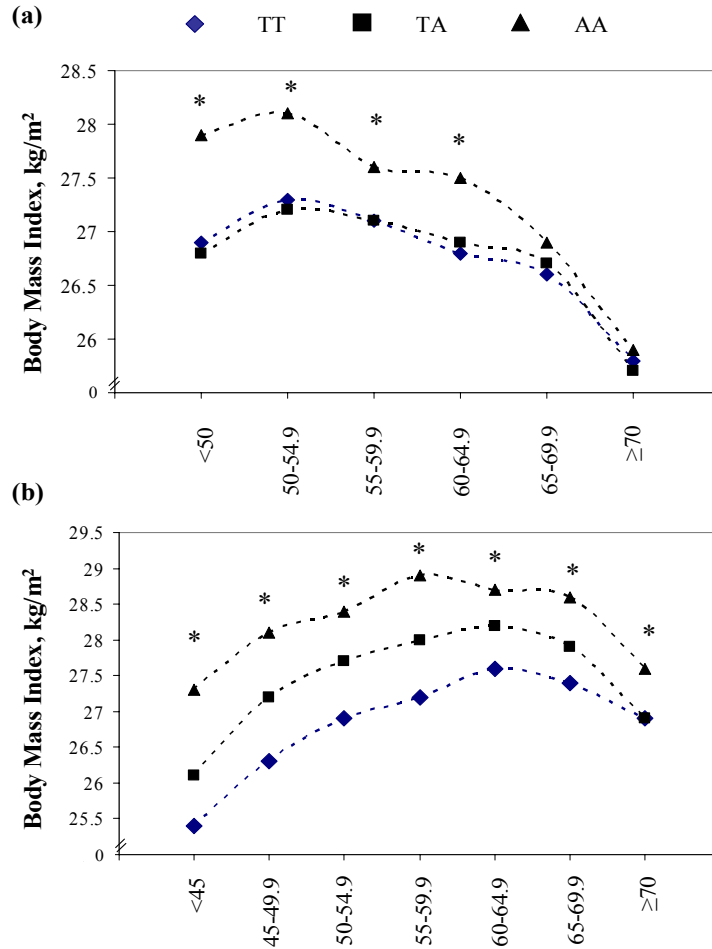
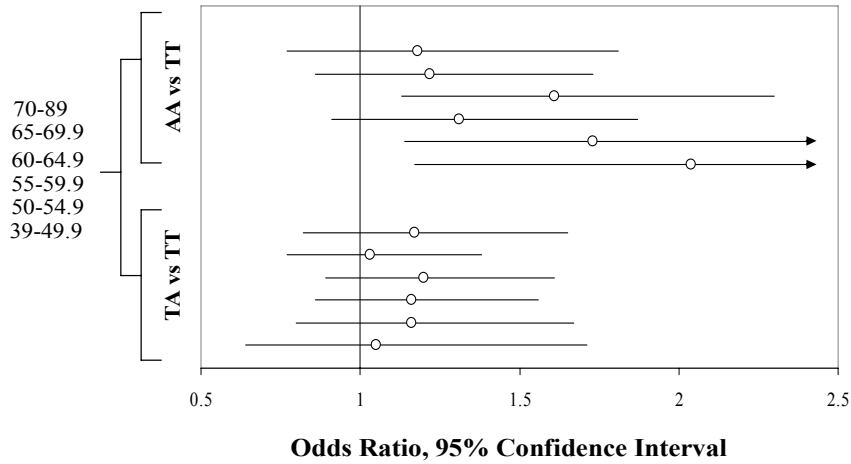


Figure 2. The geometric means of BMI by the genotypes of *FTO* SNP rs9939609 in different age groups among (a) men, <50, 50-54.9, 55-59.5, 60-64.9, 65-69.9, and ≥70 years old; and (b) women, <45, 45-49.5, 50-54.9, 55-59.5, 60-64.9, 65-69.9, and ≥70 years old. The analyses were adjusted for diabetes status. *: P<0.05.

Figure 3

(a)



(b)

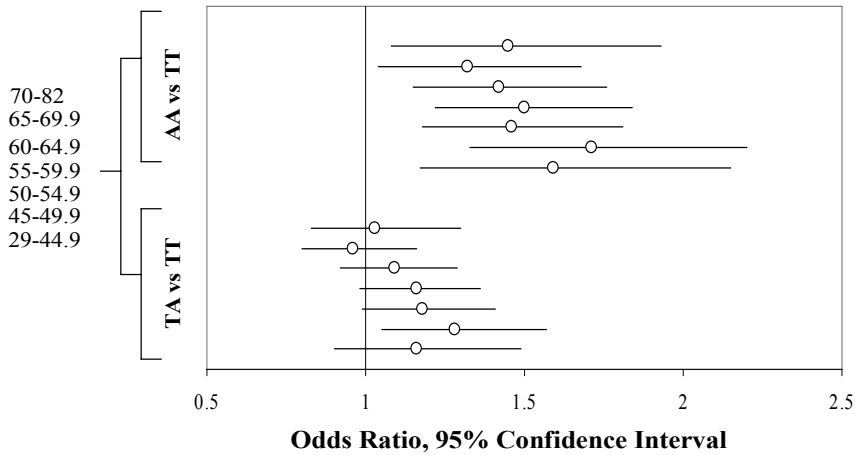


Figure 3. Odds ratios of obesity associated with the TA and AA genotypes of *FTO* SNP rs9939609 compared with TT genotype in different age groups of **(a)** men, 39-49.9, 50-54.9, 55-59.5, 60-64.9, 65-69.9, and 70-89 years old; and **(b)** women, 29-44.9, 45-49.5, 50-54.9, 55-59.5, 60-64.9, 65-69.9, and 70-82 years old. The analyses were adjusted for diabetes status. Error bars denote 95 percent confidence intervals.

Figure 4

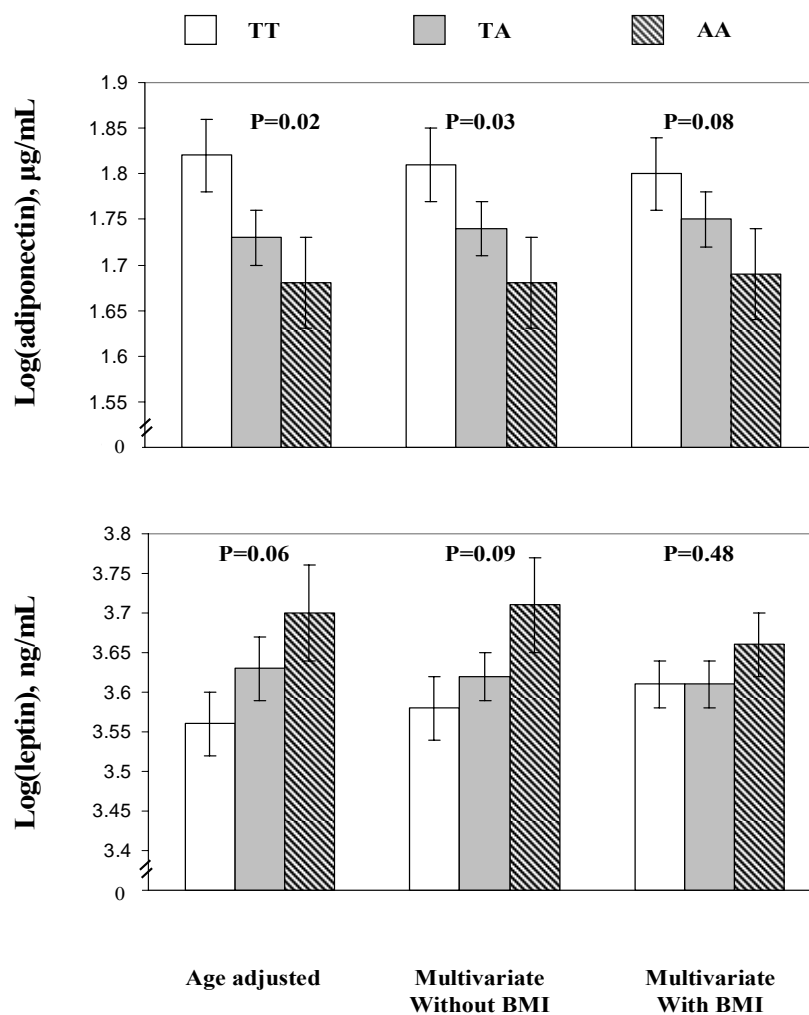


Figure 4. The geometric means (standard errors) of plasma adiponectin and leptin levels (log-transformed) by the genotypes of *FTO* SNP rs9939609 among women with type 2 diabetes. The multivariate analyses were adjusted for age, physical activity (quintiles), smoking (never, past, and current), alcohol intake (nondrinker and drinker [0.1–4.9, 5–10, or >10 g/day]), duration of diabetes, HbA_{1c}, and menopausal status [pre- or postmenopausal (never, past, or current hormone use)].

Figure 5

(a) White adipocytes

(b) Macrophages

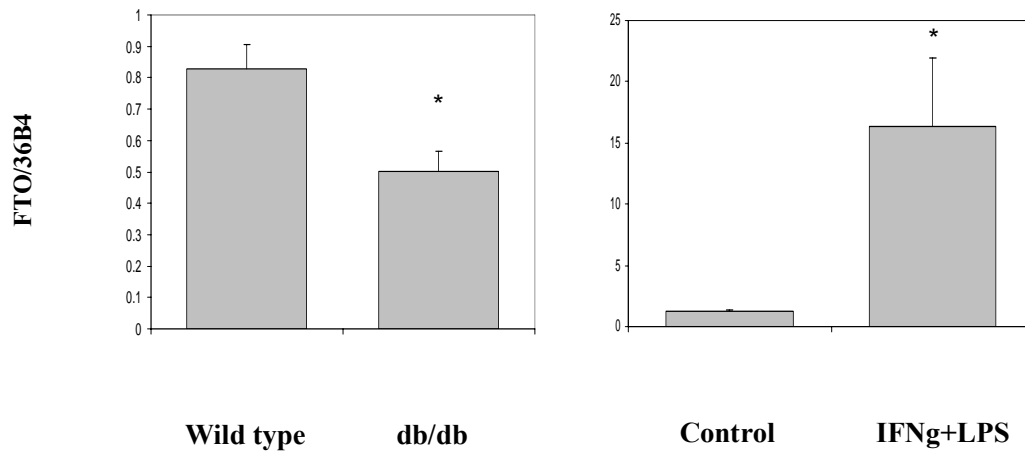


Figure 5. Regulation of *FTO* expression by genetic obesity and inflammatory stimulants. **(a)** *FTO* is down-regulated in adipose tissue of db/db mice. Adipose tissue was harvested from lean wild type (n=3) and obese, diabetic db/db mice (n=3) and gene expression was determined by real-time RT-PCR; **(b)** *FTO* is induced by pro-inflammatory stimulants in the macrophages. Bone marrow derived wild-type mouse macrophages were incubated with or without interferon γ (IFN γ) (2 ng/ml) overnight followed by lipopolysaccharide (LPS) treatment (10 ng/ml) for 8 hours. The experiments were done in triplicate. *P<0.05.