ORIGINAL RESEARCH

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Variants in the fat mass and obesity associated (FTO) gene are associated with obesity and C-reactive protein levels in Chinese Han populations

Abstract

Purpose: Various genetic variants of the fat mass and obesity associated gene (FTO) have been linked to obesity in populations of Europeans. Low-grade inflammation is a key feature of obesity, characterized by elevated plasma levels of C-reactive protein (CRP). In the present study, we have investigated whether the FTO-risk variant is associated with obesity and CRP in the Chinese population.

Methods: 1799 individuals aged 50-70 years (896 men and 903 women), including both obese (560 cases) and control individuals, (1200 cases) were recruited. The *FTO* rs9939609 polymorphism was tested using sequences retrieval. Waist circumference, body fat, hs-CRP, blood total cholesterol, triglycerides and LDL-C were also measured.

Results: The *FTO* rs9939609 polymorphism per-A allele was associated with an increased odds ratio for obesity of 1.42 (95%CI 1.23-1.64). The genotypic odds ratio for obesity was 1.92 (95%CI 1.43-2.57) for the AA genotype, 1.71 (95%CI 1.35-2.16) for the AT genotype. The association between *FTO* rs9939609 polymorphism and *CRP* levels was $r=0.78$.

Conclusion: Our data support the hypothesis that *FTO* rs9939609 polymorphism is strongly associated with obesity and *CRP* levels in the Chinese population as reported in the European population.

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Obesity and higher body mass index (BMI) are known to be associated with hypertension and related disease risks [1,2]. Obesity is a leading risk factor for multiple common diseases such as type 2 diabetes, heart disease and hypertension, and has a strong genetic component [3] although the underlying genetic causes are poorly understood [4]. Recent studies have revealed a strong association between common variants in the first intron of *FTO* and obesity in children and adults [4]. *FTO* protein expression could be a key link between the central nervous system and systemic energy balance. The amino acid sequence of the *FTO* protein shows a high homology with the enzyme AlkB, which oxidatively demethylates DNA [5,6]. Furthermore, recombinant *FTO* protein catalyzes demethylation of 3-methylthymine in single-stranded DNA [5]. *FTO* was found to be significantly up-regulated (41%) in the hypothalamus of rats after 48 hours of food deprivation [7]. Increases in hypothalamic expression of *FTO* were associated with the regulation of food intake. Studies also found a strong negative correlation between the expression of *FTO* expression and the expression of orexigenic galanin-like peptide, which is mainly synthesized in the arcuate nucleus. These results are consistent with the hypothesis that *FTO* could participate in the central control of energy homeostasis [7]. The *FTO* genetic code for 2-oxoglutarate-dependent nucleic acid demethylase is widely expressed in human tissues [5]. Researchers have observed high *FTO* expression in the brain, particularly in the hypothalamic nuclei governing energy balance. Many peripheral tissues, including the eye and skeletal muscle, have displayed equally high levels of *FTO* mRNA [7]. C-reactive protein (CRP) an acute phase protein and marker of chronic, low grade inflammation, is a reliable predictor of cardiovascular disease [8]. Low grade inflammation is a key feature of obesity and is characterized by elevated plasma levels of CRP. CRP is made by the liver in response to inflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor- α (TNF α) [8]. Adipose tissue is a major source of these inflammatory cytokines [9]. Therefore, we investigated whether the *FTO*-risk variant is associated not only with obesity but also with C-reactive protein levels in the Chinese population.

Methods

Subjects

We recruited 1799 individuals aged 50-70 years (896 men and 903 women), who are all of Han nationality from the Hubei province of China, and who are unrelated to outpatients or volunteers at the Physical Examination Center at the

ZhongNan Hospital of Wuhan University. Of these subjects, 39 were excluded because of genotyping failure. This study had been reviewed and approved by HuBei Ethics Committee. The study subjects had been given informed consent to participate in the study by letters of advice.

Obese subjects (560 individuals) were defined as having BMI ≥ 28 kg/m², according to Chinese criteria [10]. Waist circumferences, by gender, (male: WC > 90cm; female: WC > 80cm) defined as central obesity [10]. The control subjects (1200 individuals exhibited normal clinical and biochemical characteristics).

Additional selection criteria included the absence of (1) renal dysfunction (serum creatinine > 180 mmol/L), (2) liver disease, (3) severe physical or mental disease, (for example, malignancy, terminal cancer or dementia), (4) pregnancy (history and serum β -hCG ≥ 2500 mIU/ml) and (5) substance abuse, including alcohol.

Clinical measurements

Height and weight were measured with participants dressed in lightweight clothing without shoes. BMI was calculated as the weight in kilograms divided by the square of height in meters. Body fat percentage was assessed using dual energy X-ray absorptiometry. Waist circumference (in centimeters) was measured midway between the lowest rib and the iliac crest to the nearest 0.1 cm after inhalation and exhalation. Hip circumference was measured over the widest part of the gluteal region and the waist-to-hip ratio (WHR) was calculated. Plasma CRP levels were assayed using a highly sensitive turbidimetric immunoassay kit with an analytical sensitivity of 0.1 mg/l. The within and between assay coefficients of variance were <2% in standardized quality control samples and the inter- and intra-assay variability was <1% in the overall assay. Blood total cholesterol, triglycerides and LDL-C were also measured. Arterial blood pressure was measured using a mercury sphygmomanometer on the right arm with the subjects in a sitting position after a 5 minute rest.

Genotyping

Each subject donated 5 ml of blood sample for genomic DNA extraction. Genotyping was carried out as previously described [11]. Polymerase chain reaction (PCR) was used to amplify a 155-bp fragment which contains the *FTO* rs9939609 polymorphism. The reaction was performed on an automated DNA thermal cycler (Beijing Institute of Technology, China) with the primers of *FTO* rs9939609 polymorphism (FP: 5'-AAGAGATGATCTCAAATCTACTTTATGAGAT

TABLE 1. Characteristics of the study sample

	Male	Female	Total
N (%)	878(49.9)	882 (50.1)	1760
Age (years)	56.4±8.6	58.9±9.8	57.7±8.7
Weight (kg)	80.5(70-101)	65.2(50-81)	71.8(51-98)
BMI (kg/m ²)	27.9(22.2-29.8)	29.3(23.2-35.2)	28.6(22.1-34.9)
Waist circumference (cm)	91.9(76.1-109.4)	81.4(71.2-92.0)	86.7(71.4-109.1)
WHR	0.942±0.06	0.844±0.07	0.896±0.05
Body Fat (%)	24.6±5.8	33.2±5.4	29.2±8.2
hs-CRP (mg/l)	0.58(0.52-0.65)	0.72(0.67-0.80)	0.66(0.50-0.81)
TC (mmol/l)	4.30±1.66	4.32±1.61	4.31±1.20
TG (mmol/l)	2.21±1.59	2.15±1.37	2.18±1.51
LDL-cholesterol (mmol/l)	3.02±1.96	3.05±1.98	3.03±1.96
NSAID (%)	16.3	20.0	18.1
Current smoker (%)	28.0	16.3	22.0
Hypertension by doctor (%)	13.5	15.1	14.3
Diabetes diagnosed by doctor (%)	14.6	10.8	12.7

Data are means±SD, median (Inter quartile range) or percentage unless otherwise indicated.

WHR: waist-to-hip ratio; hs-CRP: high-sensitivity C-reactive protein; TC: total cholesterol; TG: total triglyceride; HDL, high-density lipoprotein; NSAID: non-steroidal anti-inflammatory drug.

A-3' and RP: 5'- TTAGAGTAACAGAGACTATCCAAGT GCATCAT-3', annealing temperature 54°C, 30 cycles) [12]. The PCR product was purified by ExoSAP-IT kit (Amersham Biosciences, UK) and Gel Extraction purification. The *FTO* rs9939609 polymorphism genotyped using sequences retrieval by ABI BigDye 3.1 kit through the ABI 3730XL sequencing equipment (SinoGenoMax, China). The sequencing results were analyzed by CHROMAS 2.01.

Statistical analysis

Statistical analysis was performed using SPSS 11.5 for Windows. Genotype and allele frequencies were compared with the Hardy-Weinberg equilibrium model and then analyzed by χ^2 testing using contingency tables respectively. The observed gene frequencies of the cases and controls were used to calculate the genotype frequency. Allelic and genotypic associations of the *FTO* rs9939609 polymorphism variant found significant were evaluated by computing odds ratios and 95% CIs. All data were presented as means±SD. The clinical and biochemical characteristics between these genotypes were compared by one-way ANOVA. The association between *FTO* polymorphism and plasma CRP levels was evaluated using correlation coefficient, standardized regression coefficients (beta), partial correlation coefficients (partial Rs) and overall coefficient of determination (R²). The association between fat measurements and

plasma CRP was assessed using linear regression analysis. CRP levels were logarithmically transformed. $P < 0.05$ was considered significant. All statistical analyses were sequentially adjusted for sex, age, BMI, WHR, hypertension and diabetes status, and use of NSAIDs, through four ANCOVA models (see recommendation below).

Results

1. Basic characteristics of the participants (Table 1)

2. Effects of the *FTO* rs9939609 polymorphism on obesity and BMI (Table 2 and Table 3)

All the genotype and allele frequencies of *FTO* rs9939609 polymorphism are in agreement with the Hardy-Weinberg equilibrium ($P > 0.05$). Genotype distribution was significantly different between obese patients and controls. The A allele frequency was significantly higher in obese patients than controls (48.4% vs. 39.8%, $P < 0.05$). The AA frequencies were 22.1% (patients) vs. 17.1% (controls). The per-A allele increases of odds ratio for obesity was 1.42 (95%CI 1.23-1.64). The genotypic odds ratio for obesity was 1.92 (95%CI 1.43-2.57) for the AA genotype and 1.71 (95%CI 1.35-2.16) for the AT genotype.

TABLE 2. *FTO* rs9939609 distributions in obesity and control groups

Groups	n	Genotypes n (frequency)			Alleles n (frequency)	
		AA	AT	TT	A	T
Obesity	560	124 (22.1)	294 (52.5)	142 (25.4)	542 (48.4)	578 (51.6)
Controls	1200	205 (17.1)	545 (45.4)	450 (37.5)	955 (39.8)	1445 (60.2)

Data are means (SD) for genotypic classes on unrelated individuals. Through Pearson χ^2 test, comparison of genotypes: obesity vs. controls, $\chi^2=26.05$, $P<0.05$; comparison of alleles: obesity vs. controls, $\chi^2=23.11$, $P<0.05$.

3. Effects of *FTO* on C-reactive protein levels (Table 4)

The *FTO* rs9939609 polymorphism A allele was found to be associated with CRP levels in men and women (Table 4): in age adjusted analyses, the associations were statistically significant in both men ($P = 0.002$) and in women ($P = 0.01$). When further adjusted for BMI, body fat and WHR, the association was slightly attenuated in women and much more obviously attenuated in men (Table 4). Further adjustment for age, BMI, waist-to-hip ratio, prevalent diseases (hypertension and diabetes), use of NSAID, statins and blood pressure lowering medication and smoking status, did not appreciably change the association (Table 4).

4. The markers of adiposity in relation to CRP concentrations (Table 5)

The association between adiposity and logCRP was also evaluated. Waist circumference and BMI were significantly correlated with CRP levels in both male and female subjects (Table 5); however, body fat was significantly related to CRP only in female subjects.

Discussion

Obesity is reaching epidemic proportions and has become a serious health problem as it contributes to the increasing burden of *T2DM*, heart disease, hypertension and dyslipidemia [12]. Although numerous gene variants have been found to be associated with obesity or obesity-related phenotypes, only a few have been consistently replicated [13-16]. Recently, genome-wide associations, followed by large-scale replication studies, have shown studies on *FTO* to be a major success in the field of obesity genetics [17,18]. Its role as a gene predisposing individuals to higher BMI, obesity [18-25], fat mass [17,18,22, 24,26], and *T2DM* risk [17,22,27-31] has been confirmed in many populations.

In this study, we confirmed the association of *FTO* with obesity in Chinese Han population. Our analysis demonstrated significant association of *FTO* with BMI and obesity in Chinese population ($P<0.05$). The *FTO* gene function is still unknown but is thought to encode a non-heme (FeII)-dioxygenase with a potential role in adaptation to hypoxia, lipolysis or DNA methylation [5,6]. The *FTO* protein is expressed in almost all tissues and is localized in the nucleus [5]. The molecular mechanisms involved in the pathogenesis of obesity as well as the role of *FTO* in other complex disorders are unknown.

Obesity is characterized by chronic subclinical inflammation, which plays an essential role in the development of atherosclerosis [32]. Prospective, epidemiological studies have shown high CRP to be a major contributor to the risk of *T2DM*, independent of measures of body adiposity [33]. There exists variability in CRP levels, which presumably follows specific patterns of several genes, such as *LEPR* polymorphisms, a triallelic SNP rs3091244 (C→T C→A) of the *CRP* gene. Wessel *et al.* showed that genetic variants in the catecholaminergic/ β -adrenergic pathway might contribute to the predictive value of CRP levels [34].

As expected, strong observational associations were found between *FTO* and C-reactive protein levels. This suggest that the *FTO* variant not only increases the size of the adipose tissue stores, but may also enhance the inflammatory state of adipose tissue stores and may increase systemic inflammation, independently of the degree of adiposity. Recent protein alignment and functional analyses have shown that *FTO* contains an active 2-oxoglutarate-dependent oxygenase domain that catalyzes the demethylation of DNA [5] but details on the functional role of the *FTO* SNP in the pathophysiology of human obesity remain unknown. It has been postulated that the mechanism may involve leptin resistance, caused by the binding of the molecule to CRP [35]: the *FTO* SNP could provoke a positive feedback on top of its underlying functional impact on BMI. Certainly, this needs addition study.

The present study has some limitations. The single time point design of this study is a limitation of our research. The CRP levels were measured only once in this study; however, we and others showed that intra-individual CRP levels are reasonably stable when compared at different points in time, even when measurements are made several years apart [36,37]. Another limitation to this study was the small sample size. Hence, our findings cannot be generalized to the greater Chinese community. It is possible that the association observed was attributable to other genetic variant(s) in strong linkage disequilibrium with the *FTO* polymorphism.

TABLE 3. Associations between the rs9939609 genotypes and obesity-related quantitative traits

Genotypes	AA	AT	TT	P(ANOVA)	P(pot-hoc test)
Men					
Age (years)	57.4±8.0	56.6±8.2	56.3±8.1	--	--
BMI (kg/m ²)	30.1±6.1	28.3±4.6	27.6±4.9	0.00091	a:0.0017 b:0.00035 c:0.00059
Waist circumference (cm)	92.7±16.4	90.3±17.2	87.9±16.1	0.0025	a:0.0089 b:0.0015 c:0.0023
WHR	0.952±0.05	0.945±0.03	0.939±0.03	0.0037	a:0.02 b:0.0021 c:0.0024
Body Fat (%)	28.5±9.1	26.2±9.0	23.9±8.6	0.0036	a:0.019 b:0.0077 c:0.0086
TC (mmol/L)	4.41±1.72	4.05±1.50	4.46±1.54	0.885	a:0.931 b:0.654 c:0.621
TG (mmol/L)	2.16±1.81	2.18±1.37	2.32±1.92	0.873	a:0.80 b:0.837 c:0.755
LDL-C (mmol/L)	2.96±1.84	3.21±1.76	2.97±1.98	0.456	a:0.588 b:0.475 c:0.532
Women					
Age (years)	59.1±9.2	59.0±9.5	58.7±9.2	--	--
BMI (kg/m ²)	31.8±5.4	28.8±5.0	26.9±4.9	0.00073	a:0.0015 b:0.00033 c:0.00052
Waist circumference (cm)	83.6±14.4	80.9±15.1	77.5±13.5	0.0018	a:0.0076 b:0.0011 c:0.0019
WHR	0.851±0.06	0.844±0.07	0.837±0.05	0.0033	a:0.026 b:0.0023 c:0.0024
Body Fat (%)	34.1±9.3	32.8±9.2	28.2±8.9	0.0012	a:0.014 b:0.0058 c:0.0062
TC (mmol/L)	4.45±1.73	4.09±1.51	4.48±1.55	0.881	a:0.94 b:0.646 c:0.628
TG (mmol/L)	2.11±1.80	2.12±1.31	2.28±1.86	0.877	a:0.825 b:0.822 c:0.824
LDL-C (mmol/L)	2.99±1.82	3.20±1.78	3.02±2.02	0.452	a:0.587 b:0.413 c:0.509

Data are mean±SD for genotypic classes on unrelated individuals. a, b, c : the P value for multiple comparisons between AA and AT, AA and TT, AT and TT with the clinical and biochemical characteristics, respectively.

TABLE 4. Associations between *FTO* rs9939609 and plasma CRP

Genotypes	AA	AT	TT	P	r	partial R	beta	R ²
Men								
Model 1	-0.28(-0.37,-0.23)	-0.22 (-0.29,-0.14)	-0.12(-0.19,-0.05)	0.001	0.78	0.34	0.61	0.6084
Model 2	-0.26 (-0.35,-0.22)	-0.22 (-0.28,-0.15)	-0.15 (-0.20,-0.07)	0.03	0.32	0.13	0.22	0.1024
Model 3	-0.26 (-0.35,-0.22)	-0.22 (-0.28,-0.15)	-0.15 (-0.21,-0.07)	0.03	0.33	0.15	0.24	0.1089
Model 4	-0.26 (-0.35,-0.22)	-0.22 (-0.28,-0.15)	-0.15 (-0.21,-0.07)	0.04	0.30	0.15	0.23	0.0900
Women								
Model 1	-0.18 (-0.27,-0.13)	-0.09 (-0.14,-0.89)	-0.06 (-0.12,-0.02)	0.02	0.55	0.25	0.40	0.3025
Model 2	-0.18 (-0.27,-0.12)	-0.10 (-0.16,-0.88)	-0.07 (-0.14,-0.03)	0.03	0.31	0.17	0.22	0.0961
Model 3	-0.18 (-0.28,-0.11)	-0.10 (-0.16,-0.88)	-0.08 (-0.14,-0.04)	0.03	0.30	0.14	0.21	0.0900
Model 4	-0.18 (-0.27,-0.11)	-0.10 (-0.15,-0.88)	-0.08 (-0.14,-0.04)	0.03	0.31	0.16	0.21	0.0961

Geometric mean (95% confidence limits) of log hs-CRP (mg/l); *FTO*, fat-mass and obesity-associated; hs-CRP, high-sensitivity C-reactive protein; model 1, adjusted for age; model 2, adjusted for age, BMI, body fat and waist-to-hip ratio; model 3, adjusted for age, BMI, waist-to-hip ratio, prevalent diseases (hypertension and diabetes); model 4, adjusted for age, BMI, waist-to-hip ratio, prevalent diseases (hypertension and diabetes), use of NSAID, statins and blood pressure lowering medication and smoking. The *r* value is correlation coefficient between *FTO* polymorphism and CRP levels. The partial *R* value is partial correlation coefficient between *FTO* polymorphism and CRP levels. Beta: Standardized regression coefficients. R²: overall coefficient of determination.

In conclusion, we found that *FTO* is a major risk factor for obesity in the Chinese Han population, and its effects play an important role in fat mass deposition and obesity prevalence. The explanation for this may be that its protein is a key link between the central nervous system and energy balance. At the same time, a significant association was found between *FTO* and C-reactive protein levels; however, this association may not be valid for the entire Chinese population.

The progress of obesity or diabetes is determined not only by genetic factors, but by factors such as environment. These

factors each exert a different impact at each step of disease development, complicating analysis. Whether a single gene can be associated with obesity or in what way is a complicated question. Thus, proof-of-concept and prospective studies are needed to further investigate the physiology and the impact of the *FTO* polymorphisms and associated gene products. From a population perspective, only the combined effects of the most potent genetic variants should be then considered.

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TABLE 5. Relation of the markers of adiposity with logCRP

parameter	P	r	partial Rs	beta	R ²
Men					
Body mass index	0.17	0.14	0.11	0.13	0.0196
Waist circumference	0.22	0.08	0.01	0.04	0.0064
WHR	0.31	0.03	0.008	0.019	0.0009
Body Fat	0.15	0.12	0.09	0.12	0.0144
Women					
Body mass index	0.13	0.16	0.12	0.15	0.0256
Waist circumference	0.18	0.10	0.02	0.07	0.0100
WHR	0.26	0.05	0.009	0.026	0.0025
Body Fat	0.04	0.33	0.18	0.24	0.1089

The *r* value is correlation coefficient between the markers of adiposity and logCRP. The partial *R* value is partial correlation coefficient between the markers of adiposity and logCRP. Beta: Standardized regression coefficients. R²: overall coefficient of determination.

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