Research Article

Association of FTO Polymorphisms with Early Age of Obesity in Obese Italian Subjects

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1. Introduction

Obesity is considered a worldwide epidemic in modern societies, affecting all ages, genders, and ethnic groups. Increased adiposity is due to the interaction between a genetic background and environmental factors, particularly excessive food intake and reduced physical activity. Although the obesity epidemic has been certainly driven by lifestyle and environmental changes, it is also clear that there is individual variation in response to these factors, suggesting a strong genetic predisposition. In recent years, of the many genes studied for obesity, the FTO (fat mass- and obesity-associated) gene has been proven consistently to be associated with increased body mass index (BMI). Since its discovery in 2007 in genomewide association studies for type 2 diabetes [1], several other studies have confirmed the association in different populations [2–4]. Of the many found associated, the FTO rs9939609 single nucleotide polymorphism (SNP), located in the first intron, is of particular interest since it was the first to be found to be associated with obesity and has been constantly replicated through independent studies of large Caucasian populations [2, 3]. It was estimated that each copy of the highly frequent (40–50% in the general population) FTO rs9939609 minor allele (i.e., A allele) corresponds to approximately 1.5-kg heavier body weight [1].

FTO is mainly expressed in the hypothalamus, and it may play important roles in the management of energy homeostasis [5], nucleic acid demethylation, and in the regulation of body fat masses by lipolysis [6].
Other than BMI, FTO gene SNPs have been shown to associate with a number of metabolic-related traits, such as higher fasting insulin, glucose, triglycerides and lower HDL cholesterol [7], waist circumference [8, 9], and body weight [10].

So far, very limited data is available on the relationship between FTO gene and obesity in the Italian adult population. The only study published in a cohort from Sardinia [10] showed association between the FTO rs9939609 SNP and BMI, total body weight, and hip circumference. Furthermore, a second FTO SNP, the rs9930506, showed in this population an even stronger association [10] compared to the rs9939609 SNP. It should be pointed out that this population is from Sardinia, an Italian island that shows peculiar genetic characteristics that may differ from the rest of the Italian population [11].

Various studies have investigated the effect of FTO variants on BMI and weight in a longitudinal perspective, and whether it influences weight gain during adult life [12, 13]. Interestingly, a stronger correlation between BMI and FTO single nucleotide polymorphisms is most commonly seen in cohorts of children and young adults [14, 15].

Aims of our study are therefore: (1) to investigate the association of the FTO gene SNPs rs9939609 and rs9930506 with BMI and obesity-related parameters in a large cohort (n = 752) of Italian obese population; (2) to examine the association between the two FTO SNPs and age of onset of obesity.

2. Methods

2.1. Study Group. A total of 950 subjects were studied. 752 consecutive unrelated obese (BMI ≥ 30 kg/m²) Caucasians were recruited from the Day-Hospital of the Department of Clinical Sciences, University of Rome “Sapienza”. They underwent complete medical evaluation and a standard 75 g oral glucose tolerance test (OGTT) with measurements of glucose and insulin at baseline and after 30, 60, 90, and 120 minutes. All individuals provided informed consent prior to inclusion in the study. The study was approved by the local research ethics committee.

Nonobese subjects (n = 198) were recruited from subjects participating in a community-based health screening. All subjects were unrelated, and the only inclusion criteria in the control group for obesity was a BMI < 27 kg/m². In nonobese subjects a complete medical history was obtained together with laboratory parameters including total cholesterol, HDL, LDL, triglycerides, blood glucose, and fasting plasma insulin.

In all subjects diagnosis of type 2 diabetes was based on either fasting plasma glucose ≥ 7.0 mmol/L (126 mg/dL), or plasma glucose ≥ 11.1 mmol/L (200 mg/dL) 2 hours after a 75 g glucose load during the OGTT. Also a previous diagnosis of diabetes and/or a history of hypoglycaemic treatment were considered.

2.2. Biochemistry. Glucose, insulin, cholesterol, HDL-cholesterol, and triglycerides (TGs) were measured as previously described [16], after an overnight fast. Glucose levels below 100 mg/dL, HDL > 40 mg/dL for men and > 50 mg/dL for women, and triglycerides < 150 mg/dL were taken as normal limits.

Serum alanine (ALT) and aspartate aminotransferase (AST) levels in fasting subjects were assayed using a Hitachi 737 analyzer (Boehringer-Mannheim Diagnostics, Indianapolis, IN). Homeostasis model assessment of insulin resistance (HOMA-IR) and insulin sensitivity index (ISI) indices were calculated as previously shown by Matthews et al. [17] and Matsuda and DeFronzo [18].

2.3. Genotyping Assay. Genomic DNA samples were extracted from peripheral blood, using a modified salting out procedure from Miller et al. [19].

In this study, we have used locked nucleic acid (LNA) hybridization probes (fluorometric method) as allele-specific tools in genotyping assays in order to analyze each SNPs (rs9930506 and rs9939609). Real time PCR was performed using iQ5 Multicolor Real-Time (Bio-Rad Laboratories), and data analysis was conducted with the corresponding software interface. In a 25 μL reaction volume 300 nM of each PCR primer, 280 nM of each LNA probe, 1X iQ Supermix (Bio-Rad Laboratories), and 10 ng of genomic DNA were added.

PCR primers and LNA probes were designed and synthesized by Sigma-Aldrich Life Science (further data on primers and probes are available upon request).

Some genomic DNA samples used for real-time PCR optimization were sequenced to validate the observed allelic discrimination curves and genotypes.

2.4. Statistical Analysis. Categorical variable distributions were compared by the Pearson χ². Differences between continuous variables across the genotype classes were evaluated by ANOVA including gender, age, and BMI as covariates. Skewed variables were logarithmically transformed prior to entering the analyses. Linkage disequilibrium between the rs9939609 and rs9930506 SNPs was assessed by calculating the disequilibrium statistics D’ [20] and D’′ [21]. The sign of D’ (positive or negative) depends on the arbitrary choice of the alleles paired at the two loci and indicates whether the same or opposite allelic association is present. The differences in the variables within the same group before and after bariatric surgery were compared with paired-samples statistics. All statistical analyses were performed with SPSS 17.0 statistical package.

3. Results

3.1. Characteristics of the Study Population. Clinical characteristics of the study subjects stratified by classes of BMI (group A, nonobese: BMI < 27 kg/m²; group B, class I/II obese: BMI = 30–39.9 kg/m²; group C, class III obese: BMI ≥ 40 kg/m²) are reported in Table 1. Overall, 752 (79%) of the 950 study subjects were obese (422 with class III obesity), whereas 209 (22%) subjects had T2D.

Generally, as expected, many clinical and biochemical data worsened significantly with increasing BMI, with class III obese subjects showing the highest levels of fasting blood
glucose and insulin, lipids, transaminases, HOMA-IR, and lower values of ISI. In the control normal-weight group all parameters were within normal range, with only 5 subjects with diabetes.

3.2. FTO Allele Frequencies and Linkage Disequilibrium. In the whole population risk-allele frequencies of the rs9939609 (A) and rs9930506 (G) FTO gene were 0.48 and 0.50, respectively, similar to HapMap (haplotype map of the human genome) population frequencies for rs9939609 (A) allele in CEPH Europeans and for rs9930506 (G) allele in CEU Europeans.

The two SNPs showed a strong linkage disequilibrium ($\Delta = 0.88, D' = 0.90, P < 0.0001$). Nevertheless, because of a different effect observed in Italian subjects [10], we analyzed the two variants separately and as haplotypes in our study cohort.

3.3. Association Study of the rs9939609 and rs9930506 Polymorphisms with BMI and T2D. Table 2 shows the association of the 2 FTO variants with obesity; the rs9939609 A-allele frequency and rs9930506 G-allele frequency in the obese subjects were significantly higher than in the lean group ($P < 0.027$ and $P < 0.013$, resp.). When the association was adjusted by age and sex, significance was maintained, although at lower $P$ values, suggesting a strong effect of these parameters ($P < 0.038$ and $P < 0.015$, resp.).

When we stratified for BMI levels, no significant difference in rs9939609 A-allele frequency was observed between class I/II obesity and class III obesity (Table 2(a)). Similar results were observed for rs9930506 G-allele frequency between class I/II obesity and class III obesity (0.51 and 0.52, resp.) (Table 2(b)). With reference to the rs9939609 (A) allele we should point out that we did not observe a significant difference between controls and class I/II obesity. This may be explained by the fact that this SNP has a weaker effect in Italian population, as also demonstrated by the study of Scuteri et al. [10]. Conversely, rs9930506 was, in the same population, significantly associated with class I/II obesity.

We next tested the association with T2D. A-allele frequencies for rs9939609 polymorphism were not significantly different between T2D and nondiabetic individuals (0.51 and 0.50, resp. $P = NS$); similarly, no significant differences between T2D and nondiabetic subjects were observed for the rs9930506 G-allele (0.51 and 0.52, resp. $P = NS$).

We then compared other clinical parameters according to genotype classes for both SNPs. There were no significant differences between the three genotypes in fasting blood glucose and insulin levels, plasma concentration of ALT

### Table 1: Clinical characteristics of study subjects stratified for BMI.

|                | Group A | Group B | Group C | Group D |
|----------------|---------|---------|---------|---------|
| N              | 198     | 330     | 422     | 301     |
| Age (yr)       | 49 (45–55) | 48 (34–58) | 42 (35–51) | 115 (35–51) |
| Body mass index (kg/m²) | 23.0 (21.3–24.7) | 35.7 (32.9–38.1) | 46.2 (42.8–51.1) | 40.5 (37.9–43.1) |
| Waist (cm)     | 95.9 (91–100) | 112 (105–120) | 132.5 (123–142) | 135.6 (127–145) |
| Blood pressure | 120 (115–135) | 130 (120–140) | 130 (120–140) | 135 (120–140) |
| Triglycerides  | 80 (70–86) | 80 (75–90) | 85 (80–90) | 85 (80–90) |
| Total cholesterol | 209 (187.2–234) | 203 (178–234) | 195 (170–218.3) | 190 (175–215) |
| HDL cholesterol | 59 (50–67) | 47.3 (40.3–56.6) | 45.8 (39.8–53) | 45.0 (39.5–52) |
| LDL cholesterol | 126 (108–147) | 124.2 (105–149) | 115 (95.1–139.6) | 120 (105–140) |
| ALT (U/L)      | 23 (13.1–34.5) | 27.5 (19.2–40) | 29.1 (20.7–45.6) | 30.2 (21.5–46.5) |
| AST (U/L)      | 20 (18–25) | 20 (16–27) | 20 (16–28) | 20 (16–28) |
| Fasting blood glucose (mg/dL) | 92 (84–100) | 93 (82–118) | 95 (85–111) | 97 (85–115) |
| Fasting blood insulin (µU/mL) | 9.9 (7–15.2) | 18.8 (13–30) | 29 (19.5–47.3) | 29 (19.5–47.3) |
| HOMA-IR (U)    | 2 (1.5–3.3) | 3.9 (2.6–6.7) | 6.2 (4.1–10.7) | 6.2 (4.1–10.7) |
| ISI (U)        | 4.6 (2.9–7.8) | 2.8 (1.8–4.4) | 1.9 (1.2–2.8) | 1.9 (1.2–2.8) |
| Type 2 diabetes n (%) | 5 (2.5) | 101 (30.6) | 103 (24.4) | 103 (24.4) |

*Abbreviations: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high density lipoprotein; LDL, low density lipoprotein; ALT, alanine transaminase; AST, aspartate transaminase; HOMA-IR, homeostatic model assessment of insulin resistance; ISI, insulin sensitivity index. Non-obese: BMI < 27 kg/m²; class I/II obese: BMI = 27–39.9 kg/m²; class III obese: BMI ≥ 40 kg/m².*
Table 2: (a) Association study of FTO rs9939609 polymorphism with obesity. (b) Association study of FTO rs9930506 polymorphism with obesity.

### (a) Association study of FTO rs9939609 polymorphism with obesity

| Genotype n (%) | A-allele frequency (%) | P  | P<sub>adj</sub> |
|----------------|------------------------|----|----------------|
| Stratified on BMI |                     |    |                |
| Nonobese       | 198                    | 68 (34.3) | 90 (45.5) | 40 (20.2) | 43.0 | <0.027 | <0.038 |
| Obese          | 752                    | 195 (25.9) | 357 (47.5) | 200 (26.6) | 50.3 |         |        |
| Stratified on BMI |                     |    |                |
| Nonobese       | 198                    | 68 (34.3) | 90 (45.5) | 40 (20.2) | 43.0 | <0.027 | <0.038 |
| Class I/II obese| 330                    | 88 (26.7) | 152 (46.1) | 90 (27.2) | 50.3 | <0.066* | <0.075* |
| Class III obese | 422                    | 107 (25.4) | 205 (48.6) | 110 (26.0) | 50.4 | <0.038* | <0.057* |

Data are n (%). P<sub>adj</sub> values were calculated using logistic regression analysis adjusted for age and sex. Nonobese: BMI < 27 kg/m<sup>2</sup>; class I/II obese: BMI = 30–39.9 kg/m<sup>2</sup>; class III obese: BMI ≥ 40 kg/m<sup>2</sup>. * versus nonobese.

### (b) Association study of FTO rs9930506 polymorphism with obesity

| Genotype n (%) | G-allele frequency (%) | P  | P<sub>adj</sub> |
|----------------|------------------------|----|----------------|
| Stratified on BMI |                     |    |                |
| Nonobese       | 198                    | 67 (33.8) | 90 (45.5) | 41 (20.7) | 43.4 | <0.013 | <0.015 |
| Obese          | 752                    | 190 (25.3) | 346 (46.0) | 216 (28.7) | 51.7 |         |        |
| Stratified on BMI |                     |    |                |
| Nonobese       | 198                    | 67 (33.8) | 90 (45.5) | 41 (20.7) | 43.4 | <0.043* | <0.038* |
| Class I/II obese| 330                    | 86 (26.1) | 149 (45.2) | 95 (28.7) | 51.4 | <0.018* | <0.019* |
| Class III obese | 422                    | 104 (24.6) | 197 (46.7) | 121 (28.7) | 52.0 |         |        |

Data are n (%). P<sub>adj</sub> values were calculated using logistic regression analysis adjusted for age and sex. Nonobese: BMI < 27 kg/m<sup>2</sup>; class I/II obese: BMI = 30–39.9 kg/m<sup>2</sup>; class III obese: BMI ≥ 40 kg/m<sup>2</sup>. * versus nonobese.

3.4. Association Study of the rs9939609 and rs9930506 Polymorphisms with Age. We further evaluated the distribution of the rs9939609 and rs9930506 FTO SNPs genotypes according to age, stratifying the study population in younger subjects (<46 years of age, median of the whole population) and older subjects (≥46 years of age). The rs9930506 G-allele frequency in younger subjects was significantly higher than in older group (0.54 versus 0.46, <0.002) and similarly for rs9939609 A-allele frequency (0.53 versus 0.45, <0.001). Further stratification in quartiles of age showed that subjects in the 75th quartile (>55 years) had a much lower frequency of “at risk” FTO alleles (rs9939609 (A) = 0.42 and rs9930506 (G) = 0.44) compared to subjects in the 25th quartile (age > 35 years, 0.53 A allele and 0.54 G allele), a difference that was highly significant (<0.01). Since class III obese were younger they might explain most of the difference in frequency distribution of the rs9930506 G-allele and of the rs9939609 A-allele between younger and older subjects. In order to analyze this point we have performed two separate analyses in our population: (1) we repeated the same analyses in Table 3 excluding the class III obese subjects, and association with age was still highly significant (data not shown, <0.01); (2) we also repeated the same analyses in
Table 3: Association of rs9930506 and rs9939609 SNPs with age, BMI, and waist.

| SNP        | Genotype Class (n.) | Age (years) | BMI (kg/m²) | Waist (cm) |
|------------|---------------------|-------------|-------------|------------|
| rs9930506  | AA (257)            | 47 ± 14     | 37.2 ± 11.4 | 117.8 ± 18.7 |
|            | GA (436)            | 45 ± 13     | 38.6 ± 10.4 | 124.2 ± 18.5 |
|            | GG (257)            | 44 ± 13     | 40 ± 9.6    | 126.7 ± 15.2 |
|            | P                   | <0.013*     | <0.029**    | <0.006*    |
| rs9939609  | TT (263)            | 47 ± 14     | 37.3 ± 11.4 | 118.4 ± 19.5 |
|            | TA (448)            | 45 ± 13     | 38.6 ± 10.4 | 125 ± 18.2  |
|            | AA (239)            | 43 ± 13     | 39.4 ± 9.8  | 125 ± 14.5  |
|            | P                   | <0.006*     | <0.043**    | <0.025*    |

All values are means ± standard deviations. BMI: body mass index, n.: number of subjects. *P values were calculated using a linear regression model including gender, age, and BMI as covariates. **Analysis adjusted for gender and age.

Table 4: Association of rs9930506 and rs9939609 SNPs with age, BMI, and waist in age-matched subjects selected from the three BMI classes.

| SNP         | Genotype Class (n.) | Age (years) | BMI (kg/m²) | Waist (cm) |
|-------------|---------------------|-------------|-------------|------------|
| rs9930506   | AA (224)            | 50 ± 11     | 36.1 ± 11.2 | 116.1 ± 18.8 |
|             | GA (364)            | 48 ± 10     | 37.6 ± 10.6 | 124.8 ± 18.6 |
|             | GG (215)            | 46 ± 12     | 38.3 ± 9.5  | 125.5 ± 15.1 |
|             | P                   | <0.010*     | <0.022**    | <0.005*    |
| rs9939609   | TT (224)            | 50 ± 11     | 36.1 ± 11.3 | 116.8 ± 20  |
|             | TA (364)            | 48 ± 11     | 37.5 ± 10.5 | 125.1 ± 18.2 |
|             | AA (215)            | 46 ± 12     | 38.2 ± 9.6  | 124 ± 14.6  |
|             | P                   | <0.011*     | <0.038**    | <0.012*    |

All values are means ± standard deviations. BMI: body mass index; n.: number of subjects. *P values were calculated using a linear regression model including gender, age, and BMI as covariates. **Analysis adjusted for gender and age.

3.5. Haplotypes Analyses from rs9939609 and rs9930506 Polymorphisms. Analyses in the haplotypes groups derived from rs9939609 and rs9930506 FTO genetic polymorphisms confirmed the lack of association of the FTO variants with fasting blood glucose and insulin levels, plasma ALT and AST levels, total cholesterol, HDL- and low density lipoprotein-(LDL-) cholesterol, triglycerides, HOMA-IR, and ISI indices (data not shown). However the AG haplotype (homozygous carriers of “at risk” alleles) was associated with increased BMI (P < 0.026) and waist circumference (P < 0.003) which is consistent with the findings observed for the two SNPs separately (data not shown). Furthermore, AG haplotype carriers were significantly younger than homozygotes for the TA haplotype (P < 0.014) (data not shown) confirming the results of the two SNPs analyzed individually.

3.6. Multivariate Analyses of FTO Polymorphisms. Due to the strong linkage disequilibrium between rs9939609 and rs9930506 FTO genetic polymorphisms, only rs9930506 (the most significant SNP) was considered for further analyses. Since univariate analyses showed a strong association of the rs9930506 FTO only with BMI, age, and waist, we entered in a multiple regression model these three variables. Multiple regression analysis with BMI as a dependent variable revealed that the rs9930506 FTO genetic polymorphisms are associated with body mass index (P < 0.029), independently of sex (P > 0.036) and age (P > 0.001).

4. Discussion

4.1. Association of FTO rs9939609 and rs9930506 Polymorphisms with BMI. Our results demonstrate in the Italian population a strong association between rs9939609 and rs9930506 SNPs of the FTO gene and BMI and waist circumference, in concordance with previously published studies in other European populations [1–3, 10]. Of the 2 SNPs, rs9930506 was the most strongly associated with BMI in our Italian population, confirming the only previous observation [10]. Furthermore, the G allele of rs9930506 was significantly associated with higher BMI in a G allele dose-dependent manner (BMI + 1.4 kg/m² per G allele).

4.2. Association of FTO rs9939609 and rs9930506 Polymorphisms with Age. We also observed that the associations of FTO rs9939609 and rs9930506 variants on body size varied with age. Particularly, the at-risk allele frequencies for both SNPs were significantly higher in younger than in older subjects, suggesting that carriers of the risk allele develop an increase in body weight earlier in life.

There have been only a few other studies exploring the association between FTO gene and BMI during the life course [12, 13]. In the study by Hardy and coworkers a longitudinal pattern of association between rs9939609 FTO genetic variant and BMI across childhood, adolescence, and adulthood was observed [13]. In particular, the associations with this FTO variant strengthened during childhood and adolescence, peaked in early adulthood, and then weakened in adult age (after 43 years). It can be hypothesized that the
effect of FTO on body composition may be less severe in adult life when weight gain may be more strongly determined or modified by psychosocial or environmental influences [22] compared to younger ages.

4.3. Lack of Association of FTO Polymorphisms with Metabolic Parameters and T2D. At variance with previous studies [7–9], in our population FTO gene polymorphisms were associated only with an increased BMI, but not with metabolic parameters such as lipids, impaired glucose tolerance, or insulin resistance. One possible explanation for these discrepancies may be ascribed to the relatively young age of our population (median 46 years), with >50% of carriers of the FTO risk allele that are under the median age and with a significant proportion of carriers that are below the 25th percentile of age (<35 years) of our cohort. Thus, these subjects may have not yet developed age-related metabolic abnormalities.

We also tested a possible association with T2D, but no significant differences between T2D and nondiabetic subjects were observed for the FTO SNPs. It has been previously observed that the association of the SNPs (located in the first intron) of the FTO gene with T2D was abolished after adjustment for BMI, indicating that the impact of FTO on T2D was primarily due to its association with BMI [1]. Our study population presented a prevalence of obesity of ∼79%, and this might explain the absence of association of FTO SNPs with T2D.

It is unclear how FTO increases the susceptibility to develop overweight and obesity. There are no obvious evidences suggesting that rs9939609 or rs9930506 are the causal variants, and, indeed, there are many other variants elsewhere in the gene or control elements of other genes that are in complete linkage disequilibrium with the FTO locus. However, based on the rapid fall-off of linkage disequilibrium with other SNPs beyond 47 kb, it has been concluded that the functional variant is likely to lie within this area of the FTO gene [1].

Through the use of bioinformatics and recombinant functional studies it has been recently demonstrated that the FTO gene encodes a nucleic acid demethylase [23]. Demethylation of DNA is essential for repairing exocyclic DNA lesions, which, if left unchecked, can lead to permanent, and sometimes deleterious, sequence changes. It is also conceivable that the nucleic acid demethylation activity of FTO on DNA might regulate the expression of genes involved in metabolism and that dysregulation of this process might lead to obesity at an epigenetic level [24].

5. Conclusion

FTO is the first and the most robust obesity susceptibility gene of the GWAS era. Although its effect size is modest, one should not underestimate the relevance of FTO at the population level. The observed association is common and consistent across multiple ethnic groups and could be influencing BMI of up to half the world’s population.

Our data confirm all these findings. In particular, we observe that the association between FTO gene and BMI strongly influences the age of onset of obesity, with the carriers of the “at risk” alleles showing a significantly higher prevalence in younger age. In conclusion, our study shows a role of the genetic variability in FTO in the modulation of BMI in a large Italian population.

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