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# Association of *FTO* gene with hyperandrogenemia and metabolic parameters in women with polycystic ovary syndrome

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**Abstract**

Variants in the fat mass and obesity–associated gene (*FTO*) are associated with obesity and type 2 diabetes mellitus. Women with polycystic ovary syndrome (PCOS) are frequently affected by obesity and impaired glucose tolerance. The aim of this study was to investigate the impact of *FTO* variants (rs9939609) on metabolic and endocrine parameters in PCOS women. We genotyped the single nucleotide polymorphism rs9939609 (T/A) in 288 PCOS women and performed metabolic and hormonal measurements, oral glucose tolerance test, hirsutism score, and lipometry. The A/T + A/A genotype showed an increased prevalence in overweight/obese PCOS patients (odds ratio [OR] = 1.91, $P = .028$) and in PCOS women with impaired glucose tolerance (OR = 3.23, $P = .009$). The A allele was associated with a significant increase in free testosterone ($P = .042$), weight ($P = .024$), body mass index ($P = .011$), 2-hour glucose ($P = .047$), 1-hour insulin ($P = .032$), and AUCins (area under the curve insulin) ($P = .038$). In a logistic regression analysis, the A allele was associated with free testosterone ($P = .025$; OR = 1.54; 95% confidence interval, 1.06-2.25; $B = 0.86$). Total body fat (percentage) ($P = .016$), total fat mass ($P = .013$), visceral adipose tissue mass ($P = .044$), and subcutaneous fat mass ($P = .011$) were significantly increased in PCOS women carrying the A allele. We demonstrated that variants within the *FTO* gene influence hyperandrogenemia and anthropometric parameters in women with PCOS, indicating an important role of *FTO* variants not only in obesity and diabetes but also in hyperandrogenism in women with PCOS.


## 1. Introduction

Polycystic ovary syndrome (PCOS) is a complex endocrine disorder, affecting approximately 5% to 10% of women of reproductive age [1-3]. It is characterized by increased ovarian and adrenal androgen secretion; hyperandrogenic symptoms such as hirsutism, acne, and/or alopecia; menstrual irregularity; and polycystic ovaries. The disorder is frequently associated with insulin resistance accompanied by compensatory hyperinsulinemia, creating an increased risk for the development of type 2 diabetes mellitus [4]. Obesity plays a key role in the development of PCOS [5,6] and affects about 50% of women with PCOS depending on ethnicity. Both PCOS and obesity are considered highly heritable complex diseases [7,8].

Recently, genomewide association studies have led to genetic discoveries of various common genetic diseases and traits [9]. The discovery of fat mass and obesity–associated gene (*FTO*) in 2 independent studies was the first major success in the field of obesity genetics [10,11]. Single nucleotide polymorphisms in the *FTO* gene predispose to type 2 diabetes mellitus through an effect related to the body mass index (BMI) [10,12]. Furthermore, there is evidence that *FTO* variants influence gestational diabetes [13].

*FTO* is a very large gene localized on chromosome 16 [14]. Up to now, the biology of *FTO* and its products is poorly understood. It has been shown that *FTO* gene encodes a 2-oxoglutarate–dependent nucleic acid demethylase [15]. Studies in rodents indicated that *FTO* messenger RNA is abundant in the brain, particularly in the hypothalamic nuclei

The study protocol was approved by the local ethics committee, and written informed consent was obtained from each patient and control.

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governing energy balance, and in many peripheral tissues [16]. Gene expression profiles show that *FTO* is expressed in several tissues, especially specific parts of the brain and the muscle [10,17].

Recent work has shown an association of *FTO* variants with impaired fasting glucose and the metabolic syndrome in PCOS [18]. There is evidence that *FTO* variants also influence the susceptibility to PCOS [19]. So far, there is no evidence for a relationship between *FTO* variants and androgen levels.

The aim of this study was to investigate the impact of *FTO* variants (rs9939609) on metabolic parameters in PCOS women. A further goal of this study was to establish whether *FTO* variants influence endocrine parameters including androgens in PCOS women, given that there is a link between *FTO* variants with PCOS [19] and that hyperandrogenemia is the most consistent endocrine feature of PCOS.

## 2. Methods

### 2.1. Subjects

The study population consisted of 288 women (with verified PCOS) aged 16 to 41 years. The diagnosis was based on the Rotterdam criteria [20]. Two out of 3 of the following are required for the diagnosis: oligo- and/or anovulation, clinical and/or biochemical signs of hyperandrogenism, and polycystic ovaries (by ultrasound). Disorders with a similar clinical presentation, such as congenital adrenal hyperplasia, Cushing syndrome, and androgen-secreting tumors, had to be excluded. *Oligo-* and/or *anovulation* was defined by the presence of oligomenorrhea or amenorrhea. *Hyperandrogenism* was defined by the clinical presence of hirsutism (Ferriman-Gallwey score $\geq$6) [21], acne, or alopecia and/or elevated androgen levels. Polycystic ovarian morphology was examined by ultrasound. Hyperprolactinemia, Cushing syndrome, congenital adrenal hyperplasia, and androgen-secreting tumors were excluded by specific laboratory analysis (cortisol, corticotropin, 17αOH-progesterone, DHEAS [dehydroepiandrosterone sulfate]). The PCOS women were taking no medication known to affect carbohydrate metabolism or endocrine parameters for at least 3 months before entering the study. The study protocol was approved by the local ethics committee, and written informed consent was obtained from each patient and control.

### 2.2. Procedures

Standard anthropometric data (height, weight, waist and hip circumference, blood pressure) were obtained from each subject. Waist circumference was measured in a standing position midway between the lower costal margin and the iliac crest. Hip circumference was measured in a standing position at the maximum circumference over the buttocks. Hirsutism was quantified with the modified Ferriman-Gallwey score [21]. Moreover, basal blood samples for hormonal (total testosterone, free testosterone, SHBG (sex hormone binding globulin), androstenedione, DHEAS, fT3 (free triiodothyronine), fT4 (free thyroxine), thyrotropin, 17αOH-progesterone, cortisol) and metabolic (glucose, insulin, C-peptide, total cholesterol, high-density lipoprotein [HDL] cholesterol, low-density lipoprotein [LDL] cholesterol, triglycerides) determinations were collected at 8:00 to 9:00 AM after overnight fast. All participants underwent a fasting 75-g oral glucose tolerance test. Blood samples were drawn after 60 and 120 minutes for glucose, insulin, and C-peptide determination. The following indices of insulin resistance were calculated: homeostatic model assessment (HOMA) index = (fasting plasma insulin [in microunits per milliliter] × fasting plasma glucose [in milligrams per deciliter])/405 [22] and quantitative insulin sensitivity check index = 1/log fasting insulin (in microunits per milliliter) + log fasting glucose (in milligrams per deciliter). The free androgen index was calculated as testosterone (in nanomoles per liter)/SHBG (in nanomoles per liter) × 100.

### 2.3. Biochemical analysis

Fasting glucose, triglycerides, total cholesterol, HDL cholesterol, and LDL cholesterol were determined using Modular Analytics SWA (Roche, Basel, Switzerland). Insulin and C-peptide were measured by enzyme-linked immunosorbent assay (DRG, Marburg, Germany). Free testosterone was determined using a radioimmunoassay (DSL, Webster, TX). The SHBG (Roche), corticotropin, cortisol, HGH (human growth hormone) (DPC Bühlmann, Salzburg, Austria), insulin-like growth factor 1, prolactin, total testosterone, and thyrotropin (Bayer, Leverkusen, Germany) were measured by luminescence immunoassay.

### 2.4. Lipometry

Measurements of subcutaneous adipose tissue (SAT) thickness were performed by means of a patented optical device, "Lipometer" (EU patent no. 0516251), in a part of the study population. To describe the complete fat distribution of a person, 15 evenly distributed and anatomically well-defined body sites were specified from neck to calf on the right side of the body. The sensor head of the Lipometer contains a set of light-emitting diodes ($\lambda$ = 660 nm) and a photodetector [23]. To measure the thickness of a SAT layer, the sensor is held perpendicular to the selected body site. The SAT layer is illuminated by different light patterns varying in time. A photodiode measures the corresponding back-scattered light intensities of the light patterns and calculates the thickness of the SAT layer in millimeters. Calibration and evaluation were done using computer tomography as the reference method [23]. A detailed SAT profile of a subject is obtained by measuring the 15 specified body sites. The complete measurement cycle takes 2 minutes and is performed with the subject in an upright standing position.

Table 1
Characteristics of PCOS women according to *FTO* genotypes

| | All (N = 288) | | T/T (n = 87) | | A/T or A/A (n = 201) | | *P* value |
|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD | |
| *Clinical characteristics* | | | | | | | |
| Age (y) | 28 | 6 | 28 | 6 | 28 | 6 | .935 |
| Weight (kg) | 72.7 | 19.7 | 68.5 | 17.6 | 74.5 | 20.3 | .024 |
| Height (cm) | 166.2 | 6.0 | 166.5 | 6.1 | 166.1 | 6.0 | .631 |
| BMI (kg/m$^2$) | 26.3 | 6.9 | 24.7 | 6.2 | 27.0 | 7.1 | .011 |
| Waist circumference (cm) | 89.6 | 18.8 | 86.8 | 18.1 | 90.7 | 19.1 | .191 |
| Hip circumference (cm) | 106.5 | 13.4 | 104.3 | 13.0 | 107.4 | 13.6 | .127 |
| WHR | 0.84 | 0.10 | 0.83 | 0.09 | 0.84 | 0.10 | .557 |
| *Biochemical characteristics* | | | | | | | |
| Fasting glucose (mg/dL) | 84.5 | 10.9 | 83.9 | 9.8 | 84.8 | 11.4 | .510 |
| 1-h glucose (mg/dL) | 121.9 | 43.2 | 115.0 | 37.5 | 125.0 | 45.3 | .157 |
| 2-h glucose (mg/dL) | 101.7 | 34.0 | 95.5 | 28.5 | 104.4 | 35.9 | .047 |
| $AUC_{gluc}$ (mg/dL) | 107.5 | 30.6 | 102.3 | 26.3 | 109.8 | 32.2 | .091 |
| HOMA | 1.9 | 2.3 | 1.6 | 1.7 | 2.0 | 2.5 | .245 |
| QUICKI | 0.38 | 0.09 | 0.39 | 0.10 | 0.38 | 0.08 | .280 |
| Fasting insulin ($\mu$U/mL) | 8.7 | 8.9 | 7.4 | 6.6 | 9.2 | 9.7 | .227 |
| 1-h insulin ($\mu$U/mL) | 64.0 | 50.6 | 54.3 | 44.2 | 68.3 | 52.8 | .032 |
| 2-h insulin ($\mu$U/mL) | 53.1 | 48.6 | 45.4 | 42.2 | 56.5 | 50.9 | .112 |
| $AUC_{ins}$ ($\mu$U/mL) | 47.5 | 36.1 | 40.5 | 31.7 | 50.7 | 37.6 | .038 |
| Fasting C-peptide (ng/mL) | 5.1 | 2.8 | 5.0 | 3.0 | 5.2 | 2.7 | .339 |
| 1-h C-peptide (ng/mL) | 13.6 | 3.5 | 13.3 | 3.6 | 13.7 | 3.4 | .408 |
| 2-h C-peptide (ng/mL) | 12.8 | 4.0 | 12.6 | 3.5 | 12.9 | 4.3 | .141 |
| Free testosterone (pg/mL) | 2.90 | 1.16 | 2.64 | 0.92 | 3.01 | 1.23 | .042 |
| SHBG (nmol/L) | 50.7 | 30.7 | 52.8 | 26.9 | 49.8 | 32.3 | .099 |
| FAI | 6.2 | 4.6 | 5.7 | 4.0 | 6.4 | 4.9 | .294 |
| Testosterone (ng/mL) | 0.66 | 0.27 | 0.65 | 0.23 | 0.67 | 0.29 | .923 |
| Androstenedione (ng/mL) | 2.7 | 1.1 | 2.7 | 1.1 | 2.7 | 1.2 | .772 |
| DHEAS ($\mu$g/dL) | 261.1 | 137.9 | 258.3 | 145.1 | 262.3 | 135.2 | .551 |
| Cholesterol (mg/dL) | 177.8 | 34.3 | 177.0 | 34.4 | 178.1 | 34.4 | .598 |
| Triglycerides (mg/dL) | 90.8 | 50.4 | 81.8 | 38.3 | 94.6 | 54.4 | .107 |
| HDL (mg/dL) | 65.5 | 16.7 | 65.5 | 14.4 | 65.5 | 17.7 | .752 |
| QChol/HDL | 2.9 | 0.8 | 2.8 | 0.8 | 2.9 | 0.9 | .651 |
| LDL (mg/dL) | 99.0 | 28.5 | 101.5 | 29.5 | 98.0 | 28.1 | .455 |
| | All (n = 157) | | T/T (n = 45) | | A/T or A/A (n = 112) | | *P* value |
| | Mean | SD | Mean | SD | Mean | SD | |
| *Fat measures* | | | | | | | |
| Body fat (%) | 25.3 | 5.4 | 23.7 | 5.7 | 25.8 | 5.2 | .016 |
| Total fat mass (kg) | 18.8 | 7.1 | 16.6 | 7.3 | 19.6 | 6.9 | .013 |
| Lean mass (kg) | 54.4 | 15.6 | 51.1 | 11.8 | 55.7 | 16.8 | .189 |
| SAT mass (kg) | 15.6 | 5.5 | 14 | 6.0 | 16.2 | 5.2 | .011 |
| VAT mass (kg) | 3.1 | 2.1 | 2.6 | 1.8 | 3.4 | 2.2 | .044 |
| Neck (mm) | 6.0 | 3.8 | 5.0 | 3.5 | 6.4 | 3.8 | .023 |
| Triceps (mm) | 12.4 | 3.8 | 11.7 | 4.4 | 12.6 | 3.6 | .024 |
| Biceps (mm) | 9.2 | 5.2 | 8.5 | 5.9 | 9.5 | 4.9 | .139 |
| Upper back (mm) | 6.9 | 3.2 | 5.5 | 2.9 | 7.4 | 3.2 | .003 |
| Front chest (mm) | 10.2 | 6.5 | 8.1 | 6.4 | 11 | 6.3 | .007 |
| Lateral chest (mm) | 11.4 | 7.1 | 9.5 | 7.7 | 12.2 | 6.7 | .018 |
| Upper abdomen (mm) | 11.2 | 5.1 | 10.2 | 5.5 | 11.6 | 5.0 | .131 |
| Lower abdomen (mm) | 11.7 | 4.5 | 11.7 | 5.6 | 11.7 | 4.1 | .709 |
| Lower back (mm) | 10.6 | 9.4 | 9.2 | 2.7 | 11.1 | 10.9 | .201 |
| Hip (mm) | 14.2 | 4.9 | 13.7 | 5.5 | 14.3 | 4.6 | .254 |
| Front thigh (mm) | 8.8 | 2.3 | 8.4 | 1.8 | 8.9 | 2.4 | .162 |
| Lateral thigh (mm) | 7.8 | 2.6 | 7.5 | 2.0 | 7.9 | 2.7 | .526 |
| Rear thigh (mm) | 6.7 | 2.2 | 6.6 | 1.9 | 6.7 | 2.4 | .748 |
| Inner thigh (mm) | 10.2 | 3.0 | 9.6 | 2.6 | 10.5 | 3.1 | .094 |
| Calf (mm) | 5.0 | 2.0 | 4.8 | 1.6 | 5.1 | 2.1 | .386 |

Differences between *FTO* genotypes were assessed using Mann-Whitney *U* test. A/A indicates homozygous carriers of the A allele; A/T, heterozygous carriers; T/T, noncarriers; WHR, waist to hip ratio; QUICKI, quantitative insulin sensitivity check index; FAI, free androgen index; QChol, ratio of total cholesterol to HDL cholesterol.

### 2.5. Genetic analysis

Blood samples were collected in tubes containing EDTA as anticoagulant. DNA was extracted using the NucleoSpin Blood method (Clontech Laboratories, Mountain View, CA). Genotyping of rs9939609 was performed by allelic discrimination real-time polymerase chain reaction using the 40× Assay Mix (Applied Biosystems, Foster City, CA). The assay (no. C__30090620_10) consist of unlabeled polymerase chain reaction primers and TaqMan MGB probes (FAM™, and VIC® dye labeled).

### 2.6. Statistical analysis

Data are presented as means ± standard deviation (SD) unless otherwise stated. Statistical analysis was performed using SPSS version 16.0 (SPSS, Chicago, IL). Because of the low number of A/A homozygotes, the genotype was analyzed using the dominant-allele model, with the A allele being dominant [24]. Nominal variables were analyzed using the $\chi^2$ and Fisher exact tests. Shapiro-Wilk test was used to examine for normal distribution. Differences in continuous parameters between genotypes were assessed using analysis of variance, Mann-Whitney *U* test, and Kruskal-Wallis test. Genetic association analyses were performed by multivariate analyses with logistic regression analysis to examine correlations between the *FTO* genotype as dependent variable and BMI, HOMA index, 2-hour stimulated glucose, 2-hour stimulated insulin, free testosterone, and LDL cholesterol as independent variables as described previously [18,25]. To avoid multicollinearity, parameters that are highly correlated ($r > 0.6$) among each other were analyzed; variables with redundant information were excluded. A *P* value <.05 was considered significant.

## 3. Results

Table 1 shows clinical and biochemical characteristics and SAT topography of PCOS women.

### 3.1. Allelic frequencies

The frequency of the A allele was 0.438 in PCOS women. Hardy-Weinberg equilibrium was stable in PCOS women.

The T/T genotype was present in 30.2% (n = 87) of PCOS women, the allelic frequency of the A/T genotype was 52.1% (n = 150) in PCOS women, and 17.7% (n = 51) of PCOS women were A/A.

### 3.2. BMI and genotype

The association of *FTO* rs9939609 with clinical and biochemical parameters in PCOS women was examined by means of the Mann-Whitney *U* test (Table 1). The A allele of rs9939609 was associated with a significant increase in weight and BMI in PCOS women.

The A/T + A/A genotype was more prevalent in overweight/obese (BMI ≥25) PCOS patients (allelic frequency of the A allele, 77.5%) when compared with lean (BMI <25) PCOS patients (allelic frequency of the A allele, 62.2%) (*P* = .028; odds ratio [OR] = 1.91; 95% confidence interval [CI], 1.07-3.42)

Table 2
Association of clinical and biochemical characteristics with the A allele of *FTO* gene in PCOS women (logistic regression analysis, *B* = 0.86)

| | *P* value | OR | CI |
|---|---|---|---|
| BMI | .101 | 1.06 | 0.99-1.14 |
| HOMA | .835 | 0.98 | 0.79-1.21 |
| 2-h glucose | .942 | 1.00 | 0.99-1.01 |
| 2-h insulin | .953 | 1.00 | 0.99-1.01 |
| LDL | .229 | 0.99 | 0.98-1.01 |
| Free testosterone | .025 | 1.54 | 1.06-2.25 |

### 3.3. Metabolic parameters and genotype

#### 3.3.1. Oral glucose tolerance test

Of the PCOS patients, 18.5% presented with a pathologic oral glucose tolerance test. In these patients, the A/T + A/A genotype was more prevalent (86.0%) compared with patients with normal glucose tolerance (65.6%) (*P* = .009; OR = 3.23; 95% CI, 1.30-8.06).

Moreover, PCOS women carrying the *FTO* A allele had significantly higher levels of 2-hour glucose, 1-hour insulin, and AUCins (area under the curve insulin) when compared with noncarriers of the A allele (Table 1).

### 3.4. Endocrine parameters and genotype

The PCOS women carrying the A allele had significantly higher levels of free testosterone (Table 1) than noncarriers.

In a logistic regression analysis including *FTO* genotype as dependent variable and BMI, HOMA, 2-hour glucose, 2-hour insulin, LDL cholesterol, and free testosterone, the A allele was significantly associated with free testosterone (Table 2).

### 3.5. Fat mass and genotype

Subcutaneous adipose tissue measurements were performed in a subgroup of 157 PCOS women. The SAT data are shown in Table 1. A significantly positive association was seen between the A/T + A/A genotype and total body fat (percentage), total fat mass, visceral adipose tissue (VAT) mass, as well as subcutaneous fat mass. Furthermore, we found a significant increase in SAT layers at measure points neck, triceps, upper back, front chest, and lateral chest.

## 4. Discussion

This study provides the first evidence that, in women with PCOS, variants of the *FTO* gene are associated with hyperandrogenemia. Circulating levels of free testosterone were significantly associated with the A allele of rs9939609, indicating an important role of *FTO* variants not only in

obesity and diabetes but also in hyperandrogenism in women with PCOS. This finding is in contrast to the observation of Barber et al [19] who did not find an association of *FTO* rs9939609 with circulating androgen levels. However, other results in the literature reported the association of *FTO* variants with android morphotype and hyperandrogenism [18], confirming our findings. Moreover, Barber et al [19] reported a significant association of *FTO* gene with PCOS. In this study, the association was not fully abolished by adjusting for BMI, which might allow speculation on a direct effect of *FTO* variants on PCOS development. The association of free testosterone with *FTO* variants supports this theory.

So far, the association of androgen levels with *FTO* variants in studies concerning obesity and diabetes has not been investigated [10,26]. Considering the high prevalence of PCOS (5%-10%) and the high risk of developing diabetes mellitus for PCOS women, it might be interesting to examine androgen levels in diabetes mellitus and *FTO* variants in further studies.

As expected, we found a significant association of the A allele with increased weight and BMI. Furthermore, the A allele was more prevalent in overweight/obese PCOS women. This finding is consistent with former results [14,27].

In agreement with a previous study, which reports the association of the A allele with increased fat mass [10], we found a significant association of the A allele with total fat mass, VAT mass, and SAT fat mass. This has been shown in our article by a new device selectively measuring SAT layers. We did not find a significant association of lean mass with *FTO* variants, which is in line with previous studies [10,27,28]. Furthermore, we detected increased SAT layers at trunk-located measure points (neck, triceps, upper back, front chest, and lateral chest) in carriers of the A allele, whereas no association was seen between the A allele and SAT layers of the legs. These results might deliver additional evidence supporting an etiologic role of *FTO* variants in the development of PCOS. Women with PCOS are known to suffer from central obesity, whereas leg fat is decreased [5,29]. Accordingly, our results suggest an association of the A allele with increased trunk-located SAT layers.

In PCOS women with impaired glucose tolerance, the A allele was more prevalent compared with women with normal glucose tolerance. In addition, PCOS women carrying the A allele had significantly higher 2-hour glucose, 1-hour insulin, and AUCins. Concordantly, Attaoua et al [18] reported the association of impaired glucose tolerance with *FTO* variants in PCOS women.

This study had limitations that should be noted. We tested a relatively low sample size of PCOS cases compared with previous studies reporting an association of *FTO* variants with PCOS [19]. Therefore, it is possible that our study was underpowered to detect small differences between *FTO* genotypes. Further studies with larger sample sizes and subgroup analysis of PCOS women are warranted to investigate the role of *FTO* variants in the development of PCOS.

In summary, we demonstrated that variants within the *FTO* gene might influence hyperandrogenemia in women with PCOS. Increased levels of free testosterone were significantly associated with the A allele of the *FTO* gene, indicating an important role of *FTO* variants not only in obesity and diabetes but also in hyperandrogenism in women with PCOS. Further studies with larger sample sizes will be performed to elucidate the role of *FTO* variants in PCOS women.

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