Gene 560 (2015) 25–29

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

FTO gene variants are not associated with polycystic ovary syndrome in women from Southern Brazil

Ramon B. Ramos [a], Poli Mara Spritzer [a,b,*]

[a] Gynecological Endocrinology Unit, Division of Endocrinology, Hospital de Clínicas de Porto Alegre (HCPA), Rua Ramiro Barcelos, 2350, Porto Alegre, RS 90035-003, Brazil
[b] Laboratory of Molecular Endocrinology, Department of Physiology, Universidade Federal do Rio Grande do Sul (UFRGS), Rua Ramiro Barcelos, 2350, Porto Alegre, RS 90035-003, Brazil

ARTICLE INFO

Article history:
Received 29 November 2014
Received in revised form 1 January 2015
Accepted 5 January 2015
Available online 12 January 2015

Keywords:
FTO gene
Single nucleotide polymorphism
Polycystic ovary syndrome
Glucose

ABSTRACT

Background and aims: Polycystic ovary syndrome (PCOS) is a common endocrine disorder, presenting polygenic traits as well as determined by environmental factors. Given the overlap between PCOS and obesity, we assessed the frequencies of SNPs rs9939609 and rs8050136 in intron 1 of the *FTO* gene and their haplotypes in women with PCOS and healthy controls with regular cycles from Southern Brazil and investigated their relationship with metabolic traits and endocrine parameters.
Subjects and methods: The sample comprised 298 women (199 with PCOS and 99 non-hirsute women with regular ovulatory cycles). *FTO* genotyping was done by real-time PCR. Haplotypes were constructed from the combination of both polymorphisms. Frequencies were inferred using PHASE 2.1.1 software.
Results: The distribution of rs9939609 (PCOS: 32.6% TT, 45.9% TA, 21.5% AA; controls: 33.3% TT, 49.0% TA, 17.7% AA) and rs8050136 (PCOS: 21.7% AA, 43.3% AC, 35.0% CC; controls: 14.9% AA, 48.9% AC, 36.2% CC) was similar between groups. The mean age of participants was 22.7 ± 7.1 years. Women with PCOS had significantly higher BMI, waist circumference, total testosterone, and FAI vs. controls. In the PCOS group, no differences between genotypes and haplotypes were found for clinical variables. The presence of at least one risk allele for polymorphisms rs9939609 and rs8050136 was associated with higher fasting glucose levels.
Conclusion: Our findings indicate that neither the *FTO* rs9939609 and rs8050136 polymorphisms nor its haplotypes are related to PCOS, but suggest an association between the presence of risk alleles of SNPs rs9939609 and rs8050136 in *FTO* and glucose levels in women from Southern Brazil.

1. Introduction

Polycystic ovary syndrome (PCOS) is a heterogeneous disease characterized mainly by chronic anovulation and manifestations of hyperandrogenism. Using the Rotterdam consensus diagnostic criteria, PCOS affects up to 18% (March et al., 2010). Obesity is one of the most prevalent features of PCOS (Graff et al., 2013; Peppard et al., 2001; ESHRE/ASRM, 2004; Apridonidze et al., 2005). A recent meta-analysis showed that women with PCOS had increased prevalence of overweight as well as obesity (Lim et al., 2012), and abdominal obesity is found in most women with the condition, in association with metabolic disturbances such as insulin resistance (IR) (Apridonidze et al., 2005). Indeed, the metabolic syndrome (MetS), defined as a cluster of central obesity, dyslipidemia, hypertension, and glucose intolerance, is found in 30–40% of women with PCOS, and 25–35% of obese women with PCOS are diagnosed with impaired glucose tolerance or diabetes around the age of 30. This prevalence is nearly twofold higher than that for age-adjusted women in the general population (Gambineri et al., 2002; Apridonidze et al., 2005; Carmina et al., 2006; Wild et al., 2010). In general, women with PCOS have 3–7 times more risk of developing diabetes mellitus type 2 (DM2) compared with the general population (Ehrmann et al., 1999; Legro et al., 1999).

Individual susceptibility to PCOS is determined by genetic and environmental factors. Among several candidate genes for obesity, the fat mass and obesity associated gene (*FTO*) has been recently identified. *FTO* is located on chromosome 16q12.2 (Frayling et al., 2007) and, statistically, remains the candidate gene with the largest effect size on obesity. The protein it encodes is a member of the nonheme dioxygenase (Fe(II)- and 2-oxoglutarate-dependent dioxygenases) superfamily (Gerken et al., 2007; Sanchez-Pulido and Andrade-Navarro, 2007), and is involved in various cellular processes, including DNA repair, fatty acid metabolism, and posttranslational modifications. However, to date, its link to body weight regulation remains unclear.

Thus far, studies have shown controversial results. Some investigations demonstrated an association with PCOS (Attaoua et al., 2008; Barber et al., 2008; Yan et al., 2009), whereas others found no such

Abbreviations: BMI, body mass index; BP, blood pressure; DM2, diabetes mellitus type 2; FAI, free androgen index; FTO, fat mass and obesity associated gene; Ht, haplotype; HDL-c, high density lipoprotein cholesterol; HWE, Hardy–Weinberg equilibrium; MetS, metabolic syndrome; OR, odds ratio; PCOS, polycystic ovary syndrome; SNP, single nucleotide polymorphism; TT, total testosterone.

* Corresponding author at: Division of Endocrinology, Hospital de Clínicas de Porto Alegre, Rua Ramiro Barcelos, 2350, Porto Alegre, RS 90035-003, Brazil.
E-mail address: spritzer@ufrgs.br (P.M. Spritzer).

http://dx.doi.org/10.1016/j.gene.2015.01.012
0378-1119/

relation (Ewens et al., 2011; Kim et al., 2012). This difference may be due to factors such as ethnicity or whether there was adjustment for body mass index (BMI) in each individual study.

A recent meta-analysis showed no association between *FTO* gene polymorphisms and PCOS susceptibility. On subgroup analysis, the association was significant in East Asians but not in Caucasians, suggesting an effect of ethnicity on the association. Further studies are required to assess the real impact of polymorphisms in the *FTO* gene on PCOS in other populations (Cai et al., 2014).

Within this context, the aim of the present study was to assess whether the prevalence of SNPs rs9939609 and rs8050136 in intron 1 of the *FTO* gene and their haplotypes differs between women with PCOS and healthy controls with regular cycles from Southern Brazil, and to investigate the relationship of these genetic factors with metabolic traits and endocrine parameters.

2. Material and methods

2.1. Patients

The study population included women of reproductive age recruited by advertisement in the local media between 2009 and 2013 and who met the inclusion criteria. The advertisement called for volunteers with hirsutism and irregular menses and also for volunteers without hirsutism and with regular menses (to be enrolled as controls). The enrollment was planned to include PCOS and controls participants in a proportion of 2:1.

The study population comprised 298 women: 199 patients with PCOS and 99 non-hirsute women with regular, ovulatory cycles (luteal phase progesterone >3.8 ng/ml). The mean age of participants was 22.7 ± 7.1 years. All participants had attended a university hospital in Southern Brazil (Hospital de Clínicas de Porto Alegre, state of Rio Grande do Sul). PCOS was diagnosed according to the Rotterdam criteria as the presence of two out of three of the following signs: 1) Oligomenorrhoea and/or chronic anovulation (≤9 cycles/year and/or luteal phase progesterone < 3.8 ng/mL), 2) clinical and/or biochemical hyperandrogenism, and 3) polycystic ovaries (PCO) on ultrasound examination. Diagnosis of PCOS also relied on exclusion of other hyperandrogenic disorders (ESHRE/ASRM, 2004). None of the PCOS or control participants had received any drugs known to interfere with hormone levels (such as oral contraceptive pills, antiandrogens, metformin, fibrates, or statins) for at least 3 months before the study. The exclusion criteria were pregnancy, liver disease, or kidney disease.

2.2. Study protocol

Anthropometric measurements included body mass index (BMI) (current weight in kg divided by the height in m squared) and waist circumference (measured at the midpoint between the lower rib margin and the iliac crest) (Toscani et al., 2007). Blood pressure was measured after a 10-minute rest, with the patient seated, with both feet on the floor, and the arm supported at heart level. Two measurements were obtained 10 min apart using an Omron HEM-742INT automatic blood pressure monitor (Rio de Janeiro, Brazil) with the correct cuff size for the arm diameter. MetS and the cutoffs for its isolated components were defined in accordance with the Joint Scientific Statement (Alberti et al., 2009). A Z score was calculated for metabolic syndrome ([value − mean] / SD) to define metabolic risk clustering on a continuous scale. The Z scores were multiplied by −1 if necessary to indicate higher metabolic risk increasing value. A lower risk score is indicative of a better metabolic profile.

Approval for this study was obtained from the Institutional Review Board and the local Research Ethics Committee, functioning according to the 3rd edition of the Guidelines on the Practice of Ethical Committees in Medical Research issued by the Royal College of Physicians of London, and written informed consent was obtained from every subject.

2.3. Laboratory measurements

All samples were obtained between the 2nd and 10th days of the menstrual cycle, or on any day if the patient was amenorrheic, between 8:00 and 10:00 am, after a 12-h overnight fast. Oral glucose tolerance test (oGTT) was also tested. Blood samples were drawn from an antecubital vein for the determination of hormonal levels. Glucose levels were determined by enzymatic colorimetric methods (Bayer 1650 Advia System). Hormonal measurements were performed using commercial kits, as previously described (Nacul et al., 2007; Toscani et al., 2007; Wiltgen et al., 2009). The free androgen index was calculated as testosterone (nmol/L) / SHBG (nmol/L) × 100.

2.4. Genotyping

In addition to serum samples, whole blood samples were collected from all participants. Genomic DNA was extracted from peripheral leukocytes as reported elsewhere (Ramos et al., 2013). The DNA samples were diluted to 2 ng/mL and genotyped for SNP rs9939609 T > A and rs8050136 A > C of the *FTO* gene by real-time polymerase chain reaction (7500 Fast Real-Time Polymerase Chain Reaction System, Applied Biosystems, CA, USA), using the allelic discrimination assay with TaqMan MGB primers and probes (Applied Biosystems, CA, USA). TaqMan Master mix (2.5 μL), TaqMAn assay (0.25 μL), and H_2O (1.25 μL) were added for a final volume of 4 μL per sample, and 1 μL of DNA was added for a total reaction volume of 5 μL. Reaction conditions for the SNP rs9939609 were: 10 min at 95 °C after 50 cycles of denaturation at 95 °C (15 s) and annealing at 61 °C (1 min). Endpoint fluorescent readings were performed in the 7500 Fast System Sequence Detection Software version 1.4 environment. The internal quality of genotype data was assessed by typing 20% of blinded samples in duplicate.

Haplotypes were constructed from the combination of the two *FTO* polymorphisms (rs9939609 and rs8050136), and their frequencies were inferred using the PHASE 2.1.1 program (Stephens et al., 2001). The first letter of each haplotype refers to the rs8050136 polymorphism, and the second to the rs9939609 polymorphism.

2.5. Statistical analysis

Results are presented as mean ± standard deviation for normally distributed variables or as median (interquartile range) for variables with a non-Gaussian distribution. The Kolmogorov–Smirnov test and descriptive statistics were used to evaluate the distribution of data. Non-Gaussian variables were log-transformed for statistical analysis with an independent Student *t* test and one-way ANOVA and reported as back-transformed into their original units. Binary logistic regression analyses were performed to examine the ORs of PCOS (dependent variable) with genotype. The chi-square test was used to compare categorical variables, and to assess deviation of the genotype frequencies from Hardy–Weinberg equilibrium. Previous studies have shown that the rs8050136 "A" allele is in strong linkage disequilibrium with the rs9939609 "A" allele (Frayling et al., 2007; Hotta et al., 2008; Ramos et al., 2011). For each studied polymorphism, patients were divided into two groups: one combined heterozygous and risk homozygous subjects into a single group and the second comprised non-risk homozygous subjects. For rs9939609, we combined TA (heterozygous) and AA (polymorphic homozygous) as one group and wild type TT (wild homozygous) as the second group, with a dominant model; for rs8050136, we combined AC (heterozygous) and AA (wild homozygous) as one group and CC (polymorphic homozygous) as the second group, with a recessive model. All analyses were carried out in SPSS version 18.0 (SPSS, Inc., Chicago, IL, USA). Findings were considered significant at $p < 0.05$.