Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy

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RAPID COMMUNICATION

Association of *FTO* and *PPARG* polymorphisms with obesity in Portuguese women

This article was published in the following Dove Press journal:
Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy
10 July 2013
Number of times this article has been viewed

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Purpose: We evaluated the association between risk of obesity in the Portuguese population and two obesity-related single-nucleotide gene polymorphisms: *fat-mass and obesity-associated* (*FTO*) rs9939609 and *peroxisome proliferator-activated receptor gamma* (*PPARG*) rs1801282.

Patients and methods: A total of 194 Portuguese premenopausal female Caucasians aged between 18 and 50 years (95 with body mass index [BMI] ≥30 g/m^2, 99 controls with BMI 18.5–24.9 kg/m^2) participated in this study. The association of the single-nucleotide polymorphisms with obesity was determined by odds ratio calculation with 95% confidence intervals.

Results: Significant differences in allelic expression of *FTO* rs9939609 ($P<0.05$) were found between control and case groups, indicating a 2.5-higher risk for obesity in the presence of both risk alleles when comparing the control group with the entire obese group. A fourfold-higher risk was found for subjects with class III obesity compared to those with classes I and II. No significant differences in BMI were found between the control and case groups for *PPARG* rs1801282 ($P>0.05$).

Conclusion: For the first time, a study involving an adult Portuguese population shows that individuals harboring both risk alleles in the *FTO* gene locus are at higher risk for obesity, which is in agreement to what has been reported for other European populations.

Keywords: rs9939609, rs1801282, BMI, SNP, odds ratio

Introduction

Obesity prevalence has grown dramatically in recent decades and shows no signs of decline. According to the World Health Organization (WHO), it is estimated that 1.5 billion people are overweight, of which 500 million are obese.[1] Obesity and overweight result from a combination of genetic background, environmental, and lifestyle factors, and are intrinsically associated with increased risk of associated disease, such as hypertension, dyslipidemia, and type 2 diabetes.[2] Several gene-association studies have led to the identification of different loci (single nucleotide polymorphisms [SNPs]) that contribute to obesity and overweight.[3] One of these SNPs, rs9939609, in the *fat-mass and obesity-associated* (*FTO*) gene, has been described as a risk factor to obesity, and strongly associated with body mass index (BMI) increments in European adults.[4] Frayling and colleagues[4] demonstrated that the presence of the risk allele A is cumulative and represent a 20% higher risk for the development of obesity and 13% for the development of overweight. This association was later confirmed by several other studies in different populations.[5–7] Another gene playing an important role in

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obesity is *peroxisome proliferator-activated receptor gamma* (*PPARG*), which regulates the adipocyte differentiation, thus influencing BMI, as well as glucose metabolism.[8] In particular, SNP rs1801282 has been associated with obesity in different populations, with a clear identification of the risk allele G.[9–11]

To date, there are no data on the involvement of either of these SNPs in obesity in the adult Portuguese population, and whether the same pattern of risk alleles is present. Here, we report on the first association study between these SNPs and obesity for the adult Portuguese population, which can provide useful data for the clinical management and risk assessment of obesity.

Materials and methods

Subjects

All 194 subjects participating in the study were premenopausal Caucasian Portuguese females between 18 and 50 years old, duly informed about the study and having signed an informed consent.

As a control group were 99 healthy subjects showing a BMI ranging between 18.5 and 24.9 kg/m^2 with body-weight variation inferior to 10% in the last year. These subjects were either selected during a routine health check or belonged to the staff of Curry Cabral Hospital (Lisbon, Portugal).

The case group was composed of 95 subjects showing a BMI ≥30 kg/m^2 with body-weight variation inferior to 10% in the last year. These subjects were all attending the Endocrinology Department of Curry Cabral Hospital.

Sample collection

Samples were collected from peripheral total blood and preserved at −80°C. For analysis, 2 mL of blood was transferred to individual FTA (Whatman, Maidstone, UK) microcards, and DNA was purified according to the manufacturer's protocol.

Polymorphism analysis

Polymerase chain reaction (PCR) amplifications were performed on a Biometra TGradient Thermocycler (Göttingen, Germany) in 25 µL final volume with Master Mix and DNA Surf Hot Taq Polymerase (10 U/µL) (Stab Vida, Lisbon, Portugal) with the following thermal cycling conditions: initial 15-minute denaturation at 96°C, followed by 30 amplification cycles of denaturation at 94°C for 1 minute, annealing at 59°C for 1 minute, elongation at 70°C for 1 minute, and a final elongation at 70°C for 5 minutes. Primers for *PPARG* locus (GenBank accession no NC_000003.11): PPARG-F 5′-CAATTCAAGCCCAGTCCTTT-3′ and PPARG-R 5′-TTATCTCTGTGCATGGCTCC-3′. Primers for *FTO* locus (GenBank accession no NC_000016.9): FTO-F 5′-GCAAAATGGCAACACACACT-3′ and FTO-R 5′-AACACCATCCTTGGGCTG-3′.

SNP identification was performed via direct sequencing. Sequencing reactions were carried out with 100 ng/100 bp of the previously PCR-amplified product using Big Dye version 3.1 technology (Life Technologies, Carlsbad, CA, USA) in an Applied Biosystems 3730XL DNA analyzer.

Statistical analysis

To determine the normality of the continuous variables (age), Student's *t*-test was used. To determine the differences between genotype groups of each SNP and anthropometric traits, one-way analyses of variance and a post hoc Bonferroni test were used. All odds ratio (OR) analysis was performed using binary logistic regression with 95% confidence interval (CI) to determine the risk of each loci to obesity and the respective *P*-value. All statistical analyses were carried out using SPSS software version 20 (IBM, Armonk, NY, USA).

Results

Table 1 presents the descriptive analyses of the subjects subdivided by their phenotype group. Figure 1 shows the population characterization by allele and genotype frequencies for *FTO* and *PPARG* SNPs. Significant differences ($P<0.05$) were found only among the different genotypes of *FTO* rs9939609 for BMI, fat mass, and waist circumference. No other anthropometric traits were statistically different for *FTO* rs9939609 or *PPARG* rs1801282. Genotype frequencies for *FTO* rs9939609 were 24.74% T/T, 56.70% A/T, and 18.56% A/A. When comparing case and control groups, no significant deviation from the Hardy–Weinberg equilibrium of allele frequencies was observed for this locus ($P=0.053$), with a majority of individuals being heterozygous (A/T). Data showed that the T allele is more frequent in subjects with BMI values between 18.5 and 24.9 kg/m^2, whereas the A allele is preeminent in subjects with BMI ≥30 kg/m^2.

For *PPARG* rs1801282, the allele frequencies were 80.93% for homozygous C/C, 1.03% for homozygous G/G, and 18.04% for heterozygous C/G. Again, no significant deviation from the Hardy–Weinberg equilibrium of allele frequencies was observed for *PPARG* rs1801282 ($P=0.97$).

The presence of the A allele in *FTO* rs9939609 does not per se confer risk for obesity in the studied population. However, significant differences in allele frequencies between

Table 1 Anthropometric data of all subjects subdivided by phenotype

Anthropometric measures	Total	Phenotype	
		Normal	Obese
n	194	99	95
Age (years)	34.19 (±8.22)	34.24 (±8.30)	34.12 (±8.14)
BMI (kg/m^2)	32.28 (±12.44)	21.42 (±1.69)	43.60 (±7.83)
Fat mass (kg)	33.59 (±22.43)	14.32 (±3.61)	54 (±14.88)
Fat mass (%)	36.22 (±12.25)	25.30 (±4.67)	47.61 (±5.30)
Waist (cm)	94.19 (±25.72)	71.75 (±5.85)	117.57 (±15.49)
Waist/hip ratio	0.80 (±0.09)	0.74 (±0.05)	0.87 (±0.08)

Notes: Obesity status: normal (BMI between 18.5 and 24.9 kg/m^2), obese (BMI ≥ 30 kg/m^2); all data presented as means ± standard deviation.
Abbreviation: BMI, body mass index.

the control and case groups were found for *FTO* rs9939609 ($P<0.05$), indicating a 2.5-fold higher risk for obesity for homozygous A/A individuals (OR=2.571, CI 1.048–6.308; $P=0.039$). Comparison of homozygous A/A individuals with T allele carriers (either homozygous T/T or heterozygous A/T) clearly shows a significant association of homozygous A/A with obesity (OR=2.451, CI 1.145–5.243; $P=0.021$) (Table 2A).

What is more striking is the allelic expression of A/A homozygosity in subjects with a BMI ≥40 kg/m^2, ie, class III obesity. Considering this subgroup of obese women compared to those with class I and class II obesity, an OR=4.044 (CI 1.099–14.878; $P=0.035$) was found (Table 2B).

Analysis of *PPARG* rs1801282 showed no association with obesity ($P>0.05$) within the studied population.

Discussion

The worldwide prevalence of obesity has been increasing dramatically in the last few decades, and Portugal is no exception, where a 13.8% prevalence of obesity has been recorded.[12] Association studies have highlighted the influence of SNPs in obesity, with particular focus on *FTO* rs9939609.[13,14] Thus far, no data on the possible association of this SNP to obesity in the adult Portuguese population has been reported. Here, for the first time, we demonstrate an association between the *FTO* rs9939609 homozygous AA genotype and increased BMI when compared to homozygous TT. Significant differences were found between control and case group confirming the increased risk for obesity of homozygous AA at this locus. Also, with the post hoc Bonferroni test, it was

Anthropometric measures	*FTO* rs9939609 TT	AT	AA	*P*-value	*PPARG* rs1801282 CC	CG	GG	*P*-value
N	48	110	36	–	157	35	2	–
Age (years)	33.40 (±8.12)	34.35 (±8.13)	34.75 (±8.79)	0.728	34.32 (±8.37)	32.89 (±7.15)	46 (±5.67)	0.307
BMI (kg/m^2)	31.42 (±13.99)	30.99 (±11.49)	37.66 (±12.12)	0.024*	32.72 (±12.56)	29.97 (±12)	38.2 (±9.33)	0.487
Fat mass (kg)	31.55 (±23.98)	31.21 (±20.60)	43.55 (±23.55)	0.021*	34.55 (±22.61)	28.74 (±21.71)	42.48 (±13.7)	0.432
Fat mass (%)	34.68 (±11.88)	35.33 (±11.78)	41.04 (±13.27)	0.053	36.87 (±11.98)	32.74 (±13.08)	45.7 (±8.63)	0.283
Waist (cm)	92.04 (±26.42)	91.61 (±24.43)	104.92 (±26.59)	0.030*	94.84 (±26)	90.51 (±24.86)	107 (±18.38)	0.406
Waist/hip ratio	0.79 (±0.08)	0.79 (±0.09)	0.84 (±0.11)	0.112	0.80 (±0.09)	0.79 (±0.09)	0.89 (±0.06)	0.641

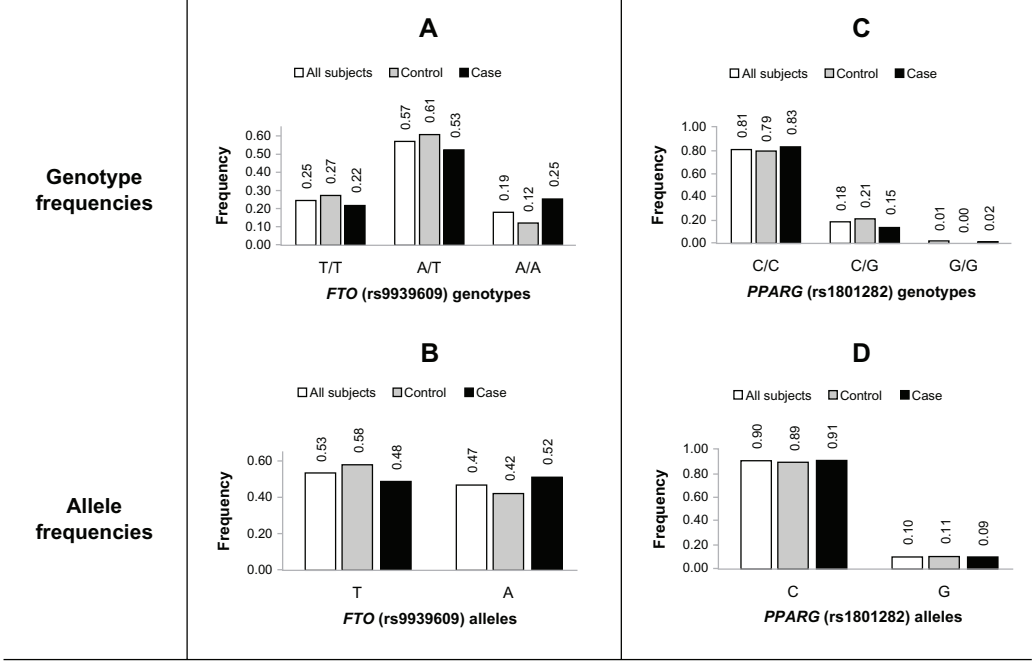

Figure 1 Population characteristics, in function of the respective genotype (upper). Genotype and allele frequencies (bottom) obtained for each single-nucleotide polymorphism. White bars – all subjects, grey bars – controls and black bars – case. (**A**) Genotype frequencies for *fat-mass and obesity-associated* (*FTO*) rs9939609; (**B**) allele frequencies for *FTO* rs9939609; (**C**) genotype frequencies for *peroxisome proliferator-activated receptor gamma* (*PPARG*) rs1801282; (**D**) allele frequencies for *PPARG* rs1801282.
Note: *Significant differences between groups were found for these cases.

Table 2 Odds ratio (OR) values between case and control groups for risk to obesity for allele A in *fat-mass and obesity-associated* (*FTO*) rs9939609 and G in *peroxisome proliferator-activated receptor gamma* (*PPARG*) rs1801282, and between BMI ≥30–<40 kg/m^2 and BMI ≥40 kg/m^2 for risk for obesity for allele A in *FTO* rs9939609 and allele G in *PPARG* rs1801282 only in the case group

	***FTO* (rs9939609)**			***PPARG* (rs1801282)**		
		OR[a] (95% CI)	***P*-value**		**OR[a] (95% CI)**	***P*-value**
Case vs control	T/T	1 (reference)	–	C/C	1 (reference)	–
	A/T	1.071 (0.541–2.121)	0.843	C/G	0.658 (0.312–1.387)	0.271
	A/A	2.571* (1.048–6.308)	0.039	C/C + G/C[b]	1 (reference)	–
	T/T + A/T[b]	1 (reference)	–	G/G	0.752 (0.366–1.548)	0.439
	A/A	2.451* (1.145–5.243)	0.021			
Case vs case	T/T + A/T[b]	1 (reference)	–	C/C + G/C[b]	1 (reference)	–
	A/A	4.044* (1.099–14.878)	0.035	G/G	0.431 (0.026–7.134)	0.556

Notes: [a]All ORs were calculated by logistic regression; [b]values were considered as reference; *significant difference found.

possible to determine that individuals with both A alleles in *FTO* rs9939609 show 6.37±2.35 (P=0.022) higher BMI, 11.99±4.86 kg (P=0.043) higher body-fat mass and 13.31±4.87 cm (P=0.020) higher waist circumference compared to T-allele carriers. These data are in clear agreement with what has been reported for other populations of European origin.[5–7,15,16] Our data show that in the adult Portuguese population, this polymorphism confers an even higher risk for class III obesity (BMI >40 kg/m^2). This may allow identification of those individuals at increased risk and target them for an earlier clinical and lifestyle intervention. This idea has been recently reinforced by the report of Albuquerque and colleagues showing a strong association of *FTO* rs9939609 with obesity in Portuguese children.[17] Together, these data may prove useful for a structured public health strategy within the European Union.

Conversely, *PPARG* rs1801282 showed no association with obesity within the studied population, as no significant difference was found between control and case subjects (P>0.05). In European populations, *PPARG* rs1801282 has shown association with obesity and higher BMI for homozygous carriers of the G allele (P<0.05).[10] Nevertheless, no association was found in other studies,[18–20] indicating that this SNP may differ between populations and probably should not be considered as a strong genetic marker to evaluate risk for obesity as *FTO* rs9939609 is. Despite reports on a relation between *PPARG* rs1801282 and diabetes type 2,[21] we observed no relation in our study (P>0.05). The relevance of *PPARG* rs1801282 as a genetic marker to assess risk for obesity and high insulin levels in premenopausal Portuguese women is, therefore, negligible.

Conclusion

Thus far, this is the first association study involving obesity-related genetic polymorphisms in the adult Portuguese population. Data show that *FTO* rs9939609 could be useful for the clinical management of obese women. Nevertheless, additional data are required, namely inclusion of males and youth subjects, to fully characterize the involvement of these loci in the development of obesity within the Portuguese population.

Acknowledgments

This work was supported by Stab Vida, Lda; FCT/MEC (PEst-OE/SAU/UI0009/2011 – CIGMH) and SFRH/BDE/51103/2010 for FFC.

Disclosure

The authors report no conflicts of interest in this work.

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