Original Paper

Effect of the *FTO* rs9930506 Polymorphism on the Main Comorbidities of the Cardiorenal Metabolic Syndrome in an Elderly Spanish Cohort

Eliecer Coto^{a, d} Beatriz Tavira^a Juan Gómez^a Salvador Tranche^c
Carmen Díaz Corte^{b, d}

^aGenética Molecular-Laboratorio Medicina-Fundación Renal (IRSIN-FRIAT), and ^bNefrología, Hospital Universitario Central de Asturias, ^cCentro de Salud El Cristo, and ^dDepartamento de Medicina, Universidad Oviedo, Oviedo, Spain

Key Words

FTO polymorphisms · Body mass index · Obesity · Glomerular filtration · Renal function

Abstract

Background/Aim: Fat mass and obesity-associated (*FTO*) gene polymorphisms have been linked to the risk of obesity and diabetes, two well-recognized risk factors for renal disease. Our aim was to determine whether a common *FTO* polymorphism was associated with a reduced estimated glomerular filtration rate (eGFR) independently of body mass index (BMI) and type 2 diabetes mellitus (T2DM) in a cohort of elderly individuals from the region of Asturias (Northern Spain; RENASTUR cohort). **Methods:** A total of 544 Spanish Caucasians aged 55–85 years were genotyped for the *FTO* rs9930506 single-nucleotide polymorphism (SNP). Individuals with a previous diagnosis of renal disease were not eligible for the study. The eGFR was calculated with the Modification of Diet in Renal Disease formula, and individuals with an eGFR of <60 ml/min/1.73 m² (n = 91) were considered as having impaired renal function. The effect of alleles and genotypes on BMI, hypertension, diabetes, eGFR and blood lipid values was statistically determined. **Results:** The rs9930506 GG genotype was significantly more common in the group with a BMI of >25 (p = 0.03; odds ratio = 2.43; 95% CI: 1.09–5.43). Age and T2DM were significant risk factors for a reduced eGFR, but neither obesity nor the *FTO* genotypes were associated with a reduced eGFR. **Conclusion:** The common *FTO* rs9930506 polymorphism was a risk factor for overweight and obesity in the RENASTUR cohort. However, this SNP was not associated with other comorbidities of the cardiorenal metabolic syndrome in this population.

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Eliecer Coto
Genética Molecular
Hospital Universitario Central de Asturias
ES–33006 Oviedo (Spain)
E-Mail eliecer.coto@sespa.princast.es

Introduction

It is a well-established fact that chronic kidney disease (CKD) is more prevalent among individuals with obesity [1, 2]. In fact, the two conditions coexist with hypertension, dyslipidaemia, insulin resistance and microalbuminuria in the so-called cardiorenal metabolic syndrome (CRS) [3–5]. Among other evidence, a follow-up of data on 177,570 individuals from a large integrated healthcare delivery system in Northern California established that proteinuria and excess weight were the most potent risk factors for end-stage renal disease (ESRD). Compared with normal weight, the adjusted hazard ratios ranged from 4.39 for severe obesity to 1.65 for overweight [6]. In a cohort of 320,252 adults followed up over a 15- to 35-year period, a total of 1,471 subjects developed ESRD, and the rate increased in a fashion proportional to their body mass index (BMI) [7]. Higher BMI was a risk factor for ESRD in multivariable models after adjustment for other CRS comorbidities. Compared with normal weight (BMI <25), the adjusted relative risk of ESRD rose from 1.87 for overweight (BMI = 25.0–29.9) over 3.57 for class I obesity (BMI = 30.0–34.9) and 6.12 for class II obesity (BMI = 35.0–39.9) to 7.07 for extreme obesity (BMI ≥40). In a large Japanese cohort, the odds ratios (ORs) of BMI for developing ESRD were 1.27 ($p = 0.0002$) for men and 0.950 (not significant) for women after adjustment for age, systolic blood pressure and proteinuria [8].

Obesity is a complex trait with both inherited and acquired (behavioural and environmental) factors defining each individual's risk. The increased prevalence of obesity should be a consequence of the exposure of genetically susceptible individuals to an unhealthy diet and a sedentary lifestyle [9]. Among the several genes that have been linked to the risk of increased BMI, the fat mass and obesity-associated gene (*FTO*) has been associated with obesity in different populations [10–14]. The *FTO* single-nucleotide polymorphism (SNP) rs9930506 showed the strongest association with BMI in some studies [14, 15]. This SNP was in almost complete linkage disequilibrium (LD) with others in intron 1, defining an obesity-risk *FTO* haplotype. It remains to be discovered whether one of these SNPs is directly responsible for the association or their effect on BMI is a consequence of LD with a different functional variant, even though recent studies did not find evidence for an increased frequency of functional amino acid missense variants among obese (compared with lean) subjects [16, 17].

FTO is a nucleic acid demethylase with a function in the sensing of nutrients and the regulation of translation and growth [18, 19]. *FTO* is ubiquitously expressed, with the highest levels seen in the brain's hypothalamic nuclei, a region that regulates energy homeostasis [19]. In vitro cellular studies showed that the concentration of *FTO* is regulated by some nutrients. In particular, the fact that *FTO* mRNA and protein levels were dramatically down-regulated by total amino acid deprivation supported a role as a cellular sensor for amino acids [20]. A reduction of *FTO* in mice resulted in increased energy expenditure and a lean phenotype, while overexpression resulted in an increase in body and fat mass irrespective of whether the mice were fed with a high-fat diet or not [21]. This suggested that the increased body mass could result from an increase in food intake.

Recently, two studies have shown a significant association between *FTO* polymorphisms and CKD, an effect that was independent of BMI and diabetes [22, 23]. Our aim was to determine whether the *FTO* rs9930506 SNP was associated with a reduced estimated glomerular filtration rate (eGFR), BMI and type 2 diabetes mellitus (T2DM) in the RENASTUR population, a cohort of elderly Caucasians from Asturias (Northern Spain).

Table 1. Main values according to *FTO* rs9930506 genotypes

	All genotypes (n = 544)	GG (n = 139)	AG (n = 270)	AA (n = 135)
Age, years		71 (56–81)	70 (56–81)	71 (56–81)
BMI		29.97 (20.64–46.78)	29.36 (18.59–46.58)	30.03 (17.63–44.46)
BMI <25, n ^a	95 (18)	16 (17)	54 (57)	25 (26)
25 ≤ BMI < 30, n	229 (42)	63 (28)	110 (48)	56 (24)
BMI ≥30, n	220 (40)	60 (27)	106 (48)	54 (25)
T2DM, n	129 (24)	34 (26)	65 (50)	30 (24)
No T2DM, n	415 (76)	105 (25)	205 (50)	105 (25)
Hypertension, n	170 (31)	46 (27)	87 (51)	37 (22)
No hypertension, n	374 (69)	93 (25)	183 (49)	98 (26)
Dyslipidaemia, n	200 (37)	50 (25)	95 (47)	55 (28)
No dyslipidaemia, n	344 (63)	89 (26)	175 (51)	80 (23)
Total cholesterol, mg/dl		212 (132–321)	212 (95–317)	214 (100–335)
HDL-C, mg/dl		59 (33–101)	59 (22–156)	59 (33–108)
LDL-C, mg/dl		131 (62–239)	130 (47–217)	131 (53–249)
Triglycerides, mg/dl		121 (50–382)	120 (41–356)	121 (42–358)
eGFR <60 ml/min/1.73 m ² , n	91 (17)	22 (24)	48 (53)	21 (23)
eGFR ≥60 ml/min/1.73 m ² , n	453 (83)	117 (26)	222 (49)	114 (25)

Continuous variables are represented as means with ranges in parentheses. For categorical variables, figures in parentheses indicate percentages.

^a GG vs. AA+AG frequencies in BMI ≤25 compared with BMI >25: p = 0.032, OR = 1.86, 95% CI = 1.05–3.31.

Subjects and Methods

Study Population and Data Collection

This study was approved by the Ethics Committee of the Hospital Universitario Central de Asturias and involved a total of 544 individuals aged 55–85 years (49% male), all Caucasian and from the region of Asturias (Northern Spain; total population: 1 million). They were recruited through the Primary Healthcare Center of the city of Oviedo to evaluate renal function in healthy elderly people, as reported [24]. Individuals with a history of renal disease or who had suffered episodes of cardiovascular disease were not eligible for the study. The main characteristics of the study cohort are summarized in table 1.

Age, sex and smoking were self-reported, and the BMI was calculated by weight and height measured at the examination. Overweight was defined as a BMI in the range of 25–30, and obesity as a BMI of ≥30. Individuals with a documented history of T2DM or who were receiving antidiabetic drugs were considered as diabetics, while those with a history of hypertension or who were receiving antihypertensive drugs were considered as hypertensives. The biochemical profiles of all the participants were obtained from fasting blood samples collected by venipuncture. Participants who had total cholesterol >240 mg/dl, triglyceride >200 mg/dl or high-density lipoprotein cholesterol (HDL-C) <40 mg/dl, who had a prior diagnosis of dyslipidaemia or who were receiving lipid-lowering drugs were considered as having dyslipidaemia. The eGFR was calculated with the Modification of Diet in Renal Disease formula [25]:

$$\text{eGFR (ml/min/1.73 m}^2\text{)} = 186 \times [\text{plasma creatinine (mg/dl)}]^{-1.154} \times (\text{age})^{-0.203} \times (0.742 \text{ if female}) \times (1.212 \text{ if black}).$$

FTO Genotyping

DNA was obtained from 5 ml of blood leukocytes and the *FTO* rs9930506 (A/G) genotypes were determined via a real-time TaqMan assay (assay ID C_29819994_10; Life Technologies). To confirm the accuracy of this genotyping method, PCR fragments from several individuals with each of the genotypes were sequenced using BigDye chemistry and an ABI3130xl device (Applied Biosystems).

Table 2. Univariate and multivariate binary logistic regression analysis

	Univariate analysis		Multivariate analysis	
	p values	OR (min–max)	p values	OR (min–max)
Age	<i>0.002</i>	<i>0.91 (0.84–0.93)</i>	<i>0.01</i>	<i>0.94 (0.82–0.92)</i>
Male	<i>0.08</i>	<i>0.89 (0.85–1.01)</i>		
BMI	0.23	0.98 (0.93–1.04)		
Obesity (BMI >30)	0.27	1.55 (0.71–3.37)		
Total cholesterol	<i>0.03</i>	<i>1.01 (1.00–1.02)</i>	<i>0.37</i>	<i>1.00 (0.98–1.02)</i>
LDL-C	0.06	1.02 (0.99–1.02)		
HDL-C	0.25	1.03 (0.99–1.04)		
Triglycerides	0.09	0.99 (0.98–1.02)		
HTA (yes)	0.13	1.27 (0.85–2.24)		
Smoker (yes)	0.15	1.85 (0.97–3.29)		
T2DM (yes)	<i>0.01</i>	<i>2.39 (1.42–4.01)</i>	<i>0.02</i>	<i>1.89 (1.21–3.45)</i>
Dyslipidaemia (yes)	0.29	1.15 (0.79–1.79)		
<i>FTO</i> rs9930506 GG	<i>0.74</i>	<i>0.92 (0.54–1.54)</i>		

The dependent variable is eGFR (<60 vs. ≥60 ml/min/1.73 m²). Significant results are set in italics.

Statistical Analysis

Fisher's exact and Student's *t* tests were used to compare categorical and continuous variables between the groups, respectively. Variables that were significantly associated with overweight, obesity or an eGFR of <60 ml/min/1.73 m² in the univariate analysis were included in the multivariate logistic regression analysis. ORs and 95% CIs were also calculated. *p* < 0.05 was considered as statistically significant.

Results

Table 1 summarizes the main characteristics of the studied cohort according to *FTO* rs9930506 genotypes. The genotype frequencies observed did not deviate from the expected Hardy-Weinberg equilibrium. The two alleles showed the same frequency (0.50), which is close to that reported in other Caucasian populations (http://www.ensembl.org/Homo_sapiens/Variation). There were no significant differences in mean BMI between the genotypes. We observed a higher frequency of the GG genotype in individuals with a BMI of ≥25 (*p* = 0.032; OR = 1.86; 95% CI: 1.05–3.31). Assuming the lower frequency in the lean group (17 vs. 28%), at *p* < 0.05 and a ratio of 4.72 between BMI <25 and BMI ≥25, a total of 168 and 724 participants would be required for a statistical power of 80.

There were no significant differences in genotype frequency between T2DM and nondiabetics, normotensives and hypertensives, or individuals with and without hyperlipidaemia. There were no significant differences between the *FTO* genotypes in mean total cholesterol, HDL-C, LDL-C and triglyceride levels (table 1).

An impaired renal function (eGFR <60 ml/min/1.73 m²) was significantly associated with older age (*p* = 0.002), higher total cholesterol (*p* = 0.03) and T2DM (*p* = 0.01; table 2). The frequency of individuals with an eGFR of <60 ml/min/1.73 m² was higher in obese than in lean individuals (18 vs. 11%), but without a significant difference. The *FTO* genotype frequencies did not differ between the group with an eGFR of <60 ml/min/1.73 m² and that with an eGFR of ≥60 ml/min/1.73 m². In the multivariate analysis, age and T2DM remained significant risk factors for an eGFR of <60 ml/min/1.73 m² (table 2).

Discussion

We confirmed the reported association between the *FTO* rs9930506 SNP and overweight (BMI >25) in a healthy elderly Northern Spanish cohort. A study based on 6,148 Sardinians showed a strong association between this SNP and BMI, with the mean BMI increasing with the number of G copies (27.9 > 26.9 > 26.4) [14]. We did not observe this strong effect in our population, mainly because there were no differences between the GG and AA individuals (27.97 vs. 30.03; mean BMI in AG+AA: 29.56). The effect of *FTO* SNPs on BMI has also been ascertained in two different Spanish cohorts of 1,425 and 869 adults (mean ages: 54.4 and 46.2 years) [26]. Although the authors of that study did not genotype the rs9930506 SNP, the SNPs analysed were in the same intron 1 LD block.

The most significant association was with allele A of the rs9939609 SNP, which produced an increase of 0.29 in BMI ($p = 0.02$) after combining the data from the two cohorts. A trend toward a decreased effect of *FTO* on BMI with an increase in age has been found in some studies (e.g. by Frayling et al. [13]). Thus, the lower effect of the *FTO* polymorphism on mean BMI found in our study could be explained in part by the higher mean age of our cohort.

We also analysed the effect of the *FTO* polymorphism on renal function measured as eGFR. At least two works have shown a significant association between *FTO* polymorphisms and CKD, an effect that was independent of BMI and diabetes [22, 23]. The mean eGFR values did not differ between the rs9930506 genotypes, and the genotype frequencies were almost identical between individuals with values of <60 ml/min/1.73 m² and those with values of ≥60 ml/min/1.73 m². Moreover, the *FTO* SNP was not associated with risk of T2DM, which was a significant predictor of an impaired renal filtration rate in our cohort, and neither the mean BMI nor obesity had a significant effect on the eGFR.

In conclusion, we showed that the *FTO* rs9930506 SNP was a modest but significant predictor of overweight in the elderly RENASTUR cohort. This polymorphism was not associated with the risk of developing other components of the CRS such as T2DM and dyslipidaemia and had no significant effect on reduced renal function.

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Disclosure Statement

None of the authors have competing interests related to this work.

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