

Dietary fatty acid distribution modifies obesity risk linked to the rs9939609 polymorphism of the FTO gene in a Spanish children case-control study.

Moleres A¹, Ochoa MC², Rendo-Urteaga T¹, Martínez-González MA³, Azcona-Sanjulián MC⁴, Martínez JA¹ and Marti A¹, on behalf of the GENOI study group.

¹ Department of Nutrition, Food Science, Physiology and Toxicology, University of Navarra, Pamplona, Spain

² Center for Applied Medical Research, University of Navarra, Pamplona, Spain

³ Department of Preventive Medicine and Public Health, University of Navarra, Pamplona, Spain

⁴ Paediatric Endocrinology Unit, Department of Pediatrics, Clínica Universidad de Navarra, Pamplona, Spain

Corresponding author: Dr. Amelia Marti. Department of Nutrition, Food Science, Physiology and Toxicology. University of Navarra. C/Irunlarrea s/n. 31008, Pamplona, Navarra, SPAIN.

Phone: +34 948425600

Fax: +34 948425649

E-mail: amarti@unav.es

Abstract

The rs9939609 polymorphism of the FTO gene has been widely associated with childhood obesity in several European cohorts. This association appears to be depending on dietary macronutrients. Therefore, our aim was to evaluate whether dietary fatty acid distribution intake could interact with this FTO genetic variation and obesity in a Spanish children and adolescents case-control study. 354 Spanish children and adolescents aged 6-18 (49% males) were genotyped for the rs9939609 variant of the FTO gene. Anthropometric parameters were taken and energy intake was measured. We observed an interaction between the percentage of saturated fatty acids (SFA) of diet over total energy intake as well as polyunsaturated/saturated (PUFA/SFA) fatty acid ratio and the association of the polymorphism with obesity risk. In our population the risk allele carriers consuming more than 12.6% SFA of their total energy value had an increased obesity risk compared with TT carriers. In the same way, A allele carriers that intake less than 0.43 PUFA/SFA ratio presented higher obesity risk than non A allele carriers. In summary, this study reports for the first time, the influence of dietary fatty acid distribution on the effect of the rs9939609 polymorphism of the FTO gene on obesity risk.

Key words: Childhood obesity, FTO, fatty acids intake, obesity risk

INTRODUCTION

According to the estimates from the International Obesity Taskforce, at least 155 million school-aged children worldwide are overweight or obese. Within that figure, around 30-45 million are classified as obese, accounting for 2-3% of the world's children aged 5-17 ⁽¹⁾. The European Association for the Study of Obesity (EASO) estimates that 16-22% of European adolescents between 14 and 17 years old are overweight or obese with an annual increase of the prevalence of around 2% in the 1990s and early 2000s ⁽²⁾. Childhood obesity is usually accompanied by associated comorbidities as type 2 diabetes mellitus, insulin resistance or the metabolic syndrome ^(3,4).

Concerning genetic origins of obesity, single-nucleotide polymorphisms (SNP) of the FTO (fat mass and obesity associated) gene have recently been strongly associated with excessive body weight for height and adiposity and, therefore, FTO has been identified as a candidate gene contributing to childhood obesity in several European cohorts ⁽⁵⁻⁷⁾. This gene, located in human chromosome 16, has been proposed to have a nucleic acid demethylation activity that might regulate the expression of genes involved in metabolism leading to obesity ⁽⁸⁾.

Carriers of minor frequency A allele of rs9939609, one of the most prevalent FTO genetic variants, have previously been identified with greater BMI in several studies ⁽⁹⁻¹¹⁾. Moreover this polymorphism has been reported to interact with energy intake patterns in children, with carriers of minor A allele consuming more fat and total energy than non-carriers ^(9,12,13).

In regard to specific macronutrient dietary composition, Sonestedt et al. (2009) have shown that fat and carbohydrate intake modify the association between the rs9939609 genetic variation in the FTO gene and obesity in a Swedish population ⁽¹⁴⁾.

Therefore our aim was to study whether dietary fat composition modifies the association between obesity risk and this FTO genetic variant (rs9939609) in a Spanish children and adolescents case-control study.

SUBJECTS AND METHODS

The study population included 354 Spanish children and adolescents (49% males) aged 6-18 years and enrolled in a case-control study (GENOI). The subjects were recruited

from the Paediatric Departments at the Virgen del Camino Hospital, Clínica Universidad de Navarra and other Primary Care Centres in Navarra (Spain). Cases were subjects with a body mass index (BMI) above the 97th percentile of the Spanish BMI reference data for age and sex ⁽¹⁵⁾. Exclusion criteria were exposure to hormonal treatment or development of secondary obesity due to endocrinopathy or serious intercurrent illness. Controls were healthy subjects with a BMI below the 97th percentile of the same reference.

Written consent to participate was requested from both parents and adolescents above 12 years old. The study protocol was performed in accordance with the ethical standards of the Declaration of Helsinki (as revised in Hong-Kong in 1989, in Edinburgh in 2000 and in South Korea in 2008), and was approved by the Ethics Committee of the University of Navarra.

Procedures

Trained researchers conducted face-to-face interviews with participants and their parents, based on standardized procedures. A semi-quantitative food-frequency questionnaire, previously validated in Spain ⁽¹⁶⁾, and containing 132 food items were filled in, in order to evaluate dietary patterns (complete data were available for 288 children and adolescents, 53% males). Familial medical history was collected by specific questionnaire. Weight and height were measured with an electronic scale (Type SECA 861) and with a telescopic height measuring instrument (Type SECA 225) respectively, to establish BMI-SDS according to Cole et al. criteria ⁽¹⁷⁾. Skinfolds were measured with a Holtain skinfold calliper and waist and hip circumferences with a flexible non-stretchable measuring tape (Type SECA 200) using validated protocols. Percentage of body fat was determined by bioelectrical impedance (TBF-300A Body Composition Analyzer/Scale, TANITA, Tokyo, Japan). Venous blood samples were collected to obtain DNA samples.

Genotyping

DNA was extracted from the buffy coat fraction using a commercial kit (Master PureTM; Epicentre, Madison, WI, USA). All the subjects were genotyped for the rs9939609 polymorphism of the FTO gene using Taqman SNP allelic discrimination (ABI PRISM 7900). The probes and the primers for these assays were designed by Applied Biosystems (Madrid, Spain). Replicate quality control samples were included in every genotyping plate with more than 99% of concordance.

Statistical Analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) software 15.0 (SPSS INC., Chicago, IL). A χ^2 test was used to evaluate the Hardy-Weinberg equilibrium. The Kolmogorov-Smirnov test was used to determine variable distribution.

The differences in anthropometric, biochemical and energy intake variables between the polymorphism genotypes were tested with analysis of the covariance (ANCOVA) adjusted for age, sex and energy intake (for normally distributed variables), or the Mann Whitney U test. Multivariate logistic regression models were fitted to assess the association between the genotypes and obesity risk after adjusting for relevant confounders. For the evaluation of the association between the FTO SNP and obesity risk, fat intake values were dichotomized by the median. The level of probability was set at $p < 0.05$ as statistically significant.

RESULTS

The distribution of genotypes for the rs9939609 polymorphism of the FTO gene variant was in Hardy-Weinberg equilibrium in the study population. The minor A allele frequency was 0.46. The prevalence of the polymorphism was higher in cases compared to controls both in the codominant and in the dominant model with a marginally statistical significance ($p = 0.060$ and $p = 0.098$ respectively, χ^2 test) (table 1).

As expected, anthropometric measurements as weight, BMI-SDS, waist and hip circumferences or fat mass, were significantly different ($p < 0.001$) between cases (208) and controls (146). Concerning energy intake, consumption of PUFA was significantly higher in the control group ($p = 0.002$), whereas obese subjects had a higher intake of MUFA ($p < 0.001$) (Table 1).

Logistic regression models were performed to study the association between this SNP of the FTO gene and obesity risk including a recessive (T carriers vs. AA), a dominant (TT vs. A carriers) and a codominant (TT vs. TA vs. AA) model adjusted for age, sex, total energy (kcal) and total fat (g) intake. The odds ratio for obesity increased for each additional A allele in the genotype (table 2). Children and adolescents homozygous for the mutation (AA) had higher risk of becoming obese than those without the mutation (OR=1.89; CI95%=1.06-3.30).

Next, we analyzed the effect of the rs9939609 mutation of the FTO gene on obesity risk depending on the fatty acid consumption. A relationship between saturated fatty acids

(SFA) intake and obesity was found (figure 1A). After dichotomizing for the median, a higher SFA intake ($\geq 12.6\%$) contributes to a greater obesity risk. An interaction term between the polymorphism and SFA consumption on BMI-SDS was also identified (p for interaction=0.035). In those subjects consuming less than 12.6% of total energy as SFA, the mutation did not seem to affect BMI-SDS, while A allele carriers with a higher intake of SFA had a significantly higher BMI-SDS ($p=0.009$) than TT carriers (Figure 2).

Besides, as seen in Figure 1B, the consumption of more than 0.43 of polyunsaturated/saturated (PUFA/SFA) fatty acids intake ratio seems to protect against obesity risk. Moreover we observed an interaction between the dominant model of the rs9939609 polymorphism of the FTO gene and the PUFA/SFA intake ratio (p for interaction =0.034) and a borderline statistical interaction between the SNP and SFA consumption (p for interaction=0.080).

DISCUSSION

In the present study, we confirm the previously reported association between the rs9939609 polymorphism of the FTO gene with obesity in a case/control children and adolescent population. Moreover, we characterize the role of dietary fat intake distribution and its interaction with this genetic variant as a risk factor for obesity.

The frequency of the minor A allele was 0.46, similar to those described in other Spanish populations⁽¹⁸⁾ and other European children and adolescents cohorts^(19,20).

Case-control studies have been demonstrated to be a suitable tool to screen the effects that a genetic polymorphism may have on obesity development. It has been previously reported that the accuracy of the phenotype measurement is crucial in the ability to detect gene-environment interactions for traits such as BMI⁽²¹⁾. On this point, our subjects were a homogeneous sample being cases and controls of similar age and belonging to the same social environment. As expected, control subjects showed statistically significant differences compared with obese children and adolescents in all measured anthropometric traits.

The rs9939609 polymorphism of the FTO gene has been largely related with greater adiposity. In regard to this observation, our data showed a significant increase in obesity risk per A allele present in the genotype. Homozygous carriers for the mutation are on greater risk of developing obesity than non carriers (OR=1.89). This increase in obesity

risk was similar to that observed by Wardle et al. ⁽²²⁾ in a British children population and slightly higher than those observed by other authors ^(10,23,24) both in children and adult cohorts.

Our results confirm that the effect of the mutation is influenced by dietary fatty acid composition. The percentage PUFA, SFA and PUFA/SFA ratio over total energy intake modulates obesity risk associated to the rs9939609 polymorphism of the FTO gene. Particularly, children and adolescents carrying the A allele of the mutation and consuming more than 12.6% of total energy as SFA or having less than 0.43 PUFA/SFA intake ratio, increased significantly the risk of being obese. In the same way, we observed an interaction between the polymorphism and the PUFA/SFA ratio; subjects with the polymorphism and having a PUFA/SFA ratio lower than 0.43 had a 2.3 times higher risk of becoming obese than those consuming a higher amount of PUFA and wild type for the mutation.

Genetic associations and fatty acid-gene interactions are widely explored in obesity onset and development ⁽²⁵⁾. In this sense, Luan et al., showed an interaction between PUFA/SFA dietary ratio intake and the Pro12Ala polymorphism of the PPAR γ gene. In this study population, Ala carriers decreased their BMI as the PUFA/SFA ratio increased, while wild-type homozygous were not affected by fatty acid intake ⁽²⁶⁾. Other genetic variants have also showed interactions with dietary fatty acid consumption on obesity risk. In 2007, Corella et al, found that a APOA5 gene variation modulates the effects of dietary fat intake on BMI and obesity risk in an adult American cohort ⁽²⁷⁾. Concerning SFA, a recent study carried out in Mediterranean and Asian populations has also shown a gene-saturated fat intake interaction on the association between the APOA2 promoter polymorphism and body weight ⁽²⁸⁾. All these studies demonstrate that fatty acid intake could affect obesity risk in a different way according to subject's individual genotype, and therefore, the study of these nutrient-gene interactions would be an important tool in obesity prevention and personalized nutrition.

In regard to the rs9939609 polymorphism of the FTO gene, there are some studies in children that link the SNP with a higher total energy and fat intake ^(9,12,13,20) and with diminished satiety sensation ⁽²²⁾. A recent study has showed that fat and carbohydrate intake modify the association between the rs9939609 genetic variation of the FTO and obesity ⁽¹⁴⁾, but specific dietary fatty acid composition and its interaction between this SNP and obesity risk has not been studied so far. A study carried out in our group by Razquin et al. ⁽¹⁸⁾ in high cardiovascular risk Spanish subjects aged 55-80 years, showed

that subjects carrying the A allele of the mutation gained significantly less weight compared to wild type subjects (TT) after 3 years of intervention with Mediterranean Diet. This diet is characterized by a high intake of PUFA and a low intake of SFA and confirms the implication of dietary fatty acid distribution in the relationship between FTO gene variant and body weight. . To our knowledge this is the first study linking fatty acid intake with the FTO rs9939609 polymorphism on obesity risk, nevertheless one limitation of our report is the sample size, and therefore, more studies in larger populations, leading to a better understanding about how dietary macronutrients and the rs9939609 polymorphism of the FTO gene could interact and modify obesity risk are needed to support these findings.

In summary, our study confirms that this variation in the FTO gene is associated with a higher obesity risk in this children and adolescents cohort. Moreover we report for the first time the influence of dietary fatty acid distribution on the effect of this polymorphism on obesity risk.

Acknowledgments

Research relating to this work was funded by grants from the Navarra Government (Health and Education Department) and Línea Especial, Nutrición y Obesidad (University of Navarra). The scholarships to A. Molerés from the Navarra Government and to T. Rendo-Urteaga from University of Navarra's Asociación de Amigos are fully acknowledged. A. Marti, JA. Martinez and MA. Martinez-Gonzalez were involved in the design, funding and writing of the manuscript. MC. Azcona-Sanjulián was involved in the design, conduct and writing of the manuscript. A. Molerés, T. Rendo-Urteaga and MC. Ochoa were involved in the conduct, analysis and writing of the manuscript. The authors have no competing interests.

REFERENCES

- 1 IOTF (2005). Global picture of childhood obesity. <http://www.who.int/dietphysicalactivity/childhoodobesity.asp>. (Accessed Decembre 2010).
- 2 Baker JL, Farpour-Lambert NJ, Nowicka P, Pietrobelli A, Weiss R. (2010) Evaluation of the overweight/obese child--practical tips for the primary health care provider: recommendations from the Childhood Obesity Task Force of the European Association for the Study of Obesity. *Obes Facts* **3**, 131-137.
- 3 Cali AM, Caprio S. (2008) Obesity in children and adolescents. *J Clin Endocrinol Metab* **93**, S31-36.
- 4 Ochoa MC, Santos JL, Azcona C *et al.* (2007) Association between obesity and insulin resistance with UCP2-UCP3 gene variants in Spanish children and adolescents. *Mol Genet Metab* **92**, 351-358.
- 5 Dina C, Meyre D, Gallina S *et al.* (2007) Variation in FTO contributes to childhood obesity and severe adult obesity. *Nat Genet* **39**, 724-726.
- 6 Frayling TM, Timpson NJ, Weedon MN *et al.* (2007) A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity. *Science* **316**, 889-894.
- 7 Haworth CM, Carnell S, Meaburn EL, Davis OS, Plomin R, Wardle J. (2008) Increasing Heritability of BMI and Stronger Associations With the FTO Gene Over Childhood. *Obesity (Silver Spring)* **16**, 2663-8.
- 8 Fawcett KA, Barroso I. (2010) The genetics of obesity: FTO leads the way. *Trends Genet* **26**, 266-274.
- 9 Cecil JE, Tavendale R, Watt P, Hetherington MM, Palmer CN. (2008) An obesity-associated FTO gene variant and increased energy intake in children. *N Engl J Med* **359**, 2558-2566.
- 10 Jacobsson JA, Danielsson P, Svensson V *et al.* (2008) Major gender difference in association of FTO gene variant among severely obese children with obesity and obesity related phenotypes. *Biochem Biophys Res Commun* **368**, 476-482.
- 11 Rendo T, Moleres A, Marti Del Moral A. (2009) Effects of the FTO Gene on Lifestyle Intervention Studies in Children. *Obes Facts* **2**, 393-399.
- 12 Timpson NJ, Emmett PM, Frayling TM *et al.* (2008) The fat mass- and obesity-associated locus and dietary intake in children. *Am J Clin Nutr* **88**, 971-978.

- 13 Wardle J, Llewellyn C, Sanderson S, Plomin R. (2009) The FTO gene and measured food intake in children. *Int J Obes (Lond)* **33**, 42-45.
- 14 Sonestedt E, Roos C, Gullberg B, Ericson U, Wirfalt E, Orho-Melander M. (2009) Fat and carbohydrate intake modify the association between genetic variation in the FTO genotype and obesity. *Am J Clin Nutr* **90**, 1418-1425.
- 15 Sobradillo B. (2004) *Curvas y tablas de crecimiento (estudios longitudinal y transversal)*. Bilbao, Fundacion Faustino Orbegozo Eizaguirre.
- 16 Martin-Moreno JM, Boyle P, Gorgojo L *et al.* (1993) Development and validation of a food frequency questionnaire in Spain. *Int J Epidemiol* **22**, 512-519.
- 17 Cole TJ, Bellizzi MC, Flegal KM, Dietz WH. (2000) Establishing a standard definition for child overweight and obesity worldwide: international survey. *Bmj* **320**, 1240-1243.
- 18 Razquin C, Martinez JA, Martinez-Gonzalez MA, Bes-Rastrollo M, Fernandez-Crehuet J, Marti A. (2010) A 3-year intervention with a Mediterranean diet modified the association between the rs9939609 gene variant in FTO and body weight changes. *Int J Obes (Lond)* **34**, 266-272.
- 19 Muller TD, Hinney A, Scherag A *et al.* (2008) 'Fat mass and obesity associated' gene (FTO): no significant association of variant rs9939609 with weight loss in a lifestyle intervention and lipid metabolism markers in German obese children and adolescents. *BMC Med Genet* **9**, 85.
- 20 Tanofsky-Kraff M, Han JC, Anandalingam K *et al.* (2009) The FTO gene rs9939609 obesity-risk allele and loss of control over eating. *Am J Clin Nutr* **90**, 1483-1488.
- 21 Andreasen CH, Stender-Petersen KL, Mogensen MS *et al.* (2008) Low physical activity accentuates the effect of the FTO rs9939609 polymorphism on body fat accumulation. *Diabetes* **57**, 95-101.
- 22 Wardle J, Carnell S, Haworth CM, Farooqi IS, O'Rahilly S, Plomin R. (2008) Obesity associated genetic variation in FTO is associated with diminished satiety. *J Clin Endocrinol Metab* **93**, 3640-3643.
- 23 Loos RJ, Bouchard C. (2008) FTO: the first gene contributing to common forms of human obesity. *Obes Rev* **9**, 246-250.

- 24 Price RA, Li WD, Zhao H. (2008) FTO gene SNPs associated with extreme obesity in cases, controls and extremely discordant sister pairs. *BMC Med Genet* **9**, 4.
- 25 Smith CE, Ordovas JM. (2010) Fatty acid interactions with genetic polymorphisms for cardiovascular disease. *Curr Opin Clin Nutr Metab Care* **13**, 139-144.
- 26 Luan J, Browne PO, Harding AH *et al.* (2001) Evidence for gene-nutrient interaction at the PPARgamma locus. *Diabetes* **50**, 686-689.
- 27 Corella D, Lai CQ, Demissie S *et al.* (2007) APOA5 gene variation modulates the effects of dietary fat intake on body mass index and obesity risk in the Framingham Heart Study. *J Mol Med* **85**, 119-128.
- 28 Corella D, Tai ES, Sorli JV *et al.* (2010) Association between the APOA2 promoter polymorphism and body weight in Mediterranean and Asian populations: replication of a gene-saturated fat interaction. *Int J Obes (Lond)*. (Epublication ahead of print versión).

TABLES

	Obese (n=208)	Control (n=146)	p
Males (%)	50	49	
Age (y)	11.6±0.20	11.5±0.17	0.608
Weight (kg)	63.7±0.70	43.1±0.77	<0.001
BMI (kg/m ²)	27.4±0.20	19.0±0.22	<0.001
BMI-SDS	3.53±0.46	0.28±0.42	0.001
Waist circumference (cm)	86.4±0.77	65.9±0.67	<0.001
Hip circumference (cm)	99.5±0.70	81.7±0.62	<0.001
Waist/hip ratio	0.87±0.005	0.81±0.004	<0.001
Fat mass (%)	34.5±0.47	18.4±0.53	<0.001
Tricipital skinfold (mm)	25.4±0.37	15.7±0.41	<0.001
Dietary fat consumption	Obese(n=155)	Control (n=133)	p
Total fat (% TEV)	37.7±0.40	36.6±0.40	0.057
PUFA (% TEV)	5.50±0.12	6.03±0.13	0.004
SFA (% TEV)	12.9±0.21	13.1±0.22	0.351
MUFA (% TEV)	16.0±0.22	14.8±0.24	<0.001
PUFA/SFA	0.44±0.01	0.47±0.01	0.079
ω3 (% TEV)	0.47±0.03	0.48±0.03	0.824
Trans Fatty Acids (% TEV)	0.98±0.07	0.98±0.07	0.986
rs9939609	n (%)	n (%)	p
TT	53 (25.5)	49 (33.6)	
TA	106 (51.0)	76 (52.1)	
AA	49 (23.6)	21 (14.4)	0.060

Table 1: Baseline characteristics and prevalence of the rs9939609 polymorphism of the FTO gene in children and adolescent obese and control subjects. Data are shown as mean±SEM, after adjusting for age and sex.

TEV: Total Energy Value.

rs9939609	OR	95%CI	p
Codominant			0.059
TT	1		
TA	1.28	0.78-2.09	0.321
AA	2.20	1.15-4.21	0.018
Dominant			
TT	1		
TA+AA	1.47	0.92-2.03	0.106
Recessive			
TT+TA	1		
AA	1.89	1.06-3.30	0.031

Table 2: OR of obesity (95% CI) associated with the rs9939609 polymorphism of the FTO gene in a codominant, dominant and recessive model. Estimates are adjusted for age and sex.

LEGENDS OF FIGURES

Figure 1: Odds Ratio (OR) for obesity risk in children and adolescents linked to the consumption of: A) Percentage of SFA of total fat intake (dichotomized by the median); B) PUFA/SFA ratio (dichotomized by the median) in a dominant model of the rs9939609 polymorphism of the FTO gene. ORs are adjusted for age and sex. TT subjects are set as reference in each analysis with a OR of 1. Data from children and adolescents with complete dietary records available.

Figure 2: BMI-SDS of children and adolescents according a dominant model of the FTO rs9939609 polymorphism and SFA consumption (% of total energy) dichotomized by the median. Mean \pm SEM.



