LIFESTYLE FACTORS MODIFY OBESITY RISK LINKED TO PPARG2 AND FTO VARIANTS IN AN ELDERLY POPULATION: A CROSS-SECTIONAL ANALYSIS IN THE SUN PROJECT

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Keywords: *PPARG*2; Pro12ala; *FTO*; rs9939609; obesity risk

ABSTRACT

Genetic factors may interact with lifestyle factors to modify obesity risk. *FTO* and *PPARG*2 are relevant obesogenes. Our aim was to explore the effect of Pro12Ala (rs1801282) of *PPARG*2 and rs9939609 of *FTO*, on obesity risk and to examine their interaction with lifestyle factors in an elderly population.

Subjects (n=978, aged 69±6) were recruited from the SUN ("Seguimiento Universidad de Navarra") Project. DNA was obtained from saliva and lifestyle and dietary data were collected by validated self-reported questionnaires. Genotyping was assessed by RT-PCR plus allele discrimination.

Subjects carrying the Ala allele of *PPARG*2 gene had a significantly increased obesity risk compared to non carrier -Pro12Pro- subjects (OR 1.66, 95% CI: 1.01-2.74, p=0.045). Greater obesity risk was also found in inactive or high carbohydrate intake subjects with the Ala12 allele of *PPARG2* gene. Interestingly, subjects carrying the Ala allele of the *PPARG*2 gene and with a high CHO (>246 g/d) intake had an increased obesity risk compared to Pro12Pro subjects (OR 2.67, 95%CI: 1.3-5.46, p=0.007, p for [CHO x*PPARG*2] interaction=0.046). Moreover, in subjects with a high CHO intake the co-presence of the Ala allele of *PPARG2* gene and one A minor allele (rs9939609) of *FTO* gene did increase obesity risk (OR 3.26, 95%CI: 1.19-8.89, p=0.021) when compared to non carrier (Pro12Pro/TT) subjects.

In conclusion, it appears that lifestyle factors may act as effect modifiers for obesity risk linked to Ala12 allele of the *PPARG*2 gene and the A minor allele of *FTO* gene in an elderly population.

BACKGROUND

Obesity is a complex disease with genetic and environmental basis (Marti 2008). *FTO* and *PPARG*2 gene variants for obesity risk have been widely studied (Razquin 2011). Several meta-analyses showed an increased BMI in subjects with the Ala allele of the *PPARG2* gene (Masud 2003; Tonjes 2006). This observation was recently confirmed**,** carriers of the Ala allele of the *PPARG*2 gene had a significant higher BMI (+0.060 kg/m^2) compared to non-carriers (Galbete et al, in press), with a total of 49,337 subjects.

As it is known the *FTO* gene harbours the stronger association with adiposity in GWAS studies. Although the physiological function of FTO remains unclear (Tung 2011). In the large meta-analysis of GWAS thus far performed with 123,865 individuals of European ancestry the *FTO* locus was confirmed as one of the 32 variants associated with BMI with *P*-values $<5\times10^{-8}$ (Speliotes 2010; Speakman 2011). A significant association between rs9939609 SNP of *FTO* gene and obesity, with an overall *odds ratio* (OR) for obesity of 1.31 under per-allele comparison was reported in another meta-analysis including 111,571 subjects (Peng 2011).

Epidemiological studies have suggested that in addition to genetic factors, a variety of lifestyle factors (e.g., dietary composition, low level of physical activity (PA)) may contribute to the epidemic of obesity and interact with genetic factors to modify obesity risk (Chung 2008; Walley 2009).

The interaction between lifestyle factors and these gene variants (Pro12Ala of *PPARG*2 (rs1801282) and rs9939609 of *FTO*) have been explored in different populations and cohorts. On one hand, some studies have reported an interaction between Ala allele of *PPARG*2 gene variant and carbohydrate (CHO) or fat intake, (Marti 2002; Lamri 2011) on obesity risk whereas in others no association was found (Memisoglu 2003; Nelson

2007). A significant interaction between food intake and rs9939609 SNP of *FTO* gene on BMI was detected in some populations (Corella 2011; Lappalainen 2012; Moleres 2012).

With regard to PA, recently, Kilpelainen (2011) meta-analyzed data from 45 studies with a total of 218,166 adults. They reported a significant interaction between the minor A allele of rs9939609 and PA, being the odds for obesity risk 27% smaller in active vs. inactive subjects.

A cohort study is the best way to identify incidence and natural history of a disease, and can be used to examine multiple outcomes after a single exposure (Grimes 2002). The SUN Project (Seguimiento Universidad de Navarra–University of Navarra Follow-up-) is a multi-purpose prospective Mediterranean dynamic cohort designed to study the prospective association of diet and other lifestyle factors with various health outcomes including cardiovascular disease, hypertension, diabetes or obesity (Martinez-Gonzalez 2002; Segui-Gomez 2006).

The aim of this study was to explore the effect of two gene variants, Pro12Ala of *PPARG*2 and rs9939609 of *FTO*, on obesity risk and to examine their interaction with lifestyle factors in an elderly population of the SUN study

SUBJECTS AND METHODS

Sample population

This work has been conducted within the framework of the SUN Project (Martinez-Gonzalez 2002). The SUN Project was initiated in December 1999 in Spain and recruitment is permanently open. All participants are university graduates and about 50% of them are health professionals themselves.

Lifestyle and dietary data is collected by self-reported biennially mailed questionnaires (Alonso 2005; Bes-Rastrollo 2005; Martinez-Gonzalez 2005). Dietary intake was assessed using a semi-quantitative food frequency questionnaire (136 food items) included at baseline. Validity and reproducibility of this questionnaire has recently been re-evaluated (de la Fuente-Arrillaga 2010). Nutrient intakes of 136 food items were calculated as frequency multiplied by nutrient composition of specified portion size for each food item, using an ad hoc computer program developed for this purpose. A trained dietician updated the nutrient data bank using the latest available information from the food composition table for Spain. Baseline intake of macronutrients was analyzed as quantitative variables (grams per day) (de la Fuente-Arrillaga 2010; Fernandez-Ballart 2010).

PA was ascertained through a baseline 17-item questionnaire. The index of metabolic equivalent task hours per week (METs-h/week) was computed by using the time spent engaging in 17 activities and multiplying the time spent by the resting metabolic rate (MET-score) specific for each activity. The METs-h/week for all activities were combined to obtain a value of total METs-h/week, which adequately correlated with the objectively measured energy expenditure in a validation study in a subsample of the cohort (Martinez-Gonzalez 2005).

For this research, elderly participants of the SUN project (more than 55 years old when the baseline questionnaire was completed) were invited to participate in a genetic study in May 2008. Each participant received a kit designed to collect saliva and 1085 participants agreed to participate but 986 kits were received back. Finally, 978 volunteers were correctly genotyped for the rs1801282 SNP (*PPARG*2) and 967 for the rs9939609 SNP (*FTO*). The mean age was 69 years (70% male). Anthropometric data

was collected from the baseline questionnaire. Self-reported information on BMI had been previously validated in a subsample of the SUN Project (Bes-Rastrollo 2005). Specific written informed consent was requested to participate in this study. 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 Institutional Ethical Review Board of the University of Navarra.

Genotyping

Saliva samples were collected with specially designed kits (Oragene®ADN Self-Collector kit-OG250) and DNA was extracted according to the manufacturer's instructions. The genotyping for the Pro12Ala SNP of *PPARG*2 gene (rs1801282) and for the rs9939609 SNP of the *FTO* gene were performed using Taqman assays with allele-specific probes on the ABI Prism 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA, USA) according to standardized laboratory protocols.

Statistical analysis

Hardy-Weinberg equilibrium was tested using a chi-square test. This test was also used to analyze if there were differences on the genotype distribution according to obesity status.

The *Odds Ratio* (OR) for obesity associated with genotypes (dominant models) were fitted with an unconditional logistic regression model after adjustment for sex, age, PA and total energy intake as covariables. To address the combined effect of these two polymorphisms, Pro12Ala (rs1801282) of *PPARG*2 and rs9939609 of *FTO* gene (dominant model) a dummy variable was created. Non carrier subjects (Pro12Pro and

TT) were considered as the reference category. Three different categories according to the genotypes were considered: having the Ala allele (rs1801282) of *PPARG2* gene, the A allele (rs9939609) of *FTO* gene, and the third, for the co-presence of the two risk alleles (Ala and A allele). The association between the different possible genotypes and BMI was analyzed using linear regression models and analysis of covariance (ANCOVA), after adjusting for potential confounders (sex, age, PA and total energy intake). We also evaluated the relationship between the genetic variants Pro12Ala (rs1801282) of *PPARG2* and rs9939609 of *FTO* and a high CHO intake or low PA practice (dichotomized at the median) on obesity risk. Indicated interactions were estimated for obesity risk with the likelihood ratio test. Product terms between the SNPs and lifestyle factors were calculated firstly, with the corresponding variables dichotomized at the median (model 1 and 3), and secondly, as continuous variables (model 2 and 4). Interactions between the SNPs and lifestyle factors on BMI (as a continuous variable) were also tested.

RESULTS

Anthropometrical and lifestyle characteristics of elderly subjects of the SUN cohort according to the two genotypes (Pro12Ala SNP (rs1801282) of *PPARG*2 and the rs9939609 SNP of *FTO* gene, dominant model) are shown in Table 1. The frequencies of these two SNPs did fulfil the Hardy-Weinberg equilibrium.

The ORs for obesity risk were calculated for each gene variant after adjustment for sex, age, PA and total energy intake. The presence of the Ala allele of *PPARG*2 gene significantly increased obesity risk in the adjusted models (including total population, subjects with high CHO intake, and those with low PA practice). The obesity risk linked

to the Ala12 allele of *PPARG*2 was 1.66 (95%CI: 1.01-2.74, p=0.045) in the total population (Table 2).

Interestingly, as shown in Table 2, obesity risk was higher in subjects with a high CHO consumption (>246 g/day) carrying the Ala allele of the *PPARG*2 gene (OR 2.67, 95%CI: 1.30-5.46, p=0.007). This *p*-value did remain statistically significant after applying the Benjamini-Hochberg multiple comparison correction. The interaction for obesity risk between CHO intake and *PPARG*2 gene was also statistically significant (p for [CHOx*PPARG*2] interaction =0.046). Similar results for this interaction were also obtained when considering CHO as a continuous trait (p for [CHOx*PPARG*2] interaction =0.030).

Furthermore, subjects with a high CHO intake and carriers of the Ala allele, had an increased obesity risk, by the co-presence of one A minor allele (rs9939609) of *FTO* gene (OR 3.26, 95%CI: 1.19-8.89, p=0.021) compared to non carriers of the two alleles (Pro12Pro and TT) subjects with a high CHO intake.

The presence of the Ala12 allele (rs1801282) of *PPARG*2 gene increased obesity risk to 2.14 (95%CI: 1.13-4.05, p=0.020) in subjects with a sedentary lifestyle (<18.6 METs-h/week) compared to Pro12Pro subjects. However, there was no evidence of statistical interaction (p for interaction = 0.243). Furthermore, in inactive carrier subjects of the Ala12 allele of *PPARG2*, the co-presence of the A minor allele (rs9939609) of *FTO* gene had a further rise in obesity risk to 2.51 (95%CI: 1.01-6.23, p=0.047) compared to inactive non carrier (Pro12Pro and TT) subjects. The interactions between the genetic variants and low PA practice for obesity risk were not statistically significant (Table 2).

Linear regression models were also fitted to confirm the association between the co-presence of the two risk alleles (Ala of the PPARG2 and A allele of FTO gene) and

BMI (as a continuous variable) in the three models undertaken: total population, high CHO intake and low PA practice (Table 2). Moreover, the same tendency was observed in the ANCOVA analysis (Figure 1).

DISCUSSION

The main finding of this work is that a high CHO consumption seems to modify the obesity risk linked to the Pro12Ala SNPs of the *PPARG2* gene in an elderly population. Some strengths of the SUN cohort deserve to be mentioned. The homogeneity of participants with regard to socioeconomic status which helps to better control confounding and the higher educational level of participants in the cohort that ensures a higher validity in self-reported information (Beunza 2010; Sayon-Orea 2011). A potential limitation in our study is the self-reported outcome, nevertheless, self-reported weight and BMI had been previously validated (Bes-Rastrollo 2005). Another limitation is that identifying interactions between genetic variants and lifestyle factors may need much larger sample size (Smith 1984).

The present work shows that the co-presence of these two risk alleles in *PPARG2* and *FTO* gene increases obesity predisposition, but, novel studies are needed to elucidate the potential mechanisms. Pro12Ala variant of *PPARG*2 gene is one of the most studied genes as potentially linked to obesity phenotypes (Razquin 2011). Previous meta-analysis had associated the Ala12 minor allele with a higher BMI (Masud 2003; Tonjes 2006; Galbete et al. in press) and this study confirmed in a larger sample of aged subject the association of the Ala12 allele with obesity risk.

Depending on the genotype the response of individuals to a dietary component or components could be different. The Pro12Ala genetic variant is probably the most studied mutation in relation to the interaction with dietary components on adiposity

features. Fatty acids are natural agonist of PPARG transcription factor; consequently most of the studies have been directed to analyze the interaction between Pro12Ala and fat intake. However, this study replicated an earlier association of this *PPARG*2 genetic variant with obesity risk linked to a high CHO intake (Marti 2002). Notably, in our study the interaction between CHO consumption and this Ala12 allele of *PPARG2* for obesity risk was statistically significant although confirmation if needed in larger sample studies.

The *PPARG* Pro12Ala genotype seems to be associated with obesity, type 2 diabetes and CHD risk (Dallongeville 2009). This variant is a diet-dependent sensor, and in the presence of a positive energy balance, the adipogenic capacity of the Ala allele exceeds that of the Pro12 genotype, being partially attributed to diet-dependent effects of the *PPARG2* Pro12Ala genotype on adiponectin signaling and on the interaction of PPARG2 with several transcriptional coregulators (Anderson 2010). From a mechanistic point of view it is shown that the Ala12 allele alters ligand interaction between PPARG2 and its cofactors (Pgc1alfa, SRC1, Ncor) leading to an effect beyond decreased DNA binding efficiency (Heikkinen 2009) .The enhancement in obesity risk linked to a high CHO intake may be partly explained by the fact that CHO are not able to activate the PPARG protein and could worsen the action of the Ala12 substitution on the receptor activity.

The impact of this rs9939609 SNP of *FTO* gene on human body weight is mainly through energy intake, however some results are contradictory (Berentzen 2008; Do 2008; Speakman 2008, Goossens 2009; Haupt 2009). In our elderly population no effect of *FTO* on obesity was found. This observation agrees with former findings in mature subjects. Hardy (2010) described a weak association between *FTO* and BMI at age of 50 years. Jacobsson (2011) suggested that the effect of *FTO* on corporal adiposity may

decrease by age. Our limited sample size could also impair our ability to find significant results.

In regard to PA it is well known that there is an inverse relationship with obesity (Levine 1999; Levine 2005; Kuliczkowska 2008). Previous studies had reported that a high PA practice was linked to a lower fasting insulin level in Pro12 homozygous subjects of *PPARG*2 gene (Franks 2004) but no studies were found on association between Pro12Ala polymorphism and inactivity on obesity risk. Nevertheless, our results suggested an association of this genetic variant with obesity risk linked a low PA practice.

To our knowledge we assessed for the first time the joint association of *PPARG*2 and *FTO* gene variants on obesity risk when modulated by lifestyle factors. Previous studies have found a higher obesity risk associated with the combined effect of several polymorphisms. Some research work reported the combined effect of *PPARG* and *ADRB3* or ACE I/D gene variants for increasing BMI (Huang 2011; Passaro 2011) stated that the combined effect of *FTO* and *MC4R* genetic variants was strongly associated with obesity risk and BMI. Similarly, Cauchi (2009) observed that these two genetic variants increased obesity risk by 24% and low PA levels did accentuate this effect. Our study showed that the effect of *PPARG*2 (Ala12 allele) and *FTO* (rs9939609) gene variants on obesity effect might depend on high CHO intake.

In summary, it seems that lifestyle factors may act as effect modifiers for obesity risk linked to Ala12 allele of the *PPARG*2 gene and the A minor allele of *FTO* gene in an elderly population.

ACKNOWLEGMENTS

The SUN Study has received funding from the Spanish Government (Grants PI01/0619, PI030678, PI040233, PI042241, PI050976, PI070240, PI070312, PI081943, PI080819, PI1002658, PI1002293, RD06/0045, G03/140 and 87/2010), the Navarra Regional Government (36/2001, 43/2002, 41/2005, 36/2008) and the University of Navarra, Línea Especial, Nutrición y Obesidad (University of Navarra), Carlos III Health Institute (CIBER project, CB06/03/1017) and RETICS network. The scholarship to C. Galbete from the Asociación de Amigos de la Universidad de Navarra is fully acknowledged. The authors have no competing interests.

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FIGURE LEGEND

Fig. 1 BMI differences according to genotype (dominant models for Pro12Ala and rs9939609 SNPs) for the three population groups (total population, only subjects with a high CHO intake or a low physical activity practice)
Adjusted for sex, age, physical activity and total energy intake
* $p<0.05$ between Ala12+TA/AA and Pro12Pro+TT genotypes

Table 1: Baseline characteristics according to genotype for elderly subjects from the SUN project.

	PPRG2 rs1801282			FTO rs9939609		
	Pro12Pro (n=814)	Ala12 (n=164)	p-vaule[a]	TT (n=336)	TA/AA (n=631)	p-vaule[a]
% Male	70%	74%	0.275	72%	70%	0.657
Age (years)	69 (6)	70 (7)	0.071	69 (6)	69 (6)	0.484
BMI (kg/m^2)	25.7 (3.2)	26.2 (3.2)	0.091	25.6 (3.1)	25.9 (3.2)	0.173
Total Energy Intake (kcal/day)	2378 (903)	2484 (1021)	0.182	2412 (1038)	2384 (862)	0.654
CHO intake (g/day)	267 (129)	281 (147)	0.200	272 (147)	267 (123)	0.647
Protein intake (g/day)	107 (40)	109 (37)	0.686	109 (46)	107 (36)	0.449
Fat intake (g/day)	91 (39)	93 (42)	0.392	91 (41)	91 (39)	0.883
Physical activity (METs-h/week)	23.9 (20.7)	24.3 (21.5)	0.828	23.3 (20.1)	24.5 (21.3)	0.387

Values are expressed as mean (SD), unless otherwise state

[a] Continuous variables were compared using a Student-*t* test. Categorical variables were compared using chi squared test.

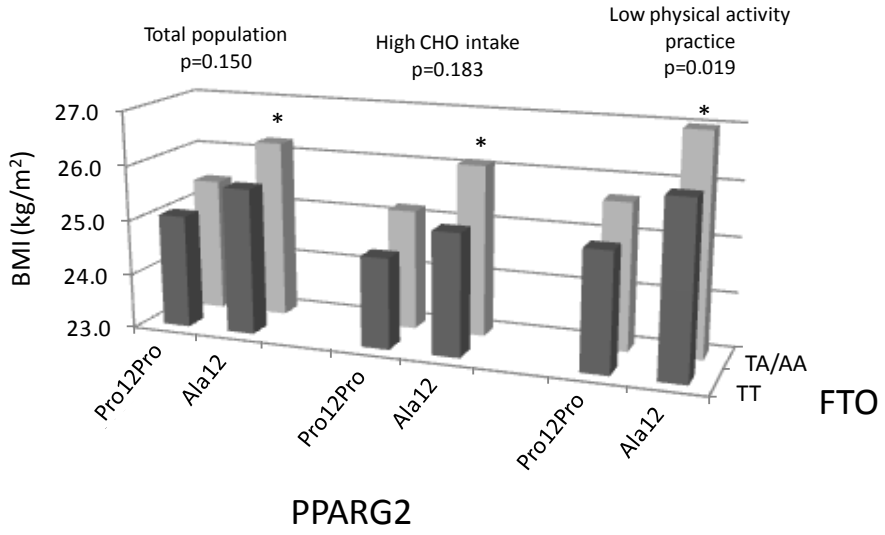
Table 2: Odds Ratios (OR) for obesity risk and linear regression coefficients for the association between the rs9939609 of *FTO* gene and Pro12Ala SNPs of the *PPARG2* gene and BMI in elderly participants of the SUN project

		OR (95% CI) for obesity	*p* value	*p* for interaction[a]	B (95% CI)[b]	*p* value	*p* for interaction[c]
PPARG2 (rs1801282)							
Pro12Pro		1 (ref)			0 (ref)		
Ala12		1.66 (1.01-2.74)	0.045		0.40 (-0.13-0.90)	0.139	
FTO (rs9939609)							
TT		1 (ref)			0 (ref)		
TA/AA		1.03 (0.66-1.60)	0.892		0.33 (-0.08-0.74)	0.112	
Genotype							
FTO[e]	*PPARG2*[e]						
-	-	1 (ref)			0 (ref)		
-	+	1.92 (0.86-4.27)	0.111		0.46 (-0.36-1.30)	0.287	
+	-	1.10 (0.66-1.84)	0.704		0.34 (-0.11-0.79)	0.135	
+	+	1.71 (0.84-3.48)	0.138		0.79 (0.08-1.50)	0.030	
High CHO intake (> 246 g/day)				model 1/model 2			
PPARG2 (rs1801282)				0.046/0.030			0.260
Pro12Pro		1 (ref)			0 (ref)		
Ala12		2.67 (1.30-5.46)	0.007[d]		0.49 (-0.21-1.20)	0.169	
FTO (rs9939609)				0.609/0.449			0.739
TT		1 (ref)			0 (ref)		
TA/AA		1.15 (0.57-2.31)	0.697		0.46 (-0.11-1.03)	0.111	
Genotype							
FTO[e]	*PPARG2*[e]			0.814/0.844			0.973
-	-	1 (ref)			0 (ref)		
-	+	1.92 (0.79-6.76)	0.312		0.28 (-0.03-1.43)	0.639	
+	-	1.04 (0.60-2.41)	0.924		0.41 (-0.22-1.03)	0.204	
+	+	3.26 (1.19-8.89)	0.021		1.07 (0.10-2-03)	0.031	
Low physical activity practice (< 18.6 METs-h/week)				model 3/model 4			
PPARG2 (rs1801282)				0.266/0.243			0.741
Pro12Pro		1 (ref)			0 (ref)		
Ala12		2.14 (1.13-4.05)	0.020		0.93 (0.16-1.69)	0.017[d]	
FTO (rs9939609)				0.366/0.152			0.417
TT		1 (ref)			0 (ref)		
TA/AA		1.14 (0.64-2.06)	0.652		0.56 (-0.05-1.16)	0.070	
Genotype							
FTO[e]	*PPARG2*[e]			0.346/0.230			0.360
-	-	1 (ref)			0 (ref)		
-	+	2.31 (0.79-6.76)	0.125		0.63 (-0.65-1.90)	0.334	
+	-	1.21 (0.60-2.41)	0.594		0.48 (-0.18-1.14)	0.156	
+	+	2.51 (1.01-6.23)	0.047		1.67 (0.63-2.71)	0.002[d]	

Adjusted for gender, age, physical activity and total energy intake

(a) p value for Likelihood Ratio Test for obesity risk. (b) Adjusted differences in average BMI (kg/m^2) between genotypes. (c) p value for interaction for BMI (as continuous variable). (d) p value < 0.05 after correcting for Benjamini-Hochberg multiple comparisons. (e) (-) Non carriers of the minor risk alleles (+) Subjects carrying the minor risk alleles, either Pro12Ala of *PPARG2* gene or rs9939609 of *FTO* gene. Model 1: interaction term = genotype*CHO (dichotomized at the median); model 2: interaction term = genotype*CHO (continuous); model 3: interaction term = genotype*PA (dichotomized at the median); model 4: interaction term = genotype*PA (continuous)

Figure 1:


Total population
p=0.150
High CHO intake
p=0.183
Low physical activity
practice
p=0.019
27.0
26.0
25.0
24.0
23.0
BMI (kg/m²)
*
*
*
Pro12Pro
Ala12
Pro12Pro
Ala12
Pro12Pro
Ala12
TA/AA
TT
FTO
PPARG2