

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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Received: 30 November 2011 / Accepted: 4 April 2012 / Published online: 24 April 2012
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Abstract Genetic factors may interact with lifestyle factors to modify obesity risk. *FTO* and *PPARG2* are relevant obesogenes. Our aim was to explore the effect of Pro12Ala (rs1801282) of *PPARG2* 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 *PPARG2* 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 *PPARG2* gene and with a high CHO (>246 g/

day) 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 \times *PPARG2*] interaction = 0.046). Moreover, in subjects with a high CHO intake, the co-presence of the Ala allele of *PPARG2* gene and one minor A 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 *PPARG2* gene and the minor A allele of *FTO* gene in an elderly population.

Keywords *PPARG2* · Pro12ala · *FTO* · rs9939609 · Obesity risk

Background

Obesity is a complex disease with genetic and environmental basis (Marti et al. 2008). *FTO* and *PPARG2* gene variants for obesity risk have been widely studied (Razquin et al. 2011). Several meta-analyses showed an increased body mass index (BMI) in subjects with the Ala allele of the *PPARG2* gene (Masud 2003; Tonjes et al. 2006). This observation was recently confirmed; carriers of the Ala allele of the *PPARG2* gene had a significant higher BMI ($+0.060 \text{ kg/m}^2$) 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 genome-wide association (GWA) 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

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of the 32 variants associated with BMI with p -values $<5 \times 10^{-8}$ (Speliotes et al. 2010; Speakman et al. 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 et al. 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 et al. 2009).

The interaction between lifestyle factors and these gene variants (Pro12Ala of *PPARG2* (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 *PPARG2* gene variant and carbohydrate (CHO) or fat intake, (Marti et al. 2002; Lamri et al. 2012) on obesity risk, whereas in others no association was found (Memisoglu et al. 2003; Nelson et al. 2007). A significant interaction between food intake and rs9939609 SNP of *FTO* gene on BMI was detected in some populations (Corella et al. 2011; Lappalainen et al. 2012; Moleres et al. 2012).

With regard to PA, recently, Kilpelainen et al. (2011) using data from 45 studies with a total of 218,166 adults conducted a meta-analysis. They reported a significant interaction between the minor A allele of rs9939609 and PA, the odds for obesity risk being 27 % smaller in active versus 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 association of diet and other lifestyle factors with various health outcomes including cardiovascular disease, hypertension, diabetes or obesity (Martinez-Gonzalez et al. 2002; Segui-Gomez et al. 2006).

The aim of this study was to explore the effect of two gene variants, Pro12Ala of *PPARG2* 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 et al. 2002). The SUN Project was initiated in December 1999 in Spain, and

recruitment was permanently open. All participants were university graduates and about 50 % of them are health professionals themselves.

Lifestyle and dietary data were collected by self-reported biennially mailed questionnaires (Alonso et al. 2005; Bes-Rastrollo et al. 2005; Martinez-Gonzalez et al. 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 et al. 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 databank using the latest available information from the food composition table for Spain. Baseline intake of macronutrients was analysed as quantitative variables (grams per day) (de la Fuente-Arrillaga et al. 2010; Fernandez-Ballart et al. 2010).

PA was ascertained through a baseline 17-item questionnaire. The index of metabolic equivalent task hours per week (METs-h/week) was computed 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 et al. 2005).

For this research, elderly participants (more than 55 years old when the baseline questionnaire was completed) of the SUN Project 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 (*PPARG2*), and 967 for the rs9939609 SNP (*FTO*). The mean age was 69 years (70 % male). Anthropometric data were collected from the baseline questionnaire. Self-reported information on BMI had been previously validated in a subsample of the SUN Project (Bes-Rastrollo et al. 2005). Specific written informed consent was required 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-Collection kit-OG250), and DNA was extracted according to the manufacturer's instructions.

The genotyping for the Pro12Ala SNP of *PPARG2* 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 χ^2 test. This test was also used to analyse 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 *PPARG2* and rs9939609 of *FTO* gene (dominant model)], dummy variables were created. Non-carrier subjects (Pro12Pro and TT) were considered as the reference category. Three different categories according to the genotypes were considered: the presence of Ala allele (rs1801282) of *PPARG2* gene, the presence of A allele (rs9939609) of *FTO* gene and the co-presence of the two risk alleles (Ala and A allele). The association between the different possible genotypes and BMI was analysed 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 interaction 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 tested 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 *PPARG2* 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 *PPARG2* 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 *PPARG2* 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 *PPARG2* 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 *PPARG2* gene was also statistically significant (p for [CHO \times *PPARG2*] interaction = 0.046). Similar results for this interaction were also obtained when considering CHO as a continuous trait (p for [CHO \times *PPARG2*] interaction = 0.030).

Table 1 Baseline characteristics according to genotype for elderly subjects from the SUN Project

	<i>PPARG2</i> rs1801282			<i>FTO</i> rs9939609		
	Pro12Pro ($n = 814$)	Ala12 ($n = 164$)	p -value ^a	TT ($n = 336$)	TA/AA ($n = 631$)	p -value ^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 stated

^a Continuous variables were compared using student's t -test. Categorical variables were compared using χ^2 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	<i>p</i> value	<i>p</i> for interaction ^a	B (95 % CI) ^b	<i>p</i> value	<i>p</i> for interaction ^c
<i>PPARG2</i> (rs1801282)							
Pro12Pro		1 (ref)			0 (ref)		
Ala12		1.66 (1.01–2.74)	0.045		0.40 (–0.13–0.90)	0.139	
<i>FTO</i> (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							
<i>FTO</i> ^e		<i>PPARG2</i> ^c					
–	–	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			
<i>PPARG2</i> (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	
<i>FTO</i> (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							
<i>FTO</i> ^e		<i>PPARG2</i> ^c		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			
<i>PPARG2</i> (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	
<i>FTO</i> (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							
<i>FTO</i> ^e		<i>PPARG2</i> ^c		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

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)

^a *p* value for likelihood ratio test for obesity risk

^b Adjusted differences in average BMI (kg/m²) 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

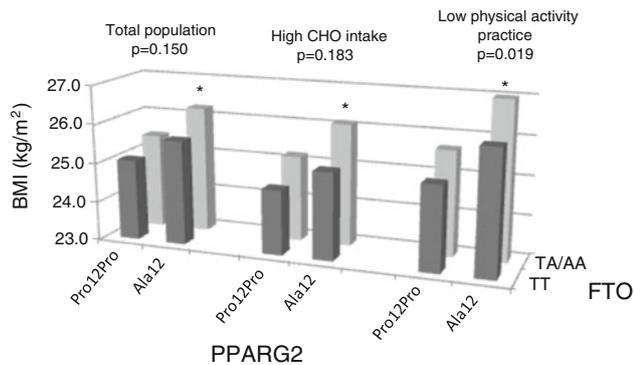


Fig. 1 BMI differences according to genotype (dominant models for Pro12Ala and rs9939609 SNPs) for the three population groups (total population, only subjects with a high CHO intake or subjects with a low physical activity practice). Adjusted for sex, age, physical activity and total energy intake.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

Furthermore, subjects with a high CHO intake and carriers of the Ala allele had an increased obesity risk by the co-presence of one minor A 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) in subjects with a high CHO intake.

The presence of the Ala12 allele (rs1801282) of *PPARG2* 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 minor A 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 (Fig. 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

socio-economic 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 et al. 2010; Sayon-Orea et al. 2011). A potential limitation in our study is the self-reported outcome; nevertheless, self-reported weight and BMI had been previously validated (Bes-Rastrollo et al. 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 *PPARG2* gene is one of the most studied genes as potentially linked to obesity phenotypes (Razquin et al. 2011). Previous meta-analysis had associated the Ala12 minor allele with a higher BMI (Masud 2003; Tonjes et al. 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 agonists of PPARG transcription factor; consequently, most of the studies have been directed to analyse the interaction between Pro12Ala and fat intake. However, this study replicated an earlier association of this *PPARG2* genetic variant with obesity risk linked to a high CHO intake (Marti et al. 2002). Notably, in our study, the interaction between CHO consumption and this Ala12 allele of *PPARG2* for obesity risk was statistically significant although confirmation is needed in larger sample studies.

The *PPARG* Pro12Ala genotype seems to be associated with obesity, type 2 diabetes and CHD risk (Dallongeville et al. 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 signalling and on the interaction of *PPARG2* with several transcriptional coregulators (Anderson et al. 2010). From a mechanistic point of view, it is shown that the Ala12 allele alters ligand interaction between *PPARG2* and its cofactors (Pgc1alpha, SRC1, Ncor), leading to an effect beyond decreased DNA binding efficiency (Heikkinen et al. 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 et al. 2008; Do et al. 2008; Speakman et al. 2008, Goossens et al. 2009; Haupt et al. 2009). In our elderly population, no effect of *FTO* on obesity was found. This observation agrees with former findings in mature subjects. Hardy et al. (2010) described a weak association between *FTO* and BMI at the age of 50. Jacobsson et al. (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.

With regard to PA, it is well known that there is an inverse relationship with obesity (Levine et al. 1999; Levine et al. 2005; Kuliczowska et al. 2008). Previous studies had reported that a high PA practice was linked to a lower fasting insulin level in Pro12 homozygous subjects of *PPARG2* gene (Franks et al. 2004), but no studies were found on interactions between Pro12Ala polymorphism and inactivity on obesity risk. Nevertheless, our results suggested an association of this genetic variant with obesity risk linked to a low PA practice.

To our knowledge, we assessed for the first time the joint association of *PPARG2* 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 which reported the combined effect of *PPARG* and *ADRB3* or *ACE I/D* gene variants for increasing BMI (Huang et al. 2011; Passaro et al. 2011) stated that the combined effect of *FTO* and *MC4R* genetic variants was strongly associated with obesity risk and BMI. Similarly, Cauchi et al. (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 *PPARG2* (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 *PPARG2* gene and the minor A allele of *FTO* gene in an elderly population.

Acknowledgments 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 and 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.

Conflict of interest The authors have no competing interests.

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