PHYSICAL ACTIVITY AND GENDER MODULATE OBESITY RISK LINKED TO 3111T/C GENE VARIANT OF *CLOCK* GENE OF AN ELDERLY POPULATION: THE SUN PROJECT

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ABSTRACT

Genetic factors may interact with physical activity levels to modify obesity risk. Our aim was to explore the effect of rs1801260 SNP (3111T/C) of *CLOCK* gene, on obesity risk and to examine their interaction with lifestyle factors in an elderly population of the SUN Project. Subjects (n=903, 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. A significant interaction between the 3111T/C SNP of *CLOCK* gene and sex for overweight/obesity risk was observed (p for interaction [Sex*CLOCK] interaction <0.001). Our results showed that women carrying the C allele of *CLOCK* gene had a decreased overweight/obesity risk compared to non carrier -TT- subjects (OR 0.61, 95% CI: 0.36-1.04, p=0.069). Moreover, the protective effect of the 3111T/C gene variant may be enhanced in those women with a high physical activity practice. We found that women practicing more than 16.8 METs-h/week had a significantly lower overweight/obesity risk (OR 0.36, 95%CI: 0.17-0.79, p=0.011). A significant interaction between the 3111T/C gene variant and physical activity for overweight/obesity risk was observed in women (p for [PA x *CLOCK*] interaction=0.015). In conclusion, it appears that physical activity levels may act as an effect modifier for overweight/obesity risk linked to the 3111T/C SNP (rs1801260) of the *CLOCK* gene in an elderly population of the SUN Project.

Keywords: *CLOCK,* rs1801260, cross-sectional study, obesity risk

BACKGROUND

Circadian rhythms are biological events generated by endogenous mechanisms composed by circadian clocks. The clocks are synchronized or adjusted to coincide with periodical environmental events such as the day/night cycle. If clocks are not well-synchronized the coordination between physiological and behavioural rhythms over the 24-h period is not guaranteed (Reppert & Weaver 2001). Clock genes are expressed in all tissues and circadian clocks participate in the daily regulation of metabolic functions such as glucose and lipid metabolism (Rudic et al., 2004; Yamamoto et al., 1987). One of the first clock genes studied was *Clock* (circadian locomotor output cycles kaput gene). Mutant mice for this gene display severe metabolic alterations, including hypercolesteronemia, hypertriglyceridemia, hepatic steatosis, and hyperglycemia.

The *CLOCK* gene codifies for the Clock protein, a positive regulatory arm of the circadian system. It belongs to a family of proteins that generate auto-regulatory mechanisms of positive and negative transcriptional feedback loops (Albrecht & Eichele 2003). Few studies have examined the association between *CLOCK* gene variants and obesity. In regard to the 3111T/C gene variant (rs1801260) results are not conclusive. Scott et al (Scott et al., 2008) observed that a haplotype including the C allele of this gene variant could protect against obesity in Caucasian men. Tortorella (Tortorella et al., 2007) observed an association of the C allele of this SNP with lifetime lower body weight in a group of subjects with eating disorders. Whereas Monteleone (Monteleone et al., 2008) did not find any association between the 3111T/C SNP and human obesity In an weight loss program based on the Mediterranean diet Garaulet, (Garaulet et al., 2010) observed that obese patients carrying the C allele participating were weight loss resistance.

It is widely recognised that there is an inverse relationship between obesity and physical activity practice (Levine 2005; Galbete et al., 2012; Kilpelainen et al., 2011). In fact, some studies have revealed association between genetic variants and physical activity (Galbete et al., 2012; Kilpelainen et al., 2011).

Our aim was to evaluate the association of the 3111T/C SNPs of *CLOCK* gene on obesity risk and examine its potential interaction with physical activity levels in an elderly population of the SUN Project.

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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 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 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).

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, 972 volunteers were correctly genotyped for the rs1801260 SNP (*CLOCK*). Among them, 69 subjects who reported total energy intake out of predefined values (<800 kcal/d for men, <500 kcal/d for women or >4000 kcal/d for men, >3500 kcal/d for women) were excluded, living a total of 903 participants available for the analysis. 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 3111C/T SNP of *CLOCK* gene (rs1801260) was 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 differences on the genotype distribution according to obesity status existed.

The *Odds Ratio* (OR) for obesity associated with genotype (dominant model) were fitted with an unconditional logistic regression model after adjustment for sex, age (years, continuous), PA (METs-h/week, continuous) and total energy intake (kcal/day, continuous) as covariables. The association between the different possible genotypes and BMI was analyzed using linear regression models and analysis of covariance (ANCOVA), after adjusting for the same potential confounders (sex, age, PA and total energy intake). We also evaluated the relationship between the genetic variants 3111C/T (rs1801260) of *CLOCK* and PA practice (sex-specific dichotomized at the median) on obesity risk. Interactions were estimated for obesity risk with the likelihood ratio test. Product terms between the SNPs and lifestyle factors were calculated with the corresponding variables as continuous. Interactions between the SNPs and lifestyle factors on BMI were also tested.

RESULTS

The frequency of the C allele of the 3111T/C gene variant of the *CLOCK* gene was 0.29 in our elderly population. Specifically, 50.8% of the subjects were wildtype (TT), 41.1% were heterozygous for the mutation (TC) and 8.1% were homozygous (CC). The allele distribution fulfilled the Hardy-Weinberg equilibrium (Table 1). Notably, in women the TC/CC genotype (dominant model) was more frequent in normoweight (55.1%) than in overweight/obese women (43.3%, (p=0.075).

Characteristics of elderly participants of the SUN Project (n=903) according to the 3111T/C genotype (dominant model) are shown in Table 2. Interestingly, subjects carrying the C allele had lower BMI than TT subjects (p=0.062). As shown in Table 3, after distributing by sex, BMI differences were marginally significant in women (p=0.059).

The linear regression analysis showed association of the C minor allele with lower BMI levels (Table 4). Moreover, a significant interaction (p<0.001) between the 3111T/C SNP and sex for obesity risk was found. Thus, men and women were analyzed separately for overweight/obesity risk. We observed that women with at least one C allele of the gene variant had a slight lower overweight/obesity risk (OR=0.61, 95%CI=0.36-1.04, p=0.069, Table 4) than TT women. Similar results were found when linear regression coefficients (Table 4) were calculated and the ANCOVA was performed (Figure 1). Women with the C allele had a lower BMI (24.10±0.65 kg/m^2) compared to those with the TT genotype (24.98±0.64 kg/m^2). Thus, in women the BMI difference was -0.89 kg/m^2 (95%CI= -1.80-0.02, p=0.056) which is equivalent to -2.43 kg for a woman 1.65 m tall.

In regard to physical activity, there is an interaction between the 3111T/C SNP and physical activity (PA) practice (p for interaction 3111T/C*PA=0.015) for overweight/obesity risk in women. In fact, women carrying the C allele and physically active (>16.8 METs-h/week, dichotomized at the median) had a significantly lower overweight/obesity risk (OR=0.36, 95%CI=0.17-0.79, p=0.011). The BMI difference for these women was -1.36 kg/m^2 (95%CI= -2.57-(-0.15), p=0.028) when they carried the C allele and practised more than 16.8 METs-h/week. It is also worthy to mention that women carrying the C allele and being physically active (>16.8 METs-h/week) had a significantly lower overweight/obesity risk (OR=0.36, 95%CI=0.17-0.79, p=0.011).

Comentario [CTI2]: Primera vez solo

Adjusted BMI means are shown in Figure 1.

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DISCUSSION

Our work showed that the 3111T/C SNP of *CLOCK* gene could protect against overweight/obesity risk in women from an elderly population of the SUN Project. Moreover, physical activity practice seemed to further increase the protective effect of this gene variant.

Sex could be considered as an "environmental" risk factor, which incorporates established anatomical, physiological, and behavioural differences between males and females (Ober et al., 2008). Thus, it is plausible that sex may interact with common genetic variants resulting in allelic effects that differ between males and females (Magi et al., 2010). Our study found an interaction between sex and the 3111T/C gene variant for overweight/obesity risk. Women presented a lower overweight/obesity risk associated to the C allele. The effects of some genetic variants on obesity have been shown to be modulated by sex. Ben Ali described that the Pro12Ala SNP of the *PPARG2* (rsXXX) gene was associated with obesity only in non-diabetic men (Ben Ali et al., 2009). Other investigators observed that a common variant (rs4712652) adjacent to the prolactin gene was related with increased BMI and fat mass only in men (Nilsson et al., 2011). It has been suggested that sex-specific lifestyle components such as diet, alcohol consumption, physical activity practice or smoking habits could act as modifiers of the effects of gene variants.

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 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 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 2005). Another limitation is that identifying interactions between genetic variants and lifestyle factors may need much larger sample size (Smith & Day 1984). However, some strengths of the SUN cohort deserve to be mentioned.

Our results suggest a protective effect of the C minor allele on overweigh/obesity risk, which is supported by some previous studies (Scott 2008, NOMBRE) but no for others NOMBRE).

Our work also identified an interaction between PA practice and the 3111T/C gene variant of *CLOCK* gene. Similar effects were observed for PA and other SNPs on obesity. For instances, a recent meta-analysis observed that the effect of rs939609 of *FTO* gene on increasing obesity risk was reduced by approximately in a 30% in physical active adults compared with non active (Kilpelainen 2011).

ALGUN OTRO SNP??

One potential explanation for the effect of PA on obesity risk linked to the CLOCK gene may come from recent works in animals. Since it has been suggested that PA interacts with circadian rhythms probably through genes involved in the circadian clock (Yamanaka 2008).

In summary, it seems that the C allele of the 3111T/C gene variant of *CLOCK* gene prevents against overweight/obesity risk in women of this elderly population. Moreover, physical activity may act as effect modifiers for overweight/obesity risk linked to the C allele by enhancing its protective effect.

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DECLARATION OF INTEREST

The authors declare no conflict of interest.

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TABLES

TABLE 1. Prevalence of the 3111T/C polymorphism of *CLOCK* gene in an elderly SUN population according to BMI

	WHOLE POPULATION			**WOMEN**			**MEN**		
	Normoweight	Overweight/ Obesity	p	Normoweigh	Overweigh/ Obesity	p	Normoweigh	Overweigh/ Obesity	p
Allele T	516 (69.5%)	789 (72.7%)		209 (67.0%)	133 (73.9%)		307 (71.4%)	640 (72.4%)	
Allele C	226 (30.5%)	297 (27.3%)		103 (33.0%)	47 (26.1%)		123 (28.6%)	255 (27.6%)	
TT	181 (48.8%)	278 (52.2%)		70 (44.9%)	51 (56.7%)		111 (51.6%)	227 (51.4%)	
CT	154 (41.5%)	217 (40.8%)		69 (44.2%)	31 (34.4%)		85 (39.5%)	186 (42.1%)	

CC	36 (9.7%)	37 (7.0%)	0.274	17 (10.9%)	8 (8.9%)	0.203	19 (8.9%)	29 (6.5%)	0.534
TT	181 (48.8%)	278 (52.3%)		70 (44.9%)	51 (56.7%)		111 (51.6%)	227 (51.4%)	
TC/CC	190 (51.2%)	254 (47.7%)	0.305	86 (55.1%)	39 (43.3%)	0.075	104 (48.4%)	215 (48.6%)	0.948

Chi-square test

TABLE 2. Characteristics of an elderly SUN Population according to 3111T/C polymorphism of *CLOCK* gene

	TT (n = 459)	**TC + CC** (n = 444)	**p**
Male (%)	74	72	0.546
Age (years)	69 ± 6	69± 6	0.916
Weigh (kg)	74.2 ± 12.0	73.1± 11.6	0.153
BMI (kg/m^2)	26.1 ± 3.3	25.7± 3.0	0.062
Total energy intake (kcal/day)	2256 ± 660	2232 ± 637	0.581
Carbohydrates (kcal/day)	1014 ± 398	981 ± 357	0.180
Proteins (kcal/day)	410 ± 114	408 ± 113	0.811
Lipids (kcal/day)	762 ± 264	777 ± 275	0.414
Physical activity (METs h/week)	24.1 ± 21.3	23.7 ± 20.4	0.770
Sleep time (hours/day)	7.7 ± 1.0	7.5 ± 1.0	0.115

Data are shown as mean±SD. n for sleep time is 402 for TT genotype and 394 for TC+CC genotype. Chi-square test for male % and t-student test for the others

TABLE 3. Characteristics according to sex of an elderly SUN population according to rs1801260 polymorphism of the CLOCK gene

	WOMEN			**MEN**		
	TT (n = 121)	**TC + CC** (n = 125)		**TT** (n =338)	**TC + CC** (n =319)	
Age (years)	67 ± 5	67 ± 5	0.908	70 ± 6	70 ± 6	0.950
Weight (kg)	63.5 ± 9.9	61.6 ± 8.8	0.112	78.1 ± 10.3	77.6 ± 9.1	0.552
BMI (kg/m^2)	24.97 ± 3.75	24.10 ± 3.45	**0.059**	26.5 ± 3.0	26.3 ± 2.6	0.444
Total energy intake (kcal/day)	2194 ± 625	2276 ± 644	0.313	2279 ± 671.7	2216 ± 634	0.216
Carbohydrates (kcal/day)	986 ± 370	1023 ±348	0.411	1024 ± 408	963 ± 359	**0.042**
Proteins (kcal/day)	415 ± 118	417 ± 116	0.868	408 ± 113	405 ± 112	0.678
Lipids (kcal/day)	763 ± 276	810 ± 315	0.211	762 ± 259	764 ± 258	0.928

Physical activity (METs h/week)	19.7 ± 14.1	20.3 ± 17.8	0.743	25.7 ± 23.1	25.1 ± 21.2	0.696
Sleep time (h/day)	7.7 ± 1.1	7.5 ± 1.1	0.114	7.6 ± 1.0	7.5 ± 1.0	0.388

Data are shown as mean±SD. T-student test.

TABLE 4: Odds Ratios (OR) for overweight/obesity risk and linear regression coefficients (B) for the association between the rs1801260 of *CLOCK* and BMI gene in elderly participants of the SUN project

	OR (95% CI)	*p* value	*p* for interaction*	B (95% CI)†	*p* value	*p* for interaction ‡
WHOLE POPULATION						
WOMEN			< 0.001			0.139
TT	1 (ref.)			0 (ref.)		
TC/CC	0.61 (0.36-1.04)	0.069		-0.89 (-1.80-0.02)	0.056	
MEN						
TT	1 (ref.)			0 (ref.)		
TC/CC	1.00 (0.72-1.39)	0.996		-0.19 (-0.62-0.24)	0.387	
PHYSICAL ACTIVITY PRACTICE (METs-h/week)						
WOMEN						
Low (< 16.8 METs-h/week)						
TT	1 (ref.)			0 (ref.)		
TC/CC	0.97 (0.47-2.06)	0.970	0.015	-0.43 (-1.80-0.95)	0.542	0.137
High (>16.8 METs-h/week)						
TT	1 (ref.)			0 (ref.)		
TC/CC	0.36 (0.17-0.79)	0.011		-1.36 (-2.57-(-0.15))	0.028	
MEN						
Low (< 20.6METs-h/week)						
TT	1 (ref.)			0 (ref.)		
TC/CC	0.77 (0.48-1.25)	0.291	0.957	-0.33 (-0.95-0.30)	0.303	0.887
High (> 20.6METs-h/week)						
TT	1 (ref.)			0 (ref.)		
TC/CC	1.26 (0.80-1.99)	0.312		0.063 (-0.66-0.53)	0.834	

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

* p value for Likelihood Ratio Test for obesity risk. † Adjusted differences in average BMI (kg/m^2) between genotypes. ‡ p value for interaction for BMI (as continuous variable)

FIGURES

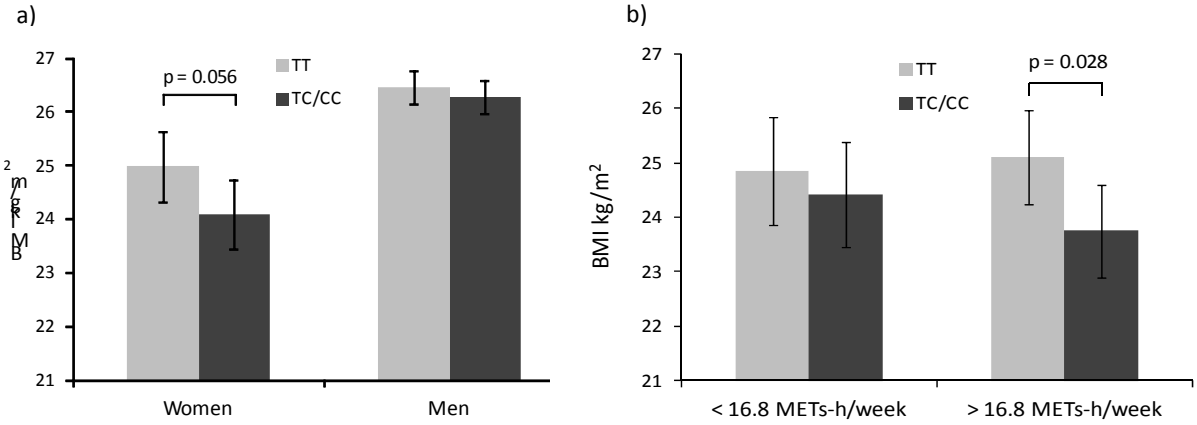

FIGURE 1: Differences on BMI adjusted by gender, age, physical activity and total energy intake according to the 3111T/C SNP of CLOCK gene. a) Differences in BMI in women and men separately. b) Differences on BMI in women according to physical activity level (lower or higher than the median).