



Association between obesity and insulin resistance with UCP2–UCP3 gene variants in Spanish children and adolescents

María C. Ochoa^a, José L. Santos^d, Cristina Azcona^b, María J. Moreno-Aliaga^a, Miguel A. Martínez-González^c, J. Alfredo Martínez^a, Amelia Marti^{a,*}, GENOI Members¹

^a Department of Nutrition and Food Sciences, Physiology and Toxicology, C/Iturrugarrea s/n, University of Navarra, 31080 Pamplona, Spain

^b Pediatric Endocrinology Unit, Department of Pediatrics, University Hospital, University of Navarra, 31080 Pamplona, Spain

^c Department of Preventive Medicine and Public Health, University of Navarra, 31080 Pamplona, Spain

^d Institute of Nutrition and Food Technology (INTA), University of Chile, Santiago, Chile

Received 23 July 2007; accepted 23 July 2007

Abstract

A number of studies have yielded controversial results on the association between polymorphisms in UCP2 and UCP3 genes with obesity and its comorbidities. The discrepancy among studies might be partially explained by the lack of consideration of the effect of adjacent loci in the same haplotype and the exclusion of key lifestyle factors in the statistical analysis. In this study, we have assessed the association between three genetic variants of the UCP2–UCP3 gene cluster, the –866G/A (rs659366) and the 45 bp insertion (in position 173247 of the AC019121) of the UCP2 gene, the –55C/T (rs1800849) polymorphism of the UCP3 gene and their estimated haplotypes with childhood obesity and insulin resistance. This research was designed as a case–control study and information about several environmental parameters such as leisure time physical activity and time spent watching television were included. The study sample consisted in 193 obese children and adolescents (cases) and 170 controls aged 6–18. We found that the individual polymorphisms were not associated with obesity, but the (–866G; rs659366) – (Del; 45 bp) – (–55T; rs1800849) haplotype was significantly associated with obesity and its presence in the control group increased about nine times the insulin resistance risk. Thus, the (–866A; rs659366) – (Ins; 45 bp) – (–55C; rs1800849) haplotype may protect against insulin resistance in the obese population group.

© 2007 Elsevier Inc. All rights reserved.

Keywords: Case–control; Childhood; Obesity; Uncoupling proteins; Insulin resistance; Haplotypes

At least 155 million school-age children worldwide are overweight or obese according to the latest estimates from the International Obesity TaskForce [1]. Moreover, these incidence rates are increasing dramatically every year [2]. There are significant health consequences linked to childhood obesity, including insulin resistance, type 2 diabetes mellitus and the metabolic syndrome [3,4].

Uncoupling protein 2 and 3 (UCP2 and UCP3) genes are located in chromosome 11q13. They play an important

role in human energy homeostasis [5] and have been considered candidate genes for obesity and insulin resistance. Thus, UCP2 and UCP3 reduce glucose-stimulated insulin secretion by pancreatic β cells and could modify fatty-acid oxidation [6–8]. Quantitative trait locus (QTL) for obesity were reported in the UCP2–UCP3 region in three mouse models [9] and three QTL studies showed evidence for the linkage between these genes with obesity in humans [10]. A number of studies have been performed seeking for an association between genetic variants in this gene cluster with obesity and/or related comorbidities [11,12].

Indeed, the –866G/A (rs659366) and the 45 bp insertion (in position 173247 of the AC019121) polymorphisms of the UCP2 gene and the –55C/T (rs1800849) polymorphism

* Corresponding author. Fax: +34 948425649.

E-mail address: amarti@unav.es (A. Marti).

¹ Other members of GENOI are M. Chueca, M. Oyarzabal, A. Patiño, R. Pelach.

of the UCP3 have been elsewhere reported to be functional variants on gene expression [13–15]. These three polymorphisms have been related to obesity and insulin resistance [16–19], although controversial outcomes have been also obtained.

The discrepancies among studies might be partially explained by the lack of consideration of the effect of adjacent loci. Haplotype analysis allows to include information about different polymorphisms in one single analysis [20] and improves the statistical power in association studies because, due to the linkage disequilibrium, the observed number of haplotypes tends to be much smaller than the theoretical number [21]. Since the rs659366, the 45 bp insertion and the rs1800849 variants expand for a small region of 40 kb, this haplotype study will give information on UCP2 and UCP3 participation, and could be a useful tool to study the association of this gene region with obesity and/or insulin resistance.

For the UCP2 gene, there are at least two investigations dealing with the association between obesity or insulin resistance risk and haplotypes [11,15]. One study reported an association between the rs659366 polymorphism and a protector effect against obesity in 596 middle-aged subjects with Northern European ancestry [15]. Specifically, haplotypes that included the A allele of the rs659366 polymorphism and the 45 bp insertion were more frequent in lean subjects compared to obese. In the other study, performed with type 2 diabetic and healthy European adults ($n = 241$), the rs659366 polymorphism and the 45 bp insertion presented a trend to be a protector factor against diabetes [11]. Moreover, a prospective cohort study in Caucasian men studied UCP2–UCP3 haplotypes including the rs659366 and the rs1800849 variants. They found that subjects carrying the two variants had a higher risk of type 2 diabetes 10 years after the follow-up [22]. All these studies did not include lifestyle factors of subjects in the analysis information. As multifactorial disease association studies could be biased by environmental factors, in our work, potential confounding by leisure time physical activity and time spent watching television were accounted for in the statistical analysis.

The aim of this study was to evaluate the association between three polymorphisms of the UCP2–UCP3 cluster gene and their estimated haplotypes with childhood obesity and insulin resistance.

Subjects and methods

The study sample, recruited from the Pediatric Departments at the Virgen del Camino Hospital, University Hospital and some Primary Care Centers, comprised 363 Spanish children and adolescents. Cases ($n = 193$) were subjects aged 5–18 years, with body mass index (BMI) above the 97th percentile of the Spanish BMI reference data for age and gender [23]. Should we have used the International Obesity TaskForce (IOTF) standards, only 73% of these Spanish obese children would have been classified as such [24]. Exclusion criteria included exposure to hormonal treatment or development of secondary obesity due to endocrinopathy or serious intercurrent illness. Controls ($n = 170$) were healthy subjects with BMI below the 97th percentile of the same reference. They were recruited when they attended the Primary Care Centers for routine medical examination or to be vacci-

nated. Our study was approved by the Ethics Committee of the University of Navarra and all parents and subjects over 12 years old provided written informed consent. Participants under 12 years old were also informed about the study and expressed their agreement to join it.

Anthropometric measurements were all collected in a medical environment by standard procedures [25]. Height was measured to the nearest centimeter with a validated stadiometer and weight to the nearest 100 g with a digital balance (TBF-300A Body Composition Analyzer/Scale, TANITA®, Tokyo, Japan). Blood samples were taken for the extraction of genomic DNA from leukocytes and the polymorphisms analyzed by PCR-RFLP methods reported elsewhere [26–29]. After 12 h fasting, venous blood samples were obtained and serum glucose was measured by an enzymatic method. Serum insulin was measured by radioimmunoassay (TKIN1 kit; Diagnostic Products, Madrid, Spain). Since few children presented glucose levels >5.6 mmol/L (the criteria for the diagnosis of impaired fasting glucose of the American Diabetes Association [30]), the upper tertile of fasting glucose was considered elevated (>5.0 mmol/L). Insulin resistance is affected by age and pubertal status, and Tanner stages were not recorded in all patients. In order to adjust insulin levels, the population was divided into three age groups (5–8, 9–11 and 12–18) and we used the upper tertile within age group to consider elevated fasting insulin. The glucose and insulin cutoffs were decided prior to analysis based on previously reported information. There were 70 subjects aged <9 years old (36 control and 34 obese subjects), 119 with ages between 9 and 11 years old (51 control and 68 obese subjects) and 174 were between 12 and 18 years old (83 control and 91 obese subjects). The homeostasis model assessment was calculated ($\text{HOMA} = \text{fasting serum insulin } (\mu\text{U/mL}) \times \text{fasting plasma glucose } (\text{mol/L}) / 22.5$) [31] and the upper tertile within each age group was used to consider insulin resistance.

Two trained research assistants conducted face-to-face interviews with participants and their parents (father, mother or tutor) following standardized protocols. A previously validated physical activity questionnaire was used [32,33]. The questionnaire included 17 activities (sports and games) and 10 response categories for frequency, ranging from “never” to “eleven hours or more per week”. A multiple of resting metabolic rate (MET score) was assigned to each activity and an activity metabolic equivalent index (METs-h/week) was computed for each participant. The activity metabolic equivalent index represents the overall leisure-time physical activity exerted in a one-week period for each participant [34]. Sedentary lifestyle was assessed through the number of hours spent watching TV and videos during school days and on weekends.

Statistical analyses

Descriptive summary statistics for quantitative variables are shown as means \pm standard error of the mean (SEM). Genotype and allele frequencies were estimated for each polymorphism and measures of linkage disequilibrium (D') were calculated [35]. The χ^2 goodness-of-fit test was used to assess the concordance of estimated genotype frequencies with Hardy–Weinberg proportions.

The comparison of allele/genotype frequencies in cases *versus* controls was performed using a simple χ^2 test followed by logistic regression techniques adjusting by relevant covariates. The analysis of haplotype–obesity association was carried out using the UNPHASED program [36] that handles the uncertainty in the construction of the haplotype phase of unrelated subjects.

Different logistic regression models were calculated using the SPSS program to assess the association between UCP2 and UCP3 genotypes and insulin resistance in obesese and controls separately: the model 1 was adjusted for sex and age, while model 2 also included leisure time physical activity and TV watching as covariates. The association between haplotypes and insulin resistance was assessed through the UNPHASED program.

The statistical power was calculated for carriers of haplotype 1 (the most frequent in our sample) was 62.8%. The assumptions to calculate power were: alpha risk = 0.05, control/case ratio = 0.89, % of exposure among controls 54% and number of cases = 193. We used the software OpenEpi 2.0 (<http://www.openepi.com/Menu/OpenEpiMenu.htm>).

Results and discussion

UCP2 and UCP3 uncouple respiration from ATP synthesis by providing an alternative route for the protons to enter into the mitochondrial matrix [6]. This decrease in ATP/ADP ratio makes that UCP2 activation will reduce glucose-stimulated insulin secretion by pancreatic β cells [7]. Moreover, UCP3 may regulate muscle substrate metabolism and fat oxidation [8,22]. Through these mechanisms, UCP2 and UCP3 may play an important role in human energy metabolism by regulating the use of fatty acids and glucose as fuels [5] and have been considered candidate genes for obesity and insulin resistance [10].

The rs659366 and the 45 bp insertion of the UCP2 and the rs1800849 polymorphism of the UCP3 could modify UCP2 and UCP3 function. The rs1800849 variant enhances UCP3 mRNA levels in skeletal muscle [13], whereas rs659366 and the 45 bp insertion alter mRNA levels *in vitro* and in intraperitoneal fat [15]. In this context, we have studied the association of these gene variants with obesity and insulin resistance in a Spanish group of 193 obese children and adolescents and 170 controls (Table 1).

Prevalence of the polymorphisms and the estimated haplotypes are reported in Table 2. The three polymorphisms fulfilled the Hardy–Weinberg expectations both obese and control subjects. The distribution of the rs659366 in obese was different from control subjects, presenting this group higher frequency of heterozygous subjects ($p = 0.038$). The prevalence of the –866A allele of the rs659366 polymorphism was 0.38 and the 45 bp insertion had a prevalence of 0.29, roughly similar to the frequencies found by Esterbauer et al. [15] in a case–control study with 596 middle-aged subjects from Austria (~ 0.35 for the rs659366 and ~ 0.28 for the 45 bp insertion). The –55T allele of the rs1800849 presented a frequency of 0.18 in the obese group and 0.14 in controls. The prevalence of this polymorphism has been reported to be different among populations. In Spanish adults the prevalence of the –55T allele was 0.21, 0.22 in French subjects and 0.26 in United States Caucasians [12,19,26]. Anthropometric, biochemical and lifestyle characteristics of subjects with different genotypes are shown in Table 3.

Table 1
Anthropometrical, biochemical and lifestyle parameters in the study group

	Obese (<i>n</i> = 193)	Control (<i>n</i> = 170)	<i>p</i>
Age (years)	11.5 (2.79)	11.8 (2.63)	0.718
% Males	51.3%	49.3%	0.720
BMI (kg/m ²)	27.9 (2.55)	19.0 (4.74)	<0.001
BMI-SDS	3.91 (0.81)	0.16 (1.64)	<0.001
Fasting glucose (mM)	4.95 (0.46)	4.69 (0.43)	<0.001
Fasting insulin (μ U/mL)	17.64 (46.3)	8.53 (62.3)	<0.001
HOMA index	3.94 (1.79)	1.87 (2.39)	<0.001
Physical activity (METs-h/week)	19.6 (21.7)	37.0 (11.8)	<0.001
TV watching (h/week)	15.2 (8.64)	12.9 (8.73)	0.068

Data are expressed in means (standard deviation). The *p*-values were calculated with χ^2 and the *U* Mann–Whitney tests.

Table 2

Distribution of the rs659366 and the 45 bp insertion of the UCP2 and the rs1800849 polymorphism of the UCP3 frequencies and estimated frequencies of the haplotypes in obese and control subjects^a

	Obese	Control	<i>p</i> [*]			
<i>Polymorphism</i>						
–866G/A of the UCP2 (rs659366)						
G/G	79 (40.9)	59 (34.7)	0.038			
G/A	80 (41.5)	92 (54.1)				
A/A	34 (17.6)	19 (11.2)				
45 bp insertion in the UCP2						
DD	103 (53.6)	79 (47.6)	0.207			
DI	71 (37.0)	76 (45.8)				
II	18 (9.4)	11 (6.6)				
–55C/T of the UCP3 (rs1800849)						
C/C	123 (66.8)	114 (72.6)	0.323			
C/T	55 (29.9)	41 (26.1)				
T/T	6 (3.3)	2 (1.3)				
<i>Haplotypes^b</i> –866G/A 45 bp –55C/T (rs659366) insertion (rs1800849)						
1	G	D	C	42%	54%	0.019
2	A	I	C	17%	26%	0.020
3	A	D	T	9%	9%	0.874
4	G	D	T	9%	6%	0.374

^a Data are given in subjects (frequency in %).

^b Only haplotypes with a frequency >5% in both study groups are shown.

* *p*-value in χ^2 test.

The three polymorphisms showed significant linkage disequilibrium ($p = 0.004$ in obese group and $p < 0.001$ in controls). The pairwise linkage disequilibrium measures $|D'|$ were estimated as 0.24 for rs659366 and rs1800849, 0.40 for rs659366 and the 45 bp insertion and 0.62 for the 45 bp insertion and rs1800849 in the obese group. In controls, the corresponding $|D'|$ estimates were 0.43, 0.83 and 0.98. The $|D'|$ values for the rs659366 and the 45 bp insertion fit in with other reported in studies that pooled obese and control subjects and found $|D'|$ values between 0.74 and 0.77 [11,37].

With these three polymorphisms, haplotype frequencies were estimated. Some of the advantages of haplotype analysis are that it pools information about the three studied variants and increases the power of the study. Four haplotypes showed a frequency higher than 5% both in obese and control groups. In the case–control study a statistically significant association was found between haplotype 4 (–866G; rs659366) – (Del; 45 bp) – (–55T; rs1800849) and obesity (Table 4). This haplotype had a prevalence of 9% in the obese group and 6% in the control group. Similarly, Esterbauer et al. [15] reported that the –866G allele of the rs659366 polymorphism increased obesity risk in middle-aged Caucasian subjects and the same tendency was reported in the haplotypes analysis. The –866G allele has been associated to reduced adipose tissue UCP2

Table 3
Anthropometrical, biochemical and lifestyle parameters in the study group distributed by genotype

	–866G/A (rs659366)				45 bp insertion				–55C/T (rs1800849)			
	GG	GA	AA	<i>p</i>	DD	DI	II	<i>p</i>	CC	CT	TT	<i>p</i>
<i>Obese subjects</i>												
BMI (kg/m ²)	28.0 (5.0)	28.2 (4.9)	27.3 (3.3)	0.684	28.7 (4.69)	27.3 (4.92)	26.0 (3.70)	0.044	28.1 (5.0)	27.8 (4.4)	26.5 (3.2)	0.658
BMI-SDS	3.92 (1.55)	4.00 (1.83)	3.74 (1.42)	0.709	4.16 (1.72)	3.79 (1.59)	3.05 (1.09)	0.011	3.91 (1.71)	3.94 (1.55)	3.74 (1.16)	0.305
Fasting glucose (mM)	4.95 (0.44)	4.95 (0.44)	4.94 (0.42)	0.972	4.97 (0.42)	4.92 (0.45)	4.95 (0.45)	0.543	4.92 (0.42)	4.99 (0.47)	5.04 (0.27)	0.087
Fasting insulin (μU/mL)	114.1 (64.4)	102.0 (57.8)	99.1 (67.3)	0.680	115.1 (60.0)	102.6 (67.4)	77.4 (42.4)	0.158	111.8 (64.6)	95.4 (55.7)	103.6 (68.4)	0.738
HOMA index	4.20 (2.45)	3.79 (2.25)	3.67 (2.56)	0.580	4.25 (2.27)	3.81 (2.62)	2.89 (1.72)	0.098	4.11 (2.46)	3.59 (2.21)	3.88 (2.61)	0.755
Physical activity (METs-h/week)	19.8 (12.3)	20.4 (10.8)	17.2 (13.1)	0.160	18.1 (10.3)	21.0 (12.3)	20.8 (15.9)	0.366	20.0 (12.8)	19.5 (10.0)	13.7 (6.1)	0.662
TV watching (h/week)	14.2 (8.4)	15.3 (8.8)	17.4 (9.3)	0.355	16.6 (8.9)	13.4 (8.22)	14.3 (8.88)	0.182	15.9 (9.2)	13.4 (7.8)	17.9 (5.3)	0.638
<i>Control subjects</i>												
BMI (kg/m ²)	18.7 (2.3)	19.0 (2.6)	19.2 (3.1)	0.598	18.9 (2.6)	19.0 (2.3)	20.3 (3.2)	0.301	19.1 (2.3)	18.8 (3.1)	20.1 (1.9)	0.854
BMI-SDS	0.16 (0.75)	0.18 (0.80)	0.05 (1.03)	0.958	0.19 (0.81)	0.16 (0.77)	0.26 (1.09)	0.919	0.21 (0.76)	0.08 (0.90)	1.10 (0.68)	0.750
Fasting glucose (mM)	4.65 (0.49)	4.68 (0.45)	4.80 (0.39)	0.266	4.65 (0.42)	4.74 (0.48)	4.75 (0.31)	0.604	4.65 (0.41)	4.80 (0.45)	5.13 (1.45)	0.538
Fasting insulin (μU/mL)	51.4 (34.1)	53.4 (53.4)	50.2 (44.2)	0.849	50.9 (31.8)	55.7 (58.6)	57.3 (33.7)	0.560	52.9 (57.1)	57.1 (47.7)	72.0 (52.6)	0.343
HOMA index	1.84 (1.45)	1.89 (2.02)	1.85 (1.68)	0.822	1.79 (1.21)	2.03 (2.30)	1.99 (1.10)	0.366	1.87 (1.76)	2.09 (1.88)	3.02 (2.77)	0.445
Physical activity (METs-h/week)	40.5 (21.9)	34.2 (21.3)	39.7 (22.9)	0.285	37.6 (22.8)	36.3 (20.6)	42.4 (26.4)	0.687	37.9 (21.3)	35.2 (23.3)	24.1 (33.1)	0.478
TV watching (h/week)	12.3 (8.2)	13.3 (8.7)	12.7 (9.0)	0.984	12.8 (8.4)	13.1 (9.2)	14.3 (8.6)	0.433	13.5 (8.6)	11.8 (9.5)	13.9 (8.7)	0.132

Data are expressed in means (standard deviation). The *p*-values were calculated with Kruskal–Wallis tests.

Table 4
Association between obesity risk with the rs659366 and the 45 bp insertion of the UCP2 and the rs1800849 polymorphism of the UCP3 gene

	OR	95% CI	95% CI
Model 1			
<i>Polymorphism</i>			
–866G/A (rs659366)			
G/A	0.66	0.42	1.04
A/A	1.35	0.70	2.60
45 bp Ins/DeI			
D/I	0.72	0.46	1.12
I/I	1.28	0.57	2.88
–55C/T (rs1800849)			
C/T	1.24	0.77	2.00
T/T	2.70	0.53	13.74
Haplotypes			
1	1		
2	0.83	0.54	1.2
3	1.27	0.93	1.73
4	1.95	1.43	2.65
Model 2			
<i>Polymorphism</i>			
–866G/A (rs659366)			
G/A	0.62	0.36	1.06
A/A	1.36	0.61	3.04
45 bp Ins/DeI			
D/I	0.90	0.53	1.53
I/I	1.62	0.60	4.38
–55C/T (rs1800849)			
C/T	1.16	0.67	2.02
T/T	1.27	0.20	8.19
Haplotypes			
1	1		
2	0.98	0.60	1.61
3	1.13	0.77	1.66
4	1.94	1.33	2.85

The reference categories were –866G/G, D/D and –55C/C carriers, respectively. Model 1 included sex and age and model 2 included sex, age, leisure time physical activity and TV watching as covariate.

mRNA expression *in vivo* and UCP2 transcriptional activity *in vitro*, but increased risk of obesity [14]. Complementary information was obtained in two metabolic studies that found that carriers of the –866A allele of the rs659366 and 45 bp insertion had an increased metabolic rate [17,38]. This impact on energy metabolism could explain the higher obesity risk of these alleles in non-carriers.

Regarding the rs1800849 polymorphism, a study in French individuals, in which the –55T/T genotype was associated with BMI showed the same trend that our findings [19], but opposite data were reported in United States and European Caucasians [12,16]. In a previous study, physical activity showed an interaction because it modified the effect of rs1800849 polymorphism on obesity [19,26]. In our study, physical and sedentary activities did not interact with obesity risk, but they behaved as confounding factors (the ORs estimated changed >10%) [39]. The adjustment

for lifestyle variables in model 2 allowed us to estimate more validly the ORs and to detect some associations that were not apparent in model 1.

We estimated insulin resistance according to HOMA index [31]. We did not use the glucose clamp test (the gold standard for quantifying insulin sensitivity/resistance *in vivo*), since it is an invasive technique not suitable for healthy children and adolescents. Currently, there are no widely accepted values to define insulin resistance in chil-

dren, and no standard values for fasting insulin or HOMA index have been validated as predictor of cardiovascular disease or diabetes. For these reasons, several studies have used upper tertiles or quartiles for HOMA index to identify insulin resistant prone subjects [40,41].

In our study, individual polymorphisms were not related to high fasting glucose levels (>5.0 mmol/L) neither in the obese group nor in the control group (Table 5). The rs659366 and the 45 bp insertion showed a tendency to

Table 5

Association between biochemical variables and HOMA index with the rs659366 and the 45 bp insertion of the UCP2 and the rs1800849 polymorphism of the UCP3 gene

	Fasting glucose				Fasting insulin				HOMA index			
	OR	95% CI		<i>p</i>	OR	95% CI		<i>p</i>	OR	95% CI		<i>p</i>
Obese												
<i>–866G/A (rs659366)</i>												
Model 1												
G/A	1.06	0.55	2.03	0.858	0.87	0.46	1.66	0.682	0.77	0.40	1.46	0.422
A/A	1.01	0.43	2.39	0.977	0.60	0.27	1.36	0.225	0.53	0.23	1.23	0.139
Model 2												
G/A	1.12	0.57	2.20	0.750	0.69	0.35	1.39	0.304	0.63	0.31	1.26	0.191
A/A	0.97	0.39	2.38	0.946	0.43	0.178	1.04	0.062	0.36	0.14	0.91	0.031
<i>45 bp Ins/Del</i>												
Model 1												
I/D	0.79	0.42	1.50	0.469	0.76	0.41	1.43	0.399	0.62	0.33	1.17	0.141
I/I	0.86	0.29	2.56	0.791	0.27	0.09	0.82	0.021	0.30	0.10	0.95	0.040
Model 2												
I/D	0.76	0.38	1.50	0.423	0.74	0.37	1.46	0.385	0.61	0.31	1.20	0.154
I/I	0.67	0.21	2.10	0.491	0.28	0.09	0.90	0.032	0.32	0.10	1.03	0.057
<i>–55C/T (rs1800849)</i>												
Model 1												
C/T	0.32	0.54	1.89	0.208	0.72	0.37	1.39	0.331	0.72	0.37	1.39	0.331
T/T	0.51	0.08	3.13	0.467	1.73	0.30	9.89	0.540	1.73	0.30	9.88	0.540
Model 2												
C/T	0.31	0.052	1.85	0.198	0.65	0.33	1.29	0.215	0.61	0.31	1.21	0.158
T/T	1.10	0.07	3.02	0.437	1.21	0.21	7.10	0.832	1.21	0.21	7.12	0.830
Control												
<i>–866G/A (rs659366)</i>												
Model 1												
G/A	1.37	0.54	3.46	0.505	0.67	0.22	2.05	0.488	0.83	0.26	2.62	0.745
A/A	1.70	0.45	6.45	0.437	1.62	0.39	6.79	0.510	1.97	0.45	8.58	0.370
Model 2												
G/A	1.46	0.56	3.81	0.436	0.74	0.23	2.41	0.616	0.92	0.27	3.19	0.899
A/A	2.11	0.54	8.24	0.282	1.92	0.38	9.80	0.433	2.38	0.44	12.77	0.312
<i>45 bp Ins/Del</i>												
Model 1												
I/D	1.41	0.61	3.27	0.428	0.73	0.25	2.14	0.571	0.86	0.29	2.58	0.792
I/I	0.72	0.13	4.03	0.710	2.15	0.46	10.16	0.333	2.54	0.51	12.77	0.257
Model 2												
I/D	1.44	0.61	3.40	0.405	0.77	0.25	2.39	0.655	0.93	0.29	2.98	0.908
I/I	0.94	0.16	5.34	0.941	2.70	0.43	16.95	0.288	3.37	0.50	22.75	0.212
<i>–55C/T (rs1800849)</i>												
Model 1												
C/T	0.16	0.10	2.90	0.218	4.83	1.64	14.20	0.004	5.48	1.78	16.88	0.003
T/T	0.39	0.02	7.10	0.523	17.54	0.91	336.3	0.057	18.7	0.95	366.45	0.054
Model 2												
C/T	0.18	0.01	3.27	0.247	4.43	1.46	13.48	0.009	5.14	1.61	16.39	0.006
T/T	0.41	0.02	7.80	0.556	11.14	0.495	250.88	0.129	11.8	0.50	275.81	0.125

The reference categories were –866G/G, DD and –55C/C carriers, respectively. Model 1 included sex and age and model 2 included sex, age, leisure time physical activity and TV watching as covariate.

protect against high fasting insulin levels ($p = 0.062$ and $p = 0.032$) and insulin resistance ($p = 0.031$ and $p = 0.057$) in homozygous obese subjects. On the contrary, the rs1800849 presented a direct association with high fasting insulin levels ($p = 0.009$) and insulin resistance ($p = 0.006$) in heterozygous subjects from the control group. There is some evidence that UCP2 and UCP3 expression levels did correlate with BMI [42,43]. For this reason, the analysis of the association between UCP2 and UCP3 variants and insulin resistance was performed separately in obese and control groups and this may explain the different outcomes found in each group.

Haplotype 4 (–866G; rs659366) – (Del; 45 bp) – (–55T; rs1800849) was associated to high fasting glucose in obese subjects (Table 6), and haplotype 2 (–866A; rs659366) – (Ins; 45 bp) – (–55C; rs1800849) exhibited an inverse association with fasting insulin and insulin resistance. In the control group, haplotype 4 increased the risk of insulin resistance ninefold. These results agree with Wang et al. [11] findings, that showed a trend toward an association between type 2 diabetes and the –866G allele of the rs659366 and the 45 bp deletion. Furthermore, in a study performed with 296 juvenile obese, Le Fur et al. [17] reported that obese children with A/A genotypes of the rs659366 did oxidize glucose better than lipids compared to subjects with G/G or G/A. However, a prospective cohort study in Caucasian men found

that subjects carrying the (–866A; rs659366) – (–55T; rs1800849) haplotype had a higher risk of type 2 diabetes, 10 years after the follow-up [22]. In our investigation, the haplotype 3 (that included the –866A and –55T alleles) did not show a statistically significant association with insulin resistance, but the –55C/T genotype of the UCP3 increased the risk of insulin resistance in obese subjects.

In the study performed by Esterbauer et al. [15], they reported that the –866G/A polymorphism was associated with enhanced adipose tissue UCP2 mRNA expression. It could be possible that the higher UCP2 content in certain tissues may be related to higher rates of fatty acid oxidation and oxidative stress protection, having an antidiabetic role.

As in previous reports [11,14–17,19], we found an effect of the UCPs gene variants on obesity or insulin resistance, but drawing solid conclusions is difficult as seen in the literature. The discrepancies may derive from differences in the design of the study (phenotypical measurements, characteristics of participants, ethnic origin, age, gender, sample size, statistical analysis, etc.).

To our knowledge this is the first case–control study in children that estimates haplotypes of the UCP2–UCP3 gene cluster. One of the advantages of our work is the multivariate analysis, taking into account different parameters that could be confounders. Moreover, the case–control design with subjects recruited from the same hospital-based population group and matched by age and gender could lead to avoid bias and to obtain more informative findings. However, in order to obtain better results, more research about the physiological function of the UCP2 and UCP3 is necessary, since other genetic factors could interact with these genetics variants. Studies performed in very large population samples (or meta-analysis) that also may allow to adjust by other variants could partly solve some of these disparities.

In conclusion, in this study we have found that after controlling for lifestyle factors, which is apparently made for the first time and it is specific feature of our study, the (–866G; rs659366) – (Del; 45 bp) – (–55T; rs1800849) haplotype is associated with obesity and insulin resistance risk, whereas the (–866A; rs659366) – (Ins; 45 bp) – (–55C; rs1800849) haplotype may protect against insulin resistance in a population of Spanish children and adolescents.

Acknowledgments

The authors are grateful for the participation of subjects and their families and the collaboration of the medical teams from Virgen del Camino Hospital, University Clinic and Health Center of Barañain. This work was supported by grants from the Navarra Government (Departamentos de Salud y Educación) and Línea Especial—Nutrición y Obesidad (University of Navarra LE/97). We are grateful for the technical assistance of Ana Lorente, Rafael Zurbano and Tamara Rojo.

Table 6
Association between biochemical variables and the HOMA index with the estimated haplotypes

Haplotype	Fasting glucose		Fasting insulin		HOMA	
	OR	95% CI	OR	95% CI	OR	95% CI
<i>Obese</i>						
Model 1						
1	1		1		1	
2	0.90	0.46–1.77	0.40	0.21–0.78	0.36	0.17–0.76
3	1.14	0.44–2.91	0.71	0.31–1.66	0.78	0.31–1.94
4	3.68	2.51–5.42	0.70	0.29–1.72	0.94	0.36–2.44
Model 2						
1	1		1		1	
2	0.83	0.36–1.91	0.41	0.20–0.85	0.35	0.16–0.75
3	1.09	0.38–3.07	0.59	0.24–1.45	0.57	0.22–1.49
4	3.98	2.62–6.04	0.77	0.27–2.19	0.81	0.28–2.30
<i>Control</i>						
Model 1						
1	1		1		1	
2	1.44	0.64–3.24	1.43	0.54–3.77	1.73	0.63–4.79
3	2.60	0.85–7.90	3.31	0.77–14.3	3.93	0.83–18.6
4	2.88	0.82–10.1	8.79	2.21–34.9	9.54	2.28–39.9
Model 2						
1	1		1		1	
2	1.31	0.54–3.18	1.46	0.51–4.12	1.76	0.56–5.52
3	2.33	0.69–7.91	2.91	0.63–13.4	3.49	0.68–17.8
4	1.97	0.49–7.86	7.96	1.89–33.5	9.52	2.11–42.9

Model 1 included adjustment by sex and, age and model 2 included adjustments by sex, age, leisure time physical activity and TV watching as covariate.

References

- [1] I.O. TaskForce. Available from: <<http://www.iotf.org/childhoodobesity.asp>> (January 2007).
- [2] T.J. Lobstein, W.P. James, T.J. Cole, Increasing levels of excess weight among children in England, *Int. J. Obes. Relat. Metab. Disord.* 27 (2003) 1136–1138.
- [3] H. Kaur, M.L. Hyder, W.S. Poston, Childhood overweight: an expanding problem, *Treat. Endocrinol.* 2 (2003) 375–388.
- [4] S. Caprio, Insulin resistance in childhood obesity, *J. Pediatr. Endocrinol. Metab.* 15 (Suppl. 1) (2002) 487–492.
- [5] M.D. Brand, C. Affourtit, T.C. Esteves, K. Green, A.J. Lambert, S. Miwa, J.L. Pakay, N. Parker, Mitochondrial superoxide: production, biological effects, and activation of uncoupling proteins, *Free Radic. Biol. Med.* 37 (2004) 755–767.
- [6] T.R. Nagy, M.L. Blaylock, W.T. Garvey, Role of UCP2 and UCP3 in nutrition and obesity, *Nutrition* 20 (2004) 139–144.
- [7] M.D. Brand, T.C. Esteves, Physiological functions of the mitochondrial uncoupling proteins UCP2 and UCP3, *Cell Metab.* 2 (2005) 85–93.
- [8] J. Himms-Hagen, M.E. Harper, Physiological role of UCP3 may be export of fatty acids from mitochondria when fatty acid oxidation predominates: an hypothesis, *Exp. Biol. Med.* (Maywood) 226 (2001) 78–84.
- [9] G. Solanes, A. Vidal-Puig, D. Grujic, J.S. Flier, B.B. Lowell, The human uncoupling protein-3 gene. Genomic structure, chromosomal localization, and genetic basis for short and long form transcripts, *J. Biol. Chem.* 272 (1997) 25433–25436.
- [10] T. Rankinen, A. Zuberi, Y.C. Chagnon, S.J. Weisnagel, G. Argyropoulos, B. Walts, L. Perusse, C. Bouchard, The human obesity gene map: the 2005 update, *Obesity (Silver Spring)* 14 (2006) 529–644.
- [11] H. Wang, W.S. Chu, T. Lu, S.J. Hasstedt, P.A. Kern, S.C. Elbein, Uncoupling protein-2 polymorphisms in type 2 diabetes, obesity, and insulin secretion, *Am. J. Physiol. Endocrinol. Metab.* 286 (2004) E1–E7.
- [12] Y.J. Liu, P.Y. Liu, J. Long, Y. Lu, L. Elze, R.R. Recker, H.W. Deng, Linkage and association analyses of the UCP3 gene with obesity phenotypes in Caucasian families, *Physiol. Genomics* 22 (2005) 197–203.
- [13] P. Schrauwen, J. Xia, K. Walder, S. Snitker, E. Ravussin, A novel polymorphism in the proximal UCP3 promoter region: effect on skeletal muscle UCP3 mRNA expression and obesity in male non-diabetic Pima Indians, *Int. J. Obes. Relat. Metab. Disord.* 23 (1999) 1242–1245.
- [14] F. Krempler, H. Esterbauer, R. Weitgasser, C. Ebenbichler, J.R. Patsch, K. Miller, M. Xie, V. Linnemayr, H. Oberkofler, W. Patsch, A functional polymorphism in the promoter of UCP2 enhances obesity risk but reduces type 2 diabetes risk in obese middle-aged humans, *Diabetes* 51 (2002) 3331–3335.
- [15] H. Esterbauer, C. Schneitler, H. Oberkofler, C. Ebenbichler, B. Paulweber, F. Sandhofer, G. Ladurner, E. Hell, A.D. Strosberg, J.R. Patsch, F. Krempler, W. Patsch, A common polymorphism in the promoter of UCP2 is associated with decreased risk of obesity in middle-aged humans, *Nat. Genet.* 28 (2001) 178–183.
- [16] D.J. Halsall, J. Luan, P. Saker, S. Huxtable, I.S. Farooqi, J. Keogh, N.J. Wareham, S. O’Rahilly, Uncoupling protein 3 genetic variants in human obesity: the c-55t promoter polymorphism is negatively correlated with body mass index in a UK Caucasian population, *Int. J. Obes. Relat. Metab. Disord.* 25 (2001) 472–477.
- [17] S. Le Fur, C. Le Stunff, C. Dos Santos, P. Bougneres, The common –866 G/A polymorphism in the promoter of uncoupling protein 2 is associated with increased carbohydrate and decreased lipid oxidation in juvenile obesity, *Diabetes* 53 (2004) 235–239.
- [18] A. Martí, M.S. Corbalan, L. Forga, M.A. Martínez-González, J.A. Martínez, Higher obesity risk associated with the exon-8 insertion of the UCP2 gene in a Spanish case-control study, *Nutrition* 20 (2004) 498–501.
- [19] S. Otabe, K. Clement, C. Dina, V. Pelloux, B. Guy-Grand, P. Froguel, F. Vasseur, A genetic variation in the 5′ flanking region of the UCP3 gene is associated with body mass index in humans in interaction with physical activity, *Diabetologia* 43 (2000) 245–249.
- [20] L.J. Palmer, L.R. Cardon, Shaking the tree: mapping complex disease genes with linkage disequilibrium, *Lancet* 366 (2005) 1223–1234.
- [21] D.Y. Lin, D. Zeng, Likelihood-based inference on haplotype effects in genetic association studies, *J. Am. Stat. Assoc.* 101 (2006) 89–104.
- [22] D.R. Gable, J.W. Stephens, J.A. Cooper, G.J. Miller, S.E. Humphries, Variation in the UCP2–UCP3 gene cluster predicts the development of type 2 diabetes in healthy middle-aged men, *Diabetes* 55 (2006) 1504–1511.
- [23] B. Sobradillo, *Curvas y tablas de crecimiento (estudios longitudinal y transversal) Bilbao, 2004.*
- [24] T.J. Cole, M.C. Bellizzi, K.M. Flegal, W.H. Dietz, Establishing a standard definition for child overweight and obesity worldwide: international survey, *BMJ* 320 (2000) 1240–1243.
- [25] L.A. Moreno, M.I. Mesana, M. Gonzalez-Gross, C.M. Gil, J. Fleta, J. Warnberg, J.R. Ruiz, A. Sarria, A. Marcos, M. Bueno, Anthropometric body fat composition reference values in Spanish adolescents. The AVENA Study, *Eur. J. Clin. Nutr.* 60 (2006) 191–196.
- [26] A. Alonso, A. Martí, M.S. Corbalan, M.A. Martínez-González, L. Forga, J.A. Martínez, Association of UCP3 gene –55C > T polymorphism and obesity in a Spanish population, *Ann. Nutr. Metab.* 49 (2005) 183–188.
- [27] G. Sesti, M. Cardellini, M.A. Marini, S. Frontoni, M. D’Adamo, S. Del Guerra, D. Lauro, P. De Nicolais, P. Sbraccia, S. Del Prato, S. Gambardella, M. Federici, P. Marchetti, R. Lauro, A common polymorphism in the promoter of UCP2 contributes to the variation in insulin secretion in glucose-tolerant subjects, *Diabetes* 52 (2003) 1280–1283.
- [28] A. Nieters, N. Becker, J. Linseisen, Polymorphisms in candidate obesity genes and their interaction with dietary intake of n-6 polyunsaturated fatty acids affect obesity risk in a sub-sample of the EPIC-Heidelberg cohort, *Eur. J. Nutr.* 41 (2002) 210–221.
- [29] L. Forga, M. Corbalan, A. Martí, C. Fuentes, M.A. Martínez-González, A. Martínez, Influence of the polymorphism 03826 A → G in the UCP1 gene on the components of metabolic syndrome, *An Sist. Sanit. Navar.* 26 (2003) 231–236.
- [30] Diagnosis and classification of diabetes mellitus, *Diabetes Care* 27 (Suppl. 1) (2004) S5–S10.
- [31] D.R. Matthews, J.P. Hosker, A.S. Rudenski, B.A. Naylor, D.F. Treacher, R.C. Turner, Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man, *Diabetologia* 28 (1985) 412–419.
- [32] M.A. Martínez-González, J.J. Varo, J.L. Santos, J. De Irala, M. Gibney, J. Kearney, J.A. Martínez, Prevalence of physical activity during leisure time in the European Union, *Med. Sci. Sports Exerc.* 33 (2001) 1142–1146.
- [33] M.C. Ochoa, A. Martí, C. Azcona, M. Chueca, M. Oyarzabal, R. Pelach, A. Patino, M.J. Moreno-Aliaga, M.A. Martínez-González, J.A. Martínez, Gene-gene interaction between PPAR gamma 2 and ADR beta 3 increases obesity risk in children and adolescents, *Int. J. Obes. Relat. Metab. Disord.* 28 (Suppl. 3) (2004) S37–S41.
- [34] B.E. Ainsworth, W.L. Haskell, A.S. Leon, D.R. Jacobs Jr., H.J. Montoye, J.F. Sallis, R.S. Paffenbarger Jr., Compendium of physical activities: classification of energy costs of human physical activities, *Med. Sci. Sports Exerc.* 25 (1993) 71–80.
- [35] P.W. Hedrick, Gametic disequilibrium measures: proceed with caution, *Genetics* 117 (1987) 331–341.
- [36] F. Dudbridge, Pedigree disequilibrium tests for multilocus haplotypes, *Genet. Epidemiol.* 25 (2003) 115–121.
- [37] H. Esterbauer, H. Oberkofler, Y.M. Liu, D. Breban, E. Hell, F. Krempler, W. Patsch, Uncoupling protein-1 mRNA expression in

- obese human subjects: the role of sequence variations at the uncoupling protein-1 gene locus, *J. Lipid Res.* 39 (1998) 834–844.
- [38] P. Kovacs, L. Ma, R.L. Hanson, P. Franks, M. Stumvoll, C. Bogardus, L.J. Baier, Genetic variation in UCP2 (uncoupling protein-2) is associated with energy metabolism in Pima Indians, *Diabetologia* 48 (2005) 2292–2295.
- [39] G. Maldonado, S. Greenland, Simulation study of confounder-selection strategies, *Am. J. Epidemiol.* 138 (1993) 923–936.
- [40] S. Dhuper, H.W. Cohen, J. Daniel, P. Gumidyala, V. Agarwalla, R. St Victor, Utility of the modified ATP III defined metabolic syndrome and severe obesity as predictors of insulin resistance in overweight children and adolescents: a cross-sectional study, *Cardiovasc. Diabetol.* 6 (2007) 4.
- [41] H.E. Resnick, K. Jones, G. Ruotolo, A.K. Jain, J. Henderson, W. Lu, B.V. Howard, Insulin resistance, the metabolic syndrome, and risk of incident cardiovascular disease in nondiabetic american indians: the strong heart study, *Diabetes Care* 26 (2003) 861–867.
- [42] H. Oberkofler, Y.M. Liu, H. Esterbauer, E. Hell, F. Krempler, W. Patsch, Uncoupling protein-2 gene: reduced mRNA expression in intraperitoneal adipose tissue of obese humans, *Diabetologia* 41 (1998) 940–946.
- [43] S. Bao, A. Kennedy, B. Wojciechowski, P. Wallace, E. Ganaway, W.T. Garvey, Expression of mRNAs encoding uncoupling proteins in human skeletal muscle: effects of obesity and diabetes, *Diabetes* 47 (1998) 1935–1940.