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ORIGINAL CONTRIBUTION

Genotype-by-nutrient interactions assessed in European obese women

A case-only study

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Abstract *Background* The development of obesity is influenced by both genetic and environmental risk factors. Whereas changes in the environment appear to be responsible for the increasing prevalence of obesity, genetic factors interacting with environmental factors would contribute to explain obesity onset and severity. *Aim* To explore epidemiologic genotype-by-nutrient interactions in obesity. *Methods* A total of 42 polymorphisms of 26 candidate genes for obesity were genotyped in 549 adult obese women recruited from eight European centres in a case-only study. The nutritional variables assessed in this study were the dietary fibre intake (grams per day), the ratio of dietary polyunsaturated fat to saturated fat (P:S ratio) and the percentage of energy derived from fat in the diet as calculated from a weighed three-day food record (%E). Under the assumption of genotype-nutrient independence in the population, the odds ratio calculated in a sample of obese women would indicate the existence of genotype-by-nutrient interactions, measured as deviations from the multiplicative effects of the genetic and the nutrient factors separately. *Results* No new but confirmaty evidences for genotype-by-nutrient interactions in obesity were detected in this case-only study. The test of interaction between fibre intake and the −514 C > T polymorphism of the hepatic lipase gene (*LIPC*) yielded *P*-values of 0.01 across different statistical models. Likewise, the −11377G > C polymorphism of the adiponectin gene (*ADIPOQ*) and the −681 C > G polymorphism of the PPARG3 gene might interact with the percentage of energy derived from fat in the diet for the development of obesity (*P*-values in the range of 0.01–0.05 across different statistical models). The *P*-values were not adjusted for multiple testing, so these results should be considered with caution. *Conclusions* Although the use of obese-only samples is theoretically a useful approach to detect interactions, few genotype-by-nutrient interactions have been suggested in obese European women after the analysis of candidate polymorphisms and the selected nutrient variables. The most remarkable multiplicative interaction found in this study refers to the combination of the hepatic lipase gene polymorphism −514 C > T and fibre intake.

Key words obesity – nutrient – genes – interaction

Introduction

Obesity has reached epidemic proportions as a consequence of the changes that have occurred in modern societies, in which rapidly available and cheap high-caloric foods are combined with sedentary lifestyles [1–4]. Although a great number of genetic variants and chromosomal regions have been associated or linked to excessive weight gain [5], there is no doubt that changes in the environment interacting with the genetic background, must be the direct cause of the rapid increase in the prevalence of obesity [6–8]. The assessment of the interactions between nutrient and genotypes appears as a relevant issue in genetic epidemiology studies of obesity since its knowledge may pave the way for more targeted prevention strategies, and hereby a better success in the prevention and treatment [9]. In this context, there is increasing evidence from observational studies that support a role for genotype-by-nutrient interactions in obesity [10–13].

In the present study, we have assessed the array of possible interactions between 42 polymorphisms of 26 candidate genes for obesity with three nutritional factors: the dietary fibre intake (g/day), the ratio of dietary polyunsaturated fat to saturated fat (P:S ratio), and the percentage of energy derived from fat in the diet (%E). The dietary fibre intake was chosen because there is convincing evidence that it is a protective factor against obesity according the WHO/FAO expert consultation [2]. Dietary P:S ratio and the percentage of energy intake derived from fat were also selected as interesting nutritional factors that may interact with genetic variants in the development of obesity as suggested by previous studies [11, 12]. Regarding dietary fat, meta-analysis about the energy intake derived from fat showed clearly a link with weight balance [14] with the existence of possible interactions with genetic variants in the development of obesity-related phenotypes [15].

We have used the case-only approach for the assessment of genotype-by-nutrient interactions in obesity. The case-only study may constitute an adequate strategy for examining potential interactions between nutrient and genes in obesity when nutrient and genotypes are independent [16–22]. As a limitation, the case-only study does not provide any information on the main effects of the susceptible genotype and the nutrient variables, but only gives information on multiplicative interactions [16–22]. The aim of this research was to explore genotype-by-nutrient interactions in obesity using a sample of obese European women.

Table 1 Polymorphisms for obesity genotyped in 549 European obese women

Name	Gene name	Locus ID	Location	Rs	Polymorphism	Allele frequency	*P*-value Hardy–Weinberg Equilibrium
Regulation of appetite							
Solute Carrier Family 6 Member 14	*SLC6A14*	11254	Xq23	2011162	+22510 C > G	0.58	0.82
Cocaine- and Amphetamine-Regulated Transcript	*CART*	9607	5q13.2	7379701	−3608 T > C	0.52	0.94
				6453132	−1702 C > T	0.42	0.68
				–	−175 A > G	0.52	0.98
				5868607	−1336 delA	0.10	0.33
Growth Hormone Secretagogue Receptor	*GHSR*	2693	3q26.3	2232169	Leu149Leu	0.02	0.99
Glutamate Decarboxylase 2	*GAD2*	2572	10p11.23	928197	+83897 T > A	0.17	0.002
				992990	+61450 C/A	0.28	0.09
				2236418	−243 A > G	0.17	0.11
Ghrelin	*GHRL*	51738	3p25.3	696217	Leu72Met	0.06	0.74
McKusick-Kaufman syndrome	*MKKS*	8195	20p12	1547	Arg517Cys	0.10	0.62
Leptin Receptor Overlapping Transcript-Like 1 (Endospanin)	*LEPROTL1*	23484	8p21.2 −1		−2625 C > T	0.05	0.94
Proprotein Convertase Subtilisin/Kexin Type 1	*PCSK1*	5122	5q15	6235	T690S C > G	0.25	0.10
Efficiency of energy expenditure							
Uncoupling Protein 2	*UCP2*	7351	11q13	6593669	−866 G > A	0.38	0.75
Uncoupling Protein 3	*UCP3*	7352	11q13	1900849	−55 C > T	0.27	0.44
Regulation of adipocyte differentiation and function							
Forkhead Box C2	*FOXC2*	2303	16q22-24	–	−512 C > T	0.61	0.87
Peroxisome Proliferative Activated Receptor (PPAR)	*PPARGC1A*	10891	4p15.1	8192678	Gly482Ser	0.67	0.59
Gamma Coactivator 1 alpha				2932963	+2962A > G	0.49	0.41
PPAR Gamma isoform 2	*PPARG2*	5468	3p25	1801282	Pro12Ala	0.13	0.84
				3856806	1431C > T	0.13	0.91
				7649970	−820 C > T	0.13	0.74
PPAR Gamma isoform 3	*PPARG3*	5468	3p25	10865710	−681C > G	0.26	0.54
Sterol Regulatory Element Binding Factor 1	*SREBF1*	6720	17p11.2	–	17 C > G	0.36	0.76
WW Domain Containing Adaptor with Coiled-Coil	*WAC*	51322	10p12.1	2807761	−829 A > G	0.50	0.36
Regulation of lipid and glucose metabolism							
Hydroxysteroid (11-beta) Dehydrogenase 1	*HSD11B1*	3290	1q32.2	846910	−2490 G > A	0.06	0.96
Hepatic Lipase C	*LIPC*	3990	15q21-23	6082	644 A > G	0.33	0.99
				1800588	−514 C > T	0.22	0.49
Insulin-like Growth Factor 2	*IGF2*	3481	11p15.5	3168310	1926 C > G	0.30	0.97
				680	ApaI A > G	0.29	0.92
				3842759	−6815 A > T	0.28	0.77
Potassium Inwardly-Rectifying Channel J11 (KIR6.2)	*KCNJ11*	3767	11p15.1	5219	Glu23Lys	0.35	0.27
Ectonucleotide Pyrophosphatase/Phosphodiesterase 1	*ENPP1*	5167	6q23.2	–	IVS20 del T-11	0.25	0.25
				1044498	Lys121Gln	0.16	0.74
				7754561	1044 A > G	0.29	0.57
Production of adipokines							
Adiponectin	*ADIPOQ*	9370	3q27.3	266729	−11377 C > G	0.24	0.29
				2241766	+45 T > G	0.13	0.22
				1501299	+276 T > G	0.70	0.19
				17300539	−311391 G > A	0.08	0.51
CD36 Antigen	*CD36*	948	7q11.2	2232169	−178 A > C	0.56	0.54
Interleukin 6	*IL6*	3569	7p21	1800795	−174 G > C	0.44	0.22
Tumor Necrosis Factor alpha	*TNFα*	7124	6p21.3	1800629	−308 G > A	0.16	0.01
Serine Proteinase Inhibitor 1	*SERPINE1*	5054	7q21.3-22	1799889	−675 insG	0.50	0.11

Subjects and methods

Subjects

A sample of 549 European obese women (BMI ≥ 30 kg/m^2; range: 30–56 kg/m^2) aged 20–50 years were recruited as a part of the NUGENOB project (NUGENOB: "Nutrient Gene Interaction in Human Obesity—Implications for Dietary Guidelines". For details of the project, see www.nugenob.org). The NUGENOB study was a randomised, parallel, two-arm, open label 10-week dietary intervention of two hypo-energetic diets for obese men and women undertaken at eight sites in seven European countries [23]: United Kingdom (Nottingham), The Netherlands (Maastricht), France (Paris and Toulouse), Spain (Pamplona), Czech Republic (Prague), Sweden (Stockholm) and Denmark (Copenhagen). Exclusion criteria were: weight change >3 kg within the 3 months prior to the study start; reported clini-

cally diagnosed hypertension, diabetes or hyperlipidaemia treated by drugs; untreated thyroid disease; surgically or drug-treated obesity; pregnancy; participation in other trials; alcohol or drug abuse. Additionally, thirty women were excluded from the present case-only study due to lack of DNA for genotyping, fasting blood glucose >7 mmol/L or due to extremely high BMI (>60 kg/m^2). Participants were recruited through local sources as available and convenient.

The baseline measurements in obese female subjects were used for the present case-only analysis. The sample sizes stratified by location were: Pamplona, Spain (n = 67); Toulouse, France (n = 30); Paris, France (n = 85); Prague, Czech Republic (n = 90); Maastricht, The Netherlands (n = 49); Copenhagen, Denmark (n = 71), Huddinge, Sweden (n = 74) and Nottingham, United Kingdom (n = 83). The ethical committee at each of the participating centres approved the study. Volunteers were informed about the nature of the study, and written consent was obtained prior to study participation.

Anthropometry and obesity-related variables

All subjects underwent a thorough standardised clinical and physiological examination described in Standard Operational Procedures applied in all participating centres (see www.nugenob.org). Each examination included blood sampling and measurement of height with a calibrated stadiometer and weight (in light indoor clothes and without shoes) with a calibrated set of scales.

Nutritional variables

Three-day weighed food records of two weekdays and one weekend were completed by all the participants. The nutrient variables selected for this study were the percentage of energy intake derived from fat (%E), the ratio of dietary polyunsaturated fat to saturated fat (P:S ratio) and the dietary fibre intake (g/day). The food intake data from the record was converted into nutrient intake data in each centre using local composition tables and computer programs after a careful coordination of a dietician core group following standardised operative procedures (see www.nugenob.org). The values of the selected nutrient variables for each participant were computed as the average across the three days of recordings.

Candidate genes

The selection of candidate genes and Single Nucleotide Polymorphisms (SNPs) was based on their potential contribution to obesity-related phenotypes, their presumed nutrient-sensitive expression and function of the gene products, and the presence of common SNPs with a presumed allele frequency above 0.05. Genotyping was carried out for 42 SNPs in 26 genes related to hypothalamic regulation of appetite, efficiency of energy expenditure, regulation of adipocyte differentiation and function, lipid and glucose metabolism, or production of several adipokines. Table 1 lists the candidate genes and the SNPs determined in this study.

Genotyping

Blood drawing and processing was performed according to international guidelines for genetic studies [24]. Forty out of the 42 SNPs in the 26 genes were genotyped by the LightCycler™ assay (the SLC6A14, CART, GHSR, GAD2, GHRL, LEPROTL1, UCP2, UCP3, WAC, LIPC, IGF2, ENPP1, ADIPOQ, CD36 genes) based on hybridization probes labeled with fluorescent dyes that allow fluorescence resonance energy transfer or by Taqman™ assay (the MKKS, PCSK1, FOXC2, PPARGC1A, PPARG2, PPARG3, SREBF1, HSD11B1, KCNJ11, IL6, TNFα, SERPINE1 genes) (Applied Biosystems®). Sequences of primers pairs, labeled with fluorescein and LC Red 640 are available on request from the authors. One SNP in IGF2 (−6815 A > T) and one in CART (−3608 T > C) were genotyped by direct sequencing. The genotyping success rate was 92.8% through 98.8% except for WAC (89.2%) and SERPINE1 (86.6%) genes.

Statistical methods

Odds Ratio for genotype-by-nutrient interaction (OR_i) refers to the cross-product ratios computed in a sample of obese subjects for each nutrient-genotype categories. In this context, OR_i is an estimate of the ratio of the relative risk for the joint gene-nutrient effects divided by the product of individual genetic or nutrient effects under the assumption of independence between genotypes and the nutrient variables in the source population [16–22]. Consequently, OR_i's can be regarded as effect measure modification of the risk ratio (RR) on a multiplicative scale.

The case-only design has been applied in the genetic epidemiology literature of complex rare diseases for assessing gene-environment interaction as departures for the multiplicative odds ratios [25] provided that gene and environment are independent in non-diseased subjects. However, it has been shown that the OR_i does not require the rare disease assumption for assessing interactions based on departures for

multiplicative *RR* [19, 21]. However, the validity of the case-only study for assessing interactions is highly sensitive to the assumption of gene-environment independence [22].

The statistical test for genotype-by-nutrient interaction (null hypothesis $OR_i = 1$) was assessed through unconditional logistic regression techniques. The genetic polymorphism was considered as the dependent variable in all logistic models. The non-carriers of the variant allele were coded as 0 while carriers of at least one copy of the variant allele were coded as 1. Nutrient variables (fibre intake, P:S ratio and percent of total energy from dietary fat) were included in different logistic equations as independent variables. Using sample tertiles, the nutrient variables were categorized in three groups of equal size (the upper third, the middle third and the lower third). Each nutrient variable was then included in logistic regressions as binary indicators leaving one category (upper third for fibre intake and P:S ratio and lower third for percent dietary fat) as the reference. Additional covariates were included using three different logistic models that incorporate different sets of covariates. Model 1 was fitted with the centre of study as a covariate. Model 2 was fitted with centre of study, age, smoking status, physical activity score and energy intake as covariates. Finally, model 3 was fitted with centre of study, age, smoking status, physical activity score and fat-free mass as covariates. These covariates were included in order to control for factors that may influence the possible non-independence between genetic polymorphisms and nutrient variables [20].

Examination of Hardy–Weinberg equilibrium was carried out by summing up the goodness-of-fit Pearson chi-square statistics for each study centre and comparing them with a chi-square distribution with 8 degrees of freedom. All Statistical analyses were carried out using the STATA 8.2 package (Stata Corp. 2004).

Results

The mean and standard deviation for BMI (35.9 ± 4.6 kg/m^2), percentage of body fat (44.2 ± 4.9%) and energy intake (8.5 ± 2.3 MJ/day) were within the expected values in accordance to the study design. Regarding the genetic variants, all allele frequencies exceeded 0.05 except for *GSHR* −447 C > G and *LEPTROTL1* −2625 C > T, which, however, were retained in the analysis (Table 1). The Hardy–Weinberg test showed that all SNPs except two (*GAD2* + 83897 T > A and *TNFα* −308 G > A) were in Hardy–Weinberg equilibrium (Table 1), but in view of the unsuspicious and minor departure from the equilibrium, these SNPs were kept in the analysis.

Tables available at http://www.nugenob/org/case-only show *P*-values, OR_i's and 95% confidence intervals for assessing the array of interactions between the 42 polymorphisms of candidate genes and the selected nutrient variables under three different statistical models. As shown in the tables, only a small number of statistical tests achieved *P*-values equal or below the standard 0.05 significance level. None of them would be considered as statistically significant after correction for multiple testing.

Only few genotype-by-nutrients were suggested in the analysis and showed consistent results across different statistical models: the interaction between fibre intake and the −514 C > T polymorphism of the hepatic lipase gene (*LIPC*) yielded *P*-values of 0.01 across the three different statistical models considered. Likewise, the −11377G > C polymorphism of the adiponectin gene (*ADIPOQ*) and the −681 C > G polymorphism of the PPARG3 gene might interact with the percentage of energy derived from fat in the diet for the development of obesity (uncorrected *P*-values in the range 0.01–0.05 across the three different statistical models). Tables 2–4 show the results obtained for assessing interactions between polymorphisms at candidate genes and the nutrient factors considered under the statistical model 3. Model 3 includes age, centre of study, smoking status, physical activity score and fat-free mass as covariates. Odds ratios for interaction estimated with other statistical models are available at http://www.nugenob.org/caseonly. *P*-values correspond to the null hypothesis of no interaction considering the joint gene-nutrient effects for the two binary variables defined for the nutrient variable. *P*-values shown in the table were not corrected for multiple comparisons. Some statistical tests are not independent since there are genetic polymorphisms located in the same gene that may exhibit different degree of linkage disequilibrium.

Discussion

The detection of genotype-by-nutrient interactions is useful for a better understanding of the multifactorial causation of complex diseases as well as for the design of prevention strategies in genetic high-risk subjects [6]. However, the assessment of genotype-by-nutrient interactions in epidemiologic studies of obesity can be regarded as a difficult issue due to limitations imposed by the uncertainty over the existence of relevant main effects of genetic polymorphisms for many candidate susceptibility loci and the assumed small magnitude of deviations from the expected values under the hypothesis of the absence of genotype-by-nutrient interactions. In this context, modest genotype-phenotype associations and lack of replication

are typical features of many association studies of complex diseases such as obesity and obesity-related disorders [26]. In the present study, we have tested multiple polymorphisms, different nutrients and different statistical models to assess interactions defined as departures from the multiplicative risk ratios. Given the lack of a consensus in choosing the most adequate strategy for correcting for multiple tests [27, 28], we have decided to show *P*-values in Tables 2–4 that would need adjustments to compensate multiple testing.

Among the genotype-by-nutrient interactions suggested across the different statistical models tested (http://www.nugenob.org/caseonly), a possible interaction is suggested for genetic variants of the *LIPC* gene (−514C > T) and fibre intake. This finding might reflect the role of fibre and the hepatic lipase in faecal biliary excretion, cholesterol metabolism and obesity [29]. It is important to note that the term "fibre" covers a range of different dietary components which have different structural and functional characteristics. On the other hand, the possible interaction between the −11377G > C polymorphism of the *ADIPOQ* gene and dietary fat may also deserve further research, especially considering the impact of fat intake on *ADIPOQ* gene expression [30, 31]. Since the type of interaction that can be assessed in a case-only study is related to the deviations from the multiplicative effects of relative risks [21], no information is directly available about other type of interaction effects.

Apart from formal statistical tests of interaction focusing on *P*-values, it is also important to put more emphasis on the evaluation of the size and consistency of the effects we are assessing, taking into account that the epidemiological genotype-by-nutrient interaction studies are in general in an exploratory phase rather than in a more confirmatory type of study where hypothesis are specified on the basis of the pre-existing knowledge. In this context, further research should be performed to assess the interactions involving genes with OR_i's that show a trend across the tertiles of dietary components such as the polymorphisms at *LEPROTL1, TNFα, ENPP1, PPARG2, PPARG3, MKKS* or *CD36* regarding their interaction with the percentage of dietary energy as fat (Table 2). In this context, genotype-by-nutrient interactions in obesity were previously suggested for the *PPARG* system, specifically for the Pro12Ala *PPARG2* polymorphism and the percentage of dietary fat [30, 31] and CHO intake [32]. Interestingly, an interaction between fat intake and obesity was found for the −681 C > G PPARG3 polymorphism.

Albert et al. [22] pointed out that inferences on gene-environment interactions derived from case-only studies heavily depend on the key assumption of independence between the genotypes and the nutrient variables in the population. In this context, some authors have suggested the use of a sample of controls for checking such assumption. However, it has been shown that using controls for this purpose may be misleading in many situations [20]. Additionally, it

Table 2 Odds ratios (95% confidence interval) and *P*-values for interaction between candidate genes and percentage of dietary fat in the obese-only sample

Gene	Genetic polymorphism	Percentage of dietary fat		
		OR_i middle third	OR_i upper third	*P*
Regulation of appetite				
SLC6A14	+22510 C > G	0.7 (0.4–1.3)	0.6 (0.4–1.1)	0.28
CART	−3608 T > C	0.7 (0.4–1.2)	0.9 (0.5–1.7)	0.41
	−1702 C > T	1.2 (0.7–2.1)	0.9 (0.5–1.5)	0.32
	−175 A > G	0.8 (0.4–1.3)	1.1 (0.6–2.1)	0.37
	−1336 delA	0.9 (0.5–1.8)	1.4 (0.7–2.8)	0.41
GHSR	Leu149Leu	0.8 (0.2–2.8)	1.0 (0.3–3.5)	0.93
GAD2	+83897 T > A	0.7 (0.4–1.1)	0.7 (0.4–1.2)	0.25
	+61450 C/A	0.8 (0.5–1.3)	0.7 (0.4–1.2)	0.48
	−243 A > G	0.7 (0.4–1.2)	0.7 (0.4–1.2)	0.31
GHRL	Leu72Met	1.0 (0.5–2.0)	0.6 (0.2–1.3)	0.33
MKKS	Arg517Cys	0.7 (0.4–1.3)	0.5 (0.3–1.0)	0.15
LEPROTL1	−2625 C > T	1.1 (0.5–2.5)	0.4 (0.2–1.2)	0.10
PCSK1	T690S C > G	0.8 (0.5–1.4)	1.2 (0.7–2.0)	0.36
Efficiency of energy expenditure				
UCP2	−866 G > A	0.9 (0.5–1.4)	1.0 (0.6–1.7)	0.79
UCP3	−55 C > T	1.3 (0.8–2.2)	0.9 (0.6–1.5)	0.22
Regulation of adipocyte differentiation and function				
FOXC2	−512 C > T	1.2 (0.6–2.4)	1.2 (0.6–2.4)	0.81
PPARGC1A	Gly482Ser	0.7 (0.3–1.6)	0.7 (0.3–1.6)	0.64
	+2962 A > G	0.8 (0.5–1.4)	0.8 (0.4–1.4)	0.63
PPARG2	Pro12Ala	1.0 (0.6–1.8)	1.2 (0.7–2.2)	0.76
	1431 C > T	1.4 (0.8–2.6)	1.7 (0.9–3.2)	0.22
	−820 C > T	1.1 (0.6–1.9)	1.5 (0.8–2.7)	0.40
PPARG3	−681 C > G	1.7 (1.0–2.7)	1.9 (1.1–3.3)	0.04
SREBF1	17 C > G	1.2 (0.7–1.9)	1.0 (0.6–1.7)	0.82
WAC	−829 A > G	0.9 (0.5–1.6)	1.1 (0.6–2.1)	0.84
Regulation of lipid and glucose metabolism				
HSD11B1	−2490 G > A	0.6 (0.3–1.2)	0.6 (0.3–1.3)	0.29
LIPC	Ans215Ser	0.8 (0.5–1.3)	0.8 (0.4–1.3)	0.52
	−514 C > T	0.8 (0.5–1.3)	1.0 (0.6–1.6)	0.64
IGF2	1926 C > G	1.3 (0.8–2.2)	0.8 (0.5–1.4)	0.11
	ApaI A > G	1.3 (0.8–2.2)	0.9 (0.5–1.5)	0.23
	−6815 A > T	1.2 (0.7–2.0)	1.1 (0.6–1.8)	0.71
KCNJ11	Glu23Lys	1.0 (0.6–1.6)	1.2 (0.7–2.1)	0.73
ENPP1	IVS20 del T-11	0.7 (0.4–1.2)	0.5 (0.3–0.9)	0.08
	Lys121Gln	0.6 (0.4–1.0)	0.6 (0.3–1.0)	0.08
	1044 A > G	0.9 (0.5–1.5)	0.6 (0.4–1.1)	0.24
Production of adipokines				
ADIPOQ	−11377 C > G	1.5 (0.9–2.4)	0.8 (0.5–1.3)	0.04
	+45 T > G	0.9 (0.5–1.6)	1.4 (0.8–2.7)	0.24
	+276 T > G	1.5 (0.7–3.2)	1.0 (0.4–2.2)	0.50
	−11391 G > A	0.9 (0.4–2.0)	1.6 (0.7–3.4)	0.25
CD36	−178 A > C	0.9 (0.5–1.7)	0.8 (0.4–1.6)	0.85
IL6	−174 G > C	1.3 (0.7–2.1)	1.2 (0.7–2.0)	0.70
TNFα	−308 G > A	1.2 (0.7–2.1)	1.5 (0.8–2.7)	0.41
SERPINE1	−675 insG	0.6 (0.3–1.1)	0.9 (0.5–1.8)	0.13

Notes: The reference category is the wild-type homozygous genotype and the lower tertile of the percentage of calories derived from fat

Table 3 Odds ratios (95% confidence interval) and *P*-values for interaction between candidate genes and P:S ratio in the obese-only sample

Gene	Genetic polymorphism	P:S ratio OR_i middle third	P:S ratio OR_i upper third	*P*
	Regulation of appetite			
SLC6A14	+22510 C > G	1.1 (0.7–1.7)	0.8 (0.5–1.3)	0.50
CART	−3608 T > C	0.8 (0.5–1.4)	0.6 (0.4–1.1)	0.30
	−1702 C > T	0.9 (0.5–1.4)	1.3 (0.8–2.1)	0.33
	−175 A > G	0.8 (0.4–1.3)	0.6 (0.3–1.0)	0.15
	−1336 delA	0.7 (0.4–1.2)	1.0 (0.6–1.9)	0.34
GHSR	Leu149Leu	0.9 (0.3–3.1)	1.6 (0.5–5.2)	0.56
GAD2	+83897 T > A	0.8 (0.5–1.3)	1.2 (0.7–2.0)	0.20
	+61450 C/A	0.7 (0.5–1.1)	0.9 (0.6–1.5)	0.35
	−243 A > G	0.8 (0.5–1.3)	1.0 (0.6–1.7)	0.60
GHRL	Leu72Met	0.8 (0.4–1.6)	0.7 (0.3–1.5)	0.60
MKKS	Arg517Cys	1.2 (0.7–2.1)	1.3 (0.7–2.4)	0.68
LEPROTL1	−2625 C > T	1.2 (0.6–2.7)	1.1 (0.4–2.6)	0.87
PCSK1	T690S C > G	1.0 (0.6–1.6)	1.0 (0.6–1.7)	0.99
	Efficiency of energy expenditure			
UCP2	−866 G > A	1.2 (0.7–1.9)	1.0 (0.6–1.6)	0.71
UCP3	−55 C > T	1.3 (0.8–2.1)	1.4 (0.9–2.3)	0.33
	Regulation of adipocyte differentiation and function			
FOXC2	−512 C > T	1.4 (0.7–2.6)	1.3 (0.7–2.4)	0.58
PPARGC1A	Gly482Ser	0.7 (0.3–1.5)	0.8 (0.4–1.9)	0.70
	+2962 A > G	1.1 (0.6–1.8)	1.0 (0.6–1.8)	0.95
PPARG2	Pro12Ala	1.1 (0.6–1.9)	1.2 (0.7–2.2)	0.79
	1431 C > T	1.5 (0.9–2.6)	1.1 (0.6–2.0)	0.28
	−820 C > T	1.0 (0.6–1.7)	1.2 (0.7–0.2)	0.72
PPARG3	−681 C > G	1.3 (0.8–2.1)	1.3 (0.8–2.1)	0.42
SREBF1	17 C > G	1.2 (0.8–2.0)	1.1 (0.7–1.7)	0.68
WAC	−829 A > G	1.8 (1.0–3.1)	1.0 (0.5–1.7)	0.09
	Regulation of lipid and glucose metabolism			
HSD11B1	−2490 G > A	0.6 (0.3–1.3)	0.7 (0.3–1.4)	0.36
LIPC	Ans215Ser	1.0 (0.6–1.6)	0.9 (0.5–1.4)	0.86
	−514 C > T	1.1 (0.7–1.8)	1.3 (0.8–2.2)	0.50
IGF2	1926 C > G	1.2 (0.7–1.9)	0.9 (0.6–1.5)	0.60
	ApaI A > G	1.1 (0.7–0.8)	0.9 (0.6–1.5)	0.75
	−6815 A > T	1.2 (0.7–1.9)	1.4 (0.9–2.4)	0.37
KCNJ11	Glu23Lys	0.8 (0.5–1.4)	0.7 (0.4–1.2)	0.40
ENPP1	IVS20 del T-11	1.0 (0.6–1.6)	0.7 (0.5–1.2)	0.41
	Lys121Gln	0.9 (0.6–1.6)	1.3 (0.7–2.1)	0.52
	1044 A > G	1.1 (0.7–1.7)	0.8 (0.5–1.3)	0.38
	Production of adipokines			
ADIPOQ	−11377 C > G	1.3 (0.8–2.1)	1.2 (0.8–2.0)	0.52
	+45 T > G	0.8 (0.5–1.4)	1.0 (0.6–1.8)	0.74
	+276 T > G	1.0 (0.5–2.0)	0.9 (0.4–2.0)	0.99
	−11391 G > A	1.1 (0.5–2.1)	1.6 (0.8–3.3)	0.31
CD36	−178 A > C	1.2 (0.7–2.2)	1.2 (0.7–2.3)	0.75
IL6	−174 G > C	1.2 (0.7–1.9)	1.7 (1.0–2.9)	0.15
TNFα	−308 G > A	1.0 (0.6–1.7)	0.6 (0.4–1.1)	0.13
SERPINE1	−675 insG	0.7 (0.4–1.3)	1.1 (0.6–2.0)	0.35

Notes: The reference category is the wild-type homozygous genotype and the upper tertile of P:S ratio

Table 4 Odds ratios (95% confidence interval) and *P*-values for interaction between candidate genes and fibre intake in the obese-only sample

Gene	Genetic polymorphism	Fibre intake (grams/day) OR_i middle third	Fibre intake (grams/day) OR_i upper third	*P*
	Regulation of appetite			
SLC6A14	+22510 C > G	0.6 (0.4–1.0)	1.0 (0.6–1.7)	0.06
CART	−3608 T > C	1.1 (0.6–1.9)	0.8 (0.5–1.5)	0.56
	−1702 C > T	1.0 (0.6–1.6)	1.1 (0.6–1.8)	0.95
	−175 A > G	1.1 (0.6–1.9)	0.8 (0.4–1.4)	0.53
	−1336 delA	1.3 (0.7–2.4)	1.4 (0.7–2.8)	0.56
GHSR	Leu149Leu	0.4 (0.1–1.3)	0.6 (0.2–2.0)	0.33
GAD2	+83897 T > A	1.0 (0.6–1.6)	1.2 (0.7–2.0)	0.79
	+61450 C/A	1.1 (0.7–1.7)	1.2 (0.7–1.9)	0.83
	−243 A > G	1.2 (0.7–1.9)	1.1 (0.6–1.9)	0.83
GHRL	Leu72Met	0.7 (0.4–1.5)	1.0 (0.5–2.2)	0.66
MKKS	Arg517Cys	1.6 (0.9–3.0)	1.7 (0.9–3.2)	0.20
LEPROTL1	−2625 C > T	0.8 (0.4–1.8)	0.6 (0.2–1.6)	0.60
PCSK1	T690S C > G	1.2 (0.7–1.9)	0.9 (0.5–1.5)	0.57
	Efficiency of energy expenditure			
UCP2	−866 G > A	1.5 (0.9–2.3)	1.4 (0.8–2.4)	0.25
UCP3	−55 C > T	1.3 (0.8–2.1)	1.0 (0.6–1.6)	0.29
	Regulation of adipocyte differentiation and function			
FOXC2	−512 C > T	1.1 (0.6–2.1)	0.5 (0.3–1.1)	0.07
PPARGC1A	Gly482Ser	0.6 (0.3–1.3)	0.4 (0.2–1.0)	0.16
	+2962A > G	0.7 (0.4–1.2)	0.8 (0.4–1.4)	0.38
PPARG2	Pro12Ala	0.9 (0.5–1.5)	0.8 (0.4–1.4)	0.68
	1431C > T	0.9 (0.5–1.5)	0.9 (0.5–1.6)	0.88
	−820 C > T	0.9 (0.2–1.5)	0.7 (0.4–1.3)	0.59
PPARG3	−681 C > G	0.9 (0.6–1.5)	0.7 (0.4–1.2)	0.43
SREBF1	17 C > G	1.0 (0.6–1.6)	0.7 (0.4–1.2)	0.40
WAC	−829 A > G	1.0 (0.6–1.7)	0.9 (0.5–1.7)	0.96
	Regulation of lipid and glucose metabolism			
HSD11B1	−2490 G > A	0.6 (0.3–1.3)	0.7 (0.3–1.5)	0.43
LIPC	Ans215Ser	1.2 (0.8–1.9)	1.2 (0.7–2.0)	0.72
	−514 C > T	0.5 (0.3–0.9)	0.5 (0.3–0.8)	0.01
IGF2	1926 C > G	1.0 (0.6–1.6)	0.7 (0.4–1.2)	0.35
	ApaI A > G	1.0 (0.6–1.5)	0.6 (0.4–1.0)	0.12
	−6815 A > T	0.8 (0.5–1.4)	0.7 (0.4–1.3)	0.54
KCNJ11	Glu23Lys	1.3 (0.8–2.0)	1.2 (0.7–2.0)	0.63
ENPP1	IVS20 del T-11	1.1 (0.7–1.8)	1.4 (0.8–2.3)	0.51
	Lys121Gln	0.9 (0.5–1.4)	0.9 (0.5–1.6)	0.82
	1044 A > G	1.0 (0.6–1.6)	1.1 (0.6–1.8)	0.97
	Production of adipokines			
ADIPO	−11377 C > G	1.1 (0.7–1.7)	1.0 (0.6–1.7)	0.94
	+45 T > G	0.7 (0.4–1.1)	0.6 (0.3–1.1)	0.19
	+276 T > G	0.5 (0.2–1.0)	0.7 (0.3–1.6)	0.13
	−11391 G > A	1.7 (0.8–3.4)	2.0 (0.9–4.2)	0.20
CD36	−178 A > C	0.7 (0.4–1.3)	0.8 (0.4–1.6)	0.60
IL6	−174 G > C	1.1 (0.6–1.7)	1.4 (0.8–2.4)	0.56
TNFα	−308 G >A	0.9 (0.5–1.4)	0.9 (0.5–1.6)	0.85
SERPINE1	−675 insG	1.3 (0.8–2.4)	1.0 (0.6–1.9)	0.54

Notes: The reference category is the wild-type homozygous genotype and the fibre intake

has been suggested that the main reason for including covariates in the logistic models using case series addressing for interactions, is to control for possible factors affecting the non-independence between genes and environment [20]. Therefore, we have incorporated different sets of covariates in the logistic equations (models 1–3). The centre of origin was included as a covariate in all logistic models to partially take into account issues such as population stratification by ethnicity in genotype frequencies [33, 34], as well as differences in nutritional habits [35] and physical activity levels [36] across European countries.

A limitation of the design of this study is directly related to its cross-sectional nature. Thus, it must be assumed that the dietary factors assessed in the obese-only sample are also reflecting the dietary factors that

might have been operating while obesity developed. Moreover, as others we have found difficulties in accurately assessing the dietary components including the energy intake in the participants, as occurs in nutritional epidemiology studies concerning obese subjects [37]. The distorting effect of the cross-sectional measurements on causal inferences is possibly not as relevant in the genetic polymorphisms if we assume no selection bias related to the genetic profile. Another concern arises from the fact that obese subjects were non-randomly recruited from the population and this procedure was potentially affected by selection forces that might distort the inferences on genotype-by-nutrient multiplicative interactions [21]. As shown in tables, not all the tests for detecting interactions are independent because there are polymorphisms that are located in the same gene and therefore they may exhibit some degree of linkage disequilibrium.

Although the use of obese-only samples is theoretically a useful approach to detect genotype-by-nutrient interactions in obesity, few interactions have been indeed suggested in the present study of obese European women addressing possible interaction between genetic polymorphisms of 26 candidate genes and three nutrient variables (dietary fibre intake, the ratio of dietary polyunsaturated fat to saturated fat, and the percentage of energy derived from fat in the diet). The detection of genotype-by-nutrient interactions in obesity is a complex issue that clearly needs further research. An interesting exploratory observation in our study refers to the possible interaction between carriers of the genetic variant of the *LIPC* gene (−514C > T) and fibre intake. The possible interactions with fat intake concerning the −11377G > C polymorphism of the adiponectin gene (*ADIPOQ*) and the −681C > G polymorphism of the *PPARG3* deserve further investigation.

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