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Variants of uncoupling protein-2 gene and obesity: interaction with peroxisome proliferator-activated receptorγ2

Francesco P. Mancini*‡[1], Lina Sabatino*[1], Vittorio Colantuoni*‡, Fabrizio Pasanisi†, Carmine Finelli†, Franco Contaldo†, Maria Masulli†, Gabriele Riccardi† and Olga Vaccaro†

**Department of Biological and Environmental Sciences, University of Sannio, Benevento and Departments of †Clinical and Experimental Medicine and ‡Biochemistry and Medical Biotechnologies, 'Federico II' University, Napoli, Italy*

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Summary

OBJECTIVE To analyse the association of the *UCP2* gene, alone or in combination with the PPARγ2 gene, with obesity.

DESIGN Cross-sectional, case–control study.

STUDY POPULATION From a working population of 4500 Italian Caucasian employees of the Italian telephone company participating in a firm-sponsored health screening programme, we selected all those with obesity [$n = 122$; body mass index (BMI) ≥ 30 kg/m^2]. For each case, three nonobese age- and sex-matched individuals were selected as controls from the same population ($n = 374$). Included in the study were also 76 severely obese (BMI ≥ 40 kg/m^2) patients consecutively admitted to the obesity clinic of the department. Diabetic individuals were excluded.

MEASUREMENTS The –866G/A UCP2 and the Pro12Ala PPARγ2 polymorphisms were determined on genomic DNA of the studied individuals. Several metabolic and anthropometric measures were also obtained, like plasma glucose, insulin, triglycerides, total cholesterol, high-density lipoprotein (HDL) cholesterol and BMI.

RESULTS BMI, plasma glucose, insulin, triglycerides, total and HDL cholesterol were not significantly different in carriers and noncarriers of the –866G/A variant. No significant association was observed between the –866G/A UCP2 gene polymorphism and moderate or severe obesity. This was also observed when the UCP2 polymorphism was analysed in combination with the PPARγ2 polymorphisms.

CONCLUSIONS The –866G/A variants of the UCP2 gene are not associated with either obesity or other features of the metabolic syndrome in the studied groups of the Italian population. This negative finding is not modified after a combined analysis of the UCP2 polymorphism and the Pro12Ala polymorphism of PPARγ2.

The genetic control of metabolism is still an open field of research both from a physiological and a pathological perspective. Genetic factors are involved in the multifactorial origin of the most important metabolic diseases. Recent knowledge about the uncoupling proteins (UCPs) has indicated possible novel molecular links among energy metabolism, obesity and diabetes (Zhang *et al*., 2001). UCPs are inner mitochondrial membrane transporters and are considered pivotal regulators of energy homeostasis; they can uncouple mitochondrial oxidative phosphorylation by dissipating the respiration-derived proton gradient across the inner mitochondrial membrane, therefore transforming energy into heat (Boss *et al*., 2000; Ricquier & Bouillaud, 2000). Differences in this mechanism could be one of the molecular bases of the genetic predisposition to obesity and type-2 diabetes in humans (Dalgaard & Pedersen, 2001; Argilés *et al*., 2002). Although a physiological, cold-induced, thermogenic role has been established for UCP1 in brown adipose tissue (BAT), it is still unclear what are the functions of UCP2, -3, -4 and -5, the other isoforms so far identified. While UCP3 is preferentially expressed in skeletal muscle and UCP4 and -5 are specifically found in brain, the ubiquitous distribution of UCP2, including pancreatic beta-cells, is particularly well-suited for a dual function in the control of energy and glucose metabolism. Very recently, a role for UCPs has been proposed in a feedback control of reactive oxygen species inside mitochondria (Echtay *et al*., 2002). Several papers have been published about the association of natural variants of UCPs and body mass index (BMI; reviewed in Schonfeld-Warden & Warden, 2001). Although not all studies draw concordant conclusions,

Correspondence: Francesco Paolo Mancini, Via Port' Arsa, 11–82100 Benevento, Italy. Tel: +39 0824 305107; Fax: +39 0824 23013; E-mail: mancini@unisannio.it

[1]These authors equally contributed to this work.

UCP2 appears the strongest contributor to BMI, as compared to UCP1 and UCP3. A recent paper about this issue is particularly convincing because reported an association among a polymorphism in the *UCP2* promoter (−866 G/A), increased level of adipose tissue *UCP2* mRNA *in vivo*, an higher transactivating capacity in *in vitro* assays and a decreased risk of obesity in humans (Esterbauer *et al*., 2001). Although the evidence provided in this paper is very strong due to the convergence of experimental and epidemiological evidence, several potential limitations apply to association studies. In fact, environmental and genetic differences between populations may account for different results of different studies. Therefore, it may be helpful to replicate a similar study in a different setting. It is also plausible that gene interactions are potentially important in the aetiology of complex diseases like obesity, and it has been suggested that multiple gene interactions may lead to a synergistic effect in the development of obesity (Hsueh *et al*., 2001). This is particularly intriguing when two or more genes are candidate genes, whose expression and function can be reciprocally influenced as it has been suggested for UCPs and peroxisome proliferator-activated receptors (PPARs). PPARs are ligand-activated nuclear receptors that regulate transcription of genes involved in lipid and glucose metabolism, adipocyte differentiation, inflammation and, probably, cancerogenesis (Desvergne & Wahli, 1999). The PPAR-γ isoform, in particular, plays an important role in adipose tissue and several reports have evaluated the relationship of genetic polymorphisms of PPAR-γ and BMI or obesity, but conflicting evidence was obtained (Beamer *et al*., 1998; Deeb *et al*., 1998; Mori *et al*., 1998; Ek *et al*., 1999; Mancini *et al*., 1999; Vaccaro *et al*., 2002). Interestingly, it has been reported that PPARs can affect UCP2 expression (Aubert *et al*., 1997; Rieusset *et al*., 1999; Viguerie-Bascands *et al*., 1999; Chevillotte *et al*., 2001). Therefore, we resolved to study the relation of the −866G/A polymorphism in the *UCP2* gene with obesity and several metabolic parameters and subsequently to repeat the analysis combining the UCP2 polymorphism with the Pro12Ala variant of *PPARγ*, in unrelated Italian Caucasian people. Furthermore, we tested the relationship between UCP2 with several metabolic parameters, like plasma lipids, glucose and insulin, which could be influenced by this gene.

Materials and methods

Study population

Of 4500 employees of the Italian Telephone Company in the age range 35–65 years, participating in a firm-sponsored health screening, all those with obesity (BMI ≥ 30 kg/m^2, 91 men, 31 women) were selected to participate in the study; this group is referred to as 'group 1'. For each case, three nonobese controls (BMI < 30 kg/m^2, 258 men, 116 women), matched on age and sex, were also selected from the same reference population. Also included in the study were 76 young adults with severe, uncomplicated obesity (BMI ≥ 40 kg/m^2, i.e. WHO class 3, 41 men, 35 women; WHO, 1997) manifesting early in life (i.e. before 40 years of age) who were consecutively seen at the obesity clinic of the department over a three-month period. This group is referred to as 'group 2'. Specific exclusion criteria were diabetes (i.e. fasting plasma glucose ≥ 7 mmol/l, or under medication for diabetes) or use of hypolipidaemic drugs. All participants were Caucasians of Italian origin residents in the Campania region in Southern Italy. None were taking drugs for weight control or affecting glucose or lipid metabolism. Informed consent was obtained from all participants; the study protocol was approved by the local ethics committee.

Measurements

Height and weight were measured in light underclothing. BMI (kg/m^2) was calculated.

Blood pressure was measured in supine position after a 5-min rest. The average of three readings taken 2 min apart was used in the analysis.

Blood samples were collected after an overnight fasting. Plasma glucose, triglycerides, total cholesterol and high-density lipoprotein (HDL) cholesterol were measured with an Ektakem DT-60 (Eastman Kodak, Rochester, NY, USA) using already validated dry chemistry methodologies (El-Deriny *et al*., 1986; Marotta *et al*., 1994). Accuracy and reproducibility were monitored by daily determination of all the above parameters on each of two reference sera (normal and high concentration). The accuracy was 1·2% for HDL cholesterol, 2·1% for total cholesterol, 3·6% for triglycerides and 3·0% for glucose. Reproducibility, as assessed by the calculation of the between-day coefficient of variation was 6·3% for HDL cholesterol, 4·0% for total cholesterol, 5·9% for triglyceride and 3·6% for plasma glucose. Plasma insulin was measured by radioimmunoassay on frozen samples (Roth *et al*., 1973); the detection limit was less than 7 pmol/l. The intra-assay and interassay coefficients of variation were 3·0% and 5·8%, respectively, at the level of 174 pmol/l. Insulin resistance was estimated with the homeostatic model assessment (HOMA), a technique validated against clamp studies, and suited for population studies (Mattheus *et al*., 1985; Bonora *et al*., 2000).

DNA assays

Genomic DNA was purified from peripheral blood leucocytes (Miller *et al*., 1988). Both UCP2 and PPARγ2 variants were determined by restriction enzyme digestion of DNA fragments generated by polymerase chain reaction. Detailed experimental procedures have been already reported (Mancini *et al*., 1999; Esterbauer *et al*., 2001). For 5% of the samples, genetic analysis

was performed on duplicates in a blinded fashion and a 100% concordance of results was obtained.

Statistical analysis

Data are given as mean and standard deviation or percentages. Log-transformed values were used in the analysis of very skewed variables like plasma insulin and triglyceride concentrations; the original values are given in Tables 1 and 2. The statistical analysis was performed with the SPSS package for Windows. Unpaired Student *t*-test and analysis of variance were used to compare means. Proportions were compared by χ^2 analysis.

Results

Pertinent clinical data of the three study groups are given in Table 1. By study design, obese people in group 2 were substantially and significantly younger and heavier as compared to obese people in group 1 (age: 31 ± 7 *vs.* 46 ± 8; BMI: 50·2 ± 9·3 *vs.* 36·2 ± 4·8). There were some expected significant differences between obese individuals and nonobese controls, such as higher fasting glucose and triglycerides in group 1, and higher insulin, HOMA and lower HDL cholesterol in both groups 1 and 2 as compared to the control group.

In Table 2, frequencies of the −866G/A polymorphism of UCP2 are given for nonobese controls and the two different groups of obese people. The prevalence of 866G/A genotypes of *UCP2* was not significantly different in obese (both group 1 and group 2) and nonobese participants ($\chi^2 = 7{\cdot}3$; $P = 0{\cdot}2$).

To explore whether the *UCP2*–866G/A polymorphism is related to BMI and other variables related to insulin resistance, we compared the mean values of BMI, plasma glucose, insulin and triglycerides in the two groups of obese and nonobese controls across UCP2 genotypes (Table 2). No significant differences were found among the three different genotypes either in obese or in nonobese participants; in particular, no differences were observed between carriers and noncarriers of the 866G/A variant in regard to BMI or metabolic variables associated with insulin resistance.

Furthermore, the −866G/A polymorphism of the *UCP2* gene was analysed in combination with the Pro12Ala variant of PPARγ2 (data not shown). No significant differences were detected in the distribution of the combined genotypes between nonobese controls and both groups of obese participants. In particular, the genotype combination hypothetically more protective against obesity (Pro/Pro with A/A + G/A) was not more frequent in nonobese (5·6%) *vs.* obese individuals (9·8 and 9·2% in the two obese groups). Similarly, the genotype combination supposedly more predisposing to obesity (Pro/Ala + Ala/Ala with G/G) was not significantly more frequent in obese (8·2 and 7·9% in the two obese groups) *vs.* nonobese individuals (7·8%).

Table 1 Characteristics of the study populations

	Males (%)	Age (years)	BMI (kg/m^2)	SBP (mmHg)	DBP (mmHg)	Glucose (mmol/l)	Insulin (pmol/l)	HOMA	HDL-chol (mmol/l)	Triglycerides (mmol/l)
Controls Nonobese ($n = 374$)	69	45 ± 6	24·2 ± 2·3	133 ± 16	85 ± 10	5·27 ± 0·44	49·31 ± 20·84 (14·58–162·51)	1·68 ± 0·2	1·27 ± 0·38	1·42 ± 0·76 (0·45–6·78)
Group 1 Moderately obese ($n = 122$)	75	46 ± 8	36·2 ± 4·8†	140 ± 21†	91 ± 16	5·55 ± 0·61	84·04 ± 51·39 (15·97–355·58)	2·9 ± 1·9	1·17 ± 0·34*	1·84 ± 0·81* (0·53–4·37)
Group 2 Severely obese ($n = 76$)	54	31 ± 7†‡	50·2 ± 9·3†‡	130 ± 14	81 ± 10	5·27 ± 0·54	170·85 ± 85·42†‡ (15·28–522·96)	5·81 ± 3·1*	1·12 ± 0·30*	1·45 ± 0·73 (0·35–5·66)

*$P < 0{\cdot}05$, †$P < 0{\cdot}001$ *vs.* nonobese controls; ‡$P < 0{\cdot}001$ *vs.* group 1. SBP, systolic blood pressure; DBP, diastolic blood pressure; (), range.

Table 2 BMI and measured plasma levels of metabolic variables by UCP2 polymorphism in obese and nonobese people

UCP2 Polymorphism	A/A	A/G	G/G	P between groups
Controls nonobese (n = 374)	(n = 26) 7·0%	(n = 165) 44·1%	(n = 183) 48·9%	
BMI (kg/m^2)	24·2 ± 2·2	24·1 ± 2·5	24·2 ± 2·0	NS
Glucose (mmol/l)	5·75 ± 1·75	6·14 ± 1·94	6·37 ± 2·63	NS
Triglycerides (mmol/l)	1·29 ± 0·61	1·57 ± 0·89	1·55 ± 0·93	NS
Cholesterol (mmol/l)	5·22 ± 0·70	5·30 ± 1·09	5·25 ± 1·01	NS
HDL cholesterol (mmol/l)	1·22 ± 0·35	1·25 ± 0·38	1·29 ± 0·38	NS
HOMA	2·0 ± 1·4	2·2 ± 1·6	2·2 ± 1·5	NS
Group 1				
Moderately obese (n = 122)	(n = 13) 10·7%	(n = 43) 35·2%	(n = 66) 54·1%	
BMI (kg/m^2)	34·6 ± 3·6	37·2 ± 3·9	35·9 ± 4·4	NS
Glucose (mmol/l)	5·77 ± 0·56	5·44 ± 0·72	5·55 ± 0·56	NS
Triglycerides (mmol/l)	2·00 ± 0·86	1·77 ± 0·71	1·80 ± 0·83	NS
Cholesterol (mmol/l)	5·92 ± 1·14	5·66 ± 1·14	5·51 ± 0·98	NS
HDL cholesterol (mmol/l)	1·08 ± 0·15	1·23 ± 0·34	1·20 ± 0·36	NS
HOMA	2·5 ± 0·9	3·4 ± 2·7	2·9 ± 1·9	NS
Group 2				
Severely obese (n = 76)	(n = 7) 9·2%	(n = 39) 51·3%	(n = 30) 39·5%	
BMI (kg/m^2)	48·7 ± 8·5	50·1 ± 10·0	50·6 ± 8.7	NS
Glucose (mmol/l)	5·66 ± 0·50	5·22 ± 0·56	5·27 ± 0·50	NS
Triglycerides (mmol/l)	2·14 ± 0·92	1·85 ± 0·75	1·94 ± 0·85	NS
Cholesterol (mmol/l)	4·62 ± 1·10	4·97 ± 0·82	4·58 ± 1·14	NS
HDL cholesterol (mmol/l)	1·13 ± 0·38	1·16 ± 0·31	1·07 ± 0·25	NS
HOMA	5·8 ± 2·2	5·8 ± 3·0	6·5 ± 3·2	NS

Discussion

The worldwide spreading of overweight and obesity in the general population is a threat for human health due to the promoting action on chronic-degenerative diseases. Although environmental factors are well known and have a profound effect on body weight, unravelling the genetic component predisposing to obesity is a very active field of research and the list of candidate genes for this condition is constantly growing (Rankinen *et al*., 2002).

UCPs have attracted a lot of attention because they can modulate energy conservation and dissipation, thus providing a potential contribution to the development of metabolic disorders like obesity and diabetes. However, the pathophysiological role of UCPs, with the exception of the thermogenic function of UCP1, has not been clearly established. Two seminal papers report concordant data about a negative association between UCP2 levels in pancreatic beta-cells and insulin secretion (Chan *et al*., 2001; Zhang *et al*., 2001). Also, the physiological role of UCP3 is still elusive. Mice overexpressing UCP3 are hyperphagic and lean, and *de novo* expression of UCP3, after fenofibrate treatment, has been associated to reduced weight gain and adiposity in diet-induced obese rats (Mancini *et al*., 2001; Lanni *et al*., 2002). However, UCP3-knockout mice are not obese (Clapham *et al*., 2000; Vidal-Puig *et al*., 2000).

In humans, several genetic studies have looked at the association of naturally occurring variants of UCP genes and metabolic parameters and syndromes (reviewed in Schonfeld-Warden & Warden, 2001). Although not all studies are concordant, especially UCP2 appears to be related with BMI. Recently, it has been demonstrated that the −866G/A common polymorphism in the promoter of *UCP2* is associated to an increased *in vivo* level of adipose tissue mRNA, increased transcription rate *in vitro* and a reduced risk of obesity in humans (Esterbauer *et al*., 2001). In the present study we have investigated the relationship of this polymorphism of the *UCP2* promoter with obesity in a sample of Italian Caucasians, all coming from the same geographical area around Naples in Southern Italy. In particular, we studied two different phenotypes of obesity: a population-based group of middle-aged, moderately obese individuals (average BMI of 36·2 kg/m^2) and a clinic-based sample of young adults with uncomplicated, severe obesity (average BMI of 50·2 kg/m^2), manifesting early in life. In this latter, more homogeneous group a stronger contribution of the genetic background to the metabolic disorder should be expected as higher heritability for severe obesity with early onset as compared to obesity acquired later in life is described (Rosenbaum *et al*., 1997). This notwithstanding, the distribution of *UCP2* genotype was not significantly different between nonobese and both groups of obese individuals.

The possibility of a type 2 error should be taken into account. With the sample size studied we have a 80% power of detecting a relative risk of about 0·6 or less for the presence of the mutated allele (A/A + A/G) in obese *vs.* nonobese controls with a significance of 0·05 (i.e. a 40% reduction in the risk of obesity associated with the A variant). A smaller contribution may be missed, and would be in line with the concept that obesity is multifactorial with a polygenic background and strong environmental component which may mask the small effect of many genes. We believe that the presence of the group of extremely obese young people, i.e. with an average BMI of 50 kg/m^2 and a mean age of 31 years, gives additional strength to the present data because it is a particularly well-suited sample to reveal a genetic basis for obesity.

Our data are at variance with those of Esterbauer *et al*. (2001); possible explanations include difference in the geographical origin of the study populations, which may result in different genetic and environmental background: other close loci with potential influence on the same phenotype, like UCP3, could be different; moreover, it is well known that nutritional habits vary in Europe from north to south. It is also known that PPARs are activated by nutrients like long-chain fatty acids. Finally, gender distribution is quite different between the two studies: male/female ratio is 2·1 in our study and 0·3 in the population studied by Esterbauer *et al*. (2001), and the same authors report that allele frequencies for UCP2–866A/G polymorphism are quite different between males and females.

We also explored the hypothesis of gene–gene interaction between the variants of the *PPARγ* and *UCP2* genes by analysing the combined effect of the two polymorphisms, Pro12Ala of PPARγ2 and −866G/A of UCP2. This hypothesis was particularly stimulating because PPARγ2 is a very strong candidate for interacting with UCP2 in the pathogenesis of obesity. In particular, the Pro12Ala substitution occurs within the ligand-independent activation domain and could have functional relevance. Moreover, PPARγ2 can affect the level of UCP2 by direct induction of gene expression or by modulating the levels of fatty acids, which in turn influence the levels of *UCP2* gene transcription. However, we did not observe a reduction of the risk of obesity associated with any combination of the −866G/A polymorphism of the *UCP2* promoter and the Pro12Ala polymorphism of PPARγ2. Although with subgroup analysis the power of the study is further reduced, this might be partly compensated for by the hypothetically stronger association of the combined genetic polymorphisms with the phenotype analysed.

In conclusion, this study does not confirm an association of the risk of obesity with the −866G/A UCP2 polymorphism in different groups of the Italian Caucasian population. In addition, no relation was found between this polymorphism and BMI or markers of insulin resistance. Moreover, no interaction was observed between the −866 UCP2 polymorphism and the Pro12Ala variant of PPARγ2 in the association with obesity. Although these are negative data, they still endorse the need for more efforts to disclose the complex genetic bases of such an important disease like obesity.

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