Aim of the study was to investigate by sequence analysis the presence of UCP1 gene variations that may be associated with obesity. We studied 100 severe obese adults (BMI $> 40$ kg/m$^2$) and 100 normal-weight control subjects (BMI range $= 19$–24.9 kg/m$^2$). We identified 7 variations in the promoter region, 4 in the intronic region and 4 in the exonic region. Globally, 72% of obese patients bore UCP1 polymorphisms. Among UCP1 variants, g.IVS4$-208T>G$ SNP was associated with obesity (OR: 1.77; 95% CI $= 1.26$–2.50; $P = .001$). Further, obese patients bearing the g.$-451C>T$ (CT+TT) or the g.$940G>A$ (GA+AA) genotypes showed a higher BMI than not polymorphic obese patients ($P = .008$ and $P = .043$, resp.). In conclusion, UCP1 SNPs could represent “thrifty” factors that promote energy storage in prone subjects.

1. Introduction

Brown adipose tissue, where Uncoupling Protein 1 (UCP1) activity uncouples mitochondrial respiration, is an important site of facultative energy expenditure. This tissue may normally function to prevent obesity. Our aim was to investigate by sequence analysis the presence of UCP1 gene variations that may be associated with obesity. We studied 100 severe obese adults (BMI $> 40$ kg/m$^2$) and 100 normal-weight control subjects (BMI range $= 19$–24.9 kg/m$^2$). We identified 7 variations in the promoter region, 4 in the intronic region and 4 in the exonic region. Globally, 72% of obese patients bore UCP1 polymorphisms. Among UCP1 variants, g.IVS4$-208T>G$ SNP was associated with obesity (OR: 1.77; 95% CI $= 1.26$–2.50; $P = .001$). Further, obese patients bearing the g.$-451C>T$ (CT+TT) or the g.$940G>A$ (GA+AA) genotypes showed a higher BMI than not polymorphic obese patients ($P = .008$ and $P = .043$, resp.). In conclusion, UCP1 SNPs could represent “thrifty” factors that promote energy storage in prone subjects.
The mean value and the standard deviation (SD) were calculated for each investigated parameter. The Mann-Whitney test and/or χ², when necessary, were used for between-group comparisons. Differences were considered significant at P level <.05. Linkage analysis was performed by using Haploview 4.0 software [15]. Binomial logistic regression analysis was used to investigate the association between the biochemical and genetic characteristics (i.e., glucose, total cholesterol and triacylglycerols concentrations and AST activity; g.−451C>T, g.940G>A, g.IVS4−208, and g.6537A>T polymorphisms) and the condition of being obese, after adjustment for age and sex.

Statistical analyses were carried out with the PASW package for Windows (Ver.18; SPSS Inc. Headquarters, Chicago, Ill).

3. Results and Discussion

Adiponectin and leptin concentrations were statistically different (P < .001) between obese and control subjects (mean level ± SD respectively: adiponectin 31.6 ± 30.0 μg/mL versus 53.8 ± 38.6 μg/mL; leptin 119.6 ± 72.4 versus 21.9 ± 18.7 ng/mL). Higher concentrations or activities of glucose, triacylglycerols, AST, ALT and GGT were measured in obese patients than in controls (P < .001) (Table 1).

We identified 15 sequence variations in UCPI gene (Table 2): 7 in the promoter region (3/7 described for the first time), 4 in the intronic regions (1/4 described for the first time) and 4 in the exonic regions (2 in the 5′ UTR; 2 in the translated region). Globally, 72% of obese patients bore one or more UCPI polymorphisms.

There were no differences in genotype frequencies between obese and control subjects at level of the detected SNPs, except for g.IVS4−208T>G polymorphism more frequent in obese than in control subjects (P = .002). After a permutation test with 100000 permutations, the association of the polymorphic allele with the obese phenotype remained statistically significant (P = .017). Subjects bearing this polymorphism (TG or GG) were at high risk for obesity (OR: 1.774; 95% CI = 1.26−2.50, P = .001). At binomial logistic regression analysis, the g.IVS4−208 (TG+GG) genotype was confirmed to be statistically associated in our patients with obesity independently of sex and age (OR: 22.0; 95% CI = 5.6−87.1). This SNP did not alter the splicing site nor the branch site [16, http://www.umd.be/HSF/], and the polymorphic allele did not change the ΔG of the predicted mRNA secondary structure by mfold analysis (http://mfold.bioinfo.rpi.edu), suggesting that the stability of the polymorphic mRNA is the same as the wild-type. The G allele may be a marker linked to other gene variants promoting energy storage as well as fat accumulation in prone subjects.

The novel UCPI variants g.−637T>C, g.−206C>A, and g.IVS2+174T>A, each of them present in a single obese patient, were not associated with differences in clinical and/or biochemical parameters measured in the obese and control populations. Among them, only the g.−206C>A occurred in a conserved region indentified by cisRED algorithm (http://www.cisred.org/) as a cis-regulatory element.

2. Materials and Methods

We studied 200 age-matched unrelated Caucasian subjects from Southern Italy: 100 adult severe obese patients (60% female, mean ± SD: BMI = 47.9 ± 6.9 kg/m²; age = 32.1 ± 10.9 years) and 100 unrelated adult normal-weight subjects (64% female, mean ± SD: BMI = 22.8 ± 2.1 kg/m²; age = 33.3 ± 8.1 years). The patients were recruited at the obesity outpatient clinic of the Department of Clinical and Experimental Medicine, University of Naples Federico II, Italy, from 2007 to 2008, whereas control subjects were recruited at the Department of Preventive Medical Science of the Federico II University Hospital. Clinical and biochemical data were obtained from each patient on their first admission. The general and biochemical characteristics of the studied populations are reported in Table 1. All patients and controls gave their informed consent to the study, which was carried out according to the Helsinki II Declaration. The research was also approved by the Ethics Committee of the School of Medicine, University of Naples Federico II.

Genomic DNA was extracted from whole blood (Nucl-eon BACC-II; Amersham Science Europe). UCPI 5′ flanking region, exons and intron-exon junction regions were amplified by ten sets of primers (primers ID: Rsa000984680, Rsa000984677, Rsa000984675, Rsa000984673, Rsa000984666, Rsa000990288, Rsa000990284, Rsa000990283, Rsa000990281, and Rsa000990278 http://www.ncbi.nlm.nih.gov/sites/entrez). PCR products were sequenced on ABI Prism 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA). PCR conditions were 96°C for 5 min; than 94°C for 30 sec, 60°C for 45 sec and 72°C for 45 sec, for 40 cycles; final extension at 72°C for 10 min; final soak at 25°C.

| Table 1: General and biochemical characteristics of obese patients and control subjects. | Obese patients | Control subjects |
|---|---|---|
| Females (%) | 60 | 64 |
| Age (years) | 32.1 ± 10.9 | 33.3 ± 8.1 |
| BMI* (kg/m²) | 47.9 ± 6.9 | 22.8 ± 2.1 |
| Adiponectin* (μg/mL) | 31.6 ± 30.0 | 53.8 ± 38.6 |
| Leptin* (ng/mL) | 119.6 ± 72.4 | 21.9 ± 18.7 |
| Resistin (ng/mL) | 12.2 ± 8.4 | 12.7 ± 7.9 |
| Glucose* (mmol/L) | 4.9 ± 0.8 | 4.5 ± 0.4 |
| Total cholesterol (mmol/L) | 4.7 ± 1.1 | 5.0 ± 0.7 |
| Triacylglycerols* (mmol/L) | 1.5 ± 0.6 | 0.9 ± 0.3 |
| AST* (U/L) | 26.5 ± 16.7 | 19.8 ± 5.6 |
| ALT* (U/L) | 39.8 ± 35.0 | 22.5 ± 12.9 |
| GGT* (U/L) | 35.3 ± 26.0 | 17.4 ± 10.4 |
| Creatinine (mg/dL) | 0.9 ± 0.2 | 0.7 ± 0.1 |

*Statistically significant difference between obese and control subjects, P < .001 at Mann-Whitney test. Biochemical parameters were measured by routine laboratory methods. Adipokines concentrations were measured by ELISA assay (LINCO Research, Mo, USA). Values are expressed as mean ± SD.
than the wild-type patients (50.6 kg/m² versus 47.6 kg/m², p.M229L polymorphism showed a slightly higher mean BMI found that patients carrying the polymorphic allele for the demonstrate such association [9, 19, 20]. In our study we a diabetes-associated SNP, while other studies failed to (g.6537A>T) polymorphism in a Japanese obese population The amino acidic substitution p.A64T (g.940G>A) in the fifth helix of the protein is due to an A>T transversion in the 5th exon of the UCP1 gene [17]. Mori and colleagues [18] found a higher frequency of the Leu allele of the p.M229L (g.6537A>T) polymorphism in a Japanese obese population with Type II diabetes, indicating this gene variation as a diabetes-associated SNP, while other studies failed to demonstrate such association [9, 19, 20]. In our study we found that patients carrying the polymorphic allele for the p.M229L polymorphism showed a slightly higher mean BMI than the wild-type patients (50.6 kg/m² versus 47.6 kg/m², resp.) while no difference were found at level of glucose and insulin concentration or regarding the homeostatic model assessment (HOMA) index (a measure of insulin sensitivity) (data not shown). This difference could be due to the lower mean age of our studied subjects (32.1 years in our patients versus 58.6 years in Mori et al. [18]), since Type II diabetes is more frequent in middle aged than in young adult patients.

Further, the haplotype investigation by Haplovip software showed a significant linkage disequilibrium among the three SNPs g.−56C>T (a), g.12A>C (b) and g.940G>A (c) (a-b: log likelihood ratio, LOD = 27.5; r² = 1; b-c and a-c: LOD = 22.6; r² = 0.9); however no statistically significant association was observed between obesity and this haplotype, the frequency of this latter being the same in obese and control subjects (8.0% versus 9.0%, resp.). The g.12A>C polymorphism is located in the insulin response sequence (IRS). In vitro experiments, the DNA mutated C allele was demonstrated to reduce the transcription of UCP1 by 40% respect to the wild-type allele. This variation was hypothesized to impair the affinity of the transcription factors for the consensus motif of IRS [18]. Further, this SNP was previously indicated as contributing to hepatic lipid accumulation and altering insulin sensitivity in Japanese individuals with Type II diabetes mellitus (NIDDM) [18]. In our population, the lack of association of this SNP with any obesity-related phenotype could be due to the younger mean age of our patients respect to those investigated by Fukuyama et al. [21] (32.1 years versus 56.6 years, resp.) and to different ethnic background of the studied groups.

The amino acidic substitution p.A64T (g.940G>A) in the first matrix loop of the protein is due to a G>A transition in the 2nd exon of the UCP1 gene [17]. Cha et al. [22] reported in a Korean female population an association between the mutated allele and a higher blood pressure. In our population, polymorphic patients compared to wild-type patients showed a higher mean BMI (52.0 ± 6.4 kg/m² versus 47.5 ± 6.9 kg/m², P = .043) but only a trend toward a higher mean systolic blood pressure (130.0 mmHg versus 124.4 mmHg, resp.). This difference does not raise the statistically significant level probably due to the lower number of patients in our examined casistic.

### Table 2: UCP1 sequence variations and their frequencies in obese and control subjects.

| Position       | rs#  | Obese patients | Control subjects |
|----------------|------|----------------|------------------|
|                |      | n = 100        | n = 100          |
| g.−637T>C      | 99   | 1              | 0                |
| g.−451C>T      | 82   | 16             | 2                |
| g.−412A>C      | 57   | 36             | 7                |
| g.−372A>C      | 97   | 3              | 0                |
| g.−206C>A      | 99   | 1              | 0                |
| g.−56C>T       | 91   | 9              | 0                |
| g.−17C>G       | 94   | 6              | 0                |
| g.12A>C        | 91   | 9              | 0                |
| g.21G>A        | 86   | 13             | 1                |
| g.940G>A       | 92   | 8              | 0                |
| g.IVS2+138C>T  | 80   | 15             | 5                |
| g.IVS2+174T>A  | 99   | 1              | 0                |
| g.IVS2+201T>G  | 79   | 21             | 0                |
| g.IVS4−208T>G  | 45   | 44             | 11               |
| g.6537A>T      | 89   | 11             | 0                |

1New variants; 2More frequent polymorphism in obese patients (P = .002) than in controls. wt: wild-type homozygous subjects; HE: heterozygous and HO: homozygous subjects at level of the detected variant.

(craHsap157022), and we could hypothesize to alter the interaction with transcriptional factors. Regarding the previously described UCP1 polymorphisms, a higher mean BMI was observed in our obese patients bearing the g.−451C>T (CT+TT) than in not polymorphic obese patients (resp., 52.6 ± 7.4 kg/m² versus 47.0 ± 6.6 kg/m², P = .008).

The amino acidic substitution p.M229L (g.6537A>T) in the fifth helix of the protein is due to a G>A transition in the 5th exon of the UCP1 gene [17]. Further, this SNP was previously indicated as contributing to hepatic lipid accumulation and altering insulin sensitivity in Japanese individuals with Type II diabetes mellitus (NIDDM) [18]. In our population, the lack of association of this SNP with any obesity-related phenotype could be due to the younger mean age of our patients respect to those investigated by Fukuyama et al. [21] (32.1 years versus 56.6 years, resp.) and to different ethnic background of the studied groups.

### 4. Conclusions

Functional activity of BAT has been recently demonstrated in adult humans [6–8] and its amount is inversely related to body fat percentage [23]. We do not have any information
in our patients about BAT amount. However, variations in the BAT marker UCP1 gene were present in most of our obese patients. These variations could represent common factors contributing to the development of obesity, particularly, g.−451C>T, g.940G>A, and g.IVS4−208T>G could represent “thrifty” factors that promote energy storage. The precise role in obesity of these variants should be investigated in a larger casistic.

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