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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

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Abstract

Aims/hypothesis. In obese French Caucasian subjects we previously described a silent *UCP3* Tyr99Tyr mutation, associated with body mass index. We hypothesised that an unknown polymorphism in the vicinity of the gene could contribute to obesity.
Methods. Morbidly obese subjects were screened for mutations in 1 kb upstream from the *UCP3* gene. Association studies were done between a variant and obesity in 401 morbidly obese and 231 control subjects.
Results. We detected three rare genetic variants and one polymorphism: a + 5 G→A in exon 1, a –155 C→T, a –439 A insertion and a –55 C→T located 6 bp from the putative TATA box. This variant was in linkage disequilibrium with the Tyr99Tyr polymorphism. Frequencies of the variant allele at the –55 locus were similar in the obese and control groups (0.23 vs 0.21). The –55 polymorphism was associated with BMI in the obese group ($p = 0.0031$): BMI was higher in TT than in CC or CT patients. Likewise control subjects with a TT genotype had a higher BMI ($p = 0.03$). In the obese group, homozygocity for this variant is a risk factor for high BMI (odds ratio: 1:75, $p = 0.02$). Obese patients were divided into tertiles according to physical activity. In the group with a wild C/C genotype, BMI was negatively associated with physical activity ($p = 0.015$).
Conclusion/interpretation. The C→T polymorphism in the 5′ sequences of the *UCP3* gene might contribute to the corpulence in obese and normal weight subjects and alter the benefit of physical activity. The *UCP3* gene can be considered as a gene modifying corpulence. [Diabetologia (2000) 43: 245–249]

Keywords Uncoupling protein (UCP), promoter, mutation, polymorphism, obesity, body mass index (BMI).

Uncoupling proteins (UCPs) are inner mitochondrial membrane transporters which reduce the efficiency of oxidative phosphorylation and ATP synthesis [1, 2]. Three *UCP* genes have been identified so far: *UCP1*, mostly expressed in brown adipose tissue, seems unlikely to play a major part in body weight regulation of most large-sized adult animals and humans living in thermal environments which do not require energy expenditure for body temperature maintenance [3], *UCP2* and *UCP3* both discovered from a human muscle cDNA library [4–6]. The *UCP2* gene is widely expressed in adult human tissues. Mutation screening of the *UCP2* gene did not detect polymorphisms associated with obesity in Caucasian cohorts [7, 8], although a variant was associated with BMI in an Indian cohort [9]. The *UCP3* gene is expressed in human skeletal muscle. We previously described an association between a Tyr99Tyr *UCP3* silent mutation and body mass index (BMI) in morbidly obese French Caucasians [10]. These results suggested that other mutation(s) in linkage disequilibrium with the Tyr99Tyr variant could exist in the vicinity of the *UCP3* gene. In this study we have

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Abbreviations: UCP, Uncoupling protein; SSCP, single strand conformation polymorphism; UTR, untranslated region.

Table 1. List of primers and annealing temperatures

	Primer sequence	Annealing temperature (°C)
TATA box region 297bp fragment	5′AAG-CGT-CCA-CAG-CTT-AAA 3′ 5′GGC-AGG-GGC-AGC-ACA-GGG 3′	52
Fragment 1 315bp fragment	5′GGG-AAC-ACA-GCA-AGA-CCT 3′ 5′TTG-ACT-TGG-GGT-GCT-TTA 3′	55
Fragment 2 337bp fragment	5′GTG-AGA-GGG-CTA-TGA-TGA-AAA 3′ 5′TGA-GAT-AGG-TAC-TAT-TAG-GA 3′	57
Fragment 3 330bp fragment	5′CCA-TTT-CCT-CCT-CAG-TAA 3′ 5′CCA-CCA-TCA-ACA-CAA-TCT-AT 3′	52
RFLP C- > T – 55	5′CCT-CCC-CTC-TCA-CCT-CAC-TG 3′ 5′GGC-ACT-GGT-CTT-ATA-CCC-AC 3′	53

screened the human *UCP3 5′* sequences. Case-control association studies were done between a prevalent polymorphism and obesity-related phenotypes.

Subjects and methods

Initial mutation screening was done by an automated fluorescent single strand conformation polymorphism (SSCP) technique [11]. We analysed the upstream region and part of exon 1 of the human *UCP3* gene from nt –1006 to + 55 according to the GenBank DNA sequence AF 032871 at the NCBI web site (http://www.ncbi.nlm.nih.gov). A set of 60 obese patients from a well-described cohort of morbidly obese French Caucasians and 60 non-obese control subjects [12] were used in the initial screening. Informed consent was obtained for all subjects and the protocols were approved by the ethics committee of Paris (CCPPRB Hôtel-Dieu). Four PCR products were required to cover the region. Primers and annealing temperatures are listed in Table 1. Every abnormal SSCP profile was ascertained by direct sequencing of the PCR product as described previously [13]. Genotyping of the –55 CT variant for case-control association studies was first done by SSCP on a set of 244 morbidly obese patients (mean age: 44.9 ± 11.4 years, mean BMI: 47.4 ± 7.5 kg/m^2, sex ratio women/men = 194/50) and 231 unrelated non-obese, non-diabetic control subjects selected from French pedigrees (mean age: 56 ± 14.0 years, mean BMI: 23 ± 2.5 kg/m^2, sex ratio women/men = 130/101). Although SSCP profiles clearly distinguished between the CC, CT and TT genotypes, quality control of the results was achieved by direct sequencing of PCR products chosen randomly from each genotype. Genotyping of this variant was extended by PCR-RFLP to a larger group of 401 morbidly obese patients, (mean age: 44 ± 12 years, mean BMI: 46.5 ± 7.5 kg/m^2, sex ratio women/men = 318/83) without (n = 182) or with (n = 219) impaired glucose tolerance or Type II (non-insulin-dependent) diabetes mellitus. These included patients previously genotyped for the Tyr99Tyr variant of the *UCP3* exon 3 gene [10] and the insertion deletion polymorphism of the *UCP2* exon 8 gene [8]. A degenerated reverse primer (Table 1) was designed to detect the –55 C→T variant. The BseDI endonuclease (New England Biolabs, Beverly, Mass., USA) restricted the wild C allele. Data concerning physical activity using the Baecke questionnaire [14] were available for 358 of the 401 obese patients. They were divided into three tertiles according to their physical activity index (calculated as the sum of the work index, leisure index and sport index). First, second and third tertiles were 2.875–5.125, 5.126–6.625 and 6.626–12.2, respectively. Categorical variables were compared between groups using the Chi-squared test and crude odds ratios. Continuous clinical and biological variables were analysed using either one-way analysis of variance or the Wilcoxon and Kruskal-Wallis test depending on the shapes of the distribution curves. Hardy-Weinberg's equilibrium was tested by the Chi-squared test. Multivariate analyses including age, sex and the –55 C→T polymorphism were done using the logistic regression model to find out whether the *UCP3* gene variant was an associated factor for a BMI over the median of BMI in the obese group. Statistics were done with the JMP software (SAS Institute, Cary, N. C., USA). The EH software [15] was modified to allow the calculation of linkage disequilibrium.

Results

Single strand conformation polymorphism screening among the first set of 60 obese patients and 60 control subjects showed two polymorphisms. A –55 C→T change located at 6 base pairs upstream from the putative TATA box was found in 25 obese and in 28 control subjects. One A insertion, at nt –439, was detected in only one normoglycaemic obese woman of 40 years of age (BMI = 41 kg/m^2). In her family, the A insertion did not cosegregate with obesity (data not shown). The extension of the initial SSCP screening to a set of 244 morbidly obese patients and 231 control subjects, allowed the detection of two additional genetic variants: a + 5 G→A change in exon 1 and a –155 C→T change. These variants were present each in one obese patient and absent from the 231 control subjects. Neither BMI nor diabetes mellitus cosegregated with the + 5 G→A or the –155 C→T variant in the families of the obese carriers (data not shown). A total of 401 morbidly obese patients and 231 control subjects were genotyped by PCR-RFLP for the prevalent –55 C→T variant. In the obese group 235 patients (58.6 %) were CC (wild), 146 (36.4 %) were CT (heterozygotes) and 20 (5 %) were TT (homozygotes). In the control group: 144 subjects (62.3 %) were CC, 78 (33.8 %) were CT and 9 (3.9 %) were TT. There was no significant deviation from the Hardy Weinberg expectation. Frequencies of the variant T allele were similar between the morbidly

Table 2. BMI of the morbidly obese patients and control subjects acccording to the genotype at the – 55 (+ 524) C- > T locus

Genotype	Morbidly obese group	Control group
C/C	46.3 ± 7.45 *n* = 235	22.6 ± 2.67 *n* = 1,44
C/T	46.0 ± 7.09 *n* = 146	22.7 ± 2.27 *n* = 78
T/T	52.6 ± 9.59 *n* = 20	25.5 ± 2.58 *n* = 9
	p = 0.0037	*p* = 0.09

Values are expressed in kg/m^2

Table 3. BMI of the morbidly obese patients according to their genotype at the – 55 (+ 524) C- > T locus and physical activity

Genotype	1st tertile	2nd tertile	3rd tertile	
CC *n* = 210	49.1 ± 9.58	45.9 ± 7.09	44.6 ± 4.64	*p* = 0.015
CT *n* = 130	46.5 ± 7.19	45.6 ± 6.14	46.6 ± 8.65	*p* = 0.85
TT *n* = 18	51.8 ± 6.39	56.2 ± 10.01	48.9 ± 9.60	*p* = 0.42

Patients were divided into tertiles according to their physical activity index as described in subjects and methods. Values are expressed in kg/m^2

obese and the control groups, (0.23 vs 0.21, $p = 0.52$). The –55 C→T variation was in linkage disequilibrium ($D' = 0.789$) with the previously described *UCP3* exon 3 Tyr99Tyr variant associated with BMI in our cohort [10]. The variant allele at the –55 (+ 524) locus was in phase with the variant allele at the Tyr99-Tyr locus. The –55 (+ 524) polymorphism was associated with BMI in the obese group ($p = 0.0037$) (Table 2). The BMI of obese patients with a TT genotype was higher than the BMI of wild type CC (52.6 ± 9.59 vs 46.3 ± 7.45 kg/m^2, $p = 0.0011$) or heterozygous CT patients (52.6 ± 9.59 vs 46.0 ± 7.09 kg/m^2, $p = 0.0012$). In addition, the maximum BMI reached during adult life was higher in the TT bearers than in the CT (53.9 ± 10.44 vs 48.8 ± 7.18 kg/m^2, $p = 0.0092$) or CC bearers (53.9 ± 10.44 vs 48.9 ± 8.68, $p = 0.021$). Likewise in our control cohort (Table 2), there was a trend towards an association of the variant with BMI ($p = 0.09$). The BMI of the control subjects with a TT genotype was higher than the BMI of the wild CC (25.5 ± 2.58 vs 22.6 ± 2.67 kg/m^2, $p = 0.041$) or heterozygous CT subjects (25.5 ± 2.58 vs 22.7 ± 2.27 kg/m^2, $p = 0.028$). These data were confirmed in a multivariate analysis when accounting for age and sex: respectively $p = 0.0002$ and $p = 0.0028$ in the obese and control groups. In the obese group the median of the BMI (44.5 kg/m^2), as a categorical-dependent variable was used in a multivariate logistic regression analysis and the T allele in the homozygous state was associated with a BMI over the median: odds ratio = 1.75; 95 % confidence interval: 1.04–2.9; $p = 0.03$. There was no association with serum lipid concentrations and with the diabetic status.

A potential effect of physical activity on BMI in obese patients was investigated according to the genotype at the –55 locus (Table 3). Among subjects carrying the wild type CC genotype, BMI was negatively associated with physical activity ($p = 0.015$). No statistically significant association between BMI and physical activity was noticed in obese patients with a CT or a TT genotype.

Discussion

The *UCP2* and *UCP3* genes are considered to be candidate genes for obesity in humans. A linkage between the UCP2/UCP3 locus on chromosome 11 (11q13) and resting metabolic rate, percentage body fat and fat mass has been reported [16]. Polymorphisms of the *UCP3* gene associated with a 50 % reduction of basal fat oxidation rate have been found in an isolated African American population [17]. In this population, rare mutations in the coding regions were detected in subjects with severe obesity and Type II diabetes [17] but were absent in Caucasian obese subjects [10, 18]. We report polymorphisms in the 5′ sequences of the human *UCP3* gene and locate them according to the beginning of exon 1 as reported in GenBank accession number AF 032 871. A recent alternative UCP3 genomic organisation [19] should, however, locate the beginning of exon 1, 579 bp upstream. For the GenBank AF 032871, we describe three rare genetic variants: a + 5 G→A, a –439 A insertion, a –155 C→T change and a previously reported [9] prevalent C→T variation at –55 that should be 6 bp upstream the putative TATA box. According to the alternate genomic organisation [19] these variants should be located in the 5′ untranslated region (UTR) of exon 1 at + 584 (G→A), + 140 (A insertion), + 424 (C→T) and + 524 (C→T). The –55 (+ 524) C→T polymorphism was in linkage disequilibrium with the previously described Tyr99Tyr polymorphism of the *UCP3* exon 3 [10], the variant C allele at Tyr99Tyr locus being in phase with the variant T allele at the –55 (+ 524) locus. The association previously detected under a recessive model between the Tyr99Tyr variant and BMI [10] could be the result of the linkage disequilibrium with a functional polymorphism in the 5′ region as the –55 (+ 524) variant, unless these variants are both in linkage disequilibrium with another functional variant still to be detected. The present data show a higher BMI (+ 6 units) for the –55 (+ 524) TT bearers, in favour of a recessive model too. Similar results were noticed for the maximum reached BMI with + 5 units for the TT bearers. Likewise in our control group homozygous

TT subjects had a statistically significantly higher BMI (+ 3 units). Our data raise the hypothesis that polymorphisms in the 5′ sequences of the *UCP3* gene could modify the *UCP3* gene expression and therefore modulate metabolic rate. A positive correlation between BMI, metabolic rate and *UCP3* expression in skeletal muscle has already been reported in Pima Indians [20]. If located in the proximal promoter, the variant at the –55 locus could alter the *UCP3* gene transcription. If located in the 5′ untranslated region (UTR), it could affect the *UCP3* mRNA stability. Genetic variants in promoter or UTR of uncoupling protein genes were associated with obesity-related phenotypes, through a possible modulation of gene expression or stability of mRNA: the *UCP1* –3826 A→G BclI polymorphism associated with weight gain [21, 22] and the 45 bp insertion deletion (I/D) polymorphism in the 3′ UTR of the *UCP2* exon 8 [8] associated with BMI in South Indian women [9]. This *UCP2* variant was not associated with obesity in a French [8] and in a British cohort [9]. It was in linkage disequilibrium (D′ = –0.883) with the –55 (+ 524) C→T variant of the *UCP3* gene (data not shown). Because *UCP2* and *UCP3* genes are adjacents on the human chromosome 11, genetic studies could have limitations in discriminating what polymorphisms are truly associated with obesity phenotype variations in humans. Physical activity could counterbalance the effect of a genetic predisposition to increase body weight conferred by a polymorphism [23]. From our results a potential beneficial effect of physical activity on BMI, is limited to obese subjects with a wild-type genotype: –55 (+ 524) C/C; the presence of one variant T allele, abolishes this beneficial effect. This dominant effect of the variant allele could be disputable as in our cohort only the TT bearers had higher BMIs fitting with a recessive model. Skeletal muscle *UCP3* mRNA is up regulated by short-term exercise in mouse [24]. On the contrary down regulation of *UCP3* by endurance training was described in rodents [25]. Unless our results are type 1 errors, we speculate that with a sedentary lifestyle, a basal expression from only one wild allele would be sufficient to offer a relative protection against weight gain and in agreement with a recessive model, only TT bearers would present higher BMIs. For physical activity, the Baecke questionnaire was developed for epidemiological studies and fits better with a general population. Thus values of activity indexes in our morbidly obese cohort could appear rather low and alternate criteria for obese patients should still be defined [26]. Because of a higher oxygen requirement, even moderate physical activity is, however, exhausting and perceived by obese patients as intense and even painful [27, 28]. We speculate that these periods of physical activity are related to an up regulation of the *UCP3* gene and requires expression from two wild alleles to offer a statistically significant benefit to BMI and obesity-related phenotypes in obese patients. Thus the effect of the –55 (+ 524) variant in relation to physical activity should fit a dominant model.

The Respiratory Quotient was not evaluated and body composition was difficult to measure in our group of extremely obese subjects because many of them were more than 150 kg in weight. As the prevalence of the –55 (+ 524) C→T variation is not statistically significantly different in the obese and control groups, genetic variations in the *UCP3* 5′ flanking regions are unlikely to be sufficient to induce obesity by itself. These variations might, however, contribute in modulating corpulence in both normal weight and obese subjects. Thus, the *UCP3* gene could be considered as a gene modifying corpulence.

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