Brief Genetics Report

Mutation Screening of the Human UCP 2 Gene in Normoglycemic and NIDDM Morbidly Obese Patients

Lack of Association Between New UCP 2 Polymorphisms and Obesity in French Caucasians

Shuichi Otabe, Karine Clement, Nathalie Rich, Craig Warden, Claire Pecqueur, Maria Neverova, Serge Raimbault, Bernard Guy-Grand, Arnaud Basdevant, Daniel Ricquier, Philippe Froguel, and Francis Vasseur

Uncoupling proteins (UCPs) are inner mitochondrial membrane transporters that dissipate the proton gradient, releasing stored energy in the form of heat (1,2), and are therefore potentially important determinants of the defense against obesity. Three distinct UCPs were identified so far. UCP 1 (2,3) is uniquely expressed in brown adipose tissue, which is an important site of energy expenditure. A polymorphism in the human UCP 1 promoter was recently suggested to play a role in exaggerated weight gain (4) and in synergy with a β_3-adrenergic receptor polymorphism (5). However, UCP 1 seems unlikely to be a major gene involved in weight regulation of most large-sized adult animals and humans living in a thermoneutral environment where little brown adipose tissue exists (6). Recently, two other human uncoupling proteins, UCP 2 and UCP 3, were consecutively identified from a human muscle cDNA library and acting as a mediator of adaptative thermogenesis in humans (7–9). The amino acid sequences of UCP 2 and UCP 3 were 59 and 57%, identical to human UCP 1, respectively. In comparison to UCP 1, UCP 2 and UCP 3 are widely expressed in adult human tissues. Concerning UCP 2, the gene is upregulated in white fat in response to fat feeding (7). Human UCP 1 was located on chromosome 4 (10), while human UCP 2 was mapped in the regions of human chromosome 11 that have been linked to hyperinsulinemia and obesity (7).

We studied the UCP 2 gene by two approaches. First, we screened by direct sequencing the eight exons and the intron/exon junctions of the human UCP 2 gene to examine whether a genetic variation of this gene is involved in human obesity. Second, we performed an association study between an insertion/deletion (Ins/Del) polymorphism of exon 8 and morbid obesity-related traits into a large population of morbidly obese subjects. For UCP sequencing, a total of 72 unrelated morbidly obese patients (BMI >40 kg/m^2) were randomly selected from a well-described collection of independent French patients recruited from the Department of Nutrition at the Hôtel-Dieu Hospital in Paris. From this group, 36 patients were diagnosed with NIDDM (mean age 48.5 ± 9.5 years; mean BMI 48.1 ± 6.3 kg/m^2; female-to-male ratio = 22/14) and 36 others were normoglycemic (mean age 39.9 ± 14.3 years; mean BMI 44.9 ± 6.8 kg/m^2; female-to-male ratio = 32/4). Patients receiving an antidiabetic oral treatment or an insulin treatment that started at least 2 years after diagnosis for hyperglycemia as well as those patients with a fasting plasma glucose >7.8 mmol/l were considered as NIDDM patients. For the remaining subjects, a standard 75-g oral glucose tolerance test was performed, and the diagnosis of diabetes was made according to the World Health Organization criteria. A larger group of 483 morbidly obese subjects were selected for the association study (mean age 46.0 ± 12 years; mean BMI 47 ± 7; male-to-female ratio = 321/121). Clinical and biological data such as weight history, obesity-related complications, glycemia, and insulinemia were available for all the subjects. Moreover, we tested for resting metabolic rate (RMR) using indirect calorimetry (DELTATRAC II; Datex, France) and body composition (using the biphotonic absorptiometry, Hologic Device QDR 1000/W; Watham, MA) for 68 morbidly obese subjects of this cohort. A control group of 120 unrelated nonobese (BMI <27 kg/m^2) nondiabetic subjects, selected from French pedigrees, has also been analyzed (mean age 55.4 ± 14.3 years; mean BMI 23.2 ± 2.7 kg/m^2; female-to-male ratio = 65/55).

The eight exons (C.P., D.R., unpublished observations), including untranslated exons 1 and 2 of the UCP 2 gene,

From the Institute of Biology (S.O., K.C., P.F., F.V.), CNRS EP10, Lille; the Department of Nutrition (K.C., N.R., A.B., P.F., B.G.-G.), Hôtel-Dieu, Paris; CNRS CEREMOD (C.P., S.R., D.R.), Meudon; the Medical Faculty and CHRU (P.F., F.V.), Lille, France; and the Department of Medicine (M.N., C.W.), Rowe Genetics, University of California, Davis, California.

Address correspondence and reprint requests to Philippe Froguel, CNRS EP10, Institut Pasteur de Lille, 1 rue du Professeur Calmette, B.P.245, 59019 Lille cedex, France. E-mail: froguel@xenope.pasteur-lille.fr.

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Ins/Del, insertion/deletion; PCR, polymerase chain reaction; QTL, quantitative trait loci; RFLP, restriction fragment length polymorphism; RMR, resting metabolic rate; UCP, uncoupling protein.

were screened for mutations by direct sequencing of nine amplified polymerase chain reaction (PCR) products. Genomic DNA was amplified on a PTC 200 thermal cycler (MJC Research, Watertown, MA) using each DNA fragment's specific oligonucleotides extended by the universal –21 M13 (TGTAAAACGACGGCCAGT) and M13 Reverse (CAGGAAACAGCTATGACC) sequences at their 5′ ends, except for primers used to detect the Ins/Del polymorphism of exon 8 by agarose gel electrophoresis. PCR amplifications were carried out in a final reaction volume of 25 µl, containing 200 ng human genomic DNA, 10 mmol/l Tris-HCl (pH 8.3), 50 mmol/l KCl, 1.5 mmol/l $MgCl_2$, 200 mmol/l of each dNTP, 150 nmoles of each primer, and 1.25 U of Taq polymerase (Perkin Elmer, Forster City, CA). All PCR cycling conditions were an initial denaturation at 94°C for 5 min, followed by 35 cycles at 94°C for 30 s, 30 s at each annealing temperature, 30 s at 72°C, and a final extension of 15 min at 72°C. Additional information about this subject can be found in the on-line appendix at www.diabetes.org/diabetes/appendix.htm. After the protocol of ABI PRISM dRhodamine Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Forster City, CA), PCR products were amplified using the universal –21 M13 and M13 Reverse oligonucleotides as a specific primer and then both strands were sequenced using an ABI Prism 377 DNA sequencer. We also carried out the direct sequencing of exon 1 and exon 4 in another 84 control subjects, as the nucleotide changes had been detected in these exons. Moreover, PCR restriction fragment length polymorphism (RFLP) was used for the detection of the Gly85Ser mutation, since the nucleotide change resulted in the loss of a site for *Msp*I (Boehringer Mannheim, Germany). Additional sets of 96 unrelated NIDDM subjects without obesity (mean age 60.7 ± 11.2 years; mean BMI 26.7 ± 4.5 kg/m^2; female-to-male ratio = 53/38) and of unrelated 238 morbidly obese patients (mean age 46 ± 12.1 years; mean BMI 48.35 ± 8.2 kg/m^2; female-to-male ratio = 166/72) have been screened by *Msp*I polymorphism for the Gly85Ser mutation in exon 4. For the Ins/Del polymorphism of the exon 8, the PCR products were resolved on a 2% agarose gel giving a 317-bp band for the fragment with the deleted allele and a 362-bp band for the fragment with the insertion allele.

Six genetic variants including three changes in untranslated exons, two changes in exon 4 and a 45-bp insertion in exon 8 were observed in the 72 obese subjects. The two sites of nucleotide change in the untranslated exon 1 were a C → T (nt19) and a C → G (nt27), and in untranslated exon 2 a C → T (nt97) was found. Two sites of aminoacid changes (the Ala55Val change, GCC-GTG and the Gly85Ser change, GGC-AGC) have been found in exon 4. A 45-bp insertion (ACC-TACCACCTTCCCTCTTTCCCCACCTCTTCCTTCCGCTC-CTTT) in exon 8 was identified in this study. The frequencies of two nucleotide changes in exon 1, one in exon 2 and the Ala55Val change in exon 4, were not different between tested obese individuals and control subjects (Table 1). When examining a large group of morbidly obese subjects, the allelic frequency of the Ins/Del exon 8 polymorphism was similar to that found in the control group (Table 1), suggesting that this polymorphism was not associated with obesity in our population. Moreover, neither the obesity-related traits (weight, BMI, weight gain) nor the obesity-related complications (NIDDM, cardiovascular disease) and the metabolic data were different between Ins and Del carriers in the whole morbidly obese population. Additionally, the resting metabolic rate and body composition characteristics were similar in 44 Del carriers and 24 Ins heterozygous carriers. No homozygous bearers for the Ins variant were found in this group. UCP 2 polymorphism frequencies were not significantly different among control subjects and the whole collection of obese subjects. Also, there was no significant difference among diabetic or nondiabetic obese subjects. The Gly85Ser change in exon 4 was absent from 120 control subjects and detected in a single morbidly obese patient (II6) with NIDDM. Six other members of the proband's family were available to clarify the relationship between this mutation and phenotype. Two of seven members in this family had been diagnosed with NIDDM at the age of 38 (proband II6) and 55 (II2), respectively. Segregation analysis of this Gly85Ser change in exon 4 was investigated by direct sequencing of exon 4 in the members of the family. Although two out of three members bearing the mutation were affected with NIDDM, Gly85Ser did not tightly cosegregate with either morbid obesity or NIDDM. Hence, the Gly85Ser mutation does not seem to have a major effect in the two afflictions mentioned above. The Gly85Ser mutation discovered in this family has been screened by *Msp*I polymorphism in two additional sets of patients: 96 nonobese subjects affected with NIDDM and 238 morbidly obese patients. None of them presented the Gly85Ser mutation. The UCP 2 gene was mapped to chromosome 7 in the mouse and the long arm of the human chromosome 11 where quantitative trait loci (QTL) for obesity and hyperinsulinemia have been mapped (7). The relationship between UCP 2 and obesity and/or NIDDM has to be clarified. Bouchard et al. (11) presented a close linkage between markers (D11S911, D11S916, and D11S1321) in the vicinity of the UCP 2 gene and RMR. Our results failed to give evidence of a relationship between RMR and polymorphisms in the UCP 2 gene. However, a role of the

TABLE 1
Frequencies of variant alleles of the UCP 2 gene in morbidly obese and control subjects

	Obese patients	Control subjects
Exon 1		
C → T (5′UTR nt19)		
C	141 (0.98)	208 (0.99)
T	3 (0.02)	2 (0.01)
C → G (5′UTR nt27)		
C	139 (0.97)	208 (0.99)
G	5 (0.03)	2 (0.01)
Exon 2		
C → T (5′UTR nt97)		
C	134 (0.93)	68 (0.94)
T	10 (0.07)	4 (0.06)
Exon 4		
C → T (Ala55Val)		
C	104 (0.72)	137 (0.62)
T	40 (0.28)	83 (0.38)
G → A (Gly85Ser)		
G	143 (0.99)	240
A	1 (0.01)	0
Exon 8		
Ins/Del 3′UTR		
Ins	239 (0.25)	50 (0.23)
Del	727 (0.75)	176 (0.77)

Data are *n* (frequency).

recently described human UCP 3 gene located between D11S911 and D11S916 and adjacent to the UCP 2 gene (12) (C.W., unpublished observations) may not be excluded.

In conclusion, mutation screening of all exons and the intron/exon junctions of the human UCP 2 gene in a morbidly obese French Caucasian population allowed us to describe three new polymorphisms in the 5′ untranslated regions of exons 1 and 2, one new mutation in exon 4 (Gly85Ser), and a 45-bp insertion/deletion polymorphism in the 3′ untranslated region of exon 8. No potential splice mutation was detected in the subjects we screened. The Ala55Val mutation recently reported in the Danish population by SSCP analyses of muscle UCP 2 cDNA (13) was also found in the population we studied. According to the similar frequencies of the Ala55Val mutation in morbidly obese and control subjects and in normoglycemic (0.27) and NIDDM (0.29) obese patients, and according to the quite minor aminoacid change, this mutation could be considered as a nonfunctional mutation. Moreover, the Ala55Val change does not alter the uncoupling activity as assayed in yeast (C. Fleury, personal communication). The role of the Gly85Ser mutation found in only one family and its possible role in NIDDM associated with morbid obesity would deserve further investigations. Because none of the polymorphisms detected in the coding sequences of the UCP 2 gene was associated with morbid obesity, it seems unlikely that a structural defect of the UCP 2 protein plays a major role in morbid obesity, at least in French Caucasians. Further characterization and screening of the UCP 2 promoter sequences, when available, will permit the testing of the hypothesis of an abnormal regulation of the UCP 2 expression in both diabetic and obese patients.

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