Human Molecular Genetics, 1997, Vol. 6, No. 11 **1887–1889**

Linkage between markers in the vicinity of the uncoupling protein 2 gene and resting metabolic rate in humans

Claude Bouchard*, Louis Pérusse, Yvon C. Chagnon, Craig Warden[1] and Daniel Ricquier[2]

Physical Activity Sciences Laboratory, Laval University, Ste-Foy, Québec, Canada G1K 7P4, [1]University of California, Davis, CA 95616, USA and [2]Centre de Recherche sur l'Endocrinologie Moléculaire et le Développement, CNRS, 92190 Meudon, France

Received May 28, 1997; Revised and Accepted July 14, 1997

The recent cloning of a gene that codes for a novel uncoupling protein, UCP2, which is expressed in a wide range of adult human tissues, has raised the possibility that it may be involved in regulation of energy balance. To explore this concept we have investigated potential linkage relationships between three microsatellite markers which encompass the *UCP2* gene location on 11q13 with resting metabolic rate (RMR), body mass index, percentage body fat (%FAT) and fat mass (FM) in 640 individuals from 155 pedigrees from the Québec Family Study. Using a linkage analysis strategy based on sibling, avuncular, grandparental and cousin pairs, strong evidence of linkage was found between the marker D11S911 (*P* = 0.000002) and RMR, with more moderate evidence for D11S916 (*P* = 0.006) and D11S1321 (*P* = 0.02). Suggestive evidence of linkage was also observed between D11S1321 and %FAT (*P* = 0.04) and FM (*P* = 0.02). It is concluded that the three markers encompassing the *UCP2* locus and spanning a 5 cM region on 11q13 are linked to resting energy expenditure in adult humans. The evidence is strong enough to warrant a search for DNA sequence variation in the gene itself.

INTRODUCTION

Uncoupling protein-1 (UCP1) is a mitochondrial protein expressed exclusively in mammalian brown adipose tissue. UCP1 dissipates the proton electrochemical gradient across the mitochondrial membrane, thereby uncoupling substrate oxidation from conversion of ADP to ATP, leading to generation of heat and thus increased energy expenditure. The role of UCP1 in regulation of energy balance in humans has been controversial because of the very small amount of brown adipose tissue commonly found in adults. However, recently a gene that codes for a novel uncoupling protein, named UCP2, has been cloned which is expressed in a wide range of adult human tissues, in contrast to UCP1 (1). Because UCP2 could play a role in energy balance, it becomes a new candidate gene for human obesity.

The mouse *ucp2* gene was recently mapped to chromosome 7, closely linked to the tubby mutation (1), a mutation known to be responsible for adult onset obesity in this mouse model. Furthermore, *UCP2* mRNA level was found to be higher in mouse strain A/J, which is resistant to diet-induced obesity, than in the obesity-prone C57BL/6J mouse (1). The evidence accumulated thus far on animal models suggests that the *UCP2* gene could play a role in development of obesity because of its potential role in energy metabolism. The human *UCP2* gene has been mapped to chromosome 11q13 at a location distinct from tubby (11p15.1), but in the same chromosomal location as Bardet–Biedl syndrome locus 1 (2), one of four loci of a Mendelian syndrome exhibiting obesity as one of its clinical features. *UCP2* is also in the proximity (~15 cM) of a locus (11q21–q22) recently uncovered through a genome-wide search and found to be linked to percent body fat in Pima Indians (3). Based on the evidence from these recent studies, we hypothesized that markers around the *UCP2* gene may exhibit a linkage relationship with metabolic rate and body fat phenotypes. To test this hypothesis we typed three markers (D11S916, D11S1321 and D11S911) on 640 individuals from 155 pedigrees from the Québec Family Study. Linkage studies were undertaken with resting metabolic rate (RMR), body mass index (BMI), percentage body fat (%FAT) and fat mass (FM) using four types of relatives. RMR was adjusted for the effects of age, sex, FM and fat-free mass (FFM), whereas BMI, %FAT and FM were adjusted only for age and sex effects.

RESULTS

Table 1 presents the linkage results with the number of relative pairs available in each case. Strong evidence of linkage was observed between D11S911 and RMR (*P* = 0.000002), while more moderate evidence for linkage was found for the other two markers. Suggestive evidence of linkage between D11S1321 and %FAT (*P* = 0.04) and FM (*P* = 0.02) was also found. D11S1321 was, however, a marker with a slightly lower level of heterozygosity. No linkage was observed between the three markers and FFM in this population.

*To whom correspondence should be addressed. Tel: +1 418 656 5174; Fax: +1 418 656 3044; Email: claude.bouchard@kin.msp.ulaval.ca

Table 1. Relative pair linkage analysis of resting metabolic rate and body fat variables with markers encompassing the *UCP2* gene in the Québec Family Study

Marker	HZ	cM	RMR	BMI	%FAT	FM
D11S916	0.72	78	0.006 (301)	0.27 (415)	0.50 (304)	0.11 (304)
D11S1321	0.64	79	0.02 (380)	0.36 (537)	0.04 (383)	0.02 (383)
D11S911	0.85	83	0.000002 (240)	0.38 (324)	0.23 (243)	0.26 (243)

Based on four different types of relative pairs: siblings (165–275 pairs); avuncular (47–134 pairs); grandparental (45–94 pairs); first degree cousins (22–34 pairs). The entries are the *P* values with total number of relative pairs given in parentheses. HZ, heterozygosity; cM, distance in centimorgans (13).

DISCUSSION

In the first paper describing *UCP2* (1) the predicted amino acid sequence as well as its activity in recombinant yeast provided support for a role of the protein in control of the ratio between energy stored as ATP and energy dissipated in the form of heat. Hence, UCP2 may be one of the biochemical mechanisms potentially involved in regulation of RMR. Overall, the results of the present study suggest that the *UCP2* gene, which is encoded within the 5 cM span covered by these markers, plays a role in determining resting energy expenditure in humans. It would be useful to extend these studies to diet-induced thermogenesis and to sequence variation in the *UCP2* gene itself.

MATERIALS AND METHODS

Sample

A total of 640 individuals (299 males and 341 females) from 155 pedigrees were available for the present study. These were randomly recruited from a larger pool of families of French descent living in the Québec city area who were invited to participate in the Québec Family Study, a population-based study of the genetics of physiological fitness and body composition. The age of individuals in the sample ranged from 18 to 94 years.

Phenotypes

BMI was obtained from height and weight measurements (BMI = weight in kg/height in m^2). FM, FFM and %FAT were determined from body density measurements obtained by weighing underwater and using the conversion factor of Siri (4). RMR was determined by indirect calorimetry measurements using an open circuit indirect calorimeter with the ventilated hood technique as described ealier (5). Measurements were taken in the morning after an overnight fast, while subjects sat quietly in a semi-reclined position for the 30 min measurement period.The last 10 min were kept for calculation of the RMR. The O_2 and CO_2 data were converted into energy as recommended by Weir (6). The phenotypes were adjusted by sex, for age and age^2 by regression procedures and RMR was further adjusted for FM and FFM. The residuals from the regressions were used for linkage analysis.

DNA typing

Genomic DNA was prepared from permanent lymphoblastoid cells (7) by the proteinase K and phenol/chloroform technique. DNA was dialysed four times against TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0) for 6 h at 4°C and ethanol precipitated (8). Amplification (EasyCycler; Ericomp, San Diego, CA) was in 96-well microtiter plates using 250 ng genomic DNA, 0.1 (D11S1321 and D11S916) or 0.25 pmol (D11S911) forward primer coupled to the infrared tag IRD41 (Licor) and 0.1 or 0.4 pmol reverse primer respectively, 125 µM dNTPs and 0.3 U Taq polymerase (Perkin Elmer, Roche Molecular Systems, Branchburg, NJ) in PCR buffer (100 mM Tris–HCl, pH 8.3, 15 mM $MgCl_2$, 0.5 M KCl, 0.01% gelatin) for a final volume of 10 µl. PCR cycles consisted of one cycle at 93°C for 5 min, 10 cycles at 94°C for 20 s, 57°C for 60 s, and 24 cycles at 94°C for 20 s, 52°C for 60 s, except for D11S911, for which the first annealing temperature was set at 55°C. PCR products were analyzed on an automatic DNA sequencer (Li-Cor) using 18 cm glass plates. Typing was computer assisted (OneDscan; Scannalytics).

Linkage analysis

We used relative pair-based methods to test for linkage between the phenotypes and the marker loci. In the presence of linkage between a marker locus and a quantitative trait (Y), relative pairs sharing a greater proportion of alleles identical by descent (π) at the marker locus tend to have more similar phenotypes than pairs who share fewer alleles. Thus, under the hypothesis of linkage, a negative relationship is expected between π and the within-pair variance. The sib pair linkage method described by Haseman and Elston (9) is the most widely used method to investigate linkage between a quantitative phenotype and a marker locus. This method has been extended to other types of relative pairs (10). Tests of linkage which combine information from different types of relative pairs have been developed and shown to be more powerful than the Haseman–Elston (9) method based only on sib pairs (11). This relative pair linkage analysis has been implemented in the program RELPAL (12), which considers the following five types of relative pairs: sibling, half-sibling, grandparent–grandchild, avuncular and first degree cousins. For each relative pair type the statistic for testing linkage is obtained by dividing the estimated regression coefficient ($\hat{\beta}$) by its standard error. Because the number (n) of relative pairs could vary among the different types of relatives depending on the complexity of the pedigrees, the contribution of each type of relative pair needs to be weighted in the overall linkage statistic, which combines information from all relative pairs. The linkage test implemented in RELPAL is:

$$T = \frac{c^T \hat{\beta}}{\sqrt{c^T\ Var(\hat{\beta})c}} \qquad (1)$$

where $\hat{\beta}$ is a vector containing the $\hat{\beta}$s for each of the five types of relatives and c^T is a weighing vector based on n and the variance of π and equal to $[\mathrm{var}(\pi_s)n_s, \mathrm{var}(\pi_h)n_h, \mathrm{var}(\pi_g)n_g, \mathrm{var}(\pi_a)n_a, \mathrm{var}(\pi_c)n_c]$, where subscripts s, h, g, a and c stand for siblings, half-siblings, grandparent–grandchild, avuncular and first degree cousins respectively. In the Québec Family Study, since there are no half-sibs, only sibling, avuncular, grandparental and cousin pairs were used in the relative pair linkage analysis.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the contribution of Sonia Roy, Michel Lacaille and Monique Chagnon for the laboratory work on the microsatellites. Thanks are also expressed to Guy Fournier,

Lucie Allard, Anne-Marie Bricault and Dr Germain Thériault for their contribution to the data collection in the Québec Family Study. The results of this paper were obtained using the program SAGE, which is supported by US Public Health Service Resource grant 1P41RR03655 from the National Center for Research Resources. This work was supported by the Medical Research Council of Canada (PG-11811). C.W. is funded by the NIH (DK52581 and HL55798).

ABBREVIATIONS

BMI, body mass index; FM, fat mass; FFM, fat-free mass; %FAT, percentage body fat; RMR, resting metabolic rate; UCP1, uncoupling protein-1; UCP2, uncoupling protein-2.

REFERENCES

1. Fleury,C., Neverova,M., Collins,S., Raimbault,S., Champigny,O., Levi-Meyruis,C., Bouillaud,F., Seldin,M.F., Surwit,R.S., Ricquier,D. and Warden,C.H. (1997) Uncoupling protein-2: a novel gene linked to obesity and hyperinsulinemia. *Naure Genet*., **15**, 269–272.
2. Online Mendelian Inheritance in Man, OMIM™ (1996) . Center for Medical Genetics, Johns Hopkins University and National Center for Biotechnology Information, National Library of Medicine, Bethesda, MD. World-wide Web URL: http://www3.ncbi.nlm.nih.gov/omim.
3. Norman,R.A., Thompson,D.B., Foroud,T., Garvey,W.T., Bennett,P.H., Bogardus,C., Ravussin,E. and other members of the Pima Diabetes Gene Group (1997) Genomewide search for genes influencing percent body fat in Pima Indians: suggestive linkage at chromosome 11q21–q22. *Am. J. Hum. Genet*., **60**, 166–173.
4. Siri,W.E. (1976) The gross composition of the body. *Adv. Biol. Med. Phys*., **4**, 239–280.
5. Dériaz,O., Dionne,F.T., Pérusse,L., Tremblay,A., Vohl,M.C., Côté,G. and Bouchard,C. (1994) DNA variation in the genes of the Na,K-adenosine triphosphatase and its relation with resting metabolic rate, respiratory quotient, and body fat. *J. Clin. Invest*., **93**, 838–843.
6. Weir,J.B. (1949) New methods for calculating metabolic rate with special reference to protein metabolism. *J. Physiol. (Lond)*, **109**, 1–9.
7. Neitzel,H. (1986) A routine method for the establishment of permanent growing lymphoblastoid cell lines. *Hum. Genet*., **73**, 320–326.
8. Sambrook,J., Fritsch,E.F. and Maniatis,T. (1989) *Molecular Cloning: A Laboratory Manual*, 2nd Edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 9.16–9.19.
9. Haseman,J.K. and Elston,R.C. (1972) The investigation of linkage between a quantitativre trait and a marker locus. *Behav. Genet*., **2**, 3–19.
10. Amos,C.I. and Elston,R.C. (1989) Robust methods for the detection of genetic linkage for quantitative data from pedigrees. *Genet. Epidemiol*., **6**, 349–360.
11. Olson,J.M. and Wijsman,E.M. (1993) Linkage between quantitative trait and marker loci: methods using all relative pairs. *Genet. Epidemiol*., **10**, 87–102.
12. SAGE, Statistical Analysis for Genetic Epidemiology, Release 3.0. (1997) Department of Epidemiology and Biostatistics, Rammelkamp Center for Education and Research, Metrohealth Campus, Case Western Reserve University, Cleveland, OH.
13. Collins,A., Frezal,J., Teague,J. and Morton,N.E. (1996) A metric map of humans: 23,500 loci in 850 bands. *Proc. Natl. Acad. Sci. USA*, **93**, 14771–14775.