Genome-Wide Search for Genes Related to the Fat-Free Body Mass in the Québec Family Study

Yvon C. Chagnon, Ingrid B. Borecki, Louis Pérusse, Sonia Roy, Michel Lacaille, Monique Chagnon, My Anh Ho-Kim, Treva Rice, Michael A. Province, D.C. Rao, and Claude Bouchard

Fat-free mass (FFM) consists mostly of skeletal muscle and bone tissues, and identification of the genes and molecular mechanisms involved in the control of FFM would have implications for the understanding of sarcopenia and potentially osteoporesis associated with aging, as well as the response to starvation, refeeding, anorexia, and any other conditions in which lean body mass is important. A genome-wide search for genes related to body leanness has been completed in the Québec Family Study (QFS). Microsatellite markers (N = 292) from the 22 autosomal chromosomes were typed. The mean spacing of the markers was 11.9 centimorgans (cM) (range, <0.1 to 41). FFM was calculated from percent body fat, derived from underwater weighing, and body weight and was adjusted by regression for age and sex effects before analysis. A maximum of 336 sib pairs or 609 pairs of extended relatives were analyzed using single-point Haseman-Elston regression (SIBPAL and RELPAL) and multipoint variance component (SEGPATH) linkage analyses. Significant linkages were observed on chromosomes 15q25-q26 for a CA repeat within the insulin-like growth factor 1 receptor (IGF1R) gene (Lod score = 3.56) and at 18q12 with D18S877 (Lod score = 3.53) and D18S535 (Lod score = 3.58), 2 markers located 10 cM apart. A moderately significant linkage was also observed on chromosome 7p15.3 with the marker D7S1808 (Lod score = 2.72). The most obvious candidate genes within the regions identified by these linkages include the IGF1R on 15q and neuropeptide Y (NPY) and growth hormone–releasing hormone (GHRH) receptor on 7p. On 18q, the melanocortin receptor 4 (MC4R) is not likely the candidate gene for the observed linkage. This study represents the first genome-wide search for genes that may be involved in the regulation of the lean component of body mass in humans.
Copyright © 2000 by W.B. Saunders Company

THE REGULATION of body weight is a complex phenomenon in which multiple factors, genetic and nongenetic, are involved. A recent review of the genes or loci potentially related to poor body weight control leading to obesity found evidence for multiple candidates on almost all chromosomes.[1] Up to now, the results from 4 genome-wide scan studies for genes related to body weight, fat mass, and energy expenditure have been reported.[2-8] From these studies, significant linkages (log of the odds [Lod] score ≥ 3.0) were observed with leptin at 2p21, obesity at 10p12, body mass index (BMI) at 11q24.1-q24.3, and BMI and 24-hour respiratory quotient at 20q11.2-q13.

The excess body weight of the obese consists primarily of fat mass, but also of lean tissue, the nature of which is largely unknown.[9] Although it is known that fat mass is correlated with fat-free mass (FFM) and that these 2 phenotypes are influenced by genetic factors,[10] we have shown that the association between the 2 phenotypes was not explained by common familial factors.[11] The lack of familial covariation between these 2 phenotypes of body composition suggests that different genes may be involved in determining them. FFM can be roughly divided into 2 subcomponents, the bone mass and the remaining mass, the latter mostly constituted by the skeletal muscle mass. Twin and family studies have shown that there is a strong genetic effect on FFM.[10,12] Race differences in FFM have also been reported.[13]

FFM is important to good health, particularly with aging. For instance, it is well known that bone mass and muscular mass decrease with age, eventually giving rise to a decrease in fitness and mobility along with growing fragility in older individuals. On the other hand, in periods of crisis such as famine or starvation or in disease states such as anorexia, it is critical to be able to preserve lean body mass. Since FFM variation is strongly influenced by the genotype, the identification of genes involved in the control of lean body mass is essential for a proper understanding of the mechanisms that modulate its variation during growth and in the presence of adverse conditions. One candidate gene potentially involved is the ornithine decarboxylase gene for which 2 different variants were found in lines of mice selected for high and low lean body mass.[14] One variant was associated with an increased peak of ornithine decarboxylase activity in embryos of all high-mass lines.[14] This variant resulted in a higher transcription of the gene but not different specific activities between the 2 forms.[15] In addition, 3 quantitative trait loci (QTL) significantly linked to bone mineral density have been reported on chromosomes 1p, 2p, and 4q[16] and 1 on chromosome 11q12-q13,[17] whereas 2 QTLs related to peak bone mass were detected from a murine model of senile osteoporosis on mice chromosomes 11 and 13.[18] Hence, several unknown genes are expected to be involved in the regulation of overall lean body mass in both animals and humans.

No genome-wide search for the lean component of body composition has been reported. Based on the Québec Family Study (QFS) data, we report the first strong significant linkages

From the Physical Activity Sciences Laboratory, Division of Kinesiology, Department of Social and Preventive Medicine, Faculty of Medicine, Laval University, Ste-Foy, Quebec, Canada; Division of Biostatistics, Washington University School of Medicine, St Louis, MO; and Pennington Biomedical Research Center, Louisiana State University, Baton Rouge, LA.

Submitted March 17, 1999; accepted July 8, 1999.

Supported by the Medical Research Council of Canada (PG-11811, MT-13960, and GR-15187), and in part by the Donald B. Brown Research Chair on Obesity funded by the Medical Research Council of Canada and Roche Canada (C.B.).

Address reprint requests to Yvon C. Chagnon, PhD, Physical Activity Sciences Laboratory, Kinesiology, PEPS 0212, Department of Social and Preventive Medicine, Laval University, Ste-Foy, Quebec, G1K 7P4 Canada.

Copyright © 2000 by W.B. Saunders Company

0026-0495/00/4902-0001$10.00/0

with the body FFM on chromosomes 15 and 18, and moderate evidence of linkage on chromosome 7.

SUBJECTS AND METHODS

Subjects and Phenotypes

The QFS is an ongoing project that has been previously described.[19] Caucasian nuclear families from the greater Québec City area representing a mixture of random sampling and ascertainment through obese probands with a BMI greater than 32 kg/m^2 are involved in the study. Subjects have been tested 1 to 3 times (phases 1 to 3) over a period of 19 years using a battery of morphologic and physiologic variables. Data from the last measures obtained in adult subjects aged 18 years and older were retained for the present study. Blood samples were obtained, and permanent lymphoblastoid cell lines were established for extraction of DNA. The study was approved by the Laval University Medical Ethics Committee, and all subjects provided written informed consent. A total of 748 subjects (46 grandparents, 334 parents, and 368 adult offspring) from 194 families (336 sib pairs and 609 pairs of relatives) were available for the study. The FFM (kilograms) was calculated by subtracting from body weight, body fat mass estimated from body density measurements obtained by underwater weighing[20] and the equation of Siri.[21] Pulmonary residual volume was assessed by the helium dilution technique.[22]

Molecular Analysis

Genomic DNA was prepared by the proteinase K and phenol/chloroform technique. DNA was dialyzed 4 times against TE buffer (10 mmol/L Tris and 1 mmol/L EDTA, pH 8.0) for 36 hours at 4°C and ethanol-precipitated. Microsatellite markers (dinucleotide, trinucleotide, and tetranucleotide repeats) have been selected from different sources, mainly the Marshfield panel version 8a. The locations of markers on the chromosomes (Kosambi distance in centimorgans [cM]) have been updated using map version 9 from the Marshfield Institute (http://www.marshmed.org/genetics/) and the Location Database (LDB) map (June 1998) from Southampton, UK (http://cedar.genetics.soton.ac.uk). The LDB summary map was also used to look for candidate genes 10 cM on each side of the markers yielding the strongest results.

Marker Analysis

The polymerase chain reaction (PCR) conditions and genotyping have been described in detail elsewhere.[23] Briefly, PCRs were performed using a forward primer coupled to the infrared tag IRD800 or IRD700 (LICOR, Lincoln, NE). PCR cycles followed a 2-step procedure in which the annealing temperature was between 52°C and 67°C, with a difference of 5°C being maintained between the 2 temperatures. Automatic infrared DNA sequencers from LICOR were used to detect PCR products. One microliter of each of 52 samples, including up to 5 different markers, interspaced with standards at each 4 samples were applied to the gel. Standards were produced by PCR from a PUC19 plasmid using an infrared-tagged M13 forward primer (LICOR) and a corresponding untagged reverse primer for each standard. The 18-cm gel was run for 1.5 to 2 hours. At the end of the run, an image of the gel was produced for use in the genotyping. Automatic genotyping was performed using the software SAGA (Rick McIndoe, Roger Bumgarner, Russ Welti, University of Washington at Seattle; LICOR). After genotyping, manual editing of the results was performed using the optical density of the PCR fragments of the different markers, and the results were stored in a local dBase IV database, GENEMARK, in which a procedure for checking Mendelian inheritance incompatibilities within nuclear families and extended pedigrees was implemented. Subjects with Mendelian incompatibilities were excluded from the database automatically by GENEMARK and retyped completely, ie, from the PCR to the genotyping. Less than 5% of the genotypes had to be retyped.

Table 1. Subject Characteristics by Generation and Gender in the QFS

	Parent				Adult Offspring			
	Male (n = 133)		Female (n = 144)		Male (n = 154)		Female (n = 204)	
Variable	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Age (yr)	54.6	37-71	51.4	32-68	28.2	18-60	29.0	18-62
BMI (kg/m^2)	28.2	19-53	27.4	18-50	25.6	17-57	26.5	17-65
FFM (kg)	59.8	44-81	43.7	31-66	61.9	40-88	47.0	33-73

Statistical Analysis

FFM was adjusted within sex for age, age^2, and age^3 by a regression procedure with outliers (±3 SD) excluded for the estimation of the regression parameters. Only significant covariates ($P < .05$) were included in the model. Residuals were computed for all subjects, including outliers and ascertained subjects, and then standardized to a mean of 0 and a SD of 1. Single-point linkage analyses using pairs of sibs from nuclear families (SIBPAL) or pairs of relatives from extended pedigrees (RELPAL)[24] and a multipoint variance component linkage analysis (SEGPATH)[25] on nuclear families were performed. The SAS package (version 6.08) was used for the other analyses (SAS Institute, Cary, NC).

RESULTS

A total of 292 microsatellite markers with a mean heterozygosity of 0.75 (range, 0.32 to 0.94) and an average spacing of 11.9 cM (range, <0.1 to 41) were used for the study. For some chromosome arms, including regions with candidate genes or loci for obesity (1p, 5q, 7q, 8p, 8q, 11p, 16p, and 20q), a higher marker density was used (mean spacing, 5.6 cM; range, <0.1 to 10). The mean age, BMI, and FFM by generation and by sex of the subjects are presented in Table 1. The QFS includes normal-weight (BMI < 25 kg/m^2, 48%), overweight (25 ≤ BMI < 30 kg/m^2, 27%), and obese (BMI ≥ 30 kg/m^2, 25%) subjects representing, in the aggregate, an overweight population with a mean BMI of 27.3 kg/m^2 (range, 17 to 65).

Table 2 presents the significant linkage results obtained from

Table 2. Linkage Results With FFM in the QFS

Chromosome	Marker	Position (cM)	Lod Score	P§
SEGPATH analysis				
1q44	D1S547	284	2.27	.0006
7p15.3	D7S1808	41	2.72	.0002
15q26.2-q26.3	IGF1RCA	101	3.56	.00003‡
	D15S652	102	2.52	.0003
	D15S657	106	2.05	.001
16p13.2	D16S748	11	2.10	.0009
18q12.1-q12.3	D18S877	54	3.53	.00003‡
	D18S535	64	3.58	.00002‡
SIBPAL† analyses				
2q22.1	D2S1290	113	—	.0002*
6q16.3	D6S1056	100	—	.001†
7p15.3	D7S1808	41	—	.0003*

*RELPAL.

†SIBPAL.

‡Significant result (Lod score ≥ 3.0).

§Moderately significant results (.001 ≥ P > .0001) are reported.

RELPAL and SIBPAL single-point and SEGPATH multipoint variance component linkage analyses. Significant linkages (Lod score ≥ 3.00) with FFM were obtained from the variance component analysis on chromosome 15 with a dinucleotide CA microsatellite repeat (Lod score = 3.56) located in the 5′ untranslated region of the insulin-like growth factor 1 receptor (IGF1R), and on chromosome 18 with the markers D18S535 (Lod score = 3.58) and D18S877 (Lod score = 3.53) located 10 cM apart (Fig 1). Additionally, lower Lod scores were observed with the neighboring markers D15S652 (Lod score = 2.52) and D15S657 (Lod score = 2.05) located, respectively, 1 and 6 cM telomeric to IGF1R, and with D18S542 (Lod score = 1.04) and D18S851 (Lod score = 1.51) located, respectively, 25 cM centromeric and 10 cM telomeric to D18S535. The linkages observed with the multipoint analysis on chromosome 18 were generally supported by the other 2 single-point linkage methods, but with lower LOD scores (Fig 1).

Moderately significant linkages with FFM were also found on chromosomes 1, 2, 6, 7, and 16 from single-point (.001 ≥ P > .0001) and/or multipoint (2.00 ≤ Lod score < 3.00) analyses (Table 2). Convincing evidence for linkage can also be inferred when positive results are obtained with different analytical linkage methods, or with several neighboring markers. Such a region was found on chromosome 7, where both RELPAL (P = .0003) and SEGPATH (Lod score = 2.72) yielded moderately significant linkages with the marker D7S1808 (Fig 1). The other linkages were observed only with 1 marker or by 1 linkage method.

DISCUSSION

A genome-wide search has been completed on data from the QFS. The 2 strongest linkages with FFM were observed on chromosomes 15 and 18 using multipoint SEGPATH analysis. The linkage on chromosome 18 is supported by the single-point results, whereas the linkage on chromosome 15 is not. The lower statistical power of the single-point methods could be at the origin of these differences. On chromosome 15, a marker within IGF1R showed the strongest signal with a Lod score of 3.56 (P = .00003). IGF1R is clearly a strong candidate gene for this linkage with FFM. For instance, we have already ob-

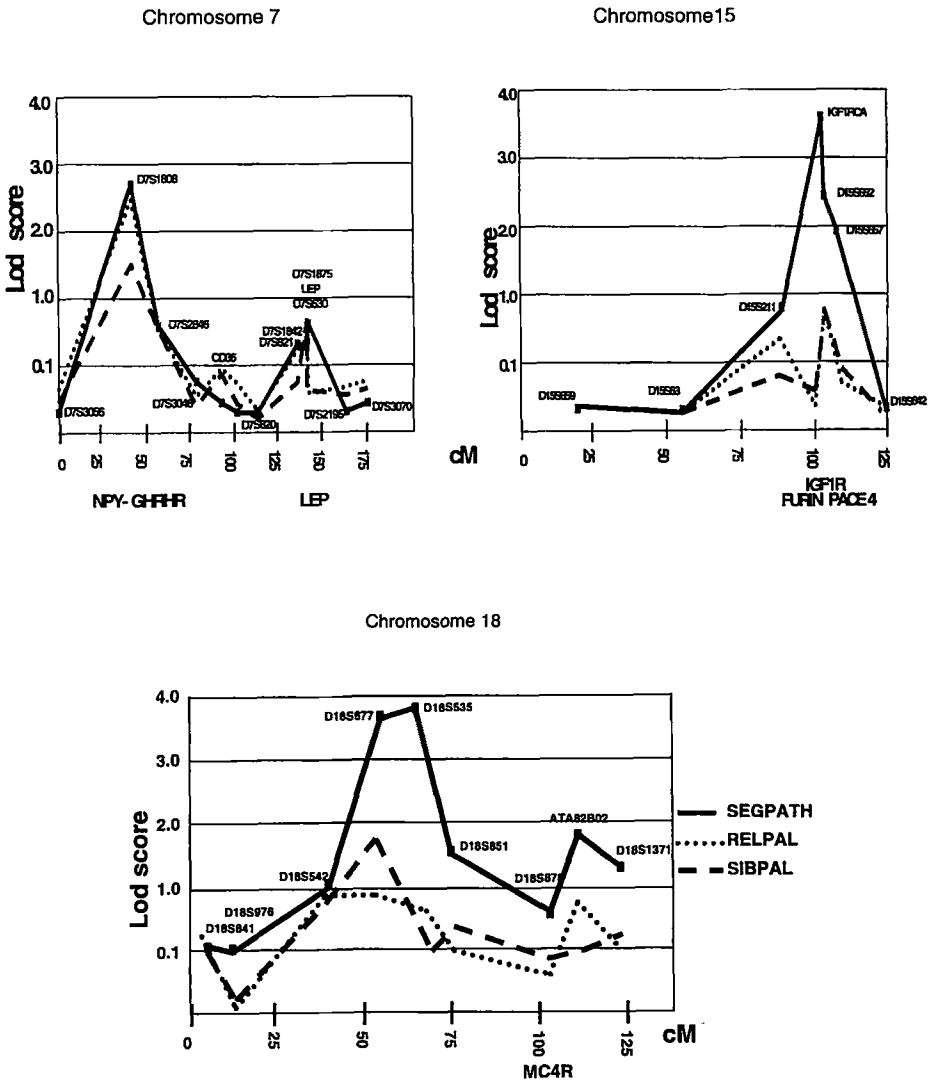

Fig 1. Significant genome-wide scan linkage results for FFM in the QFS. Results from the 3 linkage methods (SEGPATH, RELPAL, and SIBPAL) are shown. The markers and distances along the chromosome in cM are indicated.

served[26] in the HERITAGE Family Study a significant linkage ($P = .0002$) between the changes in FFM following 20 weeks of endurance exercise training and an insulin-like growth factor 1 (IGF1) marker. On the other hand, no effect of this polymorphism was observed on bone mineral density in Japanese subjects.[27] Growth delay and low levels of growth hormone, IGF1, and IGF-binding protein 3 (IGFBP3) were observed in severely obese subjects with a mutation in the leptin receptor (LEPR) gene,[28] but not in a subject with a mutation in the leptin gene.[29] We have already reported a linkage and an association between a polymorphism in LEPR and FFM in the QFS.[30] Hence, a relation between FFM, IGF elements (IGF1, IGF1R, and IGFBP3), and LEPR is emerging. But the way these genes interact to modulate FFM remains to be clarified. On the other hand, from the absence of effects of IGF1 on bone mineral density in the Japanese[27] and from the QTL previously identified on chromosomes 1p, 2p, 4q, and 11q for bone mineral density,[16,17] we can speculate that the linkages on chromosomes 7p, 15q, and 18q are probably related more to the skeletal muscle component of FFM than to bone mineral density. Additionally, ornithine decarboxylase is not a candidate for these linkages, since the human gene is located on chromosome 2p25, outside any of the linked regions on chromosomes 7, 15, or 18 of the present study.

In addition, the 2 pro-protein hormone convertases, PACE4 and furin, could be candidates for the observed linkage on 15q. Indeed, mutations in another gene involved in the processing of prohormone, the prohormone convertase 1,[31] were shown to be responsible for a case of severe loss of body weight control leading to obesity. Studies with polymorphisms within the PACE4, furin, and IGF1R genes are needed to clarify their possible involvement in FFM modulation in humans.

The strong linkages observed on chromosome 18q between FFM and both D18S535 (Lod score = 3.58, $P = .00002$) and D18S877 (Lod score = 3.53, $P = .00003$) cannot be translated into obvious candidate genes at this time. The melanocortin receptor 4 (MC4R), located at 18q21.3 (Genome Database [GDB]; http://gdbwww.gdb.org/) and previously shown to play a role in body weight regulation[32] and human obesity,[33,34] is not close to this region and therefore is an unlikely candidate. According to GDB linkage maps, markers in 18q21.3, for example, D18S851 and D18S814, are located some 26 cM telomeric to D18S535, which is not very close to the linked region. Moreover, the marker D18S851, located between MC4R at 18q21.3 and D18S535, had an intermediate Lod score of 1.51, whereas the marker D18S878, located at 18q22.1 some 28 cM from D18S535 and closer to MC4R, yielded no evidence of linkage (Lod score = 0.72). In the QFS, we have previously reported no linkage between FFM and a *Nco*I polymorphism detected by Southern blotting using a MC4R cDNA probe.[35] A Val103Ile polymorphism in MC4R reported in a British male population[36] also showed negative linkage results in the QFS (Chagnon et al, unpublished observation). On the other hand, a weak association ($P = .05$) was observed in the same study between the MC4R *Nco*I polymorphism and FFM,[35] but it is unlikely to be at the origin of the strong linkage reported here.

Weaker linkages were observed at 7p15.3 with D7S1808 based on the RELPAL ($P = .0003$) and SEGPATH (Lod score = 2.72, $P = .0002$) analytical strategies. Two possible candidate genes are present in the linked region. Neuropeptide Y (NPY) is one of the numerous neuropeptides involved in food intake regulation and is known to stimulate food intake.[37] Growth hormone–releasing hormone (GHRH), the agonist of GHRH receptor, the other candidate gene for the observed linkage between FFM and D7S1808, is also a peptide that stimulates food intake. The influence of the growth hormone axis, including IGFs, on growth is well known, but a more direct link with lean body mass seems to be emerging. On the other hand, an effect of appetite-controlling factors, such as NPY and GHRH, on lean body mass has not yet been described. Both lines of evidence could represent major clues for the factors involved in the regulation of body weight, particularly muscular mass. An understanding of the genes involved and the role they play in the modulation of FFM would be useful to prevent or correct the FFM disturbances commonly observed with some diseases and aging.

In summary, in the QFS, we have observed for the first time strong significant linkages between FFM and markers located on chromosomes 15 and 18, and good evidence of linkage on chromosome 7. Reasonable candidate genes are available for the chromosome 7 and 15 linkages, whereas there is no obvious candidate for the linkage on chromosome 18. Additional studies are needed to identify the genes responsible for these linkages, and to uncover other loci across the human genome affecting FFM.

ACKNOWLEDGMENT

The authors wish to acknowledge the contribution of Guy Fournier and Lucie Allard to the data collection in the QFS, François Michaud and Claude Leblanc for management of the database, and Anne-Marie Bricault for cell culture and preparation of the DNA. The results of this study were obtained, in part, using the program S.A.G.E., which is supported by a US Public Health Service Resource Grant (1P41RR03655) from the National Center for Research Resources.

REFERENCES

1. Pérusse L, Chagnon YC, Weisnagel J, et al: The human obesity gene map: The 1998 update. Obes Res 7:111-129, 1999

2. Norman RA, Thompson DB, Foroud T, et al: Genomewide search for genes influencing percent body fat in Pima Indians: Suggestive linkage at chromosome 11q21-q22. Am J Hum Genet 60:166-173, 1997

3. Norman RA, Tataranni PA, Pratley R, et al: Autosomal genomic scan for loci linked to obesity and energy metabolism in Pima Indians. Am J Hum Genet 62:659-668, 1998

4. Pratley RE, Thompson DB, Prochazka M, et al: An autosomal genomic scan for loci linked to prediabetic phenotypes in Pima Indians. J Clin Invest 101:1757-1764, 1998

5. Hanson RL, Ehm MG, Pettitt DJ, et al: An autosomal genomic scan for loci linked to type II diabetes mellitus and body-mass index in Pima Indians. Am J Hum Genet 63:1130-1138, 1998

6. Comuzzie AG, Hixson JE, Almasy L, et al: A major quantitative trait locus determining serum leptin levels and fat mass is located on human chromosome 2. Nat Genet 15:273-276, 1997

7. Hager J, Dina C, Francke S, et al: A genome-wide scan for human obesity genes reveals a major susceptibility locus on chromosome 10. Nat Genet 20:304-308, 1998

8. Lee JH, Reed DR, Li WD, et al: Genome scan for human obesity and linkage to markers in 20q13. Am J Hum Genet 64:196-209, 1999

9. Schutz Y, Jéquier E: Resting energy expenditure, thermic effect of food, and total energy expenditure, in Bray GA, Bouchard C, James

WPT (eds): Handbook of Obesity. New York, NY, Dekker, 1998, pp 443-455

10. Bouchard C, Pérusse L, Rice T, et al: The genetics of human obesity, in Bray GA, Bouchard C, James WPT (eds): Handbook of Obesity. New York, NY, Dekker, 1998, pp 157-190

11. Rice T, Tremblay A, Deriaz O, et al: Genetic pleiotropy for resting metabolic rate with fat-free mass and fat mass: The Quebec Family Study. Obes Res 4:125-131, 1996

12. Nguyen TV, Howard GM, Kelly PJ, et al: Bone mass, lean mass, and fat mass: Same genes or same environments? Am J Epidemiol 147:3-16, 1998

13. Nelson DA, Barondess DA: Whole body bone, fat and lean mass in children: Comparison of three ethnic groups. Am J Phys Anthropol 103:157-162, 1997

14. Gray A, Tait A: Identification of ornithine decarboxylase as a trait gene for growth in replicated mouse lines divergently selected for lean body mass. Genet Res 62:31-37, 1993

15. Gray A, Tait A, Bulfield G: Differential transcription and expression of ornithine decarboxylase in embryos of replicated mouse lines divergently selected for lean body mass. Biochem J 308:161-166, 1995

16. Deveto M, Shimoya K, Caminis J, et al: First-stage autosomal genome screen in extended pedigrees suggests genes predisposing to low bone mineral density on chromosome 1p, 2p and 4q. Eur J Hum Genet 6:151-157, 1998

17. Koller DL, Rodriguez LA, Christian JC, et al: Linkage of a QTL contributing to normal variation in bone mineral density to chromosome 11q12-13. J Bone Miner Res 13:1903-1908, 1998

18. Shimizu M, Higuchi K, Bennett B, et al: Identification of peak bone mass QTL in a spontaneously osteoporotic mouse strain. Mamm Genome 10:81-87, 1999

19. Bouchard C: Genetic epidemiology, association and sib-pair linkage: Results from the Québec Family Study, in Bray GA, Ryan DH (eds): Molecular and Genetic Aspects of Obesity. Baton Rouge, LA, Louisiana State University Press, 1996, pp 470-481

20. Behnke AR, Wilmore JH: Evaluation and Regulation of Body Build and Composition. Englewood Cliffs, NJ, Prentice-Hall, 1974

21. Siri WE: The gross composition of the body. Adv Biol Med Phys 4:239-280, 1976

22. Himes JH, Bouchard C: Validity of anthropometry in classifying youths as obese. Int J Obes 13:183-193, 1989

23. Chagnon YC, Roy S, Chagnon M, et al: High-Throughput Genotyping Using Infrared Automatic Li-Cor DNA Sequencers in the Study of the Obesity and Co-morbidities Genes. Lincoln, NE, LiCor Application Note 500. 1998

24. S.A.G.E. Statistical Analysis for Genetic Epidemiology, Release 3.0. Computer program package available from the Department of Epidemiology and Biostatistics, Rammelkamp Center for Education and Research, MetroHealth Campus, Case Western Reserve University, Cleveland, OH, 1997

25. Province MA, Rao DC: General purpose model and a computer program for combined segregation and path analysis (SEGPATH): Automatically creating computer programs from symbolic language model specifications. Genet Epidemiol 12:203-219, 1995

26. Sun G, Gagnon J, Chagnon YC, et al: Association and linkage between an insulin-like growth factor-1 gene polymorphism with changes in body composition: The HERITAGE Family Study. Int J Obes 23:929-935, 1999

27. Miyao M, Hosoi T, Inoue S, et al: Polymorphism of insulin-like growth factor I gene and bone mineral density. Calcif Tissue Int 63:306-311, 1998

28. Clément K, Vaisse C, Lahlou N, et al: A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction. Nature 392:398-401, 1998

29. Montague CT, Farooqi IS, Whitehead JP, et al: Congenital leptin deficiency is associated with severe early-onset obesity in humans. Nature 387:903-908, 1997

30. Chagnon YC, Chung WK, Pérusse L, et al: Linkages and associations between the leptin receptor LEPR gene and human body composition in the Québec Family Study (QFS). Int J Obes 23:278-286, 1999

31. Jackson RS, Creemers JWM, Ohagi S, et al: Obesity and impaired prohormone processing associated with mutations in the human prohormone convertase 1 gene. Nat Genet 16:303-306, 1997

32. Fan W, Boston B, Kesterson V, et al: Role of melanocortinergic neurons in feeding and the agouti obesity syndrome. Nature 385:165-168, 1997

33. Yeo GSH, Farooqi S, Aminian S, et al: A frameshift mutation in human MC4R associated with dominantly inherited human obesity. Nat Genet 20:111-112, 1998

34. Vaisse C, Clément K, Guy-Grand B, et al: A frameshift mutation in human MC4R is associated with a dominant form of obesity. Nat Genet 20:113-114, 1998

35. Chagnon YC, Chen WJ, Pérusse L, et al: Linkage and association studies between the melanocortin receptors 4 and 5 genes and obesity-related phenotypes in the Québec Family Study. Mol Med 3:663-673, 1997

36. Gotoda T, Scott J, Aitman TJ: Molecular screening of the human melanocortin-4 receptor gene: Identification of a missense variant showing no association with obesity, plasma glucose, or insulin. Diabetologia 40:976-979, 1997

37. Woods SC, Seely RJ, Porte D Jr, et al: Signals that regulate food intake and energy homeostasis. Science 280:1378-1383, 1998