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Genomic scan for genes affecting body composition before and after training in Caucasians from HERITAGE

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Chagnon, Yvon C., Treva Rice, Louis Pérusse, Ingrid B. Borecki, My-Anh Ho-Kim, Michel Lacaille, Chantal Paré, Luigi Bouchard, Jacques Gagnon, Arthur S. Leon, James S. Skinner, Jack H. Wilmore, D. C. Rao, and Claude Bouchard. Genomic scan for genes affecting body composition before and after training in Caucasians from HERITAGE. *J Appl Physiol* 90: 1777–1787, 2001.—An autosomal genomewide search for genes related to body composition and its changes after a 20-wk endurance-exercise training program has been completed in the HERITAGE Family Study. Phenotypes included body mass index (BMI), sum of eight skinfold thicknesses, fat mass (FM), fat-free mass, percent body fat (%Fat), and plasma leptin levels. A maximum of 364 sib-pairs from 99 Caucasian families was studied with the use of 344 markers with single-point and multipoint linkage analyses. Evidence of significant linkage was observed for changes in fat-free mass with the S100A and the insulin-like growth factor I genes ($P = 0.0001$). Suggestive evidence ($2.0 \leq \text{Lod} < 3.0$; $0.0001 < P \leq 0.001$) was also observed for the changes in FM and %Fat at 1q31 and 18q21-q23, in %Fat with the uncoupling protein 2 and 3 genes, and in BMI at 5q14-q21. At baseline, suggestive evidence was observed for BMI at 8q23-q24, 10p15, and 14q11; for FM at 14q11; and for plasma leptin levels with the low-density lipoprotein receptor gene. This is the first genomic scan on genes involved in exercise-training-induced changes in body composition that could provide information on the determinants of weight loss.

genomewide search; linkage; genetic; body mass index; body fat; fat-free mass

EXERCISE TRAINING CAN FAVORABLY alter body composition by reducing body weight and fatness, but there are individual differences in responsiveness. Our laboratory has observed small but significant changes in body composition among families trained with endurance exercise for 20 wk in the HERITAGE Family Study (36). Body composition is determined by complex phenotypes for which multiple genetic and nongenetic factors are expected to be involved. A recent review of the genes potentially related to human adiposity found evidence for several candidates pertaining to regulation of adipose tissue, body mass, energy intake, and energy expenditure (7). Probing the genome of selected individuals with informative markers can identify candidate genes. Evidence for linkage between markers and a phenotype defines chromosomal regions potentially involved in the phenotype variance and thus suggests that candidate genes be localized in these regions.

Five genomewide scans for genes related to obesity, body composition, and energy expenditure have been reported. In Pima Indians, Norman et al. (23, 24) presented such data for percent body fat (%Fat), waist-to-hip circumference ratio, 24-h metabolic rate, sleeping metabolic rate, and respiratory quotient, whereas Hanson et al. (16) showed results for body mass index (BMI). From these studies, evidence of linkages was found for BMI and %Fat at 11q24.1-q24.3 and for 24-h respiratory quotient at 20q11.2. A genomic scan was also conducted on Mexican-American subjects based on fat mass (FM) and blood leptin levels (Lep). Evidence of linkage was observed with Lep, and to a lesser extent with FM, at 2p21 near the proopiomelanocortin gene (11). Another genomic scan (13) was conducted on a French cohort using Lep, FM, and BMI status (>27 kg/m^2), and it confirmed previous linkage at 2p21 with

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leptin and has provided evidence of linkage at 10p12 with BMI. In another study, Lee et al. (19) analyzed a mixed sample of Caucasian and African-American subjects for %Fat and BMI status (BMI < 27 kg/m^2 vs. BMI > 27 kg/m^2), and linkage was observed at 20q13 for BMI. Finally, based on data from the Québec Family Study, our laboratory recently reported evidence of linkage for fat-free mass (FFM) at 15q25-q26 and 18q12 (5).

Here, we now report the results of the genomic scan for body-composition phenotypes in the sedentary state and in response to 20 wk of exercise-endurance training in the HERITAGE Family Study cohort. This is the first genomic scan reporting results on body-composition changes after exercise training.

MATERIALS AND METHODS

Subjects and Phenotypes

The HERITAGE Family Study cohort has been previously described (4). The HERITAGE Family Study includes Black and Caucasian nuclear family volunteers with no obvious major diseases from the greater Québec City, Canada; Phoenix, AZ; Minneapolis, MN; Austin, TX; and Indianapolis, IN areas. Subjects were tested for a battery of anthropometric and physiological variables before and after a 20-wk exercise program. The Institutional Review Board of each participating institution previously approved the study protocol. Informed, written consent was obtained from each subject. Data from the 522 Caucasian subjects are used in the present study (192 parents and 330 offspring from 99 families), providing a maximum of 364 sib-pairs.

Blood samples were obtained for various biochemical assays, and permanent lymphoblastoid cell lines were established for the extraction of DNA. Dependent variables include baseline levels and changes (Δ) with the 20-wk training period in BMI, %Fat, FM, FFM, sum of eight skinfold thicknesses (SF8), and Lep. BMI was estimated as the weight in kilograms divided by height in square meters. %Fat was calculated from body density measurements obtained after underwater weighing (3) and the equations of Siri (32) and Lohman (20) for men and women, respectively, with corrections for pulmonary residual volume assessed by the helium dilution (17) or oxygen dilution (35, 37) techniques. FM and FFM (in kg) were calculated from %Fat and body weight. SF8 (in mm) was estimated by the values in the abdominal, subscapular, suprailiac, medial calf, triceps, biceps, midaxillary, and thigh (36). Lep (ng/ml) was evaluated in pre- and posttraining blood samples obtained before and after, respectively, completion of a maximal exercise test on the cycle ergometer (25). A radioimmunoassay (Linco, St. Charles, MO) was used in which the lowest quantity detectable in the plasma was 0.5 ng/ml.

Molecular Analysis

Choice of markers. Microsatellite markers were selected from various sources, but mainly the Marshfield panel version 8a and the Genethon panel, using maps of the Location Database (LDB) from Southampton, UK (http://cedar.genetics.soton.ac.uk). Microsatellite and restriction fragment length polymorphism (RFLP) markers for candidate genes of obesity and comorbidities, and some microsatellites that have generated positive results in other relevant studies, were also included. The LDB summary maps were also used to identify candidate genes 10 cM on each side of the markers yielding the strongest results.

PCR conditions. Genomic DNA was prepared from permanent lymphoblastoid cells by the proteinase K and phenol-chloroform technique. DNA was dialysed four times against Tris·EDTA buffer (10 mM Tris, 1 mM EDTA, pH 8.0) for 6 h at 4°C, and ethanol was precipitated. PCR conditions and genotyping of the markers have been described in detail elsewhere (8). Briefly, PCR reactions were conducted using 100-ng genomic DNA, 0.5 pmol each of a forward universal M13 primer coupled to infrared tag IRD800 or IRD700 (LICOR) and of the specific forward M13 tailed and reverse untagged primers, 125 μM dNTPs, and 0.3 units Taq polymerase (Perkin-Elmer, Pharmacia, Quiagen) 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 followed a two-step procedure: 1 cycle at 93°C for 5 min, 10 cycles at 93°C for 20 s and 57°C for 60 s, and 24 cycles at 93°C for 20 s and 52°C for 60 s. Annealing temperature was defined according to the melting temperature of the primers used and ranged between 52 and 67°C, with a difference of 5°C being maintained between the two annealing temperatures.

Marker analysis. Infrared LICOR DNA sequencers were used to detect the PCR products. Automatic genotyping was performed simultaneously for a given marker on all of the study subjects using the computer software SAGA (LICOR). Results were exported directly to a local dBase IV database (GENEMARK) in which a procedure was used to check for Mendelian inheritance incompatibilities within families. Subjects with Mendelian incompatibilities (between 5 and 10%, depending on marker) were retyped completely, i.e., from the PCR reaction to the genotyping.

Statistical Analysis

Phenotypic variables were adjusted within gender for the effects of age, age^2, and age^3, with Lep further adjusted for FM (Lep-FM). Phenotype changes were also adjusted for baseline values, and ΔLep was adjusted for ΔFM. When the regression parameters were estimated, a regression procedure with outliers (greater than ± 3 SD) excluded was used. Only significant effects ($P < 0.05$) were included in the model. Residuals were computed for all subjects, including outliers, and were then standardized to a mean of 0 and a SD of 1. SAS (version 6.08) for personal computer was used for the regression analysis. Single-point [SIBPAL (28)] and multipoint [MAPMAKER/SIBS (18)] linkage analyses were performed by using sibships from the nuclear families, where non-independence of sib-pairs was taken into account in the analyses. The data were also analyzed with the multipoint variance components routine implemented in SEGPATH (26), in which the correlation among siblings within a family is modeled as a function of the allele sharing at a marker locus, as well as a residual polygenic component. The proportion of marker alleles shared that were identical by descent was estimated by using MAPMAKER/SIBS (18) and was input as data into the SEGPATH model. In addition to the trait locus and polygenic heritabilities, the spouse correlation and excess sibling resemblance beyond that predicted under the genetic model were estimated by maximum likelihood in SEGPATH. Linkage was tested by a likelihood ratio test distributed as a 1:1 mixture of a χ^2 with 1 df and a point mass at 0 or by Lod score. LDB maps complemented by those of the Marshfield were used to define marker distances for multipoint analyses. LDB maps compared well with other genetic maps as with the Genethon and the Marshfield ones. For instance, the majority of the markers showed the same rel-

ative position across the different maps, which is the more important criterion for accurate multipoint analyses in contrast to absolute distances on the chromosome. Significance level for the genomewide analysis was set at 0.0001 or to the equivalent Lod score of 3.0, as estimated by nominal P value targeted (0.05) divided by the number of markers used (344 markers). In fact, this value is conservative because the real value should be $M_{eff} = M\{1. . .[V(\lambda)/M]\}$, where M_{eff} is the effective marker number, M is the number of markers scored, and $V(\lambda)$ is the variance of the eigen values of the inter-marker correlation matrix for each chromosome (9). M_{eff} should be less than M because of intercorrelations among linked markers.

RESULTS

A total of 344 markers (291 microsatellites; 53 RFLPs) from the 22 autosomal chromosomes were typed. The mean spacing of the markers was 9.7 cM (range <0.1–25 cM), and the mean heterozygosity in HERITAGE was 0.69 (0.01–0.97). The mean age, BMI, FFM, FM, %Fat, SF8, and Lep values by generation and gender of the subjects are presented in Table 1. The mean BMI for both genders from the parental generation was 28.0 kg/m^2 (n = 192; range 19–48 kg/m^2). In the parental generation, 27, 42, and 37% of the subjects were normal weight (BMI < 25 kg/m^2), overweight (25 ≤ BMI < 30 kg/m^2), and obese (BMI ≥ 30 kg/m^2), respectively (22). Offspring were leaner than their parents with a mean BMI of 24.6 kg/m^2 (n = 330; range 17–44 kg/m^2) and a BMI distribution of 63, 24, and 13% as normal weight, overweight, and obese, respectively.

The results from multipoint linkage analyses using either MAPMAKER/SIBS (Fig. 1) or SEGPATH (Fig. 2) for the 22 autosomal chromosomes are presented for BMI, FM, and %Fat. FFM, SF8, Lep, and Lep-FM results were not included in Figs. 1 and 2 for clarity and because no significant results were observed with these phenotypes using multipoint analytic strategies.

Significant ($P \leq 0.0001$; Lod score ≤ 3.0) and suggestive ($0.0001 < P \leq 0.001$; 2.0 ≥ Lod score < 3.0) linkages from single-point and multipoint analyses are summarized in Tables 2 and 3, respectively. Significant linkages were observed for the ΔFFM with candidate genes S100A (P = 0.0001) and insulin-like growth factor I (IGF-I) (P = 0.0001). Suggestive evidence of linkage was observed for ΔBMI at 5q14-q21, ΔFM at 9q34, ΔFM and Δ%Fat at 1q31 and 18q21-q23, and Δ%Fat with the ATPase α_2 and uncoupling protein 2 (UCP2) genes.

The evidence for linkage could be considered as more convincing when more than one phenotype shows linkage evidence with the same marker, when different markers within a linkage group are linked with the same phenotype, or when markers yield positive results for given phenotypes with different analytic linkage methods (P values for single point; Lod scores for multipoint). Such regions were found on chromosomes 1, 5, 8, 10, 11, 14, and 18. On chromosome 1, the marker D1S1660 at 1q31.1 showed evidence of linkage with both ΔFM and Δ%Fat, using either SIBPAL (P = 0.0006 and P = 0.0007, respectively) or MAPMAKER/SIBS (Lod score of 2.2 with each). At 5q14.1-q21.1 on chromosome 5, two successive markers, D5S1725 and D5S1462, showed linkages with ΔBMI (P = 0.0004 and Lod = 2.4, respectively). At 11q13.1-q21, several polymorphisms were tested for the two neighboring genes, UCP2 and UCP3. UCP2 haplotypes of the exon 4 Ala55Val and exon 8 insertion/deletion polymorphisms and marker D11S2002 yielded suggestive linkages with Δ%Fat (Lod = 2.2, P = 0.0009, respectively). Moreover, polymorphisms in UCP3 gene also showed some evidence of linkages with Δ%Fat (P = 0.009; Lod = 1.9) and ΔFM (P = 0.01; Lod = 1.7). Finally, on chromosome 18, within a region of 16 cM, three markers (D18S38, D18S878, and D18S1371) generated evidence of linkage with ΔFM and Δ%Fat, with D18S878 giving the strongest evidence (P = 0.001; Lod scores between 1.3 and 1.9).

At baseline, suggestive evidence was observed for BMI at 9q34 and 10p15, for BMI and Lep at 8q23-q24, for BMI and FM at 12p12 and 14q11, for Lep with low-density lipoprotein receptor (LDLR) gene, and for Lep-FM at 10q21-q22. At 8q23.3-q24.12, two neighboring markers, D8S556 and D8S592, showed suggestive linkage both with BMI (P = 0.001 and Lod = 2.0, respectively) and D8S592 with Lep (P = 0.001). Two different areas of chromosome 10 yielded evidence of linkage. At 10p15.3-p15.1, neighboring markers

Table 1. *Number of subjects and mean and range by generation and gender for each phenotypic variable at baseline*

	Parent						Adult Offspring					
	Male			Female			Male			Female		
Variable	n	Mean	Range	n	Mean	Range	n	Mean	Range	n	Mean	Range
Age, yr	99	53.5	44–64	95	52.0	42–65	164	25.2	17–40	172	25.4	17–41
BMI, kg/m^2	98	28.4	21–41	94	27.6	19–48	161	25.7	17–44	169	23.7	17–39
FFM, kg	92	62.3	47–80	85	44.5	35–57	150	64.2	45–87	169	46.1	34–65
FM, kg	92	24.6	8–58	85	27.0	10–62	150	17.0	<1–53	169	18.1	3–59
%Fat	92	27.6	11–42	85	36.6	20–54	150	19.6	<1–43	169	26.7	7–53
SF8, mm	88	113.5	44–273	82	159.8	70–291	156	92.5	31–208	168	118.9	40–261
Lep, ng/ml	97	9.0	2–30	88	24.0	5–70	155	5.8	1–30	165	15.2	2–82

n, No. of subjects; BMI, body mass index; FFM, fat-free mass; FM, fat mass; %Fat, percent body fat; SF8, sum of 8 skinfold thicknesses; Lep, plasma leptin levels.

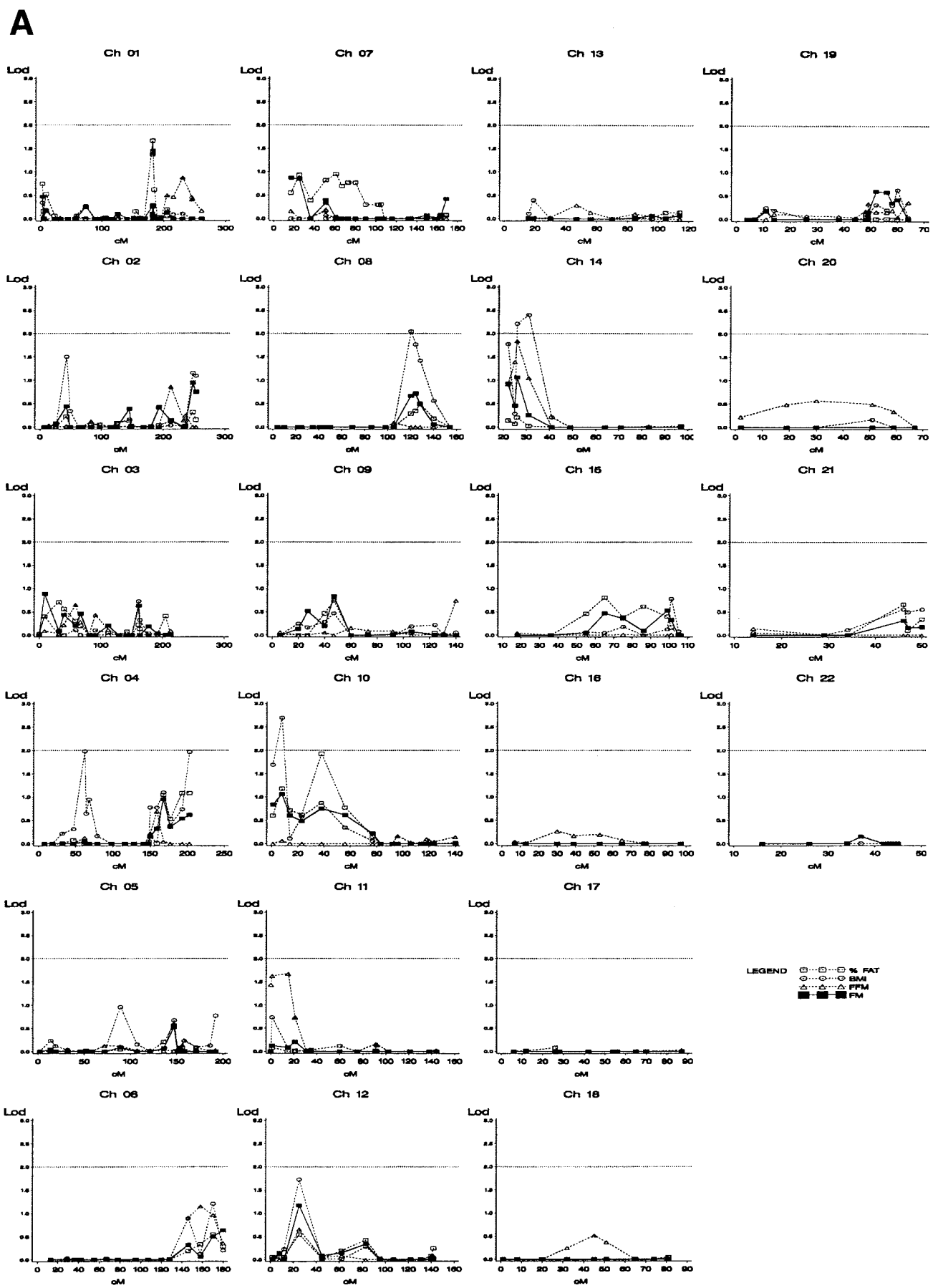

Fig. 1. Multipoint (MAPMAKER/SIBS) linkage results for body mass index (BMI), fat mass (FM), fat-free mass (FFM), and percent body fat (%Fat) in Caucasians from the HERITAGE Family Study at baseline (*A*) and for training changes (*B*). d, Changes.

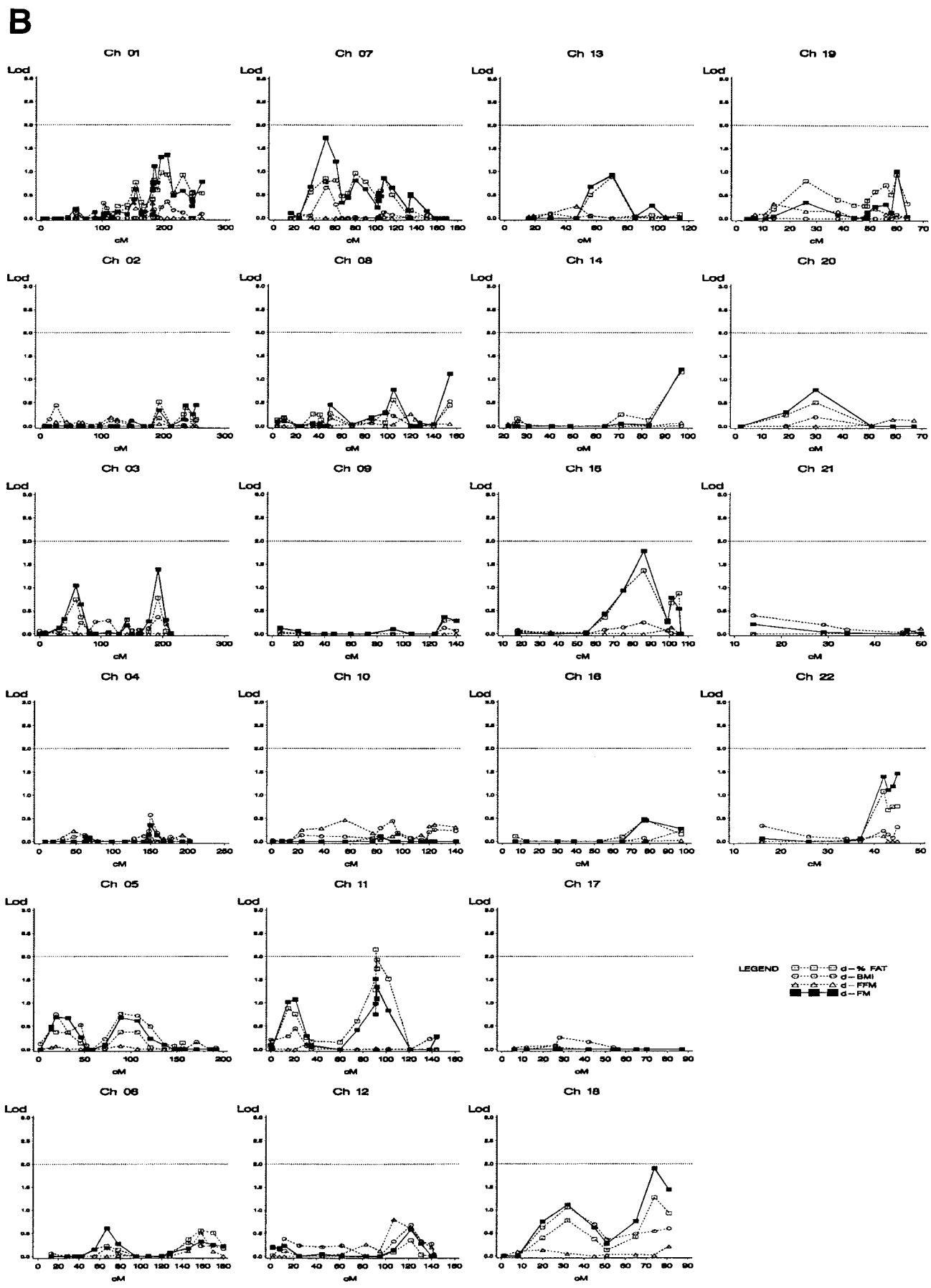

Fig. 1*B*—*Continued.*

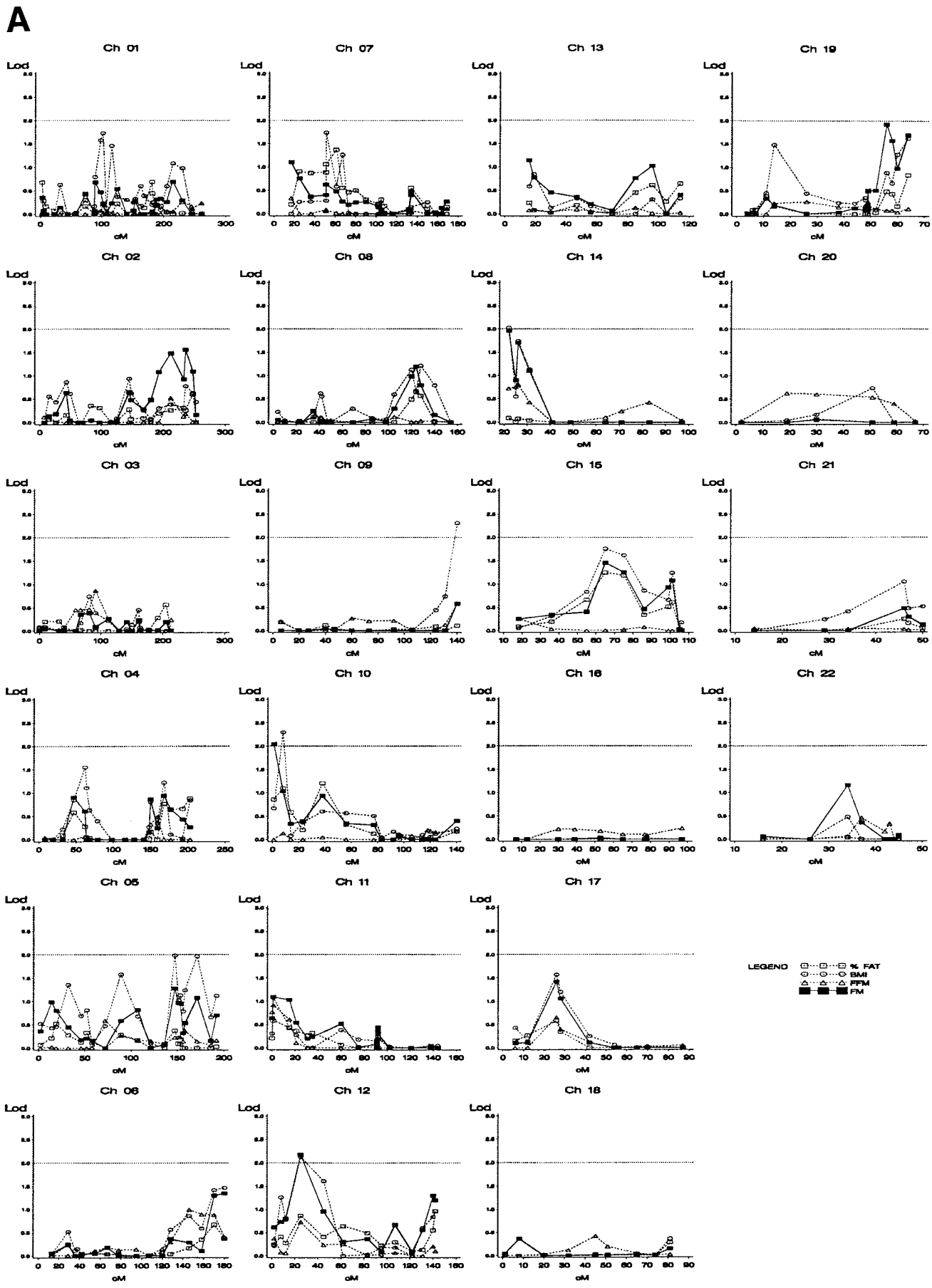

Fig. 2. Multipoint (SEGPATH) linkage results for BMI, FM, FFM, and %Fat in Caucasians from the HERITAGE Family Study at baseline (*A*) and for training changes (*B*).

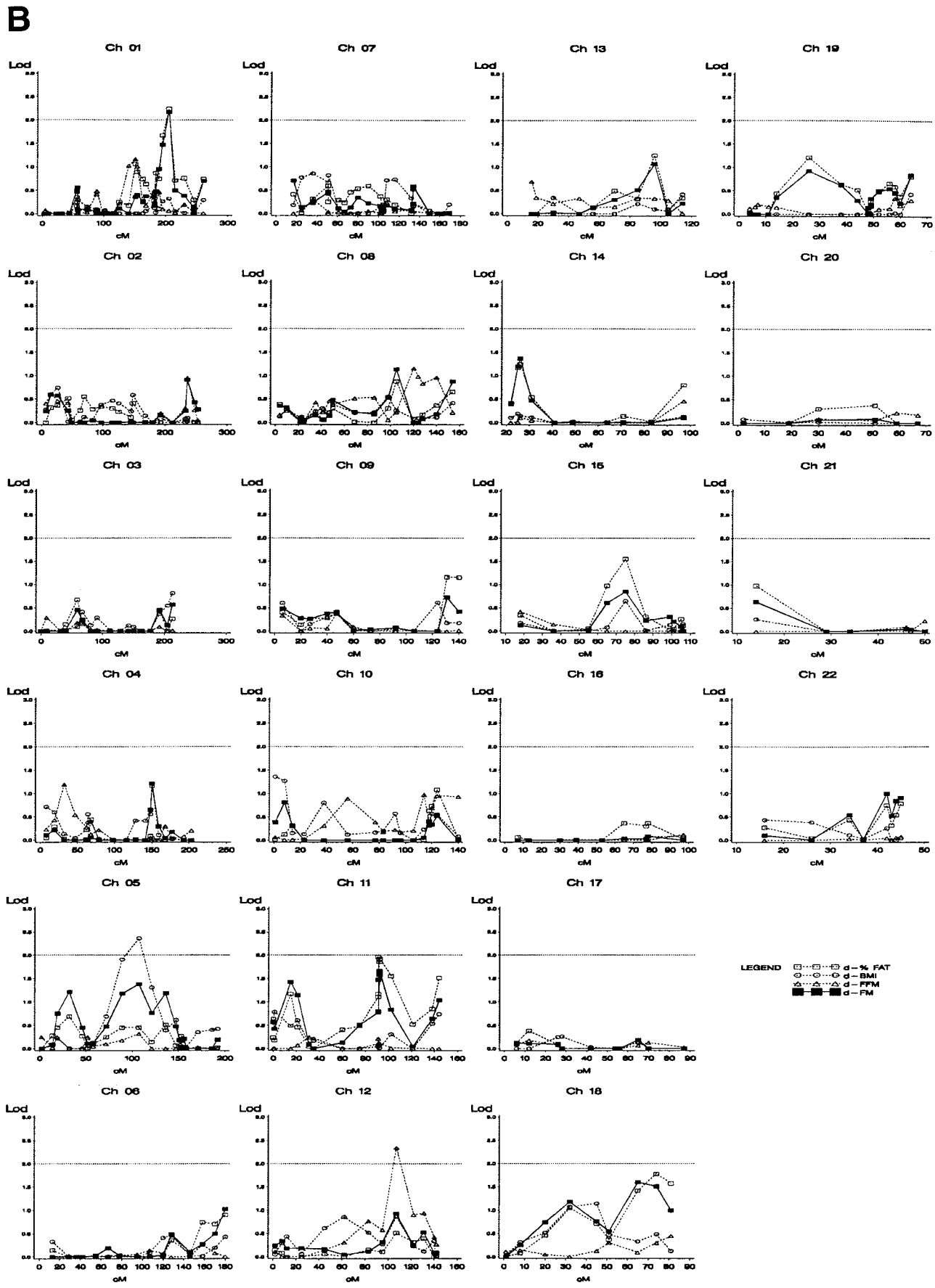

Fig. 2*B*—*Continued.*

Table 2. *Summary of linkages from single-point (SIBPAL) linkage analysis with body-composition phenotypes at baseline and for the training changes in Caucasians from the HERITAGE Family Study*

Chromosome Location	Marker	Position, cM	Phenotype (*P* values)	
			Baseline	Change
1q22	S100A	154	NS	FFM **(0.0001)**
1q24.1	ATPα_2	182	NS	%Fat (0.001)
1q31.1	D1S1660	205	NS	FM (0.0006), %Fat (0.0007)
5q14.1	D5S1725	89	BMI (0.006), SF8 (0.002)	BMI (0.0004)
8q24.12	D8S592	128	BMI (0.001), Lep (0.001), SF8 (0.02), FM (0.03), %Fat (0.02)	SF8 (0.04), FFM (0.02)
9q34.11	D9S282	131	NS	FM (0.001), %Fat (0.005), SF8 (0.04)
10q21-q22	GATA121A08	72	Lep-FM (0.001), Lep (0.03), FM (0.02), %Fat (0.03)	Lep-FM (0.05), Lep (0.04)
	D10S2327	83	Lep-FM (0.001)	NS
11q13.4-q21	UCP2	92	NS	%Fat (0.02)
	UCP3	92	FM (0.003), %Fat (0.004), Lep (0.01)	%Fat (0.009), FM (0.01)
	D11S2002	102	NS	%Fat (0.0009), FM (0.01), BMI (0.05)
12q23.2	IGF-I	107	NS	FFM **(0.0001)**
14q11	D14S283	22	FM (0.0006), BMI (0.002), Lep (0.003)	NS
18q21-q23	D18S38	65	NS	%Fat (0.02), FM (0.02)
	D18S878	74	NS	%Fat (0.001), FM (0.001)
	D18S1371	81	NS	%Fat (0.04), FM (0.02)
19p13.2	LDLR	11	Lep (0.0009), Lep-FM (0.04), SF8 (0.002), FM (0.01), %Fat (0.009)	Lep (0.02), Lep-FM (0.01)

Lep-FM, Lep adjusted for FM; UCP, uncoupling protein; IGF-I, insulin-like growth factor I; LDLR, low-density lipoprotein receptor. *P* values are in parentheses. Significant results ($P \leq 0.0001$) are in bold. Suggestive linkages ($0.001 \geq P > 0.0001$) are underlined. For markers with positive linkages, weaker results ($0.05 \geq P > 0.001$) are also shown. NS, not significant: $P > 0.05$.

D10S1435 and D10S189 showed suggestive linkages, respectively, with FM (Lod = 2.1) and BMI (Lod of 2.7 or 2.3 by the two methods). At 10q21-q22, markers GATA121A08 and D10S2327 provided evidence of linkage with Lep-FM ($P = 0.001$ for both). Finally, on chromosome 14q11-q11.2, three consecutive markers (D14S283, D14S742, and D14S1280) showed linkages with BMI ($2.2 \leq \text{Lod} \leq 2.4$), whereas D14S283 was also linked with FM ($P = 0.0006$, Lod = 2.0) and, to a lesser extent, with Lep ($P = 0.003$).

DISCUSSION

A genomewide search for quantitative trait loci influencing body composition before and in response to 20 wk of endurance training was completed in the HERITAGE Family Study. The strongest evidence of linkage, in terms of statistical significance reflected by Lod scores and *P* values, was with the training changes in FFM on chromosomes 1 and 12. On chromosome 12, positive linkage with IGF-I was observed, whereas on chromosome 1, changes in FFM were linked with a polymorphism in the S100A gene. Both results were supported by multipoint analysis (Lod scores of 2.3 and 1.0, respectively). For IGF-I, this confirmed the positive linkage reported previously using single-point analysis (33). The S100A gene encodes an acidic calcium-binding protein and is located at 1q21 within a cluster of nine calcium-binding protein coding genes (29). The S100A protein is mainly present in the slow-twitch muscle fiber of cardiac and skeletal muscles and, at a much lower level, in nervous tissue (14, 15). S100A was found to be more efficient than calmodulin in antagonizing DNA binding to MyoD, which belongs to a family of myogenic basic helix-loop-helix transcription factors that activates muscle-specific genes (2). Because FFM is composed, in large part, by muscle tissue, we can speculate that the linkage observed here between the S100A gene and the training changes in FFM might come from the S100A protein-antagonizing effects on MyoD by which numerous muscle-specific genes could be activated or inactivated after training.

Single-point and multipoint linkage with training changes in body fat was observed at 1q31 with the marker D1S1660. The genes encoding the two regulators of G-protein signaling 1 and 2 have their genes located at 1q31, <5 cM from D1S1660. On chromosome 5q14.1-q21.1, a region of 18 cM, defined by markers D5S1725 and D5S1462, was linked to training changes in BMI. The cocaine- and amphetamine-regulated transcript and the prohormone convertase 1 (PCSK1) are located in this region. The cocaine- and amphetamine-regulated transcript is a neuropeptide, expressed in the brain, that has an appetite-suppressing effect. PCSK1 is involved in the processing of numerous prehormones, like proinsulin, and one could speculate that regular exercise stimulates PCSK1.

No obvious candidate genes can be proposed for the linkages observed on chromosomes 8q and 10p with baseline values of BMI, FM, and Lep, and on 10q for Lep adjusted for body fat. These regions of chromosome 8 and 10 are not close to those previously reported to be linked to Lep or body fat (11, 13). On the other hand, chromosome 8 linkage was shown to be in a region where the human homologue of several quantitative trait loci related to control of body weight and body fat are predicted to be located (see Ref. 7). On chromosome 11, the two uncoupling protein (UCP2 and UCP3) genes are good candidates for the linkage observed.

Table 3. *Summary of linkages from multipoint linkage analyses with body-composition phenotypes at baseline and for the training changes in Caucasians from the HERITAGE Family Study*

Chromosome Location	Marker	Position, cM	Phenotype (Lod scores)	
			Baseline	Change
1q22	S100A	154	NS	FFM (1.0^{M})
1q31.1	D1S1660	205	NS	%Fat ($\underline{2.2}^{M}$), FM ($\underline{2.2}^{M}$)
5q21.1	D5S1462	107	NS	BMI ($\underline{2.4}^{M}$), FM (1.4^{M})
8q23.3	D8S556	120	BMI ($\underline{2.0}^{S}$)	NS
9q34.3	D9S158	140	BMI ($\underline{2.3}^{M}$)	%Fat (1.1^{M})
10p15.3-p15.1	D10S1435	1	BMI (1.7^{S}), FM ($\underline{2.1}^{M}$)	BMI (1.4^{M})
	D10S189	8	BMI ($\underline{2.7}^{S}$-$\underline{2.3}^{M}$), FM (1.1^{S}-1.0^{M}), %Fat (1.2^{S}-1.1^{M})	BMI (1.3^{M})
11q13.4-q21	UCP2	92	NS	%Fat ($\underline{2.2}^{S}$-$\underline{2.0}^{M}$), FM (1.5^{S}-1.5^{M})
	UCP3	92	NS	%Fat (1.9^{S}-1.9^{M}), FM (1.3^{S}-1.7^{M})
	D11S2002	102	NS	%Fat (1.6^{M}-1.5^{S})
12p12.1	D12S1042	25	BMI ($\underline{2.1}^{M}$-1.7^{S}), FM ($\underline{2.2}^{M}$-1.2^{S})	SF8 (1.3^{M})
12q23.2	IGF-I	107	NS	FFM ($\underline{2.3}^{M}$)
14q11-q11.2	D14S283	22	BMI ($\underline{2.0}^{M}$-1.8^{S}), FM ($\underline{2.0}^{M}$)	NS
	ANG	25	FFM (1.4^{S})	%Fat (1.2^{M}), FM (1.2^{M})
	D14S742	26	BMI ($\underline{2.2}^{S}$-1.7^{M}), FM (1.7^{M}-1.1^{S}), FFM (1.8^{S})	FM (1.4^{M})
	D14S1280	31	BMI ($\underline{2.4}^{S}$-1.1^{M}), FM (1.1^{M}), FFM (1.1^{S})	NS
18q21-q23	D18S38	65	NS	FM (1.6^{M}), %Fat (1.4^{M})
	D18S878	74	NS	FM (1.9^{S}-1.5^{M}), %Fat (1.8^{M}-1.3^{S})
	D18S1371	81	NS	FM (1.4^{S})

ANG, angiogenin. Suggestive results ($2.0 \leq$ Lod score < 3.0) are underlined. For markers with positive linkages, weaker results ($1.0 \leq$ Lod < 2.0) are also shown. NS: Lod < 1.0. Superscript S, variance component analysis (SEGPATH); superscript M, Mapmaker/Sibs analysis.

Positive correlation between UCP2 mRNA levels and BMI and %Fat in skeletal muscle (1) and white adipose tissue (21), as well as a negative correlation between BMI and UCP3 in skeletal muscle (31), has been reported. Moreover, it has been observed that exercise training lowered the level of UCP3 mRNA (30). These observations support the hypothesis that UCP2 and UCP3 might be related to changes in BMI and body fat content observed after the 20-wk endurance-exercise training program in the HERITAGE Family Study.

Evidence of linkage for BMI and body fat at baseline was also detected with three markers covering a region of 9 cM on chromosome 14. The angiogenin (ANG) gene encoding a protein involved in neovascularization of tissues is located in the linked region. However, only weak linkages (Lod scores < 1.5) were observed in HERITAGE for a RFLP located within ANG. Further association and functional studies are needed to clarify the possible effect of ANG on the regulation of human body composition. On chromosome 18, the observed linkages with ΔFM and Δ%Fat were 4 cM telomeric to the melanocortin 4 receptor (MC4R) gene (10). Two mutations in human MC4R have been found to be at the origin of massive obesity (34, 38), whereas our laboratory previously observed an association between MC4R polymorphism and FM and %Fat in female subjects from the Québec Family Study (6). An enhanced stimulation of MC4R produces a decrease in food intake and an increase in energy expenditure and body temperature. These observations are concordant with the observation that there was a mean body fat loss with training in the HERITAGE Family Study cohort.

Finally, evidence of linkage was observed between the LDLR gene on chromosome 19 and baseline and exercise training-induced changes in Lep and, to a lesser extent, with baseline adiposity phenotypes. LDLR is an important regulator of serum cholesterol that may have implications for the development of both hypertension and obesity. Significant associations of LDLR with obesity have been previously reported in two different normotensive populations (12, 27) with the same LDLR microsatellite marker used in the present study. The linkage observed here is more strongly related to leptin per se than to obesity phenotypes. Further studies of this LDLR polymorphism and its relationships with lipid variables and Lep are needed in the HERITAGE cohort.

In summary, we have observed significant linkages for a body-composition phenotype with changes in response to a 20-wk exercise training period and markers within S100A and IGF-I genes in the Caucasian families from the HERITAGE Family Study. Suggestive results were also observed on genomic regions from chromosomes 1, 5, 8, 10, 11, 14, 18, and 19. Candidate genes could be proposed for all chromosomes except 8 and 10. Linkages with training changes in lean body mass, body fat content, and BMI were generally different from those observed at baseline levels, suggesting that different metabolic pathways are contributing to human variation in body-composition alterations resulting from chronic exposure to exercise. This underlines the usefulness of intervention studies in which a metabolic stress, in this case exercise training, keeps uncovering new links between genes and phenotypes, which would otherwise go undetected in cross-sectional studies.

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REFERENCES

1. **Bao S, Kennedy A, Wojciechowski B, Wallace P, Ganaway E, and Garvey WT.** Expression of mRNAs encoding uncoupling proteins in human skeletal muscle: effects of obesity and diabetes. *Diabetes* 47: 1935–1940, 1998.
2. **Baudier J, Bergeret E, Bertacchi N, Weintraub H, Gagnon J, and Garin J.** Interactions of myogenic bHLH transcription factors with calcium-binding calmodulin and S100a (alpha alpha) proteins. *Biochemistry* 34: 7834–7846, 1995.
3. **Behnke AR and Wilmore JH.** *Evaluation and Regulation of Body Build and Composition*. Englewood Cliffs, NJ: Prentice-Hall, 1974.
4. **Bouchard C, Leon AS, Rao DC, Skinner JS, Wilmore JH, and Gagnon J.** The Heritage Family Study. Aims, design, and measurement protocol. *Med Sci Sports Exerc* 27: 721–729, 1995.
5. **Chagnon YC, Borecki IB, Perusse L, Roy S, Lacaille M, Chagnon M, Ho-Kim MA, Rice T, Province MA, Rao DC, and Bouchard C.** Genome-wide search for genes related to the fat-free body mass in the Quebec family study. *Metabolism* 49: 203–207, 2000.
6. **Chagnon YC, Chen WJ, Perusse L, Chagnon M, Nadeau A, Wilkison WO, and Bouchard C.** Linkage and association studies between the melanocortin receptors 4 and 5 genes and obesity-related phenotypes in the Quebec Family Study. *Mol Med* 3: 663–673, 1997.
7. **Chagnon YC, Perusse L, Weisnagel S, Rankinen T, and Bouchard C.** The human obesity gene map: the 1999 update. *Obes Res* 8: 89–117, 2000.
8. **Chagnon YC, Roy S, Chagnon M, Lacaille M, Leblanc C, and Bouchard C.** *High-throughput Genotyping Using Infrared Automatic LI-COR DNA Sequencers in the Study of the Obesity and Co-morbidities Genes.* Lincoln, NB: LiCor, 1998, p. 5.
9. **Cheverud J.** Detecting epistasis among quantitative trait loci. In: *Epistasis and the Evolutionary Process*, edited by Wolf J, Brodie E III, and Wade M. New York: Oxford University Press, 1999.
10. **Cody J, Reveles X, Hale D, Lehman D, Coon H, and Leach R.** Haplosufficiency of the melanocortin-4 receptor gene in individuals with deletions of 18q. *Hum Genet* 105: 424–427, 1999.
11. **Comuzzie AG, Hixson JE, Almasy L, Mitchell BD, Mahaney MC, Dyer TD, Stern MP, MacCluer JW, and Blangero J.** A major quantitative trait locus determining serum leptin levels and fat mass is located on human chromosome 2. *Nat Genet* 15: 273–276, 1997.
12. **Griffiths LR, Nyholt DR, Curtain RP, Gaffney PT, and Morris BJ.** Cross-sectional study of a microsatellite marker in the low density lipoprotein receptor gene in obese normotensives. *Clin Exp Pharmacol Physiol* 22: 496–498, 1995.
13. **Hager J, Dina C, Francke S, Dubois S, Houari M, Vatin V, Vaillant E, Lorentz N, Basdevant A, Clement K, Guy-Grand B, and Froguel P.** A genome-wide scan for human obesity genes reveals a major susceptibility locus on chromosome 10. *Nat Genet* 20: 304–308, 1998.
14. **Haimoto H and Kato K.** S100a0 (alpha alpha) protein in cardiac muscle. Isolation from human cardiac muscle and ultrastructural localization. *Eur J Biochem* 171: 409–415, 1988.
15. **Haimoto H and Kato K.** S100a0 (alpha alpha) protein, a calcium-binding protein, is localized in the slow-twitch muscle fiber. *J Neurochem* 48: 917–923, 1987.
16. **Hanson RL, Ehm MG, Pettitt DJ, Prochazka M, Thompson DB, Timberlake D, Foroud T, Kobes S, Baier L, Burns DK, Almasy L, Blangero J, Garvey WT, Bennett PH, and Knowler WC.** 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.
17. **Himes JH and Bouchard C.** Validity of anthropometry in classifying youths as obese. *Int J Obes* 13: 183–193, 1989.
18. **Kruglyak L and Lander ES.** Complete multipoint sib-pair analysis of qualitative and quantitative traits. *Am J Hum Genet* 57: 439–454, 1995.
19. **Lee JH, Reed DR, Li WD, Xu W, Joo EJ, Kilker RL, Nanthakumar E, North M, Sakul H, Bell C, and Price RA.** Genome scan for human obesity and linkage to markers in 20q13. *Am J Hum Genet* 64: 196–209, 1999.
20. **Lohman TG.** Skinfolds and body density and their relation to body fatness: a review. *Hum Biol* 53: 181–225, 1981.
21. **Millet L, Vidal H, Andreelli F, Larrouy D, Riou JP, Ricquier D, Laville M, and Langin D.** Increased uncoupling protein-2 and -3 mRNA expression during fasting in obese and lean humans. *J Clin Invest* 100: 2665–2670, 1997.
22. **National Institutes of Health.** *Clinical Guidelines on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults. National Institutes of Health: The Evidence Report.* Bethesda, MD: National Heart, Lung, and Blood Institute, 1998. (NIH Pub. No. 98–4083)
23. **Norman RA, Tataranni PA, Pratley R, Thompson DB, Hanson RL, Prochazka M, Baier L, Ehm MG, Sakul H, Foroud T, Garvey WT, Burns D, Knowler WC, Bennett PH, Bogardus C, and Ravussin E.** Autosomal genomic scan for loci linked to obesity and energy metabolism in Pima Indians. *Am J Hum Genet* 62: 659–668, 1998.
24. **Norman RA, Thompson DB, Foroud T, Garvey WT, Bennett PH, Bogardus C, and Ravussin E.** Genomewide search for genes influencing percent body fat in Pima Indians: suggestive linkage at chromosome 11q21-q22. Pima diabetes gene group. *Am J Hum Genet* 60: 166–173, 1997.
25. **Perusse L, Collier G, Gagnon J, Leon AS, Rao DC, Skinner JS, Wilmore JH, Nadeau A, Zimmet PZ, and Bouchard C.** Acute and chronic effects of exercise on leptin levels in humans. *J Appl Physiol* 83: 5–10, 1997.
26. **Province MA, Rice T, Borecki IB, Gu C, and Rao DC.** A multivariate and multilocus variance components approach using structural relationships to assess quantitative trait linkage via SEGPATH. *Genetic Epidemiol* in press.
27. **Rutherford S, Nyholt DR, Curtain RP, Quinlan SR, Gaffney PT, Morris BJ, and Griffiths LR.** Association of a low density lipoprotein receptor microsatellite variant with obesity. *Int J Obes Relat Metab Disord* 21: 1032–1037, 1997.
28. **SAGE.** *Statistical Analysis For Genetic Epidemiology, release 2.1.* New Orleans, LA: SAGE, 1992.
29. **Schafer BW, Wicki R, Engelkamp D, Mattei MG, and Heizmann CW.** Isolation of a YAC clone covering a cluster of nine S100 genes on human chromosome 1q21: rationale for a new nomenclature of the S100 calcium-binding protein family. *Genomics* 25: 638–643, 1995.
30. **Schrauwen P, Troost FJ, Xia J, Ravussin E, and Saris WH.** Skeletal muscle UCP2 and UCP3 expression in trained and untrained male subjects. *Int J Obes Relat Metab Disord* 23: 966–972, 1999.
31. **Schrauwen P, Xia J, Bogardus C, Pratley RE, and Ravussin E.** Skeletal muscle uncoupling protein 3 expression is a determinant of energy expenditure in Pima Indians. *Diabetes* 48: 146–149, 1999.
32. **Siri WE.** The gross composition of the body. In: *Advances in Biological and Medical Physics*, edited by Lawrence JH and Tobias CA. New York: Academic, 1956, p. 239–280.
33. **Sun G, Gagnon J, Chagnon Y, Perusse L, Despres J, Leon A, Wilmore J, Skinner J, Borecki I, Rao D, and Bouchard C.** Association and linkage between an insulin-like growth factor-1 gene polymorphism and fat free mass in the HERITAGE Family Study. *Int J Obes Relat Metab Disord* 23: 929–935, 1999.
34. **Vaisse C, Clement K, Guy-Grand B, and Froguel P.** A frameshift mutation in human MC4R is associated with a dominant form of obesity. *Nat Genet* 20: 113–114, 1998.

35. **Wilmore JH.** A simplified method for determination of residual lung volumes. *J Appl Physiol* 27: 96–100, 1969.
36. **Wilmore JH, Després J-P, Stanforth PR, Mandel S, Rice T, Gagnon J, Leon AS, Rao DC, Skinner JS, and Bouchard C.** Alterations in body weight and composition consequent to 20 wk of endurance training: the HERITAGE Family Study. *Am J Clin Nutr* 70: 346–352, 1997.
37. **Wilmore JH, Vodak PA, Parr RB, Girandola RN, and Billing JE.** Further simplification of a method for determination of residual lung volume. *Med Sci Sports Exerc* 12: 216–218, 1980.
38. **Yeo GS, Farooqi IS, Aminian S, Halsall DJ, Stanhope RG, and O'Rahilly S.** A frameshift mutation in MC4R associated with dominantly inherited human obesity. *Nat Genet* 20: 111–112, 1998.