A Genome-Wide Scan for Childhood Obesity–Associated Traits in French Families Shows Significant Linkage on Chromosome 6q22.31-q23.2

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We conducted a genome-wide search for childhood obesity–associated traits, including BMI ≥95th percentile (PCT95), 97th percentile (PCT97), and 99th percentile (PCT99) as well as age of adiposity rebound (AAR), which corresponds to the beginning of the second rise in childhood adiposity. A set of 431 microsatellite markers was genotyped in 506 subjects from 115 multiplex French Caucasian families, with at least one child with a BMI ≥95th percentile. Among these 115 pedigrees, 97 had at least two sibs with a BMI ≥95th percentile. Fine-mapping was performed in the seven most positive loci. Nonparametric multipoint analyses revealed six regions of significant or suggestive linkage on chromosomes 2q33.2-q36.3, 6q22.31-q23.2, and 17p13 for PCT95, PCT97, or PCT99 and 15q12-q15.1, 16q22.1-q24.1, and 19p13.3-p13.11 for AAR. The strongest evidence of linkage was detected on chromosome 6q22.31 for PCT97 (maximum likelihood score: 4.06) at the marker D6S287. This logarithm of odds score meets genome-wide significance tested through simulation (empirical genome-wide $P = 0.01$ [0.0027–0.0254]). Six independent genome scans in adults have reported quantitative trait loci on 6q linked to energy or glucose homeostasis-associated phenotypes. Possible candidate genes in this region include *SIM1*, *MCHR2*, and *PC-1*. *Diabetes* 53: 803–811, 2004

The available prevalence data show that not only is pediatric obesity dramatically increasing in most developed and developing countries, but there is also an increasing degree of adiposity in youth, particularly in older children and adolescents (1). Young-onset obesity is directly associated with early-onset type 2 diabetes (2) and an increase in the mortality risk of coronary heart disease once an adult (3). In childhood, several important periods of life giving higher risk for persistent obesity were pointed out: the prenatal period and early infancy, the time of adiposity rebound, and adolescence (4). BMI increases during the first year of life, after which it subsequently decreases and then begins to increase again at ~6 years of age. This second rise in childhood adiposity has been defined as the adiposity rebound (5). Six independent studies have confirmed that early adiposity rebound is associated with an increased BMI in adulthood (6,7). Early adiposity rebound could represent an indicator of a genetically determined propensity to deposit fat readily (8).

If the current epidemic of obesity clearly has its underpinnings in the changes in culture during the past half-century, the role of susceptibility genetic factors is likely to be stronger in the extreme phenotypes such as very-early-onset and/or severe forms of obesity. The rare monogenic forms of obesity were almost all identified in children or adolescents with massive obesity (9). In addition, evidence for strong inheritance of young-onset obesity has been established through family studies, investigating either parent-offspring fatness relationships (10), twins (11), or adopted children (12). These studies reported heritability estimates of 30–80% for common childhood obesity phenotypes. In adults, the risk of being overweight in relatives proportionally increases with the degree of obesity of the proband, suggesting that genetic influences are stronger in extreme phenotypes (13). Focusing on pedigrees with extreme forms of familial obesity with early age of onset can maximize our ability to detect genetic linkage by reducing the underlying genetic heterogeneity of the trait. In addition, parental genotypes are mostly available in pedigrees with obese children, therefore increasing the proportion of genetic information extracted from the dataset with substantial effects both on the power of our study to detect true linkage and on the significance of any linkage finding (14).

About 30 genome-wide searches for "obesity" genes have been published (15). Surprisingly, only three studies focused on extreme phenotypes. The group of R.A. Price reported a linkage with obesity in 10p13 and in 20q12-q13 from the analysis of families with extreme obesity (one proband with BMI ≥40 kg/m^2 and an additional obese sibling with BMI ≥30 kg/m^2) (16). Stone et al. (17), after the analysis of pedigrees containing at least three members with BMI ≥40 kg/m^2, revealed a major predisposition locus for severe obesity on chromosome 4p15-p14 and replicated the linkage with obesity in 20q12-q13. To our

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AAR, age of adiposity rebound; LOD, logarithm of odds; MLB, maximum likelihood binomial; MLS, maximum likelihood score.

TABLE 1
Family structures

Number of pedigrees	115
Pedigrees with one sib with BMI ≥95th percentile	18
Pedigrees with two sibs with BMI ≥95th percentile	74
Pedigrees with three sibs with BMI ≥95th percentile	21
Pedigrees with four sibs with BMI ≥95th percentile	2
Number of documented sibpairs	233
Number of sibpairs with BMI ≥95th percentile	149
Number of sibpairs with BMI ≥97th percentile	135
Number of sibpairs with BMI ≥99th percentile	109

knowledge, the only genome-wide search for adolescent obesity was performed in two sets of 89 and 76 German families, but no suggestive peak of linkage (logarithm of odds [LOD] >2.2) with obesity was observed (18). Here we present a genome-wide scan of childhood obesity and show evidence for linkage with obesity phenotypes or with the age of adiposity rebound at six chromosomal regions.

RESEARCH DESIGN AND METHODS

Our sample consisted of 97 Caucasian nuclear families collected through a multimedia campaign at the Institut Pasteur de Lille in France and in the Department of Pediatric Endocrinology of Jeanne de Flandres Hospital. Families with at least two minor probands with a BMI >95th percentile for age and sex before the age of 8 and two living parents were included in the study (Table 1). Moreover, 18 pedigrees with at least two minor sibs and only one proband with a BMI >95th percentile for age and sex were added for analysis of age of adiposity rebound. This pool of 115 families gave informed written consent, and family participants (sibs, parents, and grandparents) were then submitted to detailed personal and medical questionnaires and anthropometrical measurements. This study was approved by the Ethics Committee of the Paris Hôtel-Dieu Hospital. The main phenotypic characteristics of children by affection status are given in Table 2.

Phenotyping. Children with a BMI >97th percentile for age and sex in the tables of a French reference population (19) were defined as obese by the European Childhood Obesity Group (20). The European Childhood Obesity Group recommendations use the 90th percentile threshold to define overweight status. From these data, the 95th, 97th, and 99th percentiles reported in the tables by Rolland-Cachera were chosen in this study to define a range of the three worst levels of childhood adiposity. Height and weight were measured with an empty bladder and without a requirement for fasting. BMI was calculated as weight (kilograms) divided by height (meters) squared. BMI curves were individually established from data obtained from the child's health notebook when available. Age at which BMI exceeded the 97th percentile was defined as the age of obesity onset. Age of rebound was evaluated graphically as the age of upward inflection of BMI curve (5). Figure 1 shows the distribution of the age of adiposity rebound in children below and above the 95th percentile of BMI.

Genotyping. Genomic DNA was extracted from peripheral blood cells by PURE-GENE D50K DNA isolation kits (Gentra Systems). Genotyping was performed using a fluorescently labeled human linkage mapping set (PE-LMSV2) comprising 400 highly informative microsatellite markers, according

TABLE 2
Phenotypic characteristics by affection status

Phenotypes	Children with BMI ≤95th percentile	Children with BMI >95th percentile
n_{total}	47 (boys: 51.1%)	237 (boys: 51.1%)
Age (years)	11.91 ± 7.23 (47)	11.89 ± 6.05 (237)
BMI (kg/m^2)	18.30 ± 4.99 (47)	29.12 ± 13.56 (237)
Z score of BMI	0.379 ± 1.47 (47)	3.965 ± 2.57 (237)
Age of obesity onset (years)	—	4.05 ± 5.27 (219)
Age of rebound (years)	5.29 ± 2.86 (38)	2.49 ± 3.25 (207)

Data are means ± 95% CI (*n*).

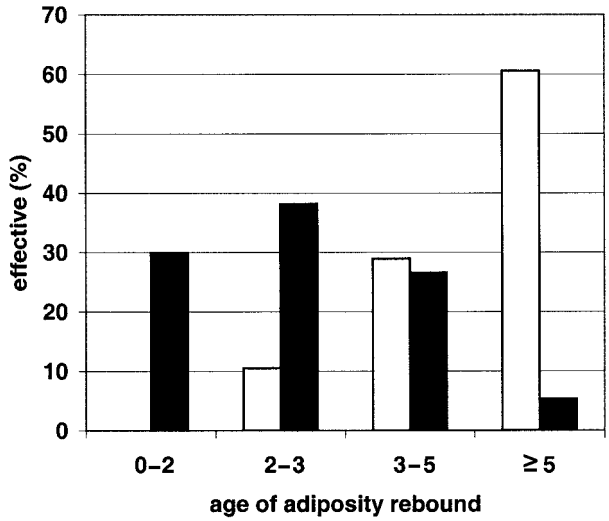

FIG. 1. Distribution of the age of adiposity rebound in children below (n = 38) or above (n = 207) the 95th percentile. The x-axis gives the age of adiposity rebound. The categories are as follows: 0 included to 2 excluded, 2 included to 3 included, 3 excluded to 5 included, and 5 excluded and more. The y-axis gives the effective in percent.

to a procedure detailed elsewhere (21). Among 400 markers in PE-LMSV2, two markers were not included because of technical problems. In addition to PE-LMSV2, 33 additional markers were genotyped. Therefore, a final total of 431 markers were genotyped with an average heterozygosity equal to 0.78. The average spacing between markers was 8.58 cM. After the first-stage genome search, seven regions were saturated with 87 additional markers to support and more accurately localize the linkage results. Six markers were discarded because of technical problems. We subsequently saturated the region of chromosomes 2q24-q36, 6q16.1-q24.1, 7q34-q36, 15q11-q22, 16q12-q24, 17p13, and 19p13.2-q12 with 15, 13, 7, 17, 13, 12, and 4 additional microsatellite markers, respectively. The average spacing between markers in the fine-mapped regions was 2.89 cM. We used Genethon data to build our maps. When markers were missing, we referred to Marshfield data to complete the maps. To detect mendelian inconsistencies, we ran the PED-CHECK 1.1 program (22). Five individuals were discarded from the analysis because at least 10% of the markers showed recurrent mendelian incompatibilities. Using the program MERLIN (23), we detected suspected double recombination for 0.31% of the genotypes (n = 826). These genotypes were removed. We checked departure from the Hardy-Weinberg equilibrium with the program HWE, and if necessary, markers were replaced or removed. Finally, we compared the position of a marker according to our data versus the one given by Genethon or Marshfield by using the program VITESSE. A marker showing a difference higher than the double was replaced or removed. Eight markers were removed after the position was checked.

Statistical analysis. Obesity is a complex disease with no clear model of inheritance; therefore, we used model-free methods to detect linkage. Three childhood obesity traits were analyzed. Children were considered affected in PCT95, PCT97, and PCT99 traits when their BMI exceeded thresholds of the 95th, 97th, and 99th percentiles, respectively, for age and sex of a French reference population (19). Only the concordant affected sibpairs from the pool of 97 obese multiplex families were taken into account for the PCT95, PCT97, and PCT99 obesity threshold analyses. We used this binary status because our ascertainment scheme resulted in reduced variability of the quantitative trait BMI in our sample compared with its variability in the general population. We analyzed the age of adiposity rebound (AAR) trait in the whole set of 115 families, taking into account both lean and obese children. We created four categories according to the distribution of data (Fig. 1). The number of categories and their probabilities were determined after visual inspection of the traits' distribution and prior epidemiological hypotheses. We used a very early age of rebound (<2 years) as the first category. We also included in this category obese individuals who failed to show a rebound. An absence of adiposity rebound or a rebound before the age of 2 years were never observed in our sample of children with a BMI <95th percentile. On the contrary, 95% of children without any rebound of adiposity or with a rebound before the age of 2 years harbored a BMI higher than the 99th percentile at the age of examination, suggesting that they both can be included in the same category of massively obese children with early onset of the disease. We put individuals whose rebound occurred between 2 and 3 years including these parameters in

TABLE 3
Pearson correlation coefficients between the AAR and the childhood obesity–associated traits PCT95, PCT97, and PCT99

	PCT95	PCT97	PCT99
PCT95	—	—	—
PCT97	0.89	—	—
PCT99	0.76	0.85	—
AAR	−0.54	−0.53	−0.53

All correlations are significant with a P value <0.001.

the second category, and we put those with rebounds between 3 and 5 years including the upper parameter in the third category. In the final fourth category, we gathered individuals with a rebound after the age of 5 years. Age of adiposity rebound could not be analyzed as a classic quantitative trait because a subgroup of obese children in our sample was characterized by the absence of an adiposity rebound. Furthermore, the advantage of the categorized trait is its robustness when the quantitative trait does not follow a normal distribution or is an ordinal score.

We used two methods to assess linkage for binary (affected status) traits. The first method, called maximum likelihood score (MLS) (24), calculated the identical by descent (IBD) status probabilities P(IBD = 0), P(IBD = 1), and P(IBD = 2) (z_0, z_1, and z_2, respectively) in affected pairs through maximum likelihood estimation. The obtained maximum log likelihood (L_{H1}) was compared with likelihood (L_{H0}) under null hypothesis of no linkage ($z_0 = 0.25$, $z_1 = 0.5$, and $z_2 = 0.25$). The difference followed a mixture of χ^2 with 1 and 2 degrees of freedom. The method was implemented in the Genehunter 2.1 package.

The maximum likelihood binomial (MLB) method (25), implemented in the MLBGH software, is based on the probability (α) that sibship members of the same phenotypic category (affected sibs, for example) had received the same allele from a heterozygous parent. In case of linkage, α is higher than 0.5. The log-likelihood difference followed a mixture of 0.5 χ^2 with 0 and 1 degree of freedom. The ASPEX program was used to test for linkage on chromosome X. MLB has been extended (26) to quantitative traits (Q). A latent binary variable ($y_i = 0.1$) is created and let $Y = \{y_1,y_2..y_n\}$ be the vector of ys for a sibship of size n. For each individual, this variable could take the value 0 or 1 with a probability dependent on the individual's value of Q [P(yi) = F (Qi)]. F can be user specified or can be the normal distribution. In our sibship, we had 2^n possible Y vectors, and the probability of each vector was the product of each individual probability. The use of categorized traits was of special interest because it was independent of distributional assumptions for the trait. The method implemented in MLS takes the sibpair as a unit, whereas the MLB method deals with sibships as a whole. Results may therefore slightly differ.

Because we conducted these multiple analyses for multiple markers, we completed a simulation study to estimate the genome-wide empirical P values. Given the familial structure that included sibships of unequal size and incomplete marker information, the use of simulation, rather than asymptotic theory, has been preferred to assess significance levels (27). Marker allele frequencies and map distances were kept as in the original sample and genotypes were dropped through the 115 families, with the program SIMULATE, under the hypothesis of no linkage between the disease and the markers. Our aim was to obtain the same information for replicates and the original set: the same allele frequencies and map distances and the same missing individuals for each marker and same phenotype for each individual. To estimate the genome-wide P values, we simulated 400 replicates for the 22 autosomes. The phenotypes were not simulated, and each individual was attributed his or her real set of phenotypes (PCT95, PCT97, PCT99, and AAR). The correlation between phenotypes was therefore preserved. We then conducted four analyses for each of the replicates for the three affected groups: 95th percentile, 97th percentile, and 99th percentile subsets and the categorized trait AAR. Thus, we completed a total of 1,600 genome-wide multipoint analyses (400 for each group), and for each replicate, we stored the maximum MLS reached (MLS_{max}) and analysis-specific maximum MLS (MLS-95, MLS-97, MLS-99, and LOD-AAR, respectively). This gave us two empirical genome-wide P values. The first one, P_{UNCORR}, was the probability that MLS_{max} exceeded an observed MLS for one trait. This P_{UNCORR} P value was therefore specific for each trait. The second one, P_{CORR}, was the probability that MLS_{max} exceeded MLS in the entire experiment: how many times the MLS_{max} > observed MLS out of 400. These P values account for multiple testing at all positions of the genome, and the latter one, P_{CORR}, also accounted for multiple testing of four correlated phenotypes according to the phenotype. This last P value can be considered the P value for the entire experiment.

RESULTS

The Pearson correlation coefficients between the traits PCT95, PCT97, PCT99, and AAR are showed in Table 3. Results of the MLS and MLB multipoint analysis of the initial genome scan for selected qualitative (PCT95, PCT97, and PCT99) and categorized traits (AAR) are summarized in Table 4. Only the regions with suggestive evidence for linkage (LOD MLS ≥2.32, LOD MLB ≥2.08, $P \leq 0.001$) are reported here. Figure 2 presents an overview of the linkage analysis results. Multipoint analyses in the initial genome scan revealed five regions with suggestive evidence of linkage on chromosomes 2q35, 6q23.3, 15q14, 16q23.1, and 19p13.12 (Table 4). No linkage to any binary trait was detected on chromosome X. Among these results, the 6q23.3 linkage is significant after controlling for multiple markers and phenotypes analyses (P_{CORR} = 0.01 [0.0027–0.0254]).

The five regions of interest (2q24-q36, 6q16.1-q24.1, 15q11-q22, 16q12-q24, and 19p13.2-q12) were chosen after the first stage mapping (Table 4) for partial saturation with additional markers. In addition, two other loci were fine-mapped: the locus 7q34–36, harboring an MLS value of 2.18 for PCT97, which is close to the selected threshold, and the locus 17p13, showing only a weak evidence of linkage for PCT95 and previously described as a quantitative trait loci for the metabolic syndrome (28). Results of the multipoint analyses for the high resolution genetic mapping at chromosomes 2, 6, 7, 15, 16, 17, and 19 are reported in Fig. 3 and Table 5. After fine-mapping, presence of suggestive linkage was observed at six loci (2q33.2-q36.3, 6q22.31-q23.2, 15q12-q15.1, 16q22.1-q24.1, 17p13, and 19p13.3-p13.11), whereas the saturation with

TABLE 4
Markers with evidence for linkage ($P \leq 0.001$) in the 10-cM genome scan

Positive markers	Interval*	Position† (cM)	Traits	MLB LOD (P)‡	MLS LOD (P)‡	P_{CORR}§	P_{UNCORR}§	1-LOD unit (CI) (cM)
D2S325	2q35	215.26	PCT99	2.77 (0.0002)	3.26 (0.0001)	0.16	0.08	20.83 (222.1–242.9)
D6S287	6q22.31	124.49	PCT97	3.61 (0.00002)	4.29 (0.000009)	0.01	0.01	17.03 (128.2–145.2)
D6S287	6q22.31	124.49	PCT95	3.08 (0.00008)	3.62 (0.00005)	0.08	0.06	17.03 (128.2–145.2)
D6S287	6q22.31	124.49	PCT99	2.07 (0.001)	2.33 (0.001)	0.45	0.37	29.00 (125.3–154.3)
D15S1007	15q14	26.78	AAR	2.11 (0.0009)	NA	0.73	0.34	17.83 (23.9–41.8)
D16S516	16q23.1	112.09	AAR	2.08 (0.001)	NA	0.74	0.35	31.43 (87.78–119.21)
D19S226	19p13.12	43.34	AAR	2.08 (0.001)	NA	0.74	0.35	23.58 (26.2–49.8)

*Chromosomes locations were determined from the map available in the Human Genome Browser. †Haldane's distances were used to determine the locations of linkage peaks. ‡P: single-test P value; §P_{CORR}: empirical genome-wide P value when considering all the traits; P_{UNCORR}: empirical genome-wide P value when considering one trait only. NA, not available. A Genethon map was used.

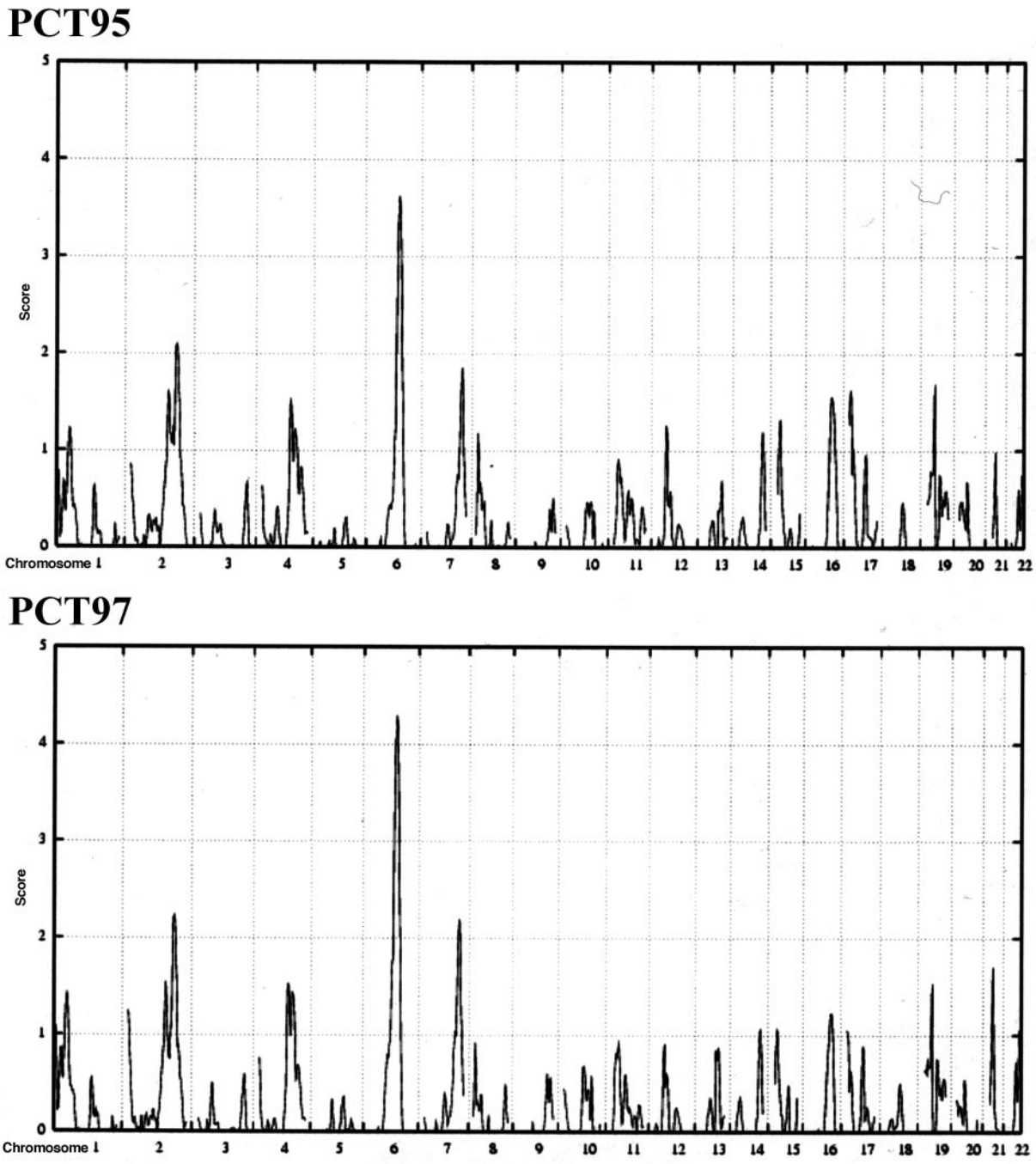

FIG. 2. Results of the multipoint analyses for PCT95, PCT97, PCT99, and AAR. The *x*-axis gives the chromosome. The *y*-axis gives the LOD score. MLS LOD scores are given for PCT95, PCT97, and PCT99, and MLB LOD scores are given for AAR.

additional markers at the locus 7q34-q36 shifted the LOD value from 2.18 to 1.66 for PCT97 (Fig. 3), under the threshold of suggestive linkage (MLS 2.32, $P = 0.001$). Increasing the number of markers led two linkage peaks to suggestive significance on chromosomes 2q33.2-q36.3 (PCT97) and 17p13 (PCT95), whereas one suggestive peak of linkage decreased under the threshold of suggestive linkage, for PCT99 on chromosome 6q16.1-q24.1 (Fig. 3). Saturation with additional markers led to an increase of the LOD score values in six peaks from eight among suggestive linkage peaks (Table 5). The saturation decreased the 1-LOD unit CI for six of the eight suggestive linkage peaks, and this decrease was of 38% in average (Table 5). On chromosome 6q22.31-q23.2, the increased density of microsatellite markers led to an LOD score MLS of 4.06 for linkage with PCT97. The saturation decreased the 1-LOD unit CI on chromosome 6q from 17.03 to 10.85 cM (Table 5). Strong evidence of linkage was supported on chromosome 2q33.2-q36.3 for PCT99. After fine-mapping, three loci harbored suggestive linkage for AAR on chromosomes 15q12-q15.1, 16q22.1-q24.1, and 19p13.3-p13.11, with simultaneous indication of linkage for PCT99 (Fig. 3).

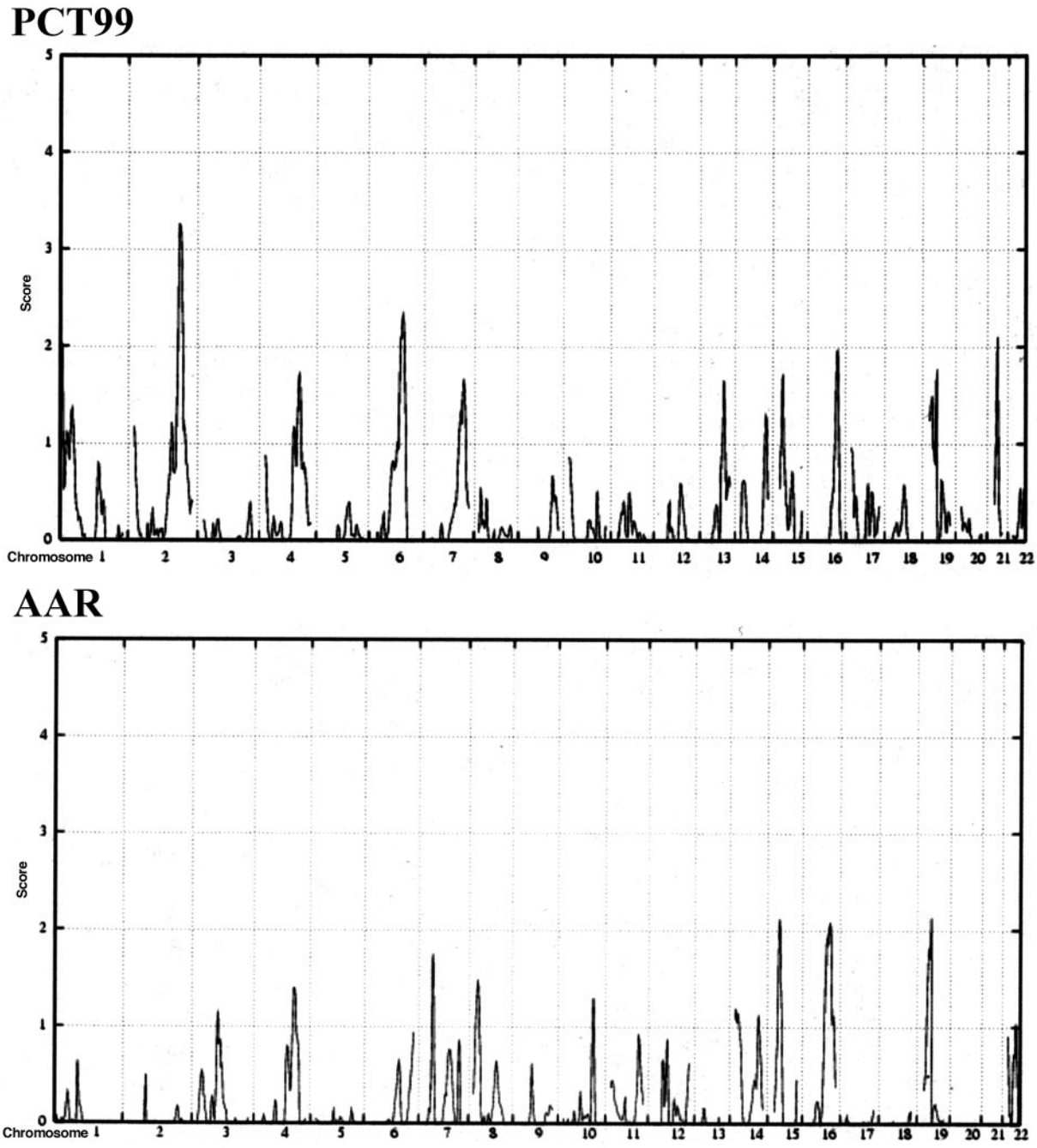

FIG. 2. Continued.

DISCUSSION

Our results show indication for linkage with childhood obesity and obesity-related traits on six chromosomes. The locus on 6q22.31-q23.2 showed the most significant evidence of linkage for PCT97 (Fig. 3), according to the criteria proposed by Lander and Kruglyak (29) and according to the empirical *P* values ($P = 0.01$). Another suggestive linkage peak (MLS 3.05) with massive obesity is located on chromosome 2q33.2-q36.3. In addition, three chromosomal regions on 15q12-q15.1, 16q22.1-q24.1, and 19p13.3-p13.11 contain quantitative trait loci modulating the age of the physiological adiposity rebound (Fig. 3). This trait was considered as a categorized trait rather than a quantitative one because it displayed clear cutoffs in the distribution. Moreover, there are individuals with no observable age of rebound who were not removed from the study but represented an additional category. Therefore, we could only use MLB method cat because other programs and especially those partitioning the variance were not well tailored for this case. Our study allows us to identify regions harboring genes with a heritability of 15%, with a power of 80%. Thus, we do not expect all these regions to harbor genes of obesity. The simulation conducted to determine empirical genome-wide *P* values also showed that an MLS LOD score >2 would occur two times [0.24–7.16] per genome scan on average. This clearly means that most of these loci need further replication and that the chromosome 6q, which reaches a satisfactory genome-wide empirical *P* value, is the major result of our study.

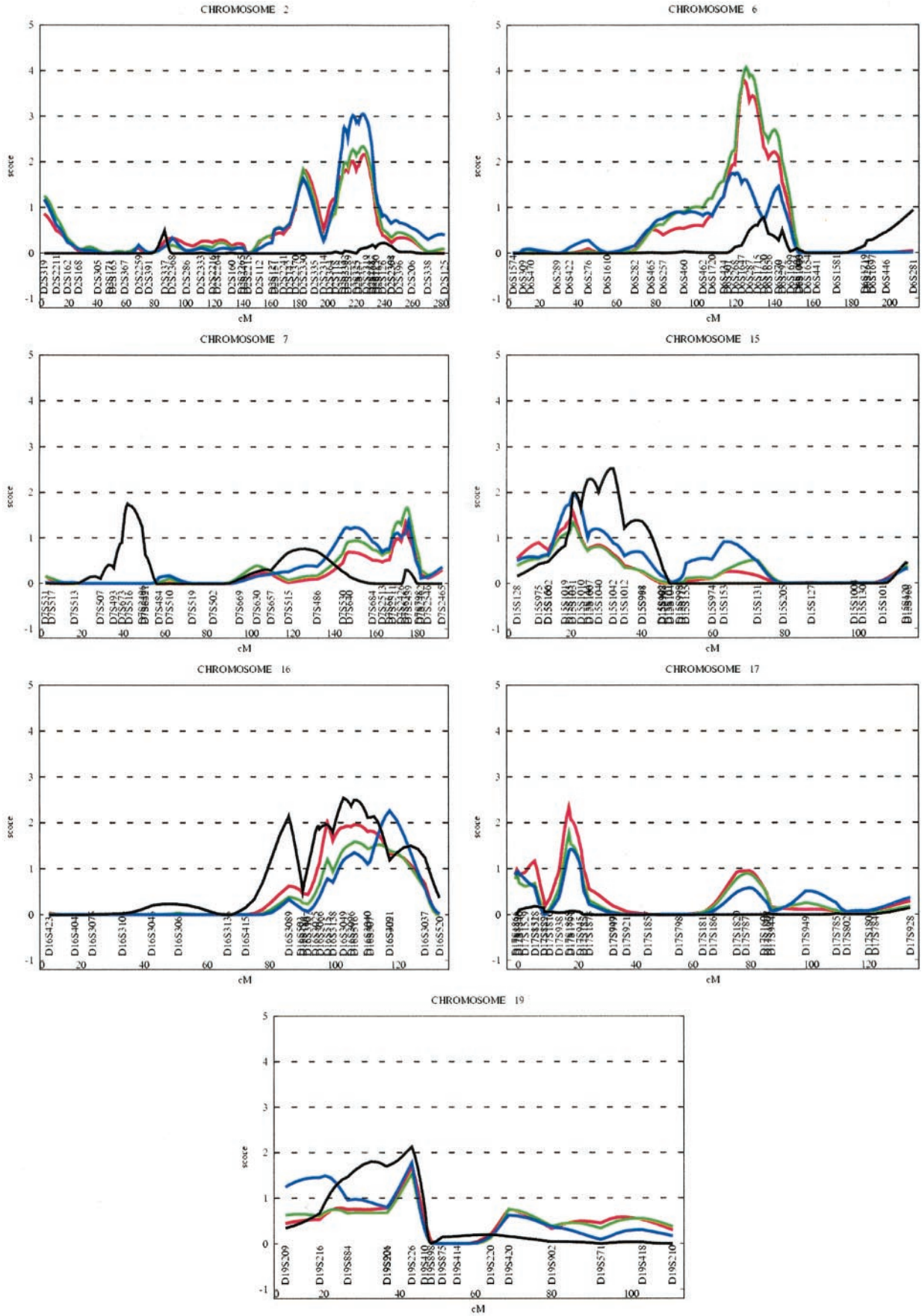

FIG. 3. Results of the MLS (PCT95, PCT97, and PCT99) and MLB (AAR) multipoint analyses after fine-mapping. The *y*-axis indicates the LOD score. Red, green, and blue curves correspond to PCT95, PCT97, and PCT99 traits, respectively. Black curves correspond to the AAR trait. A Genethon map was used.

TABLE 5
Multipoint results after the fine-mapping step

Chromosome	Fine-mapping interval (cM)	Average density	Trait	MLB LOD (*P*)*	MLS LOD (*P*)*	1-LOD unit (CI) (cM)	Location (cM)
2q33.2-q36.3	D2S112-D2S396	3.84	PCT99	2.73 (0.0002)	3.05 (0.0002)	22.34 (214.96–237.30)	230.62
			PCT97	2.08 (0.001)	2.33 (0.001)	24.46 (214.00–238.46)	230.62
6q22.31-q23.2	D6S462-D6S441	3.01	PCT97	3.27 (0.00005)	4.06 (0.00002)	10.85 (125.97–136.82)	129.76
			PCT95	3.13 (0.00007)	3.77 (0.00003)	11.53 (125.29–136.82)	128.95
15q12-q15.1	D15S128-D15S153	2.22	AAR	2.53 (0.0003)	NA	13.67 (21.71–35.38)	32.69
16q22.1-q24.1	D16S415-D16S520	3.13	AAR	2.54 (0.0003)	NA	21.68 (95.63–117.31)	105.15
17p13	D17S849-D17S799	1.96	PCT95	2.25 (0.0007)	2.35 (0.001)	6.50 (16.59–23.09)	18.70
19p13.3p13.11	D19S221-D19S414	2.58	AAR	2.13 (0.0009)	NA	22.62 (24.71–47.33)	44.60

*P: single-test *P* value. A Genethon map was used. Only the traits with suggestive evidence for linkage are reported here (LOD MLS ≥2.32, LOD MLB ≥2.08, $P \leq 0.001$).

A recent publication based on simulation data suggested that fine-mapping has little value to reduce the error in the estimated location of a locus linked to a quantitative trait with an LOD score >3 (30). In our study, the fine-mapping step enabled us to exclude one artifactual locus on 7q34-q36 and to further support two peaks with suggestive evidence for linkage that were under the corresponding threshold (LOD MLS ≥2.32, LOD MLB ≥2.08, $P = 0.001$) in the initial genome scan and to reduce the 1-LOD CI by 38% on average, in six suggestive peaks from 8. For instance, the saturation of markers on 6q16.1-q24.1 led to a decrease from 17.03 to 10.85 cM of the 1-LOD CI of our highest peak of linkage with childhood obesity (Table 5). Therefore, we believe that, at least for small sample–sized genome scans, fine-mapping is of interest.

Our results show significant evidence for linkage with childhood obesity (PCT97) on the locus 6q22.31-q23.2. Interestingly, a strong linkage with BMI (LOD score 4.64) was found on the chromosome 6q23–25 region from the study of 1,764 individuals of the Framingham Heart Study (31). A peak of linkage (LOD score = 2.2) for leptin concentrations was also reported at marker D6S1009 (141 cM on our map) in 579 nondiabetic Mexican Americans (32). The chromosome 6q16.1-q24.1 region shows additional evidence of linkage for insulin sensitivity, insulin secretion, and susceptibility to type 2 diabetes. In the same Mexican-American population (32), a strong linkage was reported with the variation of fasting insulin levels (LOD score 4.1) and with the insulin sensitivity index homeostasis model assessment-S (LOD score = 3.5) between markers D6S1009 and D6S1003. Abney et al. (33) observed a linkage in the same region (LOD score 2.0) for fasting insulin levels in the Caucasian Hutterite founder population. Ghosh et al. (34) found evidence for a type 2 diabetes susceptibility locus (LOD score 3.17) located in 6q16.3-q23.2, in a subset of 94 Finnish type 2 diabetic families with the lowest fasting glucose levels. In another subset of 74 families with the lowest age of type 2 diabetes onset, a type 2 diabetes susceptibility locus (LOD score 2.48) was detected on 6q22.31-q25 (34). Linkage with type 2 diabetes (LOD score 1.9) was also observed at the same locus in 229 African Americans (35). Evidence of linkage for type 2 diabetes was recently reported on chromosome 6q21-q23.3 (LOD score 5.08) from analysis of 257 Chinese pedigrees with a history of type 2 diabetes (36). There is much evidence to believe that the genetic bases of childhood obesity and glucose intolerance/type 2 diabetes could be partially redundant. Insulin has potent anorectic actions in the central nervous system (37). However, insulin also promotes in peripheral tissues the induction of genes involved in lipogenesis and the repression of those involved in lipolysis. In insulin-resistant states, it was suggested that hepatic lipogenesis and lipid storage are driven in excess, whereas insulin effects related to glucose homeostasis are impaired (38). Hence, insulin's antilipolytic effect is relatively preserved, resulting in maintenance or expansion of adipose stores. In Pima Indians, a population prone to obesity and type 2 diabetes, fasting hyperinsulinemia is a strong predictor for the development of obesity in young children (39). Consistent results were recently reported for a sample of Caucasian and African-American children, because a lower insulin sensitivity, a higher fasting insulin level, and a higher acute insulin response were significantly associated to higher increases in fat mass 3–6 years later (40). In addition, in a genetic study focusing on the variable number of tandem repeats (VNTR) of the *INS* gene, it was shown that French young obese patients homozygous for class I VNTR alleles secrete more insulin and gain more weight than those with other genotypes (41). Within the 6q confidence interval for linkage map are the single minded 1 (*SIM*1) and melanocortin concentrating hormone receptor 2 (*MCHR*2) and the plasma cell membrane glycoprotein (*PC-1*) genes, which are strong candidates for obesity. Mice homozygous for a null allele of *SIM*1 lack the paraventricular nucleus of the hypothalamus and die perinatally, and null heterozygous *SIM*1 mice are hyperphagic and develop early-onset obesity (42). In humans, deletion of *SIM*1 or disruption of the gene by translocation result in severe early-onset obesity (43,44). MCHR2 is a putative receptor for MCH, a peptide involved in the regulation of feeding and energy homeostasis. The expression of MCHR2 mRNA is restricted to several regions of the brain, including the arcuate nucleus and the ventral medial hypothalamus—areas implicated in regulation of body weight (45). MCHR2 shares 38% amino acid identity with MCHR1 (45). MCHR1-deficient mice are lean, hyperphagic, and resistant to diet-induced obesity (46). The plasma cell membrane glycoprotein *PC-1* gene, when overexpressed, has been shown to be an inhibitor of insulin receptor tyrosine kinase activity, and its levels are elevated in muscle, fat, and fibroblasts of subjects with insulin resistance (47,48). A polymorphism (K121Q) in exon 4 of the *PC-1* gene was

associated with insulin resistance in Caucasian subjects from Sicily, Sweden, and Finland (48,49).

Previous studies also found suggestive linkage for corpulence-associated traits on chromosomes 2q33.2-q36.3, 6q22.31-q23.2, 16q22.1-q24.1, 17p13, and 19p13.3-p13.11 in general or obese-enriched samples (15). Surprisingly, no linkage with morbid obesity in adults was reported in these regions, whereas this phenotype can be considered a logical continuation in adulthood of the early-onset obesity. However, this lack of replication may be due to the scarcity of genome-wide scans with morbidly obese sib-pairs. Alternatively, it may be possible that the molecular determinants of the severe forms of obesity in infancy (a phenotype that was very infrequent 20 years ago) are not the same that those associated with morbid obesity in adults.

An early adiposity rebound has been extensively related to higher BMI values or risk of obesity in adulthood (6,7). In this article, we show that individuals harboring a very early rebound (before age of 2 years) or an absence of BMI rebound systematically develop an overweight phenotype in childhood (Fig. 1). Indeed, a very early or absent rebound is present in 30% of children who become overweight, whereas no lean children harbor a rebound before the age of 2 years. The recognition of a very early age of adiposity rebound or the absence of BMI rebound by the examination of a BMI curve is a simple mechanism to identify an "at high risk of childhood obesity" subpopulation for clinicians.

In summary, this report provides significant evidence of a linkage for childhood obesity on chromosome 6q22.31-q23.2. Further work will be required to confirm our linkage chromosomal regions for childhood obesity in new independent samples and to test associations of positional candidate genes with occurrence of early-onset obesity.

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REFERENCES

1. Livingstone MB: Childhood obesity in Europe: a growing concern. *Public Health Nutr* 4:109–116, 2001
2. Fagot-Campagna A: Emergence of type 2 diabetes mellitus in children: epidemiological evidence. *J Pediatr Endocrinol Metab* 13:1395–1402, 2000
3. Maffeis C, Tato L: Long-term effects of childhood obesity on morbidity and mortality. *Horm Res* 55:42–45, 2001
4. Dietz WH, Gortmaker SL: Preventing obesity in children and adolescents. *Annu Rev Public Health* 22:337–353, 2001
5. Rolland-Cachera MF, Deheeger M, Bellisle F, Sempe M, Guilloud-Bataille M, Patois E: Adiposity rebound in children: a simple indicator for predicting obesity. *Am J Clin Nutr* 39:129–135, 1984
6. Dietz WH: "Adiposity rebound": reality or epiphenomenon? *Lancet* 356: 2027–2028, 2000
7. He Q, Karlberg J: Probability of adult overweight and risk change during the BMI rebound period. *Obes Res* 10:135–140, 2002
8. Poskitt EM: Obesity in the young child: whither and whence? *Acta Paediatr Scand Suppl* 323:24–32, 1986
9. O'Rahilly S, Farooqi IS, Yeo GS, Challis BG: Minireview: human obesity: lessons from monogenic disorders. *Endocrinology* 144:3757–3764, 2003
10. Whitaker RC, Wright JA, Pepe MS, Seidel KD, Dietz WH: Predicting obesity in young adulthood from childhood and parental obesity. *N Engl J Med* 337:869–873, 1997
11. Koeppen-Schomerus G, Wardle J, Plomin R: A genetic analysis of weight and overweight in 4-year-old twin pairs. *Int J Obes Relat Metab Disord* 25:838–844, 2001
12. Sorensen TI, Holst C, Stunkard AJ, Skovgaard LT: Correlations of body mass index of adult adoptees and their biological and adoptive relatives. *Int J Obes Relat Metab Disord* 16:227–236, 1992
13. Allison DB, Faith MS, Nathan JS: Risch's lambda values for human obesity. *Int J Obes Relat Metab Disord* 20:990–999, 1996
14. Wiltshire S, Cardon LR, McCarthy MI: Evaluating the results of genome-wide linkage scans of complex traits by locus counting. *Am J Hum Genet* 71:1175–1182, 2002
15. Chagnon YC, Rankinen T, Snyder EE, Weisnagel SJ, Perusse L, Bouchard C: The human obesity gene map: the 2002 update. *Obes Res* 11:313–367, 2003
16. Dong C, Wang S, Li WD, Li D, Zhao H, Price RA: Interacting genetic loci on chromosomes 20 and 10 influence extreme human obesity. *Am J Hum Genet* 72:115–124, 2003
17. Stone S, Abkevich V, Hunt SC, Gutin A, Russell DL, Neff CD, Riley R, Frech GC, Hensel CH, Jammulapati S, Potter J, Sexton D, Tran T, Gibbs D, Gress R, Bloomquist B, Amatruda J, Rae PM, Adams TD, Skolnick MH, Shattuck D: A major predisposition locus for severe obesity, at 4p15–p14. *Am J Hum Genet* 70:1459–1468, 2002
18. Saar K, Geller F, Ruschendorf F, Reis A, Friedel S, Schauble N, Nurnberg P, Siegfried W, Goldschmidt HP, Schafer H, Ziegler A, Remschmidt H, Hinney A, Hebebrand J: Genome scan for childhood and adolescent obesity in German families. *Pediatrics* 111:321–327, 2003
19. Rolland-Cachera MF, Cole TJ, Sempe M, Tichet J, Rossignol C, Charraud A: Body mass index variations: centiles from birth to 87 years. *Eur J Clin Nutr* 45:13–21, 1991
20. Poskitt EM: Defining childhood obesity: the relative body mass index (BMI): European Childhood Obesity group. *Acta Paediatr* 84:961–963, 1995
21. Mori Y, Otabe S, Dina C, Yasuda K, Populaire C, Lecoeur C, Vatin V, Durand E, Hara K, Okada T, Tobe K, Boutin P, Kadowaki T, Froguel P: Genome-wide search for type 2 diabetes in Japanese affected sib-pairs confirms susceptibility genes on 3q, 15q, and 20q and identifies two new candidate loci on 7p and 11p. *Diabetes* 51:1247–1255, 2002
22. O'Connell JR, Weeks DE: PedCheck: a program for identification of genotype incompatibilities in linkage analysis. *Am J Hum Genet* 63:259–266, 1998
23. Abecasis GR, Cherny SS, Cookson WO, Cardon LR: Merlin: rapid analysis of dense genetic maps using sparse gene flow trees. *Nat Genet* 30:97–101, 2002
24. Risch N: Linkage strategies for genetically complex traits. III. The effect of marker polymorphism on analysis of affected relative pairs. *Am J Hum Genet* 46:242–253, 1990
25. Abel L, Muller-Myhsok B: Robustness and power of the maximum-likelihood-binomial and maximum-likelihood-score methods, in multipoint linkage analysis of affected-sibship data. *Am J Hum Genet* 63:638–647, 1998
26. Alcais A, Abel L: Maximum-likelihood-binomial method for genetic model-free linkage analysis of quantitative traits in sibships. *Genet Epidemiol* 17:102–117, 1999
27. Holmans P: Likelihood-ratio affected sib-pair tests applied to multiply affected sibships: issues of power and type I error rate. *Genet Epidemiol* 20:44–56, 2001
28. Kissebah AH, Sonnenberg GE, Myklebust J, Goldstein M, Broman K, James RG, Marks JA, Krakower GR, Jacob HJ, Weber J, Martin L, Blangero J, Comuzzie AG: Quantitative trait loci on chromosomes 3 and 17 influence phenotypes of the metabolic syndrome. *Proc Natl Acad Sci U S A* 97: 14478–14483, 2000
29. Lander E, Kruglyak L: Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. *Nat Genet* 11:241–247, 1995
30. Atwood LD, Heard-Costa NL: Limits of fine-mapping a quantitative trait. *Genet Epidemiol* 24:99–106, 2003
31. Atwood LD, Heard-Costa NL, Cupples LA, Jaquish CE, Wilson PW, D'Agostino RB: Genomewide linkage analysis of body mass index across 28 years of the Framingham Heart Study. *Am J Hum Genet* 71:1044–1050, 2002
32. Duggirala R, Blangero J, Almasy L, Arya R, Dyer TD, Williams KL, Leach

RJ, O'Connell P, Stern MP: A major locus for fasting insulin concentrations and insulin resistance on chromosome 6q with strong pleiotropic effects on obesity-related phenotypes in nondiabetic Mexican Americans. *Am J Hum Genet* 68:1149–1164, 2001
33. Abney M, Ober C, McPeek MS: Quantitative-trait homozygosity and association mapping and empirical genomewide significance in large, complex pedigrees: fasting serum-insulin level in the Hutterites. *Am J Hum Genet* 70:920–934, 2002
34. Ghosh S, Watanabe RM, Valle TT, Hauser ER, Magnuson VL, Langefeld CD, Ally DS, Mohlke KL, Silander K, Kohtamaki K, Chines P, Balow J Jr, Birznieks G, Chang J, Eldridge W, Erdos MR, Karanjawala ZE, Knapp JI, Kudelko K, Martin C, Morales-Mena A, Musick A, Musick T, Pfahl C, Porter R, Rayman JB: The Finland-United States investigation of non-insulin-dependent diabetes mellitus genetics (FUSION) study. I. An autosomal genome scan for genes that predispose to type 2 diabetes. *Am J Hum Genet* 67:1174–1185, 2000
35. Ehm MG, Karnoub MC, Sakul H, Gottschalk K, Holt DC, Weber JL, Vaske D, Briley D, Briley L, Kopf J, McMillen P, Nguyen Q, Reisman M, Lai EH, Joslyn G, Shepherd NS, Bell C, Wagner MJ, Burns DK: Genomewide search for type 2 diabetes susceptibility genes in four American populations. *Am J Hum Genet* 66:1871–1881, 2000
36. Xiang K, Wang Y, Zheng T, Shen K, Jia W, Li J, Lin X, Wu S, Zhang G, Wang S, Lu H: Genome-wide scan search for type 2 diabetes susceptibility loci in Chinese. Poster presentation at the American Diabetes Association's 62nd Scientific Sessions, 14–18 June 2002, San Francisco, CA, 2002
37. Schwartz MW: Progress in the search for neuronal mechanisms coupling type 2 diabetes to obesity. *J Clin Invest* 108:963–964, 2001
38. Kahn BB, Flier JS: Obesity and insulin resistance. *J Clin Invest* 106:473–481, 2000
39. Odeleye OE, de Courten M, Pettitt DJ, Ravussin E: Fasting hyperinsulinemia is a predictor of increased body weight gain and obesity in Pima Indian children. *Diabetes* 46:1341–1345, 1997
40. Johnson MS, Figueroa-Colon R, Huang TT, Dwyer JH, Goran MI: Longitudinal changes in body fat in African American and Caucasian children: influence of fasting insulin and insulin sensitivity. *J Clin Endocrinol Metab* 86:3182–3187, 2001
41. Le Stunff C, Fallin D, Schork NJ, Bougneres P: The insulin gene VNTR is associated with fasting insulin levels and development of juvenile obesity. *Nat Genet* 26:444–446, 2000
42. Michaud JL, Boucher F, Melnyk A, Gauthier F, Goshu E, Levy E, Mitchell GA, Himms-Hagen J, Fan CM: Sim1 haploinsufficiency causes hyperphagia, obesity and reduction of the paraventricular nucleus of the hypothalamus. *Hum Mol Genet* 10:1465–1473, 2001
43. Holder JL, Butte NF, Zinn AR: Profound obesity associated with a balanced translocation that disrupts the SIM1 gene. *Hum Mol Genet* 9:101–108, 2000
44. Faivre L, Cormier-Daire V, Lapierre JM, Colleaux L, Jacquemont S, Genevieve D, Saunier P, Munnich A, Turleau C, Romana S, Prieur M, De Blois MC, Vekemans M: Deletion of the SIM1 gene (6q16.2) in a patient with a Prader-Willi-like phenotype. *J Med Genet* 39:594–596, 2002
45. Sailer AW, Sano H, Zeng Z, McDonald TP, Pan J, Pong SS, Feighner SD, Tan CP, Fukami T, Iwaasa H, Hreniuk DL, Morin NR, Sadowski SJ, Ito M, Bansal A, Ky B, Figueroa DJ, Jiang Q, Austin CP, MacNeil DJ, Ishihara A, Ihara M, Kanatani A, Van der Ploeg LH, Howard AD, Liu Q: Identification and characterization of a second melanin-concentrating hormone receptor, MCH-2R. *Proc Natl Acad Sci U S A* 98:7564–7569, 2001
46. Chen Y, Hu C, Hsu CK, Zhang Q, Bi C, Asnicar M, Hsiung HM, Fox N, Slieker LJ, Yang DD, Heiman ML, Shi Y: Targeted disruption of the melanin-concentrating hormone receptor-1 results in hyperphagia and resistance to diet-induced obesity. *Endocrinology* 143:2469–2477, 2002
47. Goldfine ID, Maddux BA, Youngren JF, Frittitta L, Trischitta V, Dohm GL: Membrane glycoprotein PC-1 and insulin resistance. *Mol Cell Biochem* 182:177–184, 1998
48. Pizzuti A, Frittitta L, Argiolas A, Baratta R, Goldfine ID, Bozzali M, Ercolino T, Scarlato G, Iacoviello L, Vigneri R, Tassi V, Trischitta V: A polymorphism (K121Q) of the human glycoprotein PC-1 gene coding region is strongly associated with insulin resistance. *Diabetes* 48:1881–1884, 1999
49. Gu HF, Almgren P, Lindholm E, Frittitta L, Pizzuti A, Trischitta V, Groop LC: Association between the human glycoprotein PC-1 gene and elevated glucose and insulin levels in a paired-sibling analysis. *Diabetes* 49:1601–1603, 2000