A Genome-Wide Scan for Abdominal Fat Assessed by Computed Tomography in the Québec Family Study

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To identify chromosomal regions harboring genes influencing the propensity to store fat in the abdominal area, a genome-wide scan for abdominal fat was performed in the Québec Family Study. Cross-sectional areas of the amount of abdominal total fat (ATF) and abdominal visceral fat (AVF) were assessed from a computed tomography scan taken at L4-L5 in 521 adult subjects. Abdominal subcutaneous fat (ASF) was obtained by computing the difference between ATF and AVF. The abdominal fat phenotypes were adjusted for age and sex effects as well as for total amount of body fat (kilogram of fat mass) measured by underwater weighing, and the adjusted phenotypes were used in linkage analyses. A total of 293 microsatellite markers spanning the 22 autosomal chromosomes were typed. The average intermarker distance was 11.9 cM. A maximum of 271 sib-pairs were available for single-point (SIBPAL) and 156 families for multipoint variance components (SEGPATH) linkage analyses. The strongest evidence of linkage was found on chromosome 12q24.3 between marker D12S2078 and ASF (logarithm of odds [LOD] = 2.88). Another marker (D12S1045) located within 2 cM of D12S2078 also provided evidence of sib-pair linkage with ASF ($P = 0.019$), ATF ($P = 0.015$), and AVF ($P = 0.0007$). Other regions with highly suggestive evidence ($P < 0.0023$ or LOD ≥1.75) of multipoint linkage and evidence ($P < 0.05$) of single-point linkage, all for ASF, included chromosomes 1p11.2, 4q32.1, 9q22.1, 12q22-q23, and 17q21.1. Three of these loci (1p11.2, 9q22.1, and 17q21.1) are close to genes involved in the regulation of sex steroid levels, whereas two others (4q32.1 and 17q21.1) are in the proximity of genes involved in the regulation of food intake. This first genome-wide scan for abdominal fat assessed by computed tomography indicates that there may be several loci determining the propensity to store fat in the abdominal depot and that some of these loci may influence the development of diabetes in obese subjects. *Diabetes* **50:614–621, 2001**

An elevated body fat content, as commonly seen in overweight or obese individuals, and particularly excess abdominal fat (1) are recognized as risk factors for type 2 diabetes and cardiovascular disease. The evidence suggesting that the amount of abdominal visceral fat is influenced by genetic factors has been recently reviewed (2). Two family studies have provided heritability estimates for abdominal total fat (ATF), abdominal subcutaneous fat (ASF), and abdominal visceral fat (AVF) areas measured by computed tomography (CT). In the first study based on 366 adult subjects from the Québec Family Study (QFS), age- and sex-adjusted heritability estimates of 70, 68, and 68% were obtained for ATF, ASF, and AVF, respectively (3). After adjustment for fat mass measured by hydrodensitometry, heritability estimates were slightly reduced for ASF (42%) and AVF (56%). For ATF, there were sex differences in the heritabilities with higher values in male (76%) than in female (69%) and cross-sex (57%) pairs. In the second study based on 483 subjects from the HERITAGE Family Study, heritability estimates of 47 and 48% were obtained for AVF before and after adjustment for fat mass, respectively (4). Segregation analyses of these two study samples have also provided tentative evidence for the role of a single gene with a major effect on AVF (5,6). The results of these family studies indicate that the amount of fat stored in the abdomen, independent of overall body fatness, is strongly influenced by genetic factors.

Despite evidence of a strong genetic component determining the amount of abdominal fat, very little is known about the nature of the genes involved. Only a few candidate genes, including the glucocorticoid receptor gene (7), the β_3-adrenergic receptor gene (8,9), and the fatty acid binding protein 2 gene (10), were found to be associated with abdominal fat. The identification of genes associated with complex phenotypes such as abdominal fat is limited when based on the candidate gene approach only. A genome-wide scan allows the identification of chromosomal regions that may harbor novel genes affecting a phenotype. Here we report the results of the first autosomal genomic scan for loci linked to abdominal fat phenotypes measured by a CT scan. Because our objective was to search for genes influencing the propensity to develop abdominal obesity, including visceral obesity, independently of total body

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ASF, abdominal subcutaneous fat; ATF, abdominal total fat; AVF, abdominal visceral fat; CRH, corticotropin-releasing hormone; CT, computed tomography; HSDB3, 3-β-hydroxy-steroid dehydrogenase; 1GF1, insulin-like growth-factor 1; LOD, logarithm of odds; NHLH2, nescient helix-loop-helix 2; NPY, neuropeptide Y; NPY2R, type 2 NPY receptor; PPARGC1, peroxisome proliferator–activated receptor-γ coactivator 1; PYY, peptide YY; QFS, Québec Family Study; QTL, quantitative trait locus.

TABLE 1
Characteristics of the subjects

Variable	Fathers	Mothers	Sons	Daughters
n	110	143	114	154
Age (yrs)	55.6 ± 6.7	52.6 ± 7.6	28.2 ± 8.5	29.0 ± 9.7
BMI (kg/m^2)	27.8 ± 4.7	27.6 ± 7.1	25.6 ± 5.1	26.7 ± 7.4
ATF (cm^2)	394.3 ± 169.3	464.6 ± 209.4	293.5 ± 201.5	393.7 ± 243.2
ASF (cm^2)	228.5 ± 107.5	338.1 ± 155.2	204.3 ± 151.7	320.1 ± 201.8
AVF (cm^2)	165.8 ± 83.0	126.5 ± 70.3	89.2 ± 60.2	73.6 ± 52.6

Data are *n* or means ± SD.

fatness, only the results adjusted for total amount of body fat are reported. It is essential to control for the total level of fatness in the present situation because the covariation between fat mass and the different abdominal fat phenotypes ranges from 30 to 50% (3,4,11).

RESEARCH DESIGN AND METHODS

Sample. A total of 521 adult (≥17.5 years) subjects from the QFS participated in the study. The QFS is a prospective family study on the genetics of obesity and its comorbidities and has been described in detail elsewhere (12). The sample available for this study included 224 men and 297 women from 156 Caucasian families with data on abdominal fat measured by CT. Half of these families were randomly ascertained with regard to obesity, whereas in the other half, one or more members of the family were required to have a BMI ≥32.0 kg/m^2. The characteristics of these subjects are presented in Table 1. A total of 25 subjects with type 2 diabetes (4.8%), defined as subjects with fasting glucose levels ≥7 mmol/l or glycemia 2 h after an oral glucose tolerance test ≥11.1 mmol/l, were present in this sample.

Phenotypes. Abdominal adipose tissue areas were assessed by CT using a Siemens Somatom DRH scanner as previously described (13). An abdominal scan was obtained between the fourth and fifth lumbar vertebrae (L4-L5) while subjects were in a supine position with arms stretched above the head. Total and visceral adipose tissue areas were delineated. The AVF area was determined by drawing a line within the muscle wall surrounding the abdominal cavity, whereas the ASF area was obtained by obtaining the difference between the ATF and AVF areas. Body fatness was determined from body density measurements obtained by underwater weighing (14). The total amount of body fat (fat mass in kilograms) was used as a covariate in the adjustment procedure to take into account the effects of fat mass on abdominal fat areas.

Genotypes. Genomic DNA was prepared from permanent lymphoblastoid cells by the proteinase K and phenol/chloroform technique. Details on DNA preparation, polymerase chain reaction conditions, and genotyping are described in detail elsewhere (15,16). A total of 293 microsatellite markers selected from different sources, but mainly from the Marshfield panel version 8a, were available for this genome scan. Map locations (Kosambi distance in centimorgans) were taken from version nine of the Marshfield Institute map (http://www.marshmed.org/genetics) and the Location Database map (http://cedar.genetics.soton.ac.uk/public_html). The marker density was highest for chromosome 20, with an average intermarker distance of 4.8 cM, and lowest for chromosome 21, with an average intermarker distance of 16.7 cM. Over all autosomes, the average intermarker distance was 11.9 cM (range 0–41). Genotypes for each marker were typed using automatic DNA sequencers from LICOR and the computer software SAGA (Rick McIndoe, Roger Bumgarner, Russ Welti, University of Washington at Seattle; LI-COR, Lincoln, NE). The genotypes of each maker were exported in a local dBase IV database (GENEMARK). Inspection for Mendelian inheritance incompatibilities in nuclear families and extended pedigrees was performed within GENEMARK. Subjects with Mendelian incompatibilities were identified and retyped completely, i.e., from the polymerase chain reaction to the genotyping. Less than 10% of the original genotyping had to be retyped.

Statistical analysis. Descriptive statistical analyses and adjustment of the phenotypes were performed using SAS (version 6.08). The abdominal fat phenotypes were adjusted for the effects of age (up to a cubic polynomial), sex, and fat mass using stepwise regression procedures performed separately in six age- (<30, 30–50, and ≥50 years) by-sex (males vs. females) groups. For estimation of regression parameters, individuals with phenotypic values ±3 SD from the mean, within each age-by-sex group, were identified and temporarily set aside. These outliers were added back to the sample for computation of residual scores. The residuals were standardized to a mean of zero and a SD of one and used in linkage analyses. The distribution of the three residualized and standardized abdominal fat phenotypes used for linkage analyses is presented in Fig. 1.

Nonparametric single-point linkage analysis was performed using the SIBPAL linkage procedure implemented in the Statistical Analysis for Genetic Epidemiology (S.A.G.E. version 3.1) package (17). The maximum number of sib-pairs available was 271. Multipoint linkage analysis was also performed using a variance component method implemented in SEGPATH (18). The phenotypic correlations between relatives in a pedigree is assumed to result from the additive effects of genetic variation at the trait locus (g), genetic variance due to a residual pseudo-polygenic background (G_R), and a residual component (r). The effects of the trait locus and the pseudo-polygenic components on the phenotypes represent the trait locus (h^2g) and residual (h^2r) heritabilities, respectively. In addition to these heritabilities, the spouse resemblance (u) and the sibling resemblance not accounted for by genetic factors (b) are also considered in the model. The proportion of marker alleles shared identical by descent (IBD) was estimated at each marker locus but was conditional on all the marker data available in a given chromosome, using MAPMAKER/SIBS (19). Thus, in the present analysis, the search for linkage was not performed over the whole chromosome but only at marker loci. The allele-sharing probabilities data file was then used as input in SEGPATH.

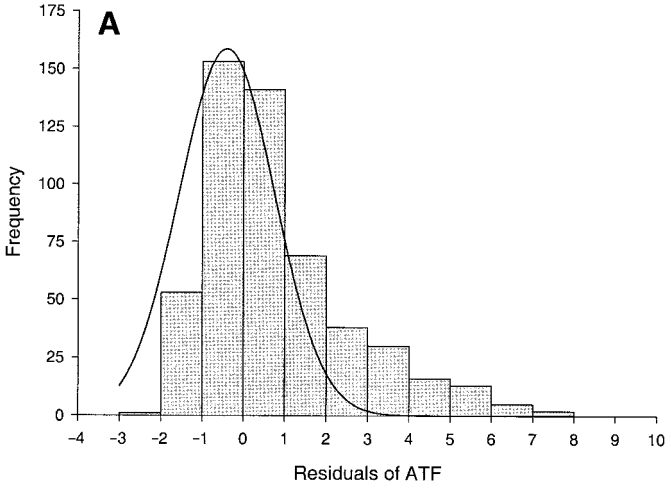
In this variance component linkage model, linkage is tested by contrasting the null hypothesis [$h^2g = 0$ (no linkage)] with the alternative hypothesis, in which h^2g is estimated. The difference in minus twice the log likelihood (-2 ln L) provides the likelihood ratio test, which is asymptotically distributed as a 50:50 mixture of χ^2 with one df and a point mass at zero (20). The logarithm of odds (LOD) score was computed as $\chi^2/(2 \times \log_e 10)$.

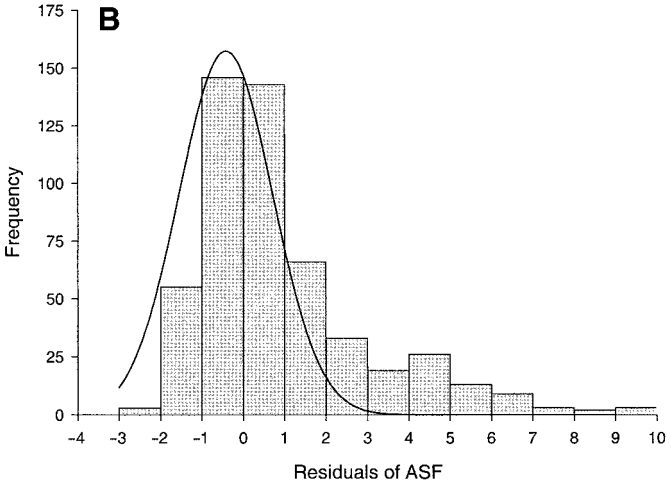
RESULTS

The mean age and unadjusted abdominal fat phenotypes by sex and by generation groups are presented in Table 1. The percentage of variance accounted for by the covariates (age and fat mass) in the six age-by-sex groups ranged from 68 to 91% for ATF, 71 to 80% for ASF, and 42 to 82% for AVF.

An overview of the multipoint linkage results for the three abdominal fat phenotypes is presented in Fig. 2. Figure 3 presents the maximum LOD scores of each phenotype by chromosome. Except for chromosomes 19 and 21, the strongest linkage signals were always observed for ASF. A total of seven chromosomes provided highly suggestive evidence of multipoint linkage ($P < 0.0023$ or LOD ≥1.75, corresponding to the horizontal line in Fig. 3). A summary of single-point and multipoint linkage results is presented in Table 2. Highly suggestive ($P < 0.0023$; LOD ≥ 1.75) and suggestive ($P < 0.01$; LOD ≥ 1.18) results are shown in bold. The remaining entries in the table are for comparison of *P* values across linkage methods.

The region with the strongest evidence of linkage was on chromosome 12q24.3 between ASF and marker D12S2078 located at 139.6 cM (LOD 2.88). Another marker (D12S1045) located within 2 cM of D12S2078 also provided evidence of linkage ($P = 0.0007$) with AVF. The other regions with highly suggestive evidence of multipoint linkage (all for ASF) included chromosomes 1p11.2





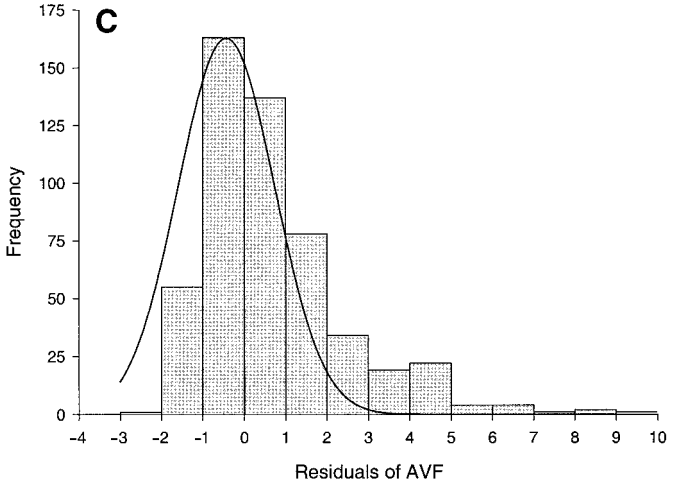


FIG. 1. Distribution of the residualized and standardized abdominal fat phenotypes submitted to linkage analyses. *A*: ATF; *B*: ASF; *C*: AVF.

(D1S534, LOD 2.31), 4p15.1 (D4S2937, LOD 2.30), 4q32.1 (D4S2417, LOD 1.76), 7q31.3 (D7S1875, LOD 1.97), 9q22.1 (markers D9S1122 and D9S257 about 4 cM apart, LOD 2.37 and 2.14), 12q22-q23 (IGF1, LOD 1.89), 13q34 (D13S285, LOD 1.92), and 17q21.1-q21.3 (a region of about 13 cM between markers D17S2180 [LOD 2.24] and D17S1301 [LOD 2.21]). Six of these regions—1p11.2, 4q32.1, 9q22.1, 12q22-q23, 12q24.3, and 17q21.1—also provided evidence ($P < 0.05$) of single-point linkage.

DISCUSSION

Our objective in the present study was to identify, through a genome-wide linkage analysis, quantitative trait loci (QTLs) affecting the amount of fat stored in the abdominal area, independent of total body fatness. Two points need to be consider in the interpretation of the results of this study. First, the three abdominal phenotypes submitted to linkage analyses were adjusted for fat mass and could therefore be considered as indicators of the propensity to develop abdominal obesity independent of total body fatness. This is clearly reflected by the very low common variance between the residualized phenotypes and percentage of body fat, which ranged from 0.5% for AVF to 2% for ASF. The correlations among the residualized phenotypes were 0.67 (ATF vs. ASF), 0.49 (ATF vs. AVF), and −0.19 (ASF vs. AVF), which also indicates that they differ from each other. The low negative correlation observed between ASF and AVF suggests that the propensity to accumulate fat in the visceral fat depot is probably not regulated by the same biologic mechanisms as those associated with the accumulation of ASF and that different genes might be involved in the determination of each phenotype.

Second, to identify chromosomal regions likely to harbor genes affecting abdominal fat without missing genes with moderate effects that could have a real impact on the determination of abdominal fat and on the development of diabetes, we adopted more liberal criteria than those proposed by Lander and Kruglyak (21) to identify regions with positive results. Thus, following the recommendations of Rao and Province (22), we considered a P value <0.0023 as "highly suggestive" evidence of linkage and a P value <0.01 as "suggestive" evidence of linkage. These P values correspond to LOD scores of 1.75 and 1.18, respectively. We think that these more liberal criteria maximize the detection of genomic regions to be considered for further investigation, while allowing true negative results to be reported. Based on these recommendations (22), the results of the present study have led to the identification of nine QTLs affecting ASF. Table 3 presents the list of these QTLs with the markers providing the evidence of linkage and the candidate genes potentially involved.

The strongest evidence of multipoint linkage (LOD 2.88; $P = 0.0001$) was found on chromosome 12q24.3 between the marker D12S2078 and ASF. Another marker (D12S1045) located within 2 cM of D12S2078 also provided evidence of multipoint linkage with ASF (LOD 1.51; $P = 0.004$). The same marker (D12S1045) also provided evidence of single-point linkage (Table 2) with ASF ($P = 0.019$), AVF ($P = 0.0007$), and ATF ($P = 0.015$). Those two markers are located within 10 cM of the NIDDM2 locus identified on 12q24.3 in a genome scan of Finnish families (23). The NIDDM2 locus was reported in families with adult-onset diabetes (mean age of onset 55 years) and low insulin secretion after an oral glucose tolerance test, suggesting that the gene was responsible for a defect in insulin secretion. This locus happens to be in the region of the gene encoding hepatic nuclear factor-1α (HNF1A), a gene that has been shown to be responsible for a form of

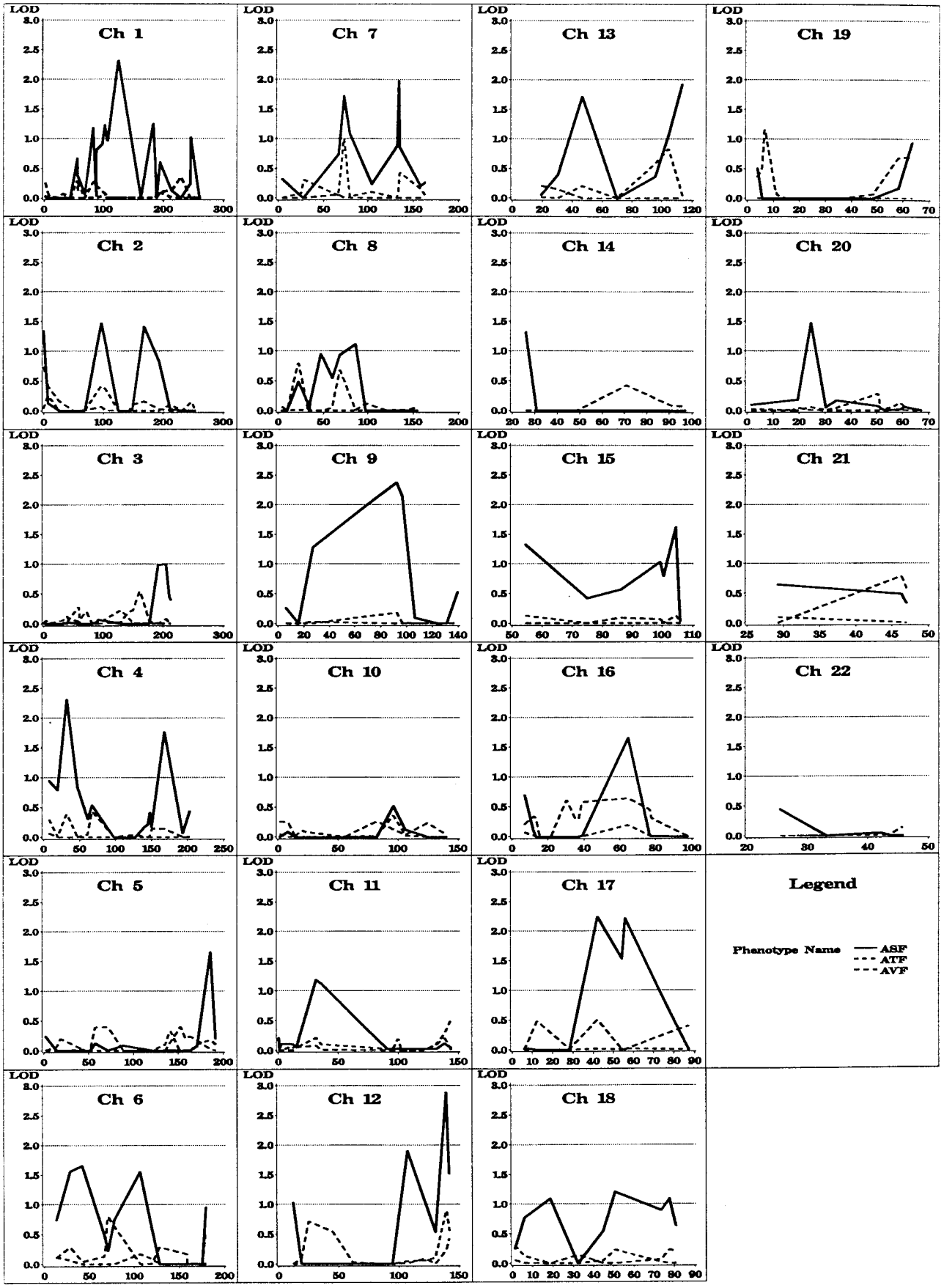


FIG. 2. Multipoint linkage results for all chromosomes (Ch) for abdominal fat phenotypes. LOD scores are presented on the *y*-axis, whereas genetic distance in centimorgans is presented on the *x*-axis.

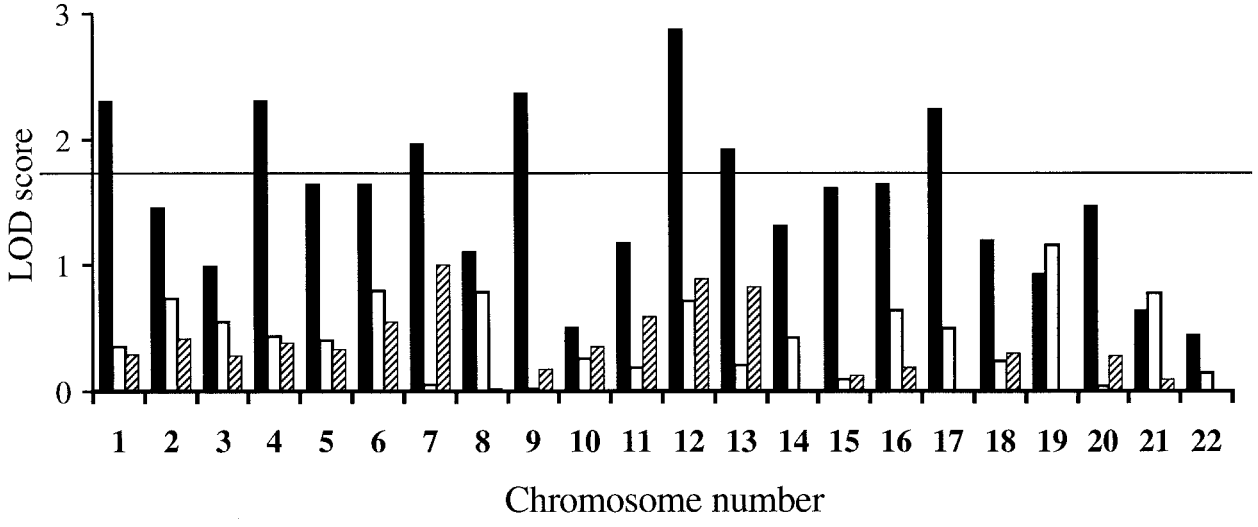


FIG. 3. Maximum LOD scores derived from multipoint linkage analyses for the abdominal fat phenotypes by chromosome. The horizontal line represents a LOD score of 1.75, which corresponds to a *P* value of 0.0023. ■, ASF; □, AVF; ▨, ATF.

maturity-onset diabetes of the young (MODY3) characterized by a severe insulin secretory defect (24–26). Suggestive evidence of multipoint (LOD 1.89) and single-point ($P = 0.005$) linkage was also found between the insulin-like growth factor 1 (IGF1) gene on 12q22-q23 and ASF. Recently, a polymorphism in the IGF1 gene was found to be associated with the amount of abdominal visceral fat as well as with the changes in body composition in response to endurance training (27).

On chromosome 1p11.2, evidence of multipoint (LOD 2.31; $P = 0.0006$) and single-point ($P = 0.003$) linkage was observed between ASF and marker D1S534. One potential candidate gene for this linkage is the nescient helix-loop-helix 2 (NHLH2) gene. The helix-loop-helix proteins are a family of putative transcription factors that have been shown to be involved in growth and development of a wide variety of tissues. The NHLH2 gene is expressed in the developing hypothalamus and in the adult pituitary. A recent study reported that mice with a deletion of this gene exhibited a disruption of the hypothalamic-pituitary axis with alterations in circulating gonadotropins and adult-onset obesity (28). The difference in body weight between normal mice and $Nhlh2^{-/-}$ mice was apparent by 11 weeks of age and reached a twofold difference by 20 weeks of age. Interestingly, the excess body weight was found to be due to an accumulation of adipose tissue in the perirenal, perigonadal, and subcutaneous areas of the body (28). These results suggest that the NHLH2 could play an important role in the regulation of body weight and in the regional distribution of the excess body fat. Another candidate gene that is located close (0.5 cM) to this QTL is 3-β-hydroxy-steroid dehydrogenase (HSDB3). The HSDB3 gene is involved in the synthesis of sex steroid hormones that have been shown to be important correlates of the adipose tissue distribution.

Two other QTLs influencing abdominal fat and located on chromosomes 9q22.1 and 17q21.1 were in the vicinity of genes involved in the regulation of sex steroid levels. On chromosome 9q22.1, we found evidence of linkage between ASF and markers D9S1122 (LOD 2.37; $P = 0.0005$) and D9S257 (LOD 2.14; $P = 0.0008$), located 4 cM apart. This region contains the 17-β-hydroxysteroid dehydrogenase 3 (HSD17B3) gene, one of the four isoforms of the enzyme responsible for the interconversion of estrone and estradiol as well as the interconversion of androstenedione and testosterone. Another isoform of this enzyme is encoded by the 17-β-hydroxysteroid dehydrogenase 1 (HSD17B1) gene, which happened to be within 0.5 cM of the marker D17S2180 on chromosome 17q21.1, which also provided evidence of linkage with ASF (LOD 2.24; $P = 0.0007$).

Two other markers—D17S1290 (LOD 1.53; $P = 0.004$) and D17S1301 (LOD 2.21; $P = 0.0007$), located 15 cM telomeric to D17S2180—also provided evidence of linkage with ASF. The genes encoding the peptide YY (PYY) and the pancreatic peptide (PPY) are located in this region of chromosome 17 ~10 kb apart (17q21.1). These two genes, which belong to the same gene family as neuropeptide Y (NPY), may play a role in the regulation of appetite and food intake. Another QTL located on chromosome 4q32.1 was found to be close to a gene involved in the regulation of food intake. The marker D4S2417, which provided evidence of linkage with ASF (LOD 1.76; $P = 0.002$), is within 10 cM of the gene encoding the type 2 neuropeptide Y receptor (NPY2R). The NPY2R shows a high affinity for NPY and PYY and could also be involved in the regulation of food intake. Another marker on chromosome 4p15.1 (D4S2397) showed highly suggestive evidence of linkage with ASF (LOD 2.3; $P = 0.0005$). This marker is located ~2 cM from the human peroxisome proliferator–activated receptor-γ coactivator 1 (PPARGC1) gene (29). The PPARGC1 gene has been shown to induce activation of the uncoupling protein 1 (UCP1) gene transcription and could therefore be involved in the regulation of energy expenditure. The PPARGC1 gene also maps to a 20-cM chromosomal region (4p15-q12) that was previously shown to be linked with fasting insulin levels after a genome-wide scan of prediabetic phenotypes in Pima Indians (30).

On chromosome 7q31.3, evidence of multipoint linkage (LOD 1.97; $P = 0.001$) was observed between ASF and marker D7S1875. The most obvious candidate gene for this QTL is the leptin (LEP) gene, which is located within 0.1

TABLE 2
Summary of suggestive ($P \geq 0.01$) single-point (SIBPAL) and multipoint (SEGPATH) linkage results

Chromosome	Cytogenetic band	Map position (cM)	Marker	SIBPAL	SEGPATH
1	p21.3	102.1	D1S1588	ASF 0.43	**ASF 0.009**
	p11.2	125.0	D1S534	**ASF 0.003**	**ASF 0.006**
	q24.2	183.5	D1S1677	ASF 0.32	**ASF 0.008**
2	p25.3	3.8	D2S2976	**ASF 0.009**	**ASF 0.006**
	p11.1	96.7	D2S1790	ASF 0.037	**ASF 0.005**
	q11.2	102.8	GATA176C	ASF 0.19	**ASF 0.01**
	q24.2	168.1	D2S1776	ASF 0.076	**ASF 0.005**
	q37.3	247.9	D2S427	**ATF 0.006**	ATF 0.21
4	p15.1	32.2	D4S2397	ASF 0.099	**ASF 0.0005**
	q32.1	167.7	D4S2417	ASF 0.025	**ASF 0.002**
5	q35.2	185.9	D5S1456	ASF 0.043	**ASF 0.003**
6	p21.33	28.8	D6S2439	ASF 0.087	**ASF 0.004**
	p21.1	42.4	D6S1017	ASF 0.399	**ASF 0.003**
	q16.3	106.6	D6S1056	ASF 0.023	**ASF 0.004**
7	q11.22	73.2	CD36	ASF 0.023	**ASF 0.0025**
	q31.3	134.2	D7S1875	ASF 0.066	**ASF 0.001**
8	q21.11	86.4	CRH	**ASF 0.003**	ASF 0.012
9	p21.1	26.8	D9S1118	ASF 0.098	**ASF 0.008**
	q22.1	92.3	D9S1122	ASF 0.040	**ASF 0.0005**
	q22.2	96.4	D9S257	ASF 0.115	**ASF 0.0008**
12	q22-q23	107.2	IGF1	**ASF 0.005**	**ASF 0.001**
	q24.33	139.6	D12S2078	ASF 0216	**ASF 0.0001**
	q24.33	142.1	D12S1045	ASF 0.019	**ASF 0.004**
				AVF 0.007	AVF 0.081
				ATF 0.015	ATF 0.054
13	q14.11	46.6	D13S325	ASF 0.174	**ASF 0.002**
	q34	113.6	D13S285	ASF 0.313	**ASF 0.001**
14	q11.2	26.2	D14S742	ASF 0.014	**ASF 0.007**
15	q21.1	54.5	D15S659	ASF 0.294	**ASF 0.007**
	q26.3	104.6	D15S657	ASF 0.082	**ASF 0.003**
16	q21	65.0	D16S3253	ASF 0.031	**ASF 0.003**
				AVF 0.008	AVF 0.04
17	q21.1	42.1	D17S2180	ASF 0.045	**ASF 0.0007**
	q21.3	54.2	D17S1290	ASF 0.079	**ASF 0.004**
		55.7	D17S1301	ASF 0.093	**ASF 0.0007**
18	q21.1	50.6	D18S851	ASF 0.643	**ASF 0.009**
	q22.3	77.8	GATA82B02	**AVF 0.003**	AVF 0.148
19	p13.3	6.7	INSR	AVF 0.138	**AVF 0.01**
20	p11.22	24.5	D20S104	ASF 0.035	**ASF 0.005**

Highly suggestive ($P < 0.0023$; LOD $\geq$1.75) and suggestive ($P < 0.01$; LOD $\geq$1.18) results are shown in bold. The remaining entries are given for comparison of multipoint and single-point linkage results for a given maker.

cM, but no linkage was observed with the LEP gene and any of the abdominal fat phenotypes. Another potential candidate gene located in this area is the gene encoding protein caveolin-2 (CAV2) located ~6 cM centromeric to the LEP gene. CAV2 is part of the caveolin gene family consisting of caveolin-1, -2, and -3, all cytoplasmic membrane proteins. In adults, caveolin-1 and -2 are coexpressed in many cell types, with particularly high levels in white adipose tissue where they represent as much as 20% of the total plasma membrane surface area, and are induced during adipocyte differentiation (31,32). Caveolins have been implicated in a variety of human diseases, including diabetes, because the localization of these proteins in the cell membrane is thought to play a role in signal transduction events (32). A last QTL affecting ASF was observed on chromosome 13q34 (marker D13S285, LOD 1.92; $P = 0.0015$), but no obvious candidate genes mapped to this region.

Because diabetes may affect the amount of abdominal fat, we repeated the linkage analyses after excluding the 25 diabetic subjects. Overall, the exclusion of subjects with type 2 diabetes had only a small impact on the linkage results because all the QTLs identified in the analyses with all subjects remained significant. As shown in Table 3, this exclusion resulted in a small reduction of the strength of linkages, as reflected in the reduction of the LOD scores for all but one of the QTLs (7q31.1). For example, the LOD score of the peak linkage on chromosome 12q24.3 was reduced from 2.88 to 2.53, but this region remained the one with the strongest evidence of linkage. The same trend was observed for the suggestive linkages presented in Table 2 (results not shown).

The results of multipoint linkage analyses reveal a large number of highly suggestive linkages (LOD $\geq$ 1.75) for ASF compared with the other two abdominal fat phenotypes, whereas the number of suggestive linkages in single-point linkage analyses is not excessively higher for ASF. This apparent discrepancy between the results from single-point and multipoint linkage analyses is probably explained by the sensitivity of the variance component

TABLE 3
Summary of the QTLs associated with abdominal subcutaneous fat in the QFS

Chromosome	Marker	LOD score		Candidate genes
		All subjects	Without diabetic subjects*	
1p11.2	D1S534	2.31	2.07	NHLH2, HSDB3
4p15.1	D4S2397	2.30	2.13	PPARGC1
4p32.1	D4S2417	1.76	1.74	NPY2R
7q31.1	D7S1875	1.97	2.01	LEP, CAV2
9q22.1	D9S1122	2.37	2.01	HSD17B3
	D9S257	2.14	1.69	
12q22-q23	IGF1	1.89	1.86	IGF1
12q24.3	D12S2078	2.88	2.53	HNF1
	D12S1045	1.51	1.09	
13q34	D13S285	1.92	1.44	None
17q21.1-q21.3	D17S2180	2.24	1.30	HSD17B1
	D17S1290	1.53	1.29	PYY
	D17S1301	2.21	1.99	PPY

*After exclusion of 25 subjects with type 2 diabetes.

linkage method to the nonnormality of the distribution of the phenotypes, which could result in spuriously high linkage results. A distribution with a coefficient of kurtosis >3.0 is considered leptokurtic and nonnormal. A close look at the detailed descriptive statistics of the three abdominal phenotypes reveal that the distribution of ASF is characterized by a coefficient of kurtosis of 8.5 compared with values of 1.3 and 3.7 for the distributions of ATF and AVF, respectively. This difference in the coefficients of kurtosis probably explains the larger number of suggestive multipoint linkages for ASF compared with the two other abdominal fat phenotypes.

A few candidate genes have been included in the panel of markers tested in the present study. Besides the linkage described above with the IGF1 gene on 12q22-q23, evidence of both single-point and multipoint linkages (Table 2) was found between ASF and the CD36 and corticotropin-releasing hormone (CRH) genes on chromosomes 7q11.2 and 8q21.1, respectively. The CD36 gene was identified as an insulin resistance gene responsible for QTLs associated with metabolic defects related to the metabolic syndrome in the spontaneously hypertensive rat (33), whereas the CRH gene could be considered a good candidate gene of abdominal fat because of the evidence showing that abdominal obesity is associated with an increased activity of the hypothalamic-pituitary-adrenocortical axis (34–36). Suggestive evidence of multipoint linkage ($P < 0.01$) was also observed between the insulin receptor gene (INSR) on 19q13.3 and AVF. Negative results were obtained with the other candidate genes investigated (LEP, LEPR, FABP2, FABP3, UCP1, UCP2, and IGF1R).

Genome-wide linkage analyses performed in Pima Indians (37,38), Mexican-Americans (39), French (40), and American (41) populations and in the QFS (Changon, Borecki, Pérusse, Roy, Lacaille, Ho-Kim, Chagnon, Rice, Province, Rao, and Bouchard, unpublished data) have led to the identification of several obesity QTLs. However, none of the linkages reported in these studies appear to be in the same regions as those described above for abdominal fat, which is not surprising considering the phenotypes analyzed in the present study were adjusted for body fatness.

In summary, the results of the present study reveal the existence of highly suggestive linkages between abdominal fat adjusted for total body fatness and markers located on nine different chromosomal regions: 1p11.2, 4p15.1 and 4q32.1, 7q31.3, 9q22.1, 12q22-q23 and 12q24.3, 13q34, and 17q21.1-q21.3. Three of these QTLs (1p11.2, 9q22.1, and 17q21.1-q21.3) are close to genes involved in the regulation of sex steroid levels, whereas two others (4q32.1 and 17q21.1-q21.3) are in the proximity of genes involved in the regulation of food intake. Moreover, six of these chromosomal regions (1p11.2, 4q32.1, 9q22.1, 12q22-q23, 12q24.3, and 17q21.*1*) provided evidence of both single-point and multipoint linkages. The QTLs and candidate genes identified in this article should be investigated in further linkage and association studies to confirm their role in regulation of abdominal fat stores and potentially in the development of insulin resistance and diabetes.

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