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Genome scan for adiposity in Dutch dyslipidemic families reveals novel quantitative trait loci for leptin, body mass index and soluble tumor necrosis factor receptor superfamily 1A

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OBJECTIVE: To search for novel genes contributing to adiposity in familial combined hyperlipidemia (FCH), a disorder characterized by abdominal obesity, hyperlipidemia and insulin resistance, using a 10 cM genome-wide scan.
DESIGN: Plasma leptin and soluble tumor necrosis factor receptor superfamily members 1A and 1B (sTNFRSF1A and sTNFRSF1B, also known as sTNFR1 and sTNFR2) were analyzed as unadjusted and adjusted quantitative phenotypes of adiposity, in addition to body mass index (BMI), in multipoint and single-point analyses. In the second stage of analysis, an important chromosome 1 positional candidate gene, the leptin receptor (LEPR), was studied.
SUBJECTS: Eighteen Dutch pedigrees with familial combined hyperlipidemia (FCH) ($n = 198$) were analyzed to search for chromosomal regions harboring genes contributing to adiposity.
RESULTS: Multipoint analysis of the genome scan data identified linkage (log of odds, LOD, 3.4) of leptin levels to a chromosomal region defined by D1S3728 and D1S1665, flanking the leptin receptor (*LEPR*) gene by approximately 9 and 3 cM, respectively. The LOD score decreased to 1.8 with age- and gender-adjusted leptin levels. Notably, BMI also mapped to this region with an LOD score of 1.2 (adjusted BMI: LOD 0.5). Two polymorphic DNA markers in *LEPR* and their haplotypes revealed linkage to unadjusted and adjusted BMI and leptin, and an association with leptin levels was found as well. In addition, the marker D8S1110 showed linkage (LOD 2.8) with unadjusted plasma concentrations of soluble TNFRSF1A. BMI gave a LOD score of 0.6. Moreover, a chromosome 10 q-ter locus, AFM198ZB, showed linkage with adjusted BMI (LOD 3.3).
CONCLUSION: These data provide evidence that a human chromosome 1 locus, harboring the *LEPR* gene, contributes to plasma leptin concentrations, adiposity and body weight in humans affected with this insulin resistant dyslipidemic syndrome. Novel loci on chromosome 8 and 10 qter need further study.
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Introduction

Human obesity predisposes to the development of chronic illnesses including type 2 diabetes mellitus, hypertension, hyperlipidemia and coronary heart disease.[1] It has been well established that traits related to adiposity, including body mass index (BMI) and skinfold thickness, are influenced by both genetic and environmental factors.[2,3] Segregation analysis, adoption and twin studies have shown that the degree of adiposity has a significant genetic component in humans.[4–6] Genes contributing to adiposity and common forms of obesity are largely unknown. This may result from underlying genetic heterogeneity. One approach to reducing such heterogeneity is to conduct gene finding efforts in families ascertained for the study of common metabolic syndromes, with associated obesity. To increase our understanding of the genetic contributions to adiposity, we conducted a genome scan (10 cM) to identify chromosomal regions that contribute to plasma leptin and soluble tumor necrosis factor-α (TNF) receptor concentrations, quantitative traits used as intermediate phenotypes of body weight. The synthesis of both leptin and TNF-α by adipocytes is reported to be upregulated with increasing fat mass.[7,8] In addition, substantial

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evidence is linking leptin and TNF-α to obesity-associated insulin resistance.[9–12]

Leptin, the product of the obese (*LEP*) gene, is predominantly produced by adipocytes, and is a marker of body fat, and therefore of body weight. Two well-defined genetic models of severe obesity in rodents are caused by loss-of-function mutations in either the obese (*LEP*) gene encoding leptin, or the leptin receptor (*LEPR*) gene, respectively.[13,14] Several linkage or association studies provide evidence for a relation between obesity and the *LEP* gene in humans.[15–17] However, results in the literature on the *LEPR* gene and obesity are less clear.[15,18,19] Until now identified mutations in the *LEP* or *LEPR* genes in humans have been rare.[20,21]

TNF-α is a pro-inflammatory cytokine, which exerts its action through two plasma membrane TNF receptors, namely TNF receptor superfamily 1A (TNFRSF1A (55 kDa)) and TNF receptor superfamily 1B (TNFRSF1B (75 kDa)).[22,23] The two TNF receptors are encoded by separate genes *TNFRSF1A* (also called *TNFR1* or *TNFRp55*, chromosome 12; 12p13) and *TNFRSF1B* (also called *TNFR2* or *TNFRp75*, chromosome 1; 1p36.2, not in the vicinity of the *LEPR* gene). Adipose tissue and cellular elements of the immune system are a source of TNF-α production. TNF-α is involved in the regulation of lipid and fatty acid metabolism, adipocyte differentiation and regulation of insulin sensitivity.[8,24] The *TNF-α* gene has been shown to be linked and associated with body weight,[25] but this is not undisputed.[26] The complete array of intracellular effects associated with activation of the TNF-α/TNFR pathway has not yet been elucidated.[22] A soluble form of both receptors is released by proteolytic cleavage of each receptor type, yielding soluble (s) sTNFRSF1A and sTNFRSF1B proteins in the plasma.[27] Elevated plasma levels of sTNFRSF1A and sTNFRSF1B are assumed to reflect activation of the immune system, but also reflect obesity.[28–30] Moreover, in obese subjects plasma leptin showed a significant association with BMI and sTNFRSF1A.[31]

In the present study, we analyzed plasma leptin, sTNFRSF1A and sTNFRSF1B levels—as intermediate phenotypes of adiposity—in addition to BMI for linkage in sibship data from 18 Dutch familial combined hyperlipidemia (FCH) pedigrees. We have recently completed a genome-wide scan for novel genes contributing to hyperlipidemia in FCH.[32] Affected FCH subjects show abdominal obesity, hyperlipidemia and insulin resistance, associated with premature coronary artery disease.[32–36] Independent FCH families selected for these phenotypes are expected to be genetically more homogeneous than the general population, and this should provide less heterogeneity and more power to identify genes contributing to adiposity. Based on multipoint and single-point linkage analyses, the results in this report provide evidence for linkage of markers in the *LEPR* region on chromosome 1 to BMI and plasma leptin concentrations, confirmed by association of plasma leptin to *LEPR*. Furthermore, linkage was found between DNA makers on chromosome 8 and plasma sTNFRSF1A concentrations. A novel locus on chromosome 10 qter showed linkage with BMI.

Methods

Subjects

Within the framework of genetic studies of insulin resistance and hyperlipidemia, we have collected 18 families with familial combined hyperlipidemia (FCH). These represent probands, spouses, as well as over 95% of their relatives.[32–34] The pedigree structure of the families has been published.[32] FCH probands met the criteria described previously,[33,34] including: (1) a primary combined hyperlipidemia with varying phenotypic expression, including a fasting plasma cholesterol concentration > 6.5 mmol/l or $>$ 90th percentile for age, defined according to tables from the Lipid Research Clinics, and fasting plasma triglyceride concentrations > 2.3 mmol/l; (2) at least one first-degree relative with a hyperlipidemic phenotype different from the of the proband; (3) a positive family history of premature coronary artery disease (before age 60); and (4) the absence of xanthomas, BMI > 30, diabetes, and E2/E2 genotype.[33,34] The spouse group represented an environment-matched, nutrition-matched and age-matched group. A subgroup of 198 siblings (364 sibpairs) from 18 Dutch FCH pedigrees was utilized in this study, representing nuclear families, five of which were three-generational and 13 of which were two-generational, with sibship sizes of $n = 1$ to $n = 9$ and a median sibship size of $n = 3$. This subgroup has been assayed for phenotypes related to obesity. The height and body weight (fasting, after voiding, in underwear) were recorded, as well as blood pressure. Body mass index (BMI: weight (kg)/height^2 (m^2)) was calculated.

Biochemical analysis

Venous blood was collected after an overnight fast (12–14 h) in EDTA-containing (1 mg/ml) tubes. Plasma samples were prepared by immediate centrifugation. Plasma triglyceride and cholesterol were measured in duplicate using a commercial colorimetric assay (GPO-PAP, Boehringer Mannheim no. 701912 and Monotest cholesterol kit, Boehringer Mannheim no. 237574). Plasma leptin concentrations were determined by a commercial radioimmunoassay (Linco Research, St Louis, MO, USA). Plasma sTNFRSF1A and sTNFRSF1B concentrations were measured as described by Froon *et al*.[37] Insulin was measured by a competitive radioimmunoassay, using the polyclonal insulin antibody Caris 46, insulin tracer labeled with 125I (IM.166, Radiochemical Center, Amersham, England), and insulin standards (WHO: first international reference preparation 66/304).

Statistical analysis

Data are expressed as mean ± s.d. Data for triglyceride, insulin and leptin levels were analyzed after log transformations. Pearson product moment correlation coefficients were calculated. Adjustments for age and gender were carried out using multiple linear regression analysis. All statistical analyses were conducted using SPSS 7.0 or SAS 6.12.

Genotyping

DNA was isolated from 10 ml of EDTA-augmented blood following standard procedures.[38] In total, 198 siblings (364 sibpairs) were genotyped for multipoint and single-point analyses. Reaction conditions for microsatellite analysis were as follows: 75 ng of genomic DNA, 0.34 mM of each primer (the forward primer was end-labeled with ^{32}P), 200 mM dNTPs (Gibco BRL), 3.0 mM $MgCl_2$, and 0.5 U Taq polymerase (Gibco BRL). PCR was carried out in 96-well plates with 25 cycles: 94°C for 30 s, 55°C for 30 s, and 72°C for 45 s. PCR products were resolved on 5% polyacrylamide gels, blotted with Whatman paper, and exposed to film overnight. Genotypes were then assigned to each individual by two independent observers. The complete linkage map, constructed of 198 individuals from 18 Dutch FCH kindred, consists of 399 markers spanning, on average, 10 cM intervals.[39] All 22 autosomes and the X and Y chromosomes were typed. The 399 markers employed in construction of the genome screen linkage map comprised the Weber 6a screening set.

Nonparametric sibpair linkage analysis

Since the modes of inheritance of the quantitative traits under analysis are unknown, nonparametric linkage analyses were conducted to test for linkage with the polymorphic marker(s).[40–42] The primary screen was done with the use of the Haseman-Elston multipoint option of MAPMAKER/SIBS program.[43] This program uses information from multiple-linked markers to estimate allele sharing at 1 cM intervals along the chromosome on the basis of the most likely genotypes.[43] The significance of the test statistic for excess allele sharing among sibpairs is expressed as the log of odds (LOD) score. In regions exhibiting a LOD > 3.0, we conducted additional single-point quantitative sibpair analyses using the Haseman–Elston algorithm of the SIBPAL subprogram in the SAGE package.[41] This method regresses the squared trait differences within sibpairs against the estimated proportion of marker alleles that they are estimated to share identical by descent (IBD). A significantly negative slope indicates that those who are phenotypically alike tend to share more alleles and those who are discordant tend to exhibit less allele sharing, thus providing evidence for linkage. If there is no evidence for linkage, the slope of the regression line is not expected to be significantly different from zero. Because sibpairs in the same sibship may be non-independent statistically, significance levels are calculated using effective degrees of freedom (edf), which reflects the number of independent sibling pairs in the analyses.[41]

Both age and gender contributed significantly to leptin concentrations, while only age contributed significantly to BMI, sTNFRSF1A and sTNFRSF1B levels (data not shown). All 399 markers were subsequently re-scanned for the four traits, adjusted as follows: leptin data were adjusted for age and gender, and BMI, sTNFRSF1A and sTNFRSF1B data were adjusted for age. The locations of the markers in the linked regions are mentioned in the Results.[44] Two polymorphic DNA markers in the leptin receptor gene (*LEPR*), an intron 4 LEPR-CA repeat[45] and an intron 16 LEPR-CTTT repeat;[45,46] as well as their haplotypes, were each tested for single point linkage and association. We found that the original D1S2852 marker[47] was not linked to the *LEPR*, and was therefore useless as a marker of LEPR; this marker is mapped to an interval defined by D1S418 (152.2 cM) and D1S514 (157.4 cM). Haplotypes of the two LEPR markers were assigned according to parsimony and the structure of the pedigrees. Association with each of the unadjusted and adjusted traits was tested using a measured genotype approach.

Results

Characteristics

Clinical and biochemical characteristics of the study population, which comprised 198 individuals from 18 Dutch Caucasian FCH pedigrees, are summarized in Table 1. In this population 10% of subjects had a BMI ≥ 30 ($n = 20$). Increased body weight was associated with greater waist circumference and a higher waist-to-hip ratio, as expected. Moreover, plasma triglycerides, insulin, leptin and sTNFRSF1B concentrations were higher in the group with BMI ≥ 30 compared to the group with BMI < 30. Men and women were equally represented in the study population. Expected gender-related differences, such as lower waist circumference and waist-to-hip ratio in women were observed. Plasma leptin concentrations were higher in women.

Genetic linkage to leptin and BMI

One hundred and ninety-eight subjects were genotyped for multipoint and single-point linkage analyses. Using polymorphic microsatellite markers spaced at approximately 10 cM intervals spanning the entire genome, we obtained evidence for genetic linkage between the chromosomal region harboring D1S1665, in the vicinity of the LEPR gene, and unadjusted plasma leptin concentrations (LOD = 3.4, multipoint analysis; Figure 1A). Following age and sex

Table 1 Clinical and biochemical characteristics of 18 studied FCH families

		Male subjects		*Female subjects*	
	Whole group	*BMI < 30*	*BMI ≥ 30*	*BMI < 30*	*BMI ≥ 30*
n	198	92	8	83	12
Relatives/spouses	175/23	79/13	7/1	77/6	9/3
Age (y)	46.4 ± 16.1	47.1 ± 16.4	51.0 ± 12.1	44.4 ± 15.6	52.3 ± 17.6
BMI[a]	25.7 ± 3.3	25.0 ± 2.5	31.7 ± 1.2	24.5 ± 2.4	32.2 ± 1.9
Waist (cm)	90.9 ± 11.6	93.5 ± 8.5	110.3 ± 6.8	83.1 ± 9.2	103.5 ± 8.7
Waist–hip ratio	0.87 ± 0.09	0.90 ± 0.06	1.00 ± 0.06	0.80 ± 0.08	0.88 ± 0.11
Triglycerides	2.4 ± 5.7	2.2 ± 1.6	3.6 ± 2.6	2.5 ± 8.6	2.4 ± 0.9
Cholesterol	6.4 ± 2.1	6.3 ± 1.7	6.8 ± 0.9	6.4 ± 2.5	6.3 ± 1.4
Glucose	4.9 ± 1.4	4.9 ± 1.3	5.6 ± 1.5	4.8 ± 1.3	5.4 ± 1.2
Insulin	7.9 ± 7.7	7.1 ± 8.1	16.6 ± 8.5	7.0 ± 6.2	14.1 ± 8.6
Leptin	10.7 ± 9.8	4.8 ± 3.3	10.6 ± 5.2	13.5 ± 6.0	35.2 ± 17.7
STNFRSF1A	1.04 ± 0.32	1.05 ± 0.34	1.11 ± 0.09	1.02 ± 0.31	1.17 ± 0.30
STNFRSF1B	1.68 ± 0.47	1.67 ± 0.45	2.03 ± 0.79	1.62 ± 0.35	1.97 ± 0.71

Results represent mean ± s.d.
[a]BMI was not available in three subjects (missing measurements).

adjustment of leptin levels, the LOD score decreased to 1.8 (Figure 1B). Single-point analyses provided concordant results with regard to the linkage of adjusted and unadjusted leptin levels to this region on chromosome 1, where highest significance again was found with marker D1S1665 ($P < 0.00002$, Table 2). Unadjusted BMI gave an LOD score of 1.2 with the same marker, D1S1665, and adjusted BMI a LOD score of 0.5. In single-point linkage analyses with BMI, the results showed $P < 0.002$ with unadjusted BMI, and $P = 0.012$ with adjusted BMI. No evidence for linkage to this chromosomal 1 region was found with other quantitative traits, including adjusted plasma sTNFRSF1A, sTNFRSF1B, insulin, glucose, lipids and fatty acid concentrations.

It is of interest that the 399 marker scan of the four adjusted traits yielded a new chromosomal region that exhibited a LOD score of 3.3 with BMI, ie chromosome 10 q-ter (Figure 2). Because no significant LOD score was observed with unadjusted BMI (LOD 0.4), a false positive result needs to be excluded.

Linkage to soluble TNF receptor superfamily member 1A

Plasma sTNFRSF1A concentrations, but not sTNFRSF1B, showed significant linkage with a region on chromosome 8 (between D8S1110 and D8S1136; Figure 3). The maximum LOD score was 2.8 with marker D8S1110. Multipoint linkage analyses of BMI yielded a LOD score of 0.6 in this region. In single-point linkage analyses, D8S1110 yielded the best *P*-value, $P = 0.013$ (Table 3). After adjustment for age, a LOD score of 2.1 for TNFRSF1A was observed with D8S1110 ($P = 0.013$ in single-point linkage). A smaller, second peak of multipoint LOD scores (approaching 2.0) was observed between sTNFRSF1A and the region between D8S1106 (27 cM) and D8S136 (46 cM) on chromosome 8 (Figure 3). This region is close to the LPL gene, mapped at 30.7–40.3 cM. No evidence for linkage was found with other quantitative traits examined, including plasma leptin, sTNFRSF1B, insulin, glucose, lipids and fatty acid concentrations.

Linkage and association study with LEPR

In the second stage of the analysis, two polymorphic DNA markers in *LEPR* and their haplotypes were each tested for linkage and association with the four unadjusted traits as well as their age- and/or gender-adjusted values (Table 4). As expected, significant linkage to adjusted and unadjusted plasma leptin levels and BMI was observed, supporting the results obtained by the multipoint genome scan. Moreover, significant associations were observed between DNA variation in LEPR and plasma leptin ($P = 0.033$ with LEPR-CTTT, and $P = 0.025$ with LEPR-haplotype) as well as sTNFRSF1B plasma levels ($P = 0.007$ with LEPR haplotype). No significant associations were observed with BMI (data not shown).

Relationship of sTNFRSF1A, sTNFRSF1B and leptin to BMI and waist circumference

Plasma sTNFRSF1A and sTNFRSF1B concentrations are correlated with BMI (Table 5). A highly significant correlation existed between leptin and BMI and between leptin and waist circumference. When analyzed by gender, the associations between leptin and BMI, sTNFRSF1A and sTNFRSF1B became more evident (Figure 4). The correlation between BMI and leptin is 0.66 ($P < 0.001$) in men, and 0.74 ($P < 0.001$) in women (Panel A), respectively. In men, the correlation coefficient between sTNFRSF1A and leptin is 0.28, and 0.46 in women ($P < 0.01$, in both groups; panel C). The correlation coefficient between sTNFRSF1B and leptin is 0.44 in men, and 0.33 in women ($P < 0.01$, in both groups; panel D).

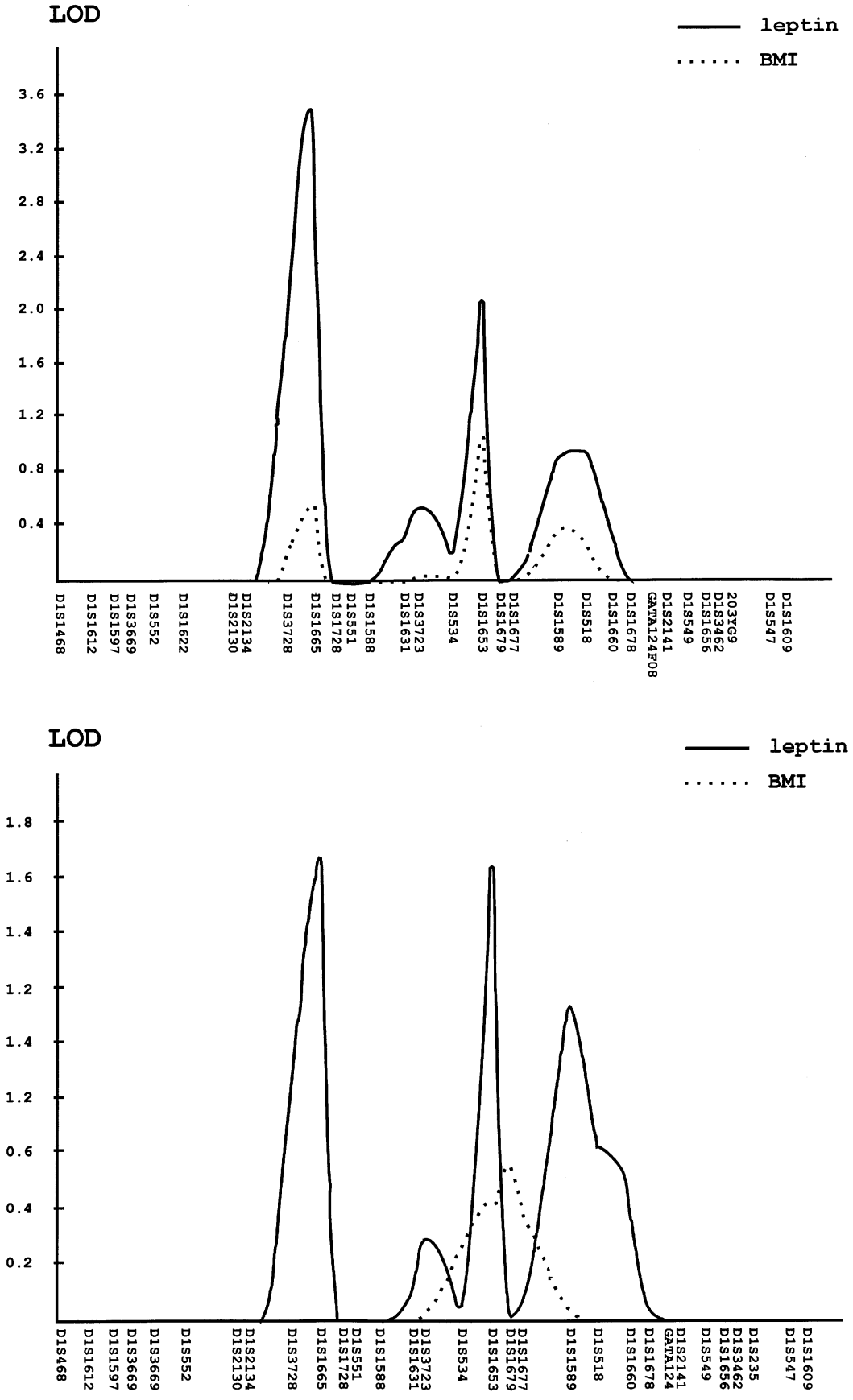

Figure 1 (A) Graphical representation of the LOD scores for chromosome 1 analyzed by multipoint quantitative linkage analysis with unadjusted plasma leptin concentrations, and unadjusted BMI. (B) LOD scores with age and sex-adjusted plasma leptin concentrations, and age-adjusted BMI (see Methods).

Table 2 Quantitative single-point linkage analysis of flanking markers on chromosome 1 with unadjusted and adjusted plasma leptin concentrations

Marker	*cM*	*Heterozygosity index*	*edf*	*P-value*	*Adjusted P-value**
D1S2134	73	0.84	89	0.55	0.74
D1S3728	91	0.74	90	0.0008	0.007
D1S1665	102.1	0.74	91	0.00002	0.005
D1S1728	111.8	0.67	92	0.368	0.88

edf: effective degrees of freedom.
*Adjusted for age and gender by multiple regression.

Discussion

This genome-wide scan for novel genes contributing to adiposity yielded evidence of genetic linkage to plasma leptin concentrations and BMI of the chromosome 1 region containing the polymorphic marker D1S1665 (at 102.1 cM), in the vicinity of the leptin receptor gene (*LEPR*), mapped at 99.3 cM.[13] The maximum LOD score with leptin was 3.4 and, with BMI, 1.2. Moreover, linkage and significant association was observed between DNA variation in *LEPR* and plasma leptin concentrations, as well as sTNFRSF1B levels. The present results add to the body of genetic, biological and statistical evidence that *LEPR* contributes to plasma leptin concentrations, and body fat and weight regulation. We also obtained evidence for linkage (LOD score 2.8) of plasma sTNFRSF1A and the polymorphic marker D8S1110 (71 cM) on chromosome 8. We did not find evidence to support the suggested linkage between the same D8S1110 marker and plasma leptin concentrations, observed in a sample of obese Mexican Americans.[48] Another novel finding is the LOD score of 3.3 with adjusted BMI to chromosome 10 q-ter. Because no significant LOD score was observed with unadjusted BMI, a false positive result needs to be excluded. At present, no obvious positional candidate gene is readily identified in that region in the public domain databases. In the present study, adjusted and unadjusted leptin concentrations and BMI yielded results in the same direction and of the same order of magnitude, except for the 10 q-ter locus. Other genome scans of obesity and adiposity have yielded interesting loci as well, and their different findings may result from the different populations studied, ie obese Mexican Americans or Pima Indians.[48–51] The present results are consistent with the concept that studies in well-defined families with common metabolic syndromes associated with adiposity—in this case, familial combined hyperlipidemia (FCH)—are a powerful tool to identify linkage, because genetic heterogeneity in such families is expected to be reduced relative to that of the population.

The linkage (maximal LOD score 3.4) of plasma leptin concentrations to the region on chromosome 1 that contains the *LEPR* gene, defined by D1S1665 and D1S3728, is a novel finding. The single-point linkage with plasma leptin and BMI and the association with

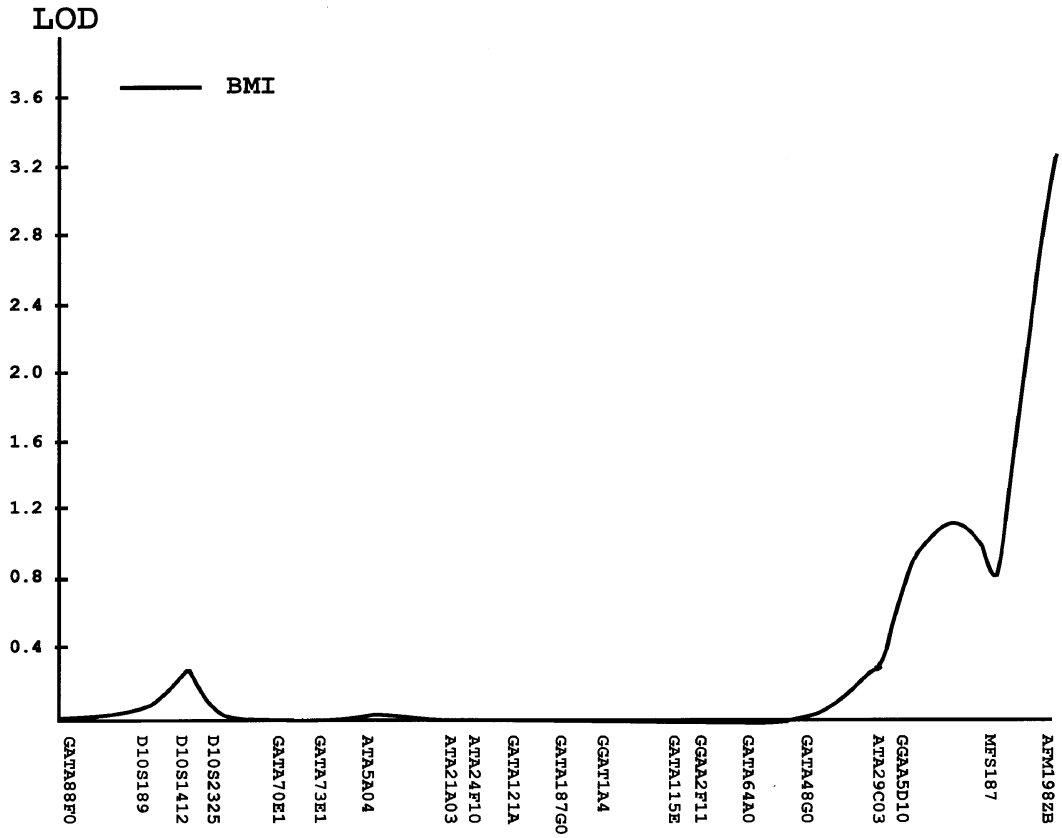

Figure 2 Graphical representation of the LOD scores for chromosome 10 analyzed by multipoint quantitative linkage analysis with adjusted BMI (unadjusted BMI did not reveal any LOD score greater than 0.5; data not shown).

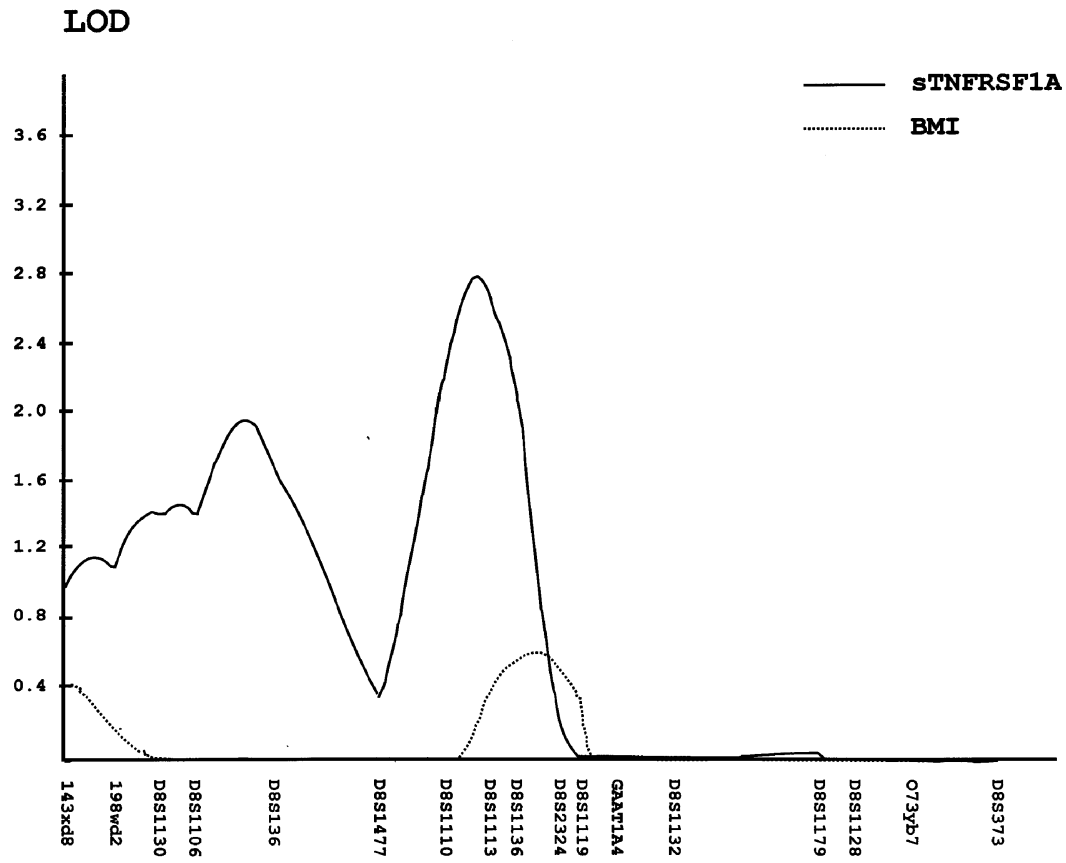

Figure 3 Graphical representation of the LOD scores for chromosome 8 analyzed by multipoint quantitative linkage analysis with unadjusted serum sTNFRSF1A levels and BMI.

Table 3 Quantitative single point linkage analysis of flanking markers on chromosome 8 with unadjusted and adjusted plasma sTNFRSF1A concentrations

Marker	*cM*	*Heterozygosity index*	*edf*	*P-value*	*Adjusted P-value**
D8S1477	62	0.86	64	0.041	0.06
D8S1110	71	0.77	62	0.005	0.013
D8S1113	79	0.81	61	0.005	0.14
D8S1136	86	0.72	66	0.006	0.023

edf: effective degrees of freedom.
*Adjusted for age by multiple regression.

Table 4 Single-point linkage of unadjusted and adjusted plasma leptin levels and BMI to DNA variation within the chromosome 1 leptin receptor gene (*LEPR*). No significant linkage was obtained with plasma sTNFRSF1A or sTNFRSF1B concentrations (data not shown)

	Tested for linkage with:			
Marker	*Unadjusted BMI; P-value*	*Adjusted BMI; P-value*	*Unadjusted leptin; P-value*	*Adjusted leptin; P-value*
LEPR-CA	0.014	0.013	0.04	NS
LEPR-CTTT	0.004	0.004	0.011	0.05
LEPR-haplotype	0.015	0.015	0.005	NS

edf: effective degrees of freedom; NS, not significant.

plasma leptin levels provide further evidence that DNA variability in *LEPR* contributes to these phenotypes, at least in FCH. The present findings corroborate and extend the recently found association between *LEPR* and plasma leptin and parameters of body composition, in two Canadian family studies.[45,52] Mutations in the *LEPR* gene are rare monogenic causes of obesity, and result in high leptin levels in the $LEPR^{db}/LEPR^{db}$-mouse and $LEPR^{fa}/LEPR^{fa}$-rat by a feedback mechanism.[53,54] *LEPR* mutations identified in these animal models lead to a short form of the LEPR or to decreased cell surface expression, both causes of leptin insensitivity. The present results are consistent with linkage and association studies that have shown evidence for a relation between adiposity or obesity and DNA variation in the *LEPR* gene in rodents[13,14] and humans.[18,19,45,52,55] However, negative results from linkage and association studies of obesity and the *LEPR* gene have been reported as well,[56–58] suggesting genetic heterogeneity. It cannot be overlooked that direct sequencing of the *LEPR* gene has failed to identify mutations as a frequent cause of human obesity. For instance, Clement *et al*[21] has identified a mutation in the human leptin receptor gene (located in intron 16), associated with obesity and pituitary dysfunction. We did not generate definitive evidence implicating these specific *LEPR* polymorphisms to BMI regulation (while evidence regarding the contribution of LEPR to plasma leptin levels is very strong).

Table 5 Pearson product moment correlation coefficients between sTNFRSF1A, sTNFRSF1B, leptin, BMI and waist in the study population ($n = 198$)

	(log) leptin (ng/ml)	*sTNFRSF1A (ng/ml)*	*STNFRSF1B (ng/ml)*
BMI	0.510**	0.261*	0.329**
Waist (cm)	0.723**	0.153	0.274***
(log)Leptin (ng/ml)	—	0.254**	0.250**
STNFRSF1A (ng/ml)	—	—	0.470***

$^{**}P < 0.01$; $^{*}P < 0.05$; $^{***}P < 0.001$.
sTNFRSF1A: plasma soluble TNFRSF1A concentration.
sTNFRSF1B: plasma soluble TNFRSF1B concentration.

Nonetheless, the present results do not rule out the LEPR as contributing to BMI as there can be an association with a mutation in this gene that is not in linkage disequilibrium with the markers used here. Additionally, there could be heterogeneity resulting in association with, as yet unidentified, multiple mutations within the human *LEPR* gene. The presence of another, unknown gene acting on satiety or coping mechanisms, or metabolism, is a theoretical possibility, but the most logical positional candidate is the LEPR.

Another novel finding is genetic linkage (LOD 2.8) of plasma sTNFRSF1A concentrations to a region of chromosome 8, defined by the markers D8S1110 and D8S1136. This region has been reported earlier to show evidence of linkage with leptin (LOD 2.2) in Mexican Americans.[48] Interestingly, the transmembrane TNFRSF1A receptor, encoded by the *TNFRSF1A* gene mapped on chromosome 12

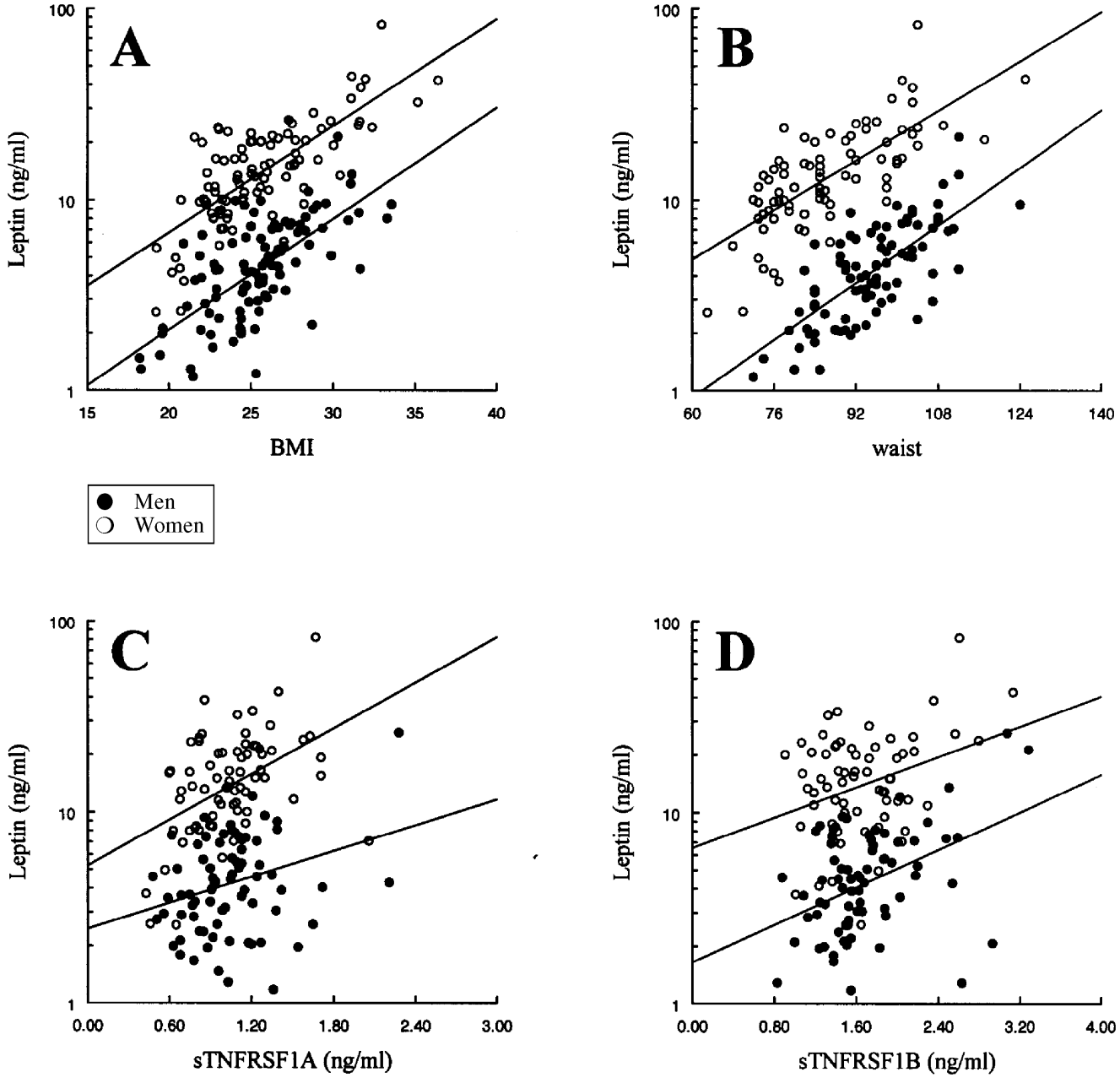

Figure 4 Relationship between leptin and BMI (A), waist (B), sTNFRSF1A (C) and sTNFRSF1B (D) in both men (●) and women (○).

(12p13), has been shown to regulate leptin secretion by adipocytes.[59] More recently, Finck *et al* described that the induction of leptin production by TNF-α requires activation of TNFRSF1A, but not TNFRSF1B.[60] The cleavage of TNFRSF1A to sTNFRSF1A is due to the action of metalloproteinases. One of these metalloproteinases (MMP) is MMP 16, which is mapped to 8q21.3–q22.1 in the vicinity to the D8S1136 marker. Therefore, MMP16 represents a potential candidate gene that can contribute to sTNFRSF1A plasma concentrations. As discussed by Comuzzie *et al*,[48] another candidate gene mapped in this region (D8S1110–D8S1136) is the β3-adrenergic receptor (*ADRB3*). ADRB3 is mapped at 60.0–65.8 cM on chromosome 8, whereas D8S1110 is mapped at 71.0 cM. Both negative (reviewed in Chagnon *et al*[61]) and positive[61–63] linkage and association results of *ADRB3* with adiposity have been reported. However, in the presently studied FCH families, plasma leptin concentrations and BMI showed no linkage to this region on chromosome 8.

It is plausible that with increasing body fat and body weight, increased activation occurs of TNF-α/TNFR pathway(s)—characterized by higher sTNFRSF1A and sTNFRSF1B plasma levels (Tables 1 and 5)—as well as the leptin/leptin receptor pathway—characterized by higher leptin levels (Figure 4 and Table 1). Individual adipocyte cell volumes may act as one of the regulators. With expanding individual adipocyte cell volume, increased TNFRSF1A activation occurs (increased TNF-α), with variable effects on leptin secretion,[59,60,64] increased intracellular lipolysis,[65,66] and higher extracellular FFA concentrations. These mechanisms all contribute to a worsening of insulin resistance. Therefore, TNFR pathway activation may act as regulator of plasma leptin concentrations,[59] whereas leptin receptor signaling represents a regulatory step to the central nervous system, ultimately contributing to regulation of plasma leptin, body fat mass and organ fat distribution.

In summary, we have reported evidence that the human chromosome 1 locus, harboring the *LEPR* gene, contributes to plasma leptin concentrations, adiposity and body weight in humans with an insulin resistant dyslipidemic syndrome, familial combined hyperlipidemia. The novel loci on chromosome 8 and 10q-ter need further study.

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