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Pleiotropic QTL on chromosome 19q13 for triglycerides and adiposity: The HERITAGE family study☆

Mary F. Feitosa[a,*], Treva Rice[a], Kari E. North[b], Aldi Kraja[a], Tuomo Rankinen[c], Arthur S. Leon[d], James S. Skinner[e], John Blangero[f], Claude Bouchard[c], D.C. Rao[a,g,h]

[a] *Division of Biostatistics, Washington University School of Medicine, Campus Box 8067, 660 S. Euclid, St. Louis, MO 63110-1093, USA*
[b] *Department of Epidemiology, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA*
[c] *Pennington Biomedical Research Center, Louisiana State University System, Baton Rouge, LA, USA*
[d] *School of Kinesiology, University of Minnesota, Minneapolis, MN, USA*
[e] *Department of Kinesiology, Indiana University, Bloomington, IN, USA*
[f] *Department of Genetics, Southwest Foundation for Biomedical Research, San Antonio, TX, USA*
[g] *Department of Genetics, Washington University School of Medicine, Campus Box 8067, 660 S. Euclid, St. Louis, MO 63110-1093, USA*
[h] *Department of Psychiatry, Washington University School of Medicine, Campus Box 8067, 660 S. Euclid, St. Louis, MO 63110-1093, USA*

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Abstract

Motivated by strong correlations between plasma levels of triglycerides (TG) and adiposity traits, we conducted a series of bivariate genome-wide linkage analyses of TG with body mass index (BMI), total fat mass (FAT), percentage of body fat (FATPC), and abdominal subcutaneous fat (ASF). Maximum lod scores of 3.3, 3.0, 2.2 and 2.4, respectively, were found on chromosome 19q13. This linkage region includes the *APOE* gene, a predictor of variation in lipid-lipoprotein levels, and the hormone-sensitive lipase (*LIPE*) gene, a key enzyme in the mobilization of fatty acids from triglyceride stores. In addition, the adiposity measures together with the *APOE* marker showed significant association with TG levels ($p = 0.02$ to $p = 0.03$). In summary, these results suggest that one or more QTLs in the 19q13 region jointly influence TG levels and adiposity. Polymorphisms in the *APOE* gene, and possibly *LIPE* gene, appear to be strong candidates for the source of this pleiotropic QTL.

Keywords: Lipids obesity; Risk factors; Quantitative trait loci; Genetic epidemiology; Exercise

1. Introduction

Coronary heart disease (CHD) is the leading cause of mortality in the United States [1]. CHD is a complex disease and is likely influenced by multiple genes as well as by gene–environment interactions. Multiple risk factors are associated with CHD, including altered lipid and lipoprotein levels, obesity, hypertension, non-insulin-dependent (type 2) diabetes mellitus, cigarette smoking and physical inactivity. Several studies have shown correlations (of ∼5–20%) between triglyceride (TG) levels and obesity in different populations [2,3], suggesting that pleiotropic genetic effects may influence both phenotypes. However, to our knowledge the presence of a common quantitative trait locus (QTL) controlling TG and adiposity levels has not been reported.

A bivariate linkage approach may substantially improve power to detect susceptibility loci that have pleiotropic effects on correlated traits and can help to localize such genes more precisely [4]. The aim of the present study is to assess whether a pleiotropic QTL affects adiposity traits and TG levels in the HERITAGE Family Study at baseline (in a sedentary state) and in response to an exercise program in White and Black families. A variance components approach to bivariate link-

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* Corresponding author. Tel.: +1 314 747 3792; fax: +1 314 362 2693.
E-mail address: maryf@wubios.wustl.edu (M.F. Feitosa).

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age analysis has been employed to test whether covariances between TG levels and adiposity phenotypes are due to a pleiotropic QTL.

2. Methods

2.1. Data

Families were recruited for the HERITAGE Family Study primarily if all members were had not engaged in regular vigorous physical activity over the previous 6 months. Individuals with confirmed or possible CHD, hypertension, chronic or recurrent respiratory problems, and uncontrolled endocrine and metabolic disorders, including diabetes and use of lipid-lowering and hypertensive drugs, were excluded from the study for all lipid phenotypes. A detailed description of the study design, inclusion and exclusion criteria, exercise training protocol and measurements has been published elsewhere [5].

Blood samples were collected from an antecubital vein into vacutainer tubes containing EDTA in the morning after a 12 h fast with participants in a semi-recumbent position. Blood was drawn twice at baseline at least 24 h apart and twice at 24 and 72 h after the last exercise session. Blood samples were taken from post-menopausal women at any time of the month, but for the remaining women blood was drawn during the early follicular stage. The response to exercise training was computed as the difference between post-training and baseline values. TG concentrations were determined in total plasma by enzymatic methods using a Technicon RA-500 Analyzer (Bayer Corporation Inc., Tarrytown, NY). Extensive quality control procedures were implemented for all assays, tests and the training program [6]. The reproducibility of the baseline plasma TG concentrations was high. The adiposity measurements included body mass index (BMI, in kg/m^2), total fat mass (FM, in kg), percentage body fat (FATPC, in %), abdominal subcutaneous fat (ASF, in cm^2). The reproducibility of these phenotypes was also high [7]. Both baseline and training response TG and baseline BMI were transformed using natural logarithms to improve distributional properties.

APOE and *LIPE C-60G* polymorphisms were typed with polymerase chain reaction (PCR). Genotyping methods and quality control of molecular data were previously outlined [8–10]. The *LIPE* polymorphism was a C-to-G single nucleotide substitution at position -60 in the *LIPE* gene promoter region (C-60G). The *APOE* gene polymorphism was for the three isoforms (E2, E3 and E4) of the ApoE protein.

2.2. Covariates and data adjustments

A stepwise regression analysis was used to remove the effects of age (age, age^2 and age^3), hormonal use (i.e., contraceptives and hormone replacement therapy: 0 = no, 1 = yes and, 2 = unknown) within sex and race group, for each phenotype. Similar adjustment procedures were used for the training responses, plus the corresponding baseline value was also included. A total of 99 White families and 101 Black families were studied using a total of 654 polymorphic markers. A total of 843 baseline and 739 training response subjects had phenotype data and were used in the estimation of means, variances, and spouse and sibling resemblances in the variance components linkage analysis. The genotype data yielded a maximum of 363 White and 106 Black sibpairs at baseline, and a maximum of 324 White and 96 Black sibpairs for the training responses.

2.3. Statistical analysis

Multipoint variance component genome-wide linkage analysis was employed using the computer program SOLAR version 2.1.2 [11]. The method utilizes information from all possible pedigree relationships simultaneously examining the genetic effect of a quantitative trait based on expected covariances between relatives as a function of their identity by descent (IBD) relationships at a marker locus. The method tests for linkage between marker loci and the trait under study. To identify a specific QTL influencing the phenotype, the total phenotypic variance attributable to the genetic effects is estimated. The total phenotypic variance can be decomposed into the environmental (e^2) and genetic components. The genetic component can be decomposed further into additive genetic variance as a result of specific QTLs (h^2_q) and residual genetic (non-QTL, h^2_r) variance. A bivariate linkage analysis is an extension of univariate analysis, in which tests whether the correlation pattern between two quantitative traits in families is due to pleiotropic genetic effects [4]. The correlations caused by a QTL (ρ_q), a residual additive genetic effect (ρ_g), and a random environmental effect (ρ_e) were also estimated. All parameters, including trait-specific means and standard deviations were estimated using maximum-likelihood methods and the hypothesis of no linkage was tested by likelihood-ratio tests.

The significance of the genetic contribution of the QTL was tested by comparing the log likelihood of the full model with that of a reduced model where the QTL specific genetic variance is fixed at zero for both traits. To assess the evidence for bivariate linkage, bivariate LODs are converted from 2 to 1 d.f. effective LODs; these are comparable to LODs for univariate models [12]. That is, the difference in likelihoods is asymptotically distributed as a 50:50 mixture of a χ^2_1 and a point mass at zero. Evidence for pleiotropy and/or coincident linkage was assessed using likelihood-ratio tests [13]. Evidence for pleiotropic effects ($\rho_q \neq 0$) occurs when two traits are influenced by the same QTL, while for coincident linkage ($\rho_q = 0$), no QTL effects both traits. To test for evidence of complete pleiotropy, the likelihood of a restricted model in which ρ_q is constrained to 1 (or -1) is compared to that of a model in which ρ_q is estimated. To test for evidence of coincident linkage, the likelihood of a restricted model in

which ρ_q is constrained to 0 ($\rho_q = 0$) is compared to that of a model in which ρ_q is estimated.

Associations between the *APOE* and *LIPE C-60G* markers and TG levels and adiposity measures were analyzed using a MIXED procedure in the SAS (Statistical Analyses System, version 9; SAS Institute, Cary, NC) software package. Non-independence among family members was adjusted for using a sandwich estimator which asymptotically yields the same parameter estimates as ordinary least squares or regression methods, but the standard errors and consequently hypothesis tests are adjusted for the dependences. This method assumes the same degree of dependency among all members within a family.

3. Results

Table 1 presents the sample characteristics for the unadjusted baseline and training response phenotypes and covariates in White and Black samples. Detailed methodologies for covariate adjustments have been reported elsewhere [8,14,15].

Genome-wide univariate linkage scans for TG levels and adiposity traits have been previously reported in the HERITAGE Study and are summarized in Table 2. As shown, suggestive linkage was observed on several chromosome regions for these univariate traits. In the current study we performed bivariate analysis to explore pleiotropic hypothesis. However evidence of a pleiotropic QTL was only found on chromosome 19 (Table 3). All baseline traits were heritable in White families. The polygenic heritability for TG levels was 48% and for adiposity traits the heritabilites ranged from 38 to 47%. The additive genetic correlations among baseline TG levels with adiposity traits were positive (from 51 to 67%), while the cross-trait correlations due to QTL effects were negative (bounded at −1.0).

Compared with the univariate linkage signals, the bivariate analysis substantially improved the LOD scores on chromosome 19 for baseline traits in White families (Table 3). Strong evidence of a pleiotropic QTL was found for baseline TG and BMI with the maximum LOD of 3.3 located on 19q13 (at 48 cM; Fig. 1).

Linkage to 19q was also observed for the covariation of TG with FAT, FATPC and ASF (LODs of 3.0, 2.2 and 2.4, respectively), about 3 cM downstream from the initial find-

Table 2
Summary of univariate linkage analyses reported for adiposity measures and TG levels in the HERITAGE study

Chromosome	Phenotypes (LOD score)	References
1p36	TG^{B_R} (1.5)	[14]
2p14	ASF^{W_B} (1.9)	[15]
2q22	AVF^{W_B} (2.3)	[15]
2q36	AVF^{W_B} (2.5)	[15]
3p26	ASF^{B_B} (2.2)	[15]
3q29	ASF^{B_B} (2.5)	[15]
4q31	ASF^{B_B} (2.3)	[15]
5q14	TG^{B_R} (1.7)	[14]
5q31	ASF^{W_B} (2.1), ATF^{W_B} (1.9)	[15]
7q36	ASF^{B_B} (1.7), ATF^{R_B} (2.5)	[15]
8q23	BMI^{W_B} (2.0)	[8]
9q34	BMI^{W_B} (2.3)	[8]
10p14	TG^{W_B} (1.5)	[14]
10p15	BMI^{W_B} (2.7), FM^{W_B} (2.1)	[8]
11p14	ASF^{B_B} (1.9)	[15]
12p12	BMI^{W_B} (2.1), FM^{W_B} (2.2)	[8]
14q11	BMI^{W_B} (2.4), FM^{W_B} (2.0)	[8]
14q24	ASF^{B_B} (2.4)	[15]
22q11	ASF^{W_B} (2.0)	[15]

Subscripts: W_B: Whites at baseline; W_R: Whites training response; B_B: Blacks at baseline; B_R: Blacks training response.

Table 1
Descriptive statistics for baseline and training responses unadjusted phenotypes and covariates in White and Black samples

Covariate/phenotype	Whites					Blacks				
	N	Mean	S.D.	Min	Max	*N*	Mean	S.D.	Min	Max
Age (years)	529	35.4	14.5	17.0	65.2	326	32.9	11.6	17.0	65.9
Hormonal use[a]	267	39%				209	22%			
Baseline										
BMI (kg/m^2)	522	25.9	5.0	17.0	47.5	321	28.0	6.2	17.4	50.9
FM (kg)	494	20.5	10.8	0.3	62.1	266	25.2	12.9	2.1	72.5
FATPC (%)	494	26.5	10.1	0.6	53.7	266	30.9	10.8	3.3	54.9
ASF (cm^2)	517	260.2	145.1	21.0	746.0	314	310.0	187.2	9.3	860.0
TG (mmol/L)	520	1.37	0.78	0.38	6.33	315	1.03	0.60	0.36	4.62
Training response										
BMI (kg/m^2)	481	−0.1	0.8	−3.5	2.9	258	−0.2	1.0	−5.2	2.9
FM (kg)	453	−0.7	1.9	−12.5	6.5	213	−0.8	2.4	−12.1	7.7
FATPC (%)	453	−0.9	1.9	−7.8	6.0	213	−0.8	1.9	−8.3	5.3
ASF (cm^2)	471	−8.7	25.5	−124.0	61.4	256	−11.5	33.3	−144.5	79.5
TG (mmol/L)	468	−0.02	0.42	−1.83	2.31	222	−0.04	0.35	−1.68	1.41

S.D.: standard deviation; min: minimum values; max: maximum values, negative training responses for adiposity traits indicate decreased mean values after training (post-baseline). To convert mmol/L to mg/dL, multiple triglyceride by 88.5.

[a] *N* and % denote the number of women.

Table 3
Summary of univariate and bivariate linkage analyses on chromosome 19q13 (45–51 cM)

Traits	Univariate analysis		Bivariate analysis			
TG–BMI	LOD (TG) = 0.40	LOD (BMI) = 0.10	LOD = 3.31	h^2_r (TG) + S.E. = 0.30 ± 0.12	h^2_r (BMI) + S.E. = 0.29 ± 0.10	$\rho_g = 0.51$	$\rho_q = -1$
TG–FAT	LOD (TG) = 0.40	LOD (FAT) = 0.20	LOD = 3.04	h^2_r (TG) + S.E. = 0.21 ± 0.14	h^2_r (FAT) + S.E. = 0.30 ± 0.11	$\rho_g = 0.67$	$\rho_q = -1$
TG–FATPC	LOD (TG) = 0.40	LOD (FATPC) = 0.10	LOD = 2.24	h^2_r (TG) + S.E. = 0.25[a]	h^2_r (FATPC) + S.E. = 0.37[a]	$\rho_g = 0.57$	$\rho_q = -1$
TG–ASF	LOD (TG) = 0.40	LOD (ASF) = 0.15	LOD = 2.38	h^2_r (TG) + S.E. = 0.24 ± 0.15	h^2_r (ASF) + S.E. = 0.40 ± 0.10	$\rho_g = 0.65$	$\rho_q = -1$

LOD = maximum likelihood score corresponding to one degree of freedom; h^2_r = residual additive genetic heritability; ρ_g = correlation due to residual additive genetic effect; ρ_q = correlation due to QTL effect; S.E. = standard error.
[a] S.E. not available.

ing for TG and BMI. The 1-LOD support interval overlaps for both peaks and extends from 46 to 54 cM (Fig. 2). The hypothesis of coincident linkage was not rejected ($p = 0.06$ for TG–BMI, $p = 0.09$ for TG–FAT, $p = 0.06$ for TG–FATPC, $p = 0.22$ for TG–ASF) nor was the hypothesis of complete pleiotropy for all four bivariate analyses conducted ($\rho_q = -1$, $p > 0.99$).

Chromosome 19q13 harbors two candidate genes (*LIPE* and *APOE*) for the variation in TG levels and adiposity and both are available in the HERITAGE marker panel. Follow-up association analyses were performed for each of the *LIPE* and *APOE* genes with each of the TG and adiposity phenotypes. Allele and genotype frequencies were consistent with those previously reported in the HERITAGE for *LIPE* [9] and for *APOE* [10]. Also as is typically reported, the E3 allele was the most prevalent for *APOE* (79%), and for *LIPE* allele C-60 was more frequent (93%). While TG was significantly associated with each of the *LIPE* ($p < 0.0001$) and *APOE* ($p = 0.0091$) polymorphisms, the adiposity measures were generally associated only with *LIPE* ($p = 0.0044$ for FATPC, $p = 0.0145$ for

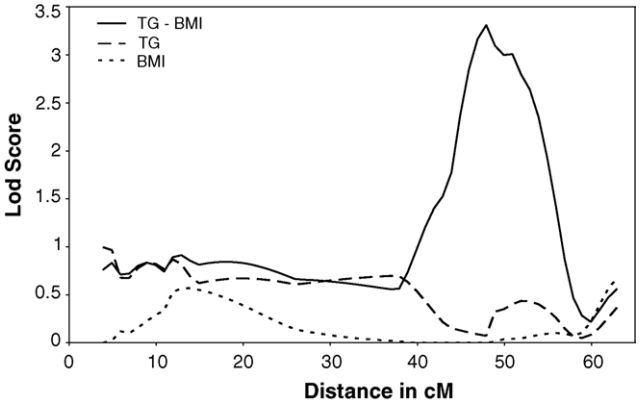

Fig. 1. Overview of the multipoint univariate and bivariate results for TG levels and BMI on chromosome 19. Lod scores are along the *Y*-axis and map locations (in cM) on the *X*-axis.

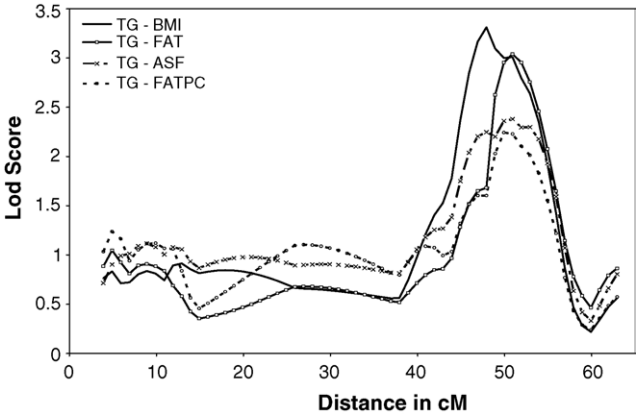

Fig. 2. Overview of the multipoint bivariate linkage results on chromosome 19 for baseline TG levels and BMI, FAT, FATPC and ASF. Lod scores are along the *Y*-axis and map locations (in cM) on the *X*-axis.

ASF). However, when both a marker and an adiposity phenotype were used to predict TG levels, only the *APOE* genotype remained independently significant. This pattern would suggest that TG levels are independently associated with *APOE* polymorphisms, that adiposity is associated with *LIPE* polymorphisms, and the original relationship between TG and LIPE is likely an artifact due to the phenotypic correlation between TG and adiposity.

For the Black families, polygenic heritabilities of 22% for baseline TG levels and ∼57% for adiposity traits were verified, as were additive genetic correlations between TG and adiposity traits that varied from 49 to 53%. Although these correlations suggested common genetic influences, there was no bivariate linkage evidence on 19q or any other chromosome in Black families at baseline, perhaps due to the small sample size. For the Heritage Study, the subjects were recruited in family units consisting of two parents and at least three offspring, however this structure requirement was relaxed in the Black families. The genotype data yielded a maximum of 363 White sibpairs and only 106 Black sibpairs, consequently decreasing the power of variance components bivariate analysis to detect linkage. Also, there was no evidence of a pleiotropic QTL for the exercise response traits in either racial group.

4. Discussion

It is well established that plasma TG levels are correlated with adiposity traits, and both are related to the metabolic syndrome, insulin resistance, and risk of non-insulin-dependent (type 2) diabetes mellitus, and may play a role in the pathogenesis of CHD [16]. Particularly in the HERITAGE Study data, suggestion of linkage for TG levels [14] and adiposity traits [8,15] has been described on several chromosome regions (see Table 2). A genome-wide bivariate linkage analyses found evidence for linkage for the covariation of lipid and adiposity phenotypes, in which the LOD values were non-significant for univariate analyses of TG and adiposity traits, only on chromosome 19q12-q13. The pleiotropic QTL located on 19q12-q13 accounted for ∼30% of total variance for TG levels and from 30 to 40% of total variance for the adiposity phenotypes (Table 3), suggesting that although this QTL may explain considerable variability there are likely other contributing loci as well.

Our findings are corroborated by studies that have reported weak evidence of linkage for TG (LOD of 0.1–1.2) [17,18] and studies that have reported genome-wide significant linkage for TG (LOD of 3.2) [19]. In addition, evidence for linkage on 19q12-q13 has been reported for LDL-cholesterol levels [17,20,21], LDL-2 particles [22], apoE levels [18], and several adiposity traits [23].

Our linkage findings emphasize the relevance of genetic variation on chromosome 19q13 for both obesity and lipid risk factors and the underlying complexity of this genetic effect. The low magnitude of univariate LOD scores for the individual phenotypes perhaps was due to the low power of linkage analysis with relatively small sample sizes. However, the novel result arising from the current study is that the bivariate linkage analysis yielded relatively strong results, as predicted by Allison and colleagues [24]. They demonstrated that multivariate linkage can markedly increase power as compared to univariates, particularly when the cross-trait QTL correlation is negative and the residual genetic correlation is positive. As shown in Table 3, this is the pattern observed for TG and adiposity phenotypes, so by jointly estimating the effects of adiposity and TG, we were able to localize important genetic effects on 19q. These results suggest that the QTL (or a cluster of linked QTLs) in this region may exert effects on both plasma TG levels and adiposity.

Chromosome 19q12-q13 harbors several prominent genes that influence lipid metabolism, adiposity and metabolic syndrome such as the apolipoprotein E (*APOE* and *APOE*/*C-I*/*C-IV*/*C-II* cluster), hormone-sensitive lipase (*LIPE*), low density lipoprotein-receptor-related protein-type3 (*LRP3*), transforming grown factor-beta 1 (TGFB1) and glycogen synthase 1 (GYS1) genes.

There is a large body of evidence supporting a major contribution of the *APOE* gene to total cholesterol, TG, apoB and HDL-cholesterol levels [25,26]. This *APOE* influence varies among subgroups defined by gender, age and body size [25]. TG levels were significantly higher in carriers of *APOE2* and *APOE4* in white men in the HERITAGE Study [10], as was verified again in the current study. Additionally the present results showed associations between the *APOE* marker and TG levels and adiposity traits, suggesting that *APOE* may be the pleiotropic QTL influencing these traits. *APOE* is the major component of chylomicrons, VLDL, and IDL particles and serves as ligand for the *LDLR*. Impaired clearance of chylomicron and VLDL remnants, as a consequence of a defect in *APOE*, results in increased plasma cholesterol and TG levels. *APOC-I* is a component of VLDL and HDL particles and inhibits the lipoprotein lipase (LPL), the enzyme that hydrolyzes triglycerides in plasma and transfers the fatty acids to tissues of the TGs from VLDL. *APOC-II* acts in opposite direction, and it is a necessary cofactor for the activation of lipoprotein lipase (LPL). The functions of *APOC-IV* and *LRP3* are unknown, but there are some suggestions that they might play a role in lipid metabolism [27].

The *LIPE* gene exhibits a broad specificity for lipid substrates such as TG, diglycerides, cholesteryl esters, and retinyl esters and the enzyme is expressed in a wide variety of tissues. The *LIPE* gene has a key role in the regulation of adipocyte lipolysis, catalyzing the breakdown of TG to glycerol and free acids [28]. The enzyme activity in adipose tissue is considered rate-limiting in the degradation of stored TG. Several polymorphisms have been found in the *LIPE* gene, some of which are associated with metabolic dysfunctions. Polymorphism in *LIPE* (HSLi6 A5) was associated with obesity (BMI $\geq 30\,kg/m^2$ and FATPC > 39.6%) in Swedish women [29]. Also a suggestive linkage between the *LIPE* locus and longitudinal changes in TG levels was observed

in American women [30]. Strong gene-by-race-interactions between the C-60G polymorphism was found in the *LIPE* gene and body composition measures, including BMI, FAT, FATPC and ASF in women, but not in men of the HERITAGE Family Study [9]. In the current study, we found that *LIPE* polymorphism effect on TG levels was mediated by these adiposity measures. This is consistent with the findings that one form of *LIPE* is associated with decreased lipolysis in abdominal subcutaneous adipocytes of obese subjects [31]. However, because the relevance of the *LIPE* gene on the variation of lipid levels and obesity we need be cautions about this implication.

In conclusion, there is suggestive evidence for a pleiotropic QTL at 19q12-q13 for the covariation of lipid levels and adiposity in White families. Further studies of the genes located on chromosome 19q12-q13 (e.g. *APOE* and *LIPE*) should probably benefit from considering both the lipid and adiposity phenotypes jointly in a multivariate setting. We believe these findings are relevant and may guide further investigation into gene discovery. Particularly, the *APOE* gene should be considered to narrow-down the chromosome 19q13 in follow-up studies.

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