patient-oriented research

Heritability and genetic loci of fatty liver in familial combined hyperlipidemia

Martijn C. G. J. Brouwers,[1,*] Rita M. Cantor,[†,§] Naoko Kono,[†] Jeong lim Yoon,[†] Carla J. H. van der Kallen,[*] Monique A. L. Bilderbeek-Beckers,[**] Marleen M. J. van Greevenbroek,[*] Aldons J. Lusis,[†,††] and Tjerk W. A. de Bruin[2,*]

Department of Medicine and Cardiovascular Research Institute Maastricht,* Academic Hospital Maastricht, Maastricht, The Netherlands; Departments of Human Genetics,† Pediatrics,§ and Medicine,†† David Geffen School of Medicine, University of California Los Angeles, Los Angeles, CA; and Department of Radiology,** VieCuri Medical Center Noord Limburg, Venlo, The Netherlands

Abstract VLDL overproduction, a process that is driven by an excess amount of hepatic fat, is a well-documented feature of familial combined hyperlipidemia (FCHL). The aims of this study were to investigate whether fatty liver, measured with ultrasound and as plasma alanine aminotransferase (ALT) levels, develops against a genetic background in FCHL and to identify chromosomal loci that are linked to these traits. In total, 157 FCHL family members and 20 spouses participated in this study. Radiological evidence of fatty liver was more prevalent not only in FCHL probands (40%) but also in their relatives (35%) compared with spouses (15%) ($P < 0.05$). Heritability calculations revealed that 20–36% of the variability in ALT levels could be attributed to genetic factors. Nonparametric quantitative trait locus (QTL) analysis revealed three significant ($P < 0.001$) loci with either the ultrasound or the ALT trait in the male sample: 1q42.3, 7p12-21, and 22p13-q11; none was found in the female sample or the entire group. Of these QTLs, the 7p region was consistent over time, because reanalysis with ALT levels that were determined during a visit 5 years earlier yielded similar results. This study shows that fatty liver is a heritable aspect of FCHL. Replication of particularly the 7p region is awaited.—Brouwers, M. C. G. J., R. M. Cantor, N. Kono, J. l. Yoon, C. J. H. van der Kallen, M. A. L. Bilderbeek-Beckers, M. M. J. van Greevenbroek, A. J. Lusis, and T. W. A. de Bruin. **Heritability and genetic loci of fatty liver in familial combined hyperlipidemia. *J. Lipid Res.* 2006.** 47: **2799–2807.**

Supplementary key words steatosis • nonalcoholic fatty liver disease • quantitative trait locus • linkage analysis • alanine aminotransferase

More than three decades ago, familial combined hyperlipidemia (FCHL) was delineated as a highly prevalent (1:100) primary hyperlipidemia that was associated with premature coronary artery disease (1). Although it was initially assumed that FCHL was inherited as an autosomal dominant disease (1), subsequent studies revealed that FCHL follows a complex segregation pattern (2, 3). However, to date, a true understanding of the genetic background of FCHL is still lacking, despite numerous genome screens and positional candidate association studies with FCHL-related traits, such as plasma triglycerides, total cholesterol, apolipoprotein B (apoB), and LDL particle size (4–7). One major breakthrough in the genetic dissection of FCHL was the recent identification and subsequent confirmation of upstream stimulatory factor 1 located on chromosome 1q21-23 (8, 9). Functional evidence is now accumulating that variants in the upstream stimulatory factor 1 gene can indeed contribute to the development of hyperlipidemia (10, 11).

Today, it is commonly accepted that the increased plasma triglycerides, total cholesterol, and apoB in FCHL are heterogeneous in origin, because they are the consequences of both an overproduction of VLDL particles and a decreased clearance of remnants (12–14). Therefore, researchers have stated that lipid levels are not the optimal phenotypical markers for elucidating the genetic background of FCHL (15). However, easily obtainable markers that directly reflect either the overproduction or clearance of lipid particles have not been available.

Previous experimental studies in human subjects have suggested that the production of VLDL particles is driven by the amount of fat that is supplied to the liver (16, 17). Adiels and colleagues (18) confirmed this assumption by reporting that, in both normal subjects and patients with type 2 diabetes mellitus, the production of VLDL particles is indeed related to the hepatic fat content. Of interest, our laboratory recently demonstrated that an increased hepatic fat content is a common feature of FCHL as well,

Manuscript received 18 July 2006 and in revised form 12 September 2006.
Published, JLR Papers in Press, September 13, 2006.
DOI 10.1194/jlr.M600312-JLR200

[1] To whom correspondence should be addressed.
e-mail: martijn.brouwers@intmed.unimaas.nl
[2] Present address of T. W. A. de Bruin: GlaxoSmithKline, Translational Medicine and Genetics, Research Triangle Park, NC 27713.

in particular in patients with the hypertriglyceridemic phenotype (19).

Therefore, this study was conducted to investigate whether an increased hepatic fat content is involved in the genetic background of FCHL. For this purpose, surrogates of fatty liver [i.e., liver ultrasound and plasma alanine aminotransferase (ALT) levels] were determined in our well-defined FCHL pedigrees and correlated with the traditional FCHL lipid traits. Subsequently, the heritability of fatty liver was estimated and a quantitative trait locus (QTL) analysis was performed to search the genome for chromosomal loci implicated in the pathogenesis of fatty liver and hyperlipidemia in FCHL.

MATERIALS AND METHODS

Study population

FCHL patients, their relatives, and spouses were ascertained as described previously (20). Because FCHL family members and their spouses share a similar environment, any observed statistical difference is likely to be explained by genetic factors. Therefore, it should be noted that the spouses do not necessarily represent a random sample from the general population.

All subjects visited our lipid clinic after an overnight fast and total abstinence from alcohol for the last three days. They were withdrawn from lipid medication 2 weeks before the visit. Furthermore, subjects did not consume more than two units of alcohol daily, did not report recent rapid weight loss, and did not use any medication associated with the development of fatty liver (21). One subject was excluded from the study because of positive serology for hepatitis C.

This study was approved by the Human Investigation Research Committee of the Academic Hospital of Maastricht/Maastricht University and the University of California Los Angeles institutional review board. All subjects gave written informed consent.

Height was determined with a stadiometer, and weight was measured while wearing only underwear. Body mass index (BMI) was calculated as weight divided by height squared (kg/m^2).

Plasma measurements

Blood was collected in precooled EDTA tubes. After centrifugation at 3,000 rpm for 15 min at 4°C, plasma aliquots were stored at −80°C. ApoB, total cholesterol, HDL-cholesterol, and triglycerides were measured as described previously (20). ALT levels were measured with a commercially available assay (Ecoline® S+; DiaSys Diagnostic Systems GmbH). ALT levels were also determined in plasma samples that were withdrawn during all subject screening visits 5 years earlier (ALT_{1999}). Preparation of subjects and handling of plasma in 1999 were similar to those described above.

Ultrasound

Ultrasound of the liver was performed with an ATL9 HDI (Bothel) ultrasound system using C7-4 and C4-2 transducers. Standardized images and movies of the liver and right kidney were recorded on videotape and examined by a radiologist unaware of the subject's clinical characteristics. Criteria for fatty liver were as described previously (22, 23). In short, the four classifications were as follows: *1*) normal liver, defined as normal hepatic echotexture and normal beam attenuation; *2*) mild steatosis, characterized by slight increase in echogenicity of liver parenchyma compared with right kidney, with minimal or no decrease of visualization of hepatic vessels and diaphragm; *3*) moderate steatosis, defined as diffuse increase in echogenicity of the liver with slightly impaired visualization of intrahepatic vessels and diaphragm; and *4*) severe steatosis, characterized by marked increase of liver echogenicity, poor visualization of intrahepatic vessels, and increased posterior beam attenuation represented by nonvisualization of the diaphragm. The intraobserver agreement expressed as kappa, determined in 30 random scans, was good ($\kappa = 0.68$) and in agreement with earlier studies (23).

Genotyping

Genotyping of the 377 informative microsatellite markers was done by the Marshfield genotyping service (http://www.marshfieldclinic.org/research/genetics/), as described previously (7). Markers of Marshfield panel 10 were used with an average intermarker distance of 9.4 centimorgan (cM). In total, 157 FCHL family members and 20 spouses were genotyped.

Statistical and genetic analyses

Population characteristics. Differences in general descriptives between FCHL probands, their relatives, and their spouses were calculated with linear regression for continuous traits and with logistic regression for binary traits, both with inclusion of age and sex. FCHL status was entered as a dummy variable into these models (FCHL proband/relative = 1, spouse = 0). Sex-specific correlations between fatty liver measured with ultrasound and ALT levels were calculated with ordinal regression. All of these analyses were conducted with the SPSS 13.0 statistical package (SPSS, Inc.).

Heritability of ALT levels. Familiality of ALT levels, the continuous trait used as a surrogate for fatty liver, was assessed in two ways. First, the FCOR subprogram of the SAGE software package (24) was used to estimate the intraclass correlation for sibling pairs. This correlation is relevant because our linkage analyses were also conducted in sibling pairs (see below). Intraclass correlations were also calculated for plasma triglycerides, total cholesterol, HDL-cholesterol, and apoB levels.

Additionally, the variance component model implemented in SOLAR (25) was used to estimate the familiality of the normalized deviates of ALT. Similar analyses were performed for ALT values adjusted for BMI.

It should be noted that the heritability estimate is strongly dependent on both the reproducibility of the assay, which was high for ALT (coefficient of variation, 3.5%), and the temporal (biological) variation in the trait value.

Identification of genetic loci. Multipoint QTL analysis at 1 cM intervals was conducted using the nonparametric model-free analysis option of the Genehunter software (26), which correlates the differences in trait levels within sibling pairs with their degree of allele sharing identical by descent along the chromosomes. The linkage map was sex-averaged. In the case of linkage in a region, similar trait values (i.e., plasma ALT levels or liver ultrasound stages) are expected to occur in sibling pairs with increased marker allele sharing, whereas those pairs that have markedly different trait values will exhibit less marker allele sharing. This is assessed with a statistical test similar to the Kruskal-Wallis test. $P < 0.001$ was regarded as significant to identify a QTL. For continuous traits with more normal distributions (i.e., ALT levels), the Haseman-Elston option of the Genehunter software was used as well. It correlates allele sharing identical by descent with the

TABLE 1. Population characteristics

Characteristic	Spouses	Relatives	FCHL Probands
Male/female	9/11	67/70	12/8
Age (years)	58.6 ± 9.2	45.4 ± 14.9[a]	57.1 ± 9.1
BMI (kg/m^2)	26.1 (22.9–28.7)	25.8 (23.1–28.9)	27.0 (24.3–28.6)
Apolipoprotein B (g/l)	1.0 ± 0.3	1.1 ± 0.3[b]	1.2 ± 0.3
Cholesterol (mmol/l)	5.6 ± 1.0	5.6 ± 1.6	6.9 ± 2.2[b]
HDL-cholesterol (mmol/l)	1.1 ± 0.3	0.9 ± 0.3	0.9 ± 0.3
Triglycerides (mmol/l)	1.1 (1.0–1.9)	1.3 (1.0–2.0)[b]	3.1 (1.4–3.8)[b]
ALT (U/l)	15.2 (13.1–17.8)	18.2 (13.8–25.6)[b]	21.7 (16.6–26.1)[b]
Fatty liver (US) (%)	15	35[c]	40[c]

ALT, alanine aminotransferase; BMI, body mass index; FCHL, familial combined hyperlipidemia; US, ultrasound. Data are presented as means ± SD or as median and (interquartile range). All analyses were Hochberg corrected.
[a] $P < 0.05$, compared with spouses, by Student's *t*-test.
[b] $P < 0.05$, compared with spouses, by linear regression, age and sex adjusted.
[c] $P < 0.05$, compared with spouses, by logistic regression, age and sex adjusted.

squares of trait differences in sibling pairs by regressing the squared trait differences against the estimated allele sharing.

RESULTS

Sample characteristics

Descriptive statistics of FCHL probands, their relatives, and their spouses are displayed in **Table 1**. FCHL probands had higher plasma triglycerides and total cholesterol levels compared with their spouses, and apoB levels were increased, but not significantly. Furthermore, both surrogates of fatty liver (i.e., plasma ALT and liver ultrasound) suggest a higher prevalence of fatty liver, not only in the FCHL probands but also in their relatives (Table 1).

Of note, in all FCHL family members (probands and relatives combined), plasma ALT levels were related to the different stages of fatty liver as measured with ultrasound. ($r = 0.49$, $P < 0.001$; ordinal regression). Furthermore, ALT levels were within the normal range. However, as shown in **Fig. 1**, a marked sex difference was observed for this ALT-ultrasound relation ($r = 0.69$, $P < 0.001$ in men versus $r = 0.27$, $P = 0.03$ in women), a phenomenon

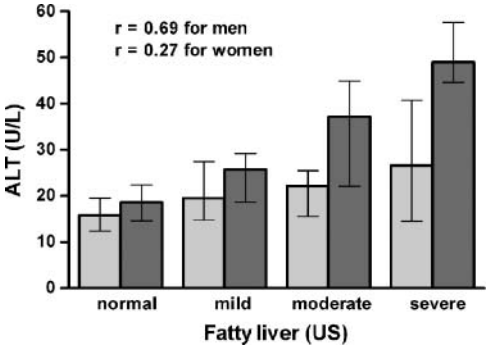

Fig. 1. Relation of fatty liver severity by ultrasound (US) versus plasma alanine aminotransferase (ALT) levels in all familial combined hyperlipidemia (FCHL) family members (n = 157), split by sex (dark bars, men; light bars, women). ALT levels are presented as medians with interquartile ranges. Correlation coefficients were calculated with ordinal regression (Nagelkerke pseudo *r*).

that has also been found for the relation ALT-fatty liver as measured with magnetic resonance spectroscopy (27).

Relation of surrogates of fatty liver with plasma lipid levels

To assess whether fatty liver is related to the plasma lipid levels used to determine the characteristic FCHL phenotype, we calculated correlations of the surrogates of fatty liver with plasma apoB, total cholesterol, and triglycerides in all FCHL family members (i.e., probands and relatives combined). As shown in **Fig. 2**, no significant correlations were observed for apoB and total cholesterol levels with the ultrasound assessment of fatty liver (A, C), and marginal associations were found with ALT levels (B, D). A much stronger correlation was observed between the surrogates of fatty liver and plasma triglyceride concentrations (E, F).

Heritability of ALT levels

The intraclass correlation estimated with SAGE FCOR (24) in 230 sibling pairs (**Table 2**) was 0.18 for log ALT (sex-adjusted), corresponding to a maximum heritability estimate of 0.36 ($2r = h^2$) (28). Intraclass correlations were 0.12 ($h^2 = 0.24$) for plasma triglycerides, 0.27 ($h^2 = 0.54$) for total cholesterol, 0.23 ($h^2 = 0.46$) for HDL-cholesterol, and 0.08 ($h^2 = 0.16$) for apoB levels, indicating that the maximum heritability for the traditional FCHL traits are in a similar range as those for ALT levels.

Heritability estimates for ALT levels when all family relationships were included were a maximum of 0.20 ($P = 0.09$, by SOLAR). Of interest, a correction for BMI by including it as a covariate in the model did not reduce the value of the maximum heritability estimates for log ALT (0.27; $P = 0.03$, by SOLAR), implying that BMI is not a major contributor to these heritability estimates.

QTL analysis for surrogates of fatty liver

Nonparametric multipoint QTL analyses were conducted for log ALT (sex-adjusted; continuous trait) and for the stages of fatty liver by ultrasound (US) (ordinal trait). This analysis did not reveal any QTLs when the stringent criterion of $P < 0.001$ was applied. Because a sex-specific relation was found for plasma ALT levels with fatty liver stages

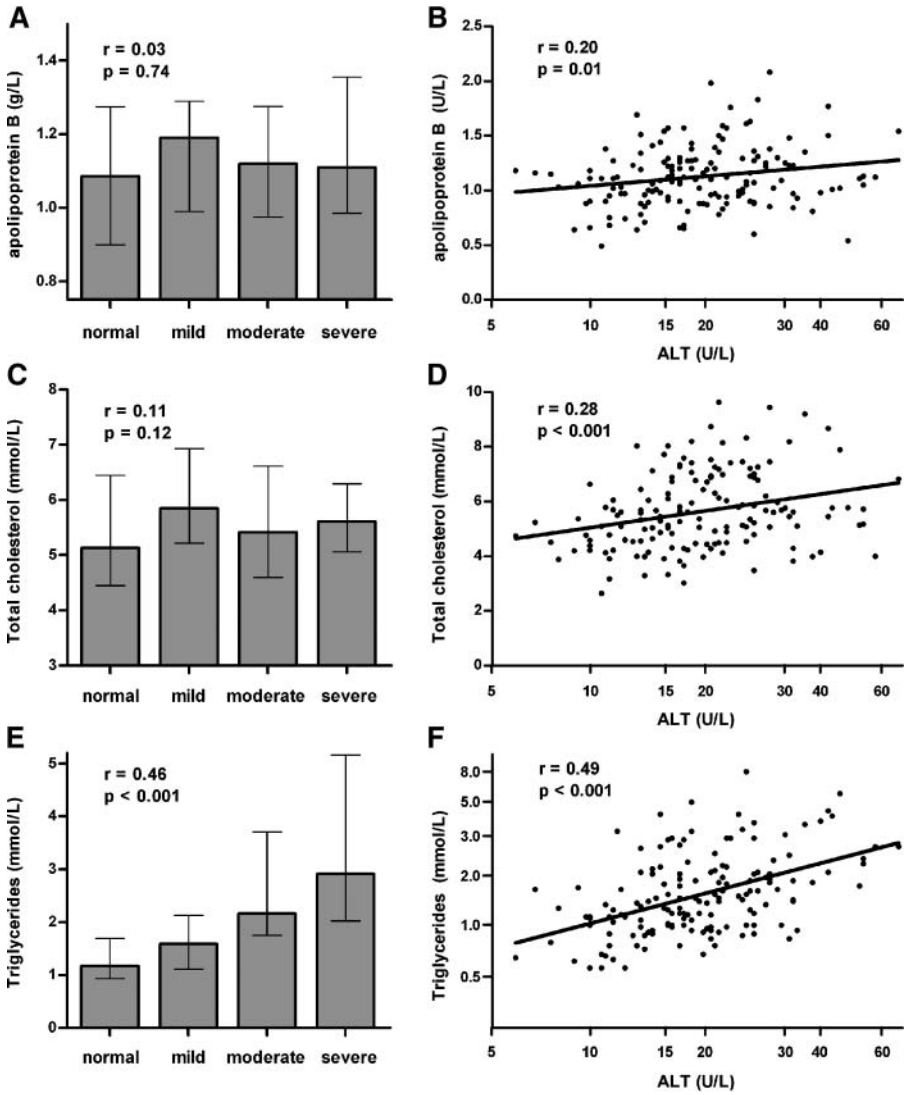

Fig. 2. Relation of ultrasound stages of fatty liver and ALT levels with apolipoprotein B (A, B), total cholesterol (C, D), and triglycerides (E, F) in all FCHL family members. Data in A, C, and E are presented as medians with interquartile ranges. ALT levels are presented on a log scale.

(US) (Fig. 1), QTL analyses were subsequently conducted by splitting the sample by sex. Although the male sample was reduced to 72 sibpairs (Table 2), one borderline significant ($P = 0.001$) and two significant ($P < 0.001$) QTLs were observed for either log ALT levels or fatty liver (US). These were located on chromosome 1q42.3 [$P = 0.001$, for fatty liver (US)], 7p12-21 ($P = 0.0002$, for log ALT), and 22p13-q11 [$P = 0.0007$, for fatty liver (US)] (**Table 3**). No QTLs were found in the female sample. The male sample contained one potentially influential large pedigree consisting of nine siblings (Table 2); however, similar QTL results were obtained when the analysis was conducted without this particular family (data not shown). The peak marker, the marker distance (from the p-terminal end of

TABLE 2. Sibships in heritability and QTL analyses

	Sibship Size								
Variable	2	3	4	5	6	7	9	11	Total
Number of sibships	28 (18)	12 (2)	5 (2)	3	2	1	(1)	1	52 (23)
Number of individuals	56 (34)	36 (6)	20 (8)	15	12	7	(9)	11	157 (57)
Number of sibpairs	28 (18)	36 (6)	30 (12)	30	30	21	(36)	55	230 (72)
Number of independent sibpairs	28 (18)	24 (4)	15 (6)	12	10	6	(8)	10	105 (36)

QTL, quantitative trait locus. Data are presented for the total FCHL population (men and women combined). The data for the male sample are presented in parentheses.

TABLE 3. QTLs for fatty liver (US) and log ALT by $P < 0.001$ in multipoint analyses of male FCHL sibships

Quantitative Trait	Marker Closest to QTL	QTL Chromosome Band	Marker Distance from Telomere	Nonparametric Z Score (P)	Haseman-Elston Log of the Odds Score
Fatty liver (US)	D1S235	1q42.3	251	3.1 (0.001)	
Log ALT	D1S3462	1q42.2	243	2.7 (0.004)	1.8
Fatty liver (US)	D7S2846	7p14.1	56	2.2 (0.01)	
Log ALT	D7S2846	7p14.1	62	3.6 (0.0002)	2.8
Fatty liver (US)	D22S686	22q11.22	12	3.2 (0.0007)	
Log ALT	D22S686	22q11.22	9	2.4 (0.008)	1.3

Haseman-Elston log of the odds score is given only for the continuous trait log ALT (see Materials and Methods).

the chromosome), and the Haseman-Elston log of the odds score for the three QTLs are also presented in Table 3. Plots of the QTLs for log ALT and fatty liver (US) in the male FCHL sample are displayed in **Fig. 3**. Of interest, the QTL plots for fatty liver (US) and log ALT are very similar, which likely reflects the correlation between these traits in the male population ($r = 0.69$), as was shown in Fig. 1. Furthermore, the QTL plots for log ALT and log ALT corrected for BMI are similar in shape in all three graphs, illustrating that BMI does not contribute to the evidence for linkage to this region (Fig. 3). Of interest, linkage peaks were observed for BMI only at 1q42.3 and 7p12-21 but not at 22p13-q11 (Fig. 3).

QTL analysis for plasma triglyceride levels in the 1q, 7p, and 22q regions

Because both surrogates of fatty liver were correlated with plasma triglycerides (Fig. 2E, F), one of the diagnostic hallmarks of FCHL, we subsequently decided to conduct QTL analyses for plasma triglycerides in the three significant regions. As shown in **Fig. 4**, the *Z* scores for plasma triglycerides were markedly lower than the original QTL for log ALT. However, the plots for triglycerides were similarly shaped, in particular for the 7p region (Fig. 4B), which probably reflects the plasma triglyceride-ALT relation. This relation was also reflected by a diminished linkage signal of the original QTL at 7p and 22q when ALT levels were corrected for plasma triglycerides (Fig. 4B, C). The unchanged QTL at 1q (Fig. 4A) probably indicates that this locus is specific for ALT levels but does not affect plasma triglycerides.

QTL analysis for ALT_{1999} levels in the 1q, 7p, and 22q regions

To further substantiate the observed linkage peaks, QTL analyses were conducted for log ALT levels that were determined in all subjects during their screening visits 5 years before the current study (further referred to as ALT_{1999}). In addition, ALT_{1999} levels were also corrected for BMI, which was determined 5 years earlier (BMI_{1999}). Similar to our previous report (29), we assumed that, because a genetic predisposition does not change over time, QTLs are not expected to change over time either, unless there is a strong environmental component that affects the trait (29). Of note, very similar intraclass correlation and heritability estimate were observed for ALT_{1999} levels in the overall FCHL population (intraclass correlation, 0.18, by SAGE; maximum heritability, 0.19, by SOLAR).

As shown in **Fig. 5A**, C, the QTL for the original ALT (ALT_{2004}) trait did not appear at the 1q and 22q regions when analyzed with the ALT_{1999} values, nor with ALT_{1999} adjusted for BMI_{1999}. In contrast, the QTL on chromosome 7 was observed with the ALT_{1999} and ALT_{1999} adjusted for BMI_{1999} traits (Fig. 5B). Furthermore, the QTL was narrowed from 7p12-21 to 7p12-15 ($Z_{max} = 2.7$ at 33 cM; $P = 0.003$ for ALT_{1999} adjusted for BMI_{1999}). We interpret this to mean that when the linkage results for ALT_{2004} and ALT_{1999} levels are compared, the observed QTL on chromosome 7 is least likely to result from a type 1 statistical error.

Of note, the linkage results for BMI_{1999} were very similar to the original results for BMI (data not shown).

DISCUSSION

This study was conducted to explore whether fatty liver develops against a genetic background in FCHL and, when affirmative, whether the fatty liver trait can be used in linkage analysis to unravel the genetic susceptibility to develop FCHL. These research questions were triggered by our previous observation of an increased prevalence of fatty liver in an outpatient FCHL population (19) and the recently reported relation between fatty liver and VLDL overproduction in normal subjects and patients with type 2 diabetes mellitus (18).

Both surrogates of fatty liver that were used in this study (i.e., liver ultrasound and plasma ALT levels) were significantly correlated with plasma triglyceride levels in all FCHL family members, analogous to what has been observed in non-FCHL populations (18). This relation probably reflects the relation between fatty liver and the overproduction of VLDL particles, a well-documented feature of FCHL (12, 13). Of interest, we very recently reported that VLDL-apoB levels are also related to the amount of hepatic fat in FCHL (30). Although this observation seems to be in contrast with the current almost absent relation between fatty liver surrogates and total apoB levels, it is likely explained by the fact that only a minor part (<10%) of the variation in total apoB levels is accounted for by VLDL-apoB (31).

This study demonstrated that the prevalence of fatty liver as measured with ultrasound was increased not only

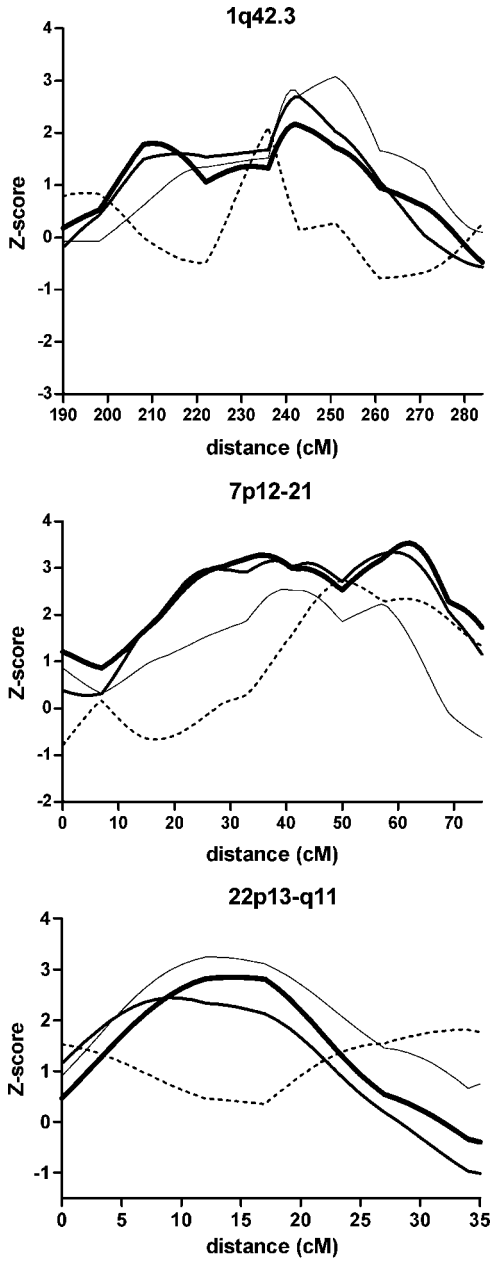

Fig. 3. Quantitative trait loci (QTLs) identified by $P < 0.001$ for either fatty liver (US) (thin lines) or log ALT (intermediate lines) in male FCHL sibships. QTLs are also shown for log ALT adjusted for body mass index (BMI; thick lines) and for BMI only (dashed lines). cM, centimorgan.

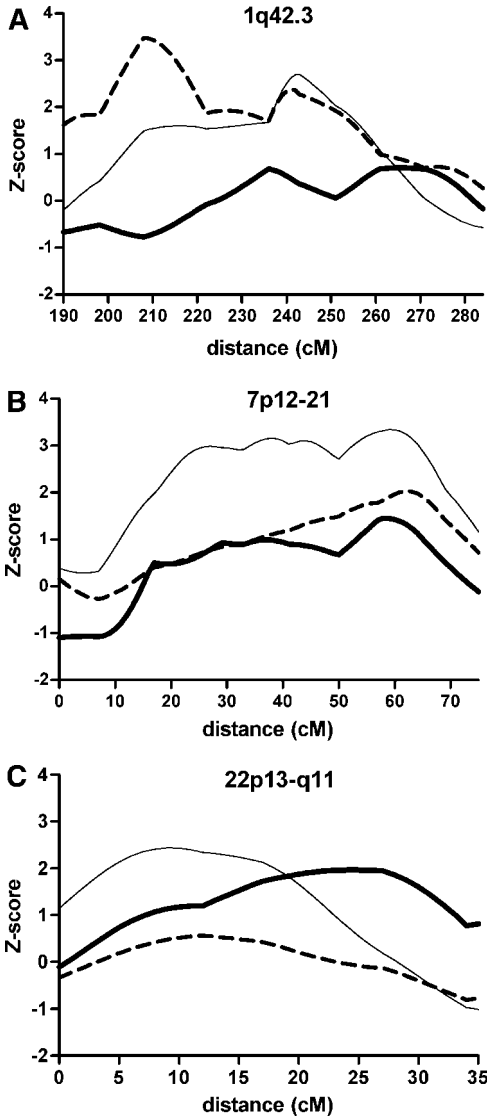

Fig. 4. QTLs for plasma triglycerides (thick lines) and for ALT levels corrected for triglycerides (dashed lines) compared with the original QTL for log ALT (thin lines) in the 1q (A), 7p (B), and 22q (C) regions.

in FCHL probands but also in their relatives, which is suggestive of the presence of a genetic component. Similar results were obtained with ALT levels, a liver-specific enzyme that was, within its normal range, associated with the ultrasound stages of fatty liver. The suggestion of a genetic component was subsequently confirmed using two different analytical strategies as implemented in SOLAR (25) and SAGE (24): 20–36% of the variability in fatty liver, measured as plasma ALT levels, can be attributed to genetic factors. This is the first study that has estimated the contribution of genetic factors to variations in fatty liver. This contribution was within the same range as the more traditional FCHL traits that were included as a reference. Of note, the high maximum heritability for total cholesterol and HDL-cholesterol and the low heritability for plasma triglycerides are in agreement with previous reports (32, 33).

A subsequent genomic search for chromosomal regions associated with surrogate markers of fatty liver (i.e., ultrasound and ALT levels) revealed three loci in the male sample that fulfilled our significance criterion: 1q42.3, 7p12-21, and 22p13-q11 [Genehunter software (26)]; no QTLs were observed in the female sample and overall population. We are reporting QTLs with $P < 0.001$, but we recognize that appropriate correction for multiple testing has not been made. Such a correction is not straightforward, because two of the samples (male and female) are nested in the third (overall population). We are reporting

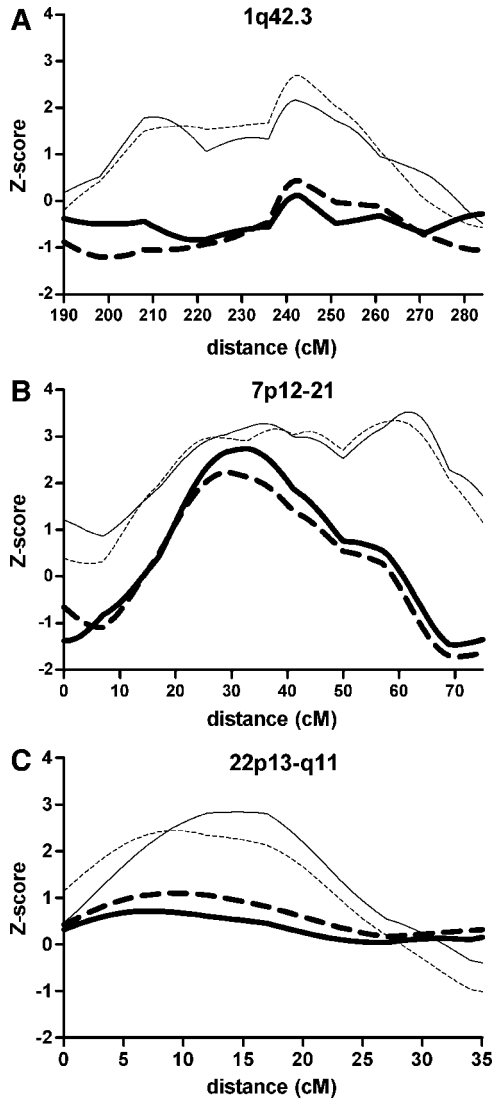

Fig. 5. QTLs for log ALT_{1999} (thick dashed lines) and ALT_{1999} adjusted for BMI (thick lines) compared with the original QTLs (2004) for log ALT (thin dashed lines) and log ALT adjusted for BMI (thin lines) in the 1q (A), 7p (B), and 22q (C) regions.

these results and suggest replication before additional gene-finding efforts in these regions.

The three observed QTLs in the male sample were not confounded by obesity, an important causal factor in the development of fatty liver (34, 35), because correction for body mass index did not affect the original QTL. Analysis with only BMI resulted in linkage peaks at 1q and 7p as well. The currently observed QTLs have not been reported before in FCHL. Although linkage has been noted for apoA-II levels in the 1q41 region (36), it is probably too far away from our observed QTL for fatty liver at 1q42.3. Because we are the first to use this refined phenotype, it is possible that we have detected QTLs that would not have been found with the traditional FCHL traits. This is illustrated by our linkage results for plasma triglycerides: although the curve was similarly shaped for triglycerides and ALT levels (Fig. 5), the results for triglycerides did not reach significance by far. Of note, a QTL for BMI has been reported before for the 7p region (37–39).

The sex specificity of our findings is not exceptional, because very recently it was shown that sex-specific genetic effects are observed for many common traits, such as HDL-cholesterol, blood pressure, eosinophilia, and lymphocyte count (40). Of note, most significant linkage results were found in the male population (40), probably because of the more homogeneous nature of that sample. Because estrogens exert well-known effects on lipid and liver metabolism (41–43), variations in estrogen concentrations among premenopausal and postmenopausal women have probably introduced substantial noise in our data set.

The aspect of false-positive results is a frequently encountered problem in the field of linkage analysis (44). Therefore, experts have proposed guidelines to reduce the occurrence of type 1 errors as much as possible (44, 45). We applied, similar to our previous report (29), an alternative strategy that can be regarded as a first validation step to avoid false-positive results (46): because the maximum heritability of ALT levels did not change over time, we assumed that a true QTL should not change over time either. Therefore, QTL analysis was repeated with ALT levels that were determined during another visit, 5 years earlier. Reanalysis revealed that of the three initially observed QTLs, the one at 7p is most worthwhile to pursue, whereas the 1q and 22q regions may be type 1 errors.

Power is an additional important criterion for QTL analyses. Given the number of sibling pairs in our sample, we have 80% power to identify QTLs with locus-specific heritabilities of 15% or greater when a 0.05 level of significance is set. Given our stringent criterion of 0.001, we have sufficient power to identify QTLs of 30% locus-specific heritabilities; thus, we are likely to have missed many QTLs of smaller effects.

In this study, ultrasound and ALT levels were used as surrogates of fatty liver. The gold standard (i.e., liver biopsy) was ethically not acceptable in this relatively healthy population. Furthermore, the use of magnetic resonance spectroscopy, an alternative method to assess the amount of hepatic fat, is limited in large-sized populations, such as the present one. Nevertheless, we are confident that we have carefully studied the role of genetic loci in fatty liver in men, because both ultrasound, a validated measure of moderate and severe stages of fatty liver (23), and ALT, a plasma marker that is, within its normal range, fairly correlated with hepatic fat by magnetic resonance spectroscopy (27), yielded similar QTL outcomes in the male population.

In summary, this study has demonstrated that fatty liver is a heritable aspect of FCHL. A subsequent genomic screen for surrogates of fatty liver revealed three QTLs in the male sample, of which the chromosomal 7p12-15 region appeared to be the most consistent QTL over time. Therefore, replication and fine mapping of this region are awaited.

This work was supported in part by National Institutes of Health Grant HL-28481. M.C.G.J.B. received a travel grant from the Netherlands Genomics Initiative (050-72-409).

REFERENCES

1. Goldstein, J. L., H. G. Schrott, W. R. Hazzard, E. L. Bierman, and A. G. Motulsky. 1973. Hyperlipidemia in coronary heart disease. II. Genetic analysis of lipid levels in 176 families and delineation of a new inherited disorder, combined hyperlipidemia. *J. Clin. Invest.* **52:** 1544–1568.
2. Jarvik, G. P., J. D. Brunzell, M. A. Austin, R. M. Krauss, A. G. Motulsky, and E. Wijsman. 1994. Genetic predictors of FCHL in four large pedigrees. Influence of apoB level major locus predicted genotype and LDL subclass phenotype. *Arterioscler. Thromb.* **14:** 1687–1694.
3. Pajukanta, P., J. D. Terwilliger, M. Perola, T. Hiekkalinna, I. Nuotio, P. Ellonen, M. Parkkonen, J. Hartiala, K. Ylitalo, J. Pihlajamaki, et al. 1999. Genomewide scan for familial combined hyperlipidemia genes in Finnish families, suggesting multiple susceptibility loci influencing triglyceride, cholesterol, and apolipoprotein B levels. *Am. J. Hum. Genet.* **64:** 1453–1463.
4. Allayee, H., K. L. Krass, P. Pajukanta, R. M. Cantor, C. J. van der Kallen, R. Mar, J. I. Rotter, T. W. de Bruin, L. Peltonen, and A. J. Lusis. 2002. Locus for elevated apolipoprotein B levels on chromosome 1p31 in families with familial combined hyperlipidemia. *Circ. Res.* **90:** 926–931.
5. Naoumova, R. P., S. A. Bonney, S. Eichenbaum-Voline, H. N. Patel, B. Jones, E. L. Jones, J. Amey, S. Colilla, C. K. Neuwirth, R. Allotey, et al. 2003. Confirmed locus on chromosome 11p and candidate loci on 6q and 8p for the triglyceride and cholesterol traits of combined hyperlipidemia. *Arterioscler. Thromb. Vasc. Biol.* **23:** 2070–2077.
6. Mar, R., P. Pajukanta, H. Allayee, M. Groenendijk, G. Dallinga-Thie, R. M. Krauss, J. S. Sinsheimer, R. M. Cantor, T. W. de Bruin, and A. J. Lusis. 2004. Association of the APOLIPOPROTEIN A1/C3/A4/A5 gene cluster with triglyceride levels and LDL particle size in familial combined hyperlipidemia. *Circ. Res.* **94:** 993–999.
7. Cantor, R. M., T. de Bruin, N. Kono, S. Napier, A. van Nas, H. Allayee, and A. J. Lusis. 2004. Quantitative trait loci for apolipoprotein B, cholesterol, and triglycerides in familial combined hyperlipidemia pedigrees. *Arterioscler. Thromb. Vasc. Biol.* **24:** 1935–1941.
8. Pajukanta, P., H. E. Lilja, J. S. Sinsheimer, R. M. Cantor, A. J. Lusis, M. Gentile, X. J. Duan, A. Soro-Paavonen, J. Naukkarinen, J. Saarela, et al. 2004. Familial combined hyperlipidemia is associated with upstream transcription factor 1 (USF1). *Nat. Genet.* **36:** 371–376.
9. Huertas-Vazquez, A., C. Aguilar-Salinas, A. J. Lusis, R. M. Cantor, S. Canizales-Quinteros, J. C. Lee, L. Mariana-Nunez, R. M. Riba-Ramirez, A. Jokiaho, T. Tusie-Luna, et al. 2005. Familial combined hyperlipidemia in Mexicans: association with upstream transcription factor 1 and linkage on chromosome 16q24.1. *Arterioscler. Thromb. Vasc. Biol.* **25:** 1985–1991.
10. Naukkarinen, J., M. Gentile, A. Soro-Paavonen, J. Saarela, H. A. Koistinen, P. Pajukanta, M. R. Taskinen, and L. Peltonen. 2005. USF1 and dyslipidemias: converging evidence for a functional intronic variant. *Hum. Mol. Genet.* **14:** 2595–2605.
11. Hoffstedt, J., M. Ryden, H. Wahrenberg, V. van Harmelen, and P. Arner. 2005. Upstream transcription factor-1 gene polymorphism is associated with increased adipocyte lipolysis. *J. Clin. Endocrinol. Metab.* **90:** 5356–5360.
12. Venkatesan, S., P. Cullen, P. Pacy, D. Halliday, and J. Scott. 1993. Stable isotopes show a direct relation between VLDL apoB overproduction and serum triglyceride levels and indicate a metabolically and biochemically coherent basis for familial combined hyperlipidemia. *Arterioscler. Thromb.* **13:** 1110–1118.
13. Kissebah, A. H., S. Alfarsi, and P. W. Adams. 1981. Integrated regulation of very low density lipoprotein triglyceride and apolipoprotein-B kinetics in man: normolipemic subjects, familial hypertriglyceridemia and familial combined hyperlipidemia. *Metabolism.* **30:** 856–868.
14. Cabezas, M. C., T. W. de Bruin, H. Jansen, L. A. Kock, W. Kortlandt, and D. W. Erkelens. 1993. Impaired chylomicron remnant clearance in familial combined hyperlipidemia. *Arterioscler. Thromb.* **13:** 804–814.
15. Porkka, K. V., I. Nuotio, P. Pajukanta, C. Ehnholm, L. Suurinkeroinen, M. Syvanne, T. Lehtimaki, A. T. Lahdenkari, S. Lahdenpera, K. Ylitalo, et al. 1997. Phenotype expression in familial combined hyperlipidemia. *Atherosclerosis.* **133:** 245–253.
16. Malmstrom, R., C. J. Packard, M. Caslake, D. Bedford, P. Stewart, H. Yki-Jarvinen, J. Shepherd, and M. R. Taskinen. 1998. Effects of insulin and acipimox on VLDL1 and VLDL2 apolipoprotein B production in normal subjects. *Diabetes.* **47:** 779–787.
17. Lewis, G. F., K. D. Uffelman, L. W. Szeto, B. Weller, and G. Steiner. 1995. Interaction between free fatty acids and insulin in the acute control of very low density lipoprotein production in humans. *J. Clin. Invest.* **95:** 158–166.
18. Adiels, M., M. R. Taskinen, C. Packard, M. J. Caslake, A. Soro-Paavonen, J. Westerbacka, S. Vehkavaara, A. Hakkinen, S. O. Olofsson, H. Yki-Jarvinen, et al. 2006. Overproduction of large VLDL particles is driven by increased liver fat content in man. *Diabetologia.* **49:** 755–765.
19. de Bruin, T. W., A. M. Georgieva, M. C. Brouwers, M. V. Heitink, C. J. van der Kallen, and M. M. van Greevenbroek. 2004. Radiological evidence of nonalcoholic fatty liver disease in familial combined hyperlipidemia. *Am. J. Med.* **116:** 847–849.
20. Voors-Pette, C., and T. W. de Bruin. 2001. Excess coronary heart disease in familial combined hyperlipidemia, in relation to genetic factors and central obesity. *Atherosclerosis.* **157:** 481–489.
21. Farrell, G. C. 2002. Drugs and steatohepatitis. *Semin. Liver Dis.* **22:** 185–194.
22. Fishbein, M., F. Castro, S. Cheruku, S. Jain, B. Webb, T. Gleason, and W. R. Stevens. 2005. Hepatic MRI for fat quantitation: its relationship to fat morphology, diagnosis, and ultrasound. *J. Clin. Gastroenterol.* **39:** 619–625.
23. Saadeh, S., Z. M. Younossi, E. M. Remer, T. Gramlich, J. P. Ong, M. Hurley, K. D. Mullen, J. N. Cooper, and M. J. Sheridan. 2002. The utility of radiological imaging in nonalcoholic fatty liver disease. *Gastroenterology.* **123:** 745–750.
24. Sorant, A. J. M., G. E. Bonney, R. C. Elstron, J. E. Bailey-Wilson, and A. F. Wilson. 1994. S.A.G.E.: Statistical Analysis for Genetic Epidemiology. Case Western Reserve University, Cleveland, OH.
25. Almasy, L., and J. Blangero. 1998. Multipoint quantitative-trait linkage analysis in general pedigrees. *Am. J. Hum. Genet.* **62:** 1198–1211.
26. Kruglyak, L., M. J. Daly, M. P. Reeve-Daly, and E. S. Lander. 1996. Parametric and nonparametric linkage analysis: a unified multipoint approach. *Am. J. Hum. Genet.* **58:** 1347–1363.
27. Westerbacka, J., A. Corner, M. Tiikkainen, M. Tamminen, S. Vehkavaara, A. M. Hakkinen, J. Fredriksson, and H. Yki-Jarvinen. 2004. Women and men have similar amounts of liver and intra-abdominal fat, despite more subcutaneous fat in women: implications for sex differences in markers of cardiovascular risk. *Diabetologia.* **47:** 1360–1369.
28. Falconer, D. S. 1967. The inheritance of liability to diseases with variable age of onset, with particular reference to diabetes mellitus. *Ann. Hum. Genet.* **31:** 1–20.
29. Brouwers, M. C., N. Kono, M. M. van Greevenbroek, C. J. van der Kallen, A. J. Lusis, T. W. de Bruin, and R. M. Cantor. 2006. Longitudinal differences in familial combined hyperlipidemia quantitative trait loci. *Arterioscler. Thromb. Vasc. Biol.* **26:** e118–e119.
30. Brouwers, M. C., M. A. Bilderbeek-Beckers, A. M. Georgieva, C. J. van der Kallen, M. M. van Greevenbroek, and T. W. de Bruin. 2006. Fatty liver is an integral feature of familial combined hyperlipidemia: relation with fat distribution and plasma lipids. *Clin. Sci. (Lond.).* In press.
31. Sniderman, A., H. Vu, and K. Cianflone. 1991. Effect of moderate hypertriglyceridemia on the relation of plasma total and LDL apo B levels. *Atherosclerosis.* **89:** 109–116.
32. Rao, D. C., P. M. Laskarzewski, J. A. Morrison, P. Khoury, K. Kelly, R. Wette, J. Russell, and C. J. Glueck. 1982. The Cincinnati Lipid Research Clinic family study: cultural and biological determinants of lipids and lipoprotein concentrations. *Am. J. Hum. Genet.* **34:** 888–903.
33. Hamsten, A., L. Iselius, G. Dahlen, and U. de Faire. 1986. Genetic and cultural inheritance of serum lipids, low and high density lipoprotein cholesterol and serum apolipoproteins A-I, A-II and B. *Atherosclerosis.* **60:** 199–208.
34. Suzuki, A., K. Lindor, J. St. Saver, J. Lymp, F. Mendes, A. Muto, T. Okada, and P. Angulo. 2005. Effect of changes on body weight and lifestyle in nonalcoholic fatty liver disease. *J. Hepatol.* **43:** 1060–1066.
35. Petersen, K. F., S. Dufour, D. Befroy, M. Lehrke, R. E. Hendler, and G. I. Shulman. 2005. Reversal of nonalcoholic hepatic steatosis, hepatic insulin resistance, and hyperglycemia by moderate weight reduction in patients with type 2 diabetes. *Diabetes.* **54:** 603–608.
36. Allayee, H., L. W. Castellani, R. M. Cantor, T. W. de Bruin, and A. J. Lusis. 2003. Biochemical and genetic association of plasma apolipoprotein A-II levels with familial combined hyperlipidemia. *Circ. Res.* **92:** 1262–1267.
37. Adeyemo, A., A. Luke, R. Cooper, X. Wu, B. Tayo, X. Zhu, C.

Rotimi, N. Bouzekri, and R. Ward. 2003. A genome-wide scan for body mass index among Nigerian families. *Obes. Res.* **11:** 266–273.
38. Chen, W., S. Li, N. R. Cook, B. A. Rosner, S. R. Srinivasan, E. Boerwinkle, and G. S. Berenson. 2004. An autosomal genome scan for loci influencing longitudinal burden of body mass index from childhood to young adulthood in white sibships: the Bogalusa Heart Study. *Int. J. Obes. Relat. Metab. Disord.* **28:** 462–469.
39. Heijmans, B. T., A. L. Beem, G. Willemsen, D. Posthuma, P. E. Slagboom, and D. Boomsma. 2004. Further evidence for a QTL influencing body mass index on chromosome 7p from a genome-wide scan in Dutch families. *Twin Res.* **7:** 192–196.
40. Weiss, L. A., L. Pan, M. Abney, and C. Ober. 2006. The sex-specific genetic architecture of quantitative traits in humans. *Nat. Genet.* **38:** 218–222.
41. Ogawa, Y., Y. Murata, A. Nishioka, T. Inomata, and S. Yoshida. 1998. Tamoxifen-induced fatty liver in patients with breast cancer. *Lancet.* **351:** 725.
42. Nemoto, Y., K. Toda, M. Ono, K. Fujikawa-Adachi, T. Saibara, S. Onishi, H. Enzan, T. Okada, and Y. Shizuta. 2000. Altered expression of fatty acid-metabolizing enzymes in aromatase-deficient mice. *J. Clin. Invest.* **105:** 1819–1825.
43. Campos, H., B. W. Walsh, H. Judge, and F. M. Sacks. 1997. Effect of estrogen on very low density lipoprotein and low density lipoprotein subclass metabolism in postmenopausal women. *J. Clin. Endocrinol. Metab.* **82:** 3955–3963.
44. Lander, E., and L. Kruglyak. 1995. Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. *Nat. Genet.* **11:** 241–247.
45. Pollex, R. L., and R. A. Hegele. 2005. Complex trait locus linkage mapping in atherosclerosis: time to take a step back before moving forward? *Arterioscler. Thromb. Vasc. Biol.* **25:** 1541–1544.
46. Pollex, R. L., and R. A. Hegele. 2006. Longitudinal differences in familial combined hyperlipidemia quantitative trait loci. *Arterioscler. Thromb. Vasc. Biol.* **26:** e120.