Common Variants in the Lipoprotein Lipase Gene, But Not Those in the Insulin Receptor Substrate–1, the β_3-Adrenergic Receptor, and the Intestinal Fatty Acid Binding Protein-2 Genes, Influence the Lipid Phenotypic Expression in Familial Combined Hyperlipidemia

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Familial combined hyperlipidemia (FCHL) is a common, atherogenic lipid disorder characterized by a variable phenotypic expression of hyperlipidemia. Variations in genes regulating fatty acid metabolism must be considered in the search for factors affecting the lipid phenotypic expression of FCHL. Therefore, we have evaluated the association of the common variants in the lipoprotein lipase (LPL) (D9N, N291S, and S447X), insulin receptor substrate-1 (IRS-1) (G972R), fatty acid binding protein-2 (FABP-2) (A54T), and β_3-adrenergic receptor (β_3-AR) (W64R) genes with lipid and lipoprotein levels in 30 Italian FCHL families (195 individuals). The transmission disequilibriun test (TDT) was used to evaluate the association between these variants and the FCHL trait. No significant differences were observed in the frequencies of the common LPL variants between affected and nonaffected FCHL family members. A significantly lower frequency of the LPL447X allele was noted only when members of the FCHL families were compared with normolipemic controls (.06 *v* .142, respectively; $P < .01$) suggesting a reduced representation of this LPL variant in FCHL families. The frequencies of variants in the IRS-1, FABP-2, and β_3-AR genes were not significantly different between affected and nonaffected FCHL family members and normolipemic controls. The TDT did not demonstrate any significant association of these gene variants with the FCHL trait. FCHL individuals carrying the LPL N291S gene showed higher plasma lipids and apolipoprotein B (apoB) levels compared with affected noncarriers. Only a marginal effect of the LPL D9N and S447X variants on lipid levels in FCHL individuals was observed. Conversely, the variants in the IRS-1, FABP2, and β_3-AR genes did not show any major influence on lipid and lipoprotein levels in FCHL family members. In conclusion, these results confirmed that none of the investigated genes were major loci for FCHL. Nevertheless, variations in genes affecting the removal rate of triglycerides (TG) from plasma, such as the LPL gene, significantly influence the lipid phenotypic expression of FCHL. Conversely, genetic variants in the IRS-1, FABP-2, and the β_3-AR gene appear not to have a major role as modifier genes in FCHL.

FAMILIAL COMBINED hyperlipidemia (FCHL) is the most common inherited hyperlipidemia and is found in up to 10% of premature myocardial infarctions.[1] Individuals affected by FCHL show variable types of hyperlipidemia characterized by elevated plasma very–low-density lipoprotein (VLDL) and/or LDL concentrations.[1,2] Other common features are reduced high-density lipoprotein (HDL), elevated apolipoprotein B (apoB) concentration, and increased prevalence of small dense LDL subfractions.[3,4] Several heterogeneous mechanisms resulting in overproduction of VLDL particles or impaired clearance of triglyceride (TG)-rich particles have been proposed to contribute to FCHL.[5] However, clinical studies indicate that reduced adipocytic insulin sensitivity, as well as disorders in fatty acid transfer and metabolism, may also be present.[6-10] The genetic defect(s) responsible for these metabolic alterations is unknown. A current genetic model suggests that FCHL might result from the combination of a dominant major gene(s) with a number of modifier genes influencing plasma lipid levels.[11] The identification of such modifier genes might help in reducing the problem of genetic heterogeneity of FCHL.[11]

It is reasonable to postulate that common variations in genes affecting fatty acid transfer and metabolism, as well as insulin action, might have a significant role as modifier genes. The lipoprotein lipase (LPL) gene has been extensively studied due to its pivotal role in the hydrolysis of VLDL-TG. Previous studies showed rare functional mutations resulting in decreased LPL activity in FCHL individuals.[12-14] However, within the coding region of the LPL gene, 3 amino acid changes have been reported to be common in the general population: D9N, N291S, and S447X. These LPL variants have been demonstrated to significantly affect lipid levels, the N291S and D9N variant being associated with increased plasma TG and decreased HDL levels and the S447X with a more favorable lipoprotein phenotype.[15] Only a few studies have investigated the effects of these LPL variants in modulating plasma lipids in FCHL.[16-18]

Given the association between FCHL and insulin resistance,[5] genes involved in insulin sensitivity may also act as modifier genes for FCHL. The insulin receptor substrate-1 (IRS-1) is known to play a key role in the cellular effects of insulin by acting as proximal signalling molecule for the insulin receptor.[19] A glycine to arginine substitution at codon 972 of the IRS-1 gene (G972R) has been reported to be associated with impaired insulin action.[20] Other investigators and ourselves demonstrated that this variant might also be associated with increased plasma TG concentrations.[21,22] Variations of both the fatty acid binding protein-2 (FABP-2) and the β_3-adrenergic

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receptor (β_3-AR) gene may influence the flux of free fatty acids (FFA) to the liver. The intestinal FABP-2 is an intracellular protein expressed only in the columnar absorptive epithelial cells of the small intestine and has a role in the absorption and intracellular transport of long chain fatty acids.[23,24] A common alanine to threonine substitution at codon 54 (A54T) of FABP2 has been reported to influence absorption and processing of fatty acids, as well as insulin sensitivity.[25-27] The β_3-AR gene regulates lipolysis in brown and white adipose tissue and therefore may affect FFA concentrations.[28] In previous studies, a tryptophan to arginine substitution in codon 64 of this gene (W64R) was found to be associated with abdominal obesity and insulin resistance.[29,30] The acylation-stimulating protein (ASP) is also an important determinant of fatty acid trapping and retention by adipose tissue.[31] However, its direct involvement in the pathogenesis of FCHL lipid abnormalities is still unclear.[32]

The aim of this study was to investigate the role of common genetic variants in LPL, IRS-1, FABP-2, and β_3-AR genes in influencing the phenotypic expression in Italian families with FCHL. None of them was confirmed to be causative, although the N291S and S447X variants in the LPL were found to significantly affect the lipid phenotypic expression of FCHL.

MATERIALS AND METHODS

Subjects

Thirty Italian families with FCHL were enrolled in the study. They were identified through probands (more than 20 years old) attending the Lipid Clinic of the University of Rome "La Sapienza" fulfilling the following criteria: (1) total cholesterol (TC) and/or total TG levels above the 90th percentile using the age- and sex-related percentile levels for the Italian population,[33] (2) isolated elevation of apoB concentration (>130 mg/dL corresponding to the 90th percentile for the Italian population),[33] (3) at least 1 first-degree relative with a different hyperlipidemic phenotype from the proband, and (4) absence of tendon xanthomas. Patients with type III hyperlipidemia, diagnosed by the presence of a broad-beta band on electrophoretogram and apoE2/E2 genotype, were excluded.

All probands were tested for plasma lipids twice when on ad libitum diet, and only if both samples were above the cut-off values were they considered affected. Other acquired causes of dyslipidemia including thyroid, liver disease, renal insufficiency, and proteinuria were excluded by standard laboratory tests. Individuals with obesity (body mass index [BMI] $>$ 30 kg/m^2), poorly controlled diabetes mellitus (blood glucose $>$ 120 mg/dL and/or glycosylated hemoglobin $>$ 6.0%), or taking lipid-affecting drugs were excluded. Probands's relatives were defined affected if they met the above criteria for FCHL and nonaffected if their lipid levels were $\leq$75th age- and sex-specific percentile.[33] Relatives with intermediate lipid levels were not considered in the study. As a whole, 195 FCHL family members (30 probands, 101 first-degree relatives, 38 second-degree relatives, and 26 spouses) were investigated.

To estimate the distribution of the genetic variants under study in the general Italian normolipemic population, unrelated individuals randomly selected from 1,023 subjects participating in a community-based screening of coronary risk factors were taken as population controls. These subjects were characterized as previously reported.[22] Those showing lipid levels less than 75th age- and sex-specific percentile[33] and no family history of hyperlipidemia were considered for the analysis. Table 1 shows the clinical and biochemical characteristics of the study groups. Other than elevated plasma lipids, FCHL affected family members showed higher mean age, higher number of males, and higher coronary heart disease (CHD) prevalence compared with nonaffected family members. No differences were observed in the prevalence of hypertension, diabetes, and smoking. As expected, nonaffected FCHL family members showed lower plasma lipids comparable to those of normolipemic population controls. However, population controls were older and showed higher BMI and lower blood glucose and smoking compared with nonaffected FCHL family members.

The ethical committee of the University of Rome "La Sapienza" approved the study protocol, and all subjects provided their informed consent.

Table 1. Comparisons of Clinical Characteristics of FCHL Family Members and Controls

Variable	FCHL		Controls (n = 114)
	Affected (n = 114)	Nonaffected (n = 81)	
Age (yr)	48.5 ± 15.0*†	40.8 ± 15.2‡	60.6 ± 11.7
Sex (M/F)	68/46§	35/46	58/56
BMI (kg/m^2)	25.4 ± 3.7‖*	24.0 ± 3.5‡	25.8 ± 3.6
Current smokers, n (%)	44 (38.5)†	26 (32.0)¶	14 (12.3)
Hypertension, n (%)	30 (26.3)	17 (20.0)	37 (32.5)
Blood glucose (mg/dL)	85.0 ± 11.7#	84.1 ± 17.3¶	75.8 ± 28.8
Diabetes, n (%)	4 (3.5)	0	1 (0.9)
CHD (%)	12 (10.5)‖§	0	2 (1.7)
Plasma lipids (mg/dL)			
TC	255.8 ± 47.9**†	191.7 ± 32.3	214.0 ± 31.8
HDL-C	47.4 ± 15.0†	54.6 ± 13.9	59.1 ± 15.0
LDL-C	162.0 ± 48.0**†	116.9 ± 27.6	131.9 ± 29.0
TG	266.6 ± 139.1**†	105.6 ± 46.4	116.8 ± 38.6
ApoB	155.4 ± 33.2**	98.6 ± 20.2	ND

NOTE. Data are reported as means ± SD. Other values represent the number of individuals (n) with percentage in parentheses.

Abbreviations: BMI, body mass index; TC, total cholesterol; TG, total triglycerides; HDL-C, high-density lipoprotein-cholesterol; CHD, coronary heart disease; ND, not determined.

§$P < .05$, *$P < .01$, and **$P < .001$ for comparison between affected *v* nonaffected subjects.

‖$P < .05$, #$P < .01$ and †$P < .001$ for comparison between affected *v* controls.

¶$P < .05$ and ‡$P < .001$ for comparison between nonaffected *v* controls.

DNA Analysis

DNA was obtained from peripheral blood leukocytes by the salting-out extraction method[34] and stored at −4°C in 10 mmol/L Tris-HCl and 1 mmol/L EDTA (pH 8.0). All genetic variants were detected by a combination of polymerase chain reaction (PCR) and digestion with restriction enzymes. Common variants in the LPL gene and the G972R variation in the IRS-1 gene were detected according to previously reported protocols.[22,35] The GCT to ACT substitution at codon 54 of the FABP-2 disrupts the sequence of a unique *HhaI* restriction site in exon 2 of the gene. Therefore, the latter was amplified using the following pair of primers: 5′-CACTTCCTATGGGATTTGACT-3′ and 5′-TT-GGGTAGAAAAATCAAGAATG-3′. PCR reaction was conducted in 20-μL volumes containing 100 to 200 ng of genomic DNA, 200 μmol/L deoxynucleotide triphosphate (dNTPs), 1.5 mmol/L MgCl2, 0.2 μmol/L primers, and 0.05 U Taq DNA polymerase (Amersham Pharmacia Biotech, Milan, Italy) in the standard buffer. PCR conditions were denaturation at 96°C for 5 minutes followed by 30 cycles of denaturation at 94°C for 1 minute, annealing at 60°C for 1 minute, and extension to 72°C for 1 minute. The obtained 274-bp fragment was digested with *HhaI* and separated through a 3.5% agarose gel. The wild-type allele was diagnosed as the presence of two 149-bp and 125-bp fragments, whereas the mutant T allele as an uncut 274-bp fragment. Exon 1 of the β_3-AR gene was amplified with the following pair of primers: 5′-ACCGCCAACACCAGTGGGCTGCCAGGGG-3′ and 5′GCCAGTGGCGCCCAACGG-3′. PCR conditions were denaturation at 94°C for 10 minutes followed by 10 cycles of denaturation at 94°C for 1 minute, annealing at 68°C for 20 seconds, and extension at 72°C for 20 seconds; annealing temperature was further reduced to 65°C for 10 cycles and to 62°C for 10 cycles; a final extension step at 72°C for 4 minutes was performed. The amplified 274-bp fragment was digested overnight at 60°C with *BstNI* restriction enzyme. The wild-type allele yielded fragments of 97, 62, 61, 24, and 15 bp in length, whereas the mutated allele lost 1 of the *BstNI* sites yielding a novel 158-bp fragment.

Other Laboratory Determinations

Blood samples were collected early in the morning after an overnight fast in EDTA-containing tubes. Total plasma and lipoprotein lipids were determined as previously reported.[22] In samples with TG greater than 400 mg/dL (n = 8), LDL-C was estimated after VLDL isolation by preparative ultracentrifugation and apoB–containing particles precipitation as described.[36] Total plasma apoB was measured by an immunoturbidimetric method (Kone Instruments, Espoo, Finland). Plasma glucose was measured with a glucose oxidase method adapted for the Technicon RA-1000 Autoanalyzer (Bayer, Milan, Italy). Apo E genotype was determined as reported.[37]

Statistical Analysis

All basic statistical analyses were performed with SPSS/WIN program (SPSS, Chicago, IL). The χ^2 or Fisher's exact test were used to compare differences between categorical variables, whereas those between continuous variables were determined by the Student's *t* test. The departure from the Hardy-Weinberg equilibrium was evaluated by the χ^2 test. The frequencies of genetic variants between the study groups were compared with the χ^2 test. Lipid and lipoprotein levels between genotypes were compared by analysis of variance (ANOVA). The independent effect of genetic variants on lipids was evaluated by the multiple regression analysis in which sex, age, and BMI were included as covariates. The association between the genetic variants and the FCHL trait was estimated by the transmission disequilibrium test (TDT),[38] which compares the number of heterozygous parents who transmitted the candidate allele to their affected offsprings with the number of those who transmitted their other allele. The comparison was performed using the χ^2 test. To lower the chance of possible biases, only FCHL families with both parents available were used. All data are presented as mean ± SD. A *P* value less than .05 was considered statistically significant.

RESULTS

The frequencies of genetic variants in FCHL subjects and controls are reported in Table 2. None of the subjects was homozygous for the LPL N291S. In population controls, frequencies of the LPL 9N, 291S, and 447X mutated alleles were .026, .017, and .162, respectively, similar to those reported in other populations.[15] Heterozygosity for the LPL D9N variant was detected in 5 probands giving an allele frequency of .083. The screening of family members did not show any significant differences in the frequency of the mutated 9N allele between affected and nonaffected relatives (.044 *v* .068, respectively), and no difference was observed when the comparison was made with population controls. The lack of a significant association between the FCHL trait and the LPL D9N variant was confirmed by the TDT analysis, which failed to show a preferential transmission of the mutated allele in FCHL individuals (Table 3). The LPL N291S variant was detected in 3 probands giving an allele frequency of .05. It showed a slightly higher prevalence in FCHL subjects as compared with nonaffected relatives, as well as to population controls, but this difference was not statistically significant. TDT analysis confirmed no preferential transmission of the LPL 291S defective allele in FCHL affected individuals (Table 3). No homozygote for the LPL S447X was found in FCHL families. A heterozygous state was detected in 2 probands giving a frequency of the mutated allele of .033. Although the frequency of the mutated 447X allele was not significantly different between affected and nonaffected FCHL family members, it was lower in comparison to that observed in population controls ($P = .003$). However, when the association was tested by the TDT analysis, a random transmission of the mutant LPL 447X allele was detected in affected subjects. Surprisingly, this variant allele was preferably nontransmitted in nonaffected FCHL individuals ($P = .02$). Overall these data do not indicate any significant association between the LPL S447X mutation and the FCHL trait.

The effects of the carrier status for the common LPL variants on lipid and lipoprotein levels are reported in Table 4. Control subjects carrying the LPL N291S showed increased TG and reduced HDL-C levels as compared with noncarriers, although neither difference reached statistical significance. Compared with noncarriers, affected FCHL individuals carrying the LPL N291S mutation showed higher TC and LDL-C ($P < .05$), TG ($P < .08$), and apoB ($P < .03$) concentrations. These effects do not appear to be related to age or BMI, as no significant differences were observed in these parameters between carriers and noncarriers of this variant (data not shown). On the other hand, no effects of the LPL N291S were observed in nonaffected FCHL family members. The affected FCHL family members carrying the LPL D9N mutation showed a tendency towards higher TG levels compared with noncarriers, but the difference was only marginally significant, probably for the low number of individuals in this group. In the other groups, no effects of this LPL variant were detected. Finally, lower levels of total TG were observed in carriers of the LPL S447X

Table 2. Frequencies of Gene Variants in Affected and Nonaffected FCHL Subjects and Controls

Variants	FCHL		Controls (n = 114)	P‖
	Affected* (n = 114)	Nonaffected (n = 81)		
LPL				
D9N	10 (8.8)	9 (11.1)	6 (5.3)	
N9N	0	1 (1.2)	0	.270
Allele 9N	.044	.068	.026	
LPL†				
N291S	9 (7.9)	2 (2.5)	4 (3.5)	.388
Allele 291S	.039	.012	.017	
LPL				
N447X	13 (11.4)	11 (13.6)	27 (23.7)	
X447X	0	0	5 (4.4)	.003
Allele 447X	.057	.068	.162	
IRS-1‡				
G972R	15 (13.8)	8 (10.7)	8 (7.0)	
R972R	1 (0.9)	0	0	.333
Allele 972R	.078	.053	.035	
FABP-2				
A54T	41 (36.0)	34 (42.0)	43 (37.7)	
T54T	3 (2.6)	8 (9.9)	9 (5.9)	.147
Allele 54T	.206	.309	.267	
β_3-AR§				
W64R	12 (11.0)	6 (7.8)	9 (7.9)	
R64R	0	0	1 (0.7)	.648
Allele 64R	.055	.039	.048	

NOTE. Data are reported as number of individuals (n) with percentage in parentheses.

Abbreviations: LPL, lipoprotein lipase; IRS-1, insulin receptor substrate-1; FABP-2, fatty acid binding protein-2; β_3-AR, β_3 adrenergic receptor.

*Probands (n = 29) were included in this group.

†No homozygous for the LPL N291S were found in the study subjects.

‡IRS-1 genotypes for 7 FCHL subjects and 3 controls were missing

§β_3-AR genotypes for 9 FCHL subjects and 11 controls were missing

‖For comparison between groups by contingency tables.

compared with noncarriers in both affected and nonaffected FCHL family members, but the differences reached statistical significance only in the latter group ($P < .03$).

Four probands were found to be heterozygous carriers for the G972R mutation in the IRS-1 gene giving an allele frequency of .066. The observed prevalence of the IRS-1 972R encoding allele among controls was similar to that previously reported in the normal population.[22] Overall, FCHL family members showed a higher frequency of the G972R variant than controls (12.3% *v* 7.0%, respectively; $P < .05$). However, when affected subjects were compared with nonaffected subjects, only marginal differences in the prevalence of this variant were found (Table 2). A tendency towards a preferential transmission of the mutated IRS-1 972R allele was observed in affected

Table 3. Linkage of Variant Alleles in the LPL, IRS-1, FABP-2, and β_3-AR Genes to the FCHL Trait Evaluated by TDT in FCHL

Gene Variants	FCHL Family Members							
	Affected				Nonaffected			
	T (n)	NT (n)	T (%)	P	T (n)	NT (n)	T (%)	P
LPL								
Allele 9N	1	0	100	.31	0	0	0	1
Allele 291S	2	2	50	1	0	0	0	1
Allele 447X	4	5	44.4	.73	0	5	0	.02
IRS-1								
Allele 972R	9	3	75	.08	2	0	100	.15
FABP-2								
Allele 54T	7	12	36.8	.25	4	3	57.1	.70
β_3-AR								
Allele 64R	1	2	33.3	.56	1	0	100	.31

Abbreviations: TDT, transmission disequilibrium test; T, transmitted; NT, nontransmitted; T(%), percentage transmitted; LPL, lipoprotein lipase; IRS-1, insulin receptor substrate-1; FABP-2, fatty acid binding protein-2; β_3-AR, β_3-adrenergic receptor.

Table 4. Lipid and Lipoprotein Levels in Affected and Nonaffected FCHL Individuals and Controls According to Common Variants in the LPL Gene

	FCHL																	
	Affected (n = 114)						Nonaffected (n = 81)						Controls (n = 114)					
Variants	n*	TC	HDL-C	TG	ApoB	LDL-C	n*	TC	HDL-C	TG	ApoB	LDL-C	n*	TC	HDL-C	TG	LDL-C
Noncarriers	83	253.3 ± 44.9	48.9 ± 16.0	256.6 ± 131.1	152.7 ± 32.0	159.3 ± 44.1	59	195.7 ± 32.2	53.5 ± 13.8	113.0 ± 49.2	98.5 ± 21.0	120.9 ± 28.1	71	208.6 ± 32.7	59.1 ± 14.3	113.9 ± 38.4	127.2 ± 29.3
Carriers†																	
D9N	9	257.0 ± 38.0	46.6 ± 15.7	334.3 ± 139.3	142.5 ± 37.6	151.0 ± 47.2	8	191.8 ± 28.0	57.9 ± 9.2	103.0 ± 37.2	101.1 ± 18.0	114.1 ± 28.3	4	239.7 ± 19.5	51.7 ± 11.0	145.5 ± 15.8	158.7 ± 20.8
N291S	6	285.6 ± 71.7‡	38.8 ± 8.5	349.0 ± 185.5	184.9 ± 40.8§	211.5 ± 73.0‖	2	207.0 ± 24.0	59.0 ± 19.8	92.0 ± 15.6	126.5 ± 12.0	129.5 ± 7.8	4	212.2 ± 32.7	45.0 ± 7.6	130.2 ± 57.9	141.2 ± 27.7
N447X	13	253.8 ± 65.1	44.4 ± 8.6	202.5 ± 105.0	154.9 ± 25.9	169.1 ± 58.9	10	171.9 ± 25.5	56.4 ± 17.6	70.8 ± 18.6§	91.9 ± 16.2	99.1 ± 18.1	20	218.6 ± 33.5	60.3 ± 19.0	122.7 ± 38.8	133.6 ± 28.1

NOTE. Probands (n = 29) were included in the affected group. TG values were log-transformed before statistical analysis; untransformed data are shown. Data are expressed as mg/dL and reported as means ± SD. Plasma concentrations of apoB were not available in population controls

*Compound heterozygotes for LPL variants were excluded from the analysis.

†Since no homozygote for the LPL X447X was found in the FCHL families, no comparison with controls was reported.

¶$P < .05$; §$P < .03$; ‖$P < .04$ for comparison with noncarriers.

subjects, although barely approaching statistical significance ($P = .08$) (Table 4). No significant differences in lipid levels were observed between carriers and noncarriers of the G972R variant in either affected or nonaffected FCHL individuals.

Eleven probands showed to be heterozygous for the A54T variant of the FABP-2 gene, giving a frequency of the variant allele of .183 and 4 for the W64R variant of the β_3-AR producing a frequency of the variant allele of .067. The frequency of both variant genes was not different between FCHL family members and controls (Table 2). This lack of association was further confirmed by the nonpreferential transmission of mutated alleles in affected, as well as in nonaffected FCHL individuals (Table 4). Variants in both FABP-2 and β_3-AR genes did not appear to significantly affect plasma lipid levels (Table 5).

DISCUSSION

In the present study, we evaluated the concurrent effects on the phenotypic expression of FCHL of several common variants in genes playing a role in fatty acids metabolism, such as LPL, IRS-1, FABP-2, and β_3-AR. Our results suggest that LPL variants significantly influence dyslipidemia in FCHL individuals. Affected LPL N291S carriers showed significantly elevated plasma TC and TG and reduced HDL-C levels as compared with affected noncarriers. Such an effect was not observed in nonaffected FCHL individuals, whereas only HDL-C levels appear to be influenced by LPL N291S in controls. Although to a lesser extent, the LPL D9N mutation appears to predispose FCHL individuals to higher TG levels, even though HDL-C is not influenced. These results are in line with previous reports[16-19] and strongly support that these genetic variants may explain part of the phenotypic variability observed in FCHL subjects. This is consistent with in vitro and in vivo studies showing that LPL D9N and N291S variants are both associated with a reduced lipolytic activity.[15] A novel observation was that the carrier status for the LPL N291S mutation causes significantly increased plasma apoB levels in FCHL individuals. Although a clear explanation is not available, this might reflect an accumulation of partially degraded VLDL in affected individuals carrying the defective LPL enzyme. Previous studies demonstrated an increased concentration of IDL in LPL N291S carriers,[16] and in FCHL families, this variant was reported to be associated with higher levels of cholesterol in the VLDL fraction, indicating accumulation of VLDL remnants.[17] Whether the presence of LPL N291S causes poorer apoB response to treatment or predisposes FCHL individuals to increased coronary artery disease (CAD) risk deserves further investigation. Only one study evaluated the role of the LPL S447X mutation in FCHL reporting no significant effects on lipid levels.[18] Conversely, we observed that FCHL subjects carrying the LPL S447X variant showed 25% lower, although not significant, TG levels compared with noncarriers. These differences reached 50% when comparison was performed with FCHL subjects carrying the N291S. These observations are in agreement with previous reports indicating that carriers of the LPL S447X mutation may present with an increased LPL-mediated removal of TG-rich lipoproteins[15] and, therefore, a reduced risk of high TG/low HDL dyslipidemia.[35] These findings may help in interpreting the lower frequency of the mutated 447X allele in FCHL family members compared with population controls. It is also possible that the TG-lowering effect of this LPL

Table 5. Lipid and Lipoprotein Levels in Affected and Nonaffected FCHL Individuals and Controls According to Common Variants in the IRS-1, FABP-2, and β_3-AR Genes

	FCHL																			
	Affected* (n = 114)						Nonaffected (n = 81)						Controls (n = 114)							
Variants	n†	TC	HDL-C	TG	ApoB	LDL-C	n†	TC	HDL-C	TG	ApoB	LDL-C	n†	TC	HDL-C	TG	LDL-C
Noncarriers	58	264.3 ± 49.1	47.8 ± 16.4	279.4 ± 134.9	162.8 ± 34.7	172.1 ± 48.9	27	196.0 ± 34.2	55.5 ± 17.2	110.0 ± 53.0	99.6 ± 20.8	119.4 ± 29.3	53	213.1 ± 34.1	55.0 ± 14.3	117.9 ± 37.5	134.2 ± 28.4
Carriers†																	
IRS-1																	
G972R	4	238.5 ± 66.2	53.0 ± 20.4	171.2 ± 43.9	135.7 ± 16.5	151.2 ± 54.8	6	200.5 ± 19.3	50.8 ± 16.9	134.7 ± 47.1	100.2 ± 17.3	122.8 ± 19.2	2	211.5 ± 9.2	49.5 ± 0.71	133.0 ± 36.7	135.5 ± 2.1
FABP-2																	
A54T	29	247.7 ± 50.7	45.8 ± 12.2	260.6 ± 141.5	148.8 ± 32.8	153.7 ± 54.8	33	183.9 ± 29.2	55.7 ± 10.1	101.0 ± 36.7	96.1 ± 16.5	110.7 ± 25.0	37	215.3 ± 33.6	59.5 ± 16.5	125.0 ± 42.5	132.2 ± 33.3
T54T	2	216.0 ± 76.4	44.0 ± 14.1	174.0 ± 114.5	120.0 ± 19.8	138.0 ± 39.6	6	183.0 ± 35.3	46.8 ± 10.2	112.0 ± 71.4	97.8 ± 33.1	116.3 ± 28.4	7	221.9 ± 22.9	63.7 ± 14.9	92.9 ± 26.1	138.1 ± 19.5
β_3-AR W64R	6	252.7 ± 25.1	51.7 ± 15.8	267.2 ± 124.4	146.4 ± 34.4	144.2 ± 30.6	6	213.8 ± 44.2	54.5 ± 17.8	88.3 ± 39.7	94.0 ± 28.1	139.7 ± 42.3‡	5	191.8 ± 30.4	66.6 ± 19.2	107.0 ± 43.4	104.0 ± 17.1

NOTE. Probands (n = 29) were included in the affected group. TG values were log-transformed before statistical analysis; untransformed data are shown. Data are expressed as mg/dL and reported as means ± SD.
*Plasma concentrations of apoB were not available in population controls.
†Compound heterozygotes were excluded from the analysis.
‡$P < .03$; ||$P < .04$ for comparison with noncarriers.

variant may weaken the dyslipidemic phenotype, thus masking the phenotypic effect of FCHL gene.

In agreement with a previous report,[39] serum lipid and lipoprotein levels in FCHL subjects were unaffected by the codon 64 polymorphism of the β_3-AR gene. Moreover, the observation that the locus does not show any significant association with the FCHL trait itself further excludes a major role of the β_3-AR gene in this disorder. The W64R variant in the β_3-AR gene has been shown to influence insulin resistance mainly in obese subjects,[30] and our negative results could be related to the normal-borderline body weight of our FCHL subjects. However, it must be borne in mind that in vitro studies showed that the expression of this polymorphism in Chinese Hamster Ovary (CHO) cells does not lead to impaired receptor function[40] and that fat cells from subjects with the Trp64Arg phenotype display normal lipolytic activity in response to a β_3-AR agonist.[41] Contrary to what has been found in Finnish FCHL subjects,[42] the A54T variant of the FABP-2 gene did not show any significant influence on lipid levels. It must be noted that the reported association was rather weak and limited to increased TG contents in HDL and LDL particles. Because the FABP-2 gene is supposed to influence plasma lipids through changes in postprandial lipemia,[27] our results might be due to population-specific differences in fat contents of the habitual diet. Nevertheless, the results of the TDT analysis seem to exclude any role of the FABP2 as a major locus for FCHL.

We observed that the G972R mutation of IRS-1 gene, consistently associated with impaired insulin action,[20] did not show any causative role for the FCHL trait. This is not a surprising finding considering that a recent study by Purnell et al[43] demonstrated that insulin resistance in FCHL is attributable to increases in intra-abdominal fat, not to the effect of a putative "FCHL gene". In addition, this IRS-1 variant did not show any major influence on the lipid phenotype of FCHL individuals. Although this might be a chance finding due to the low number of FCHL subjects carrying the IRS-1 variant, yet it appears to be in contrast with what was observed in a cohort of coronary subjects.[22] A clear explanation of such discrepancy is not readily available. A possibility might be that the IRS-1 variant has a weak effect on TG levels in individuals with normal body weight, whereas the same effect becomes apparent in overweight subjects.[44] This hypothesis is supported by the fact that the average body weight of our FCHL subjects was lower than that of coronary patients.[22]

In conclusion, our study shows that none of the investigated genes is a major locus for FCHL. Nevertheless, variations in genes affecting the removal rate of TGs from plasma, such as the LPL gene, significantly affect the phenotypic expression of FCHL, whereas genetic variants in the FABP-2 and the β3-AR genes have no major role as modifier genes. The possibility that the G972R variant in the IRS-1 may worsen insulin resistance in FCHL must be further investigated.

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