Lack of association of the apolipoprotein A-I–C-III–A-IV gene XmnI and SstI polymorphisms and of the lipoprotein lipase gene mutations in familial combined hyperlipoproteinemia in French Canadian subjects

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Abstract Familial combined hyperlipoproteinemia (FCH) is a common familial lipoprotein disorder characterized by elevated plasma cholesterol and triglyceride levels with segregation in first-degree relatives. Most affected subjects with FCH have elevated plasma levels of apolipoprotein (apo) B. The disorder results from oversecretion of hepatic apoB-containing lipoprotein particles. The genetic defect(s) are unknown. Previous work has suggested that genetic polymorphisms of the apoA-I gene and functional abnormalities of the lipoprotein lipase (LPL) gene are associated with FCH. We investigated the XmnI and SstI restriction fragment length polymorphisms (RFLP) of the apoA-I gene in FCH subjects of French Canadian descent. We also investigated three common functional mutations of the lipoprotein lipase (LPL) gene ($LPL_{Gly188Glu}$, $LPL_{Pro207Leu}$, and $LPL_{Asp250Asn}$) in French Canadians that account for approximately 97% of cases of complete LPL deficiency in the province of Québec, Canada. We identified and characterized 54 FCH probands in lipid clinics and examined at least one first-degree relative. There were 37 men and 17 women (mean age 48 ± 9 and 58 ± 8 years, respectively). None of the probands had diabetes mellitus; mean plasma glucose was 5.5 mmol/L. High blood pressure was diagnosed in 32% of men and 29% of women. The body mass index (weight (kg)/height(m^2)) was elevated in probands (27 ± 4 for men and 26 ± 4 for women). Mean plasma levels of cholesterol (C) was 7.6 ± 1.5 mmol/L, triglycerides 3.5 ± 1.6 mmol/L, LDL-C 4.9 ± 1.2 mmol/L, HDL-C 1.0 ± 0.3 mmol/L, and apoB 1.83 ± 0.67 g/L in the probands. Allele frequency of the rare alleles of the XmnI and SstI RFLP was not significantly different from a healthy reference group. In several families studied, the XmnI and SstI RFLP did not unequivocally segregate with the FCH phenotype. There was no significant effect of the presence or absence of the XmnI or SstI RFLP's on plasma lipids, lipoprotein cholesterol or apoB levels. Only one FCH proband was found to have a mutation of the LPL gene ($_{(Gly188Glu)}$), and this did not segregate with the FCH phenotype in the family. We conclude that in our highly selected group of FCH subjects of French Canadian descent, the XmnI and SstI RFLPs of the apoA-I gene and common functional mutations of the LPL gene resulting in complete LPL deficiency are not associated with FCH.—**Marcil, M., B. Boucher, E. Gagné, J. Davignon, M. Hayden, and J. Genest, Jr.** Lack of association of the apolipoprotein A-I–C-III–A-IV gene XmnI and SstI polymorphisms and of the lipoprotein lipase gene mutations in familial combined hyperlipoproteinemia in French Canadian subjects. *J. Lipid Res.* 1996. **37:** 309–319.

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Familial combined hyperlipoproteinemia (FCH) is a syndrome defined initially by elevations in total cholesterol and triglycerides, and by lipoprotein electrophoresis profile (1–3). With improvement in laboratory methods, it is generally accepted that FCH is characterized by elevations in the number of apoB-containing particles, resulting in lipoprotein phenotypes IIa (elevated

Abbreviations: FCH, familial combined hyperlipoproteinemia; apo, apolipoprotein; LPL, lipoprotein lipase; RFLP, restriction fragment length polymorphism; C, cholesterol; LDL, low density lipoprotein; HDL, high density lipoprotein; HB, hyperapobetalipoproteinemia; CAD, coronary artery disease; FH, familial hypercholesterolemia; BMI, body mass index; PCR, polymerase chain reaction.

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low density lipoprotein (LDL)-C), IIb (elevated LDL-C and triglycerides), IV (elevated triglycerides), or even normal, depending on which lipoprotein-cholesterol fraction is increased (4–10). LDL particle size is usually decreased and high density lipoprotein (HDL) cholesterol usually reduced in FCH (11); an elevated level of plasma apoB is a common denominator in affected individuals. Hyperapobetalipoproteinemia (HB), described over 15 years ago (12), shares many biochemical characteristics with FCH and the two can be considered part of the same syndrome. FCH is the most common familial lipoprotein abnormality in subjects with premature coronary artery disease (CAD) (1, 13). Understanding the molecular genetics of FCH may yield considerable insight in the fields of cardiovascular genetics and lipoprotein metabolism.

Previous reports have investigated a possible association between apoA-I–C-III–A-IV gene complex and FCH/HB (14), the rare allele of the XmnI RFLP 5′ to the apoA-I gene was seen more frequently in FCH subjects than in controls (15). Our previous work on the apoA-I–C-III–A-IV gene complex (16) or the apoB gene (17) did not suggest an association between RFLPs at these loci and alteration in apoB levels, nor did we find an association with the presence of familial lipoprotein abnormalities.

In the province of Québec, mutations within the LPL gene leading to an abnormally functioning protein are common (18). Several point mutations involving exons 5 and 6 have been described in the French Canadian population with $LPL_{Gly188Glu}$, $LPL_{Pro207Leu}$ and $LPL_{Asp250Asn}$ (19–21) accounting for the majority (97%) with complete LPL deficiency (19). The heterozygous state for LPL deficiency is seen frequently. Based on the measurement of post-heparin lipoprotein lipase activity, Babirak et al. (22) postulated that the heterozygous state for LPL deficiency could account for nearly one third of FCH cases. Subjects known to have a mutation on one allele for the LPL gene often have elevated plasma LDL apoB/cholesterol ratios (18). One large kindred with obligatory heterozygote subjects for the LPL 188 mutation revealed an association between type IV hyperlipoproteinemia and the presence of the mutation in affected individuals (23, 24). It is uncertain, however, whether the heterozygous state for functional mutations of the LPL gene is one of the genetic defects in FCH.

In an attempt to better characterize the metabolic and genetic disorders in subjects with premature CAD (25), we examined a group of well-characterized subjects with FCH. Our purpose was to examine the association between the presence of the XmnI and SstI RFLPs of the apoA-I gene, the presence of three known mutations of the LPL gene and the FCH phenotype in subjects of French Canadian descent.

METHODS

Subject selection

Subjects for this study were selected from the lipid clinics of the Clinical Research Institute of Montréal (CRIM) and from the Montréal Heart Institute (MHI). Patients at either institution are screened for the presence of lipoprotein abnormalities and standardized assays for cholesterol are available at both institutions. Lipoprotein-cholesterol and the measurement of apolipoprotein B are part of the clinical evaluation at each institution. In all subjects, at least one first-degree relative was identified as having a lipoprotein abnormality characteristic of FCH.. Careful clinical examination by experienced physicians ruled out the presence of familial hypercholesterolemia (FH) or type III dysbetalipoproteinemia. In several subjects (n = 19), a more complete family examination was performed with all available first- and second-degree relatives. The protocol for the study was reviewed and accepted by the Ethics Committees of both institutions.

The FCH phenotype was characterized as an elevation of LDL-C or triglycerides >90th percentile for age- and gender-matched subjects (13) or an apoB level >1.50 g/L. In addition, one first-degree relative had to have a similar lipoprotein abnormality. Secondary causes of dyslipoproteinemia were cause for exclusion. Specifically, no subject had diabetes mellitus, hypothyroidism (as assessed by elevated thyroid stimulating hormone), a BMI >35 or any medical condition known to alter lipoprotein levels. Familial hypercholesterolemia and type III dysbetalipoproteinemia were excluded on clinical grounds and, when appropriate, by the molecular analysis of the LDL receptor gene for common mutations causing FH in French Canadians (26) and by apoE phenotyping. LDL-receptor mutations frequently seen in subjects of French Canadian descent (26) were determined in subjects with elevated LDL-cholesterol (>95th percentile).

Clinical variables

Data were obtained on cigarette smoking, defined as a history of cigarette smoking, the presence of hypertension, defined as a history of hypertension, drug treatment specifically prescribed for the treatment of hypertension by a physician or the presence of arterial blood pressure >150/90 mmHg; diabetes is defined as a physician diagnosis of diabetes or treatment with oral hypoglycemic drugs or insulin, and a family history of premature CAD, as defined in the NCEP II (27). The use of medications was recorded; all patients on lipid-lowering medications were asked to stop their medication for 2 months prior to blood sampling (no patient was taking the drug probucol). The family history was determined

on all patients, including age of death of first- and second-degree relatives or onset of symptoms, presence of vascular disease, hypertension or diabetes and cause of death of all relatives for three generations. Height and weight were recorded on all patients and body mass index was calculated (BMI: weight (kg)/height^2 (m)). Blood pressure was recorded with the patient in the sitting position for at least 5 min.

Comparison group

Allele frequencies of apoA-I RFLPs were assessed in a population selected for health from a major utility company in Montréal, Canada. This population has previously been described (28) and allele frequencies for several apolipoprotein genes have been published (29). We used this group to examine differences in allele frequencies between a group of normal subjects and our patients with FCH. As LPL mutations 188, 207, and 250 are known to impart abnormal function in lipoprotein lipase, we sought to ascertain through family studies whether these mutations in LPL segregated with the FCH phenotype.

Plasma lipids, lipoprotein-cholesterol, and apolipoprotein B measurements

Blood was drawn in ethylenediethylaminetetraacetic acid- (EDTA) containing tubes as an anticoagulant (final concentration 1.2 mg/mL) for determination of biochemical variables. All patients were fasting. Plasma was separated by centrifugation (20 min, 4°C, 2000 g); multiple 1-mL aliquots (n = 5) were stored at -80°C for future studies. Total cholesterol and triglyceride levels were measured as previously described (25) using the Cobas Mira-S analyzer (Hoffman LaRoche Diagnostics, Basel, Switzerland). Lipoprotein-cholesterol was determined after ultracentrifugation of plasma at density d 1.006 g/mL. HDL-cholesterol was determined after precipitation of apolipoprotein B-containing lipoproteins (25); LDL-cholesterol was determined as infranatant cholesterol - HDL-cholesterol. The laboratory participates and meets the requirements of the Centers for Disease Control cholesterol standardization program. ApoB levels were determined by nephelometry on a laser nephelometer (BN-100 Behring) as described (30).

Apo A-I–C-III–A-IV XmnI and SstI RFLP analysis

Southern blot hybridization was carried out on all probands after digestion of genomic DNA with the enzymes XmnI (New England BioLabs, MA) or SstI (BRL, Bethesda, MD) and the apoA-I cDNA was used as a probe. Radiolabeling with [^{32}P]dCTP was carried out with the random primer technique (Pharmacia). In many cases, the presence of the XmnI and SstI RFLPs was also assessed by the polymerase chain reaction (PCR) as previously reported (31). There was complete agreement between Southern blotting analysis and the results obtained by PCR-based diagnosis. Allele frequencies of the XmnI and SstI RFLPs in a healthy French Canadian population of men and women have already been determined (29). We considered this group of subjects to be representative of a healthy segment of the population. In addition, these reference subjects were sampled from the same geographical region as our FCH patients.

LPL mutations

Exon 5 of the LPL gene was amplified using the polymerase chain reaction (PCR), using the following primers LPL-14 5′-TGTTCCTGCTTTTTTCCCTT -3′ (forward) and 5′-TAATTCGCTTCTAAATAATA -3′ (reverse) (20). We used 30 cycles of PCR with a deannealing temperature of 94°C for 1 min, annealing temperature of 55°C for 1 min, and a prolongation temperature of 72°C for 2 min. We used the Taq polymerase from Bethesda Research Laboratories (BLR) laboratories, with 2 μmol $MgCl_2$. For the determination of mutation LPL-188, the amplified fragments of exon 5 of the LPL gene are precipitated and resuspended in Tris-EDTA buffer and cut with the restriction enzyme AvaII (20). The fragments are resolved on an 1.5% agarose gel and visualized under ultraviolet light after staining with ethidium bromide. The presence of the LPL-188 mutation deletes an AvaII site within exon 5. For mutation LPL-207, the amplified fragment is cleaved with the enzyme BslI which cleaves the wild type but not the mutant allele. Finally, mutation LPL-250 on exon 6 of the LPL gene was assessed by PCR as previously described (32). The primers used for exon 6 are 5′-GCCGAGATACAATCTTTGGTA-3′ (forward) and 5′-

TABLE 1. Clinical characteristics of FCH probands

FCH	n	Age	Smoking (History)	HBP (Presence)	Diabetes (Presence)	Fam Hx (Presence)	BMI	Glocose
		yr	%	%	%	%	*kg/m^2*	*mmol/L*
Men	37	48 ± 9	90	32	0	86	24 ± 4	5.5 ± 0.4
Women	17	58 ± 8	53	29	0	75	26 ± 4	5.4 ± 0.8

HBP, high blood pressure; Fam Hx, family history of coronary artery disease; BMI, body mass index.

TABLE 2. Mean lipid, lipoprotein-cholesterol, triglyceride levels, and apoB levels in men and women

	Cholesterol	Triglycerides	LDL-C	HDL-C	ApoB
		mmol/L			
FCH men (n=37)	7.3 ± 0.9	3.3 ± 1.7	4.8 ± 1.0	0.9 ± 0.2	1.89 ± 0.37
LRC men (45-49 years)[a]	5.43	1.34	3.65	1.16	
FCH women (n=17)	8.1 ± 1.3	3.8 ± 1.2	5.3 ± 1.5	1.1 ± 0.2	1.71 ± 0.91
LRC women (55-59 years)[a]	5.92	1.25	3.75	1.55	
FCH combined (n=54)	7.6 ± 1.5	3.5 ± 1.6	4.9 ± 1.2	1.0 ± 0.3	1.83 ± 0.67

[a]Normal group from the Lipid Research Clinics data (33)

CTGCTTCTTTTGGCTCTGACTGT A-3′ (reverse). Mutation LPL-250 was detected after digestion with the restriction enzyme TaqI.

Statistical analysis

The data were stored on a personal computer and the analysis was performed using commercially available software (Sigma-Stat, Jandel Scientific, San Rafael, CA). Differences for continuous variables were determined by t-test or by Mann-Whitney analysis (in the case of nonparametric data). Differences in discrete variables were determined by chi-square analysis. We considered a level of $P < 0.05$ to be significant.

RESULTS

Subjects' characteristics

We identified 54 subjects of French Canadian descent who were identified as having FCH based on the proband's lipoprotein profile and at least one first-degree relative. There were 37 men, with a mean age of 48 ± 9 years, and 17 women, with a mean age of 58 ± 8 years. The age difference between genders was statistically significantly different and likely reflects differences in physician's referral to the lipid clinics or different age of expression of FCH in women. Of the 37 men, 12 (32%) had documented coronary artery disease and 4 of 17 women (24%) also had CAD. Table 1 shows the clinical features of the study subjects, separated by gender. There was a strong history of cigarette smoking especially among the men, although this includes past history of cigarette smoking as well. Hypertension was seen frequently, 12 (32%) of the men and 5 (29%) of the women had a history of high blood pressure. Although none of the patients were diagnosed as having, or being treated for, diabetes mellitus (an exclusion criterion for entry into the study), two men were told they had "borderline" diabetes but had a normal fasting glucose level at the time of sampling. Oral glucose tolerance tests were not performed. A family history of coronary artery disease was frequently encountered both in men (86%) and in women (75%). The BMI was slightly higher in men (27 ± 4) than in women (26 ± 4), P = ns. The mean fasting plasma glucose was similar in men and in women (Table 1). This elevated prevalence of cardiovascular risk factors reflects not only a population of patients targeted for being at high risk of cardiovascular disorders but also the commonly observed clustering of cardiovascular risk factors in high risk individuals.

Plasma lipids

Plasma lipid and lipoprotein-cholesterol levels are shown in **Table 2.** The apoE phenotype was obtained in 42/54 subjects (78%). None of the subjects examined were homozygous for the apoE2/2 phenotype and no study subject had a diagnosis of type III dysbetalipoproteinemia. Mean levels of plasma-cholesterol was 7.3 ± 0.9 mmol/L in men and 8.1 ± 1.3 mmol/L in women; mean triglyceride levels were 3.3 ± 1.7 mmol/L for men and 3.8 ± 1.2 mmol/L for women. Mean levels of LDL-cholesterol were 4.8 ± 1.0 mmol/L and 5.3 ± 1.5 mmol/L while mean HDL-cholesterol levels were 0.9 ± 0.2 mmol/L and 1.1 ± 0.2 mmol/L men and women, respectively. For comparison purposes, mean levels of cholesterol, triglycerides, LDL-cholesterol, and HDL-cholesterol from the Lipid Research Clinics data (33) for men aged 45–49 and women aged 55–59 are shown in Table 2. Mean level of apolipoprotein B was determined. The mean apoB level was 1.89 ± 0.37 g/L in men and 1.71 ± 0.91 g/L in women. Mean level of apoB in a random Québec population sample of men (n = 152) aged 47 ± 8 years was 1.28 ± 0.28 g/L and for women (n = 61), 1.19 ± 0.29 g/L (J. Genest, and S. Lussier-Cacan, unpublished observations). A statistical level of comparison for biochemical variables between genders is not given as such a value has little meaning in the present context.

ApoA-I XmnI RFLP

The prevalence of the apoA-I XmnI RFLP is shown in **Table 3.** Data analyses were performed separately for men and women and then combined. Absence of the cutting site was seen in 25/37 (68%) of men and 11/17 (65%) of women; overall, therefore, 36/54 (67%) of

patients with FCH were heterozygous for absence of the XmnI cutting site. Based on the study of Kessling et al. summarized in Table 3 (29), the prevalence of the absence of the XmnI RFLP was 79% (163/206) for control men and 74% (82/111) for control women (difference nonsignificant by chi-square analysis).

Family studies of two probands are shown in **Figs. 1A and B**. As can be seen from FCH kindred 21513 (Fig. 1A), all first-degree relatives of the probands are considered affected (shaded symbols). The offspring of subject II2 are not affected despite sharing at least one XmnI allele. Subjects IIIa7 and IIIa9 are more informative; the former is affected and does not have the XmnI allele of maternal origin whereas subject IIIa9 has one XmnI allele but is unaffected. Subject IIIb8 is considered affected and does not have the XmnI RFLP. Similarly, the first-degree relatives in kindred FCH 5695 (Fig. 1B) are affected but no clear association of the XmnI allele and the FCH phenotype can be unequivocally shown. For instance, subjects FCH 5695 II3, II6, II7, and II10 are considered affected but do not bear the XmnI RFLP. Conversely, subject II12 is considered unaffected but has the XmnI RFLP. Thus, we were unable to show that the FCH phenotype segregates with the XmnI RFLP.

ApoA-I–C-III SstI RFLP

The prevalence of the apoA-I SstI RFLP is shown in Table 3. In the control group, data on 327 subjects (215 men and 112 women) are available. The frequency of the common SstI RFLP allele (absence of the cutting site) in control men was 89.3% and 93.8% in control women (P = ns). Overall, the combined (men and women) prevalence of the frequent allele in control men and women was 90.8%, and 9.2% for the rare allele. In the FCH group, the frequency of the frequent allele (absence of the cutting site) was 84.9%, and of the rare allele, 15.1%. This difference in allele frequencies was not significantly different.

Effects of the XmnI and SstI RFLP on plasma levels of lipids, lipoprotein-cholesterol, and apoB

We examined the effects of the presence or the absence of the XmnI and SstI RFLPs on plasma levels of total cholesterol, tryglicerides, LDL-cholesterol, and HDL-cholesterol as well as apoB in the probands; the data are shown in **Table 4.** In the study by Kessling et al. (29), the XmnI or SstI RFLPs did not have a significant effect on plasma cholesterol, triglycerides, or lipoprotein cholesterol. In the FCH subjects, the analysis was carried out on men and women combined. The presence of either the XmnI or SstI RFLP did not alter plasma levels of total-cholesterol, triglycerides, LDL-cholesterol, HDL-cholesterol, or apoB levels. The sample size is small, however, and this study cannot determine with certainty that the RFLPs in this selected hyperlipidemic sample do not have a significant effect on lipid parameters.

Lipoprotein lipase mutations

We assessed mutations LPL-188, 207, and 250 that are seen in the homozygous state in 97% of subjects with complete LPL deficiency in the Province of Québec, Canada. This high prevalence of single mutations indicates a founder effect. We determined the presence of mutations LPL-188, LPL 207, and LPL 250 by PCR-based diagnosis in all probands. All the analyses were performed in the Vancouver (Canada) laboratory. In all but one proband with FCH, none of the aforementioned functional mutations of the LPL gene were found. Only one patient was found to carry a mutation of the LPL gene (LPLGly188Glu). This patient was previously reported in our initial study of 31 FCH patients (all of whom are included in the present study) (34). Careful family study failed to show unequivocally that the FCH phenotype was associated with the presence of the LPL 188 mutation. We did not assess the presence of known mutations of the LPL gene other than the three mentioned above. Our previous experi-

TABLE 3. ApoA-I XmnI and SstI restriction fragment polymorphisms and familial combined hyperlipoproteinemia subjects

	Men			Women		
RFLP[a]	Control	FCH	P^b	Control	FCH	P^b
XmnI 00	79% (163/206)	68% (25/37)	0.297	74% (82/111)	65% (11/17)	0.559
XmnI 01	19% (40/206)	30% (11/37)		24% (27/111)	35% (6/17)	
XmnI 11	2% (3/206)	3% (1/37)		2% (2/111)	0% (0/17)	
SstI 00	82% (177/215)	75% (27/36)[c]	0.433	88% (99/112)	71% (12/17)	0.088
SstI 01	14% (30/215)	22% (8/36)[c]		11% (12/112)	24% (4/17)	
SstI 11	4% (8/25)	3% (1/36)[c]		1% (1/112)	6% (1/17)	

[a] "0" refers to the absence of the cutting site and "1" to its presence on each allele.
[b] *P* value indicated for allele frequencies in a 2 × 3 contingency table.
[c] The SstI RFLP on one FCH proband was not determined

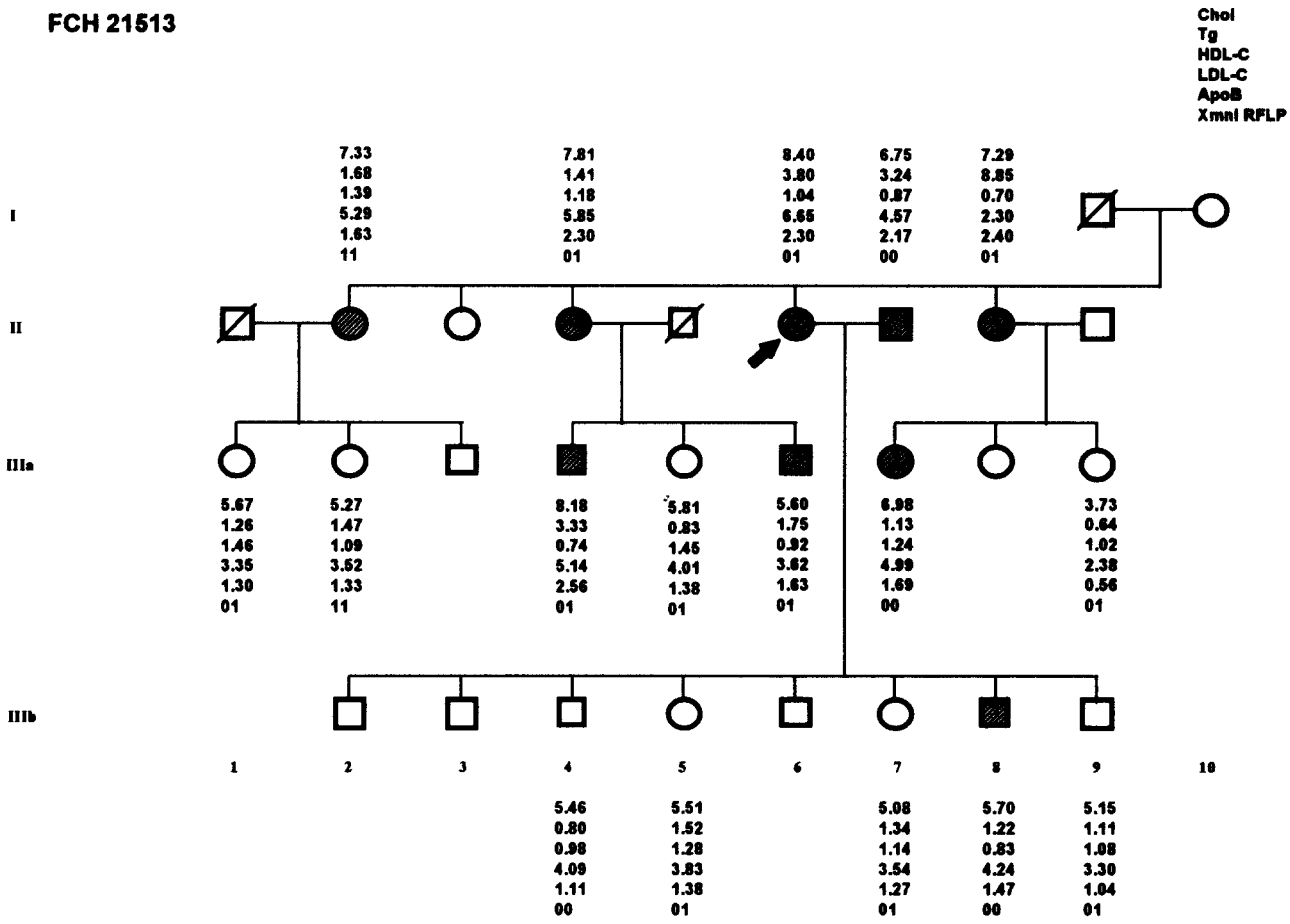

Fig. 1A. Family tree of kindred FCH 21513.

ence has revealed that other mutations of the LPL gene in these subjects (even those not affecting the catalytic site) are not associated with FCH in our subjects (34).

DISCUSSION

Familial combined hyperlipidemia is the most frequent familial lipoprotein disorder seen in subjects with premature CAD (1, 13). Despite a decade of research in genetic markers for FCH using the candidate gene approach, no consistent underlying genetic mechanism has been identified. We took advantage of a well-defined population of French Canadians where there is an enrichment of genetic lipoprotein disorders with a founder effect to examine genes that have previously been associated with the FCH phenotype in other populations. In the present study, we examined two candidate genes that have been associated or postulated to be associated with FCH in other populations. We have failed to find an association between the XmnI and SstI RFLPs of the apoA-I gene and FCH. Although the study by Wojciechowski et al. (14) strongly suggested that the apoA-I gene (or one in close linkage disequilibrium) may be associated with FCH, there is data obtained from population studies that cast doubt on the physiological effect of the XmnI RFLP on lipoprotein metabolism. First, there is little evidence in normal populations that the XmnI RFLP is associated with alterations in plasma lipoprotein cholesterol levels (29). Second, the XmnI RFLP is not seen with increasing frequency in our FCH subjects compared with healthy controls and, third, the XmnI RFLP does not segregate with the FCH phenotype in the present study.

The SstI RFLP has been associated with altered triglyceride levels in selected populations, but not in others (29, reviewed in 16). In our group of FCH subjects and controls, the SstI polymorphism was not associated with altered lipid levels, nor was it seen more frequently in FCH probands than in control subjects. Family studies performed within the context of the present study were inconclusive because of the relative rarity of the SstI RFLP in our kindred.

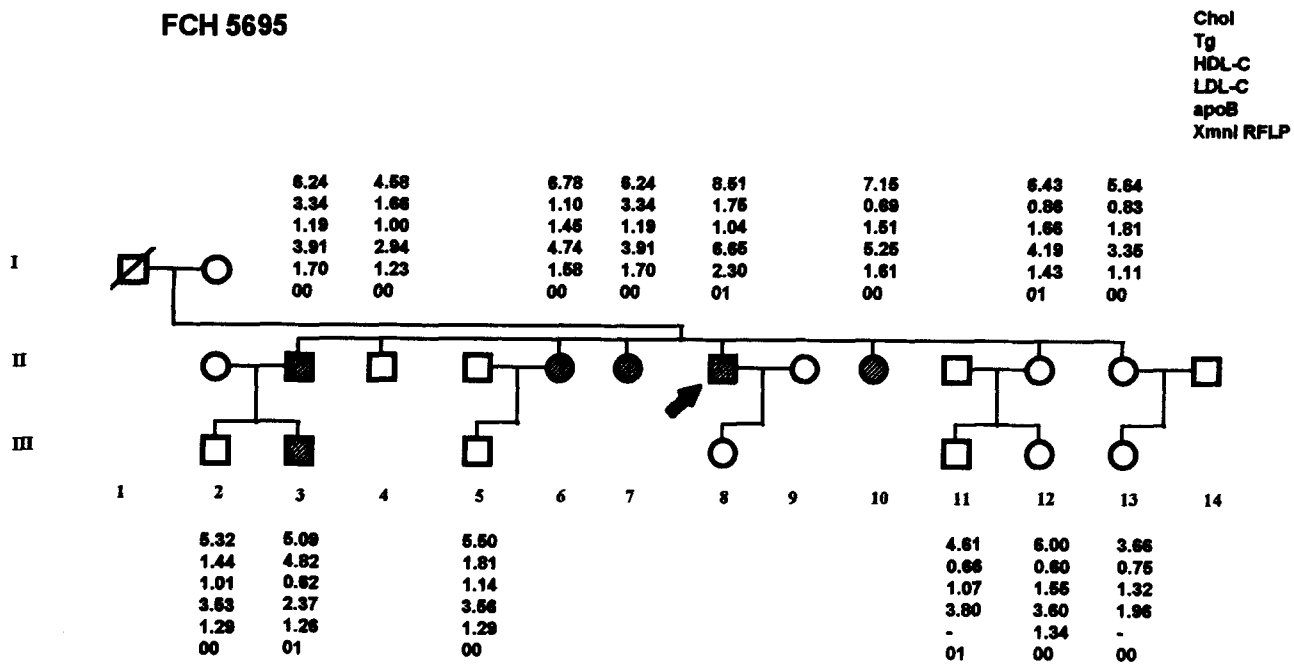

Fig. 1 B. Family tree of kindred FCH 5695.
Men are indicated by a square and women by a circle. Proband is shown by a thick arrow. Deceased subjects are marked with a diagonal line. Shaded symbols represent the subjects who are considered affected. Total cholesterol, triglyceride, HDL-cholesterol, LDL-cholesterol levels (in mmol/L), apoB values (in g/L), and XmnI RFLP (0, absence; 1, presence of the cutting site), respectively, are shown above or below the symbols of all sampled subjects.

Functional mutations of the LPL gene have been postulated to be associated with FCH (22). We have previously examined the most frequent mutations of the LPL gene causing familial LPL deficiency ($LPL_{Gly188Glu}$, $LP_{LPro207Leu}$, and $LPL_{Asp250Asn}$) in French Canadians in a group of 31 unrelated individuals with FCH as well as silent mutations detected by single-stranded conformational polymorphism (SSCP) and direct sequencing (34), and have not been able to find mutations in other domains of LPL that are associated with FCH. It is still possible that mutations other than those affecting the catalytic domain of LPL may be associated with FCH (for example, a domain responsible for the cellular uptake of triglyceride-rich lipoproteins (35, 36)). However, based on our previous study (34), this does not appear to be the case. We took advantage of the high prevalence of three mutations of the LPL gene seen in French Canadians to examine whether these mutations are associated with the FCH phenotype. Our results suggest that functional mutations of the LPL gene may not be associated with a sub-group of FCH subject, as previously suggested. Minnich et al. (32) reported that these mutations of the LPL gene are frequently seen in subjects with hypertriglyceridemia. Unfortunately, no family studies have been provided by the authors but the data are compelling to suggest that in patients with moderately severe and severe hypertriglyceridemia (triglyceride levels >6 mmol/L), LPL mutations are common in subjects of French Canadian descent.

The pathogenesis of FCH remains in dispute. Based on cell culture, perfused liver studies, animal and human studies, a consensus is emerging that FCH results from oversecretion of apoB-containing particles from the liver. The apoB gene has been cloned and sequenced and the 5′ regulatory regions have been examined (37, 38). The data shows that apoB is constitutively expressed under experimental conditions (39, 40). ApoB secretion appears to be predominantly controlled after protein synthesis and is substrate driven (41–44). Kinetic studies have shown that apoB production rates are increased in FCH (45–48).

There are important limitations to our study. First and foremost, a major difficulty lies in the assignment of the FCH phenotype. We have defined FCH based on the 90th percentile plasma levels of LDL-cholesterol and triglycerides (13), or an apoB level >1.50 g/L. These arbitrary cut-points are based on the normal distribution of these biological variables within a population. In doing so, we have dichotomized a continuous variable. Despite these caveats, the families shown in Fig. 1A and B have not presented a serious problem in the diagnosis of FCH.

TABLE 4. Effect of the XmnI and SstI RFLPs on plasma lipids and lipoprotein-cholesterol in FCH subjects.

RFLP	Cholesterol	Triglycerides	LDL-C	HDL-C	ApoB
			mmol/L		
XmnI 00 (n=36)	7.52 ± 1.09	3.44 ± 1.47	4.87 ± 1.06	0.97 ± 0.21	190 ± 30
XmnI 01 (n=17)	7.65 ± 1.3	3.69 ± 1.83	4.96 ± 0.90	0.95 ± 0.21	217 ± 48
XmnI 11 (n=1)	7.30	1.53	5.49	1.14	180
SSt 00(n=39)	7.52 ± 1.09	3.52 ± 1.76	4.85 ± 0.91	.098 ± 0.23	201 ± 41
SstI 01 (n=12)	7.66 ± 1.30	3.38 ± 1.07	5.07 ± 1.22	0.91 ± 0.14	190 ± 25
Ssti 11 (n=2)	6.69	2.99	4.28	0.87	180

Second, the familial aggregation of lipoprotein disorders, resistance to insulin-mediated glucose uptake, hypertension, fasting hyperglycemia, and abdominal obesity are seen frequently as part of the "metabolic syndrome" (syndrome X) (49), familial dyslipidemic hypertension (50), and in clustering of cardiovascular risk factors (51, 52). The genetic basis or the molecular defect(s) of this clustering of risk factors remains unknown. It is not surprising, however, that candidate genes within the genes associated with lipoprotein metabolism are not strongly associated with the phenotype under study. This is particularly relevant if the main stimulus for increased secretion of hepatic apoB-containing lipoproteins is substrate availability. Any condition that causes increased fatty acid delivery to the liver, such as a diet rich in saturated fats, peripheral insulin resistance, increased hepatic uptake of triglyceride-rich lipoproteins or decreased fatty acid utilization or incorporation into peripheral (adipose) tissues, may increase hepatic apoB (and triglyceride secretion). Under these conditions, it is difficult to associate genetic variability of the apoA-I–C-III–A-IV gene cluster and hepatic secretion of apoB.

Functional defects within LPL have been reported to be present in roughly one third of FCH probands (22). Due to the high frequency of three functional mutations of the LPL gene in the province of Québec, Canada, we have taken this opportunity to examine the role of LPL in FCH. We were unable to identify an association with these three LPL mutations in our 54 probands with FCH.

In light of these findings in our FCH probands, the data suggest the following. First, the previously reported association of FCH with the XmnI RFLP of the apoA-I gene does not appear substantiated in our selected probands. Second, three common functional mutations within the LPL gene are not associated with FCH. Third, FCH represents a poorly defined lipoprotein abnormality, very often associated with abdominal obesity (or increased BMI, as seen here), high blood pressure, and peripheral insulin resistance. Despite tremendous progress in the fields of lipoprotein metabolism and molecular biology made since the original description of FCH, we are no closer to finding an acceptable definition or a molecular defect. Our current concept is that a genetic predisposition to FCH (not yet characterized) may be expressed fully in the presence of a poor lifestyle, which includes abdominal obesity, a diet rich in saturated fats, and physical inactivity. In turn, the metabolic abnormalities seen in the context of abdominal obesity and decreased insulin-mediated glucose uptake contribute to decreased fatty acid uptake into adipose tissue and secondary increased in hepatic apoB secretion. The recently characterized acylation stimulated protein-adipsin system at the level of adipose tissue may provide important insight into the pathogenesis of FCH and other familial lipoprotein disorders (53).

The search for a genetic basis of FCH is sorely needed. Unlike monogenic disorders such as familial hypercholesterolemia, type III dyslipoproteinemia, or familial defective apoB-100 (54), the basis for FCH is likely to be polygenic and have an important environmental influence. Cullen et al. (55) recently performed complex segregation analysis in a group of 55 British families with FCH and re-examined the original data published by Goldstein et al. (1). They found evidence for a major gene acting on triglyceride levels but not on other lipoprotein fractions. This analysis confirms the genetic nature of the disorder and points to the importance of triglyceride metabolism in FCH. An association of FCH with genetic markers for the short arm of chromosome 19 (near the LDL receptor gene locus) has raised the possibility that the LDL-R gene or a gene close to it may be involved with the disorder (56). Physiologically, it is difficult to implicate the LDL-R gene in FCH. One must keep in mind, however, that overproduction of apoB is also seen in FH individuals. This association warrants further research.

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