Prevalence of alleles encoding defective lipoprotein lipase in hypertriglyceridemic patients of French Canadian descent

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Abstract It has previously been estimated that due to genetic "founder effects," 97% of lipoprotein lipase (LPL) gene alleles conferring type I hyperlipoproteinemia (HLP) in French Canadians encode one of the following mutant LPL forms: Gly188 → Glu, Pro207 → Leu, or Asp250 → Asn. Although the genetic basis of type I HLP is known to be homozygosity for LPL deficiency, that for other forms of HLP, especially types IV, and V HLP, is not clear. It is also unclear whether hypertriglyceridemia due to very low density lipoprotein (VLDL) overproduction can be distinguished phenotypically from that due to defective catabolism of plasma lipoprotein triglycerides. The present study took advantage of the unique circumstances inherent in the relatively genetically isolated French Canadian population to address these questions. This study was carried out in order to determine the prevalence of these three mutant LPL alleles, and of a fourth encoding LPL Asn291 → Ser, in French Canadian patients with hypertriglyceridemia. The prevalence of heterozygosity for one of the four LPL mutant alleles in nondiabetic, nonobese hypertriglyceridemic subjects was 16 of 95 type IV HLP (17%) and 4 of 26 type V HLP cases (15%). These alleles were not found in over 150 normotriglyceridemic subjects, supporting the likelihood that the mutant alleles were at least partially responsible for HLP. In addition, heterozygosity for LPL deficiency due to one of these mutations apparently did not contribute to hypoalphalipoproteinemia, and was observed in 3 of 39 subjects with type III HLP. The results suggest that in French Canadians, 15–20% of type IV and V HLP cases are associated with these genetic defects in plasma triglyceride catabolism.—**Minnich, A., A. Kessling, M. Roy, C. Giry, G. DeLangavant, J. Lavigne, S. Lussier-Cacan, and J. Davignon.** Prevalence of alleles encoding defective lipoprotein lipase in hypertriglyceridemic patients of French Canadian descent. *J. Lipid Res.* 1995. **36:** 117–124.

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Lipoprotein lipase (LPL), present on the luminal membrane of capillary endothelial cells, hydrolyzes plasma chylomicron and VLDL triglycerides. The most severe and rare form of genetic hypertriglyceridemia, type I hyperlipoproteinemia (HLP) or familial chylomicronemia, is caused in most cases by homozygosity for a genetic defect in LPL that renders the enzyme catalytically dysfunctional (1). Hypertriglyceridemia of types III, IV, and V represent three other disorders of triglyceride metabolism that are associated with genetic components (1). Each is characterized by the abnormal presence or concentrations, in the fasting state, of different subclasses of plasma d < 1.006 lipoproteins (β-VLDL, pre-β VLDL, and pre-β VLDL with chylomicrons, respectively).

The contribution of heterozygous LPL deficiency to these forms of hypertriglyceridemia in the general population is not known. For type III HLP, homozygosity for apolipoprotein E2, a form of apoE that is defective in binding to the LDL receptor, is a necessary but not sufficient condition (1). Other genetic factors associated with type III HLP have not been identified. Although LPL mediates the binding of apoE-containing lipoproteins to the putative chylomicron remnant receptor in vitro (2), the role of LPL in type III HLP in vivo has not been determined. The etiology of some instances of type IV HLP, such as in familial combined hyperlipoproteinemia (FCH), a syndrome characterized by hypertriglyceridemia, hypercholesterolemia, and the simultaneous presence of both within one family (3), is suspected on the basis of kinetic studies to be hepatic overproduction of apoB-containing lipoproteins. However, no molecular or genetic basis for this phenotype has been established (4). Another instance of type IV HLP is known as familial hypertriglyceridemia, a distinct monogenic disorder (3)

Abbreviations: LPL, lipoprotein lipase; HLP, hyperlipoproteinemia; VLDL, very low density lipoprotein; FCH, familial combined hyperlipoproteinemia; HDL, high density lipoprotein; RFLP, restriction fragment length polymorphism; TG, triglyceride.

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that has been attributed to overproduction of hepatic VLDL-triglyceride (5). The more severe type V HLP is thought to result from a combination of hepatic VLDL overproduction and defective catabolism of plasma lipoprotein triglyceride (1), but as in type IV HLP, no genetic or etiologic basis has been demonstrated. Family studies of heterozygous LPL deficiency in which carriers of dysfunctional LPL alleles were compared to noncarriers, (6, 7) have established that heterozygosity is associated with hypertriglyceridemia and other differences in lipoprotein profiles. However, it is unclear whether hypertriglyceridemia due to heterozygous LPL deficiency can be phenotypically distinguished from that which results from hepatic VLDL overproduction.

LPL activity may also be implicated in the rate of production of HDL, whose lipid and protein surface components are a product of VLDL or chylomicron remnant lipolysis. Indeed, there is some evidence for association of genetic variability in the LPL gene, as measured by RFLP genotypes, with differences in HDL levels (8, 9). Additional evidence for the role of LPL activity in determining HDL levels is provided by the observation that transgenic mice overexpressing the LPL gene display increased HDL levels (10). As such, normotriglyceridemic hypoalphalipoproteinema, an additional form of dyslipoproteinemia for which no genetic basis has been established, may also be related to mildly defective triglyceride catabolism.

These circumstances stimulated the investigation of the prevalence of heterozygosity for a dysfunctional LPL in type III, type IV, and type V HLP, and in normotriglyceridemic hypoalphalipoproteinemia. The aim of the present study was therefore to establish the prevalence of LPL alleles encoding defective forms of LPL in these forms of dyslipidemia. The prevalence of homozygous LPL deficiency (recessive type I HLP) in French Canadians is the highest in the world, and the frequency of defective LPL alleles is estimated to be as high as 1 in 40 in some regions (11). Furthermore, due to genetic "founder effects," 97% of defective LPL alleles in French Canadians with type I HLP can be accounted for by one of three known LPL mutations (12, 13): Gly188 → Glu (14), Pro207 → Leu (12), and Asp250 → Asn (15). In addition, a fourth, recently described LPL mutation, Asn291 → Ser (16), is found in French Canadians. This situation greatly facilitates the study of the contribution of heterozygosity for LPL deficiency to these disorders of triglyceride metabolism in the French Canadian population.

METHODS

Study subjects

Dyslipidemic subjects for this study were selected from among patients attending the lipid clinic at the Clinical Research Institute of Montreal (IRCM). Criteria for inclusion in the type IV HLP group were fasting plasma triglyceride concentrations consistently greater than 2.3 mmol/l, LDL-cholesterol concentration less than 5.0 mmol/l, and the absence of fasting chylomicronemia, apoE 2/2 phenotype, diabetes, or other known disorders associated with hypertriglyceridemia. Hypertriglyceridemic subjects were classified as type V based on the repeated presence of fasting chylomicronemia in addition to elevated VLDL as judged by agarose gel electrophoresis. Type III HLP was defined as the presence of apoE phenotype E2/2, with β-VLDL on agarose gel electrophoresis. The hypoalphalipoproteinemia group consisted of clinic patients or normal subjects with plasma triglyceride and LDL cholesterol concentrations less than 2.3 and 5.0 mmol/l, respectively, and with HDL-cholesterol levels lower than the 10th percentile for a healthy French Canadian population sample (17). The type II subject group consisted of hypercholesterolemic subjects with HDL cholesterol levels lower than the 10th percentile. Hypercholesterolemic subjects were screened for LDL receptor mutations known to occur in French Canadians (18), and those without were classified as type II based on LDL cholesterol levels greater than 5.0 mmol/l. This group consisted of 42 and 50 subjects with type IIA (normotriglyceridemic) and IIB (plasma TG > 2.3 mmol/l) HLP, respectively. Normal subjects with HDL cholesterol levels between the 40th and 60th percentile were selected from a previously described population sample selected for health (17), or from normolipidemic family members of clinic patients. For all categories, subjects for whom maternal and paternal French-Canadian origin could not be established by subject interview were excluded from the study. All subject groups contained only unrelated individuals. All values for lipoprotein lipids and protein reported herein were measured in non-medicated subjects.

Lipoprotein analysis

Plasma was isolated from venous blood of fasting subjects. Lipoproteins were isolated by ultracentrifugation at d 1.006 g/ml to obtain VLDL and precipitation of apoB-containing lipoproteins in the d > 1.006 g/ml fraction to separate LDL from HDL (19). Plasma and lipoprotein cholesterol and triglyceride concentrations were determined enzymatically on an automated analyzer (Abbott Biochromatic Analyzer model 100, Abbott Laboratories, Pasadena, CA). Plasma total and d > 1.006 g/ml apoB concentrations were determined by electroimmunoassay (20). ApoE phenotypes were determined as described (21). Plasma lipoproteins were subjected to agarose gel electrophoresis (22) with a Beckman Paragon LipoGel system and visualized by Sudan Black staining.

DNA analysis

DNA was extracted from white blood cells with an Applied Biosystems 340A extractor. Oligonucleotides were

synthesized by the solid phase triester method on a Pharmacia LKB Gene Assembler Plus DNA synthesizer. One μg genomic DNA was amplified by polymerase chain reaction (PCR) (23). For detection of point mutations in exon 5 of the LPL gene, exon 5 was amplified with 200 pmol each of primers 5'-TTCCCTTTTAAGGCCTCGAT-3' and 5'-AAGTCCTCTCTCTGCAATCAC-3'. Amplification conditions were 94°C, 1.5 min; 55°C, 2 min; 72°C, 1.25 min for 30 cycles. The LPL mutation Gly188→Glu was detected by Ava II restriction digestion of this DNA fragment (24). The mutation Pro207→Leu was detected by restriction digestion of this fragment with BslI (New England Biolabs) digestion (**Fig. 1**). The mutation Asp250→Asn was detected by Taq I restriction digestion after amplification of exon 6 of the LPL gene as described (15). LPL Asn291→Ser (resulting from an A to G substitution) was detected by mismatch PCR (method kindly communicated by Dr. Y. Ma) in which LPL exon 6 was amplified with primers 5'-GCCGAGATACAATCTTGGTA-3' and 5'-CTGCTTCTTTTGGCTCTGACTGTA-3' (mismatch underlined) at 94°C, 1 min; 51°C, 1 min; 72°C, 45 sec. Digestion of this DNA fragment with RsaI generates fragments of 238 and/or 215 base pairs for normal and mutant DNA, respectively.

Statistical analysis

Statistical analyses were carried out with Statistical Analysis System® software (SAS Institute, Inc., Cary NC).

RESULTS

Frequency of LPL mutant alleles in types IV and V hypertriglyceridemic French Canadians

In 95 unrelated French Canadian type IV HLP subjects tested, 16 (17%) were carriers of one of four mutations in the LPL gene. Six type IV subjects were heterozygous for LPL Gly188→Glu, one for Pro207→Leu, one for Asp250→Asn, and five for Asn291→Ser. One subject was a compound heterozygote for Gly188→Glu and Asn291→Ser, two for Pro207→Leu and Asn291→Ser. In 26 French Canadian type V hypertriglyceridemic subjects, four (15%) were found to carry a mutant LPL allele (one each of LPL Gly188→Glu and Asp250→Asn, two of Pro207→Leu). None of the four mutant LPL alleles were detected in a subsample of 72 normolipidemic French Canadian subjects (27 women, 45 men) with HDL levels in the 40–60th percentile. A chi-square test of the null hypothesis that the frequencies of mutant LPL alleles in normotriglyceridemic and hypertriglyceridemic subjects were equal was rejected (χ^2 vs. type IV = 12.67, ($P < 0.001$), vs. type V, 10.78, ($P < 0.01$)). No evidence for a difference in mutant LPL allele frequency between type IV and type V was detected (χ^2 = 0.03, NS).

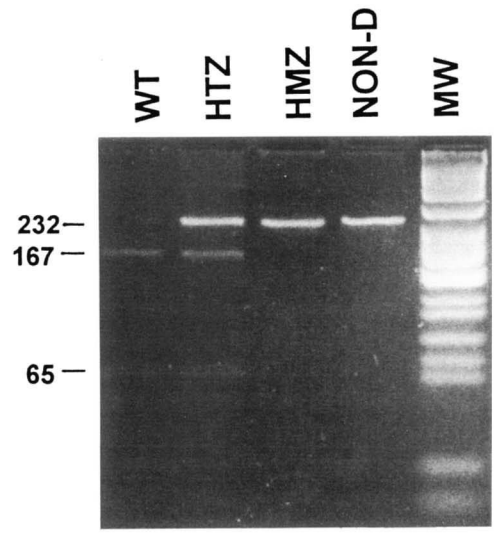

Fig. 1. Detection of the LPL Pro207→Leu mutation. Exon 5 of the LPL gene was PCR-amplified from genomic DNA and digested with BslI. DNA is from WT, a subject wild-type for the mutation LPL Pro207→Leu; HTZ, HMZ, from subjects heterozygous and homozygous, respectively, for the LPL Pro207→Leu mutation. Non-D, non-digested fragment (length = 232 base pairs). MW, MspI digested pBR322 DNA as molecular weight standard.

Potential sources of variability in plasma TG concentrations

Among type IV subjects selected for this study, a wide (almost 20-fold) range of plasma TG levels was observed. It has been noted previously (25) that the expression of hypertriglyceridemia in heterozygotes for LPL deficiency is age-dependent, with expression occurring after age 40 (6). However, among the present group of type IV subjects, no significant portion of the variability in plasma TG concentrations could be ascribed to age as the regression of log-transformed plasma TG concentrations on age gave an R^2 value of 0.02 (NS) for both males and females (n = 74 and 21, respectively). The mean age of type IV carriers of LPL mutations was significantly lower than that of noncarriers (**Table 1**), and 8 of the 15 male carriers were under the age of 40. Among male type IV subjects, body mass index accounted for a small but significant proportion of the variability in log-transformed plasma TG levels, R^2 = 0.08, n = 66, $P < 0.02$). However, the mean BMI of male carriers and noncarriers were not different (Table 1), and adjustment of TG levels for BMI did not influence mean plasma TG levels for each group. Intake of tobacco, alcohol, and saturated fat were not different among carrier and noncarrier type IV subjects, and no correlation was observed by any of these variables and log-transformed plasma TG concentrations.

Another potential source of variability in plasma TG levels is apoE phenotype (26); in particular, the E2 and E4 isoforms have been associated with a triglyceride-raising

TABLE 1. Environmental influences on plasma TG levels in hypertriglyceridemic French Canadians

Subject Group	Age	BMI	Tobacco[a]	Alcohol[b]	Saturated Fat[b]
	yr	*kg* $(m^2)^{-1}$			
Noncarrier IV, (n = 68)	45 ± 1.4[c]	26.2 ± 0.4[c]	43	3.6 ± 0.7	11.7 ± 0.4
Carrier, IV, (n = 14)	39 ± 2.3[c,d]	26.3 ± 0.9[c]	54	3.5 ± 1.0	11.9 ± 0.7
Noncarrier V, (n = 17)	40 ± 2.0	29.0 ± 0.8	59	4.8 ± 1.6	10.5 ± 0.9
Carrier V, (n = 3)	36.5 ± 4.2	25.6 ± 1.6	33	5.7 ± 5.7	13.3 ± 1.8

Noncarrier: subjects not carrying defective LPL alleles; carrier: subjects heterozygous for a mutant LPL allele (see text). The number of subjects for whom dietary information was available is indicated in parentheses. Values are mean ± SEM the day of plasma TG measurement. BMI, body mass index.

[a] Percent of subjects who were current smokers at the time of plasma TG measurement.

[b] Percent of total calories.

[c] Males only; includes all male type IV subjects (see Table 3).

[d] $P < 0.05$ versus carrier IV (Student's *t*-test, equal variances).

effect (26). **Table 2** shows the apoE phenotypes of the different groups of hypertriglyceridemic subjects. The proportion of subjects with apoE isoforms 2 and 4 were similar in carriers and noncarriers. Compared with the frequencies of alleles encoding these isoforms among French Canadians in general (17) the prevalence of the E4 isoform (35/120) was similar, while that of the E2 isoform (43/120) was considerably elevated, in our sample of type IV and type V subjects. Among type IV subjects, plasma triglyceride concentrations were not statistically significantly different in subjects with and without the apoE2 isoform (data not shown).

Comparison of plasma lipoprotein phenotypes between carriers and noncarriers of LPL mutant alleles

Of the type IV HLP carriers of LPL mutant alleles, only one was female, and due to gender differences in lipoprotein lipid and protein levels (see **Table 3**), comparisons of these levels between carriers and noncarriers was possible only in males. In male type IV subjects, mean fasting plasma TG concentrations were significantly greater in carriers than in noncarriers of LPL mutations (Table 3), as were log-transformed TG levels (data not shown). HDL levels in both groups were in the 5th percentile of the normal range for the French Canadian population (17), and these levels were significantly lower in carriers versus noncarriers. No differences in lipoprotein levels were apparent between males and females in type V subjects (Table 3), and meaningful comparisons of lipoprotein lipid levels between type V carriers and noncarriers could not be made with only four carriers.

As previously suggested, the presence of two phenotypically different forms of type IV hypertriglyceridemia may be distinguishable on the basis of plasma total apoB concentrations (4, 20, 27), LDL-cholesterol to LDL apoB ratios (28), or apoB to TG ratios (5). Carriers of LPL mutant alleles were distributed evenly over the frequency distributions for these traits (data not shown) as judged by visual observation. None of these traits were significantly different between carriers and noncarriers of mutant LPL alleles (Table 3 and data not shown).

The lower HDL cholesterol levels in LPL mutant allele carriers might be solely related to higher TG levels, or they might reflect qualitative differences in HDL metabolism between carriers and noncarriers. In fact, plasma HDL cholesterol concentrations were lower in carriers than in noncarriers (mean ± SD = 0.58 ± 0.13 vs. 0.68 ± 0.17, respectively, $P < 0.02$) even when the subjects were matched for plasma TG concentrations (9.95 ± 10.1 vs. 8.64 ± 8.9, respectively, $P < 0.6$), age, and sex (**Fig. 2,** HDL). Variability in TG levels accounted for 56% of the variability in HDL levels in carriers, but for only 15% in noncarriers (Fig. 2). A test of the identity of the regression coefficients (29) for the two equations depicted in Fig. 2 gave $F^* = 2.41$ ($F_{0.90,2,58} = 2.39$). LDL cholesterol levels were strongly inversely related to plasma TG concentrations in both groups of hypertriglyceridemic subjects (Fig. 2, LDL).

TABLE 2. Apolipoprotein E phenotypes of type IV and type V hypertriglyceridemic subjects

	ApoE Phenotype					
Group	E3/3	E3/2	E4/3	E4/2	E4/4	Total
Noncarriers	37/3	19/10	15/7	6/2	1/0	78/22
Carriers[a]	7/1	2/2	2/1	0/0	0/0	11/4
LPL Asn291→Ser[b]	2	2	1	0	0	5
Total	46/4	23/12	18/8	6/2	1/0	94/26

Noncarrier: subjects not carrying defective LPL alleles; carrier: subjects heterozygous for a mutant LPL allele (see text). Numbers to the left and right side of the slash denote the numbers of type IV and V subjects, respectively, with a given apoE phenotype.

[a] Includes subjects heterozygous for LPL Gly188→Glu, Pro207→Leu, or Asp250→Asn, and three subjects with compound heterozygosity for LPL Asn291→Ser and one of these mutations.

[b] Subjects heterozygous for LPL Asn291→Ser only. ApoE phenotype was unavailable for one type IV noncarrier subject.

TABLE 3. Plasma lipoprotein lipid and protein concentrations in type IV and type V subjects with and without LPL gene mutations

Group	n	TG	HDL-C	LDL-C	LDL-B	ApoB	VLDL-B	B/TG[a]
All Type IV								
M	74	6.6 ± 0.8	0.67 ± 0.02	2.7 ± 0.1	121 ± 3.6	165 ± 5.1	45 ± 5.8	0.4 ± 0.02
F[b]	21	7.2 ± 1.1	0.81 ± 0.05	3.2 ± 0.2	136 ± 7.8	187 ± 8.4	50 ± 9.4	0.4 ± 0.03
All Type V								
M	20	24.4 ± 4.0	0.55 ± 0.03	1.4 ± 0.2	81 ± 8.5	189 ± 14	108 ± 15	0.12 ± 0.02
F[c]	6	23.7 ± 6.9	0.47 ± 0.02	1.0 ± 0.2	78 ± 7.5	185 ± 25	106 ± 30	0.12 ± 0.02
Noncarrier IV[d]	59	5.8 ± 0.8	0.69 ± 0.02	2.7 ± 0.1	121 ± 3.7	161 ± 5.4	40 ± 5.9	0.42 ± 0.07
Carrier IV[d]	15	9.4 ± 2.5[e]	0.60 ± 0.04[e]	2.6 ± 0.3	117 ± 10.4	182 ± 12.7	65 ± 15.6	0.38 ± 0.06
Non carrier V	22	24.1 ± 3.7	0.54 ± 0.03	1.36 ± 0.2	81 ± 5.2	187 ± 12.9	106 ± 13.9	0.12 ± 0.02
Carrier V	4	25.3 ± 10.8	0.47 ± 0.02	0.94 ± 0.2	60 ± 10.8	193 ± 46.4	120 ± 50	0.10 ± 0.02

Abbreviations: n, number of subjects; TG, plasma triglyceride concentration; HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; LDL-B, LDL apolipoprotein B; apoB, total plasma apolipoprotein B; M, male; F, female. Noncarrier: subjects not carrying defective LPL alleles; carrier: subjects heterozygous for a mutant LPL allele (see text); IV, subjects with type IV HLP; V, subjects with type V HLP. Plasma lipid levels are expressed as mmol/l; apolipoprotein B levels are expressed as mg/dl. Values are means ± SEM.

[a] ApoB to TG ratio.

[b] Five, five, and eleven pre- and post-menopausal with and without hormone replacement therapy, respectively.

[c] Two, three, and one pre- and post-menopausal with and without hormone replacement therapy, respectively.

[d] Males only.

[e] $P < 0.05$ versus noncarrier IV (Student's t-test, equal variances).

Association of LPL mutant alleles in other forms of dyslipidemia

The apparently low plasma HDL cholesterol levels in LPL mutation carriers suggested that mutant LPL alleles might be detected in subjects with low HDL cholesterol levels even in the absence of hypertriglyceridemia. However, when 39 (17 women, 22 men) normotriglyceridemic subjects with HDL cholesterol levels lower than the 10th percentile for normal French Canadians (17), and 92 subjects with type II (42 type IIA and 50 type IIB) hyperlipoproteinemia and HDL levels below the 10th percentile were examined, none of the four mutant alleles was detected, with the sole exception of one type IIB carrier LPL Asn291→Ser.

It has been reported that LPL mediates the binding of remnant lipoproteins to the chylomicron remnant receptor (2). To determine whether these mutant LPL alleles contributed to the expression of type III hyperlipidemia in subjects with apoE phenotype E2/2, 39 subjects with type III HLP were screened for the LPL mutations; one carrier each of Pro207→Leu, Asp250→Asn, and Asn291→Ser were found.

DISCUSSION

Among French Canadians with homozygous LPL deficiency, or type I HLP, three alleles encoding LPL mutations Gly188→Glu, Pro207→Leu, or Asp250→Asn, account for 97% of mutant LPL alleles (12, 13). However, the contribution of LPL mutant alleles to HLP types IV and V is not known. One goal of this study was, therefore, to establish the prevalence of LPL alleles encoding defective forms of LPL in French Canadian subjects with these forms of HLP. In addition to the three mutant alleles found in type I HLP, a fourth mutation, Asn291→Ser (16) was studied. One or two mutant alleles encoding one of these mutations were present in 16 of 95 and in 4 of 26, respectively, of subjects with types IV and V HLP. We did not detect any significant difference in the frequency of LPL mutant alleles in subjects diagnosed with type IV and type V HLP. Although hypertriglyceridemia is frequently associated with diabetes, hyperglycemic subjects were excluded from this study. Thus, we cannot draw any etiologic conclusions to distinguish between types IV and V HLP based on heterozygosity for mutant LPL alleles.

The LPL variants encoded by three of these alleles have been well characterized. The catalytic inactivity of LPL mutations Gly188→Glu, Pro207→Leu, and Asp250→Asn has been demonstrated in vitro (12, 15, 30). Also in vitro, LPL Pro207→Leu is poorly secreted and LPL Gly188→Glu displays low heparin affinity. Post-heparin plasma LPL mass and activity in 13 of 14 offspring of type I subjects, i.e., obligate heterozygotes for LPL deficiency of unknown molecular basis, was below the 95% confidence limit for normals (31), and it has been reported that heterozygosity for LPL Pro207→Leu is associated with reductions of 36% and 57% of plasma LPL activity and mass, respectively (32). A previous study has demonstrated an association between heterozygosity for LPL Gly188→Glu and familial hypertriglyceridemia in one family (6). Finally, in the present study, these mutant LPL alleles were completely absent in over 150 normotriglyceridemic subjects tested. Thus, it is reasonable to suggest that, in French Canadians, heterozygosity for one of these defective alleles contributes to hypertriglyceridemia.

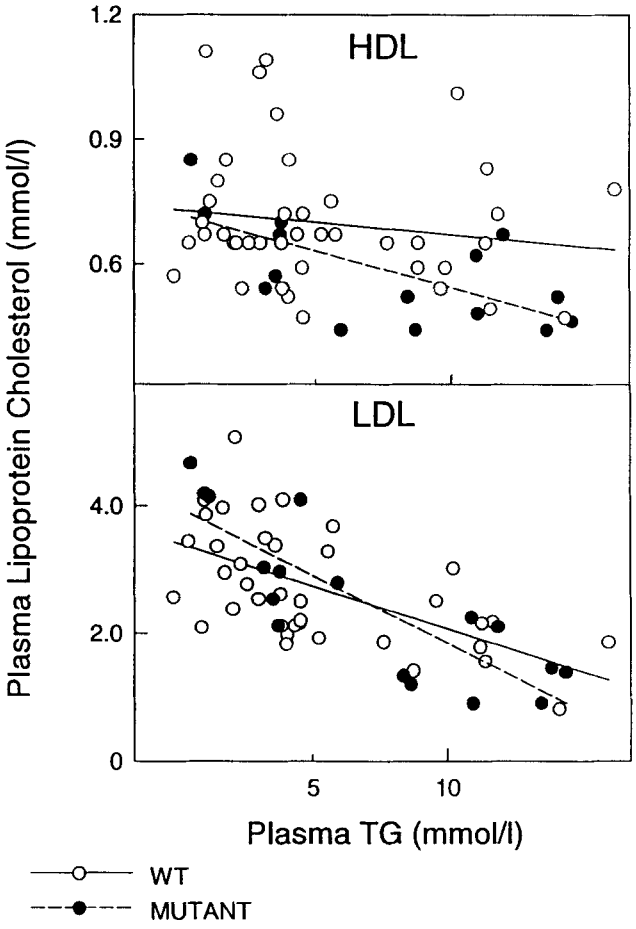

Fig. 2. Relationship between plasma TG and either HDL or LDL cholesterol levels in hypertriglyceridemic subjects. HTZ (filled symbols) are type IV or type V hypertriglyceridemic subjects heterozygous for one of the three LPL mutations (see text). WT subjects (open symbols) are all type IV or V subjects with none of these mutations, matched for age, sex, and plasma TG concentrations to the heterozygotes. One female type V heterozygote (TG 57.02 mmol/l) is not presented as no adequate control was available. Dotted and solid lines indicate regressions for carriers and noncarriers, respectively. HTZ: HDL-C = 0.84 −0.30*logTG, R^2 = 0.56 ($P < 0.001$); WT HDL-C = 0.83 − 0.20*logTG, R^2 = 0.15 ($P < 0.01$). HTZ LDL = 5.40 −3.5*logTG, R^2 = 0.70 ($P < 0.001$); WT LDL = 4.2 −2.09*logTG, R^2 = 0.49 ($P < 0.001$).

The absence of mutant LPL alleles in our sample of normotriglyceridemic French Canadians would appear to contradict a previous report that some heterozygotes for mutant LPL alleles are normotriglyceridemic (25). Part of the explanation for incomplete penetrance of the LPL mutation in these heterozygotes was hypothesized to be subjects' age, as in one large family, carriers of LPL Gly188→Glu developed hypertriglyceridemia only after age 40 (6). However, among the LPL-deficient heterozygotes with type IV or type V HLP described in the present study, 10 of 17 males were under the age of 40. These apparent differences in the penetrance of the LPL Gly188→Glu mutation could be related to differences in environmental or genetic factors between French Canadians and previously described populations sampled. One potential genetic factor is possibly the apoE2 isoform, associated with a triglyceride-raising effect (26), which was almost three times as prevalent in our sample of hypertriglyceridemic subjects (43 of 120) as in the French Canadian population (approximately 13%) (17).

The fourth LPL variant, LPL Asn291→Ser, first described by Ma et al. (16), has not yet been as well characterized biochemically as have the first three. Preliminary data (16) have suggested that this LPL variant exhibits only a moderate catalytic deficiency due to a partial impairment in dimerization. Thus, it is perhaps surprising that heterozygosity for this LPL mutation was found in five type IV subjects. However, three of these subjects were carriers for apoE isoforms E4/3 or E3/2, which isoform has been suggested to exacerbate expression of hypertriglyceridemia in the presence of LPL deficiency (33). In addition, three compound heterozygotes for this mutation and a catalytically inactive LPL variant did not consistently display type I HLP, a disease which almost invariably results from homozygosity for LPL deficiency, and LPL Asn291→Ser was not found in subjects with the more severe type V HLP. These observations are consistent with the notion that this variant is only moderately catalytically defective.

In the present study, a notable difference in the relation between plasma triglycerides and HDL cholesterol levels was observed between carriers and noncarriers of mutant LPL alleles matched for plasma triglyceride levels. Among the former group, HDL cholesterol levels were strongly inversely correlated to TG levels while a much weaker relation was observed in the latter group. In general, two mechanisms are hypothesized to be responsible for low HDL cholesterol levels in hypertriglyceridemia. One is reduced transfer of triglyceride-rich lipoprotein surface components such as phospholipids and apoC to nascent HDL. The other is loss of HDL cholesterol due to the exchange of HDL cholesteryl esters for VLDL triglycerides catalyzed by the plasma cholesteryl ester transfer protein (34, 35). If both of these mechanisms are important, a defect in hydrolysis of VLDL TG might be expected to lower HDL levels more severely than hypertriglyceridemia due merely to overproduction of VLDL triglyceride. HDL-cholesterol levels varied over a threefold range in noncarriers (0.36–1.15) but over only a twofold range in carriers (0.34–0.75), in spite of similar ranges for TG, consistent with the likelihood that LPL deficiency is the primary defect in the carriers, but that hypertriglyceridemia in at least some noncarriers results from VLDL overproduction. In addition to the higher plasma TG and lower HDL cholesterol levels, another indication of the greater severity of the primary defect in the carrier group was the younger age at which the subjects presented with hypertriglyceride-

mia. The source(s) of variability in TG levels in either group of hypertriglyceridemic subjects is unclear, but may reflect normal inter-individual variability superimposed on defective TG metabolism. Heterogeneity of HDL metabolism such as is present in our group of noncarrier hypertriglyceridemic subjects has been associated with interindividual differences in insulin resistance and abdominal obesity (36).

Though types III and IV hypertriglyceridemia are potentially associated with increased risk for cardiovascular disease (1), hyperchylomicronemia (type I HLP) is not, presumably due to the inability of the large lipoproteins to penetrate the arterial endothelium (37). LPL activity in the arterial endothelium has been hypothesized to be atherogenic, since it would promote the uptake of remnant lipoproteins (38). Thus, LPL deficiency may be an unusual genetic risk factor for cardiovascular disease in that heterozygosity might confer greater risk than does homozygosity in hypertriglyceridemia. Therefore, comparison of risk for premature cardiovascular disease in French Canadian hypertriglyceridemic subjects with and without mutant LPL alleles may clarify whether heterozygous LPL deficiency offers any protection against premature CVD via a lowering of arterial LPL activity.

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