SHORT REPORTS

Prevention of raised low-density lipoprotein cholesterol in a patient with familial hypercholesterolaemia and lipoprotein lipase deficiency

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Raised low-density lipoprotein (LDL) cholesterol is believed to predispose to development of atherosclerosis and coronary heart disease. Increased plasma LDL concentrations and premature coronary heart disease are present in familial hypercholesterolemia (FH), and the enzyme lipoprotein lipase (LPL) seems to have a key role in production of LDL. We describe a unique French Canadian individual who is both heterozygous for FH and homozygous for LPL deficiency (FH/LPL). In this patient, LDL cholesterol was strikingly low compared with both his FH (0·65 *vs* 5·84 mmol/L) and normolipidaemic (2·77 mmol/L) age-matched relatives despite the defect of LDL-receptor-mediated removal. No LDL peak was present in the cholesterol profile of the FH/LPL-deficient subject, as determined by density-gradient ultracentrifugation. Our results suggest that most LDL particles, in vivo, originate from triglyceride-rich lipoproteins, that LPL plays a vital part in this process, and that absence of LPL activity protects FH subjects against the increase in LDL cholesterol.

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Low-density lipoproteins (LDLs) are the major cholesterol-carrying particles in plasma. Epidemiological studies indicate that development of coronary heart disease is related directly to serum concentrations of LDL-cholesterol.[1] LDL is produced by stepwise hydrolysis of triglycerides in the core of very-low-density lipoproteins (VLDLs). The liver secretes triglyceride-rich VLDLs into the bloodstream where the triglyceride content is progressively removed by hydrolysis by the enzyme lipoprotein lipase (LPL) to form smaller and denser particles called intermediate-density lipoproteins (IDLs), which are potential precursor for LDLs.[2] LPL is critical for the production of LDLs[3] and in patients with complete LPL deficiency, plasma concentrations of LDLs are very low.[4] IDLs can be either degraded by the liver via receptor-mediated binding or further metabolised to LDLs. Although some steps in LDL formation are not fully understood, it is commonly accepted that LDL particles originate in part from VLDL through the metabolic pathway described above. However, kinetic studies of lipoprotein metabolism in humans and primates suggest that some LDLs are normally secreted from the liver.[5] Thus, LDL production appears to be heterogeneous and may not always be dependent on the action of the LPL on VLDL.

The catabolism of LDL via receptor-mediated and receptor-independent removal is well understood. A mutation affecting the structure and function of the cell surface receptor that normally removes LDL from plasma results in a specific form of dislipidaemia, familial hypercholesterolaemia.[6] This metabolic disorder, which is inherited as an autosomal dominant trait, is characterised by a substantial increase in plasma LDL concentration, cholesterol deposits in skin and tendons (xanthomas), and premature coronary heart disease.[6] Familial hypercholesterolaemia heterozygotes occur at a frequency of about 1 in 500 of the population.

Familial LPL deficiency is a rare autosomal recessive disorder that occurs in about 1 in every million persons. The disease is characterised by an accumulation of chylomicrons with corresponding increase of plasma triglyceride, repeated episodes of abdominal pain and pancreatitis, and eruptive cutaneous xanthomatosis.[4] From the part played by LPL in triglyceride-rich lipoprotein metabolism, it might be predicted that absence of LPL, which would result in a massive increase of plasma triglyceride concentrations, would also block conversion of VLDL to IDL and LDL with a subsequent decrease in plasma LDL concentrations.

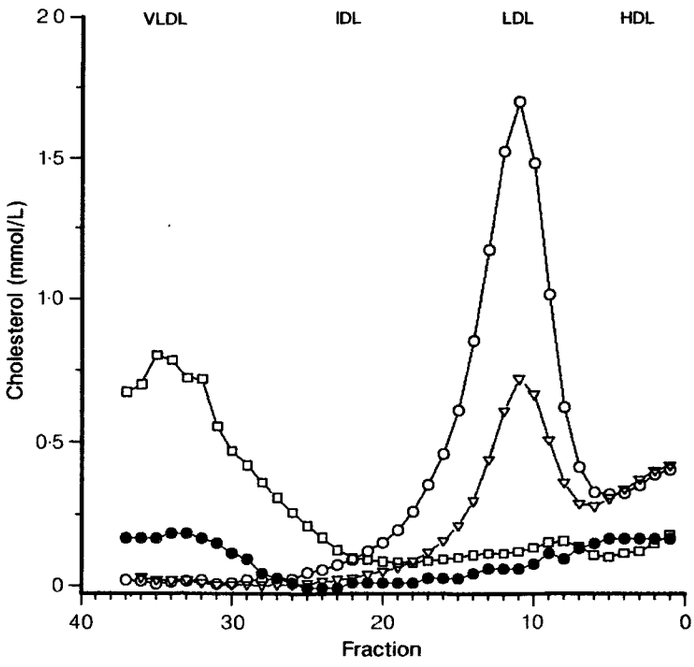
We describe a unique French Canadian individual, followed at the Lipid Center in Quebec City, who is both heterozygous for familial hypercholesterolaemia (trp_{66}→gly mutation) and homozygous for LPL deficiency (pro_{207}→leu mutation).[7]

Blood samples were collected after overnight fast from the 13-year-old male patient, from 4 age-matched relatives (mean [SE] age 10 [5] years) who are heterozygous for familial

MEAN (SE) PLASMA LIPIDS IN STUDY SUBJECTS

	Normo-lipidaemic controls (n=16)	FH/LPL-deficient patient	FH controls (n=4)	LPL-deficient subject
Cholesterol (mmol/L)				
Total	4·4 (0·2)	6·5	7·3 (1·1)	7·9
Chylomicron				
VLDL	0·4 (0·1)	5·4	0·3 (0·1)	7·0
IDL	0·1 (0·01)	0·1	0·2 (0·02)	0·2
LDL	2·8 (0·2)	0·6	5·8 (1·1)	0·6
HDL	1·3 (0·2)	0·4	1·1 (0·02)	0·3
Triglyceride (mmol/L)				
Total	2·5 (0·3)	40·4	1·9 (0·2)	99·2
Chylomicron	..	38·7	..	97·6
VLDL	..	0·2	..	0·3
IDL	..	0·8	..	1·1
HDL	..	0·9	..	0·5
LDL composition (w/w)				
TG/apoB	0·25 (0·03)	1·04	0·44 (0·06)	1·98
FC/apoB	0·49 (0·02)	0·22	0·52 (0·02)	0·5
PL/apoB	1·6 (0·1)	0·97	1·46 (0·04)	1·7
CE/apoB	2·46 (0·1)	1·15	2·83 (0·1)	1·02

FH = familial hypercholesterolaemia, HDL = high-density lipoprotein, TG = LDL triglyceride, FC = LDL free cholestrol, PL = LDL phospholipid, CE = LDL cholesteryl ester, apoB = LDL apolipoprotein B.



Density gradient ultracentrifugation profiles of lipoprotein cholesterol distribution.

Patient heterozygous for familial hypercholesterolaemia and homozygous for LPL deficiency (●), patient homozygous for LPL deficiency (□), familial hypercholesterolaemic heterozygotes (○), normolipidaemic subjects (▽).

hypercholesterolaemia, from 16 normolipidaemic, age-matched relatives (11 [1] years), and from a 43-year-old unrelated male homozygous for LPL deficiency ($gly_{188} \rightarrow glu$). None of the subjects was taking drugs known to affect lipid metabolism. Plasma was obtained by centrifugation at 4°C. Lipoprotein fractionation was by preparative ultracentrifugation.[8] Non-equilibrium density gradient ultracentrifugation for apolipoprotein-B-containing lipoproteins was done to analyse cholesterol distribution across the lipoprotein subclasses (Zambon et al, unpublished); this technique has been developed specifically to improve the resolution of apolipoprotein-B-containing lipoproteins. Cholesterol, triglyceride, and phospholipid were measured by enzymatic kits (Boehringer Mannheim and Sigma). Apolipoprotein B was analysed by immunonephelometry (Behring Diagnostic, Sommerville, New Jersey, USA). LDL-lipid composition per particle was calculated by dividing the lipid value of each LDL fraction by the apolipoprotein B content of same LDL fraction. The LPL-deficient subjects studied were affected by either the $pro_{207} \rightarrow leu^5$ or $gly_{188} \rightarrow glu^9$ mutations. These mutations were detected by polymerase chain reaction followed by dot-blot hybridisation analysis with allele-specific oligonucleotides or by restriction digestion after amplification of exon 5.[9] The point mutation in exon 3 ($trp_{66} \rightarrow gly$) of the LDL receptor was analysed by dot-blot hybridisation of amplified genomic DNA with allele-specific oligonucleotide probes.[10]

Our patient is unique because he has two genetic defects that affect LDL metabolism in different ways. The patient's relatives who are heterozygous for familial hypercholesterolaemia had LDL-cholesterol values that were twofold higher than those of normal relatives (table), as would be expected. By contrast, the man with LPL deficiency had LDL-cholesterol concentrations that were strikingly low, with most cholesterol being present in VLDL and chylomicrons associated with a substantial increase in plasma triglyceride (table). LDL-cholesterol concentrations were also strikingly low in the familial-hypercholesterolaemic/LPL-deficient patient compared with both his hypercholesterolaemic (0·65 *vs* 5·84 mmol/L) and normolipidaemic (2·77 mmol/L) relatives, despite partial deficiency of receptor-mediated LDL removal. LDL-cholesterol concentration was similar in the patient with two defects to that in the individual with LPL deficiency alone. In both these subjects, most cholesterol was found in triglyceride-rich particles, as might be expected because no LPL was available to further metabolise VLDL to IDL and LDL.

LDL compositional analysis (table) showed that LDLs from both the hypercholesterolaemic/LPL-deficient and LPL-deficient subjects were triglyceride-enriched and cholesteryl-ester-depleted compared with particles from normal subjects. LDL from the patient with a double defect was phospholipid-depleted and cholesterol-depleted (both free cholesterol and cholesteryl ester) and triglyceride-enriched compared with LDL from normal and hypercholesterolaemic relatives.

The mean cholesterol profile, determined by density-gradient ultracentrifugation, of the 4 subjects with heterozygous familial hypercholesterolaemia shows clearly a sharp and prominent peak in the LDL region (figure), reflecting the high LDL-cholesterol typical of these patients. A similar, although less prominent peak, characterises LDL distribution in 16 normal subjects. Surprisingly, no LDL peak was present in the cholesterol profile of the patient with a double defect, despite the defect in LDL-receptor-mediated removal, confirming the very low LDL-cholesterol concentrations measured in the fraction isolated by sequential ultracentrifugation (table). The cholesterol profile of the LPL-deficient subject was also characterised by absence of a clear peak in the LDL region. The abundant material found in the VLDL region of the patient with a double defect and the LPL-deficient subject reflects the high concentrations of VLDL-cholesterol and VLDL-triglyceride reported for these patients (table) and is a consequence of the absence of active LPL with subsequent metabolic block in the VLDL-IDL-LDL pathway.

Complete absence of LPL activity is associated with very low concentrations of LDL-cholesterol. Surprisingly, the plasma LDL-cholesterol concentration of the hypercholesterolaemic/LPL-deficient patient was lower than that of hypercholesterolaemic controls and of normal subjects (four and nine times, respectively) despite presence of a mutation affecting the structure and function of the cell surface receptor for LDL particles. This unique situation suggests that most LDL particles originate in vivo from the triglyceride-rich lipoproteins VLDL and IDL, and that LPL has a key role in this process. Our results also suggest that if direct secretion of LDL particles by the liver occurs in the subject with LPL deficiency and in the patient with LPL deficiency and heterozygous hypercholesterolaemia, then this process contributes only marginally to plasma LDL input. This finding confirms studies in monkeys[3] where LPL inhibition almost completely interrupted production of newly synthesised LDL particles.

In addition to providing insights into LDL metabolism, absence of LPL activity in the subject heterozygous for familial hypercholesterolaemia appears to be protective against an increase in LDL cholesterol, which is a critical risk factor for premature coronary artery disease. The massive increase in plasma triglyceride concentrations and low high-density lipoprotein cholesterol in patients with complete LPL deficiency does not seem to predispose them to atherosclerosis. Perhaps LDL-cholesterol could be lowered in individuals with homozygous familial hypercholesterolaemia by interference in the function of plasma LPL, in combination with a low-fat diet to prevent chylomicron-triglyceride-induced pancreatitis.

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Chromosome 11q13 and atopy underlying atopic eczema

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Genetic linkage has been reported between atopic respiratory disease and chromosome site 11q13. We recruited 95 multiplex families through probands with active atopic eczema. Linkage analyses between atopy and markers on 11q13 excluded a major susceptibility locus for atopy in this region. Nor was there significant deviation from the expected proportions of alleles shared by affected sib-pairs. When we analysed families according to parental atopic phenotype, we observed a positive lod score (0·8) in 19 families with unaffected fathers, in contrast to markedly negative scores for other combinations of affected parental phenotype. The possibility of a maternal influence on the inheritance of atopy cannot, therefore, be excluded.

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The atopic disorders of eczema, asthma, and allergic rhinitis are familial and associated with a raised total serum immunoglobulin E (IgE) and the development of specific IgE antibodies to common allergens. Cookson et al have reported linkage between atopy underlying asthma and allergic rhinitis and chromosome 11q13.[1]

We recruited 95 nuclear families (407 family members) on the basis of at least 2 first-degree relatives with active atopic eczema. Children under the age of one year were excluded. A detailed physician-administered questionnaire was completed for every individual. The index cases of eczema complied with the diagnostic criteria of Hanifin and Rajka.[2]

Skin prick testing to a panel of allergens was done on all individuals over the age of two years who did not have atopic eczema and also on those with eczema found to have normal total and specific serum IgE. Specific IgE was measured by fluid phase immunoenzymometry (AlaSTAT).[3] Specific antibodies to *Dermatophagoides pteronyssinus*, timothy grass, and cat dander were sought in all individuals and antibodies to hen's egg white were looked for in children aged up to three years, and other allergens where clinically indicated. Atopic status[4] was assigned prospectively.

Genomic DNA was extracted from blood and genotypes were determined by Southern blotting using the 11q13 markers pλms51 (D11S97) and CD20. Alleles at the AT repeat region of the PGYM locus were assigned by polymerase chain reaction and products were resolved on 6% denaturing polyacrylamide gels.[5] Genotypes were independently assigned by two investigators who were blind to the phenotype.

Multipoint linkage analysis was computed for an autosomal dominant model[4] using the LINKAGE package (version 5·02). Gene frequency for the disease allele was 0·25 and penetrance was set at 0·99. Affected sib-pair analysis was done, and a chi-square test was used to test for significant deviations from the expected proportions.

80% of parents (n = 196; mean age 41 [range 22–69]) and 86% of their offspring (n = 211; males/females 102/109; mean age 6 [range 1–31]) were atopic. Both, one, or neither parent was unequivocally atopic in 60, 31, and 3 families, respectively. Offspring risks of 85% for atopy were the same irrespective of whether mother, father, or both parents were affected. Clinical details will be published elsewhere.

Multipoint linkage analysis between atopy and loci at 11q13, using the marker order pms51-PGYM-CD20-centromere, gave evidence for exclusion of linkage, with location scores to −36 (lod scores to −7·8 [ie, location score ÷ 4·6]).

101 affected sib-pairs were identified, and the results of analyses with each marker independently, revealed no significant difference in sharing of paternal alleles but some excess maternally derived alleles not shared by affected sib-pairs:

	Paternal allele		*Maternal allele*	
Marker	*Sharing*	*Not sharing*	*Sharing*	*Not sharing*
pms51	21	26	23	38
PGYM	30	32	25	36
CD20	9	7	14	14

In view of previous evidence for maternal effects on the inheritance of atopy at this locus,[6] we did further analyses, dividing families according to paternal atopic status (figure). The location score for the group in which either the father or both parents were unaffected was 3·7 (lod score 0·8). Sib-pair analysis of these subgroups showed no significant deviation in the sharing of alleles, though the number in each cell was less than 6.

Linkage analysis was done with three informative markers extending over a genetic distance of 12 cM on the long arm of chromosome 11. Evidence for the exclusion of linkage between the IgE response underlying atopic eczema