Genetics of the lipoprotein lipase gene and hypertriglyceridaemia

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ABSTRACT

The aim of this study is to assess whether genetic variation at the lipoprotein lipase (LPL) gene is related to fasting triglyceride levels or to the presence of vascular disease. Hypertriglyceridaemic patients are genotyped for the N291S, G188E, and P207L variants and the *Hind*III and *Pvu*II restriction fragment length polymorphisms of the LPL gene. Sequence analysis is carried out on exons 1–9 of the LPL gene for patients with severe hypertriglyceridaemia, to search for new gene variants. No differences were found between the patient and control group for the N291S, G188E and P207L variants. The *Hind*III and *Pvu*II allelic frequencies were found to be similar for patients and controls; however, the frequency of the *PvuII* P2 allele was higher in patients with vascular disease (allele frequency: 0.56) than patients with no vascular disease (allele frequency, 0.42) (P=0.03). Sequence analysis revealed no exon sequence variants in the LPL gene but two intron sequence variants were found in intron 5 in two patients.

KEY WORDS: Gene polymorphisms. Hypertriglyceridaemia. Lipoprotein lipase. Polymerase chain reaction.

Introduction

Lipoprotein lipase (LPL) belongs to a gene family that includes hepatic lipase and pancreatic lipase. It is a glycoprotein synthesised in the parenchymal cells of many tissues, but primarily in adipose and skeletal muscle. It is secreted from these tissues and transported to the intimal surface of the vascular endothelium, to which it is anchored by a membrane-bound heparin sulphate glycosaminoglycan chain.

LPL is active as a non-covalently bound homodimer, which loses activity rapidly upon dissociation,[1] and plays an important role in the maturation of lipoprotein particles. Catabolism of very-low-density lipoprotein (VLDL) by LPL results in the formation of intermediate-density lipoprotein (IDL) and, subsequently, low-density lipoprotein (LDL). Similarly, conversion of the high-density lipoprotein (HDL) subparticles HDL_3 to HDL_2 is dependent on the LPL-catalysed hydrolysis of triglyceride-rich lipoproteins.

Hypertriglyceridaemia caused by decreased or absent LPL activity is often accompanied by decreased HDL_2 and by small, dense LDL particles that have reduced affinity for the LDL receptor. The LPL gene spans more than 35 kilobases and contains 10 exons, the first nine of which code for a 475 amino acid protein.[2] Exon 10 specifies the 3′ untranslated sequence.

Functional domains include sites for interaction with lipoprotein substrate, interaction with apolipoprotein (apo) CII, a site for subunit interaction to form the active homodimer and a site for binding to heparin sulphate. The exons that code for these domains have not been fully characterised; however, exons 4, 5 and 6 are highly conserved sequences among species and they are thought to code for regions that are part of the catalytic site.[3]

LPL deficiency is a rare recessive disorder, occurring with a frequency of one in a million, although carriers for mutations in the gene may be as frequent as one in 500.[4,5] The enzyme defect results in type I hyperlipoproteinaemia (HLP). Affected individuals are either homozygous for a single mutation or are compound heterozygotes. The majority of mutations that cause LPL deficiency are missense or non-sense mutations and most occur in exons 4, 5 and 6.

The more common forms of hypertriglyceridaemia (types IV and V HLP) may also be the result of LPL deficiencies. Altered catalytic activity or altered binding properties of the enzyme may predispose to hypertriglyceridaemia, which becomes manifest when triggered by an additional genetic or environmental component. Various studies have shown associations between variants in the LPL gene and hypertriglyceridaemia, low HDL levels and atherosclerosis.[6-14]

The objectives of the present study are to evaluate known LPL gene variants in patients with hypertriglyceridaemia, correlate genotype data with history of vascular disease and with serum lipids, and to sequence the LPL gene in patients with severe hypertriglyceridaemia to look for new DNA variants.

Materials and methods

Subjects

Hypertriglyceridaemic subjects (n=135) attending the lipid clinic at the Royal Victoria Hospital, Belfast, were recruited for the study. All subjects had fasting triglyceride values >2 mmol/L and, apart from increased body mass index (BMI),

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they had no secondary cause of hypertriglyceridaemia at the time of study.

Blood was collected onto Guthrie cards for DNA analysis, and fasting samples were collected for routine lipid analysis. Patients were not on lipid-lowering drugs at the time of lipid analysis. The presence of vascular disease was recorded for those with a history of angina, myocardial infarction (MI), carotid disease, ischaemic heart disease, or cerebrovascular accident.

Control subjects (n=113) with triglyceride levels <2 mmol/L and normal cholesterol levels were chosen from a healthy population. Blood was collected into EDTA tubes for DNA analysis and fasting samples were collected for lipid analysis.

DNA extraction

DNA was extracted from control whole blood samples according to the method of Jeanpierre.[15] DNA was extracted from dried blood spots on Guthrie cards by suspending 5 × 5 mm cut pieces of the blood spot in 250 µL sterile water and subjecting these to successive denaturation at 95°C for 3 min and cooling for 3 min.[16] Samples were stored at –70°C.

Lipid analysis

Patient and control samples were analysed for triglycerides and total cholesterol using the Ortho Clinical Diagnostics' Vitros 950 analyser. The manganese/heparin precipitation technique was used for HDL measurement.

DNA analysis

All patient and control samples were investigated for the *Hind*III and *Pvu*II restriction fragment length polymorphisms (RFLPs). A Perkin Elmer System 480 was used for thermal cycling. Polymerase chain reaction (PCR) conditions were the same for each of the known LPL variants and entailed denaturation at 96°C for 10 min, followed by 40 cycles of 1 min at 94°C, 1 min at 55°C and 1 min at 72°C. This was followed by extension at 72°C for 5 min. For Guthrie card samples, 5 µL sample was used in a 25 µL reaction mixture, while 3 µL of 50 ng/µL DNA was used for all other samples.

The reaction mixture contained 1.5 mmol/L $MgCl_2$, 20 mmol/L Tris-HCl buffer (pH 8.4), 50 mmol/L KCl, 0.125 mmol/L each dNTP (dATP, dCTP, dGTP, dTTP; Pharmacia), 0.625 units *Thermus aquaticus* (*Taq*) polymerase (Gibco BRL) and 3 pmol each relevant primer. The primers are shown in Table 1. Blank controls that contained no genomic DNA were run with each amplification, to test for DNA contamination.

Table 1. Primers and enzymes used for detection of known LPL variants

LPL variant	Primer	Restriction enzyme
G188E	F: 5'ATGATGAGCAGTGACATGCGA3'	*Ava*II
	R: 5'TACTGAGTAGGACATTGGGTC3'	
P207L	F: 5'ATGATGAGCAGTGACATGCGA3'	*Bsl*I
	R: 5' TACTGAGTAGGACATTGGGTC3'	
N291S	F: 5'GCCGAGATACAATCTTGGTG3'	*Rsa*I
	R:5'CTGCTTCTTTTGGCTCTGACTGTA3'†	
IVS 8 + 600 bp T/G	F: 5'AGCCCCACCCATGTGTACCCATAA3'	*Hind*III
	R: 5'GTGATACAAGCAAATGACTAAAGAGAA3'‡	
IVS 6 + 1.6 kb C/T	F: 5'GCTTAATTCTCAATTCAATG3'	*Pvu*II
	R: 5'CTTTAGACTCTTGTCCAGGT3'§	

†Primers derived from reference 18; ‡Primers derived from reference 27; §Primers derived from reference 28

Restriction enzyme digests

All reactions for restriction enzyme digests contained 10 µL amplification product and 5 to 25 units restriction enzyme, and were performed according to the manufacturer's recommended conditions. The enzymes used for detection of each variant are shown in Table 1. All restriction enzymes were obtained from New England Biolabs.

Digests were electrophoresed at 120 V for 1 h on 2% agarose gel comprising 1% Nusieve agarose (FMC Products), 1% standard agarose (Life Technologies) and 0.5 mg/mL ethidium bromide. DNA was visualised by transillumination of the gel with ultraviolet (UV) light.

Statistical analysis

The χ^2 test was used to compare *Hind*III and *Pvu*II genotypes and allele frequencies between the patient and control groups, and between patients with vascular disease and those without. Comparison of mean lipid levels for the *Hind*III and *Pvu*II genotypes was made using one-way ANOVA. Student's t-test was used to compare lipid parameters between patient and control groups. Triglyceride levels were log-transformed before analysis.

DNA sequencing reactions

Sequencing of exons 1–9 of the LPL gene was performed on 12 patients with triglyceride levels >10 mmol/L. Primers for exons 1–4 and exon 6 were chosen from published data. Primers were designed for amplification of exons 5, 7, 8 and 9 using a computer software package (Primer Detective; Clontech, CA, USA).

Table 2. Primers used to sequence LPL exons

Exon	Primer
1	F: 5'CACTTCTAGCTGCCCTGCCA3' R: 5' AGGGGAGTTTGCGCGCAAAA 3' §
2	F: 5' CTCCAGTTAACCTCATATCC 3' R: 5' CACCACCCCAATCCACTC 3' †
3	F: 5' GGTGGGTATTTTAAGAAAGCT 3' R: 5' AAAACACTGTTTGGACACATA 3' ‡
4	F: 5' TTTTGGCAGAACTGTAAGCA 3' R: 5' GACAGTCTTTTCACCTCTTA 3' *
5	F: 5' ATGATGAGCAGTGACATGCGA 3' R: 5' TACTGAGTAGGACATTGGGTC 3'
6	F: 5' GCCGAGATACAATCTTGGTG 3' R: 5' GCATGATGAAATAGGACTCC 3' *
7	F: 5' GTGCTAGTGAGATACTTCTGTGG 3' R: 5'GACTTGTTTTCTAGGCATCGCT 3'
8	F: 5' CGACCTTCATTTTGAGTTCTTTGTTGG 3' R: 5' TAAAGCTCTCCCTGAATTGTG 3'
9	F: 5' CAGTCCTGACAGAACTGTACC 3' R: 5' TGATGGGGTGAAGAGGAATGC 3'

§Primers derived from reference 13; †Primers derived from reference 4; ‡Primers derived from reference 29; *Primers derived from reference 30

Primer design was such that good-quality sequences were obtained for each entire exon and the splice junction region (Table 2). A Perkin Elmer System 480 was used for thermal cycling and the conditions used for each exon were the same. Initial denaturation at 96°C for 10 min was followed by 40 cycles of 94°C for 1 min, 55°C for 1 min and 72°C for 1 min. This was followed by 72°C for 5 min. For Guthrie cards, a 10 µL sample was used in a 50 µL reaction mixture, while 6 µL of 50 µg/mL DNA was used for all other samples.

The reaction mixture contained 1.5 mmol/L $MgCl_2$, 20 mmol/L Tris-HCl buffer (pH 8.4), 50 mmol/L KCl, 0.125 mmol/L each dNTP, 1.25 units *Taq* polymerase and 6 pmol of each relevant primer. The PCR products for each exon were used as templates for sequencing reactions and were purified to remove residual primer or excess PCR reaction components using Centricon-100 or Micron-100 (Amicon) purification columns.

The sequencing reactions were performed using an ABI Prism dye-terminator cycle sequencing ready reaction kit (Perkin Elmer). Primers used in the sequencing reactions were the same as those used in the original PCR reaction, with each tube containing either the forward or reverse primer for that exon.

Each reaction mix consisted of 4 µL template, 1 µL primer, 8 µL ABI Prism dye-terminator mix and 7 µL sterile distilled water. The same thermal cycling conditions were used for all the sequencing reactions and consisted of an initial denaturation at 94°C for 2 min, followed by 25 cycles of 30 sec at 94°C, 15 sec at 50°C and 4 min at 60°C. This was followed by a final cooling for 10 min at 4°C.

An ABI 373A fluorescent fragment analyser (Applied Biosystems) was used for sequencing. A pre-mixed acrylamide gel solution (Sequagel-6; National Diagnostics) was used to prepare the gel for fragment separation.

Results

Lipid profiles of patient and control groups

Table 3 shows the characteristics of the patient and control groups. As expected, the patient group showed significantly higher mean total cholesterol, triglyceride and BMI levels, and significantly lower HDL cholesterol. It was not possible to recruit enough control subjects over the age of 50 to age-match the patient and control groups.

Table 3. Characteristics of patient and control groups

Subject information	Patient group*	Control group*	*P*
n	135	113	
Total cholesterol (mmol/L)	8.41 ± 1.69	5.09 ± 1.06	<0.0001†
HDL cholesterol (mmol/L)	1.18 ± 0.34	1.34 ± 0.35	<0.001†
Triglyceride (mmol/L) (SE)	4.13 (1.06)	0.98 (1.04)	<0.0001†
Age	59 ± 0.95	39 ± 1.2	<0.0001†
BMI	28 ± 4.0	24 ± 3.3	<0.0001†
	(*n*=127)	(*n*=107)	
Men/Women	69/66	62/51	0.64‡

*Results are mean (±SD), except for triglyceride, which is geometric mean. SE = standard error. Statistical analysis was performed using †Student's *t*-test and the ‡χ^2 test

Analysis of known LPL variants

Genotype distributions for the *Hind*III and *Pvu*II RFLPs in the control group were in accordance with Hardy-Weinberg expectations. No significant differences were observed for allele frequencies between the patient and control groups for either variant. *Hind*III allele frequencies in patients and controls were H1 0.25, H2 0.75 and H1 0.24, H2 0.76, respectively. The *Pvu*II allele frequencies in patients and controls were P1 0.51, P2 0.49 and P1 0.48, P2 0.52, respectively. There were no differences in distribution of the *Hind*III alleles between patients with and without vascular disease.

Frequency of the *Pvu*II P2 allele (allele frequency: 0.56) was significantly higher in patients with vascular disease than in patients with no vascular disease (allele frequency: 0.42) (P=0.03). Comparison of mean lipid levels between the genotypes for *Hind*III and *Pvu*II showed no significant difference in either group.

Heterozygosity for the N291S variant was found in 5.3% (6/113) of the control subjects, while only 0.7% (1/135) of the patient population carried the variant. This was not a significant difference (P=0.08). The P207L variant was not detected in either the patient or control groups. One individual in the control group carried the G188E variant, but there were no carriers in the patient group.

LPL exon sequencing in patients with triglyceride levels >10 mmol/L

Characteristics of the 12 unrelated subjects used for DNA sequencing of the LPL gene are shown in Table 4. Exon sequences for all but one of the patients agreed with published complementary DNA (cDNA) sequences.[17] The only variant found in the exon sequences was N291S in exon 6 for subject 4. This agreed with the *Rsa*I digest for this patient. PCR products used to sequence exon 5 included the first 50 bases of intron 5, and two intronic variants were found in subjects 8 and 11: IVS 5 +33C → G and IVS 5 +37T → C.

Discussion

The N291S variant has been associated with moderate LPL catalytic deficiency due to partial impairment of dimerisation. Some studies have found an association between this variant and increased triglyceride levels.[18,19] The results of the present study agree with those found in a study of hypertriglyceridaemic, post-MI individuals in Northern Ireland.[20,21] There were no case-control differences for this variant, and the six controls that were carriers in the present study did not show any differences in BMI, total cholesterol, HDL cholesterol or triglyceride levels from non-carriers.

The *Hind*III polymorphism did not show any associations with lipid phenotypes. Allele frequencies for the controls and patients were similar and agreed with those found in the Northern Irish control population in the ECTIM (Etude Cas-Temoins sur l'Infarctus du Myocarde) study (n=180).[20] Chamberlain *et al*.[22] also compared a Caucasian hypertriglyceridaemic group (n=45) with normotriglyceridaemic controls (n=93) and found the

Table 4. Characteristics of patients used in sequence analysis of the LPL gene

Subject number	Sex	Age	BMI	Total cholesterol (mmol/L)	HDL cholesterol (mmol/L)	Triglyceride (mmol/L)	LPL variant found by sequencing
1	F	51	NA	20.0	NA	76.0	Normal
2	F	43	NA	14.8	NA	72.8	Normal
3	F	46	26	9.1	NA	12.4	Normal
4	M	56	26	8.4	0.66	20.2	N291S
5	M	53	NA	14.0	NA	39.2	Normal
6	M	53	32	14.0	0.61	44.3	Normal
7	M	34	29	11.2	NA	37.8	Normal
8	F	43	35	7.9	0.6	26.5	IVS5+33C/G, IVS5+37T/C
9	F	58	40	7.4	0.72	25.9	Normal
10	F	47	27	14.0	0.59	30.5	Normal
11	M	43	29	10.5	0.82	13.2	IVS5+33C/G, IVS5+37T/C
12	F	51	30	6.9	1.08	20.4	Normal

NA = details not available

*Hind*III allele frequencies of the patient group to be the same as those found in the study reported here. The frequency of the H2 allele, however, was increased relative to the control group. The control populations in both studies were of a similar size and had triglyceride and total cholesterol levels that were similar. This affect has been shown in other studies.[23-25]

Differing patient selection criteria and ethnic origins make study comparisons difficult and may explain the conflicting results. There was no association between *Hind*III alleles and vascular disease in the patient group included in the present study. Other studies have found the H2 allele to be more common in patients with CAD[1] or post-MI.[20]

Allele frequencies for the *Pvu*II polymorphism agreed with most other published values. Although there were no associations between lipid levels and this polymorphism, there was an increased frequency of the P2 allele in patients with vascular disease. There was no significant difference between lipid levels in patients with vascular disease and in those with no vascular disease. The ECTIM study also found no association between this polymorphism and lipid levels, but it did find that the P2 allele was associated with increased severity of atherosclerosis in 380 post-MI individuals from France.[20]

It would appear that the *Hind*III and *Pvu*II polymorphisms are not useful markers for hypertriglyceridaemia in the Northern Irish population. The association of the P2 allele with vascular disease may indicate that this allele is in linkage disequilibrium with a mutation, either in the LPL gene or in a neighbouring gene that predisposes to vascular damage.

The P207L variant did not occur in the patient or control groups and one individual in the control group was found to carry the G188E mutation. Although these DNA variants occur in high frequency among French Canadians with hypertriglyceridaemia, they do not appear to be prevalent among the Northern Irish population, although the numbers of patients in the present study were relatively small.

Sequencing the LPL exons in individuals with severe hypertriglyceridaemia (triglyceride >10 mmol/L) did not reveal any new mutations. This was unexpected because although the number of patients in this group was small their triglyceride levels were extremely high. The two base changes found in intron 5 for two patients may be significant and subsequently have been shown to be polymorphisms associated with coronary heart disease.[26]

It is possible that mutations in other genes are the cause of hypertriglyceridaemia in the study group. The roles of apo AIV, apo CII, and apo CIII could be examined in these patients by investigating genetic variation at the genes for these apolipoproteins.

In conclusion, some common genetic variants of the LPL gene were not found to be associated with hypertriglyceridaemia in a Northern Irish patient group. The P2 allele of the *Pvu*II polymorphism showed a significantly higher frequency in patients with vascular disease. However, further studies are required to assess the significance of sequence variants in intron 5, and to assess variations in genes other than LPL that might be responsible for producing severe hypertriglyceridaemia in patients with no apparent secondary cause. □

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