Support for founder effect for two lipoprotein lipase (LPL) gene mutations in French Canadians by analysis of GT microsatellites flanking the LPL gene

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Abstract. Mutations in the human lipoprotein lipase (LPL) gene are one of the major causes of familial chylomicronemia. We have characterized two polymorphic GT microsatellites flanking this gene. Two LPL mutations that are extremely frequent in French Canadians appear to be in complete linkage disequilibrium with specific LPL microsatellite haplotypes indicating a founder effect within this population.

Introduction

Lipoprotein lipase (LPL) hydrolyzes the triglyceride core of very low density lipoproteins (VLDL) and chylomicrons making free fatty acids available for energy metabolism or further storage. Patients with LPL deficiency, which is inherited as an autosomal recessive trait, often present with eruptive xanthomas, chylomicronemia, and recurrent pancreatitis (Brunzell 1989). This familial chylomicronemia is estimated to occur with a frequency of about 1 per million in most populations but is 100- to 200-fold more frequent in parts of French Canada (Gagne et al. 1989).

The human LPL cDNA has been cloned and sequenced (Wien et al. 1987). The LPL gene is located at 8p22 (Sparkes et al. 1987) and consists of 10 exons distributed over 30 kilobases (kb) DNA (Deeb and Peng 1989). Numerous mutations responsible for the recessive lipoprotein lipase deficiency phenotype have been characterized (Hayden and Ma 1992) including two missense mutations at residue 207 and 188 that are common in French Canadians. The Pro→Leu mutation at residue 207 accounts for over 70% of the mutant alleles while the Gly→Glu mutation at residue 188 accounts for most of the remaining mutant alleles in a group of 37 French-Canadian patients with LPL deficiency (Ma et al. 1991).

The hydrolysis of VLDL by LPL modulates plasma LDL and HDL levels (Nikkila et al. 1978). Possible associations between restriction fragment length polymorphism (RFLP) markers within the LPL gene and plasma triglyceride levels (Chamberlain et al. 1989) as well as the levels of both HDL cholesterol and total cholesterol (Heizmann et al. 1991) have been reported. To provide highly polymorphic markers for investigation of the ancestry of particular LPL mutations as well as possible association between particular LPL haplotypes and lipid profiles we have screened the LPL region for highly informative GT microsatellite polymorphisms (Weber 1990). We have isolated two GT simple sequence repeat (SSR) loci flanking the LPL gene and characterized allelic variants. We have found that each of the two common French-Canadian LPL mutants are carried by a single SSR haplotype.

Materials and methods

The flow-sorted chromosome-8 cosmid library LA08NC01 (prepared at Los Alamos as part of the National Laboratory Gene Library Project supported by the U. S. Department of Energy) was screened for cosmids containing LPL sequences (Wood et al. 1992) using the LPL35 cDNA clone (Wion et al. 1987). Four positive cosmids were isolated 114C1 I, 165B9, 178F10, and 179G2. An additional cosmid 5' from the gene, 17B7, was isolated by cosmid walking (Fig. 1).

Cosmid DNAs were digested with EcoRI, Southern blotted, and fragments containing GT microsatellites identified by hybridization with poly(dG-dT)-(dC-dA). These fragments were subcloned into pBluescript II and the GT microsatellite sequenced using a modified (Seto 1990) Sequenase (US Biochemical Corp.) protocol.

The Centre d’Etude du Polymorphisme Humain (CEPH) panel is a mixed population consisting of Utah Mormons, French, Venezuelan, and Amish individuals (Daussel et al. 1989). We also examined a total of 33 French-Canadian probands with LPL deficiency who were unrelated to each other for at least three generations (Ma et al. 1991).

Allelic variation within GT tracts was detected by polymerase chain reaction (PCR) amplification of genomic DNA using flanking oligonucleotide primers. Amplification reactions of 30 cycles consisted of 1 min at 94°C, 30 s at 58°C, and 2 min at 72°C. A 40-ng sample of genomic DNA was used with 10 pmol each primer in 25-μl reactions. The 1× reaction buffer contained 1.5 mM MgCl2, 50 mM Tris-Cl, pH 8.3, 0.02% NP 40, 0.02% Tween. Each dNTP was 200 μM final concentration. The CA strand primer was end labeled with [32P]dATP and 1 pmol (0.5 μCi) was added to each reaction. Sizes of alleles were determined with reference to the cloned allele using an M13 sequencing reaction as a secondary standard. Reactions were run out on 5% Long Ranger modified polyacrylamide denaturing gels (AT Bio-
GT GT
E EE EEEE

Fig. 1. Map of the LPL region. Distances are shown in kb. The positions of EcoRI restriction sites and the characterized GT repeats are indicated. The extent of the LPL gene is indicated by a thick line and the extent of isolated cosmids by thin lines. Isolate numbers for cosmids are shown.

Table 1. Polymerase chain reaction (PCR) primer sequences for simple sequence repeats (SSRs) flanking the lipoprotein lipase (LPL) locus

<table>
<thead>
<tr>
<th>SSR site</th>
<th>Primer[a]</th>
<th>Primer sequence</th>
</tr>
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<tbody>
<tr>
<td>LPL5GT</td>
<td>LPL5-CA</td>
<td>5'-TAGAGCACACATCCAGTGGA-3'</td>
</tr>
<tr>
<td></td>
<td>LPL5-GT</td>
<td>5'-CAGTGAGATTTGCTGGGATA-3'</td>
</tr>
<tr>
<td>LPL3GT</td>
<td>LPL3-CA</td>
<td>5'-ATCTGGTATGAAATGTACATGTG-3'</td>
</tr>
<tr>
<td></td>
<td>LPL-GT</td>
<td>5'-ATCTGGCCTCTGCAGGCTCTCA-3'</td>
</tr>
</tbody>
</table>

[a] The primers are named -CA or -GT according to the dominant composition of the strand produced by that end-labeled primer.

Fig. 2. Genotyping of CEPH family 21 for LPL5GT and LPL3GT alleles. DNA from the parents amplifies to give fragments of 118, 116 (father) and 118, 116 (mother) at the LPL5GT locus and fragments of 138, 132 (father) and 136, 134 (mother) at the LPL3GT locus (not shown). The genotypes of the six children are: 118, 116; 134, 132 (2103); 118, 116; 136, 132 (2104); 118, 116; 136, 132 (2105); 116, 116; 134, 132 (2106); 118, 116; 136, 132 (2107); and 118, 116; 134, 132 (2108). This segregation infers that the paternal haplotypes are 118–132 (6–6) and 116–138 (7–3) and the maternal haplotypes are 118–136 (6–4) and 116–134 (7–5). The additional secondary bands form a 1-bp ladder for the LPL5GT system and a 2-bp ladder for the LPL3GT system.

Results

Detection, sequencing and amplification of GT microsatellites

Over 80 kb of genomic DNA has been isolated at and surrounding the LPL locus by screening the LA08NC01 flow-sorted chromosome-8 cosmid library with the LPL35 cDNA clone followed by a cosmid walk. Three of the five isolated cosmids cover this region with minimal overlap. Three GT microsatellites were identified in these cosmids. Two of these SSRs, flanking the LPL gene, were selected for sequencing and analyzed for allelic variation. A single EcoRI fragment different in size for each of the cosmids 17B7, 165B9, and 179G2 (Fig. 1) was positive with the poly-GT probe. The 4.0-kb EcoRI fragment from 179G2 was subcloned, and a 0.8-kb PstI fragment containing the SSR was sequenced (GenBank accession no. M94221, 206 bp). This SSR, referred to as the LPL5GT site, contains a (CA)23 repeat.

Flanking primers were selected, which produced a 116-bp product with the cloned LPL5GT site and a 128-bp product with the cloned LPL3GT site (Table 1).

Allelic variation at the LPL5GT and LPL3GT sites

The CEPH panel (Dausset et al. 1990) was typed for both these flanking sites (Fig. 2). At the LPL5GT site 231 independent alleles varying from 106 to 134 bp were observed while 255 independent alleles varying from 118 to 142 bp were observed at the LPL3GT site (Table 2). Heterozygosities were calculated at 48% and 83% and polymorphic