Hepatic Lipase Deficiency
Clinical, Biochemical, and Molecular Genetic Characteristics

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Hepatic lipase (HL) is an important enzyme in the metabolism of triglyceride-rich lipoproteins and high density lipoproteins. The clinical syndrome of HL deficiency is rare and difficult to identify. We studied carriers of mutant HL to ascertain whether there are distinctive clinical and/or biochemical characteristics of the heterozygous state. In an Ontario kindred, compound heterozygosity for two HL mutations, S267F and T383M, underlies the clinical syndrome of complete HL deficiency. We report that simple heterozygotes for either HL mutant do not have a discrete lipoprotein abnormality, except for relative triglyceride enrichment of lipoprotein fractions with \(d > 1.006\) g/mL. Postheparin HL activity is depressed to a greater degree in carriers of S267F compared with carriers of T383M. Retinyl palmitate loading studies in a compound heterozygote revealed impaired clearance of chylomicron remnants. The dyslipoproteinemia in a compound heterozygote was ameliorated by lovastatin. There was no difference in the quantity and distribution of HL mRNA in the liver of a compound heterozygote when compared with that of a normal subject. Thus, HL deficiency associated with structural variation of the HL gene is characterized by premature atherosclerosis, triglyceride enrichment of lipoprotein fractions with \(d > 1.006\) g/mL, the presence of circulating \(\beta\)-very low density lipoproteins, and abnormal catabolism of postprandial triglyceride-rich lipoproteins. (Arteriosclerosis and Thrombosis 1993;13:720–728)

Key Words • lipolysis • gene mutations • chylomicrons • compound heterozygosity
assessed by the postprandial response to a retinyl palmitate [RP] challenge) of a compound heterozygote and his two sons, both S267F simple heterozygotes; 4) the response of plasma lipoproteins to pharmacological intervention in a compound heterozygote; and 5) the distribution of HL mRNA in a liver biopsy specimen from a compound heterozygote.

Methods

Subjects

Subjects from the Ontario-based HL deficiency kindred (OHLD) were ascertained through the St. Michael’s Hospital Lipid Research Clinic; this family has already been described. Members of OHLD and unrelated normal subjects were studied in accordance with the criteria of the University of Toronto Ethics Committee. The pedigree structure of kindred OHLD with biochemical and genetic features of the subjects studied are presented in Figure 1.

Lipoprotein Analyses

Biochemical assays and lipase activities in postheparin plasma were performed on OHLD family members and control subjects as described. Genotype Analyses

Whole blood was obtained from 19 family members, and typing of HL gene point mutations that affect codons 73, 133, 193, 202, 267, and 383 was done using described methods. To rule out other candidate loci as primary determinants of HL deficiency in this kindred, genotypes were established at the apoB22, LDLR23, and lipoprotein lipase (LPL)24 loci. The apoE phenotype was determined by isoelectric focusing, while the apoE genotype was determined by restriction isotyping.

Statistical and Linkage Analyses

HL activity levels in identified carriers of the mutation were compared with those in family members who were noncarriers by using the general linear models routine in SAS. Linkage analysis was performed with LIPED.

RP Fat Tolerance Tests

RP was added to oral fat tolerance tests performed on OHLD subjects B2 (a S267F/T383M compound heterozygote who had an E3/3 genotype), C5 (a S267F simple heterozygote who had an E3/2 genotype), and...
C9 (a S267F simple heterozygote who had an E3/3 genotype). All three subjects were being treated for hypercholesterolemia at the time of the turnover studies with dietary advice and 40 mg lovastatin daily. As controls, fat tolerance tests were also performed in five control subjects, all men who had neither the S267F nor the T383M mutation and who were E3/3. The control subjects had the following characteristics determined on the day that each was tested (mean±SD): age, 47±13 years; body mass index, 23.7±1.0 kg/m²; cholesterol, 5.12±1.15 mmol/L; TG, 1.73±0.61 mmol/L; HDL cholesterol, 0.99±0.19 mmol/L; and LDL cholesterol, 3.62±1.05 mmol/L.

Fat tolerance tests were performed after a 12-hour fast and consisted of 5 mL water, 0.5 g corn oil, 1.28 g skim milk powder (0.5 g protein), and 0.5 g glucose, all per kilogram of body weight, to which was added 50,000 IU RP (Hoffman–La Roche Ltd., Etobicoke, Ontario).29–35 After a fasting blood sample was drawn, subjects consumed the test meal within 15 minutes and had further blood samples drawn at hourly intervals for 10 hours. Blood samples were protected from light during removal, transport, and processing of the plasma. Subjects B2, C5, and C9 also provided a 48-hour sample. After removal of chylomicrons by centrifugation at 20,000 rpm for 30 minutes (Beckman rotor 50.3), the remainder of the plasma was separated by ultracentrifugal flotation into d 1.006 g/mL and d >1.006 g/mL fractions. RP was measured in total plasma and in the chylomicron, d <1.006 g/mL, and d >1.006 g/mL fractions. RP was measured in duplicate by high-performance liquid chromatography. Recovery of RP added to whole plasma at concentrations of 53, 263, 1,050 and 5,256 nmol/L was (in mean±SD) 97±18%, 109±17%, 101±9%, and 104±12%, respectively. The between-run coefficients of variation for repeated analyses of pooled plasma containing 101, 222, and 3,216 nmol/L RP were 18%, 6.3%, and 5.1%, respectively. The limit of detection was 20 nmol/L.

**Lipoprotein Response to Pharmacological Treatment**

Subject B2 (a compound heterozygote) had regular determinations of plasma lipoproteins during 4 years of treatment with gemfibrozil and lovastatin used both as single agents and in combination. More detailed lipoprotein analysis including Pevikon electrophoresis34 to separate the VLDL into β- and pre-β VLDL was done before, during, and after drug treatment.

**In Situ Hybridization of the HL Antisense Probe in the Liver**

HL mRNA was studied in situ as described35 using paraffin sections of a liver biopsy specimen from compound heterozygote subject B1, of another liver biopsy specimen from a normal subject, and of a normal pituitary gland. Sections were hybridized with an antisense HL DNA oligonucleotide probe spanning HL bases 1,272–1,252 (numbered as in Reference 18), labeled with the 3'-end method with deoxyadenosine 5' -32P-thiotriphosphate using terminal deoxynucleotidyl transferase, purified, and autoradiographed as described.35 An HL sense DNA oligonucleotide probe spanning HL bases 640–660 (numbered as in Reference 18) was hybridized with sections of liver as a negative control experiment.

**Results**

**DNA Variants and Haplotypes in the HL Gene**

Known HL gene variants are summarized Table 1, with cDNA and amino acid residues numbered as in Martin et al.16 Among members of OHLD, DNA variation was identified at HL codons 133, 193, 202, 267, and 383. Each allele in this family had the common DNA sequence encoding methionine at codon 73. Haplotypes were then constructed. The S267F mutation was on an allele that had GTG (encoding methionine) at codon 73, GTT at codon 133, AAT (encoding aspartate) at codon 193, and ACC at codon 202. In contrast, the T383M mutation was on an allele that had GTG (encoding methionine) at codon 73, GTG at codon 133, AAT (encoding aspartate) at codon 193, and ACC at codon 202. The T383M allele from the Quebec-based kindred (QHLD) that we reported previously9 had the same haplotype based on these flanking markers. The serine at position 193 was completely linked with T383M (maximal log of the odds score, 3.34 at 0% recombination).

**Heart Disease in Family Members**

Only the three compound heterozygotes (B1, B2, and B3) have had coronary artery disease. The proband B1 had a myocardial infarction in his late forties and a fatal myocardial infarction at age 51. B2 was symptomatic with angina at age 50 with severe multiple-vessel coronary atherosclerosis requiring coronary angioplasty at age 53. B2 then suffered a severe myocardial infarction at age 58 despite treatment with lovastatin (Figure 2). Repeat angiography in B2 in June 1992 revealed severe diffuse multiple-vessel occlusive disease. He is maintained on lovastatin 80 mg daily. B3 had a history of angina since his midfifties, with coronary bypass surgery at age 57.

**Lipoprotein Phenotypes and HL Genotypes**

Biochemical data from OHLD have been reported9,8 and are included in Figure 1. When OHLD family members (excluding unrelated spouses) were classified...
According to the presence or absence of S267F and T383M, we observed four categories of subjects: normal (n=3), simple T383M heterozygotes (n=4), simple S267F heterozygotes (n=4), and compound heterozygotes (n=3).

The Lipid Research Clinics Population Study Data Book was used to determine percentiles for plasma lipids and lipoproteins adjusted for age and sex of members of OHLD classified by HL genotype. These are shown in Table 2. There was no specific association between any of these genes and lipoprotein phenotypes in compound heterozygotes (i=3).

When compared with mean values among 78 reference subjects (41 females and 37 males; mean±SD age, 40.5±18.3 years), mean levels of HDL TG were not above the 95th percentile in carriers of T383M and S267F, whereas mean levels of LDL TG exceeded the 95th percentile for carriers of S267F but not for carriers of T383M. When “hyperbeta-triglyceridemia” and “hyperalphatriglyceridemia” are used to define the phenotype in subjects with levels of LDL TG and HDL TG, respectively, that exceed the 95th percentile for age and sex, then all compound heterozygotes have hyperalphatriglyceridemia and hyperbeta-triglyceridemia. However, only the three oldest S267F carriers (C5, C9, and C16) have hyperbeta-triglyceridemia, and only the oldest S267F carrier (C16, a female) has hyperalphatriglyceridemia. Only the oldest T383M carrier (B6, a male) has hyperalphatriglyceridemia and hyperbeta-triglyceridemia.

Among subjects with normal HL alleles, relatively more had lipoprotein concentrations that were not at the extremes of the reference range, but this was not exclusive. For example, B4 had neither T383M nor S267F but had hyperbeta-trilipoproteinemia. No subject with normal HL alleles had either hyperbeta-triglyceridemia or hyperalphatriglyceridemia.

Biochemical Determinations and HL Genotypes

Mean±SD values of lipoproteins and HL activities from the four groups, i.e., normal subjects lacking S267F and T383M, simple heterozygotes for T383M, simple heterozygotes for S267F, and compound heterozygotes for T383M and S267F, are shown in Table 3. Statistical comparisons (by analysis of variance) showed that among biochemical variables, TG, LDL TG, HDL TG, and HL activity had significant variation between subjects grouped by phenotype. Specifically, the levels of significance for between-group differences were p=0.04, 0.006, 0.0018, and 0.0068, respectively, for TG, LDL TG, HDL TG, and HL activity. Mean±SD HL activity values in normal subjects, simple T383M heterozygotes, and simple S267F heterozygotes were, respectively, 9.34±1.21, 7.18±3.77, and 3.83±2.22 micromoles of free fatty acids released per milliliter per hour (U/hr). The three compound heterozygotes each had no detectable HL activity. Mean HL activity in simple T383M heterozygotes was intermediate between normal subjects and simple S267F heterozygotes and covered a wide range: from 2.6 U/hr in a 65-year-old man (B6) to 10.6 U/hr in a 23-year-old man (C3). HL activity in simple heterozygotes did not differ significantly from each other.

No subject other than the three compound heterozygotes had detectable β-VLDL. TG content of the LDL fraction varied significantly according to HL genotype (p=0.006), with the lowest mean concentrations in normal family members and simple T383M heterozygotes (0.42±0.18 and 0.47±0.23 mmol/L, respectively), an intermediate mean concentration in simple S267F heterozygotes (0.68±0.35 mmol/L), and the highest concentrations in compound heterozygotes (1.50±0.46 mmol/L). TG content of the HDL fractions also varied significantly according to HL genotype (p=0.0018), with the lowest mean concentrations in normal family members and simple T383M heterozygotes (0.19±0.06 and 0.19±0.08 mmol/L, respectively), slightly higher mean concentrations in simple S267F heterozygotes (0.25±0.18 mmol/L), and the highest mean concentrations in compound heterozygotes (0.63±0.06 mmol/L).

Typing of Other Candidate Genes in Lipid Metabolism

Genotypes of apoE, apoB, LDLR, and LPL are shown in Figure 1. Since apoE genotypes and isoelectrofocusing phenotypes were in complete concordance, there was likely no unusual or abnormal apoE isoform. At a recombination fraction of 0%, no allele of any of these genes was linked with HL deficiency. Among the three HL-deficient subjects, there were
### Table 2. Percentiles for Plasma Lipoproteins in the Ontario-Based Hepatic Lipase–Deficient Family, Adjusted for Age and Sex

<table>
<thead>
<tr>
<th>Subject</th>
<th>HL genotype</th>
<th>Chol</th>
<th>TG</th>
<th>LDLC</th>
<th>HDLC</th>
<th>LDLTG</th>
<th>HDLTG</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1</td>
<td>S267F/T383M</td>
<td>&gt;95%</td>
<td>&gt;95%</td>
<td>N</td>
<td>&lt;10%</td>
<td>&gt;95%</td>
<td>&gt;95%</td>
<td>Hyperpreβ, borderline hyperapoB, hyperapoB-TG, hyperapoA-TG</td>
</tr>
<tr>
<td>B2</td>
<td>S267F/T383M</td>
<td>&gt;95%</td>
<td>&gt;95%</td>
<td>N</td>
<td>&gt;95%</td>
<td>&gt;95%</td>
<td>&gt;95%</td>
<td>Hyperpreβ, borderline hyperapoB, hyperapoA, hyperapoA-TG, hyperapoB-TG, hyperapoB-AI, hyperapoB-AI-TG</td>
</tr>
<tr>
<td>B3</td>
<td>S267F/T383M</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>&gt;95%</td>
<td>&gt;95%</td>
<td>&gt;95%</td>
<td>Hypera, hyperapoA, hyperapoB-AI, hyperapoB-AI-TG, hyperapoA-TG</td>
</tr>
<tr>
<td>C4</td>
<td>S267F</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>&gt;95%</td>
<td>&gt;95%</td>
<td>&gt;95%</td>
<td>HyperapoA, hyperapoA-TG</td>
</tr>
<tr>
<td>C5</td>
<td>S267F</td>
<td>&gt;95%</td>
<td>&gt;95%</td>
<td>&gt;95%</td>
<td>&gt;95%</td>
<td>&gt;95%</td>
<td>&gt;95%</td>
<td>HyperapoB, hyperapoA, hyperapoB-AI, hyperapoB-AI-TG, hyperapoA-TG</td>
</tr>
<tr>
<td>C9</td>
<td>S267F</td>
<td>&gt;95%</td>
<td>&gt;95%</td>
<td>&gt;95%</td>
<td>&gt;95%</td>
<td>&gt;95%</td>
<td>&gt;95%</td>
<td>HyperapoB-AI, hyperapoB-AI-TG</td>
</tr>
<tr>
<td>C16</td>
<td>S267F</td>
<td>&gt;95%</td>
<td>&gt;95%</td>
<td>&gt;95%</td>
<td>&gt;95%</td>
<td>&gt;95%</td>
<td>&gt;95%</td>
<td>Hyperpreβ, borderline hyperapoB, hyperapoB-AI, hyperapoB-AI-TG, hyperapoA-TG</td>
</tr>
<tr>
<td>B6</td>
<td>T383M</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>&gt;95%</td>
<td>&gt;95%</td>
<td>&gt;95%</td>
<td>HyperapoB-AI-TG, hyperapoA-TG</td>
</tr>
<tr>
<td>C2</td>
<td>T383M</td>
<td>&gt;95%</td>
<td>N</td>
<td>&gt;95%</td>
<td>&gt;95%</td>
<td>&gt;95%</td>
<td>&gt;95%</td>
<td>HyperapoB, hyperapoA, hyperapoB-AI, hyperapoB-AI-TG, hyperapoA-TG</td>
</tr>
<tr>
<td>C3</td>
<td>T383M</td>
<td>&gt;95%</td>
<td>&gt;95%</td>
<td>&gt;95%</td>
<td>&gt;95%</td>
<td>&gt;25%</td>
<td>&gt;95%</td>
<td>Hyperpreβ, hyperapoB</td>
</tr>
<tr>
<td>C17</td>
<td>T383M</td>
<td>&gt;95%</td>
<td>N</td>
<td>&gt;95%</td>
<td>&gt;95%</td>
<td>&gt;95%</td>
<td>&gt;95%</td>
<td>Hypera, hyperapoA</td>
</tr>
<tr>
<td>B4</td>
<td>Normal OHLD</td>
<td>&gt;90%</td>
<td>&gt;95%</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>HyperapoA, hyperapoA-TG</td>
</tr>
<tr>
<td>C12</td>
<td>Normal OHLD</td>
<td>&gt;95%</td>
<td>&gt;95%</td>
<td>&gt;95%</td>
<td>&gt;25%</td>
<td>N</td>
<td>N</td>
<td>HyperapoB, borderline hyperapoB</td>
</tr>
<tr>
<td>C14</td>
<td>Normal OHLD</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>Normal</td>
</tr>
<tr>
<td>L1</td>
<td>Normal spouse</td>
<td>&gt;95%</td>
<td>&gt;95%</td>
<td>N</td>
<td>&gt;25%</td>
<td>N</td>
<td>N</td>
<td>Hyperpreβ, borderline hyperapoB</td>
</tr>
<tr>
<td>L2</td>
<td>Normal spouse</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>&gt;95%</td>
<td>N</td>
<td>N</td>
<td>HyperapoA</td>
</tr>
<tr>
<td>L3</td>
<td>Normal spouse</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>Normal</td>
</tr>
<tr>
<td>L4</td>
<td>Normal spouse</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>Normal</td>
</tr>
</tbody>
</table>

Hepatic lipase (HL) genotype: S267F/T383M, compound heterozygotes; S267F, simple S267F heterozygote; T383M, simple T383M heterozygote; normal OHLD, normal first-degree relative of a compound heterozygote; OHLD, Ontario-based hepatic lipase–deficient family; normal spouse, normal unrelated spouse.

Biochemical phenotype: hyperpreβ, hyperprebetalipoproteinemia; hyperapoB, hyperapoB-lipoproteinemia; hyperapoA, hyperapoA-lipoproteinemia; hyperapoA-TG, hyperapoA-TG-lipoproteinemia (see "Results" section); hyperapoB-AI, hyperapoB-AI-lipoproteinemia; hyperapoB-AI-TG, hyperapoB-AI-TG-lipoproteinemia (see "Results" section); N, within normal limits for age and sex (values shown refer to percentile for age and sex).

Three different alleles of the apoB variable number of tandem repeats. Among the three affected subjects, two (B2 and B3) were LDLR allele 1 homozygotes, while one (B3) was an LDLR allele 2 homozygote. Among the three affected subjects, one (B3) was an LPL 1/2 heterozygote, while two (B1 and B2) were LPL 1/1 homozygotes.

**RP Fat Tolerance Tests**

RP levels in chylomicrons and chylomicron remnants from OHLD subjects B2, C5, and C9 and the mean ± SD values for the five control subjects are shown in Figure 3. Subjects B2 and C5 appear to have profiles that deviate significantly from the control curve, with peaks of RP in both the chylomicron and chylomicron remnant fractions occurring at 10 hours. In contrast, the RP in chylomicrons and chylomicron remnants for C9 and the control subjects at 10 hours were clearly in decline.

RP in chylomicrons at 10 hours in C5 and B2 were, respectively, 4.7 and 7.1 SD greater than the control value. Furthermore, RP was detectable at 48 hours in the chylomicron fraction in B2 but in neither C5 nor C9. RP in chylomicron remnants in C5, C9, and B2 remained elevated at 48 hours.

**Lipoprotein Response to Drug Treatment**

In subject B2, lovastatin reduced the plasma cholesterol level by 65% and the TG level by 62% (Figure 2). The decrease in total cholesterol was due to reductions in VLDL cholesterol (56%) and LDL cholesterol (68%) (data not shown). Plasma apoB decreased by 25%, but HDL cholesterol and apoA-I were unchanged (data not shown). The decrease in TG was found in VLDL, LDL, and HDL but did not persist throughout the treatment period (data not shown). With substitution of gemfibrozil for lovastatin, TG fell and cholesterol rose. There
TABLE 3. Biochemical Features in the Ontario-Based Hepatic Lipase-Deficient Family Classified by Hepatic Lipase Genotype

<table>
<thead>
<tr>
<th></th>
<th>Normal (n=3)</th>
<th>T383M (n=4)</th>
<th>S267F (n=4)</th>
<th>S267F/T383M (n=3)</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>42.7±15.5</td>
<td>36.5±19.3</td>
<td>32.0±3.6</td>
<td>55.3±5.8</td>
<td>0.004</td>
</tr>
<tr>
<td>TC (mmol/L)</td>
<td>6.07±0.88</td>
<td>6.36±0.64</td>
<td>6.25±0.49</td>
<td>7.20±1.05</td>
<td>NS</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>2.97±1.29</td>
<td>1.91±1.36</td>
<td>1.47±0.78</td>
<td>5.77±3.10</td>
<td>0.04</td>
</tr>
<tr>
<td>VLDLC (mmol/L)</td>
<td>1.34±0.51</td>
<td>0.78±0.68</td>
<td>0.27±0.35</td>
<td>2.77±2.47</td>
<td>NS</td>
</tr>
<tr>
<td>VLDLTG (mmol/L)</td>
<td>2.34±1.16</td>
<td>1.11±0.91</td>
<td>0.56±0.62</td>
<td>3.23±2.50</td>
<td>NS</td>
</tr>
<tr>
<td>LDLC (mmol/L)</td>
<td>3.52±0.31</td>
<td>4.10±0.58</td>
<td>4.16±0.61</td>
<td>2.93±0.81</td>
<td>NS</td>
</tr>
<tr>
<td>LDLTG (mmol/L)</td>
<td>0.42±0.18</td>
<td>0.47±0.23</td>
<td>0.68±0.35</td>
<td>1.50±0.46</td>
<td>0.006</td>
</tr>
<tr>
<td>HDLC (mmol/L)</td>
<td>1.37±0.62</td>
<td>1.48±0.54</td>
<td>1.82±0.39</td>
<td>1.53±0.67</td>
<td>NS</td>
</tr>
<tr>
<td>HDLTG (mmol/L)</td>
<td>0.19±0.06</td>
<td>0.19±0.08</td>
<td>0.25±0.18</td>
<td>0.63±0.06</td>
<td>0.0018</td>
</tr>
<tr>
<td>ApoAI (g/L)</td>
<td>1.58±0.22</td>
<td>1.50±0.16</td>
<td>1.78±0.22</td>
<td>1.86±0.23</td>
<td>NS</td>
</tr>
<tr>
<td>ApoB (g/L)</td>
<td>1.46±0.20</td>
<td>1.37±0.29</td>
<td>1.35±0.23</td>
<td>1.75±0.29</td>
<td>NS</td>
</tr>
<tr>
<td>LPL (U/hr)</td>
<td>7.72±3.80</td>
<td>11.15±4.0</td>
<td>11.65±5.21</td>
<td>13.07±3.35</td>
<td>NS</td>
</tr>
<tr>
<td>HL (U/hr)</td>
<td>9.34±1.21</td>
<td>7.18±3.77</td>
<td>3.83±2.22</td>
<td>Not detectable</td>
<td>0.0068</td>
</tr>
</tbody>
</table>

TC, total cholesterol; TG, triglycerides; VLDLC, very low density lipoprotein cholesterol; VLDLTG, very low density lipoprotein triglyceride; LDLC, low density lipoprotein cholesterol; LDLTG, very low density lipoprotein triglyceride; HDLC, high density lipoprotein cholesterol; HDLTG, high density lipoprotein triglyceride; apo, apolipoprotein; LPL, lipoprotein lipase; HL, hepatic lipase; U, micromoles of free fatty acids released per milliliter. 

*Probability of a greater between-group F value using analysis of variance (SAS). NS, not significant at p>0.05.

was no advantage when lovastatin and gemfibrozil were used in combination. Lovastatin reduced β-VLDL separated by Pevikon electrophoresis. The cholesterol/TG ratio of VLDL was also reduced (data not shown).

In Situ Hybridization of HL mRNA in an HL-Deficient Subject

Routine histological and electron microscopic examination of the liver from B1 showed no gross structural abnormalities. Hybridization of the HL antisense probe to sections of liver from subject B1 and a control subject and to a section of normal pituitary gland are shown in Figure 4. Specific hybridization signals were observed throughout the hepatic parenchyma, with no enrichment in either central venous or periportal areas in either B1 or the control. The signal intensity and number of hybridization grains were comparable, but these were not quantified. Minimal background signal was seen with the sense probe in the section of liver and with the antisense probe in the section of pituitary.

Discussion

The principal findings of this study are the clinical, biochemical, and pathological features of HL deficiency examined in the context of defined molecular variation of HL. Significantly, the HL variants S267F and T383M were coinherited in each of three brothers with complete HL deficiency. We observed that 1) compound heterozygotes had absent HL activity, increased TG content of LDL and HDL, detectable β-VLDL, and premature coronary artery disease; 2) simple heterozygotes did not have a specific lipoprotein abnormality; 3) the plasma RP fat tolerance test in a compound heterozygote deviated from that of the control subjects; 4)
FIGURE 4. In situ hybridization of hepatic lipase (HL) mRNA. The HL antisense DNA oligonucleotide probe was hybridized with paraffin sections of liver from subject B1 (panel A), a normal control subject (panel B), and a normal pituitary gland (panel D). The HL sense DNA probe was hybridized with liver from subject B1 and is shown in panel C. Positive signals (points of hybridization between mRNA and the probe) are indicated by small, dark intracellular dots.

the lipoprotein abnormalities in a compound heterozygote were ameliorated by lovastatin; and 5) HL mRNA distribution in the liver parenchyma of a compound heterozygote was not different from normal.

Plasma postheparin HL activities varied significantly according to HL genotypic class, with activities highest in normal subjects, lower in T383M heterozygotes, lower still in S267F heterozygotes, and absent in compound heterozygotes (Table 3). This implies that the deficiency imparted by S267F is clinically more severe than the deficiency imparted by T383M. Preliminary data from in vitro expression of HL support this clinical prediction (J.M. Lalouel and R.A. Hegele, unpublished data). In parallel transformations of primate renal cells with wild-type HL and T383M or S267F, the HL activity in the culture medium from cells transfected with T383M was approximately 10% of the wild type, and the HL activity in the medium of S267F was not detectable. It may also be significant that mutagenesis to alanine of the homologous serine residue in LPL resulted in a variant with impaired function.37

Among T383M heterozygotes there was considerable variability in HL activity. For example, two young male T383M heterozygotes (C2 and C3) had normal HL activity. This suggests that the HL deficiency imparted by T383M might be modulated by secondary factors (e.g., age, gender, or other variants of other genes). Since T383M has reduced but detectable in vitro activity (J.M. Lalouel and R.A. Hegele, unpublished data), it is possible that simple T383M heterozygotes may in most clinical situations have adequate lipolytic activity and no phenotypic abnormality. However, in the presence of secondary factors that modulate HL (e.g., estrogen or alcohol) and thus impair an HL mutant with marginal activity, there could be a significant impact at the clinical level. Variability in expression of a biochemical phenotype in heterozygotes for a genetic variant has also been reported in families with mutant LPL38 and with familial hypercholesterolemia.39 A secondary factor, either genetic or environmental, was postulated to affect the severity of expression of the phenotype in patients heterozygous for a mutant allele in these cases, as was observed in heterozygotes for a mutant apoCII.40

The lipoprotein fractions that varied significantly according to genotype were LDL TG and HDL TG. The highest concentrations of LDL TG and HDL TG were found in the compound heterozygotes, and these subjects could be phenotypically designated as having hyperbeta-triglyceridemia (LDL TG exceeding the 95th percentile for age and sex) and hyperalphatriglyceridemia (HDL TG exceeding the 95th percentile for age and sex). The lowest concentrations of LDL TG and HDL TG were found in normal subjects. The youngest S267F heterozygote (C4, a 28-year-old woman) did not have TG enrichment of LDL or HDL. The three older S267F heterozygotes (C5, C9, and C16) had hyperbeta-triglyceridemia. The eldest S267F heterozygote (C16) also had hyperalphatriglyceridemia. Of the T383M heterozygotes, only the oldest (B6, a 65-year-old man) had TG enrichment of LDL and HDL. The three older S267F heterozygotes (C5, C9, and C16) had hyperbeta-triglyceridemia. Of the T383M heterozygotes, only the oldest (B6, a 65-year-old man) had TG enrichment of LDL and HDL. The others had neither. The phenotype of heterozygotes involves varying degrees of TG enrichment of LDL and/or HDL. S267F appears to be associated with TG-rich LDL in older heterozygotes. T383M appears to be associated with TG-rich LDL and HDL but only in the oldest heterozygote studied. Thus, age and gender in heterozygotes may affect phenotypes (HL activity and/or TG content of LDL and HDL).

There were subjects in OHLD who were not HL deficient (e.g., B4) and who had the normal HL sequence at codons 267 and 383, but who had an abnormal lipoprotein phenotype. This suggests that a second dyslipidemia, perhaps unrelated to HL deficiency, segregates through OHLD, particularly through the "unaf-
fected" branch comprising the offspring of B4. The small number of subjects precludes a more definitive analysis.

Published cDNA and genomic HL sequence data\textsuperscript{16-20} and analysis in normal and hyperlipidemic populations\textsuperscript{21} reveal that both asparagine and serine are common variants of HL at position 193. Furthermore, the "silent" DNA variants at positions 133 and 202 are also common, with each allele having a frequency of approximately 50% in the general population. None of these variants is associated with dyslipidemia.\textsuperscript{21} After screening more than 500 HL alleles, T383M was found only in OHLD and QHLD, whereas S267F was found only in OHLD.

Genotype analysis for apoB, LPL, and LDLR in the three HL-deficient subjects ruled out both homozygosity and compound heterozygosity for mutations at these loci as etiological in HL deficiency. Genotype analysis for the LPL gene was consistent with a dominant effect of this locus on the HL-deficient phenotype in the three HL-deficient subjects but was inconsistent with the observed recessive inheritance and phenotypic expression of HL deficiency. ApoE isotyping in the affected subjects did not rule out homozygosity for variants at the closely linked apoCII and apoCI genes that might underlie or contribute to HL deficiency. However, the identification of the HL mutants means that variation at another locus is unlikely to be etiological in HL deficiency. The findings do not rule out the possibility that variants of other genes are associated with other lipoprotein abnormalities in OHLD.

We observed a delayed rise of plasma RP in the chylomicron fraction in B2 and C5 and delayed disappearance of RP from chylomicron and chylomicron remnant fractions in B2 and C5. The delayed rise of RP in chylomicrons in B2 and C5 could be due to factors other than HL deficiency. In B2, the presence of RP in chylomicron and chylomicron remnant fractions 48 hours after the fat load suggests defective TG-rich remnant catabolism imparted by HL deficiency, either in the conversion of chylomicrons to chylomicron remnants and/or in the receptor-mediated catabolism of chylomicron remnants.

A catabolic defect in TG-rich lipoprotein metabolism due to absent HL activity can be explained in the case of the compound heterozygote B2. An explanation in C5 is less straightforward but may be due to the fact that C5 is heterozygous for both S267F and E2. E2/2 homozygosity affects TG-rich lipoprotein catabolism, as in type III hyperlipidemia. C5 is thus a double heterozygote for variants of two proteins involved in the catabolism of TG-rich lipoproteins. It is possible that expression of this catabolic abnormality requires at least one mutant HL and either a single binding-defective E2 isoform or another mutant HL. Alternatively, in C5 there might be another second genetic factor such as the variants of LDLR\textsuperscript{41} or apoCI\textsuperscript{42} that have been reported to be associated with type III hyperlipidemia.

The abnormal metabolism of RP in the chylomicron and chylomicron remnant fractions of B2 and C5 compared with C9 and the control subjects must be interpreted with caution, since each of the three family members was taking lovastatin for hyperbetalipoproteinemia. In previous studies of RP in subjects taking lovastatin, no delay was seen in the rate of appearance, in the time to peak, or in the rate of disappearance of RP from either TG-rich fraction.\textsuperscript{31,43} Thus, the abnormal metabolism of RP in the TG-rich lipoprotein fraction in B2 and C5 was not likely an artifact due to lovastatin. We plan to study several simple heterozygotes of both types to determine whether this test can be used to screen for carriers of mutant HL.

The role of HL in chylomicron and chylomicron remnant metabolism remains unclear. Experiments in rats indicated that treatment with anti-HL antisera inhibits the removal of PL from chylomicrons and HDL.\textsuperscript{44} Rat HL was shown to participate in the hydrolysis of chylomicron PL.\textsuperscript{45} The observations in B2 and C5 would be compatible with a role for HL in facilitating the conversion of chylomicrons to chylomicron remnants and/or their clearance by receptor-mediated endocytosis. The persistence of significant RP in chylomicron remnants in B2, 48 hours after the fat load, suggests an important role for HL in the clearance of chylomicron remnants. However, the abnormal profiles of RP in the chylomicron fraction in B2 and C5 also suggest that HL may affect both appearance and removal of RP in the chylomicron fraction.

While gemfibrozil in B2 mainly affected plasma TG, lovastatin treatment resulted in desirable changes in all the major lipoprotein classes. It is possible that the reduction in LDL and VLDL resulted from increased LPLR expression and/or increased ligand affinity due to changes in the number and lipid composition of TG-rich lipoproteins.

The in situ hybridization experiments indicate that the mutations in HL deficiency do not reduce the amount of total HL mRNA in the liver. The results suggest that at least one allele encodes normal quantities of a stable mRNA. It is likely that the mutations result in stable mRNA species that encode poorly functioning proteins. To determine whether these proteins are detectable in the circulation will require immunoassay, with specific anti-HL monoclonal antibodies, of the HL mass in the sera of affected subjects.

In summary, subjects in OHLD with complete HL deficiency are compound heterozygotes for mutant HL. The phenotype of complete HL deficiency is clinically and biochemically distinct from other hyperlipoproteinemias and includes TG-rich LDL and HDL. HL deficiency is associated with abnormalities in chylomicron metabolism. S267F heterozygotes appear to be clinically worse off than T383M heterozygotes, but both, especially older subjects and females, tend to have depressed HL activity with TG-rich LDL and HDL. However, the phenotype in heterozygotes is variable and appears to be modulated by secondary factors, both genetic and environmental. The true prevalence of HL deficiency is not known because the phenotype is difficult to diagnose. DNA-based screening might reveal HL variants in other dyslipidemic subjects.

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