A compound heterozygote for hepatic lipase gene mutations Leu334 → Phe and Thr383 → Met: correlation between hepatic lipase activity and phenotypic expression

Petteri Knudsen,* Marjatta Antikainen,† Sonja Ehnholm,§ Mikko Uusi-Oukari,§ Heli Tenkanen,§ Sanni Lahdenperä,* Juhani Kahri,* Marju Tilly-Kiesi,* Andre Bensadoun, Marja-Riitta Taskinen,* and Christian Ehnholm[1],§**

Department of Medicine,* Helsinki University Central Hospital, Helsinki, Finland; Children's Hospital,† University of Helsinki, Helsinki, Finland; National Public Health Institute,§ Mannerheimintie 166, 00300 Helsinki, Finland; and Department of Nutritional Sciences,** Cornell University, Ithaca, NY

Abstract We have characterized the molecular basis for familial hepatic lipase (HL) deficiency in a Finnish family. In the propositus, the HL deficiency results from compound heterozygosity for two rare HL gene mutations, a previously unknown missense mutation designated L334F and the previously reported T383M mutation. These mutations were introduced into human HL cDNA by site-directed mutagenesis and the constructs expressed in COS-1 cells. In the homogenate of COS-1 cell transfected with the L334F mutant cDNA, a high amount of inactive protein accumulated. In the media of L334F transfected cells, 30% of the wild type activity and 80% of wild type mass were detected. The lysates of COS-1 cells transfected with the T383M mutant cDNA contained 39% of wild type HL activity and 34% of wild type HL mass. In the media of COS-1 cells transfected with the T383M cDNA construct, 50% of wild type HL mass but only 6% of wild type activity was present. The single amino acid substitutions present in L334F and T383M are therefore sufficient to severely affect the HL enzyme. These defects explain the HL-deficient phenotype of the individual carrying the two mutations. The lipoprotein phenotype associated with compound heterozygosity for L334F and T383M mutations is characterized by a slight increase in the buoyant low density lipoprotein (LDL) fraction and an increase in the light high density lipoprotein (HDL) fractions, HDL_{2a} and HDL_{2b}. These results demonstrate that lipoprotein changes occurring in HL deficiency are difficult to identify and support the hypothesis that HL is important in HDL remodeling and metabolism in vivo.—**Knudsen, P., M. Antikainen, S. Ehnholm, M. Uusi-Oukari, H. Tenkanen, S. Lahdenperä, J. Kahri, M. Tilly-Kiesi, A. Bensadoun, M-R. Taskinen, and C. Ehnholm.** A compound heterozygote for hepatic lipase gene mutations Leu334 → Phe and Thr383 → Met: correlation between hepatic lipase activity and phenotypic expression. *J. Lipid Res.* 1996. **37:** 825–834.

Supplementary key words hepatic lipase • HL gene • mutation • HDL-cholesterol • HL deficiency

Hepatic lipase (HL; triacylglycerol lipase EC 3.1.1.3) is a lipolytic enzyme synthesized in and secreted from hepatocytes and localized mainly to the hepatic sinusoidal endothelial surface (1, 2). The enzyme has both triglyceride hydrolase and phospholipase A_1 activities (3). The human HL gene has been assigned to chromosome 15 q 21 and spans over 35 kb containing 9 exons and 8 introns (4). The mature protein consists of 477 amino acids and has a molecular weight of about 60000 Da (3, 5). The enzyme can be released from the hepatic sinusoidal surface into the circulation by intravenous injection of heparin enabling the measurement of its activity in post-heparin plasma (6). HL has been reported to participate in the conversion of intermediate density lipoproteins (IDL) to low density lipoproteins (LDL) (7–10), in chylomicron remnant catabolism (11–15), and in high density lipoprotein (HDL) metabolism in humans (16–18), and in transgenic animals (19, 20). Targeted inactivation of the hepatic lipase in mice leads to mild dyslipidemia characterized by an increase in HDL (21). Some studies suggest that HL may have an effect on the delivery of HDL cholesterol to the liver (22, 23). However, there is no consensus regarding the precise physiological function of HL.

Abbreviations: HL, hepatic lipase; LDL, low density lipoprotein; HDL, high density lipoprotein; IDL, intermediate density lipoprotein; apo, apolipoprotein; ELISA, enzyme-linked immunosorbent assay; PCR, polymerase chain reaction; SSCP, single-strand conformation polymorphism.

[1]To whom correspondence should be addressed.

Heritable deficiency of HL has provided insights into the physiological function of HL in humans. So far, only five families with HL deficiency have been identified (24–28). Affected individuals with heterozygous state for HL mutations do not have specific lipoprotein abnormalities and even patients with complete HL deficiency display a variable phenotype (29). The most consistent findings in the lipoprotein pattern of HL-deficient subjects are an elevation of HDL_2-cholesterol (24, 25, 30) and an enrichment in triglyceride in LDL and HDL particles (25, 29, 31). Auwerx et al. (26) reported the appearance of large buoyant LDL-like particles in affected individuals.

Two missense mutations in the human HL gene, substitutions of serine for phenylalanine at amino acid 267 (S267F) and threonine for methionine at amino acid 383 (T383M) have been identified and demonstrated to be responsible for HL-deficient phenotypes (29, 32). In vitro expression studies have confirmed that the S267F mutation results in the production of a catalytically inactive enzyme protein while the T383M-mutant protein retains partial activity but is poorly secreted (32). The present investigation describes a Finnish patient with HL deficiency and his family. We report a previously unknown missense mutation, L334F, of the human HL gene and demonstrate that the affected individual is a compound heterozygote for this mutation and the T383M mutation. In addition, we report three new polymorphisms of the HL gene. We also describe the effects of HL deficiency on lipoprotein phenotype, in particular on HDL subclasses.

METHODS

Subjects

The proband is a 40-year-old, married man who has two sisters, a son aged 7 and daughter aged 9; both his parents are alive (Fig. 1). The father of the proband has type II diabetes mellitus and hypertension. His medication consists of glibenclamide 3.5 mg once daily and pindolol 2.5 mg once daily. The other members of the family are healthy and are not on any medication. No family members are smokers.

In order to investigate the allele frequencies of the HL gene variants and observed HL gene mutations, 85 healthy Finnish volunteers were recruited into this study. None of these population control subjects were relatives of the proband.

Lipid and lipoprotein analyses

Serum lipid and lipoprotein analyses were carried out on blood samples collected after an overnight fast. Lipoprotein fractions were isolated by sequential ultracentrifugation (33). The density distribution of LDL and HDL particles was examined using density gradient ultracentrifugation (34). The size of HDL particles was determined using gradient gel electrophoresis (35). Cholesterol and triglyceride concentrations were determined by an enzymatic method (36, 37) using commercial kits (No. 187313 and No. 297771, Boehringer Mannheim, Germany).

ApoA-I and apoA-II concentrations were determined by an immunoturbidometric method using commercial kits (Boehringer GmbH Kits nos 726 478 and 726 486 Mannheim, Germany). The interassay variations for apoA-I and apoA-II measurements were 3.6% and 3.7%, respectively. ApoB concentrations were determined using radial immunodiffusion (Behringswerke GmbH, Germany). ApoE phenotyping was done using isoelectric focusing and immunoblotting (38).

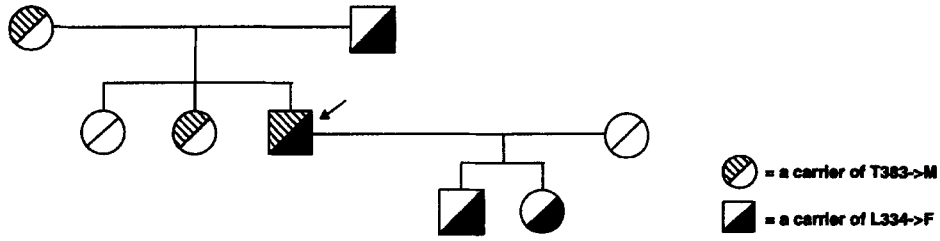
The concentration of LpA-I particles was quantified using differential electroimmunophoresis with hydrated agarose gels containing monospecific antibodies against apoA-I and apoA-II (Sebia, Issy-les Molineaux, France) (39). The interassay variation for Lp-A-I was 7.3%. The concentration of LpA-I:LpA-II particles was calculated by subtracting the concentration of LpA-I particles from turbidometrically determined total concentration of apoA-I in serum. Fasting insulin levels were determined using a radioimmunoassay (Pharmacia Diagnostics AB, S-751 82 Uppsala, Sweden). The interassay variations for insulin determined using two standard specimens (93 and 323 pmol/l) were 7% and 4.8%, respectively.

Lipolytic enzyme activities and mass determinations

After an overnight fast, plasma samples were collected 15 min after a heparin bolus injection (100 IU/kg body weight, Leiras, Huhtamäki Oy, Turku, Finland) into chilled tubes containing lithium heparin. Lipoprotein lipase and HL activities were determined using an antiserum inhibition method (6). The HL protein concentration was determined by enzyme-linked immunosorbent assay (ELISA) using two different monoclonal antihuman HL antibodies (40). To measure HL protein in cell homogenates, the cell extracts were treated with 2 ml acetone (-20°C) twice and once with 2 ml ethyl ether. The dry cell extracts were solubilized in 10 mM SDS, 1% BSA, 0.05% Tween-20, 10 mM phosphate, pH 7.4.

Amplification of the exons of the hepatic lipase gene by the polymerase chain reaction (PCR)

All of the 9 exons of the human HL gene were amplified by the PCR technique (41). PCR primers, homologous to intron sequences flanking the exons of the HL gene and containing 19–22 nucleotides, were designed based on published HL gene structure (4). The



									Control subjects	
									Men	Women
Number									41	38
Age (years)	72	37	33	40	72	9	7	36	42±7	43±6
BMI (kg/m^2)	16	22	18	21	24	20	19	23	25±5	23±3
Tg (mmol/l)	0.80	0.75	0.80	0.82	1.08	0.70	0.91	0.91	1.18±0.66	0.96±0.29
VLDL-Tg	0.24	0.26	0.34	0.15	0.63	0.23	0.42	0.45	0.64±0.57	0.42±0.18
IDL-Tg	0.09	0.07	0.08	0.13	0.10	0.10	0.11	0.10	0.10±0.04	0.10±0.04
LDL-Tg	0.26	0.20	0.18	0.30	0.16	0.18	0.18	0.19	0.24±0.07	0.23±0.07
HDL-Tg	0.22	0.21	0.20	0.30	0.20	0.18	0.20	0.18	0.19±0.06	0.21±0.06
HDL_2-Tg	0.11	0.13	0.10	0.20	0.09	0.12	0.09	0.12	0.10±0.03	0.12±0.05
HDL_3-Tg	0.11	0.08	0.10	0.10	0.11	0.06	0.11	0.06	0.09±0.04	0.09±0.03
Chol (mmol/l)	6.15	5.17	6.74	5.01	3.93	5.40	5.74	4.70	5.66±1.11	5.24±1.05
VLDL-Chol	0.11	0.22	0.19	0.07	0.44	0.15	0.24	0.27	0.23±0.15	0.23±0.13
IDL-Chol	0.14	0.10	0.09	0.33	0.20	0.22	0.12	0.13	0.20±0.14	0.17±0.13
LDL-Chol	4.05	3.81	4.27	2.70	2.25	3.31	3.45	2.73	3.45±0.98	3.24±0.84
HDL-Chol	1.85	1.67	2.19	1.91	1.04	1.72	1.93	1.57	1.51±0.38	1.60±0.32
HDL_2-Chol	1.05	0.84	1.38	1.37	0.47	0.92	0.97	0.97	0.80±0.33	0.95±0.30
HDL_3-Chol	0.80	0.83	0.81	0.54	0.57	0.80	0.96	0.60	0.71±0.11	0.65±0.11
HDL_2-C/HDL_3-C	1.30	1.01	1.70	2.54	0.82	1.15	1.01	1.62	1.13±0.47	1.51±0.56
Apo A-I (mg/dl)	166	155	180	151	126	154	143	153	150±25	158±25
Apo A-II	44	37	36	35	30	42	34	31	30±5	35±5
Apo B	87	77	91	77	71	86	70	75	102±30	83±26
LpAI (mg/dl)	60	64	72	51	45	58	62	75	54±13	61±12
LpAI/AII (mg/dl)	106	91	108	100	81	96	81	78	96±19	95±18
Apo E phenotype	4/4	4/3	4/3	4/3	3/2	3/3	4/4	4/3		
LPL activity (mU/ml)	283	281	275	230	173	311	265	212	262±63	303±95
HL activity (mU/ml)	80	291	134	55	122	194	196	167	312±102	179±83
HL mass (ng/ml)	185	316	300	196	191	264	218	222	359±53	234±53
Specific activity-HL (mU/ng)	0.43	0.92	0.45	0.28	0.64	0.73	0.89	0.75	0.86±0.20	0.73±0.19

Fig. 1. Pedigree of the hepatic lipase (HL)-deficient family. Some clinical and laboratory data for the family members and for control subjects. Squares represent males and the circles females. The arrow indicates the proband. The lipid values are expressed as mmol/l, the concentrations of apoproteins as mg/dl, the activities of the lipases as mU/ml, and their masses as ng/ml. The abbreviations used are: BMI, body mass index; Tg, triglyceride; Chol, cholesterol; VLDL, very low density lipoprotein; IDL, intermediate low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; Apo, apolipoprotein; HL, hepatic lipase; LPL, lipoprotein lipase.

following primers were used: exon 1, I:5′-(AGG GTC TGA ACA GTG GAT AAC)-3′, II:5′-(AAA GTT CAT GCC CAT CTC TGG)-3′, exon 2, I:5′-(GAT GAA GCA CAT GCC AGG CT)-3′ II:5′-(ACA CGC TAG TGA AAT CGG AGA)-3′; exon 3, I:5′-(GAA GGG TAG CGG GGA GAA AGG G)-3′, II:5′-(GGA GGG AAG GGA GGT GAA G)-3′; exon 4, I:5′-(GGC TTT CAT CCA GGC AGC CTT C)-3′, II:5′-(TGG AGT GAG ATC AGT GTG TGA)-3′; exon 5, I:5′-(CTT GCT CCC GCG TAA CCC T)-3′, II:5′-(GCT CGA ATT CCA GAG GCC C)-3′; exon 6, I:5′-(GGT AAG GGT GAT AAC GTC CT)-3′, II:5′(CTG CAT CCT GCA TCC TGC C)-3′; exon 7, I:5′-(CTT CCC TCT GTG CAT GTT TAA A)-3′, II:5′-(GAG TCC ATT TAT GTT CTG CAA G)-3′; exon 8, I:5′-(GCT GTT ACG ACT AAA CTG ATT G)-3′, II:5′-(CTG CCT GGC ACA AGT GGG T)-3′, and exon 9, I:5′-(CCA CCT AAA ACT TAA TGC TGT G)-3′, II:5′-(TGG CTT CTA AAT AAG GCA GCC)-3′. The primers were synthesized on an Applied Biosystems Model 381A DNA synthesizer (42). The PCR was carried out as described (43) in a total volume of 50 µl, except that 1 Ci of [α-^{32}P]dCTP (Amersham, U.K.) was included to each reaction for SSCP. The cycles of denaturation (1 min at 95°C), annealing (1 min at 56°C) and elongation (2.5 min at 72°C) were repeated 30 times.

Analysis of single-strand conformation polymorphism (SSCP)

The PCR products were diluted 1:5 in 0.1% SDS, 10 mM EDTA, and mixed with an equal volume of 95% formamide, 20 mM EDTA, containing 0.05% bromophenol blue and 0.05% xylene cyanol. The samples were denatured at 90°C for 2 min and cooled on ice. Aliquots of 2–4 µl were analyzed for SSCPs (44) on nondenaturing 5% polyacrylamide gel containing 10% glycerol at 360 V for 18 h at room temperature. After electrophoresis the gel was transferred to Whatman 3 MM paper and dried in a vacuum slab dryer. Autoradiographs were developed for 1–3 days at -70°C using Kodak XAR film (Rochester, NY).

DNA sequencing

All exons of the human HL gene were amplified for sequencing. The PCR products were sequenced by the dideoxy chain termination method (45) with modifications (46).

Solid-phase minisequencing

The principle of the method has been described by Syvänen et al. (47). The upstream and downstream PCR primers for detecting L334F and T383M mutations were the same as used for the amplification of exon 7 and 8 of the HL gene. The downstream primer was biotinylated as described (48). The use of these primers resulted in an amplification of 283 and 184 bp DNA fragments of the HL gene respectively. Five ng of DNA was amplified under conditions given for the amplification of exons of the HL gene except that the biotinylated primer was used at 4 µM.

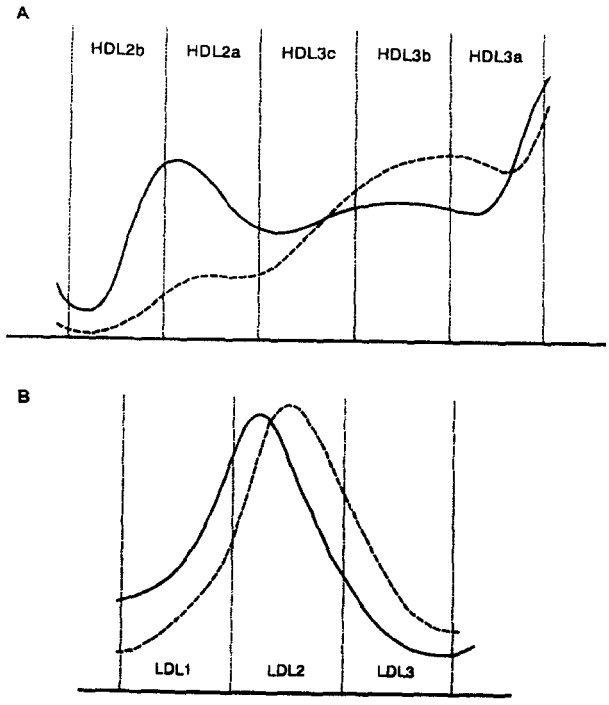


Fig. 2. A: Distribution of HDL subclasses following gradient ultracentrifugation. The solid lane depicts the relative protein concentration (OD_{280}) of HDL of the proband. The dotted line illustrates the mean HDL subclass distribution of 40 normolipidemic males. B: Distribution of LDL subclasses after gradient ultracentrifugation. The solid line depicts the relative protein concentration (OD_{280}) of LDL of the proband. The dotted line illustrates the mean LDL subclass distribution of 40 normolipidemic males.

For each minisequencing reaction, 10 µl of the PCR mixture and 40 µl of 20 mM sodium phosphate buffer, pH 7.5, containing 0.1% Tween 20, were added to microtitration wells (Maxisorb Nunc, Labsystems, Finland) coated with streptavidin. The samples were incubated with gentle shaking at 37°C for 1.5 h and washed three times with 200 µl of 40 mM Tris-HCl buffer, pH 8.8, containing 1 mM EDTA, 50 mM NaCl, and 0.1% Tween 20. The wells were treated two times with 100 µl of 50 mM NaOH for 5 min at room temperature and washed as above. A reaction mixture consisting of 0.2 µM detection primer L334F: 5′-(CTA GTT TAT CAT TAC CAG TT)-3′ and T383M: 5′-(TAA AAC GTA TTC CTT TCT TAT CA)-3′ and 0.4 µM [^{3}H]dATP/dCTP (TRK 627, 37 Ci/mmol) to detect the mutation in codon 334 or 0.4 µM [^{3}H]dCTP/dTTP (TRK 625, 67 Ci/mmol, Amersham, U.K.) to detect the mutation in codon 383. The samples were incubated at 50°C for 10 min and washed as above. The microtitration plates were treated

with 50 µl of 50 mM NaOH for 5 min at room temperature and the radioactivity was measured in a liquid scintillation counter.

Site-directed mutagenesis of hepatic lipase cDNA

The human HL cDNA was kindly provided by Dr. Lawrence Chan (4). The HL cDNA was amplified by the PCR using Vent polymerase (New England Biolabs). BamH I and Xba I restriction sites were added to the primers Primer I:5′-(GTA TGG ATC CAA AAT GGA CAC AAG TCC CCT GTG)-3′ Primer II: 5′-(CTG GGG ATC CTC TAG AAC TCA TCT GAT CTT TCG CTT TG)-3′. The PCR product was subcloned to the BamH I site of the replicative form of M13mp18. Oligonucleotide directed site-specific mutagenesis of HL-cDNA in M13mp18 single-stranded DNA was carried out by the phosphorothioate DNA selection method (49) using a commercial kit (Amersham, RPN 1526, U.K.). The oligonucleotides used for mutagenesis were 5′-(CAT TAC CAG TTC* AAG ATC CAG)-3′ (L334F), and 5′-(CCT TTC TTA TCA TG*C TGG ATG TGG)-3′ (T383M). Mutants were verified by dideoxynucleotide sequencing (45).

Expression of the wild-type and mutant HL cDNAs in COS-1 cells

Wild-type and mutant HL cDNAs were inserted into the BamH I site of expression vector pSVL (Pharmacia, Uppsala, Sweden). COS-1 cells (ATCC, CRL 1650), maintained in Dulbecco's modified Eagle's medium supplemented with antibiotics and 10% fetal calf serum, were seeded 1 day prior to transfection at a confluence of 5×10^5 cells/6 cm petri dish. The cells were transfected with 10 µg of the pSVL plasmid DNA constructs using the liposome transfection method (50) using lipofectin (Lipofectin reagent, Gibco BRL, Life Technologies, Gaithersburg, MD) as described (43). For HL activity and mass determinations, culture medium containing heparin (20 U/ml) was collected at 48 h after transfection. Cells were collected, washed in PBS, solubilized in 1 ml of 50 mM NH_3/NH_4Cl (pH 8.1) containing heparin, and sonicated. Media and cell extracts were stored at -70°C until assayed.

RESULTS

Serum lipid and lipoprotein concentrations

The mean concentrations of cholesterol and triglycerides in plasma and lipoprotein fractions of the family members and control subjects are given in **Fig. 1.** In the family members there were no clear abnormalities in the levels of cholesterol and triglycerides in the different lipoprotein classes (Fig. 1). The concentration of HDL_2-triglycerides in the proband differed more than ± 2 SD from the means of age- and gender-matched subjects. An exceptionally high HDL_2/HDL_3-cholesterol ratio was observed in the proband (Fig. 1). No compositional changes in protein, cholesteryl ester, free cholesterol, phospholipid, or triglyceride concentrations in VLDL, IDL, HDL_2 and HDL_3 particles were observed in the proband or the other family members (data not shown).

HDL and LDL subfractions in the proband

The HDL density distribution curve of the proband and the mean HDL density distribution curve of 40 healthy men are shown in **Fig. 2A.** The proband had a very prominent HDL_2 peak and smaller HDL_3 peak as compared to healthy men. The percentages of total HDL mass of the proband and of healthy men present in subfractions HDL_{2a}, HDL_{2b}, HDL_{3a}, HDL_{3b}, and HDL_{3c} were 21 versus 10 ± 3%, 24 versus 14 ± 3.4%, 10 versus 19 ± 2.6%, 19 versus 27 ± 2.4%, and 17 versus 29 ± 5.4%, respectively, indicating a preponderance of large HDL_{2a} and HDL_{2b} particles in the proband. Gradient gel electrophoresis confirmed that the proband had an excess of large HDL particles representing the HDL_{2b} subclass (data not shown). The percentage of the serum apoA-I present in LpA-I particles was 34% in the proband and 36% in control men.

The LDL density distribution curve of the probands' LDL as compared to the mean LDL density distribution of 40 healthy normolipidemic males of similar age (44.6 ± 6.8 years) and plasma triglyceride concentration (0.98 ± 0.26 mmol/l) was shifted towards larger and more buoyant LDL particles (Fig. 2B). In the proband, LDL_1, LDL_2, and LDL_3 represented 55%, 35%, and 10% of the total LDL particle mass, respectively, while in healthy men, the respective percentages were 41%, 43%, and 16%. In the proband the LDL_1/LDL_3 ratio of 4.4 was markedly higher than that observed in healthy men 2.7 ± 1.0 (mean ± SD).

Lipolytic enzymes in post-heparin plasma

The post-heparin hepatic lipase (HL) activities of the proband, his mother, and his father were reduced being 18–40% of the respective age- and gender-adjusted normal values (Fig. 1). The son and the daughter of the proband had low HL activities, which however were within the normal range. The HL activities of one of the sisters and the wife of the proband were within normal range. In the proband and his father the immunoreactive HL mass was decreased to about 50% of the respective age- and gender-adjusted normal values (Fig. 1).

Single-strand conformation polymorphism (SSCP) analysis and sequencing of the exons of the HL gene

All nine coding exons of the HL gene were amplified and analyzed for SSCPs. This analysis revealed PCR products with aberrant electrophoretic migration derived from exon 4, 5, and 8. Sequencing of the PCR products revealed eight variable sites in the human HL gene. A novel single nucleotide mutation in exon 7 resulting in the substitution of leucine for phenylalanine at amino acid 334 was identified in the propositus, his father, and two children. The T383M missense mutation in exon 8 was observed in the propositus and his mother and sister. In the family the DNA sequence polymorphism encoding either serine or asparagine at codon 193 could also be demonstrated. In addition, four silent mutations located in the codons 133, 175, 344, and 457 were observed (**Fig. 3**). Based on the genetic variants observed, five different haplotypes can be identified in the Finnish pedigree. These five haplotypes, designated A–E, and their inheritance in the family are illustrated in Fig. 3. It can be noted that the mutation T383M and the novel mutation L334F are on different haplotypes.

Solid phase minisequencing

To study the inheritance of all the genetic variants observed we analyzed the family using the minisequencing technique. Mendelian inheritance could be demonstrated for all of the genetic variants. The results for the L334F mutation are depicted in **Table 1.** The data demonstrate that the L334F mutation is inherited with haplotype C present in the proband, his father, and his two children. The minisequencing results confirm that the proband, his father, and two children are heterozygotes for the A to C change at the third base of codon 334. It also confirms that the proband, his mother, and his sister are heterozygotes for the C to T transition at the second base of codon 383.

Functional significance of the L334F and T383M substitutions

Mutant cDNA constructs containing the nucleotide change corresponding to the mutants L334F and T383M were generated by in vitro mutagenesis from wild type HL cDNA. These constructs were transiently expressed in COS-1 cells, and the culture media and cell homogenates were analyzed for HL enzyme activity and immunoreactive protein mass. Two different expression studies of L334F and T383M substitutions were carried out with triplicate expressions and measurements. The results of HL activities and immunoreactive protein

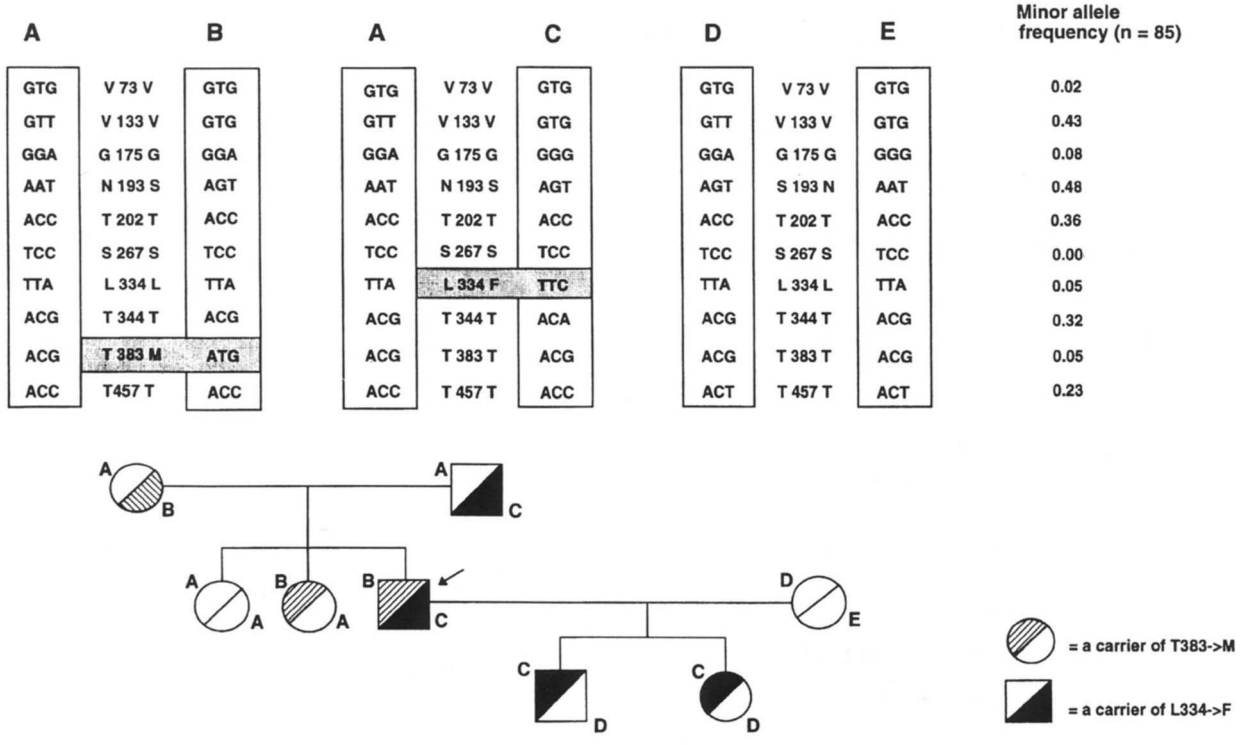


Fig. 3. Segregation of hepatic lipase gene variants in the Finnish HL-deficient family. The variants are designated according to their codon. The minor allele frequencies given are determined in a group of normolipidaemic Finns (n = 85). The five allelic haplotypes constructed based on the gene variants are boxed and designated A to E. Carriers of the mutants L334F and T383M that have an impact on disease expression are indicated by black and shaded areas.

TABLE 1. Detection of a single nucleotide mutation (A to C) in the exon 7 of the human HL gene resulting in a substitution of the leucine for phenylalanine at the amino acid 334 (L334F)

	Hapoltype	Incorporated Radioactivity		Ratio (R*)
		A	C	A/C
		cpm		
Proband	(B/C)	2334	2246	1.04
Mother	(A/B)	3595	15	239.67
Father	(A/C)	3124	3852	0.81
Sister I	(B/A)	3609	10	360.96
Sister II	(A/A)	3685	15	245.67
Wife	(D/E)	3552	13	273.26
Son	(C/D)	3588	3957	0.91
Daughter	(C/D)	1660	1595	1.04

R* > 10 for normal individuals; R* 0.1–10 for heterozygotes; R* < 0.1 for homozygotes.

masses are presented in **Table 2** and illustrated in **Fig. 4.** In COS-1 cells transfected with L334F mutant cDNA, a high amount of inactive protein accumulated into the cells. In the media of cells transfected with L334F cDNA, 30% of the wild type activity and 80% of wild type mass were detected. The COS-1 cell lysates transfected with T383M mutant cDNA contained 39% of wild type HL activity and 34% of wild type HL mass. In the media of COS-1 cells transfected with the T383M cDNA construct, 50% of the wild type HL mass but only 6% of the wild type HL activity was present.

DISCUSSION

In this study we describe a Finnish pedigree in which the HL-deficient proband is a heterozygote for two mutations in exons 7 and 8 of the human HL gene. One is a novel missense mutation L334F and the other a T383M mutation previously reported in two Canadian families with heritable HL deficiency (27, 31). In addition to the two functional mutations L334F and T383M, three novel silent variations were identified in the family studied. Together with the previously reported polymorphisms (51), at least nine variable sites are present in the human HL gene.

To confirm the roles of the two mutations L334F and T383M in HL deficiency, we introduced these mutations into human HL cDNA by in vitro site-directed mutagenesis and expressed the constructs in COS-1 cells. Our results demonstrate that the novel L334F mutation leads to the secretion of an almost normal amount of HL protein with only about 30% of the activity of the wild type enzyme. A high amount of inactive protein accumulated into the cells. In adult individuals heterozygous for the L334F mutation, postheparin plasma HL activities were decreased compared to control values. The mutant allele L334F cosegregates with diminished postheparin plasma HL activity in the Finnish family studied. It seems to be rather common in the Finnish population as among 170 HL alleles studied 8 mutants were found (10% of the subjects studied were carriers of the rare allele).

Our experiments with the T383M mutant cDNA confirm the in vitro expression studies of Durstenfeld et al. (32) and demonstrate that the T383M mutation results in the secretion of a reduced amount of enzyme protein with decreased catalytic activity. Our affected individuals with heterozygous state for the T383M mutation exhibited decreased HL activities compared to controls. Also, the T383M mutation seems to be rather common in the Finnish population as about 10% of the subjects

TABLE 2. Expression studies of wild type HL cDNA and L334F and T383M mutant cDNAs in the COS-1 cells

	Intracellular		In the Medium	
DNA Transfected	Activity	Mass	Activity	Mass
Wild-type HL cDNA	0.15 ± 0.17	2.13 ± 1.13	1.58 ± 0.30	53.70 ± 8.52
L334F cDNA	0.18 ± 0.12	57.63 ± 5.05	0.48 ± 0.14	43.01 ± 7.34
T383M cDNA	0.62 ± 0.28	18.33 ± 1.94	0.10 ± 0.09	26.83 ± 5.60

The mean HL activity (mU/ml) and mass (ng/ml) (± SD) from six measurements (two different experiments with three parallel expressions) are presented.

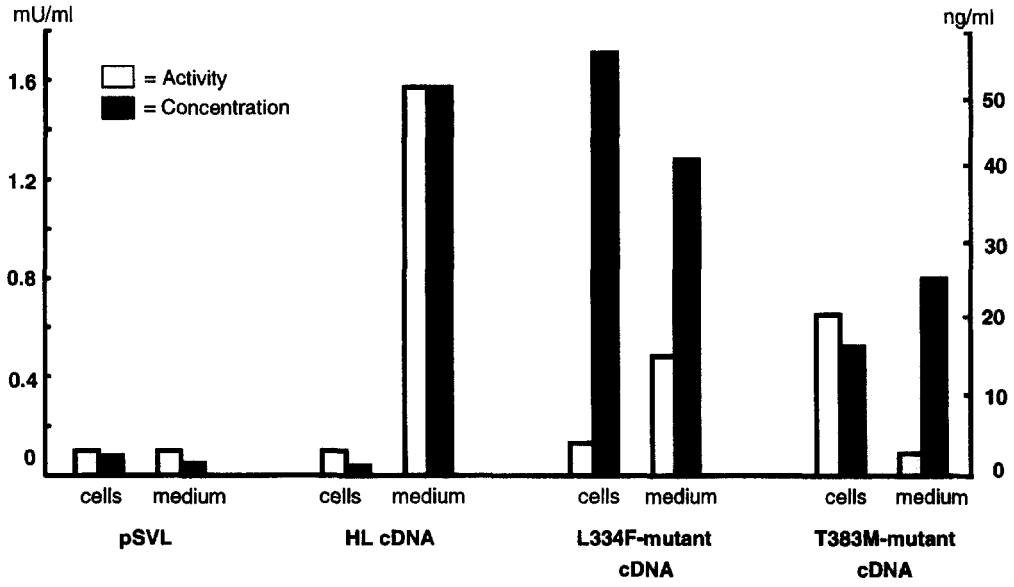


Fig. 4. Expressions of leucine 334 to phenylalanine (L334F) and threonine 383 to methionine (T383M) substitutions in the COS-1 cells. HL activity (light columns, mU/ml) and immunoreactive mass (dark columns, ng/ml) were measured in the culture medium and in cell homogenates. The means of six measurements (two different experiments with three parallel expressions) are presented.

studied were carriers of the rare allele. T383M mutation segregates in a different haplotype than L334F mutation in the Finnish pedigree studied.

In vitro HL hydrolyzes the *sn*-1 fatty acyl ester bonds of phospholipids as well as the ester bonds of mono-, di-, and triacylglycerols (2). However, the lipoprotein substrates and the role of HL in lipoprotein metabolism in vivo is unclear. The main and most consistent effect of HL has been on large HDL (24–28). It also plays an important role in the conversion of IDL into LDL (52). A similar effect has also been observed in transgenic animals. Thus, expression of human HL in mice was associated with a 34% lowering of plasma HDL-cholesterol and a decrease in HDL particle size while it had no effect on the cholesterol in the 1.006–1.063 g/ml density fraction (19). Similarly, the expression of a human HL gene in rabbits (20), an animal known to have low levels of HL (53), resulted in a dramatic reduction in the level of large HDL_2 as well as dense HDL_3. A modest decline of IDL was also observed in the transgenic rabbits (20). The recent work by Homanics et al. (21) demonstrated that the lack of HL gene in mice resulted in a 30% elevation of total cholesterol concentration, increased phospholipid and HDL-cholesterol levels but no change in triglyceride metabolism.

The lipoprotein changes observed in our HL-deficient proband are in line with those seen in animal models. The most significant lipoprotein change we observed was an increase in the amount of large buoyant HDL_2. This observation is similar to findings reported by Carlsson, Holmquist, and Nilsson-Ehle (25). However, the high content of triglycerides in LDL reported by Carlsson was not observed in the present study. The main lipoprotein changes, observed in two Canadian brothers with HL deficiency were, besides an increase in HDL_2, enrichment of LDL with triglycerides and the presence of β-VLDL (24). We observed no β-VLDL nor did we see any enrichment of LDL triglycerides in our pedigree.

Recent studies have indicated that hepatic lipase is a major determinant of LDL subclass distribution (54, 55). Watson et al. (54) reported an inverse correlation between HL activity and light LDL. Our observation that the proband had preponderance of large buoyant LDL (LDL-I) is consistent with the role of HL in the conversion of LDL-I into LDL-II and LDL-III (55). Accordingly, Auwerx et al. (26) reported that LDL particles are large in HL deficiency.

The reason for the differences in phenotypic expression of HL deficiency is at present not known. One reason for the mild symptoms of dyslipidemia observed in our patients may be due to the fact that they are not completely deficient in HL activity. Recent studies demonstrating that HL may affect the metabolism of several lipoprotein classes independently of its hydrolytic action by acting as a ligand in the uptake of lipoprotein particles may offer an explanation (56). It can be speculated that the structural changes induced by different mutations might affect the ligand properties of HL differently or might also influence the secretion of HL in such a way that no HL protein is available.

Several studies have demonstrated that the endothelial lipolytic enzymes LPL and HL both influence HDL levels (16–18) in such a way that high HL activity and low LPL activity both contribute to low HDL levels. Because of the powerful correlation between low HDL and an increased risk of coronary heart disease, the activity and regulation of HL may prove to be of importance in atherogenesis.

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