Two novel mutations in the lipoprotein lipase gene in a family with marked hypertriglyceridemia in heterozygous carriers: potential interaction with the polymorphic marker D1S104 on chromosome 1q21–q23

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Abstract Two novel mutations in the lipoprotein lipase (LPL) gene are described in an Austrian family: a splice site mutation in intron 1 (3 bp deletion of nucleotides −2 to −4) which results in skipping of exon 2, and a missense mutation in exon 5 which causes an asparagine for histidine substitution in codon 183 and complete loss of enzyme activity. A 5-year-old boy who exhibited all the clinical features of primary hyperchylomicronemia was a compound heterozygote for these two mutations. Nine other family members were investigated: seven were heterozygotes for the splice site mutation, one was a heterozygote for the missense mutation, and one had two wild-type alleles of the LPL gene. LPL activity in the post-heparin plasma of the heterozygotes was reduced to 49–79% of the mean observed in normal individuals. Two of the heterozygotes had extremely high plasma triglyceride levels; in three of the other heterozygotes the plasma triglycerides were also elevated. As plasma triglycerides in carriers of one defective LPL allele can be normal or elevated, the heterozygotes of this family have been studied for a possible additional cause of the expression of hypertriglyceridemia in these subjects. Body mass index, insulin resistance, mutations in other candidate genes (Asn291Ser and Asp9Asn in the LPL gene, apoE isoforms, polymorphisms in the apoA-II gene and in the apoAI-CIII-AIV gene cluster, and in the IRS-1 gene) could be ruled out as possible factors contributing to the expression of hypertriglyceridemia in this family. A linkage analysis using the allelic marker D1S104 on chromosome 1q21–q23 suggested that a gene in this region could play a role in the expression of hypertriglyceridemia in the heterozygous carriers of this family, but the evidence was not sufficiently strong to prove this assumption. Nevertheless, this polymorphic marker seems to be a good candidate for further studies.—Hölzl, B., H. G. Kraft, H. Wiebusch, A. Sandhofer, J. Patsch, F. Sandhofer, and B. Paulweber. **Two novel mutations in the lipoprotein lipase gene in a family with marked hypertriglyceridemia in heterozygous carriers: potential interaction with the polymorphic marker D1S104 on chromosome 1q21–q23.** ***J. Lipid Res.*** **2000.** 41: **734–741.**

Supplementary key words lipoprotein lipase • gene mutation • heterozygosity • hypertriglyceridemia • insulin resistance • D1S104

Primary hyperchylomicronemia is a rare inherited metabolic disorder characterized by extremely high triglyceride levels due to accumulation of chylomicrons in the blood. In patients with this disease, lipoprotein lipase (LPL) activity is absent or markedly decreased (1). The metabolic defect is caused by a mutation within the LPL gene or the apoC-II gene (1). Patients with two defective LPL alleles exhibit all the clinical features of the disease (2, 3), whereas in heterozygous carriers of one defective allele, fasting triglyceride levels can be both normal or moderately increased (4–9). The phenotypic expression of heterozygous LPL deficiency has been the subject of numerous studies (6, 7, 9, 10). Age, obesity, pregnancy, insulin resistance, and lipid-raising drugs were reported to play a role (6). Variability of phenotypic expression, however, cannot be entirely explained by these factors, making it likely that other as yet unknown mechanisms contribute to the phenotypic expression in heterozygous carriers of one mutant allele. As the lipoprotein phenotype in heterozygous carriers of a mutant LPL allele has been considered as atherogenic (9, 11, 12), both detection of the mutation and disclosure of additional factors that contribute to the phenotypic expression are of clinical importance.

Since the identification of the LPL gene (13, 14), a large number of mutations within the LPL gene have been described (15). Some of them give rise to a completely inactive enzyme, others result in only a slight to moderate decrease of enzyme activity. In this study we describe two novel mutations that are responsible for a com-

Abbreviations: LPL, lipoprotein lipase; IRS-1, insulin receptor substrate-1; FFA, free fatty acid; BMI, body mass index; TG, triglycerides; HTG, hypertriglyceridemia; HDL, high density lipoproteins; LDL, low density lipoproteins; FCH, familial combined hyperlipemia; apo, apolipoprotein; k_{ITT}, slope of blood glucose decrease in the short insulin tolerance test.

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pletely inactive gene product. Both mutations, a missense and a splice site mutation, occur in a family in which the hyperchylomicronemic patient is a compound heterozygote, and five of the eight heterozygotes identified in the family present with marked hypertriglyceridemia (HTG).

METHODS

Subjects

The proband was a 5-year-old boy with milky appearance of his fasting serum. He showed hepatomegaly and splenomegaly, but xanthomas were absent. His plasma lipid profile was characteristic for primary hyperchylomicronemia. Nine additional members of the family could be investigated. None of them had a history of a major disease or reported any complaints during the time of investigation. Alcohol abuse or intake of drugs affecting lipid metabolism were not present in any of the study subjects. Age, sex, body mass index (BMI), and results of the laboratory tests are given in **Table 1**.

Plasma lipids

Blood was drawn after an overnight fast. Total serum cholesterol, triglycerides, HDL cholesterol, and LDL cholesterol were determined enzymatically with commercially available kits from Hoffmann-LaRoche GmbH, Vienna, Austria. Normal values were defined as described previously (16).

Measurement of post-heparin LPL activity

For determination of LPL activity, 60 IU of heparin per kg body weight was injected intravenously. Ten minutes later blood was collected on ice. LPL activity was measured as described previously (17).

DNA preparation and sequencing

Total genomic DNA was prepared from blood leukocytes using a Nucleon BACC1 kit (Amersham International plc, Buckinghamshire, England). Fragments containing the individual exons of the LPL gene including all exon-intron boundaries were amplified by polymerase chain reaction (PCR). The oligonucleotide primers were those described by Gotoda et al. (18) and Paulweber et al. (17).

RNA isolation, cDNA synthesis, and RT-PCR analysis

Total cellular RNA was isolated from peripheral mononuclear blood cells using the acid-guanidinium-thiocyanate-phenol-chloroform-extraction method described by Chomczynski and Sacchi (19). cDNA synthesis was performed from 2 μg of total RNA using the You Prime Kit (Pharmacia, Uppsala, Sweden). Oligo(dT) was used for priming. To study the effect of the 3 bp deletion at the acceptor splice site of intron 2, cDNA was amplified with primers located in exons 1 and 5 of the LPL gene. The last base at the 3′end of the antisense primer in exon 5 includes the variant cDNA position 802; the primer sequence was identical with the wild-type sequence. With this primer design, amplification of the allele carrying the C→G missense mutation was suppressed. In order to estimate the amount of the aberrantly spliced transcript, normal cDNA was used in a dilution series and compared with the aberrantly spliced transcript.

Site directed mutagenesis, expression of the mutant LPL protein, measurement of mass and activity of the mutant LPL protein

Mutant LPL cDNA containing the C→G mutation at cDNA position 802 in exon 5 was generated by use of overlapping PCR fragments containing this mutation as described by Higuchi (20). To create EcoRI sites for cloning, the primers used for amplification contained EcoRI sites at their 5′ ends. Wild-type and mutant LPL cDNAs were inserted into the EcoRI site of the expression plasmid pMT2. COS cells were trypsinized and seeded into T75 tissue culture dishes 24 h prior to transfection. The cells were transfected with 5 μg of the pMT2 expression plasmid by a calcium chloride precipitation method. Normal and mutant LPL cDNA were expressed in COS cells in transient transfection experiments (21). COS medium was collected for determination of LPL mass and activity. LPL mass was determined in the supernatants of the transfected cells using the commercially available sandwich enzyme immunoassay (EIA) Markit-F LPL (Dainippon Pharmaceutical Co., Ltd; Osaka, Japan) essentially according to the manufacturer's instructions.

Determination of heterozygous carrier status

The 3 bp deletion in intron 1 was detected by PCR amplification of exon 2 including a 3′ portion of intron 1. This fragment was analyzed on an automated DNA sequencer (ALFexpress, Pharmacia, Uppsala, Sweden) using the fragment manager software. The missense mutation in exon 5 creates a new DdeI site, which was used for detection of the carrier status in the family members. To detect this mutation, PCR fragments containing exon 5 were digested with DdeI and subsequently separated on gels containing 1% agarose (Life Technologies, Paisley, Scotland) and 1% NuSieve® GTG agarose (FMC BioProducts, Rockland, ME).

TABLE 1. Age, sex, BMI, serum lipids, LPL activity, and insulin-sensitivity of family members

Subject ID	Age	Sex	BMI	Chol.	LDL-chol.	HDL-chol.	TG	LPL Act.[a]	k_{ITT}
	yr		*kg/m²*	*mg/dl*	*mg/dl*	*mg/dl*	*mg/dl*	*nmol FFA min⁻¹ ml⁻¹*	*%/min*
KL	5	m	18	431	n.d.	11	5492	1.1	n.d.
KM	24	f	25	201	139	48	69	227	n.d.
HM	30	m	23	199	n.d.	24	812	164	3.84
HE	49	f	26	236	118	69	95	294	3.58
MJ	50	m	22	255	n.d.	29	789	178	3.64
KK	52	f	27	284	173	39	257	216	3.94
GM	68	f	28	258	157	42	216	202	4.16
HB	59	f	25	241	144	38	324	254	3.1
HP	26	f	19	196	119	59	91	158	n.d.
FA	37	f	21	202	104	87	54	141	n.d.

The initials correspond to the initials in Fig. 3.
[a] Mean ± SD in 50 controls: 287.3 ± 71.0 nmol FFA $min^{-1}ml^{-1}$.

Determination of the apoE genotype

The apoE genotype was determined by PCR amplification of an apoE gene fragment followed by digestion with HhaI as described by Kontula et al. (22).

Detection of the mutations Asn291Ser and Asp9Asn in the LPL gene, typing for the Gly972Arg polymorphism in the IRS-1 gene, various polymorphisms within the apoA-II gene and the apoAI-CIII-AIV gene cluster, and the D1S104 microsatellite marker on chromosome 1q21–q23

The Asn291Ser and Asp9Asn mutations in the LPL gene were determined by PCR and restriction fragment analysis as described previously (8, 11).

The Gly972Arg polymorphism in the IRS-1 gene (23) was detected as follows. The DNA segment containing codon 972 of the IRS-1 gene was amplified by PCR using oligonucleotide primers 5′-CTT CTG TCA GGT GTC CAT CCC AGC-3′ (forward) and 5′-GGC GAG GTG TCC ACG TAG CTC TG-3′(reverse). The PCR product was desalted using Ultrafree®-MC columns (Millipore Corporation, Bedford, MA) prior to digestion with MvaI (MBI Fermentas GmbH, St. Leon-Rot, Germany) at 37°C for a minimum of 3 h. Restriction fragments were analyzed on high resolution agarose gels (2% NuSieve GTG® agarose (FMC Bioproducts, Rockland, ME) and 1% agarose ultra pure (Life Technologies, Paisley, Scotland)).

The MspI polymorphism in the apoA-II gene was determined as described by previously (24).

Typing of the SstI polymorphism in the apoC-III gene and the XmnI and MspI polymorphisms in the apoAI-CIII-AIV cluster (25) was performed following the protocol described by Dallinga-Thie et al. (26).

The microsatellite marker D1S104 on chromosome 1 was studied as described by Pajukanta et al. (27) by fragment length analysis on an ALFexpress automated DNA sequencer using the primer pair 5′-ATC CTG CCC TTA TGG AGT GC-3′(forward) and 5′-CCC ACT CCT CTG TCA TTG TA-3′(reverse).

Measurement of insulin sensitivity

Insulin sensitivity was measured by the short insulin tolerance test as described by Akinmokun et al. (28) with some minor modifications. In brief, after an overnight fast, Actrapid (Novo Laboratories, Basingstoke, UK) at a dose of 0.1 IU/kg body weight was injected intravenously into an antecubital vein. Blood was drawn from a contralateral antecubital vein immediately prior to insulin injection and every minute for the first 15 min after insulin injection. Insulin sensitivity was calculated from the decline (%) of venous blood glucose concentrations from minutes 5 to 13 using a linear regression model. The correlation coefficient was postulated to be at least 0.9. The mean value of glucose concentrations observed at minutes 0, 1, and 2 was set at 100%. The Minitab® (Minitab Inc.) statistical software was used for calculation. Insulin sensitivity was estimated from the slope of the linear regression line (k_{ITT} value, Table 1).

Linkage analysis

Two-point linkage analysis was performed using the MLINK procedure from the FASTLINK package (version 4.1P) (29–32). An autosomal dominant mode (frequency 0.6%) was assumed for the genetic transmission of the hypertriglyceridemic trait which was implemented as affected or unaffected (or unknown) state in the pedigree. To account for the influence of age, six liability classes were introduced as described by Helio et al. (33). For the AI-CIII-AIV gene cluster the haplotypes were used in the analysis and the frequencies of these as well as of the other tested markers were taken from the Genome Database (http://www.gdb.org/).

RESULTS

Genetic analyses

The entire coding region and all exon-intron boundaries of the LPL gene were sequenced in the hyperchylomicronemic patient. The results revealed that the patient was a compound heterozygote: one allele carried a 3 bp deletion (CCA) comprising nucleotides −2 to −4 of intron 1 (**Fig. 1B**). As this deletion affects a splice acceptor site, mRNA was isolated from peripheral mononuclear blood cells and cDNA was synthesized with primers in exon 1 and 5 of the LPL gene. In addition to the normal fragment which had the expected length, a second shorter fragment was obtained (**Fig. 2**). Sequence analysis of the shorter fragment showed that exon 2 had been skipped, resulting in a frameshift, which creates a premature TGA stop codon 6 codons downstream in

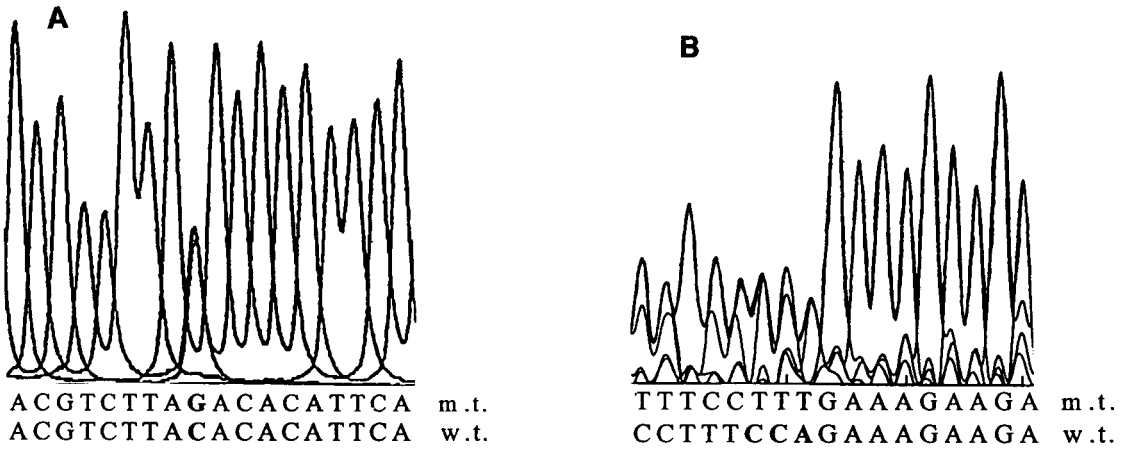


Fig. 1. Sequences showing the CG base exchange at codon 183 (A) and the 3 bp CCA deletion at the acceptor splice site of intron 1 (B). Affected positions are indicated by bold letters; m.t., mutant type; w.t., wild-type.

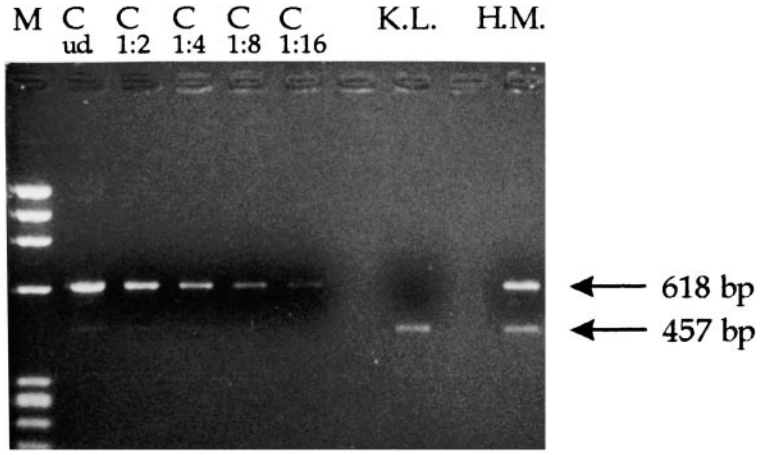


Fig. 2. Quantitative RT-PCR analyses of monocyte RNA from the compound heterozygote (KL), a heterozygote for the splice site mutation (HM), and a normal control (C) with primers in exon 1 (5′ CAA AGC CCT GCT CGT GCT GAC TCT G 3′) and exon 5 (5′ CTG GTT TCT GGA TTC CAA TGC TTC G 3′) .The last base at the 3′ end of the antisense primer in exon 5 included the variant cDNA position 802; the primer sequence was identical with the wild-type sequence. With this primer design, amplification of the allele carrying the CG missense mutation was suppressed. In addition to the normal fragment which had the expected length of 618 bp, a second shorter fragment of 457 bp was obtained. In order to estimate the amount of the aberrantly spliced transcript, cDNA of the normal control subject (C) was used in a dilution series and compared with the aberrantly spliced transcript. The amount of the aberrantly spliced transcript was reduced to less than 25% of the normal transcript. ud., undiluted. Sequence analysis of the shorter fragment showed that exon 2 had been skipped, proving that this deletion results in a splicing defect.

exon 3. The amount of the aberrantly spliced transcript was reduced to less than 25% of the normal transcript (Fig. 2).

In the second allele, a C→G missense mutation was found in codon 183 (exon 5) (Fig. 1A). This mutation results in the substitution of a histidine residue by asparagine. As this mutation had not been described so far, it was necessary to investigate the effect of this amino acid substitution on the function of the gene product. For this purpose, cDNA containing the C→G mutation in codon 183 was created by site directed mutagenesis and LPL protein was generated by transient transfection of COS cells with the mutant cDNA. The mutant LPL protein showed a complete loss of lipolytic activity (**Fig. 3**), demonstrating that the CG mutation in codon 183 was responsible for the enzymatic inactivity of the gene product derived from this allele.

Subsequently, all available family members were tested for the presence of these two mutations. As shown in **Fig. 4**, seven of the nine relatives were heterozygous for the splice site mutation in intron 2, one was a heterozygote carrier of the missense mutation in exon 5, and only one family member had two wild-type alleles.

Plasma lipoproteins and LPL activity

In the hyperchylomicronemic patient, fasting triglycerides were grossly elevated and total cholesterol was only moderately increased, while HDL cholesterol was markedly reduced. In five of the eight heterozygous subjects with either of the two mutations (MJ, HM, KK, HB, GM)

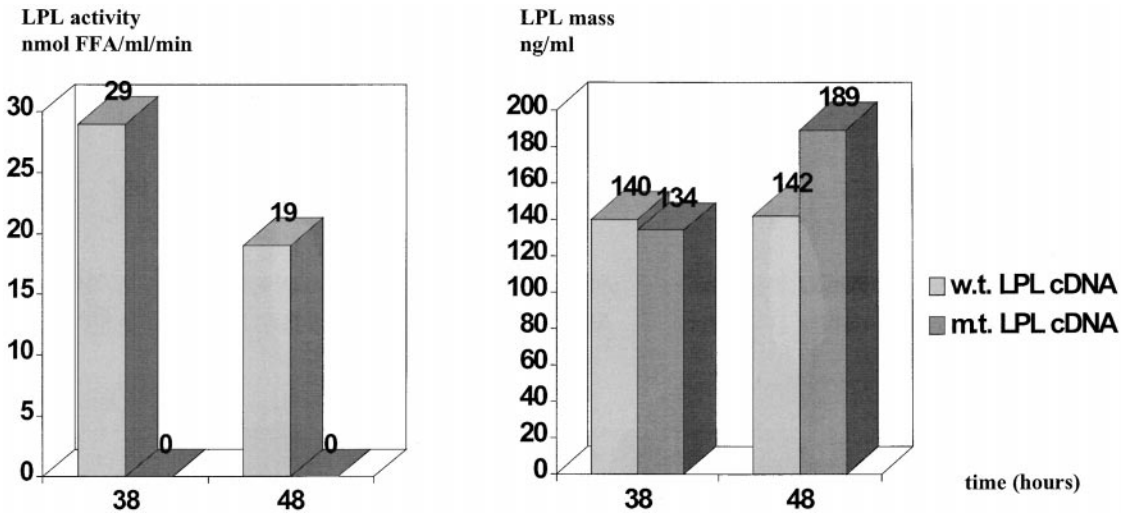
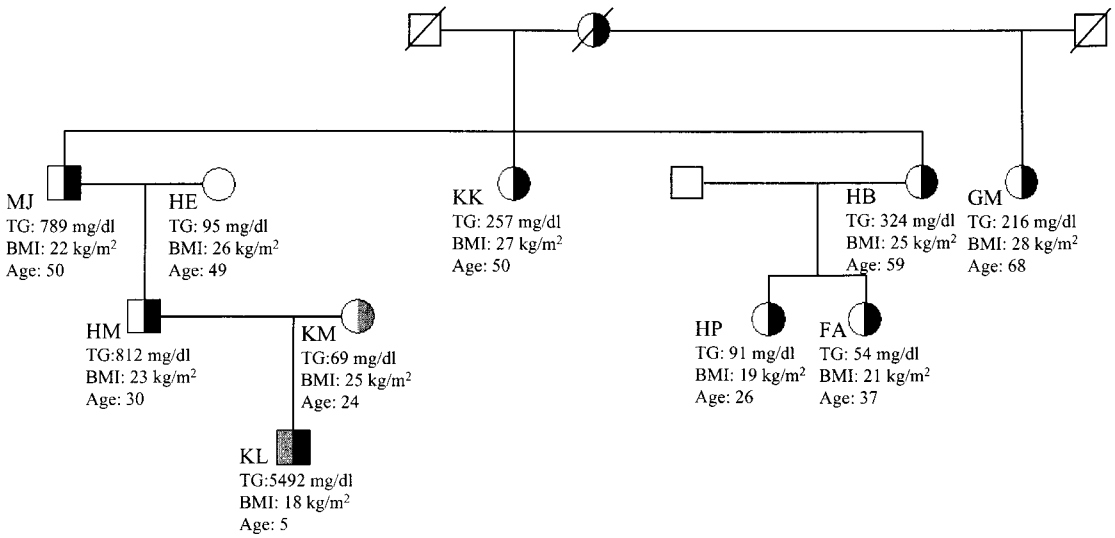


Fig. 3. Expression of the mutant LPL cDNA carrying the His183Asn mutation in COS cells. LPL activity and immunoreactive mass were measured in the culture medium 38 h and 48 h after transient transfection of COS cells with wild-type (light columns) and mutant (dark columns) LPL cDNA. LPL mass of the normal and mutant enzyme did not differ significantly. The data represent means of two different experiments; w.t., wild-type; m.t. mutant type.



AI-CIII-AIV (haplotype)	X1|X2 M2|M2 S1|S2	X1|X1 M2|M2 S1|S1	X1|X1 M1|M2 S1|S1	X1|X1 M2|M2 S1|S2	X1|X1 M2|M2 S1|S2		X1|X2 M1|M2 S1|S2			X1|X1 M2|M2 S1|S1	X1|X1 M2|M2 S1|S1	X1|X1 M2|M2 S1|S1	X1|X1 M2|M1 S1|S2	
Apo E	E3 E3	E2 E3	E2 E3	E2 E3	E3 E4		E3 E3			E2 E3	E3 E4	E3 E3	E3 E3	
D1S104	156 162	156 158	156 158	156 162	162 162		156 164			156 158	158 162	156 162	156 164	
Apo AII (MspI)	M1 M2	M2 M2	M2 M2	M2 M2	M2 M2		M2 M2			M2 M2	M1 M2	M1 M2	M2 M2	
IRS-1 (Gly972Arg)	– –	– –	– –	– –	+ –		– –			– –	– –	– –	+ –	

Fig. 4. Pedigree of the family under study. Black symbols identify the splice site mutation in intron 1 and shaded symbols represent the Gly972Arg mutation in exon 5. The initials beside the symbols correspond to the initials in Table 1; below the initials serum triglyceride levels, BMI, and age are shown. The table below the pedigree shows the results of genotyping for various polymorphisms in candidate genes whose gene products are involved in the regulation of the metabolism of triglyceride-rich lipoproteins.

fasting triglyceride levels were markedly increased. Their total cholesterol level was normal or moderately increased. HDL cholesterol was decreased in two heterozygous subjects (HM, MJ), who also had markedly increased triglyceride concentrations (Table 1). In heterozygotes no correlation between LPL activity and HDL cholesterol ($r = 0.001$, $P = 0.945$) or plasma triglycerides ($r = 0.069$, $P = 0.495$) was found.

In the hyperchylomicronemic patient, LPL activity in the post-heparin plasma was close to zero. In the eight heterozygous subjects, post-heparin LPL activity was reduced to 49–79% of the mean LPL activity observed in 50 normal control subjects (mean 287.3, SD 71.0 nmol FFA/min/ml) (17) (Table 1).

Potential factors influencing plasma triglyceride concentration in heterozygous carriers of a defective LPL allele

Obesity and age above 40 years have been discussed as possible factors affecting HTG in heterozygote carriers of a Gly188Glu mutation in a large family (2, 6). In another large family, however, a significant increase in plasma triglycerides was observed in heterozygous carriers of a P207L mutation below 40 years and even below 20 years of age (9). In our study, four carriers were above 40 years of age, all of them had elevated plasma triglyceride levels. Four carriers were aged less than 40 years, one of them had a very high triglyceride level, in the other three the triglyceride concentration was normal. Thus, in our family, age could have influenced the expression of HTG; one heterozygote with only 30 years of age, however, presented with extremely high plasma triglycerides.

Four carriers had a BMI of 25 or more, three of them had elevated and one had normal plasma triglycerides. From the four carriers with a BMI below 25, two had elevated and the other two had normal plasma triglycerides. Therefore, in this family, overweight does not seem to have a major influence on plasma triglycerides.

Insulin resistance was determined in five of the eight carriers. None of them was found to be insulin-resistant according to the test used. Normal insulin responsiveness was defined as a $k_{ITT} > 3.0$ as determined in 176 control subjects (unpublished data). The Gly972Arg mutation in the IRS-1 gene was only present in two subjects, one having plasma triglyceride levels of 69 mg/dl (KM) and the other 216 mg/dl (GM).

A common mutation in the lipoprotein lipase gene (Asn291Ser), which has been found to occur in the gen-

TABLE 2. Two-point linkage analysis (lodscores) between hypertriglyceridemia and genetic markers using the MLINK procedure from the FASTLINK package (version 4.1P) assuming an autosomal dominant mode of inheritance

Theta	LPL Mut	D1S104	ApoE	ApoA-II	AI-CIII-AIV	IRS-1
0.00	0.984168	0.739133	−1.781998	0.160789	−1.055217	−0.043464
0.01	0.970119	0.719910	−1.398402	0.155530	−0.803772	−0.039862
0.10	0.825218	0.545544	−0.625054	0.109442	−0.237411	−0.016889
0.20	0.626653	0.361410	−0.361004	0.067230	−0.100802	−0.005013
0.30	0.395811	0.208693	−0.205569	0.039314	−0.045489	−0.000840
0.40	0.163044	0.095270	−0.092367	0.021244	−0.013289	−0.000003

eral Danish population with an allele frequency of 0.024 in men and 0.026 in women (12), has been reported to be associated with elevated triglycerides (12) and reduced HDL-cholesterol levels (11, 12). Another common variant in the lipoprotein lipase gene (Asp9Asn), which occurs with a frequency between 1.6 to 4.4% (mean 3.0%) in a Dutch, Swedish, English, and Scottish population and which reduces lipoprotein lipase activity by 20–30%, was found to be associated in heterozygous carriers with increased plasma triglyceride levels (8). In the family of our study, neither the Asn291Ser nor the Asp9Asn mutation could be detected.

Among the various candidate genes whose products could play a role in the regulation of the metabolism of triglyceride-rich lipoproteins, are the apoE gene, the apoA-II gene, the genes of the apolipoprotein AI-CIII-AIV cluster, the IRS-1 gene, and the gene region around the polymorphic marker D1S104 on chromosome 1. Analysis of linkage between HTG and these markers was performed. As age could potentially influence expression of HTG, age-dependent penetrance has been taken into account. The results of the linkage analysis between HTG and the analyzed markers (two-point lod scores) are given in **Table 2** assuming an autosomal dominant mode of inheritance. Linkage between HTG and any marker could neither be significantly excluded nor proven. Negative lod scores were obtained between HTG and apoE, the haplotypes of the AI-CIII-AIV cluster and IRS-1 with the apoE lod score being almost significant. Positive lod scores were found between HTG and LPL, D1S104, and apoA-II. The highest lod scores were observed for a recombination fraction $\theta = 0.00$ (Table 2).

DISCUSSION

In this study, two novel mutations in the lipoprotein lipase gene are identified as the molecular basis of type I hyperlipemia in an Austrian family: a splice acceptor site mutation in intron 2 which results in skipping of exon 2, and a missense mutation in exon 5 which causes an asparagine for histidine substitution in codon 183 and a complete loss of lipolytic activity of the gene product. The affected family member, a 5-year-old boy, was a compound heterozygote for the two mutations, and presented with grossly elevated plasma triglyceride levels, a minor increase in total cholesterol, and extremely low HDL cholesterol. Interestingly, in this family, two heterozygous carriers of the splice site mutation also had extremely high triglyceride levels (812 and 789 mg/dl) and significantly decreased HDL cholesterol (24 and 29 mg/dl). Three other carriers of this mutation also showed an elevation of their triglyceride concentration (324, 257, 216 mg/dl), whereas two heterozygotes carrying the splice site mutation and one heterozygote with the missense mutation in exon 5 had normal plasma triglycerides. There was no relationship between LPL activity in post-heparin plasma and fasting triglyceride concentrations.

Heterozygous carriers with one defective LPL allele are not rare. The frequency is approximately the same as that of heterozygote carriers of an LDL receptor gene mutation, but can be much higher in inbred populations (34). The expression of HTG and low HDL cholesterol in heterozygotes with one defective LPL allele has been studied in numerous families (6–7, 9–12). The results of these studies reveal that many of these heterozygotes have normal plasma triglyceride concentrations, whereas in others plasma triglycerides are moderately elevated. Therefore, one can assume that on principal a half-normal LPL activity found in subjects with one defective LPL allele is able to maintain a normal fasting triglyceride concentration. An elevated plasma triglyceride level, however, could result from the presence of additional environmental or genetic factors in heterozygous carriers of one defective LPL allele.

Several studies have been performed to identify the factors that contribute to the expression of HTG in heterozygous carriers of one defective LPL allele. Obesity, age over 40 years, pregnancy, and insulin resistance have been implicated in elevation of plasma triglyceride levels in heterozygotes (6). In the family presented in this study, five of the eight heterozygotes have moderately to severely elevated plasma triglycerides. Obesity and insulin resistance were clearly not related to HTG in these subjects. The three heterozygotes with normal plasma triglycerides were below 40 years of age. Therefore, age could have some influence on the expression of HTG in this family. One 30-year-old heterozygote, however, had extremely high plasma triglycerides. Two of the heterozygotes with HTG were males and three were females, with the two males showing the highest values. From these data, an influence of gender can neither be proven nor excluded. Environmental factors such as alcohol abuse or drugs affecting triglyceride concentrations in the blood were not present in any of the study subjects.

Therefore, we proceeded to study a possible influence of other candidate genes on the expression of HTG in heterozygotes of this family. Because apolipoprotein E plays an important role in the catabolism of triglyceride-rich lipoproteins and an interaction of apoE and LPL in binding and uptake of chylomicrons has been demonstrated (35), the apoE genotype was determined in all members of this family, but linkage between the apoE genotype and plasma triglyceride levels was excluded at a level of probability reaching almost significance. An SstI polymorphism of the apoC-III gene, an XmnI polymorphism of the apoA-I gene, and the haplotype X1M1S2/X2M2S1 have been found to be associated with HTG in various populations (36–38). In our family, however, the haplotypes of this gene cluster showed no linkage with HTG. The Asn291Ser and the Asp9Asn mutation, which are two common variants of the lipoprotein lipase gene with a possible influence on plasma triglycerides, could not be detected in any of the family members.

Low levels of LPL activity have been found in some patients with familial combined hyperlipidemia (FCH) (39), which is the most common genetic lipid abnormality in premature coronary heart disease (40). Cullen et al. (41) performed a univariate complex segregation analysis in families with FCH; their results were consistent with a major gene acting on plasma triglyceride levels. Recently, Pajukanta et al. (27) performed linkage analyses with markers from various chromosomal regions; one marker, D1S104, adjacent to the apoA-II gene showed a lod score of Z = 3.50 in 31 extended FCH families. The results of this study suggest a novel locus for FCH in the chromosomal region 1q21–q23. We examined this polymorphic marker and detected four different alleles in the family under study. A positive but not significant linkage with HTG was found for this marker. However, only two families in the Finnish FCH study (27) had a similarly high lod score as the one detected here between HTG and D1S104.

This positive lod score between HTG and D1S104 is indicative of the presence of linkage which might be proven by the analysis of additional families. Our finding is consistent with the assumption that a gene in the region of 1q21–q23 plays a role in the expression of HTG in heterozygous carriers of a defective LPL allele in the family under investigation, but the evidence is not sufficiently strong to prove this assumption.

In conclusion, in this study two novel mutations in the LPL gene, both of which result in lack of enzyme activity, could be identified as the molecular basis of type I hyperlipemia in an Austrian family. The study, however, could not establish a second gene, which may be the cause of HTG observed in some of the heterozygous family members. It suggests that the allelic marker D1S104 in the region 1q21–q23 may be a good candidate for further studies evaluating the phenotypic expression of hyperlipidemia in heterozygote carriers of mutations in the LPL gene.

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