Atherosclerosis 214 (2011) 386–390

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

Rare variants in the lipoprotein lipase (LPL) gene are common in hypertriglyceridemia but rare in Type III hyperlipidemia

D. Evans*, J. Arzer, J. Aberle, F.U. Beil

Endokrinologie und Stoffwechsel, Medizinische Klinik III, Zentrum für Innere Medizin, Universitätsklinikum Hamburg-Eppendorf, Martinistrasse 52, 20246 Hamburg, Germany

ARTICLE INFO

Article history:
Received 13 September 2010
Received in revised form
17 November 2010
Accepted 17 November 2010
Available online 26 November 2010

Keywords:
Hypertriglyceridemia
Rare variants
Lipoprotein lipase
Type III hyperlipidemia

ABSTRACT

Objective: Genomewide association studies (GWAS) have shown that variation in the lipoprotein lipase gene (LPL) is associated with plasma triglyceride levels but that common variants account for only 1.25% of the variance. The aim of this study was to determine the frequency of rare variants in the LPL gene in patients with various forms of hypertriglyceridemia.
Methods: The DNA sequence of the exons plus exon/intron boundaries of the LPL gene of 313 patients with triglycerides above the 95th percentile for age and sex (107 of whom had triglycerides above 875 mg/dl) and 121 patients with Type III hyperlipidemia was determined.
Results: Twenty rare variants were detected of which seven have been previously reported. All of the rare variants were present as heterozygotes. Sixteen were missense mutations, two were short deletion mutants and there were single nonsense and insertion mutations. Fifteen of the missense mutations resulted in an amino acid change. There were 13 patients (12.1%) with triglycerides above 875 mg/dl and 10 patients (4.9%) with moderately elevated triglycerides, who were carriers of at least one rare, non-synonymous mutation in the LPL gene. Of the patients with Type III HLP, two were carriers of rare variants.
Conclusion: Rare mutations in the LPL gene are frequent in patients with elevated triglycerides.

1. Introduction

Several genomewide association studies (GWAS) have identified a number of loci which contribute to plasma lipid levels (for a meta-analysis of seven studies see [1]). Recently the identification of 95 loci for blood lipids have been reported in a collaborative GWAS study involving over 100,000 probands [2]. However the amount of variance in plasma lipids accounted for by common variants in these studies is relatively small. The 95 loci identified in [2] could explain only 10–12% of the total variance in blood lipids and thus 25–30% of the genetic variance. Bodmer and Bonilla [3] have shown that rare variants may make a substantial contribution to the multifactoral inheritance of common complex disease [3] giving rise to a "resequencing imperative" [4]. This approach has been successfully employed in a number of genes associated with dyslipidemia [5–7].

Lipoprotein lipase (LPL) is a key enzyme in triglyceride metabolism and variants in its gene have been associated with triglyceride levels in all the GWAS studies cited above. Functional SNPs in the LPL gene have been extensively investigated with the D9N and N291S [8–10] being associated with elevated and the S447X with lower triglycerides [10]. LPL deficiency, with a frequency of approximately 1 in a million, is associated with massively elevated triglycerides and to date approximately one hundred individual mutations have been identified [11,12]. The LPL gene is thus a good candidate gene to investigate the relative importance of common and rare variants in determining plasma triglyceride levels.

By sequencing the LPL gene of 110 patients with triglycerides above 10 mmol Wang et al. [13] identified six patients who were heterozygous for rare mutations. This group later extended this study to include 438 probands of European ancestry with mean triglycerides of 14.3 mmol [14]. They identified 19 individuals with rare variants in their LPL gene. In a resequencing study of 19 individuals with extreme hypertriglyceridemia (TG > 14 mmol), Wright et al. [15] identified two rare variants in the LPL gene. These findings suggest that, at least in probands with extremely high levels of triglycerides, rare variants in the LPL gene are frequent.

The aims of this study were, firstly to confirm the high frequency of mutations in the LPL gene in an independently recruited group of patients with extremely high plasma triglycerides (triglycerides > 10 mmol/dl or 875 mg/dl, severe HTG). We then extended the scope of the study by determining the incidence of rare mutations in the LPL gene in the much more numerous group of patients with moderately elevated triglycerides (moderate HTG) which we defined as above the 95th percentile for age and sex but below 875 mg/dl. Thirdly we determined the sequence of the LPL gene

* Corresponding author. Tel.: +49 40 741056978; fax: +49 40 741059036.
E-mail address: evans@uke.uni-hamburg.de (D. Evans).

0021-9150/$ – see front matter © 2010 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.atherosclerosis.2010.11.026

Table 1
Clinical characteristics of patients included in the study.

	Extremely elevated triglycerides >875 mg/dl	Moderately elevated triglycerides <875 mg/dl >95th percentile	Type III HLP APOE2/2 APOB/TC < 0.15	APOE2/2 APOB/TC > 0.15	Low triglycerides <25th percentile
n	107	206	109	12	182
M/F	76/31	151/55	74/35	8/4	74/108
Age (years) mean (±SD)	46.4 ± 10.8	44.4 ± 12.5	49.3 ± 11.3	51.0 ± 14.6	47.4 ± 13.9
BMI (kg/m^2) mean (±SD)	30.2 ± 4.3	27.7 ± 4.1	29.1 ± 8.2	27.2 ± 4.8	24.2 ± 3.9
Obese (BMI above 30) *n*	52	54	31	3	9
DM2 *n*	16	19	9	4	6
CHD *n*	7	10	14	2	12
Triglycerides (mg/dl) mean (±SD)	1768 ± 1186	549 ± 168	549 ± 356	413 ± 219	64 ± 13
Total cholesterol (mg/dl) mean (±SD)	382 ± 170	265 ± 67	340 ± 123	323 ± 136	250 ± 61
HDL (mg/dl) mean (±SD)	35 ± 14	41 ± 11	48 ± 14	38 ± 12	71 ± 61
Non HDL cholesterol (mg/dl) mean (±SD)	337 ± 160	224 ± 62	291 ± 120	285 ± 137	178 ± 59

of 121 patients with APOE2/2 genotype, 109 of whom had Type III hyperlipidemia (Type III HLP) in order to determine whether mutation in the LPL gene can act as a genetic co-factor in the development of the condition.

2. Methods

2.1. Subjects

Probands were recruited from patients who attended the lipid outpatient clinic, Universitätsklinikum Hamburg–Eppendorf (UKE) between 1997 and 2007. Informed consent was obtained and the study was approved by the local ethics committee. This patient group has been previously described [16]. Probands were unrelated and reflect the multi-ethnic nature of the city of Hamburg and the surrounding area. Four hundred and thirty four patients, divided into four groups, were selected for DNA sequence analysis. There were 107 patients with severe HTG defined as triglycerides above 875 mg/dl (10 mmol), equivalent to the patients investigated by Wang et al. [13] and Wright et al. [15]. The second group consisted of 206 patients with moderate HTG defined as triglycerides above the 95th percentile for age and sex but below 875 mg/dl. Patients with APOE2/2 genotype were excluded from the first two groups forming a third group of 121 patients, 109 of whom had Type III HLP following the criteria of Blom et al. [17], i.e. an APOB/TC ratio of below 0.15. The clinical characteristics of these patients are presented in Table 1. A fifth group of 182 patients with triglycerides below the 25th percentile for age and sex was utilized as a control group.

2.1.1. Biochemical measurements

Blood samples were taken after an overnight fast Plasma cholesterol (TC) and triglycerides (TG) were determined using the GPO-PAP and CHOD-PAP kits respectively from Boehringer Mannheim. HDL was determined following precipitation of apo B containing lipoproteins with phosphotungstate (Boehringer Mannheim).

2.1.2. DNA analysis

DNA was isolated from 10 ml blood using the QIAamp DNA Blood Kit from Qiagen, Hilden, Germany. The APOE genotype was determined as described [18]. The coding regions and exon/intron boundaries of the LPL gene were amplified by PCR using primers as described in [19]. PCR products were purified using QIAquick PCR purification kit (Qiagen) and sequenced using the Big Dye Terminator v1.1 Cycle Sequencing Kit from Applied Biosystems with the PCR primers as sequencing primers. PCR-RFLP assays were designed for each rare variant detected. Primers and enzymes are presented in Supplementary Table 1. The two deletion mutations were detected by direct analysis of the PCR amplified exon using the QIAxel System from Qiagen.

2.2. Bioinformatics

DNA sequence was analyzed for variants using Mutation Surveyor DNA Variant Analysis Software, Version 3.23 from SoftGenetics. The effect of mutation on function was analyzed using POLYPHEN [20] and PANTHER [21] software.

3. Results

3.1. Variants identified

The DNA sequence of the exons and exon/intron boundaries of the LPL gene of 434 individuals was determined. A total of 27 variants were identified the details of which are presented in Table 2. Seven of the variants were known SNPs and 20 were rare variants, of which 7 have been previously reported [11,12], the remaining 13 being, as far as we are aware, novel. All of the rare variants were present as heterozygotes. 16 of the rare variants were missense mutations, there were two short deletion mutations and one insertion mutation and a single nonsense mutation. Fifteen of the missense mutations resulted in an amino acid change and there was a single synonymous variant. The variant V69L was identified in five and G188E in three and the I225T in two probands. All the remaining rare variants were identified only once. The effect of mutation on LPL function was estimated using POLYPHEN [20] and PANTHER [21] software and summarized in Table 2 and where known a summary of the effect of mutation on LPL synthesis and activity in *in vitro* systems is presented.

3.2. Variants in patients with severe hypertriglyceridemia (>875 mg/dl)

Of the 107 patients with severe HTG, 13 (12.1%) were carriers of at least one rare, non-synonymous mutation in the LPL gene (Table 3). Of these two, namely S278N and T352I, were classified as benign in Polyphen and had scores of 0.55431 and 0.62298 respectively in Panther and so can probably be excluded as contributing to hypertriglyceridemia. Six patients were carriers of variants, which have been shown to be functional in previous publications [11,12], two were deletion mutants leading to frameshifts in exons 3 and 4 and one a nonsense mutation in exon 6 and are therefore certainly functional. One replaced the initiation methionine with isoleucine and so is probably also deleterious. The remaining variant, M301I, was classified as probably deleterious in Polyphen and had a Panther score of 0.70229. This gives a frequency of 11/107 (10.3%) or 10/107 (9.3%) if M301I is excluded of rare mutations in the