Clinica Chimica Acta 429 (2014) 61–68

Contents lists available at ScienceDirect

Clinica Chimica Acta

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

Molecular analysis of chylomicronemia in a clinical laboratory setting: Diagnosis of 13 cases of lipoprotein lipase deficiency

Jesús M. Martín-Campos [a,b,c,1], Josep Julve [a,b,c,1], Rosa Roig [a,b,d,1], Susana Martínez [b,d], Teresa Laura Errico [a,c], Silvia Martínez-Couselo [a,c], Joan Carles Escolà-Gil [a,b,c], Jesús Méndez-González [a], Francisco Blanco-Vaca [a,b,c,d,*]

[a] Institut de Recerca del HSCSP, Institut d'Investigacions Biomèdiques (IIB) Sant Pau, Barcelona, Spain
[b] CIBER de Diabetes y Enfermedades Metabólicas Asociadas (CIBERDEM), Barcelona, Spain
[c] Departament de Bioquímica i Biologia Molecular, Universitat Autònoma de Barcelona, Spain
[d] Hospital de la Santa Creu i Sant Pau, Servei de Bioquímica, Barcelona, Spain

ARTICLE INFO

Article history:
Received 12 July 2013
Received in revised form 9 November 2013
Accepted 22 November 2013
Available online 1 December 2013

Keywords:
Severe hypertriglyceridemia
Type I hyperlipidemia
Type V hyperlipidemia
LPL
GPIHBP1
Apolipoprotein A-V

ABSTRACT

Background: Familial chylomicronemia (type I hyperlipidemia) is a rare autosomal recessive disease due mainly to rare variants in the lipoprotein lipase (*LPL*) gene sequence. Molecular diagnosis of LPL deficiency is now a requirement for the first gene therapy treatment approved in the European Union. Altered coding sequence variants in *APOC2*, *APOA5* or *GPIHBP-1* can also cause familial chylomicronemia. Herein, we report the results of our molecular diagnostic activity in this topic, carried out in the setting of a Spanish clinical practice hospital laboratory, which was also extended to some patients who were more likely to have type V hyperlipidemia.
Methods: Samples from twenty-nine unrelated probands with severe hypertriglyceridemia were referred for molecular diagnosis. Samples were first screened for *LPL* sequence variants by DNA sequencing and, in the absence of alterations, subsequent analysis of *APOC2*, *APOA5*, and *GPIHBP1* genes was undertaken. Analysis of LPL function in vitro was further studied in two previously uncharacterized *LPL* sequence variants.
Results: Fourteen different, loss-of-function variants were found in the *LPL* gene: 4 were novel or uncharacterized allelic variants, and of these, 2 were directly shown to affect function. Twenty of 29 probands presented at least one *LPL* gene allele variant: 8 were homozygous, 9 compound heterozygous and 3 heterozygous. In 13 probands, the finding of two loss-of-function variants supported the diagnosis of LPL deficiency. None of the probands presented sequence variants in the *APOC2* gene, whereas 3 presented rare variants within the *APOA5* gene. Four of the five patients heterozygous for a common variant in the *GPIHBP-1* gene also carried *APOA5* sequence variants.
Conclusions: Loss-of-function *LPL* variants leading to familial chylomicronemia were found in 13 patients, accounting for a significant proportion of the LPL-deficient patients predicted to live in Spain.
© 2013 Elsevier B.V. All rights reserved.

1. Introduction

The chylomicronemia syndrome [1,2], a disorder characterized by severe hypertriglyceridemia (HTG) and fasting accumulations of chylomicrons in plasma, is accompanied by one or more of the following clinical manifestations: eruptive xanthomas, lipemia retinalis, hepatosplenomegaly, recurrent abdominal pain and/or acute pancreatitis. Although chylomicrons are considered to be present when plasma triglyceride concentrations are between 1000 and 2000 mg/dl, the symptoms associated with the chylomicronemia syndrome almost always occur at higher triglyceride levels [3]. Early diagnosis of severe chylomicronemia syndrome is crucial to avoid pancreatitis-derived consequences, including abdominal pain and diabetes mellitus, for which dietary approaches [4,5] consisting of restricted fat consumption and/or intake of medium-chain triglyceride-rich foods, or pharmacologic ones, including therapies with antioxidants, fibrates or nicotinic acid [6,7], can be prescribed.

The chylomicronemia syndrome is a feature of Frederickson type I and type V hyperlipidemias [8], which have a partially common phenotype consisting of fasting serum triglyceride >1000 mg/dl, concomitant with the presence of chylomicrons. Type I hyperlipidemia, or familial chylomicronemia (OMIM 238600), is characterized by the early onset of severe HTG associated with chylomicronemia but without a very low density lipoprotein (VLDL) increase, with a prevalence of ~1 case in 1,000,000 inhabitants [9], and is mainly due to rare homozygous loss-of-function *LPL* gene variants. Less common causes of familial hyperchylomicronemia are rare, loss-of-function variants in one of the following genes, *APOC2* (OMIM 207750) [2], *APOA5*, *GPIHBP1* or *LMF1*, as well as the presence in plasma of circulating LPL inhibitors (OMIM 118830) [10,11].

Type V hyperlipidemia (OMIM 144650) is also characterized by the presence of fasting chylomicrons, but presents with a concomitant

* Corresponding author at: Servei de Bioquímica, Hospital de la Santa Creu i Sant Pau, C/Sant Quintí 89, 08041 Barcelona, Spain. Tel.: +34 93 5537358; fax: +34 93 5537287.
E-mail address: fblancova@santpau.cat (F. Blanco-Vaca).
[1] The first three authors contributed equally to this paper.

0009-8981/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.cca.2013.11.025

increase in VLDL and is usually observed in adults rather than children. The genetic determinants of type V hyperlipidemia are less defined; however, heterozygous, loss-of function mutations of *LPL*, *APOA5* have been identified in some cases. Type V hyperlipidemia is, nevertheless, thought to be of a polygenic nature in most cases, including variations in genes such as *APOA5*, *GPIHBP1* and *LMF1* [12]. In contrast to *APOA5*, few sequence variants in *GPIHBP1* [13–21], *LMF1* and *CREB3L3* genes have been reported in humans with chylomicronemia [20,22]. Environment is obviously relevant and patients with genetic susceptibility (e.g. those with heterozygous mutations in the *LPL* gene) in combination with pregnancy, excessive alcohol intake, obesity, uncontrolled type 1 or 2 diabetes mellitus or different medications (i.e. estrogens, glucocorticoids, tamoxifen, 13-cis-retinoic acid, antiretroviral therapies) account for some cases of chylomicronemia with the type V Frederickson phenotype [9,23].

Lipoprotein lipase (LPL) is a ubiquitous, endothelially-bound lipolytic enzyme, mainly responsible for triglyceride hydrolysis in chylomicrons and VLDL. LPL activity requires the presence of functional apolipoprotein (apo) C-II as a cofactor present in triglyceride-rich lipoproteins [23]. Both genetic (i.e. *LPL*, *APOC2*) and lifestyle factors are known to determine plasma triglyceride concentrations in the population; however, this remains to be fully defined to include the potential role of the new players involved in triglyceride metabolism recently reported (i.e. *APOA5*, *GPIHBP1*, *LMF1* and *CREB3L3*) [12].

Historically, LPL and apoC-II deficiency was confirmed by in vitro determination of the enzyme activity in postheparin plasma [3,24]. Molecular diagnosis consisting of direct sequencing of target genes replaced the in vitro biochemical analysis, permitting non-invasive analysis (i.e. in saliva), better reproducibility and result comparability among different laboratories, as well as carrier detection and prenatal counseling. Herein, we report the review of our experience in the molecular diagnosis of probands with a history of severe hypertriglyceridemia and/or fasting chylomicronemia referred to our Unit for genetic confirmation of the diagnosis. In a first step, *LPL* and *APOC2* genetic analyses were applied to study the patients. This information is now of special interest since the molecular diagnosis of LPL deficiency is a requirement for the first gene therapy treatment approved in the European Union for LPL-deficient patients with recurrent pancreatitis (www.lpldeficiency.com) [25]. If a negative result was obtained, other genes such as *APOA5* (results of a thorough structure-function analysis of the three patients included in the present study in whom *APOA5* sequence variants were found have recently been reported [26]) and *GPIHBP1* were studied.

2. Materials and methods

2.1. Subjects

Twenty-nine subjects with biochemical and/or clinical traits of chylomicronemia referred to our Unit were studied. Clinical manifestations varied from asymptomatic severe HTG in newborns to recurrent attacks of acute pancreatitis, including eruptive xanthomas, hepatosplenomegaly and mild to severe abdominal pain. Only 3 of the subjects were followed at our hospital; thus, clinical and/or biochemical data were provided, in summarized form, by the medical centers of origin dispersed throughout the Spanish territory. However, the clinical and biochemical information provided was heterogeneous and sometimes incomplete. Fifty-seven different individuals, including probands, were finally analyzed. The Ethics Committee of the Hospital de la Santa Creu i Sant Pau reviewed and approved the study protocol, and all individuals provided their written informed consent.

2.2. APOE genotyping

Genomic DNA was extracted from leukocytes of peripheral whole blood samples using a QIAamp DNA Blood Mini Kit according to the manufacturer's instructions (Qiagen, Hilden, Germany). *APOE* genotype was determined by polymerase chain reaction (PCR) amplification and fluorescence detection of variability at positions 112 and 158 with specific TaqMan® single-base variation genotyping assays (Applied Biosystems, Foster City, CA, USA), and a CFX96™ real-time PCR detection system (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

2.3. LPL, APOC2, APOA5, and GPIHBP1 amplification and sequencing

PCR amplification of the 9 coding *LPL* exons, the 3 coding *APOC2* exons, the 3 *APOA5* exons and the 4 *GPIHBP1* exons was performed using exon-flanking intronic primers (Supplementary Table 1). PCR products were purified using a GFX PCR DNA and Gel Band Purification Kit (GE Healthcare, Buckinghamshire, UK), and sequencing was performed using a Big Dye Terminator cycle sequencing kit v.3.1 (Applied Biosystems) on a Genetic Analyzer 3100 (Applied Biosystems). The nomenclature of the allelic variants follows the recommendations of the Human Genome Variation Society. A sequential analysis was performed to optimize the diagnostic process. First, exons 5 and 6 of the *LPL* gene were studied, as this is the region where more frequent changes have been reported (>50%). When no sequence variation was found, the rest of the gene was studied. Second, if the result was negative for *LPL*, the *APOC2* gene was analyzed. Finally, if no sequence variant was found, the *APOA5* and *GPIHBP1* genes were also sequenced. When more than one mutation was found, familial genetic analyses or, more frequently, two allele-specific PCR amplifications and sequencing were performed to confirm compound heterozygosis. All the changes found in the sequencing analyses were confirmed, when possible, by restriction fragment length polymorphism (RFLP) analyses. All variants were checked in the HGMD and 1000-genome databases. For non-synonymous variants, an in silico prediction of the functional effect of the amino acid substitutions was made with the PolyPhen and SIFT programs and with the Human Splicing Finder for variants affecting splicing signals.

2.4. In vitro mutagenesis of LPL

A human *LPL* cDNA cloned in the pCMV·Sport 6 eukaryotic expression vector was purchased from Invitrogen (Carlsbad, CA, USA). This clone came from the Mammalian Gene Collection (MGC) of the NIH and presented the common p.318Ser allelic variant, which was first reverted to wild type prior to mutant generation. Site-directed mutagenesis was performed using a modification of the in vitro overlap-extension method [24]. Briefly, the mutagenesis was performed in 3 steps. First, two complementary primers carrying the sequence variant and two contiguous and opposite directed non-overlapping primers, located just after the ampicillin resistance gene of the vector, were designed (Supplementary Table 2). Two overlapping fragments were amplified (25 cycles) separately using the forward primer of one couple and the reverse primer of the other in order to produce mutated sequences. For example, PCR amplifications with H273Rf and pCMVr and with pCMVf and H273Rr were performed to create a p.His273Arg mutant. Second, the resulting amplified fragments were used as megaprimers in a short PCR reaction (10 cycles) to produce a full-length mutated plasmid. Third, a final PCR amplification (30 cycles) was performed using the two 5′ phosphorylated primers of the vector to produce enough material to proceed to subsequent steps. All PCR amplifications were performed using a high-fidelity thermostable DNA polymerase (*pfuUltra*®, Stratagene, La Jolla, CA, USA). Finally, the product was electrophoresed, purified from a gel band using a GFX PCR DNA and Gel Band Purification Kit (GE Healthcare) and subsequently religated with T4 DNA ligase (New England BioLabs, Ipswich, MA) in a 20-μL reaction under blunt end conditions. An aliquot of 50 μL of XL1-blue competent cells (Stratagene) was transformed with 5 μL of ligation, plated on LB agar plates containing ampicillin and incubated overnight at 37 °C. Between 100 and 500 colonies were obtained from each reaction. Single colonies were inoculated in 5 mL of LB containing

Download English Version:

https://daneshyari.com/en/article/8312302

Download Persian Version:

https://daneshyari.com/article/8312302

Daneshyari.com