Considerations for Familial Chylomicronemia Diagnosis in the Era of Next-Generation Sequencing: A Latin American Perspective

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Abstract

Familial chylomicronemia syndrome (FCS) is an autosomal recessive disorder, characterized by alterations in the catabolism of chylomicrons and by increased levels of plasma triglycerides. It has been shown that about 60-90% of FCS patients have biallelic mutations in the *LPL* gene and the remaining patients have mutations in genes encoding proteins closely related to LPL function. The objective of this manuscript is to illustrate the different clinical scenarios of FCS presentation, and to guide practitioners on the usefulness of genetic tests in each of them. To this end, several published papers about recommendations for the diagnosis of FCS are discussed briefly, in addition to the presentation of several hypothetical cases, highlighting different clinical presentations and possible associated genetic findings. These cases illustrate the multiplicity of potential aspects of family history, clinical manifestations, biochemical parameters, and patterns of genetic variants found in genomic analyses of FCS.

Keywords

Familial chylomicronemia syndrome, genomics, genetic analyses.

Introduction

Familial chylomicronemia syndrome (FCS) is an autosomal recessive disorder (OMIM # 238600), which is rare in the population (1 per 300:000 to 1 per million people) [1]. FCS is characterized by alterations in the catabolism of chylomicrons and by increased levels of plasma triglycerides (TG), i.e.: > 10 mmol/l or 880 mg/dL [2]. FCS patients also present a higher rate of pancreatitis and its associated complications [3–4] than normolipidemic individuals. The classical clinical findings in FCS patients may also include abdominal pain, eruptive xanthomas (Figure 1), *lipemia retinalis* (Figure 2) and hepatosplenomegaly [5–6]. The plasma of FCS patients commonly has a lipemic appearance (Figure 3), and their serum may show a chylomicron layer on top after being stored overnight at 4 °C or after ultracentrifugation (Figure 4). These visible changes are modified after treatment (Figure 5) [7,8,9].

A highly fat-restricted diet has been used as the main therapy for FCS patients, since hypertriglyceridemia usually remains refractory to treatment with fibrates and omega-3 fatty acids [10]. In terms of novel treatments, volanesorsen, a second-generation antisense oligonucleotide that binds the mRNA of apo C-III, has been approved in Europe for control of hypertriglyceridemia in FCS patients at high risk of acute pancreatitis [10].

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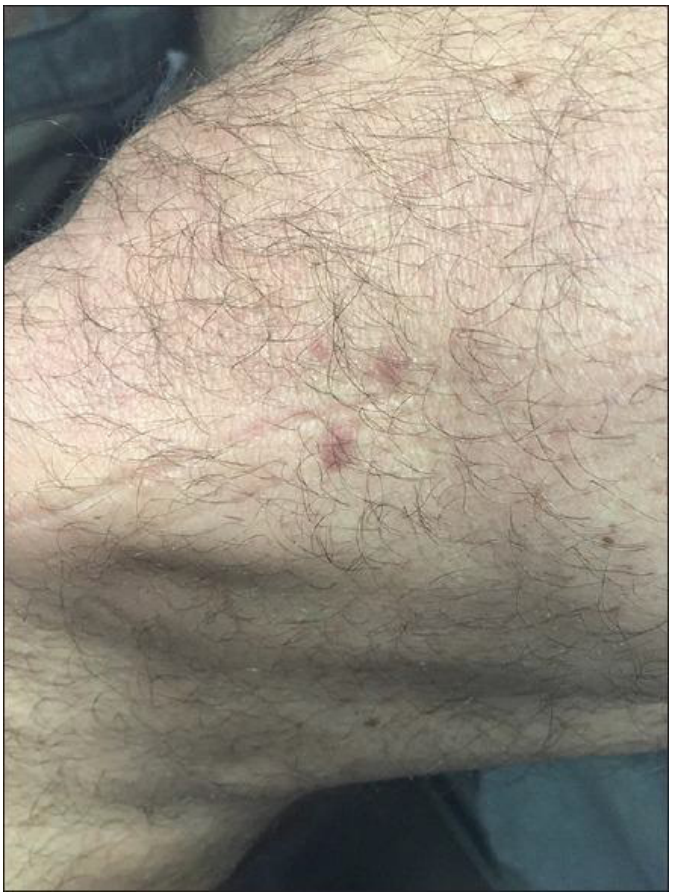

Figure 1. Crops of small, red-yellow dome-shaped papules of approx. 6 mm with well-defined borders located on the anterior medial thigh [5].

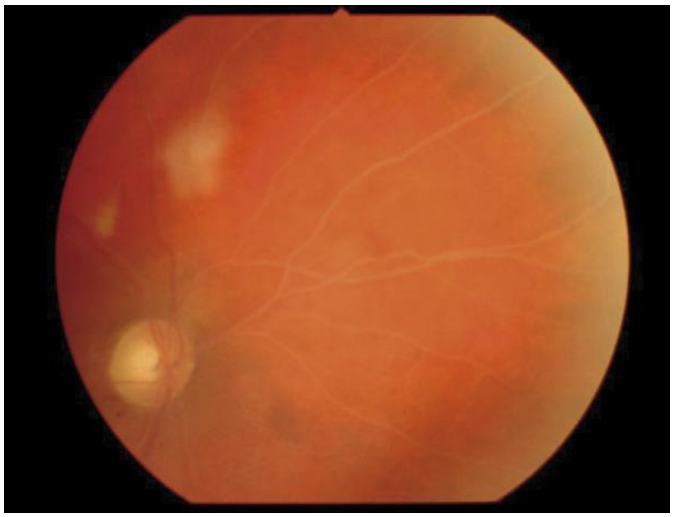

Figure 2. Right eye fundus with signs of *lipemia retinalis* (optic disc and nasal part of retina). Characteristic whitish vessels are visible. It is difficult to distinguish the arteries from the veins [6].

For the identification of relevant literature, we conducted a bibliographic search in PubMed, EMBASE, and SciELO (the Scientific Electronic Library Online) using the terms chylomicronemia, severe hypertriglyceridemia, genetic diagnosis, LPL, APOC2, LMF1, APOA5, GPIHBP1 and mutation, and their Boolean combinations. All authors were equally involved in the retrieval, selection, analysis, and interpretation of the identified sources.

Genomic Approaches

The possibility of studying a large number of genetic variants, in the entire genome or in a panel of candidate genes, has revolutionized the diagnosis of multiple human diseases [11]. In this context, approaches based on Next-Generation Sequencing (NGS) platforms have allowed the study of common and rare genetic variants in patients with hereditary diseases [12]. In addition, genomics has facilitated the study of copy number variations (CNVs) associated with human disorders [13].

The Joint Consensus Recommendation of the American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology provide a series of guidelines for the classification of sequence variants found in the genomic analyses of patients [14]. In terms of pathogenic variants, they proposed the following levels of evidence of pathogenicity: Very Strong, Strong, Moderate and Supporting. In addition, they proposed the following levels of benign impact of variants: Stand-alone, Strong, and Supporting [14]. In this context, a combination of criteria leads to the following classification of variants: Pathogenic, Likely Pathogenic, Likely Benign, Benign and Uncertain Significance [14]. Zhang et al [15] have proposed a workflow for the analysis of variants, which involves the verification of the nomenclature of the variants, the evaluation of population frequency data, the query of databases, literature and in-house evidence (involving experimental evidence, allelic data, *de novo* occurrence, segregation data and family history, among others), and the analysis of variant types, depending on whether they are null, silent or intronic, missense or in-frame deletions or insertions [15].

Clinical Features and Pathophysiology

Recently, the APPROACH study of 66 FCS patients found a median age at diagnosis of 24 years, and a median fasting triglyceride concentration of 1985 mg/dL. In the same study, 79% of patients had an identified causal mutation, and a lifetime episode of acute pancreatitis had occurred in 76% of patients [16].

It has been shown that about 60-90% of FCS patients have biallelic mutations in the *LPL* gene [2]. The *LPL* gene is located on 8p21.3 and encodes the protein lipoprotein lipase, which has 475 amino acids. The remaining patients have mutations in genes encoding proteins closely related to LPL function, such as *APOC2* (19q13.32, encoding apolipoprotein C2), *GPIHBP1* (8q24.3, encoding glycosylphosphatidylinositol-anchored, high density lipoprotein-binding protein 1), *APOA5* (11q23.3, encoding apolipoprotein A-V) or *LMF1* (16p13.3, encoding lipase maturation factor 1) [2, 17].

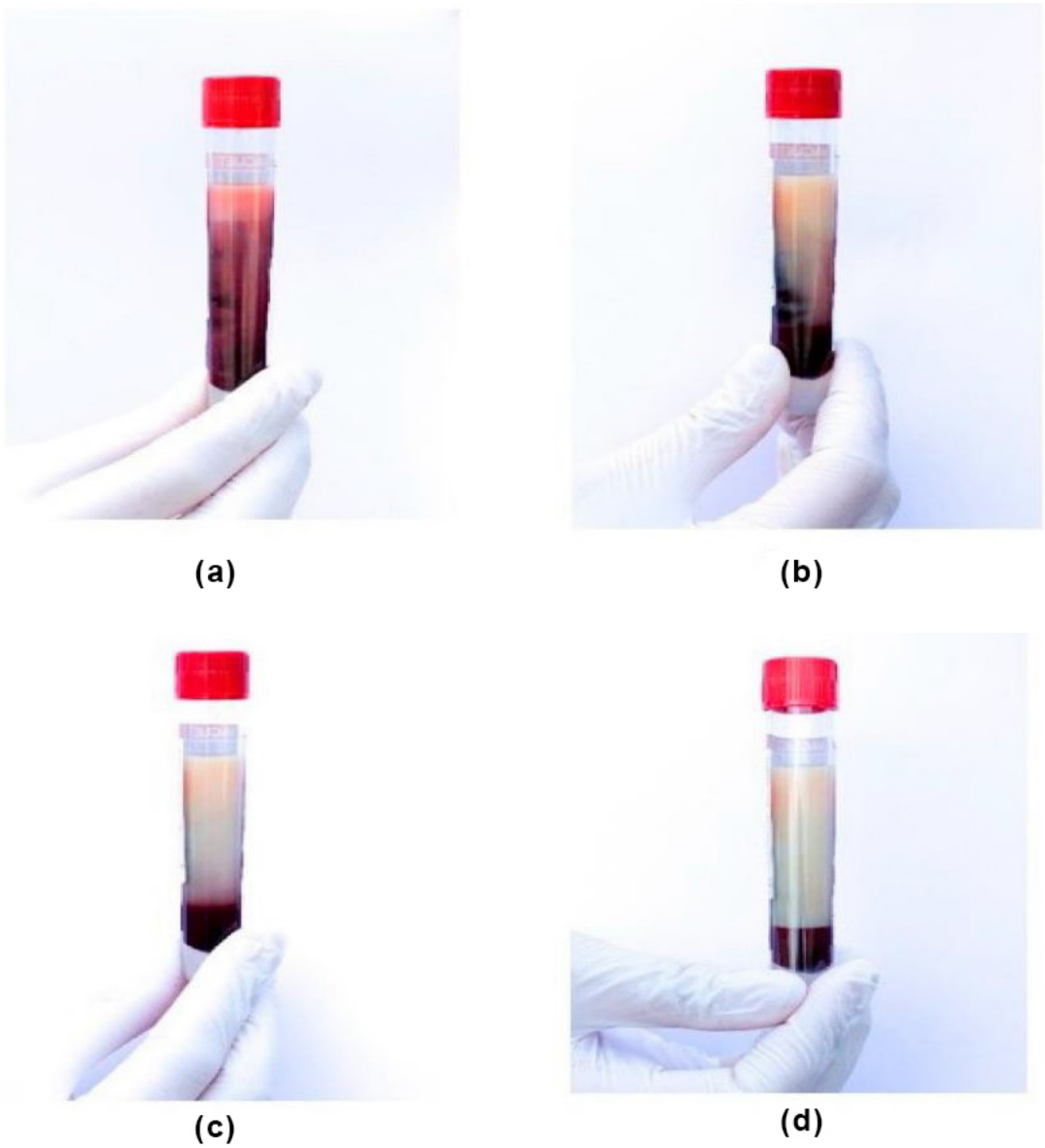

Figure 3. Lipemic plasma distribution in an EDTA tube (a) immediately after blood sampling (b) after 1 h (c) after 2 h (d) after 3 h [7].

Hegele et al. [18] studied 67 FCS patients recruited for a clinical trial of volanesorsen, using targeted next-generation DNA sequencing to identify variants in known causative genes for monogenic chylomicronemia. They found that 41 patients exhibited pathogenic biallelic mutations in the *LPL* gene. In addition, they found 11 patients with biallelic mutations in *APOA5, GPIHBP1, LMF1* or *APOC2* genes. Although they found similar phenotypes among patients with and without mutations in *LPL*, patients with mutations in *LPL* had lower levels of post heparin LPL activity [18].

Diagnosis of Familial Chylomicronemia

In this section, several published papers about recommendations for the diagnosis of FCS will be briefly discussed. These existing international guidelines incorporate aspects of clinical and biochemical features of patients, to be followed by genetic analyses. Stroes et al. [1] have proposed a diagnostic algorithm for FCS (Figure S1), which involves starting with patients in acute or non-acute events, to be followed by an evaluation of biochemical parameters, analysis of major candidate genes and identification of novel or known mutations [1].

Falko [3] has proposed an algorithm for the diagnosis of FCS (Figure S2) that involves the examination of the presence of severe refractory hypertriglyceridemia in the patient, followed by assessment of the history of acute pancreatitis or recurrent abdominal, and then genetic analysis of the main candidate genes [3].

Moulin et al. [19] recently proposed a pragmatic diagnostic score for FCS, based on eight items involving clinical and

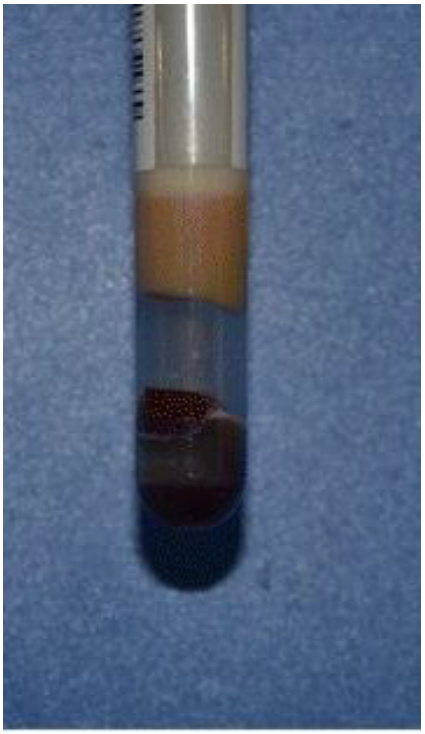

Figure 4. Serum opacity with a chylomicron layer on top after overnight refrigeration at 4 °C [8].

biochemical information (Figure 6). The authors tested their score in 53 FCS patients from three different cohorts in Europe, and found the sensitivity and specificity for a score ≥10 to be 88% and 85%, respectively. They proposed that this pragmatic clinical scoring would be helpful for the identification of patients of high interest for molecular genetic analyses [19]. Hegele et al. [20] led a European consensus statement that proposed an approach for the diagnosis of severe hypertriglyceridemia (Figure 7) that considers clinical and biochemical aspects, to be followed by a targeted NGS analysis.

Corral et al [21] developed a position paper from Argentina for the diagnosis of severe hypertriglyceridemia that also incorporated the diagnostic score proposed by Moulin et al. [19]. In addition, they developed a diagnostic algorithm for FCS [21]. An expert panel on the diagnosis and treatment of dyslipidemias from Colombia [22] included several aspects relevant to patients with FCS.

Clinical Vignettes

In this section, several hypothetical cases will be presented, to highlight different clinical presentations and the possible genetic findings associated with them. These cases illustrate the

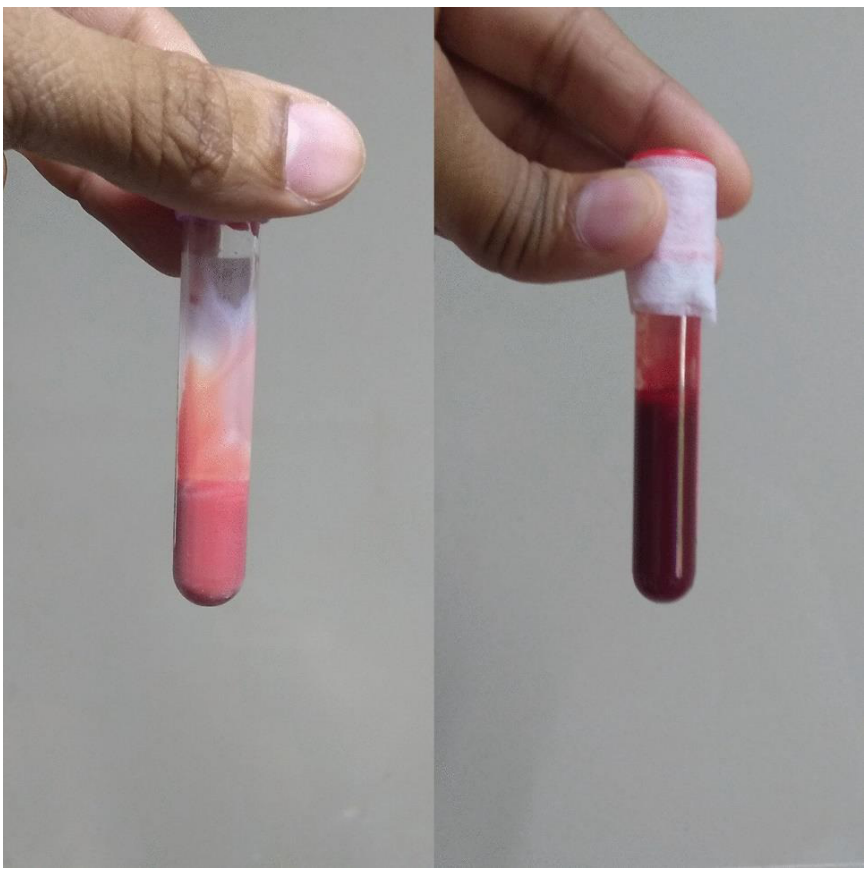

Figure 5. The figure shows the color change from pink to red of the blood of the neonate following the treatment of familial chylomicronemia syndrome [9].

Item	Description
1	Fasting TGs >10 mmol/L for 3 consecutive blood analyses (+5) or Fasting TGs >20 mmol/L at least once (+1)
2	Previous TGs <2 mmol/L (-5)
3	No secondary factor (except pregnancy and ethinylestradiol) (+2)
4	History of pancreatitis (+1)
5	Unexplained recurrent abdominal pain (+1)
6	No history of familial combined hyperlipidaemia (+1)
7	No response (TG decrease <20%) to hypolipidaemic treatment (+1)
8	Onset of symptoms at age: - <40 years (+1) - <20 years (+2) - <10 years (+3)
FCS Score	**≥10: FCS very likely** **≤9: FCS unlikely** **≤8: FCS very unlikely**

Figure 6. The FCS score. Modified from: [19].

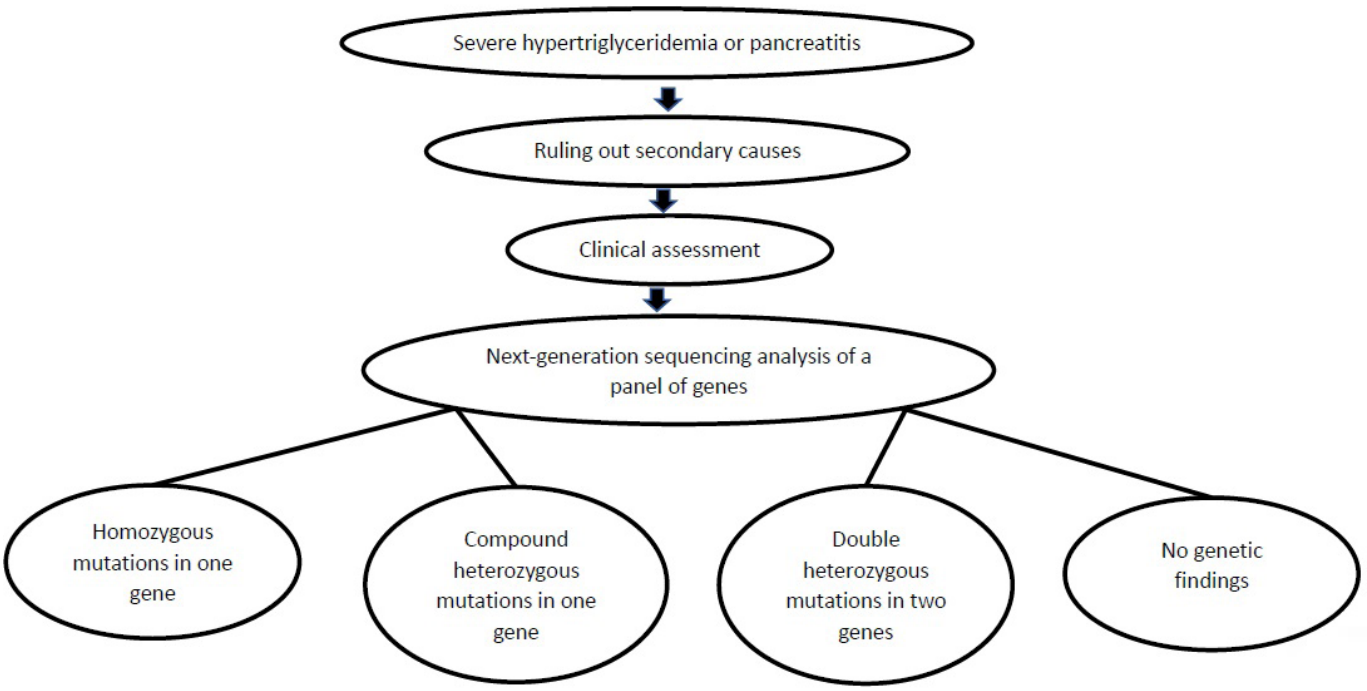

Figure 7. European Consensus algorithm for FCS diagnosis. Modified from: [20].

multiplicity of aspects of family history, clinical manifestations, biochemical parameters, and patterns of genetic variants found in NGS analyses.

Case 1

- A 43-year-old-man, born to consanguineous parents (first degree cousins), presented to the Lipid Clinic after being diagnosed with hypertriglyceridemia (HTG) during his first episode of pancreatitis at age 30.
- His highest reported TG level was 7,112 mg/dL (80.3 mmol/L), total cholesterol (TC) 455 mg/dL (11.8 mmol/L), and high-density lipoprotein cholesterol (HDL-C) 12 mg/dL (0.31 mmol/L).
- Around the same time, he was also diagnosed with diabetes mellitus (DM), independent of pancreatitis.
- A homozygous missense *APOC2* variant, c.215G > C, p.R72T, was identified in the patient, one mutated allele being inherited from each parent.

This vignette exemplifies the case of a patient with two pathogenic variants in the *APOC2* gene, in the context of familial history of consanguinity.

Case 2

- A 46-day-old female was referred to the family physician with complaints of irritability and feeding problems.
- The patient was born to third-degree consanguineous parents after an uneventful full-term pregnancy.
- Her medical history during the postnatal and early infancy period was unremarkable.
- Due to the lipemic appearance of the blood sample, she was referred to the hospital for evaluation.

Laboratory examinations

- TG level of 6295 mg/dL (71.5mmol/L)
- Low density lipoprotein cholesterol (LDLc) 50 mg/dL (1.85mmol/L)
- HDLc 48 mg/dL (1.21mmol/L)
- Amylase 52 U/L (40–140 U/L)
- Lipase 43 U/L (<50 U/L)
- C-reactive protein 4 mg/L (0–5 mg/L)

A directed NGS panel revealed two novel mutations, both in heterozygosis in the *LPL* gene, (compound heterozygous genotype):

- c.88 +2dupT mutation in intron 1, which disrupts the intron 1 splice donor site, from the father
- c.721C>T, p.P214S, in exon 5, from the mother

This vignette highlights a case associated with the presence of two variants of uncertain significance in the *LPL* gene, one inherited from the mother and the another inherited from the father.

Case 3

- A 24-day old baby was presented to the hospital with irritability, refusal to eat and vomiting. There was no history of seizures, jaundice, fever, bleeding manifestations or skin rash. This baby is the third born to non-consanguineous parents. Since the time of birth to present, the child was exclusively breastfed.
- Family history is significant for similar symptoms in an older sibling.
- Physical examination was not significant for any findings. There were no eruptive xanthomas.
- Biochemical analyses found elevated levels of plasma cholesterol and TG at 36 and 41 days of age.
- Gene panel for chylomicronemia genes did not reveal any mutation.

This vignette exemplifies a clinical case showing the classical features of FCS but without genetic findings in the DNA sequence analysis of a panel of candidate genes.

Case 4

- A 44-year-old woman presented with pancreatitis and high levels of TG.
- She was overweight and reported use of oral contraceptives.
- She did not have diabetes mellitus, alcohol abuse or nephrotic syndrome.
- Genetic analysis revealed mutations in *LPL* (c.173C>G, p.P58R, exon 2, heterozygous), and *APOA5* (c.161+5 G>C, intron 2, heterozygous). Neither mutation had been described before.

This vignette highlights a case associated with a double heterozygous in two genes: *LPL* and *APOA5.*

The previous four cases illustrate different types of genetic findings in the NGS analysis (Figure 7). The first case highlights the classical presentation with a homozygous variant in a major gene and associated with familial history of consanguinity. The second vignette shows two variants in the same gene (a compound heterozygous form); one inherited from the father and the another inherited from the mother. The third case highlights the case when no genetic findings are observed but the classical clinical and biochemical features of FCS are seen. The fourth vignette exemplifies the situation in which variants in two different genes are found in the NGS analysis of a patient.

Additional Considerations

Several aspects of the molecular analysis of FCS would deserve additional studies in the future in Latin America. One is the cost-effectiveness, in the clinical setting, of genome-wide analyses (such as exome sequencing) [23] of FCS cases without genetic findings in the DNA sequencing of a candidate genes panel. Another key aspect is the analysis of copy number variations in candidate genes [24] in those cases in which there are no findings of relevant sequence variants. A third aspect is the perspective for a broader implementation of biochemical assays, in the clinical setting, for the functional analysis of the lipoprotein lipase protein [18]. A further consideration is the need for strategies aimed at guaranteeing the constant update of clinical annotation of variants (derived from *in silico* predictions and experimental evidence from *in vitro* studies, among others) [18] found in the genetic analysis of FCS patients in Latin America. Finally, one should consider the restricted access to molecular diagnosis in the region that is a barrier to FCS adequate diagnosis and therapy [25].

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Supplementary Material

The following online material is available for this article:

Figure S1 – FCS diagnosis algorithm, based on presentation. Modified from: Stroes E, Moulin P, Parhofer KG, Rebours V, Löhr JM, Averna M. Diagnostic algorithm for familial chylomicronemia syndrome.
Figure S2 – FCS diagnosis algorithm, based on characteristics of hypertriglyceridemia. Modified from: Falko JM. Familial Chylomicronemia Syndrome: A Clinical Guide For Endocrinologists.

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