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PII: S0021-9150(18)31182-1

DOI: 10.1016/j.atherosclerosis.2018.06.870

Reference: ATH 15593

To appear in: *Atherosclerosis*

Received Date: 4 February 2018

Revised Date: 6 June 2018

Accepted Date: 19 June 2018

Please cite this article as: Marco-Benedí V, Lamiquiz-Moneo I, Álvarez-Sala LA, Civeira F, Disappearance of recurrent pancreatitis after splenectomy in Familial Chylomicronemia Syndrome, *Atherosclerosis* (2018), doi: 10.1016/j.atherosclerosis.2018.06.870.

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**Title**

Disappearance of recurrent pancreatitis after splenectomy in Familial Chylomicronemia Syndrome

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**Abstract:**

**Background and aims**: Recurrent pancreatitis is a severe complication of Familial Chylomicronemia Syndrome (FCS) mainly secondary to lipoprotein lipase deficiency. The mechanism and interindividual variability of pancreatitis in FCS is not fully understood, but abnormalities in the drainage system of pancreatic veins could be involved.

**Methods and results:** Two cases of typical FCS are described with a past history of recurrent pancreatitis that dramatically improved after splenectomy performed in both cases for reasons non-related to FCS.

**Conclusion:** These are the first reports of the disappearance of pancreatitis after splenectomy in FCS and they should be considered of anecdotal nature at this time. The disappearance of pancreatitis following splenectomy could be in part due to subsequent improvements in pancreatic drainage. Extrahepatic portal hypertension induced by hypertriglyceridemic splenomegaly leading to pancreatic congestion could also be a contributing factor.

**Keywords:**

Familial Chylomicronemia; Lipoprotein lipase deficiency; Pancreatitis; Splenectomy

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## Introduction

Familial Chylomicronemia Syndrome (FCS) deficiency is a rare autosomal recessive disorder characterized by the absence or the severe reduction of lipoprotein lipase (LPL) activity and a massive accumulation of chylomicrons in plasma leading to a large increase of plasma triglyceride concentration, usually greater than 22.5 mmol/L (2000 mg/dl) in the fasting state (1). FCS is clinically characterized by repeated abdominal pain episodes, recurrent acute pancreatitis, eruptive cutaneous xanthomatosis, *lipemia retinalis* and hepatosplenomegaly. FCS is mostly identified in childhood due to recurrent episodes of pancreatitis and high fasting triglycerides (2). Pancreatitis is the most serious complication of LPL deficiency. The underlying pathophysiological mechanisms are not fully understood (3). FCS is a very rare disease with a frequency at about one per million in the general population, although it may be higher in some populations due to a founder effect (4).

The degree of hyperchylomicronemia in FCS depends, at least in part, on dietary fat intake, but genetic heterogeneity can play a role (5). It has been observed that a severe restriction of dietary fat to less than 20 g/day is enough to control the symptoms in some cases (6). In contrast, FCS is usually not responsive to conventional lipid-lowering therapy. Acute pancreatitis characterizes early stages of the disease while some patients may develop recurrent abdominal pain and chronic pancreatitis as the disease progresses. Hepatomegaly and splenomegaly could appear over time when the chylomicronemia becomes chronic. Splenomegaly is less frequently observed than hepatomegaly and can be notably hard. The organomegaly occurs as result of triglyceride uptake by macrophages. These individuals might show anemia and/or thrombocytopenia due to secondary hypersplenism (1,7).

In this study we describe two unrelated cases of typical FCS with a past history of recurrent pancreatitis controlled in two different lipid clinics in Spain. Both cases have shown a dramatic improvement in pancreatitis after a splenectomy was performed due to reasons non-related to FCS.

**Material and methods**

Lipoprotein Activity. The LPL activity assay was done on post-heparin samples on an Intralipid 10% emulsion as previously described (8). Each sample for LPL activity was assayed in triplicate and two standard samples were analyzed in each assay.

Genetic analysis. DNA was extracted by standard methods. Promoters, coding regions and intron-exon boundaries of *LPL*, *APOA5*, *APOC2*, and *GPIHBP1* were amplified by PCR and purified by ExoSap-IT (USB) using primers previously described (9). Amplified fragments were sequenced by Sanger method using the BigDye 3.1 sequencing kit (Applied Biosystems) in an automated ABI 3500xL sequencer (Applied Biosystems).

**Results**

The first case is a 68-year-old man who first visited our Lipid Unit at the Hospital Universitario Miguel Servet, Zaragoza, Spain, approximately 20 years ago. He was referred by his physician for the study of severe hypertriglyceridemia (HTG) with fasting plasma concentrations between 22.5-45 mmol/l (2000-4000 mg/dl from, at least, the age of 30 years old. At his first visit the patient was asymptomatic and the physical exam was normal except for low weight and body mass index (61.4 Kg and 18 kg/m², respectively). He was following a low-fat diet and his fasting lipid profile showed triglycerides 39.2 mmol/l (3473 mg/dl); total cholesterol 7.8 mmol/l (303 mg/dl); HDL

cholesterol 0.62 mmol/l (24 mg/dl), apolipoprotein B (apoB) 90 mg/dl, and plasma Lp(a) 3.56 mg/dl. Other biochemical parameters including thyroid hormones, glucose, liver enzymes, and creatinine were in the normal range. His post-heparin lipoprotein lipase activity was undetectable in two occasions (<10 µU/ml, normal values 22-47.6 µU/ml). Years later, the sequencing analysis of *LPL*, *APOC2*, *APOA5*, *LMF1* and *GPIHBP1* showed that the patient was double heterozygous with a pathogenic mutation in *LPL* gene (p.Pro234Leu) and two different pathogenic mutations in *LMF1* (p.Arg354Trp) and (p.Arg364Gln) (9). He was diagnosed of FCS secondary to LPL deficiency. He has been reviewed periodically in our unit, remaining asymptomatic and without substantial changes in his physical exam or lipid phenotype.

He began at the age of 22 with recurrent episodes of abdominal pain. These episodes repeated once or twice a year, lasting 4-12 hours, ceasing after prolonged fasting. At the age of 28, the patient was admitted to our hospital because of another episode of diffuse abdominal pain, more intense and prolonged than previous, with maximal pain in the upper left quadrant of the abdomen. His physical exam was normal except for mild splenomegaly. He was treated with fasting, analgesia and intravenous fluids and the pain resolved within a few hours. Triglycerides and pancreatic enzymes were not analyzed during his hospital stay. However, mild leukocytosis and lipemic plasma was observed at admission. He was discharged with the diagnosis of abdominal pain secondary to splenomegaly.

At the age of 31 years and following a parachuting accident, he began to experience severe abdominal pain and underwent emergency splenectomy in another center under the suspicion of a spleen rupture. The pathological study of the spleen was not performed. After the splenectomy, the pain disappeared and he has remained

asymptomatic ever since. His current lipid profile shows: triglycerides 31.0 mmol/l (2748 mg/dl); total cholesterol 10.9 mmol/l (423 mg/dl); and apo B 82.6 mg/dl.

**Second case presentation**

A 43 year old woman was reviewed at the Lipid Unit, Hospital Universitario Gregorio Marañon in Madrid, Spain, where she was diagnosed with FCS due to severe HTG from birth, recurrent episodes of acute pancreatitis and very low post-heparin LPL activity: 5.2 µU/ml. Sequencing analysis of the *LPL* gene showed that the patient was homozygous for a functional *LPL* mutation (G188E/G188E). Her parents had a pattern of mild combined hyperlipidemia and were heterozygous to G188E mutation in *LPL*.

The patient started suffering from abdominal pain at 2 months of age, with triglycerides above 22.5 mmol/l (2000 mg/dl) in several controls. Breast-feeding was stopped and she started receiving an artificial milk formula.

Along her infancy she experienced several episodes of abdominal pain. No registries of clear pancreatitis were reported up to the age of 13 years. In analytical controls she always had chylomicronemia with values of triglycerides higher than 22.5 mmol/l (2000 mg/dl). In two reports at the age of 12 and 13 she presented eruptive xanthomas in the shoulders, neck and thorax. At least six cases of acute pancreatitis were documented between the ages of 13-26. Additionally, she reported frequent episodes of mild to moderate abdominal pain.

She became pregnant at 26 years old and suffered a mild pancreatitis after 4 months of pregnancy. During her 5$^{th}$ month of pregnancy she reported severe pancreatitis and developed a pseudocyst and later a pancreatic abscess. An elective cesarean was performed during her 25$^{th}$ week of pregnancy. The child who is nowadays alive and healthy (no lipids data are available). Image studies following the caesarean

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showed multiple gallstones. Part of the body and tail of the pancreas had disappeared due to autodigestion following the last severe pancreatic episode. Ten months after the caesarean, an elective surgery was done due to mechanical small bowel obstruction secondary to abdominal adhesions. Y Roux gastro-jejunostomy, cholecystectomy and splenectomy were performed. Splenectomy was due to suspected spleen vein thrombosis in presence of splenomegaly and spleen congestion. The spleen was well encapsulated, 15 cm long and 500 g weight. Histopathologically, the capsule was thickened; red pulp was predominant, there was endothelial hyperplasia, enlargement of the splenic sinuses, and fibrosis of intersinusal spaces. Penicilliary arterioles showed marked sclerosis. Lipid-laded macrophages were not observed. The pathological diagnosis was chronic congestive splenomegaly (Figure). Few months later she was admitted in the hospital due to mild episodes of pancreatitis twice (the last one not clearly confirmed). Since then, 11 years later, the patient has had no further pancreatitis episodes in spite of chylomicronemia with high triglycerides values 29.6 mmol/l (2629 mg/dl) found on Oct 29th, 2015 and 37.4 mmol/l (3316 mg/dl) on Dec 18th, 2015).

**Discussion**

The two cases presented in this report illustrates the disappearance of abdominal pain after performing a splenectomy in patients with FCS. It is the first description in the literature of this favorable evolution in this disease and thus generates hypotheses that can help to identify some of the mechanisms associated with the onset of pancreatitis and chronic pain in these patients.

Abdominal pain is very common in FCS and is due in most cases to episodes of recurrent acute pancreatitis or subsequent complications (10). Approximately 50% of subjects with FCS have at least one episode of severe pancreatitis (11). Alcohol

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consumption, pregnancy and poor compliance with a low-fat diet are risk factors for the development of pancreatitis in FCS; however, even in absence of these factors, pancreatitis may develop with large differences among subjects, even those sharing the same pathogenic mutation (11). Recently, two loci: the chymotrypsinogen C (CTRC) and serine protease inhibitor Kazal-type1 (SPINK1) have been associated with a risk of recurrent hospitalization for acute pancreatitis in severe HTG due to FCS (17). However, these genes are poorly studied and their plausible association with pancreatitis is not related to pancreatic secretion or drainage, or to spleen function. Furthermore, the improvement of pancreatic episodes after splenectomy in our cases does not support a genetic predisposition to pancreatitis in our patients (12).

In the two cases reported here, episodes of recurrent pancreatitis seem evident given the characteristics of the episodes of pain and the confirmation of LPL deficiency. However, because the first case happened 40 years ago, it is difficult to verify at present time regardless of the episodes very suggestive nature.

The mechanism by which very severe HTG leads to pancreatitis has not been fully elucidated, but it is likely related to the liberation by pancreatic lipase of free fatty acids (FFA) from triglycerides and lysophosphatidylcholine from phosphatidycholine, when the pancreas is exposed to severe hyperchylomicronemia in the pancreatic capillaries (7). High local concentrations of FFA overwhelm the binding capacity of albumin with resultant aggregation into micellar structures with detergent properties (13).

The association of chronic pancreatitis with splenomegaly has been well-documented for some time (14). On the one hand, splenomegaly and pancreatitis are more frequent in the presence of very high triglyceride levels, so they could be concomitant manifestations in nature without having a causal association between them.

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Splenomegaly can also be the result of complications from pancreatitis such as pseudocysts, abscesses, infarcts, hemorrhages or vascular lesions causing splenic portal hypertension (15). However, the association between splenomegaly and pancreatitis appears even in the absence of HTG and sometimes precedes the development of pancreatitis in the presence of splenic venous thrombosis, and so other mechanisms may be involved. As pointed out by Francesco and cols (16), it is difficult to establish which came first, the pancreatitis or the splenomegaly, what they call the chicken or the egg causality dilemma.

The most plausible explanation for the disappearance of episodes of pancreatitis in the exposed cases would be a hemodynamic mechanism. The pancreatic veins drain into the splenic vein and the latter into the vena cava. Splenomegaly frequently associates extrahepatic portal hypertension that hinders pancreatic drainage (17). In a retrospective analysis, Ramesh et al reported the effect of different surgical techniques to get pain relief in patients with chronic pancreatitis and signs of portal hypertension. In their work, 15 out of 57 patients had chronic pancreatitis with extrahepatic portal hypertension secondary to thrombosis of the splenic vein, and in these cases, splenectomy was performed along with pancreatic drainage. The authors observed that the surgery significantly improved the symptoms of these patients (18). It would be possible that in the presence of defective pancreatic venous drainage increased FFA accumulation and pancreatic aggression may occur. Splenectomy improves pancreatic venous drainage and could favor the decrease of pancreatitis in certain cases of hypertriglyceridemic pancreatitis associated with extra hepatic portal hypertension. It would be interesting to know if there are more cases of hypertriglyceridemic pancreatitis and splenectomy worldwide, in order to further confirm our observation.

These two observations cannot induce one to perform splenectomy in FCS patients with recurrent pancreatitis. They should be considered of anecdotal nature and without cause-and-effect relationship between splenectomy and recurrent pancreatitis. This association would require a definite demonstration of the deleterious effect of elevated extrahepatic portal pressure in FCS subjects. Furthermore, the effect of splenectomy in FCS animal models (19,20) should be profoundly studied before any human intervention study should commence.

**Conflicts of interest**

The authors declared that they do not have any conflict of interest to disclose with respect to this research.

**Funding**

This study was supported in part by grants from the Carlos III Research Institute: CIBERCV (co-supported by the European Regional Development Fund (ERDF) which is allocated by the European Union; IIS16/0114, PI15/01983 and PIE16/00022.

**Contribution Statement.**

All authors have made substantial contributions to all of the following: the conception and design of the study (FC); acquisition of data and analysis and interpretation of data (VM-B, LA A-S, I L-M, FC), drafting the article (V M-B, FC), and revising it critically for important intellectual content (LA A-S, I L-M). All authors have approved the final the version of the manuscript. Authors report no conflicts of interest.

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**Acknowledgement**

Authors thank Chanel A. Ramirez for her English language editing.

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**Figure legend**

Histopathological findings of the spleen of the case 2 stained with hematoxylin and eosin. A (x 100) and B (x 400). Red pulp is predominant. Endothelial hyperplasia, enlargement of the splenic sinuses, and brown areas indicating hemosiderin deposits (arrows) are present. The pathological diagnosis was chronic congestive splenomegaly.

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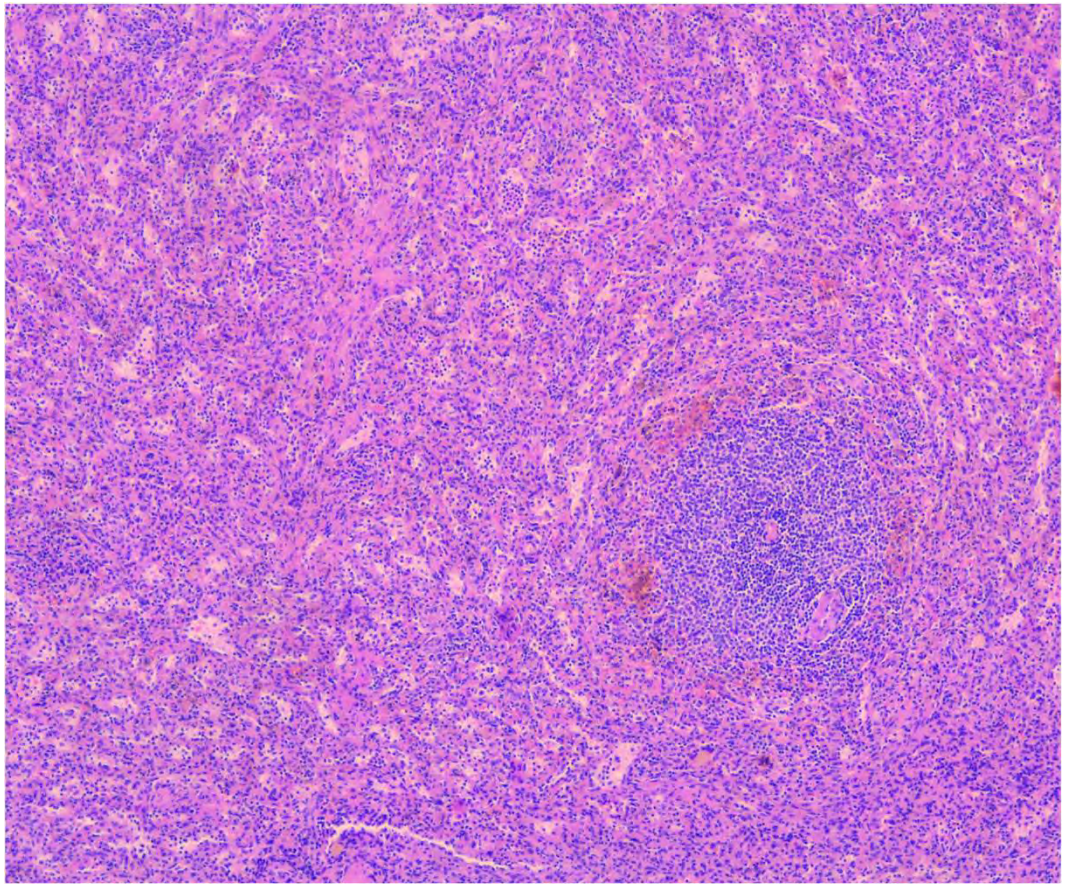
A



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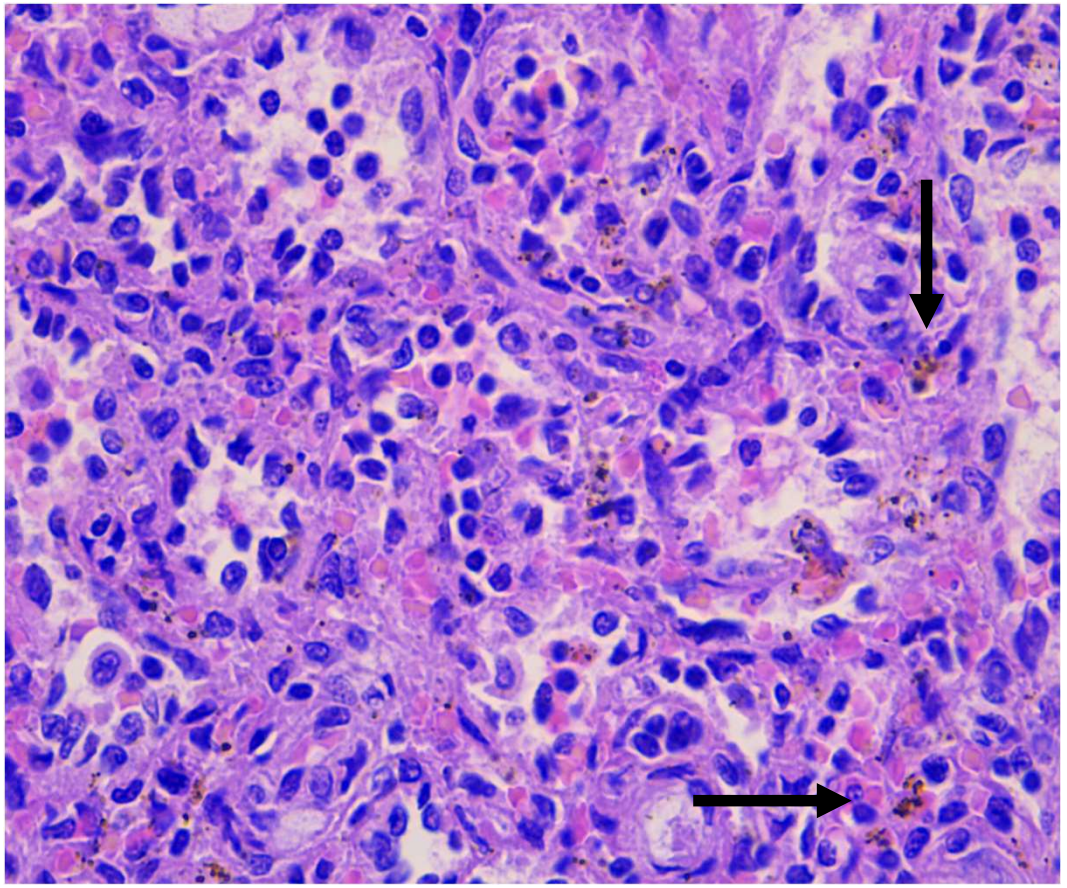

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- Pancreatitis is a frequent and serious complication in familial chylomicronemia
- We present two cases in which recurrent pancreatitis disappeared after splenectomy
- Extrahepatic portal hypertension favors the development of pancreatitis
- Improvement of portal drainage could explain this favorable evolution

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