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Chylomicronemia: Differences between familial chylomicronemia syndrome and multifactorial chylomicronemia

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HIGHLIGHTS

- Familial chylomicronemia syndrome (FCS) is a rare autosomal recessive disease.
- Multifactorial chylomicronemia (MCM) is a polygenic disorder.
- Both FCS and MCM patients have very high and variable triglycerides concentration.
- FCS patients presented a higher frequency of pancreatitis than MCM patients.
- FCS patients presented less metabolic abnormalities than MCM patients.

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ABSTRACT

Background and aims: Chylomicronemia can be either monogenic or multifactorial. The monogenic form, namely familial chylomicronemia syndrome (FCS), is a rare autosomal recessive disease that strongly predisposes to pancreatitis. However, the clinical variables differentiating FCS from multifactorial chylomicronemia (MCM) are not well established. The aims of the present study were to describe a well-defined cohort of FCS subjects and to investigate the differences between patients with FCS and MCM.

Methods: A total of 25 FCS and 36 MCM patients were included in the present study. FCS patients were genetically confirmed, whereas MCM patients had negative genetic testing, triglycerides above 10 mmol/L at least once and the presence of both chylomicrons and VLDL in plasma.

Results: FCS patients presented a significant higher frequency of pancreatitis (60% *vs.* 6%), multiple pancreatitis (48% *vs.* 3%) and abdominal pain (63% *vs.* 19%) and a lower frequency of metabolic abnormalities than in the MCM group ($p < 0.0001$). In addition, the frequency of cardiovascular events was higher in the MCM group than in the FCS group (17% *vs.* 0%), although the difference was not statistically significant ($p = 0.07$). In a univariate regression model, the significant predictors of FCS were age at first manifestation ($\beta = -2.11$, $p = 0.0005$), body mass index (BMI) ($\beta = -1.82$, $p < 0.001$) and gamma-glutamyl transferase (GGT) ($\beta = -1.64$, $p = 0.001$).

Conclusions: Our study identified several variables that significantly differentiates FCS from MCM patients. These results need to be replicated in larger cohorts to identify the independent predictors of FCS.

1. Introduction

Familial chylomicronemia syndrome (FCS), traditionally known as Type I hyperlipoproteinemia (T1HLP) (OMIM# 238600), is a rare autosomal recessive disease of chylomicron metabolism. To date, five genes involved in chylomicron lipolysis have been associated with FCS, namely lipoprotein lipase (*LPL*) (> 90% of cases), apolipoprotein C2 (*APOC2*), apolipoprotein A5 (*APOA5*), lipase maturation factor 1 (*LMF1*) and glycosylphosphatidylinositol anchored high density lipoprotein binding protein 1 (*GPIHBP1*) [1]. Homozygous or compound

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heterozygous mutations in these genes lead to a drastic reduction of chylomicrons clearance. Therefore, very high concentrations of serum triglycerides (TG) (> 10 mmol/l (> 880 md/dL)) can be observed in the fasting state [1]. The worldwide prevalence of FCS is estimated to be approximately one to 10 per million [1]. However, the prevalence is higher in some populations such as the French Canadians, where the LPL deficiency (FCS caused by *LPL* mutations) prevalence reaches 100 to 200 cases per million in some regions [2]. Acute pancreatitis, which can be recurrent and potentially fatal, represents the most severe complication associated with this monogenic disease. In addition, further complications related to repeated acute pancreatitis such as chronic pancreatitis and exocrine and endocrine pancreatic insufficiency can occur [3]. Other clinical characteristics can be present in patients with FCS such as eruptive xanthomas, lipaemia retinalis, failure to thrive, hepatosplenomegaly, abdominal pain, nausea and vomiting [1].

However, there exists two different forms of chylomicronemia, namely the FCS and the multifactorial chylomicronemia (MCM) and it remains a challenge for the clinician to distinguish these two disorders. MCM (sometimes called type V hyperlipoproteinaemia) is an oligogenic or polygenic disorder exacerbated by the presence of secondary factors such as a diet rich in fats and simple sugars, obesity, alcohol intake and uncontrolled diabetes. In patients with MCM, chylomicronemia typically occur later in life. It has been estimated that chylomicronemia can be found in 1:600 adults, but that FCS patients represent only 5% of these individuals [1]. Furthermore, it is likely that the prevalence of MCM may increase in the future due to the increasing prevalence of obesity, metabolic syndrome and type 2 diabetes. It is possible to differentiate these two forms of chylomicronemia based on lipoprotein electrophoresis or ultracentrifugation (presence of very low-density lipoprotein (VLDL) and chylomicron in MCM versus only chylomicrons in FCS). The current gold standard procedure to identify FCS patients remains genetic testing or post-heparin LPL activity. Interestingly, an expert panel has recently proposed a practical diagnostic scoring system for FCS [4].

Because the treatment of these two forms of chylomicronemia is very different, making an appropriate diagnosis is important. Indeed, MCM patients generally respond well to modifications in lifestyle and treatment of secondary factors. Furthermore, a good response to triglyceride-lowering pharmacotherapies is generally observed. In contrast, FCS patients do not respond to triglyceride-lowering therapies such as fibrates. Therefore, an extremely severe diet that restricts the consumption of long-chain fatty acids (maximum 10–30g of fat intake/day) represent the most effective treatment for these patients [1]. Thankfully, novel therapies such as inhibitors of apolipoprotein C-III are under development to lower triglycerides in FCS subjects [5].

The aim of the present study is therefore to investigate the clinical and biochemical differences between patients with FCS and MCM.

2. Materials and methods

2.1. Study population and data collection

In this study, we investigated the prevalence of selected clinical features in confirmed FCS compared to patients with MCM. All patients have been recruited at the Montreal Clinical Research Institute (IRCM) lipid clinic. The IRCM lipid clinic is a reference center for the diagnosis of genetic dyslipidemias. Data for each patient were collected retrospectively at their first visit to the clinic (Baseline visit). Inclusion criteria for the FCS group include a genetic confirmation of FCS or a null LPL activity (post-heparin LPL activity < 5%) [6]. To be included in the MCM group, the criteria were the following: a self-reported French Canadian origin, the absence of a homozygous or compound heterozygous French Canadian FCS mutation in the *LPL* gene (p.G215E, p.P234L and p.D277 N), TG above 10 mmol/L at least once and the presence of both chylomicrons and VLDL on lipoprotein electrophoresis or ultracentrifugation. Our criteria to initiate a genetic screening in hypertriglyceridemic patients is the presence of fasting TG ≥ 10 mmol/L at the baseline visit. Patients with thyroid or hepatic diseases as well as taking corticotherapy were excluded.

The cardiovascular endpoints that were included in the study were coronary artery disease (angina, myocardial infarction, coronary angioplasty, and coronary bypass surgery), peripheral vascular disease (claudication, peripheral angioplasty, and peripheral arterial surgery), and cerebrovascular disease (transient ischemic attack, stroke, and carotid endarterectomy). Physical findings including eruptive xanthomas, lipaemia retinalis and hepatosplenomegaly were assessed during the physical examination of the first consultation. Abdominal pain was self-reported by the patient. Only the episodes of pancreatitis leading to a hospitalization were quantified in the present study. The International Diabetes Federation (IDF) 2009 consensus statement criteria were used to assess the presence of features of the metabolic syndrome (MetS) [7]. Since hypertriglyceridemia and low high-density lipoprotein cholesterol (HDL-C) are primary features of chylomicronemia, these criteria were not considered in the present analysis. Furthermore, waist circumference was not available and then body mass index (BMI) ≥ 27 kg/m^2 was used as a surrogate marker of excess body weight. Written informed consent, approved by the IRCM institutional review board was obtained from all patients. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki.

2.2. Biochemical and DNA analysis

Blood samples for lipid profile were collected after a 12-h overnight fast. Serum cholesterol and triglycerides concentrations were determined by an enzymatic method (Abbott Biochromatic Analyzer model 100, Abbott Laboratories, Pasadena, CA), whereas low-density lipoprotein cholesterol (LDL-C), VLDL-C and HDL-C concentrations were measured through ultracentrifugation. Lipoprotein (a) concentrations were measured using a commercial ELISA kit (Macra EIA Kit, Strategic Diagnostics Industries, Inc., Newark).

DNA was extracted from white blood cells using an automated 340A DNA extractor (Applied Biosystems, CA) or QIAmp Blood Maxi Kit (Qiagen, Ontario, Canada). In brief, Sanger sequencing was used to detect the presence of the three classical French Canadian FCS-causing mutations (in exon 5: p.G215E (also known as *LPL*188), p.P234L (also known as *LPL*207) and in exon 6: p.D277 N (also known as *LPL*250)). PCR amplifications of both exon 5 and exon 6 were performed on 25 ng of genomic DNA using AmpliTaq Gold (ThermoFisher, cat. n°4311806) and then purified using ExoSAP-IT (ThermoFisher, cat. n°78201.1). Purified products were then sequenced using BigDye Terminator V3.1 sequencing kit (ThermoFisher, cat. n°4337455), with the specific primers for each exons. The sequencing reactions were purified using BigDye XTerminator™ Purification Kit (cat. n°4376484). The capillary eletrophoresis of the fragments was then performed on the Genetic Analyzer 3130xl (ThermoFisher, cat. n°3130XLR), and the sequences obtained analyzed by Sequencing Analysis software (ThermoFisher, cat n°4360967). The fragments have been aligned on the reference sequence of the *LPL* gene (Genebank accession NG_008855), with Sequencher Software (Gene Code Corporation) to detect the presence of the mutations.

For FCS patients not carrying the p.G215E, p.P234L or p.D277 N *LPL* mutations, whole blood samples were sent to the Robarts Research Institute. Briefly, sequencing of genes potentially implicated in the phenotype of severe hypertriglyceridemia (*LPL*, *APOC2*, *APOA5*, *LMF1*, *GPIHBP1*, *GCKR*, *CREB3L3*, *GPD1*, *APOC3*, *APOE*, *TRIB1*, *MTTP*, *APOB*, *PCSK9*, *SAR1B*, and *ANGPTL3* genes) was performed.

2.3. Statistical analysis

The data analysis for this paper was generated using SAS software version 9.4 (SAS Institute Inc., Cary, NC, USA). and SPSS (IBM Corp.

Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.). All reported p values are based on two-sided tests of significance and statistical significance was assessed at the 5% level. Continuous variables with a skewed distribution were normalized by log-transformation prior the analysis. If the logarithmic transformation did not normalize the distribution, a non-parametric test was used (Mann-Whitney U test).

Differences in baseline clinical features between the FCS and the MCM groups were evaluated by Student's t-test for continuous variables or Chi^2 test (or Fisher's exact test) for categorical variables. Logistic regression models were used to evaluate predictors of FCS and to compute the standardized β-coefficient values. Model 1 identified univariate predictors (uncorrected model), whereas model 2 was corrected for age and sex. Finally, Chi^2 analysis was used to compare the prevalence of complications of hypertriglyceridemia, clinical manifestations of hypertriglyceridemia, as well as the prevalence of metabolic features between FCS and MCM patients.

3. Results

3.1. Description of the study cohort

The causal mutations found in FCS patients are shown in Supplemental Fig. 1. The majority of the subjects were homozygous for the p.P234L mutation of *LPL* gene (32%) or carried the p.G215E mutation of *LPL* gene (28%) or were compound heterozygotes for both (24%). A total of 25 FCS and 36 MCM patients were included in this study. Clinical features of both groups are presented in Table 1. On average, the first manifestation associated with chylomicronemia occurred 25 years earlier in FCS patients compared to MCM patients. Furthermore, the FCS patients were referred to the IRCM lipid clinic on average 9 years before the MCM patients. There were a significantly lower proportion of women in the MCM group (11%) compared to the FCS group (64%). Other significant differences in clinical characteristics between the groups include higher BMI, systolic and diastolic blood pressure (SBP and DBP), higher proportion of smokers (past or current) and higher prevalence of diabetes in the MCM group. Significant differences have been observed in the general biochemistry parameters, including higher fasting glucose, higher creatinine and higher liver enzymes (alanine transaminase [ALT] and gamma glutaryl transaminase [GGT]) and creatine kinase (CK) in the MCM group. Some significant differences in the lipid profile have also been observed between the groups. In MCM patients, total cholesterol, HDL-C and non-HDL-C were significantly higher than in the FCS group (+2.54 mmol/L, + 0.10 mmol/L and +2.45 mmol/L, respectively). Interestingly, the mean TG value was also higher in the MCM group (+5.55 mmol/L), although this difference was not statistically significant. Apolipoprotein E 3 (*APOE* 3) allele frequency was lower in MCM group (57%) than in FCS group (88%), $p = 0.001$, whereas *APOE* 4 allele frequency was higher in the MCM group (25%) than in the FCS group (3%), $p = 0.006$. There was no statistical difference between the two groups concerning the *APOE* 2 allele frequency ($p = 0.21$). Concerning the clinical manifestations of chylomicronemia (eruptive xanthomas, lipaemia retinalis, hepatosplenomegaly and abdominal pain), only the prevalence of abdominal pain was significantly different between the two groups and occurred more frequently in FCS patients (63%) than in MCM patients (19%), $p = 0.001$. The prevalence of fibrate and statin use at baseline was not statistically difference between groups. However, a significantly higher proportion of FCS patients had history of medium chain triglycerides (MCT) oil use ($p < 0.001$), whereas a higher proportion of MCM patients had a history of hormone replacement therapy (HRT) ($p = 0.01$). As shown in the Supplemental Table 1, the response to fibrate treatment during the follow-up was significantly better in the MCM group compared to the FCS group ($p = 0.0002$).

Table 1
Subjects characteristics.

Characteristics	FCS patients N = 25	MCM patients N = 36	p value
Clinical characteristics			
Age at referral (y)	33 ± 13	42 ± 10	0.004
Age at first manifestation (y)	11 ± 10	36 ± 10	< 0.001
Sex (women (%))	16 (64%)	4 (11%)	< 0.001
BMI (kg/m^2)	21.7 ± 3.0	28.5 ± 3.2	< 0.001
Systolic blood pressure (mmHg)	116.6 ± 12.8	132.2 ± 21.5	0.004
Diastolic blood pressure (mmHg)	75.9 ± 12.1	83.8 ± 13.6	0.03
Smoking (yes (%))[a]	12 (48%)	29 (81%)	0.008
High alcohol intake (yes (%))	0 (0%)	4 (11%)	0.14
Diabetes (yes (%))	0 (0%)	8 (22%)	0.02
Hypertension (yes (%))	3 (12%)	10 (28%)	0.21
General biochemistry			
Fasting glucose (mmol/L)	5.18 ± 0.98	7.07 ± 3.08	< 0.001
TSH (mIU/L)	1.82 ± 1.01	2.14 ± 1.00	0.26
Creatinine (μmol/L)	70.9 ± 18.7	86.5 ± 15.7	0.001
Total bilirubin (μmol/L)	10.4 ± 5.1	7.8 ± 5.5	0.10
Alkaline phosphatase (U/L)[b]	77.8 ± 30.8	86.1 ± 19.1	0.28
AST (U/L)[b]	20.1 ± 5.7	21.8 ± 6.9	0.35
ALT (U/L)[b]	17.8 ± 14.7	27.2 ± 14.3	0.001
GGT (U/L)[b]	16.7 ± 12.0	41.0 ± 27.6	< 0.001
CK (U/L)[b]	62.3 ± 32.7	110.2 ± 50.9	< 0.001
APOE allele frequency (n(%))			
E3	30 (88%)	41 (57%)	0.001
E2	3 (9%)	13 (18%)	0.21
E4	1 (3%)	18 (25%)	0.006
Lipids			
Triglycerides (mmol/L)	19.57 (10.08–28.37)	25.12 (14.98–33.39)	0.15
Total cholesterol (mmol/L)	6.28 (3.62–7.29)	8.82 (6.41–12.75)	< 0.001
LDL-C (mmol/L)	0.93 (0.61–1.43)	1.29 (0.81–1.62)	0.02
HDL-C (mmol/L)	0.39 ± 0.11	0.49 ± 0.12	0.002
Non-HDL-C (mmol/L)	5.84 (3.25–6.72)	8.29 (5.84–12.23)	< 0.001
Lipoprotein (a) (g/L)	0.03 (0.03–0.06)	0.02 (0.01–0.15)	0.40
Clinical manifestations of hypertriglyceridemia			
Eruptive xanthomas (yes (%))	3 (12%)	4 (11%)	1.00
Lipaemia retinalis (yes (%))	9 (36%)	9 (25%)	0.35
Hepatosplenomegaly (yes (%))	3 (12%)	6 (18%)	0.72
Abdominal pain (yes (%))	15 (63%)	7 (19%)	0.001
Medication			
Fibrate at baseline (yes (%))	8 (32%)	14 (39%)	0.58
Statin at baseline (yes (%))	2 (8%)	7 (19%)	0.29
Hx of MCT oil use (yes (%))	12 (48%)	0 (0%)	< 0.001
Hx of OCT (yes (% of women)	5 (31%)	0 (0%)	0.20
Hx of HRT (yes (%of women)	2 (13%)	3 (75%)	0.01

The non-parametric Mann-Whitney U test was used for age at first manifestation, fasting glucose, ALT and GGT. LDL-C, total cholesterol, TG, Lp(a) and non-HDL-C were log-transformed prior analysis. For fibrate or statin, past or current use was considered. High alcohol intake was defined by an intake ≥22 consumptions/week. Blood pressure was measured in seated or standing position. ALT: alanine aminotransferase; *APOE*: apolipoprotein E; AST: aspartate aminotransferase; BMI: body mass index; CK: creatine kinase; FCS: familial chylomicronemia syndrome; GGT: gamma-glutamyl transferase; HDL-C: high-density lipoprotein cholesterol; HRT: hormone replacement therapy; Hx: history; LDL-C: low-density lipoprotein cholesterol; MCM: multifactorial chylomicronemia; MCT: medium chain triglycerides; OCT: oral contraceptive therapy; TSH: thyroid stimulating hormone.

[a] Past or current smoker.

[b] The conversion factor to transform U/L in μkat/L (SI unit) is 0.0167.

3.2. Reason for chylomicronemia ascertainment

Fig. 1 presents the reason for chylomicronemia discovery. The main difference between both groups is the higher proportion of incidental

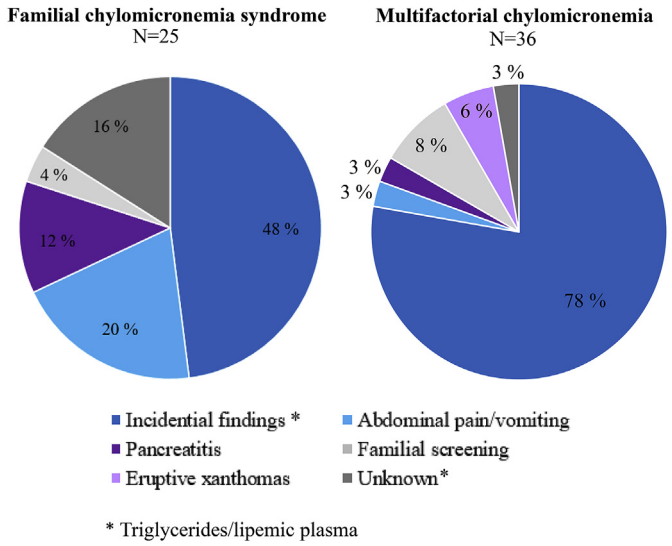

Fig. 1. Reason of discovery in FCS patients compared to MCM patients.
FCS: familial chylomicronemia syndrome; MCM: multifactorial chylomicronemia.

finding (e.g: high TG levels at the annual medical checkup) in the MCM group (78%) than in the FCS group (48%). Furthermore, abdominal pain and pancreatitis accounted for a third of the FCS discovery whereas this accounted for only 6% of MCM discoveries.

3.3. Complications of hypertriglyceridemia and metabolic features prevalence

The prevalence of three metabolic features, namely BMI ≥27 kg/m^2, SBP ≥130 mmHg or DBP ≥85 mmHg (or treatment for hypertension) and fasting glucose ≥ 5.6 mmol/L (or treatment for diabetes) was also studied. As presented in Fig. 2, a higher proportion of FCS patients were free of metabolic abnormality compared to MCM patients (55% vs 6%, respectively). Contrarily, the proportion of MCM patients carrying 2 or 3 metabolic features was higher than in the FCS patients (67% *vs*. 10%, respectively). The global *p* value for the difference of proportion between 0, 1 or ≥2 metabolic features was significant ($p < 0.0001$).

Differences in the prevalence of pancreatitis, multiple pancreatitis and cardiovascular disease (CVD) are presented in Fig. 3. A history of pancreatitis and multiple pancreatitis were more prevalent in the FCS group (60% and 48%, respectively) than in the MCM group (6% and 3%, respectively, $p < 0.0001$ for both associations). The prevalence of CVD (0% *vs*. 17%) was not statistically different between groups although a trend was observed ($p = 0.07$).

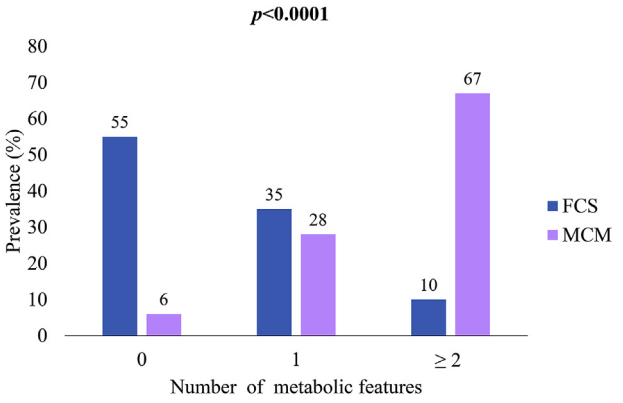

Fig. 2. Metabolic features prevalence.
FCS: familial chylomicronemia syndrome; MCM: multifactorial chylomicronemia. *p* value for Chi^2.

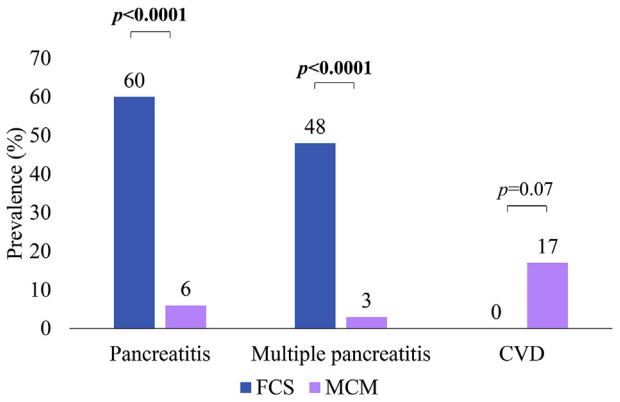

Fig. 3. Complications of hypertriglyceridemia.
Multiple pancreatitis for ≥2 episodes of pancreatitis. CVD: cardiovascular disease; FCS: familial chylomicronemia syndrome; MCM: multifactorial chylomicronemia. *p* values for Chi^2.

Table 2
Predictors of FCS.

Characteristics	Model 1			Model 2		
	β	Rank	*p* value	β	Rank	*p* value
Age at first manifestation	−2.11	1	0.0005	−2.45	1	0.003
BMI	−1.82	2	< 0.001	−1.51	2	0.004
GGT	−1.64	3	0.001	−1.17	3	0.03
Glycemia	−1.35	4	0.02	−0.65	7	0.18
Total cholesterol	−1.04	5	0.002	−0.63	8	0.09
Non-HDL-C	−0.99	6	0.002	−0.57	9	0.11
History of pancreatitis	0.81	7	0.0001	0.96	4	0.0003
CK	−0.80	8	0.001	−0.53	11	0.07
Systolic blood pressure	−0.65	9	0.009	−0.48	13	0.06
Creatinine	−0.55	10	0.004	−0.35	14	0.15
HDL-C	−0.54	11	0.004	−0.88	5	0.005
ALT	−0.50	12	0.03	−0.24	17	0.26
Abdominal pain	0.49	13	0.001	0.70	6	0.002
LDL-C	−0.39	14	0.04	−0.49	12	0.08
Diastolic blood pressure	−0.36	15	0.04	−0.15	21	0.44
Total bilirubin	−0.26	16	0.10	0.57	10	0.01
Alkaline phosphatase	−0.21	17	0.22	−0.22	18	0.28
TSH	−0.19	18	0.26	−0.29	15	0.22
AST	−0.15	19	0.35	0.05	24	0.82
Lipaemia retinalis	0.13	20	0.36	0.27	16	0.14
Lipoprotein (a)	−0.13	21	0.50	−0.14	22	0.56
Triglycerides	−0.12	22	0.46	0.16	20	0.36
Hepatosplenomegaly	−0.09	23	0.55	0.13	23	0.44
Eruptive xanthomas	0.02	24	0.92	0.19	19	0.29

Model 1: Uncorrected logistic regression. Model 2: Logistic regression corrected for age and sex.
ALT: alanine aminotransferase; AST: aspartate aminotransferase; BMI: body mass index; CK: creatine kinase; FCS: familial chylomicronemia syndrome; GGT: gamma-glutamyl transferase; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; TSH: thyroid stimulating hormone.

3.4. Predictors of FCS

Logistic regression analysis was performed to predict the FCS diagnosis. As presented in Table 2, predictors have been classified in descending order according to their absolute β-coefficient value. In a univariate model, age at first manifestation presented the strongest association with FCS (β-coefficient of −2.11) followed by BMI (β = −1.82) and GGT (β = −1.64). The top 3 variables significantly associated with FCS in the model corrected for age and sex appeared ranked in the same order as in the uncorrected model: age at first manifestation (β = −2.45), BMI (β = −1.51) and GGT (β = -1.17).

4. Discussion

FCS is a rare autosomal recessive disorder characterized by very high fasting TG levels and a high lifelong risk of life-threatening acute pancreatitis. This disease is also associated with considerable psychosocial burdens and a reduced quality of life [8]. However, it remains difficult to differentiate between the monogenic and the multifactorial form of chylomicronemia when genetic testing is not available.

In the present study, we describe the clinical profile of 25 genetically confirmed FCS patients compared to 36 MCM patients. The novel aspect of this study is the fact that we identified variables that clearly differentiate FCS from MCM subjects. Furthermore, our study used a strict definition for both of these diseases limiting the heterogeneity due to an erroneous diagnosis. It is interesting to note that, on average, MCM patients were identified later in life and through incidental findings on laboratory testing whereas many FCS patients were identified at a younger age and more frequently because of clinical findings such as pancreatitis and abdominal pain. Indeed, these clinical findings are much more prevalent in FCS compared to MCM subjects. This could be explained by the fact that FCS patients have a much earlier onset of disease compared to MCM and therefore a much longer exposure to extremely elevated TG. Furthermore, it is possible that the underlying mechanism of the disease (monogenic mutation in *LPL* vs polygenic contribution) explains in part the differences in phenotype.

Unexpectedly, the levels of triglycerides did not significantly differentiate FCS from MCM patients. Indeed, both presented TG levels that were comparable and extremely high. High triglycerides level represents an important risk factor for pancreatitis. Indeed, the risk of acute pancreatitis is 10–20% for TG levels > 2000 mg/dL (22.58 mmol/L) [9]. Interestingly for the same levels of TG, FCS patients had a 10-fold (60% in the FCS group *vs*. 6% in the MCM group) higher frequency of pancreatitis compared to MCM patients. Again, this is most likely due to the fact that FCS patients present a lifelong increase in TG, putting them at risk of pancreatitis for a much longer period than MCM patients who tend to have increased TG later in life. The higher prevalence of pancreatitis in FCS than in MCM have been previously reported [10].

GGT represents a biomarker of non-alcoholic fatty liver disease (NASH) and high GGT levels have also been associated with an increased risk of cardiometabolic diseases in prospective studies [11]. Indeed, in our cohort, MCM patients presented significantly more metabolic abnormalities than FCS patients. Previous studies have clearly established a link between diabetes, obesity, the metabolic syndrome and NASH [12]. Therefore, this could explain the higher levels of GGT observed in MCM compared to FCS patients in our cohort.

Interestingly, in the present study, the *APOE* 4 allele frequency was 8-time more elevated in the MCM group than in the FCS group. This increased frequency of *APOE* 4 allele in MCM patients compared to the general population has been reported previously [13].

There exists a controversy concerning the risk of CVD in patients with chylomicronemia. Current evidence does not support a causal role of chylomicron in atherosclerosis [1]. However, earlier studies did describe FCS subjects with premature atherosclerosis [14]. In contrast, MCM patients often present with other lipid disturbances as well as a worst metabolic profile that predispose to a proatherogenic state. No patient in our FCS cohort presented cardiovascular events. Our study tends to indicate that the risk of CVD is much lower in FCS compared to MCM subjects although our study lacked the power to answer this question. Interestingly, a previous study showed that MCM patients present a 2-fold higher risk of CVD than normolipemic controls [10].

Some limitations of the present study should be kept in mind. Firstly, the vast majority of FCS patients in our cohort are homozygous or compound heterozygous carriers of *LPL* mutations. However, it has been shown recently that there exist phenotypic differences between *LPL*-FCS and non-*LPL*-FCS [15]. Therefore, it would be interesting to verify if the clinical variables associated with FCS in our cohort present a similar association in the different FCS subtypes. In addition, because of the limited genotype variability and the higher proportion of FCS found in the Province of Quebec, the results of the present study may not be fully generalizable. Secondly, MCM patients were not tested for the non-French Canadian mutations in the *LPL* gene or in other FCS-causing mutations. Therefore, it is possible, but very unlikely due to the presence of increased VLDL that some MCM patients may have been misclassified. However, such misclassification would mean that the differences observed between groups would be smaller than that would be observed with a “pure” MCM group. Furthermore, because of the small sample size of the present study, the multivariate regression analysis of FCS predictor was not possible. A larger cohort of FCS and MCM patients should be established in order to go further with the analysis and to assess the independent predictors of FCS. Finally, insulin (required to calculate indexes of insulin resistance) and apolipoprotein B were not available in the medical files of these patients but would have been of major interest in this study.

In conclusion, our study is the first that systematically compare the clinical and biochemical characteristics of genetically confirmed FCS patients *vs*. MCM patients. The frequencies of abdominal pain, pancreatitis and multiple episodes of pancreatitis were all greater in the FCS group, whereas the prevalence of patients with 2 or more metabolic abnormalities was higher in the MCM group. In addition, there exists a trend towards a higher prevalence of CVD in the MCM compared to the FCS groups. We have identified several clinical variables that significantly differentiate these two disorders in a univariate model, including age at first manifestation, BMI and GGT. However, these findings need to be replicated in larger cohorts, which will allow the identification of independent predictors of FCS.

Conflict of interest

A.B. received research grants from Merck Frosst, Amgen, Sanofi, Astra Zeneca and the Fondation Leducq. He has participated in clinical research protocols from Pfizer, Regeneron Pharmaceuticals Inc., The Medecines Company, Amgen, Acasti Pharma Inc., Novartis, Sanofi, Ionis Pharmaceuticals, Inc., Astra Zeneca, Akcea and Merck Frosst. He has served on advisory boards and received honoraria for symposia from Amgen, Akcea and Sanofi.

S.B. has participated in clinical research protocols from Akcea, The Medecines Company and Sanofi. She has served on advisory boards for Novo Nordisk, Merck Frosst, Valeant Pharmaceuticals, Eli Lilly and Amgen and received honoraria for symposia from Sanofi-aventis, Merck Frosst, Amgen, Novo Nordisk, Valeant Pharmaceuticals and Boehringer Ingelheim.

R.A.H. has received honoraria for membership on advisory boards and speakers' bureaus for Aegerion, Akcea/Ionis, Amgen, Gemphire, Lilly, Merck, Pfizer, Regeneron, and Sanofi.

M.P. has nothing to declare.

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Author contributions

All authors contributed to the discussion, analysis and interpretation of data and have drafted and reviewed the article for the intellectual content. All authors have approved the final article. A.B. had primary responsibility for final content.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.atherosclerosis.2018.12.019.

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