Differentiating Familial Chylomicronemia Syndrome From Multifactorial Severe Hypertriglyceridemia by Clinical Profiles

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Context: Differentiation between familial chylomicronemia syndrome (FCS, type 1 hyperlipoproteinemia), a rare metabolic disorder, and the more common multifactorial severe hypertriglyceridemia (sHTG, type 5 hyperlipoproteinemia) is challenging because of their overlapping symptoms but important in patient management.

Objective: To assess whether readily obtainable clinical information beyond triglycerides can effectively diagnose and differentiate patients with FCS from those with sHTG, based on well-curated data from two intervention studies of these conditions.

Methods: The analysis included 154 patients from two phase 3 clinical trials of patients with sHTG, one cohort with genetically confirmed FCS (n = 49) and one with multifactorial sHTG (n = 105). Logistic regression analyses were performed to determine the ability of variables (patient demographics, medical history, and baseline lipids, individually or in sets) to differentiate the patient populations. Receiver operating characteristics were used to determine the variable sets with the highest accuracy (percentage of times actual values matched predicted) and optimal sensitivity and specificity.

Results: The primary model diagnosed 45 of 49 patients with FCS and 99 of 105 patients with sHTG correctly. Optimal sensitivity for all available parameters (n = 17) was 91.8%, optimal specificity was 94.3%, and accuracy was 93.5%. Fasting low-density lipoprotein cholesterol (LDL-C) provided the highest individual predictability. However, a three-variable set of ultracentrifugally measured LDL-C, body mass index, and pancreatitis history differentiated the diseases with a near similar accuracy of 91.0%, and adding high-density lipoprotein cholesterol and very low-density lipoprotein cholesterol for a five-variable set provided a small incremental increase in accuracy (92.2%).

Conclusions: In the absence of genetic testing, hypertriglyceridemic patients with FCS and sHTG can be differentiated with a high degree of accuracy by analyzing readily obtainable clinical information.
Overlapping serum triglyceride (TG) levels and similar symptoms make differentiating the more common severe hypertriglyceridemia (sHTG) from the far more rare and serious disorder of familial chylomicronemia syndrome (FCS) a challenging clinical proposition. However, an accurate diagnosis is paramount for appropriate disease management given the high frequency of acute and life-threatening episodes of pancreatitis associated with FCS.

FCS is an ultra-rare metabolic disorder with identifiable genetic underpinnings related to defective or absent lipoprotein lipase (LPL) activity [1–5]. LPL is a key enzyme in the hydrolysis of TGs in triglyceride-rich lipoprotein (TRL) particles [1, 4]. A biallelic genetic deficiency of LPL leads to sustained plasma accumulation of chylomicrons (large TRL of intestinal origin), lipid absorption resulting in plasma TG levels 10 to 100 times the normal level (>150 mg/dL) [6].

Physical manifestations of FCS interfere with normal life and include recurrent episodes of severe abdominal pain, with or without pancreatitis. Other clinical manifestations include eruptive xanthomata, lipemia retinalis, and hepatosplenomegaly [1, 2, 7]. Patients with FCS may experience symptoms from the early postnatal period onward, with a clustering in late adolescence; however, symptoms can present at any point in a patient’s life, and there may be a prolonged delay before a confirmed diagnosis is made [8]. Symptom severity may also vary across patients, with a minority of patients remaining free from episodes of acute pancreatitis. Pancreatitis is the most serious clinical complication of FCS and may be fatal [4]. In general, frequency and severity correlate with serum TG levels [9–11]. Treatment of patients with FCS is by severe restriction of dietary fat (<15 to 20 g of fat per day) and avoidance of alcohol and conventional TG-lowering medications including omega-3 fatty acids, fibrates, and niacin, even though their effect in these patients is extremely limited [1, 2, 12].

High serum TG levels and chylomicronemia are also characteristic of sHTG, a disease with similar presentation to FCS but different in underlying etiology [13]. sHTG is a polygenic disease that results from an accumulation of genetic TG-raising variants together with modulating secondary factors, including certain drugs, obesity, uncontrolled diabetes mellitus, poor diet, and alcohol intake, which drives susceptibility to higher TGs and generally presents in adulthood [1, 2]. As with FCS, patients with sHTG can have fasting TG levels >880 mg/dL, putting them at an increased risk of acute pancreatitis [14–16]. Most genetically defined patients with sHTG have polygenic susceptibility consisting of accumulation of rare heterozygous DNA variants and common small-effect DNA polymorphisms [17].

FCS has a global prevalence of 1 to 2 in 1,000,000 [1], which is significantly lower than the global prevalence of sHTG (1:600) [1], but it occurs much more frequently in certain areas because of regional founder effects. Although health care providers may infrequently see patients with FCS, enabling physicians to more easily differentiate between these two conditions is essential to facilitate appropriate therapy selection and disease management. Differentiating FCS from sHTG can be challenging, and although genetic testing may be helpful, it is not always readily available. Moreover, some patients with FCS lack the currently identified genetic markers of the disease and may therefore pose an additional challenge in differential diagnosis. A diagnostic tool based on readily available clinical data, independent of genetic data, would be an attractive option in differentiating these two conditions. A recently released diagnostic scoring system for FCS provided some insight into differentiating FCS from sHTG [18]. It considered a number of biochemical and clinical parameters but relied heavily on serum TG levels. This diagnostic scoring system was
developed as an initiative of a panel of experts arising from discussions at a sponsored meeting. Nevertheless, it has been reported that FCS is also characterized by abnormalities in other components of lipid profile and in several clinical variables [2]. Therefore, it was thought that an alternative differentiation tool that incorporates the full spectrum of lipid parameters and clinical variables would be of greater utility in differentiating FCS from sHTG. To this end, the clinical data from two phase 3 studies that evaluated the efficacy of volanesorsen (a second-generation antisense oligonucleotide inhibitor of apolipoprotein C-III production) for reduction of serum TG levels in patients with FCS (APPROACH) and sHTG (COMPASS) [19, 20] were analyzed to determine whether clinical data, other than fasting TG levels, can differentiate these two diseases. Clinical variables such as body mass index (BMI), pancreatitis history, and components of the lipid profile were analyzed individually and in groups to develop a predictive diagnostic tool that could provide physicians with a highly accurate preliminary differentiation between FCS and sHTG to assist clinical decision making and patient care.

1. Methods

A. Study Participants: APPROACH and COMPASS

Patients included in this analysis were from the phase 3, multicenter, randomized, double-blind, placebo-controlled APPROACH and COMPASS studies of subcutaneous volanesorsen treatment in adult patients [19, 20]. In brief, the APPROACH study (NCT02211209) [21] (n = 67) evaluated 300 mg volanesorsen once weekly for 52 weeks in patients with FCS, with 13 weeks of follow-up. Only patients who had genetically confirmed FCS were included in the analysis [22]. Patients had fasting TG levels \( \geq 750 \text{ mg/dL} \) (\( \geq 8.5 \text{ mmol/L} \)) at screening and a documented history of acute pancreatitis and were willing to follow a restrictive diet limited to \( \leq 20 \text{ g of fat per day} \) during the study lead-in and treatment periods. The COMPASS study (NCT02300233) [23] (n = 114) evaluated 300 mg volanesorsen once weekly for 26 weeks for patients with polygenic sHTG (non-FCS), with 13 weeks of follow-up. Patients had fasting TG levels \( \geq 500 \text{ mg/dL} \) (\( \geq 5.7 \text{ mmol/L} \)) at screening, BMI \( \leq 45 \text{ kg/m}^2 \), with stable weight for \( >6 \text{ weeks} \) before screening, and were willing to follow a restricted fat diet. Patients with FCS from COMPASS were excluded from the analysis. Also of note, ultracentrifugation was used to measure lipid parameters, including low-density lipoprotein cholesterol (LDL-C) (i.e., instead of the Friedewald equation). Additional key inclusion and exclusion criteria for both studies are shown in Table 1.

B. Statistical Analyses

Demographic variables, baseline lipid and laboratory values, previous medications, and medical history were included in the analysis (Table 2). Importantly, values shown in Table 2 include all patients who met the inclusion requirements for the analysis; however, one patient from each disease state was missing one or more of the variables to be analyzed and therefore was not included. Logistic regression methods were performed to determine the ability of these variables to differentiate between the FCS and sHTG (non-FCS) populations, that is, to provide the estimated probability of a patient being in the FCS population, based on the listed predictor variables. A log transformation was applied to the lipid values to provide a more normal distribution of the variables. For each variable, a cutoff value was determined, based on maximizing the sum of sensitivity and specificity (i.e., Youden index), to enable classification of patients as having either FCS or sHTG.

Sensitivity was defined as correctly predicting patients with FCS, and specificity was defined as correctly predicting patients with sHTG. Positive predictive value (PPV) is the probability a patient was in the FCS population, given that the patient was predicted to be in the FCS population. Negative predictive value (NPV) is the probability a patient was in the non-FCS population, given that the patient was predicted to be in the non-FCS population. Accuracy is the percentage of times a patient was correctly classified.
The accuracy of a test to discriminate between cases with and without a disorder may be evaluated via receiver operating characteristic (ROC) curve analysis [24], with an area under the curve (AUC) value approaching 1.0 indicating a high sensitivity and specificity. This technique was used for each logistic regression analysis, to determine the highest accuracy (i.e., sensitivity + specificity) by using the predicted probability of being in the FCS population.

### Table 1. Key Inclusion and Exclusion Criteria for the APPROACH and COMPASS Studies

| Key Inclusion Criteria | Key Exclusion Criteria |
|------------------------|------------------------|
| **APPROACH**           |                        |
| Adults aged ≥18 y providing written, informed consent | Active pancreatitis within 4 wk of screening |
| History of chylomicronemia evidenced by documentation of: | Diagnosis of diabetes mellitus within 12 wk of screening |
| - Lactescent serum | sHTG not due to FCS |
| - Fasting TG measurement ≥880 mg/dL (10 mmol/L) | |
| Diagnosis of FCS (type 1 hyperlipoproteinemia) evidenced by ≥1 of the following: | |
| - Confirmed homozygote, compound heterozygote, or double heterozygote for known loss-of-function mutations in type 1-causing genes (e.g., LPL, APOC2, GPIHBP1, or LMF1) | |
| - Postheparin plasma LPL activity ≥20% of normal | |
| Fasting TG ≥750 mg/dL (≥8.4 mmol/L) at screening | |
| Willingness to follow a restricted diet of ≤20 g of fat per day | |
| Documented history of pancreatitis diagnosis or hospitalization for severe abdominal pain consistent with acute pancreatitis and for which no alternative diagnosis was made | |
| **COMPASS**            |                        |
| Adults aged ≥18 y providing written, informed consent | Type 1 diabetes mellitus |
| BMI ≤45 kg/m² | Type 2 diabetes mellitus within 12 wk of screening |
| Stable weight (±4 kg) for >6 wk before screening | Acute pancreatitis within 3 mo of screening |
| Fasting TG ≥500 mg/dL (≥5.7 mmol/L) at screening | |
| **Section 1.01:** Patients receiving statin or fibrate were required to be on stable, labeled dose for ≥3 mo before screening, which was not expected to change during the treatment period | |
| **Section 1.02:** Fasting TG ≥500 mg/dL at qualification visit | |
| Willing to maintain their customary activity level and to follow NCEP ATP III TLC diet, or similar, with weight maintenance during the study | |

For full inclusion and exclusion criteria, see [21, 23].
Abbreviations: APOC2, apolipoprotein C2; GPIHBP1, glycosylphosphatidylinositol-anchored high-density lipoprotein-binding protein 1; LMF1, lipase maturation factor 1; NCEP ATP III TLC, National Cholesterol Education Program Adult Treatment Panel III Therapeutic Lifestyle Changes.

*a* If fasting TG was <750 mg/dL, ≤2 additional tests could be performed to qualify.

*b* Patients without a documented history of pancreatitis were also eligible, but their enrollment was capped at 28% (i.e., ≤20 of the 70 planned patients).

*c* If the fasting TG value at screening was ≥500 mg/dL (≥5.7 mmol/L) but ≤350 mg/dL (≥4.0 mmol/L), ≤2 additional tests could be performed to qualify.

*d* Patients discontinuing these drugs within 4 wk before screening were also eligible to enroll.
The primary logistic regression model included all predictor variables (as continuous variables) in Table 2, excluding fasting TGs because it is known that both of these patient populations have severely elevated TGs.

Limiting the set of dichotomous variables (i.e., sets of three, four, and five dichotomous variables) was evaluated to determine the most efficient algorithm to differentiate FCS and sHTG populations. Patients were predicted to be in the FCS population if the majority of a patient’s dichotomous variables were predictive of being in the FCS population (e.g., for the three-variable model, if the patient had two or three variables associated with FCS, the patient would be predicted to be in the FCS population, and if the patient had zero or one variable associated with FCS, the patient would be predicted to be in the non-FCS population). For the four-variable scenario, patients meeting three or four variables were predicted to be in the FCS population, and those meeting zero, one, or two variables were predicted to be in the non-FCS population.

2. Results

A. Demographic and Clinical Characteristics

Key demographic and clinical characteristics of patients included in the analysis are shown in Table 2. Of note, patients with sHTG (COMPASS) were predominantly male (79.2%), in contrast to patients with FCS (46.0%) (APPROACH). Mean fasting TG levels were >1000 mg/dL for both sample populations, putting both at increased risk of acute pancreatitis. The fasting levels for numerous parameters were substantially different between the two populations. Aside from total TG values, patients with FCS have lower ultracentrifugally determined lipoprotein profile values (LDL-C, very low-density lipoprotein cholesterol (VLDL-C), high-density lipoprotein cholesterol (HDL-C), very low-density lipoprotein triglyceride (VLDL-TG)) and lower BMI compared with patients with sHTG. Regarding medical history, patients with sHTG had a higher rate of diabetes than those with FCS (34.9% vs 12.0%); all had type 2 diabetes. However, patients with FCS had a much higher rate of previous pancreatitis (86% vs 21.7%). Patients from both disease states reported use of

| Variable                              | APPROACH (n = 49) | COMPASS (n = 105) |
|----------------------------------------|-------------------|-------------------|
| Age, y, mean (SD)                      | 45.4 (14)         | 51.5 (10)         |
| Male, %                                | 46.0a             | 79.2b             |
| Mean baseline BMI, kg/m²               | 23.9              | 31.6b             |
| Mean fasting TG, mg/dL                | 2320.8            | 1193.6            |
| Mean fasting LDL-C, mg/dL             | 25.6              | 63.5b             |
| Mean fasting VLDL-C, mg/dL            | 38.5              | 65.9b             |
| Mean fasting cholesterol, mg/dL       | 294.2             | 263.5b            |
| Mean fasting HDL-C, mg/dL             | 16.3              | 25.1b             |
| Mean fasting non-HDL-C, mg/dL         | 277.9             | 238.4b            |
| Mean fasting APOB100, mg/dL           | 64.9              | 99.6b             |
| Mean fasting APOA1, mg/dL             | 95.1              | 128.3b            |
| Mean fasting VLDL-TGs, mg/dL          | 291.2             | 405.5             |
| Prior use of fibrate, %               | 38.0a             | 39.6b             |
| Prior use of statin, %                | 12.0a             | 48.1b             |
| Prior use of other lipid-modifying agents, % | 32.0a             | 34.9b             |
| Cardiac medical history, %            | 2.0a              | 9.4b              |
| Pancreatitis medical history, %       | 86.0a             | 21.7b             |
| Diabetes medical history, %           | 12.0a             | 34.9b             |

Abbreviations: APOA1, apolipoprotein A1; APOB100, apolipoprotein B100.

aBased on n = 50.
bBased on n = 106.
lipid-lowering medications, such as fibrates and statins (38% and 12.0% of patients with FCS and 39.6% and 48.1% of patients with sHTG, respectively).

### B. Differentiation Analysis

Figure 1 illustrates the predicted probabilities of being in the FCS or sHTG sample population, derived from the primary logistic regression model that includes all predictor variables (N = 17) as continuous variables (Table 2), excluding fasting TGs, which are severely elevated in both populations. There is a distinct separation between the FCS and sHTG populations. The optimal results of the logistic regression were defined as maximizing both sensitivity and specificity. The optimal sensitivity was 91.8%, based on 45 of 49 patients with FCS being correctly classified by the model. The optimal specificity was 94.3%, based on 99 of 105 patients with sHTG being correctly classified by the model. The overall accuracy was 93.5%, based on these combined results, reflecting the percentage of times the predicted values matched the actual values.

When the ability of individual variables to differentiate between the two populations was evaluated, a dichotomous cutoff point was used based on ROC values to classify patients into the FCS or sHTG populations (Table 3). The parameter with the highest sensitivity plus specificity outcome (170.0%) and highest ROC AUC (0.902) was fasting ultracentrifugally determined LDL-C (Table 4). Apolipoprotein A1 and apolipoprotein B100 also had high sensitivity and specificity (Table 4). The remaining variables tended to have high values for either sensitivity or specificity but not both, indicating that they were effective at predicting only one disease state.

The dichotomous variables listed in Table 3 were analyzed in sets of three to five to assess which combination of variables would provide the highest differentiation between FCS and sHTG disease states. When three variables were analyzed (Table 5), the top five sets, when sorted based on the highest sensitivity plus specificity value, all involved BMI and pancreatitis. These clinical parameters did not have the highest values when evaluated as individual variables (Table 3). Other parameters that featured in the top five three-variable sets were fasting apolipoprotein level (A1, B-100) and cholesterol level (LDL-C, VLDL-C).

When sets of four variables were considered (Table 6), accuracy levels >86% were observed in all sets. However, none of these was as high as the leading three-variable sets, which had accuracy levels >90% (Table 5). The top four-variable set (Table 6, Obs 1) included the same parameters as one of the top five three-variable sets (BMI, pancreatitis, ultracentrifugally determined LDL-C, Table 5, Obs 5) with the addition of VLDL-C.

Results from analysis of sets of five variables (Table 7) indicate that the top two performing sets (Obs 1 and Obs 2) include the same parameters from the best four-variable set (BMI, pancreatitis, ultracentrifugally determined LDL-C, VLDL-C) with the addition of HDL-C or apolipoprotein A1. Overall, these groups of parameters were those that had the highest ROC AUC values, in the analysis of individual variables (Table 3).

In summary, the best performing three-, four-, and five-variable sets were able to differentiate between FCS and sHTG samples with accuracy values of 92.9%, 89.0%, and 92.9%, respectively.

### 3. Discussion

Based on the study populations, our analysis suggests that readily obtainable clinical information may be used with a high degree of certainty to differentiate patients with FCS from those with sHTG. These specific clinical features have the potential to be a preliminary differential diagnostic tool, particularly when genetic testing is not available or when testing may be obtained only after a long delay. Additionally, when genetic testing is more readily available, these data may help prioritize or deprioritize patients for testing. Earlier diagnosis could also facilitate the implementation of appropriate treatment strategies in the most expedient way possible.
Figure 1. Predicted probabilities of being in FCS or sHTG sample populations. Probabilities closest to 1.0 indicate a greater likelihood of having FCS, and probabilities closest to 0.0 indicate a greater likelihood of having sHTG.
In our analysis, patients with genetically confirmed FCS and sHTG were differentiated with >90% accuracy by using only three readily available clinical variables. The five most accurate three-variable analysis sets all included two factors that can be obtained from clinical evaluation and medical history (BMI, history of pancreatitis). Which of the five third variables is used changes the accuracy by 2% and depends on what is available to the clinician. This study used ultracentrifugally determined LDL-C, which is not widely available, and so the analysis was repeated with the more readily available apolipoprotein B, which provided comparable accuracy to ultracentrifugally determined LDL-C: 91.6% vs 91.0%, respectively. A five-variable analysis set, comprising three variables in addition to BMI and pancreatitis history, also differentiated patients with FCS and sHTG with >90% accuracy. The primary analysis had the greatest accuracy at 93.5% but required 17 variables and therefore had minimal incremental gain over the above data. The four-variable analysis had a lower accuracy than the three-variable but also required the highest percentage of variables predicting FCS for a majority (75%, 3 of 4), compared with the three- (67%, 2 of 3) and five- (60%, 3 of 5) variable analyses. The accuracy of these analyses is even more impressive when compared with reports of familial hypercholesterolemia studies that used predominantly calculated LDL-C values, age, tendon xanthomas, and corneal arcus, with an accuracy of <75% [25, 26].

### Table 3. Dichotomous Variable Cutoff Points for Classification as FCS

| Variable                  | Cutoff Point for FCS |
|---------------------------|----------------------|
| Fasting LDL-C, mg/dL      | <39.2                |
| Fasting APOA1, mg/dL      | <118.3               |
| Fasting APOB100, mg/dL    | <77.2                |
| BMI, kg/m²                | <26.1                |
| Fasting HDL-C, mg/dL      | <21.9                |
| Pancreatitis history      | Yes                  |
| Fasting TG, mg/dL         | >1406.6              |
| Fasting VLDL-C, mg/dL     | <37.9                |
| Fasting VLDL-TG, mg/dL    | <247.9               |

Table 4. Ability of Individual Variables to Differentiate Between FCS and sHTG Populations

| Variable                  | ROC AUC | Sensitivity, % (n) | Specificity, % (n) | PPV, % (n) | NPV, % (n) | Accuracy, % (n) | Sensitivity + Specificity, % |
|---------------------------|---------|--------------------|--------------------|------------|------------|-----------------|-----------------------------|
| Log fasting LDL-C         | 0.902   | 89.8 (44/49)       | 80.2 (44/556)      | 67.7 (44/65) | 94.4 (85/90) | 83.2 (129/155) | 170.0                      |
| Log fasting APOA1         | 0.8971  | 89.8 (44/49)       | 73.6 (78/106)      | 61.1 (44/72) | 94.0 (78/83) | 78.7 (122/155) | 163.4                      |
| Log fasting APOB100       | 0.8852  | 83.7 (41/49)       | 83.0 (88/106)      | 69.5 (41/59) | 91.7 (88/96) | 83.2 (129/155) | 166.7                      |
| BMI                       | 0.873   | 73.5 (36/49)       | 91.5 (97/106)      | 80.0 (36/45) | 88.2 (97/110) | 85.8 (133/155) | 165.0                      |
| Log fasting HDL-C         | 0.8673  | 93.9 (46/49)       | 67.0 (71/106)      | 56.8 (46/81) | 95.9 (71/74) | 75.5 (117/155) | 160.9                      |
| Pancreatitis history      | 0.8215  | 86.0 (43/50)       | 78.3 (83/106)      | 65.2 (43/66) | 92.2 (83/90) | 80.8 (126/156) | 164.3                      |
| Log fasting TG            | 0.8154  | 93.9 (46/49)       | 57.5 (61/106)      | 50.5 (46/91) | 95.3 (61/64) | 69.0 (107/155) | 151.4                      |
| Log fasting VLDL-C        | 0.7442  | 65.3 (32/49)       | 81.0 (85/105)      | 61.5 (32/52) | 83.3 (85/102) | 76.0 (117/154) | 146.3                      |
| Log fasting VLDL-TG       | 0.7233  | 57.1 (28/49)       | 88.6 (93/105)      | 70.0 (28/40) | 81.6 (93/114) | 78.6 (121/154) | 145.7                      |

Results are sorted based on highest sensitivity + specificity value.
Table 5. Sets of Three Dichotomous Variables for Differentiating FCS

| Obs | Variable 1     | Variable 2              | Variable 3       | Sensitivity, % | Specificity, % | PPV, %  | NPV, %  | Accuracy, % | Sensitivity + Specificity, % |
|-----|----------------|-------------------------|------------------|----------------|----------------|---------|---------|-------------|-------------------------------|
| 1   | Baseline BMI   | Pancreatitis history    | Fasting VLDL-C   | 89.8           | 94.3           | 88.0    | 95.2    | 92.9        | 184.1                         |
| 2   | Fasting APOA1  | Baseline BMI            | Pancreatitis history | 93.9           | 89.6           | 80.7    | 96.9    | 91.0        | 183.5                         |
| 3   | Fasting APOB100| Baseline BMI            | Pancreatitis history | 91.8           | 91.5           | 83.3    | 96.0    | 91.6        | 183.3                         |
| 4   | Baseline BMI   | Pancreatitis history    | Fasting VLDL-TGs | 87.8           | 95.2           | 89.6    | 94.3    | 92.9        | 183.0                         |
| 5   | Fasting LDL-C  | Baseline BMI            | Pancreatitis history | 91.8           | 90.6           | 81.8    | 96.0    | 91.0        | 182.4                         |
| 6   | Fasting LDL-C  | Pancreatitis history    | Fasting VLDL-C   | 91.8           | 88.6           | 78.9    | 95.9    | 89.6        | 180.4                         |
| 7   | Fasting LDL-C  | Pancreatitis history    | Fasting VLDL-TG  | 91.8           | 88.6           | 78.9    | 95.9    | 89.6        | 180.4                         |
| 8   | Fasting HDL-C  | Pancreatitis history    | Fasting VLDL-C   | 95.9           | 83.8           | 73.4    | 97.8    | 87.7        | 179.7                         |
| 9   | Fasting HDL-C  | Pancreatitis history    | Fasting VLDL-TG  | 93.9           | 85.7           | 75.4    | 96.8    | 88.3        | 179.6                         |
| 10  | Fasting LDL-C  | Fasting APOA1           | Baseline BMI     | 91.8           | 87.7           | 77.6    | 95.9    | 89.0        | 179.6                         |

Results are sorted based on highest sensitivity + specificity value.
Abbreviation: Obs, observation.
Table 6. Sets of Four Dichotomous Variables for Differentiating FCS

| Obs | Variable 1          | Variable 2       | Variable 3                | Variable 4          | Sensitivity, % | Specificity, % | PPV, % | NPV, % | Accuracy, % | Sensitivity + Specificity, % |
|-----|---------------------|------------------|---------------------------|---------------------|----------------|----------------|--------|--------|-------------|-----------------------------|
| 1   | Fasting LDL-C       | Baseline BMI     | Pancreatitis history      | Fasting VLDL-C      | 95.9           | 85.7           | 75.8   | 97.8   | 89.0        | 181.6                       |
| 2   | Fasting LDL-C       | Baseline BMI     | Pancreatitis history      | Fasting VLDL-TG     | 93.9           | 86.7           | 76.7   | 96.8   | 89.0        | 180.5                       |
| 3   | Fasting APOB100     | Baseline BMI     | Pancreatitis history      | Fasting VLDL-TG     | 91.8           | 87.6           | 77.6   | 95.8   | 89.0        | 179.5                       |
| 4   | Fasting LDL-C       | Fasting APOA1    | Fasting APOB100           | Baseline BMI        | 95.9           | 83.0           | 72.3   | 97.8   | 87.1        | 178.9                       |
| 5   | Baseline BMI        | Fasting HDL-C    | Pancreatitis history      | Fasting VLDL-TG     | 95.9           | 82.9           | 72.3   | 97.8   | 87.0        | 178.8                       |
| 6   | Fasting LDL-C       | Fasting APOA1    | Baseline BMI              | Fasting VLDL-TG     | 93.9           | 84.8           | 74.2   | 96.7   | 87.7        | 178.6                       |
| 7   | Fasting LDL-C       | Baseline BMI     | Fasting HDL-C             | Fasting VLDL-TG     | 93.9           | 84.8           | 74.2   | 96.7   | 87.7        | 178.6                       |
| 8   | Fasting APOB100     | Baseline BMI     | Pancreatitis history      | Fasting VLDL-C      | 93.9           | 84.8           | 74.2   | 96.7   | 87.7        | 178.6                       |
| 9   | Fasting LDL-C       | Fasting APOA1    | Baseline BMI              | Pancreatitis history| 95.9           | 82.1           | 71.2   | 97.8   | 86.5        | 178.0                       |

Results are sorted based on highest sensitivity + specificity value.
Table 7. Sets of Five Dichotomous Variables for Differentiating FCS

| Obs | Variable 1 | Variable 2 | Variable 3 | Variable 4 | Variable 5 | Sensitivity, % | Specificity, % | PPV, % | NPV, % | Accuracy, % | Sensitivity + Specificity, % |
|-----|------------|------------|------------|------------|------------|----------------|----------------|--------|--------|-------------|-------------------------------|
| 1   | Fasting LDL-C | APOA1      | Baseline   | Pancreatitis history | Fasting VLDL-C | 93.9           | 92.4           | 85.2   | 97.0   | 92.9        | 186.3                          |
| 2   | Fasting LDL-C | Baseline   | Fasting    | Pancreatitis history | Fasting VLDL-C | 93.9           | 91.4           | 83.6   | 97.0   | 92.2        | 185.3                          |
| 3   | Fasting LDL-C | APOA1      | Fasting    | Pancreatitis history | Fasting VLDL-TG | 91.8           | 92.4           | 84.9   | 96.0   | 92.2        | 184.2                          |
| 4   | Fasting LDL-C | Baseline   | Fasting    | Pancreatitis history | Fasting VLDL-TG | 91.8           | 91.4           | 83.3   | 96.0   | 91.6        | 183.3                          |
| 5   | Fasting LDL-C | Baseline   | Pancreatitis history | Fasting TG | Fasting VLDL-C | 91.8           | 91.4           | 83.3   | 96.0   | 91.6        | 183.3                          |
| 6   | Fasting LDL-C | Fasting    | Fasting    | Pancreatitis history | Fasting VLDL-C | 95.9           | 86.7           | 77.0   | 97.8   | 89.6        | 182.6                          |
| 7   | Fasting LDL-C | APOA1      | Fasting    | Pancreatitis history | Fasting VLDL-C | 93.9           | 88.7           | 79.3   | 96.9   | 90.3        | 182.6                          |
| 8   | Fasting LDL-C | APOA1      | Fasting    | Pancreatitis history | Fasting VLDL-TG | 93.9           | 88.7           | 79.3   | 96.9   | 90.3        | 182.6                          |
| 9   | Fasting LDL-C | Fasting    | Pancreatitis history | Fasting TG | Fasting VLDL-C | 93.9           | 88.6           | 79.3   | 96.9   | 90.3        | 182.4                          |

Results are sorted based on highest sensitivity + specificity value.
Patients from both disease states can present with fasting TG levels >880 mg/dL [15, 16]. Although fasting TG levels were higher for study patients with FCS than those with sHTG, they did not prove to be an effective parameter in differentiating the two disease states. Additionally, the goal of this study was to provide information beyond elevated TG levels for physicians to facilitate greater confidence in differentiating the two patient populations. The key identified variables from this study will work synergistically with other recently proposed diagnostic criteria [18] to maximize physician confidence in preliminary diagnosis of patients with FCS.

A reduced body mass is often associated with patients with FCS, as was observed in the phase 3 APPROACH study [20, 27]. Patients with FCS report numerous recurrent symptoms that could contribute to lower BMI, such as the severely low-fat diet they require and the postprandial pain they commonly report. Less well recognized are the eating disorders reported by a quarter of patients, which include self-induced emesis, to mitigate abdominal symptoms, particularly if incipient symptoms of pancreatitis are suspected [8, 28].

Elevated TG levels are associated with an increased risk of pancreatitis, especially as levels that exceed 880 mg/dL. Hypertriglyceridemia is the third leading cause of pancreatitis, but patients with FCS are at an even greater risk, with 65% to 80% experiencing at least one episode of pancreatitis [29]. The significantly higher risk of pancreatitis for patients with FCS has been proposed to be caused by the predominance of chylomicrons in these patients, which are involved in multiple mechanisms of pancreatitis [28, 29].

Despite the severely elevated chylomicron levels of patients with FCS, other lipoprotein levels are often reduced. All the variable analyses included lipoprotein or apolipoprotein parameters, with the five-variable analysis using LDL-C, VLDL-C, and HDL-C. In our analysis, apolipoprotein B was interchangeable with the less available determination of LDL-C by ultracentrifugation, facilitating its choice to make this differentiation of FCS from sHTG in clinical practice. Patients with FCS tend to have lower values for these variables because of their abnormal lipoprotein metabolism secondary to their LPL deficiency.

Limitations of this analysis include the fact that the sensitivity and specificity estimates are from the data used to create the logistic regression model and may not be as high from an independent data set. The data sets used in this analysis were also tightly regulated by the inclusion and exclusion criteria for the two phase 3 studies. Notably, the number of patients with FCS not reporting a history of pancreatitis was capped at 28%, and there were no pancreatitis criteria for patients with sHTG. Although pancreatitis is a primary and serious comorbidity, its reported frequency varies [8, 15, 16, 28, 29]. Pancreatitis medical history featured in the leading three-, four-, and five-variable data sets. Validating these analyses in a larger patient population could clarify the extent of the predictiveness of pancreatitis.

In conclusion, this analysis identifies several clinical variables, beyond fasting TG levels, that achieved >90% accuracy with as few as three variables, classifying a patient as having FCS or sHTG. Using these clinical variables in conjunction with previously proposed diagnostic scoring systems provides a robust platform for efficient differentiation and diagnosis of these two disease states for all health care professionals. Expediting this process is critical for proper disease management and could provide substantial benefit for these patients.

Acknowledgments

The authors thank the patients who participated in the clinical trials and their families. Medical writing assistance was provided by Complete HealthVizion, which was contracted and compensated by Akcea Therapeutics, Inc.

Financial Support: The APPROACH and COMPASS studies were funded by Ionis Pharmaceuticals and Akcea Therapeutics Inc.

Clinical Trial Information: APPROACH, ClinicalTrials.gov no. NCT02211209 (registered 7 August 2014); COMPASS, ClinicalTrials.gov no. NCT02300233 (registered 24 November 2014).

Author Contributions: The authors meet the criteria for authorship as recommended by the International Committee of Medical Journal Editors. They take full responsibility for the scope, direction, and content of and editorial decisions relating to the manuscript.
Additional Information

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**Disclosure Summary:** L.S.O. and B.H. are employees of Akcea Therapeutics. V.J.A. is an employee of Ionis Pharmaceuticals. J.M. was compensated by Akcea Therapeutics for biostatistical services. A.D. was an employee of Akcea Therapeutics at the time this work was completed. M.A. has received grants and honoraria from Amgen, Sanofi, Pfizer, Aegerion, Amryt, Akcea Therapeutics, Alfasigma, Mylan, and Ionis Pharmaceuticals. P.M.M. has received honoraria for speaker fees, research, or consultation from Genzyme, Regeneron, Sanofi, Amgen, Duke, Esperion, Kowa, IONIS, Eliza, Alexion, Lilly, Catabasis, Stage 2, Kaneka, and Novartis. J.J.P.K. has received honoraria for speaker fees, research, or consultation from Sanofi-Aventis, Amgen, Akarna Therapeutics, CSL Behring, Regeneron, Staton Biotech, Matigual, The Medicines Company, Kowa, Eli-Lily, Esperion Therapeutics, Genephize, Ionis Pharmaceuticals, and Akcea Therapeutics. E.B. has served on speakers’ bureaus for Amgen, Genfit, MSD, Sanofi, Regeneron, Unilever, Institute Benjamion Delessert, Aegerion, Chiesi, Rottapharm-MEDA, Lilly, Ionis Pharmaceuticals, and Akcea Therapeutics. H.S. reports personal fees from Akcea Therapeutics UK Ltd, Amgen, Sanofi, Alexion Pharmaceuticals, and Chiesi. J.L.W. is a consultant for IONIS Pharmaceuticals. R.A.H. has received personal fees for membership on advisory boards and speakers’ bureaus for Akcea/Ionis, Amgen, Gemphire, HLS Therapeutics, Regeneron, and Sanofi. D.G. has received grants and personal fees from Akcea, Ionis Pharmaceuticals, Aegerion, Amgen, AstraZeneca, Boehringer Ingelheim, Esperion, Gemphire, grants and personal HDL therapeutics, Ironwood, Lilly, Nestlé, Novartis, Pfizer, Regeneron, and Sanofi.

**Data Availability:** The datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

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