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Genetic Variants Associated with Severe Hypertriglyceridemia: *LPL*, *APOC2*, *APOA5*, *GPIHBP1*, *LMF1*, and *APOE*

Ciddi Hipertrigliseridemi ile İlişkili Genetik Varyantlar: *LPL*, *APOC2*, *APOA5*, *GPIHBP1*, *LMF1* ve *APOE*

ABSTRACT

Objective: High triglyceride (TG) levels are associated with an increased risk for atherosclerotic cardiovascular disease (ASCVD) and pancreatitis. The objectives for this study were to evaluate for the coexistence of severe HTG and pancreatitis in two different geographic regions of Turkey and to identify rare variants that cause monogenic HTG in our country.

Methods: In our study from 2014 to 2019, patients with severe HTG who presented to the endocrinology outpatient clinics with TG levels >500 mg/dL (5.7 mmol/L) were evaluated. The *LPL, APOC2, APOA5, GPIHBP1, LMF1*, and *APOE* genes were sequenced using next generation sequencing to screen for potentially pathogenic variants.

Results: Potentially pathogenic variants were identified in 64 (47.1%) of 136 patients. Variants in LPL were seen in 42 (30.9%) cases, APOA5 variants in 10 (7.4%) cases, APOC2 variants in 5 (3.7%) cases, LMF1 variants in 5 (3.7%) cases, and APOE mutations in 2 (1.5%) cases. In the subgroup that experienced pancreatitis (n = 76, 56.3%), LPL variants were seen at higher frequency (*P* <0.001) than in the subgroup with no history of pancreatitis (n = 60, 43.7%). Patients who developed pancreatitis (56.3%) demonstrated a median TG of 2083 mg/dL (23.5 mmol/L), and patients without pancreatitis (43.7%) demonstrated a median TG of 1244.5 mg/dL (14.1 mmol/L) (*P* <0.001).

Conclusion: Accurate approach to HTG diagnosis is important for the prevention of pancreatitis and ASCVD. Evaluation of variants in primary HTG after excluding secondary causes may help provide a patient-centric precision treatment plan.

Keywords: Apolipoprotein A5, apolipoprotein C2, apolipoprotein E, hypertriglyceridemia, lipoprotein lipase, lipase maturation factor 1, pancreatitis

ÖZET

Amaç: Yüksek trigliserid (TG) düzeyleri; aterosklerotik kardiyovasküler hastalık (ASKVH) ve pankreatit riskinde artma ile ilişkilidir. Amacımız, Türkiye'nin iki farklı coğrafi bölgesinde ciddi hipertrigliseridemi (HTG) ve pankreatit birlikteliğini değerlendirmek ve ülkemizdeki monogenik HTG'ye yol açan varyantları tanımlamaktır.

Yöntemler: Çalışmamızda 2014-2019 yıllarında endokrinoloji polikniklerine başvuran, TG düzeyi ≥500 mg/dL (5,7 mmol/L) olan HTG vakaları incelenmiştir. *LPL, APOC2, APOA5, GPIHBP1, LMF1* ve *APOE* genleri, potansiyel olarak patojenik varyantları taramak için yeni nesil dizileme kullanılarak sekanslanmıştır.

Bulgular: Yüz otuz altı hastanın 64'ünde (%47,1) potansiyel olarak patojenik varyantlar tespit edildi. 42 (%30,9) vakada LPL, 10 (%7,4) vakada APOA5, 5 (%3,7) vakada APOC2, 5 (%3,7) vakada LMF1 ve 2 (%1,5) vakada APOE varyantları saptandı. Pankreatit geçiren grupta (n = 76, %56,3) LPL varyantları, pankreatit öyküsü olmayan (n = 60, %43,7) gruba göre daha yüksek sıklıkta (*P* <0,001) görüldü. Pankreatit geçiren hastaların medyan TG'si 2083 mg/dL (23,5 mmol/L) ve pankreatit geçirmeyen hastaların medyan TG'si 1244,5 mg/dL (14,1 mmol/L) idi

ORIGINAL ARTICLE
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(*P* <0,001).

Sonuç: Pankreatit ve ASKVH'ın önlenmesi için HTG tanısına doğru bir yaklaşım önemlidir. Sekonder nedenleri dışladıktan sonra primer HTG için varyantların değerlendirilmesi, hasta merkezli hassas bir tedavi planının yapılmasına yardımcı olabilir.

Anahtar Kelimeler: Apolipoprotein A5, apolipoprotein C2, apolipoprotein E, hipertrigliseridemi, lipoprotein lipaz, lipaz maturasyon faktörü 1, pankreatit

Hypertriglyceridemia (HTG) is a common disorder of lipid metabolism that develops as a result of excess dietary fat intake, increased hepatic biosynthesis, and impaired metabolism of triglyceride (TG)-enriched lipoproteins, or a variety of secondary causes like poor diet, alcohol intake, obesity, insulin resistance, and diabetes mellitus (DM). Monogenic and polygenic factors contribute to both mild-moderate and severe HTG.[1-4]

Rare loss-of-function (LOF) variants most often affect lipolysis, whereas common variants can affect both production and catabolism of TG-rich lipoproteins.[5-8] In severe cases of HTG, derangements in chylomicron (CM) metabolism predominate; genetic etiologies are biallelic (i.e., homozygous or compound heterozygous) and result in complete deficiency of activity lipoprotein lipase (LPL) or one of its interacting factors, including apolipoprotein (apo) C-II, apo A-V, apo E, lipase maturation factor 1 (LMF1) or glycosylphosphatidylinositol anchored high density lipoprotein binding protein 1 (GPIHBP1).[1] This rare condition is now referred to as familial chylomicronemia syndrome (FCS), but it was formerly referred to as Fredrickson hyperlipoproteinemia (HLP) type I. The more common form of severe HTG is now called "multifactorial chylomicronemia" (MCM, formerly Frederickson HLP type V), characterized by concurrent elevations in chylomicrons and VLDL. This condition exhibits a much more complex etiology including susceptibility imparted by both monoallelic (i.e., heterozygous) rare variants in genes encoding LPL and the four cofactors listed above, as well as common small-effect variants (i.e., common polymorphisms) that add together creating strong polygenic susceptibility. HTG is a risk factor for the development of atherosclerotic cardiovascular disease (ASCVD) and acute pancreatitis.[1,4-9]

In many populations, mild-to-moderate HTG is seen in about 15%–30% of individuals,[1] while severe HTG demonstrates a population prevalence of about 1 in 400 to 600; FCS is a very small subgroup of the latter.[1] Observational studies show that pancreatitis is increased with TG >886 mg/dL (>10 mmol/L),[2] and it further dramatically increases when TG >1772 mg/dL (>20 mmol/L).[10] A limited number of studies exist quantifying the prevalence of HTG in Turkey.[11-13] Kayıkçıoğlu et al.[13] reported a prevalence of HTG (>150 mg/dL) in Turkey 36.5% in general. Onat A.[12] reported a prevalence of mild-to-moderate HTG in Turkey of 39.6% and 29.2% in men and women, respectively, and Bayram et al.[11] reported the population prevalence of HTG as 35.7% (33.5% for women and 38.3% for men). Analysis of rare LOF variants in Turkish patients with HTG has not been previously performed.

The objectives for this study were the following: (a) screen for the frequency of severe HTG and pancreatitis in two different geographic regions of Turkey; (b) to identify rare LOF variants of monogenic forms of HTG.

ABBREVIATIONS

ADA	American Diabetes Association
AIDS	Acquired immunodeficiency syndrome
APOA5	Apolipoprotein A5
APOC2	Apolipoprotein C2
APOE	Apolipoprotein E
ASCVD	Atherosclerotic cardiovascular disease
AUC	Area under the curve
BMI	Body mass index
C	Cholesterol
CI	Cardiac index
CM	Chylomicron
DM	Diabetes mellitus
FCS	Familial chylomicronemia syndrome
GPIHBP1	Glycosylphosphatidylinositol anchored high density lipoprotein binding protein 1
HbA1c	Hemoglobin A1c
HDL	High density lipoprotein
HLP	Hyperkeratosis lenticularis perstans
HTG	Hypertriglyceridemia
HTGP	HTG-induced pancreatitis
LMF1	Lipase maturation factor 1
LOF	Loss-of-function
LPL	Lipoprotein lipase
Max	Maximum
MCM	Multifactorial chylomicronemia
Med	Median
Min	Minimum
ROC	Receiver Operating Characteristic
SD	Standard deviation
SIFT	Sorting Intolerant from Tolerant
apoB	Apolipoprotein B
TG	Triglyceride
VLDL	Very-low-density lipoprotein

Methods

Study Population

In our two-center study from 2014 to 2019, patients who were admitted to the endocrinology outpatient clinics with TG level of ≥500 mg/dL (>5.7 mmol/L) were reviewed. The status of *LPL, APOC2, APOE, APOA5, LMF1,* and *GPHIBP1* rare variants were examined. The inclusion criteria for the patients were TG level of ≥500 mg/dL (>5.7 mmol/L) and being ≥18 years old. Of the patients who met the inclusion criteria, 136 patients participated in the study. The exclusion criteria were the presence of increased alcohol consumption without any discrimination (>2 and >1 drink(s) per day in men and women, respectively), overt hypothyroidism, nephrotic syndrome, Cushing's syndrome, rheumatoid arthritis, systemic lupus erythematosus, acquired immunodeficiency syndrome (AIDS), Gaucher disease, lipodystrophy syndromes, progeroid syndromes, and using of certain drugs such as estrogen, glucocorticoids, protease inhibitors, atypical antipsychotics, immunosuppressants, isotretinoin, tamoxifen, high dose thiazide diuretics, and nonselective beta blockers. Next, 44 patients prospectively included in the study were informed in detail, and informed consent form was read and signed by each participant. With archive scanning, retrospective data from 92 patients were included in the study. Then, triglyceride levels were measured randomly in prospective cases, regardless of fasting or satiety. Patients were evaluated according to the American Diabetes Association (ADA) 2011 criteria for the diagnosis of diabetes. The diagnosis of acute pancreatitis was made with a history of abdominal pain supported by lipase or amylase values and imaging results or with an epicrisis report. Additionally, the study was conducted in accordance with the "Helsinki Declaration" principles, with full conformity to the laws and regulations of Turkish Republic, and it fully adhered to the principles described in the "Good Clinical Practices."

Molecular Genetic Analyses

Among the cases included in the study, *LPL, APOC2, APOA5, APOE, LMF1,* and *GPIHBP1* genes were sequenced by the next generation sequence technique. According to the kit's protocol, DNA isolation was performed using the EZ1 DNA Blood kit (Catalog No: 951034 QIAGEN, Germany). Next, coding regions and exon intron boundaries of the genes were amplified with PCR with our in-house designed primers. Nextera XT DNA Library Prep Kit (Illumina, San Diego, CA) was used for library preparation. The final products were analyzed by loading into the next generation sequencing device (Miseq, Illumina). The results were confirmed by Sanger sequence analysis.

To evaluate the samples, the Integrative Genomics Viewer (Borad Institute) was used. The human genome "Hg38" was used as a reference. Next, the Human Gene Mutation Database (http://www.hgmd.cf.ac.uk/ac/index.php) and ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) databases were used to evaluate the variants detected in the study. In this analysis, only rare variants with minor allele frequency <1% were considered. Variations detected for the first time were evaluated with MutationTaster, PolyPhen-2, Sorting Intolerant from Tolerant (SIFT), and Varsome modeling programs.

Statistical Analyses

The data were analyzed by IBM SPSS V25 (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp). The assumption of normality was tested by the Kolmogorov Smirnov test. Continuous variables with normal distribution were presented as the mean (standard deviation [SD]); non-normal variables were reported as median (minimum–maximum [min–max]) values. Categorical variables were presented by frequency and percentages. Next, means of two continuous normally distributed variables were compared by independent samples Student's t test, and the Mann Whitney U test was used for non-normally distributed data. The frequencies of categorical variables were compared using Pearson χ^2 or Fisher's exact test, when appropriate. Next, the Receiver Operating Characteristic (ROC) curve method was used to determine effectiveness of a diagnostic test; area under the curve, corresponding 95% CI, cut-off point, sensitivity, and specificity values were presented. According to sex and age, propensity score matching was performed for comparison of those with and without diabetes. IBM SPSS V25 PS Matching was used for propensity matching. Also, 1:1 nearest neighbor matching was selected, and the caliper value was determined as 0.2.

Results

A total of 136 cases with HTG were included in the study. Eighty-seven (64%) of 136 patients were male, and 49 (36%) were female. Six of 49 female patients were pregnant. Since lipid levels increase physiologically during pregnancy, in order to avoid bias in the average results, they were calculated by excluding pregnant cases. The mean age of the patients was 42.3 ± 12.0 years, and the mean body mass index (BMI) was 28.1 ± 4.6 kg/m^2. Out of 136 patients, 76 (56.3%) presented with a history of acute pancreatitis, and 47 (34.8%) of them underwent plasmapheresis. In the study group, 56 (41.2%) patients presented with diabetes with a mean hemoglobin A1c (HbA1c) of 9.4 ± 2.5%. Consanguinity was found between the parents of 42 (34.7%) patients (Table 1).

Next, the median TG value of the cohort of cases was determined to be 1579 mg/dL (min–max 500–6678). The median total cholesterol (C), high density lipoprotein (HDL)-C,

Table 1. Clinical and demographic features of cases (n = 136)

Variables	Findings
Gender, female$_{nonpregnant}$, n (%)	43 (31.6)
Pregnant, n (%)	6 (4.4)
Age (year) (mean ± SD)	42.3 ± 12.0
TG (mg/dL) (med [min–max])	1579.0 [500.0-6678.0]
Total cholesterol (mg/dL) (med [min–max])	311.0 [122.0-839.0]
HDL cholesterol (mg/dL) (med [min–max])	25.0 [3.0-107.0]
Non-HDL cholesterol (mg/dL) (med [min–max])	285.0 [96.0-768.0]
Consanguinity, n (%)	42 (34.7)
Pancreatitis, n (%)	76 (56.3)
Plasmapheresis, n (%)	47 (34.8)
DM, n (%)	56 (41.2)
HbA1c (%) (mean ± SD)	9.4 ± 2.5
BMI (kg/m^2) (mean ± SD)	28.1 ± 4.6
Rare variants in genes for primary HTG, n (%)	64 (47.1)
LPL, n (%)	42 (30.9)
APOA5, n (%)	10 (7.4)
APOC2, n (%)	5 (3.7)
LMF1, n (%)	5 (3.7)
GPIHBP1, n (%)	0 (0.0)
APOE, n (%)	2 (1.5)

APOA5: Apolipoprotein A5; APOC2: Apolipoprotein C2; APOE: Apolipoprotein E; BMI: Body mass index; DM: Diabetes mellitus; GPIHBP1: Glycosylphosphatidylinositol anchored high density lipoprotein binding protein 1; HbA1c: Hemoglobin A1c; HDL: High density lipoprotein; HTG: Hypertriglyceridemia; LMF1: Lipase maturation factor 1; LPL: Lipoprotein lipase; med: Median; max: Maximum; min: Minimum; SD: Standard deviation; TG: Triglycerides.

Table 2. Rare variant distribution (n = 64)

	Homozygous (n = 37)	Heterozygous (n = 27)
LPL, n (%)	24 (57.1)	18 (42.9)
APOA5, n (%)	7 (70.0)	3 (30.0)
APOC2, n (%)	4 (80.0)	1 (20.0)
LMF1, n (%)	2 (40.0)	3 (60.0)
APOE, n (%)	0 (0.0)	2 (100.0)

APOA5: Apolipoprotein A5; APOC2: Apolipoprotein C2; APOE: Apolipoprotein E; LMF1: Lipase maturation factor 1; LPL: Lipoprotein lipase.

and non-HDL-C values were 311 (min–max 122–839), 25 (min–max 3–107), and 285 (min–max 96–768) mg/dL, respectively (Table 1). Low density lipoprotein (LDL)-C levels were not calculated because all patients demonstrated TG levels ≥500 mg/dL. Demographic data of cases with rare variants are listed on Tables 1–2, Supplementary Table 1, and Figure 1.

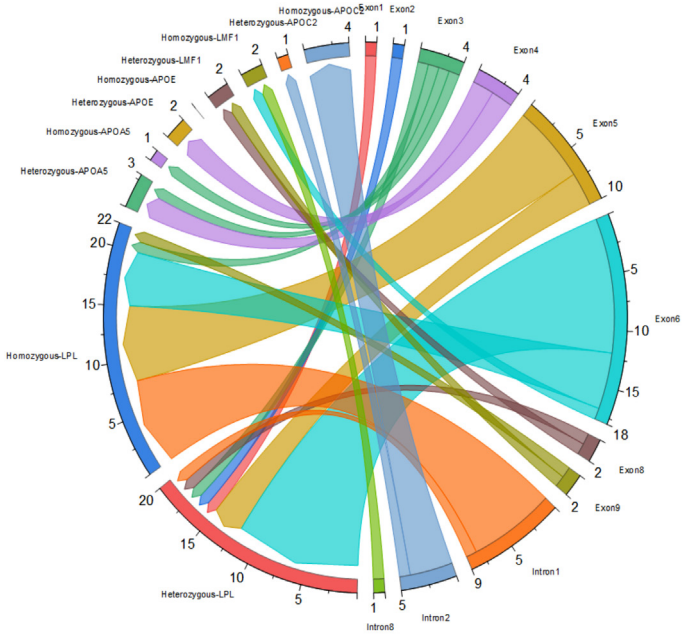

Figure 1. Genotype distribution of exon and intron regions that detected variations in *LPL*, *APOA5*, *LMF1*, *APOC2*, *APOE* genes. APOA5: Apolipoprotein A5; APOC2: Apolipoprotein C2; APOE: Apolipoprotein E; LMF1: Lipase maturation factor 1; LPL: Lipoprotein lipase.

When we compared the groups with and without variant, the median TG level was statistically significantly higher in the variant positive group: 2375 mg/dL vs. 1265 mg/dL (P <0.001). Also, the consanguinity status (61.9% vs. 38.1%) and pancreatitis history (60.5% vs. 39.5%) were significantly higher in the group with variant (P = 0.002 and P <0.01, respectively). The BMI (26.8 ± 5.0 vs. 29.4 ± 3.7 kg/m^2) was lower in the group with variant (P <0.001) (Table 3).

When we compared the cases according to their gender, use of plasmapheresis and the frequency of LPL variants were higher in nonpregnant women (Supplementary Table 2).

As nondiabetic patients most likely reflect the early form of the disease, age and gender were matched by propensity matching score analysis for a true comparison. Consanguinity and BMI differed between diabetic and nondiabetic subgroups. However, TG values and pancreatitis rates were not statistically different (Supplementary Table 3).

Next, age, gender, consanguinity, DM status, and HbA1c did not differ significantly (P >0.05) in patients with and without pancreatitis. In the group with pancreatitis, median TG levels were significantly higher (P <0.001) than the nonpancreatitis group (2083.0 mg/dL vs. 1244.5 mg/dL, respectively). The mean BMI was lower in the group with pancreatitis (P = 0.213). Also, variant rate and positivity for *LPL* rare variants were found more frequently in the pancreatitis group (Supplementary Table 4).

Table 3. Comparison of clinical features by rare variant status (n = 136)

	Variant positive (n = 64)	Variant negative (n = 72)	*p*
Gender, female, n (%)	29 (59.2)	20 (40.8)	0.034*
Age (year) (mean ± SD)	40.8 ± 14.1	43.6 ± 9.6	0.194
TG (mg/dL) (med [min–max])	2375.0 [500.0-6678.0]	1265.0 [542.0-5000.0]	<0.001*
Consanguinity (n(%))	26 (61.9)	16 (38.1)	0.002*
Pregnant, n (%)	6 (100.0)	0 (0.0)	0.030*
Pancreatitis, n (%)	46 (60.5)	30 (39.5)	<0.001*
Plasmapheresis, n (%)	31 (66.0)	16 (34.0)	0.001*
DM, n (%)	25 (44.6)	31 (55.4)	0.637
HbA1c (%) (med [min–max])	10.4 [5.8-14.5]	9.1 [5.9-15.3]	0.269
BMI (kg/m^2) (mean ± SD)	26.8 ± 5.0	29.4 ± 3.7	<0.001*

BMI: Body mass index; DM: Diabetes mellitus; HbA1c: Hemoglobin A1c; HDL: High density lipoprotein; HTG: Hypertriglyceridemia; med: Median; max: Maximum; min: Minimum; SD: Standard deviation; TG: Triglyceride.

Table 4: TG cut-off values for pancreatitis

	AUC	Standard error	*P*	95% Confidence interval		Sensitivity %	Specificity %	Correct classification %	TG cut-off point (mg/dL)
TG value for pancreatitis	0.7074	0.046	<0.001	0.618	0.797	70.42	67.80	69.23	1487.0
TG value for pancreatitis in variant positive patients	0.617	0.086	0.164	0.447	0.786	90.24	41.18	75.86	1367.5
TG value for pancreatitis in variant negative patients	0.669	0.069	0.015	0.536	0.802	46.67	85.71	69.44	1747.5

AUC: Area under the curve; TG: Triglyceride.

Next, a TG level of 1487 mg/dL (AUC 0.7074 [0.618–0.797]) was identified in the discrimination of those with and without pancreatitis. Sensitivity and specificity were 70.42% and 67.80%, respectively. In the variant positive group, a TG value of 1367.5 mg/dL was found with a sensitivity of 90.24% (Table 4, Supplementary Table 5, Supplementary Figures 1–3).

Discussion

Our results represent a preliminary study of rare genetic variants associated with severe HTG and pancreatitis from two endocrinology clinics in Turkey. After the exclusion of secondary causes, identification of the genetic etiologies of severe HTG may facilitate implementation of appropriate treatments, including certain medications and investigational treatments.[14]

Although we do not present baseline rare variant frequency data from normolipidemic Turkish controls, our findings indicate that a relatively high proportion of Turkish individuals with severe HTG present with at least one copy of a rare LOF variant in one of the five canonical genes for FCS, in addition to APOE. In studies of European, North American Caucasian, Hispanic, and East Asian patients with severe HTG, these proportions were ~35%, ~15%, ~25%, and ~35%, respectively.[15,16] Additionally, the relatively higher proportion of cases with rare variants in our Turkish cohorts could reflect ascertainment bias or possible founder effects for one or more of these variants. Also, we recognize the controversy regarding the true pathogenic status of *LPL* p.D36N (c.106G >A) and p.N318S (c.953A >G), which are each classified as "benign" or "variant of uncertain significance" or "risk factor" in the ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/variation/1550/ and https://www.ncbi.nlm.nih.gov/clinvar/variation/1552/); the expressed proteins at most demonstrate only partially impaired lipolytic function. However, we included them anyway in our tally to allow for comparison with previous analyses that also performed this.[15]

Severe HTG is an independent risk factor for pancreatitis and probably for ASCVD but not in FCS.[17-19] To date, the absolute risk of pancreatitis based on serum TG level has not been clearly defined in studies or in guidelines. In a Swedish study of 33260 adults, the risk of acute pancreatitis based on baseline serum TG levels was evaluated, but no threshold could be established.[20] In the study of Sandhu et al.,[10] the lowest TG level was reported as 1815 mg/dL (20.5 mmol/L) during pancreatitis attacks in patients with HTG-induced pancreatitis (HTGP). Similar to our study results, Rashid et al.[21] showed that among 5550 patients with baseline TG >1000 mg/dL, patients in the acute pancreatitis group demonstrated higher baseline median TG levels (2148 ±

1578 mg/dL) vs. the no acute pancreatitis group (1559 ± 861 mg/dL) (*P* <0.0001). Moreover, for each 100 mg/dL increase in TG levels above 1000 mg/dL, a 3% rise in risk of developing acute pancreatitis occurred. For the first time in Turkey, we ascertained the frequency of variants for HTG patients in two different geographic regions, investigated the inheritance, and identified a TG threshold value for pancreatitis as 1487 mg/dL (16.8 mmol/L). The TG value for pancreatitis was 1367.5 mg/dL (15.5 mmol/L) in patients with variant, and it was 1747.5 mg/dL (19.8 mmol/L) in the group without a variant. Although Gonzales KM et al.[22] reported that the serum TG / apoB ratio may guide the risk of pancreatitis, we cannot comment on the TG / apoB ratio since apoB was not measured in all patients in our study. Further, our findings indicate that a TG value of 1487 mg/dL or above increases the risk of acute pancreatitis. Over the years, different TG cut-off values have been recommended by professional groups in terms of pancreatitis risk. Endocrine Society guidelines defined fasting TG levels of 1000–1999 mg/dL as severe HTG and ≥2000 mg/dL as very severe HTG, emphasizing that a significant increase in the risk of acute pancreatitis occurs in these situations.[23] The data obtained from our study are valid only for the patients included in the study, and generalization may lead to false results. It is proper to generalize with studies to be conducted with a larger number of patients. In our study, 76 (56.3%) of 136 patients presented with a history of pancreatitis. The number of patients with LPL variant in the pancreatitis group was significantly higher than the nonpancreatitis group (*P* <0.001). Among patients with an identifiable variant and a history of pancreatitis, initiating cascade screening of family members is important so that appropriate interventions can be undertaken and risk of pancreatic and other long-term complications reduced.

In the prospective Cardiovascular Munster study, TG levels were slightly higher in men than in women. TG levels increase in men until the age of 45 and then decrease slightly, whereas in women, it continues to increase with age.[24] In the study of Ferrières et al.,[25] 297.909 lipid panels were examined in 2006–2017. Severe HTG was detected in 403 patients, and 303 (75%) of these cases were reported as male and 100 (25%) as female. Next, variant frequencies of female and male cases were determined at a similar rate, 59.2% vs. 40.2%, respectively.

In the study of dyslipidemia prevalence and risk factors in Turkish adults conducted by Bayram et al.,[11] positive associations were found between dyslipidemia and age, BMI, waist circumference, fasting blood sugar, and blood pressure. In our study, the mean BMI was 28.1 ± 4.6 kg/m^2. Then, the BMI value was significantly lower in the variant positive group than the variant negative group (*P* <0.001). This is simply consistent with the fact that the variant positive group exhibits HTG independent of insulin resistance. This HTG could, however, be exacerbated by insulin resistance in patients who gain weight and become obese.

In a cohort of 563 patients with TG ≥885 mg/dL, Dron JS et al.[26] reported that 1.1% demonstrated rare biallelic variants indicating FCS. Despite the low rate in this study, the high rate of 47.1% (27.2% homozygous-biallelic-, 19.2% heterozygous) in our group can at least partly be explained by consanguineous marriage. Multicenter studies with large patient groups are needed to reveal the true population-wide frequency of variant giving rise to HTG in Turkey. This frequency may differ from other regions as a result of genetic differences and high rates of consanguineous marriage in Turkey. The high rate of consanguineous marriage in Turkey (18.5%) results in an increase in the burden of recessive diseases.[27] However, this rate has not yet been comprehensively studies in dyslipidemic states.

Researchers reported that primary chylomicronemia affects 1:400 to 1:600 adult individuals; of these, >95% are MCM, and 1% monogenic or FCS.[1] Greater than 90% of monogenic chylomicronaemia cases are caused by variants in *LPL*.[28] LPL is responsible for the intravascular hydrolysis of TG-rich lipoproteins such as CM and VLDL. ApoA-V is thought to bind to GPIHBP1 in vitro and facilitate LPL-mediated hydrolysis of CM.[29] Human apoC-II is a necessary cofactor for the activation of LPL.[30] To promote LPL maturation, LMF1 acts as a chaperone.[31] LMF1 dimerizes and activates LPL.[32] GPIHBP1 is responsible for the transport and attachment of mature LPL to the vascular lumen surface where it is fully activated.[31] Next, among the eight molecular targets we screened, *LPL, APOA5, APOC2, LMF1,* and *GPIHBP1* cause hyperchylomicronemia and pancreatitis. In summary, besides *LPL*, other genes associated with monogenic HTG encode products that affect the activity, assembly, or transport of *LPL*. As in many other studies, *LPL* constituted the majority of variant in our study.[16,33-36] The genetics of HTG is highly heterogeneous, caused by rare variants in five canonical genes, although *LPL* underlies the large majority of cases.[28,37] Other genes associated with monogenic HTG have been reported at a lower prevalence than LPL polymorphism in several studies. Also, only about 20 families with *APOC2* deficiency have been reported worldwide.[38] Unlike the rates in our results, the *GPIHBP1* variant, which was not detected in our country, was reported more frequently, while the *APOA5* variant was reported less frequently.[28,37,39,40] Further, as the number of patients screened in Turkey increases, detection of the number of rare variants will also likely increase.

Unfortunately, the limitations of our study are the inability to provide sufficient data due to partial retrospective evaluation, the lack of regular follow-up of the patients, the low

number of cases, incomplete information such as coronary artery disease and response to treatment, and that no defined standard time interval exists for the measurement of TG levels. TG levels at the time of admission and the acute pancreatitis status in their history were recorded, so a TG value was calculated only for patients included in the study. In our future work, it will be considered. Since the study population consisted of not only those who admitted for TG elevation, this situation creates a "selection bias." Next, another limitation that should be taken into account in our further studies, which will be planned entirely prospectively, is that we evaluated TG levels with a single measurement, but TG levels vary daily-hourly according to seasons, meal content, fasting duration, carbohydrate consumption, and calorie expenditure. Since the number of our patients was insufficient to determine a cut-off value for pancreatitis, we only presented analyzes with available data.

Conclusion

A systematic approach to HTG diagnosis is important for the prevention of pancreatitis and ASCVD. A younger age of onset, family history, absence of secondary factors, low apo B level, severely elevated TG level, resistance to antihyperlipidemic therapy, and acute pancreatitis should be considered in the differential diagnosis of FCS from MCM. The prevalence of consanguineous marriage in Turkey likely contributes to genetic predisposition to HTG. Further, the fact that enzyme deficiency is not reflected in the clinic in all patients can be explained by the terms "variable penetrance" and "incomplete penetrance." Evaluation of variants in primary HTG after excluding secondary causes may help provide a patient-centric precision treatment plan.

Ethics Committee Approval: The study was approved by the Erciyes University Faculty of Medicine Ethics Committee (decision dated 10.17.2018 and numbered 2018/514).

Informed Consent: Written informed consent was obtained from the participants of this study.

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Supplementary Table 1. Demographic data of cases with mutations (n = 64)

		Exon/Intron	Sex	Age	Pancreatitis	TG (mg/dL)	BMI (kg/m²)
	LPL						
1.	**Homozygous p.G215E (p.G188E)(c.644G>A)**	Exon 5	F	36	Yes	1571	20.8
2.	**Homozygous p.G186E (c.557G>A)**	Exon 5	F	41	Yes	3527	22.7
3.	**Homozygous p.I221T (c.662T>C)**	Exon 5	F	36	Yes	3023	21.8
4.	**Homozygous p.N318S (c.953A>G)**	Exon 6	F	55	Yes	3033	28.6
5.	**Homozygous p.G215E (p.G188E)(c.644G>A)**	Exon 5	M	79	Yes	1487	21.6
6.	**Homozygous p.R333C (c.997C>T)**	Exon 6	M	33	Yes	3200	23.3
7.	**Homozygous p.L99P (c.296T>C)**	Exon 3	M	53	No	2045	23.9
8.	**Homozygous p.C465Y (c.1394G>A)**	Exon 9	F	18	Yes	2772	19.1
9.	**Homozygous p.V227A (c.680T>C)**	Exon 5	M	38	Yes	500	28.3
10.	**Homozygous p.G186E (c.557G>A)**	Exon 5	F	18	Yes	6025	18.2
11.	**Homozygous c.88+2dup (IVS1+2insT)**	Intron 1	F	31	Yes	3138	23.4
12.	**Homozygous c.88+2dup (IVS1+2insT)**	Intron 1	M	29	Yes	2286	19.8
13.	**Homozygous c.89-1G>A (IVS1-1G>A)**	Intron 1	M	42	Yes	942	22.3
14.	**Homozygous c.89-2A>G (IVS1-2A>G)**	Intron 1	F	34	Yes	1278	28.0
15.	**Homozygous c.89-2A>G (IVS1-2A>G)**	Intron 1	F	43	Yes	3400	25.5
16.	**Homozygous p.R270C (c.808C>T)**	Exon 6	F	47	Yes	4161	35.2
17.	**Homozygous p.R270C (c.808C>T)**	Exon 6	F	44	Yes	1245	23.4
18.	**Homozygous p.D183N (c.547G>A)**	Exon 5	M	60	No	3198	19.2
19.	**Homozygous p.R270C (c.808C>T)**	Exon 6	F	31	Yes	2498	21.3
20.	**Homozygous c.89-1G>A (IVS1-1G>A)**	Intron 1	M	23	Yes	5150	19,9
21.	**Homozygous c.89-1G>A (IVS1-1G>A)**	Intron 1	M	24	Yes	6390	18,4
22.	**Homozygous c.89-1G>A (IVS1-1G>A)**	Intron 1	F	25	Yes	2300	28.1
23.	**Compound heterozygous p.G215E (p.G188E) (c.644G>A)/p.D36N (c.106G>A)**	Exon 5/ Exon1	F	51	Yes	2008	30.5
24.	**Compound heterozygous p.G215E (c.644G>A)/p.L392P (c.1175T>C)**	Exon 5/ Exon8	F	44	Yes	1587	23.0
25.	**Heterozygous p.R270C (c.808C>T)**	Exon 6	M	52	Yes	6678	30.1
26.	**Heterozygous c.89-1G>A (IVS1-1G>A)**	Intron 1	M	62	Yes	2242	26.6
27.	**Heterozygous p.N318S (c.953A>G)**	Exon 6	F	42	No	1020	30.4
28.	**Heterozygous p.A125T (c.373G>A)**	Exon 3	F	51	Yes	2273	32.8
29.	**Heterozygous p.N318S (c.953A>G)**	Exon 6	F	42	Yes	6236	25.8
30.	**Heterozygous p.N318S (c.953A>G)**	Exon 6	F	44	Yes	1509	28.3
31.	**Heterozygous p.N318S (c.953A>G)**	Exon 6	M	61	No	3167	32.9
32.	**Heterozygous p.H273R (c.818A>G)**	Exon 6	M	34	No	500	28.6
33.	**Heterozygous p.N318S (c.953A>G)**	Exon 6	M	68	No	500	30.4
34.	**Heterozygous p.T75N (c.224C>A)**	Exon 2	M	52	Yes	2492	39.0
35.	**Heterozygous p.N318S (c.953A>G)**	Exon 6	F	22	No	2450	36.3
36.	**Heterozygous p.G215E (p.G188E) (c.644G>A)**	Exon 5	M	54	No	4886	29.4
37.	**Heterozygous p.N318S (c.953A>G)**	Exon 6	M	44	Yes	1495	30.9
38.	**Heterozygous p.N318S (c.953A>G)**	Exon 6	F	48	Yes	3702	29.3
39.	**Heterozygous p.D36N (c.106G>A)**	Exon 2	M	37	Yes	1883	31.9
40.	**Heterozygous p.D36N (c.106G>A)**	Exon 2	M	53	Yes	5503	24.3
41.	**Heterozygous p.N318S (c.953A>G)**	Exon 6	F	30	Yes	1926	23.1
42.	**Heterozygous p.N318S (c.953A>G)**	Exon 6	M	33	No	3973	24.1

Supplementary Table 1. Demographic data of cases with mutations (n = 64) *(continue)*

		Exon/Intron	Sex	Age	Pancreatitis	TG (mg/dL)	BMI (kg/m²)
	APOA5						
1.	**Homozygous c.16_39delGCCGTGCTCACCTGGGCTCTGGCT (p.A6_A13del)**	Exon 2	F	24	Yes	2546	26.6
2.	**Homozygous c.16_39delGCCGTGCTCACCTGGGCTCTGGCT (p.A6_A13del)**	Exon 2	M	22	Yes	1995	28.4
3.	**Homozygous p.Q22X (c.64C>T)**	Exon 3	F	21	Yes	4087	24.9
4.	**Homozygous c.C56G (p.S19W)**	Exon 2	M	57	No	2941	41.0
5.	**Homozygous c.16_39del (p.Ala6_Ala13del)**	Exon 1	F	23	No	1384	30.0
6.	**Homozygous c.16_39del (p.Ala6_Ala13del)**	Exon 1	M	50	No	3618	35.0
7.	**Homozygous c.64C>T (p.Gln22Ter)**	Exon 2	M	57	No	2313	29.4
8.	**Heterozygous p.Q22X (c.64C>T)**	Exon 3	M	49	Yes	3256	30.5
9.	**Heterozygous c.427delC (p.R143AfsX57)**	Exon 4	M	41	Yes	1351	32.9
10.	**Heterozygous p.Y110C (c.329A>G)**	Exon 4	F	39	No	818	31.0
	APOC2						
1.	**Homozygous c.55+1G>A (IVS2+1G>C)**	Intron 2	M	23	No	2529	26.0
2.	**Homozygous c.55+1G>A (IVS2+1G>C)**	Intron 2	F	21	Yes	510	14.0
3.	**Homozygous c.55+6T>G(IVS2+6T>G)**	Intron 2	M	18	Yes	3400	20.6
4.	**Homozygous c.55+6T>G(IVS2+6T>G)**	Intron 2	F	19	No	2014	19.7
5.	**Heterozygous c.55+6T>G(IVS2+6T>G)**	Intron 2	M	28	Yes	3284	25.3
	LMF1						
1.	**Homozygous c.1079-2A>C (IVS8-2 A>C)**	Intron 8	M	59	Yes	2437	26.9
2.	**Homozygous p.G292R (c.874G>A)**	Exon 6	M	44	Yes	1659	26.0
3.	**Heterozygous p.V362G (c.1085T>G)**	Exon 8	M	52	No	1143	25.1
4.	**Heterozygous p.R461C (c.1381C>T)**	Exon 9	M	59	No	751	29.0
5.	**Heterozygous c.1121 T>A (p.Leu374Glu)**	Exon 8	M	39	Yes	4570	28.7
	APOE						
1.	**Heterozygous p.R110P (c.329G>C)**	Exon 4	F	50	Yes	1100	28.1
2.	**Heterozygous p.P102R (c.305C>G)**	Exon 4	M	53	No	967	29.4

APOA5: Apolipoprotein A5; APOC2: Apolipoprotein C2; APOE: Apolipoprotein E; LMF1: Lipase maturation factor 1; LPL: Lipoprotein lipase; TG: Triglyceride.

Supplementary Table 2. Comparison by gender (n = 130)

	Nonpregnant Female (n = 43)	Male (n = 87)	*P*
Age (year) (mean ± SD)	42.4 ± 10.0	43.6 ± 12.0	0.547
TG (mg/dL) (med [min–max])	1384 [510-6236]	1504[500-6678]	0.338
Consanguinity, n (%)	14 (35.9)	24 (31.6)	0.641
Pancreatitis, n (%)	26 (60.5)	45 (51.7)	0.346
Plasmapheresis, n (%)	19 (44.2)	22 (25.3)	0.029*
DM, n (%)	22 (51.2)	33 (37.9)	0.151
HbA1c (%) (mean ± SD)	9.4 ± 2.6	10.0 ± 2.5	0.434
BMI (kg/m²) (mean ± SD)	28.4 ± 5.1	28.3 ± 4.1	0.942
Variant positive, n (%)	23 (53.5)	35 (40.2)	0.152
LPL, n (%)	19 (48.7)	20 (27.8)	0.027*
APOA5, n (%)	2 (9.1)	6 (10.3)	0.867
APOC2, n (%)	1 (4.8)	3 (5.5)	0.904
LMF1, n (%)	0 (0.0)	5 (8.8)	0.171
APOE, n (%)	1 (4.8)	1 (1.9)	0.492

APOA5: Apolipoprotein A5; APOC2: Apolipoprotein C2; APOE: Apolipoprotein E; BMI: Body mass index; DM: Diabetes mellitus; HbA1c: hemoglobin A1c; LMF1: Lipase maturation factor 1; LPL: Lipoprotein lipase; med: Median; max: Maximum; min: Minimum; SD: Standard deviation; TG: Triglyceride.

Supplementary Table 3. Propensity matching according to sex and age in evaluating diabetes (n = 90)

	Patients with DM (n = 45)	Patients without DM (n = 45)	*P*
Gender, female, n (%)	16 (47.1)	18 (52.9)	0.664
Age (year) (mean ± SD)	43.7 ± 10.3	42.4 ± 11.2	0.541
TG (mg/dL) (med [min-max])	1787 [500-6678]	1587 [500-6025]	0.974
Consanguinity, n (%)	7 (29.2)	17 (70.8)	0.038*
Pancreatitis, n (%)	29 (54.7)	24 (45.3)	0.284
Plasmapheresis, n (%)	15 (46.9)	17 (53.1)	0.660
HbA1c (%) (mean ± SD)	9.9 ± 2.4	NA	NA
BMI (kg/m^2) (mean ± SD)	29.5 ± 4.0	26.6 ± 5.0	0.003*
Variant positive, n (%)	21 (46.7)	24 (53.3)	0.527
LPL, n (%)	16 (50.0)	16 (50.0)	0.773
APOA5, n (%)	1 (20.0)	4 (80.0)	0.157
APOC2, n (%)	0 (0.0)	2 (100.0)	0.140
LMF1, n (%)	3 (60.0)	2 (40.0)	0.777
APOE, n (%)	1 (100.0)	0 (0.0)	0.354

APOA5: Apolipoprotein A5; APOC2: Apolipoprotein C2; APOE: Apolipoprotein E; BMI: Body mass index; DM: Diabetes mellitus; HbA1c: hemoglobin A1c; LMF1: Lipase maturation factor 1; LPL: Lipoprotein lipase; med: Median; max: Maximum; min: Minimum; SD: Standard deviation; TG: Triglyceride.

Supplementary Table 4. Comparison of clinical features by pancreatitis (n = 130)

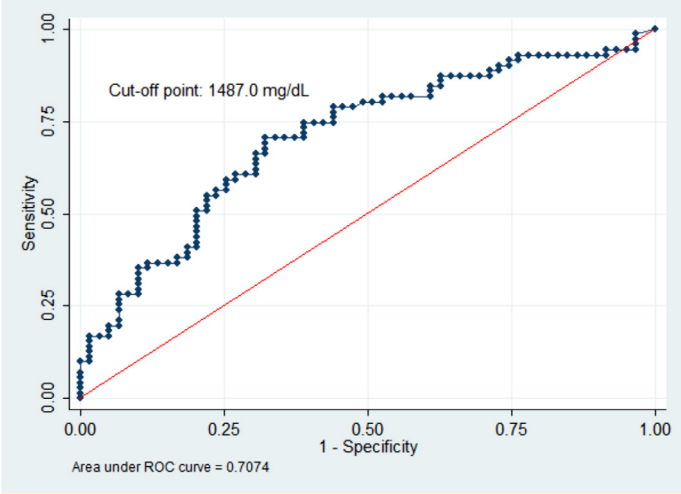
	Positive pancreatitis history (n = 71)	Negative pancreatitis history (n = 59)	*P*
Gender, male, n (%)	45 (51.7)	42 (48.3)	0.346
Age (year) (mean ± SD)	42.1 ± 10.8	44.6 ± 12.0	0.207
TG (mg/dL) (med [min-max])	2083.0 [500-6678]	1244.5 [500-4886]	<0.001*
Plasmapheresis, n (%)	40 (97.6)	2 (2.4)	<0.001*
Consanguinity, n (%)	25 (65.8)	13 (34.2)	0.054
DM, n (%)	35 (63.6)	20 (36.4)	0.077
HbA1c (%) (med [min-max])	10.4 [5.8-15.2]	9.0 [5.9-15.3]	0.937
BMI (kg/m^2) (mean ± SD)	27.7 ± 4.7	29.1 ± 4.0	0.213
Variant positive, n (%)	41 (70.7)	17 (29.3)	0.001*
LPL, n (%)	33 (76.9)	9 (23.1)	<0.001*
APOA5, n (%)	4 (40.0)	4 (40.0)	0.651
APOC2, n (%)	3 (75.0)	1 (25.0)	0.190
LMF1, n (%)	3 (60.0)	2 (40.0)	0.423
APOE, n (%)	1 (50.0)	1 (50.0)	0.814

APOA5: Apolipoprotein A5; APOC2: Apolipoprotein C2; APOE: Apolipoprotein E; BMI: Body mass index; DM: Diabetes mellitus; HbA1c: hemoglobin A1c; LMF1: Lipase maturation factor 1; LPL: Lipoprotein lipase; med: Median; max: Maximum; min: Minimum; SD: Standard deviation; TG: Triglyceride.

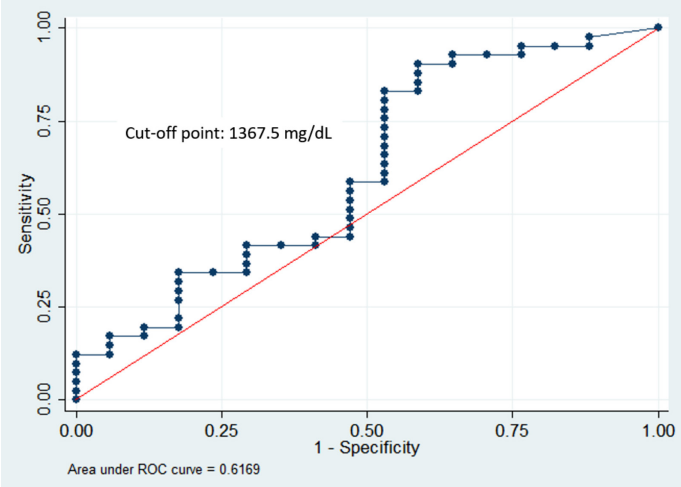
Supplementary Table 5. Comparison by TG levels (n = 130)

	TG ≥1487 mg/dL (n = 69)	TG <1487 mg/dL (n = 61)	*P*
Gender, female, n (%)	21 (48.8)	22 (51.2)	0.496
Age (year) (mean ± SD)	42.6 ± 11.9	43.8 ± 10.8	0.546
Consanguinity, n (%)	22 (52.6)	18 (47.4)	0.841
Pancreatitis, n (%)	50 (70.4)	21 (29.6)	<0.001*
Plasmapheresis, n (%)	31 (75.6)	10 (24.4)	<0.001*
DM, n (%)	32 (58.2)	23 (41.8)	0.318
HbA1c (%) (mean ± SD)	9.8 ± 2.4	9.7 ± 2.7	0.859
BMI (kg/m^2) (mean ± SD)	27.5 ± 4.6	29.3 ± 4.1	0.017*
Variant positive (n (%))	43 (74.1)	15 (25.9)	<0.001*
LPL, n (%)	32 (82.1)	7 (17.9)	<0.001*
APOA5, n (%)	5 (62.5)	3 (37.5)	0.146
APOC2, n (%)	3 (75.0)	1 (25.0)	0.119
LMF1, n (%)	2 (40.0)	3 (60.0)	0.286
APOE, n (%)	0 (0.0)	2 (100)	0.291

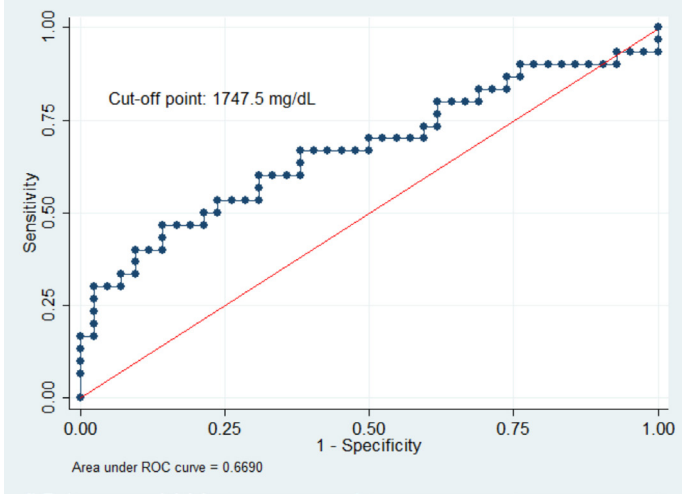
APOA5: Apolipoprotein A5; APOC2: Apolipoprotein C2; APOE: Apolipoprotein E; BMI: Body mass index; DM: Diabetes mellitus; HbA1c: hemoglobin A1c; LMF1: Lipase maturation factor 1; LPL: Lipoprotein lipase; med: Median; max: Maximum; min: Minimum; SD: Standard deviation; TG: Triglyceride.



Supplementary Figure 1. A ROC curve for the use of TG as a predictor of pancreatitis in all subjects.



Supplementary Figure 2. A ROC curve of the use of TG as a predictor of pancreatitis in variant (+) subjects.



Supplementary Figure 3. A ROC curve of the use of TG as a predictor of pancreatitis in variant (−) subjects.