Research paper

Intensive genetic analysis for Chinese patients with very high triglyceride levels: Relations of mutations to triglyceride levels and acute pancreatitis

Jing-Lu Jin, Di Sun, Ye-Xuan Cao, Hui-Wen Zhang, Yuan-Lin Guo, Na-Qiong Wu, Cheng-Gang Zhu, Ying Gao, Qiu-Ting Dong, Geng Liu, Qian Dong, Jian-Jun Li *

Division of Dyslipidemia, State Key Laboratory of Cardiovascular Disease, Fu Wai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences, Peking Union Medical College, BeiLISHi Road 167, Beijing 100037, China

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A B S T R A C T

Background: Severe hypertriglyceridemia (SHTG, TG ≥5-65 mmol/L), a disease, usually resulting from a combination of genetic and environmental factors, may increase the risk of acute pancreatitis (AP). However, previous genetic analysis has been limited by lacking of related observation of gene to AP.

Methods: The expanding genetic sequencing including 15 TG-related genes (LPL, LMF1, APOC2, GPIHBP1, GCKR, ANGPTL3, APOB, APOA1-A4-C3-A5, TRIB1, CETP, APOE, and LIP) was performed within 103 patients who were diagnosed with primary SHTG and 46 age- and sex-matched normal controls.

Findings: Rare variants were found in 46 patients and 12 controls. The detection rate of rare variants in SHTG group increased by 19-5% via intensive genetic analysis. Presence of rare variants in LPL, APOA5, five LPL molecular regulating genes and all the sequenced genes were found to be associated with SHTG (p < 0.05). Of noted, patients with history of AP presented higher frequency of rare variants in LPL gene and all the LPL molecular regulating genes (27-8% vs.4-7% and 50-0% vs. 20-0%). The risk scores for SHTG determined by common TG-associated variants were increased in subgroups according to the extent of SHTG when they were compared with that of controls. Finally, patients without rare variants within SHTG group also presented higher risk scores than control group (p < 0.05).

Interpretation: Expanding genetic analysis had a higher detection rate of rare variants in patients with SHTG. Rare variants in LPL and its molecular regulating genes could increase the risk of AP among Chinese patients with SHTG.

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1. Introduction

Hypertriglyceridemia (HTG) has been established as a common lipid disorder in association with many comorbidities including acute pancreatitis (AP) [1-3]. Severe hypertriglyceridemia [SHTG, fasting plasma triglyceride (TG) ≥5-65 mmol/L or 500 mg/dL] in adults is an especially unfavorable state with a high risk of fatal complications, which is worthy of further investigation [4]. However, the causal studies on SHTG have been relatively lagging because of its complexity, which was mainly dependent on the genetic and environmental interactions [5]. Additionally, the measurement of plasma TG has unavoidable intra-individual biological variation and is easily affected by drugs and diet, which increase the difficulty in the diagnosis of SHTG [6,7]. Furthermore, the presence of SHTG is also correlated with a series of the secondary causes, such as extreme obesity, uncontrolled diabetes, severe liver/renal insufficiency, thyroid disease, chemotherapy et al. However, in some patients, their SHTG cannot clinically controlled even if the secondary causes were eliminated, and the genetic features may be an explanation for such patients.

It has been demonstrated that Lipoprotein lipase (LPL) is a critical enzyme in determining plasma triglyceride levels and the defect in LPL gene may result in SHTG [8,9]. Moreover, Apoprotein(apo)A-V, apoC-II, glycosylphosphatidylinositol-anchored high-density lipoprotein binding protein 1 (GPIHBP1), and lipase maturation factor 1 (LMF1) are also co-factors involved in the activation, transportation or maturation of LPL [10-14]. Recently, several studies have reported that defects in LPL, APOA5, APOC2, GPIHBP1, and LMF1 are closely associated with type 1 hyperlipoproteinemia [15,16]. Most patients in this phenotype have monogenic feature but only account for a minority of SHTG cases. In fact, the majority of patients with SHTG usually present polygenic phenotypes and carry a complex burden of rare and common
variants in a broader spectrum of genes [17]. For example, APOA5/C4/A3/A1 gene cluster has been reported to be associated with 38% of genetic variance of triglyceride [18]. Moreover, previous studies have demonstrated that significant accumulation of rare variants in the APOA5/C4/A3/A1 cluster exists in patients with SHTG [19]. Additionally, other genes including APOB, glucokinase regulatory protein (GCKR), angiopoietin-like protein 3 (ANGPTL3), tribbles-1 (TRIB1), cholesteryl ester transfer protein (CETP), APOE, and LIPI are also identified to be essential for triglyceride-modulating but their attributions on the presence of SHTG cases have less been determined [17,20,21]. Thus, to find the susceptibility variants, we performed next generation sequencing (NGS) on promoters, exons, and exon–intron boundaries in 15 TG-related genes (LPL, LMF1, APOC2, GPHBP1, GCKR, ANGPTL3, APOB, APOA1-A4-C3-A5, TRIB1, CETP, APOE, and LIPI) among 103 patients and 46 age- and sex matched normal controls (TG < 1.7 mmol/L). We also conducted a survey of common single nucleotide polymorphisms (SNPs) for the associations with triglyceride levels. Finally, we tested the hypothesis that the presence of pathogenic variant may increase the risk of AP among Chinese patients with SHTG.

2. Methods

2.1. Study design and population

Our study complied with the Declaration of Helsinki and was approved by the hospital’s ethical review board (Fu Wai Hospital & National Center for Cardiovascular Diseases, Beijing, China). Informed written consents were obtained from all patients enrolled in this study. The sequencing cohort included 103 SHTG individuals and 46 controls of Chinese Han population. As is shown in Fig. 1, from April 2011 to January 2018, 10,908 patients attended the division of dyslipidemia of Fu Wai Hospital. 187 (1.7%) patients with SHTG were unrelated subjects who had fasting plasma TG ≥5.65 mmol/L at least twice from a single medical center. TG levels were measured when they were in normal diet (without alcohol intake). Patients with liver or renal insufficiency, thyroid dysfunction, BMI > 30 kg/m², and uncontrolled type 2 diabetes mellitus (T2DM) were excluded. Patients with other secondary causes of HTG such as chemotherapy, hormonal drugs, and alcohol abusers were also ruled out. Controls were recruited from individuals who had no history of HTG, were without exclusion criteria and with TG < 1.7 mmol/L. Finally, 46 controls were each matched with up to 3 HTG cases based on age within 5 years and sex.

Clinical data of each individual who entered the study were collected by experienced physicians and nurses. Coronary artery disease was defined as the presence of coronary stenosis≥50% at least one major artery segment assessed by two experienced physicians according to coronary angiography. Hypertension (HT) was recognized as systolic blood pressure (SBP) ≥140 and/or diastolic blood pressure (DBP) ≥90 mmHg for at least three separate measurement or currently using anti-hypertension drugs. Diabetes mellitus (DM) was diagnosed as fasting glucose ≥7.0 mmol/L or random glucose ≥11.0 mmol/L or hypoglycemic treatments. Family history of SHTG was identified as TG ≥5.65 mmol/L in at least 1 related family members. Alcohol consumption was assessed according to the definition by National Institute on Alcohol Abuse and Alcoholism [22].

2.2. Laboratory examination

Blood samples were collected from cubital vein into EDTA-containing tubes for biochemical measurements after overnight fast. Concentrations of Total cholesterol (TC), TG, high density lipoprotein cholesterol (HDL-C) were measured using automatic biochemistry analyzer (Hitachi 7150, Japan) using enzymatic assay.

2.3. Genetic sequencing

The blood samples for DNA extraction were well preserved at −80 °C after centrifugation for 10 min at 3500 rpm, 4 °C. The genomic DNA was isolated form peripheral blood leukocytes using standard extraction protocols. Each DNA sample is quantified by agarose gel electrophoresis and Nanodrop (Thermo). The amplification of all target genes was performed by PCR using Platinum Taq DNA polymerase (Invitrogen, Carlsbad, CA) with a Hypertriglyceridemia Gene Panel using biotinylated oligo-probes (MyGenostics GenCap Enrichment technologies). The probes were designed according to 15 TG-modulating related genes covering the coding exons, intron-exon boundaries and promoters, exons, exon–intron boundaries and 3′/5′ untranslated region (UTR) of in 15 TG-related genes (LPL (NM_000037), LMF1 (NM_000237), APOC2 (NM_000483), GPHBP1 (NM_178172), GCKR (NM_001486), ANGPTL3 (NM_014495), APOA1 (NM_000039), APOA4 (NM_000482), APOC3 (NM_000040), APOA5 (NM_052968), TRIB1 (NM_001282985), CETP (NM_000078), APOE (NM_000041), and LIPI (NM_198996)). The capture experiment was conducted according to manufacturer’s protocol. In brief, 1 mg DNA library was mixed with Buffer BL and GenCap probe (MyGenostics, Beijing), heated at 95°C for 7 min and 65°C for 2 min on a PCR machine; 23 ml of the 65°C prewarmed Buffer HY (MyGenostics, Beijing) was then added to the mix, and the mixture was hold at 65°C with PCR lid heat on for 22 h for hybridization. 50 ml MyOne beads (Life Technology) was washed in 500 ml 1X binding buffer for 3 times and resuspended in 80 ml 1X binding buffer. Sixty-four ml 2X binding buffer was added to the hybrid mix, and transferred to the tube with 80 ml MyOne beads. The mix was rotated for 1 h on a rotator. The beads were then washed with WB1 buffer at room temperature for 15 min once and WB3 buffer at 65°C for 15 min three times. The bound DNA was eluted with Buffer Elute. The eluted DNA was finally amplified for 15 cycles using the following program: 98°C for 30 s (1 cycle); 98°C for 25 s, 65°C for 30 s, 72°C for 30 s (15 cycles) and 72°C for 5 min (1 cycle). The PCR product was purified using SPRI beads (Beckman Coulter) according to manufacturer's
2.4. Bioinformatics analysis

After HiSeq 2000 sequencing, high-quality reads were retrieved from raw reads by filtering out the low quality reads and adaptor sequences using the Solexa QA package and the cutadapt program, respectively. The clean read sequences were then aligned to the human genome reference sequence (hg19) using SOAP aligner program. The reads to the reference genome using BWA and identified the insertions or deletions (InDels) using the GATK program. The identified SNPs and InDels were annotated using the Exome-assistant program. Magic Viewer was used to view the short read alignment and validate the candidate SNPs and InDels. Annotated variants were first filtered and defined as uncommon variants according to minor allele frequencies (MAF) <1% in the general population of 1000 Genomes Project, Exome Aggregation Consortium (ExAC), Exome Sequencing Project 6500 (ESP6500) and Inhouse databases. PolyPhen-2, Sorting Tolerant From Intolerant (SIFT), Mutation Taster were used to determine pathogenicity. A variant was determined to be pathogenic or likely pathogenic if above mentioned system or ACMG criteria provided a deleterious prediction.

2.5. SNP selection and risk score

We preliminary selected SNPs which were previously reported to be associated with hypertriglyceridemia and tested the dose effect of the risk alleles (Supplemental Table S1). The risk score was calculated on the 9 SNPs that were significantly associated with the presence of SHTG. Each individual was given different score as 0, 1 and 2 for number of risk alleles. The cumulative number of risk alleles was calculated for each patient.

2.6. Statistical analysis

The values were expressed as the mean ± SD, number (percentage) for the categorical variables. The differences of clinical characteristics between groups were analyzed using Student t-test, Mann–Whitney U test, χ²-tests, or Fisher’s exact test where appropriate. A p-value <0.05 was considered statistically significant. The statistical analysis was performed with SPSS version 21·0 software (SPSS Inc., Chicago, IL, USA).

3. Result

3.1. Baseline characters

Data for the 103 patients with SHTG and the 46 control subjects were shown in Table 1. The levels TG and TC were higher and the levels of HDL-C were lower (P < 0·05) in SHTG subjects than in controls. No other significant differences were found between two groups.

3.2. Carrier frequencies for rare variants found in the study

We found 46 patients and 12 controls with rare pathogenic/potentially pathogenic variants (9 in LPL, 7 in APOA5, 13 in LMF1, 4 in GPIHB1, 10 in GCKR1 in CETP, 3 in TRIB1, 5 in APOE, 22 in APOB, 3 in APOA4, 2 in APOA1, and 2 in ANGPTL3) after the exclusion of variants that did not produce amino acid substitution (synonymous variants). The details of rare variants were shown in Supplemental Table S2. Types and gene distributions of those rare variants were listed in Table 2. The results indicated that the rare variants in LPL or APOA5 genes were strongly associated with SHTG (8-7% vs. 0% and 6-8% vs. 0%, p < 0·05 respectively). LPL molecular regulating genes cumulatively existed in 25.2% of the SHTG group but did in 8-7% in controls. And also, patients with SHTG had higher frequencies of all the sequenced genes compared to controls. Moreover, it showed significantly higher
frequencies of the rare variants in patients with TG ≥ 11·30 mmol/L (subgroup 2). As shown in Fig. 2, the detection rate of the rare variants in SHTG group, subgroup1 and subgroup 2 increased by 19·5%, 22·2%, and 15·9% respectively, which was higher than that in merely sequenced LPL related genes (LPL, APOA5, LMF1, GPIHBP1, APOC2, gene cluster).

### 3.3. Clinical and genetic features in patients with and without acute pancreatitis

When SHTG patients were classified according to previous history of AP, no major clinical differences emerged for gender, TG admission, BMI, body mass index; TC, total cholesterol; HDL-C, high density lipoprotein cholesterol.

### 3.4. Associations between genetic risk score with plasma triglyceride levels

As presented in Fig. 3a, the risk scores for SHTG, determined by common TG-associated variants, were increased in the subgroup 1 and subgroup 2 compared with that in controls (p < 0·05). We also separately evaluated genetic risk scores (GRS) in both control and SHTG groups (Fig. 3b and c). Data suggested that the GRS was significantly increased according to the tertiles of TG in SHTG group (p for trend = 0·013). Patients with relative higher TG levels (classified with the median) had higher GRS than that with normal controls (p < 0·05, Fig. 3c). Patients without rare variants within SHTG group also presented higher GRS than that within control group (p < 0·05 Fig. 3d). Furthermore, as shown in Fig. 4, we compared the variation attributable to clinical and genetic variables in different subgroups. Among SHTG patients, the total proportion of variations explained by the analytical model was 33·6% (common variants explained 31·0% and rare variants explained 2·6%). In subgroup analysis, common and rare variants explained 42·0% and 4·0% of variation in subgroup 2 while only common variants explained 28·8% of variation in subgroup 1.

### Table 1
Baseline characteristics.

| Variables                  | Severe HTG n = 103 | Control N = 46 | P value |
|---------------------------|--------------------|----------------|---------|
| Age, years                | 50·1 ± 10·0        | 53·2 ± 9·8     | 0·191   |
| Sex, n (%)                | 79(76·7)           | 33(71·7)       | 0·517   |
| HT, n (%)                 | 53(51·5)           | 27(58·7)       | 0·415   |
| DM, n (%)                 | 22(19·6)           | 9(21·8)        | 0·760   |
| CAD, n(%)                 | 57(53·3)           | 30(65·2)       | 0·258   |
| BMI, kg/m²                | 25·2 ± 2·9         | 26·3 ± 2·6     | 0·227   |
| Pancreatitis,n (%)        | 18(17·5)           | 0              | 0·001   |
| Smoke,n (%)               | 60(58·3)           | 25(50·0)       | 0·394   |
| Alcohol (g/d)             | 45(37·0)           | 17(43·7)       | 0·441   |
| TG (mmol/L)               | 9·6 ± 3·9          | 1·2 ± 0·3      | < 0·001 |
| HDL-C (mmol/L)            | 0·80 ± 0·22        | 1·14 ± 0·32    | < 0·001 |

Data were expressed as mean ± SD or n (%). HT, hypertension; DM: diabetes mellitus; TG, triglyceride; BMI, body mass index; TC, total cholesterol; HDL-C, high density lipoprotein cholesterol.

### Table 2
Carrier frequencies for rare variants found in the study.

| Variables                      | Control n = 46 | Severe HTG n = 103 | Subgroup 1 n = 58 | Subgroup 2 n = 45 |
|--------------------------------|----------------|--------------------|-------------------|-------------------|
| LPL variant                    | 0(0)           | 9(8·7)             | 4(6·9)            | 5(11·1)           |
| LMF1 variant                   | 2(4·3)         | 11(10·7)           | 6(10·3)           | 5(11·1)           |
| GPHBP1 variant                 | 2(4·3)         | 2(1·9)             | 1(1·7)            | 1(2·2)            |
| APOA5 variant                  | 0(0)           | 7(6·8)             | 3(5·2)            | 4(8·9)            |
| GCKR variant                   | 2(4·3)         | 8(7·8)             | 4(6·9)            | 4(8·9)            |
| CETP variant                   | 0              | 1(1·0)             | 1(1·7)            | 0                 |
| TRIB1 variant                  | 0              | 3(2·9)             | 2(3·4)            | 1(2·2)            |
| APOE variant                   | 1(2·2)         | 4(3·9)             | 1(1·7)            | 3(6·7)            |
| APOB variant                   | 7(15·2)        | 15(14·6)           | 9(15·5)           | 6(13·3)           |
| APOA4 variant                  | 1(2·2)         | 2(1·9)             | 1(1·7)            | 2(4·4)            |
| APOAI variant                  | 0              | 2(1·9)             | 0                 | 2(4·4)            |
| ANGPTL3 variant                | 0              | 2(1·9)             | 1(1·7)            | 2(4·4)            |
| LPL molecular regulating variant | 4(8·7)       | 20(22·2)           | 13(22·4)          | 13(28·9)*         |

### Table 3
Clinical and genetic features in patients with and without acute pancreatitis.

| Variables                      | With AP n = 18 | Without AP n = 85 | P value |
|--------------------------------|----------------|-------------------|---------|
| Age, years                     | 45·0 ± 11·4    | 51·3 ± 9·6        | 0·130   |
| Sex, n(%)                      | 12(66·7)       | 66(77·6)          | 0·324   |
| TGadmission (mmol/L)           | 10·9 ± 3·8     | 9·4 ± 4·0         | 0·147   |
| TG (mmol/L)                    | 16·6 ± 6·7     | 11·3 ± 5·7        | 0·005   |
| Subgroup 1                     | 8(44·4)        | 50(58·8)          | 0·264   |
| Subgroup 2                     | 10(55·6)       | 35(41·1)          | 0·265   |
| BMI (kg/m²)                    | 25·4 ± 3·0     | 26·2 ± 2·6        | 0·651   |
| Alcohol, n (%)                 | 7(38·9)        | 46(55·3)          | 0·651   |
| Abstainers                     |                |                   |         |
| age, years                     | 45·0 ± 11·4    | 51·3 ± 9·6        | 0·130   |
| Sex, n(%)                      | 12(66·7)       | 66(77·6)          | 0·324   |
| TGadmission (mmol/L)           | 10·9 ± 3·8     | 9·4 ± 4·0         | 0·147   |
| TG (mmol/L)                    | 16·6 ± 6·7     | 11·3 ± 5·7        | 0·005   |
| Subgroup 1                     | 8(44·4)        | 50(58·8)          | 0·264   |
| Subgroup 2                     | 10(55·6)       | 35(41·1)          | 0·265   |
| BMI (kg/m²)                    | 25·4 ± 3·0     | 26·2 ± 2·6        | 0·651   |
| Alcohol, n (%)                 | 7(38·9)        | 46(55·3)          | 0·651   |
| Abstainers                     |                |                   |         |
4. Discussion

SHTG has become a major challenge in China due to its high prevalence in populations, its complexity in causes, fewer strategies in treatment, and high risk in AP development. Until recently, the most of genetic studies on patients with SHTG mainly focused on LPL molecular regulating genes, namely LPL, APOA5, APOC2, GPHLBP1 and LMF1, which might be in neglect of many other genetic causes [23–26]. In the current study, we performed NGS in 15 reported TG-regulating genes in a cohort of 103 Chinese patients with primary SHTG and 46 age- and sex- matched controls. Overall, we found that pathogenic rare variants were detected in 58 subjects (3·0%). Among these subjects, 46 patients were within SHTG group while 12 individuals were within control cases. The major findings of the present study were: 1) Presence of rare variants in LPL, APOA5, LPL molecular regulating genes and all the sequenced genes were associated with SHTG; 2) Extensive analysis of 15 genes increased the detection rate of the rare variants in SHTG group by 19·5% compared to the analysis of merely sequencing LPL molecular regulating genes; 3) Patients with history of AP presented higher frequency of the rare variants in LPL gene and all the LPL molecular regulating genes; 4) The GRS of some common variants were increased in subgroups according to the extent of SHTG.

It has been demonstrated that SHTG can be induced by both primary or secondary causes. A previous study on 215 Japanese patients with SHTG (TG > 1000 mg/dL) suggested that 74% of them were induced by secondary causes including diabetes, alcohol intake, and drug causes [27]. Before analyzing the genetic basis, therefore, we excluded 39·9%
(70 of 173) patients who were clinically considered as secondary cases. Subsequently, for other 103 patients, the 15 genes were sequenced, some of which had been analyzed in previous studies. For example, a study including 110 non-diabetic SHTG patients of European geographic ancestry indicated that 6-4%, 0-9%, and 3-6% of patients had rare variants in LPL, APOA5, and APOC2, respectively [23]. Khovidhunkit and his colleagues reported that in a cohort of Thai patients, 13% vs. 0% and 2% vs. 0% of patients in SHTG (TG > 10 mmol/L) vs. control group (TG < 1-7 mmol/L) had rare variants in LPL and APOA5 while no rare variants in APOC2 was detected [24]. Other studies showed LPL frequency ranging from 1-5% to 36% [19,25,28]. In our study, the detection rate of rare variants in LPL was relatively higher than that in other genes and such variants were found only in SHTG group. However, regarding other genes, rare variants in LMF1, GCKR and APOB accounted for 10-7%, 7-8%, and 14-6% of SHTG subjects in our study, which was similar to those of previous studies.

Recent studies have revealed that other candidate genes except for LPL, APOA5, APOC2, GPHB1, and LMF1 are also involved in the manifestation of SHTG in some individuals [20,21]. The association of rare variants in APOB with SHTG has previously been reported in several studies [17]. Gene APOA5 is located on a well-known gene cluster at chromosome 11, namely APOA5/A4/C3/A1, and the accumulating evidence has shown that single nucleotide polymorphisms (SNPs) in the APOA5/A4/C3/A1 gene cluster are associated with high triglyceride levels [19]. Furthermore, in genome-wide association studies, the possible association of polymorphisms in GCKR gene with hypertriglyceridemia has been analyzed [29]. Besides, Santoro et al. has demonstrated that GCKR has a facilitating effect for the manifestation of HTG in young obese individuals [30]. Additionally, TRIB1 is another polygenic determinant of SHTG whose protein facilitates the proteasome-dependent protein degradation [31]. Moreover, ANGPTL3 and APOE genotype may also explain additional variation in certain HTG phenotype. In our genetic analysis, detection rate of rare variants in SHTG group, subgroup 1 and subgroup 2 increased by 19-5%, 22-2%, and 15-9%, respectively, which may suggest the necessity for the expanding sequencing.

As is well known, SHTG has many fatal complications among which AP is the most common one. However, the genetic features of patients with SHTG-induced AP is not adequately studied. Recently, the study by Khovidhunkit et al. in Thai individuals reported that among 13 patients who had history of AP, 4 had a heterozygous p.Gly185Cys common variant in APOA5, 3 only had rare variants in LPL (p.Ala98Thr, p.Leu279Val, and p.Asp308Glyfs*3), and the other 6 had no identifiable variants contributing to SHTG [24]. In their study, accurate relation of genetic profile to AP could not identified due to the fact that only limited number of genes were sequenced. Another study performed by Zhu et al. among Chinese patients in emergency department sequenced APOA5, APOC2, APOE, BLK, LPL, GPHB1, and LMF1 in 11 patients with SHTG and suggested that 6 rare variants in LPL molecular regulating genes (APOA5, GPHB1, LMF1) and 1 rare variant in APOE were found in patients with AP. Similarly, their study was also limited by small sample size besides their study had no controls [32]. More importantly, in these studies, the high frequency of LPL molecular regulating genes in SHTG patients was visible but not statistically provable. Interestingly, in our study, patients with history of AP presented statistically higher frequency of rare variants in LPL gene and all the LPL molecular regulating genes (27-8% vs. 4-7% and 50-0% vs. 20-0% p < 0-05 respectively), indicating that rare variants in LPL and its molecular regulating genes could increase the risk of AP among Chinese patients with SHTG.

Besides, the present study showed that the risk scores for SHTG determined by common TG-associated variants were increased in subgroups according to the extent of SHTG, which was in coincidence with previous study. The data also indicated that only a small portion of TG variance could be explained by rare variants. Interestingly, it appeared that TG variance in patients with less extent SHTG (< 11·30 mmol/L) could be explained by only common variants, which might affect the future sequencing strategy.

Although our study might add new information regarding the relation of genetic analysis to SHTG as well as AP, the study also had several limitations. Firstly, the sample size of the present study might be not large enough to reflect the whole patterns of genetic and clinical phenotypes in patients with SHTG. Secondly, the AP history was recognized by patients’ oral account and medical records, some patients with mild symptoms might be underdiagnosed. Finally, pathogenic analysis of genes was mainly based on risk prediction software, which might limit its accuracy of the analysis. Hence, a large sample size study and further functional testing may be needed to confirm our findings.

In conclusion, expanding genetic analysis had a higher detection rate of rare variants in patients with SHTG. Detecting rare variants in LPL and its molecular regulating genes might be a tool in estimating risk of AP among Chinese patients with SHTG.

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**Conflicts of interest**

We declare that we have no conflict of interest.

**Authors’ contributions**

Dr. Jing-Lu Jin completed the project, analyzed the data, and wrote the manuscript. Dr. Jian-Jun Li designed the study, interpreted the data and contributed to critically revising the manuscript. Dr. Di Sun, Dr. Ye Xuan-Cao and Dr. Hui-Wen Zhang contributed to data collection. Drs Wu, Zhu and Guo contributed to recruitment of patients and clinical diagnosis of disease and data collection. Drs Gao and Dong, and Ms. Liu and Dong contributed to the collections of clinical data and procedure of laboratory examination. All authors have approved the final article.

**Appendix A. Supplementary data**

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ebiom.2018.11.001.

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