Original article

Hypertriglyceridemic acute pancreatitis in emergency department: Typical clinical features and genetic variants

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OBJECTIVE: To investigate the clinical characteristics of patients with hypertriglyceridemic acute pancreatitis (HTGAP), and the molecular foundation contributing to hypertriglyceridemia in such patients.

METHODS: Clinical data from 329 patients with acute pancreatitis (AP) were analyzed. The patients were divided into the HTGAP group, with fasting serum triglyceride (TG) levels ≥500 mg/dL (5.65 mmol/L), and the non-HTGAP (NHTGAP) group. Targeted next-generation sequencing was applied to 11 HTGAP patients to identify the genetic mutations associated with hypertriglyceridemia, including apolipoprotein A-V (APOA5), APOC2, APOC3 and APOE, BLK, LPL, GPIHBP1 and LMF1.

RESULTS: Patients in the HTGAP group, compared with those in the NHTGAP group, had a higher mortality rate (7.5% vs 0.7%, P = 0.001), more commonly seen severe AP (17.5% vs 5.2%, P = 0.004) as well as a higher recurrence rate (32.4% vs 19.9%, P = 0.070). DNA sequencing showed that two patients carried the same compound of p.G185C and p.V153M heterozygous mutations located in the APOA5 gene. Two patients carried a homozygous variation of p.C14F, in the GPIHBP1 gene. One patient had a homozygous variation of p.R176C in the APOE gene. And a rare heterozygous LMF1 gene mutation of p.P562R was detected in two patients.

CONCLUSIONS: HTGAP was significantly severe than NHTGAP, with a high recurrence rate. Genetic information may be useful in the clinical setting for the investigation of the pathogenesis of HTGAP and its interventions.

KEY WORDS: acute pancreatitis, hypertriglyceridemia, gene mutation.

INTRODUCTION

Acute pancreatitis (AP) is a common gastrointestinal emergency with an increasing incidence in China during the past decades. Because of its severity ranging from mild to severe or even fatal, or with systemic inflammatory conditions, AP continues to arouse the interest of clinical researchers.1,2 Although AP in most patients is mild, moderate to severe as well as recurrent AP remains a tremendous burden on both public healthcare system and the
The most common etiologies of AP are cholelithiasis with obstruction of common bile duct or main pancreatic duct and alcohol abuse. Currently, a rapid increase in obesity nationwide and worldwide is associated with an elevated incidence of metabolic diseases and changes in disease spectra. Hyperlipidemia, characterized by serum hypertriglyceridemia, has become the third leading cause of AP and contributed to 1–10% of patients with AP. Moreover, mild to moderate hyperlipidemia can be regarded as an underlying phenomenon or comorbidity of pancreatitis.11–13 Previous cohort studies have indicated that serum triglyceride (TG) of ≥1000 mg/dl (11.3 mmol/L) is a high risk factor for patients with hypertriglyceridemic acute pancreatitis (HTGAP). It has also been considered necessary to diagnose HTGAP when the patient’s fasting TG level is 5.65–11.3 mmol/L with chylous blood which occurs in about 20% of all patients with AP.11,12 Clinical studies have demonstrated that HTGAP may contribute to increased severity and mortality, higher frequencies of comorbidities and systemic complications, longer length of hospitalization, and more frequent recurrence, than other subtypes of AP.10,13

Our emergency department has accepted many patients with AP and provided them with an early diagnosis and effective interventions. We thus conducted a retrospective study on AP patients who were admitted to our emergency department between 2012 and 2016, focusing on HTGAP. Considering that notable dyslipidemias have a strong genetic component despite the important role that secondary dietary factors play in the clinical phenotype, we further performed gene mutation detection for 11 patients with HTGAP to determine the molecular genetic characteristics of this special subtype of AP.14–16 In this study, we aimed to predict the severity and recurrence risk in HTGAP with the new-generation sequencing technology based upon clinical information of the patients.

MATERIALS AND METHODS

Patients

Patients who were admitted to the Emergency Medical Ward and Emergency Intensive Care Unit (EICU) of Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University (Shanghai, China) due to AP between January 2012 and December 2016 were retrospectively included in the study. The data of the patients was anonymous and their medical insurance numbers were encrypted. Those with insufficient medical records or who were transferred to another hospital before their completion of the treatment were excluded. Patients’ data and clinical details were collected from databases such as hospital inpatient enquiry, digital medical record and the hospital information management system, and the characteristics of the patients were reviewed. The clinical data of patients having AP with well-documented medical records were collected and analyzed, including their characteristics, clinical presentations, disease etiologies and severity, underlying diseases, comorbidities, length of hospital stay, laboratory test results, frequency of recurrence and outcomes, and so on. The protocol for the study was approved by the Institutional Ethics Committee of Ren Ji Hospital, and the study was conducted in accordance with the Declaration of Helsinki (2013).

Diagnostic criteria, severity classification and the etiologies of AP

The diagnosis and severity classification of AP (mild [MAP], moderately severe [MSAP] and severe AP [SAP]) were made according to the 2012 revision of the Atlanta classification of AP.2 AP was diagnosed when the patient met at least two of the following three criteria: (i) acute upper abdominal pain that usually radiating to the back; (ii) increased serum amylase or lipase to over thrice upper limit of normal (ULN); and (iii) evidence of AP on imaging examinations. MAP was defined as AP in the absence of organ failure or local/systemic complications. MSAP was defined as transient (<48 h) organ failure, with or without local/systemic complications or the exacerbation of coexisting diseases. SAP was diagnosed when organ failure (single or multiple) was persistent for at least 48 h. Organ failure included pulmonary failure (arterial oxygen partial pressure [PO2] <60 mmHg at room temperature or a need for mechanical ventilation), cardiovascular failure (the persistence of shock [systolic blood pressure <90 mmHg] after fluid resuscitation), renal failure (serum creatinine [Cr] level >176.8 μmol/L [2 mg/dL] after rehydration or the need for hemodialysis in patients without pre-existing renal diseases).

Three main etiologies of AP (biliary origin, alcohol abuse and hypertriglyceridemia) were identified. When cholelithiasis was identified by diagnostic imaging modalities including ultrasonography, plain or
contrast-enhanced computed tomography (CT) and/or magnetic resonance imaging (MRI) scan, biliary AP was diagnosed. Alcohol-related AP was defined as AP related to an average alcohol consumption of 80 g daily for men and 50 g daily for women for at least 5 years or an excessive amount (≥200 g for men and ≥150 g for women) of alcohol consumed immediately before the acute disease attack. Other types of AP were classified as idiopathic AP with multiple etiologies.

Peripheral blood sample was collected from the patients after overnight fasting. Serum amylase levels, liver and renal function tests, blood sugar and hemoglobin A1c (HbA1c) concentrations were analyzed using a Biochemical Analyzer (Roche, Basel, Switzerland). Serum TG level was determined at day 2 or 3 of their admission. As reported previously, serum TG with a threshold of 500 mg/dL (5.65 mmol/L) has been reported to be associated with a high incidence of AP.\textsuperscript{11,17–19} HTGAP was diagnosed in this study when: (i) TG ≥11.3 mmol/L; or (ii) TG ≥5.65 mmol/L with visible chylomicronemia. Fatty liver disease was identified by ultrasonography showing a “bright” liver with increased echogenicity and/or CT/MRI scan showing a low density in the liver than in the spleen. Diabetes was diagnosed according to a clear medical history of diabetes and/or HbA1c ≥6.5%. Hypertension was diagnosed in patients with a blood pressure of over 140/90 mmHg after measured for three times under resting state or a clear history of hypertension. The patients who had at least two or three AP attacks were defined as having recurrent or repeated recurrent AP.

**Targeted sequencing**

**Patients and samples**

Among the AP patients with a fasting serum TG level ≥5.65 mmol/L, 11 (including 7 men and 4 women) agreed to provide their peripheral blood samples for gene detection. Written informed consent was obtained before the collection of the blood from each patient.

Genomic DNA from blood samples was extracted using a High Pure PCR Template Preparation Kit (Roche) according to the manufacturer’s instructions. Solution-based hybridization capture was used to enrich DNA fragments for sequencing on a MiSeq Sequencing Platform with a base paired-end read module (2 × 300; Illumina, San Diego, CA, USA). The procedure of hybridization capture was fulfilled by a commercial SureSelect Library Prep Kit (Agilent, Santa Clara, CA, USA) to capture a region of 97.49 kb, containing 325 regions of 32 genes known to cause hyperlipidemia. We selected eight target genes that are known to be associated with hypertriglyceridemia or familial combined hypertriglyceridemia for analysis, including APOA5, APOC3, APOE, BLK, LPL, APOC2, GPIHP1, and LMF1. Oligonucleotide baits were designed to cover total coding exons, untranslated regions and at least 10 intronic nucleotides and all intron-exon boundaries nearby, excluding deep intronic sequences to avoid enlarging the sequence target size and subsequently reducing the sequence coverage. DNA was first quantified by applying a Qubit 2.0 (Thermo Fisher Scientific, Waltham, MA, USA), followed by 0.3-ng genomic DNA sonication sheared with a Diagenode Bioruptor Plus (Diagenode, Liege, Belgium), hybridization was then performed between the biotinylated RNA oligonucleotide baits and sheared DNA. After removing the captured fragments from the solution via a streptavidin-coated magnetic microbeads and elution step (Dynabeads MyOne Streptavidin T1; Thermo Fisher Scientific), polymerase chain reaction (PCR) amplification was performed using the enriched fragment library and primers specific to the linked Illumina adaptors. Then the PCR-produced libraries were further processed by the MiSeq Sequencing Platform (Illumina) after quantification by a quantitative PCR. All the samples could be sequenced together depending on the 6-bp index sequences (Illumina) used to differentiate different samples.

**Variant analysis**

After MiSeq sequencing, the raw data of each sample were sorted automatically by index sequences and the adapter sequences were trimmed by Cutadapt 1.13 (https://cutadapt.readthedocs.io/en/stable/).\textsuperscript{20} SolexaQA\textsuperscript{21} V3.1.2 was used to remove low-quality bases (<Q20). The clean results were aligned to the database of the human reference genome (hg19) by applying the Burrows-Wheeler Aligner 0.7.11.\textsuperscript{22} After alignment, the PCR duplicates were removed using the Mark Duplicates package of Picard 1.109 (https://broadinstitute.github.io/picard/). Realignment around indel sites known and base quality score recalibration were performed by GATK 3.3.\textsuperscript{23} GATK HaplotypeCaller was used to call raw variants. Indels and single nuclear polymorphisms were annotated with ANNOVAR.\textsuperscript{24} Public databases including dbSNP138, 1000 Genome project, Exome Sequencing Project, Clinic Var and the Human Gene Mutation Database\textsuperscript{25} were used to screen variants. The prediction of functional effect was evaluated by PolyPhen and SIFT scores.\textsuperscript{26,27} To detect the copy number variant, the sequencing depth of each

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region covered by the probes was calculated according to the alignment files. An Exomeddepth package\textsuperscript{28} was also used to find potential copy number variants. During copy number variant analysis, confirmed point mutation samples in the same sequence run served as controls.

**Statistical analysis**

Statistical analyses were performed using SPSS 17.0 (IBM, Armonk, NY, USA). Continuous variables were expressed as mean $\pm$ standard deviation (SD), whereas categorical variables were expressed as numbers and percentages. Comparisons between the groups were performed using Student’s t-test (for independent samples), Pearson’s $\chi^2$ test and Fisher’s exact $\chi^2$ test when appropriate. Difference was considered as statistically significant at $P < 0.05$.

**RESULTS**

**Characteristics of the patients**

A total of 329 patients with AP were admitted to the Emergency Department (either the ward or ICU) of Ren Ji Hospital with detailed medical records. Fasting TG level was over 1.7 mmol/L, or without hypercholesterolemia, in 118 patients, all of whom were diagnosed as having hyperlipidemia. Among them, those with a fasting serum TG of $\geq$11.3 mmol/L or of $\geq$5.65 mmol/L together with visible chylomicronemia were enrolled in the HTGAP group ($n = 40$, 12.2%). Other patients were enrolled in the non-HTGAP (NHTGAP) group ($n = 289$, 87.8%), and were further divided into three subgroups based on their etiologies: biliary AP ($n = 76$, 23.1%), alcohol-induced AP ($n = 32$, 9.7%) and idiopathic AP ($n = 181$, 55.0%) (Fig. 1a). Serum TG level was 2.80 $\pm$ 4.62 mmol/L among all the patients, with a significantly higher level of 11.45 $\pm$ 8.90 mmol/L in the HTGAP group compared with that of 1.58 $\pm$ 1.24 mmol/L in the NHTGAP group. A peak serum TG concentration was 50.54 mmol/L (Table 1).

The NHGTAP patients were significantly elder than those in the HTGAP group (53.14 $\pm$ 16.01 years vs 37.38 $\pm$ 11.02 years, $P = 0.000$). There was a slight male predominance in both groups (27 [67.5%] and 180 [62.3%], respectively, in the HTGAP and NHTGAP groups, $P = 0.523$). Although the average length of hospitalization was slightly longer in the HTGAP group than in the NHTGAP group (14.48 $\pm$ 7.45 days vs 12.85 $\pm$ 6.28 days, $P = 0.194$), the difference was not statistically significant. The overall mortality rate was 1.5% in all the 329 AP patients; HTGAP patients had a notably higher mortality compared with the NHTGAP group (7.5% [3/40] vs 0.7% [2/289], $P = 0.001$).

MAP, MSAP and SAP were found in 7 (17.5%), 26 (65.0%) and 7 (17.5%) of the HTGAP patients, and 142 (49.1%), 132 (45.7%) and 15 (5.2%) of the NHTGAP patients, respectively. Difference was observed in the frequency of MAP ($P = 0.001$) and SAP ($P = 0.004$) between the two groups (Table 1).

Diabetes (21/40 [52.5%] vs 43/289 [14.9%], $P = 0.000$) and fatty liver disease (28/40 [70.0%] vs 116/289 [40.1%], $P = 0.014$) were more common comorbidities in the HTGAP group than in the NHTGAP group; while there was no significant difference between the HTGAP and the NHTGAP groups with respect to hypertension (11/40 [27.5%] vs 91/289 [31.5%], $P = 0.671$). There was a higher recurrence rate of AP in the HTGAP group than in the NHTGAP group (12/37 [32.4%] vs 57/287 [19.9%], $P = 0.070$) and repeated recurrence rate (> two episodes in one patient) was higher in the HTGAP group than in the NHTGAP group (6/12 [50.0%] vs 20/57 [35.1%], $P = 0.040$; Table 1, Fig. 1b).

**Detection of gene mutation in HTGAP and clinical outcomes**

DNA sequencing showed that two patients (nos. 3 and 5) carried the same heterozygous mutation (p.G185C) and four (nos. 1, 7, 8 and 10) had another heterozygous mutation (p.V153M) of the APOA5 gene. Two patients (nos. 2 and 6) with compound heterozygous variants had p.G185C and p.V153M in the APOA5 gene presented with marked hypertriglyceridemia, and patient no. 2 with the peak concentration died of SAP. Two patients (nos. 4 and 5) carried a same homozygous variation of p.C14F in the GPIHBP1 gene and two (nos. 1 and 7) carried the same heterozygous mutation of p.C14F in the GPIHBP1 gene. One patient (no. 9), who carried a homozygous variation of p.R176C (rs7412) in the APOE gene, presented with a high TG level of 19.12 mmol/L. Two patients (nos. 1 and 10) carried the same heterozygous mutation of p.R176C in the APOE gene also had high TG levels. Two patients (nos. 3 and 5) carried the same rare heterozygous mutation, p.P562R, in the LMF1 gene. One patient (no. 11) had another rare heterozygous mutation of p.N249T in the LMF1 gene (Table 2, Fig. 2 and Supplementary Fig. 1). No mutation was seen in APOC2, APOC3, LPL or BLK genes.
Table 1. Clinical characteristics of 329 patients with acute pancreatitis (AP)

|                | HTGAP (n = 40) | BAP (n = 76) | P value | AAP (n = 32) | P value | IAP (n = 181) | P value | Total (n = 289) | P value |
|----------------|---------------|--------------|---------|--------------|---------|---------------|---------|----------------|---------|
| Age, years (mean ± SD) | 37.38 ± 11.02 | 58.59 ± 16.43 | 0.000   | 47.63 ± 13.18 | 0.000   | 51.82 ± 15.75 | 0.000   | 53.14 ± 16.01 | 0.000   |
| Male sex, n (%)      | 27 (67.5)     | 36 (47.4)    | 0.039   | 32 (100)     | 0.000   | 112 (61.9)    | 0.506   | 180 (62.3)     | 0.523   |
| Serum TG, mmol/L (mean ± SD) | 11.45 ± 8.90 | 1.13 ± 0.60  | 0.000   | 1.77 ± 1.38  | 0.000   | 1.74 ± 1.37   | 0.000   | 1.58 ± 1.24   | 0.000   |
| Diabetes, n (%)      | 21 (52.5)     | 8 (10.5)     | 0.000   | 8 (25.0)     | 0.019   | 27 (14.9)     | 0.000   | 43 (14.9)     | 0.000   |
| Hypertension, n (%)  | 11 (27.5)     | 26 (34.2)    | 0.552   | 10 (31.3)    | 0.730   | 55 (30.4)     | 0.719   | 91 (31.5)     | 0.671   |
| Fatty liver disease, n (%) | 28 (70.0)  | 17 (22.4)    | 0.000   | 15 (46.9)    | 0.048   | 84 (46.4)     | 0.007   | 116 (40.1)    | 0.014   |
| Length of hospitalization, days (mean ± SD) | 14.48 ± 7.45 | 13.84 ± 5.90 | 0.618   | 11.84 ± 3.93 | 0.059   | 12.53 ± 6.62  | 0.102   | 12.85 ± 6.28  | 0.194   |
| MAP, n (%)           | 7 (17.5)      | 29 (38.2)    | 0.023   | 16 (50.0)    | 0.004   | 97 (53.6)     | 0.005   | 142 (49.1)    | 0.001   |
| MSAP, n (%)          | 26 (65.0)     | 43 (56.6)    | 0.638   | 15 (46.9)    | 0.311   | 74 (40.9)     | 0.170   | 132 (45.7)    | 0.060   |
| SAP, n (%)           | 7 (17.5)      | 4 (5.3)      | 0.033   | 1 (3.1)      | 0.015   | 10 (5.5)      | 0.010   | 15 (5.2)      | 0.004   |
| Recurrence, n/N (%)  | 12/37 (32.4)  | 17/76 (22.4) | 0.369   | 9/32 (28.1)  | 0.642   | 31/179 (17.3) | 0.037   | 57/287 (19.9) | 0.070   |
| RR, n/N (%)          | 6/12 (50.0)   | 3/17 (17.6)  | 0.035   | 4/9 (44.4)   | 0.762   | 13/31 (41.9)  | 0.081   | 20/57 (35.1)  | 0.040   |
| Mortality, n (%)     | 3 (7.5)       | 0 (0)        | 0.016   | 0 (0)        | 0.116   | 2 (1.1)       | 0.014   | 2 (0.7)       | 0.001   |

All P values compared with the hypertriglyceridemic AP (HTGAP) group.
Mild (MAP), moderately severe (MSAP) and severe AP (SAP) are classified according to the 2012 revision of the Atlanta classification.\(^2\)
AAP, alcohol-induced acute pancreatitis; BAP, biliary acute pancreatitis; IAP, idiopathic acute pancreatitis; NHTGAP, non-HTGAP; RR, repeated recurrence (at least two episodes of recurrence in one patient); SD, standard deviation; TG, triglyceride.
patients with HTGAP accounted for 12.2% (40/329) of all patients with AP. We found that younger age, diabetes mellitus, fatty liver, a high recurrence rate and a more severe prognosis were more commonly observed in HTGAP, a finding similar to that reported in recent studies.\textsuperscript{10,12,29,30}

Elevated serum TG levels are known to be associated with an increased incidence of acute or recurrent pancreatitis.\textsuperscript{6,11} Patients whose serum TG level remained ≥5.65 mmol/L had a higher probability of AP attacks. Thus we chose a fasting serum TG level of ≥5.65 mmol/L with visible chylomicronemia or TG ≥11.3 mmol/L to obtain an accurate diagnosis of hypertriglyceridemia. Population-based studies from Christian \textit{et al.}\textsuperscript{17} and Murphy \textit{et al.}\textsuperscript{11} defined TG levels of ≥5.65 mmol/L (500 mg/dL) and 1.7 mmol/L as cut-off values, respectively. The incidence of AP in individuals with serum TG levels of above 5.65 mmol/L was significantly higher than in those with a TG level of 150–500 mg/dL. Although there are controversies about the severity of HTGAP compared with other subsets of AP,\textsuperscript{3,11,30} our study showed that HTGAP was associated with a higher incidence of MSAP (65.0%) and SAP (17.5%), more common comorbidities such as diabetes and fatty liver as well as a higher mortality (7.5%). High recurrence rates or repeat recurrence rates were also found in the HTGAP group. It should be noted that mild to moderate hypertriglyceridemia (TG 175–500 mg/dL) could be considered as a comorbidity of pancreatitis, and choosing a lower cut-off value for hypertriglyceridemia might bear major flaws. This might explain the results of a British study in 43 patients with different types of AP using a cut-off value of TG >175 mg/dL (2 mmol/L) that higher severity was not found in the HTGAP group compared with other subtypes of AP.\textsuperscript{8,30}

Among the 11 patients who underwent genetic detection, we found that eight carried heterozygous APOA5 gene mutations. Genome-wide association studies have shown that genetic variations existing in the APOA5 loci, mainly \textit{p.G185C}, \textit{p.V153M} and \textit{p.S19W}, are definitely associated with abnormal blood TG levels in humans, each with compound monogenic or polygenetic effects.\textsuperscript{31} Although the \textit{p.G185C} variant located in the APOA5 gene was first reported in 2003, it has attracted great attention from researchers during the past years because its homozygous mutation contributes strongly to severe hypertriglyceridemia and resultant HTGAP.\textsuperscript{32–34} In particular, the \textit{p.G185C} polymorphism in the APOA5 gene is more commonly distributed in Asians, including Chinese populations,

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{(a) The flowchart of patient grouping and subtypes of patients with acute pancreatitis (AP). (b) The histogram graph showing the grouping, subtypes, serum triglyceride (TG) levels, and mortality of AP. AAP, alcohol-induced acute pancreatitis; AIP, autoimmune pancreatitis; BAP, biliary acute pancreatitis; IAP, idiopathic acute pancreatitis; HTGAP, hypertriglyceridemic acute pancreatitis; MAP, mild acute pancreatitis; MSAP, moderately severe acute pancreatitis; SAP, severe acute pancreatitis. *\textit{P} < 0.05 and **\textit{P} < 0.01 compared with HTGAP.}
\end{figure}

Among the 11 patients, two were classified as having MAP (nos. 6 and 10), seven had moderate AP (nos. 1, 3, 4, 5, 7, 8 and 11) and the other two had SAP (nos. 2 and 9); one of them died of SAP (no. 2), and four (nos. 3, 4, 5 and 7) had recurrence. Notably, patient no. 5, who had hypertriglyceridemia and carried the homozygous variation of \textit{p.C14F} in the GPIHBP1 gene and heterozygous mutation of \textit{p.G185C} in the APOA5 gene, was readmitted nine times to our hospital.

\section*{DISCUSSION}

In this retrospective exploratory study on AP, we conducted a cohort analysis in 329 patients who were admitted to our emergency department and focused on those with HTGAP. Additionally, we obtained genetic information on hyperlipidemia from 11 patients with HTGAP. In the present study,
than in others.\textsuperscript{15,35,36} It has been speculated that the functional mechanism of \textit{APOA5} can decrease the concentration of blood TG by increasing lipoprotein lipase activity.\textsuperscript{37} We inferred that a functional loss of single nucleotide polymorphisms in the \textit{APOA5} gene would result in reduced TG lipolysis and remnant accumulation, hence causing hypertriglyceridemia.\textsuperscript{38,39} However, all the eight patients identified in this study were heterozygous for \textit{p.G185C} and \textit{p.V153M}, or compound heterozygous for \textit{p.G185C} and \textit{p.V153M}, which may have contributed partially to the abnormal serum TG levels.\textsuperscript{40–42}

It is interesting that two (nos. 4 and 5) of the 11 patients carried a same homozygous \textit{p.C14F} variation located in the \textit{GPIHBP1} gene and two had the same heterozygous mutation of \textit{p.C14F} in the \textit{GPIHBP1} gene. \textit{GPIHBP1} has been found to be expressed on capillary endothelial cells to bind lipoprotein lipase and shuttle it to its site of action in the

| Patient nos. | Mutant gene | Position | SNP | Exon | Nucleotide change | Protein change | Status |
|--------------|-------------|----------|-----|------|-------------------|----------------|--------|
| 3, 5         | APOA5       | NM_052968| chr11-116661392 | rs2075291 | exon4 | c.G553T | p.G185C | het    |
| 1, 7, 8, 10  | APOA5       | NM_052968| chr11-116661488 | rs3135507 | exon4 | c.G457A | p.V153M | het    |
| 2, 6         | APOA5       | NM_052968| chr11-116661392 | rs2075291 | exon4 | c.G553T | p.G185C | com.   |
| 9            | APOE        | NM_052968| chr11-116661488 | rs3135507 | exon4 | c.G457A | p.V153M | het    |
| 1, 10        | APOE        | NM_00041 | chr19-45412079 | rs7412    | exon4 | c.C526T | p.R176C | hom    |
| 4, 5         | GPIHBP1     | NM_178172| chr8-144295183 | rs11538389| exon1 | c.G41T | p.C14F | hom    |
| 1, 7         | GPIHBP1     | NM_178172| chr8-144295183 | rs11538389| exon1 | c.G41T | p.C14F | het    |
| 3, 5         | LMF1        | NM_022773| chr16-904551 | rs4984948| exon11 | c.C1685G| p.P562R | het    |
| 11           | LMF1        | NM_022773| chr16-929721 | -         | exon6  | c.A746C | p.N249T | het    |

Table 2. Main genetic variants in 11 patients with acute pancreatitis and severe hypertriglyceridemia

\textit{APOA5}, apolipoprotein A-V; \textit{APOE}, apolipoprotein E; com. het, compound heterozygous mutation; \textit{GPIHBP1}, glycosylphosphatidylinositol anchored high density lipoprotein binding protein 1; hom, homozygous mutation; het, heterozygous mutation; \textit{LMF1}, lipase maturation factor 1.
capillary lumen, which is crucial in the process of releasing fatty acid from blood TG for uptake in tissues. Homozygous GPIHBP1 genetic mutations may interfere with protein folding and disable the capacity of GPIHBP1 to combine and transport lipoprotein lipase, resulting in severe hypertriglyceridemia. This can help to explain the pathogenesis of hypertriglyceridemia in some patients with AP in this study.

Among the 11 patients, one (no. 9) had a homogenous APOE mutation of p.R176C (rs7412), and two had the same heterozygous mutations, which were variants that are more often found in isolated hypertriglyceridemia in APOE-associated dyslipidemia. Recent studies have also suggested that dyslipidemia due to a polymorphism allele of the APOE gene or APOE deficiency can be identified by the accumulation of chylomicrons in the blood. This was similar to the finding in our study that one patient carried a homogeneous APOE mutation with a high TG level of 19.12 mmol/L. This indicates that dysfunctional APOE contributes to the occurrence of HTGAP, while the relationship between APOE mutations and AP needs further investigation. One unique finding in the HTGAP group was that two patients carried the same heterozygous mutation (p.P562R in the LMF1 gene), and one carried a heterozygous mutation (p.N249T in the LMF1 gene). These have been considered rare gene variants up to now and the relationship between LMF1 and HTGAP is still unclear and needs further research.

AP-associated dyslipidemias are heterogeneous disorders with a strong genetic component, characterized by the moderate to severe elevation of serum TG in combination with or without hypercholesterolemia. It results from the functional loss of mutations in a single gene or more genetic loci that damage the process of lipolysis and intravascular chylomicron clearance, with an inherited autosomal recessive mode caused by mutations in LPL, APOC2, LMF1, APOA5, GPIHBP1, APOE. However, limited genetic information is available among individuals with HTGAP admitted to the emergency department. The present study identified several common and rare variants across the human genomes that are associated with HTGAP, indicating that genetic variants may have an important influence on the incidence of HTGAP. Once HTGAP attack has been resolved in these patients, we should focus on the strategies for early intervention and the prevention of recurrence, including early diagnosis, dietary restrictions and close monitoring of serum lipid levels. Therefore, we recommend routine genetic test combined with treatment of HTGAP, providing genetic information to patients with hyperlipemia type 1 or combined hypertriglyceridemia, and those with inherited mutations in APOA5, GPIHBP1, APOE and other candidate genes related to HTGAP. Pivotal factors for better outcomes include educating patients and encouraging them to adhere strictly to a low-fat diet, and using antihyperlipidemic agents to control their TG levels in order to avoid further episodes of AP.

The present study had some limitations. First, it was based on a single emergency medical center and some relevant details might not have been documented. Second, only 11 patients consented to gene detection. Further characterizations of the genetic profile influencing lipid levels with a larger sample size combined with the biological information of their family members are required to validate these findings.

In conclusion, patients with HTGAP presented with notably more severe disease process with a higher rate of recurrence compared with those without, although the differences were not statistically significant. Genetic mutations including APOA5, GPIHBP1 and APOE variants might have contributed to the occurrence of hypertriglyceridemia in patients with AP. Genetic information may be useful to investigate the pathogenesis of HTGAP and to predict the prognosis of patients with HTGAP, enabling them to offer a relevant management plan for prevention and early intervention of this disease. New-generation sequencing technology may change the current clinical diagnostic process.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher’s website:

Supplementary Fig. 1. Typical graphs of the second-generation sequencing coverage of gene mutations associated with hypertriglyceridemic acute pancreatitis (HTGAP). The typical peak map graphs of the second-generation sequencing coverage of the (A) APOA5, (B) APOE, (C) GPIHBP1 and (D) LMF1 genes. The bottom dark-blue region represents the exon regions of the genes and the areas between the blue regions are intron regions, respectively; each of the gray bars represents a read length of the second-generation sequencing, and the peak pattern above the gray bar represents the overall coverage of the region.