Gene-environment interaction between *APOA5* c.553G>T and pregnancy in hypertriglyceridemia-induced acute pancreatitis

Running title: *APOA5* c.553G>T and pregnancy in HTG-AP

Na Pu[1,†], BM, Qi Yang[1,†,*], PhD, Xiao-Lei Shi[1], BM, Wei-Wei Chen[1,2], MD, Xiao-Yao Li[1,3], PhD, Guo-Fu Zhang[1], BM, Gang Li[1], MD, Bai-Qiang Li[1], MD, Lu Ke[1], PhD, Zhi-Hui Tong[1], PhD, David N. Cooper[4], PhD, Jian-Min Chen[5], MD, PhD, Wei-Qin Li[1,*], PhD, Jie-Shou Li[1], PhD

[1]Surgical Intensive Care Unit (SICU), Department of General Surgery, Jinling Hospital, Medical School of Nanjing University, Nanjing, China.

[2]Department of Gastroenterology, Clinical Medical College, Yangzhou University, Yangzhou, China.

[3]Department of Intensive Care Unit, The Affiliated Drum Tower Hospital, Medical School of Nanjing University, Nanjing, China.

[4]Institute of Medical Genetics, School of Medicine, Cardiff University, Cardiff, United Kingdom.

[5]EFS, Univ Brest, Inserm, UMR 1078, GGB, F-29200 Brest, France.

Na Pu, punayeah@163.com

Qi Yang, yangqi_nj@163.com

Xiao-Lei Shi, 15950561608@163.com

Wei-Wei Chen, cww1984@126.com

Xiao-Yao Li, lixiaoyaonju@163.com

Guo-Fu Zhang, njuzgf@163.com

Gang Li, nju8icu@126.com

Bai-Qiang Li, li_baiqiang@aliyun.com

Lu Ke, kkb9832@163.com

Zhi-Hui Tong, njzyantol@hotmail.com

David N. Cooper, CooperDN@cardiff.ac.uk

Jian-Min Chen, jian-min.chen@univ-brest.fr

Wei-Qin Li, njzy_pancrea@163.com

Jie-Shou Li, lijieshou2013@sohu.com

†Na Pu and Qi Yang contributed equally to this work.

***Corresponding authors:**

Qi Yang, Department of General Surgery, Jinling Hospital, Nanjing University School of Medicine, Nanjing 210002, China. E-Mail: yangqi_nj@163.com

Wei-Qin Li, Department of General Surgery, Jinling Hospital, Nanjing University School of Medicine, Nanjing 210002, China. njzy_pancrea@163.com

Highlights (maximum 85 characters including space, for each point)

- *APOA5* c.553G>T has been previously associated with altered triglyceride levels.
- Here we report for the first time an association of *APOA5* c.553G>T with HTG-AP.
- We provide evidence that *APOA5* c.553G>T interacts with pregnancy in causing HTG-AP.
- Our findings provide novel insights into the complex etiology of HTG-AP.

Abstract (maximum 250 words)

BACKGROUND: The etiology of hypertriglyceridemia (HTG) and, consequently HTG-induced acute pancreatitis (HTG-AP), is complex.

OBJECTIVE: Herein, we explore a possible gene-environment interaction between *APOA5* c.553G>T (p.185Gly>Cys, rs2075291), a common variant associated with altered triglyceride levels, and pregnancy in HTG-AP.

METHODS: We enrolled 318 Han Chinese HTG-AP patients and divided them into three distinct groups: group 1, male patients (n =183); group 2, female patients whose disease was unrelated to pregnancy (n = 105); and group 3, female patients whose disease was related to pregnancy (n =30). *APOA5* rs2075291 genotype status was determined by Sanger sequencing. 362 healthy Han Chinese subjects were used as controls. Data on body mass index, peak triglyceride level, age of disease onset, episode number and clinical severity of HTG-AP were collected from each patient. Multiple comparisons, either between patient groups, between patient groups and controls, or within each patient group, were performed.

RESULTS: A robust association of *APOA5* rs2075291 with HTG-AP in general, and HTG-APIP in particular, was demonstrated. The minor T allele showed a stronger association with group 3 patients than with either group 1 or group 2 patients. This stronger association was due mainly to the much higher frequency of TT genotype in group 3 patients (20%) than that (<6%) in group 1 and group 2 patients. Moreover, the TT genotype was associated with a significantly higher peak triglyceride level in group 3 patients as compared to the GG genotype.

CONCLUSION: Our findings provide evidence for an interaction between *APOA5* rs2075291 and pregnancy in HTG-AP.

KEYWORDS: hypertriglyceridemia-induced acute pancreatitis (HTG-AP); acute pancreatitis in pregnancy (APIP); triglyceride; apolipoprotein A5; *APOA5* c.553G>T variant; gene-environment interaction

Introduction

Acute pancreatitis (AP) occurs with an annual incidence of 4.9–73.4 per 100,000 individuals worldwide.[1] Its incidence is increasing in recent years and its mean mortality rate has reached 2%.[2] Although biliary diseases, excessive alcohol consumption and hypertriglyceridemia (HTG) are generally thought to constitute the three leading etiologies of AP worldwide,[3, 4] in China more cases have been reported to be caused by HTG than by alcohol abuse.[5-7] Irrespective of which population is studied, HTG is often associated with more severe disease than other etiological factors.[4, 8, 9]

The etiology of HTG can be broadly divided into two categories, primary and secondary. Primary factors refer to genetic defects that cause or predispose to HTG whereas secondary etiological factors include obesity, alcohol abuse, diabetes mellitus and chronic renal failure.[10, 11] Nonetheless, in most cases, the etiology of HTG is complex.[12] Thus, for example, Dron and colleagues have recently shown that even severe HTG is primarily polygenic.[13] Moreover, it is increasingly appreciated that there is an interplay between primary and secondary etiological factors in causing severe HTG.[14, 15]

Pregnancy is a physiological state that is normally associated with a 2- to 4-fold increase in serum triglyceride (TG) levels in late gestation.[16] This increase is well tolerated by most women with normal baseline TG levels but may render those with genetic defects in TG metabolism prone to severe HTG and consequently HTG-induced AP (HTG-AP). In this regard, numerous studies have reported the identification of rare pathogenic variants in the lipoprotein lipase (*LPL*; OMIM# 609708),[17-26] glycosylphosphatidylinositol-anchored high-density lipoprotein binding protein 1 (*GPIHBP1*; OMIM# 612757) and apolipoprotein C2 (*APOC2*; OMIM# 608083) genes[24, 26] in patients experiencing HTG-AP during pregnancy (HTG-APIP). *LPL*, *GPIHBP1* and *APOC2*, together with *APOA5* (apolipoprotein A5; OMIM# 606368) and *LMF1* (lipase maturation factor 1; OMIM# 611761), constitute the five primary TG-related genes.[12, 27-29] Specifically, LPL plays an essential role in TG metabolism; APOC2 and APOA5 are essential LPL activators; LMF1 is involved in the folding and expression of LPL; GPIHBP1 mediates the transmembrane transport and binding of LPL.

By contrast, data on the possible interactions between pregnancy and common genetic risk factors in any of the five primary TG-related genes in HTG-AP have so far been scarce. We are aware of only

one such study, involving a single-nucleotide polymorphism in the *APOA5* gene, c.553G>T (p.Gly185>Cys; rs2075291), which was found in three (all homozygotes) of five Chinese patients with HTG-APIP.[24] *APOA5* c.553G>T is common in the Chinese population [minor allele frequency (MAF) = ~4%[30, 31]] and other Asian populations [e.g., MAF of 6.8% in East Asians according to gnomAD (https://gnomad.broadinstitute.org/)], but it is very rare in European populations (MAF of 0.03% according to gnomAD). Importantly, the minor T allele of *APOA5* rs2075291 has been firmly established as being associated with altered TG levels in normal controls and/or HTG patients in different Asian populations;[30-43] and its functionality and pathogenicity have been supported by different lines of experimental evidence.[44-47]

Data on the role of *APOA5* c.553G>T in HTG-AP unrelated to pregnancy are also scarce; this is reflected by the very limited number of patients so far analyzed for this variant [one in Arai et al. (2014)[48] and 11 in Chen et al. (2017)[49]]. Given the high frequency of this variant in the Chinese population and its direct role in altering TG levels, we sought to explore its potential interaction with pregnancy in HTG-AP in particular, and its relationship with the disease in general, using three well-defined Chinese patient cohorts.

Patients and Methods

Ethical compliance

This study was approved by the Ethics Committee of Jinling Hospital. Informed consent was obtained from all participating patients. Blood samples were obtained from the Biobank of Acute Pancreatitis in Jinling Hospital with the approval of the Biobank's Management Committee.

Study subjects

318 HTG-AP patients were included for final analysis (see Fig. 1 for detailed patient selection procedures). The electronic medical records of each patient were systematically evaluated for peak TG level measurement within the first-72 h post-onset of AP, body mass index (BMI), family history of HTG, family history of AP, number of episodes of HTG-AP and clinical severity of the disease.

Diagnosis of AP and classification of disease severity as mild (MAP), moderate severe (MSAP) and severe (SAP) were made in accordance with the Modified Atlanta Classification Standard in 2012.[50] HTG-AP was diagnosed in AP patients with fasting serum TG level of >1000 mg/dL (>11.3 mmol/L) alone or >500 mg/dL (>5.65 mmol/L) with coincidentally detected milky serum, with secondary factors such as gallstones and alcohol abuse being excluded as etiologies.[51] 362 healthy Han Chinese subjects (60 newly recruited here and 302 published elsewhere[31]) were used as controls.

DNA sequencing

Genomic DNA was extracted from 0.5 ml whole blood by the TIANamp Blood DNA Kit (TIANGEN Biotech, Beijing, China). All coding and proximal intronic regions of the *LPL*, *APOC2*, *APOA5*, *GPIHBP1* and *LMF1* genes were PCR amplified from genomic DNA and subsequently Sanger sequenced as previously described.[14] DNA extraction and PCR amplification were performed in our lab at the Jinling Hospital whilst Sanger sequencing was conducted by the Genewiz Life Science Company (Nanjing, China). All splice-site, missense, nonsense and frameshifting variants that had an allele frequency of <1% in East Asians (according to gnomAD) were considered to be potentially rare pathogenic variants (these data will be published elsewhere); all their corresponding carriers (n = 49) were excluded from this study (Fig. 1).

Statistical analysis

All data were analyzed using the SPSS 24.0 software package. Continuous variables were expressed as mean ± S.D. and tested using the Student's t test. *Chi*-Square and F-testing were performed to examine categorical variables. Benjamini-Hochberg test was subjected to a correction for multiple comparisons. *P* value < 0.05 was defined as statistically significant. Haldane's correction (i.e., adding 0.5 to all the cells of a contingency table) was applied for comparisons of the TT genotype frequencies in patients and controls because of the absence of TT homozygotes in controls.

Results

Division of patients into three groups

A total of 318 HTG-AP patients, all of Han Chinese origin, were included for final analysis in this study (Fig. 1). To assess the contribution of *APOA5* c.553G>T (rs2075291) to HTG-AP and its possible interaction with pregnancy, we divided the patients into three groups, firstly on the basis of gender and secondly, in the female patients, on the basis of disease occurrence during pregnancy or not (Fig. 1). The main demographic and clinical characteristics of the three groups of patients are summarized in Table 1. It should be noted that if a female patient experienced both HTG-APIP and HTG-AP not related to pregnancy, she was classified as group 3.

Comparisons of demographic and clinical characteristics between patient groups

We compared the demographic and clinical characteristics between the different patient groups. To increase biological relevance and, for the sake of simplicity, we performed comparisons only between group 1 and 2 patients and between group 2 and 3 patients. Only BMI displayed a significant difference between group 1 and 2 patients. By contrast, BMI did not exhibit any difference between group 2 and 3 patients. However, group 3 patients had a higher peak TG level approaching borderline statistical significance ($P = 0.058$), significantly earlier mean ages at disease onset, a significantly higher rate of SAP but a significantly lower rate of disease recurrence as compared to group 2 patients (Table 1). Differences in terms of age of onset of AP and disease recurrence could well have been related to the selection bias inherent in group 3 patients and will therefore not be considered further.

Comparisons of genotype and allele frequencies of rs2075291 between patient groups and between patients and controls

All exons and exon/intron boundaries of the five primary TG-related genes (*LPL*, *APOC2*, *APOA5*, *GPIHBP1* and *LMF1*) were analyzed by Sanger sequencing initially in 367 HTG-AP patients. Of these, 49 patients were found to carry rare putatively pathogenic variants in the five TG-related genes and excluded from analysis. All remaining 318 patients were informative with respect to the genotype status of rs2075291, and all participated in this study (Fig. 1). Genotype and minor T allele frequencies of rs2075291 in each of the three patient groups are summarized in Table 2. We also

sequenced the five primary TG-related genes in 60 Han Chinese healthy controls. The minor T allele frequency of rs2075291 in these controls showed no significant difference with that in 302 Han Chinese controls (0.07% (8/120) vs. 0.04% (24/604), $P = 0.22$) from Tang and colleagues[31] (for details, see Supplementary Table 1). We therefore combined our data with those of Tang and colleagues[31] to generate a single control dataset (Table 2).

We first compared the genotype and minor T allele frequencies of rs2075291 between patient groups. As in the preceding section, we performed comparisons only between group 1 and 2 patients and between group 2 and 3 patients. No significant difference was found for any parameters in the context of the group 1 and 2 comparisons. By contrast, both the TT genotype and the minor T allele of rs2075291 were significantly overrepresented in group 3 patients as compared to group 2 patients in the unadjusted analysis ($P = 0.015$ and 0.023) and approached or reached borderline statistical significance after Benjamini-Hochberg Correction ($P = 0.060$ and 0.046) (Table 2).

We then compared the minor T allele frequencies of rs2075291 and minor T-containing genotypes between each patient group and the control group, respectively. The minor T allele frequency of rs2075291 was significantly enriched in all three groups of patients as compared to controls, with the strongest enrichment being observed in group 3 (OR = 10.02, adjusted $P = 3.4E-9$; Table 2). Both the GT and TT genotypes were significantly enriched in all three groups of patients as compared to the control group, with the TT genotype invariably showing a much stronger effect than the GT genotype and the strongest enrichment being observed in group 3 patients (OR = 192.35, adjusted $P = 2.7E-7$) (Table 2).

As shown in Table 2, the TT genotype was absent in the 362 controls but present in approximately 6% of both group 1 and group 2 patients and in up to 20% of group 3 patients. For comparison, the frequency of TT homozygotes in East Asians was 0.56% (55/9875) in accordance with the gnomAD database (accessed 03 March 2020).

Evaluation of the possible impact of rs2075291 on peak TG level, age of AP onset, disease severity and recurrence

We tested whether rs2075291 could possibly impact on peak TG level, age of AP onset, disease severity and recurrence in the patients. To this end, we compared these features between the GG, GT and TT genotype carriers in each of the three groups of patients. No significant difference was found for any parameter comparisons in either group 1 or group 2 patients (data not shown). These findings, together with the similar findings between the two groups observed in preceding sections, made it permissible to combine data from groups 1 and 2 (Fig. 2). In group 3 patients, TT genotype carriers exhibited significantly higher peak TG levels than GG genotype carriers (adjusted $P = 0.0042$; Fig. 3a). Although no other comparison showed any significant difference, two observations are worth mentioning. First, the GT genotype showed a trend toward an increase in peak TG level as compared to the GG genotype (Fig. 3a). Second, the TT genotype appeared to be associated with more severe disease as compared to the GG and GT genotypes (Fig. 3c). Impact on disease recurrence was not analyzed in group 3 patients owing to the very small number of patients who had more than one episode of AP in this group (n = 3), which was also deemed to be at least partly related to the selection bias inherent in group 3 patients.

Discussion

The etiology of HTG and, consequently HTG-AP, is complex and may involve multiple gene-gene and/or gene-environment interactions. *LPL*, *APOC2*, *APOA5*, *GPIHBP1* and *LMF1* are by far the most extensively studied HTG genes and pregnancy is a well-established environmental factor predisposing to HTG. Interaction between genetic risk factors in the five primary HTG genes and pregnancy in the etiology of HTG-AP has been frequently described in the literature but these studies have almost invariably been anecdotal case reports that have implicated rare pathogenic variants. The sole exception was a report of a possible interaction between the common *APOA5* c.553G>T variant (rs2075291) and pregnancy in HTG-AP;[24] this study was however somewhat limited in scope since only five patients with HTG-APIP (all Chinese) were analyzed.

The high frequency of the rs2075291 T allele in the Chinese population (~4%)[30, 31] and its established association with altered TG levels made rs2075291 a good candidate (with sufficient

236 statistical power) to evaluate any interaction between this common genetic risk factor and pregnancy
237 in HTG-AP. Therefore, we retrospectively recruited and sequenced a large cohort of Chinese HTG-AP
238 patients for analysis. To perform this analysis properly, we first divided the patients into three groups.
239 Groups 1 (male) and 2 (female; disease not related to pregnancy) served not only as controls *vis à vis*
240 group 3 (HTG-APIP patients) but also as cohorts for evaluating the role of rs2075291 in HTG-AP
241 unrelated to pregnancy. Since it is almost a truism that rare pathogenic variants have strong genetic
242 effects whereas common pathogenic variants generally have only mild or modest genetic effects, to
243 avoid interference from rare pathogenic variants, patients carrying such variants in the five primary
244 HTG genes were excluded from this study.

245 Group 1 and 2 patients were initially treated as independent cohorts. The two groups showed
246 remarkable similarity in almost all studied parameters, the only exception being a difference of 1.47 in
247 terms of BMI value (Table 1). This suggested that groups 1 and 2 could effectively be considered
248 together as a single group, as exemplified in Fig. 2. By contrast, biologically meaningful differences in
249 terms of peak TG level and disease severity were apparent between group 3 patients and group 2
250 patients (as well as group 1 patients), with a higher peak TG level and a higher rate of SAP being
251 observed in group 3 patients with HTG-APIP (Table 1).

252 Intuitively, an interaction between rs2075291 and pregnancy in HTG-AP should be reflected by a
253 higher detection rate of the risk rs2075291 T allele and genotypes in HTG-APIP patients as compared
254 to HTG-AP patients unrelated to pregnancy. Employing 362 healthy Han Chinese subjects as
255 population controls, we demonstrated for the first time a robust association of rs2075291 with HTG-
256 AP in general and HTG-APIP in particular (Table 2). Importantly, a stronger association was observed
257 in group 3 patients than in either group 1 or group 2 patients in terms of the minor T allele and minor
258 T allele-harboring genotype frequencies. This stronger association was mainly due to the much higher
259 frequency of the TT genotype in group 3 patients (20%) than was found in groups 1 and 2 patients
260 (<6%; Table 2). These findings provide the first evidence for an interaction between rs2075291 and
261 pregnancy in HTG-AP.

262 To delve deeper into the interaction between rs2075291 and pregnancy in HTG-AP, we evaluated
263 the possible impact of minor T allele-harboring genotypes on peak TG level, age of AP onset, disease

severity and recurrence by reference to the GG genotype within each patient group. We found no significant differences in any comparison between group 1 or 2 patients, with the combined data being provided in Fig. 2. However, we found that the TT genotype was associated with a significantly higher peak TG level in group 3 patients as compared to the GG genotype (adjusted $P = 0.0042$; Fig. 3); this constitutes a new line of evidence supporting an interaction between rs2075291 and pregnancy in HTG-AP.

Whilst we provided strong evidence to support an interaction between rs2075291 and pregnancy in HTG-AP, the most remarkable finding emerging from this study was related to the TT genotype. The TT genotype was found to be present at a much higher frequency (20%) in group 3 patients than in either group 1 or 2 patients (<6%) and it was also associated with a significantly higher peak TG level in group 3 patients. This may be understood in terms of the TT genotype comprising two identical risk alleles. Put simply, the homozygous TT genotype might be expected to exert double the genetic effect of a GT genotype, thereby rendering the corresponding carrier more prone to high HTG during pregnancy. Following this line of reasoning, our findings lend support to the mosaic genetic model of HTG, first proposed by Johansen, Kathiresan and Hegele in 2011.[52] In essence, this model postulates the "stacking" of additional HTG risk alleles on top of a minimum number of genetic risk alleles and highlights a variable combination of genetic determinants and presence of environmental factors in causing HTG and HTG-related diseases.

The strength of this study lies in the analysis of three well-characterized patient groups. However, our study has various limitations. For example, we did not search for copy number variants in the five primary TG-related genes; although this is a very rare type of variant, it can have strong genetic effects on predisposing to HTG.[13, 53, 54] The number of our group 3 patients is still relatively small and hence the corresponding findings should still be regarded as exploratory at this stage. Interaction between rs2075291 and pregnancy in HTG-AP remains to be confirmed in larger studies.

Conclusion

Here, on the basis of sequencing the five primary TG-related genes in three groups of well-defined Han Chinese participants, we have for the first time demonstrated a robust association of rs2075291

with HTG-AP in general and HTG-APIP in particular. We have also provided strong evidence for an interaction between rs2075291 and pregnancy in HTG-AP. Our findings provide novel insights into the complex etiology of HTG as well as HTG-AP and suggest that analysis of both rare and common pathogenic variants in TG-related genes is likely to be of key importance in risk assessment for HTG-AP in pregnancy.

Authorship contribution statement

Na Pu: Study design, data curation, and paper writing. Qi Yang: Study design, and paper writing. Xiao-Lei Shi and Wei-Wei Chen: Investigation and methodology. Xiao-Yao Li and Guo-Fu Zhang: Methodology and visualization. Gang Li and Bai-Qiang Li: Investigation and data curation. Lu Ke and Zhi-Hui Tong: Formal analysis and software. David N. Cooper: Paper review and critical revision of the manuscript. Jian-Min Chen: Data interpretation and paper writing. Wei-Qin Li and Jie-Shou Li: Study design, conceptualization and funding acquisition. All authors contributed to revision of the manuscript and approved the final manuscript.

Declarations of Competing Interests

The authors have no conflicts of interest to declare.

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Li, had full access to all data in the study and had final responsibility for the decision to submit for publication.

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457 **Table 1**. Main demographic and clinical characteristics of the three groups of Han Chinese HTG-AP

458 patients

	Group 1 (Male)	Group 2 (Female; disease unrelated to pregnancy)	Group 3 (Female; HTG-APIP)
Number	183	105	30
Age (year)	39.8 ± 8.9	39.2 ± 9.6	29.2 ± 4.1***
Family history of HTG	4 (2.19%)	2 (1.90%)	0 (0)
Family history of AP	5 (2.73%)	1 (0.95%)	0 (0)
BMI (kg/m^2)	26.97 ± 13.58	25.50 ± 3.90**	25.33 ± 3.59
Peak TG (mmol/L)	20.00 ± 17.93	21.11 ± 18.87	29.45 ± 29.35[a]
Age at AP onset (year)[b]	37.0 ± 8.5	37.6 ± 9.6	29.0 ± 4.0***
Disease severity			
MAP	60 (32.79%)	26 (24.76%)	1 (3.33%)
MSAP	61 (33.33%)	39 (37.14%)	10 (33.33%)
SAP	62 (33.88%)	40 (38.10%)	19 (63.34%)*
Episodes			
1	103 (56.28%)	67 (63.81%)	27 (90%)
>1	80 (43.72%)	38 (36.19%)	3 (10%)**

459 Comparisons between patient groups were performed only between group 1 and group 2 patients and

460 between group 2 and group 3 patients. No significant difference was observed unless specifically

461 indicated. *, $P < 0.05$. **, $P < 0.01$. ***, $P < 0.001$.

462 [a] $P = 0.058$.

463 [b] First episode of disease in case of recurrence in groups 1 and 2 patients; first episode of HTG-APIP

464 in case of group 3 patients.

465 AP, acute pancreatitis; BMI, body mass index; HTG, hypertriglyceridemia; HTG-AP, HTG-induced

466 AP; HTG-APIP, HTG-AP in pregnancy; MAP, mild AP; MSAP, moderate severe AP; SAP, severe

467 AP.

468

Table 2. Frequency of the *APOA5* c.553G>T polymorphism and associated genotypes in the three groups of Chinese HTG-AP patients and controls

Genotype	Patient	Control (n = 362)	*P* value (unadjusted)	*P* value (after Benjamini-Hochberg correction)	OR
	Group 1 (n =183)				
GG	134 (73.22%)	330 (91.16%)			
GT	39 (21.31%)	32 (8.84%)	1.2E-5	2.1E-5	3.00
TT	10 (5.47%)	0 (0.00%)	4.4E-5	6.6E-5	43.88
GT+TT	49 (26.78%)	32 (8.84%)	2.7E-8	1.18E-7	3.77
Minor T allele	59 (16.12%)	32 (4.42%)	0.0040	0.0048	4.16
	Group 2 (n = 105)				
GG	73 (69.52%)	330 (91.16%)			
GT	26 (24.76%)	32 (8.84%)	4.0E-6	8.0E-6	3.67
TT	6 (5.72%)	0 (0.00%)	1.7E-4	2.3E-4	47.36
GT+TT	32 (30.48%)	32 (8.84%)	1.4E-8	8.3E-8	4.52
Minor T allele	38 (18.10%)	32 (4.42%)	0.023	0.023	4.78
	Group 3 (n =30)				
GG	17 (56.67%)	330 (91.16%)			
GT	7 (23.33%)	32 (8.84%)	0.0060	0.0065	4.25
TT	6 (20.00%)[b]	0 (0.00%)	8.9E-8	2.7E-7	192.35
GT+TT	13 (43.33%)	32 (8.84%)	3.0E-6	7.2E-6	7.89
Minor T allele	19 (31.67%)[c]	32 (4.42%)	2.8E-10	3.4E-9	10.02

Genotype and allele frequency comparisons between patient groups were performed either between group 1 and group 2 or between group 2 and group 3. No significant difference was observed unless specifically indicated. Haldane' correction was applied for comparisons of TT genotype frequencies in patients and controls.

[b] Unadjusted $P = 0.015$; adjusted $P = 0.060$ (Group 3 vs. Group 2).

[c] Unadjusted $P = 0.023$; adjusted $P = 0.046$ (Group 3 vs. Group 2).

OR, odds ratio.

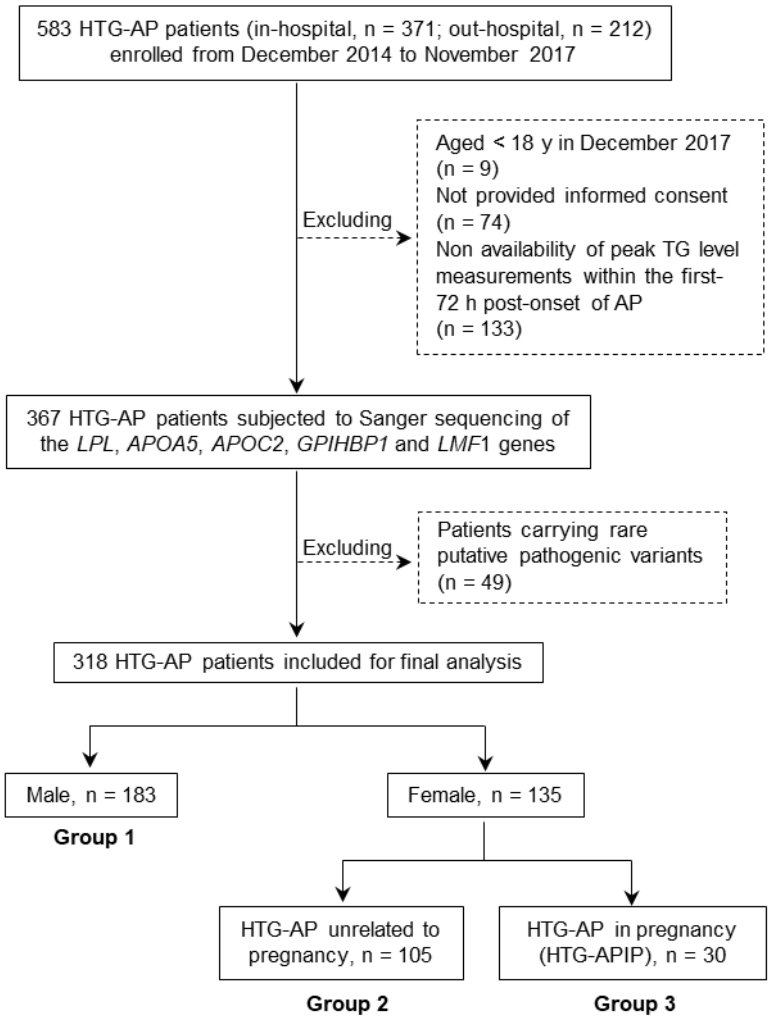

Figure 1 Flow chart of patient selection procedures. HTG-AP, hypertriglyceridemia-induced acute pancreatitis; HTG-APIP, HTG-AP in pregnancy. TG, triglyceride.

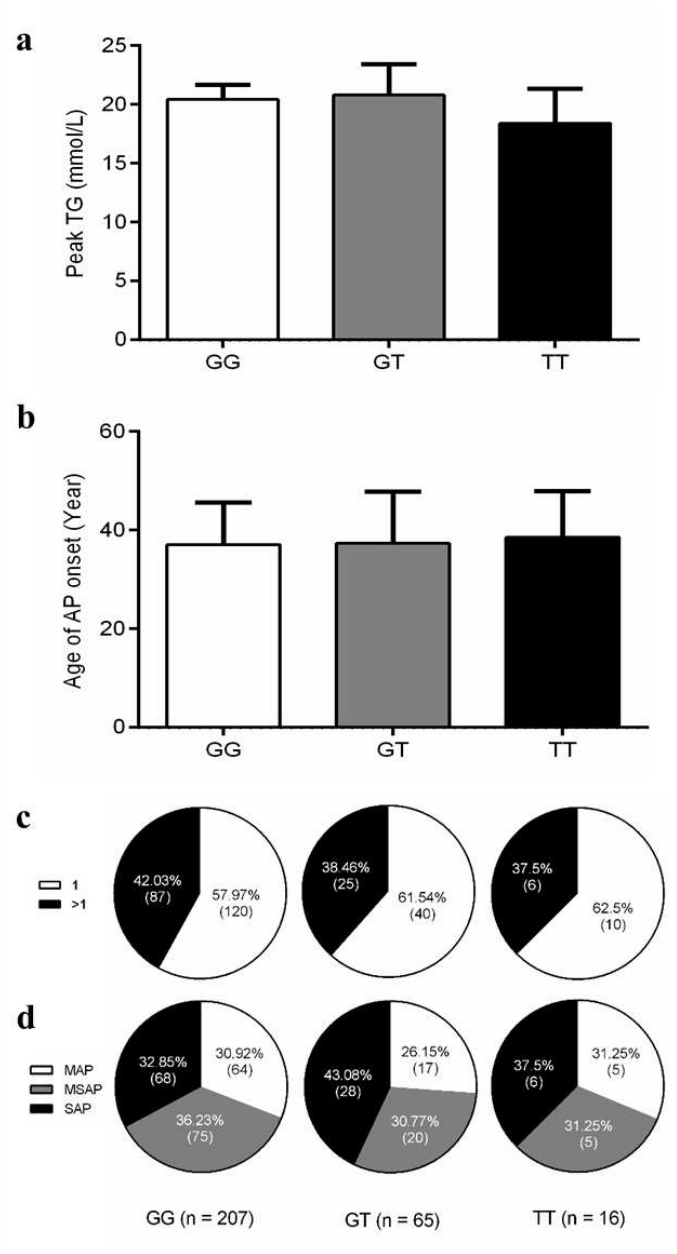

Figure 2 Comparison of peak TG level (a), age of onset (b), number of episodes (c) and clinical severity (d) of HTG-AP patients who carried the different *APOA5* c.553G>T genotypes in the combined group 1 and 2 patients. Numbers of the GG, GT and TT genotype patients were 207, 65 and 16, respectively. In panels **c** and **d**, percentages and exact numbers (in brackets) of the subjects with the indicated clinical characteristics are provided in the context of each genotype. AP, acute pancreatitis; MAP, mild AP; MSAP, moderate severe AP; SAP, severe AP; TG, triglyceride.

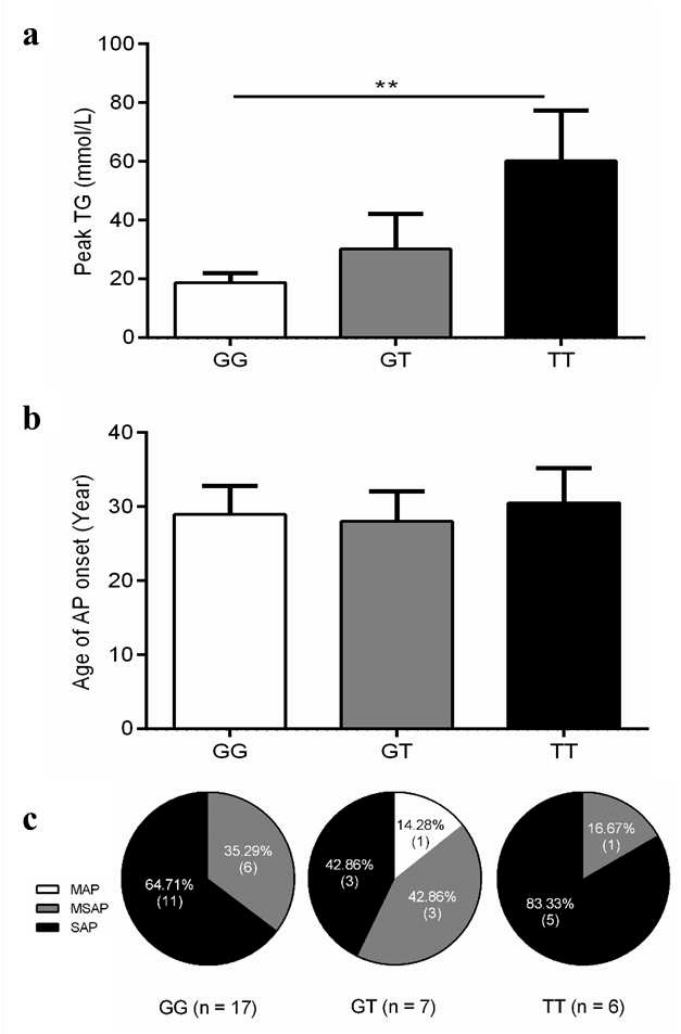

Figure 3 Comparison of peak TG level (a), age of onset (b) and clinical severity (c) of group 3 (HTG-APIP) patients who carried the different *APOA5* c.553G>T genotypes. Numbers of the GG, GT and TT genotype carriers were 17, 7 and 6, respectively. In panel **c**, percentages and exact numbers (in brackets) of the subjects with the indicated clinical characteristics are provided in the context of each genotype. **, adjusted $P = 0.0042$ (TT vs. GG). AP, acute pancreatitis; MAP, mild AP; MSAP, moderate severe AP; SAP, severe AP.