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VARIANTS *APOA5* P.G185C AND P.S19W ASSOCIATED WITH EARLY-ONSET SEVERE HYPERTRIGLYCERIDEMIA-INDUCED PANCREATITIS AND DIABETES COMPLICATIONS IN A VIETNAMESE GIRL

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SUMMARY

An imbalance of glucose and severely high triglycerides are the key characteristics driving hypertriglyceridemia and hyperlipidemic pancreatitis. Hereditary factors considerably promote the increase of triglyceride levels and secondary complications. This study explored the clinical characteristics and genetic causes of a young girl with recurrent severe hypertriglyceridemia-developed pancreatitis at 9 years old and diabetes mellitus appearance at 14 years old. At 20 years old, she was admitted to the hospital in critical condition and had pancreatitis with a triglyceride level of 26.22 mmol/L, HbA1c level of 15%, and a glucose level of 21.3 mmol/L. Whole exome sequencing analysis showed that she had compound heterozygous variants, p.G185C and p.S19W, in the *APOA5* gene, which were reported as risk factors for hypertriglyceridemia, and other variants of uncertainty in the *APOE* and *CFRT* genes. The results and analysis propose that these variations were the underlying causes of elevated plasma triglycerides and led to the onset of metabolic complications at a juvenile age. These findings shed light on the molecular mechanism of elevated plasma triglyceride and the early onset of diabetes following hyperlipidemic pancreatitis in the patient.

Keywords: *APOA5*, *APOE*, hyperlipidemic pancreatitis, hypertriglyceridemia, WES

INTRODUCTION

Hypertriglyceridemia (HTG) occurs when the human body exhibits an increased concentration of serum triglycerides (TG). The TG level reaches from 1.7 to 5.6 mmol/L to be considered moderate and severe HTG above 5.6 mmol/L (Grundy *et al.*, 2019). HTG is elicited by a variety of factors, including genetic disorders, obesity, diabetes, alcohol

consumption, smoking, and the intake of certain medications. Elevated levels of lipids in the bloodstream can result in complications of heart disease, pancreatitis, and diabetes (Shemesh, Zafrir, 2019).

Hypertriglyceridemia is multifaceted due to the intricate interplay of both genetic predispositions and environmental factors. *APOE,* an apolipoprotein E gene, plays a central role in lipoprotein metabolism. The protein apolipoprotein E binds with lipid particles to form a lipoprotein-mediated complex for lipid transport throughout the body. APOE is also essential for the efficient clearance of chylomicron remnants after enzymatic lipolysis in the circulation (Marais, 2019). Variants of APOE (APOE4 protein) can impair the processing of very low-density lipoproteins (VLDL), leading to an imbalance in cholesterol distribution and potentially causing hypertriglyceridemia (Phillips, 2014). *APOA5* encodes for an apolipoprotein A5 that significantly contributes to the regulation of triglyceride levels in the bloodstream. APOA5 acts as a stimulator of lipoprotein lipase, which is a crucial enzyme in the breakdown of triglycerides, thereby amplifying the metabolism of TG-rich particles (Su *et al.*, 2018). *APOA5* variants have been implicated by countless previous studies as genetically essential in the mechanism of hypertriglyceridemia and the control of plasma triglyceride concentrations (Martin *et al.*, 2003).

High TG levels (≥ 20 mmol/L) significantly increase the risk of acute pancreatitis (Valdivielso *et al.*, 2014). It is the third main cause, accounting for up to 10% of acute pancreatitis cases to be reported. A genetic susceptibility to dyslipidemia could potentially lead to elevated triglyceride levels. Such individuals develop pancreatitis more quickly due to their innate incapacity to control their triglyceride levels, underscoring the hereditary role in hastening this condition (Weiss *et al.*, 2021). Multiple genetic variants positioned in *LPL* (Lipoprotein lipase), *APOC2* (Apolipoprotein C-II), *APOA5, APOE*, *LMF1* (Lipase maturation factor 1), *GPIHBP1* (glycosylphosphatidylinositol-anchored high-density lipoprotein binding protein 1) were reported to be associated with HTG and a predisposition to pancreatitis risk (Dron, Hegele, 2020). The *CFTR* gene encoding for cystic fibrosis transmembrane regulator has also been reported to be a gene that is associated with pancreatic and respiratory diseases (Wang *et al.*, 2014; Wertheim-Tysarowska *et al.*, 2021). Recently, Fujita et al. (2022) were the first to use whole-exome sequencing (WES) analysis on an HTGP (hypertriglyceridemia-induced pancreatitis) complication and revealed genetic materials in *LPL, APOA5, APOE,* and *CFTR* (Cystic fibrosis transmembrane conductance regulator) involved in the condition (Fujita *et al.*, 2022).

This study presented the clinical and genetic characteristics of a young Vietnamese patient with severe hypertriglyceridemia and multiple complications, including pancreatitis and diabetes.

CASE PRESENTATION

Patient's clinical characteristics

A 20-year-old Vietnamese female patient was admitted to the Emergency Department of Hanoi Medical University Hospital. The patient was first diagnosed with acute pancreatitis at the age of 9 and was subsequently diagnosed with diabetes mellitus five years later. She has been

managing her diabetes with an insulin injection regimen consisting of 8-8-8-14 units. There is no known history of diabetes, metabolic disorders, premature cardiovascular disease, or allergies in her family. No consanguineous marriages were reported. The patient does not have a history of tobacco or alcohol use and has not been on steroid therapy.

At the Emergency Department of Hanoi Medical University Hospital, the patient presented with symptoms of severe abdominal pain and pronounced fatigue. Upon admission, the patient was diagnosed with diabetic ketoacidosis, as evidenced by an arterial blood gas (ABG) test showing hyperglycemia (glucose = 21.3 mmol/L), acidosis (pH = 7.24), and metabolic acidosis (HCO3 = 5.30 mEq/L) (Table 1). Immediate treatment comprised of fluid administration and insulin therapy was provided. As the patient's blood gas levels improved, her abdominal pain subsided. The patient was transferred to the Department of Endocrinology - Respiratory Medicine for further management.

The patient's electrocardiogram (ECG) revealed a normal sinus rhythm (112 bpm) and an intermediate cardiac axis, consistent with normal heart orientation. The cardio-ankle vascular index (CAVI) was within the normal range on both sides, indicating no significant risk of arterial stiffness or atherosclerosis. Left ventricular size and systolic function were within normal limits as determined by the echocardiogram. Carotid Doppler ultrasound did not find abnormalities in the carotid and spinal arteries on both sides.

The patient's lipid profile was severe HTG, with a TG level of 26.22 mmol/L, cholesterol of 12.35 mmol/L, HDL-cholesterol of 0.59 mmol/L, and LDL-cholesterol of 3.21 mmol/L. The patient's episode of acute pancreatitis symptoms was expressed in abdominal pain, elevated serum 39.8 g/L, a high total white blood cell (WBC) count of 472, and hyperglycemia (glucose = 21.3 mmol/L) (Table 1). An abdominal ultrasound revealed acute pancreatitis, hepatic steatosis, and gallstones. The pancreatic, intrahepatic, and extrahepatic bile ducts were mildly dilated, possibly indicating obstruction or inflammation due to compression by the enlarged pancreatic head or gallstones. The scan also revealed a fatty liver, mild hepatomegaly, and a left renal angiomyolipoma. A subsequent computed tomography (CT) scan of the upper abdomen showed an enlarged pancreatic head with surrounding fat infiltration. The severity of the condition was assessed using the Balthazar score (C), the computed tomography severity index (CTSI, 4), and the Glasgow score (2), all of which indicated moderate severity.

The patient received glycemic control and fluid infusions and underwent lipid-lowering therapy with omega-3 fatty acid treatment during inpatient treatment. After 10 days of hospitalization, her serum TG has declined from a severe to a moderate level (5.04 mmol/L). The levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were within the normal range, indicating no liver damage (13/14 U/L). The serum concentrations of sodium (Na) and potassium (K) were also normal, reflecting adequate electrolyte balance (141/3.8 mmol/L). The activities of the digestive enzyme amylase (83 U/L) and lipase (21.3 U/L) were controlled and below the threshold, suggesting no pancreatic inflammation. The index on the ABG test improved when with condition was stable

and the occurrence of ketoacidosis ceased (pH = 7.411). The metabolic acidosis condition was resolved with $paCO_2$ raised to 29.2 mmHg and HCO3 enhanced to 18.1 mEq/L (Table 2). Blood glucose levels were gradually normalized under appropriate management, no indications of pancreatitis were observed, and the patient was discharged after 10 days of hospital treatment.

Table 1. Patient's clinical indicators on admission day.

Biochemical							
	Index	Normal			Index	Normal	
Glucose	21.3	4.0-5.4	mml/L	Ca	2.38	2.15 - 2.55	mmol/L
HbA1c	15.00%	≤ 5.7%		Cholesterol	12.35	< 5.5	mmol/L
C-peptid	0.21	0.26-1.27	nmol/L	Triglyceride	26.22	< 1.69	mmol/L
Creatinin	39	52.2-91.9	µmol/l	HDL	0.59	> 1.69	mmol/L
Ure	5.4	2.1-8.5	mmol/L	LDL	3.21	> 2.0	mmol/L
Na	121	135-145	mmol/L	GOT/GPT	10/9	>35	U/L
K	4.7	3.5-5.0	mmol/L	Amlylase	27	30-110	U/L
hs-CRP	2.68	<3.0	mg/dL				
Arterial blood gas							
	Index	Normal			Index	Normal	
pH	7.24	7.35 -7.45		paO2	131	75-100	mmHg
paCO2	12.7	35-45	mmHg	HCO3	5.3	22-26	mEq/L
Endocrine							
	Index	Normal			Index	Normal	
Cortisol	633	140-690	nmol/L	TSH	0.567	0.4 - 4.0	µUI/mL
ACTH	142	7.2-63.3	pg/mL	Albumin	39.8	34-45	g/L
FT4	13.3	12.0-22.0	pmol/L				
Hematological							
	Index	Normal			Index	Normal	
HC	4.56	36-44	T/L	PT	89%	70-140%	
Hb	135	116-150	g/L	APTT	1.03	0.85-1.25	
BC	9.7 g/L	115-165	g/L	INR	1.06	<1.5	
TC	472	4.5-11	(x 10^9/L)	Fibrinogen	8.78	2.0-4.0	g/L
Urine							
Ketones	(-)			Protein	(-)		

HbA1c: Hemoglobin Ac1; Na: Sodium; K: Potassium; Ca: Calcium; HDL: high-density lipoprotein; LDL: low-density lipoprotein; GOT: Glutamic Oxaloacetic Transaminase; GPT: Glutamic Pyruvic Transaminase; hs-CRP: High-sensitivity C-reactive protein; ACTH: Adrenocorticotropic hormone; FT4: Free Thyroxine; TSH: Thyroid Stimulating Hormone; HC: Hematocrit; HB: Hemoglobin; BC: Blood count; TC: Total count; PT: Prothrombin time; APTT: Activated partial thromboplastin time; INR: International normalized ratio.

Table 2. Clinical progress of the patient at discharge.

	Admission	Discharge	Normal range	
Biochemical				
Na	121	141	135 - 145	mmol/L
K	4.7	3.8	3.6-5.2	mmol/L
Triglyceride	26.22	5.04	<1.7	mmol/L
GOT	10	13	7 - 56	U/L
GPT	9	14	7 - 35	U/L
Amylase	27	83	30 - 110	U/L
Lipase	47.2	21.3	10 - 140	U/L
hs-CRP	2.68	0.45	≤ 3.0	mg/dL
ABG				
pH	7.24	7.411	7.35 - 7.45	
paCO2	12.7	29.2	35 - 45	mmHg
paO2	131	110.9	85 - 100	mmHg
HCO3	5.3	18.1	21 - 28	mEq/L

Na: Sodium; K: Potassium; GOT: Glutamic Oxaloacetic Transaminase; GPT: Glutamic Pyruvic Transaminase; hs-CRP: High-sensitivity C-reactive protein; ABG: Arterial blood gas.

Genetic findings

A genetic test was performed after the patient received counseling from medical professionals (Supplements 1). The consent documents were written and signed. The genomic DNA of the patient was extracted from peripheral blood and underwent whole-exome sequencing (WES) analysis. The variants of metabolism-related genes were concentrated on screening causative factors according to criteria described in the Supplement methods. As a result, one homozygous variant in the *APOE*, two heterozygous variants in the *APOA5*, and two heterozygous variants in the *CFTR* genes were detected (Table 3). Among these variants, only two heterozygous variants, c.553G>T (p.G185C) and c.56C>G (p.S19W), of the *APOA5* gene were classified as the risk factors for hypertriglyceridemia condition, according to the ClinVar Database. The presence of these two variants in the patient was validated by Sanger sequencing (Fig. 1).

Table 3. Variants of metabolism-related genes extracted from WES.

Gene	Variant		Zygosity	Allele Frequency		ClinVar	*In silico* prediction
	cDNA	Amino acid		gnomAD	VN GVDB		SIFT/ MutationTaster / PROVEAN
APOE	c.466T>C	p.C156R	hom	0.1485	.	Conflicting	T/P/N
APOA5	c.553G>T	p.G185C	het	0.0062	0.037	Risk factor	D/N/D
	c.56C>G	p.S19W	het	0.0676	0.003	Risk factor	D/N/N
CFTR	c.1666A>G	p.I556V	het	0.0034	0.047	Conflicting	T/A/N
	c.1251C>A	p.N417K	het	0.0026	.	Conflicting	T/N/N

VN GVDB: Vietnamese Genetic Variation Database (NCBI Bioproject ID: 515199); hom: homozygous; het: heterozygous; A: disease-causing automatic; D: Damaging/ Deleterious; P: polymorphism automatic; T: Tolerate; N: Polymorphism - probably harmless (MutationTaster)/ Neutral (Provean); (.): unknown

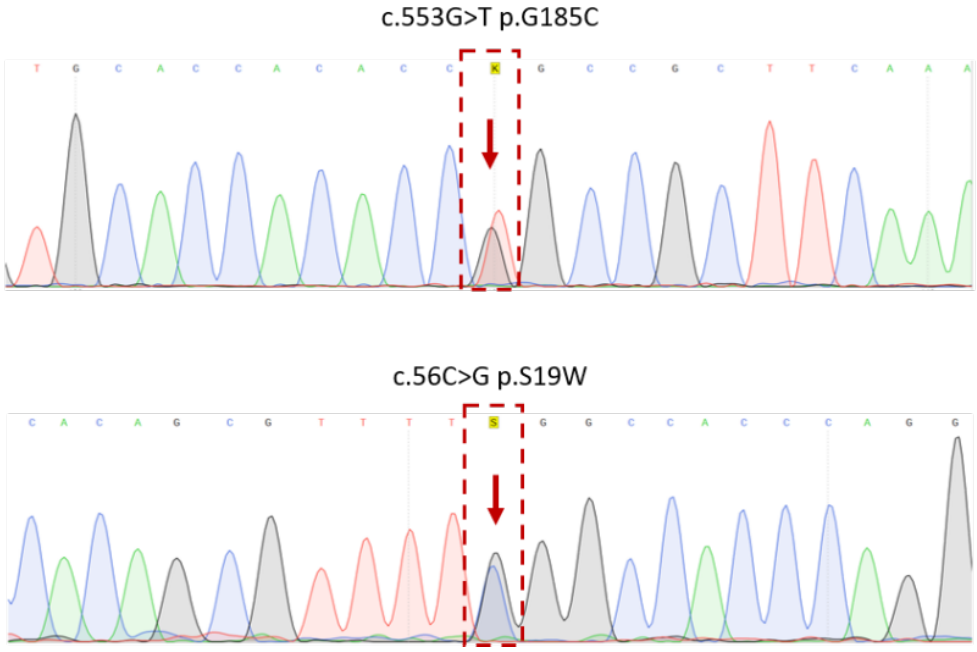

Figure 1. Sanger sequencing results of the *APOA5* p.G185C and p.S19W variants detected in the patient.

DISCUSSION

In this report, the patient presented with the main symptoms of diabetic ketoacidosis, HTG, and pancreatitis. Besides that, she also harbored the compound heterozygous variants p.G185C and p.S19W of the *APOA5* gene, which are associated with hypertriglyceridemia, and variants of the *APOE* and *CFTR* genes with

conflicting interpretations of pathogenicity. Her clinical expression and genetic findings are quite similar to two those in previous reports. The first one has described two patients, at the age of 12, who suffered acute pancreatitis from diabetic ketoacidosis and HTG. However, this report lacked a comprehensive analysis of the patients' genetic aspects, despite their young age and clean, healthy medical history (Bouchaala *et al.*, 2020). The second one reported the genetic finding of a young HTG patient from Japan with recurrent acute pancreatitis (Fujita *et al.*, 2022). All the patients first sustained high-level TG at a young age, then complications onset later, and they have no smoking or alcohol history. The Japanese patient was found to have two heterozygous variants in the *APOA5* gene, including the p.G185C, three heterozygous in the *CFTR* gene, and one in the *APOE* gene. They indicated variants in HTG-linked genes (*APOA5* and *APOE*) and *CFTR* associated with acute pancreatitis could be attributed to the patient severe pathology.

Meta-analysis studies showed that the common *APOA5* p.G185C (rs2075291) variant is an HTG risk factor with an impact on TG lipid levels, specifically in Asians (He *et al.*, 2016; Qian *et al.*, 2018). Heterozygous variant p.G185C was also found among subjects with a history of acute pancreatitis (Khovidhunkit *et al.*, 2016). Functional analysis demonstrated that the homozygous variant p.G185C reduces the hydrolysis of VLDL by decreasing the activation of lipoprotein lipase (LPL) (Huang *et al.*, 2012). The accumulation of unprocessed TG-rich lipoproteins suggests the pathogenic impact of p.G185C on patients with severe HTG conditions. The *APOA5* p.S19W variant was also reported to be positively correlated with upraised TG and severe HTG in the Caucasian and Asian populations (Dallongeville *et al.*, 2008; Evans *et al.*, 2011). It has also been considered a biomarker for hyperlipoproteinemia (Wang *et al.*, 2008). With the above evidence, the simultaneous appearance of two variants, *APOA5* p.G185C, and p.S19W, could be considered the main cause of the patient's hyperlipoproteinemia condition.

In this study, the appearance of "Variants of Uncertain Significance, VUS" on the *APOE* and *CFTR* genes might be followed up. The *APOE* p.C156R (rs429358) variant appeared widely and was found to be associated with HDL-cholesterol and lipid levels in Europe and southern China (Deng *et al.*, 2021; Richardson *et al.*, 2020). The altered cysteine-arginine interchanges were shown to exert an influence on the configuration of human E apoprotein isoforms ε-4, ε-3, and ε-2 (Weisgraber *et al.*, 1981). Hence, the homozygous variant p.C156R in the *APOE* gene may synergistically affect lipid metabolism together with variants p.G185C and p.S19W of the *APOA5* gene, resulting in serious development of HTG in our patient. In addition, *CFTR* polymorphisms were believed to contribute to the genetic etiology of acute pancreatitis in young HTG Japanese patients (Fujita *et al.*, 2022). The *CFTR* p.I566V was established most frequently in idiopathic and acute pancreatitis patients in the Chinese and Japanese populations (Chang *et al.*, 2007; Iso *et al.*, 2019). In the first study of the relationship between *CFTR* variants and hyperlipidemic pancreatitis, p.I566V was the most common variant present in patients with HTG with the onset of hyperlipidemic pancreatitis (HLP) compared with patients with HTG who do not have HLP (Chang *et al.*, 2008). p.I566V may cause conditions like pancreatitis through lowered open probability channel activity, resulting

in a reduction in current density in the chloride (Cl^-) current throughout the cell (Lee *et al.*, 2003). p.N417K was predicted as "deleterious" via computational analysis and appeared in cystic fibrosis (CF), and CFTR-related patients on clinical genetic screening (Trujillano *et al.*, 2015; George Priya Doss *et al.*, 2008). Impaired chloride transport could disturb the pancreas' fluid transport and electrolyte balance, resulting in pancreatitis (Hegyi *et al.*, 2016). In people with CF, the pancreatic transport tube thickens blocked digestive enzymes and produces sticky mucus. This evinces a combination of p.I566V and p.N417K that could defect CFTR protein function and provoke pancreatitis progression. While the existing information on the CFTR variant is inconclusive, our future focus will center on considering and vigilantly monitoring the patient's clinical symptoms. Gallstones detected by abdominal ultrasound could indeed be one of the causes of pancreatitis. However, it was unclear when the gallstone formation occurred since the patient presented symptoms of pancreatitis-induced TG at 9 years old.

Hypertriglyceridemia-induced pancreatitis presents TG levels exceeding 20 mmol/L; our patient reached the condition threshold at 26.22 mmol/L (Valdivielso *et al.*, 2014). The patient has also been on insulin treatment for diabetes mellitus since 2017. Diabetes is reported as the most commonly seen secondary factor in individuals with HTGP cohorts (Zafrir *et al.*, 2019). Furthermore, diabetes is listed as the primary metabolic driver of HLP due to the association between HTG and diabetes (Shemesh, Zafrir, 2019). Treatment of severe HTG with pancreatitis and diabetes mellitus complications includes both lifestyle innovation with diet and the uptake of manageable medications. The patient is counseled on a diet rich in dietary fiber and physical activity. She received guidelines for self-administering insulin and recognizing and managing hypoglycemia before discharge. A regular examination is required for her every 2–3 months. Fibrates, Omega-3 fatty acids, and niacin are the cornerstones of HTG therapy, with proficiency in reducing TG levels, hepatic VLDL production, and lipid-regulating agents (Shemesh, Zafrir, 2019). HTG-induced pancreatitis is thought to be treated similarly to various types of inflammation. Interestingly, some studies suggested that anti-diabetes drugs and heparin lower the risk of pancreatitis by promoting LDL activity (Lai *et al.*, 2011).

In this study, we documented a young Vietnamese patient who suffered from complications caused by severe HTG and analyzed the genetic aspect. Applying whole-exome sequencing analysis, we detected two molecular genetic variants in the *APOA5* gene, p.G185, and p.S19W, and a homozygous variant, p.C156R, in the *APOE* gene as the underlying cause of the patient's susceptibility to high serum TG levels. The compound heterozygous variants p.I556V and p.N417K in the *CFTR* gene could predispose to developing early hyperlipidemic pancreatitis. Further observation of the patient's pancreatic and respiratory conditions is required to draw a definitive conclusion. The identification of variants related to HTG and complications is valuable in confirming clinical diagnoses as well as identifying potential high-risk profiles among HTG cases.

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