BMC Medical Genomics

RESEARCH

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Identification of a novel heterozygous *GPD1* missense variant in a Chinese adult patient with recurrent HTG-AP consuming a high-fat diet and heavy smoking

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Abstract

Background Glycerol-3-phosphate dehydrogenase 1 (*GPD1*) gene defect can cause hypertriglyceridemia (HTG), which usually occurs in infants. The gene defect has rarely been reported in adult HTG patients. In the present study, we described the clinical and functional analyses of a novel *GPD1* missense variant in a Chinese adult patient with recurrent hypertriglyceridemia-related acute pancreatitis (HTG-AP), consuming a high-fat diet and smoking heavily.

Methods Exome sequencing was used to analyze the DNA of the adult patient's blood sample. It was found that there was a new variant of *GPD1* gene-p.K327N, which was verified by gold standard-sanger sequencing method. In *vitro*, the corresponding plasmid was constructed and transfected into human renal HEK-293T cells, and GPD1 protein levels were detected. A biogenic analysis was performed to study the population frequency, conservation, and electric potential diagram of the new variant p.K327N. Finally, the previously reported *GPD1* variants were sorted and their phenotypic relationships were compared.

Results A novel heterozygous variant of *GPD1*, p.K327N (c.981G > C), was found in the proband. Furthermore, the patient's daughter carried this variant, whereas his wife did not carry the variant. The proband with obesity suffered eight episodes of HTG-AP from the age of 36 years, and each onset of AP was correlated to high-fat diet consumption and heavy smoking. In vitro, this variant exerted a relatively mild effect on GPD1 functions, which were associated with its effect upon secretion (~ 25% of secretion decreased compared with that of the wild-type); thus, eventually impairing protein synthesis. Additionally, 36 patients with *GPD1* variants found in previous studies showed significant transient HTG in infancy. The proband carrying the *GDP1* variant was the first reported adult with recurrent HTG-AP.

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Conclusion We identified a novel *GPD1* variant, p.K327N, in a Chinese adult male patient with recurrent HTG-AP. The variant probably exerted a mild effect on GPD1 functions. The heterozygosity of this *GPD1* variant, in addition to high-fat diet consumption and heavy smoking, probably triggered HTG-AP in the patient.

Keywords Glycerol-3-phosphate dehydrogenase 1, Gene-environment interaction, Hypertriglyceridemia, Missense variant, Hypertriglyceridemia-induced acute pancreatitis

Introduction

Hypertriglyceridemia(HTG) is a common clinical condition that affects approximately 10% of the population worldwide [1]. Based on etiology, HTG is divided into primary and secondary HTG. Primary HTG is caused by gene defects related to lipid metabolism, including lipoprotein lipase(*LPL*), apolipoprotein C-II (*APOC2*), apolipoprotein A-V (*APOA5*), glycerol-3-phosphate dehydrogenase 1 (*GPD1*), glycosylphosphatidylinositol-anchored high density lipoprotein-binding protein 1(*GPIHBP1*), and lipase maturation factor 1 (*LMF1*), whereas secondary HTG is mostly caused by other diseases (E.g., obesity, diabetes, and metabolic diseases), drugs (E.g., oral drugs), unhealthy lifestyle choices (E.g., smoking), and high-fat diet consumption [2]. Moreover, an interplay has been observed between primary and secondary etiologic factors related to the occurrence of severe HTG [3]. Severe HTG increases the risk of acute pancreatitis (AP) and is the second leading cause of AP in China; thus, owing to its higher severity and recurrence rate, HTG is of great research importance [1]. Data from Western countries show that hypertriglyceridemia-induced acute pancreatitis (HTG-AP) accounts for 1.3–9% of total AP cases [4, 5], whereas clinical research data show that HTG occurs in 10.36–35.5% of patients with AP in China [6, 7].

The genetic map of HTG has not been completely identified; thus, studying the genome of patients with HTG is of critical importance. *GPD1* gene defect can lead to HTG. GPD1 catalyzes the reversible conversion of dihydroxyacetone phosphate (DHAP) and nicotine adenine dinucleotide (NADH) to glycerol-3-phosphate (G3P) and NAD + in the cytoplasm and participates in the synthesis of glycogen, whereas owing to excess calorie intake, G3P can enter the triglyceride (TG) synthesis pathway and increase TG levels [8], indicating that gene-environment interactions are vital for disease pathogenesis.

Individuals with frameshift, nonsense, or biallelic *GPD1* variants that result in almost complete loss of function develop transient infantile HTG; thus, the pathogenicity of the clinically identified *GPD1* missense heterozygous variant should be experimentally determined. To date, 36 patients carrying *GPD1* variants have been reported in infants in most cases, whereas adults with HTG carrying *GPD1* variants have rarely been reported. Hence, in the present study, we described the clinical and functional analyses of a novel *GPD1* missense variant in a Chinese adult patient with recurrent HTG-AP who consumed a high-fat diet and smoked heavily to investigate the potential of gene–environment interactions.

Methods

Patients

The proband was a 48-year-old male patient without a history of hypertension but with a 13-year history of fatty liver and a 29-year history of smoking. He suffered from AP for the first time in 2012 at the age of 36 years. He first developed AP in 2012 at the age of 36, at which time obesity was present with a body mass index (BMI) of 31.1 kg/m2. In addition, he smoked approximately 60 cigarettes per day and consumed a high-fat diet. Chylous blood samples were collected, which showed significantly increased TG levels; consequently, the condition was confirmed as severe HTG. Initially, in 2012, the condition was confirmed as HTG-AP by excluding factors such as biliary system diseases and alcohol consumption.

The patient suffered from five bouts of recurrent HTG-AP until 2021, and his TG levels remained high (Fig. 1A). His BMI fluctuated between 25.2 and 29.2 kg/m^2 afterward. Moreover, the patient smoked 30 cigarettes a day and consumed 100 mg of fenofibrate a day irregularly. During the sixth episode of HTG-AP, he was admitted to the Department of Critical Care Medicine, Nanjing Drum Tower Hospital, for treatment on May 21, 2021. The patient suffered from two episodes of mild acute pancreatitis (MAP) in August 2021 and May 2022, and each time, the onset of AP was related to high-fat diet consumption and heavy smoking. Later, his smoking frequency decreased (10 cigarettes a day), and he was administered a drug (fenofibrate). He kept exercising, which resulted in a normal BMI of 24 kg/m^2, and consumed a healthy low-fat diet. All these factors played an important role in controlling his TG levels, and no episode of AP occurred.

The patient's mother had a history of pancreatitis (suffered from it twice and passed away), the patient's wife and daughter(19-year-old) had no history of pancreatitis, and the family denied the history of consanguineous marriage.

Exome sequencing

2 mL of peripheral EDTA anticoagulant blood was drawn from the family members and genomic DNA was extracted from the blood using the Gentra Puregene blood kit (Qiagen, Dusseldorf, Germany) according to

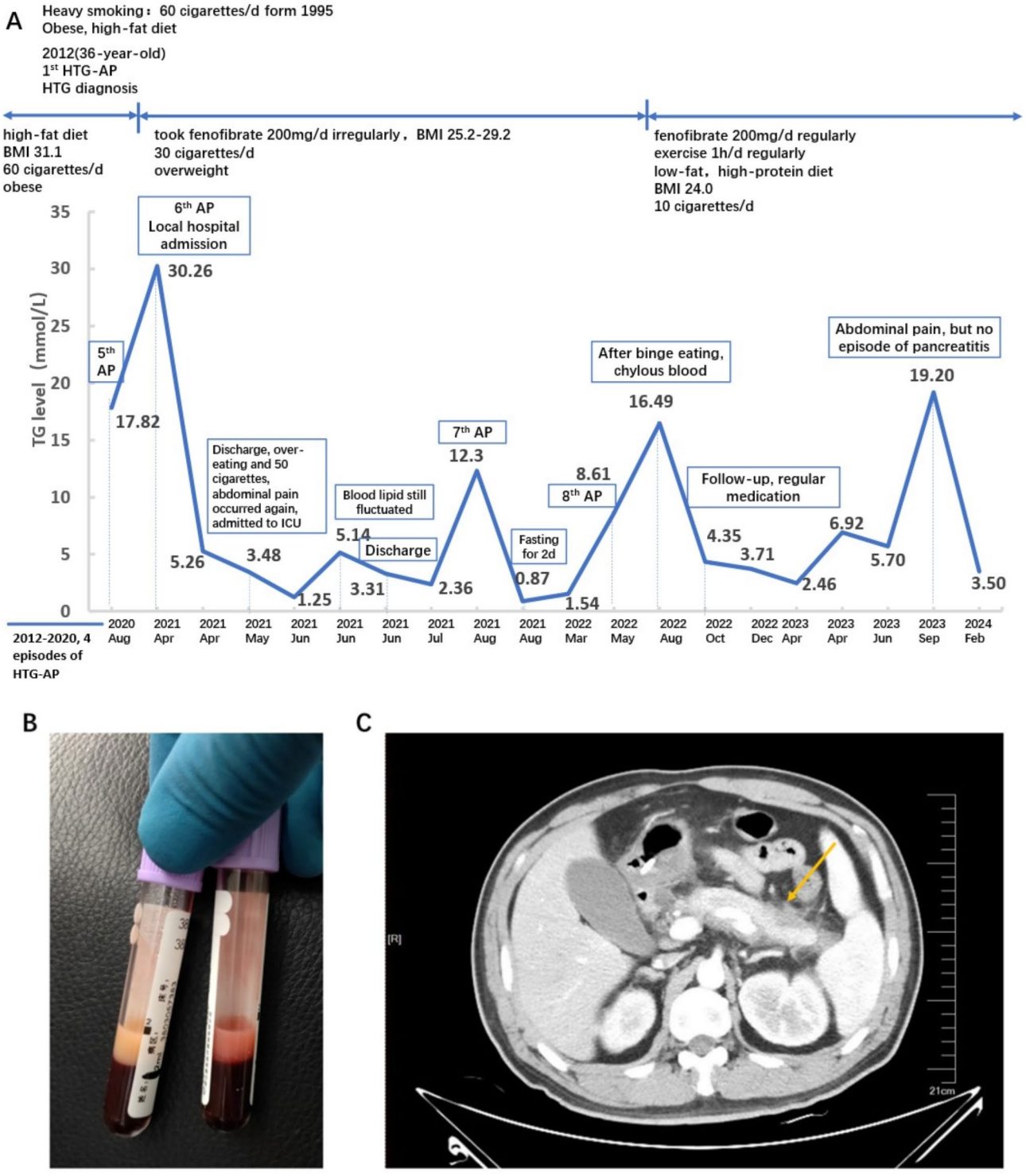

Fig. 1 **A.** Details of TG levels and disease time points of the proband; **B.** Blood samples showing chylemia; **C**. Computed tomography scan of the patient captured during his sixth episode of HTG-AP
TG: triglycerides; AP: acute pancreatitis; HTG-AP: hypertriglyceridemia-induced acute pancreatitis

the manufacturer's instructions. Shanghai Biotecan Company was commissioned to carry out exome sequencing. After the library was constructed, Nova6000 gene sequencer was used for on-board sequencing. The effective sequencing data were compared to the reference genome (UCSC hg19) through Burrows-Wheeler Aligner (BWA). Then the Haplo type Caller module of Genome Analysis TK was used for SNP/Indel detection.

It was found that the patient and his daughter had a novel variant of GPD1 gene p.K327N (c.981G > C), which was a heterozygous variant.

Polymerase chain reaction (PCR) amplification and *GPD1* gene Sanger sequencing

Exome sequencing revealed that the patient had a new variant in the GPD1 gene p.K327N (c.981G > C). PCR primer sequences primer F sequence CCAGTTGGCA CAGAAAATCC and primer R sequence CCTGTCCTC CAGTGAAAAGA were designed. Anhui General Company was commissioned to perform gold standard sanger sequencing on the DNA of the patient's blood samples, and repeated verification sequencing (3 times) was performed at the GPD1 gene locus p.K327N to verify the results.

Plasma lipid profile analysis

Blood samples were taken from the proband and family members after fasting for 12 h. Serum TG, TC total cholesterol (TC), high density lipoprotein cholesterol (HDL) and other biochemical indexes were measured enzymatically on an automatic analyzer (Hitachi High-Tech, 7600–120, Japan).

In vitro cell assay

Genewiz Company was commissioned to synthesize the GPD1 coding sequence of wild type and p.K327N mutant, and cloned into the overexpressed plasmid vector pcDNA3.1, respectively. Sanger sequencing confirmed the accuracy of the insertion sequence, and constructed GPD1 WT and GPD1 K327N plasmids. HEK-293T cells (ATCC, CRL-3216) were cultured in a medium containing 10% fetal bovine serum and 1% penicillin streptomycin. The plasmids (1.5 μg/mL) were transiently transfected into HEK-293T cells in a 6-well plate using Lipofectamine 3000 (Thermo, L3000015). After 6 h, the cells were transferred to DMEM medium containing 2% fetal bovine serum.

48 h after transfection with plasmids (wild-type GPD1 WT, mutant GPD1 K327N, empty vector), HEK 293T cells were cleaned with ice PBS once, and then lysed with lysis buffer to extract proteins. SDS-PAGE and immunoblotting of samples were performed using the standard Western blotting procedure, strips were visualized using the Chemidoc XRS system (Clinx Scientific Instruments, Shanghai, China) and Image Lab software (CLINX Scientific Instruments, Shanghai, China) for analysis. The antibodies used were anti-Flag antibody (Abcam, ab109732) and anti-GAPDH (Santa, sc-69,778).

In *silico* analyses

Population allele frequencies of variants found in this study were evaluated using the Genome Aggregation Database (gnomAD) genome dataset [9] via VarSome [10]. Variant nomenclature was in accordance with Human Genome Variation Society (HGVS) recommendations [11]. NM_005276.4 was used as the *GPD1* mRNA reference sequence.

We examined the evolutionary conservation of *GPD1* K327 amino acids across various species, from chimpanzee (a close evolutionary relative) to Norway rat(distant evolutionary relatives).

Then we used the data in UCSC Genome Browser (http://genome.ucsc.edu/) and ENCODE (http://genome.ucsc.edu/ENCODE/) to show the regulatory elements with the information of histone modifications of epigenetic markers (H3K4Me1, H3K4Me3, and H3K27Ac) data.

The three-dimensional (3D) structures and the electric potential diagram of the wild-type, mutant GPD1 proteins were predicted using PyMOL software [12].

Review of *GPD1* variant carriers

Keywords, including "glycerol 3-phosphate dehydrogenase-1 variant", "glycerol 3-phosphate dehydrogenase-1 variants", "*GPD1* variant" and "*GPD1* mutation" were used for searching reviews in PubMed and CNH databases up to September 30, 2023. The clinical phenotype, liver function, and lipid levels of variant carriers were reviewed. The heterozygous variant carriers in the family were also recorded.

Results

Clinical findings and treatment of the proband

The patient suffered from AP for the first time in 2012 at the age of 36 years. His BMI was 31.1 kg/m^2, which indicated obesity, and the patient smoked about 60 cigarettes a day and consumed a high-fat diet. He suffered from eight bouts of recurrent HTG-AP until Feb, 2024, recalling that he ate greasy food and smoked excessively right before the onset of the eight episodes of HTG-AP.

During the sixth bout of AP on April 18, 2021, the patient experienced upper abdominal pain accompanied by nausea after overeating and heavy smoking; thus, he visited the local hospital for treatment. His BMI fluctuated between 25.2 kg/m^2 and 29.2 kg/m^2; he smoked 30 cigarettes a day and consumed 100 mg of fenofibrate a day irregularly. Furthermore, the TG level was 30.26 mmol/L(2681.6 mg/dL), and his plasma was milky (Fig. 1B); these observations fulfilled the definition of extreme HTG by the Endocrine Society [13]. The amylase level was 403 U/L, and the computed tomography (CT) scan showed pancreatic swelling; thus, the diagnosis was confirmed to be HTG-AP. Physicians at the local hospital asked the patient to fast and abstain from drinking. Additionally, they treated him with fluid rehydration and administered rabeprazole to inhibit acids, octreotide, and ulinastatin to inhibit enzymes, and fenofibrate to manage

hyperlipidemia. His TG level gradually decreased to 5.26 mmol/L (466.13 mg/dL). The infection index still fluctuated because the C-reactive protein level was up to 57.65 mg/L. Moreover, the CT scan showed pancreatic swelling. However, the patient himself decided to get a self-willed discharge on May 17, 2021. After consuming a small amount of greasy food and smoking 50 cigarettes a day, the patient again experienced abdominal pain accompanied by nausea, vomiting, and high fever (39.2 °C). Then, he was admitted to our department for treatment on May 21, 2021.

At admission, his laboratory tests showed increased levels of amylase (133 U/L), C-reactive protein (inflammatory marker) (111.3 mg/L), and procalcitonin (0.097 ng/mL), as well as levels of abnormal liver function-total bilirubin, alanine aminotransferase, and aspartate aminotransferase were 38.4 U/L, 154 U/L, and 204 U/L, respectively. Despite the decreased leukocyte count (0.7×10^9/L), hemoglobin level (72 g/L), and platelet count (40×10^9/L), the results associated with severe infections were considered after excluding hematological diseases. A recombinant human granulocyte colony-stimulating factor was injected, and blood products were transfused. The abdominal CT scan showed pancreatic swelling and necrosis with local bleeding (Fig. 1C). During the patient's admission to our department, he was asked to fast, treated with gastrointestinal decompression, and administered with omeprazole and somatostatin to inhibit acids and enzymes. Additionally, fenofibrate was administered to manage hyperlipidemia. Imipenem cilastatin sodium and teicoplanin caspofungin were used for anti-infective therapy. Additionally, nadroparin calcium was used for anticoagulation therapy, and magnesium isoglycyrrhizinate was used for liver protection therapy. His TG level still fluctuated, with the highest being 5.14 mmol/L (454.89 mg/dL). The patient was provided with fat-free formula nutrition support until the discharge on June 17, 2021, when his condition improved.

The patient had another two episodes of MAP in August 2021 and May 2022. Additionally, he experienced exocrine pancreatic difficulties and suffered from symptoms such as nausea, vomiting, and diarrhea. Moreover, he was diagnosed with diabetes mellitus in 2023. Since October 2022, his smoking frequency decreased (10 cigarettes per day), he was administered a drug (fenofibrate), and he began to exercise and consume a healthy low-fat diet, which resulted in a normal BMI (24 kg/m^2) and played an important role in controlling TG levels. No episodes of AP were observed. During most of the follow-up time, his TG levels were maintained in the mild-to-moderate range (defined as 2–9.9 mmol/L according to Dron et al. [14]). In Sep 2023, the TG level raised to 19.20 mmol/L (1692.33 mg/dL), he suffered slight abdominal pain but there was no episode of pancreatitis. His TG level was 3.50 mmol/L (308.49 mg/dL) at the follow-up visit. The missense variant may increase the hepatic synthesis of TGs and is a risk factor for HTG, even when the lifestyle is relatively healthy.

Genetic findings

Exome sequencing revealed a novel heterozygous variant, p.K327N (c.981 g>C, p.Lys327Asn) at exon 8 of *GPD1*, and the mRNA reference was NM_005277.4. Sanger sequencing was performed thrice to verify the presence of the *GPD1* variant (Fig. 2). After testing the patient's family members (Fig. 2A), the variant was not detected in his wife but detected in his daughter(Figure 2C&D). The biochemical detection indices of the family members are shown in Table 1. His daughter and wife showed normal serum TG levels, 1.1 mmol/L (97.35 mg/dL) and 1.6 mmol/L (141.52 mg/dL), respectively. The daughter is 19 years old now and has a normal BMI of 19.5 kg/m^2, which was achieved by consuming a low-fat diet and avoiding cigarettes and alcohol. The proband and the family members carried no other variants in HTG-related genes such as *LPL*, *APOA5*, *APOC2*, *LMF1*, and *GPIHBP1*.

Functional characterization of the *GPD1* variant p.K327N

For in vitro analysis, the wild type, *GPD1* p.K327N, and an empty vector were transiently transfected into HEK-293 T cells (Fig. 3A). After transfecting the mutant plasmid, *GPD1* expression was downregulated (74.9%) compared with that of the wild-type plasmid. The western blotting results are presented as the mean ± standard deviation and were analyzed using the SPSS 25.0 software package (IBM Analytics, Armonk, NY). A probability (*P*-value) of less than 0.05 was defined as statistically significant.

The *GPD1* variant K327N was not reported in the East Asian population included in the Genome Aggregation Database, validating that it is a novel variant. Moreover, K327N was conserved across the species (Fig. 3B and C), suggesting that amino acids may play a role in *GPD1* maturity or function. The active epigenetic marker H3K4Me1 was enriched in seven cell lines, namely GM12878, H1-hESC, K562, HSMM, HUVEC, NHEK, and NHLF (Fig. 3D). The predicted partial 3D structures of the wild-type (p.K327) and variant (p.N327) are presented in Fig. 3E. The electric potential diagram (Fig. 3F) showed that the potential changed, affecting the protein function. In summary, the novel variant *GPD1* p. K327N may exert a mild effect on GPD1 protein function.

Review of *GPD1* gene map

The search terms "glycerol 3-phosphate dehydrogenase-1 variant," "glycerol 3-phosphate dehydrogenase-1 variants," "*GPD1* mutation," and "*GPD1* variant" were used to search the PubMed, CNGI, and CNH databases

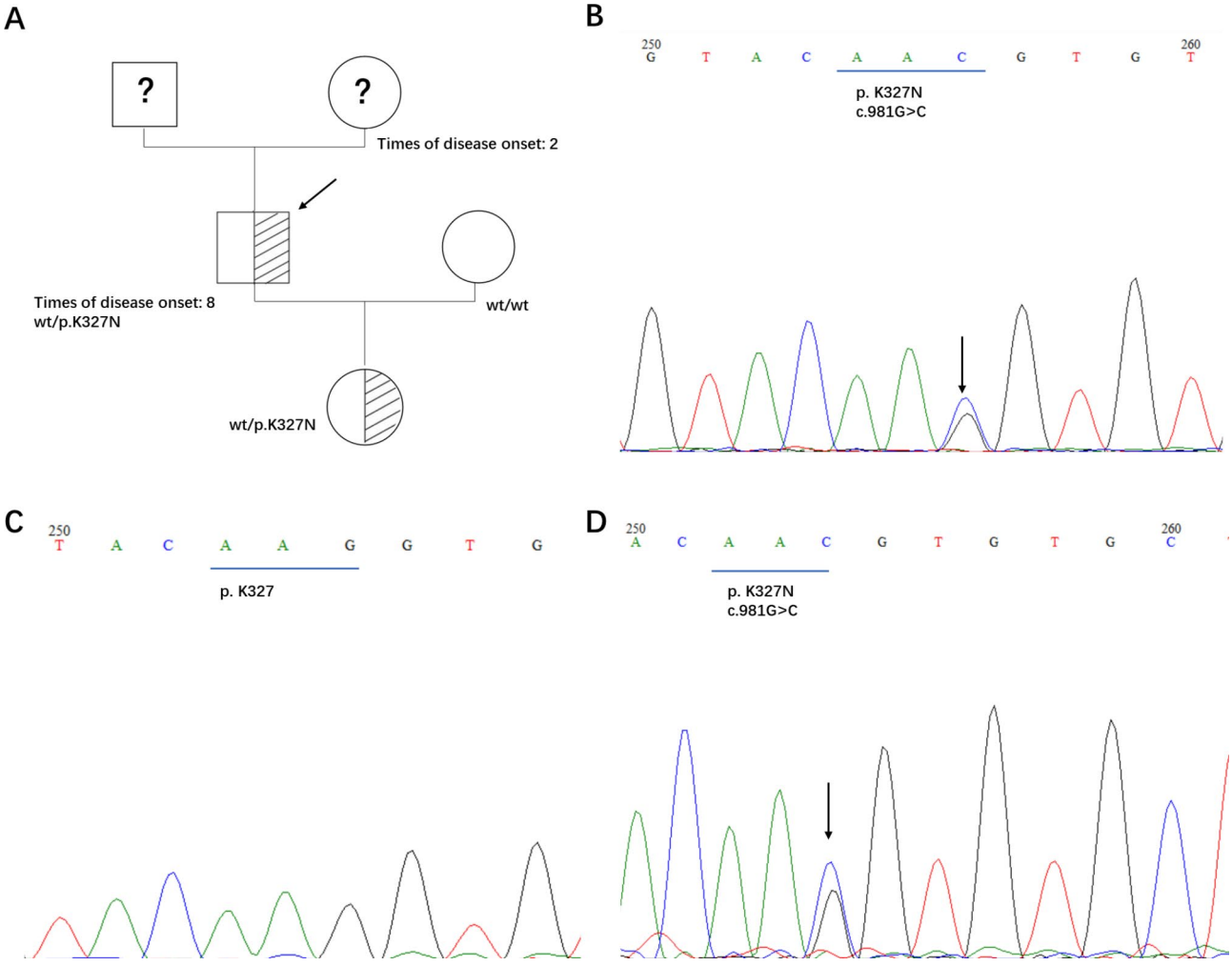

Fig. 2 Identification of a novel variant in *GPD1*. **(A)** Family pedigree. The arrow indicates the proband. *GPD1* genotypes are provided for all patients. wt, wild-type; **(B)** Sanger sequencing electropherogram of the proband showing the heterozygous G > C single nucleotide substitution at position c.981 of *GPD1* (indicated by the arrow), which would change the codon for lysine at position p.327 (underlined) to asparagine. (i.e., p.K327N); **(C)** p.K327N was not detected in the wife of the proband; **(D)** The daughter of the proband was heterozygous for the nucleotide substitution, which resulted in p.K327N. The arrow shows the nucleotide substitution from G to C

Table 1 Biochemical detection index of the family

Physiological Indexes	Proband	The wife of proband	The daughter of proband	Normal range
Alanine aminotransferase (ALT)	29.847	18.855	12.671	≤ 40U/L
Aspartate aminotransferase (AST)	29.712	22.751	22.027	≤ 40U/L
Total bilirubin (TBIL)	9.164	11.726	8.793	2–18μmol/L
Direct bilirubin (DBIL)	8.538	10.865	8.225	2–8μmol/L
Albumin (ALB)	45.93	42.22	47.02	33–55 g/L
Alkaline phosphatase (ALP)	144.702	54.918	70.965	53-185U/L
Gamma-glutamyltransferase (γ-GT)	62.599	14.635	16.006	Men:≤50U/L Women:≤32U/L
Total bile acids (TBA)	6.0	1.4	2.0	≤ 14μmol/L
Triglyceride (TG)	2.685	1.629	1.108	0.56-1.7mmol/L
Cholesterol (CHO)	3.956	4.285	3.274	3.6-6.5mmol/L
High density lipoprotein cholesterol (HDL)	0.777	1.735	1.409	0.83-1.96mmol/L
Low density lipoprotein cholesterol (LDL)	2.459	1.879	1.425	2.07-3.10mmol/L
Glucose (GLU)	5.8	5.9	4.6	3.9-6.1mmol/L
Glycosylated serum protein(GSP)	1.749	1.912	1.724	0.8-2.0mmol/L

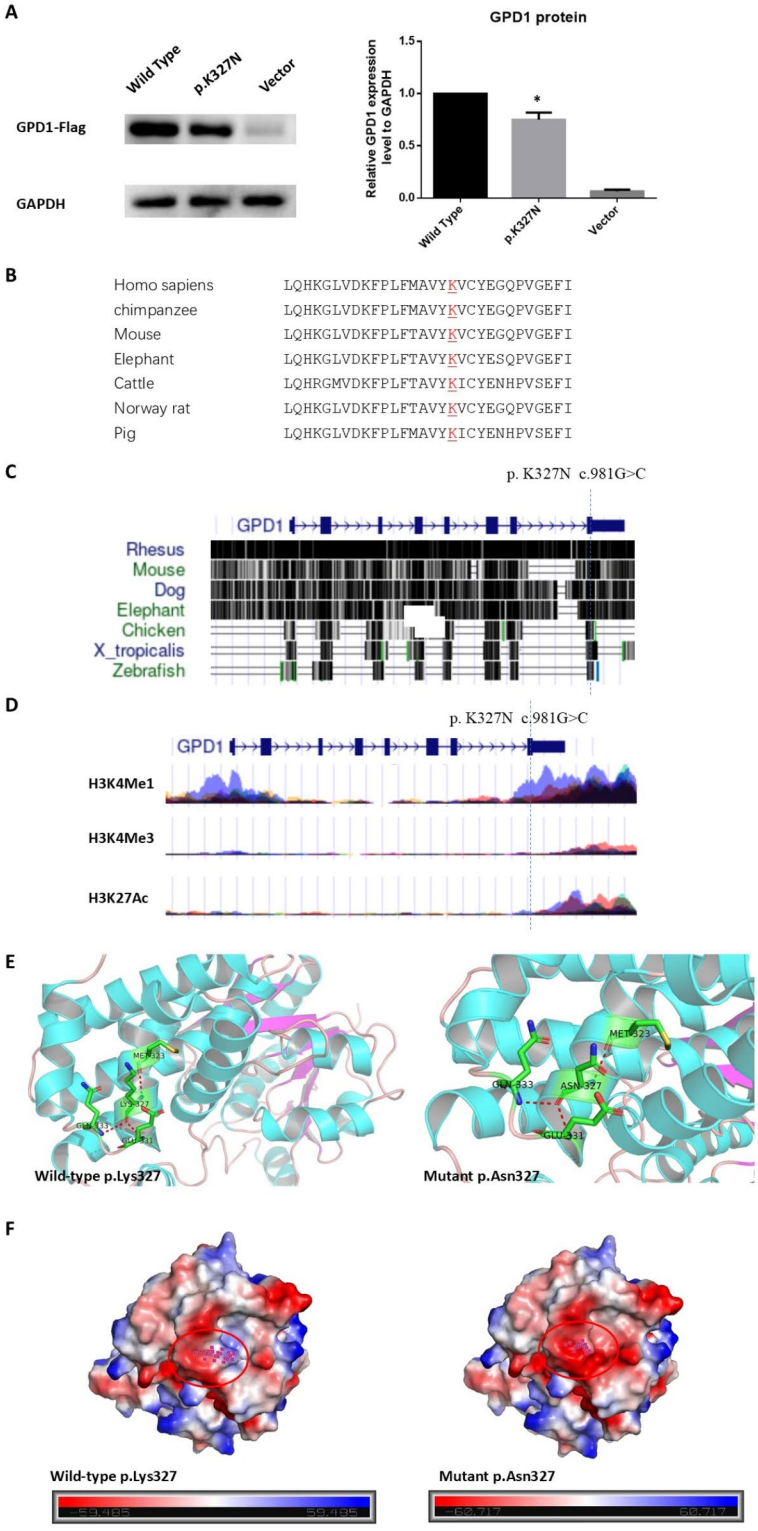

Fig. 3 (See legend on next page.)

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Fig. 3 **A.** Functional characterization of the K327N variant. Proteins obtained from HEK293T cells that were transiently transfected with the wild-type (WT) expression vector, the mutant expression vector, and the empty vector (EV) were used for western blotting. GAPDH, loading control. The results are presented as the mean ± SD from three independent transfections, and all assays were performed in triplicates. **B** and **C.** Evolutionary conservation of K327N amino acid residues in various species. **D.** Functional annotation of rs28539249. The epigenetic landscape showed the enrichment of the transcription regulatory histone markers H3K4 me1, H3K4 me3, and H3K27ac and chromatin state annotation from seven cell lines in ENCODE. (ChromHMM color coding is as follows: orange, strong enhancer; yellow, weak enhancer; light green, weak transcribed; light gray, low signal). **E.** Predicted partial 3D structures of the wild-type (p.Lys327) and variant (p.Asn327). **F.** The electric potential diagram of the wild-type (p. Lys327) and variant (p. Asn327)

from their inception to September 30, 2023. Eleven English [8, 15–24] and two Chinese articles [25, 26] were retrieved, involving 36 patients with the *GPD1* variant who were not heterozygous. Six heterozygous parents of the patients were reported to suffer related clinical manifestation.

A review of previous literature reports and the present study included 36 patients with the *GPD1* variant(Table 2); among them, 10 were females, 17 were males, and the gender of the remaining 9 was not indicated, and all were not heterozygous. Among them, 35 patients (97.2%) showed increased TG levels, with the highest value ranging from 1.92 to 70.56 mmol/L (169.92 to 6244.56 mg/dL) in infancy, and suffered constant state of HTG, but less than 30.0% had returned to normal during follow-up. 34 patients (94.4%) showed abnormal liver function, and 28 patients (77.8%) showed hepatic steatosis. The pathogenicity of frameshift, nonsense, or the biallelic *GPD1* variants in these patients was often self-evident, which resulted in the complete or almost complete loss of function, leading to the development of transient infantile hypertriglyceridemia. *GPD1* variants have a long term infection on TG level, although severe HTG is transient at an early age in few patients, but TG levels may not maintain normal with age spontaneously for most of these patients.

In addition to the proband, only two other patients were followed up to adulthood (23 years old and 31 years old); all of them still showed persistently high TG levels. These two patients suffered from transient HTG in infancy; however, their TG levels improved without special treatment, and AP was not observed until adulthood. The proband was the oldest patient who consumed a high-fat diet and smoked heavily, thus presenting with persistent HTG and recurrent HTG-AP and is the first reported adult patient with HTG-AP. Six heterozygous parents were reported to suffer from HTG, fatty liver, or short stature as listed in Table 3. The monogenic variant may be a previously unrecognized risk factor of dyslipidemias.

Discussion

In the present study, we reported the novel variant *GPD1* p.K327N in a Chinese adult male patient with recurrent HTG-AP, and in *vivo* and in *vitro* analyses revealed that the variant may exert a mild effect on GPD1 protein function. The patient's medical history revealed that each onset of AP was related to high-fat diet consumption and heavy smoking, as well as gene-environment interactions played a vital role in the pathogenicity of the variant. HTG is a polygenic disorder, it doesn't conform to the typical dominant inheritance pattern. Instead, it is a disease manifestation triggered by the intertwined and synergistic effects of genetic factors and external environmental factors. The HTG phenotype is regulated by the complex networks of multiple gene variation and secondary factors. Heterozygosity for this *GPD1* variant may have combined with high-fat diet and heavy smoking to trigger HTG and HTG-AP in the patient.

GPD1, located on chromosome 12q13.12, encodes GDP1 and is a member of the NAD-dependent GPD family. GPD1 catalyzes the reversible conversion of DHAP and NADH to G3P and NAD + in the cytoplasm and participates in carbohydrate and lipid metabolism [22, 27]. Currently, the exact mechanism underlying HTG caused by *GPD1* deficiency remains unclear, and the mainstream theory suggests that the *GPD1* variant may increase the G3P amount available for TG synthesis in the liver by restricting the G3P-to-DHAP conversion when the body suffers an overabundance of calories; thus, increasing TG levels [15]. In *vivo* experiment has confirmed that this novel mutation *GPD1* p.K327N can lead to a decrease in protein expression levels resulting the restriction of G3P-to-DHAP conversion and an increase in TG levels.

Previous studies reported 35 biallelic patients with the *GPD1* variant, characterized by transient infantile hypertriglyceridemia (HTGTI) and presented mild or moderate persisting HTG, abnormal liver function, and hepatic steatosis. The primary variant sites of the *GPD1* gene were missense variants, followed by splicing variants and nonsense variants. Part of the articles clarified the reasons for HTG caused by the biallelic or homozygous variants. Basel-Vanagaite et al. reported the splice-site homozygous variant in intro 3 (c.361-1G > C) creates a truncated protein of 213 residues and missing some major secondary structures and active sites [8]. While compound heterozygous missense variant in exon 6 (c.820G > A) and splicing variant in exon 3 (c.220–2 A > G) generated a decreased expression of the protein and the loss of bases [15]. Most of the reported patients were identified in infancy and presented long-term abnormal TG levels, only about 30.0% had returned to normal during follow-up. However, only two patients were followed up to adulthood (23 years old and 31 years

old), who were accompanied by persistently high TG levels without HTG-AP onset. The fitting TG curve of these patients carrying the *GPD1* variant showed that HTG in early infants decreased rapidly with age, especially within 1 year of age. However, its average level exceeded the normal upper limit during the later period [28]. The characteristics of mild or moderate persisting HTG in some patients indicated that the influence of *GPD1* defect was a prolonged process rather than temporary, reflecting the persistent disorder in lipid metabolism, which may exert long-term adverse effects on health, as well as HTG may relapse in most patients.

HTG is a polygenic disease, which may not be explained by a simple monogenic disease inheritance model. The HTG phenotype is regulated by a complex network of multiple gene variations and secondary factors. Previous studies have demonstrated that monogenic HTG in patients with severe HTG or HTGTI displays classic autosomal recessive hereditary disorders. Affected individuals are often homozygous or compound heterozygous for large-effect loss-off function variants in genes that regulate catabolism of TG-rich lipoproteins, such as *LPL*, *APOC2*, *APOA5*, *LMF1*, *GPIHBP1*, and *GPD1* [8]. Fasilaas et al. reported homozygous mutations of LPL lead to the onset of HTG in infancy [29], while heterozygous patients develop the disease in adulthood synergized with environmental factors [30]. Heterozygous mutations, which possess the potential to collaborate with environmental elements and subsequently trigger the onset of diseases, urgently demand the attention of clinicians. Gene-environment interactions are vital for the pathogenicity of many diseases, including HTG-AP. The heterozygous monogenic variant of *GPD1* may be a risk factor of dyslipidemias. The six related parents, who were identified as *GPD1* heterozygous carriers, suffered from HTG, fatty liver. Unlike the pathogenicity of frameshift, nonsense, or biallelic *GPD1* variants resulting in the complete or almost complete loss of function and leading to HTG, a heterozygous missense variant associated with a mild functional effect may be identified by clinicians if it synergistically interacts with other genetic and environmental factors [30, 31]. The HTG phenotype is regulated by a complex network of multiple gene variations and secondary factors, and HTG-related diseases may be caused by a variable combination of genetic determinants and environmental factors present. The present functional analyses showed that *GPD1* p.K327N was a mild variant in terms of its functional effects.

The proband was obese in the AP onset period, with a long history of high-fat diet consumption and heavy smoking. Additionally, the patient had been overeating and smoking excessively right before the onset of HTG-AP (Fig. 1). Tobacco can increase free fatty acid delivery into plasma by affecting lipid metabolism in the liver, thus inducing an increase in TG levels owing to the defective lipolytic system. Heavy smoking is correlated to plasma TG levels at the population level [32]; thus, the combination of high-fat diet consumption, heavy smoking, and underlying genetic risk factors for HTG may worsen or even trigger HTG-AP development. Additionally, specific triggers of HTG related to *GPD1* mutation cannot be excluded and should be identified in the future. It is impossible to trace back or determine whether the patient and his daughter have suffered HTGTI. The patient's daughter is 19 years old now and has a normal BMI and normal TG level, consuming a low-fat diet and avoiding cigarettes and alcohol. But as the variant carrier, she still needs close follow-up and has a relatively high probability of developing HTG over time as lack of GPD1 may interfere with the glycerol phosphate shuttle. Related expert panel advises that people aged 10–21 years with lipid abnormalities should be handled for 3–6 months with diet adjustments at least. A diet rich in low-fat medium-chain TG may be effective in some people for lowering TG levels [30, 33]. As the long-term effects of *GPD1* deficiency on transient HTG remain unclear, strengthening the long-term follow-up of these patients, maintaining a healthy lifestyle, avoiding alcohol and tobacco intake and paying attention to changes in lipid levels, liver functions, and pancreatitis are crucial.

The proband, who consumed a high-fat diet and smoked heavily, carried a novel *GPD1* variant and thus was diagnosed with HTG. Furthermore, he suffered from repeated episodes of HTG-AP since adulthood (32 years old). The present patient is the first reported case of a *GPD1* heterozygous mutation carrier with recurrent HTG and HTG-AP in adulthood. Drug administration (fenofibrate), less smoking, and healthy diet consumption were effective in controlling HTG-AP attacks, indicating supervision on the patient's lifestyle have a significant impact on clinical outcomes. This result indicated that the missense variant associated with a mild functional effect may interact synergistically with other genetic and environmental factors to affect normal protein functions. The present study highlights the importance of screening *GPD1* in patients with recurrent HTG. Patients with recurrent HTG should be considered gene-screening and provided individualized lifestyle guidance. Additionally, the study shows that gene-environment interactions play a key role in the pathogenicity of the disease, thereby deepening the existing knowledge of this rare disorder, both concerning the phenotype and underlying molecular mechanism.

Conclusion

In conclusion, we reported a Chinese male adult patient with recurrent HTG-AP carrying a novel variant of *GPD1*, p.K327N (c.981G > C). It is the first reported case

Table 2 Summary of the key clinical and genetic data of the 36 reported biallelic patients with the *GPD1* variants

Reference	Case	Age of onset	Follow up until	Gender	Country	GPD1 Variants	Location	Zygosity	Elevated TG	Highest TG level(mmol/L)	Elevated transaminases	Hepatic steatosis	Clinical features
Present	1	36y	47y	male	China	c.981G > C, p. K327N	exon8	Heterozygote	Y	17.82	Y	Y	8 episodes of AP. Overweight
Basel-Vanagaite et al. (2012)	2	1 m	14y	male	Israel	c.361-1G > C, p.I119fs*94	intron3	Homozygote	Y	70.56	Y	Y	Case2-4 were from the same family; Short in stature; Obesity; Insulin resistance
Basel-Vanagaite et al. (2012)	3	1 m	10y	male	Israel	c.361-1G > C, p.I119fs*94	intron3	Homozygote	Y	2.83	Y	Y	Vomiting
Basel-Vanagaite et al. (2012)	4	4 m	12y	male	Israel	c.361-1G > C, p.I119fs*94	intron3	Homozygote	Y	11.18	Y	Y	Short in stature
Basel-Vanagaite et al. (2012)	5	at birth	23y	male	Israel	c.361-1G > C, p.I119fs*94	intron3	Homozygote	Y	5.87	Y	Y	case5-6 were from the same family; Short in stature; splenomegaly
Basel-Vanagaite et al. (2012)	6	6 m	3y	female	Israel	c.361-1G > C, p.I119fs*94	intron3	Homozygote	Y	13.65	Y	Y	Developmental retardation
Basel-Vanagaite et al. (2012)	7	2.5 m	4y	female	Israel	c.361-1G > C, p.I119fs*94	intron3	Homozygote	Y	3.94	Y	Y	Case7-9 were from the same family; Vomiting
Basel-Vanagaite et al. (2012)	8	7 m	1y	female	Israel	c.361-1G > C, p.I119fs*94	intron3	Homozygote	Y	2.92	Y	Y	Splenomegaly
Basel-Vanagaite et al. (2012)	9	7 m	1y	female	Israel	c.361-1G > C, p.I119fs*94	intron3	Homozygote	Y	2.91	Y	Y	Splenomegaly
Basel-Vanagaite et al. (2012)	10	9 m	12y	male	Israel	c.361-1G > C, p.I119fs*94	intron3	Homozygote	Y	3.73	Y	Y	Case10-11 were from the same family; Short in stature; horseshoe kidney
Basel-Vanagaite et al. (2012)	11	3.5 m	12y	male	Israel	c.361-1G > C, p.I119fs*94	intron3	Homozygote	Y	2.54	Y	Y	Short in stature;
Li et al. (2017)	12	7y	13y	male	China	c.220–2 A > G;c.820G > A,p. A274T	intron2;exon6	Compound heterozygote	N	/	N	N	Short in stature; Obesity; Insulin resistance
Li et al. (2018)	13	3.5 m	NA	male	China	c.523 C > T, p. Q175*	exon4	Homozygote	Y	10.94	Y	Y	Splenomegaly

Table 2 (continued)

Reference	Case	Age of onset	Follow up until	Gender	Country	GPD1 Variants	Location	Zygosity	Elevated TG	Highest TG level(mmol/L)	Elevated transaminases	Hepatic steatosis	Clinical features
Ma et al. (2021)	14	1 m	1y	male	China	c.901G > T,p.E301*;c.220–2 A > G	exon7;intron2	Compound heterozygote	Y	19.08	Y	Y	Liver fibrosis
Xie et al. (2021)	15	1 m	12 m	female	China	c.901G > T,p.E301*; Deletion > 5.1 kb at a different locus;	exon7	Hemizygous	Y	9.2	Y	N	
Xie et al. (2021)	16	13 m	19 m	male	China	c.931 C > T,p.Q311*;c.901G > T,p.E301*	exon7;exon7	Compound heterozygote	Y	15.14	Y	N	
Dionisi-Vici et al. (2016)	17	10 m	14 m	male	Arab Muslim	c.806G > A, p.R269Q	exon6	Homozygote	Y	1.92	Y	Y	Consanguineous marriage; Liver fibrosis
Dionisi-Vici et al. (2016)	18	1y	3y	female	NA	c.361-1G > C, p.I119fs*94	intron3	Homozygote	Y	13.33	Y	Y	
Dionisi-Vici et al. (2016)	19	5 m	7y	male	Italy	c.640T > C, p.C214R	exon5	Homozygote	Y	5.27	Y	Y	Consanguineous marriage, cirrhosis of the liver, dicarboxyuria
Dionisi-Vici et al. (2016)	20	2y	31y	male	Italy	c.640T > C, p.C214R	exon5	Homozygote	Y	2.41	Y	Y	Persistent hypertriglyceridemia;
Joshi et al. (2014)	21	5 m	1.5y	female	America	c.686G > A, p.R229Q; Deletion > 1.85 kb at a different locus;	exon6	Hemizygous	Y	9.48	Y	Y	Developmental retardation; vomiting
Matarazzo et al.(2020)	22	1y	16y	male	Russia	c.895G > A, p.G299R	exon7	Homozygote	Y	11.49	Y	Y	Persistent hypertriglyceridemia, fenofibrate treatment is effective
Iin et al.(2021)	23	4 m	4y	female	China	c.454 C > T,p.Q152*	exon4	Homozygote	Y	4.39	Y	Y	Short in stature;
Wang et al.(2021)	24	1 m	13 m	female	China	c.901G > T,p.E301*; A short fragment heterozygous deficiency	exon7	Hemizygous	Y	6.36	Y	Y	Persisted jaundice and hepatomegaly
Leo Polchar et al.(2022)	25	28 m	6y	female	South Asian	c.500G > A, p.G167D	exon4	Homozygote	Y	8.9	Y	Y	Faltering growth, hepatomegaly and raised transaminases; consanguineous marriage
Pawan Kumar et al.(2021)	26	5 m	11 m	male	India	c.500G > A, p.G167D	exon4	Homozygote	Y	NA	N	Y	Massive hepatomegaly and mild splenomegaly

Table 2 (continued)

Reference	Case	Age of onset	Follow up until	Gender	Country	GPD1 Variants	Location	Zygosity	Elevated TG	Highest TG level(mmol/L)	Elevated transaminases	Hepatic steatosis	Clinical features
Tesarova et al.(2021)	27–35	2-17y	NA	NA	Roma	c.895G > A, p.G299R	exon7	Homozygote	Y	2.13-12	Y	6 of 10 patients	Early onset moderate to severe hepatomegaly (9 of 10 patients)
Tesarova et al.(2021)	36			male	Palestinian Arab	c.116G > A, p.Trp39*	exon2	Homozygote					

Abbreviations: AP- acute pancreatitis; m-months; y-years; NA-not informative; Y-yes; N-no

of a *GPD1* heterozygous mutation carrier with recurrent HTG and HTG-AP. The *GPD1* p.K327N missense variant exerted a mild effect on GPD1 secretion, highlighting an association of this variant with high-fat diet consumption and heavy smoking. In future clinical diagnosis, genetic screening should be considered for the presence of variants of HTG-related genes such as *GPD1* in patients with recurrent HTG-AP. The pathogenesis of *GPD1*-related diseases and gene-environment interactions associated with them need further clinical and basic medical research.

Table 3 Summary of the key clinical and genetic data of the 6 mentioned heterozygous parents with the *GPD1* variants

Reference	Case	Relation	Age	Consanguineous	Country	GPD1 Variants	Location	Zygosity	Elevated TG	Clinical features
Basel-Vanagaite et al. (2012)	1	Mother of F2-II6	47y	NA	Israel	c.361-1G > C, p.I119fs*94	intron3	Heterozygote	Y	Fatty liver; obesity; normal liver enzymes; HTG
Li et al. (2017)	2	The father	40y	N	China	c.220–2 A > G	Intron2	Heterozygote	N	Short in stature; (the height:160 cm, BMI:17.6),
Li et al. (2017)	3	The mother	37y	N	China	c.820G > A, p. A274T	exon6	Heterozygote	N	Short in stature; (the height:153 cm, BMI:27.8) Obesity
Dionisi-Vici et al. (2016)	4	The father	NA	N	NA	c.361-1G > C, p.I119fs*94	intron3	Heterozygote	Y	Fatty liver
Dionisi-Vici et al. (2016)	5	The mother of patient B	NA	N	NA	c.361-1G > C, p.I119fs*94	intron3	Heterozygote	Y	Fatty liver
Li et al. (2018)	6	The father of patient B	NA	N	China	c.523 C > T, p. Q175*	exon4	Heterozygote	Y	Obesity(BMI:31.3); Elevated liver enzymes

Abbreviations: y-years; NA-not informative; Y-yes; N-no; BMI-body mass index

Abbreviations

TG	Triglyceride
HTG	Hypertriglyceridemia
AP	Acute pancreatitis
LPL	Lipoprotein lipase
APOA5	Apolipoprotein A-V
GPD1	Glycerol-3-phosphate dehydrogenase 1
GPIHBP1	Glycosylphosphatidylinositol-anchored high density lipoprotein-binding protein 1
LMF1	Lipase maturation factor 1
HTG-AP	Hypertriglyceridemia-related acute pancreatitis
DNA	Deoxyribonucleic acid
SAP	Severe acute pancreatitis
APOC2	Apolipoprotein C-II
DHAP	Dihydroxyacetone phosphate
G3P	Glycerol-3-phosphate
NADH	Nicotine adenine dinucleotide
BMI	Body mass index
PCR	Polymerase chain reaction
TC	Total cholesterol
HDL	High density lipoprotein cholesterol
LDL	Low density lipoprotein cholesterol
PCT	Procalcitonin
CT	Computed tomography
MAP	Mild acute pancreatitis
HTGTI	Transient infantile hypertriglyceridemia
NA	Not informative

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12920-025-02088-6.

Supplementary Material 1

Supplementary Material 2

Author contributions

X.L., J.D. and W.Y. designed the study. X.L., B.Z. and X.L. identified the novel variants, performed the experiments. Y.H., M.C., K.C. and M.W. obtained the clinical data. X.L., X.C. and M.C. wrote the manuscript. J.D. and W.Y. critically revised the manuscript with important intellectual input. All authors reviewed the manuscript.

Funding

This work was supported by the National Natural Science Foundation of China (No.82302442), the fundings for Clinical Trials from the Affiliated Drum Tower Hospital, Medical School of Nanjing University(grant numbers 2021-LCYJ-PY-40).

Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Nanjing Drum Tower Hospital (2021-370-02) and was conducted under the Declaration of Helsinki ethical principles for medical research involving human subjects. Informed consent was obtained from the children's parents. All participants provided both written and verbal consent to be part of this study.

Consent for publication

Written informed consent for publication of clinical details was obtained from the guardians of the patient.

Competing interests

The authors declare no competing interests.

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Received: 16 November 2024 / Accepted: 13 January 2025
Published online: 23 January 2025

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