Novel homozygous mutation of PNLIP gene in congenital pancreatic lipase deficiency: an extended family study

Naglaa M. Kamal, Omar I. Saadah, Shahad S. Alheraiti, Ruwayd Attar, Asmaa D. Alsufyani, Moratda H.F. El-Shabrawi and Laila M. Sherief

Abstract

Introduction: Congenital pancreatic lipase deficiency (MIM 614338) is a rare genetic disorder caused by homozygous mutation in the PNLIP gene. Few cases have been reported worldwide and among them, few cases were genetically confirmed.

Patients and methods: A 3-year-old girl presented with abundant greasy diarrhea started at the age of 2 years. Work up of steatorrhea including molecular testing of PNLIP gene in the patient and her family was done.

Results: A novel homozygous variant c.1257G > A (p. Trp419Ter) of the PNLIP gene was detected in the patient. Her parents and two siblings were carriers for the same mutation. Pancreatic enzyme therapy was introduced, and a multidisciplinary team was involved with the education for the need for the lifelong use of pancreatic enzymes, and genetic counseling was carried out. There was a great improvement of steatorrhea with pancreatic enzymes treatment.

Conclusions: PNLIP deficiency should be suspected in patients with steatorrhea who have low pancreatic lipase and an otherwise normal health and appropriate growth.

Keywords: novel, pancreatic lipase deficiency, PNLIP gene

Received: 20 September 2021; revised manuscript accepted: 20 January 2022.

Introduction

Fat is an important source of energy in the human body; it contributes to 30%–35% of the human energy sources. Loss of excessive fat in the stool more than 5% of the daily intake is considered abnormal and defined as ‘steatorrhea’.1

Fat digestion requires the action of digestive lipase enzymes such as lingual lipase, gastric lipase, and pancreatic lipase (PNLIP). The pancreatic lipase enzyme requires pancreatic colipase (CLPS) to augment its action.2 Congenital deficiency of PNLIP is a very rare condition that has been reported in a few studies.3–11 Most of these studies reported the clinical and laboratory findings,3–11 but only two studies reported the associated genetic mutations.12,14

Here we report a family with an index case of congenital pancreatic lipase deficiency with a novel mutation affecting the PNLIP gene and four heterozygous carriers.

Case presentation

A 3-year-old female patient born to consanguineous parents presented to our hospital at the age of 2 years with a history of the passage of steatorrhea stool that was difficult to flush away. The daily stool frequency was about 1–2 times per day, mostly related to fatty meals with liquid oil separating from the stool sometimes observed. She had no other associated symptoms, and her growth pattern and development were reported normal by her parents.

On physical examination, she was thriving well, with weight and height being on the 50th percentile of appropriate growth charts, and she had no associated dysmorphic features. The physical
examination of the remaining body systems was unremarkable.

Her laboratory investigations revealed a normal complete blood count (WBC, Hb, MCV, MCH, Platelets) and a normal coagulation profile (PT, APTT, INR). Repeated stool analysis showed an abundance of fat globules. Her fasting lipid profile revealed: low LDL: 1.4 mmol/L (nv. 3.0–3.6 mmol/L), normal HDL 1.09 mmol/L (nv. 1.04–1.55 mmol/L), cholesterol and triglycerides level of 3.1 mmol/L (nv. 3.1–5.1 mmol/L) and 1.38 mmol/L (nv. 0.00–1.70 mmol/L) respectively. The pancreatic panel was normal except for persistently low serum pancreatic lipase 4 U/L (8–78 U/L) on multiple occasions. She had a low 25-hydroxy-vitamin-D level of 9 ng/mL (30–100 ng/mL) that was refractory to correction by vitamin D supplementation.

Her sweat chloride test showed no abnormality. Fecal elastase level was 384 which was within the normal range of (200–500 mcg/g), along with normal alpha-1-antitrypsin in the stool.

Abdominal ultrasonography (US), computed tomography (CT) of the abdomen, and magnetic resonance cholangiopancreatography (MRCP) demonstrated normal pancreas and pancreatic ducts with no obvious pathology. The imaging studies showed no abnormalities in other abdominal organs.

Genetic testing of the patient confirmed novel homozygous mutation c.1257G > A (p. Trp419Ter) of the PLNIP gene. Parents and two other siblings were heterozygous for the same mutation. The parents and the two heterozygous siblings had normal CBC, coagulation profile, lipid profile, and pancreatic lipase and amylase level with negative stool fat globules (Table 1).

### Molecular genetic analysis of the PLNIP gene for the patient and her family

**Method.** The coding exons 2–13, as well as the corresponding exon-intron boundaries of the PLNIP gene (OMIM 246600) on chromosome 10q25.3, were enriched using Roche/NimbleGen sequence capture technology and sequenced on a Illumina HiSeq 1500-system (next-generation sequencing. NGs) (Figure 1).

**Results.** Sequencing analysis revealed a homozygous substitution from G to A at position c.1257 in exon 12 of the PLNIP gene (e.1257G > A), leading to the introduction of a premature stop codon at position 419 of the protein sequence (p. Trp419Ter) (chr10-118321071; hg19). This probably results in early translation termination by the introduction of TGA stop codon. This nonsense mutation is named as W419X. All four bioinformatic analysis programs used predict a pathogenic effect variant. Taken together, the detected variant can be classified as likely pathogenic.

Pancreatic lipase deficiency is inherited in an autosomal recessive manner. Therefore, with the detection of the likely pathogenic variant c.1257G > A (p. Trp419Ter) in the PLNIP gene homozygous state molecular genetic cause for the

### Table 1. Laboratory results of the patient and her family.

| Laboratory test       | Patient | Mother | Father | Sister 1 | Sister 2 | Brother |
|-----------------------|---------|--------|--------|----------|----------|---------|
| PNLIP gene            | c.1257G > A (p. Trp419Ter) | Homozygous | Heterozygous | Heterozygous | Heterozygous | Normal (no mutation) | Heterozygous |
| Stool fat globules    | Present | Absent | Absent | Absent | Absent | Absent | Absent |
| Lipase (8–78 U/L)     | < 4     | 25     | 32     | 19      | 31       | 27      |
| Amylase (25–125U/L)   | 51      | 56     | 61     | 44      | 49       | 52      |
| HDL (1.04–1.55 mmol/L)| 1.09    | 1.33   | 1.21   | 1.12    | 1.18     | 1.15    |
| LDL (3–3.6 mmol/L)    | 1.40    | 3.51   | 3.55   | 3.10    | 3        | 3.15    |
| Cholesterol (3.1–5.1 mmol/L) | 3.10 | 5.10 | 5.00 | 3.15 | 3.22 | 3.25 |
| Triglycerides (0–1.7 mmol/L) | 1.38 | 1.7 | 1.7 | 1.48 | 1.45 | 1.39 |
Figure 1. Molecular genetic testing of the index case and her family. (a) Affected Patient. (b) Carrier Mother. (c) Carrier Father. (d) Carrier Brother. (e) Carrier Sister. (f) Normal Sister.
clinical phenotype of the patient has most likely been identified.

A copy number variation analysis of the NSG data did not indicate a large deletion in the PNLIP gene in trans variant.

A deletion in trans to c.1257G > A would lead to hemizygosity of c.1257G > A. Both homozygosity and hemizygosity of c.1257G > A in the PNLIP gene would be compatible with the clinical diagnosis of pancreatic lipase deficiency in the patient.

**Interpretation:** The novel homozygous likely pathogenic variant c.1257G > A (p. Trp419Ter) in the PNLIP gene was detected.

To differentiate between homozygosity and hemizygosity, a segregation analysis of both parents was done and detected that both parents were heterozygous for the same mutation. Targeted molecular genetic testing was also done for other family members and detected two siblings carrying the offending gene in a heterozygous pattern with one normal sibling (Figure 1). The family pedigree is shown in Figure 2.

This mutation was deposited in the database after diagnosing this patient and her family. The patient was treated with pancreatic lipase supplementation that resulted in normalization of her stool consistency with the disappearance of steatorrhea and improvement of her growth during the follow-up period of 4 years after treatment. Genetic counseling was provided to the family with an explanation of the condition, the inheritance pattern, and the possibilities for further affected children in future pregnancies.

**Discussion**

To the best of our knowledge, this case is considered the first case from an extended family reported from Saudi Arabia in a child with congenital pancreatic lipase deficiency, that was confirmed with genetic testing with a novel homozygous mutation c.1257G > A (p. Trp419Ter) of the PLNIP gene. Table 2 summarizes previously reported cases of congenital lipase deficiency.

The reported patient presented with steatorrhea that was typical for all patients with congenital pancreatic lipase deficiency and was reported in all previously published cases (Table 2).

The disease onset manifested in early infancy at the age of 1 year which was concordant with the onset of the presentation of most reported cases. However, one of the reported patients has presented in adulthood at the age of 46 years and had steatorrhea started at 20 years of age.8

Growth and development have been normal in almost all the reported cases despite persistent steatorrhea. No additional clinical manifestations or symptoms were observed in our patient throughout clinical follow up which was consistent with other reports.3,4,5,9

The differential diagnosis of steatorrhea is wide and requires extensive diagnostic work up to exclude conditions such as celiac disease, cystic fibrosis, exocrine pancreatic insufficiency, and cholestasis. Therefore, abdominal imaging including abdominal computed tomography and magnetic resonance imaging and upper endoscopy may be required.3,9 Our patient had magnetic resonance cholangiopancreatography that showed a normal result.

As expected for patients with fat malabsorption, laboratory investigation of fat-soluble vitamins and serum fasting lipid profile are usually required as in the case of our patient and the patient reported by Behar et al.12
Table 2. Previously reported cases of congenital pancreatic lipase deficiency.

| Patients | Onset of steatorrhea | Pancreatic lipase level / activity in duodenal fluid analysis/ and other tests | Growth and development | Genetic testing | Reference |
|----------|----------------------|---------------------------------------------------------------------------------|------------------------|-----------------|-----------|
| 4 children, 2 sisters and 2 brothers from unrelated non consanguineous Families | Early infancy | Total absence of pancreatic lipase in 1 boy and less than half the normal amount in the other 3 children. | Normal | Not performed | 3 |
| 10-year-old boy | At 3 years of age | Absent activity and normal secretion of bicarbonates, slightly decreased trypsin and amylase activity. Sweat chloride: normal. | Normal | Not performed | 4 |
| 8-year-old girl | Infancy | Absent activity with slightly reduced protease activity and normal amylase activity | Normal | Not performed | 5 |
| 9-year-old boy | At 2 years of age | Decreased pancreatic lipase activity. Treatment measures: high doses of pancreatic extract and extreme reduction of dietary fat, to 20 g of corn oil per day. | Normal | Not performed | 6 |
| Reported patient | | Absent activity | Normal | Not performed | 7 |
| 9 years | 3 months | Absent activity | Normal | Not performed | 8 |
| 46-year-old man | at least age 20 years | Absent activity | Normal | Not performed | 9 |
| 5.5-year-old boy of German parentage | infancy | Absent activity | Normal | Not performed | 10 |
| 10-year-old girl | 10 months | Absent activity | Normal | Not performed | 11 |
| 15- and 19-year-old brothers, born to healthy first-cousin parents of Arab Muslim origin from central Israel | First postnatal days of feeding | Decrease in PNLIP activity. Normal sweat chloride test. Normal lipid profile. Low vitamin A, D, E | Normal | Thr221Met [c.662C > T] | 12 |
| Identical twin brothers | Early in infancy | Data not available. The paper by Gottesman-Katz et al. This should be indicated in Table 1 last row. | | Whole exome sequencing: W102X and R188C compound heterozygous mutations in the PNLIP gene associated with lipase deficiency | 14 |

Our patient and all reported cases of congenital pancreatic lipase deficiency had low or undetectable pancreatic lipase on multiple occasions, which was the clue to the diagnosis. Most cases were clinically suspected based on the symptom of steatorrhea and confirmed by assessment of bicarbonates’ secretion, trypsin level, and pancreatic lipase level in the duodenal aspirate.
The confirmation through genetic testing was reported by Behar et al., who detected the novel mutation Thr221Met [c.662 C > T] in the PNLIP gene. This mutation was studied in 2015 by Szabo and colleagues in cell models demonstrating protein misfolding and intracellular aggregation of the pancreatic lipase molecule.

In 2020, Gottesman-Katz and colleagues presented 2 novel compound heterozygous variants of uncertain significance in the PNLIP gene in two identical twin male brothers. The first mutation (maternally inherited) was c.562 C > T p.R188 C and the other one (unknown inheritance) was c.305G > A p.W102X. Variant R188 C has an allele frequency of 0.0115% in individuals of African ancestry and is a nonconservative missense variant. Variant W102X is predicted to cause a loss of function via premature truncation and has not been previously reported in large population cohorts. They presumed that these mutations are in trans, thus causing the observed clinical picture.

Our patient carried the novel c.1257G > A (p. Trp419Ter) mutation in the PNLIP gene in a homozygous state with both parents and two siblings being heterozygous for it. One sibling was genetically normal.

Following the confirmation of the diagnosis, family counseling was carried with a thorough explanation of the disease and its prognosis and further pregnancies planning.

A multidisciplinary team was involved to provide the optimal care needed for this patient as lifelong treatment is a cornerstone.

An important limitation of the study is the need for further research to confirm these mutations' specific effects on pancreatic lipase structure and function.

Conclusion
Congenital pancreatic lipase deficiency is a very rare condition; thus, a high index of suspicion is needed to diagnose it in patients with steatorrhea and an otherwise normal health and appropriate growth and development. Low pancreatic lipase level is suggestive and PNLIP gene testing is confirmatory.

Acknowledgements
To Bioscientia Laboratories where the genetic testing was done.
To Dr. Nemer S Aljuaid, Saudi Board in Pediatrics, Saudi Arabia, for his support to the current work.
To Miss Sara AS Abosabie, Medical Student, 4th Semester, Charite’ Universitatsmedizen Berlin, Berlin, Germany. We thank her for her help in collecting patient’s data and for her help in the laboratory part of the manuscript. She also helped in editing the manuscript and English proofreading.

Author contributions
Naglaa M. Kamal: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Project administration; Supervision; Validation; Visualization; Writing – original draft; Writing – review & editing.
Omar I. Saadah: Writing – original draft; Writing – review & editing.
Shahad S. Alheraiti: Data curation; Investigation; Methodology; Project administration; Writing – original draft.
Ruwayd, Attar: Data curation; Formal analysis; Investigation; Methodology.
Asmaa D. Alsufyani: Data curation; Writing – original draft.
Moratda H.F. El-Shabrawi: Writing – original draft; Writing – review & editing.
Laila M. Sherief: Writing – original draft; Writing – review & editing.

Availability of data and material
All data generated or analyzed during this study are included in this published article.

Funding
The authors received no financial support for the research, authorship, and/or publication of this article.

Conflict of interest statement
The authors declare that there is no conflict of interest.

Ethics approval and consent to participate
The study was approved by Alhada Armed Forces Hospital research and ethical committee. Written informed consent was obtained from the patient’s parents for the contribution of their child in the study.
Consent for publication
Written informed consent was obtained from the patient’s parents for publication of details of the case report. Personal information was not mentioned in a way that can lead to the identification of the patient or family.

ORCID iDs
Naglaa M. Kamal https://orcid.org/0000-0002-8535-3838
Laila Sherief https://orcid.org/0000-0003-2088-4213

References
1. Mu H and Høy CE. The digestion of dietary triacylglycerols. Prog Lipid Res 2004; 43: 105–133.
2. Lindquist S and Hernell O. Lipid digestion and absorption in early life: an update. Curr Opin Clin Nutr Metab Care 2010; 13: 314–320.
3. Sheldon W. congenital lipase deficiency. Arch Dis Child 1964; 39: 268–271.
4. Rey J, Frezal J, Royer P, et al. L’absence congenitale de lipase pancréatique. Arch Franc Pediat 1966; 23: 5–14.
5. Balzer E. Congenital lack of pancreatic lipase. Z Gastroent 1967; 5: 239–246.
6. Verger P, Babin R, Guillard J-M, et al. Steatorrhée chronique de l’enfant par insuffisance congenitale de la lipase pancréatique. Arch Franc Pediat 1971; 28: 992.
7. Muller DPR, McCollum JPK, Trompeter RS, et al. Studies on the mechanism of fat absorption in congenital isolated lipase deficiency. Gut 1975; 16: 838.
8. Figarella C, Negri GA and Sarles H. Presence of colipase in a congenital lipase deficiency. Biochim Biophys Acta 1972; 280: 205–210.
9. Figarella C, De Caro A, Deprez P, et al. Un nouveau cas de déficience congénitale en lipase pancréatique avec présence de colipase. Gastroent Clin Biol 1979; 3: 43–46.
10. Figarella C, De Caro A, Leupold D, et al. Congenital pancreatic lipase deficiency. J Pediat 1980; 96: 412–416.
11. Larbre F, Hartemann E, Cotton J-B, et al. Diarrhee chronique par absence de lipase pancréatique. Pediatr June 1969; 24: 807–813.
12. Behar DM, Basel-Vanagaite L, Glaser F, et al. Identification of a novel mutation in the PNLIP gene in two brothers with congenital pancreatic lipase deficiency. J Lipid Res 2014; 55: 307–312.
13. Szabo A, Xiao X, Haughney M, et al. A novel mutation in PNLIP causes pancreatic triglyceride lipase deficiency through protein misfolding. Biochim Biophys Acta 2015; 1852: 1372–1379.
14. Gottesman-Katz L, Chung W, Hernan R, et al. Two novel PNLIP mutations causing congenital lipase deficiency in identical twin boys. J Pediatr Gastroenterol Nutr 2020; 70: e85–e86.
15. NM_000936.4(PNLIP):c.1257G>A (p.Trp419Ter). ClinVar Genomic variation as it relates to human health. https://www.ncbi.nlm.nih.gov/clinvar/variation/522506/ (accessed 6 November 2021).
16. Single nucleotide variant:10-118321071-G-A(GRCh37). gnomAD database. https://gnomad.broadinstitute.org/variant/10-118321071-G-A?dataset=gnomad_r2_1 (accessed 6 November 2021).