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CASE REPORT

A novel mutation in TRMT5 associated with idiopathic non-cirrhotic portal hypertension and hepatopulmonary syndrome: Case report of two siblings

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Abstract Non-cirrhotic portal hypertension (NCPH) is a rare clinical entity in children. Familial clusters of idiopathic non-cirrhotic portal hypertension (INCPH) were previously reported in cases with deoxyguanosine kinase (DGOUK) and potassium calcium-activated channel subfamily N member 3 (KCNN3) mutations. Herein, we report two siblings who had a novel mutation in mitochondrial tRNA methyltransferase 5 (TRMT5) gene and presented with hepatopulmonary syndrome and later diagnosed as INCPH. Autosomal recessive inheritance of this mutation may suggest a role of TRMT5 mutations in the development of NCPH. Screening of TRMT5 mutations could be considered when familial INCPH is suspected.

Introduction

Noncirrhotic portal hypertension (NCPH) is a rare clinical syndrome characterized by the presence of portal hypertension in the absence of liver cirrhosis, liver synthetic dysfunction and portal vein thrombosis. Congenital hepatic fibrosis (CHF), nodular regenerative hyperplasia (NRH), non-alcoholic fatty liver disease (NAFLD), sinusoidal obstruction syndrome (SOS), rarely metabolic diseases, schistosomiasis, and hepatoportal sclerosis are the main causes of NCPH. Failure to identify the underlying etiology of NCPH defined as idiopathic NCPH (INCPH), is another challenging clinical entity in pediatric patients. Idiopathic noncirrhotic portal hypertension accounts for 4.6% cases of portal hypertension

Abbreviations: TRMT5, TRNA Methyltransferase 5; INCPH, Idiopathic noncirrhotic portal hypertension.

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in children and more probably associated with underlying hereditary predisposition and malignancy in contrast to adults [1]. Recently, the umbrella term of porto-sinusoidal vascular disorder, accepted by Baveno VII consensus, encompasses a variety of clinicopathological entities like non-cirrhotic portal fibrosis, non-cirrhotic intrahepatic portal hypertension and idiopathic portal hypertension. Histological entities like nodular regenerative hyperplasia, obliterative portal venopathy and hepatoportal sclerosis are also included [2]. Although the exact mechanism of INCPH is still enigmatic, the potential involvement of immunological disorders (common variable immunodeficiency syndrome, connective tissue diseases, Crohn's disease, solid organ transplantation), infections (bacterial intestinal infections, HIV), toxins, genetic disorders (Adams-Olivier syndrome, Turner syndrome, phospho-mannose isomerase deficiency) and prothrombotic conditions were reported [3]. HLA-DR3 haplotype was reported in six patients with noncirrhotic portal fibrosis [4]. Many reports suggest a potential role of mitochondrial toxicity in the development of INCPH. A previous report identified rare homozygous p.N46S mutation in deoxyguanosine kinase (DGOUK) gene, which encodes an enzyme required for faithful replication of mitochondrial DNA, in three patients with INCPH [5]. Familial cluster of INCPH was also reported in a family with de novo mutation in KCNN3 which fits autosomal dominant inheritance [6].

Mitochondrial tRNA mutations (MTT) are associated with a wide phenotypic spectrum from isolated disorders affecting specific organs to multisystemic involvement and account for the majority of mitochondrial diseases. Mitochondrial tRNAs (mt-tRNAs) have a unique secondary structure different from the classical cloverleaf of cytosolic t-RNAs. Modifications at specific bases mediated by different enzymes are vital for the functionality of mt-tRNA. These modifications create identity elements needed for aminoacylation, binding domains of translation factors and tertiary structure formation. Nuclear TRMT 5 gene encodes for TRMT5, a methyltransferase responsible for the methylation of Guanosine at 37 positions (G^{37}) at mt-tRNA, which is essential for the accuracy and fidelity of mitochondrial DNA translation [7]. Hereditary spastic paresis, MELAS like features, lactic acidosis, neuropathy, cardiomyopathy, spasticity, optic atrophy and cirrhosis were previously reported in patients with TRMT5 mutations [8,9]. Herein, we report two siblings with INCPH presented with hepatopulmonary syndrome due to novel compound heterozygous mutations in TRMT5 gene. Up to our knowledge, the association of INCPH and hepatopulmonary syndrome with TRMT5 mutations has not yet been reported.

Case 1

A 12-year-old girl was the first child born with a birth weight of 3500 g by term cesarean section to non-consanguineous parents. She was followed after birth for non-recurrent hypoglycemia. She developed jaundice and was treated with phototherapy. Both of her prenatal and postnatal histories were unremarkable. She started walking at 18 months and by age two she was able to use two-word sentences. Her family history was significant for both grandmothers diagnosed with dilated cardiomyopathy and hypertension while was unremarkable for metabolic and neurological diseases.

At age four, the patient presented with thrombocytopenia and splenomegaly with hypoechoic and heterogeneous areas in the liver. She was assessed for possible chronic liver disease. Liver function tests, creatine kinase, alpha-1 antitrypsin, 24-h copper urine test, and serum ceruloplasmin levels were normal. Hepatitis C, B, and autoimmune hepatitis markers were negative. The patient was screened for suspected metabolic diseases. Results of urine organic acid test, sweat test, tandem mass spectrometry test, serum chitotriosidase, glucocerebrosidase, sphingomyelinase, and β-galactosidase levels were normal. Lipid profile test, folic acid, vitamin A, E, and B12 were within the normal range. Liver biopsy findings were unremarkable. Upper gastrointestinal endoscopy revealed grade 1 esophageal varices. At age 7, the patient was referred to the department of pediatric cardiology for clinically evident cyanosis and clubbing. Echocardiography demonstrated a pulmonary arteriovenous fistula. Cardiac catheterization revealed multiple pulmonary arteriovenous fistulas and mild degree pulmonary hypertension which were considered to be compatible with the diagnosis of hepatopulmonary syndrome. At age 11, the patient developed epilepsy, and treatment with levetiracetam was initiated. Brain MRI findings were unremarkable.

The patient was referred to our department after her 5-year brother displayed similar complaints. Her physical examination revealed the following: weight 55 kg (75−90p), height 162 cm (90−97p), blood pressure 120/70 mmHg. Neurological examination revealed abnormal gait, joint laxity, low muscle tone and strength with positive Gower's sign. The detailed cerebellar examination was normal. The spleen was palpable (3 cm below the costal margin) without hepatomegaly. She had blue sclera. Partial oxygen pressure was 63 mmHg in arterial blood gas test with high alveolar-arterial gradient (50 mmHg). Serum lactate level was mildly elevated (2.4 mmol/L).

To investigate the etiology of hepatopulmonary syndrome we re-performed a liver biopsy at our hospital. Liver biopsy revealed minimal fibrous dilation of portal areas, focal sinusoidal dilation, and congestion while cirrhosis was not identified (Fig. 2). Hepatic CT-CT angiography revealed prominent diffuse splenomegaly, multiple varices, and venous collaterals at the lower third of the esophagus, stomach, and adjacent to the spleen with patent hepatic venous and arterial vascular systems. Evaluation of the patient for non-cirrhotic portal hypertension was considered. Upper gastrointestinal endoscopy revealed portal hypertensive gastropathy while esophagus and duodenum were normal. Further investigations to exclude the possibility of underlying metabolic diseases were performed. Screening tests for peroxisomal diseases, Wolman disease, Niemann pick disease, Gaucher disease, and bile acid synthesis disorders were reported normal. The clinical evaluation further raised the suspicion of neurometabolic disease since the patient had a history of epileptic seizures. Brain MRI performed at our hospital revealed bilateral manganese deposits in globus pallidus caused by underlying chronic parenchymal liver disease. Magnetic resonance spectroscopy of the brain was unremarkable. Ophthalmological examination showed no findings suggestive of neuro-metabolic disorders. Control

echocardiography showed pulmonary arteriovenous fistula, moderate degree tricuspid insufficiency, and pulmonary hypertension. The patient was discharged and scheduled follow-up by the departments of pediatric gastroenterology and metabolic diseases was arranged.

Whole-exome sequencing was performed in our patient and revealed novel compound heterozygous mutations c.617T>C (p.I206T) (p.Ile206Thr) and c.899A>T (p.D300V) (p.Asp300Val) in NM_020810.3 transcript of TRMT5 gene. Both parents were heterozygous and asymptomatic (Fig. 1). p.I206T variant was found in GnomAD exomes but p.D300V was not. These two variants were classified as likely pathogenic due to ACMG criteria. There is no data regarding these variants in the literature. Treatment with mitochondrial drug cocktail (Vitamin B1, B2, and coenzyme Q10) was initiated.

Case 2

The younger brother of case 1, presented with complaints of dyspnea and fatigue at the age of 3.5 years. The patient was evaluated by the departments of pediatric cardiology and pulmonology. Patient's medical history revealed cyanosis, clubbing and low oxygen saturation measured by pulse oximetry. Thorax CT scan at the age of 5 years was unremarkable for lung parenchymal pathologies. Contrast echocardiography revealed pulmonary arteriovenous (AV) shunt. The patient was diagnosed with hepatopulmonary syndrome and followed at home with oxygen therapy (3 L/min).

At the age of 5 years, the patient was referred to our center for further evaluation. At the time of admission, his weight and height were 16.6 kg (10p) and 106 cm (10−25 p), respectively. Physical examination revealed clubbing, cyanosis, pectus carinatum deformity, abdominal venous collaterals and splenomegaly (3 cm palpable under costal margin). His neurological examination was unremarkable. Pulse oximetric oxygen saturation levels were 40−50%. Serum lactate level was mildly elevated (3.3 mmol/L). Biochemical tests revealed ALT 49 U/L, AST 78 U/L, GGT 34 U/L, total bilirubin/direct bilirubin 1.8/0.6 mg/dL, INR 1.54, platelets 135 $\times$ $10^3/\mu$L, ceruloplasmin 25 mg/dL. Hepatic viral markers (HBV, HCV and HAV) and autoimmune liver markers were negative. During follow up, platelet number decreased to 101 $\times$ $10^3/\mu$L. Hepatic Doppler USG revealed heterogenous liver parenchyma and hypoechoic nodular structures with median diameter of 10 mm. Hepatic and portal veins were patent. Abdominal CT scan revealed irregular hepatic contour, splenomegaly along with gastric and venous collaterals. The patient underwent selective pulmonary angiography. There were no detectable shunts for angiographic occlusion since shunts were at the capillary bed level.

The patient was diagnosed with hepatopulmonary syndrome associated with underlying liver disease. Liver biopsy was declined by the family since they preferred to wait for the genetic tests results of their siblings.

Patient's genetic analysis revealed the same novel mutation in TRMT5 gene [(c.617T>C) ((p. I206T) (p. Ile206Thr)] and [(c.899A>T) (p. D300V) (p. Asp300Val)] that was detected in his sister. Treatment with vitamin cocktail (B12, thiamine, riboflavin, coenzyme Q10 and alpha lipoic acid) was initiated. During his follow up, there was a mild amelioration of oxygen saturation levels (81%) and clubbing. The patient is currently followed up by the departments of pediatric gastroenterology and metabolic diseases.

Discussion

In this report, we described two siblings with INCPH presented to our clinic with hepatopulmonary syndrome. Laboratory and histological findings excluded common causes of chronic liver disease in both patients. Although the clinical pictures of both cases were non-specific, neurological findings in case 1 and lactic acidosis were highly suggestive of a mitochondrial disorder. Suspected diagnosis of a familial cluster of INCPH was confirmed by the detection of TRMT5 gene mutations in whole-exome sequencing test.

TRMT5 is a methyltransferase responsible for the methylation of Guanosine at 37th position (G37) at mt-tRNA. m^1G methylation is essential for the efficiency and accuracy of mitochondrial DNA translation. On one hand, N^1-methylation of G37 decreases base-pairing potential by preventing the

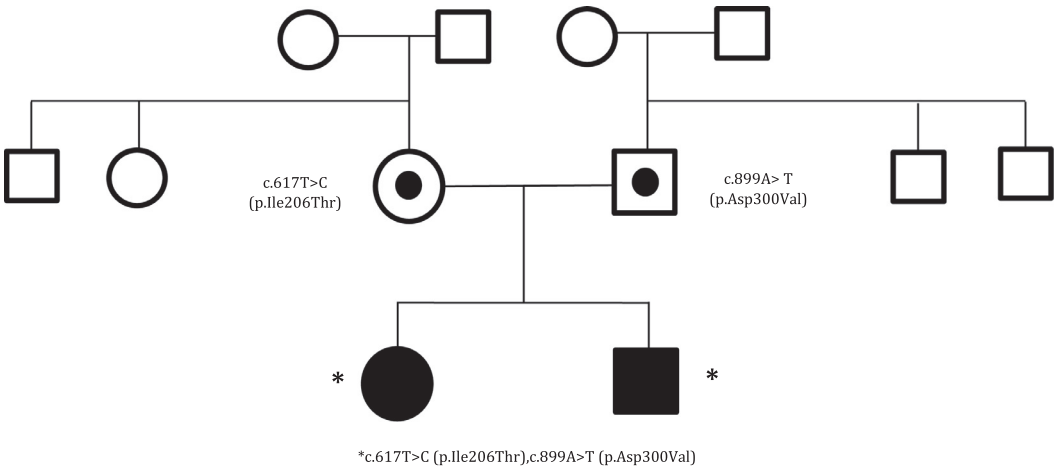

Fig. 1 Familial pedigree of the two reported patients.

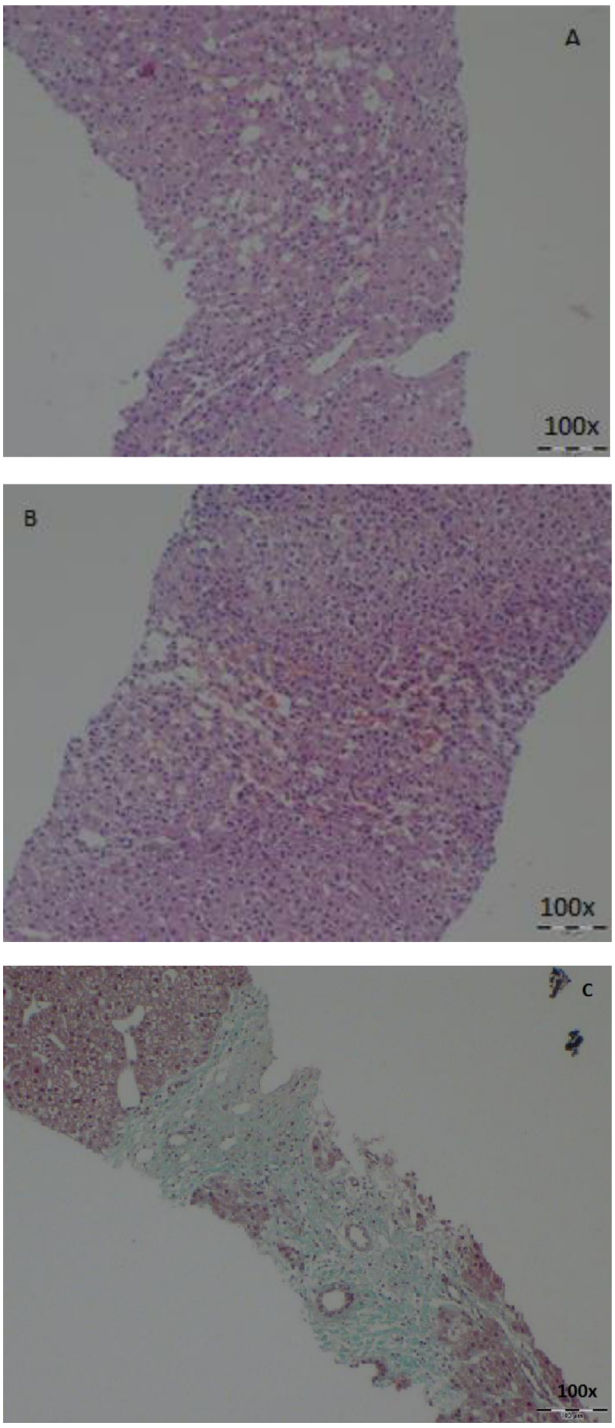

Fig. 2 (A, B) Localized sinusoidal dilatation and congestion around the central vein (HE). (C) Mild-moderate fibrosis in portal area (Trichrome).

interaction of anticodon loop with nearby mitochondrial tRNA nucleotides and maintaining conformational dynamics of the loop. On the other hand, methylation of G37 maintains ribosome translational fidelity by impeding +1 frameshift that results from erroneous tRNA-mRNA interactions [10]. TRMT5 mutations were associated with hypomethylation of guanosine residue at position 37 in mitochondrial tRNA. m^1G37 hypomodification has been associated with decreased accuracy of aminoacyl-tRNA selection, reduced polypeptide elongation, and increased tRNA mis-acylation [9].

Although the clinical presentation was remarkably different among subjects reported, lactic acidosis and findings of multiple mitochondrial respiratory chain complex deficiencies in skeletal muscle were detected. Protean clinical phenotypes seen may be related to the degree of mt-tRNA hypomodification and consequent mitochondrial translation impairment [9]. TRMT5 mutations were previously associated with hereditary spastic paraparesis, cerebral palsy, and exercise intolerance, and MELAS-like features (acute encephalopathy, seizures, stroke, and visual loss with optic atrophy) [8]. Mitochondrial mutations associated with m^1G37 modification were associated with systemic hypertension. $tRNA_{Met}$ 4435A→G mutation was identified in four genetically unrelated Chinese families with maternally transmitted hypertension. $tRNA_{Met}$ 4435A→G mutation created a tRNA methyltransferase 5 (TRMT5)-catalyzed site at $tRNA_{Met}$ altering its structure and function. Aberrant m^1G37 modification at $tRNA_{Met}$ was associated with increased generation of reactive oxygen radicals, decreased ATP production and membrane potential [11].

Our patients displayed different phenotypes from previously reported patients with TRMT5 mutations. While cirrhosis was previously reported by Powell et al. [9] in one patient, our patients presented with INCPH and hepatopulmonary syndrome. Although the same TRMT5 mutation was detected in both of our patients, they display different clinical phenotypes since epilepsy, muscle weakness and gait abnormalities were detected in case 1 but not case 2.

Diseases caused by mitochondrial tRNA gene mutations (MTT) display a poorly understood genotype-phenotype relationship. Patients with the same mutation may display heterogeneous phenotypes due to the complex interaction between genotype and phenotype. The highly polymorphic nature of mitochondrial DNA adds another level of complexity. Most MTT mutations are heteroplasmic in nature although some homoplasmic MTT mutations were reported [7]. Exhibition of the disease phenotype varies between tissues and depends on the mtDNA mutation threshold. Mutation levels and mt-tRNA hypomodification degree may explain for the variation in disease severity and phenotype between our patients.

In conclusion, we report for the first time a possible association of INCPH with TRMT5 mutations. Screening of TRMT5 mutations should be considered in patients with familial INCPH.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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