Heliyon 9 (2023) e21760

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Citrullinemia type II accompanied by mental derangement combined with multidrug resistance 3 decrease, case report

Hong Zhao [1], Xia Yu [1], Jinlin Cheng, Deying Chen, Qiaomai Xu, Jifang Sheng [**], Yu Shi [*]

State Key Laboratory for Diagnosis and Treatment of Infectious Diseases, National Clinical Research Center for Infectious Diseases, National Medical Center for Infectious Diseases, Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, The First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, 310003, China

ARTICLE INFO

Keywords:
Citrullinemia type II
Multidrug resistance 3
SLC25A13
Mutation
Case report

ABSTRACT

Background: Here we report a rare case of citrullinemia type II (CTLN2) accompanied by mental derangement with a deficiency of multidrug resistance 3 (MDR3) in the liver.
Case presentation: The clinical data of a 17-year-old girl were collected. Liver puncture was performed, and hepatic expression of MDR3 was determined by immunohistochemistry. Serum amino acids of the patient and her parents wwere determined by a chemical isotope labeling liquid chromatography−mass spectrometry (CIL LC-MS). Genetic mutations of *ABCB4* and *SLC25A13* were screened by whole-exome sequencing. Immunohistochemical analysis showed a remarkably lower expression of MDR3. Mutation in *ABCB4* gene was not found and whole-exome sequencing revealed the *SLC25A13* mutation 852–855 del. Elevated serum levels of citrulline, homocitrulline, and homoarginine in the patient and her mother were found.
Conclusions: We reported a rare case of CTLN2 combined with MDR3 deficiency, without mutation of *ABCB4*. The link between MDR3 down-expression and CTLN2 warrants further investigation. Meanwhile, clinicians need to further rule out the possibility of CTLN2 if MDR3 decreases in adolescent patients with mental disorders and abnormal liver function.

1. Introduction

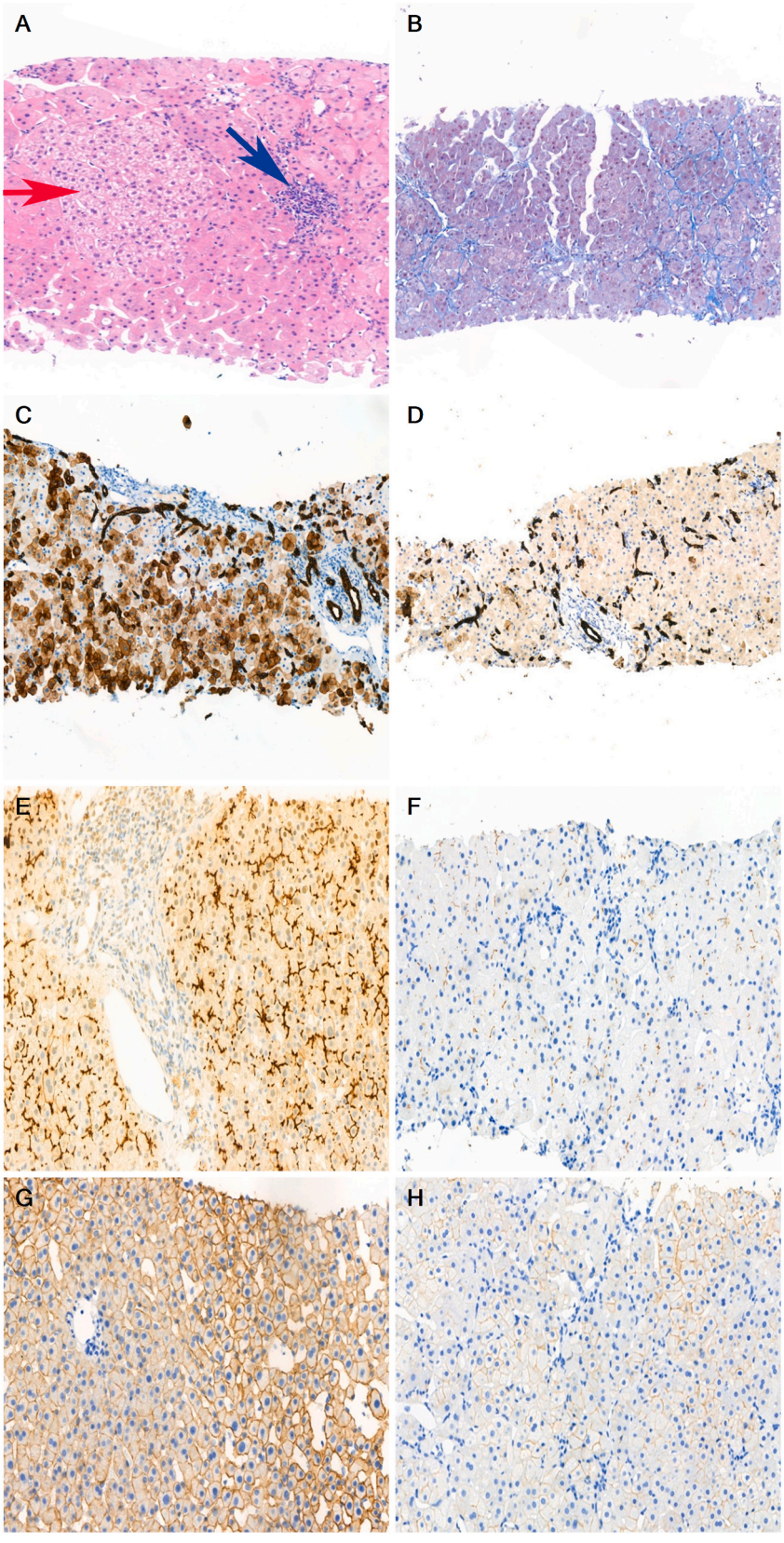
Citrullinemia type II (CTLN2), is often accompanied by mental derangement. CTLN2 is a rare autosomal recessive disorder caused by a genetic mutation of citrin (SLC25A13), which leads to malfunction of the urea cycle and elevated concentrations of citrulline in the blood [1]. Here we report a case of a 17-year-old girl who initially presented with neuropsychological symptoms and was finally diagnosed with CTLN2. Interestingly, a hepatic deficiency of multidrug resistance 3 (MDR3) expression, which is a classical mark of progressive familial intrahepatic cholestasis (PFIC) type 3, was concurrently found in this patient.

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; ASS1, argininosuccinate synthase 1; AST, aspartate aminotransferase; CTLN1, Citrullinemia type I; CTLN2, Citrullinemia type II; GGT, gamma glutamyltransferase; IHC, immunohistochemical; MDR3, Multidrug Resistance 3; NGS, next-generation sequencing; PFIC, progressive familial intrahepatic cholestasis.

* Corresponding author.
** Corresponding author.
E-mail addresses: jifang_sheng@zju.edu.cn (J. Sheng), zjushiyu@zju.edu.cn (Y. Shi).

[1] Hong Zhao and Xia Yu contributed equally to this manuscript.

https://doi.org/10.1016/j.heliyon.2023.e21760
Received 5 September 2022; Received in revised form 2 June 2023; Accepted 27 October 2023
Available online 1 November 2023



(caption on next page)

Fig. 1. Basic pathologic features of our patient in liver biopsy specimens with HE and IHC **(FB × 100)**. The lobules were marked with ballooned changes (indicated by the red arrow) with inflammatory cell infiltration (indicated by the blue arrow) under hematoxylin and eosin (H&E) staining (Fig. 1A). Portal and sinuses fibrosis were both observed under Masson's trichrome and reticulin staining (Fig. 1B). There was no obvious damage of the small bile duct (CK19) (Fig. 1D). There was ductal reaction and a large number of intermediate bile duct hyperplasia (CK7) (Fig. 1C), indicating that the patient had cholestasis. The normal expression of MDR3 in the control patient (E) and the significantly decreased expression of MDR3 in our patient (F); The normal expression of N3C2 in a control patient (G) and the absent expression of N3C2 in our patient (H). magnification: 200 × in A, B, C, D, E, F, G and H.

2. Methods

2.1. Measurement of serum amino acid

The Chemical Isotope Labeling Liquid Chromatography−Mass Spectrometry (CIL LC-MS) technology was used to target the amine/phenol metabolites as follows: 12C-dansyl chloride (Dns-Cl) was used to label individual samples and 13C-Dns-Cl was used to label the pooled sample, sample-wise normalization was conducted by measuring the total concentration of Dns-labeled amine/phenol-containing metabolites using LC-UV at 338 nm [2]. Then, each 12C-labeled individual sample was then mixed with the 13C-labeled pooled sample in 1:1 mole-ratio. The mixed labeled sample was analyzed using UltiMate 3000 UHPLC (Thermo Scientific, MA) combined with the Impact II Quadrupole Time-of-flight (QTOF) mass spectrometer (Bruker, Billerica, MA). Data processing and analysis were conducted by using IsoMS Pro (www.novamt.com). Amine/phenol metabolites are detected in the form of peak pairs. Metabolite identification was carried out using a three-tier approach reported previously [3].

2.2 Whole-exome sequencing Exome sequencing was carried out on an Illumina HiSeq X ten platform. Variants were further annotated by ANNOVAR and associated with multiple databases, such as, 1000 genome, ESP6500, dbSNP, EXAC, Inhouse (MyGenostics), HGMD, and predicted by SIFT, PolyPhen-2, MutationTaster, GERP++ [4]. The potential pathogenic mutations were selected by five steps as previously described [5]. Filtered candidate variants were confirmed by Sanger sequencing by an ABI 3730 Genetic Analyzer (Applied Biosystems,CA). The mutations of family members were confirmed by the same procedure.

3. Case presentation

A 17-year-old girl was admitted to our hospital due to abnormal liver function that occurred one month after sertraline treatment. This patient had suffered from disorientation, delirium, and mental derangement for three months. She was diagnosed as schizophrenic in a local hospital two months ago, based on the clinical symptoms, her mother's history of schizophrenia, a normal neurological examination, a brain MRI, and an electroencephalogram during her first visit.

Her mother was diagnosed with schizophrenia at the age of 28 years. The patient showed signs of growth retardation, including delayed occurrence of first menstruation (at the age of 16 years), slow breast development, and thinness (with a body mass index of 15). But no abnormalities were found either in serum gonadal and growth hormones or in the ultrasonography of uterine and appendages.

The routine laboratory tests disclosed an elevated alkaline phosphatase (ALP) of 257 IU/L, γ-glutamyl transferase (GGT) of 84 IU/L, bile acid of 28.3 μmol/L, alanine aminotransferase (ALT) of 76 IU/L, aspartate aminotransferase (AST) of 109 IU/L, whereas serum bilirubin was normally at 5.7 μmol/L. Other laboratory analyses, including a complete blood count, plasma levels of ammonia, creatinine, coagulation, urine and stool test, showed no abnormalities. The etiology-specific tests, including serology of hepatotropic virus, herpes simplex virus, cytomegalovirus, rubella and Epstein–Barr virus, antibodies for autoimmune liver diseases, and serum ceruloplasmin, did not reveal any cause of liver injury, and the liver ultrasonography showed no abnormality.

A liver biopsy was performed three days after hospitalization. The lobules were marked with ballooned changes (indicated by the red arrow) with inflammatory cell infiltration (indicated by the blue arrow) (Fig. 1A). Portal fibrosis or bile ductular reaction was observed by Masson's trichrome (Fig. 1B) and reticulin stains (Fig. 1C and D). Biliary atresia, autoimmune liver disease, copper, and iron metabolic disease were unlikely diagnoses based on the findings of the liver histology.

According to the immunohistochemical (IHC) staining without damage to the small bile duct, bile duct reaction and a large number of intermediate bile duct hyperplasia, it was indicated that the patient had long-term cholestasis. Considering the presence of cholestasis, we next performed an IHC analysis of the liver biopsy tissue, which showed a remarkably lower expression of MDR3 than control patients (Fig. 1E and F). However, there was no mutation in the *ABCB4* gene as determined by PCR and sequencing. To explore the presence of other cholestatic liver diseases, we further performed a sequencing of the specific related genes (*ABCC2, UGT1A1, SLCO1B3, SLCO1B1*), which did not reveal genetic mutations associated with cholestatic liver diseases, including glucose-6-phosphate dehydrogenase deficiency, phenylketonuria, homocystinuria, galactosemia, glutaric aciduria type I, glutaric acidemia type II, isovaleric acidemia, methylmalonic acidemia and propionic acidemia. Thus, whole-exome, sequencing by the next-generation sequencing (NGS) test using the Illumina NovaSeq 6000 sequencing system, was further performed to screen genetic diseases and revealed the *SLC25A13* homozygous mutation 852–855 del (Supplementary Fig. 1A) one month later. The girl's biological parents (both, mother and father) were also verified by sequencing using Sanger's method as a compound heterozygote for the mutations. (Supplementary Figs. 1B and 1C).

As *SLC25A13* mutation is linked to citrullinemia, we further measured changes in serum amino acids of the patient and her parents by a proteomic approach and an elevation of citrulline, homocitrulline, and homoarginine in the patient and her mother was shown (Supplementary material, Fig. 2). In addition, the IHC analysis of the liver biopsy tissue showed an absence of expression of N3C2 (*Anti-*

SLC25A13/citrin antibody, Abcam, UK). The cloning site was encoded by *SLC25A13* gene (Fig. 1G and H). Based on the genetics of biallelic pathogenic variants in *SLC25A13* and biological findings, a diagnosis of CTLN2 was established for the patient. Also, the abnormal liver enzymes (ALP: 57 IU/L, GGT: 34 IU/L, bile acid: 8.3 μmol/L, ALT: 16 IU/L, AST: 29 IU/L) and mental symptoms (disorientation, delirium, and mental derangement) returned to normal after 3-month treatment of ursodeoxycholic acid capsules (LosanPharmaGmbH, Germany) and arginine administration during the hospital stay. A supplemental diet with lipid- and protein-rich low-carbohydrates was prescribed for the girl in order to prevent the occurrence of the mental symptoms (disorientation, delirium, and mental derangement).

Ethics and informed consent: The study was performed following the Declaration of Helsinki and was approved by the Ethics Committee of the First Affiliated Hospital, Zhejiang University School of Medicine. Because the girl is under 18 years old and has immaturity, we obtained informed consent from the girl's mother (the girl's mother is her legal guardian).

4. Discussion

Citrullinemia, first reported in 1962, is two distinct well-recognized types: citrullinemia type 1 (CTLN1), caused by a genetic mutation of argininosuccinate synthase 1 (ASS1) on chromosome 9 p34.11; and CTLN2, caused by genetic mutation of SLC25A13 on chromosome 7q21.3 [6–8]. The characteristics of CTLN2 include recurring episodes of hyperammonemia and associated neuropsychiatric symptoms from childhood to adulthood, especially in people with thinness and delayed menarche (first period after the age of 15 years) [6,7,9]. The clinical mental symptoms include nocturnal delirium, confusion, restlessness, memory loss, somnipathy,

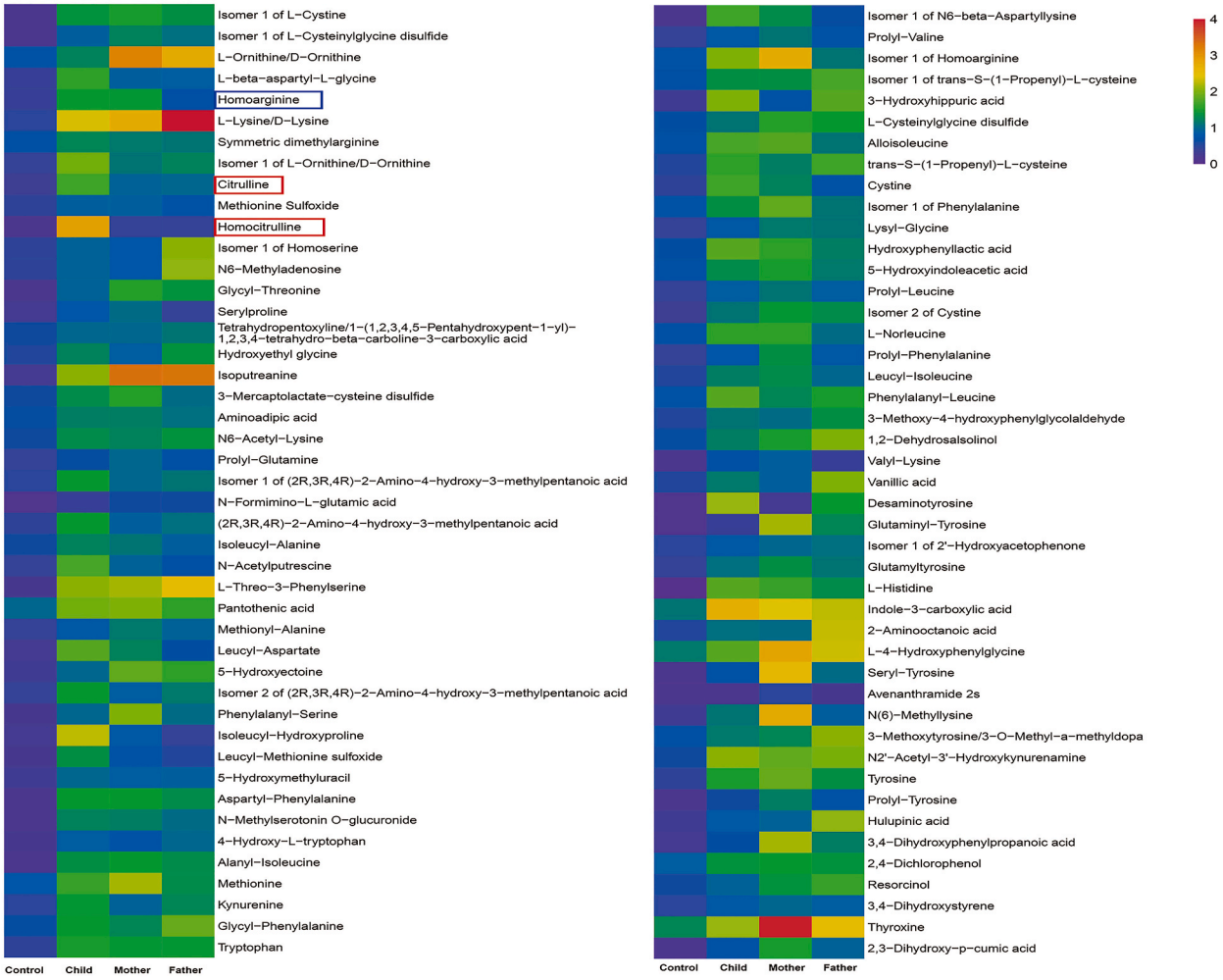


Fig. 2. Profile of serum amino acids from the patient and her parents. The levels of the citrulline, homocitrulline, and homoarginine in the girl and her mother were significantly increased (color box marking).Heat map shows the levels of the citrulline, homocitrulline, and homoarginine in the girl and her mother were significantly increased. Columns represent different groups (Control/Child/Mother/Father). Rows represent different ammo acid sequence levels. Colors represent ammo acid sequence levels, with red indicating high level of ammo acid sequence and blue indicating low level of ammo acid sequence.

abnormal behavior, and seizures [7]. Patients with CTLN2 often have specific food preferences for lipid- and protein-rich low--carbohydrates, especially during childhood [10]. The clinical signs and symptoms are often provoked suddenly by certain medications, infections, mood swings, surgery, sugar, and/or alcohol intake in older children and adults (ranging from 11 to 79 years; mean age: 34.4 ± 12.8 years), especially in thin persons (90 % BMI <20, and 40 % < 17, range: 15.6–19.1) [7,11]. In some individuals, CTLN2 also causes pancreatitis, hyperlipidemia, fatty liver, and hepatoma [12,13]. The pathologic findings were not characteristic except with fatty infiltration and mild fibrosis of the liver [7,11]. CTLN2 is confirmed to be resistant to serial clinical and biochemical changes and biallelic pathogenic variants in *SLC25A13* [7,9]. Supplementing the diet with lipid- and protein-rich low-carbohydrates is a common treatment. However, liver transplantation has been a successful therapy to date, but the best time for liver transplantation remains unclear [7,10].

In our report, the patient was thin (BMI = 15) and exhibited delayed menarche (first period at the age of 16 years). Her symptoms of mental derangement were provoked suddenly by mood swings from examination pressure in school. As in our reported case, more than 30 % of patients with CTLN2 have been misdiagnosed with psychological disorders [1,6,14]. The diagnosis of hyperammonemia for the girl at admission was not supported because of the normal level of plasma ammonia in the patient. The girl was misdiagnosed as having drug-induced liver damage caused by sertraline, but it was not suitable to perform the liver biopsy pathology because of ballooning and chronic fibrosis. Therefore, we needed to further screen other liver-damaging diseases. MDR3 in liver tissue was significantly reduced, and MDR3 glycoprotein, encoded by the *ABCB4* gene, is mainly expressed in the capillary bile duct membrane of hepatocytes [15]. The functional product of the *ABCB4* gene, phosphatidylcholine transferase, regulates the outward movement of phospholipids from the bilayer and is a phospholipid transporter[16,17]. Deficiency of phosphatidylcholine transferase leads to cholesterol crystallization, increased formation of bile stones and obstruction of the small biliary tract. MDR3 mutations are common in progressive family intrahepatic cholestasis-3 (PFIC3), and the following diseases such as intrahepatic cholestasis of pregnancy, drug-induced cholestasis, transient cholestasis of the newborn, gallstones, biliary cirrhosis are also observed [15]. The clinical pathological changes of PFIC3 liver tissue include bile duct hyperplasia and fibrosis. Our patient was without the bile duct hyperplasia and mutations in *ABCB4*. Therefore, we ruled out PFIC3.

As the patient showed clinical characteristics of thinness, delayed menarche, mental symptoms with delirium and mental derangement, we considered whether there were other inherited disorder(s). We performed whole-exome sequencing to screen other genetic diseases and detected mutation in *SLC25A13* 852–855 del in the patient's peripheral blood. We also conducted whole-exome sequencing for her parents and found that both, her biological father and mother carried the *SLC25A13* mutation 852–855 del. We conducted a Sanger's sequencing analysis and the results showed that the patient was homozygous for the mutations, and her parents were a compound heterozygote for the mutations. Finally, we measured changes of serum amino acids of the patient and her parents by a CIL LC-MS approach and found an elevation in serum citrulline, homocitrulline, homoarginine in the patient and her parents. By the mental symptoms of delirium and mental derangement, thinness, delayed menarche, along with *SLC25A13* mutation 852–855 del, levels of citrulline, homocitrulline, and homoarginine and the hepatic N3C2 expression, we confirmed the diagnosis of CTLN2.

5. Conclusion

Here we reported a rare case of CTLN2 combined with down-regulated expression of MDR3 in the liver but not with PFIC3. However, whether MDR3 contributes to the pathogenesis of CTLN2 or is the consequence of CTLN2 needs further investigation. Clinicians should pay particular attention to the possibility of atypical CTNS when older children or adults with thinness suffer from mental disorders due to drug, infection, mood fluctuation and other stimulating factors even if there is no typical abnormal eating behavior and hyperammonemia. Meanwhile, clinicians need to further rule out the possibility of CTLN2 if MDR3 decreases in adolescent patients with mental disorders and abnormal liver function.

Author contribution statement

All authors listed have significantly contributed to the investigation, development and writing of this article.

Funding

The study was funded by the National Key Research and Development Program of China (No. 2021YFC2301800, No. 2022YFC2304501), the Fundamental Research Funds for the Central Universities (2021FZZX001-41, 226-2023-00127) and Medical Health Science and Technology Project of Zhejiang Provincial Health Commission (No. 2022RC141).

Data availability statement

No data was used for the research described in the article.

Additional information

No additional information is available for this paper.

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2023.139645.

References

[1] T. Kakiuchi, et al., Chronic pancreatitis and pancreatic pseudocyst with adult-onset type II citrullinemia, Clin J Gastroenterol 13 (1) (2020) 97–101.
[2] Y. Wu, L. Li, Determination of total concentration of chemically labeled metabolites as a means of metabolome sample normalization and sample loading optimization in mass spectrometry-based metabolomics, Anal. Chem. 84 (24) (2012) 10723–10731.
[3] S. Zhao, et al., Metabolomic coverage of chemical-group-submetabolome analysis: group classification and four-channel chemical isotope labeling LC-MS, Anal. Chem. 91 (18) (2019) 12108–12115.
[4] K. Wang, M. Li, H. Hakonarson, ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data, Nucleic Acids Res. 38 (16) (2010) e164.
[5] L. Wu, et al., Case-only exome sequencing and complex disease susceptibility gene discovery: study design considerations, J. Med. Genet. 52 (1) (2015) 10–16.
[6] S. Bijarnia-Mahay, et al., Citrin deficiency: a treatable cause of acute psychosis in adults, Neurol. India 63 (2) (2015) 220–222.
[7] K. Hayasaka, C. Numakura, Adult-onset type II citrullinemia: current insights and therapy, Appl. Clin. Genet. 11 (2018) 163–170.
[8] N. Boy, et al., Recommendations for diagnosing and managing individuals with glutaric aciduria type 1: third revision, J. Inherit. Metab. Dis. (2022) 1–38.
[9] Y. Okano, et al., Current treatment for citrin deficiency during NICCD and adaptation/compensation stages: strategy to prevent CTLN2, Mol. Genet. Metabol. 127 (3) (2019) 175–183.
[10] K. Hayasaka, Metabolic basis and treatment of citrin deficiency, J. Inherit. Metab. Dis. 44 (1) (2021) 110–117.
[11] M.T. Nguyen, et al., The mutation spectrum of SLC25A13 gene in citrin deficiency: identification of novel mutations in Vietnamese pediatric cohort with neonatal intrahepatic cholestasis, J. Hum. Genet. 68 (5) (2023) 305–312.
[12] M. Komatsu, et al., Citrin deficiency as a cause of chronic liver disorder mimicking non-alcoholic fatty liver disease, J. Hepatol. 49 (5) (2008) 810–820.
[13] S. Tavoulari, et al., Pathogenic variants of the mitochondrial aspartate/glutamate carrier causing citrin deficiency, Trends Endocrinol. Metabol. 33 (8) (2022) 539–553.
[14] T. Saheki, K. Kobayashi, Mitochondrial aspartate glutamate carrier (citrin) deficiency as the cause of adult-onset type II citrullinemia (CTLN2) and idiopathic neonatal hepatitis (NICCD), J. Hum. Genet. 47 (7) (2002) 333–341.
[15] A.F. Stättermayer, et al., Variants in ABCB4 (MDR3) across the spectrum of cholestatic liver diseases in adults, J. Hepatol. 73 (3) (2020) 651–663.
[16] G.M. Hirschfield, et al., The genetics of complex cholestatic disorders, Gastroenterology 144 (7) (2013) 1357–1374.
[17] D. Degiorgio, et al., ABCB4 mutations in adult patients with cholestatic liver disease: impact and phenotypic expression, J. Gastroenterol. 51 (3) (2016) 271–280.