Citrullinemia type I is associated with a novel splicing variant, c.773 + 4A > C, in ASS1: a case report and literature review

Yiming Lin1, Hongzhi Gao2, Bin Lu3, Shuang Zhou2, Tianwen Zheng4, Weihua Lin1, Lin Zhu3, Mengyi Jiang3* and Qingliu Fu1*

Abstract

Background: Citrullinemia type I (CTLN1) is a rare autosomal recessive disorder of the urea cycle caused by a deficiency in the argininosuccinate synthetase (ASS1) enzyme due to mutations in the ASS1 gene. Only a few Chinese patients with CTLN1 have been reported, and ASS1 gene mutations have been identified sporadically in China.

Case presentation: A Chinese family with one member affected with mild CTLN1 was enrolled. Targeted exome sequencing was performed on the proband, and Sanger sequencing was used to validate the detected mutation. We also reviewed the genetic and clinical characteristics of CTLN1 in Chinese patients that have been published to date. Newborn screening showed remarkably increased concentrations of citrulline with elevated ratios of citrulline/arginine and citrulline/phenylalanine, and the patient presented with a speech delay at age three. The urinary organic acid profiles were normal. A novel homozygous splicing variant c.773 + 4A > C in the ASS1 gene was identified in the proband, and it was predicted to affect splicing by in silico analysis. To date, only nine Chinese patients with CTLN1 have been reported, with a total of 15 ASS1 mutations identified and no high frequency or hot spot mutations found; the mutation spectrum of Chinese patients with CTLN1 was heterogeneous.

Conclusions: We described a mild Chinese CTLN1 case with a novel homozygous splicing variant c.773 + 4A > C and reviewed previous genotypes and phenotypes in Chinese patients with CTLN1. Thus, our findings contribute to understanding the molecular genetic background and clinical phenotype of CTLN1 in this population.

Keywords: Citrullinemia type I, ASS1, Novel variant, Mutation spectrum

Background

Citrullinemia type I (CTLN1, MIM# 215700) is a rare autosomal recessive disorder of the urea cycle caused by a deficiency of the argininosuccinate synthetase (ASS, EC 6.3.4.5) enzyme due to mutations in the ASS1 gene [1]. CTLN1 encompasses a spectrum of varying clinical phenotypes. Patients that present with fatal neonatal hyperammonemia are said to have classical citrullinemia, patients with late onset and/or mild symptoms are said to have mild citrullinemia, and a considerable number of asymptomatic individuals detected by expanded newborn screening (NBS) have only a biochemical phenotype [2, 3].

Biochemically, CTLN1 with elevated citrulline concentrations can be detected by NBS. Nonetheless, increased levels of citrulline can also be found in other inherited metabolic disorders such as citrullinemia type II, argininosuccinate lyase deficiency, and pyruvate carboxylase deficiency [4, 5]. Therefore, definitive diagnosis of CTLN1 mainly relies on an ASS enzyme assay and identification of ASS1 gene mutations. However, determination of enzyme activity in liver tissue requires an invasive procedure, and direct measurement of ASS activity or indirect measurement using a C14 incorporation assay in fibroblasts have not yet been evaluated in patients with mild CTLN1 [6]. Therefore, molecular genetic testing is paramount,
not only for clinical diagnosis but also for future prenatal testing and family member screening [7]. The ASS1 gene is located on chromosome 9q34.1 and contains 16 exons, with the translation start codon in exon 3, encoding 412 amino acids [2, 8]. To date, at least 137 mutations that cause CTLN1 have been reported in the ASS1 gene [9]. However, only a few Chinese patients with CTLN1 have been reported, and ASS1 gene mutations have been identified only sporadically in China [10–17]. In this study, we present biochemical, clinical, and genetic characteristics of a new Chinese patient with CTLN1. In addition, we reviewed previous genotypes and phenotypes of Chinese patients with CTLN1, to help better understand the genetic background of this disease in the Chinese population.

**Case presentation**

**Case report**

This study was approved by the Ethical Committee of Quanzhou Maternity and Children’s Hospital. Written informed consent was obtained from the parents of the patient, who agreed to join this study, with the intent of using the resulting medical data for scientific research and publication. The proband was born at a gestational age of 38 weeks and 1 day via caesarean section; his weight at birth was 3700 g. He was the third born child of consanguineous parents of Chinese descent. There was no significant family history of inherited metabolic diseases. NBS via ACQUITY TQD tandem mass spectrometry (MS/MS) (Waters, Milford, MA, USA) analysis on dried blood spots (DBS) was performed on the proband after birth. The initial NBS results showed an elevated citrulline concentration with increased ratios of citrulline/arginine and citrulline/phenylalanine. The hypercitrullinemia and increased citrulline/phenylalanine ratios persisted, and the concentrations of citrulline fluctuated between 71.82–120.99 μmol/L during follow up, while the citrulline/arginine ratios were persistent within the reference range (Table 1). Subsequently, urinary organic acid analysis by gas chromatography-mass spectrometry (7890B/5977A, Agilent Technologies, Santa Clara, CA, USA) and auxiliary biochemical tests were carried out. Increased levels of orotic acid were not observed in urinary organic acid analysis. The blood ammonia levels were slightly elevated at 1 month and 11 months, which may have been transitional, and returned to the normal reference range later. The patient exhibited normal growth and development during follow up, but a speech delay was noted at 3 years of age.

**Genetic analysis**

Genomic DNA was extracted from whole blood of the proband and his family members using Qiagen Blood DNA mini kit (Qiagen*, Hilden, Germany). The DNA of the proband was used for NGS. Targeted enrichment of target region sequences was performed by multiple probe hybridization using metabolic abnormality of common amino acids capture oligo, which was designed by Genuine Diagnostics (Zhejiang, China), and included 40 genes (ABCD4, ACSF3, ARG1, AMT, ASS1, ASL, BCAT1, BCAT2, BCKDHA, BCKDHB, CBS, CPS1, CTH, D2HGDH, ETHE1, GCH1, GCSH, GLDC, GPHN, GRHPR, HGD, HPD, L2HGDH, LMBRD1, MOCS1, MOCS2, MTR, OAT, OGDH, OPA3, PAH, PCBD1, PRODH, PTS, QDPR, SERAC1, SLC25A13, SLC25A15, SPR, and SUOX). The sequencing libraries were quantified using the Illumina DNA Standads and Primer Premix Kit (Kapa Biosystems, Boston, MA, USA), and then massively parallel sequenced using the Illumina HiSeq 2500 platform (Illumina, San Diego, CA, USA). After sequencing and filtering out low-quality reads, high-quality reads were compared to the human genome reference sequence (GRCh37.p13, hg19). The quality control data are listed in Additional file 1: Table S1. Variants were called using the GATK software. Next, candidate variants were further confirmed by Sanger sequencing.

| Table 1 Detection results of MS/MS and biochemical testing in the patient |
|-----------------------------------------------|
| **Testing time** | **MS/MS analysis in dried blood spots** | **Biochemical testing** |
| | MS/MS analysis in dried blood spots | Arginine (1–50 μmol/L) | Blood ammonia (10–70 μmol/L) | Total bilirubin (5.1–19 μmol/L) | Direct bilirubin (0–68 μmol/L) | AFP (ng/ml) |
| | Citrulline (5.5–30 μmol/L) | Arginine (0.35–15) | Citrulline/Phenylalanine (0.12–0.83) | | | |
| 2015.9.21a | 90.05 | 46.18 | 1.17 | 1.95 | | |
| 2015.10.9 | 89.87 | 6.58 | 2.26 | 13.65 | | |
| 2015.10.19 | 88.14 | 4.72 | 2.82 | 18.69 | 70 | 142 | 15.6 | 6508.14 |
| 2016.8.26 | 118.24 | 3.9 | 2.56 | 30.3 | 49 | 4.2 | 0.9 | 2.37 |
| 2016.10.10 | 87.66 | 4.8 | 1.9 | 18.25 | 33 | | |
| 2016.11.8 | 81.25 | 3.52 | 2.25 | 23.05 | | | |
| 2017.8.4 | 71.82 | 5.19 | 1.37 | 13.85 | 29 | 6.2 | 1.6 | <1.3 |
| 2018.9.7 | 120.99 | 3.4 | 1.45 | 35.63 | 45 | 3.2 | 2.1 | |

*aNewborn screening results*
Sequencing was performed on ABI 3500xL (Applied Biosystems, Foster City, CA, USA), and the results were analysed using DNASTAR software. The primers used for polymerase chain reaction (PCR) and Sanger sequencing are listed in Additional file 2: Table S2.

The identified variants were annotated to public databases, such as the Human Gene Mutation Database (HGMD, http://www.hgmd.cf.ac.uk/ac/index.php), ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/), the Leiden Open Variation Database (LOVD, http://www.lovd.nl/3.0/home), dbSNP (https://www.ncbi.nlm.nih.gov/projects/SNP/), the 1000 Genome Project (http://www.1000genomes.org/), and the ExAC consortium (http://exac.broadinstitute.org/). In addition, the variant was further assessed for possible pathogenicity using HSF (http://www.umd.be/HSF3/), MutationTaster (http://www.mutationtaster.org/), and regSNP-intron (http://clark.compbio.iupui.edu/regsnp_intron_web/). To exclude any polymorphisms, 100 healthy controls underwent Sanger sequencing of the ASS1 exon 11. Pathogenicity analysis of the variant was performed to comply with the American College of Medical Genetics and Genomics (ACMG) guidelines [18].

**Mutation analysis**

We identified a homozygous ASS1 gene variant c.773 + 4A > C in the proband, which was inherited from the parents. In addition, no ASS1 mutations were detected in the proband’s older brother via family genetic screening (Fig. 1). The c.773 + 4A > C variant is located in intron 11 of the ASS1 gene, with the 5th nucleotide changed from adenine to cytosine. This variant has not been previously reported in the literature and was not detected in 100 healthy controls. It was found only in a heterozygous state in the East Asian population with an allele frequency of 1.387e-03 in the ExAC database, and the allele frequency in the overall population was 9.892e-05, indicating that individuals in the population with this heterozygous variation were just carriers. It was absent from the dbSNP and 1000 Genomes databases, and was also absent from disease databases such as HGMD, ClinVar, and LOVD. Furthermore, in silico analysis by HSF, MutationTaster, and regSNP-intron all suggested that the variant most likely affects splicing (Table 2). According to the ACMG guidelines, the c.773 + 4A > C variant was classified as variant of uncertain clinical significance.

In a review of Chinese CTLN1 cases published to date, we found that only ten Chinese patients with CTLN1 received genetic testing. The mutations in the ASS1 gene from our study and from previously reported Chinese patients are summarized in Table 3. In total, 15 ASS1 mutations have been identified in Chinese patients to date. Among them, 11 are missense mutations, three are splice mutations, and one is a deletion mutation. 50% of the mutant alleles are clustered in exons 7, 13, and 14. Four mutations (p.Arg127Gln, p.Arg265Cys, p.Gly324Ser, and p.Gly390Arg) proved to be disease-causing. Four patients had homozygous or compound heterozygous splicing/frameshift mutations, resulting in neonatal onsets and with poor outcomes, and thus were classified as having neonatal CTLN1. Three patients developed symptoms after the neonatal period with moderate

![Fig. 1](#)
outcomes and were classified as having late-onset CTLN1. The other three patients without obvious clinical symptoms and with good prognosis were classified as having a mild form of CTLN1.

Discussion and conclusions

In this study, we described a Chinese family with one child having a mild form of CTLN1. The patient had an elevated citrulline level, which was detected by MS-based NBS. No abnormalities were found in urinary organic acid analysis. The patient had normal growth and development during follow up, and the main clinical manifestation was a speech delay. A homozygous ASS1 gene variant c.773 + 4A > C was identified in the patient. This variant has not been previously reported in the literature, and was predicted through bioinformatics analysis to cause a broken WT donor site, and to affect splicing. Furthermore, it is likely to truncate the monomers, impairing the synthetase-binding domain. Similar variants leading to protein truncation have been reported previously [13, 19, 20]. Therefore, we believe that the variant c.773 + 4A > C is associated with the pathogenesis of CTLN1. However, further functional studies are needed to validate the pathogenicity of this variant.

After combining our findings with those from previously reported Chinese patients with CTLN1, we performed mutation spectrum analysis. The results showed that the mutation spectrum of Chinese patients with CTLN1 was heterogeneous, with no high frequency or hot spot mutations. In comparison, many mutations have been documented at high frequencies in various other populations and the mutation spectra differ among different ethnic groups. p.Gly390Arg is by far the most common mutation and is widely distributed around the world [9]. It has been proposed that a CpG dinucleotide in the coding region could be the cause of recurring mutations in this region [21], and therefore the recurrent nature of this variant could be explained by its location in a CpG dinucleotide. For instance, the allelic frequencies of p.Gly390Arg in Indian and Turkish patients are 42.7 and 50%, respectively [22, 23]. Likewise, 17.3% of German patients carry at least one p.Gly390Arg allele. p.Gly390Arg is also regarded as a recurrent mutation in a limited geographic area of Argentina [24]. Similarly, the p.Val263Met variant seems to be common in the Pacific Island population [25]. The most frequent mutations in Korean patients are c.421-2A > G, p.Gly324Ser, and c.1128-6_1188dup67, and in Japanese patients the predominant mutations are c.421-2A > G, p. Arg265His, and p.Arg304Trp [26–30]. However, it is surprising that the particularly frequent mutation c.421-2A > G, reported in Korean and Japanese patients, has not yet been detected in Chinese patients.

To date, some mutations have been elucidated with clear genotype-phenotype correlations [9], while most Chinese patients were in a compound heterozygous state, rendering it more difficult to investigate the relationship between genotype and phenotype. Though p.Arg127Gln was proven to be inactive in previous studies, in the neonatal CTLN1 group, a patient (no.2) in our study homozygous for p.Arg127Gln died shortly after birth, confirming previous reports and highlighting the severity of this mutation [31]. Previous enzyme studies revealed that both p.Arg265Cys and p.Gly324Ser yielded < 2% of ASS wild-type activity, and both are known to be associated with a severe phenotype [32]. Supporting these findings, a patient (no.4) who was compound heterozygous for p.Arg265Cys with a splicing mutation presented with early onset neonatal citrullinemia; of note is that an older sibling in this family progressed to severe encephalopathy and died 4 days after birth. A patient (no.10) homozygous for p.Gly324Ser presented with acute hyperammonemia and encephalopathy, again confirming previous studies. p.Arg363Trp was reported to be associated with neonatal CTLN1; consistent with this, a patient (no.9) with p.Arg363Trp in combination with a frameshift mutation died shortly after birth [9]. Regarding mild/late-onset form CTLN1, a patient (no.1) homozygous for c.773 + 4A > C presented no clinical symptoms until 3 years of age, indicating that this variant may be related to mild symptoms. The remaining patients are all compound heterozygotes, and it is likely that the mutations p.Ser18Leu, p.Val141Gly, p.Pro144Arg, and p.Cys337Arg may allow for some residual ASS function, because the second allelic mutations in these patients are known to drastically impair ASS activity [9, 13, 32].

In summary, we described one mild Chinese CTLN1 case with a novel splicing variant c.773 + 4A > C. We also reviewed previous genotypes and phenotypes of Chinese patients with CTLN1, hereby adding to our understanding of the molecular genetic background and clinical phenotype of CTLN1 in this population. The mutation spectrum of Chinese patients with CTLN1 was heterogeneous. More functional research is needed to elucidate the genotype-phenotype correlation in patients with CTLN1.
| Patient no. | Gender | Age of onset | Clinical presentation | Citrulline levels (μmol/L) | Blood ammonia (μmol/L) | Mutation 1 Location | c.DNA | Protein | Mutation 2 Location | c.DNA | Protein | Outcome | Ref. |
|------------|--------|-------------|----------------------|---------------------------|------------------------|----------------------|--------|---------|----------------------|--------|---------|---------|-----|
| 1          | Male   | 3 y         | Mild form            | 9005                      | 70                     | Intron 11            | c.773 + 4A > C     |         |         | Intron 11            | c.773 + 4A > C     |         | Well    | This study |
| 2          | n.a.   | 2 d         | Neonatal form        | 487.69                    | 286                    | Exon 6               | c.380G > A         | p.Arg127Gln | Exon 6               | c.380G > A         | p.Arg127Gln | Died    | [12] |
| 3          | Male   | n.p.        | Mild form            | 961.42                    | 91                     | Intron 4             | c.174 + 1G > A     | p.Arg127Gln | Exon 7               | c.422 T > C         | p.Val141Gly | Well    | [13] |
| 4          | Female | 4 d         | Neonatal form        | 1085.41                   | 231                    | Intron 11            | c.773 + 1G > A     | p.Arg127Gln | Exon 12               | c.793C > T          | p.Arg265Cys | Moderate | [13] |
| 5          | Female | 3 m         | Late-onset form      | n.a.                      | 311                    | Exon 7               | c.431C > G         | p.Pro144Arg | Exon 14               | c.1087C > T         | p.Arg363Trp | Moderate | [17] |
| 6          | Male   | n.p.        | Mild form            | 111.21                    | 17                     | Exon 3               | c.53C > T          | p.Ser18Leu | Exon 15               | c.1168G > A         | p.Gly390Arg | Well    | [16] |
| 7          | Female | 1 y, 3 m    | Late-onset form      | 928.77                    | 160                    | Exon 13              | c.847G > A         | p.Glu283lys | Exon 14               | c.1009T > C         | p.Cys337Arg | Moderate | [10] |
| 8          | Female | 1 y, 5 m    | Late-onset form      | 653                       | 126                    | Exon 5               | c.236C > T         | p.Ser79Phe | Exon 7               | c.431C > G         | p.Pro144Arg | n.a.    | [14] |
| 9          | Female | 2 d         | Late-onset form      | 15777                     | 670                    | Exon 13              | c.951delT          | p.F317fsX375 | Exon 14               | c.1087C > T         | p.Arg363Trp | Died    | [11] |
| 10         | Male   | 2 d         | Neonatal form        | 2513.5                    | n.a.                   | Exon 13              | c.970G > A         | p.Gly324Ser | Exon 13               | c.970G > A         | p.Gly324Ser | n.a.    | [15] |

*Reference range: 5.5–30 μmol/L; **Reference range: 10–47 μmol/L; *novel mutations are in bold character.
n.p. not present, n.a. not available, d day, y year
Additional files

**Additional file 1**: Table S1. Summary of targeted gene sequencing data in the proband. (DOCX 13 kb)

**Additional file 2**: Table S2. Primers used for PCR and Sanger sequencing of exon 11 of ASS1. (DOCX 14 kb)

Abbreviations

ACMG: American College of Medical Genetics Association of Clinical Genetics; CTN1: Citrullinemia type I; MAF: Minor allele frequency; MS/ MS: Tandem mass spectrometry; NBS: Newborn screening; NGS: Next-generation sequencing; PCR: Polymerase chain reaction

Acknowledgements

We thank all the participants for their co-operation.

Authors’ contributions

YL designed the study, performed experimental work, wrote the paper, and conducted the literature review. HG and SZ helped with data collection and interpretation of the data. BL, LZ, and MJ carried out the genetic tests, mutation analysis, and paper editing. TZ and WL followed the patients and collected the clinical data. QF conceived the study design. All authors read and approved the final manuscript.

Funding

This study was supported by the Quanzhou Municipal Science and Technology Plan Project (Grant No. 2018Z160 and 2018N085S). The role of the funding body was to sponsor the two-generation pedigree analysis, the NGS, and the other tests we conducted for this case.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author upon reasonable request.

Ethics approval and consent to participate

This study was approved by the ethics committee of The Maternal and University, Quanzhou 362000, Fujian Province, China. 3Genuine Diagnostics Company Limited, 859 Shixiang West Road, Hangzhou 310007, Zhejiang Province, China. 4Department of Pediatrics, Quanzhou Maternity and Children's Hospital, 700 Fengze Street, Quanzhou 362000, Fujian Province, China.

Consent for publication

We confirm that this family has signed written informed consent for publication of their own and their children’s research. We confirm that this family has signed written informed consent for themselves and their children to take part in this study.

Competing interests

The authors declare that they have no competing interests.

Author details

1Neonatal Disease Screening Center, Quanzhou Maternity and Children's Hospital, 700 Fengze Street, Quanzhou 362000, Fujian Province, China. 2Department of Central Laboratory, 2nd Affiliated Hospital of Fujian Medical University, Quanzhou 362000, Fujian Province, China. 3Genuine Diagnostics Company Limited, 859 Shixiang West Road, Hangzhou 310007, Zhejiang Province, China. 4Department of Pediatrics, Quanzhou Maternity and Children's Hospital, 700 Fengze Street, Quanzhou 362000, Fujian Province, China.

Received: 1 March 2019 Accepted: 27 May 2019

Published online: 17 June 2019

References

1.  Beaudet AL, O'Brien WE, Bock HG, Freytag SQ, Su TS. The human argininosuccinate synthetase locus and citrullinemia. Adv Hum Genet. 1986;15:161–96.291–162.

2.  Haberle J, Pauli S, Linnebank M, Kleijer WJ, Bakker HD, Wanders RJ, Harms E, Koch HG. Structure of the human argininosuccinate synthetase gene and an improved system for molecular diagnostics in patients with classical and mild citrullinemia. Hum Genet. 2002;110(4):327–33.

3.  Haberle J, Pauli S, Schmidt E, Schulze-Elfing B, Berning C, Koch HG. Mild citrullinemia in Caucasians is an allelic variant of argininosuccinate synthetase deficiency (citrullinemia type I). Mol Genet Metab. 2003;80(3):302–6.

4.  Haberle J, Boddaert N, Burlina A, Chakrappari A, Dixon M, Huemer M, Karall D, Martellini D, Crespo PS, Santer R, et al. Suggested guidelines for the diagnosis and management of urea cycle disorders. Orphanet J Rare Dis. 2011;6:32.

5.  Sander J, Janzen N, Sander S, Steuerwald U, Das AM, Scholl S, Trefz FK, Koch HG, Haberle J, Korall H, et al. Neonatal screening for citrullinemia. Eur J Pediatr. 2003;162(6):417–20.

6.  Engel K, Hohne W, Haberle J. Mutations and polymorphisms in the human argininosuccinate synthetase (ASS1) gene. Hum Mutat. 2009;30(3):300–7.

7.  Miller MJ, Soler-Alfonso CR, Grund JE, Fang P, Sun Q, Eisele SH, Sutton VR. Improved standards for prenatal diagnosis of citrullinemia. Mol Genet Metab. 2014;113(1):205–9.

8.  Freytag SQ, Beaudet AL, Bock HG, O'Brien WE. Molecular structure of the human argininosuccinate synthetase gene: occurrence of alternative mRNA splicing. Mol Cell Biol. 1984;10(10):1978–84.

9.  Díez-Fernández C, Rufenacht V, Haberle J. Mutations in the human Argininosuccinate Synthetase (ASS1) gene, impact on patients, common changes, and structural considerations. Hum Mutat. 2017;38(5):471–84.

10. Wu TF, Liu YP, Li XY, Wang Q, Song JQ, Yang YL. Prenatal diagnosis of citrullinemia type 1: a Chinese family with a novel mutation of the ASS1 gene. Brain Dev. 2014;36(3):264–7.

11. Xie B, Chen R, Wang J, Luo J, Li W, Chen S. ASS1 gene mutation in a neonate with citrullinemia I type. Chinese J Pediatr. 2014;52(10):788–91.

12. Sun J, Shen Y, Yan C, Gong X. [Identification of a homozygous ASS1 mutation in a child with citrullinemia type with high-melting curve method]. Chinese J Med Genet. 2018;35(3):429–33.

13. Kimani JK, Wei T, Chol K, Li Y, Yu P, Ye S, Huang X, Qi M. Functional analysis of novel splicing and missense mutations identified in the ASS1 gene in classical citrullinemia patients. Clin Chim Acta. 2015;438:323–9.

14. Wen P, Chen Z, Wang G, Liu X, Chen L, Chen S, Wan L, Cui D, Shang Y, Li C. [Genetic analysis of ASS1, ASP and SLC25A13 in citrullinemia patients]. Chinese J Med Genet. 2014;31(3):268–71.

15. Hu P, Zhou XY, Ma DY, Sun Y, Zhang XJ, Han SP, Yu ZB, Jiang T, Chen YL, Xu Z. [ASS1 mutation leading to citrullinemia I in a Chinese Han family]. Chinese J Med Genet. 2011;28(6):630–3.

16. Sun Y, Ma DY, Yang GJ, Wang YY, Yang B, Jiang T. [Clinical features and ASS1 gene mutation analysis of a case of citrullinemia]. Chinese J Med Genet. 2015;32(5):745–7.

17. Cheng AL, Han LS, Feng Y, Jin B. [The magnetic resonance imaging features and clinical manifestations of citrullinemia]. Journal of Clin Pediatr. 2015;33(5):466–9.

18. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology, Genet Med. 2015;17(5):405–24.

19. Laine CM, Chung BD, Susic M, Prescott T, Semler O, Fikerstrand T, D'Eufemia P, Castori M, Pekkinen M, Sochett E, et al. Novel mutations affecting LRPS splicing in patients with osteopetrosis-pseudoglioma syndrome (OPPG). Eur J Hum Genet. 2011;19(8):785–81.

20. Ohnani R, Inazu A, Noj Y, Wakasugi T, Miwa K, Tada H, Kawashiri MA, Noguchi T, Nohara A, Kobayashi Y, et al. Novel mutations of cholesterol ester transfer protein (CETP) gene in Japanese hyperalphalipoproteinemic subjects. Clin Chim Acta. 2012;413(5–6):337–43.

21. Morgan GE, Figueiredo MS, Winship PR, Baker R, Bolton-Magens PH, Brownlee GG. The high frequency of the -6G-->a factor IX promoter CpG dinucleotide. Br J Haematol. 1995;89(3):672–8.

22. Kose E, Unal O, Bulbul S, Gunduz M, Haberle J, Arslan N. Identification of three novel mutations in fourteen patients with citrullinemia type 1. Clin Biochem. 2017;50(12):686–9.

23. Bijnia-Mahay S, Haberle J, Jalan AB, Pur I, Kohli S, Kudalkar K, Rufenacht V, Gupta D, Maurya D, Verma J, et al. Urea cycle disorders in India: clinical course, biochemical and genetic investigations, and prenatal testing. Orphanet J Rare Dis. 2018;13(1):174.

24. Larovere LE, Angaroni CJ, Antonozzi SL, Bezard MB, Shimohama M, de Kremer RD. Citrullinemia type I, classical variant. Identification of ASS-p>G390R (c.1168G>a) mutation in families of a limited geographic area of Argentina: a possible population cluster. Clin Biochem. 2009;42(10–11):1166–8.
25. Glamuzina E, Marquis-Nicholson R, Knoll D, Love DR, Wilson C. Citrullinaemia type I: a common mutation in the Pacific Island population. J Paediatr Child Health. 2011;47(5):262–5.
26. Woo HI, Kim CS, Lee SY, Kim JW, Song J, Jin DK, Park WS, Lee DH, Lee YW, Park HD. Mutation spectrum of the ASS1 gene in Korean patients with citrullinemia type I. Clin Biochem. 2013;46(3):209–13.
27. Lee BH, Kim YM, Heo SH, Kim GH, Choi IH, Lee BS, Kim EA, Kim KS, Jhang WK, Park SJ, et al. High prevalence of neonatal presentation in Korean patients with citrullinemia type 1, and their shared mutations. Mol Genet Metab. 2013;108(1):18–24.
28. Gao HZ, Kobayashi K, Tabata A, Tsuge H, Iijima M, Yasuda T, Kalkanoglu HS, Dursun A, Tokatli A, Coskun T, et al. Identification of 16 novel mutations in the argininosuccinate synthetase gene and genotype-phenotype correlation in 38 classical citrullinemia patients. Hum Mutat. 2003;22(1):24–34.
29. Hong KM, Shin CH, Choi YB, Song WK, Lee SD, Rhee KI, Jang P, Pak GS, Kim JK, Paik MK, et al. Mutation analysis of Korean patients with citrullinemia. Mol Cells. 2000;10(4):465–8.
30. Kobayashi K, Kakinoki H, Fukushige T, Shaheen N, Terazono H, Saheki T. Nature and frequency of mutations in the argininosuccinate synthetase gene that cause classical citrullinemia. Hum Genet. 1995;96(4):454–63.
31. Diez-Fernandez C, Wellauer O, Gemperle C, Rufenacht V, Fingerhut R, Haberle J. Kinetic mutations in argininosuccinate synthetase deficiency: characterisation and in vitro correction by substrate supplementation. J Med Genet. 2016;53(10):710–9.
32. Berning C, Bieger I, Pauli S, Vermeulen T, Vogl T, Rummel T, Hohne W, Koch HG, Rolinski B, Gempel K, et al. Investigation of citrullinemia type I variants by in vitro expression studies. Hum Mutat. 2008;29(10):1222–7.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.