

p.Val452Ile mutation of the SLC25A13 gene in a Turkish patient with citrin deficiency

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Citrin deficiency is an autosomal recessive metabolic disorder, which is caused by pathogenic mutations in the SLC25A13 gene on chromosome 7q21.3, as the causative gene that encodes the liver type aspartate/glutamate carrier isoform 2 (AGC2). One of the main clinical presentations is neonatal intrahepatic cholestatic hepatitis caused by citrin deficiency.

We report a Turkish child presented with prolonged neonatal jaundice associated with elevated plasma citrulline and galactosuria. NICCD was suspected at this point and mutation study of SLC25A13 showed that she was homozygous for the missense NM_014251.2:c.1354G>A (NP_055066.1:p.Val452Ile) (dbSNP: rs143877538) mutation. Dramatic response was observed to the dietary treatment with medium-chain triglycerides containing formula, ursodeoxycholic acid and fat-soluble vitamin supplementation.

The minor allele frequency of this variant was given as nearly as 0.01 in the South Asian population; it seems like a disease causing variant. This is the first report of this variant in the Turkish and European population.

Key words: citrin deficiency, prolonged neonatal jaundice, novel mutation.

Citrin deficiency, caused by mutations in the SLC25A13 gene on chromosome 7q21.3, is an autosomal recessive disease that was first discovered in Japan and thereafter identified worldwide¹. Deficiency of citrin, which is the liver-specific isoform of the mitochondrial aspartate/glutamate carrier (AGC2), has three major age dependent clinical phenotypes: Neonatal Intrahepatic Cholestasis caused by Citrin Deficiency (NICCD, OMIM#605814) in infants, adult-onset citrullinemia type II (CTLN2, OMIM#603471) in adolescents and adults, and Failure to Thrive and Dyslipidemia caused by Citrin Deficiency (FTTDCD) in between these age groups². NICCD patients present in the first few months of life with milder symptoms characterized by intrahepatic cholestasis, diffuse fatty liver, parenchymal cellular infiltration associated with hepatic fibrosis, hypoalbuminemia, coagulopathy, liver

dysfunction with or without hypoglycemia, galactosuria, and increased α -fetoprotein, multiple aminoacidemia including elevated citrulline, arginine, threonine, methionine, phenylalanine, and tyrosine concentrations and threonine/serine ratio³. In this report we describe a new Turkish case of NICCD with a SLC25A13 mutation which is very frequent in South Asian population like a polymorphism to emphasize its probable disease causing nature and the effectiveness of the treatment.

Case Report

A three-month-old boy of consanguineous Turkish parents was referred to our clinic due to prolonged neonatal jaundice. There was no family history. After an uneventful antenatal and perinatal period at the 3rd day of life he presented with neonatal jaundice and received phototherapy. At the initial

administration icterus with pale colored stools, failure to thrive and mild hepatomegaly were detected. Developmental milestones were normal and he had no dysmorphic features. The rest of the physical examination findings were unremarkable. Hepatic sonography showed features of fatty liver but normal biliary tract. Laboratory examination revealed hyperbilirubinemia (total bilirubin 6.3 mg/dl, conjugated bilirubin 2.7 mg/dl). Liver transaminases were mildly elevated and serum gamma glutamyl transferase was normal (serum alanine aminotransferase 125 IU/L (control range 11-45 IU/L), serum aspartate aminotransferase 171 IU/L (control range 22-63 IU/L) and serum gamma glutamyl transferase 89 (control range 8-90 IU/L)). High AST/ ALT

ratio was remarkable. He had no coagulopathy or hypoglycemia. His serum α -fetoprotein (α FP) level was markedly elevated (41420 ng/ml control range: 8.5 ± 5.5). Thyroid hormone profile, serum alpha-1 antitrypsin levels and glucose-6-phosphatase dehydrogenase screening were normal. Infectious causes of hepatitis were excluded. Although massive galactosuria was detected in metabolic urinary screening, galactose-1-phosphate uridyl transferase assay was normal. Ammonia levels were mildly elevated $38 \mu\text{mol/L}$ (N:11-35). Plasma amino acids analysis showed moderately elevated plasma citrulline and threonine and mildly elevated tyrosine. Increased threonine/serine ratio was detected. Normal urinary succinylacetone levels ruled out tyrosinemia. Serum bile

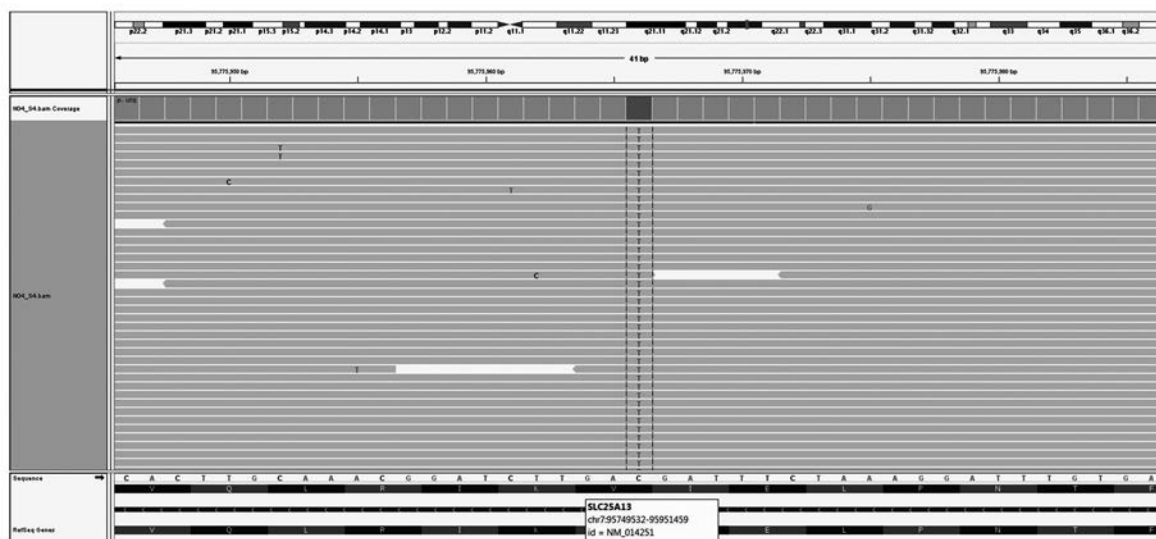


Fig. 1. Next generation sequencing of the SLC25A13 gene revealed homozygous p.Val452Ile mutation

Table I. The Course of Laboratory Data of the Patient from Diagnosis to the Last Visit.

	At diagnosis	1 month	3 month	6 months	12 months	18 months
Total/conjugated bilirubin (<1 mg/dl)	6.3/2.79	3.8/1.6	2.3/1.1	0.5/0.2	0.4/0.1	0.4/0.07
α -Fetoprotein (8.5 ± 5.5 ng/ml)	41420	17270	892	101	2.6	2.2
ALT (12-45 IU/L)/AST (22-63 IU/L)	125/171	96/103	53/72	45/58	24/62	22/51
Citrulline ($6-35 \mu\text{mol/L}$)	255	15.9	13	9	-	22
Threonine ($33-160 \mu\text{mol/L}$)	363	653	143	49	-	59
Threonine/Serine (1.1)	3.36	8.8	2.0	1.4	-	1.2
Galactosuria	Positive	Negative	Negative	Negative	Negative	Negative

acid levels were normal. Based on these metabolic abnormalities, neonatal intrahepatic cholestatic hepatitis caused by citrin deficiency was strongly suspected. Mutation analysis of SLC25A13 gene confirmed the diagnosis. A missense NM_014251.2:c.1354G>A(dbSNP: rs143877538) causes a protein 68 defect; NP_055066.1:p.Val452Ile (homozygous) mutation was detected by next generation sequencing, Miseq, Illumina (Illumina, San Diego, CA, USA) (Fig. 1), the minor allele frequency of this variant was given as nearly 0.01 in South Asian population. As this frequency supports the possibility of being a polymorphism, some in silico evaluations predict it as a disease causing variant (Mutation Taster prob: 0.999999993576759, Polyphen2 score: 1), while SIFT predicts it as a tolerated variant (SIFT score: 0.13). The child was treated with formulas containing medium-chain triglycerides, ursodeoxycholic acid and fat-soluble vitamin supplementation due to his clinical features strongly supports the diagnosis. As shown in Table I, we observed the progressive normalization of liver function tests, aminoacid levels and recovery of galactosuria. In the 18 month follow-up the patient no longer presented with jaundice and showed normal mental development and weight–height gain. Bilirubin, citrulline and alpha-fetoprotein levels remained in the normal range.

The study was reviewed and approved by an institutional review board. Written informed consent were provided by both of the parents.

Discussion

Although NICCD is mostly a benign condition, some of the cases could develop chronic liver failure and need liver transplantation to survive⁴. Major presentations of NICCD are cholestatic jaundice, and hepatomegaly. Coagulopathy, hypoglycemia and hypoalbuminemia could be the other presenting features. Splenomegaly has been rarely reported as an initial presenting finding³. Elevated citrulline levels, alpha fetoprotein levels and AST/ALT ratio are the other important markers of the disease⁵. Plasma aminoacids analysis showed moderately elevated plasma citrulline and threonine and mildly elevated tyrosine (Table I). High citrulline, alpha-fetoprotein level and AST/ALT ratio were significant in our patient.

Galactosemia is often previously considered rather than NICCD and these patients may be treated incidentally for galactosemia with lactose-free formula⁶. Markedly elevated levels of citrulline and alpha-fetoprotein are determinative. Tyrosinemia and mitochondrial DNA depletion syndrome are the other inherited metabolic diseases that should be considered in the differential diagnosis of NICCD². Due to the findings of galactosuria and cholestasis, galactosemia was one of the major causes of the differential diagnosis. With the absence of the cataract and normal activity of the galactose-1-phosphate uridyl transferase enzyme, we ruled out the diagnosis of galactosemia. Similarly, normal levels of tyrosine and absence of succinylacetone in the urine organic acid analysis excluded the diagnosis of tyrosinemia.

Although the NP_055066.1:p.Val452Ile variant is not clear a mutation due to its high frequency in South Asia and in silico evaluation results, clinical picture of our patient most probably supports its disease causing nature.⁷ It is firstly reported in the Turkish and European populations. Our team has the data of 512 exome analysis and this variant was not detected in that population. This variant is in the conserved area of the gene and protein in different species like *M. mulatta*, *F. catus*, *M. musculus*, *G. gallus*, *D. melanogaster*, *C. elegans* and *X. tropicalis*. It is considered to be a disease-causing variant⁷.

NICCD is not a self-limiting condition; progressive liver failure or adult-onset type II citrullinemia (CTLN2) may develop⁸. NICCD should be considered in the cases of neonatal cholestasis, especially in differential diagnosis of galactosemia due to galactosuria and tyrosinemia due to elevated levels of alpha-feto-protein. Also dietary treatment should be applied early, as a dramatic response is to be expected.

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