Citrin deficiency in a Romanian child living in Spain highlights the worldwide distribution of this defect and illustrates the value of nutritional therapy

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Abstract.

We report citrin deficiency in a neonatal non-East-Asian patient, the ninth Caucasian reported with this disease. The association of intrahepatic cholestasis, galactosuria, very high alpha-fetoprotein and increased plasma and urine citrulline, tyrosine, methionine and threonine levels suggested citrin deficiency. Identification of a protein-truncating mutation (c.1078C>T;p.Arg360*) in the *SLC25A13* gene confirmed the diagnosis. An immediate response to a high-protein, lactose-free, low-carbohydrate formula was observed. Our report illustrates the need for awareness on citrin deficiency in Western countries.

Keywords

Citrullinemia type 2; *SLC25A13*; NICCD; CTLN2; aspartate/glutamate antiporter; malate-aspartate shuttle; cholestasis, intrahepatic; neonatal cholestasis.

Deficiencies of the liver mitochondrial aspartate/glutamate antiporter citrin cause either neonatal intrahepatic cholestasis (NICCD; MIM #605814) or, in adults, the urea cycle disease citrullinemia type 2 (CTLN2; MIM #603471)[1,2]. These diseases, which are associated with mutations in the citrin-encoding gene *SLC25A13* (MIM 603859), were reported in Japan and are generally believed to affect only East-Asian populations, thus being neglected in the western world, where, for example, CTLN2 was not included in a recent urea cycle disorders guideline[3]. The relevance of citrin deficiency in western countries is highlighted here by reporting NICCD in a Romanian child living in Spain. We also provide detailed data supporting the immediate efficacy of nutritional NICCD treatment. The finding in this patient in homozygosis of the c.1078C>T nonsense (p.Arg360*) mutation in the *SLC25A13* gene, and the previous report of the same mutation in one Czech and one Japanese patient [4] suggests that this mutation might be relatively widespread.

Case report.

The patient, a male, was the third child of healthy non-consanguineous Romanian parents. He was admitted to our hospital at age 22 days with jaundice and inadequate thriving with breastfeeding. Modest hepatomegaly (2 cm) was found, and laboratory tests confirmed the jaundice (total bilirubin, 182 μ mol/L; nv <10) and revealed lowered total plasma proteins and albumin levels (34 and 26 g/L, respectively), profound anemia (67 g hemoglobin/L) requiring packed red blood cells transfusion, and impaired coagulation reflected in lengthened prothrombin time, due to decreased protein K-dependent coagulation factors VII and IX (respective levels, 30% and 48%; nv >70% for both). He presented also hypoglycemia (32 mg/L), galactosuria (10 mmol/L urine) and modestly elevated plasma aspartate aminotransferase (AST) (42 IU/L; nv <34 IU/L). Blood ammonia was normal. The jaundice was attributed to

intrahepatic cholestasis since plasma gamma glutamyl transpeptidase (GGT, 245 IU/L; nv<74 IU/L) and alkaline phosphatase (900 IU/L; nv <450 IU/L) were increased, whereas there was no ecographic evidence of alterations in the liver parenchima or the biliary tract. Consistent with persistent inadequate biliary function, levels of liposoluble vitamins A, D and E were low (respective values, 7, 170 and 36 µg/L; corresponding normal values, >30, >420 and >86 µg/L). Tyrosinemia type 1 was suspected because of the findings of, respectively, very high and high plasma levels of alpha-fetoprotein (AFP) and L-tyrosine (AFP, 58,344 µg/L; nv <10 µg/L; L-tyrosine, 383 µmol/L; nv <91 µmol/L), coexisting with tyrosinuria (188 mmol/mol creatinine) and increased urinary excretions of 4-hydroxyphenylpyruvate, 4-hydroxyphenyllactate and 4-hydroxyphenylacetate (4.9, 1.2 and 0.18 mol/mol creatinine, respectively; corresponding normal values, <0.074, <0.017 and <0.099 mol/mol creatinine). However, succinylacetone was undetectable in the urine, rendering unlikely type 1 tyrosinemia [5]. L-Citrulline, L-methionine and L-threonine were elevated in plasma (242, 110 and 673 µmol/L, respectively; corresponding normal values, <36, <44 and <222 µmol/L) and urine (943, 110 and 514 mmol/mol creatinine, respectively). On basic newborn screening (not measuring citrulline) on day 6, blood phenylalanine was elevated (366 µmol/L), and later re-screening (day 17) revealed elevated tyrosine (258 µmol/L) with normal phenylalanine. The coexistence of intrahepatic cholestasis with galactosuria and multiple hyperaminoacidemia including elevated L-citrulline, with increased AFP levels, raised a strong suspicion of NICCD [6], and thus breast milk was replaced on day 40 of life by a lactose-free, carbohydrate-low infant formula enriched in protein and medium chain triglycerides, providing per day 2.5-3.5 gr protein/kg body weight [7]. The *SLC25A13* gene (reference Genbank sequence, NM_014251.2) was analyzed for mutations by Sanger automated sequencing of the PCR-amplified (from genomic blood DNA) exons and flanking intronic sequences (oligonucleotide sequences used for amplification will be provided on request). The patient was homozygous for the transition affecting exon 11, c.1078C>T (cDNA numbering: base 1

is the A of the translation initiation ATG, corresponding to base 192 of the reference sequence), replacing codon 360 by a stop codon. This p.Arg360* mutation should truncate the enzyme approximately at the middle. Upon replacement of breast milk by the formula there was rapid clinical and laboratory improvement (Fig. 1), including the disappearance of galactosuria (not shown), with normalization of albumin and coagulation parameters and near-normalization of citrulline levels in 1-2 weeks, while the normalization of the levels of other elevated amino acids (tyrosine, methionine, threonine) and of cholestasis parameters (bilirubin, GGT) took somewhat longer (1-2 months). At 9 months of age the child is growing well (weight and height in the 85 and 90 centiles, respectively) and does not show obvious abnormalities of psychomotor development, having normal analytical values, although AFP levels, despite a precipitous decrease with initiation of the diet, slightly exceed (<2-fold) the upper limit of normality. Characteristically, he avoids carbohydrate-rich food and craves for high protein food.

Discussion

The p.Arg360* mutation found in this child is obviously disease-causing. The fact that the child is homozygous for it although his parents are non-consanguineous, and the earlier report of this mutation [4] in a Caucasian Czech NICCD patient and in a Japanese baby, suggests that this mutation may be widespread, particularly in central Europe. Nevertheless, the only Romanian patient among the eight previously reported unrelated Caucasian patients with NICCD [4,7-10] carried in homozygosis a different *SLC25A13* mutation than that reported here [10].

The apparently immediate effectiveness of the lactose-free, low-carbohydrate protein-enriched formula (Fig. 1) highlights the need to be aware of this very rare disease in Western countries, particularly since NICCD not always follows a benign course [11], what might be related with inappropriate treatment. Indeed, NICCD should always be considered in the differential diagnosis of neonatal cholestasis [12], with a

strong suspicion of NICCD being aroused by the triad of galactosuria, increased AFP and increased plasma amino acids (citrulline, threonine, methionine and tyrosine) [6]. Confirmatory diagnosis requires molecular analysis of the *SLC25A13* gene, although the failure of anti-citrin antibodies (available commercially) to reveal citrin in western blots of lymphocytes or fibroblasts is also diagnostic [13]. However, this last trait is not observed in all patients [9]. In view of the prompt therapeutic response, in cases of strong suspicion of citrin deficiency, dietary treatment under close patient monitoring can be initiated without waiting for molecular diagnosis.

About half of the NICDD patients are identified In Japan by newborn mass screening because of hypergalactosemia, hyperphenylalaninemia, and/or hypermethioninemia [14]. The progressive generalization of neonatal mass screening in countries where it was not available, together with the worldwide spread of East Asian populations, will increase the likelihood of finding more cases of NICCD outside the classical distribution area for citrin deficiency, highlighting the need for pediatricians and other physicians (particularly those dealing with metabolic diseases) to be aware of the presentations and management of this deficiency.

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Figure Legends

Figure 1. Evolution of selected analytical parameters in the patient. For representation of all analytes in the same graphs, relative values are given for the different analytes. The value of 1 corresponds to: bilirubin, 182 $\mu\text{mol/L}$; AFP, 58.3 mg/L; GGT, 245 IU/L; albumin, 26 g/L; prothrombin time, 28.2 secs; citrulline, 242 $\mu\text{mol/L}$; threonine, 673 $\mu\text{mol/L}$; tyrosine, 383 $\mu\text{mol/L}$; methionine, 110 $\mu\text{mol/L}$. The horizontal lines intersecting the lines for the change in each analyte give the higher or the lower (as appropriate) limit for normality for this given analyte. The arrow indicates the day at which feeding with maternal milk was replaced by lactose-free formula having high protein and high fat content.

Fig. 1