

Atypical clinical presentation and successful treatment with oral cholic acid of a child with defective bile acid synthesis due to a novel mutation in the *HSD3B7* gene

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Abstract

We report definitive diagnosis and effective treatment with oral cholic acid in one Italian male child affected by 3 β -hydroxy- Δ^5 -C₂₇-steroid dehydrogenase (3 β -HSD) deficiency. He presented with failure to thrive, hepatomegaly and multiple cystic images in kidneys; no biochemical evidence of cholestasis. Large amounts of bile acid metabolites was detected in urine by fast atom bombardment ionization mass spectrometry (FAB-MS). *HSDH3B7* gene analysis identified one mutation in intron 4, at nucleotide 432, G>A substitution that has never been reported before. The replacement therapy with oral cholic acid started early after the diagnosis and is still ongoing. Three years later hepatomegaly is no longer evident, liver function is normal and the child is growing regularly. In our experience, clinical features of 3 β -HSD deficiency can be very poor and even cholestasis can lack at diagnosis. Early replacement therapy with cholic acid is safe and leads to clinical and biochemical control of the disease.

Introduction

Congenital errors of bile acids (BAs)

synthesis (CBAS) are inherited autosomal recessive diseases, due to deficient activity of one of the 16 specific enzymes involved in the multistep pathway that leads from cholesterol to the synthesis of the two primary BAs, *i.e.* cholic acid (CA; 3 α ,7 α ,12 α -trihydroxy-5 β -cholanoic acid) and chenodeoxycholic acid (CDCA; 3 α ,7 α ,dihydroxy-5 β -cholanoic acid).¹ CBAS defects account for about 2% of liver disorders, and currently nine anomalies in BAs biosynthetic pathway have been definitely characterized, including the description of their genetic basis.² The deficient activity of specific enzymes involved in BAs synthesis results in marked reduction or complete lack of CA and CDCA in bile, serum and urine and concomitant production of large amounts of atypical BAs metabolites, causing progressive liver injury and cirrhosis.³ These metabolites are detected in biological fluids by mass spectrometry (MS) techniques, that currently provide the most appropriate means for characterizing each form of CBAS disorders.⁴ The 3 β -hydroxy- Δ^5 -C₂₇-steroid dehydrogenase (3 β -HSD) enzyme catalyzes the second step of the cascade that leads to BAs synthesis, *i.e.* the oxido-reduction of 3 β -hydroxy moiety to 7 α -hydroxycholesterol.²

The deficiency of 3 β -HSD (CBAS type 1 [CBAS1]; OMIM#607764) was described in 1987 by Clayton *et al.*⁵ and is very rare;⁶ its estimated prevalence in Europe is 0.99 cases per 10 million,⁷ and only 53 cases were reported in the literature.^{5,6,8,9} The CBAS1 disorder results from homozygous or compound heterozygous mutation in the *HSDH3B7* gene on chromosome 16p11.2-12, and 13 different mutations have been described so far.^{10,11} Age at diagnosis is variable: although most cases are identified in infancy or early childhood, other are diagnosed in young or even middle-aged adults.^{12,13} The most common clinical sign at presentation is persisting neonatal cholestatic jaundice, but the clinical features and the symptom progression are quite heterogeneous, some carriers of this disorder being asymptomatic.^{12,14} Additional clinical features may include hepatomegaly with or without splenomegaly, steatorrhea with fat-soluble vitamin malabsorption and failure to thrive; pruritus is absent in most instances.¹⁵ The CBAS1 disorder should be considered also in cases of rickets unrelated to malnutrition and without overt liver disease.¹⁶ Serum liver enzymes can be normal in the early stages of the disease, but later show progressive increases; despite hyperbilirubinemia, γ -glutamyltransferase (GGT) and total bile acid values remain within normal values (n.v.). The liver histology shows a generalized hepatitis, with cholestasis and

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varying degrees of giant cell transformation, probably secondary to the toxic injury.¹⁷ In the absence of specific treatment, the disease progresses to end-stage liver failure with cirrhosis, requiring liver transplantation. The combination of a cholestatic condition with normal GGT and without pruritus should alert physicians to investigate the possibility of the CBAS1 disorder.^{14,15} Specific diagnosis is based on metabolic study of urine metabolites by MS and molecular analysis.⁴ Oral therapy with primary BAs is required in all patients to down-regulate endogenous bile acid synthesis, allowing gradual resolution of biochemical and histologic abnormalities, with a favorable long-term prognosis.^{14,15,18}

Case Report

A 27-month old male, second child from unrelated healthy Italian parents, was referred for progressively worsening hepatomegaly during the last year, and failure to gain weight. The rest of his medical history was unremarkable, with no jaundice in the neonatal period or thereafter, no pruritus or steatorrhea. Physical examination showed a weight below the 3rd percentile and hepatomegaly (about 5 cm below the rib), without splenomegaly or jaundice. Urine analysis was normal. Abdominal ultrasonography (US) ruled out signs of liver damage, bile duct malformation or obstruction; yet, it surprisingly showed multiple cystic images in both kidneys, suggestive of nephrocalcinosis (Figure 1).

Except for a slight increase of aspartate aminotransferase (AST, 53 mU/mL n.v.<45 mU/mL), other routine tests were normal, including alanine aminotransferase, total and direct bilirubin, GGT, total bile acids, albumin, cholesterol, triglycerides. A slightly elevated international normalized ratio (1.41; n.v.<1.2) with low prothrombin ratio (58%; n.v. 70-120%) prompted us to investigate the coagulation factors, finding low levels of factor VII (61.3%; n.v. 70-120%) and factor X (47.5%; n.v. 70-120%), which were subsequently normalized with oral vitamin K supplementation. The clinical finding of low coagulation factors and their subsequent normalization with oral intake of vitamin K made us suspect the possible malabsorption of other fat soluble vitamins. Our suspect was confirmed by a very low level of vitamin E (37 mcg/dL; n.v. 300-1200), while vitamin D and vitamin A values were in the normal range. Tests for conventional pathogens (Epstein Barr, Cytomegalovirus, Herpes simplex 1 and 2, *Toxoplasma gondii*, Parvovirus B19, Hepatitis A, B and C, Human Immunodeficiency Virus) excluded a possible infective origin of the disease. Celiac serology was negative, as well as liver autoimmunity workup, alpha-1-antitrypsin blood levels and cystic fibrosis phenotype and genetic study. Other metabolic diseases (glycogenosis VI, IX, mucopolysaccharidosis, galactosialidosis) were ruled out, too. Based on mild liver dysfunction with normal GGT and normal serum BAs levels, together with fat soluble vitamins K and E malabsorption, a CBAS disease was suspected. At the age of 32 months, a liver gene panel analysis was performed and showed a novel single homozygous variant (NM_02519.3:c.432-2A>G) in the *HSD3B7* gene by Sanger sequencing. Both parents were heterozygous carriers, while the older

sibling was wild type. Qualitative and quantitative BAs analysis in urine by liquid chromatography-tandem MS showed high levels of unusual metabolites and the absence of primary BAs.

While the definitive diagnosis was pending, ursodeoxycholic acid (UDCA) treatment (5 mg/kg b.i.d) as well as vitamin E and K supplementation, were initiated and led to the normalization of coagulation parameters. Upon confirmation of the diagnosis of CBAS1 disorder, UDCA was replaced with

CA at increasing doses up to the current dosage (12 mg/kg/day). Dose adjustments were guided by titration against the biological response assessed by the reduction or disappearance of atypical urinary metabolites. The treatment with CA was well tolerated with no adverse effects. A remarkable clinical improvement was noted since the first months of treatment, including weight gain and regression of hepatomegaly. After more than 2 years of CA therapy, the child is in good health, his growth in weight and height



Figure 1. Kidney microcysts evidence by the abdominal ultrasound (arrows).

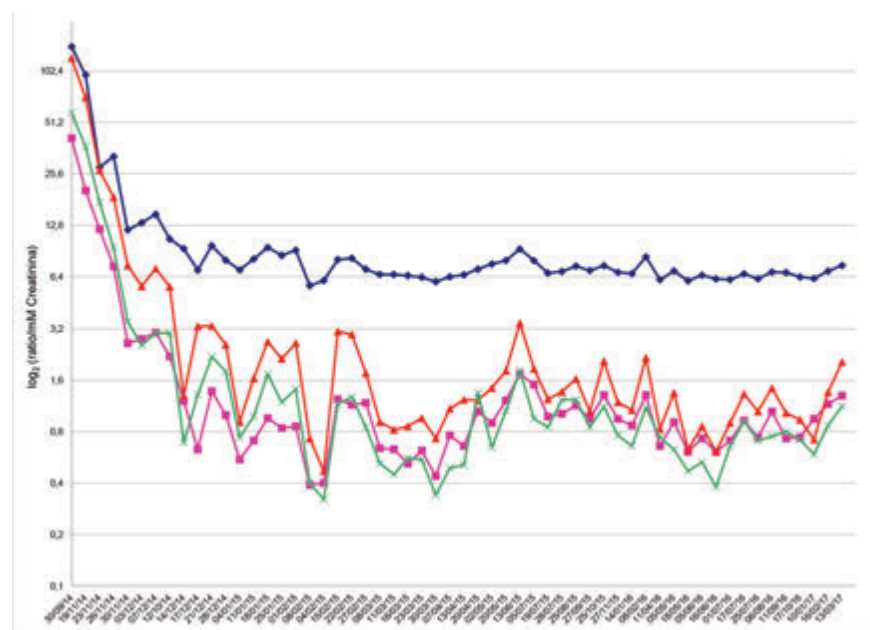


Figure 2. Mass spectrometry analyses of urinary bile acids before and during cholic acid therapy at cholic acid therapy beginning and during the follow-up. ◆ Di-OH-5-C (m/z 469); ■ Tri-OH-5-C (m/z 485); ▲ Glico-Di-OH-5-C (m/z 526); × Glico-Tri-OH-5-C (m/z 542).

is excellent and he has no clinical symptoms or laboratory findings suggestive of cholestasis or fat malabsorption; the coagulation profile and fat-soluble vitamin levels remain normal even after vitamin K and E supplementation withdrawal. Repeated US showed normal liver volume and almost complete disappearance of renal microcysts. Urinary MS analysis showed a normalization of BA metabolites within the first two months of CA therapy, and subsequent periodic analyses consistently found values of urinary metabolites below threshold limits (Figure 2).

Discussion

This observation illustrates atypical clinical features, definitive diagnosis by urine MS, genetic analysis and successful oral CA treatment of a child with β 3-HSD deficiency due to a novel homozygous splicing mutation in the *HSD3B7* gene.

The CBAS1 disorder is the first described and the most common form of a group of inherited disorders of BAs biosynthesis that, if left untreated, evolve to cirrhosis and end-stage liver disease, requiring liver transplantation. Its clinical presentation is known to be very variable:^{14,15} in our case, the child was nearly asymptomatic apart from hepatomegaly and failure to gain weight, and none of other suggestive clinical signs – especially neonatal or delayed jaundice – was present. Cholestasis is usually the biochemical and histologic hallmark of the CBAS1 disorder: our patient had no clinical or laboratory evidence of cholestasis, neither in the first months of life, nor at diagnosis. The fat-soluble vitamins malabsorption was clinically asymptomatic and could be demonstrated only by targeted laboratory tests, triggered by mild coagulation abnormalities. Abdominal US at diagnosis showed kidney hyperdense spots, initially misdiagnosed as nephrocalcinosis. It should be emphasized that such kidney microcysts possibly result from the accumulation of toxic metabolites, and are a rare finding in patients with the CBAS1 disorder,¹⁵ but when seen even in a minimally suggestive clinical context, they should be regarded as strongly evocative of this diagnosis.

The CBAS1 disorder is an inherited condition with an autosomal recessive mode of transmission, and most patients reported in the literature – but not all – belong to consanguineous families. Our patient is the only affected child of unrelated healthy parents and is homozygous for the mutation 432 G>A substitution of the *HSDH3B7* gene, not described yet in the lit-

erature or any public database. The A>G transition falls in the canonical acceptor splice site of exon 5 and the predicted consequence is the skipping of this exon. Given the peculiar clinical presentation of our patient and his unique genetic mutation, it may be hypothesized that the variable clinical patterns of the CBAS1 disorder reported in the literature could result from genotype-phenotype correlations, which remain to be disentangled. The recent introduction in clinical practice of the next-generation sequencing gene panel analysis may prove to be very useful in the identification of cases with uncommon presentations.

Noteworthy, our observation clearly shows that the liver biopsy is not mandatory for formal CBAS1 diagnosis.

While the definitive diagnosis is pending, initial treatment with UDCA could be of temporary benefit, but once CBAS1 is confirmed, UDCA should be replaced with primary BAs,¹⁵ such as CA or CDCA given as monotherapy. Yet, CDCA can be hepatotoxic at high doses,¹⁹ and is not approved for the treatment of the CBAS1 disorder. Thus, CA is now recognized as the BA of choice,^{8,15} because it is highly effective in normalizing biochemical and clinical abnormalities, with potential for prevention or reversal of morphological liver damage and proven long-term effect.¹⁵ CA was approved for the treatment of the CBAS1 disorder in Europe in 2013.

The potential hepatotoxic effect of BAs has been shown to be dose-dependent:²⁰ therefore, the treatment should be individualized by giving the minimal dose of CA able to suppress the defective metabolic pathway. As other authors proposed,^{14,15} we adjusted doses by titration against the biological response, ascertained by the reduction or disappearance of toxic metabolites in urine on repeated MS measurements. While this report is being written, the child has been on CA treatment for more than two years without any adverse effect. His growth in weight and height is excellent, hepatomegaly disappeared, serum liver biochemistries are within the normal range and atypical metabolites are undetectable in the urine.

Conclusions

Pediatricians should suspect CBAS1 even in the absence of cholestasis, especially in the context of hepatomegaly and failure to thrive with fat malabsorption. The lipids malabsorption can be evident at diagnosis as steatorrhea, but, depending on the high variability of clinical presentation of the disease, especially in younger children,

steatorrhea can not be regarded as an essential requirement to arise the suspect of fat malabsorption. Thus, also in cases without overt steatorrhea, in suspicion of CBAS 1, it is mandatory to search for fat soluble vitamins defects. Early diagnosis and prompt treatment of the disease are important to prevent progression to cirrhosis and permanent neurological damage, as a result of vitamin E malabsorption. Specific diagnosis is based on MS analysis of the urine, with gene sequencing as confirmatory test. Liver biopsy and histology are not mandatory. Repeated urine MS analyses allow monitoring the biochemical response to treatment and dose adjustments. Oral CA-based treatment leads to complete control of the abnormal metabolic pathway, disappearance of toxic metabolites, normalization of liver size and function, and recovery of normal growth, with no remarkable adverse effects. Its efficacy and safety are maintained over time,¹⁵ offering patients relief of clinical symptoms and restoration of normal quality of life.

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