Two Case Reports of Successful Treatment of Cholestasis With Steroids in Patients With PFIC-2

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Abstract

Mutations in the gene encoding the canalicular bile salt export pump (BSEP) can result in progressive familial intrahepatic cholestasis type 2 (PFIC-2). Treatment options are limited, and PFIC-2 often necessitates liver transplantation. We report on a young woman and a boy who clinically presented with PFIC-2 phenotypes and dramatically improved with steroid treatment. Gene sequencing of ABCB11 encoding for BSEP revealed 2 relevant mutations in both patients. The young woman was compound heterozygous for p.T919del and p.R1235X. At the age of 5 years, partial biliary diversion was performed and rescued liver function but left serum bile salt levels elevated. At age 23 she developed systemic lupus erythematosus. Unexpectedly, steroid therapy normalized serum bile salt levels, with a strong correlation with the steroid dose. She is currently in clinical remission. The boy was compound heterozygous for the ABCB11 mutations c.150+3A>C and p.R832C and presented with intractable pruritus. When he developed colitis, he was treated with steroids. The pruritus completely disappeared and relapsed when steroids were withdrawn. To date, with low-dose budesonide, the boy has been symptom-free for >3 years. In conclusion, the clinical courses suggest that patients with BSEP deficiency and residual BSEP activity may benefit from steroid-based therapy, which represents a new treatment option.

Familial intrahepatic cholestasis syndromes comprise a group of liver diseases that are caused by the failure of hepatobiliary transporter proteins. Certain mutations in the ABCB11 gene1–3 lead to deficient expression of the canalicular bile salt export pump (BSEP), causing hepatocellular bile salt (BS) accumulation and subsequent liver cirrhosis. The residual transport activity of BSEP inversely correlates with the severity of the patient’s phenotype. Complete absence of BSEP activity is associated with progressive familial intrahepatic cholestasis type 2 (PFIC-2). Patients with PFIC-2 suffer from continuously evolving pruritus, wasting jaundice, and sequelae of liver cirrhosis, often necessitating liver transplantation4 in early childhood. In large studies, PFIC represented 10% to 15% of causes of childhood cholestasis and is the reason for up to 15% of liver transplantsations in children. Treatment focuses on symptom relief and normalization of liver function tests. It is based on ursodeoxycholic acid (UDCA), with improvement of symptoms in 75% of cases,5,6 rifampicin, and partial biliary diversion. We here report on 2 patients with PFIC-2 phenotypes and sustained clinical relief of symptoms and unexpected improvement in hypercholeemia in response to oral steroids.

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PATIENT PRESENTATION

Patient 1

A girl (born 1989) presented with vitamin K deficiency (8 weeks of age), pruritus (8 months), followed by jaundice (10 months) with a BS concentration of 583 μmol/L (normal: <8 μmol/L). Aminotransferases and gamma-glutamyl transferase were normal. A liver biopsy revealed severe intrahepatic cholestasis and PFIC-2 was suspected. UDCA treatment was started, and a partial external biliary diversion procedure was performed at the age of 5 years, which led to good control of pruritus for 15 years.

Sequencing of ABCB11 (BSEP) revealed compound heterozygosity for the deletion of a coding triplet (c.2756_2758delCCA), resulting in the loss of a single threonine at amino acid position 919 (p.T919del, inherited by the mother) and the non-sense mutation c.3703C>T/p. R1235X, resulting in a premature stop-codon at amino acid position 1235 (inherited by the father) (Fig 1). The initial liver biopsy revealed an apparently normal expression of BSEP (Fig 1). The anti-BSEP antibody, which was used for immunofluorescence, was directed against the 13C-terminal amino acids of BSEP. BSEP immunoreactivity in the patient’s liver biopsy sample must therefore have been due to expression of the allele carrying the T919del variant, because in the presence of p.R1235X (premature stop codon) the 86 C-terminal amino acids are lacking.

At the age of 23 years the patient’s general condition deteriorated, and she presented with polyarthritis, polyserositis, splenomegaly, anemia, leukocytopenia, and pruritus. Antinuclear antibody titer was >1: 5120, and anti-double-strandedDNA antibodies were 6738 U/mL (ELISA). Systemic lupus erythematosus was diagnosed.

Treatment was started with prednisolone at a dose of 30 mg/day followed by complete symptom relief. Serositis recovered, and the arthritis improved thereafter. Steroids were tapered to 7.5 mg/day, with a flare up of arthritis and increasing anti-double-strandedDNA antibodies to 4549 U/mL. Prednisolone was increased to 15 mg/day, once again resulting

FIGURE 1
Immunofluorescence of liver tissue and DNA sequencing in patient 1. Liver tissue of patient 1 (taken at the age of 10 months) reveals a clear canalicular staining pattern for BSEP and MRP2 (scale bar = 10 μm), as visualized by immunofluorescent staining and confocal laser scanning microscopy. The young woman is compound heterozygous for a triplet deletion in exon 21 c.2756_2758delCCA (p.T919del) and a non-sense mutation c.3703C>T (p.R1235X) in coding exon 26 of ABCB11. Sequences are shown on the nucleotide and protein level.
in clinical improvement in arthritis and pruritus.

Serum BS levels normalized during the initial higher dose of systemic steroid therapy (Fig 2). Interestingly, total BS concentration in bile collected from the partial external biliary diversion increased to 9.56 mmol/L (August 2012) compared with 1.53 mmol/L before steroid treatment (May 2009). Three years after the introduction of steroids (November 2011), she is still free of symptoms of cholestasis.

Patient 2
Patient 2 (born 2004) presented at an age of 2.5 years with severe pruritus despite adequate therapy with rifampicin, phenobarbital, and naloxone; interrupted night sleep; and fatigue. Blood examinations revealed a mild cholestasis with total bilirubin of 23.4 μmol/L and direct bilirubin of 9 μmol/L. Serum BS concentration was 148.6 μmol/L, whereas aminotransferases, gamma-glutamyl-transferase, alkaline phosphatase, and cholesterol were within normal ranges. PFIC-2 was suspected, and a liver biopsy was obtained. Histology revealed chronic cholestasis, disorganization of portal structures, and degenerative bile duct alterations. Treatment with UDCA and naltrexon improved pruritus.

At the age of 4.5 years pruritus and jaundice worsened, with a direct bilirubin of 230 μmol/L. In addition, diarrhea and painful bowel movements developed. Endoscopic retrograde cholangiography revealed no evidence of primary sclerosing cholangitis but showed a secretion of pale-appearing bile. On colonoscopy, a left-sided colitis with superficial granulocyte infiltration of the mucosa in the proximal parts of the colon was diagnosed.

Genetic analysis revealed mutations in the ABCB11 gene. In the 20th coding exon a heterozygous missense mutation c.2494C>T (p.R832C, inherited by the mother) was detected together with the heterozygous splice site mutation c.150+3A>C distally to exon 3 (inherited by the father). No mutations of the ATP8B1 gene or of the JAG1 gene (Alagille syndrome) were detected. Immunofluorescence revealed normal distribution of BSEP and of the bilirubin transporter multidrug resistance-associated protein 2 (MRP 2) used as a canalicular marker (Fig 3).

The course of the disease was progressive, with increasing hepatomegaly and advancing liver fibrosis (FibroScan [Echosense, Paris, France] was 10.4 kPa at the age of 4.75 years and 14.6 kPa at the age of 5 years). The patient was listed for liver transplantation and a living related transplantation was considered.

The administration of prednisolone led to relief of the diarrhea and pruritus. BSs and bilirubin normalized completely (Fig 4). Budesonide had the same benefit but without side effects. Liver stiffness improved to almost normal values6 (6.3 kPa, age 7 years). The patient was taken off the transplant list and is currently free of symptoms (as of 2014).

DISCUSSION
We here report on complete symptom relief and sustained normalization of
liver function tests in 2 unrelated patients with PFIC-2 phenotypes.
In most PFIC-2 patients, BSEP expression is absent or severely reduced in liver tissue.9 However, our patients showed clear immunoreactivity for BSEP in their livers. Detection of significant amounts of BSs (between 1.5 and 9.5 mmol/L) in the bile of patient 1 suggests residual BSEP activity, which may be an important requirement for successful steroid therapy. It is believed that the extent of expression inversely correlates with phenotype severity (reviewed in ref 10), with some exceptions.5

In patient 2, the course of liver and intestinal disease could be positively influenced by prednisolone. Tapering of prednisolone was associated with worsening of cholestasis and intestinal disease. Eventually, budesonide at a minimal effective dosage of 3 mg every second day resulted in complete clinical and biochemical remission within 6 months for >3 years.

The mechanism of action of the steroids in the context of PFIC-2 is
not yet clear but could possibly involve upregulation of BSEP transporter activity, although experimental data are conflicting. In primary rat hepatocytes, BSEP-mRNA as well as Mrp2-mRNA and protein expression were upregulated by dexamethasone in a concentration-dependent manner. In line with this finding, treatment of rats with glucocorticoids including budesonide was associated with the upregulation of BSEP, Mrp2, and cytochrome P450 oxidase. On the other hand, Liu et al. showed that dexamethasone administration to neonatal rats in vivo had a stimulating effect on Mdr2 (the ortholog to the human MDR3) and Na+-taurocholate cotransporting polypeptide (Ntcp) gene expression, but decreased the gene expression of Bsep, Mrp2, and Fic1, a P-type ATPase protein. However, most differences were not significant in that study. The effect of steroids on canine bile flow has been demonstrated by Brems et al. Dogs given high dosages of hydrocortisone at 5 mg/kg per hour showed a significant increase in bile flow (from 258 μL/minute to 357 μL/minute) independent of BS concentration.

The ABCB11 splice site mutation c.150+3A>C results in partial exon skipping of exon 3 in vivo, and approximately one-third of mRNA transcripts are correctly spliced (C.D., R.K., D.H., unpublished data). Therefore, in patient 2, steroids may upregulate transcription and could potentially improve correct mRNA splicing or recover transport activity of BSEP<sup>R332C</sup>.

Another steroid-dependent mechanism of regulated BS transport may involve Na<sup>+</sup>-dependent cellular bile acid uptake via NTCP, which is localized at the sinusoidal membrane of hepatocytes. It has been shown that, under cholestatic conditions such as biliary atresia, inflammation-induced cholestasis, or progressive familial intrahepatic cholestasis, NTCP is downregulated in human livers. This downregulation may serve to protect hepatocytes from toxic BS concentration but may contribute to the retention of BSs in the blood under cholestatic conditions. An essential glucocorticoid response element (GRE) has been identified within the NTCP promoter at a nucleotide position of −32 to −12 relative to the transcription start site. Therefore, glucocorticoids may improve transepithelial BS transport by upregulation of NTCP, providing an increased gradient for BSEP, thus counteracting cholestasis.

Budesonide is a steroid with rapid intestinal reabsorption and a high first-pass clearance of ~90%. Special formulations of budesonide, however, may reach the distal colon. In line with this, colitis in patient 2 remained in remission under alternating dosages of 3 mg budesonide. It is possible that cholestasis might be perpetuated due to extrahepatic inflammation as the primary insult. On the other hand, patient 1 had a slight decrease in BS coincident with active systemic lupus erythematosus even before initiation of steroid therapy. It is well known that lipopolysaccharides induce cholestasis and downregulate BSEP in a model of human liver slices. However, primary sclerosing...
cholangitis in the context of ulcerative colitis in general is not sensitive to steroid therapy,26,29 a fact that demonstrates the completely different pathomechanisms of BSEP deficiency and primary sclerosing cholangitis. Clearly, steroid therapy in patients with PFIC is only indicated if prompt effect (within 4 weeks) is detected because the steroid therapy itself has relevant side effects if given for a long time.30

To our knowledge, this is the first clinical report in which, in vivo, a beneficial effect of steroid administration on the disease course of BSEP deficiency/PFIC-2 was shown. For future patient management it would be worth further classifying the PFIC population via genetic analysis, immunohistochemistry or immunofluorescence, and clinical profile to determine those patients who would benefit from steroid administration.

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