Severe hepatocellular dysfunction in obstetric cholestasis related to combined genetic variation in hepatobiliary transporters

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Summary

Obstetric cholestasis (OC) is a cholestatic disorder with a prominent genetic background including variation in diverse hepatobiliary lipid transporters, such as *ABCB4* (phospholipids) and ABCB11 (bile salts). Given a marked hepatocellular dysfunction in an OC patient indicated by > 40-fold rise in alanine aminotransferase activity and minor γ-glutamyl transpeptidase increases, we performed genotyping of candidate gene variants associated with adult cholestatic phenotypes. Genetic analysis revealed the heterozygous *ABCB4* mutation p.R590Q, the *ABCB11* variant p.V444A and the lithogenic *ABCG8* variant p.D19H. Aggregation of multiple hepatobiliary transporter variants is rare in OC, and may cooperate to negatively modulate hepatobiliary transport capacities.

Key words: Obstetric cholestasis; Hepatobiliary transporters; *ABCB4; ABCB11; ABCG8*; Low phospholipid-associated cholelithiasis; Cholelithiasis.

Introduction

Obstetric cholestasis (OC) is the most common pregnancy-related liver disorder estimated to affect up to 1% of pregnant women in European countries typically in their third trimester. Patients present with troublesome pruritus due to elevated serum bile acid levels (> 11 μM/l) and mild to moderate elevations in aminotransferases. In general, increases in liver function tests do not exceed a 10- to 20-fold rise in alanine aminotransferase (ALT), representing the most specific marker of hepatocellular injury in OC [1]. Maternal prognosis in OC is considered benign, whereas OC may entail a considerable perinatal risk and fetal complication rate, e.g. preterm delivery and fetal distress, in particular, if fasting serum bile acid levels surpass 40 μM/l. As for clinical management, ursodeoxycholic acid (UDCA) has become the therapeutic mainstay, confering beneficial symptomatic and biochemical effects. Close fetal monitoring with early delivery in severe cases is essential.

Though the precise molecular mechanisms in OC pathogenesis are as yet poorly understood, cholestatic decompensation of the hepatobiliary transport machinery in genetically susceptible individuals challenged by pregnancy-related systemic and hepatic hormonal loads seems to be key to OC development. Indeed, modulation of hepatobiliary transporter expression by sex hormones has been shown in vitro. Specifically, estrogen glucuronides and specific progesterone metabolites negatively regulate expression of canalicular ATP-binding cassette (ABC) transporters, i.e., the bile salt export pump *ABCB11* (BSEP) and the phospholipid transporter *ABCB4* at the posttransciptional level.

Up to now, an increasing number of hepatobiliary transporter gene variants have been associated with OC. Of note, coincidence of OC in mothers of patients with progressive familial intrahepatic cholestasis type 3 (PFIC-3), closely related to homozygous *ABCB4* mutations, first suggested a genetic basis for OC. In the meantime, sequence variance of the hepatocellular phospholipid transporter *ABCB4* (MDR3) has been linked to OC, and is considered to account for up to 15% of all OC cases.[2] ABCB4-deficient OC may represent a clinically distinct subset characterized by elevations of γ–glutamyl transpeptidase (γ–GT) activity. Similarly, functional variants of *ABCB11*, and the putative aminophospholipid translocase *ATP8B1* (FIC1) have been implicated in the genetic predisposition to OC [3]. Genetic variation in the heterodimeric cholesterol transporter *ABCG5/8*, more specifically the D19H variant in the *ABCG8* gene, has been shown to increase the risk of cholesterol gall stone disease, which is also associated with OC, and might, thus, contribute to modulation of cholestatic phenotypes [4].

We report on the complex genetic findings in an individual with severe OC as indicated by a > 40-fold rise in ALT activity harboring combined coding variants in hepatobiliary transporters *ABCB4* and *ABCB11* coupled with co-inheritance of the primarily lithogenic cholesterol transporter *ABCG8* variant D19H.

Methods

To better dissect the genetic basis underlying this severe OC phenotype, we performed genotyping of several candidate variants related to adult cholestatic phenotypes and/or OC after obtaining written informed consent from the patient (Table 1). Genomic DNA was extracted from peripheral venous blood leukocytes by standard procedures (Qia Amp DNA Blood Mini Kit, Qiagen, Hilden, Germany). Five single-nucleotide poly-

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Table 1. — *Summary of the candidate gene variants and genotyping methodology*.

Gene	Variant	Method
PATP8B1	I661T	RFLP/PCR
	N45T	PCR
	E429A	TaqMan
ABCB11	E297G	PCR
	A444V	TaqMan
	D482G	PCR
	A1028A	TaqMan
ABCB4	R590Q	PCR
ABCG8	D19H	TaqMan

RFLP, restriction fragment length polymorphisms; PCR, polymerase chain reaction, TaqMan, solution-phase hybridization assay with 5'-nuclease and fluorescence detection.

morphisms (SNPs), including ABCB11 E297G *(rs11568372), ABCB11* D482G *(rs72549402), ATP8B1* N45T and *ABCB4* R590Q (rs45575636) were detected by direct sequencing using *BigDye Terminator Kit* (Applied Biosystems, Darmstadt, Germany). Four variants were analyzed using solution-phase hybridization reactions with 5′-nuclease and subsequent fluorescence detection (TaqMan SNP Genotyping Assays, Applied Biosystems, Darmstadt, Germany). The polymorphism V444A *(rs2287622)* in the *ABCB11* gene was genotyped by assay C__16182459_10, while the synonymous *ABCB11* variant A1028A *(rs497692),* previously demonstrated to be associated with exon skipping, was detected by assay C__881357230. The coding variant *ATP8B1* E429A *(rs34018205)* was analyzed by a self-designed assay. Finally, the *ATP8B1* mutation I661T *(rs28939686)* was analyzed by restriction fragment length polymorphism (RFLP) and subsequent polymerase chain reaction (PCR). The PCR product contained 281 bp and was digested with *Pst1*. The major allele was present when the PCR product remained undigested, for the minor allele the PCR product was cut into two fragments of 261 bp and 19 bp. Subsequently, the samples were analyzed on a 4% agarose gel (Biozym, Hess. Oldendorf, Germany). Primer and probe sequences for the respective SNPs can be provided on request.

Results

A 30-year-old primigravida was referred in her 29th gestational week for progressive "pruritus sine materia" with nocturnal exacerbations and markedly elevated liver enzymes. Laboratory data were as follows (maximum values indicated; normal values in parentheses): ALT 1450 U/l (< 35), aspartate aminotransferase (AST) 766 U/l (< 35), γ-GT 46 U/l (< 40), alkaline phosphatase (AP) 207 U/l (35-104), total serum bilirubin 0.7 mg/dl (< 1.2). Fasting serum bile acids were elevated to a maximum of 33 μM/l (pregnancy-specific normal < 11). Abdominal ultrasound revealed an uncomplicated, as yet unknown, cholecystolithiasis and an inconspicuous liver without evidence of chronic liver disease and/or mechanical cholestasis. A dedicated history of medications including recent use of over-the-counter and herbal preparations remained unremarkable. After exclusion of alternative diagnoses, such as viral hepatitis, hereditary, metabolic and/or autoimmune liver disease and ongoing preeclampsia or *hemolysis, elevated liver tests and low platelet count* (HELLP) syndrome, an unequivocal diagnosis of OC was established. Family history was negative for OC or pregnancy-related itching as well as gall stone disease. The patient was treated with escalating UDCA doses up to 20 mg/kg resulting in satisfactory symptomatic and biochemical control. Due to cardiotocographic irregularities during labor, the patient underwent secondary cesarean section in the 39th week of gestation, giving birth to a healthy female neonate (APGAR scores 9-10-10). After delivery the patient experienced complete symptomatic and laboratory relief (Figure 1).

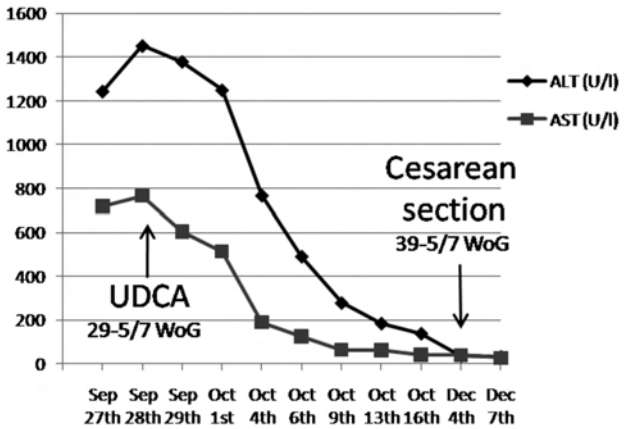

Figure 1. — Liver function tests in the course of pregnancy. The start of therapeutic intervention with 20 mg/kg UDCA and the time point of cesarean section are indicated (UDCA ursodeoxycholic acid, WoG weeks of gestation, ALT alanine aminotransferase, AST aspartate aminotransferase).

The genetic analysis was significant for a heterozygous G>A transition in nucleotide position 1769 in exon 15 of the *ABCB4* gene, resulting in a substitution from wild-type arginine to glutamine (p.R590Q). In addition, a homozygous state was identified for the *ABCB11* variant in exon 13, codon 1331, with thymidine replaced by cytidine (c.1331T>C), leading to an exchange of alanine to valine (p.V444A). The *ABCG8* variant D19H representing a risk allele for cholesterol gall stone disease by enhanced hepatobiliary cholesterol secretion (putative *"gain-of-function"*) was identified in its heterozygous state, while for the other variants investigated the respective wild types were detected.

Discussion

Pregnancy is an exceptional physiological state challenging the individual's hepatobiliary transporter capacity by virtue of excessive hormonal levels. Genetic variation in hepatobiliary transporters in association with environmental factors may determine whether cholestatic decompensation occurs, i.e., whether OC manifests clinically. Despite the general appreciation of a complex genetic background in OC, where single gene polymor-

phisms may only have a small impact on clinical phenotype, combined genetic variation in OC-related loci has rarely been reported [5, 6]. The genetic findings in the presented patient are novel as to the co-inheritance of the lithogenic *ABCG8* D19H variant with combined variation within *ABCB4* and *ABCB11* as functionally coupled OC-associated genes.

The non-synonymous *ABCB11* SNP p.V444A (c.1331T>C) located in the nucleotide binding fold (NBF) in the intracellular loop of BSEP is a very common variant occuring in up to 50% of the general population, yet a robust association with OC has been established. Though allele-specific functional analyses assessing taurocholate transport capacity yielded similar results under basal conditions, available data demonstrate reduced canalicular membrane BSEP staining and lowered mature BSEP production by the SNP variant 444A, supporting the notion that basal BSEP abundancy may be overridden in cholestatic conditions, such as pregnancy where sex hormone levels peak, in genetically susceptible individuals. In our patient, canalicular BSEP deficiency may have contributed to the profoundly elevated liver enzymes as a consequence of hepatocellular bile salt toxicity.

However, the detectable γ-GT elevation in addition raised the possibility of aberrant ABCB4 function underlying her cholestatic phenotype. In fact, she proved heterozygous for the missense mutation p.R590Q in the *ABCB4* gene, which has been previously documented in OC [7, 8]. Located in the evolutionary conserved nucleotide binding domain (NBD) 1 engaged in energy transfer from ATP hydrolysis to substrate transport, altered biological function of the variant ABCB4 protein may be assumed. However, as yet, no functional data assessing alterations in transporter activities are available for this specific coding variant. In the report by Ziol *et al*. liver samples from a single R590Q mutation carrier were available for ABCB4 immunohistochemistry revealing "faint and discontinuous canalicular staining" [8]. In the same direction, potential disruption of targeted protein routing giving rise to functional lack of ABCB4 protein on the canalicular membrane appears plausible, and has been demonstrated previously for another missense mutation in the same NBD. In a study by Tavian *et al*. the mutation was not detected in 43 parous controls, while previous data from France had indicated that up to 3% of healthy controls may harbor this sequence alteration heterozygously [8, 9]. In the most recent study sequencing the entire *ABCB4* coding sequence in 50 OC patients, however, the R590Q variant was identified in a total of five individuals, of interest, in two of them homozygously, compared to one heterozygous carrier among 107 pregnant control women [10]. Of note, some of the patients carrying the R590Q mutation were also homozygous for a distinct haplotype comprising three SNPs (c.175C>T, c.504T>C and c.711A>T), pointing to a possible founder effect of the mutation. The overrepresentation of the R590Q variant in the OC cohort proved statistically significant, and all women carrying the risk allele showed normalization of liver dysfunction after delivery [10]. Likewise, the complete resolution of laboratory evidence for chronic cholestasis postpartum and after subsequent UDCA withdrawal in conjunction with the as yet uncomplicated gall stone disease in the presented patient is confirmative of true OC in its strictest definition. However, as chronic liver disease in the setting of *ABCB4* sequence variation representing a spectrum from LPAC syndrome to biliary cirrhosis may occur at a later date, it is mandatory to follow up the patient [8]. However, most of the patients reported so far with co-inheritance of multiple OC risk alleles including *ABCB4* mutations exhibited clinical signs of chronic ABCB4 deficiency at the time of OC diagnosis, which does not apply to our patient, suggesting a more subtle effect of the R590Q variant on ABCB4 biological function outside pregnancy. With this perspective, the detection of uncomplicated cholelithiasis, not meeting the diagnostic criteria for LPAC syndrome, may represent a mere coincidence in a pregnant female with an *ABCG8* 19H carrier status. Conversely, there are no experimental data available up to now to support a pathogenic involvement of the *ABCG8* D19G variant in OC, although, given the multitude of complex interaction among the different components of the hepatobiliary transport system, it may be suggested that there might be a potential for modulation of cholestatic phenotypes. Notwithstanding, preliminary data from a small-sized, European-based association study do not indicate an overrepresentation of the *ABCG8* risk allele in individuals with advanced OC as indicated by bile acid concentrations in excess of 40 μM/l (*unpublished data*).

Taken together, considering the unusual extent of OC-related hepatocellular dysfunction, representing one of the most profoundly raised ALT activities reported so far in OC, we conclude that the cholestatic phenotype might have been mediated by combined genetic variations in *ABCB4* and *ABCB11* synergistically impacting on hepatobiliary transport capacities. To what extent, if any, the acquisition of the heterozygous *ABCG8* 19H variant may have contributed to the advance clinical phenotype remains yet to be determined. Increased recognition of combined genetic variation in functionally related susceptibility and/or modifier loci is expected to provide further insights into the complex genetic etiology of OC and related cholestatic liver diseases, and may, in the future, guide targeted medical intervention and follow-up strategies.

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