

## Letter to the Editor

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# An unexpectedly prolonged severe hyperbilirubinemia in a patient with pre-existing hepatitis A: a role of genetic predisposition?

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To the Editor,

Acute hepatitis A virus (AHAV) infection is a self-limited condition that usually spontaneously recovers in 2–8 weeks. While in children under 6 years of age AHAV is often asymptomatic, >70% of adults manifest symptoms together with typical laboratory findings [1]. AHAV is serologically diagnosed by the detection of anti-HAV IgM, which persists 2–6 months after infection. Laboratory results during AHAV show a marked increase (usually 10 to 40 times the upper reference limit [URL]) in serum alanine aminotransferase (ALT) activity, together with high total bilirubin (TB) (average peak of 171.0  $\mu\text{mol/L}$ , mainly consisting of the conjugated form) and moderately (<3 URL) increased alkaline phosphatase (ALP) and  $\gamma$ -glutamyltransferase (GGT) [2]. With resolution of AHAV, conjugated bilirubin (CB) is rapidly cleared and its serum concentrations return to normal quickly. However, 10%–20% of patients develop prolonged cholestasis lasting for >6 months [1].

Bilirubin, which is a major end-product of heme breakdown, is physiologically glucuronidated in the liver and then excreted into the bile. The mechanisms of bilirubin excretion, and of hyperbilirubinemia causes, are

largely understood. Unconjugated bilirubin (UB) enters the hepatocyte by passive transmembrane diffusion combined with active mediated transport. In the hepatocyte, UB is bound to ligandin and transported to the endoplasmic reticulum, where it is conjugated into bilirubin glucuronide by UDP glucuronosyltransferase (UGT) 1A1, and excreted into the bile by ATP-binding cassette (ABC) transporters ABCC2 and, to a lesser extent, by ABCG2. Under pathophysiological conditions, such as acute hepatitis or cholestasis, the CB back into blood by the basolateral ABCC3 transporter and then the solute carrier organic anion (SLCO) transporters 1B1 and 1B3 mediate its hepatic reuptake [3]. Several inherited disorders characterized by impaired bilirubin conjugation or transport result in various degrees of unconjugated or conjugated hyperbilirubinemia, respectively [4].

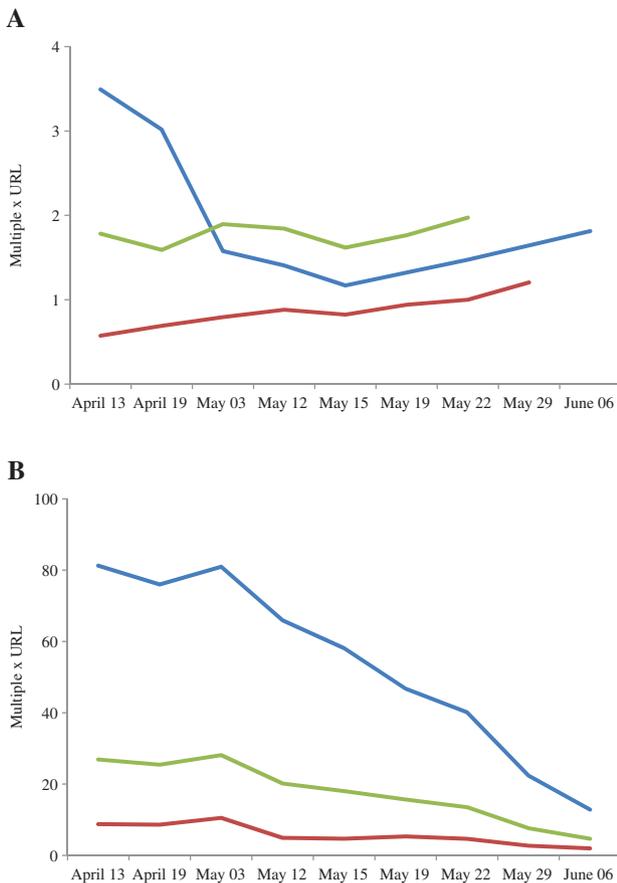
Here, we report a case of prolonged severe hyperbilirubinemia in a patient with pre-existing AHAV, in which we were however, unable to show an evident persistence of cholestasis. A 33-year-old man was admitted at the Emergency Department of our hospital on April 7, 2017, 6 weeks after an AHAV diagnosis performed in another hospital. The patient, strongly jaundiced, presented asthenia, nausea, diarrhea, pruritus and hyperchromic urine. Blood tests revealed a moderate increase of serum ALT activity (202 U/L; URL: 59 U/L), in line with the expected AHAV resolution, but an unexpectedly markedly elevated serum TB (625.9  $\mu\text{mol/L}$ , with CB of 480.5  $\mu\text{mol/L}$ ; TB URL: 20.5  $\mu\text{mol/L}$ , CB URL: 5.1  $\mu\text{mol/L}$ ). The presence of anti-HAV IgM (slightly positive) and anti-HAV IgG confirmed the previous AHAV diagnosis. Other concomitant conditions (such as hepatitis B and C infection, syphilis, autoimmune disease and drug toxicity) were excluded. Steroid therapy was immediately started, but without any clinical improvement or significant decrease in serum TB/CB concentrations (Figure 1). A magnetic resonance cholangiopancreatography (MRCP) was therefore carried out, revealing no signs of intra- or extra-hepatic cholestasis, besides those already suggested by the steadily normal values of GGT and the only slight ( $\leq 2$  times the URL)

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**Figure 1:** Trend of laboratory tests in the studied patient. (A) Serum liver enzymes: alanine aminotransferase (blue line), alkaline phosphatase (green line) and  $\gamma$ -glutamyltransferase (red line). (B) Total bilirubin (green line), conjugated bilirubin (blue line) and unconjugated bilirubin (red line) in serum, respectively. URL, upper reference limit.

elevation of ALP in the serum (Figure 1). In the following weeks, bilirubin in the serum started to slowly decrease, reaching 157.3  $\mu\text{mol/L}$  for TB and 114.6  $\mu\text{mol/L}$  for CB, respectively, the day of hospital discharge (May 29, 2017). Seven days later (15 weeks after the AHAV onset), a further laboratory evaluation showed a TB concentration of 95.8  $\mu\text{mol/L}$  and a CB concentration of 66.7  $\mu\text{mol/L}$ , indicating their normalization trend (Figure 1).

To explore the possible contribution of genetics on the anomalous persistence of marked hyperbilirubinemia, we conducted a genetic analysis. The patient signed a written informed consent before genotyping. We investigated the major functional variants that map into the genes involved in the glucuronidation and in the cycle of bilirubin efflux and bilirubin re-uptake. Genomic DNA was isolated from the peripheral blood cells using an automatic DNA extraction system (Maxwell 16 System,

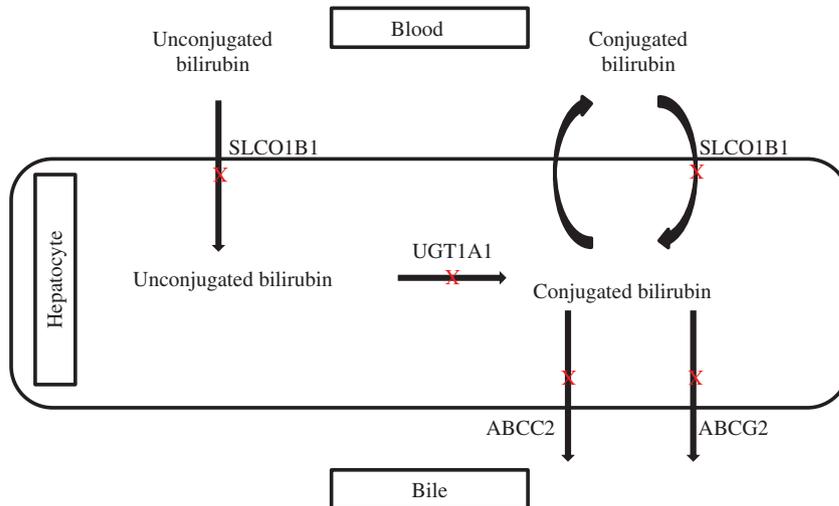
Promega), according to the manufacturer's instructions. All genotypes were determined by real-time PCR, using a panel of LightSNiP from TIB-MolBiol on LightCycler 480 (Roche Diagnostics). First, we investigated the presence of the *UGT1A1*\*28 (rs8175347) allele, which contains an extra-TA repeat in the TATA box promoter region: 7 (TA) in \*28 allele, compared to six (TA) repeats in the common \*1 allele. This extra-TA repeat decreases the rate of transcription of the *UGT1A1* gene and thus decreases the enzyme activity and the glucuronidation of bilirubin [5]. Regarding the efflux transporters, we checked the presence of the functional single nucleotide polymorphisms (SNP) in the *ABCC2* (-24C>T/rs717620) and *ABCG2* (c.421C>A/rs2231142) genes, which have been found to influence transport activity in several studies [6, 7]. Finally, as *SLCO1B1* is predominantly expressed at the basolateral membrane of hepatocytes, we investigated the presence of the 521T>C variant, located in exon 5 of the gene (rs4149056), resulting in decreased uptake transport activity [8]. The genetic test of the patient revealed the presence of *UGT1A1*\*28 in the homozygous state and *ABCC2* -24C>T, *ABCG2* c.421C>A and *SLCO1B1* 521T>C in the heterozygous state (Figure 2A). The simultaneous presence of all the defective alleles may explain the occurrence of the extremely high levels of bilirubin and of their unexpectedly prolonged persistence observed in our patient. We hypothesize that the impaired apical membrane efflux may cause an activation of compensatory pathway for CB efflux into sinusoidal blood across the basolateral membrane, but, due to the presence of SNP in the *SLCO1B1*, the hepatic reuptake may be reduced (Figure 2B). Moreover, functional SNP mapping into the *SLCO1B1* gene may also contribute to the reduction of the active transport of UB in the hepatocyte and therefore influence serum bilirubin concentrations in association with the well-known effect of the *UGT1A1*\*28 allele (Figure 2B).

Krawczyk et al. previously described a possible association between two polymorphisms in the hepatocanalicular bile salt transporter ABCB11 and phospholipids transporter ABCB4 and severe liver cholestasis following AHAV [9]. In our patient, due to the absence of cholestasis as suggested by unchanged or slightly modified serum concentrations of enzymatic markers of cholestasis (ALP and GGT) and confirmed by MRCP, we speculated for the first time that the severe and prolonged mixed hyperbilirubinemia may derive from a genetic predisposition causing a metabolic deficit. A dual hereditary jaundice, due to a compound defect of bilirubin conjugation (Gilbert syndrome/*UGT1A1*) and transport (Dubin-Johnson syndrome/*ABCC2*) was previously described [10]; however, our case presented two

A

Gene	Genetic variant	MAF (%)	Case genotype	Effect of genetic variant on bilirubin pathway
<i>UGT1A1</i>	*28/rs8175347	31	*28/*28	↓ bilirubin conjugation
<i>ABCC2</i>	-24C>T/rs717620	21	CT	↓ bilirubin excretion into the bile
<i>ABCG2</i>	c.421C>A/rs2231142	9	CA	↓ bilirubin excretion into the bile
<i>SLCO1B1</i>	c.521T>C/rs4149056	16	TC	↓ uptake into the hepatocyte

B



**Figure 2:** Bilirubin metabolism in studied patient.

(A) Results of performed genetic analyses. (B) Liver bilirubin pathway, with genetic defects detected in the studied hyperbilirubinemic patient. Unconjugated bilirubin is passively and actively absorbed into the liver. Defective *SLCO1B1* may reduce the active transport of unconjugated bilirubin into hepatocytes. In the hepatocytes, bilirubin is conjugated to form bilirubin glucuronide by *UGT1A1* and then excreted into the bile by *ABCC2* and *ABCG2*. Due to the decreased activity of *ABCC2* and *ABCG2*, conjugated bilirubin back into the blood. Here, the defective activity of *SLCO1B1* cannot mediate its hepatic reuptake. Red crosses indicate defective gene activity. MAF, minor allele frequency ([www.ensembl.org](http://www.ensembl.org)).

other additional hereditary defects in the *SLCO1B1* and *ABCG2* genes.

In conclusion, in our opinion the concomitant detection of defective variants of genes involved in the bilirubin pathway was able to explain the hampered resolution of the hyperbilirubinemia in our patient with pre-existing AHAV. Although other genetic variants or other genes involved in the hepatic cycle of bilirubin can add to our findings, our observation highlights the importance of the molecular mechanisms underlying a hyperbilirubinemia condition. Further investigations are needed to define the proportion of patients with this specific genetic profile, who may show prolonged severe hyperbilirubinemia after AHAV infection.

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