Identification of a novel deletion in UDP-glucuronosyltransferase gene in a patient with Crigler–Najjar syndrome type I

Dear Editor:

Unconjugated bilirubin (UCB) is formed by the catabolism of heme and owing to its very low water solubility (<70 nM), over 99.9% is tightly bound to albumin. During hyperbilirubinemia, slightly elevated unbound concentrations may become neurotoxic, if UCB hepatic clearance is impaired. After uptake by the liver, UCB is conjugated with either one or two molecules of glucuronic acid converting UCB in water soluble compounds [1]. Hepatic glucuronidation of bilirubin is catalyzed by isoenzyme 1A1 of uridine 5'-diphosphate-(UDP)-glucuronosyltransferase (UGT1A1), which is essential for its efficient biliary excretion. Genetic alterations causing absence, or severe reduction, of UGT1A1 enzymatic activity result respectively in Crigler–Najjar syndrome (CNS) type I and type II [12]. CNS types I and II (MIM #218800 and MIM #606785) can be differentiated by the bile profile of bilirubin species and by evaluating the response to phenobarbital treatment or other enzyme-inducing agents [2]. CNS type I patients have higher UCB and lower percentages of bilirubin monoglucuronide (MGB) and diglucuronide (DGB) in bile than is the case CNS type II (CNS type I: 91±10% UCB, 9±11% MGB and 1±1% DGB; CNS type II: 37±9% UCB, 56±13% MGB and 8±5% DGB) [3]. In addition, serum bilirubin levels in patients with CNS type II can be lowered by phenobarbital treatment while CNS type I hyperbilirubinemia is unresponsive to that therapy [2]. Diagnosis of CNS type I patients is of major relevance because at the present time, liver transplantation is the sole curative treatment.

The UGT1A1 enzyme is encoded by five exons located at the 3' end of the UGT1A locus and is expressed mainly in the liver, and to a lesser extent in the biliary tract, duodenum and colon. The first mutation described in this gene, in 1992 [4], was a nonsense mutation found in homozygosity in a patient with CNS type I. To date, more than 90 mutations causing CNS have been identified, most of which are missense or nonsense mutations [5]. The phenotypes of CNS types I and II are usually associated with homozygosity or compound heterozygosity for nonsense and/or missense mutations. A combination of point mutations and abnormal TA repeat numbers in the promoter region of the UGT1A1 gene has also been described in these forms. This TA duplication in the repetitive TATA-box sequence of the gene promoter, which normally consists of six TA repeats, was found to be the main cause of Gilbert syndrome (associated with a small reduction in UGT1A1 enzymatic activity) in all reports based on population studies. Deletions in the UGT1A1 gene are rarely described in the literature [5].

We report the molecular characterization of a Portuguese patient presenting clinical and biochemical features of CNS type I since neonatal period.

The infant was born at full term after an uneventful pregnancy, by eutocic delivery, weighing 3360 g, from non-consanguineous parents and without family history of jaundice. Serum total bilirubin was 15 mg/dL on the second day of life. The child was exposed to intensive phototherapy on the 15th day, when she presented 28.8 mg/dL of serum total bilirubin and 0.8 mg/dL of serum conjugated bilirubin, attesting that the unconjugated species was the predominant one.

At 5 weeks of age, total/conjugated bilirubin in serum was 34.7/0.9 mg/dL (maximal detected value). Enzymatic induction by phenobarbital (20 mg/kg followed by daily administration of 5 mg/kg) was inefficient in significantly reducing the serum UCB concentration. To better characterize the patient, and since determination of hepatic glucuronyl transferase does not differentiate CNS type I and type II patients, determination of the bile profile of bilirubin species was performed. The relative amounts of biliary UCB and bilirubin conjugates were measured by alkaline methanolysis followed by thin-layer chromatography [6] and expressed as % of total bilirubin. We found 90% of UCB, 6% MGB and 4% DGB supporting the diagnosis of CNS type I, despite the slight elevation in DGB percentage when compared with the reported values for CNS type I patients.

Thus, after informed consent, genetic analysis of the patient and the parents was performed as previously described [7]. Sequencing of the five exons of the UGT1A1 gene from the patient revealed the presence of a novel 24 bp deletion (c.609_632del) encompassing the first exon. Both parents were found to be carriers, thereby confirming homozygosity in the patient (Fig. 1). Screening for the TA duplication in the promoter region of UGT1A1 showed a normal number of repeats in the patient and in her parents. None of the three presented the c.-3279T→G polymorphism in the phenobarbital-responsive enhancer module of UGT1A1 gene, known to be associated with Gilbert syndrome.

This novel gross c.609_632del mutation predictably gives rise to an internally deleted polypeptide (p.His203_Lys211delinsGln). The deletion is located in exon 1, encoding the substrate specific region of bilirubin-UDP-glucuronosyltransferase may result in an unstructured protein [8].

The patient has been treated with daily phototherapy (NeoBLUE®) and ursodesoxycholic acid (15 mg/kg/day) and the value of the last serum total bilirubin concentration was 10.6 mg/dL. The child is presently 2 years old and does not reveal any psychomotor or neurological impairment. Nevertheless, since kernicterus may suddenly develop, liver transplantation should be considered as a therapeutic option.

Additionally to extend our knowledge of the spectrum of disease variants, the identification of this deletion in exon 1 of the UGT1A1 gene may contribute towards a better understanding of the molecular pathology of the disorders characterized by severe unconjugated hyperbilirubinemia, and potentially be used as an antenatal screening tool to identify the affected offspring.

References

Fig. 1. Novel homozygous mutation in exon 1 of the UGT1A1 gene (c.609_632del) found in a Portuguese patient with Crigler–Najjar type I. (A) Family pedigree. (B) Sequence analysis revealing homozygosity in the patient and heterozygosity in the mother for the 24 bp deletion (c.609_632delCATGACCTTCTGCAGCGGGTGAA).