Crigler-Najjar syndrome: a case report

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Background: Crigler-Najjar syndrome (CN) clinically manifests as intense unconjugated hyperbilirubinemia without evidence of hemolysis. At present, over 90 genetic variations such as mutations, insertions, or deletions have been described in the five exons of the UDP-glucuronosyltransferase (UGT1A1) gene responsible for defect of bilirubin conjugation.

Objective: To report a case of a female CN type I child who presented with unconjugated hyperbilirubinemia, normal liver function tests, and normal ultrasonographic images of the liver.

Results: Peak total bilirubin in this patient was 27.6 mg/dL. She developed Kernicterus despite prolonged daily home phototherapy. Exons 1-5 of the UGT1A1 gene were amplified by polymerase chain reaction (PCR) and the UGT1A1 gene was screened for mutations by direct DNA sequencing. Molecular genetic analysis showed that this patient was homozygous for a nonsense mutation at nucleotide number 715 (715C→T) in exon 1 resulting in the replacement of glutamine (CAG, amino acid 239) by a stop codon (TAG).

Conclusion: Detection of this genetic defect is essential for gene therapy and can be used as a prenatal screening test to identify the affected offspring.

Keywords: Crigler Najjar, unconjugated hyperbilirubinemia, UGT1A1 gene.

Crigler-Najjar syndrome (CN) was first described in 1952 in Maryland, USA by Crigler JF and Najjar VA as congenital familial non-hemolytic jaundice with kernicterus [1]. During the first days of life, the syndrome clinically manifests as intense unconjugated hyperbilirubinemia without evidence of hemolysis. Autosomal recessive inheritance has been postulated in CN syndrome [2-6]. The syndrome is caused by mutations in the UDP-glucuronosyltransferase gene (UGT1A1), which is located on chromosome locus 2q37.

Two types of CN syndrome, type I and II, have been described. In type I patients, bilirubin UDP-glucuronosyltransferase (UDPGT) is totally lacking while in type II, it is partially deficient. Consequently, patients with CN type II suffer from less jaundice, less neurological impairment, and show a fair response to phenobarbital in that their serum bilirubin levels decrease by at least 25% [7]. In contrast, CN type I is usually fatal with kernicterus as the common cause of death at the age of 1-2 years [8-10]. In type I, total bilirubin levels usually range from 20-45 mg/dL, which is mainly unconjugated hyperbilirubinemia. In type II, on the other hand, total bilirubin amounts to 6-20mg/dL. In addition, low UDPGT synthesis due to a defect in the promoter of the UGT1A, causes Gilbert’s syndrome with total bilirubin levels of 1-6 mg/dL. Neurological impairment of CN type I patients includes deafness, oculomotor palsy, ataxia, choreoathetosis, spasticity, mental retardation, motor impairment, seizure, and death [11]. Histopathology shows bilirubin staining of basal ganglia (globus pallidus), subthalamic nuclei, and cranial nerve nuclei. Electroencephalograms (EEG) are abnormal displaying slow background activity with paroxysmal discharge and multimodal evoked potentials. EEG is more sensitive in neurotoxicity and kernicterus evaluation for unconjugated hyperbilirubinemia.
The prevention of neurological sequelae needs to be evaluated. Prior to neurological impairment, liver transplantation and gene therapy may be considered as part of the patients' treatment.

Herein, we report a case of CN type I with molecular genetic methods used to ascertain the diagnosis and review the novel treatment modules.

Case report

A 53-day-old female infant was referred from Songkla provincial hospital, in the south of Thailand. She was admitted at the Pediatric ward of King Chulalongkorn Memorial hospital with a history of severe prolonged hyperbilirubinemia since the first two days of life.

The pregnancy had been uncomplicated, the fetus was carried to term and born by vaginal delivery at the local hospital. At prenatal screening, her mother's blood group was noted as A, Rh-negative. Serum test for hepatitis B surface antigen was negative. She had no family history of liver and gastrointestinal diseases, no history of consanguinous marriage. On the second day of life, the infant developed jaundice. She had been feeding well, had good urine output, and had not been febrile. On physical examination, her weight was 2,940 grams. The infant was a non-dysmorphic female. The abdomen was soft without palpable mass. All other examination results were normal.

Single phototherapy was administered for two days. She was discharged home receiving no medication. At the age of 10 days, the infant developed deep jaundice, with serum total bilirubin up to 25 mg/dL. Subsequently, single phototherapy was administered for three days. The urine color was normal. The stool color was yellow. Except for profound jaundice, no other abnormal findings became apparent on physical examination. Complete blood count showed normal hemoglobin, adequate platelet count and no evidence of hemolysis. Specimens of blood obtained for culture proved negative. The infant’s blood type was O, Rh-positve. Reticulocyte count was within the normal limit. Glucose-6-phosphate dehydrogenase was within the normal limit. Coombs’ test was negative. Thyroid function was within the normal range. Screening for antibodies to toxoplasmosis, rubella, and cytomegalovirus infections produced negative IgM results.

On day 20, she still had severe jaundice and was transferred to a secondary care hospital where intensive phototherapy was administered for 10 days. Abdominal ultrasonography did not show any abnormality nor evidence of intrahepatic biliary dilatation. Phototherapy was discontinued, and phenobarbital was started at 5 mg per kilogram per day. After ten days of double surface phototherapy, the patient’s total bilirubin decreased to 13.8 mg/dL.

On day 53, she was still jaundiced and was transferred to a tertiary care center, King Chulalongkorn Memorial Hospital. Her weight was 4,215 grams (50th percentile), length 51 cm (40th percentile) and head circumference 36 cm (50th percentile). She was exclusively breast-fed. Complete blood count showed Hb 12.3 g/dL, Hct 37.5%, WBC 11,670/μL (PMN 14% L73% Mo 8% Eo 3% Ba 2%), and platelets 461,000 /μL. Blood chemistry revealed BUN 5 mg/dL, creatinine 0.4 mg/dL, Na 135 mEq/L, K 4.5 mEq/L, Cl 108 mEq/L, and HCO3 15 mEq/L. Liver function test results were SGOT 21 U/L, SGPT 14 U/L, total bilirubin 23 mg/dL, direct bilirubin 2 mg/dL, alkaline phosphatase 387 U/L, albumin 3.8 g/dL, and globulin 4.2g/dL. Blood coagulation was within the normal range. Peak total bilirubin was 27.6 mg/dL. Intensive phototherapy was administered. Once her total bilirubin decreased to 18.3 mg/dL, she was discharged at the age of 73 days and received phototherapy at home.

The persistent unconjugated hyperbilirubinemia in the absence of hemolysis and liver dysfunction suggested Crigler-Najjar syndrome type I. Her blood samples were subjected to molecular genetic diagnosis.

Molecular genetic diagnosis

After informed consent had been granted by the parents, peripheral blood (3 mL) was obtained from the infant and DNA was extracted by standard methods. All entire coding regions of UGT1A1 were amplified by standard PCR and nested PCR (Table 1). The PCR products were treated with ExoSAP-IT (USB Corporation, Cleveland, USA), according to the manufacturer’s recommendations, and sent for direct sequencing at Macrogen Inc. (Seoul, Korea). Analyses were performed by Sequencer 4.2 (Gene Codes Corporation, Ann Arbor, USA).

Direct sequencing revealed that the patient was homozygous for a C>T substitution at nucleotide 715 (c.715C>T) in exon 1, resulting in a change from glutamine to codon 239 to a premature stop codon (Q239X) [12]. The mutation at this position has been previously reported in a patient with Crigler-Najjar syndrome (Fig. 1).
Clinical course

At the age of three years, she still received phototherapy at night-time. She had clinical symptoms and signs of kernicterus. She was able to walk without being assisted, was able to talk only 6-8 unintelligible words without meaning. She suffered from choreoathetosis in both hands and generalized seizures two-three times per month, and had developed clinical kernicterus. Electroencephalogram during awakening revealed generalized slow spike and waves supporting the diagnosis of generalized epileptic disorder and mild diffuse encephalopathy. Her epileptic disorder was controlled with phenobarbital. Our teams (general pediatrician, pediatric hepatologist, and care team nurses) have discussed the pathology, clinical course, disease prognosis, and treatment plan with her parents. We have raised the option of orthotopic liver transplantation for this child. Kernicterus had caused irreversible brain damage and thus, the treatment was only supportive treatment in addition to life-long phototherapy and seizure control medication.
Discussion

Cloning of the UGT1 gene in region q37 of chromosome 2 [13, 14] made genetic diagnosis of the CN syndrome possible. It was found to result from mutations in one of the five exons of the gene coding for bilirubin exon 1 of UDP-glucuronosyltransferase and exons 2-5 of the UDP-glucuronosyltransferase 1 locus, the bilirubin glucuronidation isofrom of UDP glucuronosyltransferase 1, the bilirubin glucuronidation isofrom of UDP glucuronosyltransferase. In CN type I, mutations were detected in exon 1, 2, 3, at the 3/4 intron-exon boundary, and exon 4. The mutations comprise missense mutations, nonsense mutations, nucleotide deletions, mutations leading to a premature stop codon, mutations leading to frameshifts, and splice site mutations. As a result of these mutations, the enzymes encoded by the respective exons could not be produced. Phenobarbital could not enhance the expression of bilirubin UDP-glucuronosyltransferase 1 in CN type I and thus, patients with the type I syndrome did not respond to phenobarbital therapy. In this case, we found a premature stop codon at codon 239 (Q239X) or C715T causing a CAG to TAG mutation. In CN type II, the mutations have been located in exons 1, 2, and 5. In Gilbert’s syndrome, the genetic abnormality was located at the promoter of the gene, in that the TATA box in the promoter region of exon 1 contains 7 instead of 6 TA repeats, hence [TA] 7TAA [15, 16]. There have been reports of missense mutations at nucleotide 211 (G211A) in exon 1 resulting in G71R and a C to A mutation at nucleotide 686 translated into P229Q [17, 18].

CN type II patients respond to medicinal treatment in the form of phenobarbital administration, which reduces bilirubinemia after two-three weeks of treatment [19], whereas CN type I patients show no response to phenobarbital and require phototherapy (10-12 hours/day) until they receive a liver transplant [20, 21]. Liver transplantation is a curative treatment. Bilirubin encephalopathy could be reversed after liver transplantation within the first few months. Prior to transplantation, total bilirubin in CN type I patients should be kept below 20.3 mg/dL with daily phototherapy. Full blown kernicterus is irreversible and these children should not be considered for liver transplantation. Orthotopic liver transplantation (OLT) presents a curative for CN [22, 23] yet, up to 15% of OLT patients require re-transplantation because of chronic rejection and fibrosis of the graft in the long term [24]. Auxiliary liver transplantation (ALT) is another curative procedure but it constitutes an invasive and difficult procedure and the functional mass of liver transplanted does not suffice to cure the liver impairment [25]. Liver cell therapy (LCT) represents a new alternative treatment for metabolic liver disease such as CN, and constitutes an intermediate between whole organ transplantation and gene therapy. The new cells can be infused into the diseased liver, thus establishing enzyme activity and restoring bilirubin metabolism [26]. Subsequent to liver cell therapy, the duration of phototherapy could be reduced (10 to 8 hours). Yet, LCT has its limits due to incomplete and time dependent metabolic control due to immunological cell interaction, impaired donor cell quality, and poor repopulation rates [27]. Other medical treatments include agar [28] as a bilirubin-trapping agent. Oral calcium supplements augment the efficiency of phototherapy [29]. Treatment with tinstepotoporphyrin (or zinc-mesoporphyrin) helps decrease the serum bilirubin temporarily and thus, shortens the duration of phototherapy [30].

Research conducted on a model CN syndrome in Gunn rats demonstrated that after intraperitoneal injection of antilymphocyte serum followed by intrathyemic inoculation of recombinant adenovirus carrying the human UGT1A1 gene, the rats became tolerant and we observed that the UGT1 enzyme was expressed in the liver [31]. After one year, serum bilirubin levels were reduced to 64% [32].

In conclusion, we have reported a case of CN type I with the clinical syndrome of severe unconjugated hyperbilirubinemia since birth. She was treated by phototherapy and developed kernicterus. Diagnosis was ascertained by molecular genetics, which detected a premature stop codon at exon 1. Prompt diagnosis of this syndrome at a very young age (newborn) prior to the onset of neurological complications and treatment with liver transplantation can help the patient lead a normal live.

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References


