

# Mutation Analysis in Crigler–Najjar Syndrome Type II—Case Report and Literature Review

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**Crigler–Najjar syndrome (CN) is a congenital defect in bilirubin conjugation due to complete or partial deficiency of uridine 5'-diphosphate-glucuronosyltransferase (UGT). It is of two types: CN type I and CN type II. Patients with CN type II present with indirect hyperbilirubinemia in adulthood. We report a CN type II with homozygous mutation in UGT1A1 gene. This is the first case report of mutation analysis in CN type II from India. (J CLIN EXP HEPATOL 2011;1:204–206)**

Crigler–Najjar syndrome (CN) is an inborn error of metabolism due to defect in the activity of the hepatic enzyme bilirubin uridine 5'-diphosphate-glucuronosyltransferase (UGT). It is characterized by unconjugated hyperbilirubinemia without the evidence of hemolysis. There are two types, CN type I is severe and CN type II is milder in severity.

## CASE REPORT

A 16-year-old male presented to the outpatient department with complaints of recurrent jaundice noticed since age of 10 years. There was no history of pruritus, clay colored stools, or high colored urine. There was no history of easy fatigability, pain abdomen, or fever. Patient was icteric, there was no pallor, hepatosplenomegaly, or features of liver cirrhosis.

Investigations revealed total serum bilirubin—13.4 mg/dL (conjugated fraction 0.7 mg/dL and unconjugated fraction 12.7 mg/dL), aspartate aminotransferase (32 U/L, normal <40 U/L), alanine aminotransferase (normal 40 U/L), alkaline phosphatase (125 U/L, normal <390 U/L),  $\gamma$ -glutamyl transpeptidase (24 U/L, normal <49 U/L), and prothrombin time (13.9 s, control 13.2 s). There was no evidence of intravascular hemolysis and

serological markers (HBsAg, anti-HCV, anti-nuclear antibody, smooth muscle antibody, liver-kidney microsomal antibody) were absent. Serum ceruloplasmin level was normal. Ultrasound abdomen showed normal liver and there was no evidence of biliary obstruction.

In view of the raised indirect bilirubin with normal liver function tests and no hemolysis, a clinical diagnosis of Gilbert's disease or CN type II was made. The CN type II was considered more likely because serum bilirubin was >6 mg/dL. Mutation analysis of UGT1A1 gene was done to confirm the diagnosis.

## Mutation Analysis of UGT1A1 Gene

Genomic deoxyribonucleic acid (DNA) was extracted from peripheral blood sample. Sequencing of UGT1A1 gene was done as described earlier.<sup>1–3</sup> Mutation analysis of UGT showed him to be a homozygote for Y486D in exon 5 (Figure 1), which confirmed the clinical diagnosis of CN. Polymorphism in TATA promoter region was also studied by previously described methods with slight modifications.<sup>4</sup> The promoter TA repeat region was carrying normal TA repeats (TA)<sub>6</sub>/(TA)<sub>6</sub>.

He was given phenobarbital in the dose of 30 mg twice daily for 6 months. Serum bilirubin decreased to 4.5 mg/dL at 6 months.

**Keywords:** Crigler–Najjar syndrome, indirect hyperbilirubinemia, UGT1A1 gene

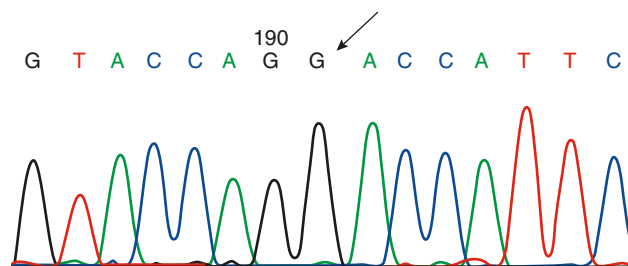
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**Abbreviations:** CN: Crigler–Najjar syndrome; DNA: deoxyribonucleic acid; HBsAg: hepatitis B surface antigen; HCV: hepatitis C virus; UGT: uridine 5'-diphosphate-glucuronosyltransferase

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**Figure 1** Mutation analysis of UGT1A1 gene, c.1456T >G; p.Y486D.

## DISCUSSION

Crigler–Najjar syndrome is an inborn error of metabolism due to defect in enzyme UGT. It is an autosomal recessive disorder and the clinical hallmark is unconjugated hyperbilirubinemia in adults without the evidence of hemolysis and with normal liver function.

The hereditary hyperbilirubinemias are divided into those resulting in predominantly unconjugated hyperbilirubinemia, such as Gilbert or Arias syndrome and CN type I and type II and those resulting in predominantly conjugated hyperbilirubinemia, such as Dubin–Johnson syndrome and Rotor syndrome.<sup>5</sup>

Crigler–Najjar syndrome types I and II are rare diseases with only few 100 cases described in the literature.<sup>5</sup> Its incidence is estimated to be 1 in 1,000,000 births. There is no particular racial or gender predisposition. The more common inherited unconjugated hyperbilirubinemia, Gilbert syndrome, affects approximately 3–7% of adult population.<sup>6</sup>

All three types of hereditary unconjugated hyperbilirubinemia are distinguished on the basis of serum bilirubin level, response to phenobarbitone, and the presence of kernicterus. Total serum bilirubin level ranges between 1 mg/dL and 6 mg/dL in Gilbert syndrome, between 20 mg/dL and 45 mg/dL in CN type I, and between 6 mg/dL and 20 mg/dL CN type II. The CN type I patients do not respond to phenobarbital treatment and only traces of bilirubin glucuronides can be found in their bile. In CN type II, phenobarbital treatment lowers the serum bilirubin levels by >30% and bilirubin glucuronides are present in bile. Analysis of liver tissue reveals residual activity of UGT activity in CN type II and the absent activity in CN type I. Our patient had bilirubin level of 13.4 mg/dL, which came down after treatment with phenobarbital.

Both Gilbert syndrome and CN are caused by the mutation in UGT1A1 gene and UGT1A subfamily enzymes are encoded by a single gene locus that spans approximately 200 kb on chromosome 2q37.<sup>7</sup> The UGT1A1 locus includes 13 unique ‘first’ exons (four of which are pseudogenes) and their associated promoters, which are spliced separately to four common exons (exons 2–5). As a result, the nine translated UGT1A proteins (UGT1A1 and 1A3–10) have unique amino-terminal domains but share an identical carboxyl-terminus domain of 245 residues.

Our patient had mutation in the homozygous state (Y486D) (UGT1A1\*7). This results in the substitution of asparagine instead of tyrosine. This mutation has been described earlier in Asian population.<sup>8</sup> The promoter region of this patient was carrying a normal number of repeat elements. Homozygosity for UGT1A1\*7 (often together with homozygosity for UGT1A1\*6) has been observed in Asian subjects with the more severe CN type II.<sup>9,10</sup>

Mutation analysis in the patient confirmed the clinical diagnosis. The clinical importance of diagnosing CN type II is in reassuring these patients because high bilirubin levels can be considered as a symptom of liver failure and

hence, related anxiety. From the point of view of physicians, it may present a diagnostic challenge. Phenobarbitone is of some help in reducing bilirubin levels.

There has been case reports of CN type II from India.<sup>11</sup> Pregnancy in CN type II patients has also been described from India.<sup>12,13</sup> However, this is the first case report of CN type II along with mutation analysis.

Mutation analysis of UGT1A1 gene has been described earlier in an Indian girl with CN type I.<sup>14</sup> The patient was homozygous for a novel mutation at nucleotide 392 in exon 1. Substitution of thymine for cytosine changed the codon from leucine to proline at position 131 of the corresponding protein (c. 392T > C: p. L131P).

Farheen et al<sup>15</sup> in a large study in patients with Gilbert syndrome have described the pattern of mutations in Indian population. Among Gilbert syndrome patients, 80% were homozygous for the TA insertion, which was several-fold higher than reports from other ethnic groups. The mean unconjugated bilirubin level was elevated among individuals with only one copy of this insertion, which was not significantly different from those with two copies. Many new DNA variants in UGT1A1 gene were discovered, including a trinucleotide (CAT) insertion in the promoter found in a subset (10%) of Gilbert’s syndrome patients, but not among normal controls.

Crigler–Najjar syndrome type I is caused by nonsense (or frameshift) and missense mutations both in homozygosity and in the compound heterozygous state.<sup>16</sup> The milder phenotype in CN type II patients seems to be mainly the result of homozygosity for missense mutations,<sup>17</sup> and more rarely of the genetic compound for nonsense (or frameshift) and missense mutations or an interaction between missense mutations and a homozygous TA insertion in the TATAA promoter element. The normal TA promoter element is A (TA)<sub>6</sub> TAA whereas after insertion this becomes A(TA)<sub>7</sub> TAA. The presence of the TA insertion in the TATAA promoter element of the UGT1A1 gene reduces the expression of UGT. An inverse relationship exists between the number of TA repeats and the UGT1A1 promoter activity.<sup>18</sup> Patients with Gilbert syndrome have polymorphism in the promoter region. In addition to the promoter polymorphism, coding region mutations that result in reduced bilirubin–glucuronidation activity are also known to be associated with the Gilbert syndrome. The most prevalent of these (13–23%) is UGT1A1\*6, which results in the substitution of glycine for arginine at position 71 (G71R) of the UGT1A1 protein.<sup>19–22</sup> Other missense mutations linked to the Gilbert syndrome in Asians include UGT1A1\*7 (Y486D; frequency ca. 3%), UGT1A1\*27 (P229Q; frequency <1%), and UGT1A1\*62 (F83L; frequency ca. 1%).<sup>19–24</sup>

Genetic heterogeneity and the presence of compound heterozygotes complicate any direct approach in prenatal diagnosis. Prenatal diagnosis can be done only in cases where the genotype of the affected family is precisely

known. Prenatal diagnosis by UGT activity is not determinable from the usual fetal tissues used for prenatal diagnosis like amniocentesis, trophoblasts, or fetal blood.

In conclusion, CN type II is a rare disease. Mutation analysis confirms the diagnosis and thus a definite management and counseling can be done.

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