

UGT1a1 Gene Mutations in a Tibetan Patient with Crigler-Najjar Syndrome Type II - Case Report and Literature Review



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Abstract

Crigler-Najjar Syndrome (CNS) is a conjugated hyperbilirubinemia caused by mutations in bilirubin UDP-glucuronosyl transferase (UGT1A1) gene which can transform the unconjugated bilirubin by conjugating it with glucuronic acid. There are two types of CNS. CNS-I is fatal because of the bilirubin encephalopathy combined with severe unconjugated hyperbilirubinemia, while CNS-II is a moderate form of hyperbilirubinemia and can be treated with phenobarbital therapy. The CNS-II has never been reported in Tibetan population. This is the first reported case of Tibetan patient with CNS-II based on the UGT1A1 gene analysis.

Keywords: Crigler-Najjar Syndrome; UGT1A1; Tibetan

Abbreviations: CNS-I: Crigler-Najjar Syndrome type I; CNS-II: Crigler-Najjar Syndrome type II; GS: Gilbert Syndrome; UGT1A1: UDP-Glucuronosyl Transferase

Introduction

Crigler-Najjar Syndrome (CNS) is an autosomal recessive hereditary disorder characterized by moderate to severe unconjugated hyperbilirubinemia without hemolysis and caused by a deficiency of bilirubin UDP-glucuronosyltransferase (UGT1A1) enzyme due to mutations of UGT1A1 gene [1]. CNS can be classified into type I or type II based on the hyperbilirubinemia level and patients' response to phenobarbital treatment. CNS-I is fatal because of the bilirubin encephalopathy with the presence of severe unconjugated hyperbilirubinemia. CNS-II is a moderate form of hyperbilirubinemia and patients have good responses to the phenobarbital treatment [2].

Case Presentation

A 17-year-old Tibetan male presented at the inpatient department with sustained jaundice since his birth. There was no history of fatigability, fever, abdomen pain, pruritus, clay-colored stools, or soy-sauce-colored urine. Physical examination identified severe yellowing staining of skin and sclera. There was no pallor, hepatomegaly, splenomegaly, or feature of liver cirrhosis.

The laboratory examination results for blood stool and urine routines were normal. Biochemical analysis of blood showed that

total serum bilirubin was 321.3 μmol/L (conjugated bilirubin 17.6 μmol/L and unconjugated bilirubin 303.7 μmol/L), alanine amino transferase was 25 IU/L (normal 50 IU/L), aspartate amino transferase was 26 IU/L (normal <40 IU/L), alkaline phosphatase was 125 IU/L (normal <125 IU/L), γ-glutamyl transpeptidase was 18 IU/L (normal <60 IU/L), and prothrombin time was 11.9 s (control 12.8 s).

There was no evidence of intravascular hemolysis. The diagnostic results were negative for all viral markers including HBsAg, anti-HCV, anti-HEV IgA, anti-HAV IgM, anti-CMV, anti-EBV. The testing results with serological antibodies of nuclear, mitochondrial, liver-kidney microsomal and liver cytoplasm were negative too. Serum ceruloplasmin level was 284.0 mg/L. MRI showed no evidence of biliary obstruction and normal liver form. The patient's mother had mild jaundice while his father didn't have any.

Because the patient had the elevated unconjugated bilirubin but had normal liver function and no evidence of hemolysis, a clinical diagnosis of Gilbert's disease or CNS-II was made. CNS-II was more likely because serum bilirubin was >103 μmol/L. Mutation analysis of UGT1A1 gene was carried out for this purpose.

Mutation Analysis of UGT1A1 Gene

The direct sequencing of the UGT1A1 gene was reported previously. This patient carried several point mutations on UGT1A1 Gene (c.211G→A on exon 1 and c.1456T→G on exon 5), mutations that changed glycerine to argine and tyrosine

to aspartic acid (positions 71 and 486 of the UGT1A1 protein) respectively (Figure 1). The promoter TA repeat region in exon 1 carried normal TA repeats A(TA)₆TAA. The combination of homozygous G71R and Y486D mutations in UGT1A1 confirmed the diagnosis of CNS-II.

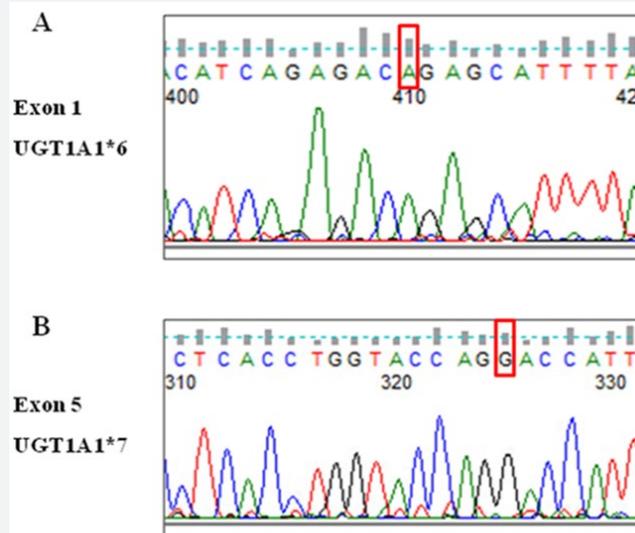


Figure 1: Mutation analysis of UGT1A1 gene. (A) Nucleotide sequence of the mutated section of exon 1. The point mutations of c.211G→A on exon 1, which changed glycerine to argine at position 71. (B) Nucleotide sequence of the mutated section of exon 5. The point mutations of c.1456T→G on exon 5, which changed tyrosine to aspartic acid at position 486.

Discussion

Hereditary hyperbilirubinemias the direct result of genetic defects in enzymes that control the bilirubin metabolism. There are two major types of hereditary hyperbilirubinemias, the un-conjugated hyperbilirubinemia such as CNS-I, CNS-II, and Gilbert syndrome (GS) and the conjugated hyperbilirubinemia such as Dubin-Johnson syndrome and Rotor syndrome [3].

CNS-I, CNS-II, GS are familial un-conjugated hyperbilirubinemias resulted from genetic mutations of UGT1A1 which transforms the un-conjugated bilirubin in to a nontoxic form with the insertion of one glucuronic acid. Genetic mutations of UGT1A1 lead to the absence, severe or moderate reduction of UGT1A1 enzymatic activities (corresponding to CNS-I, CNS-II or GS disease respectively) [4].

The clinical classifications of CNS-I, CNS-II and GS are based on several metrics such as the bilirubin levels, the presence of kernicterus, and the response to phenobarbitone. CNS-I patients' bilirubin level is above 342 μmol/L or higher and the patients do not respond to the Phenobarbital treatment due to the complete absence of UGT1A1 enzyme activity. The patients usually die of kernicterus at early ages. There were case reports of liver transplantations for CNS-I patients. CNS-II patients' bilirubin level is in the range of 103-342 μmol/L due to severe deficiency of UGT1A1 enzyme activity (usually less than 10% of

normal activity). This bilirubin level can be cut by more than 30% after Phenobarbital treatment. GS patients' bilirubin level is usually lower than 103 μmol/L and the patients also respond to Phenobarbital treatment very well. UGT1A1 enzyme activity in those patients is approximately 30 percent of that in normal population.

Crigler-Najjar syndromes are rare diseases, with only 100 cases reported in literatures to date. The incidence of CNS is estimated to be 1 in 1,000,000 births. There were a few cases of CNS-II reported in china [5-7]. Qing Mao's research showed the spectrum of UGT1A1 mutations in 11 CNS-II patients in China [6]. However, CNS-II case has never been reported in Tibetan population. This is the first such case of Tibetan patient with CNS-II and UGT1A1 gene analysis. The incidence of GS is approximately 3-7% of adults.

Both CNS and GS are caused by the mutations of UGT1A1 gene, a single gene at chromosome 2q37. UGT1 gene consists of 13 unique alternate first exons (exons 1) and four common exons (exons 2-5) that encode nine function enzymes involved in the transformation of small lipophilic molecules, such as steroids, bilirubin, hormones, and drugs, into water-soluble and excretable metabolites. UGT1A1 is the only enzyme from the glucuronidation pathway that transforms un-conjugated bilirubin into a nontoxic form by attaching one glucuronic acid [8]. Genetic mutations of UGT1A1 can lead to the absence, severe

or moderate reduction of UGT1A1 enzymatic activity, which eventually result in CNS-I, CNS-II and GS, respectively.

The patient in this study was diagnosed with un-conjugated hyperbilirubinemia and had a compound homozygous mutation in the UGT1A1 gene (p.G71R and p.Y486D). The promoter region of the gene carried normal TA repeats A(TA)₆TAA. The patient was diagnosed as CNS-II based on his medical history and biochemical and genetic analysis. The level of bilirubin fluctuated between 231 μmol/L and 324 μmol/L in this patient, in consistent with the reports that bilirubin levels were always greater than 200 μmol/L for CNS-II patients with compound homozygous mutations (p.G71R and p.Y486D) [6]. The phenotype of this patient was classified as UGT1A1 genotype. Previous study demonstrated that UGT1A1 activity of the heterozygous model of G71R was 60.2 ± 3.5% of that for the wild-type model, while UGT1A1 activities of single homozygous models of G71R and Y486D were 32.2 ± 1.6% and 7.6 ± 0.5% respectively and those of double homozygous models of G71R and Y486D were 6.2 ± 1.6%, respectively [9]. The mutation G71R was mild, since it was observed in the homozygous state in Gilbert syndrome. When this mutation was observed in CNS-II, it always came with

homozygous Y486D mutation [2].

To date, there were almost 40 reported UGT1A1 mutations related to CNS-II. CNS-II diagnosis is usually associated with compound homozygosity or heterozygosity for missense mutations or deletion mutations [10,11]. The compound homozygous mutations (p.G71R and p.Y486D) were the most common mutations in Asian CNS-II patients, and were detected in 14 of 27 East Asian patients, while there were detected only in 1 of 58 Caucasian patients, and in 1 of 14 other races including India and Pakistani [6]. Previous study discovered that the mutation rate in coding region of UGT1A1 was higher among Asians than among Caucasians [12]. The mutation in UGT1A1 might be different among different races. Since there was no previously reported data in Tibetan patient with CNS-II, this case would provide a highly valuable reference. The mutations of UGT1A1 in CNS-II were listed in Figure 2.

To date there were almost 50 UGT1A1 mutations found to be related to CNS-I. CNS-I is caused by nonsense (or frame-shift) or missense mutations or deletion mutations in both homozygosity and compound heterozygous state [10]. The mutations of UGT1A1 in CNS-I were also listed in Figure 2.

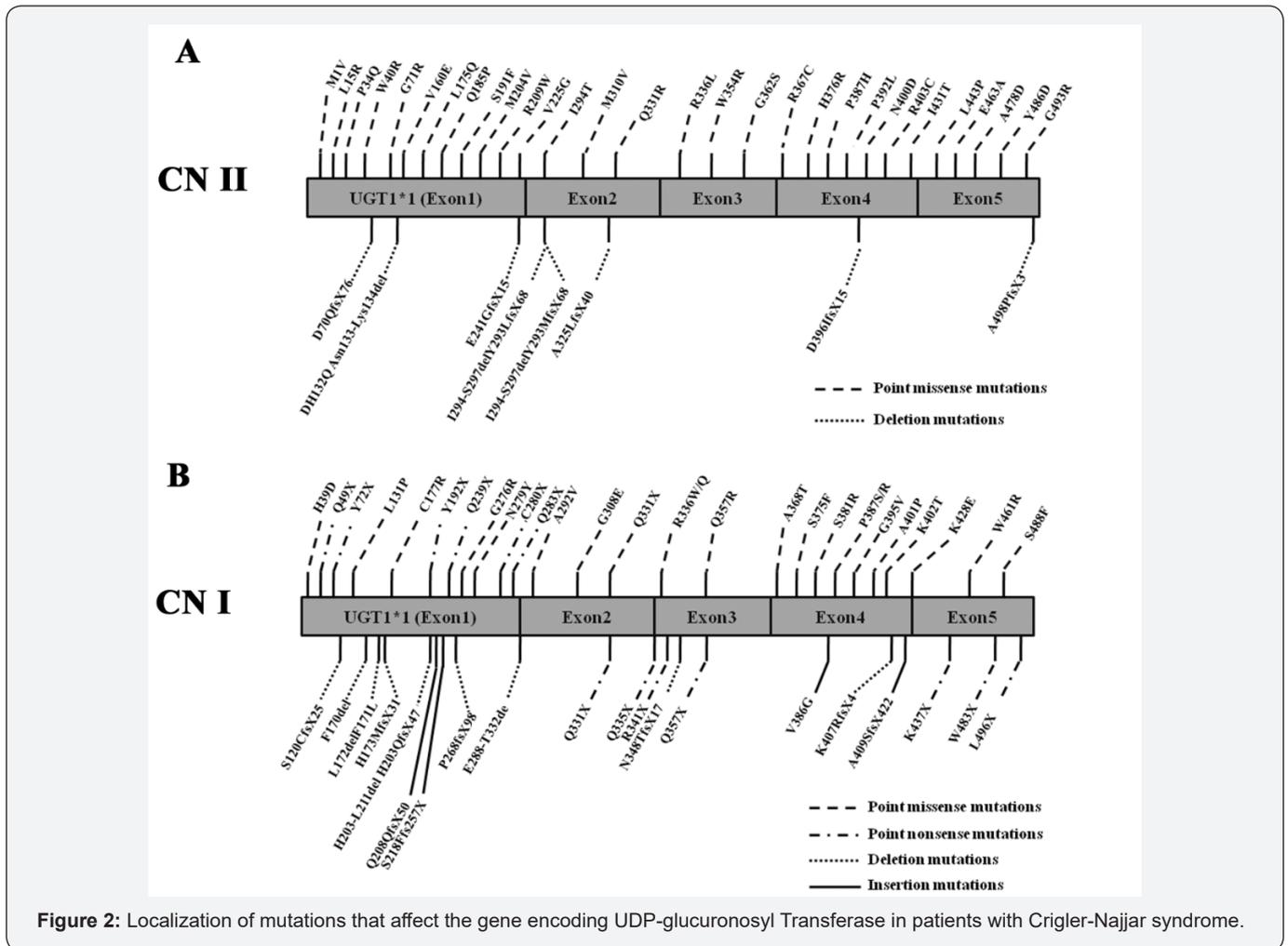


Figure 2: Localization of mutations that affect the gene encoding UDP-glucuronosyl Transferase in patients with Crigler-Najjar syndrome.

80%GS patients were homozygous for the TA insertion in the promoter upstream to the coding sequences. While the normal TATAA element in the promoter has the sequence A(TA)₆TAA, the Gilbert-type promoter has the sequence A(TA)₇TAA [13]. Many mutations (G71R, P229G etc) reported in the coding sequences of UGT1A1 may also cause mild un-conjugated hyperbilirubinemia, consistent with the clinical diagnosis of Gilbert syndrome [10]. El'sio Costa's study suggested that c.-3279T >G polymorphism in phenobarbital responsive enhancer module of UGT1A1 gene could be an additional risk factor for the development of GS [14].

In conclusions, this is the first reported case of Tibetan patient with CNS-II based on the UGT1A1 gene analysis despite the compound homozygous mutations (p.G71R and p.Y486D) in UGT1A1 were reported. Mutation analysis confirmed the diagnosis and a definite management would be carried out.

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