

# Two Different *UGT1A1* Mutations causing Crigler–Najjar Syndrome types I and II in an Iranian Family

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## ABSTRACT

**Background** : Crigler–Najjar syndrome type I (CN-1) and type II (CN-2) are rare hereditary unconjugated hyperbilirubinemia disorders. However, there have been no reports regarding the co-existence of CN-1 and CN-2 in one family. We experienced a case of an Iranian family that included members with either CN-1 or CN-2. Genetic analysis revealed a mutation in the bilirubin UDP-glucuronosyltransferase (*UGT1A1*) gene that resulted in residual enzymatic activity.

**Case report**: The female proband developed severe hyperbilirubinemia [total serum bilirubin concentration (TB) = 34.8 mg/dL] with bilirubin encephalopathy (kernicterus) and died after liver transplantation. Her family history included a cousin with kernicterus (TB = 30.0 mg/dL) diagnosed as CN-1. Her great grandfather (TB unknown) and uncle (TB = 23.0 mg/dL) developed jaundice, but without any treatment, they remained healthy as CN-2.

**Results**: The affected cousin was homozygous for a novel frameshift mutation (c.381insGG, p.C127WfsX23). The affected uncle was compound heterozygous for p.C127WfsX23 and p.V225G linked with A(TA)7TAA. p.V225G-*UGT1A1* reduced glucuronidation activity to 60% of wild-type. Thus, linkage of A(TA)7TAA and p.V225G might reduce *UGT1A1* activity to 18%–36 % of the wild-type.

**Conclusion**: Genetic and in vitro expression analyses are useful for accurate genetic counseling for a family with a history of both CN-1 and CN-2.

**Key words**: Crigler–Najjar syndrome – Gilbert syndrome – bilirubin UDP-glucuronosyltransferase – *UGT1A1*.

**Abbreviations**: CN-1: Crigler–Najjar syndrome type I; CN-2: Crigler–Najjar syndrome type II; GS: Gilbert syndrome; *UGT1A1*: bilirubin UDP-glucuronosyltransferase; WT: Wild type; TB: total serum bilirubin.

## INTRODUCTION

Crigler–Najjar syndrome type I (CN-1, MIM #21880), the manifestation of a defect to the bilirubin UDP-glucuronosyltransferase (*UGT1A1*) gene, is extremely rare and the most severe phenotype of hereditary unconjugated hyperbilirubinemia [1]. Patients with CN-1 develop severe unconjugated hyperbilirubinemia at birth with total serum bilirubin (TB) concentrations >30 mg/dL. In such cases, daily phototherapy is necessary and exchange blood transfusion is sometimes required to avoid bilirubin

encephalopathy (kernicterus), a lethal complication of CN-1. After puberty, hyperbilirubinemia usually worsens, eventually necessitating liver transplantation. A defect to *UGT1A1* also causes mild to moderate phenotypes of hereditary unconjugated hyperbilirubinemia, namely Crigler–Najjar syndrome type II (CN-2, MIM #606785) and Gilbert syndrome (GS, MIM #143500). The ranges of TB in CN-2 (6–20 mg/dL) and GS (1–5 mg/dL) have been reported [2]. Newborns with CN-2 or GS develop severe neonatal hyperbilirubinemia in the early neonatal period and prolonged unconjugated hyperbilirubinemia associated with breast milk feeding (breast milk jaundice) in the late neonatal period, sometimes requiring phototherapy and exchange blood transfusion [3–5]. After the neonatal period, additional therapy is usually not necessary. To evaluate patient prognosis and offer genetic counseling, it is important to differentiate CN-1 from CN-2. Here, we report the case of a family that included some members with CN-1 and others with CN-2. For genetic counseling to the proband's parents, the genetic diagnosis of *UGT1A1* was useful.

## PATIENTS

Our clients (Fig. 1) were a non-consanguineous couple (IV-4 and IV-5) who visited the outpatient clinic of the Medical Genetics Laboratory of Genome, Isfahan University of Medical Sciences (Isfahan, Iran) for genetic counseling because their first female child was diagnosed with severe unconjugated hyperbilirubinemia at the age of 3 days and died at liver transplantation (V-2). Her peak TB was 34.8 mg/dL, and she developed symptoms of kernicterus (i.e., lethargy and infantile hypotonia) and was ultimately diagnosed with CN-1.

The proband's family history included a paternal 4-year-old male cousin who was also diagnosed with CN-1 (V-1). His peak TB was 30 mg/dL. Although he had already undergone liver transplantation, he also developed kernicterus. The proband's paternal great grandfather (I-1) and uncle (VI-1) were also diagnosed with Crigler–Najjar syndrome. However, both patients are healthy. The TB of the paternal uncle was reportedly 23.0 mg/dL, consistent with CN-2.

## METHODS

### Gene analysis of *UGT1A1*

Genomic DNA was isolated from the leukocytes of both clients (IV-4 and IV-5), affected patients (V-1 and V-2), and relatives (III-1, III-2, III-7, IV-2, and IV-3) with their informed consent, and exons, particularly the promoter and enhancer regions of *UGT1A1*, were amplified by polymerase chain reaction, as described elsewhere [5]. The amplified DNA fragments were then sequenced [5].

### Construction of expression vectors and expression of *UGT1A1* in COS-7 cells

The methods used to construct the pCR3.1 expression vector (Invitrogen Corporation, Carlsbad, CA, USA) and conduct the expression study are described elsewhere [6]. The following primers were used to introduce mutations into

the expression vector (the mutation points are underlined): 5'-CTGTGCGACGGGGTTTATTCC-3' for the T to G transversion at nucleotide 674 (c.674T>G) for p.V225G by means of site-directed mutagenesis using the PrimeSTAR mutagenesis basal kit (TaKaRa, Kyoto, Japan). Three models were generated: wild-type (WT) *UGT1A1*, p.V225G *UGT1A1*, and non-transfected cells as controls. Protein content was measured using a Protein Assay Bicinchoninate kit (Nacalai Tesque, Inc., Kyoto, Japan).

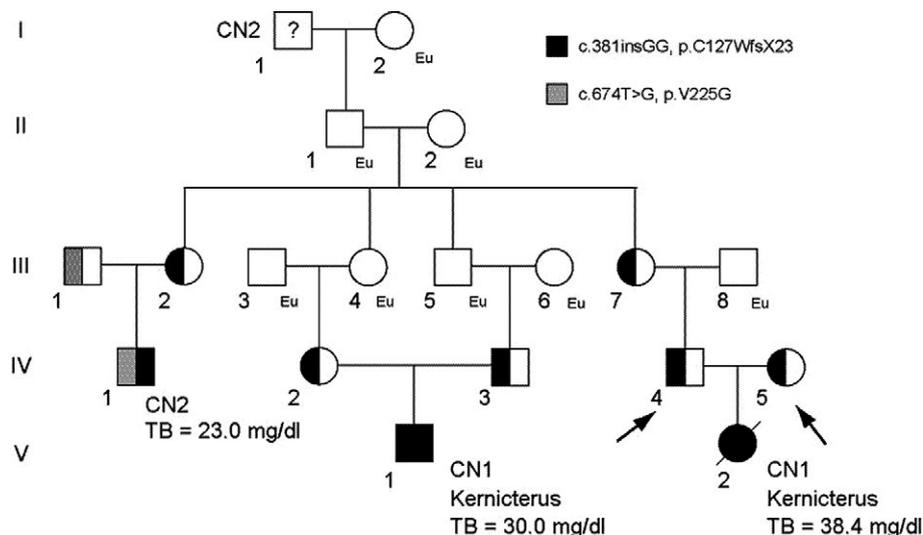
### *UGT1A1* activity and Western blot analyses

The methods used in the *in vitro* expression study and assay for normal and mutated *UGT1A1* activity were conducted as reported previously, with a minor modification. Bilirubin glucuronidation was determined using reverse-phase HPLC analysis, as described by Odell et al. [7]. Enzyme expression was quantitated by Western blot analysis using anti-*UGT1A1* antibody to normalize the enzymatic activity of normal and mutated *UGT1A1*.

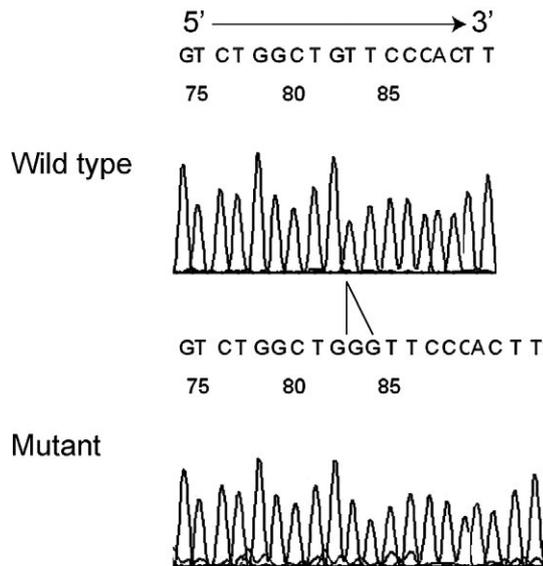
## RESULTS

### Gene analysis

The client couple (IV-4 and IV-5) were heterozygous for a novel insertion mutation, c.381insGG (Fig. 2). The insertion of two guanines at nucleotide positions 381 and 382 in exon 1 changed the codon from cysteine to tryptophan at position 127 of the corresponding protein and generated a stop codon at position 150 (p.C127WfsX23). Thus, their deceased daughter (V-2) should have been homozygous for this mutation. The CN-1 patient (V-1) was homozygous for c.381insGG, and his mother and father (IV-2 and IV-3) were both heterozygous for c.381insGG. The proband's uncle (IV-1) with Crigler–Najjar syndrome was compound heterozygous for c.381insGG and c.674T>G (p.V225G) with A(TA)7TAA in exon 1, which is a TATA box variation in the promoter that recognizes *UGT1A1*\*28 and a known cause of GS. The proband's other



**Fig. 1.** Pedigree of an Iranian family with two different *UGT1A1* mutations. The clients are indicated by arrowheads. Serum bilirubin concentrations, expressed in mg/dL, are shown beneath the symbols.



**Fig. 2.** *UGT1A1* nucleotide sequences amplified from the genomic DNA of the patient. The mutation, an insertion of “GG” at positions 381 and 382 in *UGT1A1* cDNA (c.381insGG), changes the codon from cysteine to tryptophan at position 127 of the corresponding protein and generates a stop codon at 150 (p.C127WfsX23).

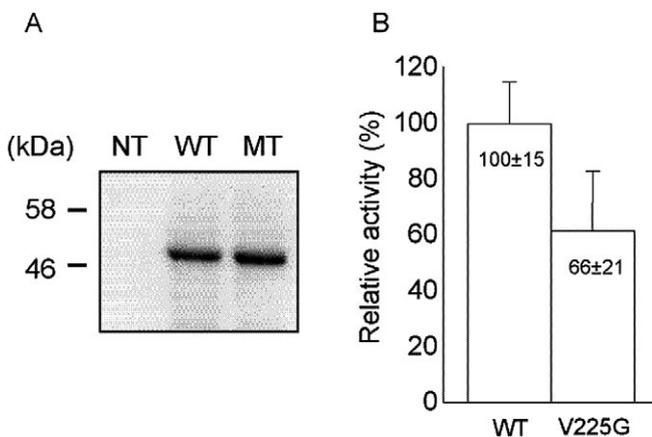
relatives (III-2, III-7, IV-2 and IV-3) were all heterozygous for c.381insGG.

**Expression and identification of UGT1A1**

Western blot analysis revealed a 52-kDa protein band in the WT expression model and p.V225G mutant model, but not in the mock transfection model (Fig. 3A). The relative expression of mutant UGT1A1 was 1.19-fold greater than that of WT UGT1A1. We adjusted enzymatic activity based on these results.

**Enzymatic activity analysis**

The relative glucuronidation activity of p.V225G-UGT1A1 toward bilirubin was reduced to 61% of that of WT UGT1A1 (Fig. 3B).



**Fig. 3.** *In vitro* expression study of p.V225G UGT1A1. A. Western blot analysis revealed a 52-kDa protein band in the WT and p.V225G mutant models, but not in the mock transfection model. B. Relative enzymatic activity of p.V225G-UGT1A1 toward bilirubin was reduced to 61% of that of WT UGT1A1.

**DISCUSSION**

The prognoses of CN-1 and CN-2 greatly differ; thus, a differential diagnosis is dependent on the residual UGT1A1 activities of the two syndromes. Patients with CN-2 have residual UGT1A1 activity (<10% of WT). In contrast, CN-1 mutations extinguish UGT1A1 activity. UGT1A1 is the only enzyme that can catalyze bilirubin. Hence, CN-1 is a lethal condition, and patients with CN-1 require daily phototherapy and possibly exchange transfusions throughout their life to prevent bilirubin encephalopathy. To achieve complete remission, liver transplantation is necessary. In contrast, the prognosis of CN-2 is good. In the neonatal period, infants with CN-2 should receive phototherapy and exchange transfusion, as with CN-1, because these patients usually develop neonatal hyperbilirubinemia and prolonged unconjugated hyperbilirubinemia associated with breast milk feeding (breast milk jaundice). During the neonatal period, UGT1A1 expression in the liver is 1% of that in adults [8]. By the age of 4 months, liver UGT1A1 levels almost reach adult levels, whereas infants with CN-2 develop severe unconjugated hyperbilirubinemia during the neonatal period. After the induction of liver UGT1A1, elevations in serum bilirubin concentration are not severe (6–20 mg/dL). After the neonatal period, treatment is not necessary for patients with CN-2, indicating that an accurate diagnosis to differentiate the two syndromes is necessary for genetic counseling.

In this family, the proband likely had CN-1, as her paternal cousin also had CN-1. However, her paternal uncle and great grandfather should also have had CN-2. For accurate diagnosis and genetic counseling, the genetic analysis of *UGT1A1* among family members was performed. Our clients were heterozygous for p.C127WfsX23 and the affected nephew was homozygous for p.C127WfsX23, suggesting that the clients’ child must have been homozygous for this mutation. On the other hand, the uncle with CN-2 was compound heterozygous for p.C127WfsX23 and p.V225G with A(TA)TAA7. p.V225G UGT1A1 maintains residual enzymatic activity. Thus, the compound heterozygous uncle might be positive for CN-2.

The p.V225G mutation is reportedly the cause of GS [9]. In that report, the *in silico* functional analysis of p.V225G showed that this mutation is benign, suggesting that p.V225G causes GS. However, *in vitro* analysis performed in this study indicated that p.V225G-UGT1A1 maintained 60% residual activity for bilirubin glucuronidation compared to the WT enzyme, suggesting the presence of GS. However, the p.V225G mutation is also linked with the A(TA)7TAA promoter (*UGT1A1*\*28), which is associated with the reduced transcriptional activity of 30%–60% of the WT promoter [10,11]. Thus, the linkage of p.V225G and *UGT1A1*\*28 might reduce UGT1A1 activity to 18%–36%. One of the uncle’s alleles was p.C127WfsX23, which had no glucuronidation activity, whereas the other was p.V225G with A(TA)7TAA. The UGT1A1 activity of a compound heterozygous patient should be about 10% of that of the WT, which is compatible with CN-2. The measurement of residual enzymatic activity via *in vitro* expression activity is helpful to achieve an accurate differential diagnosis.

## CONCLUSION

The genetic diagnosis of *UGT1A1* and measurement of *UGT1A1* activity was useful for the accurate diagnosis and genetic counseling of the Iranian family, which included some members with CN-1 and others with CN-2. The co-existence of p.C127WfsX23 and p.V225G with A(TA)<sub>7</sub> resulted in both syndromes in this family.

**Conflicts of interest:** None to declare.

**Authors' contribution:** Y.M.: concept and design of the study, *UGT1A1* analysis, manuscript drafting; S.N.: *UGT1A1* analysis; S.I.: measurement of *UGT1A1* enzyme activity, Western blotting; M.B.: genetic counseling for clients and family; N.M. and M.S.: treatment of patients. All authors reviewed and revised the manuscript and approved the final version.

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