Novel combined UGT1A1 mutations in Crigler Najjar Syndrome type I

Nawel Abdellaoui1 | Balkiss Abdelmoula1 | Rania Abdelhedi2 | Najla Kharrat2 | Mouna Tabebi3 | Ahmed Rebai2 | Nouha Bouayed Abdelmoula1

1Genomics of Signalopathies at the service of Medicine, Medical University of Sfax, Sfax, Tunisia
2Laboratory of Screening Cellular and Molecular Process, Center of Biotechnology of Sfax, University of Sfax, Sfax, Tunisia
3Department of Biomedical and Clinical Sciences (BKV), Linköping University, Linköping, Sweden

Abstract

Background: Uridine diphosphate-glucuronosyl transferase 1A1 (UGT1A1), which is the major UGT1 gene product, is located on chromosome 2q37. The expression of UGT1A1 is relatively managed by a polymorphic dinucleotide repeat inside the promoter TATA box consisting of 5–8 copies of a TA repeat. A (TA) 6TAA is considered as the wild type. The A (TA) 7TAA allele has been identified as the most frequent allele in the Caucasian populations while A (TA) 8TAA allele remains the rarest allele worldwide in North Africa, including the Arab populations.

Methods: The spectrum of UGT1A1 genetic mutations in seventeen Tunisian children affected by persistent unconjugated hyperbilirubinemias is represented in addition to their relatives, notably parents, sisters, and brothers. Tunisian children, from 16 unrelated families as well as a 17th family without CN1 affected child, were originated from the West Center of Tunisia. The promoter region and coding exons of the UGT1A1 were PCR amplified, subsequently subjected to Sanger sequencing.

Results: The frequencies of genotypes in CN1 patients were as follows (TA) (7/7) (12/17: 70.6%) and (TA) (8/8) (5/17: 29.4%). All patients harbored the c.1070A>G mutation of exon 3 (UGT1A1*16) in the homozygous state. Among relatives of our patients (n = 16), who were all heterozygotes for UGT1A1*16, 13/16 (81.25%) had a heterozygous state for UGT1A1*1/UGT1A1*28 or (TA) (6/7) and, 18.75% (3/16) were heterozygous for UGT1A1*28/UGT1A1*37 or (TA) (7/8) of the promoter polymorphisms.

Conclusion: UGT1A1*16 accompanied with UGT1A1*28 or UGT1A1*37 had a specific geographic and ethnic distribution for CN pathogenesis in this Tunisian cohort.

KEYWORDS
(TA) 8, crigler-najjar syndrome type I, hereditary unconjugated hyperbilirubinemia, UDP-glucuronosyl transferase, UGT1A1
1 | INTRODUCTION

Crigler Najjar syndrome (CNS), first described in 1952 by John Crigler and Victor Najjar, is an autosomal recessive disorder that affects the liver. It is considered as a severe unconjugated hyperbilirubinemia. CNS presents two distinct forms: type I (CN-1; OMIM#218800) is very severe and possibly fatal due to either brain damage or kernicterus; and type II (CN-2; OMIM#606785) is associated with intermediate levels of hyperbilirubinemia, and low risk to develop kernicterus. CN-1 syndrome affects less than 1/10^6 live births.1

In CNS, UDP-glucuronyltransferase (UGT) activity is markedly reduced (CN-II) or alternatively absent (CN-I). The bilirubin is unable to be effectively conjugated. These severe hyperbilirubinemias are caused by variations in the UGT1A1 gene (OMIM#191740), which is a member of the UGT1 superfamily located on chromosome 2 at 2q37. Encoded by UGT1A1, the UGT is the only enzyme in the liver responsible for bilirubin glucuronidation. Accordingly, the reduced activity of the UGT leads to a significant increase at the unconjugated bilirubin levels.2

To date, more than 130 UGT1A1 variants have been detected in both coding and non-coding regions.3 As far as the hereditary unconjugated hyperbilirubinemia is concerned, the spectrum of UGT1A1 variants varies markedly between different ethnic populations.

The expression of UGT1A1 is relatively managed by a polymorphic dinucleotide repeat inside the promoter TATA box, consisting of between 5 and 8 copies of a TA repeat, with A(TA)6TAA considered as the wild type (UGT1A1*1). The UGT1A1*28 (a single TA insertion in the TATA box: A (TA) 7TAA) allele has been identified as the most frequent allele in the Caucasian populations while UGT1A1*37 allele (or two TA insertion in the TATA box: A (TA) 8TAA) remains the rarest allele worldwide, including the North African and Arab populations. The UGT1A1*37 alleles were described for the first time by Beutler E et al. (1998) at the beginning in the Afro-American populations and later detected in the Sub-Saharan African populations.5

Population genetic studies have shown that throughout history, in the Tunisian populations there is a founding effect with a specific mutation, coming from a founding ancestor.6 Correspondingly, for all Tunisian CN1 patients, the c.1070A>G (p.Q357R) mutation in exon 3 is the most common one, described for the first time by Labrune P in 1994 and constantly associated with the UGT1A1*28 or A (TA) 7TAA promoter polymorphism.7,8

The current study evaluated exon 3 mutations and TATA box polymorphisms in the UGT1A1 gene, using bidirectional sequencing in CN patients from the West Center of Tunisia.

2 | MATERIALS AND METHODS

2.1 | Patients

Seventeen patients, with unconjugated hyperbilirubinemia from the West Center of Tunisia, were diagnosed as affected by CN1 syndrome and submitted to a genetic etiological assessment of persistent mucous cutaneous jaundice and hereditary unconjugated hyperbilirubinemia.

Epidemiological, clinical, and biological data were collected from the medical files and 10 ml of blood was obtained from patients as well as their available relatives who represented 16 unrelated families. Another family from the same geographic region was also considered, despite the absence of an affected child.

2.2 | Ethics approval and consent to participation

All participants were consented to participate in the investigative stage of the study, which was conducted in accordance with the Declaration of Helsinki. Its protocol was approved by the Ethics Committee entitled “Comité de Protection des Personnes SUD” (CPPS SUD) (approval number: 0398/2022).

2.3 | Methods

Genomic DNA was extracted from venous blood according to phenol-chloroform protocol (isoamyl alcohol (25:24:1)). Designed primers were used to amplify exon 3: forward primer CCTCCCACTGTGTTAAAGACTGTTTC, reverse primer AGTGTTACTCACATGCCTTGCG and TATA box: forward primer CTTGGGTATCGATTGTTTTTG, reverse primer ACACGCTGCAGGAAAGAATC in first place and then the rest of the exons (Table 1) using Polymerase Chain Reaction (PCR) in a thermocycler “Geneamp PCR System 9700” (Applied Biosystem). PCR was performed using ~100 ng genomic DNA under the following conditions: initial denaturation for 5 min at 95°C, followed by 95°C for 20 seconds, 62°C for 30 seconds, and 72°C for 30 seconds during 30 cycles, with a final elongation at 72°C for 7 min.

PCR products were subjected to gel migration, purified by a commercial purification kit (Wizard SV Gel) and PCR clean up system Promega. Subsequently, forward and reverse primers were used for sequencing by the automated capillary sequencer: 3500xLGenetic Analyzer, Applied Biosystems, Foster City, California, USA.

The blast homology analysis was implemented using the program available in the National Center for Biotechnology Information web site in comparison with the consensus Cambridge sequence (GenBank Accession No. NC_000002.12).

3 | RESULTS

3.1 | Patients’ characteristics

The age of the hyperbilirubinemic patients ranged from one month to nine years old. They presented with neonatal jaundice, which quickly became complicated or prolonged by the nuclear jaundice. The bilirubin levels in the majority of the patients were exceeding 200 μmol/L. All patients appeared to have the CN type I (Table 2).
3.2 | Genetic study results

Seventeen CN patients and 16 of their relatives (parents, brothers, and sisters) were included in our study. Seventeen patients bore an A-to-G transition at codon 357 (CAA CGA), changing a glutamine residue into an arginine one (Q357R). No other nucleotide changes were detected in the entire coding sequence. Indeed, all patients were found to be homozygous for the Q357R mutation (c.1070A>G) within exon 3 of the UGT1A1 gene. However, their parents and relatives were all heterozygous for the same mutation. More importantly, this mutation, along with others, were absent in patient S18.

Using sequencing, the screening for the A (TA)n TAA polymorphism in UGT1A1 promoter was performed for all subjects. Our results revealed the presence of four different genotypes, which are as follows: TA7/7, TA8/8, TA6/7, and TA7/8.

Genotype (TA) 7/7 was predominant in the patients’ cohort. Twelve patients (70.6%) were homozygous for the [UGT1A1*28/UGT1A1*28] polymorphism. A two TA insertion within the promoter of the gene (TA8) was detected in the homozygous state, resulting in (TA) 8/8 among five patients (29.4%). Interestingly, we detected the heterozygous (TA) 6/7 polymorphism in thirteen (81.25%) and the (TA) 7/8 variant in three (18.75%) relatives of our patients (Tables 3 and 4).

Table 3 shows that, out of the CN patients homozygous for the c.1070A>G (UGT1A1*16/UGT1A1*16) variation, 70.6% (12/17) also harbored (UGT1A1*28/UGT1A1*28) in the homozygous state while 29.4% (5/17) were homozygous for (UGT1A1*37/UGT1A1*37). These results indicated that all CN1 patients, homozygous for the c.1070A>G variation, also harbored the homozygous variation TA (7/7) or TA (8/8) in the UGT1A1 promoter region. This finding unveils that c.1070A>G homozygosity is mostly accompanied by homozygous variations in the UGT1A1 promoter in our patients.

Moreover, we detected that all the patients’ relatives were heterozygotes compound for two different variations. On the one hand, they were all heterozygous for c.1070A>G mutation UGT1A1*1/UGT1A1*16. On the other hand, for the TATA box promoter polymorphism, thirteen were (TA) 6/7 or (UGT1A1*1/UGT1A1*28) and only three were either (TA) 7/8 or (UGT1A1*28/UGT1A1*37) (Table 4).

In addition to the c.1070A>G mutation, the TATA box variants, A (TA) 7TAA and A (TA) 8TAA represented the principal associated genotypes in CNS patients in this cohort. Contrary to what has been stated in the literature concerning CNS in Tunisia, where the c.1070A>G mutation has always been reported to be associated with the A (TA) 7TAA polymorphism and considered as a founding effect mutated allele, only 70.6% (12/17) of our CN1 patients harbored the usual TATA box profile (TA) (7/7) associated with the c.1070A>G mutation while 29.4% (5/17) had the c.1070A>G mutation associated with (TA) (8/8) polymorphism.

4 | DISCUSSION

In this study, we identified the genetic profiles of UGT1A1 gene variations in CNS Tunisian patients and their relatives. The geographical origin of our population at the Tunisian West Center includes the areas of Gafsa, Sidi Bouzid, and Kasserine. These regions are characterized by their specific ethnicity. The sequencing exploration of the entire UGT1A1 gene identified four genetic variants. These variations were distributed predominantly in the TATA box promoter.

**TABLE 1** Primers used to amplify UGT1A1 gene

| Amplified region | Primer sequences | PCR product size (bp) |
|------------------|------------------|----------------------|
| TATA box and 5’ end of exon 1 | 5’-CTTGGTGTATCGATTGGTTTTTG-3’ | 405 |
| forward primer | 5’-ACACGCTGCAGAAAGAATC-3’ | 702 |
| Reverse primer | 5’-TGTGCCATTCCAAGGAG-3’ | 409 |
| Rest of Exon 1 forward primer | 5’-TCTGGGGCTAGTTAATCAGG-3’ | 402 |
| Reverse primer | 5’-GAAGCTGGAAGTCTGGGATTAG-3’ | 434 |
| Exon 2 forward primer | 5’-TGTAAGCAGGAACCTTCCCTC-3’ | 429 |
| Reverse primer | 5’-CCCTCACTCTGTTAAAGACTGTTC-3’ | |
| Exon 3 forward primer | 5’-AGTGTATCTACATGCCCTTGC-3’ | |
| Reverse primer | 5’-TGCAAGGGCATGTGAGTAACAC-3’ | |
| Exon 4 forward primer | 5’-TGAAACAACGCTATTAAATGCT-3’ | |
| Reverse primer | 5’-AGTGTTACTCACATGCCCTTGC-3’ | |
| Exon 5 forward primer | 5’-GAGAGGATTGTTCTACACAGG-3’ | |
| Reverse primer | 5’-CACTGATTTCTGTTTTTCAAGG-3’ | |
# Table 2: Presentation of the studied population

| Patients | Geographic origin | Age at the discovery of jaundice /Age at hospitalization | Levels of BT/BC/UCB(µmol/L) | Karyotype | Neurological signs | Kernicterus | Associated pathologies | Evolution |
|----------|-------------------|----------------------------------------------------------|-----------------------------|-----------|-------------------|-------------|------------------------|-----------|
| P1       | Sidi Bouzid       | Day 3/1 months                                           | 470/89/379                 | Unavailable | Axial hypotonia   | +           | Umbilical hernia        | Death     |
| P2       | Sidi Bouzid       | Day 6/1 months                                           | 460/20/239                 | 46,XX      | Axial hypotonia   | -           |                        |           |
| P3       | Sidi Bouzid       | Day 6/day 9                                              | 608/8/-                    | 46,XX      | Convulsions       | -           | Enlarged clitoris       |           |
| P4       | Gafsa             | Day 6/1 months                                           | 354/3/-                    | 46,XY      | No                | -           | Enophthalmos colobomeirian microphthalmia | --        |
| P5       | Sidi Bouzid       | Day 3/7 months                                           | --Under phenobarbital      | 46,XY      | Febrile status epilepticus | +           | Speech and walking disorders Sharp deep tendon reflexes and hypertonia of the limbs | Alive + at school without cognitive and motor sequelae |
| P6       | Sidi Bouzid       | Day 4/3 months                                           | 590/8/--                   | 46,XY      | Seizures          | +           | Hyperthyroidism Hypertrophic pyloric stenosis | Death    |
| P7       | Gafsa             | Day 3/day20                                              | --                         | Unavailable | No                | --          | --                     |           |
| P8       | Gafsa             | H10/H36                                                  | 139/14,65/124,3            | Unavailable | No                | -           | Suspected G6PD deficiency | --        |
| P9       | Gafsa             | Not known/Day 10                                          | 192,7/12,7/-496/1,84/-    | Unavailable | Axial hypotonia   | -           | Fetal distress (tinted amniotic fluid) | --        |
| P10      | Gafsa             | Not known/Day 10                                          | 269/18 418/7,3/366        | Unavailable | Axial hypotonia   | Sunset eyes | hepatosplenomegaly | Death |
| P11      | Sidi Bouzid       | Day 3/3 months                                           | 540/28/512                 | Unavailable | Axial hypotonia   | -           |                        |           |
| P12      | Gafsa             | Day 5/3 months                                           | 248/22/-310/21/-442,6/21,58/- | Unavailable | Axial hypotonia Generalized convulsions Rolled eyes Lack of eye pursuit | +           |                        | Death    |
| P13      | Gafsa             | Day 7/Day 20                                             | ?                          | Unavailable | ?                 | ?           | -                      | --        |
| P14      | Gafsa             | Day 3/Day 7                                              | 383/30/353 437/30 /407    | Unavailable | No                | -           |                        | --        |
| P15      | Gafsa             | Day 1/3 months                                           | 800/-600/10               | Unavailable | No                | -           |                        | Alive + at school without cognitive and motor sequelae |
| P16      | Kasserine         | Day 1/1 month                                            | 580/180/--                 | Unavailable | No                | -           | Hepatosplenomegaly      | Death    |
| P17      | Sidi Bouzid       | Day 3/Day 8                                              | 272/17/255                 | Unavailable | No                | -           | Neonatal infection      | --        |

**Abbreviations:** BT, total Bilirubin; BC, Conjugated bilirubin; UCB, unconjugated bilirubin; H, hour; -, absence; --, unknown
region followed by the exon 3. Despite the limited number of patients volunteered in this group, it is the first study in which high frequency of (TA) 8 allele of the TATA box was reported particularly with the association to the c.1070A>G mutation of the exon 3 of UGT1A1.

In CN1, several founding effects have been reported in isolated communities such as Portugal, France, and Sardinia with the presence of a limited number of mutations. It was highlighted in a previous work of Philippe Labrune team that a recurrent mutation c.1070A>G [UGT1A1*16] was responsible for p. Glu357Arg or p.Q357R modification, in Tunisia. The resulting protein is inactive by total loss of catalytic activity. Notably, c.1070A>G is reported as always associated with the A (TA) 7TAAA anomaly of the promoter, which is responsible for the homozygous state [UGT1A1*16/UGT1A1*16] of CN1 syndrome. This mutation has been described with reference to Tunisian patients taken from all over Tunisia without any geographical specifications, suggesting a founder effect.

The founder p.Q357R mutation in the UGT1A1 gene of CNS type I observed in Tunisian has been also reported in the Middle Eastern Kuwaiti population. Several studies claim that this mutation (Q365C), responsible for CNS, probably originated in the Middle East. It came into Tunisia with the Banu Hilal invasions during which middle easterners settled in the Maghreb in the 11th century. Haplotype analysis performed by François M Petit et al. in 2008 confirmed the founder effect hypothesis, and ascertained that the appearance of the c.1070A>G mutation in the UGT1A1 gene occurred 32 generations ago in the Tunisian population. Hence, in our cohort, UGT1A1*16 (c.1070A>G) was the most commonly reported, with an allelic frequency of 76%. However, all CN1 patients (17/17) were identified as homozygous for UGT1A1*16 [UGT1A1*16/UGT1A1*16] while all their relatives were heterozygous for this variant [UGT1A1*1/UGT1A1*16]. These findings corroborated that the large prevalence of c.1070A>G variant of the exon 3 was the main cause of hereditary unconjugated hyperbilirubinemia among our patients, thus suggesting an ancestral common origin and reinforcing the Tunisian founder effect for CN1. It seems to be impossible to determine whether this homozygous mutation totally abolishes UGT1A1 activity by itself or whether it is associated with the A (TA) 7TAAA/A (TA) 7TAAG genotype in the TATA box.

Furthermore, the c.1070A>G mutation conventionally reported as being systematically associated with homozygous polymorphism UGT1A1*28 was found in our study in only 70.6% of the mutated patients. We identified a novel association of c.1070A>G with UGT1A1*37 homozygous polymorphism (A (TA)8 TAA /A (TA)8 TAA) in 29, 4% of CNS type I. No significant association between these alleles was previously found. Nevertheless, in the Caucasian populations, the (TA) 8 allele is extremely rare. It is rather described to be more common in African populations, with a frequency of 6.9%. The presence of this rare allele in the West Center of Tunisia (particularly in Gafsa’s region) with an allelic frequency of 19.7% suggests a common ancestral mutation and not a recent genetic event. These results were, consequently, consistent with the founding effect of the Tunisian mutation and revealed an ancestral common origin. Table 5 shows that, as the A (TA)n TAA repeat number increases, UGT1A1 enzymatic activity decreases. Subsequently, promoters with (TA)7 or (TA)8 replicates exhibit a reduction in gene transcription, which in turn results in UGT1A1 enzyme activity reduction (Table 5). As shown in Table 4, patients presenting CNS type I and carrying missense mutation p.Q357R manifested an additional homozygous in the TATA box. The combination of missense mutations and variants of UGT1A1 promoter region, TA (7)/TA (7) or TA (8)/TA (8) probably abolishes instead of reducing enzymatic activity. A higher frequency of A (TA)7 TAA and A (TA)8 TAA in the presence of p.Q357R variant was observed among the patients of CNS type 1, proving that the promoter polymorphisms improved the effect of the associated variant.

Our patients presenting the c.1070A>G mutations were all geographically from the South and the West Center of Tunisia, including the regions of Gafsa, Sidi Bouzid, and Kasserine. The geographical distribution of the novel association [UGT1A1*16/ UGT1A1*16 and UGT1A1*37/UGT1A1*37] seems to be specific to the region of Gafsa, whereas the classical reported combination [UGT1A1*16/UGT1A1*16 and UGT1A1*28/UGT1A1*28] was detected in the regions of Sidi Bouzid and Kasserine. This is the consequence of consanguineous and endogamous marriages as a social habit, especially in the South and the Central West Center of Tunisia.

A (TA)7 TAA polymorphism is the most common polymorphism in the world, especially in the Caucasian and African populations, including Tunisia. This fact was further emphasized by the current study. However, A (TA)8 TAA allele was described for the first time by Beutler E et al. (1998) in Afro-Americans with different genotype combinations (TA8/TAA or TA8/T8A or TA6/T8A) and later on detected in other groups Sub-Saharan Africans, remaining the rarest allele. UGT1A1*37 allele leads to a lower level reduction in promoter activity. It is unlike the one in the promoter with UGT1A1*28 allele. A first Caucasian TA7/T8A case was reported in an Italian girl in 1999. Several other cases were subsequently announced as either homozygous TA8/T8A or heterozygous (TA7/T8A or TA6/T8A) in several ethnic groups and populations while remaining a fairly rare allele compared to the others. TA8 allele was reported in the Caucasian population.

| Table 3 Association of c.1070A>G in Exon 3 with TA insertion in promoter region of UGT1A1 in CN patients |
|---------------------------------------------------------------|
| CN (n = 33) | c.1070A>G in Exon 3 | c.1070A>G in Exon 3 |
|             | Homo (n = 17) | Heter (n = 16) |
| A(TA)7TAA   |               |               |
| Heter       | 0             | 13 (81.25%) |
| Homo        | 12 (70.6%)   | 0             |
| A(TA)8TAA   |               |               |
| Heter       | 0             | 3 (18.75%) |
| Homo        | 05 (29.4%)   | 0             |

Abbreviations: Homo, Homozygous; Heter, Heterozygous.
population in Croatia as heterozygous (TA7/TA8 and TA6/TA8), in India in the three forms, 13 in Saudi Arabia (one case TA8/TA8)14 and in Kuwait (only 4/270 cases have TA6/TA8).15 Isolated cases have also been described in the context of CNS in a girl of Moroccan origin16 and Gilbert syndrome in Algeria and Turkey.17 Generally, (TA) 8 is present at low frequency.5,17,18 29.4% of our patients harbored the TA8 genotype in a context of CNS. In the present study, we discovered the highest frequency of (TA) 8 allele compared to other ethnic groups, which was 19.7%. UGT1A1*16 accompanied with UGT1A1*28 or UGT1A1*37 was essential for CN pathogenesis in this cohort.

### 5 | CONCLUSION

A significant association was observed between the Q357R homozygous mutation of exon 3 and the genotypes (TA) 7/7 and (TA) 8/8 of the TATA box promoter of UGT1A1 in CNS Tunisian patients. The frequency of the extremely rare UGT1A1 (TA) 8 promoter polymorphism genotype was determined for the first time in association with the Tunisian Q357R mutation of exon 3 among CN1 Tunisian children.
ACKNOWLEDGEMENTS
This study was financially supported by the Tunisian government program “Federated research project” (PRF2017D3P1) funded by the Ministry of Higher Education and Scientific Research. The authors are grateful to Doctor Mohamed El Behi for his precious contribution.

CONFLICTS OF INTEREST
The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT
The data that support the findings of the current study are available from the corresponding author on reasonable request.

ORCID
Nawel Abdellaoui https://orcid.org/0000-0002-6205-5811
Nouha Bouayed Abdelmoula https://orcid.org/0000-0002-0102-4405

REFERENCES
1. Crigler JF Jr, Najjar VA. Congenital familial non hemolytic jaundice with kernicterus. Pediatrics. 1952;10:169-180.
2. Erlinger S, Arias IM, Dhumeaux D. Inherited disorders of bilirubin transport and conjugation: new insights into molecular mechanisms and consequences. Gastroenterology. 2014;146:1625-1638.
3. Pharmacogenomics Knowledge. Implementation [PharmGKB] (web-page on the Internet). Haplotypes for UGT1A1 (UGT Alleles Nomenclature Page); 2017. Available from: https://www.pharmgkb.org/haploTypeSet/. PA166115840. Accessed January 12, 2017.
4. Bosma PJ, Chowdhury JR, Huang TJ, et al. Mechanisms of inherited deficiencies of multiple UDP-glucuronosyltransferase isoforms in two patients with Crigler-Najjar syndrome, type I. Faseb J. 1992;6:2859-2863.
5. Premawardhena A, Fisher CA, Liu YT, et al. The global distribution of length polymorphisms of the promoters of the glucuronosyltransferase 1 gene (UGT1A1): hematologic and evolutionary implications. Blood Cells Mol Dis. 2003;31(1):98-101.
6. Francoual J, Rivierre A, Mokrani C, et al. Crigler-Najjar syndrome type I in Tunisia may be associated with a founder effect related to the Q357R mutation within the UGT1 gene. Hum Mutat. 2002;19(5):570-571.
7. Petit FM. Aspects Moléculaires Des Maladies Rares du Métabolisme Hépatique à Propos de la Maladie de Crigler-Najjar. theses.fr; 2008.
8. Labrune P, Myara A, Hadchouel M, et al. Genetic heterogeneity of Crigler-Najjar syndrome type I: a study of 14 cases. Hum Genet. 1994;94(6):693-697.
9. Rosatelli MC, Meloni A, Faa V, et al. Molecular analysis of patients of Sardinian descent with Crigler-Najjar syndrome type I. J Med Genet. 1997;34:122-125.
10. Meech R, Mackenzie PI. Structure and function of uridine diphosphateglucuronosyltransferases. Clin. Exp Pharmacol Physiol. 1997;24:907-915.
11. Petit FM, Bézieau S, Gajdos V, et al. The Tunisian population history through the Crigler-Najjar type I syndrome. Eur J Hum Genet. 2008;16:848-853.
12. Petit FM, Gajdos V, Parisot F, et al. Paternal isodisomy for chromosome 2 as the cause of Crigler-Najjar type I syndrome. Eur J Hum Genet. 2005;13:278-282.
13. Agrawal SK, Kumar P, Rathi R, Sharma N, DAS R, Prasad R, Narang A. UGT1A1 gene polymorphisms in North Indian neonates presenting with unconjugated hyper-bilirubinemia. Pediatr Res. 2009;65:675-680.
14. Alkhafry KM, Alghamdi AM, Bagulb KM, et al. Distribution of selected gene polymorphisms of UGT1A1 in a Saudi population. Arch Med Sci. 2013;9:731-738.
15. AlFadhili5 AJ, Hadi M, Al-Mutairi M, Nizam R. The effect of UGT1A1 promoter polymorphism in the development of hyperbilirubinemia and cholelithiasis in hemoglobinopathy patients. PloS One. 2013;8(10):e77681.
16. Labrune P. Exploration d’un ictère néonatal. Méd Thér Pédiatr. 2001;4:127-132.
17. Horsfall LJ, Zeitlyn D, Tarekegn A, et al. Prevalence of clinically relevant UGT1A alleles and haplotypes in African populations. Ann Hum Genet. 2011;75:236-246.
18. Beutler E, Gelbart T, Demina A. Racial variability in the UDPglucuronosyltransferase 1 (UGT1A1) promoter: a balanced polymorphism for regulation of bilirubin metabolism? Proc Natl Acad Sci U S A. 1998;95:8170-8174.
19. Romdhane L, Kefi R, Azaiez H, Ben Halim N, Dellagi K, Abdelhak S. Founder mutations in Tunisia: implications for diagnosis in North Africa and Middle East. Orphanet J Rare Dis. 2012;21(7):52.
20. Romdhane L, Mezzi N, Hamdi Y, El-Kamah G, Barakat A, Abdelhak S. Consanguinity and Inbreeding in Health and Disease in North African Populations. Annu Rev Genomics Hum Genet. 2019;31(20):155-179. doi:10.1146/annurev-genom-083118-014954. Epub 2019 Apr 30.
21. Iolascon A, Faienza MF, Centra M, Storelli S, Zelante L, Savoia A. (TA)8 allele in the UGT1A1 gene promoter of a caucasian with Gilbert’s syndrome. Haematologica. 1999;84(2):106-109.
22. Tzezou A, Tzetis M, Kitsiou S, Kavazarakis E, Galla A, Kanavakis E. A Caucasian boy with Gilbert’s syndrome heterozygous for the (TA)8 allele. Haematologica. 2000;85(3):319.

How to cite this article: Abdellaoui N, Abdelmoula B, Abdelhedhi R, et al. Novel combined UGT1A1 mutations in Crigler Najjar Syndrome type I. J Clin Lab Anal. 2022;36:e24482. doi:10.1002/jcla.24482