



RESEARCH ARTICLE

Novel combined UGT1A1 mutations in Crigler Najjar Syndrome type I

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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*

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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 1/10⁶ live births.¹

In CNS, UDP-glucuronyl transferase (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).⁴ 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-Sahara 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 CCTCCCACTCTGTAAAGACTGTTTC, reverse primer AGTGTACTCACATGCCCTTGC and TATA box: forward primer CTTGGTGTATCGATTGGTTTTTG, reverse primer ACACGCTGCAG GAAAGAATC 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: 3500xL Genetic 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).

TABLE 1 Primers used to amplify UGT1A1 gene

Amplified region	Primer sequences	PCR product size (bp)
TATA box and 5' end of exon 1		405
forward primer	5'-CTTGGTGTATCGATTGGTTTTTG-3'	
Reverse primer	5'-ACACGCTGCAGGAAAGAATC-3'	
Rest of Exon 1		702
forward primer	5'-TGTGCCATTCCAAAGGGAG-3'	
Reverse primer	5'-TCTGGGGCTAGTTAATCGATCC-3'	
Exon 2		409
forward primer	5'-TGTAAGCAGGAACCCTTCCTCC-3'	
Reverse primer	5'-GAAGCTGGAAGTCTGGGATTAG-3'	
Exon 3		402
forward primer	5'-CCTCCCACTCTGTAAAGACTGTTC-3'	
Reverse primer	5'-AGTGTTACTCACATGCCCTTGC-3'	
Exon 4		434
forward primer	5'-TGCAAGGGCATGTGAGTAACAC-3'	
Reverse primer	5'-TTGAAACAACGCTATTAATGCTACG-3'	
Exon 5		429
forward primer	5'-GAGAGGATTGTTACATACCACAGG-3'	
Reverse primer	5'-CACTGATTCTGTTTTCAAGTTGG-3'	

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 2 Presentation of the studied population

Patients	Geographic origin	Age at the discovery of the jaundice /Age at hospitalization	Levels of BT/BC/UCB($\mu\text{mol/L}$)	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 Consciousness disorders Axial hypotonia Weak cry	+	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 /--314,5/-- /286,4	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

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 Homo (n = 17)	c.1070A>G in Exon 3 heter (n = 16)
A(TA)7TAA		
Heter	0	13(81.25%) (TA)6/7
Homo	12(70,6%) (TA)7/7	0
A(TA)8TAA		
Heter	0	3(18.75%) (TA)7/8
Homo	05(29.4%) (TA)8/8	0

Abbreviations: Homo, Homozygous; Heter, Heterozygous.

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.^{8,9} It was highlighted in a previous work of Philippe Labrune team that a recurrent mutation c.1070A>G [*UGT1A1*16*] was responsible for p. Gln357Arg or p.Q357R modification, in Tunisia.⁸ The resulting protein is inactive by total loss of catalytic activity.¹⁰ Notably, c.1070A>G is reported as always associated with the A (TA) 7TAA 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.^{6,8,11,12}

The founder p.Q357R mutation in the *UGT1A1* gene of CNS type I observed in Tunisia has been also reported in the Middle Eastern Kuwaiti population.¹¹ 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.^{11,19,20} Haplotypic 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.¹¹ 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) 7TAA/A (TA) 7TAA genotype in the TATA box.

Furthermore, the c.1070A>G mutation conventionally reported as being systematically associated with homozygous polymorphism *UGT1A1*28*¹¹ 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.^{21,22} It is rather described to be more common in African populations, with a frequency of 6.9%.¹⁸ 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).^{5,17,18} 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/TA8 or TA7/TA8 or TA6/TA8) and later on detected in Sub-Saharan Africans, remaining the rarest allele.¹⁷

*UGT1A1*37* allele leads to a lower level reduction in promoter activity. It is unlike the one in the promoter with *UGT1A1*28* allele.¹⁸ A first Caucasian TA7/TA8 case was reported in an Italian girl in 1999. Several other cases were subsequently announced as either homozygous TA8/TA8 or heterozygous (TA7/TA8 or TA6/TA8) in several ethnic groups and populations while remaining a fairly rare allele compared to the others. TA8 allele was reported in the Caucasian

TABLE 4 Results of Exon 3 and TATA box sequencing in CN patients

Subjects	Mutation Exon 3	Genotype Exon 3	UGT1A1*16 state	Polymorphism A(TA)nTAA	Genotype	Polymorphism A(TA)nTAA state
S1	c.1070A>G	UGT1A1*16/ UGT1A1*16	Homozygous	A(TA)7TAA	UGT1A1*28/ UGT1A1*28	Homozygous 7/7 TA
S2	c.1070A>G	UGT1A1*16/ UGT1A1*16	Homozygous	A(TA)7TAA	UGT1A1*28/ UGT1A1*28	Homozygous 7/7 TA
S3	c.1070A>G	UGT1A1*16/ UGT1A1*16	Homozygous	A(TA)7TAA	UGT1A1*28/ UGT1A1*28	Homozygous 7/7 TA
S4	c.1070A>G	UGT1A1*16/ UGT1A1*16	Homozygous	A(TA)7TAA	UGT1A1*28/ UGT1A1*28	Homozygous 7/7 TA
S5	c.1070A>G	UGT1A1*16/ UGT1A1*16	Homozygous	A(TA)7TAA	UGT1A1*28/ UGT1A1*28	Homozygous 7/7 TA
S6	c.1070A>G	UGT1A1*16/ UGT1A1*16	Homozygous	A(TA)7TAA	UGT1A1*28/ UGT1A1*28	Homozygous 7/7 TA
S7	c.1070A>G	UGT1A1*16/ UGT1A1*16	Homozygous	A(TA)7TAA	UGT1A1*28/ UGT1A1*28	Homozygous 7/7 TA
S8	c.1070A>G	UGT1A1*16/ UGT1A1*16	Homozygous	A(TA)7TAA	UGT1A1*37/ UGT1A1*37	Homozygous 8/8 TA
S9	c.1070A>G	UGT1A1*16/ UGT1A1*16	Homozygous	A(TA)7TAA	UGT1A1*28/ UGT1A1*28	Homozygous 7/7 TA
S10	c.1070A>G	UGT1A1*16/ UGT1A1*16	Homozygous	A(TA)7TAA	UGT1A1*28/ UGT1A1*28	Homozygous 7/7 TA
S11	c.1070A>G	UGT1A1*16/ UGT1A1*16	Homozygous	A(TA)7TAA	UGT1A1*28/ UGT1A1*28	Homozygous 7/7 TA
S12	c.1070A>G	UGT1A1*16/ UGT1A1*16	Homozygous	A(TA)8TAA	UGT1A1*37/ UGT1A1*37	Homozygous 8/8TA
S13	c.1070A>G	UGT1A1*16/ UGT1A1*16	Homozygous	A(TA)8TAA	UGT1A1*37/ UGT1A1*37	Homozygous 8/8TA
S14	c.1070A>G	UGT1A1*16/ UGT1A1*16	Homozygous	A(TA)8TAA	UGT1A1*37/ UGT1A1*37	Homozygous 8/8TA
S15	c.1070A>G	UGT1A1*16/ UGT1A1*16	Homozygous	A(TA)7TAA	UGT1A1*28/ UGT1A1*28	Homozygous 7/7 TA
S16	c.1070A>G	UGT1A1*16/ UGT1A1*16	Homozygous	A(TA)8TAA	UGT1A1*37/ UGT1A1*37	Homozygous 8/8TA
S17	c.1070A>G	UGT1A1*16/ UGT1A1*16	Homozygous	A(TA)7TAA	UGT1A1*28/ UGT1A1*28	Homozygous 7/7 TA

TABLE 5 UGT1A1 alleles and enzymatic activity^{5,17}

Metabolic capacity /enzymatic activity	Allele number
Increased functional	UGT1A1*36
Fully functional	UGT1A1*1(wt)
Reduced functionality	UGT1A1*37 and UGT1A1*28

Abbreviation: Wt, wild type.

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 been also 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 TA8/8 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.

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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.

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