Seven novel mutations of the *UGT1A1* gene in patients with unconjugated hyperbilirubinemia

The aim of this study was to identify new pathogenic variations of the *UGT1A1* gene in 11 patients diagnosed with neonatal unconjugated hyperbilirubinemia. We describe two cases in which clinically unapparent heterozygotic mutations in the *UGT1A1* gene may become evident in combination with certain environmental conditions or additional genetic defects.

Haematologica 2007; 92:133-134

Genetic alterations of the *UGT1A1* gene result in Crigler-Najjar syndrome type I (CN1) and type II (CN2) and Gilbert's syndrome, autosomal recessive conditions that are characterized by non-hemolytic unconjugated hyperbilirubinemia.[1-3] Several other disorders associated with hemolysis caused by premature destruction of erythrocytes are also characterized by hyperbiliribinemia.[4,5] Therefore, the expression of *UGT1A1* may be a major factor responsible for bilirubin variability (a modifier gene) in inherited hemolytic diseases.

The aim of this study was to analyze the mutation pattern causing unconjugated hyperbilirubinemia in 11 unrelated patients with neonatal hyperbilirubinemia. For the analysis of mutations of *UGT1A1* genes, the coding and promoter region and the splicing sites were analyzed by direct sequencing.[6]

Nine patients had a clinical diagnosis of CN1 (patients #1 to #4) and CN2 (patients #5 to #9). Two subjects (patients #10 and #11) presented with transient hyperbilirubinemia caused by hemolysis. Patient #10 had neonatal kernicterus associated with hyperbilirubinemia due to a skull hematoma. Phototherapy and phenobarbital were started in this neonate, whose bilirubin levels decreased shortly afterwards. The phototherapy was suspended when the bilirubin concentration was under 10 mg/dL following resorption of the hematoma. Patient #11, affected by glucose-6-phosphate dehydrogenase (G6PD) deficiency, showed increased bilirubin production due to hemolysis. Direct sequencing of *G6PD* revealed a change from cytosine to thymine at base position 563 (in exon 6) causing a change from serine to phenylalanine in amino acid position 188. Results of the analysis of *UGT1A1* are summarized in Table 1.

We identified a total of 12 sequence variations, seven of which are described for the first time.

Two out of the four CN1 patients had a frameshift mutation, p.Q239fsX256, a recurrent change already found in a cohort of Italian patients.[7] Patients #3 and #4 showed novel variations. Patient #3 had a homozygous tri-nucleotide deletion c.513_515 del CTT causing a deletion of leucine codon at position 172 and the substitution of a phenylalanine residue by a leucine at position 171 [p.L172delF171L]. Although, this mutation eliminates a single amino acid (leucine), we believe that the substitution of a phenylalanine in a conserved diphenylalanine region of an aglycone binding domain may abolish the enzymatic activity of *UGT1A1*. Patient #4 was heterozygous for two novel mutations: a c.652insT insertion causing a frameshift and a C→T transition at nucleotide 847 in exon 1 that introduces a stop codon (p. Q283X).

Table 1. The patients' clinical features and mutations.

Patient	*Clinical manifestation*	*Biliribun levels (mg/dL)*	*Gestational age weeks*	*Birth weight (Kg)*	*Mutation*	*Mutant Protein*	*(TA) Polymorphism*	*References*
#1	CN1	30	38	3,00[	c.717-718 del AG] [c.717-718 del AG]	[p.Q239fsX256]+ [p.Q239fsX256]	$(TA)^7(TA)^7$	Iolascon, 2000
#2	CN1	28,8	38	3,30	[c.717-718 del AG]+ [c.717-718 del AG]	[p.Q239fsX256]+ [p.Q239fsX256]	$(TA)^7(TA)^7$	Iolascon, 2000
# 3	CN1	32,05	40	3,45	[c.513-515 del CTT]+ [c.513-515 del CTT]	[p.L172delF171L]+ [p.L172delF171L]	$(TA)^7(TA)^7$	Novel
# 4	CN1	31,2	37	3,05	[c.652insT]+[c.847C→T]	[p.S218Ffs257X]+ [p.Q283X]	$(TA)^6(TA)^6$	Novel+Novel
# 5	CN2	19	38	3,25	[c.835 A→T]	[p.N279Y]	$(TA)^6(TA)^7$	Elferink, 1994
# 6	CN2	16,21	39	3,10	[c.1381 T→C]	[p.W461R]	$(TA)^6(TA)^6$	Maruo, 2003
# 7	CN2	17,9	40	3,60	[c.1223-1224 ins G]+ [c.1184G→T]	[p.A409SfsX422]+ [p.G395V]	$(TA)^6(TA)^6$	Labrune, 1994+ Servedio, 2005
# 8	CN2	10	37	2,95	[c.1328 T→C]	[p.L443P]	$(TA)^6(TA)^7$	Novel
# 9	CN2	7,29	38	3,10	[c.1060 T→A]	[p.W354R]	$(TA)^6(TA)^7$	Novel
#10	hyperbil. due to hematoma	27,7	40	3,60	[c.1108 A→G]	[p.I370V]	$(TA)^6(TA)^6$	Novel
#11	G6PD	11,9	38	3,15	[c.210delC]	[p.D70QfsX76]	$(TA)^6(TA)^6$	Novel

GT1A1 cDNA sequence from GenBank accession number NM_000463NM was used as the reference sequence: the A of the ATG translation initiation start site represents nucleotide +1. CN1: Crigler-Najjar type I; CN2: Crigler-Najjar type I; G6PD: glucose-6-phosphate dehydrogenase deficiency.

The milder phenotype of CN2 patients is usually associated with homozygosity or compound heterozygosity for missense mutations. In addition to known causative alterations (p.N279Y, p.W461R), two missense mutations were identified in heterozygous subjects affected by CN2. In patient # 8, a novel missense mutation c.1328 T→C was found in exon 5 which affected residue 443 (p. L443P) involving a polar amino acid substituition. In patient #9 a T→A heterozygous transition at nucleotide 1060 of the *UGT1A1* gene resulted in the substitution of a tryptophan residue by arginine at position 354 (p.W354R) in the carboxy-terminal domain of the *UGT1A1* protein. Recently, missense p.W354R was reported to be caused by substitution of T to C at nucleotide 1060.[7] These results suggest that nucleotide 1060 in *UGT1A1* may be a mutational hot spot. In our cohort, all the individuals affected by CN2, except patient #7, were heterozygous for one *UGT1A1* coding region variation. However, the *in trans* presence of a c.-41_-40dupTA polymorphism [(TA)7] can explain the CN2 phenotype. This polymorphism can play a role in enhancing the effect of the heterozygous coding mutation.

Here, we describe two cases in which clinically unapparent heterozygotic mutations in *UGT1A1* may become evident when combined with certain environmental conditions or additional genetic defects. In patient #10 a novel missense mutation, c.1108 A→G, was detected in a heterozygous state. This change caused an amino acid substitution (p.I370V) in the catalytic core of the *UGT1A1* protein. This patient's severe hyperbilirubinemia and kernicterus were consequences of the imbalance between bilirubin production and conjugation. In patient #11, affected by G6PD deficiency, a novel heterozygous small deletion of one nucleotide at position 210 (c.210delC) of the *UGT1A1* gene was found in combination with the Mediterranean variant, c.563 C→T of the *G6PD* gene. This represents an example of co-inherited modifying gene causing clinical heterogeneity in monogenic disorders. The expression of *UGT1A1* is a major modifying factor in inherited hemolytic diseases being responsible for a large proportion of the bilirubin variability in these conditions.

In conclusion, the identification of these novel mutations in the *UGT1A1* gene, increasing the mutational spectrum of *UGT1A1* allelic variants, contributes to a better understanding of the molecular pathology of disorders characterized by unconiugated hyperbilirubinemia.

Maria D'Apolito, Agnese Marrone,° Veronica Servedio,* Pietro Vajro,# Luigia De Falco,° Achille Iolascon°*

**Dipartimento di Scienze Mediche e del Lavoro, Università degli Studi di Foggia, Italy; °Dept of Biochemistry and Biomedical Technologies University Federico II, CEINGE, Advanced Biotechnologies, Napoli, Italy; #Dipartimento di Pediatria Università degli Studi di Napoli Federico II, Napoli*

Key words: Bilirubin, jaundice, Crigler-Najjar syndrome, Gilbert syndrome, bilirubin UDP-glucuronosyltransferase; UGT1A1.

Correspondence: Achille Iolascon, Chair of Medical Genetics, Dept. of Biochemistry and Biomedical Technologies, University Federico II , Naples, CEINGE, Advanced Biotechnologies 80145 Naples. E-mail: iolascon@dbbm.unina.it

References

1. Bosma PJ, Chowdhury JR, Huang TJ, Lahiri P, Elferink RP, Van Es HH, et al. Mechanisms of inherited deficiencies of multiple UDP-glucuronosyltransferase isoforms in two patients with Crigler-Najjar syndrome, type I. FASEB J 1992;6:2859-63.
2. Bosma PJ, Goldhoorn B, Oude Elferink RP, Sinaasappel M, Oostra BA, Jansen PL. A mutation in bilirubin uridine 5'-diphosphate-glucuronosyltransferase isoform 1 causing Crigler-Najjar syndrome type II. Gastroenterology 1993; 105:216-20.
3. Bosma PJ, Chowdhury JR, Bakker C, Gantla S, de Boer A, Oostra BA, et al. The genetic basis of the reduced expression of bilirubin UDP-glucuronosyltransferase 1 in Gilbert's syndrome. N Engl J Med 1995;333:1171-5.
4. Kaplan M, Renbaum P, Levy-Lahad E, Hammerman C, Lahad A, Beutler E. Gilbert syndrome and glucose-6-phosphate dehydrogenase deficiency: a dose-dependent genetic interaction crucial to neonatal hyperbilirubinemia. Proc Natl Acad Sci USA 1997;94:12128-32.
5. Sampietro M, Lupica L, Perrero L, Comino A, Martinez di Montemuros F, Cappellini MD, et al. The expression of uridine diphosphate glucuronosyltransferase gene is a major determinant of bilirubin level in heterozygous beta-thalassaemia and in glucose-6-phosphate dehydrogenase deficiency. Br J Haematol 1997;99:437-9.
6. Bosma PJ, Chowdhury NR, Goldhoorn BG, Hofker MH, Oude Elferink RP, Jansen PL, et al. Sequence of exons and the flanking regions of human bilirubin-UDP-glucuronosyltransferase gene complex and identification of a genetic mutation in a patient with Crigler-Najjar syndrome, type I. Hepatology 1992;15:941-7.
7. Servedio V, d'Apolito M, Maiorano N, Minuti B, Torricelli F, Ronchi F, et al., Spectrum of UGT1A1 mutations in Crigler– Najjar (CN) syndrome patients: identification of twelve novel alleles and genotype–phenotype correlation, Hum Mutat 2005;25:325.
8. Iolascon A, Meloni A, Coppola B, Rosatelli MC. Crigler-Najjar syndrome type II resulting from three different mutations in the bilirubin uridine 5'-diphosphate-glucuronosyltransferase (UGT1A1) gene. J Med Genet 2000;37:712-3.
9. Labrune P, Myara A, Hadchouel M, Ronchi F, Bernard O, Trivin F, et al. Genetic heterogeneity of Crigler–Najjar type I: a study of 14 cases, Hum. Genet. 1994;94:693-7.
10. Maruo Y, Poon KK, Ito M, Iwai M, Takahashi H, Mori A, et al. Co-occurrence of three different mutations in the bilirubin UDP-glucuronosyltransferase gene in a Chinese family with Crigler-Najjar syndrome type I and Gilbert's syndrome. Clin Genet 2003;64:420-3.