UGT1A1 Genetic Analysis as a Diagnostic Aid for Individuals with Unconjugated Hyperbilirubinemia

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Objective To assess the clinical utility of *UGT1A1* genetic testing and describe the spectrum and prevalence of *UGT1A1* variations identified in pediatric unconjugated hyperbilirubinemia (UCH), and to characterize specific genotype-phenotype relationships in suspected Gilbert and Crigler–Najjar syndromes.

Study design A retrospective study was conducted to review clinical information and *UGT1A1* genotyping data from 181 pediatric patients referred for UCH. In silico analyses were performed to aid in the assessment of novel *UGT1A1* variants.

Results Overall, 146/181 pediatric patients had at least one heterozygous *UGT1A1* functional variant. Identified *UGT1A1* variants included 17 novel variants, 7 rare star alleles, and 1 rare variant. There were 129 individuals who possessed the TA7 (*28) promoter repeat and 15 individuals who possessed the *6 (c.211G > A) variation. Out of the 104 individuals with accompanying bilirubin levels, 41 individuals did not have identifiable *UGT1A1* variants that explained their UCH, although glucose-6-phosphate dehydrogenase deficiency and other causes of UCH could not be ruled out.

Conclusion Much of the observed UCH could be attributed to variation at the *UGT1A1* locus, and *UGT1A1* testing helped to substantiate a genetic diagnosis, thereby aiding in individual and family disease management. Although *UGT1A1* variation plays a large role in UCH, genetic assessment of *UGT1A1* alone may not be comprehensive. Assessment of additional genes may also be useful to evaluate genetic causes for UCH. *(J Pediatr 2013;162:1146-52)*.

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The processes of uptake, conjugation, and excretion of bilirubin can be affected by a variety of acquired and heritable conditions. Gilbert syndrome (GS) and Crigler–Najjar types I and II syndromes (CN I and CN II, respectively) are autosomal recessive unconjugated hyperbilirubinemia (UCH) disorders caused by genetic variation in *UGT1A1*, which encodes for *UGT1A1*, a key enzyme involved in the conjugation of bilirubin.

CN I is the most severe of the *UGT1A1*-associated hereditary disorders and is characterized by a complete (or near complete) absence of *UGT1A1* activity (caused by biallelic truncating or nonsense mutations that abolish enzyme activity).[1,2] Individuals with CN I typically develop total serum bilirubin levels between 20 and 40 mg/dL and require aggressive management with phototherapy and, when necessary, exchange transfusion to prevent bilirubin encephalopathy and kernicterus. With careful management and intensive phototherapy, intact survival into adolescence can be achieved, although the risk of bilirubin encephalopathy is always present.[3]

Individuals with CN II have low, but detectable levels of *UGT1A1* activity, usually due to missense or other mutations that are less severe than CN I mutations, and typically present with a total serum bilirubin range between 6 and 20 mg/dL.[1,2,4] Individuals with CN II may respond to phenobarbital treatment, which induces residual *UGT1A1* activity and can reduce plasma unconjugated bilirubin (UCB) concentrations by 30% or more.[5,6] However, events such as trauma and sepsis have been shown to increase UCB levels and lead to irreversible neurological damage in CN II individuals.[7-9]

GS is the mildest form of these three hereditary *UGT1A1*-associated syndromes and occurs at a frequency of 5%-10% in the US and Europe.[10] It is frequently associated with genetic variation in the *UGT1A1* promoter TATAA box and characterized by mild, chronic UCH, with serum bilirubin levels between 1-6 mg/dL, and hepatic *UGT1A1* activity reduced by approximately 70%.[11] Individuals with GS may experience hyperbilirubinemia during episodes of illness and fasting, but treatment is generally not necessary.[12] Individuals with GS must take caution when taking certain medications (eg, irinotecan) that are metabolized by glucuronidation.[13,14]

Methods

A total of 181 pediatric patients with UCH had *UGT1A1* full gene sequencing from 2009 through 2011 in our clinical molecular genetics laboratory (Mayo

CN I	Crigler–Najjar type I syndrome
CN II	Crigler–Najjar type II syndrome
GS	Gilbert syndrome
UCB	Unconjugated bilirubin
UCH	Unconjugated hyperbilirubinemia

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Clinic, Rochester, Minnesota). Additional clinical information (age, sex, ethnicity, bilirubin levels, suspected diagnosis, and/or family history) was provided for most of the individuals. The study was approved by the Mayo Foundation Institutional Review Board.

Total bilirubin references values are 0.1 to 0.9 mg/dL for males 1 to 2 years old (0.1-1.0 mg/dL for >2 years old), and for females 0.1 to 0.9 mg/dL for 1 to 11 years old and 0.1 to 1.0 mg/dL when 12 years or older.[15] Reference ranges have not been established for infants <12 months old, and are dependent on several risk factors as outlined by the American Academy of Pediatrics Subcommittee on Hyperbilirubinemia's clinical practice guidelines for the management of hyperbilirubinemia in newborns.[16,17]

Genomic DNA was extracted from EDTA-anticoagulated whole blood samples on the Qiagen EZ1 BioRobot using the manufacturer's recommended protocol (Qiagen Inc, Valencia, California). The promoter, all 5 exons, exon-intron boundaries, and a region in the distal promoter (the phenobarbital response enhancer module) of *UGT1A1* were sequenced, as previously described.[18]

Sequence traces for each region were compared with the *UGT1A1* sequence from GenBank accession number NM_000463.2. Variations were identified and star alleles were assigned according to the home page for uridine 5′-diphospho-glucuronosyltransferase allele nomenclature.[19] For *UGT1A1*, the star allele designation describes a specific variation which has already been described in the literature.[20] Novel variations were assessed by in silico analyses using SIFT software (J. Craig Venter Institute San Diego, California) and PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/). Amino acid conservation was analyzed considering 20 species (down to *Saccharomyces cerevisiae*). Splicing prediction algorithms used were SpliceSiteFinder-like, MaxEntScan (http://genes.mit.edu/burgelab/maxent/Xmaxentscan_scoreseq.html), and NNSPLICE (Berkley Drosophila Genome Project, Berkeley, California). These bioinformatics tools are computerized prediction programs that help to aid in the predicted phenotype of novel and rare variations, and were mainly accessed when the variants were initially identified clinically (2009-2011), but in some cases were accessed during the preparation of the manuscript.[21]

Results

The age range of patients tested was 5 days old to 17 years old (median age = 7 years old). Pediatric patients, <1 year old, comprised 43% of the patients tested. A total of 98/181 patients provided bilirubin levels. **Figure 1** illustrates an age-based comparison of the total bilirubin levels and identified *UGT1A1* variation(s).

In the age group of <8 days old, there was one Hispanic female who carried a heterozygous *UGT1A1* *28 (TA7) variant, which alone did not account for a reported total bilirubin level of 11.7 mg/dL. The patient had a diagnosis of Turner syndrome and coarctation of the aorta, which the ordering

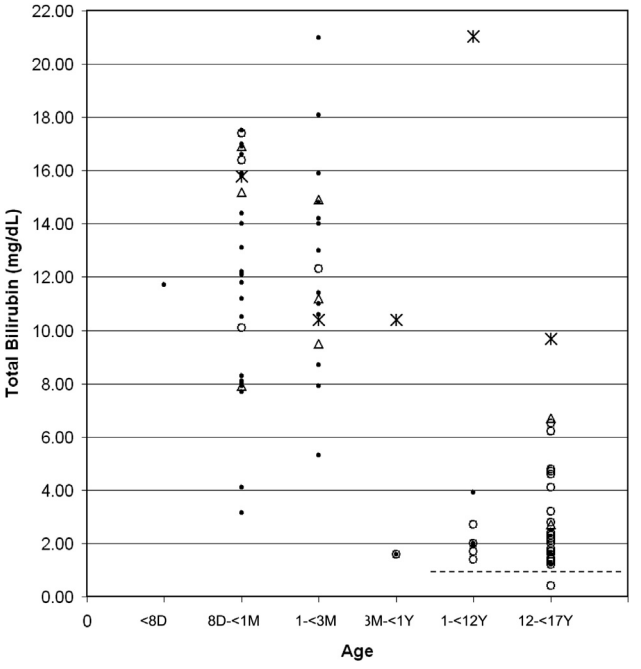

Figure 1. Age group analysis of patients for whom bilirubin levels were provided. *D*, Days; *M*, Month; *Y*, Year. *Small dot*, no variation or heterozygous functional variation only. *Open circle*, homozygous *28 (TA7/7) or *6. *Open triangle*, compound heterozygous *28 or *6 plus a functional variation. *Asterisk*, homozygous or compound heterozygous functional variants. A reference range cut-point of 1.0 mg/dL for total bilirubin was utilized for patients one year and older (*dashed line*).

physician speculated may have caused reduced liver perfusion and potentially contributed to the observed hyperbilirubinemia. Idiopathic liver involvement is relatively common in patients with Turner syndrome, occurring in 20%-80% of patients.[22] It is not known whether any of these factors may have contributed to this patient's presentation of hyperbilirubinemia.

Of the patients aged 8 days old to <1 month old, 21 out of 28 had elevated bilirubin levels without identifiable *UGT1A1* variation that could account for their hyperbilirubinemia. Seven patients in this age group had *UGT1A1* variation that likely explained their hyperbilirubinemia: 3 had homozygous variation at *28 (TA7) or *6 (c.211G > A, p.Gly71Arg) and 3 were compound heterozygous for *28 or *6 plus a functional variant; and 1 was compound heterozygous for 2 likely functional variations (c.827G > T, p.Gly276Val, and c.992A > G, p.Glu331Arg).

In the patient age group ranging from 1 month to <3 months old, 13/17 did not have *UGT1A1* variation that could account for their hyperbilirubinemia. Four patients harbored *UGT1A1* variation that likely explained their hyperbilirubinemia: 3 were compound heterozygous for *28 or *6 plus a functional variant, and 1 patient was homozygous *28.