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CASE REPORT

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Coexistence of mutations of Gilbert's syndrome and Crigler-Najjar syndrome in an infant with unconjugated hyperbilirubinemia—a case report

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Abstract

Background Jaundice in the newborn period is a very common entity; rare inherited causes are often forgotten. Persistent unconjugated hyperbilirubinemia in the intermediate levels with non-hemolytic features must prompt the necessity for evaluating for genetic defects in bilirubin metabolism.

Case presentation Three-and-a-half-month-old first-born girl of consanguineous marriage presented with jaundice from day 5 of life. Dark yellow color urine or pale stools were not present. Antenatal and birth history was normal. She had mild pallor and icterus and no hepatosplenomegaly. Total serum bilirubin was 8.2 mg/dl, and direct was 0.4 mg/dl. Workup for hemolytic anemia, thyroid function test, and sonography of abdomen was normal. Syrup phenobarbitone was started, and bilirubin levels after dropped to 2 mg/dl. Crigler-Najjar type II syndrome (CN II) or Gilbert's syndrome (GS) was suspected. Next-generation sequencing for *UGT1A1* gene mutation showed homozygous missense mutation consistent with CN II and 7 *TA* repeats in the promoter region consistent with GS. Bilirubin levels gradually fell after starting oral phenobarbitone syrup, and at 5 years of age, a trial of withholding phenobarbitone was given, and bilirubin levels remained lower, and she is asked to follow-up with bilirubin levels every 15 days to assess the need for reintroducing the therapy. Parents are planning for a second pregnancy, and a preconception genetic counseling has been done.

Conclusion Genetic confirmation of coexistence of mutations causing GS and CN II have an implication on long-term neurological complications of unconjugated hyperbilirubinemia in stress or crisis situations. Prenatal diagnostic testing must be advised for detecting homozygous UGT1A1 mutations to diagnose CN II and Gilbert mutations for each of the future pregnancies. Considering the side effects of long-term phenobarbitone therapy, the decision can be taken on case-to-case basis of stopping the therapy while monitoring TSB levels.

Keywords Crigler-Najjar syndrome type II, Gilbert's syndrome, *UGT1A1*, Intermediate hyperbilirubinemia, Double mutation, Genetic counseling

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Background

Pathological hyperbilirubinemia in neonatal period can be classified into unconjugated and conjugated hyperbilirubinemias [1]. The indirect hyperbilirubinemias mainly comprise groups of hemolytic anemias or disorders of bilirubin metabolism. JF Crigler and VA Najjar in 1952 studied seven patients with congenital familial non-hemolytic jaundice and kernicterus and proved that the abnormality was inherited most likely in an autosomal recessive manner [2]. Crigler-Najjar syndrome is a rare genetic disease with an estimated incidence of 0.6–1.0 per million live births around the world [3], and Gilbert's syndrome (GS) is one of the commoner genetic but benign cause of unconjugated hyperbilirubinemia worldwide with a population frequency of 5–10% [4]. Uridine diphospho-glucuronosyltransferase (UDPGT) is the primary enzyme needed for bilirubin glucuronidation in the liver and the gene *UGT1A1* coding for it is located on the long (q) arm of chromosome 2 at position 37.1 [5], mutations of which causes abnormal accumulation of the unconjugated bilirubin which can cause bilirubin induced neurotoxicity (BIND) [1]. The complete absence of the enzyme activity (stop codon or frameshift mutation) causes Crigler-Najjar I syndrome (OMIM: 218,800) and decreased activity causes CN II (homozygous missense mutation) (OMIM: 606,785) [6]. CN II usually presents in infants or children with intermediate hyperbilirubinemia with bilirubin levels ranging from 1.5 to 22 mg/dl [6]. They usually respond well to oral lifelong phenobarbitone therapy. Gilbert's syndrome (GS) (OMIM: 143,500) is a quite common benign condition which shows up in adolescence caused by polymorphisms in the promoter region of *UGT1A1* gene (7 tandem repeats) which reduces its expression and decreases the enzyme activity. The bilirubin levels reach up to 3 mg/dl and usually does not require any treatment [7]. We present a case of coexistence of both Crigler-Najjar syndrome and Gilbert's syndrome mutations in an infant presenting with indirect hyperbilirubinemia.

Case presentation

Three-and-a-half-month-old full-term baby girl born to third-degree consanguineous parents presented with yellow discoloration of the eyes and skin since day 5 of life. The parents hail from the western central part of India. Antenatal and birth history was uneventful. There was no history of clay-colored stools or dark yellow urine, bleeding manifestations, lethargy, seizures, and blood or blood product transfusion. Her family history was not significant. She was exclusively breastfed. She had developed a social smile, could recognize her mother, could hold her neck well and make cooing sounds, used to quieten when spoken to, and could visualize toys and reach out for them. On general examination, the baby was active and alert, weight at presentation was 5.5 kg, height was 62 cm, head circumference was 39 cm, and as per WHO growth chart for girls (0 to 5 years), she was on 0 SD. She had icterus and mild pallor. There was no obvious dysmorphisms or any other signs of liver cell failure. The liver was 2 cm palpable (span normal) with no free fluid. Thus, we had a 3.5-month-old well grown, developmentally normal, girl child with jaundice since day 5 of life, with no organomegaly, no facial dysmorphisms, and normal family history. She received double surface phototherapy in newborn period for 4 days after which the jaundice reduced on day 10 just to appear again on day 15. The child was readmitted for phototherapy once again at day 20. The jaundice reduced clinically on day 25 and reappeared on day 30 after which it was fairly persistent until 3 months of age when she presented to us. A complete blood count, bilirubin, and peripheral smear were done during this period which was within normal limits.

On presentation to us, the child had a total bilirubin of 8 mg/dl with indirect component of 7.6 mg/dl. The mother's blood group was B positive, and the baby was O positive with direct and indirect Coombs test negative. Other liver function tests, complete blood count, urine routine examination, thyroid function tests, peripheral smear, reticulocyte count, sickling test, hemoglobin electrophoresis of parents, RBC membrane and enzyme studies, glucose six phosphate dehydrogenase enzyme levels, and ultrasonography of abdomen were all normal. Hence, inherited disorders of bilirubin metabolism were suspected, and a next-generation sequencing (NGS) of the child's DNA sample was sent. Meanwhile, the child was started on oral phenobarbitone (5 mg/kg/day) after which there was a gradual decrease in jaundice (total bilirubin/indirect 4/3.6 mg/dl). The absence of kernicterus and hyperbilirubinemia responding to phenobarbitone favored a clinical diagnosis of CN II. NGS was done which revealed a homozygous 2 bp insertion of TA nucleotides [c.-55_-54insTA], leading to 7 TA repeats [A(TA)7TAA] in the promoter region of *UGT1A1* gene known as *UGT1A1*28* variation, which was pathogenic and another homozygous missense variation (chr2:234669097A > G; c.164A > G) in exon 1 of the *UGT1A1* gene, that results in the amino acid substitution of arginine for histidine at codon 55 (p.His55Arg) which was pathogenic as was detected in this subject (Fig. 1). The variant analysis in Sanger sequencing was based on the *UGT1A1* reference sequence ENST00000305208.5. This was consistent with the diagnosis of CN syndrome II and Gilbert's syndrome (Table 1). The gene studies of the parents could not be done due to financial reasons. She was on daily oral phenobarbitone therapy (compliant) till the age of 5.5 years with bilirubin levels in the range of

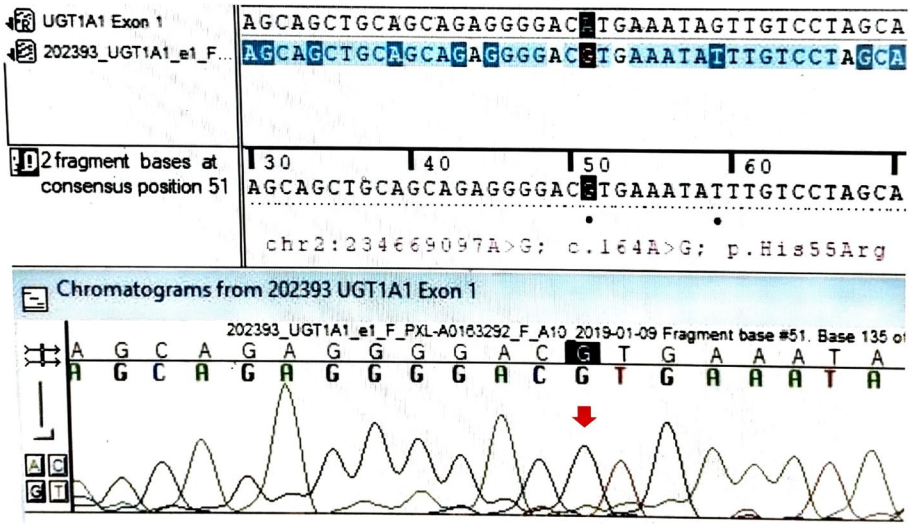

Fig. 1 Sequence chromatogram and alignment to the reference sequence showing the variation in exon 1 of the *UGT1A1* gene (chr2:234669097A > G;c.164A > G;p.His55arg) detected in homozygous condition in the baby

Table 1 Next-generation sequencing report of the child

Gene transcription	Location	Variant	Zygosity	Disease	Inheritance	Classification
UGT1A1 (+)	Exon 1	c.164A > G (p.His55Arg)	Homozygous	Crigler-Najjar syndrome type II	Autosomal recessive	Pathogenic
UGT1A1 (+)	Promoter region		Homozygous	Gilbert's syndrome	Autosomal recessive	Pathogenic

total bilirubin/indirect 2.6/1.49 mg/dl, and she is developmentally normal and growing well. The serial bilirubin values of the child are depicted in Table 2.

However, keeping in mind the effect of long-term phenobarbitone on the cognitive function and behavior of the child, a trial of withholding phenobarbitone therapy follows (total/indirect 3.6/2.4 mg/dl). She has been advised to repeat serum bilirubin levels every 2 weeks for the first few months and to restart phenobarbitone syrup if the TSB levels exceed 8 mg/dl. The parents are planning for a second pregnancy, and a preconception genetic counseling has been done.

Discussion

While clinically diagnosing CN II in an infant with bilirubin levels up to 20 mg/dl in the absence of Kernicterus is easier, diagnosing GS at this age without genetic studies is difficult. Comparison of genetic patterns of our patient along with other similar studies is elicited in Table 3.

Usually, children having Gilbert's mutations remain asymptomatic, and the jaundice becomes apparent during early to late adolescence along with an intercurrent infection, stress, or crisis situation [11]. However, a combination with a Crigler-Najjar type 2 mutation could be the reason for early presentation of our child from neonatal period. Also, levels of total serum bilirubin (TSB) were thought to be around 3 mg/dl only in olden days; however, Gilbert's syndrome in the current era presents with higher bilirubin levels which may confuse the picture. A large study (170 children with genetically proven Gilbert mutations) among the Indian population by Sood V et al. had 133 cases with homozygous and 37 with heterozygous status. The median serum total bilirubin (TB) levels were around 3.3 mg/dl with maximum levels reaching up to 18 mg/dl. The mean age of diagnosis in this study was around 13.6 years. They also showed that bilirubin levels were higher in heterozygous status than homozygous status [12].

The presence of Gilbert mutations along with homozygous mutations of *UGT1A1* gene for CN II causes varied presentation in infants. Laura CozzI et al. reported two Chinese neonates who presented with prolonged severe jaundice > 15 mg/dl and found homozygous for a variant site within the coding region of the gene in the 4 exon, c.1091C > T, p. Pro364Leu. the P364L mutation. Jaundice was persistent and unresponsive to phototherapy. The infants however responded well to phenobarbitone, TSB levels gradually fell, and jaundice resolved in a few

Table 2 Bilirubin levels of the child from the neonatal period till date

	Day 5 of life	Day 10	Day 20	Day 23	Day 25	Day 40	3 months	3.5 months	5 months (1 month after starting phenobarbitone)	12 months	18 months	2 years	3 years	4 years	5 years	5.5 years (1 month after stopping phenobarbitone)
Total bilirubin (mg/dl)	22.7	17.9	21.3	17.3	13.7	10.1	12.5	8	4	3	2.5	2	2.5	3	2.6	3.6
Direct bilirubin (mg/dl)	1.9	1	1.5	1	0.4	0.3	0.4	0.4	0.3	0.2	0.2	0.2	0.3	0.2	1.49	2.4

Table 3 Comparison of pattern of genetic mutations among other similar studies [8–10]

Study	No. of patients	Presentation	Peak total serum bilirubin Levels	Response to phenobarbitone	Crigler-Najjar II (*UGT1A1* coding region missense mutation)	Gilbert's syndrome (promoter region mutation)
Our case (2019–2023)	One Indian infant	Persistent unconjugated hyperbilirubinemia a from birth	22.7 mg/dl	Significant > 80% reduction	Homozygous c.164A > G (p. His55Arg)	Homozygous (TA)7TAA/(TA)7TAA
Abell R et al. (2012) [8]	One (6-day-old American)	Pronounced neonatal hyperbilirubinemia	20.1 mg/dl	Not significant	*3 mutations* Heterozygous *28 (TA 6/7) (c. 40-39insTA) Heterozygous *60 (c-3275 T > G) Heterozygous *93 (c.-3152G > A)	Heterozygous (TA)6TAA/(TA)7TAA
Chalasani N et al. (1997) [9]	One American boy diagnosed as CN II 3 weeks of age	Kernicterus at 23 years of age	356 µmol/l	50% reduction	Heterozygous Single base substitution in one allele of exon 5 at nucleotide 1391 (A–C) and other alleles normal	Homozygous (TA)7TAA/(TA)7TAA
Kadakol A et al. (2001) [10](four American families)	**Family A**					
	18-month twin 1	Persistent unconjugated hyperbilirubinemia since birth	456 µmol	Significant response	1223delA/normal	(TA)6TAA/(TA)7TAA
	18-month twin 2	Persistent unconjugated hyperbilirubinemia since birth	410 µmol/l		1223delA/normal	(TA)6TAA/(TA)7TAA
	Mother	Asymptomatic	10 µmol/l		Normal	(TA)6TAA/(TA)7TAA
	Father	Asymptomatic	15 µmol/l		1223delA/normal	(TA)6TAA/(TA)6TAA
	Family B					
	10-year boy	Jaundice	205 µmol/l	No response	1490 T > A/normal	(TA)6TAA/(TA)7TAA
	18-year sister	Mild jaundice	85 µmol/l		1490 T > A/normal	(TA)6TAA/(TA)7TAA
	Mother	Asymptomatic	7 µmol/l		Normal	(TA)6TAA/(TA)7TAA
	Father	Asymptomatic	9 µmol/l		1490 T > A/normal	(TA)6TAA/(TA)6TAA
	Family C					
	Infant	Persistent unconjugated hyperbilirubinemia since birth	359 µmol/l	80% reduction	1452G > A/1452G > A	(TA)6TAA/(TA)7TAA
	Mother	Asymptomatic	51 µmol/l		1452G > A/Normal	(TA)6TAA/(TA)7TAA
	Father	Asymptomatic	10 µmol/l		1452G > A/Normal	(TA)6TAA/(TA)6TAA
	Family D					
	Sister 1	Unconjugated hyperbilirubinemia since birth	282 µmol/l	Not tried	524 T > A/524 T > A	(TA)7TAA/(TA)7TAA
	Sister 2	Unconjugated hyperbilirubinemia since birth	203 µmol/l		524 T > A/524 T > A	(TA)7TAA/(TA)7TAA
	Father	Asymptomatic	60 µmol/l		524 T > A/Normal	(TA)7TAA/(TA)7TAA

months, and the therapy was stopped. These infants had intermediate levels of TSB between Gilbert's syndrome and Crigler-Najjar syndrome and responded completely to phenobarbitone therapy [13].

Studies have also shown that cases with both Gilbert and CN II mutations suffer from liver parenchymal changes, hence the need to identify this double mutation earlier. In a study from China, percutaneous liver biopsy of 59 patients of unconjugated hyperbilirubinemia was done for assessing inflammation and extent of fibrosis. They showed that the linked polymorphic mutations, A(TA)7TAA and c.-3279 T > G in UGT1A1, were

most strongly associated with GS, whereas mutations in the coding region, especially p.G71R and p.Y486D, were more strongly associated with CNS-II. Iron deposition was more common in liver biopsies from patients with CNS-II than in those with GS [14].

After ruling out hemolytic conditions, the absence of kernicterus and response to phenobarbitone makes the clinical diagnosis of CN II in newborn period obvious; however, these children may still carry a GS mutation. Gailite L et al. described a 17-year-old boy born to non-consanguineous parents who presented with neonatal jaundice and diagnosed as GS by fragment analysis (A(TA)7TAA allele in homozygous state). He required periodical phenobarbitone therapy and was asymptomatic until puberty when his bilirubin levels significantly raised, and CN II was suspected. A bidirectional sequencing of five exons and exon/intron boundaries of the gene UGT1A1 (OMIM: 191,740) was performed. Four different variants in the UGT1A1 gene were identified in the patient: g.3664A>C (c.1352A>C, rs3755319); g.4963_4964TA [7] (c.-53_-52insTA, A(TA)7TAA, UGT 1A1*28, rs8175347); g.5884G>T (c.864+5G>T, IVS1+5G>T); and g.11895_11898del (c.996+2_996+5del). In the ClinVar database, the variant g.4963_4964TA [7] is described as a variant affecting response to drug treatment. This is the most common variant identified in patients with GS. The second intronic variant g.11895_11898del was reported for the first time [15]. In pediatric patients, Maruo et al [16] showed that the levels of serum bilirubin varied continuously within the spectrum from GS to CN II depending on genotypes. Serum bilirubin concentrations of typical CN II was 12.9 ± 5.1 mg/dl, the intermediate group was 5.2 ± 2.2 mg/dl, and typical GS was 2.8 ± 1.1 mg/dL ($P < 0.0001$) [16].

Drug therapy with phenobarbitone to reduce the levels of unconjugated bilirubin in CN II is well known; however, studies have reported that phenobarbital may cause hyperactivity, behavioral problems, sedation, and even dementia; these effects are dose related to some extent [17]. One of the reviews showed that phenobarbitone was significantly more likely to be associated with withdrawl than phenytoin in epileptic patients [18]. However, most of the studies with regard to safety profile of phenobarbitone are in the context of epileptic patients. There is hardly any data on the safety profile of phenobarbitone in patients with CN syndrome II, and data is lacking regarding the long-term neurodevelopmental outcome of those taking phenobarbitone.

Conclusion

Early suspicion of inherited causes of jaundice must prompt the evaluation of genetic mutations to look for coexistence of mutations for both GS and CN II. Coexistence of these mutations have implication on long-term neurological complications of unconjugated hyperbilirubinemia in stress or crisis situations. Prenatal diagnostic testing must be advised for detecting homozygous *UGT1A1* mutations to diagnose CN II and Gilbert mutations for each of the future pregnancies. Although there are no clear guidelines as to when to stop phenobarbitone therapy in CN II patients, considering the side effects of long-term phenobarbitone therapy, the decision can be taken on case-to-case basis of stopping the therapy while monitoring TSB levels.

Patient perspective

The child's father expresses the following: "We were quite anxious regarding the prolonged jaundice of our daughter in the early infancy. After the diagnosis was made by genetic tests, the presence of double mutations was scary; however, soon after the treatment was started (oral phenobarbitone syrup), she has been as normal as other children, growing well, is able to do all her regular activities, and is good at academics too; we wished to discontinue the medication soon and look for the recurrence of jaundice. We have been counseled regarding the recurrence of similar jaundice causing mutations in the next pregnancy and advised to undergo prenatal genetic diagnosis."

Abbreviations

CN II	Crigler-Najjar syndrome type II
CN I	Crigler-Najjar syndrome type I
GS	Gilbert's syndrome
UDPGT	Uridine diphospho-glucuronosyltransferase
BIND	Bilirubin-induced neurological damage
NGS	Next-generation sequencing
TSB	Total serum bilirubin

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None

Authors' contributions

RS and SS have made substantial contributions to the conception of the case report; RS and SS designed the work. SS and CTD contributed to the case follow-up and in collecting the case details. RS and SS contributed to the literature search and case analysis and drafted the work. SS and CTD substantially revised it, and RS, SS, and CTD have approved the submitted version and have agreed to be personally accountable for the author's own contributions to the case report.

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The data that support the case findings are available with the corresponding author on request.

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Not applicable.

Consent for publication
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Competing interests
The authors declare no competing interests.

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