

Case Report

Long-standing unconjugated hyperbilirubinaemia disproportionate to β -thalassaemia trait: unmasking coexisting homozygous UGT1A1*28 Gilbert syndrome through stepwise diagnostic reasoning

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ABSTRACT

A man in his late twenties presented with a 13-year history of icterus and dark urine, together with a short history of abdominal pain for 2.5 months. Biochemical evaluation demonstrated a total serum bilirubin of 9.46 mg/dL with an unconjugated fraction of 9.04 mg/dL, normal hepatocellular enzymes, and splenomegaly on abdominal ultrasonography. The peripheral blood film showed a microcytic, hypochromic picture with anisopoikilocytosis and mild red-cell fragmentation. Whole-exome sequencing identified a heterozygous pathogenic HBB c.92+5G>C splice-donor variant (IVS-I-5 G>C), confirming β -thalassaemia carrier status. The magnitude of unconjugated hyperbilirubinaemia was, however, disproportionate to trait alone, prompting a targeted search for a coexisting inherited disorder of bilirubin conjugation. UGT1A1 promoter fragment analysis revealed a homozygous (TA)₇/(TA)₇ (*28/*28) genotype, confirming Gilbert syndrome. Recognising the coexistence of the two disorders, rather than accepting a single incomplete diagnosis, altered counselling, drug-safety advice and reproductive planning. This case underscores that when unconjugated hyperbilirubinaemia is disproportionate to the demonstrable degree of haemolysis, a second inherited disorder of bilirubin metabolism should be actively sought.

Keywords: Unconjugated hyperbilirubinaemia, Gilbert syndrome, β -thalassaemia trait, Diagnostic reasoning

INTRODUCTION

Unconjugated hyperbilirubinaemia has two broad mechanisms: increased bilirubin production (haemolysis, ineffective erythropoiesis) and impaired hepatic conjugation due to inherited defects of the UDP-glucuronosyltransferase 1A1 (UGT1A1) enzyme.¹ Their coexistence in a single patient is under-recognised and a frequent source of diagnostic error either through attributing the unconjugated bilirubin elevation entirely to a haemolytic disorder, or through missing an accompanying disorder of conjugation such as Gilbert syndrome.²

The problem is particularly relevant in South Asia. β -thalassaemia trait affects 3–4% of the Indian population, with the IVS-I-5 (intervening sequence I, nucleotide 5; G>C) variant in the haemoglobin subunit beta (HBB) gene accounting for 25–60% of pathogenic mutations across most regions.^{3,4} Gilbert syndrome, driven principally by homozygosity for the UGT1A1*28 promoter polymorphism seven thymine–adenine dinucleotide repeats [(TA)₇] in the TATA box occurs in 10–25% of the Indian subcontinent, among the highest global frequencies.⁵ Despite the plausibility of their co-existence, the paired diagnosis is seldom pursued.

We describe a young man with more than a decade of persistent icterus initially attributed to β -thalassaemia trait,

in whom the severity of unconjugated hyperbilirubinaemia was disproportionate to trait alone. The diagnostic reasoning that uncovered coexisting Gilbert syndrome is the substance of this report.

CASE REPORT

A man in his late twenties, previously in reasonable general health, presented to our centre with a long-standing history of jaundice. He described yellowish discolouration of the eyes for 13 years, first noticed in his mid-teens and persistent since then, with dark, high-coloured urine that worsened during febrile illnesses and periods of reduced oral intake. He did not report pale stools, pruritus, weight loss, easy bruising, altered sensorium or bleeding tendency.

Two-and-a-half months before presentation, he developed intermittent, dull aching left upper-quadrant abdominal pain without radiation or postprandial aggravation, and without fever, vomiting, haematemesis or melaena. Three years earlier, during an acute febrile illness associated with worsening pallor and jaundice, he had received a single unit of packed red-cell transfusion at another facility.

He was a non-smoker with no alcohol or drug use, and was not taking any prescription, over-the-counter, or herbal

medications. None of his family members had complaints of jaundice, blood transfusion, cholelithiasis, or splenomegaly. On examination, he was afebrile and haemodynamically stable, with scleral icterus evident in natural light and mild pallor. There was no lymphadenopathy or stigmata of

chronic liver disease. A firm, non-tender spleen was palpable approximately 3 finger-breadths below the left costal margin; the liver edge was borderline palpable in the mid-clavicular line with a smooth surface. There was no ascites. Cardiovascular, respiratory and neurological examinations were unremarkable.

Investigations

Biochemistry confirmed a predominantly unconjugated hyperbilirubinaemia: total serum bilirubin 9.46 mg/dl (reference 0.3-1.2 mg/dl), with unconjugated 9.04 mg/dl and conjugated 0.42 mg/dl the unconjugated component comprising approximately 96% of the total. Aminotransferases, alkaline phosphatase, gamma-glutamyl transferase, serum albumin and prothrombin time were within normal limits, providing no evidence of hepatocellular injury, cholestasis or synthetic dysfunction of the liver.

Table 1: Key haematological and biochemical investigations.

Parameter	Reference range	On admission	Later during admission
Haemoglobin (g/dL)	12-15	8.9	9.2 (at HPLC)
Mean corpuscular volume (fL)	78-98	64	68.7
Red cell distribution width (%)	11-14	16.9	19.0
Reticulocyte count / reticulocyte production index	-	1.82% / 0.77	-
Lactate dehydrogenase (U/L)	225-450	619	-
Total bilirubin (mg/dL)	0.3-1.2	9.66	9.46
Direct (conjugated) bilirubin (mg/dL)	0-0.4	0.42	0.42
Alanine aminotransferase (U/L)	0-35	22	19
Aspartate aminotransferase (U/L)	0-35	21	26
Serum albumin (g/dL)	3.5-5.2	4.0	4.0
Direct / indirect antiglobulin test	Negative	Negative	-
Serum ferritin (ng/ml)	7-140	516	-

HPLC: high-performance liquid chromatography.

Table 2: Haemoglobin high-performance liquid chromatography fractions.

Haemoglobin fraction	Result (%)
HbF	3.50
HbA0	80.40
HbA2	5.90

HbF: fetal haemoglobin; HbA0: adult haemoglobin A; HbA2: haemoglobin A2.

The peripheral blood film demonstrated anisopoikilocytosis with a microcytic, hypochromic

picture, a few ovalocytes, occasional teardrop cells and fragmented red cells consistent with a hypoproliferative

microcytic anaemia accompanied by mild ineffective erythropoiesis and only low-grade extravascular haemolysis, rather than a florid haemolytic phenotype. Serum lactate dehydrogenase was 619 U/L (reference 225–450 U/L). Hepatitis B, hepatitis C and human immunodeficiency virus serologies were negative. Ultrasonography demonstrated splenomegaly with borderline hepatomegaly; the gallbladder was not distended, no gallstones were identified, and the biliary tree was of normal calibre. Haemoglobin high-performance liquid chromatography (HPLC) showed a profile consistent with β -thalassaemia trait, with a raised HbA2 fraction (Table 1, Table 2 and Figure 1).

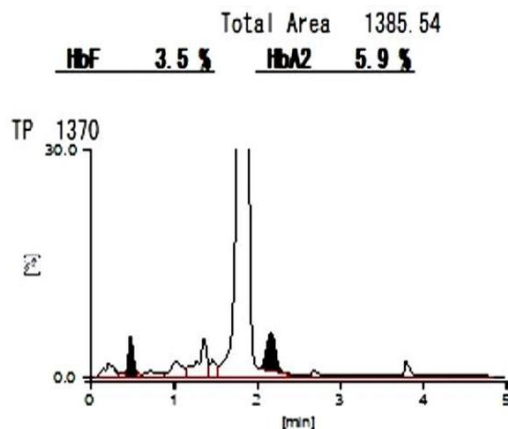


Figure 1: Haemoglobin high-performance liquid chromatography chromatogram showing an elevated HbA2 fraction (5.9%) with HbF 3.5%, a pattern consistent with β -thalassaemia trait.

Given the disproportion between the severity of hyperbilirubinaemia and the modest markers of haemolysis, further molecular evaluation was pursued. Whole-exome next-generation sequencing identified a heterozygous pathogenic splice-site variant in HBB (NM_000518.5: c.92+5G>C; p.), consistent with β -thalassaemia carrier status, with no other pathogenic, likely pathogenic, secondary or incidental variant identified on the same panel. UGT1A1 promoter genotyping by polymerase chain reaction and fragment analysis showed a 7/7 TA-repeat genotype (UGT1A1*28/*28), the genotype associated with severely reduced (approximately 70% reduction) UGT1A1 glucuronidation activity and diagnostic of Gilbert syndrome.

Differential diagnosis

A predominantly unconjugated hyperbilirubinaemia (>95% of total) sustained over 13 years localised the pathophysiology to bilirubin overproduction or defective conjugation. Preserved liver enzymes, normal synthetic function and a normal biliary tree excluded hepatocellular injury, cholestasis and biliary obstruction.

The initial consideration was whether a single haematological disorder could unify the presentation. The peripheral blood film findings and the heterozygous HBB IVS-1-5 splice-site variant confirmed β -thalassaemia trait. However, trait is characteristically clinically silent or associated with only mild anaemia; unconjugated bilirubin from trait alone rarely exceeds 2–3 mg/dL, arising from low-grade ineffective erythropoiesis and a mildly shortened red-cell life span.⁶ A sustained total bilirubin approaching 10 mg/dL is not consistent with β -thalassaemia trait alone. The absence of transfusion-dependence, the absence of affected first-degree relatives, and the smear morphology together made β -thalassaemia intermedia unlikely, though a structural HBB variant could not be entirely excluded by clinical exome sequencing.

Other coexisting haemolytic disorders were considered. Hereditary spherocytosis was not favoured given the absence of spherocytes and the atypical microcytic–hypochromic morphology. Glucose-6-phosphate dehydrogenase deficiency was not biochemically excluded, but the sustained (rather than episodic, post-oxidant) hyperbilirubinaemia argued against it. Autoimmune haemolytic anaemia was clinically implausible given the 13-year chronicity and negative antiglobulin test.

Although β -thalassaemia trait may be associated with mild chronic haemolysis, the degree of unconjugated hyperbilirubinaemia was disproportionate to this mechanism. Attributing 9 mg/dL of unconjugated bilirubin to an incompletely characterised haemolytic process would have led to an incomplete diagnostic evaluation.⁷ We therefore refocused on inherited disorders of bilirubin conjugation. The first considered was Gilbert syndrome, in which homozygous UGT1A1*28 promoter polymorphism reduces UGT1A1 transcription by approximately 70% and typically produces bilirubin of 1–4 mg/dL, occasionally up to 5–6 mg/dL under provocation from fasting, dehydration, intercurrent illness or a coexisting haemolytic disorder.^{5,8} These levels rise further when an additional bilirubin production load is present.

Another possibility was Crigler–Najjar syndrome type II, in which biallelic UGT1A1 coding missense variants reduce enzyme activity to <10% of normal, producing sustained bilirubin of 6–20 mg/dL that characteristically responds to phenobarbital.⁹ This bilirubin range overlaps substantially with Gilbert syndrome plus a superimposed haemolytic load, making biochemical differentiation very difficult. Our patient's total bilirubin of 9.46 mg/dL fell within this overlap zone, in which Crigler–Najjar type II must be actively excluded.⁹ Crigler–Najjar type I was clinically implausible given the age at presentation, the absence of neonatal kernicterus, and bilirubin far below the 20–45 mg/dL range seen in that condition.

Genotyping of both the UGT1A1 promoter and coding region was therefore essential. Targeted analysis identified homozygous (TA)7/(TA)7 (*28/*28) in the promoter,

while coding-region interrogation of the exome data revealed no pathogenic variant. Homozygous UGT1A1*28/*28 with the superimposed bilirubin load from coexisting β -thalassaemia trait adequately explained the observed hyperbilirubinaemia; the absence of a coding UGT1A1 variant argued against Crigler–Najjar type II. The final diagnosis was β -thalassaemia trait (heterozygous pathogenic HBB c.92+5G>C) coexisting with Gilbert syndrome (homozygous UGT1A1*28/*28).

Management

Management centred on correct labelling, reassurance and structured counselling; no pharmacological treatment was required for either diagnosis in isolation. The patient was counselled that Gilbert syndrome is a benign condition not associated with progressive liver disease or increased mortality and was advised regarding triggers for exacerbation of unconjugated hyperbilirubinaemia prolonged fasting, dehydration, intercurrent infections and physical exertion with encouragement to maintain hydration and regular meals during febrile illnesses.¹⁰

The pharmacogenomic implications of homozygous UGT1A1*28 were emphasised. This genotype confers an approximately 50% risk of severe neutropenia and delayed-onset diarrhoea with the antineoplastic agent irinotecan.¹¹ Similar considerations apply to nilotinib, atazanavir, indinavir, sorafenib and high-dose paracetamol.¹² The patient was provided with written documentation of his UGT1A1 genotype for future prescribers.

For β -thalassaemia trait, iron supplementation was specifically avoided in the absence of biochemical iron deficiency, given the well-recognised risk of iron loading in carrier states.³ The importance of prenatal genetic counselling before family planning was emphasised, and partner testing (complete blood count with mean corpuscular volume, haemoglobin HPLC, and HBB sequencing where indicated) was advised.³ Given the modestly increased lifetime risk of pigment gallstones with chronic unconjugated hyperbilirubinaemia and low-grade haemolysis, periodic ultrasonographic surveillance was also advised.¹³

Outcome and follow-up

The patient remained haemodynamically stable throughout the index admission and was discharged in a clinically stable condition after approximately one week, with the supportive measures and dietary and lifestyle advice detailed above. He was advised outpatient follow-up two weeks after discharge, with a repeat full blood count, liver function tests, and the UGT1A1 and next-generation sequencing reports for review.

He continues to be followed up in our medicine outpatient department. At his most recent review, he remained vitally and clinically stable, with no recurrence of abdominal

pain, worsening jaundice, dark urine, pale stools or constitutional symptoms since discharge. He was reassured that no further invasive investigation or specific treatment was required for the hyperbilirubinaemia itself, and was advised to continue periodic outpatient review.

DISCUSSION

This case exemplifies an under-recognised problem: the coexistence of two common inherited disorders a haemoglobinopathy and a bilirubin-conjugation defect producing a phenotype that neither disorder produces in isolation.

The mechanistic rationale is straightforward. β -thalassaemia trait produces low-grade ineffective erythropoiesis and a modestly shortened red-cell life span, imposing a persistent additional bilirubin load on the liver.⁶ With intact UGT1A1 activity, this load is comfortably handled, and unconjugated bilirubin does not rise significantly. With homozygous UGT1A1*28 (transcription reduced by ~70%, residual enzyme activity ~30% of normal), the same load precipitates jaundice out of proportion to either the trait or the syndrome alone.⁸ Galanello and colleagues first quantified this synergy in β -thalassaemia carriers and Shrestha and colleagues, in a large Indian transfusion-dependent β -thalassaemia series, identified UGT1A1*28/*28 in a substantial minority of patients and demonstrated its independent contribution to hyperbilirubinaemia across thalassaemia genotypes.^{2,5} The reverse also holds: a patient with severe hyperbilirubinaemia, anaemia or premature gallstones labelled as Gilbert syndrome should prompt a search for a coexistent haemolytic disorder.¹

In our case, the diagnostic evaluation proceeded through three sequential steps: predominant unconjugated hyperbilirubinaemia localised the abnormality to overproduction or under-conjugation; whole-exome sequencing established β -thalassaemia trait but, on its own, failed to account for the degree of hyperbilirubinaemia; and targeted UGT1A1 promoter genotyping identified the missing mechanistic piece. It was the willingness to reject a partial explanation for the severity of jaundice, rather than any single elaborate test, that made the complete diagnosis possible.

Two aspects of the evaluation warrant emphasis. First, whole-exome sequencing is an incomplete test for this phenotype. The Gilbert syndrome (TA)n promoter polymorphism lies outside the protein-coding regions targeted by standard clinical exome capture, and short-tandem-repeat variants are poorly resolved by short-read variant callers even when captured. Standard exome will therefore miss the commonest genetic cause of chronic unconjugated hyperbilirubinaemia unless UGT1A1 promoter fragment analysis or long-read sequencing is specifically requested.¹ Second, in the bilirubin range 6–20 mg/dL, Crigler–Najjar type II cannot be excluded on biochemical grounds alone; the overlap with Gilbert

syndrome plus a haemolytic load is substantial, and only genotyping of the UGT1A1 coding region reliably distinguishes the two.⁹ This distinction has therapeutic implications, as patients with Crigler–Najjar type II generally require phenobarbital and carry a small but real risk of kernicterus in adulthood when provoked.

The clinical consequences of correctly identifying the coexistence are non-trivial: a coherent explanation for 13 years of jaundice; a slightly increased lifetime risk of pigment gallstones requiring periodic surveillance; substantially increased pharmacogenomic risk from UGT1A1 substrates, needing documentation for future prescribers; and reproductive counselling that depends on the partner's HBB genotype.^{3,11-13}

CONCLUSION

When unconjugated hyperbilirubinaemia is disproportionate to the demonstrable degree of haemolysis, clinicians should actively search for a second inherited disorder of bilirubin metabolism rather than attributing all findings to a single haemolytic process. Recognising the coexistence of β -thalassaemia trait and Gilbert syndrome, as in this patient, carries direct consequences for counselling, drug safety and reproductive planning, and depends on genotyping strategies that extend beyond standard clinical exome sequencing.

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