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Unconjugated hyperbilirubinaemia due to Hb Hofu (HBB: c.380T>A) with Co-inherited UGT1A1 Promoter Polymorphism: A Familial Case Series from India

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ABSTRACT

Background: Evaluation of unconjugated hyperbilirubinemia can be challenging due to contributions from multiple environmental and genetic factors. Certain hemoglobinopathy like HbE syndrome and inherited defect of bilirubin metabolism are various etiologies for persistent unconjugated hyperbilirubinemia. Therefore, hyperbilirubinaemia, persistent or disproportionate to the degree of hemolysis warrants evaluation for additional genetic causes affecting bilirubin metabolism.
Method: We describe case series involving four siblings evaluated for unexplained unconjugated hyperbilirubinaemia. Hematological, hemoglobin (Hb)-cation-exchange high-performance liquid chromatography (HPLC), and molecular analyses for unconjugated hyperbilirubinaemia including HBB and UGT1A1 sequencing were performed.
Results: Two siblings demonstrated an abnormal hump in the ascending limb of HbA0 on HPLC comprising 25.6% of total hemoglobin. Both were heterozygous for HBB: c.380T>A consistent with Hb Hofu. All four siblings demonstrated heterozygous UGT1A1 promoter polymorphism [A(TA)6TAA] [A(TA)7TAA] associated with Gilbert syndrome spectrum. The affected siblings had mild microcytosis with preserved hemoglobin levels without evidence of hemolysis. However, the remaining siblings lacked the HBB variant.
Conclusion: This report of co-inheritance of Hb Hofu with compound heterozygosity for Gilbert and Criggler-Najjar syndromes highlights the need for sequential hematological and molecular work-up for inherited defect of bilirubin metabolism in patients where finding a variant hemoglobin only partially explains the observed clinical phenotype.

Keywords*: Hb Hofu, unstable hemoglobin, Gilbert syndrome, Crigler–Najjar syndrome, UGT1A1, unconjugated hyperbilirubinemia.*

INTRODUCTION

Work-up of adult patients with unconjugated hyperbilirubinemia can be challenging due to contributions from multiple environmental and genetic factors **[1]**. We recently encountered coexisting relatively rare hemoglobinopathy with inherited variants influencing both bilirubin production as well as handling as cause of unconjugated hyperbilirubinemia in two siblings.

Inherited disorders of bilirubin metabolism, particularly Gilbert syndrome caused by polymorphisms in the promoter region of the UGT1A1 gene, may contribute to disproportionate unconjugated hyperbilirubinemia in patients with otherwise mild hemolytic disorders.

Unconjugated hyperbilirubinaemia is also a feature of transfusion dependent thalassemia resulting from mutation in both the beta globin gene. However, HbE hemoglobinopathy in homozygous and heterzygous state also presents with mild unconjugated hyperbilirubinaemia in some individuals.

We report a familial case series involving four siblings in whom the unconjugated hyperbilirubinaemia in two siblings were found be due to an uncommon hemoglobinopathy, Hb Hofu associated with heterozygous UGT1A1 promoter polymorphism and variant in exon 3 while in another two, UGT1A1 promoter sequence polymorphism alone was responsible.

METHODS

Four siblings from a family originating in northern India underwent hematological evaluation for jaundice and abnormal hematological parameters. Clinical assessment included complete blood counts, peripheral blood smear examination, reticulocyte count, and hemoglobin analysis by cation-exchange high-performance liquid chromatography (CE-HPLC). Qualitative screening for G6PD deficiency was performed using methemoglobin reduction test to rule out common enzymopathies. Molecular studies included sanger sequencing of the HBB gene to rule out hemoglobinopathies and UGT1A1 promoter region for polymorphism associated with Gilbert syndrome. It was followed by targeted next generation sequencing (NGS) for hemolytic and dyserythropoietic anaemias.

Results: The hematological and molecular findings of the four siblings are summarized in Table 1

Table 1: Hematological findings of the siblings:

	Brother (case 1)	**Sister (case 2)**	**Sibling (case 3)**	**Sibling (case 4)**
Age/Sex	18 Y/M	20Y/F	14Y/F	21Y/F
Hb (g/dl)	13.5	11.4	10.5	8.7
TLC ($x10^9$/L)	6.2	7.9	6.0	7.4
Platelet count ($x10^9$ / L)	203	100	123	211
HCT (%)	41.4	33.7	31.6	27
MCV (fl)	76.6	77.1	82.9	69.7
MCH (pg)	25.1	25.9	27.6	22.5
MCHC (g/l)	32.7	33.6	33.3	32.2
RBC ($x10^9$ / µL)	5.4	4.3	3.8	-
Reticulocyte count (%) (uncorrected)	1.6	2.4	-	3.8
RDW %	14.6	15.3	15.4	19.4

PBS	Normocytic normochromic to microcytic hypochromic red cells	Normocytic normochromic to microcytic hypochromic red cells	Normocytic normochromic red cells	Microcytic hypochromic red cells
HbF (%)	0.6	1.0	0.3	0.2
HbA2 (%)	3.4	3.7	2.7	2.2
HbA + Hump in ascending limb of HbA0 (%)	25.6	25.6	No hump	No hump
G6PD qualitative assay (methemoglobin reduction test)	Negative	Negative	Negative	Negative
Sequencing of UGT1A1 for Gilbert syndrome UGT1A1, NM_000463.3	[A(TA)6TAA][A(TA)7TAA]	[A(TA)6TAA][A(TA)7TAA]	[A(TA)6TAA][A(TA)7TAA]	[A(TA)6TAA][A(TA)7TAA]
HBB gene sequencing	Hetetozygous for NM_000518.5: c.380T>A,p.12 7V>E	Hetetozygous for NM_000518.5:c. 380T>A,p.127V> E	Normal	Normal

The index case (Case 1), an 18-year-old male, presented with persistent unconjugated hyperbilirubinemia (total and unconjugated bilirubin 12.3 and 7 mg% respectively) Liver enzymes were minimally elevated (AST/ALT/ALP 31.4/54.2/155.4 U/L) with normal serum proteins and renal parameters. Serological studies revealed presence of anti-HEV IgM, but tests for hepatitis A, B and C were negative. Hemoglobin level was preserved (13.5g/dL) with mild

microcytosis and hypochromia. CE-HPLC demonstrated an abnormal hump in the ascending limb of HbA0 (with retention time of 2.25 min) constituting 25.6% of total hemoglobin. (Figure 1a) This variant was faster-moving than HbA on alkaline pH electrophoresis **(Figure 1b)**. DNA sequencing of the β-globin gene (*HBB*)'s exons 1 and 2 revealed heterozygosity for Hb Hofu, a rare but previously described variant hemoglobin, *NM_000518.5(HBB):c.380T>A (p.Val127Glu)* **(Figure 1c) [2-6]** Hb Hofu despite being mildly unstable, is not associated with severe jaundice **[2-4]**, hence, sequencing of the *UGTI1A1* gene promoter to exclude coexisting Gilbert syndrome was done. It showed a heterozygous (TA6/TA7) pattern **(Figure 1D)**.

Since his jaundice still remained unexplained, targeted sequencing was performed using a customized panel of 55 genes implicated in hereditary hemolytic and dyserythropoietic anemias (1,525 amplicons of total length 360,381 bp, TruSeq Custom Amplicon Panel, Illumina) on a HiSeq sequencer. This revealed a heterozygous nonsense mutation in *UGT1A1* exon 3 (NM_000463.3(*UGT1A1*):c.1021C>T, p.Arg341Ter). Homozygosity for this mutation was previously implicated in a cohort of Pakistani children with Crigler- Najjar syndrome, type 1 **[7]**. The same mutation was also reported as causing Crigler- Najjar syndrome type 2 when occurring in a compound heterozygous state with another coding sequence variant of *UGT1A1* **[7]**. In addition, the co-inheritance in *trans* of compound heterozygosity for a Gilbert type promoter sequence and a coding sequence variation causing Criggler-Najjar syndrome is known to result in intermediate level of hyperbilirubinemia (i.e., a CN2 phenotype) **[8]**. In our patient, this last combination, compounded by Hb Hofu was therefore the most likely multifactorial cause of his jaundice.

His 20-year-old sister **(Case 2)** with mild jaundice and mild microcytic hypochromic anaemia also demonstrated similar chromatographic findings with identical abnormal fraction constituting 25.6% of total hemoglobin and heterozygous (TA6/TA7) polymorphism in UGT1A1 promoter sequence like case 1. NGS was not done in this case but mild jaundice can be explained by presence of promoter polymorphism, which usually presents with mild jaundice exaggerated by presence Hb Hofu may explain her clinical phenotype. **(Table 1)**

The remaining two siblings **(Cases 3 and 4)** presented with moderate anaemia but lacked the abnormal HPLC hump and had normal HBB sequencing. However, both demonstrated heterozygous UGT1A1 promoter polymorphism similar to the affected siblings. Peripheral smear findings ranged from normocytic normochromic to microcytic hypochromic morphology. **(Table 1)** Reticulocyte counts was minimally elevated in fourth sibling. G6PD screening was negative in all siblings.

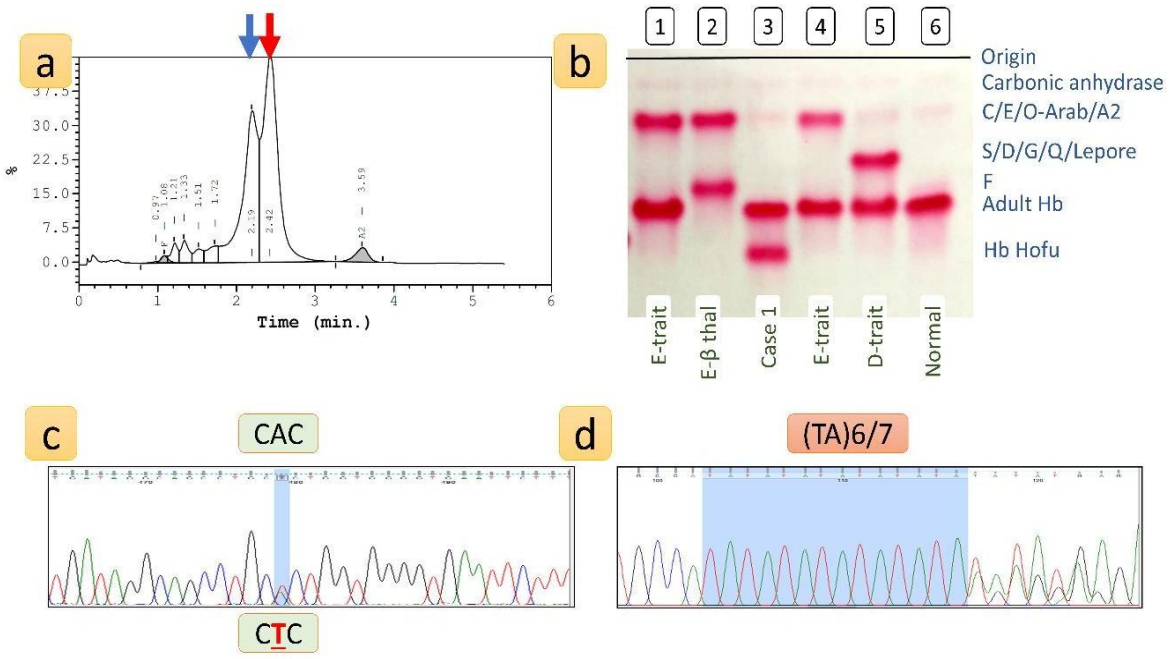

Figure 1: a. Hemoglobin CE-HPLC of case 1 shows an unknown peak adjacent to HbA0 (Variant-II™ analyzer, β-Thal Short program, Bio-Rad Laboratories, Hercules, USA). **b**. Hemoglobin electrophoresis at alkaline pH shows adult Hb and a fast- migrating band (arrow). **c.** Sanger sequencing shows heterozygous state for Hb Hofu, NM.000518.5 (*HBB*):c.380 T>A,p.V127E. **d**. Sanger sequencing of *UGT1A1* promoter shows heterozygous state for TA6/TA7 repeats. (NM_000463.3 (*UGT1A1*):[A(TA)6TAA][A(TA)7TAA].

DISCUSSION

Hb Hofu is a rare negatively-charged Hb variant that was first discovered in the Yamaguchi Prefecture of Japan in 1968.**[2]**. An early Indian report in 1978 described 10 cases (8 Hofu heterozygotes and two double heterozygotes for Hofu and Sickle Hb) in two Valmiki families **[4].** The mutation results in β16(H4) Val→Glu substitution and produces a negatively charged unstable hemoglobin molecule. The clinical phenotype is usually mild or even asymptomatic in the heterozygous state **[2-4]**. Two previously reported cases where Hb Hofu was co-inherited in a compound heterozygous state with β^0-thalassemia had a clinical presentation of mild thalassemia intermedia (Hb ranging from 9.0-9.5 g%). One of the patients required two transfusions during pregnancy **[5,6]**.

The present familial series highlights several important observations. First, the characteristic abnormal hump in the ascending limb of HbA0 on CE-HPLC was reproducibly identified in both affected siblings, emphasizing the importance of careful chromatographic interpretation in rare hemoglobin variants.

Second, despite harboring the Hb Hofu mutation, the affected siblings (case 1 & 2) demonstrated relatively preserved hemoglobin levels and only mild microcytosis without marked evidence of ongoing hemolysis. This observation supports the generally mild phenotype previously described with heterozygous Hb Hofu.

Third, all siblings demonstrated heterozygous UGT1A1 promoter polymorphism [A(TA)6TAA][A(TA)7TAA], suggesting co-inheritance of Gilbert syndrome spectrum abnormality within the family. Presence of UGT1A1 polymorphism may contribute to disproportionate indirect hyperbilirubinemia in patients with even mild hemoglobin instability.

The coexistence of inherited bilirubin conjugation defects and unstable hemoglobin variants may complicate clinical interpretation of jaundice. Recognition of such combined genetic abnormalities is important to avoid unnecessary evaluation for acquired liver disease or hemolytic conditions.

CONCLUSION

We report a familial case series of Hb Hofu associated with heterozygous UGT1A1 promoter polymorphism in Indian siblings. Characteristic HPLC findings combined with molecular confirmation facilitated diagnosis of this rare unstable hemoglobin variant. The study highlights the importance of integrated hematological and molecular evaluation in patients with unexplained indirect hyperbilirubinemia and uncommon hemoglobin variants.

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