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Hazardous hyperbilirubinemia in a neonate with novel homozygous biallelic GSR (glutathione reductase) mutations

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Abstract

A six-day-old term male presented to our hospital emergency department with a total serum bilirubin (TSB) of 34.8 mg/dL. Double volume exchange transfusion resulted in a fall to 20.6 mg/dL, and intensive phototherapy resulted in a gradual further fall to 5.4 mg/dL by discharge home eight days later. This was the first child of a consanguineous couple from Southern India, with no family history of anemia or jaundice. Extensive evaluation for the etiology of the hyperbilirubinemia revealed homozygous biallelic mutations in *GSR*, the gene encoding Glutathione Reductase and the heterozygous polymorphism UGT1A1*28. The Brainstem Auditory Evoked Response test and Magnetic Resonance Imaging seeking evidence of kernicterus one week later were normal. When examined at two and four months of age he had no hearing loss or other signs of neurological abnormalities. This case is similar to one reported from the Netherlands where compound heterozygous mutations in *GSR* were identified and postulated to be the underlying cause of significant neonatal jaundice. We speculate that these two cases support the theory that *GSR* mutations that result in significantly diminished enzyme function can be associated with hazardous neonatal hyperbilirubinemia with a good outcome after exchange transfusion.

Introduction

Neonatal hyperbilirubinemia is termed "hazardous" if the Total Serum Bilirubin (TSB) exceeds 30 mg/dL (513 µmol/L) [1]. The term "hazardous" is used because of the risk of acute and chronic encephalopathy, including hearing loss [2,3]. Unfortunately, many cases of neonatal hazardous hyperbilirubinemia are termed "idiopathic" because there is no clear explanation for the hyperbilirubinemia [4]. However, there is value in discovering the underlying etiology because this not only gives families clarity, but can provide information relevant to future pregnancies [5,6]. Next generation DNA sequencing panels can assist in identifying the cause in puzzling cases [7]. We report a neonate who was very similar in presentation, appearance, sequencing, and outcome, to a neonate previously reported from the Netherlands, where biallelic mutations in *GSR* were also identified.

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Case Report

A six-day old male was admitted to the Primary Children's Hospital NICU with a total serum bilirubin (TSB) of 34.8 mg/dL. He was born to a healthy primagravida mother at 37 weeks gestation after normal pregnancy, labor, and vaginal delivery, with no family history of jaundice, anemia, splenectomy or cholecystectomy. Parents were immigrants from Southern India and were first cousins. Birth weight was 2693 g (<3rd percentile), length 46 cm (<3rd percentile) and OFC 33.5 cm (3rd percentile). In the birth hospital a TSB was 5.6 mg/dL between 24 and 36 hours after birth. He was discharged home after 48 hours, and seen by a physician on the following day, where no problems were identified and a routine follow-up was planned at 2 weeks. During the next two days the parents reported that he breast-fed well with 6 to 8 wet diapers per day, but on day five, they noted his eyes were yellow and he was sleepier. They took him to the children's hospital emergency department because of the increasing yellow appearance.

Examination on admission to the NICU revealed intense jaundice but was otherwise normal. His weight on admission was 2400 g. He had normal tone and motor activity and BIND score was zero [8]. Umbilical catheters were placed and a double volume exchange transfusion performed. His TSB immediately before the exchange transfusion was 35.3 mg/dL (direct fraction 0.8 mg/dL) and was 20.6 mg/dL after exchange transfusion. His TSB continued to gradually fall under phototherapy to 5.4 mg/dL by discharge home on day of life 14.

Laboratory evaluation performed to determine the cause of the hyperbilirubinemia included a blood film with marked anisocytosis and poikilocytosis. Leukocytes and platelets appeared normal. His serum haptoglobin was below the lower limit of detectability (< 8mg/dL) and his end-tidal carbon monoxide measurement (a measurement of the hemolytic rate) on day of life eight was 1.9 ppm (mildly elevated). The reticulocyte count was 1.69% and the hematocrit 53%. Mother and infant were blood group O (+) and DAT was negative. G6PD 18.6 U/g hgb was elevated. No evidence was found for thyroid dysfunction, liver dysfunction or cholestasis. We performed a next-generation sequencing panel of 28 genes involved in hereditary hemolytic jaundice (ARUP Laboratories, SLC, UT, USA).

Abdominal ultrasound examination revealed sludge in the bladder, but was otherwise normal. Prior to discharge home he passed his hearing screen, including BAER, and an MRI did not reveal evidence of kernicterus. He has continued to do well at home with normal growth and development at two and four months of age. The genetic testing results (shown in table 1) revealed homozygous biallelic mutations in *GSR*, the gene encoding Glutathione Reductase, which was judged by *in silico* programs (SIFT and PolyPhen-2) as deleterious. In addition he has a heterozygous promotor polymorphism for *UGT1A1**28, plus variants in both *SPTA1* and *SPTB* that likely have no clinical significance.

Discussion

Human glutathione reductase is encoded by the *GSR* gene (MIM 138300) which is located on chromosome 8p21.1. *GSR* spans 50 kb and consists of 13 exons. The enzyme generated by *GSR* is essential for cellular well-being because it maintains a concentration of reduced glutathione needed as a cellular antioxidant (figure 1) [9].

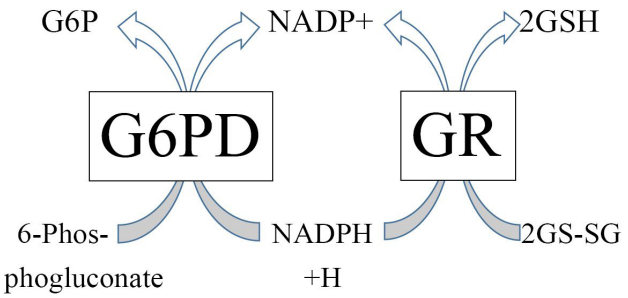

Figure 1: Schematic representation of the role of Glutathione Reductase in preventing oxidative stress in erythrocytes. A deficiency in the enzyme glutathione reductase (GR) is expected to decrease the concentration of reduced glutathione (GSH). G6P, Glucose 6-Phosphate; NADP+, Nicotinamide Adenine Dinucleotide Phosphate; NADPH, Reduced Nicotinamide Adenine Dinucleotide Phosphate; GS-SG, Glutathoine Disulfide.

Kamerbeek *et al* described a neonate with a presentation, hospital course, and outcome somewhat similar to our case [9]. Their patient had biallelic *GSR* mutations. Their patient was a compound heterozygote, where one allele had a premature stop codon and the other had a point mutation at amino acid position 330, which alters a highly conserved amino acid in a binding domain, and impairs thermostability of the enzyme. This case has biallelic (homozygous) *GSR* mutation at position 330, in a first-cousin marriage where both parents were asymptomatic carriers of the mutant allele.

One perplexing aspect of both cases is the lack of evidence for hemolysis; specifically a normal reticulocyte count, hematocrit, and hemoglobin at presentation on day six. This case had undetectable serum haptoglobin and a slightly elevated end-tidal carbon monoxide measurement, suggesting at least mild hemolysis, and had a distinctly abnormal blood smear before the exchange transfusion (haptoglobin and echinocytes were not reported in the Dutch case). Thus, it is not clear whether hemolysis played some role in the hyperbilirubinemia in these two cases, but an alternative explanation is lack of bilirubin uptake into hepatocytes, impaired conjugation, or retarded bilirubin excretion. The lack of marked hemolysis in this patient is similar to some cases of neonatal hyperbilirubinemia due to G6PD deficiency, where hazardous hyperbilirubinemia can be seen with minimal evidence of hemolysis. Luzzarro and Arese recently reported that hemolysis may not explain hyperbilirubinia in fauvism [10]. In contrast, Kaplan recorded elevated carboxyhemoglobin concentrations and elevated end tidal carbon monoxide in jaundiced G6PD patients [11,12]. Thus the mechanisms for the jaundice in cases of neonatal G6PD deficiency and GR deficiency might be complex. Kammerbeck *et al.* postulated that bilirubin conjugated to glutathione normally plays a role in the first few days after birth, when conjugation of bilirubin to glucuronic acid is still immature, and that lower levels of reduced glutathoine might retard bilirubin metabolism [9].

Another similarity in these two cases is no evidence of kernicterus or damage to the cochlea or auditory nerve. Hemolysis appears to be common in cases where hyperbilirubinemia results in kernicterus, and the lack of hemolysis in these cases may explain the good outcomes.

Table 1: DNA variants, and interpretations, based on next generation sequencing.

Gene	Nucleic Acid Change	Amino Acid Alteration	*In silico* prediction of damage		Interpretation
			SIFT	PolyPhen2	
GSR (glutathione reductase)	c.9891>C Homozygous	p.Leu330Pro	deleterious	Probably damaging	Novel homozygous variant, likely to significantly diminish glutathione reductase activity in erythrocytes
SPTB (beta spectrin)	c.5486G>A Heterozygous	p.Ser1829Asn	tolerated	benign	Likely benign
SPTA1 (alpha spectrin)	Alpha LELY Heterozygous	None	tolerated	benign	Frequency of 20-30% in general population, only damaging when coupled *in trans* with an alpha spectrin mutation
UGT1A1	*28 allele (TA)7 Heterozygous	None	tolerated	benign	In the homozygous state, it results in Gilbert's syndrome. Heterozygotes can have moderate impairment in bilirubin conjugation and clearance, particularly as neonates.

Table 2: Comparison of features of two neonates with hazardous hyperbilirubinemia subsequently diagnosed with Glutathione Reductase Deficiency due to damaging mutations in *GSR*.

Feature	Case 1	Case 2
Year and Country	2007 Netherlands	2018 USA
Gestational age at birth	term	37 weeks
Birth weight and Gender	Not given/female	2693 g/male
Parent's country of origin/Ethnicity	Netherlands/ Caucasian	Southern India/Indo-Aryan
Consanguinity	No	Parents are first cousins
DOL at presentation to hospital with hazardous hyperbilirubinemia	7	6
Highest serum bilirubin recorded (total, direct, indirect)	44 mg/dL	35.3 mg/dL (1.1 and 34.2)
Exchange transfusion	Double volume	Double volume
Hgb/Hct	normal	18.8 g/dL/ 53.1%
MCV/ MCHC	normal	100.7 fL.35.4 g/dL
Reticulocytes (%)	1.6%	1.9%
G6PD level	Normal	Normal – elevated (18.6 u/g hgb)
Haptoglobin	Not reported	Below detection
Blood group Mother/Baby, DAT	O(+)/O(+), DAT (-)	O(+)/O(+), DAT (-)
State at presentation	Lethargic and hypotonic	Sleeping more frequently but normal when awake
GSR mutations	Compound heterozygous GSR mutations. G861A (premature stop codon) and G989C (missense mutation altering highly conserved sequence in the FAD-binding motif).	Homozygous GSR mutations. Biallelic G989C (missense mutation altering highly conserved sequence in the FAD-binding motif).
Inheritance	Autosomal recessive	Autosomal recessive
In silico prediction of mutation effect on protein	Damaging mutation	Damaging mutation
UGT1A1 polymorphism	Heterozygous TA(6) and TA(7)	Heterozygous TA (6) and TA (7)
Studies for other causes of neonatal hyperbilirubinemia (metabolic diseases, and specific sequencing for Crigler-Najjar I and II)	Negative	Negative
BAER	Normal	Normal
MRI	No markers of kernicterus	No markers of kernicterus
Clinical condition at one to two months of age	Normal	Normal

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