

Jaundice with hypertrophic pyloric stenosis as an early manifestation of Gilbert syndrome

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Abstract

Jaundice associated with hypertrophic pyloric stenosis was recognised in three patients; previous reports have suggested that this is a possible early manifestation of Gilbert syndrome. Most patients with Gilbert syndrome are homozygous for a (TA)₇TAA polymorphism in the gene promoter coding for bilirubin glucuronosyltransferase. Two of the reported patients were homozygous for the (TA)₇TAA polymorphism whereas the third was heterozygous for the same polymorphism. Furthermore, no other factors contributing to jaundice in the three patients were found. These results suggest that jaundice associated with hypertrophic pyloric stenosis is due to molecular defects within the gene promoter.

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Jaundice is sometimes observed in infants with hypertrophic pyloric stenosis.¹ Previous reports favoured the hypothesis that most cases of jaundice associated with pyloric stenosis could represent an early manifestation of Gilbert syndrome.² Moreover, the low activity of bilirubin glucuronosyltransferase (BGT) observed in two patients, 4–5 months after they had been operated on for pyloric stenosis while jaundiced, suggested a permanent deficiency in bilirubin glucuronidation.³

Recent molecular studies of patients with Gilbert syndrome have shown the presence of a homozygous dinucleotide insertion of the TATA box in most cases^{4,5}; conversely, few patients bore either heterozygous missense mutations,⁶ or rarely, homozygous missense mutations⁷ in the coding sequence of the gene encoding BGT.

We report three patients who had jaundice associated with hypertrophic pyloric stenosis. Two of them had a homozygous TA dinucleotide insertion in the TATA box of the BGT gene, and the third one was heterozygous for the same TA dinucleotide insertion (whereas the 5 exons and the exon–intron junctions were normal). These results suggest that jaundice associated with pyloric stenosis is due to molecular defects within the TATA box of the BGT gene and suggest that this condition is an early manifestation of Gilbert syndrome.

Case reports

CASE 1

This 3500 g male infant was born after a full term uneventful pregnancy. His parents were healthy and unrelated and had no history of

icteric episodes. He was bottle fed and not jaundiced when he was discharged. At 3 weeks old he was admitted to hospital because of projectile vomiting. He then weighed 4100 g and was mildly icteric. Serum bilirubin was 135 µmol/l, entirely unconjugated. Pyloric stenosis was diagnosed and pyloromyotomy performed. The jaundice cleared within two days and the infant was discharged.

CASE 2

This 3300 g male infant was born after a full term uneventful pregnancy. His parents were healthy and unrelated and had no history of icteric episodes. He was bottle fed and jaundice did not develop during the first 10 days. At 4 weeks old he was admitted to hospital because of projectile vomiting. He then weighed 3950 g and was mildly icteric. Serum bilirubin was 165 µmol/l, entirely unconjugated. Pyloric stenosis was diagnosed and pyloromyotomy was performed. The jaundice cleared within three days and the infant was discharged.

CASE 3

This 30 year old woman presented with mild jaundice. She had been operated on for pyloric stenosis at the age of 4 weeks and jaundice was noted at that time. She had been bottle fed until weaning, and had several icteric episodes since the age of 6 years, often triggered by infections. Except for mild jaundice, physical examination was normal. Laboratory data showed isolated unconjugated hyperbilirubinaemia (80 µmol/l).

Methods

Ten infants with hypertrophic pyloric stenosis but without jaundice were studied as controls. Genomic DNA was extracted from peripheral leukocytes. In the three patients, the 5 exons (including the exon–intron junctions and the splice–donor sites) and the promoter (including the TATA box) of the BGT gene were PCR (polymerase chain reaction) amplified; primers and experimental conditions have been reported previously.^{4,8} The amplified DNA fragments were then directly sequenced. In controls, only the promoter of the BGT gene was PCR amplified and sequenced.

Results

Patients 1 and 3 were homozygous for (TA)₇TAA, whereas patient 2 was heterozygous for (TA)₇TAA (fig 1). In the three patients, the entire coding sequence, including the exon–intron junctions and the splice donor sites, was normal. The 10 control infants were homozygous for the (TA)₆TAA sequence (fig 1).

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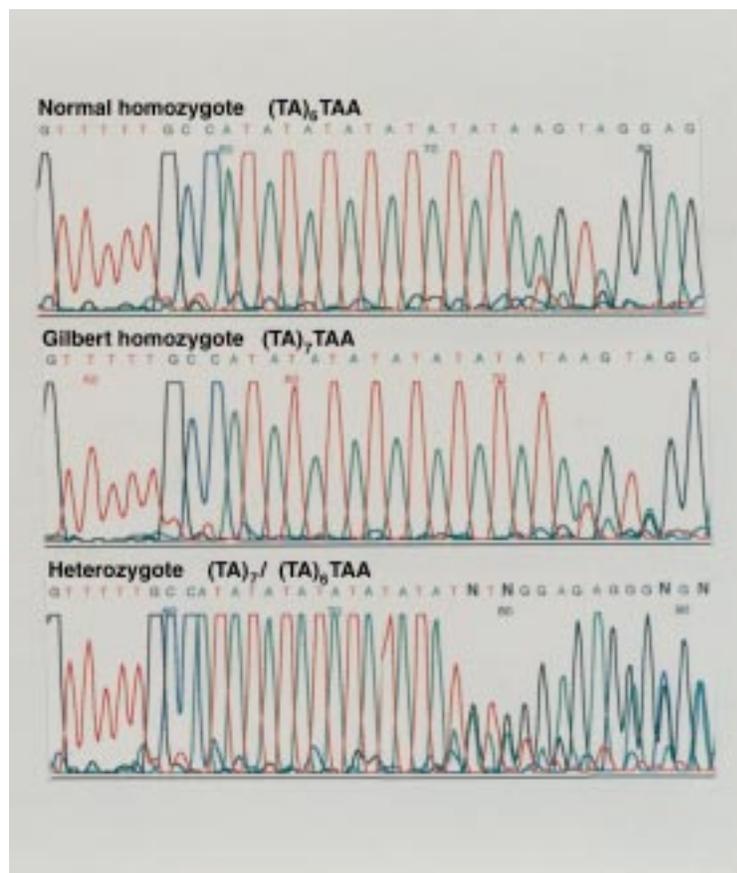


Figure 1 Nucleotide sequence of the BGT gene promoter. The first lane shows the normal sequence of the TATA box ($(TA)_6TAA$). The second shows the homozygote Gilbert sequence of the TATA box ($(TA)_7TAA$). The third shows the heterozygous Gilbert sequence of the TATA box ($(TA)_7/(TA)_6TAA$).

Discussion

We report three patients with hypertrophic pyloric stenosis and unconjugated hyperbilirubinaemia. Two of them were homozygous for the $(TA)_7TAA$ polymorphism associated with Gilbert syndrome while the third was heterozygous for the same polymorphism. Conversely, none of the controls bore the polymorphism within the promoter of the BGT gene.

Gilbert syndrome is a benign condition. Most cases have been shown to be due to a polymorphism in the promoter of the BGT gene, with seven instead of six TA repeats in this region.^{4,5} In a few cases, missense mutations of the coding sequence of the BGT gene have been identified.^{6,7} Moreover, recent studies have indicated a racial variability in the gene promoter.⁹ Other studies have shown that Gilbert syndrome is likely to accelerate the development of neonatal jaundice,¹⁰ to contribute to prolonged neonatal unconjugated hyperbilirubinaemia,¹¹ and to interact with hereditary haemolysis, such as glucose-6 phosphate dehydrogenase deficiency¹² or hereditary spherocytosis,¹³ to trigger the development of jaundice in newborns.

Previous reports have provided strong arguments favouring the hypothesis that jaundice associated with hypertrophic pyloric stenosis is an early manifestation of Gilbert syndrome. In our two patients homozygous for the $(TA)_7TAA$ polymorphism, the diagnosis of

Gilbert syndrome was confirmed as this polymorphism is found in most patients with this condition. The third infant was heterozygous for the $(TA)_7TAA$ polymorphism and a heterozygous missense mutation was thus carefully sought. The 5 exons (including the exon–intron junctions) were sequenced three times, following three independent PCR reactions, and no base substitution was identified. It is possible we missed a nucleotide substitution within an intron, however, such findings have never been observed in either Gilbert or Crigler-Najjar syndromes.¹⁴ Thus, it is likely that the heterozygous $(TA)_7TAA$ polymorphism is the only nucleotide variant in the BGT gene in this infant.

Bosma and colleagues⁴ suggested that $(TA)_7TAA$ homozygosity was necessary but not sufficient for the development of clinically apparent Gilbert syndrome; they speculated that additional factors disturbing the metabolism of bilirubin were necessary (increase in bilirubin load, defective hepatic uptake); however, this report concerned adult patients. Bancroft and colleagues¹⁰ have shown that even though peak jaundice levels did not differ among groups, neonates with homozygous $(TA)_7TAA$ had an accelerated increase in jaundice during the first 2 days of life, whereas neonates with heterozygous $(TA)_7TAA$ were not significantly different in this respect from $(TA)_6TAA$ homozygotes. However, this report did not consider the consequences of the polymorphism on the duration of jaundice in the neonatal period. In our three patients, other factors contributing to jaundice were carefully sought but were not found: all of them were full term, bottle fed infants with no clinical or biological evidence of infection, haemolysis (blood groups and Coombs tests) or hypothyroidism. Thus, these findings suggest that the promoter polymorphism was the only factor likely to contribute to jaundice in these three patients; this polymorphism has been showed to reduce the expression of the BGT gene by in vitro expression studies⁴ and fasting (induced by hypertrophic pyloric stenosis) is contributing to the increase of bilirubin production.

Our findings indicate an association between the promoter polymorphism of the BGT gene and jaundice associated with hypertrophic pyloric stenosis. It appears that this condition may be due to either homozygous or heterozygous $(TA)_7TAA$ polymorphism.

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