Two familial cases of high blood galactose of unknown aetiology
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Summary We report two male siblings presenting as newborns with increased blood galactose, urinary excretion of galactitol, and normal galactose 1-phosphate on a breast milk diet. A lactose-free diet led to normalization of all metabolites, while reintroduction of galactose in the diet resulted in an accumulation of metabolites. Potential causes of galactosaemia include: (1) activities of three enzymes of galactose metabolism: galactokinase (GALK), galactose-1-phosphate uridyltransferase (GALT), and uridine diphosphate galactose 4′-epimerase (GALE), (2) portosystemic shunting, (3) Fanconi–Bickel syndrome, (4) tyrosinaemia. Each was excluded with appropriate tests. These two familial cases may represent a novel autosomal or X-linked recessive disorder of galactose metabolism, possibly due to a novel defect in the transport of galactose across the plasma membrane.

Abbreviations
GALE uridine diphosphate galactose 4′-epimerase
GALK galactokinase
GALT galactose-1-phosphate uridyltransferase
MRA magnetic resonance angiography

Introduction
Increased blood galactose is observed in inborn errors of the Leloir pathway of galactose metabolism, tyrosinaemia (OMIM 276700), end-stage liver disease (Lindskov et al 1982), portosystemic shunt (Gitzelmann et al 1992, 1997; Kim et al 1998; Ono et al 1998; Uchino et al 1996) and Fanconi–Bickel syndrome (OMIM 227810) (Aperia et al 1981; Brivet et al 1983; Muller et al 1997; Peduto et al 2004; Yoo et al 2002). Galactosaemia (OMIM 230400), the most common disorder of galactose metabolism, is an autosomal recessive disorder caused by deficiency of galactose-1-phosphate uridyltransferase (GALT) (EC 2.7.7.12) that leads to the accumulation of galactose and galactose 1-phosphate. Patients with classic galactosaemia have near complete absence of enzymatic activity and, often before a diagnosis is established, may develop a life-threatening multiorgan system disease following lactose ingestion. Early treatment prevents the development of the acute toxicity syndrome but it does not prevent the long-term complications such as mental retardation, dysarthria, ovarian failure and ataxia observed in a subset of patients (Ridel et al 2005; Waggoner et al 1990).

In North America and in a number of European countries, galactosaemia is currently detected by newborn screening programmes, determining from blood spots on filter paper either GALT enzymatic activity or total galactose content.

We report two male siblings with abnormal galactose metabolism of unknown aetiology characterized by an increase in blood galactose and in urinary galactitol excretion responsive to dietary galactose restriction.
Case reports

The first patient was born in the United States at term to unrelated healthy Nigerian parents following an uncomplicated pregnancy. Newborn screening by blood galactose determination showed an increased blood galactose level. On a follow-up sample the galactose level was found to be 2572 μmol/L (normal 0–56) and red blood cell galactose 1-phosphate was 0.14 μmol/g Hb (normal 0–0.17). In urine, the galactose concentration was 20070 mmol/mol creatinine (normal 0–377) and galactitol was 2147 mmol/mol creatinine (normal 2.0–93). The family history revealed no consanguinity but was notable for a paternal aunt of the two siblings who experienced premature ovarian failure at 18 years of age. Based upon the high galactose levels the patient was placed on a galactose-free diet. Urinary galactitol was reduced to 295 mmol/mol creatinine (reference range for galactosaemia patients on lactose-free diet 140–400) after approximately 1 month of therapy. No glucosuria, aminoaciduria or other metabolic derangement was observed; serum amino acids, glucose, ammonia, liver and renal function tests were all normal. The patient was maintained on a lactose-free diet and followed with periodic determination of urinary excretion of galactitol, which was normal or slightly elevated. Red blood cell galactose 1-phosphate was also normal on several determinations. Following a dietary challenge with ~8 g (360 ml of cow milk) per day for one week, performed at the age of 18 months, the child again excreted high levels of urinary galactitol with normal red blood cell galactose 1-phosphate. The galactitol levels increased from 46 mmol/mol creatinine before the challenge to 103 mmol/mol creatinine (normal 2.0–36) after challenge. He was therefore maintained on diet restricted for dairy products.

Activities of all three major galactose-metabolising enzymes – GALT, galactokinase (GALK) (EC 2.7.1.6) and uridine diphosphate galactose 4-epimerase (GALE) (EC 5.1.3.2) – were measured in erythrocytes and they were all normal: GALT activity was 25.3 μmol/h/g Hb (controls 17–37); GALE activity was 26.9 μmol/h/g Hb (controls 14.8–30.9); and GALK was 72.5 nmol/min/g Hb (controls 25–40). Analysis by agarose gel electrofocusing (Elsas et al 1994) revealed normal GALT isozymes.

The patient was further investigated with in vivo [13C]galactose oxidation analysis. Following administration of 7 mg/kg of [13C]galactose, breath samples were collected for 120 min at 10–15 min intervals. In each air sample the enrichment of 13C in expired CO2 was measured by automated gas-isotope-ratio mass spectrometry (Berry et al 1995, 2000; Schoeller and Klein 1979). An impaired total body galactose oxidation was evidenced by a reduced ability to metabolize [13C]galactose to 13CO2 in comparison with normal newborns.

To address the possibility of a portosystemic shunt as a cause of the high galactose levels, abdominal ultrasonography with Doppler and MRA of intrahepatic vessels were performed. Neither test found evidence for a patent ductus venosus or aberrant vessels causing a portosystemic shunt.

The patient has been followed to the age of 7 years, with normal physical growth and normal psychomotor development with only transient language delay. His physical examination showed no external anomalies and the ophthalmological evaluation revealed no signs of lens opacity and a normal fundus.

This patient (patient 1) has two younger male siblings and one of them (patient 2) was also recognized to have high galactose levels. Patient 2, the youngest brother of patient 1, had a normal newborn screen in a state where GALT activity was measured, but he was recognized to have high galactose because of his family history. This sibling was subsequently maintained on a lactose-free diet. While under dietary restrictions, his blood galactose and urinary galactitol levels were normal, as was his physical growth. His psychomotor development was characterized by a slight delay in gross motor skills and, as in his brother, by mild language delay. However, the delay was mild and he eventually caught up with his peers.

Discussion

We describe two siblings exhibiting high blood galactose and increased urinary excretion of galactitol while on a normal diet. On the basis of metabolite testing we presume that both siblings are affected by the same disorder. Whole-body galactose oxidation studies confirmed the presence of abnormal galactose metabolism. Besides galactosaemia, we considered in our patients other defects that can potentially cause an elevation in blood galactose, including other inborn errors of galactose metabolism such as deficiencies of GALK (OMIM 230200) and GALE (OMIM 230350), portosystemic shunting such that the liver cannot take up and metabolize galactose, Fanconi–Bickel syndrome, and tyrosinaemia. Despite an extensive clinical and biochemical evaluation, we were not able to identify the precise aetiology of the elevated galactose in our patients. The presence of high galactose and normal galactose 1-phosphate made the diagnosis of galactokinase deficiency the most likely. Clinically, this was untenable since neither of our patients had evidence of a nuclear cataract, which is the cardinal feature of GALK deficiency. While we cannot exclude that cataract formation has been prevented by the lactose-free diet (Holton et al 2001), GALK enzyme activity measured in red blood cells was normal and GALK deficiency has been readily detected by enzymatic test on red blood cells in all reported cases (Bosch et al 2002). We also considered GALT and GALE deficiencies as potential
causes in our patients. However, the enzymatic activity of both GALT and GALE was within the normal range. It is important to note that GALK, GALT and GALE enzyme assays are performed in vitro using artificial substrates and therefore it is possible that their in vivo activities may be more aberrant than they appear in the in vitro analysis. Moreover, the enzyme assays were performed on erythrocytes and the degree of enzymatic activity in those cells does not necessarily accurately reflect the level of activity in the liver, which is a major site of galactose metabolism (Tygstrup 1961). For example, patients carrying the S135L mutation in GALT gene have 10% of residual enzymatic activity in the liver and none in their red blood cells (Segal et al 1971), and one may hypothesize that the opposite situation, in which erythrocyte enzymatic activity is normal while no functional enzyme is present in hepatocytes, may occur.

The family history was remarkable for an aunt of the two patients with premature ovarian failure of unknown aetiology. She was not available for biochemical investigation. Premature ovarian failure has not been reported in galactokinaase deficiency and it is a well-known complication of galactosaemia. Although its presence in this family is intriguing, it is most likely simply coincidental.

We cannot rule out that the two familial cases we report may represent a novel autosomal or X-linked recessive disorder of galactose metabolism, possibly due to a defect in the transport of galactose across the plasma membrane. Free galactose is transported into cells through a variety of transporters that are members of the glucose transporter (GLUT) family. Deficiency of the GLUT2 transporter is responsible for Fanconi–Bickel syndrome (Brown 2000), a disorder excluded on the basis of absent glucosuria and aminoaciduria. A defect in a galactose transporter distinct from GLUT2 could potentially explain the accumulation of galactose in the blood, the increased urinary excretion of galactitol, the normal level of enzymatic activity in those cells does not necessarily accurately reflect the level of activity in the liver, which is a major site of galactose metabolism. Moreover, the enzyme assays were performed on erythrocytes and the degree of enzymatic activity in those cells does not necessarily accurately reflect the level of activity in the liver, which is a major site of galactose metabolism (Tygstrup 1961). For example, patients carrying the S135L mutation in GALT gene have 10% of residual enzymatic activity in the liver and none in their red blood cells (Segal et al 1971), and one may hypothesize that the opposite situation, in which erythrocyte enzymatic activity is normal while no functional enzyme is present in hepatocytes, may occur.

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