J Inherit Metab Dis (2006) 29:516–525
DOI 10.1007/s10545-006-0382-0

METABOLIC DISSERTATION

Classical galactosaemia revisited

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Received: 30 March 2006 / Accepted: 30 May 2006 / Published online: 11 July 2006

Summary Classical galactosaemia (McKusick 230400) is an: autosomal recessive disorder of galactose metabolism, caused by a deficiency of the enzyme galactose-1-phosphate uridyltransferase (GALT; EC 2.7.712). Most patients present in the neonatal period, after ingestion of galactose, with jaundice, hepatosplenomegaly, hepatocellular insufficiency, food intolerance, hypoglycaemia, renal tubular dysfunction, muscle hypotonia, sepsis and cataract. The gold standard for diagnosis of classical galactosaemia is measurement of GALT activity in erythrocytes. Gas-chromatographic determination of urinary sugars and sugar alcohols demonstrates elevated concentrations of galactose and galactitol. The only therapy for patients with classical galactosaemia is a galactose-restricted diet, and initially all galactose must be removed from the diet as soon as the diagnosis is suspected. After the neonatal period, a lactose-free diet is advised in most countries, without restriction of galactose-containing fruit and vegetables. In spite of the strict diet, long-term complications such as retarded mental development, verbal dyspraxia, motor abnormalities and hypergonadotrophic hypogonadism are frequently seen in patients with classical galactosaemia. It has been suggested that these complications may result from endogenous galactose synthesis or from abnormal galactosylation. Novel therapeutic strategies, aiming at the prevention of galactose 1-phosphate production, should be developed. In the meantime, the follow-up protocol for patients with GALT deficiency should focus on early detection, evaluation and, if possible, early intervention in problems of motor, speech and cognitive development.

Abbreviations

EGS	endogenous galactose synthesis
FSH	follicle-stimulating hormone
Gal-1-P	galactose 1-phosphate
GALE	UDP-galactose epimerase
GALK	galactokinase
GALM	galactose mutarotase
GALP	galactose-1-phosphatase
GALT	galactose-1-phosphate uridyltransferase
HRQoL	Health Related Quality of Life

Communicating editor: Georg Hoffmann

Competing interests: None declared

This article is based on a PhD thesis accepted by the University of Amsterdam, The Netherlands. Promotores: Prof. Dr. H. S. A. Heymans, Prof. Dr. R. J. Wanders. Co-promotores: Dr. H. D. Bakker, Prof. Dr. F. A. Wijburg

Electronic databases: Classical galactosaemia OMIM #230400
Galactose-1-phosphate uridyltransferase (GALT): EC 2.7.712

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Introduction

Classical galactosaemia (OMIM 230400) is an autosomal recessive disorder of galactose metabolism caused by a deficiency of the enzyme galactose-1-phosphate uridyltransferase (GALT; EC 2.7.712). The incidence in Western Europe has been estimated to be between 1:23 000 and 1:44 000 (Bosch et al 2005; Honeyman et al 1993; Schweitzer-Krantz 2003). Most patients present in the neonatal period, after ingestion of galactose-containing feeds, with jaundice, hepatosplenomegaly, hepatocellular insufficiency, food intolerance, hypoglycaemia, renal tubular dysfunction, muscle hypotonia, sepsis and cataract. Treatment, consisting of a

severe restriction of dietary galactose, is life-saving (Holton et al 2001). For many years, elimination of galactose from the diet was considered to be an effective therapy to prevent complications. However, long-term follow-up of patients with classical galactosaemia has shown that, despite a strict diet, most patients develop symptoms such as retarded mental development, verbal dyspraxia, motor abnormalities and hypergonadotrophic hypogonadism (Kaufman et al 1981, 1995; Komrower and Lee 1970; Levy et al 1994; Nelson 1995; Ridel et al 2005; Schweitzer et al 1993; Segal 1998; Waggoner et al 1990). Endogenous production of galactose, amounting to 1 gram per day in adults, has been suggested to be a major cause of the late complications (Berry et al 1995; Schadewaldt et al 2004a).

Galactose metabolism

In the human body, most of the ingested galactose is rapidly metabolized to glucose 1-phosphate by the action of four consecutive enzymes (Holden et al 2003): galactose mutarotase (GALM), galactokinase (GALK), galactose-1-phosphate uridyltransferase (GALT), and UDP-galactose epimerase (GALE). These enzymes allow the subsequent conversion of β-D-galactose into α-D-galactose (GALM), of α-D-galactose into galactose 1-phosphate (GALK), of galactose 1-phosphate and uridine diphosphate-glucose (UDP-glucose) into glucose 1-phosphate and UDP-galactose (GALT), and the interconversion of UDP-glucose and UDP-galactose (GALE) (Fig. 1). These enzymes constitute the Leloir pathway, named after one of the major contributors to the identification of this pathway in yeast and bacteria. Mutations in each of the genes coding for the three last enzymes of the Leloir pathway may cause a major decrease in enzyme activity, resulting in variable clinical phenotypes. Of these three types of galactosaemia, GALT deficiency is by far the most prevalent, and is called classical galactosaemia (OMIM 230400).

Enzymes of the Leloir pathway are present in many tissues and cell types in the body including the erythrocyte. Besides the Leloir pathway, three accessory pathways for galactose metabolism have been described.

The pyrophosphorylase pathway was first speculated upon by Isselbacher in 1957. This pathway involves a reversible UTP-dependent pyrophosphorylase reaction converting galactose 1-phosphate into UDP-galactose and can metabolize galactose at a rate of only 1% of that of the Leloir pathway. The activity of the pyrophosphorylase pathway increases with age in most tissues. Activity is highest in adult liver, amounting to about 5% of the liver GALT activity (Shin et al 1987). Probably the most important function of the pyrophosphorylase pathway is the generation of UDP-galactose and UDP-glucose for incorporation into glycoproteins and glycolipids.

The second accessory pathway is catalysed by the enzyme aldose reductase, reducing galactose to galactitol. As galactitol cannot be further metabolized by sorbitol dehydrogenase, it is excreted in the urine. However, galactitol can also accumulate in tissues, probably contributing to the development of both the cataract and the pseudotumor cerebri observed in classical galactosaemia.

A third metabolic route is revealed by the observation that patients with classical galactosaemia produce galactonate from galactose and excrete it in the urine (Cuatrecasas and Segal 1996; Wehrli et al 1997). The exact metabolic mechanism of the production of galactonate remains unclear. Further proof for the existence of one or more alternative pathways for galactose oxidation has come from a study demonstrating galactose oxidation in a patient homozygous for a large deletion in the GALT gene (Berry et al 2001).

Molecular biology

Classical galactosaemia is inherited as an autosomal recessive disorder and the gene encoding GALT is located on chromosome 9p13 and spans 4.3 kb of DNA arranged into 11 exons. It was cloned in 1992 by Leslie and colleagues, and over 180 different mutations have been identified (Tyfield and Carmichael, GALTdB: http://www.ich.bris.ac.uk/galtdb).

The most common mutation in classical galactosaemia is the p.Q188R mutation, changing the glutamine at position 188 into arginine. It is the most frequent mutation in all caucasian populations, with the highest frequency (65%) in Western Europe (Tyfield et al 1999). The p.S135L mutation (replacing serine with leucine) is found almost exclusively in the African American population and is the most frequently reported mutation (50%) in this population (Lai et al 1996). Neither the p.Q188R nor the p.S135L mutation was detected in Japan, where the incidence of classical galactosaemia is very low (1:1 000 000). Ten mutations, not reported in caucasians, were detected in 15 Japanese patients with classical galactosaemia (Hirokawa et al 1999). The p.N314D mutation, however, occurs in caucasians and Asians as well as African Americans (Tyfield et al 1999). The differences in frequency and spectrum of mutations in the different populations suggest the occurrence of a few very ancient mutations such as p.N314D, with most other mutations occurring after racial divergence (Novelli and Reichardt 2000).

Previously, the p.N314D allele was thought to give rise to two clinically relevant GALT enzyme variants, as it was associated with an increased enzyme activity in some (Los Angeles type, D1) and a reduced enzyme activity in others (Duarte type, D2) (Elsas et al 1994; Podskarbi et al 1996). In the Los Angeles type, the p.N314D mutation is coupled with a polymorphic variant, p.L218L, which has been speculated to

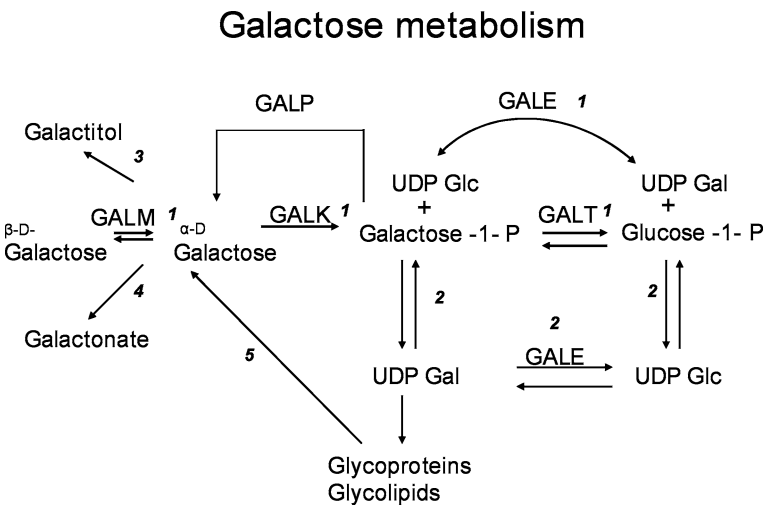

Fig. 1 The pathways of galactose metabolism: (1) Leloir pathway; (2) pyrophosphorylase pathway; (3) production of galactitol by aldose reductase; (4) production of galactonate; (5) production of galactose arising from the intracellular breakdown (notably in lysosomes) of glycoproteins and glycolipids. GALM, galactose mutarotase; GALK, galactokinase; GALT, galactose-1-phosphate uridyltransferase; GALE, UDP-galactose epimerase; GALP, galactose-1-phosphatase

increase the rate of translation of the p.N314D-GALT protein (Langley et al 1997). The reduced GALT activity associated with the Duarte allele was recently demonstrated to be caused by a 5′UTR−119_−116delGTCA deletion in the GALT promoter region (Elsas et al 2001).

Recent data suggest a clear genotype–phenotype relationship. p.Q188R is associated with a severe biochemical and clinical genotype, with nearly undetectable residual erythrocyte GALT activity in homozygotes (Tyfield et al 1999), whereas the p.S135L mutation is associated with a milder clinical outcome, with no detectable GALT activity in erythrocytes but a residual activity of 5% in leukocytes (Lai et al 1996).

The enzyme defect

In classical galactosaemia, the enzyme galactose-1-phosphate uridyltransferase is deficient, as first demonstrated by Isselbacher and colleagues in 1956. In most patients some residual activity of the enzyme can be demonstrated, which is related to the mutation involved (Lai et al 1996; Schadewaldt et al 2004a; Tyfield et al 1999).

Metabolites

Normal infants rapidly metabolize galactose to glucose. In patients with classical galactosaemia, however, galactose accumulates and is excreted in high concentrations in the urine. During treatment with a galactose-restricted diet, urinary galactose excretion returns to normal (Jakobs et al 1995).

As a result of the GALT deficiency, galactose 1-phosphate cannot be further metabolized and accumulates in red blood cells as well as in many other cells and tissues. While the high concentrations detected at the time of diagnosis fall after the start of the galactose-restricted diet, galactose 1-phosphate concentrations in patients always remain elevated compared to healthy controls (Holton et al 2001). In the Netherlands, the upper limit considered acceptable in treated patients with classical galactosaemia is 0.58 μmol/g Hb in red blood cells.

In untreated patients with classical galactosaemia, markedly elevated concentrations of galactitol are detected in plasma as well as in urine. After treatment, the plasma galactitol and the urinary excretion of galactitol rapidly decrease, but again remain above the upper limit of normal (Jakobs et al 1995).

Measurements of both galactose 1-phosphate in erythrocytes and galactitol in urine demonstrate a large intra- and inter-individual variation. Furthermore, no correlation between galactose 1-phosphate in erythrocytes and urinary galactitol excretion has been found (Hutchesson et al 1999).

Clinical presentation, diagnosis and initial treatment

Newborn screening

Classical galactosaemia is part of the newborn screening programmes of many countries. If performed on, or before, the 5th day of life, neonatal screening may prevent the acute morbidity and mortality of the disease. However, it does not change the long-term outcome (Schweitzer-Krantz 2003).

Clinical presentation

Classical galactosaemia usually presents as a life-threatening disease within the first weeks of life after ingestion of galactose, predominantly derived from lactose. The first clinical symptoms are generally feeding difficulties, vomiting and diarrhoea, lethargy and hypotonia. Jaundice, hepatomegaly and bruising indicate liver disease with clotting abnormalities. Pseudotumor cerebri may occur and may cause a bulging fontanel (Huttenlocher et al 1970). Sepsis, mostly due to

E. coli, is frequent in the neonatal crisis of classical galactosaemia. Cataract may be detected on slit lamp examination.

Laboratory investigations generally demonstrate liver disease (conjugated and unconjugated hyperbilirubinaemia, elevated transaminases, elevated amino acids, clotting abnormalities, and hypoglycaemia), renal tubular dysfunction (metabolic acidosis, galactosuria and glucosuria, phosphaturia and hypophosphataemia, aminoaciduria and albuminuria), and haematological abnormalities (haemolytic anaemia) (Holton et al 2001).

Diagnostic strategy

Galactose is a reducing sugar that is readily excreted in the urine. Although determination of reducing substances in the urine can be used as a first simple screening test for classical galactosaemia, this test should not be used either to confirm or to reject a diagnosis. No galactose will be present in the urine if the child is on intravenous fluids, as will be often the case during a neonatal crisis. In addition, galactosuria is frequently found in patients with liver disease (Holton et al 2001). Moreover, other reducing sugars (glucose) also give a positive test. This test, accordingly, should always be accompanied by a glucose dipstick test.

A more specific approach is measurement of galactose and galactitol by gas chromatographic determination of urinary sugars and sugar alcohols. Although galactose may no longer be detectable, galactitol will always be detected in the urine of a patient with GALT deficiency, in spite of the elimination of galactose from the diet and in spite of red blood cell transfusions (Holton et al 2001). Not every biochemical genetics laboratory will have this test available as an emergency test.

The gold standard for diagnosis of classical galactosaemia is the measurement of galactose-1-phosphate uridyltransferase activity in erythrocytes (isolated from either heparin or EDTA whole blood). This can be done by a variety of different methods, including the sensitive radiometric method developed by Shin and colleagues (Shin-Buehring and Schaub 1980).

Mutation analysis is performed by sequencing all coding exons and flanking intron sequences of the GALT gene. The enzyme assay is laborious. As its final result will not determine the treatment regimen, reports are usually expected within a few days.

Initial treatment

The most important step in the initial management of patients with classical galactosaemia is the immediate removal of all galactose from the diet as soon as the diagnosis is suspected. Additional therapies may be indicated in the case of complications such as sepsis, liver failure with clotting abnormalities, or hyperbilirubinaemia.

Most infants will tolerate enteral feeding, in which case breast milk or cow's milk formula should be completely replaced by soy milk formula. Infant formula based on casein hydrolysates and dextrin maltose as carbohydrate source is also used in the initial management, although this formula still contains a considerable amount of galactose (Holton et al 2001).

Long-term complications

In spite of the galactose-restricted diet, long-term complications such as retarded mental development, verbal dyspraxia, motor abnormalities and hypergonadotrophic hypogonadism are frequently seen in patients with classical galactosaemia. Although concerns were already expressed by Komrower in 1970, the extent of these long-term complications in patients with classical galactosaemia became fully clear only after extended follow-up studies (Kaufman et al 1981, 1995; Komrower and Lee 1970; Levy et al 1994; Nelson 1995; Rubio-Gozalbo et al 2002; Schweitzer et al 1993; Waggoner et al 1990). The exact origin of these sequelae is still unknown. It has been suggested that long term complications may result from endogenous galactose synthesis (Berry et al 1993, 2004; Ning et al 2000; Schadewaldt et al 2004a). Furthermore, as abnormalities in various glycoproteins have been reported, abnormal galactosylation may also be involved (Jaeken et al 1992; Lai et al 2003; Lebea and Pretorius 2005; Ornstein et al 1992; Prestoz et al 1997).

It is not clear whether damage already occurs *in utero* or only later in life. The enzymatic pathways of galactose metabolism develop around the tenth week of gestation and abnormal concentrations of metabolites were found in fetuses from the age of 20 weeks (Holton 1995). Cataractous changes were detected by electron microscopy in the eyes of a 5-month-old fetus with classical galactosaemia (Vannas 1975). However, most newborns develop cataract only after exposure to galactose from the diet. Also, ovarian failure seems to develop later in life. One patient was found to have normal ovaries at the age of 7 years but streak gonads when she was 17 years old, while another patient was found to develop ovarian failure after giving birth (Kaufman et al 1981). A patient who died with *E. coli* sepsis has been reported with normal ovaries at the age of 5 days (Levy et al 1984). A decrease in IQ with increasing age has been reported in some publications (Schweitzer et al 1993; Waggoner et al 1990). However, no studies with individual longitudinal testing have been reported in the literature.

In conclusion, although some of the damage probably occurs *in utero*, it appears that a substantial part of the long-term complications originate from continuous toxicity during life (Kaufman et al 1981; Schweitzer et al 1993; Waggoner et al 1990).

Variables associated with long-term complications

Recent studies have revealed that, except for a diagnosis after 2 months of age, neither the age at the time of diagnosis nor the severity of clinical illness at the time of diagnosis correlate with the presence and severity of late complications (Guerrero et al 2000; Kaufman et al 1995; Schweitzer et al 1993; Shield et al 2000; Waggoner et al 1990). In contrast, the presence and severity of verbal dyspraxia as well as of premature ovarian failure are clearly correlated with a higher mean erythrocyte galactose 1-phosphate after the age of 1 year, a lower total body galactose oxidation, and the genotype, with the worst outcome demonstrated in p.Q188R homozygotes (Guerrero et al 2000; Robertson et al 2000; Webb et al 2003). Finally, cognitive problems were found to be correlated with the genotype, again with the worst outcome for p.Q188R homozygotes, but not with galactose 1-phosphate concentrations (Shield et al 2000).

Neuropsychological outcome

Neuropsychological development is affected in many patients with classical galactosaemia. Two large long-term outcome studies revealed below-average IQ scores for galactosaemic patients as a group, with a decline in group scores in groups of increasing age (Schweitzer et al 1993; Waggoner et al 1990).

Defects in speech and language development are frequent, as articulation problems and delayed vocabulary are reported in approximately 60% of the patients (Schweitzer et al 2003; Waggoner et al 1990). Verbal dyspraxia, a rare speech disorder due to a sensory motor disturbance of articulation, has been reported in 15–50% of patients in several studies (Nelson 1995; Robertson et al 2000; Schweitzer et al 2003; Waggoner et al 1990; Webb et al 2003). Lower IQs are found in the patients with the most severe speech disorders (Waggoner et al 1990).

Health-related quality of life

We recently evaluated the Health Related Quality of Life (HRQoL) and the educational attainment of a group of 63 Dutch patients with classical galactosaemia. Significant differences between patients with classical galactosaemia and healthy controls aged 1–5 years were found on the domains of abdominal complaints and communication. Patients aged 8–15 years had lower scores on the domain of cognitive function than their healthy peers, and mothers of patients aged 6–15 years reported a lower HRQoL on the domains of motor and cognitive function. Patients aged 16 years and older scored significantly lower on the domains of cognitive and social function. Furthermore, significantly more patients than controls attended special schools, and the educational attainment was significantly lower in patients with classical galactosaemia. It is clear that classical galactosaemia negatively influences the HRQoL of the patients, with a significant effect on cognition in all age groups, and on social functioning in patients over 16 years (Bosch et al 2004b).

Motor development and neurological complications

Abnormalities in motor development are frequent in classical galactosaemia (Schweitzer et al 1993; Waggoner et al 1990). Also, progressive neurological disease with ataxia, tremor and extrapyramidal motor disturbances has been reported in some patients (Boehles et al 1986; Lo et al 1984). Epilepsy and microcephaly are reported as rare complications in classical galactosaemia (Schweitzer et al 1993).

Little is known about the neuropathology in classical galactosaemia. Haberland (1971) demonstrated cerebral cortical neuronal degeneration, cerebral white-matter atrophy and sclerosis, pallidonigral pigmentary degeneration and cerebellar degeneration in one untreated patient.

Brain imaging in early-treated patients frequently revealed abnormalities. Abnormal white-matter signal, focal white-matter lesions, large ventricular size and mild cerebral atrophy were described by Nelson and colleagues in 1982. In patients with progressive neurological disease, CT scans showed cerebellar and cortical atrophy and periventricular changes in the white matter with ventricular enlargement (Boehles et al 1986; Lo et al 1984). Recently, it was hypothesized that defective galactosylation of vital sphingolipids due to a low UDP-galactose concentration may cause the long-term neurological consequences in galactosaemia (Lebea and Pretorius 2005).

Growth

In many patients, growth is delayed in childhood and early adolescence, but the final height is usually normal. It is unclear whether this is related to dietary insufficiencies during childhood. As growth delay has been reported more frequently in females than in males, there may be an association with the endocrine abnormalities described in galactosaemic patients (Waggoner et al 1990).

Endocrinology

Females with classical galactosaemia frequently suffer from hypergonadotrophic hypogonadism. Waggoner and colleagues (1990) demonstrated elevated FSH levels, indicating ovarian dysfunction, in 80% of girls aged 1–12 years. In the same study, 8 out of 34 women over the age of 17 years had primary amenorrhoea, and many women developed secondary amenorrhea within a few years of menarche. On the other hand, 30% of women over 22 years had normal

menstruations and 14 pregnancies were reported among 9 of 37 women over 17 years of age.

In contrast, male testicular function seems to be normal (Holton et al 2001; Waggoner et al 1990).

Cataract

In the literature, no new cataracts after the neonatal period have been reported in patients who were compliant with their diet.

Bone mineral density

Diminished bone mineral density due to premature ovarian failure is a well-known complication in women with classical galactosaemia. However, a significantly decreased bone mineral density was already detected in a group of 40 galactosaemic children (Panis et al 2004). The dietary calcium intake, plasma calcium concentrations and vitamin D metabolites were all found to be normal. However, the IGF1 Z-score as well as markers for bone resorption and bone formation were significantly lower in galactosaemic children compared with healthy controls. These results suggest that decreased bone metabolism is the cause of the observed decrease in bone mineral density. Another possible explanation was given by Kaufman and colleagues (1993), who suggested an intrinsic defect in the galactosylation of the collagen matrix of bone, resulting in decreased mineralization.

Pathophysiology

Metabolites

The pathogenic mechanisms in classical galactosaemia are still not resolved. Probably the accumulation of galactose 1-phosphate and of galactitol is the most important factor. In the GALT-deficient mice studied by Ning and colleagues (2000), galactose 1-phosphate was found to accumulate in liver, kidney and brain, with very high concentrations of galactose 1-phosphate in red blood cells, comparable to the findings in GALT-deficient humans. Surprisingly, these mice showed no evidence of galactose toxicity. The concentration of galactitol in these GALT-deficient mice, however, was significantly lower than observed in humans. This is probably caused by the low levels of aldose reductase in normal mouse tissues (Ai et al 2000). It has therefore been suggested that it is the combination of both high concentrations of galactitol and high concentrations of galactose 1-phosphate that causes the pathological abnormalities found in classical galactosaemia (Ning et al 2000).

Accumulation of galactitol is considered to cause the cataracts and the pseudotumor cerebri in the neonatal period (Stambolian 1988). The cause of the increased incidence of sepsis observed in galactosaemic neonates is not known, but laboratory investigations have demonstrated a depressed function of neutrophils of patients with classical galactosaemia, when they were incubated with galactose, with a greater extent of depression of neonatal neutrophils than of adult neutrophils (Kobayashi et al 1983).

Endogenous production of galactose

For a long time the cause of the persistent elevation of metabolites despite a galactose-restricted diet was not clear. The presence of endogenous galactose synthesis, in patients as well as in controls, was first demonstrated by Berry and colleagues in 1995. Subsequent studies have shown that the endogenous production of galactose is age-related, being the highest in young children and decreasing more than 50% with age (Schadewaldt et al 2004a). Another study detected much lower endogenous galactose production rates in healthy controls than in patients with classical galactosaemia, and confirmed the age dependency (Berry et al 2004). With the rate of endogenous synthesis detected in these studies, a 70 kg adult patient will produce more than 900 mg of galactose per day. In comparison, the intake of galactose in patients on a galactose-restricted diet will usually be less than 50 mg per day (Berry et al 1993). Therefore, the endogenous production and the resulting persistent elevation of galactose metabolites, in particular galactose 1-phosphate, is probably a major cause of the long-term complications in patients with classical galactosaemia.

Until recently there were no data on the regulation of the endogenous galactose synthesis (EGS). Our team decided to evaluate the effect of exogenous galactose supplementation on the EGS. If the EGS were suppressed, a higher dietary galactose intake might be safe for patients with classical galactosaemia, improving the quality of life. The EGS was measured, in the basal state and during additional exogenous galactose supplementation, in 2 adult patients with classical galactosaemia and in 3 healthy controls. Basal EGS was significantly higher in the patients than in the healthy control subjects, which confirms an earlier report (Berry et al 2004). No significant differences were found between basal EGS and EGS during exogenous galactose supplementation. We conclude that the EGS is a constant in patients as well as in controls, and is not influenced by exogenous galactose supplementation (Huidekoper et al 2005).

Abnormal glycosylation

As mentioned above, abnormalities in various glycoproteins have been reported in patients with classical galactosaemia and abnormal glycosylation may be an aetiological factor in the long-term complications of GALT deficiency.

This abnormal glycosylation may be related to the accumulation of galactose 1-phosphate, the precursor of UDP-galactose, which is the substrate for the galactosyltransferases that incorporate galactose into glycoproteins and glycolipids (Jaeken et al 1992; Lai et al 2003; Lebea and Pretorius 2005; Ornstein et al 1992; Prestoz et al 1997). In this respect it is important to mention that elevated intracellular concentrations of galactose 1-phosphate inhibit UDP-hexose pyrophosphorylase and thus may reduce the intracellular concentrations of UDP-hexoses (Lai et al 2003).

Pathophysiology of reproductive abnormalities

The severity of the endocrine problems in classical galactosaemia varies widely among the patients. Abnormal glycosylation of hormones and toxic damage to the ovaries have been reported in patients with classical galactosaemia (Bandyopadhyay et al 2003; Menezo et al 2004; Meyer et al 1992; Prestoz et al 1997; Xu et al 1989).

Females receive a finite pool of follicles during fetal development, and after puberty there is loss of follicles by ovulation and by apoptotic degradation. Premature depletion of the ovarian follicular reserve may therefore result either from a deficient initial pool of follicles or from an accelerated rate of degradation as a result of galactose toxicity.

Bandyopadhyay and colleagues (2003) demonstrated that prenatal exposure to high galactose impaired germ cell migration from the extra-embryonic mesoderm to the developing gonads in rats, resulting in a deficient initial pool of germ cells. Interestingly, in the active migration of primordial germ cells in rats, a glycoconjugate with terminal GalNAc probably plays an important role. Abnormal galactosylation of this glycoconjugate causes a deficient migration of germ cells. In the rat, galactitol accumulation also affects ovarian function (Meyer et al 1992). Remarkably, administration of an aldose reductase inhibitor prevented most of these abnormalities.

In female patients, altered FSH isoforms have been detected, which are speculated to bind to FSH receptors but appear unable to induce cyclic AMP concentrations (Prestoz et al 1997). Menezo and colleagues (2004) reported an uncomplicated pregnancy and delivery in a female galactosaemia patient with premature ovarian failure, after hormone substitution therapy and stimulation with recombinant FSH, suggesting that the infertility in classical galactosaemia is related to abnormal glycosylation of FSH rather than to toxic alterations of the ovaries.

Further studies on the pathophysiology of the endocrine abnormalities in patients with classical galactosaemia are necessary in order to develop new therapeutic approaches. If ongoing damage to the ovaries is the primary cause of the fertility problems, isolation and freezing of follicles at an early age, a procedure developed for females undergoing highly cytotoxic chemotherapy, might enable females with classical galactosaemia to conceive later in life. As the abnormal galactosylation appears to play a role in decreasing the initial follicular pool as well as in causing defective hormones, reduction of the formation of galactose 1-phosphate, perhaps by inhibiting galactokinase activity, might be beneficial. Supplementation of FSH might compensate for abnormal galactosylation of FSH (Menezo et al 2004).

Long-term treatment and follow-up

The diet

Soy formula and a formula based on of casein hydrolysates and dextrin maltose as carbohydrate source contain very little galactose. However, some galactose will inevitably be introduced into the diet as many foods, such as fruits and vegetables, bread, legumes and offal, contain trace amounts of galactose (Acosta and Gross 1995; Gross and Acosta 1991). Berry and colleagues (1993) demonstrated that in two patients on a lactose-free diet the intake of galactose from fruits and vegetables was 27 mg of galactose per day at most, which doubled with a diet enriched in galactose-containing fruit and vegetables.

There are controversies concerning the daily allowance of galactose during long-term treatment. Some European metabolic centres recommend a very strict diet, also restricting galactose-containing fruits and vegetables. Other centres, e.g. those in the UK, Germany and The Netherlands, as well as centres in the USA, are more liberal, advising only a lactose-free diet. As mature hardened cheeses such Gouda, Emmentaler and Gruyere contain no galactose because of the action fermenting microorganisms (Fox et al 1990), many centres now allow these types of cheese as they are an excellent source of calcium and can replace calcium supplements.

Galactose tolerance

It is not clear how much exogenous galactose patients with classical galactosaemia can tolerate, and whether galactose tolerance may increase with age. In our study, 3 adolescent patients with classical galactosaemia received oral supplementation with galactose to a maximum of 600 mg per day (which equals the amount of galactose in 7 kg of apples, 2.5 kg of tomatoes or 12 kg of peas) during a period of 6 weeks. No significant changes were found in any of the studied clinical or biochemical parameters (Bosch et al 2004a). On the basis of these findings, the diet in The Netherlands was changed to a lactose-restricted diet without restrictions of fruit and vegetables. Our results were confirmed by the fact that no increase of galactose 1-phosphate was found in Australian patients during 6 months following the introduction

of a less-restricted diet (Thompson et al 2003). Finally, in the UK, where fruit and vegetables are not restricted in the diet, no new cataracts or liver disease have been reported in the literature. Thus, there probably is no good argument for restriction of fruit and vegetables in the galactosaemic diet.

An as-yet unsolved issue is the role of the age of the patients in the susceptibility to galactose toxicity. A recent paper demonstrated a good outcome in an adult woman with classical galactosaemia (p.Q188R homozygous) who discontinued her diet at the age of 3 years (Lee et al 2003). It may well be possible that the galactose tolerance of patients with classical galactosaemia increases with age as a result of the age-related decrease of endogenous galactose production (Schadewaldt et al 2004a), as we demonstrated a galactose tolerance up to 600 mg per day in adolescents (Bosch et al 2004a). However, this one patient may have a greater capacity to dispose of galactose by pathways yet unknown.

At present one should be very cautious in further relaxing the dietary control in adult patients with classical galactosaemia (Segal 2004). Further long-term galactose tolerance studies are necessary, in adults as well as in children and preferably after developing a better marker for galactose toxicity.

Follow-up

Until recently, evaluation of biochemical parameters had a very prominent place in most of the follow-up protocols, whereas less attention was given to the evaluation of developmental problems. As we are so far unable to prevent the late complications in classical galactosaemia, we feel that it is highly relevant to try to provide better support and to try to facilitate as normal development as possible. Therefore, the focus in the follow-up protocol should shift to early detection, evaluation, and if possible early intervention for problems of motor, speech and cognitive development. Evaluation by a speech therapist should focus on detection of verbal dyspraxia, a typical complication in classical galactosaemia, which should be treated at a young age and demands a specific intervention (Nelson 1995). In addition, all girls with classical galactosaemia should be properly evaluated for hypergonadotrophic hypogonadism at approximately 10–12 years of age. The indication for, and timing of, hormonal replacement therapy will be determined individually, depending on pubertal development, the height of the girl and hormonal evaluation.

Regular ophthalmological evaluation is not necessary for patients without cataract who are compliant with the diet. In those patients with cataract, regular ophthalmological follow-up is needed until the abnormalities have disappeared or stabilized.

Most metabolic centres monitor patients with classical galactosaemia by regular measurements of their red blood cell galactose 1-phosphate and/or urinary galactitol excretion. As these parameters show large intra- and inter-individual variation, the clinical implications of these measurements are not clear (Hutchesson et al 1999). It is also not clear how dietary variations affect these values (Berry et al 1993). We therefore feel that the only use of these tests in the regular follow-up of patients with classical galactosaemia is to monitor compliance, only detecting severe noncompliance.

Future therapeutic strategies

Since many patients with classical galactosaemia suffer from long-term complications despite early detection and the current treatment, novel therapeutic strategies need to be developed. Such new therapies should either increase the GALT enzyme activity or decrease the accumulation of toxic metabolites. Possible ways to increase the GALT enzyme activity are administration of exogenous GALT enzyme, liver cell transplantation, or liver transplantation. The first two options are not available for clinical use in classical galactosaemia at this time. Considering the risks of liver transplantation, with a high morbidity and mortality, we feel that this is not a treatment option for patients with classical galactosaemia. Therefore, interventions aimed to decrease the accumulation of toxic metabolites should be evaluated. One potential approach is the development of a specific inhibitor of the galactokinase enzyme. We performed a literature search to evaluate the clinical spectrum in galactokinase deficiency. A total of 55 galactokinase-deficient patients had been reported in the literature. We found that cataract and pseudotumor cerebri were the only complications of galactokinase deficiency, and therefore these patients have a much better outcome than patients with classical galactosaemia. Prevention of the formation of galactose 1-phosphate by inhibiting galactokinase in patients with classical galactosaemia may prevent the toxic effects of this metabolite (Bosch et al 2002). Another option might be inhibition of the enzyme aldose reductase, since the galactosaemic mouse, which has low concentrations of galactitol due to a very low aldose reductase activity, does not present with a clear clinical phenotype. However, although the mouse does not present with an acute metabolic derangement as humans do in the neonatal period, subtle long-term effects such as mild mental retardation and the equivalent of verbal dyspraxia may very well remain undiagnosed in these mice. Before therapeutic studies with new forms of treatment can be initiated, more insight into the pathophysiology of long-term galactose toxicity as well as a better biomarker for galactose toxicity are needed.

In summary, since the first clinical description of classical galactosaemia in the literature, much insight into this disorder has been gained. However, many questions regarding

the true pathophysiology of galactose toxicity remain unanswered. In order for patients with classical galactosaemia to live a normal life, these problems need to be resolved in the foreseeable future.

Acknowledgements My thesis 'Classical Galactosaemia Revisited' could not have been written without the inspiration and encouragement of my promotores: I would like to thank Frits A. Wijburg, Ronald J. Wanders, Henk D. Bakker and Hugo S. A. Heymans for their support. Also, I wish to thank Frits A. Wijburg, Ronald J. Wanders and Ries Duran for critical reading of this manuscript.

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