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The molecular basis of galactosemia – past, present and future

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Abstract

Galactosemia, an inborn error of galactose metabolism, was first described in the 1900s by von Ruess. The subsequent 100 years have seen considerable progress in understanding the underlying genetics and biochemistry of this condition. Initial studies concentrated on increasing the understanding of the clinical manifestations of the disease. However, Leloir’s discovery of the pathway of galactose catabolism in the 1940s and 1950s enabled other scientists, notably Kalckar, to link the disease to a specific enzymatic step in the pathway. Kalckar’s work established that defects in galactose 1-phosphate uridylyltransferase (GALT) were responsible for the majority of cases of galactosemia. However, over the next three decades it became clear that there were two other forms of galactosemia: type II resulting from deficiencies in galactokinase (GALK1) and type III where the affected enzyme is UDP-galactose 4′-epimerase (GALE). From the 1970s, molecular biology approaches were applied to galactosemia. The chromosomal locations and DNA sequences of the three genes were determined. These studies enabled modern biochemical studies. Structures of the proteins have been determined and biochemical studies have shown that enzymatic impairment often results from misfolding and consequent protein instability. Cellular and model organism studies have demonstrated that reduced GALT or GALE activity results in increased oxidative stress. Thus, after a century of progress, it is possible to conceive of improved therapies including drugs to manipulate the pathway to reduce potentially toxic intermediates, antioxidants to reduce the oxidative stress of cells or use of “pharmacological chaperones” to stabilise the affected proteins.

Keywords: Galactose 1-phosphate uridylyltransferase; galactokinase; UDP-galactose 4′-epimerase; Leloir pathway; inherited metabolic disease
Introduction: galactosemia

Galactosemia is a group of three inherited metabolic diseases characterised by the inability to metabolise the aldose monosaccharide galactose (Fridovich-Keil, 2006; Fridovich-Keil & Walter, 2008). This is especially important in young mammals since the main sugar present in milk is lactose, a disaccharide of galactose and glucose. In the most severe forms, the disease manifests as a life-threatening, progressive loss of function of a number of tissues and organs including the ovaries and brain (Waggoner et al., 1990; Schweitzer et al., 1993; Ridel et al., 2005; Rubio-Gozalbo et al., 2010; Fridovich-Keil et al., 2011; Berry, 2012; Waisbren et al., 2012; Karadag et al., 2013; Potter et al., 2013; Timmers et al., 2015). As a consequence it can be associated with significant pathology and cognitive disability in childhood (Bosch, 2006; Timmers et al., 2011). However, these outcomes vary widely and the mildest forms of the diseases are essentially asymptomatic. Currently, the only treatment is the restriction of galactose (and lactose) from the diet (Holton, 1996; Gleason et al., 2000). This treatment is unsatisfactory in many cases, especially in childhood; however, in many countries, it is relaxed in adult patients and this is generally considered to be safe (Van Calcar et al., 2014; Adam et al., 2015). In severe cases of the disease, it tends to slow or reduce the development of symptoms but does not always prevent them (Gitzelmann & Steinmann, 1984; Widhalm et al., 1997).

In the majority of organisms, galactose is mainly metabolised by the Leloir pathway (Figure 1). This short metabolic pathway converts α-D-galactose into glucose 1-phosphate (Frey, 1996). This compound can be isomerised into glucose 6-phosphate by the action of phosphoglucomutase (PGM, EC 5.4.2.2). Thus, galactose is converted into a glycolytic intermediate at the cost of one molecule of ATP per molecule of galactose. Since the first enzyme of the pathway is highly specific for the α-anomer of D-galactose, another enzyme aldose 1-epimerase (GALM; EC 5.1.3.3) catalyses the equilibrium between the α- and β-forms of the sugar (Bailey et al., 1969; Timson & Reece, 2003b;
Three types of galactosemia are recognised. The most common, type I or classical galactosemia (OMIM #230400), was the first to be discovered. It has an estimated incidence of approximately 1/30,000; however it is much higher in some groups most notably Irish travellers for whom the frequency is 1/480 (Murphy et al., 1999; J. M. Flanagan et al., 2010; Coss et al., 2013). This disease results from mutations in the gene encoding galactose 1-phosphate uridylyltransferase (GALT; EC 2.7.7.12) (Leslie et al., 1992; Tyfield et al., 1999; T. J. McCorvie & Timson, 2011a; T. J. McCorvie & Timson, 2011b). The range of symptoms is wide ranging from relatively mild to life threatening (Fridovich-Keil & Walter, 2008). In contrast, type II galactosemia (OMIM#230200) is the mildest form of the disease with only early onset cataracts confirmed as a consequence of the disease (Bosc, 2002). Dietary restriction of galactose often resolves these cataracts, particularly if the disease is detected early through a screening programme (Hennermann et al., 2011; Janzen et al., 2011). Type II galactosemia is caused by mutations in the gene encoding galactokinase (GALK1; EC 2.7.1.6) (Stambolian et al., 1995; Bergsma et al., 1996; Holden et al., 2004; Timson et al., 2009). Type III galactosemia (OMIM#230250), most likely the rarest and currently the least studied form of the disease, results from mutations in the gene encoding UDP-galactose 4’-epimerase (GALE; EC 5.1.3.2) (Timson, 2006). It is still common to see this disease described as occurring in two forms: a very mild (or “peripheral”) form or a severe (or “generalised”) form. This concept was decisively debunked almost a decade ago: like the other two types of galactosemia, type III is a continuum disease in which the precise manifestations in each patient are determined by a combination of genotype and environment (Openo et al., 2006).

The discovery of type I galactosemia: from disease to gene

The first recognised report of galactosemia was made by the Austrian ophthalmologist August von Ruess in 1908 (Von Reuss, 1908). However, the first detailed report was made in 1917 by Friedrich Göppert (Göppert, 1917). (As an interesting aside, Friedrich Göppert’s scientific achievements have
been largely overshadowed by those of his daughter, the Nobel Prize winning physicist Maria Goeppert-Mayer (Goeppert-Mayer, 1963). This report of excess galactose in the urine of a patient recognised that the disease had an inherited element. The child concerned had reduced cognitive development and it was observed that feeding him cottage cheese, in which the bulk of the lactose and galactose have been partly metabolised by the bacteria present, reduced the concentration of galactose in the urine (Göppert, 1917; Shahani & Chandan, 1979). In the first half of the twentieth century a number of reports of the disease (sometimes misleadingly called “galactose diabetes”) appeared. These established that the disease tends to manifest in early childhood, can be partially reversed by the removal of galactose from the diet and that the liver is one the main organs affected (Mason & Turner, 1935; Bruck & Rapoport, 1945; Mellinkoff et al., 1945; Goldbloom & Brickman, 1946; Greenman & Rathbun, 1948; Bell et al., 1950).

The bulk of these studies were conducted before the metabolic pathway for the catabolism of galactose was fully elucidated. This discovery of this pathway was almost entirely due to the pioneering work of the Argentinian biochemist, Louis Leloir who earned the Nobel Prize in 1970 for this and related work on sugar-nucleotides (Cabib, 1970; L. F. Leloir, 1983). Prior to the elucidation of the pathway, Leloir and others had investigated the phosphorylation of galactose at the expense of ATP in a reaction catalysed by galactokinase (Reiner, 1947; Trucco et al., 1948; Spratt, 1949; Wilkinson, 1949). Leloir’s key observation was that galactose is transformed into a glucose derivative, most likely glucose 6-phosphate, in a reaction with required at least one other enzyme and a heat-resistant cofactor (Caputto et al., 1949). This overall conversion required a Walden inversion (i.e. the reversal of stereochemistry at a specific position in the molecule) and the enzyme was tentatively named galactowaldenase. Subsequent work demonstrated that the cofactor was UDP-glucose (Caputto et al., 1950; Cardini et al., 1950). This compound is a representative of a group of sugar derivatives important not only in this pathway, but also in the synthesis of polysaccharides and the oligosaccharide moieties of glycoproteins and glycolipids. Leloir’s critical role in their discovery is recognised by these compounds sometimes being referred to as “Leloir
sugars”. The transformation of UDP-glucose into UDP-galactose was shown to be part of the overall process (L. F. Leloir, 1951; Paladini & Leloir, 1952). It became clear that the enzyme “galactowaldenase” catalyses two distinct reactions: the epimerisation of the galactose moiety in UDP-galactose and the transfer of the uridyl group onto galactose 1-phosphate (L. F. Leloir, 1951). The interconversion of UDP-galactose and UDP-glucose (the step of the Leloir pathway which alters the sugar stereochemistry) is now known to be catalysed by UDP-galactose 4’epimerase (GALE, Figure 1) and galactowaldenase was increasingly used to refer to this enzyme. GALE was shown to require NAD⁺ (then known as diphosphopyridine nucleotide, DPN) as an essential cofactor (Maxwell, 1956). The other aspect of the “galactowaldenase” reaction, the transfer of a uridyl group to galactose 1-phosphate, is catalysed by galactose 1-phosphate uridylyltransferase (GALT, Figure 1).

It was not until 1956 that the genetic nature of the disease was elucidated by Kalckar and co-workers (Isselbacher et al., 1956). The same research group had already demonstrated that galactosemic patients lacked GALT and GALE activity and accumulated the intermediate galactose 1-phosphate (Kalckar Anderson Isselbacher, 1956a; Kalckar Anderson Isselbacher, 1956b). However, GALE activity could be restored in cell extracts by addition of NAD⁺ (Isselbacher et al., 1956). On this basis, it was concluded that galactosemia was a single gene disorder resulting from one or more mutations in the gene coding for GALT. This work also provided the basis for definitive tests for the disease – measurement of either galactose 1-phosphate accumulation or lack of GALT activity (Donnell et al., 1963; W. G. Ng et al., 1964). Since GALT activity was reduced in otherwise asymptomatic relatives of patients it was concluded that galactosemia is normally a recessive condition (Hsia et al., 1958; Hugh-Jones et al., 1960).

The human GALT gene was assigned to chromosome 3 in 1974, to chromosome 2 in 1975 and to chromosome 9 in 1978 (Tedesco et al., 1974; Chu et al., 1975; Meera Khan et al., 1978; Westerveld et al., 1978; Benn et al., 1979; Mohandas et al., 1979). The GALT genes (GAL7) from two yeast species (Saccharomyces cerevisiae and Kluyveromyces lactis) were among the first to be sequenced,
providing useful information to enable to the search for homologues in other species (Citron & Donelson, 1984; Riley & Dickson, 1984). The coding sequence of the human gene was determined in 1988 (Reichardt & Berg, 1988) and the first disease-associated mutations identified in 1991 (Reichardt & Woo, 1991). Genomic sequencing revealed that human GALT is arranged into 11 exons (Leslie et al., 1992). A mutation which changes glutamine 188 to arginine (p.Q188R) was shown to be the most common cause of galactosemia in Caucasians (Reichardt et al., 1991; Leslie et al., 1992). This mutation accounts for 63-90% of cases of type I galactosemia in this ethnic group (Suzuki et al., 2001; Coss et al., 2013). In African populations (and groups descended therefrom) the p.S135L variant is the most common (Lai et al., 1996). The GALT gene’s location on chromosome 9 was finally confirmed by the human genome project (Lander et al., 2001; Venter et al., 2001).

There are now over 200 disease-associated mutations in the GALT gene (Calderon et al., 2007; d’Acierno et al., 2009). Of these, the vast majority of these result in single amino acid changes. Two databases of these mutations have been created. One focuses on documenting disease-associated mutations (www.arup.utah.edu/database/galt/galt_welcome.php (Calderon et al., 2007)) and the other on the effects of these mutations on GALT’s structure and function (http://bioinformatica.isa.cnr.it/galactosemia-proteins-db/index3.html (d’Acierno et al., 2009; d’Acierno et al., 2014)).

**Two more types of galactosemia: types II and III**

The belief that all cases of galactosemia resulted from dysfunction of GALT was challenged by the discovery, in 1967, of two children with cataracts and high blood galactose concentrations (Gitzelmann, 1967). Clinical chemistry investigations demonstrated that GALT activity was normal and extracts from blood cells were able to metabolise galactose 1-phosphate. However, galactokinase activity was not detectable (Gitzelmann, 1967). Therefore, galactokinase deficiency must also result in a form of galactosemia. Further cases were reported in the following years, some
being detected through large-scale screening programmes (Thalhammer et al., 1968; Olambiwonnu et al., 1974). Like type I galactosemia, heterozygotes were largely asymptomatic except for reduced blood enzyme activity (Mayes & Guthrie, 1968; Pickering & Howell, 1972).

The galactokinase gene was located to chromosome 17 (S. Elsevier et al., 1974; Orkiewicz et al., 1974; S. M. Elsevier et al., 1975). Cloning and sequencing of the gene was complicated by the unexpected existence of a second galactokinase-like sequence in the human genome, GALK2. This gene encodes the structurally and functionally related protein N-acetylgalactosamine kinase (EC 2.7.1.157), an enzyme which has only minimal activity towards galactose (Lee et al., 1992; Ai et al., 1995; Thoden & Holden, 2005; Agnew & Timson, 2010). The coding sequence for GALK2 was determined three years before that the GALK1 was elucidated in 1995 (Stambolian et al., 1995). This study also identified two disease-associated mutations (Stambolian et al., 1995). The genomic sequence of GALK1 showed that the gene spans eight exons on chromosome 17 (Bergsma et al., 1996). Approximately 40 disease-associated mutations in GALK1 are now known (Holden et al., 2004; Timson et al., 2009).

In 1981, Holton and coworkers reported the case of a child who had similar symptoms to patients with classical galactosemia, but with normal GALT activity and diminished GALE activity (Holton et al., 1981). It was noted that treatment of this third form of galactosemia by dietary galactose restriction might be particularly problematic. In unaffected individuals, UDP-galactose (a key precursor in glycoprotein and glycolipid synthesis) can be synthesised either from galactose through part of the Leloir pathway or from glucose which is converted to UDP-glucose and then epimerised to UDP-galactose by GALE. In type III galactosemia the second route is not available and a balance needs to be made between restricting galactose intake and providing enough for the synthesis of UDP-galactose (Holton et al., 1981). Interestingly, Holton was not the first to describe a case of reduced GALE activity. Almost a decade before, Gitzelmann described the case of a patient with
reduced GALE activity but no symptoms of galactosemia (Gitzelmann, 1972; Gitzelmann et al., 1977).

Further patients with type III galactosemia were identified and the disease was classified clinically into two forms – an essentially benign peripheral form and a severe, generalised form (Garibaldi et al., 1983; Henderson et al., 1983; Sardharwalla et al., 1988; W. G. Ng et al., 1993; Walter et al., 1999). In the case of the peripheral form, the only manifestation was altered levels of galactose and some metabolites in the blood and no intervention was normally recommended. The division between the two forms was challenged by the identification of a number of mutations associated with an “intermediate” form of the disease (Openo et al., 2006). Of the three types of galactosemia, type III has the smallest number (~25) of identified disease-associated mutations (Timson, 2006; T. J. McCorvie & Timson, 2013; T. J. McCorvie & Timson, 2014). However, these mutations result in a range of severity of symptoms demonstrating that rather than being a binary (or tertiary) condition, type III galactosemia results in a range of outcomes from the almost benign to life-threatening (Openo et al., 2006).

The human GALE gene was mapped to chromosome 1 (Benn Shows et al., 1979; Lin et al., 1979). The coding sequence was determined in 1995 (Daude et al., 1995). Genomic DNA sequencing showed that the gene is organised into 11 exons and five mutations associated with type III galactosemia were identified (Maceratesi et al., 1998). The most common mutation associated with a severe form of the disease, which codes for p.V94M, was discovered in 1999 (Wohlers et al., 1999). In addition to its role in the Leloir pathway, human GALE also catalyses the interconversion of N-acetylgalactosamine and N-acetylglucosamine (Piller et al., 1983; Schulz et al., 2004). This reaction is important in maintaining the pools of UDP-sugars used in the synthesis of glycoproteins and glycolipids and loss of this activity may explain the abnormal glycosylation patterns seen in some cell culture and animal models of type III galactosemia (Kingsley et al., 1986; Rosoff, 1995; Brokate-Llanos et al., 2014).
The present: modern molecular methods applied to galactosemia

The discovery of the coding sequences for GALT, GALK1 and GALE opened the door to the application of molecular biology studies. Of particular note, it enabled determination of protein structures, detailed biochemical studies using recombinant proteins and the use of “model organisms” to study the disease. To date, the structure of human GALT has not been reported. The structure of the enzyme from Escherichia coli was the first to be determined (Wedekind et al., 1995; Wedekind et al., 1996; Thoden et al., 1997). This structure has been used to develop homology models of the human enzyme and all known disease-associated variants (Marabotti & Facchiano, 2005; Facchiano & Marabotti, 2010; d'Acierno et al., 2014). The structure of human GALK1 was solved in 2005 and that of human GALE in 2000 (Thoden et al., 2000; Thoden Wohlers Fridovich-Keil Holden, 2001a; Thoden et al., 2005). In addition to the wild-type structure of GALE, the disease-associated variant p.V94M has also been solved (Thoden Wohlers Fridovich-Keil Holden, 2001b). This is the only variant associated with any type of galactosemia for which an experimental structure is currently known.

All three of the enzymes have been subjected to detailed biochemical studies. Disease-associated variants of GALT tend to have lower enzymatic activity and some are less able to dimerise when compared to the wild-type (Wells & Fridovich-Keil, 1997; Lai et al., 1999; T. J. McCorvie et al., 2013). Underlying these defects is a failure of the variant enzymes to fold correctly (T. J. McCorvie et al., 2013). Misfolding is often accompanied by aggregation of the disease-associated variants (Coelho et al., 2014). In the case of galactokinase, defects in enzymatic activity approximately correlate with disease severity (Timson & Reece, 2003a; Sangiuolo et al., 2004). To date, no detailed studies on the effects of disease-associated variants on the folding of the enzyme have been completed. The story is similar for GALE: disease-associated variants tend to have lower activity than the wild-type and this reduction in activity is generally greater in variants associated with
severe forms of the disease (Wohlers & Fridovich-Keil, 2000; Timson, 2005). Some disease-associated variants aggregate when expressed in cultured mammalian cells (Bang et al., 2009). The loss of activity often results from a failure to fold correctly and, in some cases, reduced affinity for the catalytically vital NAD$^+$ cofactor (Quimby et al., 1997; T. J. McCorvie et al., 2012).

Although studies on isolated enzymes have been useful for understanding the fundamental, molecular basis of the disease, it is also necessary to understand the effects on cells, organs and whole organisms. Over the years, the budding yeast *S. cerevisiae* has proved to be a useful model system for studying both type I and type III galactosemia (Wells & Fridovich-Keil, 1996). This organism is well-suited to the task since it does not require galactose to grow and reproduce.

Therefore, strains which lack the genes encoding GALT or GALE (or which carry disease-associated mutations) will be unaffected while growing in glucose. However, if the yeast are switched into media in which galactose is the main carbon source then they may exhibit a phenotype depending on the allele(s) present. The human GALT and GALE genes are able to complement their yeast orthologues (*GAL7* and *GAL10* respectively) (Fridovich-Keil et al., 1995; Quimby et al., 1997). *S. cerevisiae* has been used a variety of studies on the cellular effects of human GALT and GALE mutations including the effects of various mutations on cellular metabolite concentrations (for examples see (Riehman et al., 2001; Mumma et al., 2008)). Since heterodimers can form in a heterozygous yeast strain expressing both wild-type and variant GALT, the system is also useful for investigating the effects of heterozygosity. For heterodimers of wild-type and either p.Q188R or p.R333W enzymatic activity was reduced to around 14% and 45% respectively of the wild-type homodimer level (J. P. Elsevier & Fridovich-Keil, 1996; J. P. Elsevier et al., 1996). Homodimers of either p.Q188R or p.R333W had essentially no detectable activity under the same assay conditions (J. P. Elsevier et al., 1996). Heterodimers were also less stable to thermal denaturation than wild-type homodimers (J. P. Elsevier & Fridovich-Keil, 1996). These data suggest that these alleles may be partially dominant notwithstanding the observation that in patients’ families heterozygotes normally do not present with the disease, and that the degree of dominance varies with the
mutations present (J. P. Elsevier & Fridovich-Keil, 1996). Similar results were seen with GALE heterodimers expressed in yeast cells. While a wild-type/p.V94M heterodimer had approximately 50% activity (compared to a wild-type homodimer), heterodimers of the wild-type with either p.N34S or p.L183P showed less than 50% activity (Quimby et al., 1997; Wohlers et al., 1999). Homodimers of p.V94M or p.L183P had no detectable activity under similar assay conditions, whereas homodimers of p.N34S had approximately 70% of wild-type activity (Quimby et al., 1997; Wohlers et al., 1999). One intriguing result from yeast is that the endogenous GALT and GALE proteins (Gal10p and Gal7p) colocalise in the cytoplasm indicating that the Leloir pathway enzymes may form a complex (or metabolon) (Christacos et al., 2000). When human GALT is substituted for GAL7, the GALT protein also colocalises with Gal10p suggesting that metabolon formation is conserved from yeast to humans (Christacos et al., 2000). The consequences of this for galactose metabolism in vivo or how it has is affected by disease-associated mutations has not yet been investigated.

Despite its many advantages, S. cerevisiae is ultimately limited as a model organism for understanding galactosemia since it is unicellular. In recent years a number of important multicellular models have been developed and have been used to generate interesting results. A mouse model for type I galactosemia was generated in 1996. Although the model recapitulated many of the biochemical phenotypes of galactosemia, acute galactose toxicity and consequent pathology were not observed (Leslie et al., 1996; Ning et al., 2000; Ning et al., 2001). The reasons for this were unclear. However, it has been suggested that upregulation of human tumour suppressor gene aplysia ras homolog I (ARHI) in response to the metabolic disturbances associated with galactosemia may be implicated (Lai et al., 2008). The ARHI protein causes increased apoptosis and reduced growth; the gene is not present in rodents potentially explaining the lack of effect in this mouse model (Yu et al., 1999; Bao et al., 2002; Fitzgerald & Bateman, 2004). In the last twelve months a second mouse model for GALT deficiency has been reported. In this case, pathology was observed with the majority of galt-null pups fed by mothers on a high galactose diet dying before
weaning (Tang et al., 2014). These pups also showed altered ratios of oxidised:reduced glutathione, consistent with increased oxidative stress and adult females showed reduced numbers of ovarian follicles (Tang et al., 2014). The difference in results between these two studies most likely results from the early feeding of pups with high concentrations of galactose. Increased oxidative stress was also observed in a *Drosophila melanogaster* model of type I galactosemia (Kushner et al., 2010; P. P. Jumbo-Lucioni et al., 2013). This model also showed defects in the nervous system with consequent impacts on locomotion of the flies (Ryan et al., 2012; P. Jumbo-Lucioni et al., 2014).

A mouse model for type II galactosemia in which the *galk1* gene was disrupted showed no phenotype. However, when the mice were further modified so that they expressed aldose reductase they developed cataracts (Ai et al., 2000). A key difference between mice and humans is that mice have much lower expression of aldose reductase in the lens cells of the eye. This enzyme catalyses the conversion of galactose to its corresponding sugar alcohol galactitol (dulcitol) and it is this compound which appears to be responsible for the damaging osmotic influx of water into the lens cells (Hayman & Kinoshita, 1965; Dvornik et al., 1973; Ai et al., 2000). Reactive oxygen species are also implicated in the formation of galactosemic cataracts (Mulhern et al., 2006; Mulhern et al., 2007; Abdul Nasir et al., 2014). *D. melanogaster* and *Caenorhabditis elegans* models of type III galactosemia have also been developed (Sanders et al., 2010; Brokate-Llanos et al., 2014). The fruit fly model demonstrated that GALE is essential for development of the organism (Sanders et al., 2010). It also demonstrated that the two physiologically important activities of GALE (epimerisation of UDP-galactose and UDP-N-acetylgalactosamine) were vital and played different roles in development (Daenzer et al., 2012). Developmental defects were also been observed in the *C. elegans* model (Brokate-Llanos et al., 2014).

It is becoming increasingly apparent that disruption of normal glycosylation of proteins and lipids is also a feature of the pathology of type I and type III galactosemia. Defects in the glycosylation of neuronal cells from a galactosemic patient was first noted in the early 1970s (Haberland et al.,
Reduction of galactosylation of proteins from cells and serum derived from patients with type I galactosemia has been observed in several studies (Dobbie et al., 1990; Ornstein et al., 1992; Stibler et al., 1997; Charlwood et al., 1998; Coss et al., 2014). It was hypothesised that this is caused by the reduced levels of UDP-galactose often observed in cells derived from galactosemia patients (W. G. Ng et al., 1989). In addition to decreased galactosylation, increased inappropriate incorporation of other monosaccharide moieties such as fucose has been observed (Sturiale et al., 2005). Similarly, glycolipids from galactosemic patients were shown to have reduced levels of galactose and N-acetylgalactosamine compared to healthy patients; this effect was not reversed by a low galactose diet (Petry et al., 1991). In galactosemic patients, N-linked protein glycosylation is associated with increased amounts of mannose and increased numbers of truncated oligosaccharide chains (Y. Liu et al., 2012; Staubach et al., 2012). O-linked glycosylation of proteins is also affected with increased numbers of shorter oligosaccharides (Y. Liu et al., 2012). Recently, it has been suggested that N-glycosylation patterns could be a valuable biomarker for monitoring the severity of the disease and the effectiveness of treatment regimes (Coss et al., 2012; Coss et al., 2014; Knerr et al., 2015).

The future: a realistic chance for therapy?

Recent biochemical work suggests a number of strategies for improved therapy for galactosemia. It is believed that galactose 1-phosphate build-up in types I and III contributes to toxicity. Therefore, blocking the activity of galactokinase which would prevent the accumulation of this compound may be beneficial (Bosch et al., 2002). The availability of a high resolution structure of GALK1 has enabled structure-based drug design and the discovery of some high affinity specific inhibitors of the enzyme (Wierenga et al., 2008; Tang et al., 2010; Odejinmi et al., 2011; Chiapori et al., 2013; Lai et al., 2014; L. Liu et al., 2015). The observation that GALT deficiency is accompanied by increased oxidative stress suggests that antioxidants may be beneficial. A manganese containing porphyrin
compound which mimics the activity of superoxide dismutase has been shown to be effective in the fruit fly model (P. P. Jumbo-Lucioni Ryan et al., 2013). The use of dietary antioxidants has also been suggested (Timson, 2014). Since protein misfolding is likely to be the fundamental cause of most cases of galactosemia, it may be possible to discover molecules which stabilise and promote proper folding of the variant proteins thus increasing enzymatic activity and reducing the tendency to aggregate. Such “pharmacological chaperones” have the potential to restore enzyme activity and alleviate or prevent the bulk of the symptoms (Ringe & Petsko, 2009; Muntau et al., 2014; Brandvold & Morimoto, 2015). This approach has identified compounds which are being used in the successful treatment of cystic fibrosis and transthyretin amyloidoses (Sampson et al., 2011; Bulawa et al., 2012; Hanrahan et al., 2013). Similar approaches are also being developed for a range of other inherited metabolic diseases including Fabry disease, Pompe disease, methylmalonic aciduria, hyperoxaluria, and phenylketonuria (J. J. Flanagan et al., 2009; Pey et al., 2011; Santos-Sierra et al., 2012; Underhaug et al., 2012; Cammisa et al., 2013; Jorge-Finnigan et al., 2013; Mesa-Torres et al., 2013). Discovering pharmacological chaperones for galactosemia will be challenging; however, the existence of good quality experimental structures or models of the three enzymes together with robust assays for their stability will assist the process. Recently, it has been shown that arginine stabilises GALT, including the variant forms p.Q188R and p.K285N supporting the concept that small molecules can enhance the stability and activity of this protein (Coelho et al., 2015). Since misfolded proteins are likely to be targeted for proteosomal degradation, thus further reducing cellular activity, an alternative approach is to inhibit these degradation processes using proteostasis modulators. These can increase the cellular half-lives of misfolded proteins (Vij, 2011). In a mouse model, proteasome inhibitors were able to partially correct cystathionine β-synthase deficiency (Gupta et al., 2013).

Thus after over a century of scientific progress in the understanding of galactosemia, we are finally poised to put this knowledge into practice and develop better treatments for this inherited metabolic disease. It is unlikely that any one of the treatment strategies outlined above will provide
an adequate therapy for all patients with galactosemia. The existence of three types of the disease and the wide range of disease-associated mutations combined with environmental variability, results in considerable diversity of disease phenotypes. There is also a need to treat altered metabolite levels, disturbed glycosylation patterns and the increase in free radical concentrations. Therefore, combinations of treatment approaches and careful monitoring of patients using a variety of biomarkers is likely to be required. In all cases, dietary restriction of galactose will probably continue to be needed. However, the next 100 years should see impressive advances in the treatment of galactosemic patients and there is potential for therapies to be developed which enable near-normal quality of life for these people.

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Figure legends

Figure 1: The Leloir pathway of galactose catabolism. In this pathway, α-D-galactose is first phosphorylated at the expense of ATP in a reaction catalysed by galactokinase (GALK1). Impairment of this reaction can result in type II galactosemia. α-D-Galactose 1-phosphate then reacts with UDP-glucose, forming glucose 1-phosphate and UDP-galactose. This reaction is catalysed by galactose 1-phosphate uridylyltransferase (GALT) and deficiency of this enzyme can cause type I galactosemia. The glucose 1-phosphate produced in this reaction can be isomerised to glucose 6-phosphate by phosphoglucomutase and thus enter glycolysis. The reversal of stereochemistry occurs when UDP-galactose is recycled to UDP-glucose in a reaction catalysed by UDP-galactose 4’-epimerase (GALE). Mutations in the gene coding for this enzyme can lead to type III galactosemia. This enzyme also catalyses the interconversion of UDP-N-acetylgalactosamine and UDP-N-acetylglicosamine. Both reactions are important in maintaining the cellular pools of UDP-sugars for use in the synthesis of glycolipids and glycoproteins.
Figure 1

\[
\begin{align*}
\alpha-d\text{-galactose} & \xrightarrow{\text{MgATP}} \alpha-d\text{-galactose-1-phosphate} \\
\text{Type II galactosemia} & \xrightarrow{\text{GALK1}} \text{MgADP} \\
\alpha-d\text{-galactose-1-phosphate} & \xrightarrow{\text{UDP-Glc}} \text{Type III galactosemia} \\
\text{Type I galactosemia} & \xrightarrow{\text{GALT}} \text{UDP-Gal} \\
\end{align*}
\]
Graphical abstract
Galactosemia – past, present and future

**Abbreviations list**

- **GALK1**: Galactokinase
- **GALK2**: N-acetylgalactosamine kinase
- **GALT**: Galactose 1-phosphate uridylyltransferase
- **GALE**: UDP-galactose 4’-epimerase
- **PGM**: Phosphoglucomutase
- **GALM**: Galactose mutarotase
- **DPN**: Diphosphopyridine nucleotide (former name for NAD^+^)
The molecular basis of galactosemia: past, present and future

Highlights

- Galactosemia was first described in 1908 by von Ruess
- Leloir discovered the pathway of galactose catabolism
- Kalckar determined that GALT deficiency is the cause of classic galactosemia
- Two other forms exist: GALK1 and GALE deficiency
- Modern molecular approaches are suggesting ways to improve treatment