Type IV galactosemia

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This Commentary explores some of the implications of the recent discovery of a novel form of galactosemia [1]. This disease has been recognised since 1908. During the twentieth century, the molecular basis of galactosemia was revealed, through two, parallel lines of enquiry. First, the pioneering work of Leloir and colleagues elucidated the pathway by which the monosaccharide galactose is metabolised. Second, various genetic studies established that galactosemia is an inherited metabolic disease [2].

Galactose cannot enter the glycolytic pathway directly. In order to be metabolised it is converted to glucose 6-phosphate in four, enzyme catalysed steps. First, the sugar is phosphorylated at the expense of ATP in a reaction catalysed by galactokinase (GALK1). The resulting galactose 1-phosphate reacts with UDP-glucose producing glucose 1-phosphate and UDP-galactose. This reaction is catalysed by galactose 1-phosphate uridylyltransferase (GALT). UDP-galactose 4'-epimerase (GALE) enables the regeneration of UDP-glucose by catalysing the isomerisation of UDP-galactose. A second isomerisation reaction (catalysed by phosphoglucomutase, PGM) converts glucose 1-phosphate to the glycolytic intermediate glucose 6-phosphate. The reactions catalysed by GALK1, GALT and GALE are the core reactions of the Leloir pathway.

GALK1 is highly site- and stereo specific. It acts only on the α-anomer of D-galactose producing α-D-galactose 1-phosphate. However, in solution, galactose exists in equilibrium between the α- and β-anomers. Although the two anomers do interconvert in aqueous solution, the rate of utilisation of α-D-galactose can exceed its rate of generation. Galactose mutarotase (aldose 1-epimerase, GALM) catalyses this reaction with impressive turnover numbers (12,000 s^{-1} for the human enzyme) [3]. This ensures that the Leloir pathway is supplied with sufficient amounts of α-D-galactose.

In 1956, it was demonstrated that galactosemia can associated with inherited mutations in the *GALT* gene. While this is the most common form of the disease, mutations in genes encoding other enzymes in the Leloir pathway can also cause galactosemia. Thus, GALT-deficiency has become known as classical galactosemia or type I galactosemia (OMIM #230400). Type II galactosemia

(OMIM #230200; discovered in 1967) results from mutations in the *GALK1* gene. In 1981, type III galactosemia, or GALE deficiency (OMIM #230350) was discovered. Galatosemia has a very wide spectrum of symptoms ranging from almost no phenotype to life-threatening metabolic disturbances. The most severe forms, even if treated by the removal of galactose and its precursors from the diet, almost always result in severe physical disability and cognitive impairment. This variation results primarily from the large number of mutations which can cause the disease – over 300 are currently known. Typically, mutations in *GALT* are associated with more severe forms of the disease which manifest early in childhood and result in liver, brain and ovarian damage. In contrast, type II galactosemia is associated with much milder consequences of which early-onset cataracts is the most common. Type III galactosemia probably has the widest range of symptoms. The mildest forms are almost asymptomatic, whereas the most severe have similar symptoms to type I.

It has been over 35 years since a new form of galactosemia has been discovered. While the human *GALM* gene (and its corresponding protein) have been known for over a decade, it has not previously been associated with galactosemia [3]. Wada and coworkers have now conclusively demonstrated that at least five mutations in the *GALM* gene are associated with galactosemia-like symptoms [1]. These symptoms are most like type II galactosemia: increased blood galactose concentrations with no change in the levels of galactose 1-phosphate. Two of the patients studied had developed cataracts. None had any detectable symptoms affecting the liver or gastrointestinal system. Since all these patients are currently children, the long-term, adult consequences of GALM deficiency remain unknown. The genetic changes result in point mutations which introduce stop codons or cause amino acid changes in GALM, or frameshifts which cause premature termination. The two changes to in the protein sequence occur at widely separated points (p.G142R and p.R267G). Neither of the affected residues forms part of the active site and the authors suggest that their effects result from subtle alterations to the protein's overall structure and/or destabilisation of this structure [1,4]. Both hypotheses would explain the observed loss of GALM activity in cell extracts from patients with these mutations. Loss of stability is consistent with reduced yield of the protein

following recombinant expression, shorter cellular half-lives and reduced GALM levels detected in patient-derived cells [1]. These causes of loss of enzymatic activity have been observed in the other three types of galactosemia, and other inherited metabolic diseases [5].

Interestingly, this is not the first report of a potentially clinically relevant mutation in the *GALM* gene. A polymorphism which gives rise to a single amino acid change in the GALM protein is associated with serotonin transporter binding potential [6]. This is associated with increased risk-taking in HIV positive patients [7]. No studies on non-infected individuals have been reported. Like the two variants which cause type IV galactosemia, this change (p.N190Y) does not, directly, affect the active site of the enzyme. To date, no studies on this variant's activity or stability have been reported. Furthermore, the biochemical link between potentially altered GALM activity and serotonin transport is not clear. It would be interesting to determine if the mutations reported by Wada *et al* are associated to altered serotonin uptake or behaviour.

Studies in micro-organisms have demonstrated that galactose mutarotase activity is necessary for optimum growth, at least under conditions where galactose in a major carbon source. Deletion of the *galM* gene in *Escherichia coli* results in substantially slower growth in media which lack glucose but contain lactose. There is no effect in cells growing on glucose [8]. The yeast *GAL10* gene encodes a GALE-GALM fusion protein. Yeast which are deleted for this gene and complemented with a truncated version of *GAL10* which includes only the UDP-galactose 4'-epimerase encoding sequence cannot grow on galactose, but grow normally on glucose [9]. In yeasts, the enzyme is also important in catalysing the equilibrium between the α- and β-anomers of D-glucose: strains deleted for *GAL10* grow poorly on cellubiose (a disaccharide of β-D-glucose) [10]. Human GALM is active with D-glucose as a substrate [3]. However, it is not known if this reaction is important physiologically. If it is, then this could be another cause of pathology in type IV galactosemia.

For some time, scientists and clinicians who work on galactosemia have wondered if GALM deficiency might result in another type of galactosemia. Wada *et al* have definitively shown that it

does. Metabolically, type IV galactosemia is likely to result in a build-up of β-D-galactose. In type I and type III galactosemia, the build-up of galactose 1-phosphate has been proposed to be responsible for many of the manifestations of these diseases. The link between excess amounts of this metabolite and pathology is not clear. Furthermore, other factors are important, including disruption of protein and lipid galactosylation. In type II galactosemia, loss of GALK1 activity prevents the formation of galactose 1-phosphate and results, instead, in a build-up of D-galactose. This may be (partly) the cause of the much milder symptoms seen in type II galactosemia compared to the other types. The build-up of α-D-galactose results in formation of cataracts. This compound enters the lens cells of the eyes where it is reduced by aldose reductase to form the sugar alcohol galactitol (dulcitol) [11]. This compound is not readily transported across the cell membrane and so builds up causing osmotic stress. (The mechanism is similar to the formation of cataracts in diabetic patients where glucose is reduced to sorbitol.) Aldose reductase acts on the aldehyde ("straight chain") form of the sugar, not the pyranose ("ring") forms [12]. Since the aldehyde form is the intermediate in the conversion between the α- and β-anomers of galactose, aldose reductase will catalyse the conversion to galactitol regardless of which anomer predominates in solution.

The prevalence of GALM deficiency remains to be established. Given that it has not been detected before, despite the diagnosis and genotyping of many thousands of galactosemia patients worldwide, it is a reasonable assumption that it is relatively rare. However, the relatively mild symptoms may mean that there are individuals who have mutations which result in reduced GALM activity who will not be diagnosed with type IV galactosemia. These individuals may be asymptomatic for many years with the disease manifesting as an increased risk of cataracts in middle and old age. (This is similar to the mildest known form of type II galactosemia.) Nevertheless, in cases where type II galactosemia-like symptoms are detected in childhood and sequencing of *GALK1* reveals no pathogenic mutations, consideration should be given to testing for GALM deficiency. Since the biochemical analysis of GALM activity is unlikely to be possible in most clinical chemistry laboratories (it requires either specialist equipment or specific coupling enzymes),

sequencing of the *GALM* gene is likely to be the quickest and most cost-effective way to identify possible cases of type IV galactosemia.

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