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RESEARCH REPORT

Classical Galactosaemia and CDG, the N-Glycosylation Interface. A Review

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Abstract Classical galactosaemia is a rare disorder of carbohydrate metabolism caused by galactose-1-phosphate uridyltransferase (GALT) deficiency (EC 2.7.7.12). The disease is life threatening if left untreated in neonates and the only available treatment option is a long-term galactose restricted diet. While this is lifesaving in the neonate, complications persist in treated individuals, and the cause of these, despite early initiation of treatment, and shared *GALT* genotypes remain poorly understood. Systemic abnormal glycosylation has been proposed to contribute substantially to the ongoing pathophysiology. The gross N-glycosylation assembly defects observed in the untreated neonate correct over time with treatment. However, N-glycosylation processing defects persist in treated children and adults.

Congenital disorders of glycosylation (CDG) are a large group of over 100 inherited disorders affecting largely N- and O-glycosylation.

In this review, we compare the clinical features observed in galactosaemia with a number of predominant CDG conditions.

We also summarize the N-glycosylation abnormalities, which we have described in galactosaemia adult and paediatric patients, using an automated high-throughput HILIC-UPLC analysis of galactose incorporation into serum IgG with analysis of the corresponding N-glycan gene expression patterns and the affected pathways.

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Introduction

Inborn errors of metabolism to include the primary Congenital Disorders of Glycosylation (CDG) and galactosaemia can provide vast information regarding disordered metabolic pathways involving glycosylation and potentially modifiable steps (Brinkman et al. 2006; Morava et al. 2015; Sun et al. 2015).

The number of CDG has increased dramatically over the last few years. Over 100 disorders are now described with on-going characterization of new subsets (Rymen and Jaeken 2014; Scott et al. 2014; Cartault et al. 2015; Freeze et al. 2015). While different CDG have well characterized defects in glycosylation, disorders such as galactosaemia, often termed secondary disorders of glycosylation, are less well defined (Morava et al. 2015). An understanding of the shared disturbed metabolic pathways could lead to improved understanding of the pathophysiology of these disorders of glycosylation and possibly improve therapeutic approaches.

Galactosaemia is a group of rare autosomal recessive carbohydrate metabolism disorders caused by deficiency of enzymes involved in the metabolism of the aldose monosaccharide galactose (Fridovich-Keil and Walter

2008). The most severe type of galactosaemia, Classical galactosaemia (OMIM #230400) (subsequently referred to as galactosaemia in this review), is caused by profound deficiency of the galactose-1-phosphate uridyltransferase (GALT) enzyme (EC 2.7.7.12). Following galactose intake in the affected neonate, there is a toxic build-up of intermediates of galactose metabolism. Strict dietary restriction of galactose is lifesaving in the neonate, but mild to severe long-term complications persist, including significant cognitive impairment and infertility in females, regardless of genotype or age at onset of treatment (Schweitzer et al. 1993; Fridovich-Keil and Walter 2008; Krabbi et al. 2011; Coss et al. 2013; Timson 2015). The cause of this pathophysiology is currently under review.

The toxic build-up of galactose intermediates coupled with deficiency of pathway product is proposed to contribute to the development of these complications. These intermediates can result in competitive inhibition of glycosyltransferases (Lai et al. 2003). A shortage of end-product UDP-hexose sugars could also lead to disruption of glycosylation in the posttranslational modification (PTM) of proteins and lipids (Ng et al. 1989; Ornstein et al. 1992).

Dysregulation of a number of genes and pathways has been observed in galactosaemia (Coman et al. 2010; Coss et al. 2014; Maratha et al. 2016), along with abnormal glycosylation profiles of glycoproteins in both treated and untreated patients (Charlwood et al. 1998; Quintana et al. 2009; Coman et al. 2010; Berry 2011).

Early stage perturbations of glycosylation, gene expression and inositol signaling during prenatal galactose intoxication, in combination with long-term galactose restriction and individual endogenous galactose production, likely have a substantial role in determining the long-term complications seen in galactosaemia (Berry et al. 2004; Huidekoper et al. 2005; Coss et al. 2014; Schadewaldt et al. 2014; Maratha et al. 2016). Understanding the role of glycosylation in the development of these complications is essential.

Congenital Disorders of Glycosylation

CDG are a large group of mainly autosomal recessive inherited disorders affecting the glycan synthesis. These can be divided into the following major categories: disorders of protein N-glycosylation or O-glycosylation, disorders of lipid and glycosylphosphatidylinositol (GPI) anchor glycosylation and disorders of multiple glycosylation pathways (Freeze 2006; Hennet 2012), with variable symptomatic severity. Almost all organs are affected with a particular impact on nervous development, immune, hepatic and gastrointestinal systems (Freeze and Aebi 2005; Freeze et al. 2015).

The majority of CDG disorders are caused by defects in the N-glycosylation pathway in which N-glycans are attached to arginine on proteins. N-glycan synthesis starts in the endoplasmic reticulum (ER), where the assembled product is attached. The processing of N-glycans into complex and hybrid structures continues in the Golgi apparatus. CDG-type I (CDG-I) abnormalities result from an abnormality of N-glycan assembly in the ER and CDG-type II (CDG-II) abnormalities result from abnormalities in N-glycan processing after transfer to the protein in the ER or steps occurring in the Golgi apparatus (Freeze 2013; Freeze et al. 2015), as depicted in Fig. 1.

Approximately 1% of the human genome encodes genes involved in glycosylation and over half of all proteins are N-glycosylated (Freeze 1998; Pivac et al. 2011). Glycoproteins are central to many key biological systems such as cell–cell signaling, and are important in coagulation, immunity, fertility, etc. (Zoldos et al. 2010).

Galactosaemia has been reported as a secondary disorder of glycosylation, displaying characteristics of both CDG-I and CDG-II defects with both glycan assembly and processing defects observed (Charlwood et al. 1998; Sturiale et al. 2005; Quintana et al. 2009; Coman et al. 2010; Coss et al. 2012).

CDG and galactosaemia share multiple clinical characteristics. Tables 1 and 2 summarize a number of CDG syndromes (I and II) with symptoms which may also be observed in galactosaemia including neurological involvement, coagulopathies and liver disease. For example, PMM2-CDG, the most common subtype of CDG, is an N-glycan assembly defect caused by lack of phosphomannomutase 2 (PMM2) which converts mannose-6-phosphate to mannose-1-phosphate. The enzymatic deficiency results in reduced GDP-mannose required for the synthesis of the lipid-linked oligosaccharide (LLO) precursor. The symptoms observed are commonly intellectual disability, hypotonia, cerebellar dysfunction, polyneuropathy and stroke-like episodes (Dinopoulos et al. 2007).

The key clinical adverse outcomes seen in galactosaemia include intellectual disabilities, speech abnormalities and primary ovarian insufficiency (POI) in females. Intellectual disability occurs in at least 50% of affected individuals (Waggoner et al. 1990; Schweitzer et al. 1993; Shield et al. 2000; Doyle et al. 2010; Waisbren et al. 2012; Coss et al. 2013; Rubio-Agusti et al. 2013). Abnormal myelination was first documented in galactosaemia in 1971 (Haberland et al. 1971; Lebea and Pretorius 2005). There is considerable variability in IQs documented between galactosaemia patients with scores ranging from very low to well above average.

The presence of speech and language abnormalities (commonly verbal dyspraxia) is well documented in galactosaemia. One well-described entity is Childhood Apraxia of Speech (CAS). Overall, speech and language

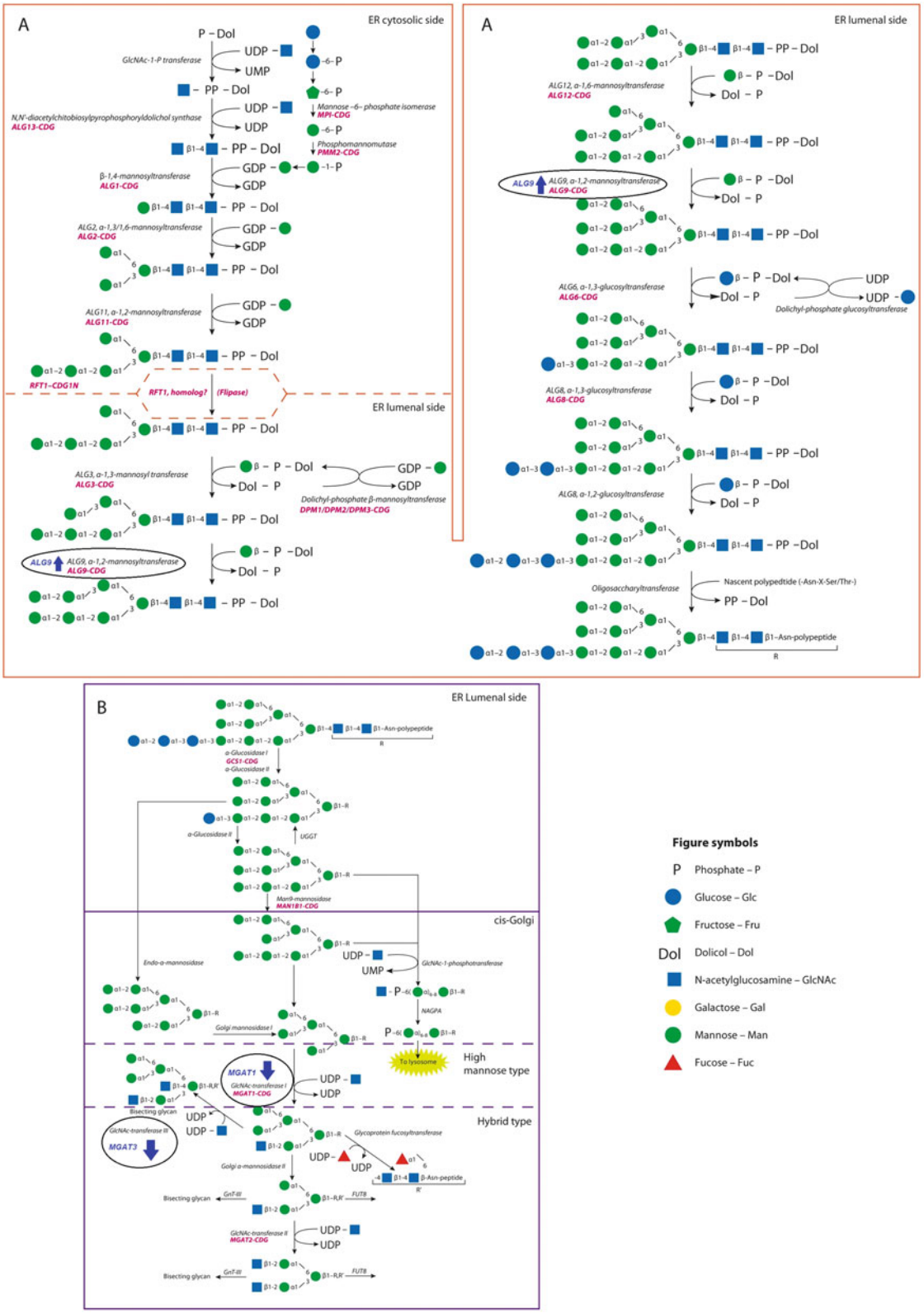

Fig. 1 Schematic representation of: (**a**) N-glycan assembly in rER (Congenital Disorders of Glycosylation-type I, CDG-I) and (**b**) N-glycan processing in Golgi apparatus (Congenital Disorders of Glycosylation-type II, CDG-II). Blue arrows for *ALG9* (Alpha-1, 2-mannosyltransferase), *MGAT1* and *MGAT3* represent respective gene expression pattern observed in our recent study (Maratha et al. 2016)

Table 1 CDG-I conditions and common symptoms

CDG name	Affected protein/gene	Common symptoms
PMM2-CDG	Phosphomannomutase 2	Cognitive/motor dysfunction (de Lonlay et al. 2001), failure to thrive, liver disease, developmental delay (Drouin-Garraud et al. 2001), coagulopathy (Van Geet and Jaeken 1993) and infection (Matthijs et al. 1997)
MPI-CDG	Mannose-6-phosphate-isomerase	Coagulopathy (Marquardt and Denecke 2003)
ALG3-CDG	Dolichyl-P-Man: Man(5)GlcNAc(2)-PP-dolichyl mannosyltransferase	Cognitive/motor dysfunction (de Lonlay et al. 2001) and failure to thrive (Kranz et al. 2007)
ALG12-CDG	Dolichyl-P-Man: Man(7)GlcNAc(2)-PP-dolichyl-alpha-1, 6-mannosyltransferase	Cognitive/motor dysfunction, (Thiel et al. 2002), failure to thrive and infection (Chantret et al. 2002)
ALG8-CDG	Dolichyl-pyrophosphate Glc Man9GlcNAc2alpha-1,3-glucosyltransferase	Coagulopathy (Chantret et al. 2003)
ALG2-CDG	Alpha-1,3-mannosyltransferase	Cognitive/motor dysfunction, coagulopathy and liver disease (Thiel et al. 2003)
ALG1-CDG	Chitobiosyldiphosphodolichol beta-mannosyltransferase	Cognitive/motor dysfunction (Dupré et al. 2010) and coagulopathy (Kranz et al. 2004)
ALG9-CDG	Alpha-1,2-mannosyltransferase	Cognitive/motor dysfunction, cerebral atrophy, delayed myelination, epilepsy, failure to thrive, liver disease and skeletal dysplasia (Weinstein et al. 2005; Frank et al. 2004; AlSubhi et al. 2016)
PGM1-CDG	Phosphoglucomutase 1	Hypergonadotropic hypogonadism and growth retardation (Tegtmeyer et al. 2014)
MAN1B1-CDG	Alpha-1, 2-mannosidase	Cognitive/motor dysfunction (Rafiq et al. 2011)

Data collated from (Sparks and Krasnewich 1993) with 2013 updates (Freeze 2013)

Table 2 CDG-II conditions and common symptoms

CDG name	Affected protein/gene	Common symptoms
SLC335C1-CDG; Leukocyte adhesion deficiency II	GDP-fucose transporter 1	Cognitive dysfunction (Etzioni et al. 1992) and infections (Lübke et al. 1999)
B4GALT1-CDG	Beta-1,4-galactosyltransferase 1	Coagulopathy and developmental disability (Peters et al. 2002)
COG7-CDG	COG complex subunit 7	Cognitive dysfunction (Zeevaert et al. 2009) and failure to thrive (Morava et al. 2007)
COG4-CDG	COG complex subunit 4	Cognitive dysfunction, failure to thrive and developmental delay (Ng et al. 2011)

Data collated from (Sparks and Krasnewich 1993) with 2013 updates (Freeze 2013)

disorders are estimated to affect at least 25% of individuals with galactosaemia, commonly presenting in childhood (Schweitzer et al. 1993; Potter et al. 2008, 2013; Timmers et al. 2012; Waisbren et al. 2012; Coss et al. 2013), with pathophysiological correlates studied in fMRI brain studies (Timmers et al. 2015).

Over 80% of galactosaemia females suffer from POI (91% in Irish female patients) (Waggoner et al. 1990; Sanders et al. 2009; Coss et al. 2013). The clinical presentation varies from primary amenorrhea to delayed pubertal development followed by irregular menses or secondary amenorrhea. This results in infertility or subfertility as a predominant feature in galactosaemia females of childbearing age (Rubio-Gozalbo et al. 2010).

The pathophysiology for this presentation is still unknown. Mechanisms proposed include prenatal toxicity with galactose and metabolites possibly causing premature follicular apoptosis/atresia, abnormal cell signaling and hormone/receptor glycosylation abnormalities. Hypoglycosylation of follicle stimulating hormone (FSH) could theoretically alter its function and lead to POI in galactosaemia. However biochemical tests have shown inconclusive results (Prestoz et al. 1997; Gubbels et al. 2011).

Male galactosaemia patients seem to be less affected and have successfully fathered offspring, reaching puberty spontaneously although the age of onset can be delayed (Rubio-Gozalbo et al. 2010). PMM2-CDG patients also virilize normally through puberty but with occurrences of

decreased testicular volume and increased serum FSH concentrations (de Zegher and Jaeken 1995).

Altered leptin signaling secondary to glycosylation abnormalities may be contributory (Knerr et al. 2013). Leptin is a key energy and fat storage regulator (Kratzsch et al. 2002) and abnormal leptin signaling due to hypoglycosylation has also been considered in abnormal fat distribution in PMM2-CDG (Wolthuis et al. 2013).

Osteopenia is another long-term complication associated with galactosaemia. Osteopenia and other skeletal abnormalities are common clinical findings in CDG (Coman et al. 2008; Rimella-Le-Huu et al. 2008). It has been suggested that hypoglycosylation of noncollagenous bone proteins may be responsible for decreased bone mass and increased osteocalcin levels in PMM2-CDG patients (Barone et al. 2002).

Decreased bone mineral density has been consistently observed in galactosaemia patients (Rubio-Gozalbo et al. 2002; Panis et al. 2004; Waisbren et al. 2012; Batey et al. 2013; Coss et al. 2013; Doulgeraki et al. 2014). Decreased bone metabolism has been suggested as the mechanism of reduced bone mineral density in galactosaemia as well as abnormal galactosylation of the collagen matrix (Kaufman et al. 1993).

Reduced levels of insulin-like growth factor 1 (IGF-1), carboxylated osteocalcin, N-terminal telopeptide and C-terminal telopeptide have been reported in serum samples from galactosaemia patients (Panis et al. 2004; Fridovich-Keil and Walter 2008).

Serum IgG N-Glycosylation Abnormalities in Galactosaemia

As discussed earlier, substantial N-glycan abnormalities have been demonstrated in serum transferrin IEF patterns in galactosaemia (Charlwood et al. 1998; Sturiale et al. 2005; Quintana et al. 2009). UDP-galactose substrate deficiency is one of the proposed contributing pathophysiological mechanisms (Gibson et al. 1995; Lai et al. 2003; Parkinson et al. 2013; Jumbo-Lucioni et al. 2014).

We have documented hypoglycosylation and gross N-glycan assembly defects in the whole serum of untreated neonates with galactosaemia similar to what has been observed in CDG-I defects (Coman et al. 2010; Coss et al. 2014). While the N-glycan assembly defects resolve within the first 6 months of life with galactose restriction, it is apparent that, after this initial effect, N-glycan processing defects persist, even in young children (Coss et al. 2014).

We also performed a study of N-glycan processing defects in 10 treated galactosaemia adults on a restricted galactose diet in comparison to matched controls using serum IgG analysis (the most abundant circulating N-glycan glycoprotein), analyzed by NP-HPLC to monitor the effects and potential benefits of galactose supplementation with galactosylation of IgG used as a specific biomarker of dietary galactose tolerance. We demonstrated an increase in non-galactosylated (G0) and monogalactosylated (G1) structures with decreased digalactosylated structures (G2) in diet-restricted galactosaemia patients, indicating continued N-glycan processing defects despite treatment (Coss et al. 2012). Five subjects followed a moderate galactose liberalization trial over 16 weeks. Their IgG N-glycan profiles showed consistent individual alterations in response to diet liberalization with improvement of profiles for three of the five subjects at a galactose intake of 1000 mg/day.

We recently also published a study of 13 children with galactosaemia which indicated that a moderate increase in galactose intake may be well tolerated in children and may improve glycosylation (Knerr et al. 2015).

We previously also identified that children with galactosaemia had lower serum leptin levels than normal controls, expressed as SDS for gender and pubertal age (Knerr et al. 2013). In the above diet relaxation study, there was no statistical significant difference noted in serum leptin levels in the patient control group and the diet relaxation groups at the baseline point. However, patients in the galactose supplementation group had, as a trend, slightly higher leptin levels at the end of the study than patient controls ($p < 0.05$), but within the normal range.

We have now established a rapid automated robotic hydrophilic interaction ultra-performance liquid chromatography N-glycan analysis for the measurement of IgG N-glycan galactose incorporation applied to adult galactosaemia patients which has demonstrated significant differences between the G0/G1 and G0/G2 incorporation ratios and controls (Stockmann et al. 2016).

This analysis of IgG glycosylation has also recently been applied to the CDG condition MAN1B1-CDG using this methodology (Saldova et al. 2015).

To further identify the specific N-glycosylation steps that are affected in galactosaemia, we performed further glycan subset analysis in the IgG glycosylation study of 40 galactosaemia treated patients compared to controls. In this work, we identified a significant increase in core fucosylated neutral glycans and a significant decrease in core fucosylated, afucosylated bisected glycans and N-linked mannose-5 glycans in circulating serum IgG N-glycans (Maratha et al. 2016). Figure 1, amended from this study, illustrates the steps in N-glycan synthesis, which may be affected in this pathway.

Abnormal Gene Expression and Cell Signaling in Galactosaemia

In a pilot microarray study of T-lymphocyte RNA expression from four galactosaemia patients (Coman et al. 2010), we identified extensive dysregulation of genes affecting many signaling pathways including MAP kinase, regulation of actin cytoskeleton, ubiquitin mediated proteolysis, inositol signaling, inflammatory pathways and glycan biosynthesis pathways (Coman et al. 2010), and we subsequently validated dysregulation of a number of N-linked glycosylation biosynthesis genes linked to CDG-1 and CDG-II, e.g. *ALG* (*1, 2, 8* and *9*) in a larger study (Coss et al. 2014).

We also confirmed and noted the dysregulation of the genes *ANXA1* and *ALG9* (Alpha-1,2-mannosyltransferase) (which also responded to differing levels of galactose exposure), in cultured galactosaemia patient fibroblast cells (Coss et al. 2014).

We have subsequently studied the expression of a number of these genes and other related relevant N-glycan biosynthesis genes in peripheral blood mononuclear cells from affected galactosaemia adult patients. We noted significant dysregulation of two key N-glycan biosynthesis genes *ALG9,* which was up-regulated ($p < 0.001$), and *MGAT1,* which was down-regulated ($p < 0.01$) with additional dysregulation of the genes *FUT8,* and *MGAT3* (Maratha et al. 2016). The site of action of these genes is illustrated in Fig. 1.

The *ALG9* (Alpha-1,2-mannosyltransferase) gene product is involved in the addition of the seventh and ninth mannose sugar to the growing N-glycan, essential for the formation of the initial oligosaccharide chain. It has been proposed that the interaction of ALG9 with ALG12 is required for the ultimate formation of the disaccharide glycan, which influences further downstream processing of N-glycans, indicating a potential regulatory role for the *ALG9* gene in glycosylation (Coss et al. 2014). The clinical phenotype for ALG9-CDG has recently been expanded (see Table 1) (AlSubhi et al. 2016).

The decreased expression of the *MGAT1* gene also has significant pathological correlates. The *MGAT1* gene encodes GlcNAc transferase I (Alpha-1,3-mannosyl-glycoprotein 2-beta-N-acetylglucosaminyltransferase), which adds GlcNAc to high-mannose sites, an essential early step in producing all branched complex and hybrid N-glycans.

Inactivation of the *MGAT1* gene in mice was shown to impair oogenesis, and mouse *MGAT1* knockouts were unviable (Shi et al. 2004).

Galactosaemia and CDG: Dietary Treatment Approaches

Our studies of IgG N-glycosylation with varying effects of galactose exposure in galactosaemia adults and children have indicated the presence of significant interindividual tolerance of exogenous galactose in galactosaemia patients. There are reports on individuals who have relaxed the diet at an early age with good outcomes (Lee et al. 2003; Panis et al. 2006). It appears that some affected individuals with galactosaemia may have more ability to utilize alternative, accessory pathways to metabolize galactose and its metabolites than others and may tolerate moderate amounts of exogenous dietary galactose (Coss et al. 2014). This may be influenced by epigenetic regulation (Lauc and Zoldos 2009). As an illustrative example, the overexpression of human UDP-glucose pyrophosphorylase (hUGP2), an 'accessory pathway' enzyme, using both galactose-1-phosphate and glucose-1-phosphate as substrates, rescued GALT-deficient yeast cells from galactose toxicity (Lai and Elsas 2000). The UGP2 reaction may not be relevant under normal physiological conditions as high toxic levels of galactose-1-phosphate seem to be required (Lai et al. 2003), as glucose-1-phosphate is the preferred substrate (Leslie et al. 2005). As referred earlier, it is possible that some patients may have the ability to generate more UDP-galactose, using excess galactose-1-phosphate as a substrate (Lai and Elsas 2000; Fridovich-Keil and Walter 2008).

Considering this variability in accessory pathways of galactose metabolism and linked glycosylation, we propose that the clinical outcomes observed in galactosaemia are multifactorial, influenced by prenatal toxicity and postnatal variation in accessory glycosylation pathways (Coss et al. 2012; Knerr et al. 2015).

Also, while the severe restriction of dietary galactose in the affected newborn is life saving and largely reverses the N-glycan assembly defect, our studies suggest that over restriction of galactose in the long-term may contribute to ongoing N-glycan processing defects, evident in all the galactosaemia patients whom we have studied to date (Coman et al. 2010; Coss et al. 2012; Knerr et al. 2015; Stockmann et al. 2016).

The manipulation of exogenous provided sugar substrates in CDG is informative. At least four subtypes of CDG have been treated with dietary modulation of sugars: MPI-CDG, SLC55C1-CDG, PGM1-CDG (Hendriksz et al. 2001; Harms et al. 2002; Penel-Capelle et al. 2003; de Lonlay and Seta 2009) and SLC35A2-CDG (Ng et al. 2013; Dorre et al. 2015).

SLC55C1-CDG is caused by a decreased affinity of the GDP-fucose transporters resulting in decreased fucose, resulting in immunological defects and severe psychomotor delay (Goreta et al. 2012). Supplementation with oral fucose challenges the defective transporters leading to clinical improvements in some patients, with correction of immunological dysfunction and psychomotor improvement (Marquardt et al. 1999; Jaeken 2010).

PGM1-CDG phosphoglucomutase 1 deficiency (E.C 5.4.2.2) is caused by disruption of the glucose metabolism pathway whereby phosphoglucomutase catalyzes the bidirectional transfer of phosphate from position 1 to 6 on glucose. Deficiency of this enzyme, now characterized clinically by hypoglycaemia, liver disease, cardiomyopathy, short stature, cleft palate and normal intelligence, has previously been associated with a primary muscle disease, Glycogen Storage Disease, XIV (Morava 2014). There are a limited number of patients reported in the literature; one of the first reported suffered from exercise-induced rhabdomyolysis and muscular glycogenosis (Stojkovic et al. 2009; Timal et al. 2012; Morava 2014). The disruption of this pathway (caused by reduced PGM1) results in dysregulation of glycolysis and disruption of the galactose metabolism pathway. It has been suggested that the build-up of glucose-1-phosphate competes with galactose-1-phosphate for the UDP-glucose pyrophosphorylase enzyme. This drives the product of the pyrophosphorylase pathway towards UDP-glucose, reducing the level of UDP-galactose (Perez et al. 2013). If this is the dysregulated pathway of PGM1-CDG, then it would indicate there is some biologically relevant level of UDP-galactose produced from the UDP-glucose pyrophosphorylase pathway, which has direct relevance for galactosaemia. A study of PGM1-CDG patients treated with a combination of D-galactose and complex carbohydrate supplementation improved serum transferrin hypoglycosylation and ameliorated clinical symptoms. This study indicated increased levels of activated UDP-galactose in the treated patients which improved glycosylation (Morava 2014).

In addition, Ng et al. in 2013 reported a disorder of the X-linked gene UDP-galactose transporter SLC35A2. This disorder leads to galactose-deficient glycoproteins as measured by N-glycans from whole serum using MALDI-TOF. This showed increased levels of hypogalactosylated glycans, particularly biantennary species (Ng et al. 2013). Interestingly, in 3 affected children, the neonatal profile improved and normalized during the first few years of life. In a recently reported child with this transporter deficiency, dietary galactose supplementation resulted in nearly complete normalization of the abnormal transferrin glycosylation pattern (Dorre et al. 2015).

The beneficial effect of galactose supplementation for PGM1-CDG and SLC35A2 deficiency suggests the physiological need for supplementary exogenous galactose in the presence of UDP galactose limited bioavailability. This is of possible relevance to galactosaemia.

Conclusion

There are many biochemical and clinical similarities between galactosaemia and CDG syndromes. Early (prenatal and perinatal) dysregulation of glycosylation in galactosaemia must be a major determinant of both neurological/cognitive and reproductive deficits, while ongoing abnormalities in glycosylation and associated gene dysregulation and associated cell signaling abnormalities may also have relevant pathophysiological consequences.

The persistence of aberrant glycosylation and disruption of CDG-related genes in long-term treated galactosaemia patients suggest that this is a major area in galactosaemia research. This requires further investigation which may offer new biomarkers to monitor affected individuals and enhance our understanding of this and related conditions.

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Synopsis

An understanding of the link between galactosaemia and CDG, with a focus on abnormal N-glycosylation.

Compliance with Ethics Guidelines

This article does not contain any studies with human or animal subjects performed by any of the authors.

Ashwini Maratha and Eileen Treacy have been involved in the planning, conception, writing, drafting and reviewing this review. Hugh-Owen Colhoun assisted in writing this review. Ina Knerr, Karen Coss and Peter Doran have assisted in reviewing this review.

Conflict of Interest

The authors Ashwini Maratha, Hugh-Owen Colhoun, Ina Knerr, Karen Coss, Peter Doran and Eileen Treacy declare that they have no conflict of interest.

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