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J.L. Doyle *and* ***L. Stubbs*** *were in the Life Sciences Division, Oak Ridge National Laboratory, PO Box 2009, Oak Ridge, TN 37831-8077, USA.*
J.L. Doyle *is now in the National Institute of Human Genome Research, NIH, Bethesda, MD, USA.*
L. Stubbs *is now in the Human Genome Center, Lawrence Livermore National Laboratory, 7000 East Avenue, Livermore, CA 94550, USA.*

The fundamental importance of human galactose metabolism: lessons from genetics and biochemistry

KLAUS G. PETRY (klaus.petry@bordeaux.inserm.fr)

JUERGEN K.V. REICHARDT (reichard@zygote.hsc.usc.edu)

Cloning and characterization of all three human galactose-metabolic genes (GALK, GALT and GALE) has led to the identification of a number of mutations which are generally of the missense type in patients with galactosemia, an inborn error of metabolism. The predominance of missense mutations is interesting, considering the general importance of galactose metabolism for cellular energy production and proper modification of glycoproteins and glycolipids. Abnormalities in both of these macromolecules have been described in transferase-deficiency galactosemia, the most common and best-studied form of galactosemia. Thus, the parallel biochemical and molecular genetic analyses of human galactose metabolism are shedding light on this under-appreciated metabolic pathway that is critical for cellular energy production, modification of cellular macromolecules and normal human development.

Galactose metabolism from bacteria to humans is carried out in three enzymatic steps (Fig. 1) that are catalyzed sequentially by galactokinase (encoded in humans by the *GALK* gene), galactose-1-phosphate uridyl transferase (*GALT*) and UDP-galactose 4′ epimerase (*GALE*)[1]. Galactose can be converted into energy by entering the glycolytic pathway because glucose-1-phosphate is one of the *GALT* reaction products[1,2] (Fig. 1). Galactose can also be incorporated into glycoproteins and glycolipids because UDP-galactose, the second product of the *GALT* reaction, is the substrate of all galactosylation reactions[1] (Fig. 1). Deficient activity of each of the three galactose-metabolic enzymes results in a human disease[1]. Here we review how the interplay of biochemical and molecular genetic investigations has shed new light on the importance of this critical metabolic pathway and revitalized a 30-year old hypothesis.

The galactose metabolic defects are inborn errors of metabolism that can result in a variety of symptoms, including cataracts, in the case of kinase deficiency, and severe neonatal symptoms, such as failure to thrive, hepatomegaly and bacterial sepsis in transferase-deficiency galactosemia[1]. Transferase-deficiency galactosemia (also termed 'classic galactosemia') is the most common form of the disease and can be treated by dietary-lactose restriction[1]. Epimerase deficiency can be benign and occurs only in blood cells (the 'peripheral' form) or it can be as severe as transferase deficiency (in the 'general' form), which is expressed ubiquitously[1].

Biochemical and genetic studies of bacterial galactose metabolism have shaped our views of molecular genetics: the *Escherichia coli gal* operon has been a popular model system for four decades[1]. The fundamental importance of human galactose metabolism is now coming into focus again, promising exciting insights into human physiology, development and genetics.

The human *GALK* cDNA and gene were cloned and characterized in 1995 (Ref. 3). Molecular analysis of the human gene revealed two point mutations that cause cataracts: a missense mutation (Val32Met, which replaces valine at codon 32 with methionine) and a nonsense mutation (at codon 80) (Table 1)[3]. This allelic heterogeneity suggests that multiple molecular lesions can lead to the cataracts that are commonly seen in kinase-deficient patients. The pathogenetic mechanism for the cataracts in this and other forms of galactosemia is currently unknown. However, their common occurrence implies a similar etiology in all three forms of the disease.

GALT

Transferase-deficiency galactosemia is the most common, well known and best studied of the three disorders of galactose metabolism[1]. A full-length, expressible *GALT* cDNA was cloned a number of years ago and at least 91 mutations have been identified to date (www.ich.bris.ac.uk/galtdb/), including about five protein polymorphisms

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that substitute amino acids but do not lead to disease phenotypes (Table 1)[4]. Most of the disease-causing mutations and all *GALT* protein polymorphisms are missense substitutions, which might be because of the small mutational target size of the *GALT* gene (which is only about 4 kb) or might indicate the vital role of this gene. Therefore, only certain mutations, such as missense, which often retain residual protein and enzyme activity, are tolerated in galactosemia patients, because more severe mutations might not be compatible with life. The mouse *Galt* gene was recently 'knocked out' and these *Galt*-deficient mice apparently have no discernible phenotype[5]. This lack of a phenotype in *Galt* knock-out mice could argue against the latter hypothesis for the human *GALT* gene. However, the complete lack of symptoms (including those of 'classic' galactosemia) in these mice suggests that galactose metabolic phenotypes are controlled differently in mice and humans[5]. Therefore, *Galt*-deficient mice might be a better model for investigating potential novel galactosemia therapies by unraveling the lack of symptoms in mice, rather than studying the human disease.

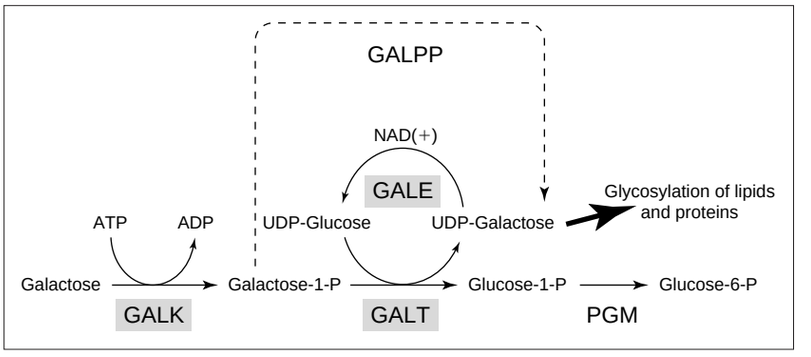

FIGURE 1. The galactose metabolic pathway is encoded by three genes: *GALK* (galactokinase), *GALT* (galactose-1-phosphate uridyl transferase) and *GALE* (UDP-galactose 4′ epimerase) (marked in gray). Deficient activity of each of the three galactose-metabolic enzymes results in a human disease. In addition, uridine diphosphate galactose pyrophosphorylase (GALPP) could have a limited role in bypassing the *GALT* reaction to convert galactose-1-phosphate to UDP-galactose. Phosphoglucomutase (PGM) converts glucose-1-phosphate to glucose-6-phosphate, which can then enter the glycolytic pathway (modified from Refs 1, 2).

The most common classic galactosemia mutation is the substitution of glutamine at codon 188 with arginine (Gln188Arg; Table 1)[4]. This mutation is the predominant mutation in all Caucasian patients[6,7]. It appears that African-American patients harbor a specific set of galactosemia mutations that includes Gln188Arg, although at a reduced frequency, as well as other race-specific mutations (e.g. Ser135Leu, which replaces serine at codon 135 with leucine)[7–9]. Preliminary studies suggest that other racial or ethnic groups in the USA also have their own set of galactosemia mutations[7], while there might also be ethnic differences in mutational spectra across various European populations[10,11].

The two most common *GALT* enzyme variants are named after the two cities in Southern California [Duarte (D) and Los Angeles (LA)], where they were first reported[1,4]. Interestingly, both variants are characterized by similar, faster electrophoretic mobility but very different enzyme activities; the D variant has about half the normal *GALT* activity while the LA variant has well-above normal activity[1,4]. It was shown that both variants bear the same amino acid substitution: asparagine-314 mutated to aspartate (Asn314Asp; Table 1)[6,12]. Thus, this amino acid substitution could account for the increased electrophoretic mobility of the D and LA enzyme variants by increasing the overall negative charge of the mutant *GALT* protein. It has been recently hypothesized that the increased activity of the LA variant could be the result of increased amounts of protein, owing to more-favorable codon usage[13]. The D variant is commonly identified in newborn screening programs[1], although it usually does not require treatment[1]. Therefore, rapid molecular confirmation of the D variant by testing for presence of the Asn314Asp amino acid substitution could be considered for newborn screening programs.

GALE

The human *GALE* cDNA is 51% identical to the homologous *E. coli* galE enzyme[14]. A total of seven missense mutations have been reported for 'peripheral' epimerase-deficiency galactosemia (Table 1)[15,16]. Thus, this disease also seems to be caused by a preponderance of point mutations and allelic heterogeneity[14,15], just like kinase- and transferase-deficiency. Historically, it is noteworthy that Kalckar, one of the pioneer investigators of human galactose metabolism, had predicted that there would be no *GALE*-deficient mammals[17]. His provocative suggestion that epimerase deficiency might be lethal can, today, be recast in modern molecular terms: galactose metabolism must be very important, if not vitally so, because all seven *GALE* mutations reported to

TABLE 1. Common galactosemia mutations and important features of human galactose-metabolic genes

Gene	Mutation	Comments
GALK		
Location: 17q24	Val32Met	In one cataract family
Gene size: 7 kb	Glu80Stop	In one cataract family
GALT		
Location: 9p23	Ser135Leu	Common in African-Americans
Gene size: 4 kb	Gln188Arg	Common in all Caucasian populations examined
	Asn314Asp	Found in D and LA variants
GALE		
Location: 1p36	Asp103Gly	Common in Japanese
Gene size: 4 kb	Lys257Arg	Common in African-Americans

Note: only selected, common mutations are listed in this Table.

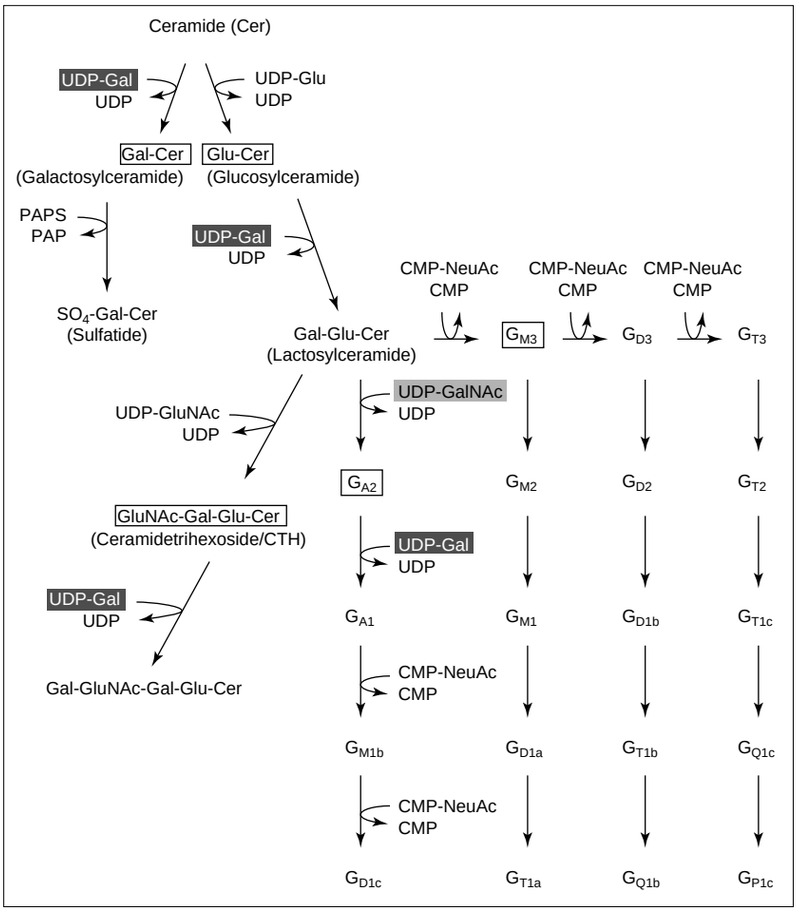

FIGURE 2. Glycosphingolipid biosynthesis model (modified from Ref. 21). The principal glycosphingolipid groups are cerebrosides, sulphatides, globosides and gangliosides. The cerebrosides are a group of monohexosides, of which the most common are galactosylceramide (Gal-Cer) and glucosylceramide (Glu-Cer). Sulfatide (or galactosyl-ceramide 3-sulfate) is a major myelin compound of the central and peripheral nervous system. Globosides are ceramides containing oligosaccharides. Gangliosides (letter G) contain neuraminic acid and are major compounds of the nervous system. To specify the different gangliosides the subscript M, D, T, Q, P refers to mono-, di-, tri-, tetra- (or quatra-) and penta-neuraminic acid-containing gangliosides. The principal gangliosides in the brain are G_{M1}, G_{D1a}, G_{D1b} and G_{T1b}. The glycolipid compounds which were identified to be modified quantitatively in galactosemic brain and B lymphocytes are shaded gray. Metabolic reactions which implicate galactose or GalNAc are highlighted in red or pink, respectively. Abbreviations: UDP-Gal, uridine 5′-diphosphogalactose; UDP-Glu, uridine 5′-diphosphoglucose; UDP-GalNAc, uridine 5′-diphospho-N-acetylgalactosamine; UDP-GluNAc, uridine 5′-diphospho-N-acetylglucosamine; CMP-NeuAc, cytidine 5′monophospho-N-acetylneuraminic acid; PAPS, 3′-phosphoadenosine-5′-phosphosulfate ('activated sulfate').

date are of the missense type[15,16]. Thus, retaining some *GALE* protein and/or activity, which is often associated with missense mutations, could be critical to human life.

Biochemical basis of transferase-deficiency galactosemia

The symptoms of transferase-deficiency galactosemia, the most common and best studied form of the trio of diseases, are usually attributed to the toxic accumulation of galactose-1-phosphate and galactitol in patient tissues, and dietary restriction of galactose has been the basis for treatment for nearly 40 years[1]. Although galactose restriction leads to rapid clinical improvement in the neonate, it is now clearly established that the neurological and intellectual prognosis of galactosemic patients is often poor and ovarian failure is frequently observed[1]. Dystrophic motor and growth retardation have also been reported in these patients[1]. To understand the biochemical bases for these pathophysiological phenomena, considerable attention has focused on galactosylated compounds obtained from patient tissues, on the expression of *GALT* enzyme activity and on *GALT* mRNA and protein synthesis in animal tissues. Galactose is a major compound of complex carbohydrates of glycoproteins and glycosphingolipids that are involved in cell–cell recognition, regulation of cell-cycle control, receptor function and many other biological functions[1].

Neurological dysfunction

In the terminal position of complex carbohydrate structures, a sulfate group or N-acetyl neuraminic acid can be attached to galactose, endowing a negative charge on these macromolecules. Thus, *GALT* deficiency might cause alterations in these carbohydrate structures, leading to completely nonfunctional or substantially less functional molecules. The analysis of various tissue samples from galactosemic patients revealed abnormalities of several galactose-containing compounds. The first suggestion of a glycosylation defect in galactosemia was based on the observation of an abnormal glycoprotein pattern in the brain of a galactosemic patient[18]. Investigations of galactosemic fibroblasts detected defective incorporation of [35S]-sulfate[19], which is most probably secondary to a deficient incorporation of galactose into glycoproteins. The deficit of galactose incorporation could also be observed in serum samples from galactosemic patients. These data reflect a galactosylation defect caused by intracellular galactose metabolism abnormalities.

The analysis of the glycolipid content in the brain of a galactosemic infant compared with two other infants who had died of other causes also revealed several quantitative alterations[20]. Along with an increase in glucosylceramides and GM3 gangliosides, we also observed a decrease in galactosyl-ceramides (Gal-Cer) and GA2 asialo-gangliosides[20]. This glycolipid study was extended to B lymphocytes where ceramide trihexosides were increased in galactosemic samples[20]. Following the different reactions involved in the glycolipid biosynthesis[21] (Fig. 2), the amounts of the identified glycolipid compounds in the galactosemic brain allowed the general observation that galactose or N-acetyl galactosamine (GalNAc)-containing glycosphingolipids are reduced,

while their precursors accumulate[20]. This glycolipid analysis revealed a decrease in Gal-Cer but also an increase in glucosyl-ceramides[20].

Glycolipid analyses have also been recently carried out in knock-out mice lacking the enzyme UDP-galactose:ceramide galactosyltransferase (CGT), which is required for galactosyl-ceramide synthesis[22]. CGT-deficient mice do not synthesize Gal-Cer nor its sulfated derivative sulfatide but, surprisingly, form myelin-containing glucosyl-ceramide, a lipid not previously identified in myelin[22]. The biochemical alterations of the CGT-deficient mouse cause a thinner myelin sheath, leading to electrophysiological-conduction deficits, generalized tremoring and mild ataxia[22].

The Gal-Cer deficit observed in the galactosemic brain led us to study *GALT* expression during myelination in a rat model system[23]. In the central (CNS) and the peripheral nervous system (PNS) the most prominent *GALT* expression was observed during myelination[23]. *In vitro* studies of PNS myelination revealed that *GALT* is specifically expressed in the Schwann cells that form the myelin sheath[24]. These data indicate a major role of this enzyme at a precise step of development of the nervous system, allowing the understanding of pathologically altered white-matter signal observed in galactosemic patients[1,23,24].

In humans, myelination of nerve tracts is a major process during the maturation of the nervous system that is critical for normal development. Myelination takes place during a relatively short postnatal period in rodents, and during late fetal life and the postnatal period in humans. A major step in myelination is the differential synthesis of galactosyl-ceramides and sulfatide by myelinating cells. These galacto-lipids represent more than 20% of myelin glycolipids, and both are early markers for embryonic oligodendrocyte and Schwann cell development. The synthesis of these two glycolipids depends on UDP-galactose, and so the whole process of myelination might be seriously impaired in galactosemic patients, leading to the characteristic cognitive dysfunction, including low IQ, verbal apraxia, memory deficits, and lack of visuo-motor coordination observed in more than half of the patients[1]. Ataxia, tremor and seizures have also been reported[1].

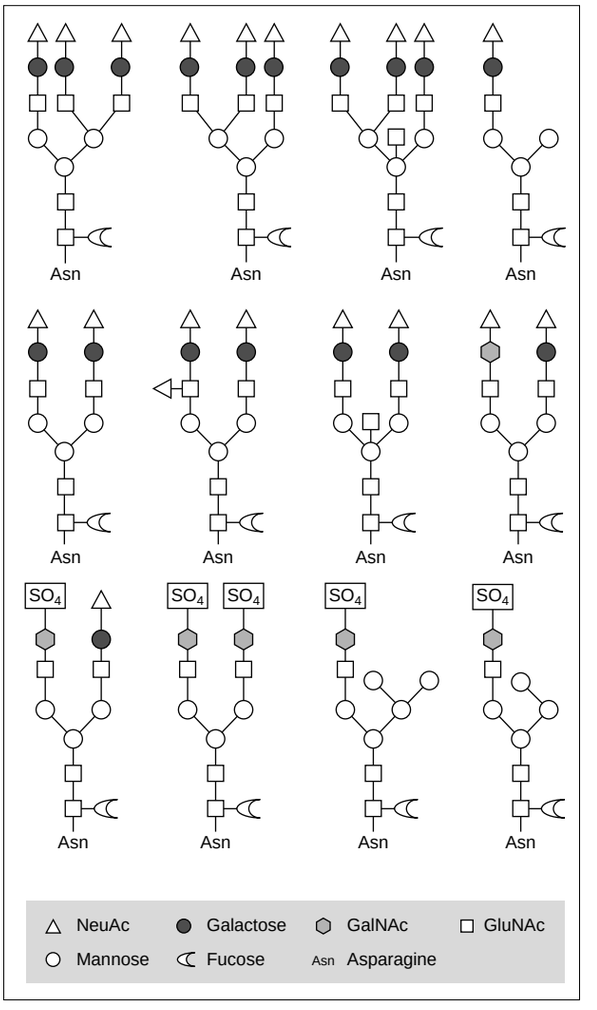

FIGURE 3. Structures of the oligosaccharide chains of human follicle stimulating hormone (FSH) (modified from Ref. 26). The different composition of the oligosaccharides leads to at least 11 distinguished acidic isoforms. In the terminal position a sulfate group or neuraminic acid is attached to galactose (gray circles) or GalNAc (gray hexagons), endowing a negative charge on these glycoprotein hormones.

Ovarian failure

Gonadal dysfunction, specifically hypergonadotrophic hypoganadism, is an almost universal finding in female galactosemia patients[1]. Even when neonatal symptoms were prevented by a galactose-free diet, galactosemic females have elevated serum levels of follicle stimulating hormone (FSH) and of luteinizing hormone (LH)[1]. *GALT* deficiency in the ovary might result in toxic metabolite accumulation but also UDP-galactose deficiency in this tissue leading to gonadal endocrinopathy[1]. These observations indicate that *GALT* expression in the ovary and in indirectly associated tissues is critical for normal ovarian development and function. The defective galactosylation and the elevated FSH serum levels generally observed in female galactosemic patients led us to analyze the FSH-glycoprotein isoforms in the serum of these patients[25] that contain normally galactose-rich carbohydrate side chains[26] (Fig. 3). In the three galactosemic patients with ovarian insufficiency we examined, the composition of the FSH-glycoprotein isoforms was abnormal[25]. Some of the FSH carbohydrate structures were deficient in galactose and N-acetyl neuraminic acid, resulting in relatively neutral FSH isoforms[25]. Furthermore, studies of *GALT* expression in the rat anterior pituitary at different stages of the estrous cycle indicate that *GALT* expression is closely related to gonadotropin synthesis[24].

In galactosemia the abnormal FSH isoforms might produce antagonistic effects at the FSH receptor, as observed with deglycosylated FSH, which has a higher binding affinity to its receptor than the glycosylated form, although it is unable to activate cyclic AMP (Ref. 14). Thus, together with toxic effects caused by galactose metabolites, the altered FSH isoforms might contribute indirectly to ovarian failure in galactosemia. These abnormal FSH isoforms can bind to the FSH receptor but they might fail to stimulate intracellular signaling.

Unlike female patients, galactosemic males have no apparent fertility problems. Studies on mice 'knocked out' for the FSH-β subunit[27] might help to explain this

phenomenon. The FSH-deficient female mice are infertile owing to a block in follicologenesis before antral follicle formation. FSH-efficient males, however, are fertile, despite having small testes. Although there are no comparative studies in humans, this difference in the physiological role of FSH during gonadal development may help to explain the observed different outcome between girls and boys affected with galactosemia.

The importance of human galactose metabolism

Current research into human galactose metabolism has predominantly focused on cloning all three genes and dissecting galactosemia pathophysiology at the molecular genetic level. These investigations have revealed extensive allelic heterogenity in three compact genes (Table 1). Furthermore, this molecular genetic research has rekindled biochemical investigations into human galactose metabolism by asking fundamental questions concerning the pathway itself. The interplay of two distinct disciplines, molecular genetics and biochemistry, has not only been fruitful but has also shed new light on the fundamental importance of this metabolic pathway in humans. Allelic heterogeneity is a feature of all three forms of galactosemia (Table 1), which has complicated genotype–phenotype correlations in the case of transferase-deficiency galactosemia, the best studied of the three diseases[7]. It is noteworthy that all three forms of galactosemia are pan-ethnic, but most populations have their own specific set of mutations (Table 1). This suggests founder effects, genetic drift and/or heterozygote advantage, particularly for the common D and LA variants. The predominance of missense mutations in human galactose-metabolic genes (Table 1) has led us to hypothesize that some galactose-metabolic activity could be of vital importance in humans. Over 30 years ago, H. Kalckar[16] hypothesized that mammalian galactose metabolism, and particularly *GALE*, would be important for 'cell sociology'. Recent investigations of glycolipids and glycohormones support this notion. The current molecular data restate the classic Kalckar hypothesis in molecular terms. In summary, human galactose metabolism provides a useful paradigm for interdisciplinary investigations of a pathway that many scientists have come to feel is so well-studied that it is now relegated to the textbooks, instead of at the forefront of current science.

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K.G. Petry *is at the Institut François Magendie, INSERM U.394 Neurobiologie intégrative, Université Victor Segalen/Bordeaux 2, Bordeaux, France.*
J.K.V. Reichardt *is at the Department of Biochemistry and Molecular Biology and Institute for Genetic Medicine, USC School of Medicine, Los Angeles, CA, USA.*

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