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Galactose Toxicity in Animals

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Introduction

Galactose is a hexose that differs from glucose only by the configuration of the hydroxyl group at the carbon-4 position. Often present as an anomeric mixture of α -D-galactose and β -D-galactose, this monosaccharide exists abundantly in milk, dairy products and many other food types such as fruits and vegetables [1,2]. In humans, absorption of galactose from food across the brush border membrane of the proximal jejunum and renal epithelium is mediated by the Na⁺/glucose co-transporters SGLT1 and SGLT2 [3–7]. Other sources of galactose include endogenous production and natural turnover of glycolipids and glycoproteins. Using the technique of isotopic labeling, Berry *et al.* elegantly demonstrated that a 70kg adult male could produce up to 2 grams of galactose per day [8–10]. Once present inside the cells, β -D-galactose is epimerized to α -D-galactose through the action of a mutarotase [11]. α -D-galactose is subsequently converted to galactose-1-phosphate (gal-1-P) by the enzyme galactokinase (GALK) (E.C. 2.7.1.6). In the presence of galactose-1-phosphate uridylyltransferase (GALT) (E.C. 2.7.7.12), gal-1-P reacts with UDP-glucose to form UDP-galactose and glucose-1-phosphate (Fig. 1). Glucose-1-phosphate produced can enter the glycolytic pathway or react with UTP in the presence of UDP-glucose pyrophosphorylase (UGP) to form a new molecule of UDP-glucose [12]. The other product, UDP-galactose, can act as a galactosyl donor for the biosynthesis of glycoproteins and glycolipids, or be converted back to UDP-glucose by UDP-galactose-4-epimerase (GALE) (E.C. 5.1.3.2) [13,14]. It is worth mentioning that in addition to UDP-glucose/galactose, human GALE can also recognize UDP-*N*-acetylgluco/galactosamine [15]. Moreover, gal-1-P can also be dephosphorylated by inositol monophosphatase to form galactose [16]. Because of these reactions and endogenous galactose production, galactose is a non-essential nutrient. GALK, GALT and GALE comprise the evolutionarily conserved Leloir pathway of galactose metabolism [17] (Fig. 1). If the flow of galactose through the Leloir pathway is perturbed either due to congenital deficiency of any of the above-mentioned enzymes or an overwhelming presence of this hexose, toxicity syndromes will be observed. Over the years, there have been dozens of book chapters and reviews on human disorders of galactose metabolism [18–29] and experimental galactosemia in animals [30–34]. Therefore, the primary goal of this review is not intended to reiterate the known, but to update recent developments in the field and offer our insights into the as yet unknown.

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Congenital Disorders of Galactose Metabolism

Inherited deficiencies of GALK, GALT, and GALE activities in humans have all been observed and studied extensively [18–29]. The clinical manifestations of each enzyme deficiency, however, differ markedly. For instance, patients with GALK deficiency (MIM 230200) (Type II Galactosemia) have the mildest clinical consequences as they may present only with cataracts [35–37]. On the other hand, GALT-deficiency (MIM 230400) (Type I Galactosemia) [38,39] is potentially lethal and demonstrates long-term, organ-specific complications [40,41]. GALE-deficiency (MIM 230350) (Type III Galactosemia) has been somewhat controversial with regards clinical manifestations as clinical information is limited, and based mostly on case reports [42–44]. Until newborn screening for GALE deficiency is available, the natural history will be unknown. The differences in clinical outcome between GALT and GALK deficiencies reflect the differences in tissue response to the characteristic changes in the levels of galactose metabolites from the respective enzyme deficiencies.

Many reported cases of deficiencies were identified as being caused by missense mutations in the corresponding genes, which resulted in reduced enzyme activities. The genes encoding all three human enzymes have been isolated [45–47], and mutations associated with decreased enzymes activities have been reported [47–49]. Unlike the *GAL* genes in the Baker's yeast [50–54], the expression of the three genes in humans has not been examined in great detail. Elsas *et al.* conducted a functional analysis of the human GALT gene promoter and found that the human GALT gene is regulated in the first –165bp of its promoter region by positive regulators of GALT gene expression, and that a GTCA micro-deletion in a carbohydrate response element explained the functional differences between the D1 and D2 variants of the Duarte N314D mutation in GALT [55]. Recently, Park *et al.* reported that a novel c.-22T>C mutation in human GALK1 gene promoter is associated with elevated galactokinase phenotype [56]. In contrast to the yeast *GAL* genes, any up-regulation seen in the human genes observed so far was less than 3-fold. Using rodents as models, several groups noticed that abundant GALT gene expression was found in liver, ovaries, heart, lung and kidneys, while the lowest levels of GALT gene mRNA were detected in testis and skeletal muscle [57–59]. In addition, Daude *et al.* observed that the GALT gene was expressed maximally in the anterior pituitary during the pro-estrous and estrous phases of the estrous cycle [60]. These findings may be relevant to the pathophysiology of primary ovarian insufficiency (POI) in GALT-deficiency galactosemia.

Only human GALK and GALE have been crystallized [61,62]. Although the human GALT enzyme has not been crystallized, the *Escherichia coli* GALT crystal was solved by Wedekind *et al.* [63]. Several laboratories have conducted prolific functional analyses of wild type and mutant human GALT enzymes [64–70]. The most common human GALT mutation, Q188R is associated with a poor clinical outcome [71–73], while the S135L mutation [74–76], has a good outcome only if it is identified and treated in the newborn period with a galactose-restricted diet. Mutations in the GALT gene have ethnic-specific distributions; the Q188R is prevalent in Caucasians of Northern European origin [49], while the S135L is prevalent in patients of African descent [74–76]. A 5kb-deletion is found so far exclusively in the Ashkenazi Jewish patients [77]. Similarly, the V94M mutation is associated with the severe, generalized form of GALE-deficiency galactosemia [78], while intermediate phenotypes for GALE deficiency are associated with S81R, T150M and P293L mutations [42,79]. There are about 20 missense mutations identified in the human GALK1 gene thus far [61], most of which are confined to single families [80], and all but one, A198V, will almost certainly cause cataracts within the first two years of life [81]. The P28T-GALK1 missense mutation is found in people of Roma (Gypsy) descent, likely through a founder effect [82].

Cellular Toxicity of Galactose in Galactosemia – a work in progress

Patients with any type of galactosemia who are on galactose-restricted diet are never truly free from galactose intoxication, as significant amounts of bio-available galactose moieties come from non-dairy foodstuffs [1,2], endogenous synthesis from UDP-glucose [8–10], and natural turnover of glycoproteins/glycolipids. At least four mechanisms producing toxicity in human galactosemia at the cellular level are proposed:

(a) Accumulation of toxic metabolites in the blocked Leloir pathway

Since patients with galactosemia are constantly exposed to galactose, they are continually subjected to the potential toxic effects of the intermediates amassed in the blocked Leloir pathway. Accumulation of galactose is seen in all three types of galactosemia, but abnormal accumulation of gal-1-P occurs only in GALT-deficiency and the generalized form of GALE-deficiency [83,84]. In fact, ingestion of a 60ml-bottle of cow's milk by GALT-deficient patients results in a rapid accumulation of 10–20mM (18–36mg/100ml) galactose in blood and other tissues [85]. Even on a galactose-restricted diet, such patient's erythrocytes continue with an intracellular concentration of up to 100–200 μ M of galactose and gal-1-P [25]. In contrast, GALK-deficient patients also accumulate galactose, galactitol and galactonate, but not gal-1-P. GALK-deficient patients manifest neither acute toxicity syndrome nor chronic complications such as POI, ataxia and growth failure as seen in the GALT-deficiency. Since the only difference is the elevated gal-1-P, gal-1-P must be the major, if not sole, pathogenic agent for these organ specific failures in GALT-deficiency. Indeed, retrospective studies showed that the median gal-1-P level is the best predictor for the development of POI and dyspraxic speech in GALT-deficiency galactosemia [72,73]. Additionally, gal-1-P accumulation inhibited cellular growth in the model system *Saccharomyces cerevisiae* [86–89]. Yet, despite reports that gal-1-P potentially interferes with enzymes such as phosphoglucomutase [89,90], glycogen phosphorylase [91], UDP-glucopyrophosphorylase [92–95], and inositol monophosphatase [16,95,96], the *in vivo* target(s) for gal-1-P toxicity have not been confirmed in humans. Pourci and coworkers proposed that gal-1-P might not be toxic, because when adding 2.5mM inosine to the growth medium of GALT-deficient fibroblasts, these cells grew in the presence of 5mM galactose, despite the accumulation of significant amount gal-1-P [97]. We suggest that the added inosine might have overcome or suppressed the toxic effects of the gal-1-P in fibroblasts.

To date, there are no well-controlled human studies that correlate between GALT mutations and gal-1-P levels, as the latter vary with the degree of dietary compliance and the level of individual's GALK1 gene expression. One can, therefore, only assume that more gal-1-P will be accumulated in patients with more deleterious GALT mutations.

(b) Accumulation of toxic products of alternate galactose catabolism

If galactose is not metabolized efficiently, whether due to a block in the Leloir pathway, excess galactose will be catabolized by alternate pathways to form (a) galactitol and (b) galactonate (Fig.1) [25,36,84,98]. Galactitol cannot be further metabolized, and is predominantly excreted in the urine [25]. Yet, some galactitol accumulates in lens fibers and other tissues. Galactonate is metabolized *via* the pentose phosphate shunt, and it remains unclear whether the accumulated galactonate is toxic [25]. Recent observations dispute the traditional view of the intracellular osmotic effect of galactitol on lens epithelial cell permeability with consequent cell death and cataract formation in both GALK- and GALT-deficient patients [99–101]. Kubo and colleagues suggested that cataract formation is caused by free radical production [102,103]. These investigators proposed that the concentration of galactitol in the target tissues might not be high enough to evoke significant osmotic stress. Instead, they showed that excess galactose resulted in activation of aldose reductase in

producing galactitol, thus depleting NADPH and leading to lowered glutathione reductase activity. As a result, hydrogen peroxide or other free radicals accumulate causing serious oxidative damage to the cells. Change in redox potential in red blood cells has also been reported by Berry *et al.* in their study of galactonate formation in Type I Galactosemia [104]. In addition to accumulation in lens, high galactitol concentrations are found by MRS in brains of GALT-deficient patients with *pseudotumor cerebri* [105,106]. It remains unclear whether this plays a role in the long-term cerebellar dysfunctions and/or mental retardation subsequently seen in these patients.

However, even if galactitol/galactonate metabolism plays a synergistic role with gal-1-P in the pathophysiology of GALT- and GALE-deficiency, it by no means diminishes the pathogenic role played by increased gal-1-P.

(c) Deficiency of UDP-galactose (and UDP-glucose) with implications in protein glycosylation and galactosylation

The Leloir pathway emphasizes the uridylation of gal-1-P. For each molecule of glucose-1-phosphate produced, one molecule of UDP-glucose and one molecule of gal-1-P are consumed, and one molecule of UDP-galactose is formed without energy expenditure (Fig. 1). UDP-glucose can come from epimerization of UDP-galactose or the pyrophosphorylase reaction (Fig. 1). Therefore, the production of glucose-1-phosphate from each gal-1-P molecule can take place at the expense of UDP-galactose formation, and does not necessarily result in a net gain of glucose-1-phosphate if UDP-glucose used originates from the pyrophosphorylase reaction (Fig. 1). In the latter case, UDP-galactose, not glucose-1-phosphate, is the net product of the conversion. It is thus logical to assume that if the galactose metabolic pathway is blocked, it will lead to a potential deficit of UDP-galactose [107]. Since UDP-galactose is a galactosyl donor in glycoproteins/glycolipids biosynthesis, UDP-galactose deficiency can theoretically impair the production of these macromolecules. Yet some investigators suggested that even if the Leloir pathway were blocked at the GALT reaction, UDP-galactose could be formed *via* the epimerization of UDP-glucose in the presence of GALE (Fig. 1). Still, we and others found that high level of gal-1-P competed with glucose-1-phosphate for the enzyme UDP-glucose pyrophosphorylase (UGP) *in vitro* [92–95]. As the UGP reaction is necessary to produce UDP-glucose (Fig. 1), we found that the natural inhibition of this enzyme in GALT-deficient fibroblasts by gal-1-P lead to reduced availability of UDP-glucose and UDP-galactose [93]. Such decline in UDP-glucose availability would further jeopardize the formation of UDP-galactose from the GALE reaction, and this in turn would lead to the production of abnormal glycoproteins and glycolipids. Several groups have identified aberrantly galactosylated glycoproteins such as serum transferrins, lysosomal enzymes, and circulating follicle stimulating hormone (FSH) in GALT-deficient patients [108–111]. At first glance, the oligosaccharide chains of the circulating glycoproteins were found to be deficient in their penultimate galactose and terminal sialic acids [108–111], suggesting galactosemia is a secondary congenital disorders of glycosylation (CDG) characterized by galactose deficiency of glycoproteins and glycolipids (processing defect or CDG-II). However, a more in-depth study published recently by Sturiale *et al.* showed the perturbation of *N*-linked glycosylation in galactosemia is more complex than originally thought. The authors showed that in untreated galactosemia, there was also a partial deficiency of whole glycans of serum transferrin associated with increased fucosylation and branching as seen in genetic glycosylation assembly defects (CDG-I). It thus suggested that galactosemia is a secondary "dual" CDG causing a processing as well as an assembly *N*-glycosylation defect [112].

It should be noted that UDP-galactose deficiency has also been observed in mouse cells with defects in the Golgi UDP-galactose translocator (UGT) [113,114]

(d) Perturbation of inositol metabolism

Wells and Wells reported decreased free and lipid-bound inositol in the tissues of both GALT-deficient patients [115] and galactose-intoxicated rats [116]. Recently, over-expression of human inositol monophosphatase was found to overcome galactose toxicity in GALT-deficient yeast cells [95]. Furthermore, gal-1-P competitively inhibited human inositol monophosphatase [117]. These recent findings suggest a pathogenic role of reduced inositol pools in GALT-deficiency.

These proposed mechanisms for gal-1-P toxicity are not mutually exclusive. For instance, it is possible that the loss of sialic acids in some of the glycoproteins detected under GALT-deficiency resulted from both UDP-galactose deficiency [107,118] and from excess galactitol formation [119]. Similarly, *myo*-inositol deficiency could be due to both excess galactitol accumulation [30] and inhibition of inositol phosphatases by excess gal-1-P [117].

Organ-specific Toxicities in Classic (Type I) Galactosemia

GALK-deficient patients manifest only cataracts. They do not have failures of liver, brain, ovary and growth seen in GALT-deficiency. Reports of patients with generalized GALE-deficiency suggested a phenotype similar to that of GALT-deficiency. Thus, we will focus on Type I Galactosemia in this section. Consequent to newborn screening programs [120,121], most cases of GALT-deficiency are now diagnosed before acute manifestations of the disease advance from prolonged neonatal jaundice to end-stage liver failure. However, very little is known about the pathophysiology of the acute, life-threatening syndromes, including hepatotoxicity [25,26].

Concerning long-term complications, neurological disorders [122–127] and POI [72,128–154] have long been subjects of intense research. More recently osteoporosis [151,155–160] and skeletal muscle weakness were recognized [161,162]. To date, studies of organ toxicities in GALT deficiency have been mostly descriptive with little known about the precise pathogenic mechanisms. For instance, depletion of Purkinje cells is observed in the cerebellum [122], but nothing is known about the cause for their reduction in number, or why Purkinje cells are more susceptible to galactose toxicity than others in cerebellum. POI associated with hypergonadotrophic hypogonadism has a prevalence of 85% among galactosemic females, and on-going research focused mainly on the followings: (1) Does the pathophysiology occur during the prenatal and/ or postnatal period [141,145]? (2) Do galactose and/or its metabolites induce apoptosis of ovarian tissues (follicles and granulosa cells) [135,137]? (3) Does UDP-galactose deficiency decrease viability and function of ovarian tissues [140]? (4) Does aberrant glycosylation of FSH (and LH) molecules impede maturation of follicles [111]? (5) Does aberrant glycosylation adversely affect migration of germ cells [146,147]? To date, these inquiries have not provided a uniform theory for the cause of POI. For example, there is no solid evidence for prenatal damaging effects of galactose upon ovarian tissues, as Levy *et al.* reported normal histological findings of ovaries in a female galactosemic patient who died of sepsis [163]. Such anecdotal observations suggest that galactose and/or its metabolites exert their toxicities upon follicles after birth. Indeed, some patients with severe GALT mutations with low MSH, high FSH and LH have conceived, with or without exogenous FSH treatment [144,149,154].

Animal Models for Galactosemia and Experimental Hypergalactosemia

One major obstacle in delineating the organ toxicity for GALT-deficiency galactosemia is the lack of an animal model that recapitulates patient symptoms. Leslie and coworkers constructed GALT-knockout mice [164,165]. When these mice were fed with a high galactose diet (40% galactose by weight), they showed mildly elevated levels of cellular

gal-1-P (~30% of the level seen in untreated human patients), galactitol and galactose. Moreover, they remained symptom-free and the female mice were fertile. The fact that these mice accumulated significant levels of gal-1-P suggested that the Leloir pathway of galactose metabolism remained the predominant route of galactose metabolism in these animals. The investigators noted that galactitol was excreted at only 10% of humans with GALT deficiency and considered the low aldose reductase to be protective of organ failure in GALT deficient mice. These authors did not consider the possibility that the target(s) of galactose toxicity in human galactosemic patients could be absent in rodents, or the likelihood that these toxicity targets in mice are less susceptible to the toxic galactose metabolites. We recently identified a human gene called ARHI (aplysia *ras* homolog I) as a new target of galactose toxicity in galactosemic patients [166]. We found that this gene was over-expressed in cultured dermal fibroblasts from patients with no GALT genes [77]. When these cells (and controls with GALT) were challenged with galactose, ARHI was increased 10-fold over a 24-hour period [166] in the GALT-deficient cells. Interestingly, over-expression of the ARHI transgene in a normal mouse model caused failure of folliculogenesis, loss of Purkinje cells in the cerebellar cortex, and stunted growth [167], all prevalent clinical complications seen in GALT-deficient patients [25]. Even more significant is that this gene is evolutionarily lost in rodents [168], which may explain why the GALT-knockout mice did not show the expected organ-specific failures [164,165].

Other investigators of Classic Galactosemia have routinely “poisoned” normal rodents or cell models with excess galactose to create ‘experimental hypergalactosemia’, and although some of these studies offered interesting insights [135,137,141,142,145,169–172], one must recognize that ‘experimental hypergalactosemia’ does not perturb any genes in the Leloir pathway. This model is reminiscent of rodent models for human type 2 diabetes mellitus by stressing with excess glucose to examine the effect of exaggerated polyol pathway activity on the target tissues as an analogy for human, organ-specific complications [33,173–180]. With normal insulin responses, these rodent models had little analogy to type 2 human diabetes [30,181,182]. Recently, ‘experimental hypergalactosemia’ has been used to study aging in wild type fruitfly *Drosophila melanogaster* [183] and age-related neuronal changes in mice [184–187], both of which suggested the involvement of oxidative stress. It is therefore, unclear to what extent that one can extrapolate the results obtained in ‘experimental hypergalactosemia’ to studies of GALT-deficiency galactosemia in humans. Fridovich-Keil’s group recently observed that loss of *Drosophila* GALT (DgalT) or GALE (DgalE) genes recapitulated significant aspects of the acute human phenotype of galactosemia (<http://crisp.cit.nih.gov>), and therefore, proposed a new *D. melanogaster* model for Classic Galactosemia. This new genetic model may help advance our understanding of the role of Leloir pathway in organ function in eukaryotes.

A GALK1-knockout mouse model for galactokinase deficiency has been constructed, but surprisingly, the GALK1-knockout mice did not form cataracts even when fed a high galactose diet [188]. Introduction of a human aldose reductase transgene into these animals resulted in cataract formation in the first postnatal day. No GALE-knockout mouse models have been reported.

Advances in Clinical Management for Galactosemia

Few advances in the clinical management of GALK, GALT and GALE deficiency have been made in recent years. GALK deficiency is detected in newborn screening programs that screen for elevation of galactose. It should be considered in such cases, as well as in cases of neonatal bilateral cataracts [35,189,190], which can occur as early as 4 weeks after birth [191]. Laboratory studies will confirm galactosuria, increased urinary galactitol [37,192]. GALK activity in erythrocytes will be low, while GALT activity in erythrocytes

will be normal. Intervention includes (ga-) lactose restriction, and cataract extraction if needed [193]. Mutation studies for GALK1 gene are clinically available.

The most important advance in treating GALT deficiency galactosemia has come from public health based newborn screening and the concept of prediction, intervention and prevention of this autosomal recessive inherited disorder. All states in the USA now screen for GALT deficiency in newborns using dried blood on filter paper either for the GALT enzyme or for total galactose concentrations (galactose plus gal-1-P). Immediate follow-up with direct measurement of gal-1-P and galactose-1-P uridylyltransferase in erythrocytes from whole blood and change of breast or milk formula to soy-based formulas have enabled prevention of liver failure, cataracts, and lethal *E. coli* sepsis. Further analysis of the GALT gene for mutations aids in developing a long term prognosis, and is clinically available. Severe mutations such as the Q188R, K285N and 5kb-deletion may still develop ataxia, POI, speech dyspraxia and osteoporosis. Recent therapies are aimed at preventing these chronic disabilities and in maintaining the gal-1-P in patients at as low a level as possible. Nutrition is important to provide adequate calories from non-galactose containing foods, to supplement with Vitamin D and Calcium to prevent osteoporosis [159], and to anticipate POI/estrogen deficiency, and provide exogenous sources if needed [152,154]. Assessment of gal-1-P concentration in erythrocytes in intervals can help determine dietary compliance. Some women with galactosemia have conceived despite having primary amenorrhea [144,149,154]. These case reports provide anecdotal evidence that ovarian pathology involves failure of follicle maturation, but the presence of immature ovarian follicles. As discussed above, most evidence supports a primary role of excess gal-1-P in the pathophysiology of galactosemia and the most exciting new approach to treatment would be to develop a non-toxic, enzyme-specific inhibitor of galactokinase to reduce gal-1-P accumulation [27,194–196]. A recent report documenting significant abnormalities on cerebral PET scans in patients with GALT deficiency may lead to further developments in imaging and management of this condition [197]. Finally, clinical management of partial GALT deficiency, especially patients with a D/G biochemical phenotype, has been a topic of interest, at least up to age 5 years [198]. Ficicioglu and coworkers, however, showed that clinical and development outcomes in D/G galactosemics are good independent of any dietary treatment [199].

Reports of patients with generalized GALE deficiency suggest a phenotype comparable to that of GALT deficiency, but also with significant infantile hypotonia, mental deficiency and sensorineural hearing loss in a few cases [78,200–205]. Acquired dysmorphic features have been described, as well as splenomegaly and contractures [204]. At least one patient was reported to have normal ovarian function [78]. GALE deficiency may be detected in newborn screening programs that screen for elevation of galactose [200,202,204]. It should be considered in such cases, as well in cases that resemble Classic Galactosemia [78,201]. Laboratory studies will confirm galactosuria; GALT activity in erythrocytes will be normal, while GALE activity will be reduced. Intervention includes (ga-)lactose restriction. Mutation studies for GALE are clinically available.

Looking to the future

Due to the relatively benign nature of GALK deficiency and the severe effects of GALT-/GALE-deficiencies, it is reasonable to assume that GALT-/GALE- deficiencies will continue to dominate the research field of galactosemia. It is also clear that early dietary treatment for GALT-deficiency fails to prevent the chronic complications and negatively affect the health-related quality of life of galactosemic patients [126,206–210]. Several of us have recognized the specific toxicity of accumulated galactose-1-phosphate in human GALT-deficiency. Therefore a rational and unique approach to therapy would be to reduce

the accumulation of gal-1-P by inhibiting the GALK enzyme with specific, non-toxic, low molecular weight compound [27,194–196]. To date, our group has screened different chemical compound libraries composed of about 50,000 small molecules with diverse structural scaffolds for their inhibitory properties against activity of purified GALK. Thus far, we have identified nearly 150 small molecules (or hits) that inhibit human GALK activity *in vitro* at the level of 86.5% or more [195]. We have selected 34 compounds for further characterization, and results so far are promising (Fig. 2).

In addition to the much-needed research for improved therapy, there is also great need to advance our understanding of the pathophysiology of this condition. Are the toxic effects of GALT-deficiency initiated *in utero* or post-natally? Are there dual effects? What are the toxicity targets of gal-1-P *in vivo*? Why are some organs more susceptible to galactose toxicity? The answers will not only dictate the treatment options, but also uncover new tissue-specific therapeutic targets.

Lastly, human GALT- and GALE- deficiencies represent natural models for the study of single gene effects on a pleiotrophic phenotype with perturbation of an essential metabolic pathway. In fact, all hypotheses concerning pathogenic mechanisms listed above implicated many other cellular processes such as inositol metabolism and protein glycosylation, further indicating the time is ripe to study human galactose metabolism and the disorders associated with it from the Systems Biology perspective [211,212].

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References

1. Acosta PB, Gross KC. Hidden sources of galactose in the environment. *Eur J Pediatr.* 1995; 154(7 Suppl 2):S87–S92. [PubMed: 7671974]
2. Berry GT, et al. The effect of dietary fruits and vegetables on urinary galactitol excretion in galactose-1-phosphate uridyltransferase deficiency. *J Inherit Metab Dis.* 1993; 16(1):91–100. [PubMed: 8487507]
3. Martin MG, et al. Defects in Na⁺/glucose cotransporter (SGLT1) trafficking and function cause glucose-galactose malabsorption. *Nat Genet.* 1996; 12(2):216–220. [PubMed: 8563765]
4. Wright EM, et al. The Na⁺/glucose cotransporter (SGLT1). *Acta Physiol Scand Suppl.* 1992; 607:201–207. [PubMed: 1449065]
5. Longo N, Elsas LJ. Human glucose transporters. *Adv Pediatr.* 1998; 45:293–313. [PubMed: 9742306]
6. Elsas LJ, Busse D, Rosenberg LE. Autosomal recessive inheritance of renal glycosuria. *Metabolism.* 1971; 20(10):968–975. [PubMed: 5097684]
7. Elsas LJ, Rosenberg LE. Familial renal glycosuria: a genetic reappraisal of hexose transport by kidney and intestine. *J Clin Invest.* 1969; 48(10):1845–1854. [PubMed: 5822589]
8. Berry GT, et al. The rate of de novo galactose synthesis in patients with galactose-1-phosphate uridyltransferase deficiency. *Mol Genet Metab.* 2004; 81(1):22–30. [PubMed: 14728988]
9. Berry GT, et al. Quantitative assessment of whole body galactose metabolism in galactosemic patients. *Eur J Pediatr.* 1997; 156 Suppl 1:S43–S49. [PubMed: 9266215]
10. Berry GT, et al. Endogenous synthesis of galactose in normal men and patients with hereditary galactosaemia. *Lancet.* 1995; 346(8982):1073–1074. [PubMed: 7564790]
11. Thoden JB, et al. Molecular structure of human galactose mutarotase. *J Biol Chem.* 2004; 279(22): 23431–23437. [PubMed: 15026423]

12. Duggleby RG, et al. Sequence differences between human muscle and liver cDNAs for UDPglucose pyrophosphorylase and kinetic properties of the recombinant enzymes expressed in *Escherichia coli*. *Eur J Biochem*. 1996; 235(1–2):173–179. [PubMed: 8631325]
13. Holden HM, Rayment I, Thoden JB. Structure and function of enzymes of the Leloir pathway for galactose metabolism. *J Biol Chem*. 2003; 278(45):43885–43888. [PubMed: 12923184]
14. Salo WL, et al. The specificity of UDP-glucose 4-epimerase from the yeast *Saccharomyces fragilis*. *Biochim Biophys Acta*. 1968; 151(2):484–492. [PubMed: 5636379]
15. Thoden JB, et al. Human UDP-galactose 4-epimerase. Accommodation of UDP-N-acetylglucosamine within the active site. *J Biol Chem*. 2001; 276(18):15131–15136. [PubMed: 11279032]
16. Parthasarathy R, Parthasarathy L, Vadnal R. Brain inositol monophosphatase identified as a galactose 1-phosphatase. *Brain Res*. 1997; 778(1):99–106. [PubMed: 9462881]
17. Leloir LF. The enzymatic transformation of uridine diphosphate glucose into a galactose derivative. *Arch Biochem*. 1951; 33(2):186–190. [PubMed: 14885999]
18. Berry, GT.; Segal, S.; Gitzelmann, R. Disorders of Galactose Metabolism. In: Fernandes, J., et al., editors. *Inborn Metabolic Diseases - Diagnosis and Treatment*. New York: Springer-Verlag; 2006.
19. Fridovich-Keil, J.; Walter, J. Chapter 72: Galactosemia. In: Valle, D., et al., editors. *Online Metabolic and Molecular Bases of Inherited Diseases-OMMBID*. New York: McGraw-Hill; 2008. www.ommbid.com
20. Fridovich-Keil JL. Galactosemia: the good, the bad, and the unknown. *J Cell Physiol*. 2006; 209(3):701–705. [PubMed: 17001680]
21. Leslie ND. Insights into the pathogenesis of galactosemia. *Annu Rev Nutr*. 2003; 23:59–80. [PubMed: 12704219]
22. Novelli G, Reichardt JK. Molecular basis of disorders of human galactose metabolism: past, present, and future. *Mol Genet Metab*. 2000; 71(1–2):62–65. [PubMed: 11001796]
23. Petry KG, Reichardt JK. The fundamental importance of human galactose metabolism: lessons from genetics and biochemistry. *Trends Genet*. 1998; 14(3):98–102. [PubMed: 9540406]
24. Segal S. The challenge of Galactosemia. *International Pediatrics*. 1993; 8(1)
25. Segal, S.; Berry, GT. Disorders of galactose metabolism. In: BA Scriver, D.; Sly, W.; Valle, D., editors. *The Metabolic Basis of Inherited Diseases*. New York: McGraw-Hill; 1995. p. 967-1000.
26. Segal S. Galactosemia unsolved. *Eur J Pediatr*. 1995; 154(7 Suppl 2):S97–S102. [PubMed: 7671976]
27. Bosch AM. Classical galactosaemia revisited. *J Inherit Metab Dis*. 2006; 29(4):516–525. [PubMed: 16838075]
28. Reichardt JK. Genetic basis of galactosemia. *Hum Mutat*. 1992; 1(3):190–196. [PubMed: 1301925]
29. Elsas, LJ. Galactosemia. In: Goldman, L.; Ausiello, D., editors. *Cecil Medicine*. Philadelphia: Saunders; 2008. p. 1555-1558.
30. Berry GT. The role of polyols in the pathophysiology of hypergalactosemia. *Eur J Pediatr*. 1995; 154(7 Suppl 2):S53–S64. [PubMed: 7671966]
31. Cogan DG, et al. NIH conference. Aldose reductase and complications of diabetes. *Ann Intern Med*. 1984; 101(1):82–91. [PubMed: 6203454]
32. Engerman RL. Pathogenesis of diabetic retinopathy. *Diabetes*. 1989; 38(10):1203–1206. [PubMed: 2676655]
33. Engerman RL, Kern TS. Hyperglycemia and development of glomerular pathology: diabetes compared with galactosemia. *Kidney Int*. 1989; 36(1):41–45. [PubMed: 2811056]
34. Kador PF, Kinoshita JH. Diabetic and galactosaemic cataracts. *Ciba Found Symp*. 1984; 106:110–131. [PubMed: 6439498]
35. Bosch AM, et al. Clinical features of galactokinase deficiency: a review of the literature. *J Inherit Metab Dis*. 2002; 25(8):629–634. [PubMed: 12705493]
36. Gitzelmann R, Wells HJ, Segal S. Galactose metabolism in a patient with hereditary galactokinase deficiency. *Eur J Clin Invest*. 1974; 4(2):79–84. [PubMed: 4365005]

37. Gitzelmann R. Deficiency of erythrocyte galactokinase in a patient with galactose diabetes. *Lancet*. 1965; 2(7414):670–671. [PubMed: 4158218]
38. Goppert F. Galaktosurie nach Milchzuckergabe bei angeborenem, familiaerem chronischem Leberleiden. *Klin. Wschr*. 1917; 54:473–477.
39. Mason H, Turne M. Chronic galactosemia: report of case with studies on carbohydrates. *Am. J. Dis. Child*. 1935; 50:359–374.
40. Waggoner DD, Buist NRM, Donnell GN. Long-term prognosis in Galactosemia: results of a survey of 350 cases. *J Inherit Metab Dis*. 1990; 13:802–818. [PubMed: 1706789]
41. Waggoner D, Buist NRM. Long-term complications in treated galactosemia - 175 U.S. cases. *International Pediatrics*. 1993; 8:97–100.
42. Openo KK, et al. Epimerase-deficiency galactosemia is not a binary condition. *Am J Hum Genet*. 2006; 78(1):89–102. [PubMed: 16385452]
43. Gitzelmann R. Deficiency of uridine diphosphate galactose 4-epimerase in blood cells of an apparently healthy infant. Preliminary communication. *Helv Paediatr Acta*. 1972; 27(2):125–130. [PubMed: 4644860]
44. Kalckar HM. Galactose metabolism and cell "sociology". *Science*. 1965; 150(694):305–313. [PubMed: 5319435]
45. Bergsma DJ, et al. Fine structure of the human galactokinase GALK1 gene. *Genome Res*. 1996; 6(10):980–985. [PubMed: 8908517]
46. Leslie ND, et al. The human galactose-1-phosphate uridylyltransferase gene. *Genomics*. 1992; 14(2):474–480. [PubMed: 1427861]
47. Maceratesi P, et al. Human UDP-galactose 4' epimerase (GALE) gene and identification of five missense mutations in patients with epimerase-deficiency galactosemia. *Mol Genet Metab*. 1998; 63(1):26–30. [PubMed: 9538513]
48. Timson DJ, Reece RJ. Functional analysis of disease-causing mutations in human galactokinase. *Eur J Biochem*. 2003; 270(8):1767–1774. [PubMed: 12694189]
49. Reichardt JK, Packman S, Woo SL. Molecular characterization of two galactosemia mutations: correlation of mutations with highly conserved domains in galactose-1-phosphate uridylyl transferase. *Am J Hum Genet*. 1991; 49(4):860–867. [PubMed: 1897530]
50. Johnston M. A model fungal gene regulatory mechanism: the GAL genes of *Saccharomyces cerevisiae*. *Microbiol Rev*. 1987; 51(4):458–476. [PubMed: 2830478]
51. Timson DJ, Ross HC, Reece RJ. Gal3p and Gal1p interact with the transcriptional repressor Gal80p to form a complex of 1:1 stoichiometry. *Biochem J*. 2002; 363(Pt 3):515–520. [PubMed: 11964151]
52. Platt A, Reece RJ. The yeast galactose genetic switch is mediated by the formation of a Gal4p-Gal80p-Gal3p complex. *Embo J*. 1998; 17(14):4086–4091. [PubMed: 9670023]
53. Platt A, et al. The insertion of two amino acids into a transcriptional inducer converts it into a galactokinase. *Proc Natl Acad Sci U S A*. 2000; 97(7):3154–3159. [PubMed: 10737789]
54. Sellick CA, Campbell RN, Reece RJ. Galactose metabolism in yeast-structure and regulation of the leloir pathway enzymes and the genes encoding them. *Int Rev Cell Mol Biol*. 2008; 269:111–150. [PubMed: 18779058]
55. Elsas LJ, et al. Functional analysis of the human galactose-1-phosphate uridylyltransferase promoter in Duarte and LA variant galactosemia. *Mol Genet Metab*. 2001; 72(4):297–305. [PubMed: 11286503]
56. Park HD, et al. A novel c.-22T>C mutation in GALK1 promoter is associated with elevated galactokinase phenotype. *BMC Med Genet*. 2009; 10:29. [PubMed: 19309526]
57. Bertoli D, Segal S. Developmental aspects and some characteristics of mammalian galactose 1-phosphate uridylyltransferase. *J Biol Chem*. 1966; 241(17):4023–4029. [PubMed: 5920808]
58. Heidenreich RA. Regulation of galactose-1-phosphate uridylyltransferase gene expression. *Eur J Pediatr*. 1995; 154(7 Suppl 2):S28–S32. [PubMed: 7671960]
59. Heidenreich RA, et al. Developmental and tissue-specific modulation of rat galactose-1-phosphate uridylyltransferase steady state messenger RNA and specific activity levels. *Pediatr Res*. 1993; 34(4):416–419. [PubMed: 8255669]

60. Denude N, et al. Expression of galactose-1-phosphate uridylyltransferase in the anterior pituitary of rat during the estrous cycle. *Neuroendocrinology*. 1996; 64(1):42–48. [PubMed: 8811665]
61. Thoden JB, et al. Molecular structure of human galactokinase: implications for type II galactosemia. *J Biol Chem*. 2005; 280(10):9662–9670. [PubMed: 15590630]
62. Thoden JB, et al. Crystallographic evidence for Tyr 157 functioning as the active site base in human UDP-galactose 4-epimerase. *Biochemistry*. 2000; 39(19):5691–5701. [PubMed: 10801319]
63. Wedekind JE, Frey PA, Rayment I. Three-dimensional structure of galactose-1-phosphate uridylyltransferase from *Escherichia coli* at 1.8 Å resolution. *Biochemistry*. 1995; 34(35):11049–11061. [PubMed: 7669762]
64. Elsevier JP, Fridovich-Keil JL. The Q188R mutation in human galactose-1-phosphate uridylyltransferase acts as a partial dominant negative. *J Biol Chem*. 1996; 271(50):32002–32007. [PubMed: 8943248]
65. Elsevier JP, et al. Heterodimer formation and activity in the human enzyme galactose-1-phosphate uridylyltransferase. *Proc Natl Acad Sci U S A*. 1996; 93(14):7166–7171. [PubMed: 8692963]
66. Fridovich-Keil JL, Jinks-Robertson S. A yeast expression system for human galactose-1-phosphate uridylyltransferase. *Proc Natl Acad Sci U S A*. 1993; 90(2):398–402. [PubMed: 8421669]
67. Fridovich-Keil JL, et al. Identification and functional analysis of three distinct mutations in the human galactose-1-phosphate uridylyltransferase gene associated with galactosemia in a single family. *Am J Hum Genet*. 1995; 56(3):640–646. [PubMed: 7887417]
68. Lai K, Willis AC, Elsas LJ. The biochemical role of glutamine 188 in human galactose-1-phosphate uridylyltransferase. *J Biol Chem*. 1999; 274(10):6559–6566. [PubMed: 10037750]
69. Quimby BB, et al. Functional requirements of the active site position 185 in the human enzyme galactose-1-phosphate uridylyltransferase. *J Biol Chem*. 1996; 271(43):26835–26842. [PubMed: 8900165]
70. Marabotti A, Facchiano AM. Homology modeling studies on human galactose-1-phosphate uridylyltransferase and on its galactosemia-related mutant Q188R provide an explanation of molecular effects of the mutation on homo- and heterodimers. *J Med Chem*. 2005; 48(3):773–779. [PubMed: 15689161]
71. Elsas LJ, et al. Galactosemia: a strategy to identify new biochemical phenotypes and molecular genotypes. *Am J Hum Genet*. 1995; 56(3):630–639. [PubMed: 7887416]
72. Guerrero NV, et al. Risk factors for premature ovarian failure in females with galactosemia. *J Pediatr*. 2000; 137(6):833–841. [PubMed: 11113841]
73. Webb AL, et al. Verbal dyspraxia and galactosemia. *Pediatr Res*. 2003; 53(3):396–402. [PubMed: 12595586]
74. Lai K, et al. A prevalent mutation for galactosemia among black Americans. *J Pediatr*. 1996; 128(1):89–95. [PubMed: 8551426]
75. Landt M, et al. Black children deficient in galactose 1-phosphate uridylyltransferase: correlation of activity and immunoreactive protein in erythrocytes and leukocytes. *J Pediatr*. 1997; 130(6):972–980. [PubMed: 9202622]
76. Lai K, Elsas LJ. Structure-function analyses of a common mutation in blacks with transferase-deficiency galactosemia. *Mol Genet Metab*. 2001; 74(1–2):264–272. [PubMed: 11592823]
77. Coffee B, et al. Characterization of an unusual deletion of the galactose-1-phosphate uridylyltransferase (GALT) gene. *Genet Med*. 2006; 8(10):635–640. [PubMed: 17079880]
78. Wohlers TM, et al. Identification and characterization of a mutation, in the human UDP-galactose-4-epimerase gene, associated with generalized epimerase-deficiency galactosemia. *Am J Hum Genet*. 1999; 64(2):462–470. [PubMed: 9973283]
79. Chhay JS, et al. Analysis of UDP-galactose 4'-epimerase mutations associated with the intermediate form of type III galactosaemia. *J Inher Metab Dis*. 2008; 31(1):108–116. [PubMed: 18188677]
80. Sangiuolo F, et al. Biochemical characterization of two GALK1 mutations in patients with galactokinase deficiency. *Hum Mutat*. 2004; 23(4):396. [PubMed: 15024738]
81. Okano Y, et al. A genetic factor for age-related cataract: identification and characterization of a novel galactokinase variant, "Osaka," in Asians. *Am J Hum Genet*. 2001; 68(4):1036–1042. [PubMed: 11231902]

82. Kalaydjieva L, et al. A founder mutation in the GK1 gene is responsible for galactokinase deficiency in Roma (Gypsies). *Am J Hum Genet.* 1999; 65(5):1299–1307. [PubMed: 10521295]
83. Donnell GN, et al. Galactose-1-phosphate in galactosemia. *Pediatrics.* 1963; 31:802–810. [PubMed: 14028648]
84. Gitzelmann R. Galactose-1-phosphate in the pathophysiology of galactosemia. *Eur J Pediatr.* 1995; 154(7 Suppl 2):S45–S49. [PubMed: 7671964]
85. Siegel CD, Sparks JW, Battaglia FC. Patterns of serum glucose and galactose concentrations in term newborn infants after milk feeding. *Biol Neonate.* 1988; 54(6):301–306. [PubMed: 3228558]
86. Douglas HC, Hawthorne DC. Enzymatic Expression And Genetic Linkage Of Genes Controlling Galactose Utilization In *Saccharomyces*. *Genetics.* 1964; 49:837–844. [PubMed: 14158615]
87. Ross KL, Davis CN, Fridovich-Keil JL. Differential roles of the Leloir pathway enzymes and metabolites in defining galactose sensitivity in yeast. *Mol Genet Metab.* 2004; 83(1–2):103–116. [PubMed: 15464425]
88. Slepak T, et al. Intracellular galactose-1-phosphate accumulation leads to environmental stress response in yeast model. *Mol Genet Metab.* 2005; 86(3):360–371. [PubMed: 16169270]
89. de Jongh WA, et al. The roles of galactitol, galactose-1-phosphate, and phosphoglucomutase in galactose-induced toxicity in *Saccharomyces cerevisiae*. *Biotechnol Bioeng.* 2008
90. Schwarz V, et al. Some disturbances of erythrocyte metabolism in galactosaemia. *Biochem J.* 1956; 62(1):34–40. [PubMed: 13293149]
91. Maddaiah VT, Madsen NB. Kinetics of purified liver phosphorylase. *J Biol Chem.* 1966; 241(17):3873–3881. [PubMed: 5920799]
92. Lai K, Elsas LJ. Overexpression of human UDP-glucose pyrophosphorylase rescues galactose-1-phosphate uridylyltransferase-deficient yeast. *Biochem Biophys Res Commun.* 2000; 271(2):392–400. [PubMed: 10799308]
93. Lai K, et al. GALT deficiency causes UDP-hexose deficit in human galactosemic cells. *Glycobiology.* 2003; 13(4):285–294. [PubMed: 12626383]
94. Oliver IT. Inhibitor studies on uridine diphosphoglucose pyrophosphorylase. *Biochim Biophys Acta.* 1961; 52:75–81. [PubMed: 14481810]
95. Mehta DV, Kabir A, Bhat PJ. Expression of human inositol monophosphatase suppresses galactose toxicity in *Saccharomyces cerevisiae*: possible implications in galactosemia. *Biochim Biophys Acta.* 1999; 1454(3):217–226. [PubMed: 10452956]
96. Bhat PJ. Galactose-1-phosphate is a regulator of inositol monophosphatase: a fact or a fiction? *Med Hypotheses.* 2003; 60(1):123–128. [PubMed: 12450779]
97. Pourci ML, et al. Culture of galactosaemic fibroblasts in the presence of galactose: effect of inosine. *J Inher Metab Dis.* 1990; 13(6):819–828. [PubMed: 2079832]
98. Gitzelmann R. Formation of galactose-1-phosphate from uridine diphosphate galactose in erythrocytes from patients with galactosemia. *Pediatr Res.* 1969; 3(4):279–286. [PubMed: 5807057]
99. Dvornik E, et al. Polyol accumulation in galactosemic and diabetic rats: control by an aldose reductase inhibitor. *Science.* 1973; 182(117):1146–1148. [PubMed: 4270794]
100. Kinoshita JH, et al. The effect of an aldose reductase inhibitor on the galactose-exposed rabbit lens. *Biochim Biophys Acta.* 1968; 158(3):472–475. [PubMed: 5660111]
101. Kinoshita JH, Merola LO, Dikmak E. Osmotic changes in experimental galactose cataracts. *Exp Eye Res.* 1962; 1:405–410. [PubMed: 14032902]
102. Kubo E, et al. Cataract formation through the polyol pathway is associated with free radical production. *Exp Eye Res.* 1999; 68(4):457–464. [PubMed: 10192803]
103. Kubo E, et al. Polyol pathway-dependent osmotic and oxidative stresses in aldose reductase-mediated apoptosis in human lens epithelial cells: role of AOP2. *Biochem Biophys Res Commun.* 2004; 314(4):1050–1056. [PubMed: 14751239]
104. Berry GT, et al. Elevation of erythrocyte redox potential linked to galactonate biosynthesis: elimination by Tolrestat. *Metabolism.* 1998; 47(11):1423–1428. [PubMed: 9826225]
105. Berry GT, et al. In vivo evidence of brain galactitol accumulation in an infant with galactosemia and encephalopathy. *J Pediatr.* 2001; 138(2):260–262. [PubMed: 11174626]

106. Wang ZJ, et al. Proton magnetic resonance spectroscopy of brain metabolites in galactosemia. *Ann Neurol.* 2001; 50(2):266–269. [PubMed: 11506413]
107. Ng WG, et al. Deficit of uridine diphosphate galactose in galactosaemia. *J Inherit Metab Dis.* 1989; 12(3):257–266. [PubMed: 2515367]
108. Charlwood J, et al. Defective galactosylation of serum transferrin in galactosemia. *Glycobiology.* 1998; 8(4):351–357. [PubMed: 9499382]
109. Dobbie JA, Holton JB, Clamp JR. Defective galactosylation of proteins in cultured skin fibroblasts from galactosaemic patients. *Ann Clin Biochem.* 1990; 27(Pt 3):274–275. [PubMed: 2382964]
110. Jaeken J, Kint J, Spaepen L. Serum lysosomal enzyme abnormalities in galactosaemia. *Lancet.* 1992; 340(8833):1472–1473. [PubMed: 1360589]
111. Prestoz LL, et al. Altered follicle stimulating hormone isoforms in female galactosaemia patients. *Eur J Pediatr.* 1997; 156(2):116–120. [PubMed: 9039515]
112. Sturiale L, et al. Hypoglycosylation with increased fucosylation and branching of serum transferrin N-glycans in untreated galactosemia. *Glycobiology.* 2005
113. Hara T, et al. The UDP-galactose translocator gene is mapped to band Xp11.23–p11.22 containing the Wiskott-Aldrich syndrome locus. *Somat Cell Mol Genet.* 1993; 19(6):571–575. [PubMed: 8128316]
114. Miura N, et al. Human UDP-galactose translocator: molecular cloning of a complementary DNA that complements the genetic defect of a mutant cell line deficient in UDP-galactose translocator. *J Biochem.* 1996; 120(2):236–241. [PubMed: 8889805]
115. Wells WW, et al. The Isolation And Identification Of Galactitol From The Brains Of Galactosemia Patients. *J Biol Chem.* 1965; 240:1002–1004. [PubMed: 14284692]
116. Quan-Ma R, Wells WW. The distribution of galactitol in tissues of rats fed galactose. *Biochem Biophys Res Commun.* 1965; 20(4):486–490. [PubMed: 5860165]
117. Slepak TI, et al. Involvement of endoplasmic reticulum stress in a novel Classic Galactosemia model. *Mol Genet Metab.* 2007; 92(1–2):78–87. [PubMed: 17643331]
118. Ng WG, et al. Measurements of uridine diphosphate hexoses in galactosemia. *J Pediatr.* 1993; 123(6):1015–1016. [PubMed: 8229509]
119. Yasuda J, et al. Reactive oxygen species modify oligosaccharides of glycoproteins in vivo: a study of a spontaneous acute hepatitis model rat (LEC rat). *Biochem Biophys Res Commun.* 2006; 342(1):127–134. [PubMed: 16480686]
120. Gitzelmann R. [Screening of newborns for inborn errors of galactose metabolism. Methods and results]. *Monatsschr Kinderheilkd.* 1976; 129(9):654–657. [PubMed: 185513]
121. Kaye CI, et al. Newborn screening fact sheets. *Pediatrics.* 2006; 118(3):e934–e963. [PubMed: 16950973]
122. Friedman JH, Levy HL, Boustany RM. Late onset of distinct neurologic syndromes in galactosemic siblings. *Neurology.* 1989; 39(5):741–742. [PubMed: 2710367]
123. Haberland C, et al. The neuropathology of galactosemia. A histopathological and biochemical study. *J Neuropathol Exp Neurol.* 1971; 30(3):431–447. [PubMed: 4105426]
124. Lo W, et al. Curious neurologic sequelae in galactosemia. *Pediatrics.* 1984; 73(3):309–312. [PubMed: 6701054]
125. Nelson CD, et al. Verbal dyspraxia in treated galactosemia. *Pediatrics.* 1991; 88(2):346–350. [PubMed: 1861938]
126. Ridel KR, Leslie ND, Gilbert DL. An updated review of the long-term neurological effects of galactosemia. *Pediatr Neurol.* 2005; 33(3):153–161. [PubMed: 16087312]
127. Kaufman FR, et al. Abnormal somatosensory evoked potentials in patients with classic galactosemia: correlation with neurologic outcome. *J Child Neurol.* 1995; 10(1):32–36. [PubMed: 7769175]
128. Forges T, Monnier-Barbarino P. [Premature ovarian failure in galactosaemia: pathophysiology and clinical management]. *Pathol Biol (Paris).* 2003; 51(1):47–56. [PubMed: 12628293]
129. Forges T, et al. Pathophysiology of impaired ovarian function in galactosaemia. *Hum Reprod Update.* 2006; 12(5):573–584. [PubMed: 16835432]

130. Kaufman FR, Donnell GN, Lobo RA. Ovarian androgen secretion in patients with galactosemia and premature ovarian failure. *Fertil Steril.* 1987; 47(6):1033–1034. [PubMed: 2954857]
131. Kaufman FR, et al. Hypergonadotropic hypogonadism in female patients with galactosemia. *N Engl J Med.* 1981; 304(17):994–998. [PubMed: 6782485]
132. Kaufman FR, et al. Correlation of cognitive, neurologic, and ovarian outcome with the Q188R mutation of the galactose-1-phosphate uridylyltransferase gene. *J Pediatr.* 1994; 125(2):225–227. [PubMed: 8040766]
133. Kaufman FR, et al. Correlation of ovarian function with galactose-1-phosphate uridylyl transferase levels in galactosemia. *J Pediatr.* 1988; 112(5):754–756. [PubMed: 2834527]
134. Kaufman FR, et al. Gonadal function and ovarian galactose metabolism in classic galactosemia. *Acta Endocrinol (Copenh).* 1989; 120(2):129–133. [PubMed: 2492704]
135. Lai KW, et al. Inhibitor of apoptosis proteins and ovarian dysfunction in galactosemic rats. *Cell Tissue Res.* 2003; 311(3):417–425. [PubMed: 12658449]
136. Liu G, Hale GE, Hughes CL. Galactose metabolism and ovarian toxicity. *Reprod Toxicol.* 2000; 14(5):377–384. [PubMed: 11020650]
137. Liu G, et al. Dietary galactose inhibits GDF-9 mediated follicular development in the rat ovary. *Reprod Toxicol.* 2005
138. Meyer WR, et al. Aldose reductase inhibition prevents galactose-induced ovarian dysfunction in the Sprague-Dawley rat. *Am J Obstet Gynecol.* 1992; 167(6):1837–1843. [PubMed: 1471707]
139. Odievre MH, Labrune P, Odievre M. [Hypergonadotrophic hypogonadism and congenital galactosemia]. *Arch Pediatr.* 2008; 15(6):1124–1125. [PubMed: 18400478]
140. Xu YK, et al. Galactose metabolism in human ovarian tissue. *Pediatr Res.* 1989; 25(2):151–155. [PubMed: 2919130]
141. Bandyopadhyay S, et al. Prenatal exposure to high galactose adversely affects initial gonadal pool of germ cells in rats. *Hum Reprod.* 2003; 18(2):276–282. [PubMed: 12571162]
142. Bandyopadhyay S, et al. Galactose toxicity in the rat as a model for premature ovarian failure: an experimental approach readdressed. *Hum Reprod.* 2003; 18(10):2031–2038. [PubMed: 14507817]
143. Berry GT. Galactosemia and amenorrhea in the adolescent. *Ann N Y Acad Sci.* 2008; 1135:112–117. [PubMed: 18574215]
144. Briones P, Giros M, Martinez V. Second spontaneous pregnancy in a galactosaemic woman homozygous for the Q188R mutation. *J Inherit Metab Dis.* 2001; 24(1):79–80. [PubMed: 11286387]
145. Chen YT, et al. Reduction in oocyte number following prenatal exposure to a diet high in galactose. *Science.* 1981; 214(4525):1145–1147. [PubMed: 7302587]
146. Fazel AR, Schulte BA, Spicer SS. Glycoconjugate unique to migrating primordial germ cells differs with genera. *Anat Rec.* 1990; 228(2):177–184. [PubMed: 2240610]
147. Fazel AR, et al. Presence of a unique glycoconjugate on the surface of rat primordial germ cells during migration. *Cell Differ.* 1987; 21(3):199–211. [PubMed: 3652214]
148. Gibson JB. Gonadal function in galactosemics and in galactose-intoxicated animals. *Eur J Pediatr.* 1995; 154(7 Suppl 2):S14–S20. [PubMed: 7671957]
149. Gubbels CS, et al. Pregnancy in classic galactosemia despite undetectable anti-Mullerian hormone. *Fertil Steril.* 2009; 91(4):1293, e13–e16. [PubMed: 19200962]
150. Gubbels CS, Land JA, Rubio-Gozalbo ME. Fertility and impact of pregnancies on the mother and child in classic galactosemia. *Obstet Gynecol Surv.* 2008; 63(5):334–343. [PubMed: 18419833]
151. Rubio-Gozalbo ME, et al. The endocrine system in treated patients with classical galactosemia. *Mol Genet Metab.* 2006; 89(4):316–322. [PubMed: 16935538]
152. Sanders RD, et al. Biomarkers of ovarian function in girls and women with classic galactosemia. *Fertil Steril.* 2008
153. Schadowaldt P, et al. Biochemical monitoring of pregnancy and breast feeding in five patients with classical galactosaemia--and review of the literature. *Eur J Pediatr.* 2009; 168(6):721–729. [PubMed: 18813948]

154. Menezo YJ, et al. Pregnancy and delivery after stimulation with rFSH of a galactosemia patient suffering hypergonadotropic hypogonadism: case report. *J Assist Reprod Genet.* 2004; 21(3):89–90. [PubMed: 15202737]
155. Fernandez Espuelas C, et al. [Bone mineral turnover and bone densitometry in patients with a high-risk diet: hyperphenylalaninemia and galactosemia]. *An Pediatr (Barc).* 2005; 63(3):224–229. [PubMed: 16219275]
156. Gajewska J, et al. Serum markers of bone turnover in children and adolescents with classic galactosemia. *Adv Med Sci.* 2008; 53(2):214–220. [PubMed: 18650146]
157. Gajewska J, et al. Bone turnover markers in prepubertal children with classical galactosemia. *Indian J Gastroenterol.* 2006; 25(4):221–222. [PubMed: 16974051]
158. Moreno Villares JM, Oliveros Leal L. [Bone mineral turnover and bone densitometry in patients with dietary risk: hyperphenylalaninemia and galactosemia]. *An Pediatr (Barc).* 2006; 64(3):284. author reply 284–5. [PubMed: 16527101]
159. Panis B, et al. Bone metabolism in galactosemia. *Bone.* 2004; 35(4):982–987. [PubMed: 15454106]
160. Kaufman FR, et al. Effect of hypogonadism and deficient calcium intake on bone density in patients with galactosemia. *J Pediatr.* 1993; 123(3):365–370. [PubMed: 8355111]
161. Bresolin N, et al. Clinical and biochemical evidence of skeletal muscle involvement in galactose-1-phosphate uridyl transferase deficiency. *J Neurol.* 1993; 240(5):272–277. [PubMed: 8326330]
162. Ninfali P, et al. Molecular basis of galactose-1-phosphate uridyltransferase deficiency involving skeletal muscle. *J Neurol.* 1996; 243(1):102–103. [PubMed: 8869397]
163. Levy HL, et al. Ovarian failure in galactosemia. *N Engl J Med.* 1984; 310(1):50. [PubMed: 6689742]
164. Leslie ND, et al. A mouse model of galactose-1-phosphate uridyl transferase deficiency. *Biochem Mol Med.* 1996; 59(1):7–12. [PubMed: 8902187]
165. Ning C, et al. Galactose metabolism by the mouse with galactose-1-phosphate uridyltransferase deficiency. *Pediatr Res.* 2000; 48(2):211–217. [PubMed: 10926297]
166. Lai K, et al. ARHI: A new target of galactose toxicity in Classic Galactosemia. *Biosci Hypotheses.* 2008; 1(5):263–271. [PubMed: 19122833]
167. Xu F, et al. The human ARHI tumor suppressor gene inhibits lactation and growth in transgenic mice. *Cancer Res.* 2000; 60(17):4913–4920. [PubMed: 10987306]
168. Fitzgerald J, Bateman JF. Why mice have lost genes for COL21A1, STK17A, GPR145 and AHRI: evidence for gene deletion at evolutionary breakpoints in the rodent lineage. *Trends Genet.* 2004; 20(9):408–412. [PubMed: 15313548]
169. Haworth JC, Ford JD, Younoszai MK. Effect of galactose toxicity on growth of the rat fetus and brain. *Pediatr Res.* 1969; 3(5):441–447. [PubMed: 5343540]
170. Litchfield WJ, Wells WW. Effect of galactose on free radical reactions of polymorphonuclear leukocytes. *Arch Biochem Biophys.* 1978; 188(1):26–30. [PubMed: 209746]
171. Rosenberg LE, Weinberg AN, Segal S. The effect of high galactose diets on urinary excretion of amino acids in the rat. *Biochim Biophys Acta.* 1961; 48:500–505. [PubMed: 13743394]
172. Tsakiris S, et al. Protective effect of L-cysteine and glutathione on the modulated suckling rat brain Na⁺, K⁺, -ATPase and Mg²⁺ -ATPase activities induced by the in vitro galactosaemia. *Pharmacol Res.* 2004; 49(5):475–479. [PubMed: 14998558]
173. Unakar NJ, et al. In utero and milk-mediated effect of aldose reductase inhibitor on galactose cataracts. *Exp Eye Res.* 1991; 53(5):665–676. [PubMed: 1743265]
174. Engerman RL, Kern TS. Experimental galactosemia produces diabetic-like retinopathy. *Diabetes.* 1984; 33(1):97–100. [PubMed: 6360771]
175. Engerman RL, Kern TS. Hyperglycemia as a cause of diabetic retinopathy. *Metabolism.* 1986; 35(4 Suppl 1):20–23. [PubMed: 3083205]
176. Kern TS, Engerman RL. Galactose-induced retinal microangiopathy in rats. *Invest Ophthalmol Vis Sci.* 1995; 36(2):490–496. [PubMed: 7843917]

177. Kern TS, Engerman RL. Kidney morphology in experimental hyperglycemia. *Diabetes*. 1987; 36(2):244–249. [PubMed: 3803734]
178. Kern TS, Engerman RL. Microvascular metabolism in diabetes. *Metabolism*. 1986; 35(4 Suppl 1):24–27. [PubMed: 3083206]
179. Kern TS, Engerman RL. Renal hemodynamics in experimentally galactosemic dogs and diabetic dogs. *Metabolism*. 1991; 40(5):450–454. [PubMed: 1902544]
180. Kern TS, Engerman RL. Retinal polyol and myo-inositol in galactosemic dogs given an aldose-reductase inhibitor. *Invest Ophthalmol Vis Sci*. 1991; 32(13):3175–3177. [PubMed: 1748548]
181. Sato S, Kador PF. NADPH-dependent reductases of the dog lens. *Exp Eye Res*. 1990; 50(6):629–634. [PubMed: 2115452]
182. Yagihashi S, et al. Galactosemic neuropathy in transgenic mice for human aldose reductase. *Diabetes*. 1996; 45(1):56–59. [PubMed: 8522060]
183. Cui X, et al. D-galactose-caused life shortening in *Drosophila melanogaster* and *Musca domestica* is associated with oxidative stress. *Biogerontology*. 2004; 5(5):317–325. [PubMed: 15547319]
184. Zhang Q, et al. GM1 ganglioside prevented the decline of hippocampal neurogenesis associated with D-galactose. *Neuroreport*. 2005; 16(12):1297–1301. [PubMed: 16056128]
185. Zhang Q, et al. D-galactose injured neurogenesis in the hippocampus of adult mice. *Neurol Res*. 2005; 27(5):552–556. [PubMed: 15978184]
186. He M, et al. Neuroprotective effects of (–)-epigallocatechin-3-gallate on aging mice induced by D-galactose. *Biol Pharm Bull*. 2009; 32(1):55–60. [PubMed: 19122281]
187. Wei H, et al. Temporal gene expression profile in hippocampus of mice treated with D-galactose. *Cell Mol Neurobiol*. 2008; 28(5):781–794. [PubMed: 17710534]
188. Ai Y, et al. A mouse model of galactose-induced cataracts. *Hum Mol Genet*. 2000; 9(12):1821–1827. [PubMed: 10915771]
189. Beutler E, et al. Galactokinase deficiency as a cause of cataracts. *N Engl J Med*. 1973; 288(23):1203–1206. [PubMed: 4700553]
190. Levy NS, Krill AE, Beutler E. Galactokinase deficiency and cataracts. *Am J Ophthalmol*. 1972; 74(1):41–48. [PubMed: 5036447]
191. Kerr MM, et al. Galactokinase deficiency in a newborn infant. *Arch Dis Child*. 1971; 46(250):864–866. [PubMed: 5129188]
192. Gitzelmann R. Letter: Additional findings in galactokinase deficiency. *J Pediatr*. 1975; 87(6 Pt 1):1007–1008. [PubMed: 171359]
193. Stambolian D. Galactose and cataract. *Surv Ophthalmol*. 1988; 32(5):333–349. [PubMed: 3043741]
194. Timson DJ. GHMP Kinases - Structures, Mechanisms and Potential for Therapeutically Relevant Inhibition. *Current Enzyme Inhibition*. 2007; 3(1):77–94.
195. Wierenga KJ, et al. High-throughput screening for human galactokinase inhibitors. *J Biomol Screen*. 2008; 13(5):415–423. [PubMed: 18490662]
196. Fridovich-Keil, J. Toward Improved Intervention for Classic Galactosemia. 2007. http://www.galactosemia.org/PGC_awards.asp
197. Dubroff JG, et al. FDG-PET findings in patients with galactosaemia. *J Inherit Metab Dis*. 2008
198. Gitzelmann R, Bosshard NU. Partial deficiency of galactose-1-phosphate uridylyltransferase. *Eur J Pediatr*. 1995; 154(7 Suppl 2):S40–S44. [PubMed: 7671963]
199. Ficicioglu C, et al. Duarte (DG) galactosemia: a pilot study of biochemical and neurodevelopmental assessment in children detected by newborn screening. *Mol Genet Metab*. 2008; 95(4):206–212. [PubMed: 18976948]
200. Alano A, et al. Molecular characterization of a unique patient with epimerase-deficiency galactosaemia. *J Inherit Metab Dis*. 1998; 21(4):341–350. [PubMed: 9700591]
201. Henderson MJ, Holton JB, MacFaul R. Further observations in a case of uridine diphosphate galactose-4-epimerase deficiency with a severe clinical presentation. *J Inherit Metab Dis*. 1983; 6(1):17–20. [PubMed: 6408303]
202. Holton JB, et al. Galactosaemia: a new severe variant due to uridine diphosphate galactose-4-epimerase deficiency. *Arch Dis Child*. 1981; 56(11):885–887. [PubMed: 7305435]

203. Quimby BB, et al. Characterization of two mutations associated with epimerase-deficiency galactosemia, by use of a yeast expression system for human UDP-galactose-4-epimerase. *Am J Hum Genet.* 1997; 61(3):590–598. [PubMed: 9326324]
204. Walter JH, et al. Generalised uridine diphosphate galactose-4-epimerase deficiency. *Arch Dis Child.* 1999; 80(4):374–376. [PubMed: 10086948]
205. Sardharwalla IB, et al. A patient with severe type of epimerase deficiency galactosaemia. *J Inherit Metab Dis.* 1988; 11 Suppl 2:249–51. [PubMed: 3141714]
206. Antshel KM, Epstein IO, Waisbren SE. Cognitive strengths and weaknesses in children and adolescents homozygous for the galactosemia Q188R mutation: a descriptive study. *Neuropsychology.* 2004; 18(4):658–664. [PubMed: 15506833]
207. Bosch AM, et al. Living with classical galactosemia: health-related quality of life consequences. *Pediatrics.* 2004; 113(5):e423–e428. [PubMed: 15121984]
208. Lambert C, Boneh A. The impact of galactosaemia on quality of life--a pilot study. *J Inherit Metab Dis.* 2004; 27(5):601–608. [PubMed: 15669675]
209. Waisbren SE, et al. Effect of expanded newborn screening for biochemical genetic disorders on child outcomes and parental stress. *Jama.* 2003; 290(19):2564–2572. [PubMed: 14625333]
210. Waisbren SE, et al. Brief report: Predictors of parenting stress among parents of children with biochemical genetic disorders. *J Pediatr Psychol.* 2004; 29(7):565–570. [PubMed: 15347704]
211. Lai K, Klapa MI. Alternative pathways of galactose assimilation: could inverse metabolic engineering provide an alternative to galactosemic patients? *Metab Eng.* 2004; 6(3):239–244. [PubMed: 15256214]
212. Ideker T, et al. Integrated genomic and proteomic analyses of a systematically perturbed metabolic network. *Science.* 2001; 292(5518):929–934. [PubMed: 11340206]

Fig. 1. The galactose metabolic pathway is linked to uridine nucleosides and inositol metabolism [166]

Galactose metabolism is linked to UDP-glucose, UDP-galactose and inositol biosyntheses and to post-translational control of protein glycosylation and galactosylation.

Fig. 2. Thirty-four selected human GALK inhibitors [195]