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MINIREVIEW

Molecular Basis of Disorders of Human Galactose Metabolism: Past, Present, and Future

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Molecular cloning and characterization of all three human galactose-metabolic genes have led to the identification of a number of mutations which result in three forms of galactosemia which are caused by kinase (GALK), transferase (GALT), or epimerase (GALE) deficiency. We review here recent developments in the molecular characterization of all three disorders of human galactose metabolism. Recent progress in the biochemical and/or structural analyses of the GALT and GALE proteins has complemented human mutational studies. Interestingly, genotype/phenotype correlations have been modest as in some other Mendelian disorders. We discuss possible reasons for this apparent paradox. Finally, we note the panethnic nature of galactosemia and suggest a hypothesis for it. © 2000 Academic Press

Key Words: **mutation analysis; structure/function correlations; genotype/phenotype correlations; Mendelian disorder; heterozygote advantage; galactosemia.**

Galactose metabolism is universally carried out in three enzymatic steps that are catalyzed sequentially by the enzymes galactokinase (EC 2.7.1.6; encoded by the *GALK* gene in humans), galactose-1-phosphate uridyl transferase (or UTP-hexose-1-phosphate uridylyltransferase; EC 2.7.7.10; *GALT*), and UDP-galactose 4′-epimerase (EC 5.1.3.2; *GALE*) (Fig. 1) (1). Galactose can be converted into energy by entering the glycolytic pathway since glucose 1-phosphate is one of the GALT reaction products (Fig. 1) (1). Galactose can also be incorporated into glycoproteins and glycolipids because UDP-galactose, the second product of the GALT reaction, is the only substrate donor of all galactosylation reactions (Fig. 1) (1). Furthermore, the GALE reaction interconverts the two nucleotide sugars UDP-glucose and UDP-galactose (Fig. 1) (1).

Deficiency of each one of the three galactose-metabolic enzymes results in Mendelian human disease conditions that are collectively referred to as the galactosemias (1). These three diseases are inborn errors of metabolism that can result in a variety of symptoms. Cataracts are most prominent in the case of kinase deficiency (OMIM 230200) (1) and are accompanied by severe neonatal symptoms such as failure to thrive, hepatomegaly, and *Escherichia coli* sepsis in transferase deficiency galactosemia (OMIM 230400) (1). The latter is the most common form of the disease, which is also often referred to as classic galactosemia and can be treated by dietary galactose restriction (1). Epimerase deficiency (OMIM 230350) can be mild and expressed only in blood cells (the peripheral form) or as severe as transferase deficiency (in the general form), since the epimerase is ubiquitously expressed, and this disorder is extremely rare (1).

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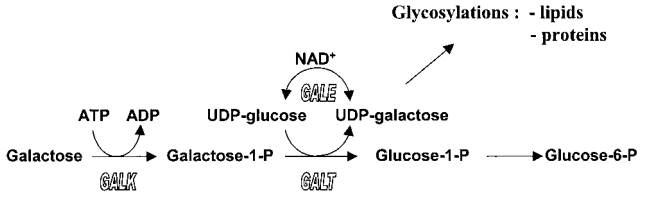

FIG. 1. Human galactose metabolism. Galactosemia can be caused by deficiency of GALK, GALT, or GALE.

GALACTOKINASE

Galactokinase catalyzes the first step in the metabolism of galactose by phosphorylating the galactose at the first carbon (Fig. 1) (1). Galactokinase deficiency (OMIM 230200) causes congenital cataracts during infancy and presenile cataracts in adults. Cataracts are the leading cause of blindness worldwide and the most frequently listed reason for hospital eye admission in the United States (2). Hereditary deficiency of galactokinase also results in galactosemia and galactosuria. The galactokinase enzyme has been partially purified and characterized from yeast, *E. coli,* pig liver, and adult and fetal human red blood cells. The human galactokinase sequence shares 45% sequence identity with *E. coli* galE (2). Three regions containing the galactokinase signature sequence and two different ATP binding motifs are conserved among all galactokinases (2). The human GALK1 gene contains eight exons and spans approximately 7.3 kb of genomic DNA (Table 1). The GALK1 promoter was found to have many features in common with other housekeeping genes, including high GC content, several copies of the binding site for the Sp1 transcription factor, and absence of TATA-box and CCAAT-box motifs typically present in eukaryotic polymerase II promoters. *In vitro* translation experiments of the GALK1 cDNA indicated that the protein is cytosolic and not associated with the endoplasmic reticulum membrane (3).

Galactokinase deficiency is generally considered to be rare when compared to classical galactosemia. However, the estimates of gene frequency vary widely with an east-to-west gradient across Europe (from 1:1,000,000 to 1:52,000). A high incidence of this disorder is found among Roma, an endogamous Gypsies population coming from some regions of east Europe. The high incidence is attributable to a founder effect as demonstrated by the segregation of a single nucleotide mutation (P28T) which is present in about 5% of the Roma population (4, Table 1). A total of 23 different mutations have been described within the GALK gene (Table 1). A majority of these mutations are of missense type and cause amino acid changes of conserved residues, which have an active role in the stability and activity of the enzyme (2, 4, 5).

A second galactokinase gene (*GALK2;* OMIM 137028), which maps to chromosome 15, called into question if galactosemia due to GALK deficiency is genetically heterogeneous (6). However, enzyme assays showed that human GK2 is a highly efficient GalNAc kinase with galactokinase activity when this sugar is present at high concentrations. Thus, Pastuszak *et al.* (7) stated that although human GK2 was identified based on its galactokinase activity, it is actually a GalNAc kinase.

GALACTOSE-1-PHOSPHATE URIDYL TRANSFERASE

Transferase deficiency galactosemia is the most common, best known, and best studied of the three galactosemias (1). The galactose-1-phosphate uridyl transferase enzyme catalyzes the conversion of galactose 1-phosphate, the product of the kinase reaction, with UDP-glucose into UDP-galactose and glucose-1-phosphate (Fig. 1) (1). A full-length, expressible human GALT cDNA was cloned some 12 years ago and a significant number of disease-causing mutations, polymorphisms, and other allelic variants have been reported over the past 10 years (8). The mutational basis of classic galactosemia (OMIM 230400) has been recently reviewed elsewhere in comprehensive detail. The interested reader is therefore referred to this report and/or the authoritative mutation website (9; http://www.ich.bris.ac.uk/galtdb/) (Table 1). The mouse GALT gene has been "knocked out" and these GALT-deficient mice apparently have no discernible phenotype (10). 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 (10).

The most common classic galactosemia mutation reported to date is the substitution of glutamine at codon 188 with arginine (Q188R) (Table 1) (9). This mutation is predominant in all Caucasian patient populations examined (9). It appears that African–American patients have a specific set of galactosemia mutations that includes Q188R although at a reduced frequency (9). Studies of several other ethnic groups in the United States and around the world suggest that they also have their own set of

TABLE 1
Important Characteristics of Human Galactose-Metabolic Genes

Gene	Mutation	Comments
GALK		
Location: 17q24	Q832X	Common in Costa Rican infants of European extraction
Gene size: 7 kb	P28T	Common in Romani (Gypsy) families
GALT		
Location: 9p23	S135L	Common in African–American galactosemia patients
Gene size: 4 kb	Q188R	Common in all Caucasian galactosemia populations examined
	N314D	Found on D and LA enzyme variants
	Mutation website: http://www.ich.bris.ac.uk/galtdb/	
GALE		
Location: 1p36	V94M	Reported in generalized disease
Gene size: 4 kb	K257R	Common in African–Americans with peripheral deficiency

galactosemia mutations (9). These data suggest that galactosemia is a panethnic disease but that many if not most of the underlying mutations often occurred after ethnic divergence. This hypothesis suggests that galactosemia carrier status may offer or may have offered universal heterozygote advantage against some common threat, e.g., infection. It is worth remembering in this context that one of the products of the GALT reaction is UDP-galactose which is required for the biosynthesis of all galactose-containing glycoproteins and glycolipids (11). The amount and/or composition of these generally extracellular or exposed compounds could be slightly altered in heterozygotes which may affect infection rates. Classically, heterozygote advantage has been reported in carriers of hemoglobin disorders, such as sickle cell disease and the thalassemias, and resistance to malaria (12).

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 observed (1). Interestingly both variants are characterized by similar electrophoretic properties but very different enzyme activities. It was shown by two independent groups that both variants bear the same amino acid substitution: asparagine-314 mutated to aspartate (N314D) (Table 1) (9). Thus, this single 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 bearing aspartate-314. The functionally relevant molecular basis of the activity differences between the D and LA variants remains somewhat controversial to date.

Biochemical investigations have confirmed the importance of GALT residue 188 in the catalysis of the transferase reaction since it appears to be critical in stabilizing the uridylate reaction intermediate (13).

Genotype/phenotye correlations in classic galactosemia have unfortunately been very difficult to establish thus far despite adequate sample sizes.

UDP-GALACTOSE 4′-EPIMERASE

UDP-galactose 4′-epimerase catalyzes the interconversion of UDP-glucose and UDP-galactose with NAD^+ as a cofactor (Fig. 1) (1). The human GALE cDNA was cloned and shown to be 51% identical to the homologous *E. coli* gal*E* enzyme (14). Data on rare missense mutations causing epimerase deficiency (OMIM 230350) have been reported for the peripheral and generalized form of epimerase deficiency galactosemia (Table 1) (14, 15).

Recently structural studies highlighting certain GALE protein residues were reported (16). There do not appear to be any such striking correlations as with the GALT protein yet, possibly because of the mild phenotype of the common peripheral form of the deficiency. It may affect structurally and/or functionally relatively irrelevant mutations resulting in a mild phenotype.

OUTLOOK

The galactosemias have been a mainstay of human genetic investigations for well over half a century (1). Substantial progress has been made but many questions remain, particularly about improv-

ing the life of patients with the classic form of the disease.

Genotype/phenotype correlations in classic galactosemia have been generally disappointing. Such analyses have been even more difficult in kinase and epimerase deficiency because of the small number of patients genotyped. We note that the GALT-KO mice have no phenotype comparable to the human disease condition of classic galactosemia (10). These two datasets on difficult human genotype/phenotype correlations and essentially normal knock-out mice may suggest that alternative metabolic pathways and/or modifier loci in both humans and mice significantly influence galactose metabolism. Identification and characterization of these possible alternatives will bring added benefits to diagnosis and treatment of this classic inborn error of metabolism. These alternatives also suggest that this inborn error of metabolism is not a strictly monogenic phenotype but may be much more complex. This finding is echoed by those made in several other "Mendelian" disorders which were recently discussed by others (17). Finally, we note that it may be possible that galactosemia carrier status might confer now or might have conferred in the past some heterozygote advantage in various racial/ethnic groups as discussed under galactose-1-phosphate uridyl transferase. These intriguing hypotheses would warrant further inquiry into the molecular basis of the galactosemias not only to fully understand the disease phenotypes but also as a model for other human Mendelian conditions.

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