INSIGHTS INTO THE PATHOPHYSIOLOGY OF GALACTOSEMIA

Dr. Zahra Beyzaei, PhD

Shiraz Transplant Research Center, Shiraz University of Medical Sciences

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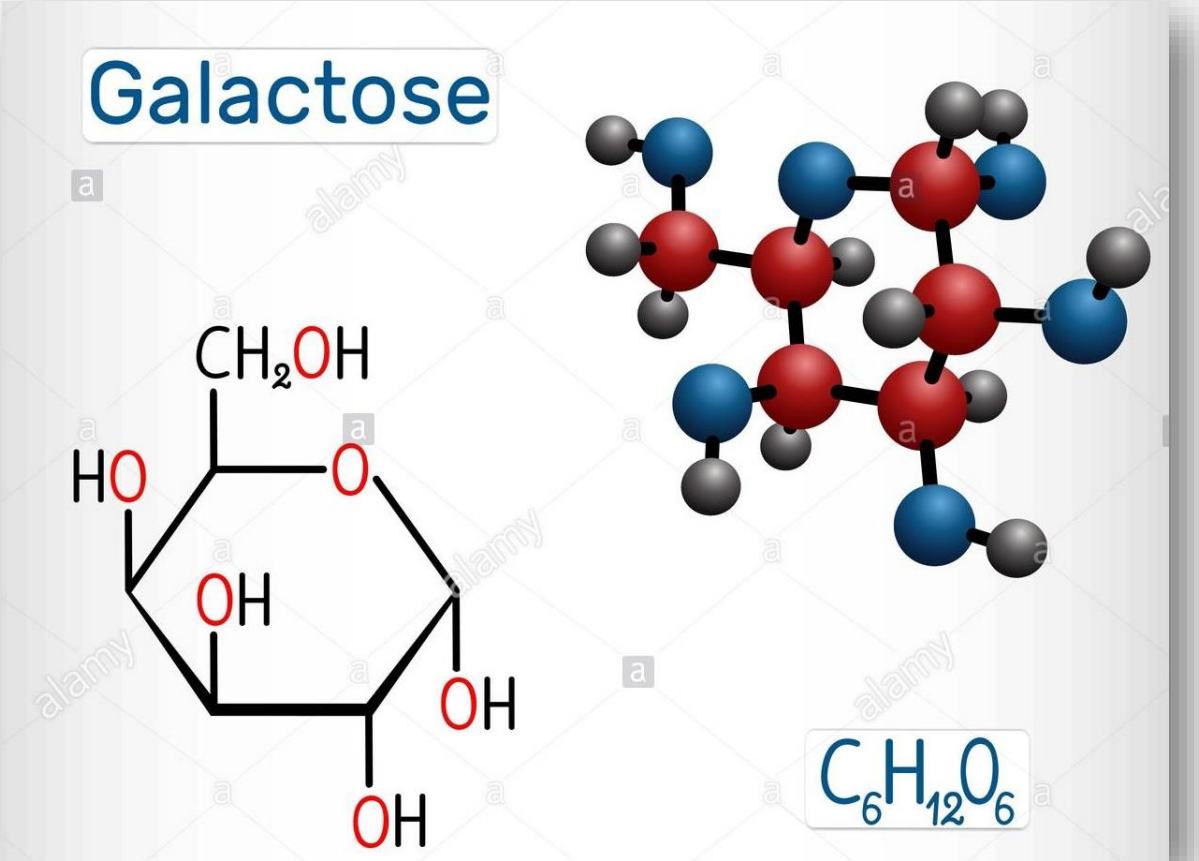
GALACTOSEMIA

- Galactosemia is a family of inherited autosomal recessive disorders of carbohydrate metabolism that result from impaired metabolism of galactose.

- Despite that the first description of galactosemia that dates from more than 100 years ago (1908) and decades of research, the pathophysiology of this disease is complex and not yet fully understood.

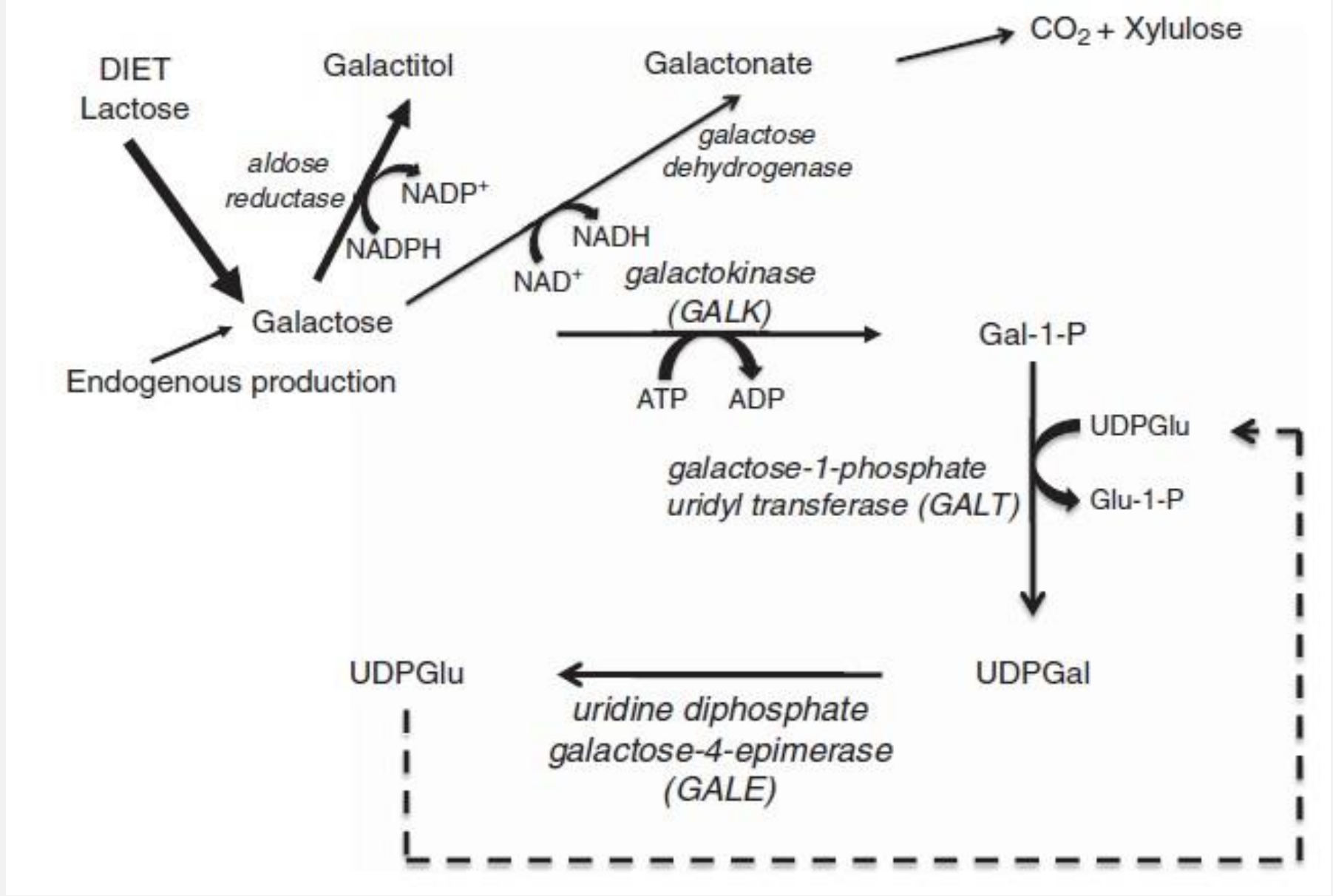
GALACTOSEMIA

- Lactose accounts for almost all sugar present in breast milk or formula and is a major source of energy for newborns and infants. Lactose is cleaved into D-glucose and β-D-galactose on the intestinal villi by β-D-galactosidase. β-D-Galactose is taken up into the liver via the portal vein and primarily metabolized by the Leloir pathway.



LELOIR PATHWAY OF GALACTOSE METABOLISM

- This pathway plays a critical role in converting D-galactose to glucose-1-phoshate for entry into glycolysis, as well as supplying uridine diphosphate galactose (UDP-galactose) for the galactosylation of carbohydrates and lipids.



Metabolism of galactose in Leloir pathway

Pasquali et al. Genetics in Medicine, 2018

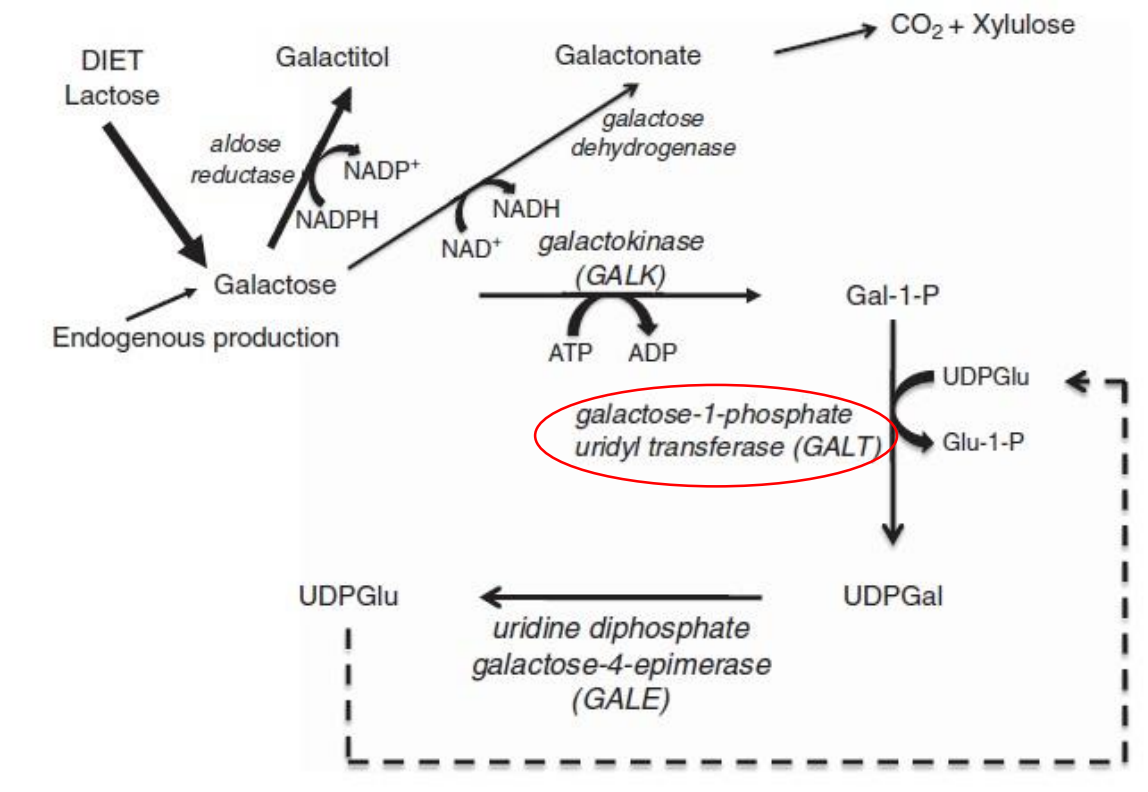
TYPES OF GALACTOSEMIA

- Galactosemia is caused by a severe impairment of any of the enzymes involved in the Leloir pathway and comprises four subtypes.

Name	Enzyme deficiency
Type 1 classic galactosemia	Galactose-1-phosphate uridyltransferase (GALT) enzyme
Type 2 galactosemia	Galactokinase (GALK) enzyme
Type 3 galactosemia	UDP-galactose 4'-epimerase (GALE) enzyme
Type 4 galactosemia	Galactose mutarotase (GALM) enzyme

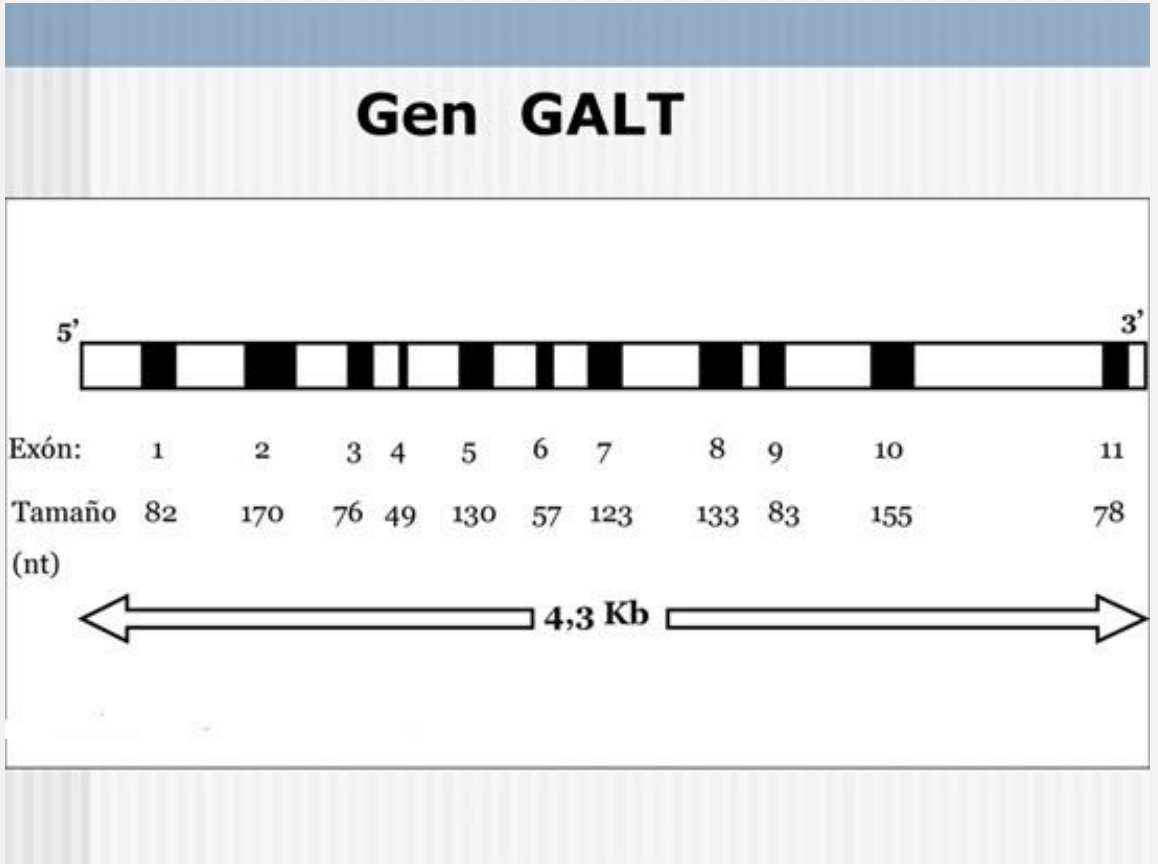
GALACTOSE-1-PHOSPHATE URIDYLTRANSFERASE (GALT)

- Galactose-1-phosphate uridyltransferase (GALT; EC 2.7.7.12), the second enzyme of the Leloir pathway, is a member of the histidine triad superfamily and is a homodimer composed of two 43-kDa subunits.



GALACTOSE-1-PHOSPHATE URIDYLTRANSFERASE (GALT)

- The GALT gene encompasses 11 exons spanning 4.3 kb of chromosome 9p13. An open-access database has curated more than 250 pathogenic variants including nonsense, missense, frameshift, splice site variants, and large deletions. The vast majority (~85%) are classified as causing profound enzyme impairment consistent with classic galactosemia; of these, 61% are missense variants.



DUARTE VARIANT GALACTOSEMIA

- Duarte variant (DG) galactosemia results from compound heterozygosity for a profound GALT pathogenic variant (G) and a second allele known as Duarte 2 (D2).
- D2 was originally characterized by an abnormal banding pattern on gel electrophoresis or isoelectric focusing. It is now known to encompass a haplotype of four variants:

✓Two intronic changes,

✓A 4-bp deletion in the promoter of the gene, (c.-119_-116delGTCA)

✓ c.940A >G (p.N314D)

DUARTE VARIANT GALACTOSEMIA

- One GALT pathogenic variant (G allele) present in the heterozygous state plus the GALT Duarte (D2) variant allele present in either the heterozygous state (in trans to the G allele) or in the homozygous state (both in cis and in trans to the G allele).
- Classic galactosemia pathogenic variants can occur in cis with D2 resulting in complete loss of activity on that allele.
- Duarte variant galactosemia is inherited in an autosomal recessive manner.

DUARTE VARIANT GALACTOSEMIA

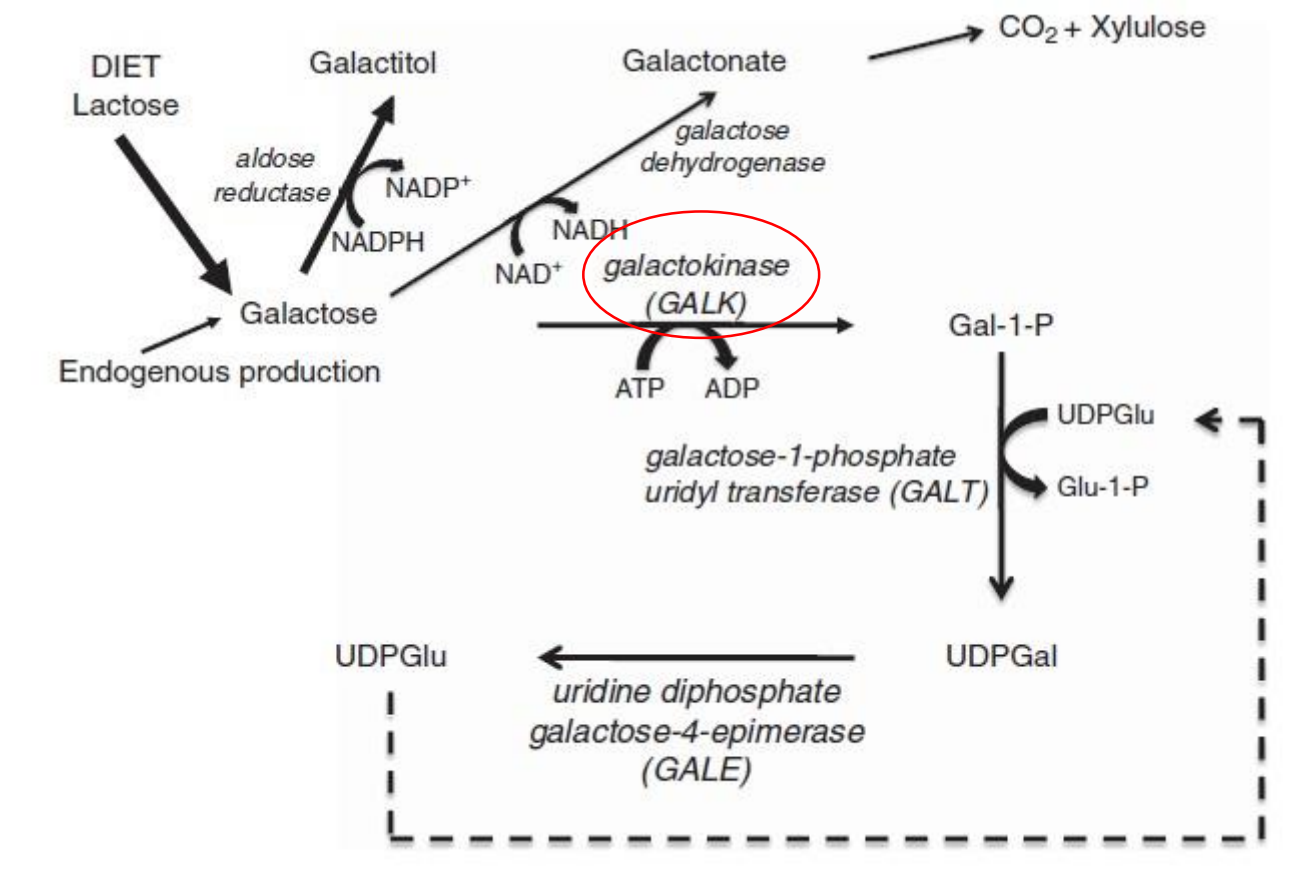
- Current evidence supports the hypothesis that the 4-bp deletion is responsible for the reduction in enzyme activity.
- A second variant, Duarte 1 (D1, also known as the Los Angeles, or LA, variant), shares the D2 electrophoretic banding pattern but is not associated with reduced GALT activity. The D1 allele contains c.940A >G (p.N314D), a synonymous nucleotide change in exon 7, c.652C > T, but not the 4-bp promoter deletion. For Duarte variant galactosemia suspicious cases, it is most appropriate to test for the 4-bp GALT promoter deletion to distinguish between the D1 and D2 variants.

GALT GENOTYPES AND BIOCHEMICAL/CLINICAL PHENOTYPES

Classic Galactosemia	Clinical Variant Galactosemia	Biochemical Variant Galactosemia
p.[Gln188Arg]+[Gln188Arg] (Q188R/Q188R)	p.[Ser135Leu]+[Ser135Leu] (S135L/S135L)	c.[940A>G;-16_119delGTCA] (4bp 5' del + N314D/Q188R)
p.[Lys285Asn]+[Lys285Asn] (K285N/K285N)		
p.[Leu195Pro]+[Leu195Pro] (L195P/L195P)		
(Δ5.2 kb del/ Δ5.2 kb del)		

GALACTOKINASE (GALK)

- Deficiency of GALK results in type II galactosemia or galactokinase deficiency (MIM 230200).
- Galactokinase (GALK; EC 2.7.1.6) is the first enzyme in the Leloir pathway.
- It catalyzes the MgATP-dependent phosphorylation of D-galactose to form Gal-1-P.

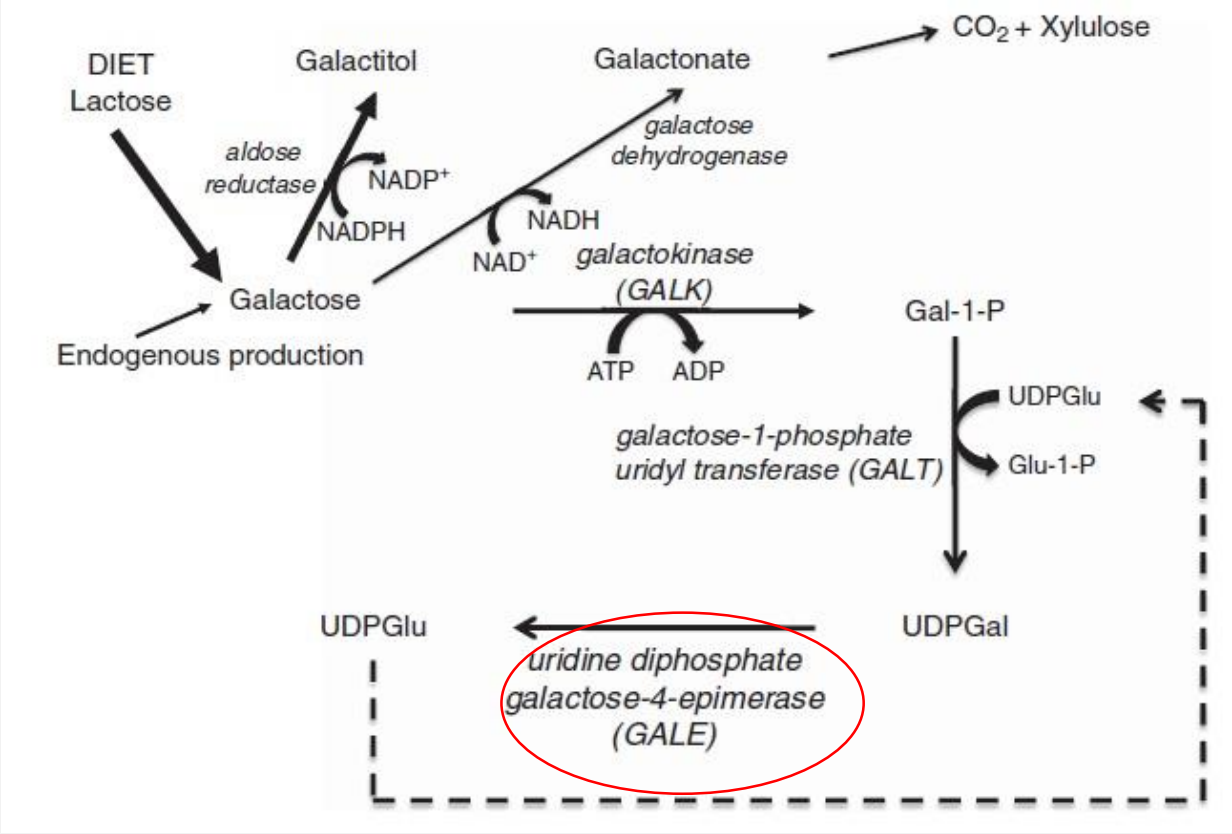


GALACTOKINASE (GALK)

- It is encoded by GALK1, a gene spanning 8 exons and 7.3 kb on chromosome 17q25.1.
- Several GALK pathogenic variants have been characterized, including insertions, deletions, and single base changes in RBCs. The most severe phenotype is associated with an insoluble enzyme, while milder phenotypes are characterized by soluble enzyme with impaired catalytic function.

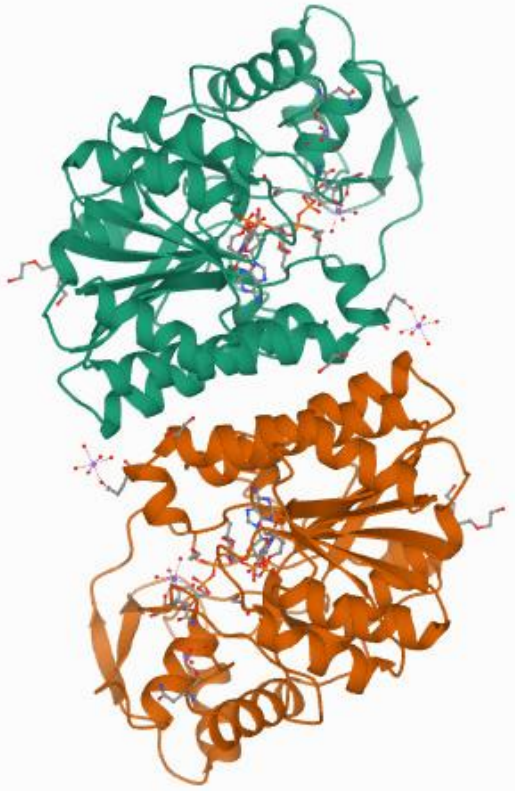
UDP-GALACTOSE-4′-EPIMERASE (GALE)

- UDP-galactose-4′-epimerase (GALE; EC 5.1.3.2) is the third enzyme of the Leloir pathway and catalyzes the reversible conversion of UDP-galactose to UDP-glucose and of UDP-N-acetylgalactosamine to UDP-N-acetylglucosamine.



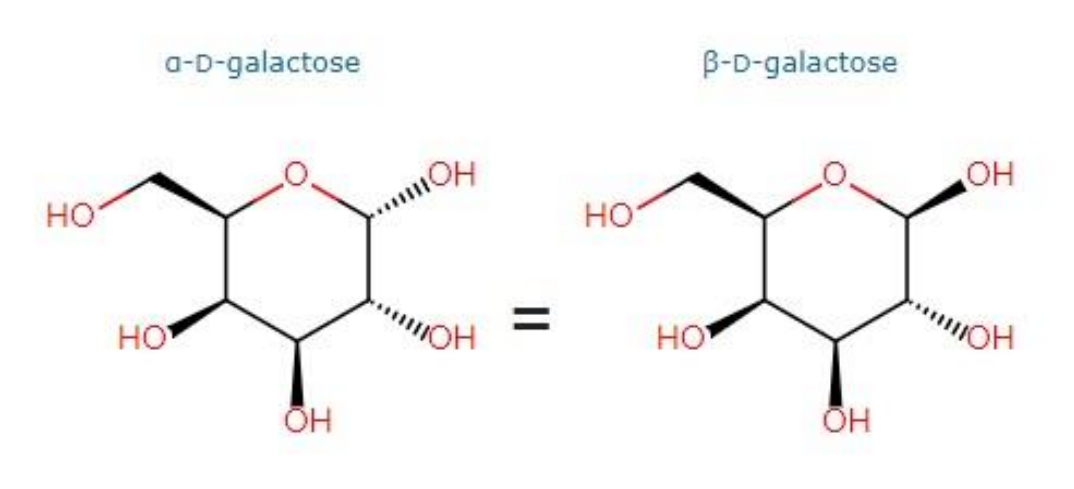
UDP-GALACTOSE-4′-EPIMERASE (GALE)

- GALE is a member of the short-chain dehydrogenase/reductase family of enzymes and is encoded by GALE, a gene spanning 13 exons and 5.1 kb on chromosome 1p36.11. In solution, GALE is a dimer and each subunit contains a binding site with one molecule of the cofactor NAD+. Impaired GALE activity results in type III galactosemia or epimerase deficiency (MIM 230350).



GALACTOSE MUTAROTASE (GALM)

- The first step before the Leloir pathway involves epimerization between β- and α-D-galactose, which is catalyzed by galactose mutarotase (or aldose 1-epimerase) (GALM, EC 5.1.3.3).



Wada et al. Genetics in Medicine, 2019

GALACTOSE MUTAROTASE (GALM)

ARTICLE | Genetics inMedicine

Biallelic *GALM* pathogenic variants cause a novel type of galactosemia

Yoichi Wada, MD[1], Atsuo Kikuchi, MD, PhD[1], Natsuko Arai-Ichinoi, MD, PhD[1], Osamu Sakamoto, MD, PhD[1], Yusuke Takezawa, MD[1], Shinya Iwasawa, MD[1], Tetsuya Niihori, MD, PhD[2], Hiromi Nyuzuki, MD[3], Yoko Nakajima, MD, PhD[4], Erika Ogawa, MD, PhD[5], Mika Ishige, MD, PhD[5], Hiroki Hirai, MD, PhD[6], Hideo Sasai, MD, PhD[7], Ryoji Fujiki, MS[8], Matsuyuki Shirota, MD, PhD[9], Ryo Funayama, PhD[10], Masayuki Yamamoto, MD, PhD[11], Tetsuya Ito, MD, PhD[4], Osamu Ohara, PhD[8], Keiko Nakayama, MD, PhD[10], Yoko Aoki, MD, PhD[2], Seizo Koshiba, PhD[11], Toshiyuki Fukao, MD, PhD[7] and Shigeo Kure, MD, PhD[1,11]

Purpose: Galactosemia is caused by metabolic disturbances at various stages of galactose metabolism, including deficiencies in enzymes involved in the Leloir pathway (GALT, GALK1, and GALE). Nevertheless, the etiology of galactosemia has not been identified in a subset of patients. This study aimed to explore the causes of unexplained galactosemia.

Methods: Trio-based exome sequencing and/or Sanger sequencing catalyzes epimerization between β- and α-D-galactose in the first step of the Leloir pathway. GALM enzyme activities were undetectable in lymphoblastoid cell lines established from two patients. Immunoblot analysis showed the absence of the GALM protein in the patients' peripheral blood mononuclear cells. In vitro GALM expression and protein stability assays revealed altered stabilities of the variant GALM proteins.

GALACTOSE MUTAROTASE (GALM)

- The biallelic pathogenic variants in GALM in patients with unexplained galactosemia, suggesting the presence of a novel type of galactosemia, namely, type IV galactosemia.
- GALM deficiency leads to persistent galactosemia, which should be treated appropriately.
- The phenotypes of GALM deficiency are similar to those of GALK1 deficiency, which abolishes the next step in the Leloir pathway.

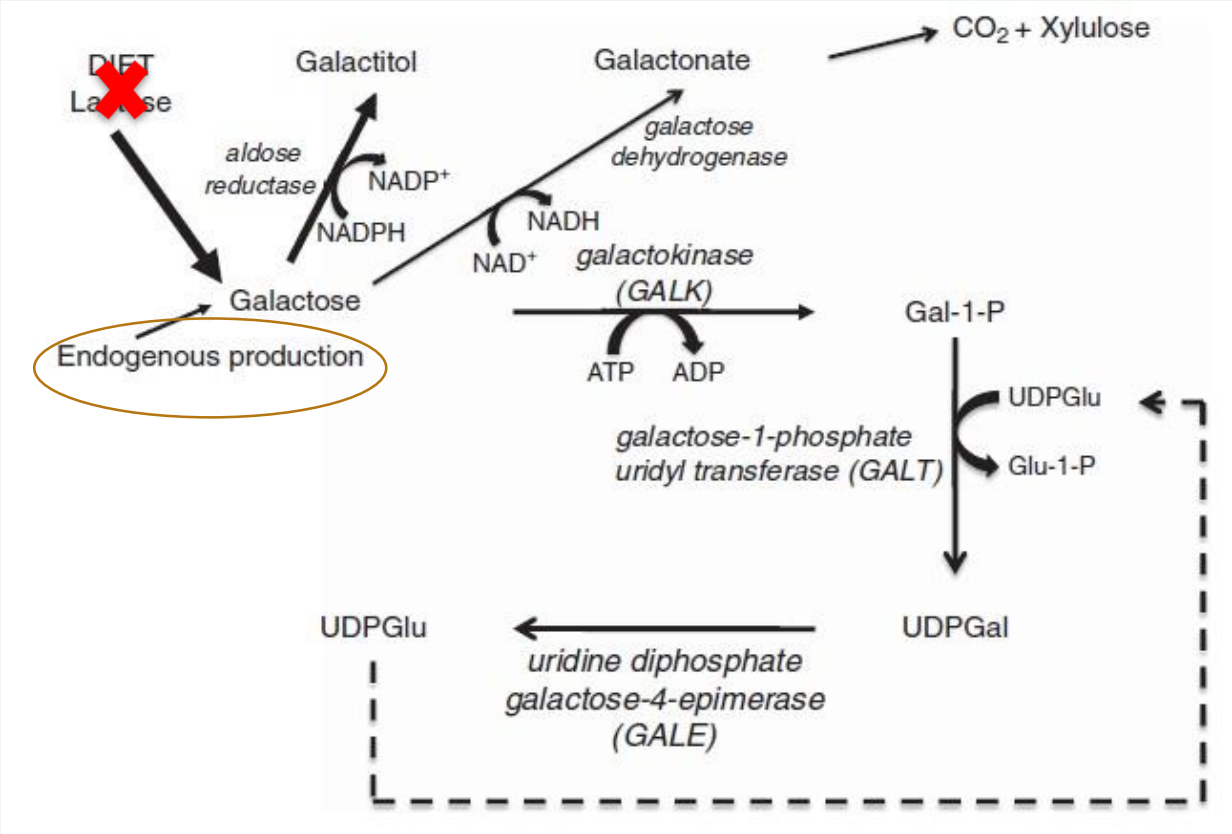
Wada et al. Genetics in Medicine, 2019

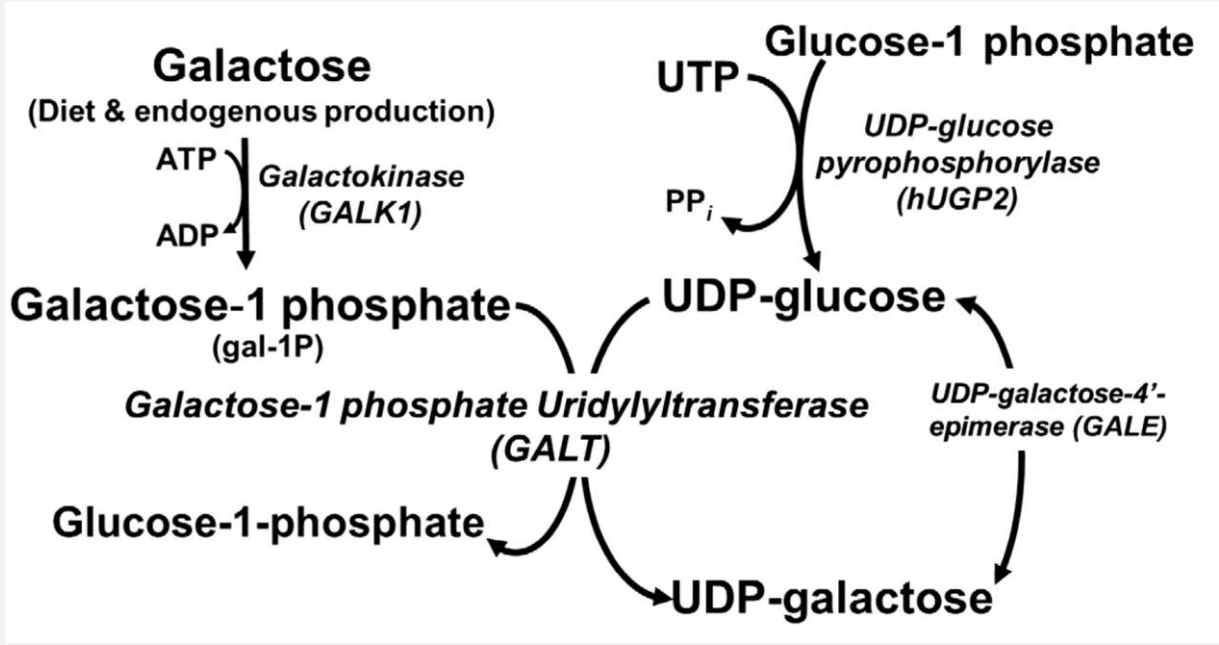
THE GENETIC HYPERGALACTOSEMIAS

Gene	Hypergalactosemia		Comments
GALK	Galactokinase deficiency		
GALE	Epimerase deficiency galactosemia		
GALM	Galactose mutarotase deficiency galactosemia		
		Classic galactosemia	Severe GALT enzyme deficiency w/absent or barely detectable activity in erythrocytes & liver (aka G/G; carriers aka G/N)
GALT	Galactose-1-phosphate uridylyltranserase deficiency	Clinical variant galactosemia	1%-10% residual GALT enzyme activity in erythrocytes &/or liver
		Biochemical variant galactosemia	15%-33% residual GALT enzyme activity in erythrocytes; incls the D_2 Duarte biochemical variant state (aka G/D)

Gene Reviews, 1993-2021, University of Washington, Seattle.

PATHOPHYSIOLOGY





PREVALENCE OF GALACTOSEMIA

- Classic galactosemia is diagnosed in the range of 1/16,000 to 1/48,000 births through newborn screening programs around the world, depending on the diagnostic criteria used by the program. The disorder has been reported in all ethnic groups. An increased frequency of galactosemia occurs in individuals of Irish ancestry. Clinical variant galactosemia occurs most often in African Americans and native Africans in South Africa who have a specific *GALT* gene mutation.

PREVALENCE OF GALACTOSEMIA

- The prevalence of GALK deficiency from 1:2,200,000 to 1:50,000 with the highest incidence in the Romani population of Bulgaria and Bosnia.
- Generalized GALE deficiency is very rare; epimerase deficiency identified by newborn screening, comprising mostly peripheral/intermediate, is 1:70,000 in infants of European descent and 1:6,700 in African Americans.


Thank you For
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