

Galactosemia : A Genetic Disease of Leloir Pathway

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ABSTRACT

Galactosemia is a genetic disorder which causes inability to metabolize galactose in the body. Our body converts galactose into glycolytic intermediate by Leloir pathway. Galactosemia is caused by the mutation in the gene encoding enzymes of Leloir pathway or non-functioning of these enzymes. Types of galactosemia are due to different enzyme deficiencies. Type I is for GALT (galactose-1-phosphate uridylyl transferases) enzyme deficiency, type II is for GALK (galactose kinase) and type III is for GALE (uridine-diphosphate galactose-4-epimerase) deficiency in the body. Structural alterations in the enzyme conformation, due to defective gene, cause loss of enzyme activity by disrupting enzyme active site. These cause accumulation of galactose and its derivatives causing many problems like cataracts, brain damage and liver damage etc. Their symptoms appear like low weight, jaundice, vomiting etc. This disease is diagnosed via number of test like genetic test, blood test, urine test, new born screening etc. For treatment of galactosemia, strict diet plan is recommended to patients containing galactose free diet such as fermented food etc. Research related to drug treatment is the scope in galactosemia.

Keywords : Galactosemia, Leloir pathway, galactokinase (GALK), UDP-galactose-4-epimerase (GALE), Galactose-1-phosphate uridylyl transferase (GALT)

I. INTRODUCTION

Galactosemia is a genetic disease in which body is unable to metabolize galactose which is a hexose sugar (Harris, 1998). Literal meaning of galactosemia is excessive galactose in the blood. Any defect in galactose metabolic pathway will result in galactosemia generally it is caused by the inability of the body to produce the enzyme of the metabolic pathway (McCorvie and Timson, 2011). History of galactosemic patients is shown in Fig 1. In 1908, in a publication named “sugar excretion in infancy”, Von Ruess reported about an infant, who due to breast feeding failed to thrive and got enlarged liver and spleen. He called it “galactosuria”. When the infant’s diet is completely devoid of milk products, he did not excrete galactose in urine later he died due to further complications (Harris, 1998). Autopsy of the infants liver showed Cirrhosis but it was said that its reason is alcohol not milk products. However, at that time due unavailability of the advanced diagnostic tools, diagnosis of galactosemia was not possible. Generally Von Ruess was accepted as the first person who discovered the disease. By the time “galactosuria” started recognizing as an inherited disease

by Goppert as he diagnosed the disease in infant and his siblings and in 1917 it was largely classified as an inherited disorder which can only be controlled if victim stopped consuming milk products (Goppert, 1917). In 1935 Mason and Turner for the first time ever published a detailed description on the disease by the name “chronic galactosemia Report of case with studies of carbohydrates” (Mason & Turner, 1935). Leloir and his co-workers established metabolic pathway of conversion of galactose to glucose in 1950s and later on due to his work on this pathway and sugar nucleotides he received Nobel Prize in chemistry in 1970.

Galactosemia was recognized very early in clinics but its cause remained a mystery until 1956 by Schwarz as he shows galactose-1-phosphate accumulated in blood cells. In the same year Isselbacher demonstrates that red blood cells of are devoid of GALT. Later GALK and GALE abnormalities are also identified in patients (Isselbacher *et al.*, 1956). In 1963 Guthrie and Paigen developed a method called new born screening to detect galactosemic infants. In 1990 Waggoner, Buist and Donnell finds chronic outcome unrelated to delayed diagnosis or poor dietary compliance.

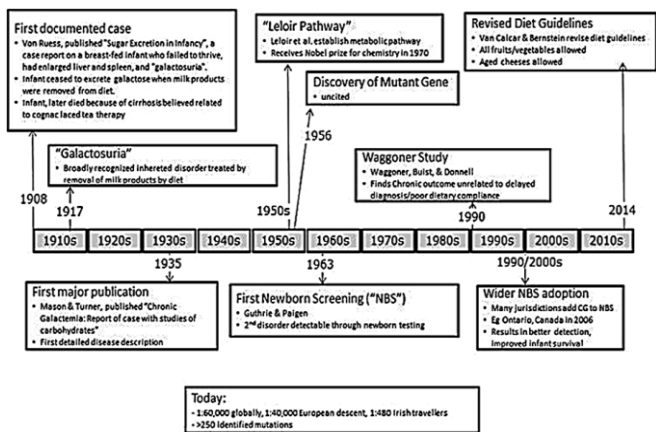


Figure 1: History of Galactosemia

Galactose and glucose have same molecular formulas but they have different structural formulas. They are C4 epimer of each other. In spite of this large similarity their inter conversion needs enzymatic steps. These enzymes are conserved even after years of evolution. These enzymes are cytoplasmic and the pathway is termed as Leloir pathway (Leloir, 1951). Our diet contains galactose especially dairy products of our diet are its great source. Dairy products contain lactose (a disaccharide made up of galactose and glucose) but non-dairy products could also contain some galactose (Gross, 1995). Galactose produces in the body by the enzymatic inter conversion of uridine diphosphate glucose and uridine diphosphate galactose and by the production of glycoproteins and glycolipids (Berry, 2004). In case of any stoppage in any metabolic pathway, it will carry out accumulation of precursors. Now all the symptoms of a metabolic disease could be the result of the above possibility (Tang *et al.*, 2012). To find out the details of any metabolic disease, one must know the details of any metabolic pathway. Galactosemia is related to Leloir pathway or galactose metabolic pathway as shown in Fig 2. Glycolysis (metabolic pathway of glucose degradation) contains highly specific enzymes that do not act upon galactose monomer. Thus to enter glycolysis, galactose must be changed into glycolytic intermediate. This needs conversion of galactose into uridine phosphate derivative. This entire pathway is explained by Luis Leloir and thus called Leloir pathway (Voet, 2011). This pathway constitutes four reactions in general. The first one is catalysed by galactose mutarotase which catalyse β -D-galactose into α -D-galactose (Holden *et al.*, 2003). In this reaction galactose is converted to galactose-1-phosphate by phosphorylation at C1 of galactose due to dissociation of ATP into ADP with the release of

phosphate group. In the second step catalysis occurs by galactose-1-phosphate uridylyl transferases. It is a reaction in which uridylyl group of uridine diphosphate glucose transfers to galactose-1-phosphate which as a result produces to products glucose-1-phosphate and uridine diphosphate galactose. This reaction also involves reversible cleavage of phosphoryl bond of UDP-glucose (Frey, 1996). The third step is catalysed by UDP-galactose-4-epimerase. NAD molecule is associated with this enzyme. UDP galactose is converted back to UDP-glucose by redox reactions on C4 atom of both the hexoses due to presence of NAD. Fourth and final reaction involves catalysis by phosphoglucomutase. Glucose-6-phosphate (G6P) is a glycolytic intermediate so glucose-1-phosphate (G1P) produced in second step reaction is converted to G6P by the action of this enzyme (Voet, 2011).

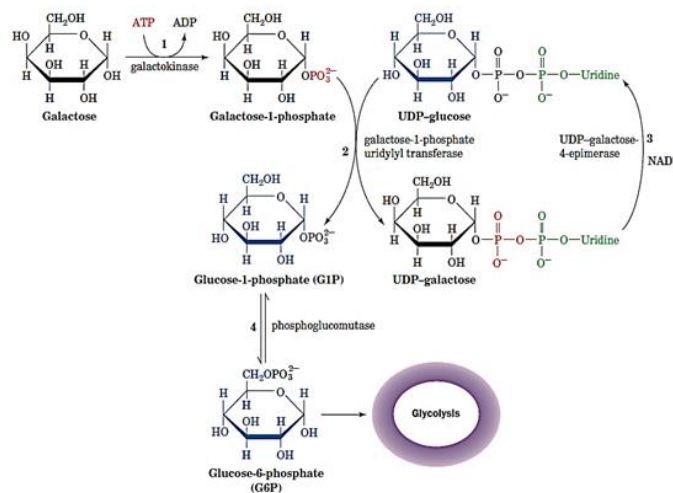


Figure 2: Leloir Pathway

II. CAUSES OF GALACTOSEMIA

Major cause of galactosemia is inheritance. Children with galactosemia usually inherit disease or we can say allele from both the parents. The disease is an autosomal recessive one so, galactosemic child contains two copies of defective gene one from each parent. In case of carrier parents there is $\frac{1}{4}$ chance for each pregnancy to have galactosemia, $\frac{2}{4}$ chance for each pregnancy to have carrier child and $\frac{1}{4}$ chance for each pregnancy to have a normal child (Haldeman-Englert *et al.*, 2015). Late diagnosis of the disease cause harsh effects thus many areas of the world have made new born screening mandatory. When enzymes of galactose metabolism are missing or non-functional then galactose and its derivatives (galactose-1-phosphate and UDP-galactose)

accumulate in tissues and blood and cause disastrous effects on the body (Berry *et al.*, 2012). Elevation of galactose level in the blood arises questions that whether the disease is primary hypergalactosemia or secondary hypergalactosemia. Primary hypergalactosemia occurs due to deficiencies of enzymes of Leloir pathway as shown in Table 1.

Table 1: Causes of galactosemia

Sr. No	Causes of primary hypergalactosemia	Causes of secondary hypergalactosemia
1.	Galactokinase (GALK) deficiency	Congenital hepatitis
2.	Galactose-1-phosphate uridylyltransferase (GALT) deficiency	Congenital hepatic arterio-venous malformations
3.	UDP-galactose-4'-epimerase (GALE) deficiency	Patent-ductus venosus
4.	Benign ("peripheral")	Tyrosinemia, type 1, citrin deficiency (citrullinemia, type 2), and others producing hepatocellular disease

On the other hand secondary hypergalactosemia is related to liver. Liver is the organ which is mainly responsible for galactose metabolic pathway (Fridovich-keil & Walter, 1993) galactosemia when it's new born screening. When the liver is dysfunctional, galactose fails to metabolize and thus causing secondary hypergalactosemia includes congenital hepatitis, patent-ductus venosus and others shown in table 1. Galactokinase (GALK) is clinically associated with cataracts, pseudotumor cerebri, and high levels of blood galactose and significantly reduced activity of GALK in red blood cells (Bosch *et al.*, 2002)galactosemia when it is anew born screening. Uridine-diphosphate galactose-4-epimerase (GALE) deficiency in case when it has no clinical associations thus called benign or peripheral deficiency. In this deficiency enzyme activity is significantly reduced or absent in red blood cells but normal in tissues. It includes high levels of blood galactose& galactose-1-phosphate but it does not affect GALT enzyme activity (Openo *et al.*, 2006). Several patients have been described which has low levels of GALE in cells other than red blood cells. These patients

have delayed growth, cataracts formation, seizures, emesis and hypoglycaemia but no cause and effect relationship has been established between this enzyme and above clinical findings (Berry, 2012). Deficiency of GALT is caused by mutations in both the allele of GALT gene. GALT deficiency has 3 basic forms i.e. classic galactosemia, clinical variant galactosemia, and biochemical variant galactosemia. Basis for this classification is phenotype as well as genetic potential for acute and chronic complications (Tang *et al.*, 2012).

GALE (EC. 5.1.3.2) converts UDP-galactose into UDP-glucose. It is member of SDR family of proteins (Short-chain dehydrogenase reductase). GALE and related enzymes contain a number of properties like activation of NAD by uridine nucleotide or irreversible binding of NAD. This family consists of UDP-glucose and UDP-galactose epimerase activity, N-acetylated forms of UDP-galactose and UDP-glucose like UDP-GlcNAc/UDP-GalNAc, 16 uronic acid forms of UDP-galactose and UDP-glucose like UDP-GlcUA/UDP-GalUA, 17 forms of UDP pentoses i.e. with other pentose sugar instead of galactose and glucose like UDP-L-Ara and UDP-Xyl (Demendi *et al.*, 2005). GALE is a homodimers or monomer in case of *E. coli* as shown in Fig 3. It consists of 338 amino acids with 37.3kDa molecular weight in *E. coli* and 348 amino acids in 76.6kDa molecular weight of dimer in case of human GALE. It consists of characteristics motifs with sequences YxxxK and GxxGxxG (Hongjie Guo, 2006). First one contains Tyr/Lys couple that is significant in catalysis. In the second one first 2 glycine plays role in bin out close binding of NAD⁺ and the third carries out close packaging of helix to B-strand (Lesk, 1995). First step of mechanism is that phenolic residue which is facilitated by serine carries out removal of hydroxyl group at carbon no 4 and transformation of hydride group from C4 of sugar to NAD making NADH and also a temporary ketone sugar is formed (Liu, 1997). Next step is of temporary ketone sugar which is still retained and 40-ketopyranose intermediate carries out rotation in the active site. Now hydride transfers back to C4 of sugar but on opposite side forming inversion of configuration at carbon no 4 which was earlier extracted by tyrosine is added on sugar at C4 but on the side opposite to hydride (Beerens, 2015). A UMP-group is transferred from UDP-glucose to galactose-1-phosphate. The reaction is catalysed by GALT or galactose-1-phosphate uridylyl transferees. This enzyme has a great

specificity for UDP-glucose and UDP-galactose, while it could not act upon other substrates like GDP-glucose, ADP-glucose, TDP-glucose, CDP-glucose, UDP-xylose and UDP-mannose (Bertoli, 1996). Mammalia GALT action can be inhibited by Uracil and its derivatives like UTP, UDP, UMP, uridine, UDP-xylose, UDP-glucuronic acid and UDP-mannose.

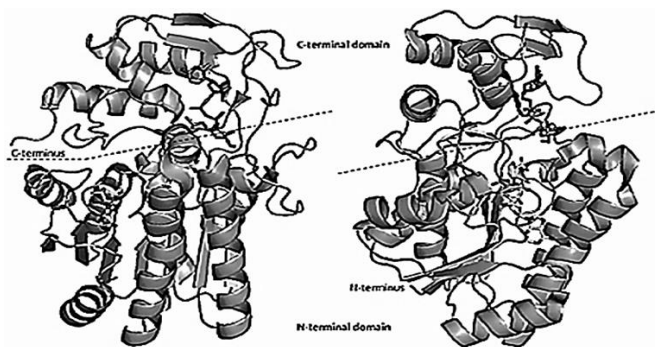


Figure 3: Structure of *E. coli* GALE

There was a report that GALT activity was less in patients of lymphocytic leukemia. This could probably be done by unidentified inhibitor (Sega & Rogers, 1971). GALT is the member of family of transferases which contains histidine triad. This mechanism of action is named as substituted enzyme mechanism or Ping Pong mechanism. In GALT-UDP glucose complex, UMP group from UDP-glucose is transferred to his-186 residue in the active site of enzyme as shown in Fig 4 also; glucose-1-phosphate diffused and replaced by galactose-1-phosphate on which UMP moiety is transferred yielding UDP-galactose. With this step enzyme again becomes freely available for catalysis action (Johnson, 1979).

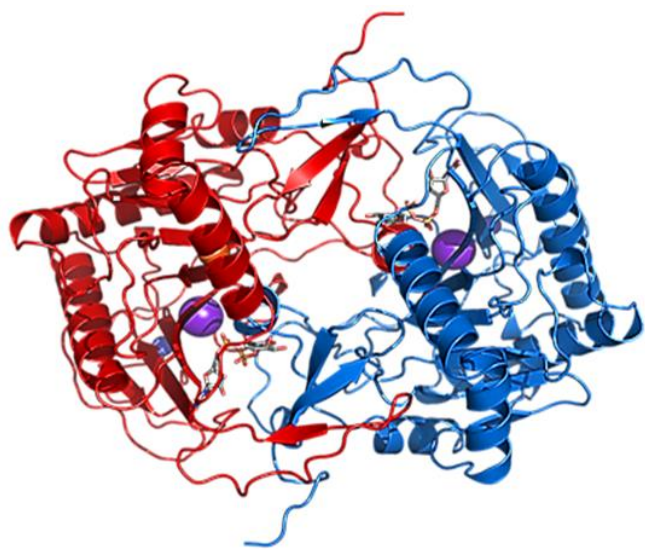


Figure 4: Structure of *E. coli* GALT

Galactose kinase (GALK) is the member of GHMP superfamily of enzyme on the basis amino acid sequence similarity. It carries out the conversion of D-galactose to galactose-1-phosphate. Kinetic properties of this enzyme differ on the basis of source of enzyme for example reaction GALK carries out ATP binding first (Ballard 1966). According to Timson and Reece human galactokinase used 2-deoxy-D-galactose as a substrate. The enzyme from *L. lactis* shows that it is 34% identical and 47% similar with galactokinase in human. It is a three amino acid residue and has two domains namely N-terminal and C-terminal domain. N-terminal Domain contains five α -helices which surrounds five mixed B-sheets. C-terminal domain constitutes bi layers of b-sheets along with α -helices which are six in number as shown in Fig 5. Structure of *L. lactis* suggests that Asp-183 and Arg-36 plays role in hydroxyl hydrogen abstraction and pKa lowering of hydroxyl group respectively. The structure also suggests that deprotonation carries out by carboxylic side chain of Asp-183. In human galactokinase, according to Timson and Reece, transfer of proton is not involved rate detrmng step (Holden et al., 2003)

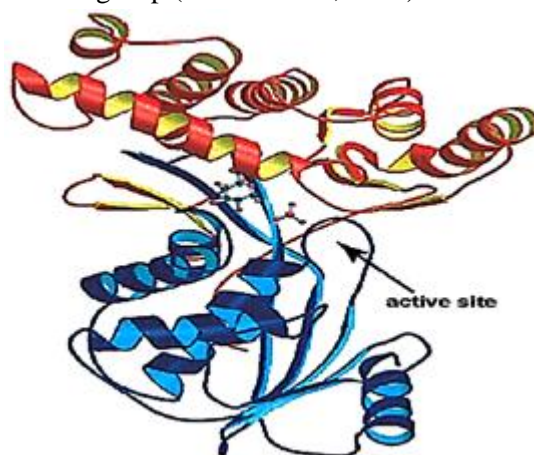


Figure 5: Structure of *E. coli* GALK

III. TYPES OF GALACTOSEMIA

Galactosemia disease is the result of defects in metabolic pathway of galactose and their derivatives. It has three types: type I, type II and type III. In the population galactosemia persistence differ very remarkably. Frequency of galactosemic patients in some countries and areas are shown in the table 2. The first and most common type of galactosemia is due to mutations in the gene encoding the enzyme galactose 1-phosphate uridylyl transferases that are involved in Leloir pathway

(Isselbacher *et al.*, 1956). Galactosemia type I anatomy is different. Its effects are very severe on the human being; it damages various organs of the body like kidney, ovaries, liver and brain. Galactosemia patients face these symptoms like mental and developmental problems in their childhood and reproductive disorder in the adulthood. In case of improper treatment the disease may cause death in childhood. We can reduce their causes and symptoms by preventing the galactose from the diet (Bosch, 2006).

Table 2: Frequency of galactosemic patients in different countries

Values	Countries or population
1/23,000 to 1/50,000	Europeans and north America
1/400,000 to 1/100,000	Chinese, Japanese, Koreans
Much higher frequency	Irish travellers

Type-1 causes build-up of galactose-1-phosphate in cells. Accumulation of galactose-1-phosphate causes many diseased effects on cells as it is well known that galactose-1-phosphate is toxic towards the cell. How it is toxic to cells is still vague. Scientists hypothesize many theories, among them the most common suggests that it deplete the phosphate stock of cell (especially ATP and other nucleotide phosphate). Later on this hypothesis proved incorrect when HepG2 hepatocytes were grown on galactose (as a sole carbon source) medium. This is because it reveals slow growth but sufficient level of ATP in cells (Davitt-spraul *et al.*, 1994). Fibroblasts of type-1 galactosemia shown stress in endoplasmic reticulum causes accumulation of unfolded proteins (Slepek, 2007). Galactose-1-phosphate also causes formation of glycoproteins and glycolipids with low levels of galactose and N-acetylgalactosamine residues (Petry *et al.*, 1991). But scientists do not get evidence on low levels of UDP-galactose in type-1-galactosemic cells (Timson, 2011).

The other type II of galactosemia is due the deficiency of another enzyme that is galactokinase (Holden *et al.*, 2004). It is delicate form of diseases. The enzyme deficiency results in change of the gene that encodes the enzyme GALTK1. In the human 20 different types of mutations are observed related to galactokinase. Due to this enzyme deficiency the more of galactitol increase in the urine and level plasma galactose elevated. Cataracts

develop in the patients and many other symptoms appear like jaundice, growth, feeding difficulties, lethargy, and poor weight gain.

Another enzyme deficiency UDP-galactose-4-epimerase results type III galactosemia (Timson, 2006). The enzyme deficiency results in mutations of the gene that encodes the enzyme UDP-galactose 4 epimerase. Different types of point mutations throughout the gene are located that are responsible for the type III. It is a genetic, sequence disease. Recently it is observed that environmental factors and many types of specific mutation with different anatomy cause this disease. Pathology of this type is variable depending upon the type of mutation present. Many types of mutation that results in decreased amount of catalytic rate constant is known as *k_{cat}* and the increased amount of protolytic sensitivity of protein. The mutation mainly occur in sequence that codes for active site of the enzyme that results in defects in protein folding due to structural change. Formerly disease was classified in the generalized, benign, severe and peripheral that has been changed due to the recent work. These types only depend on the mutation that is produced in the individuals. The treatment of this disease is very significant and it is necessary that patient must be monitored for long term (Timson, 2011).

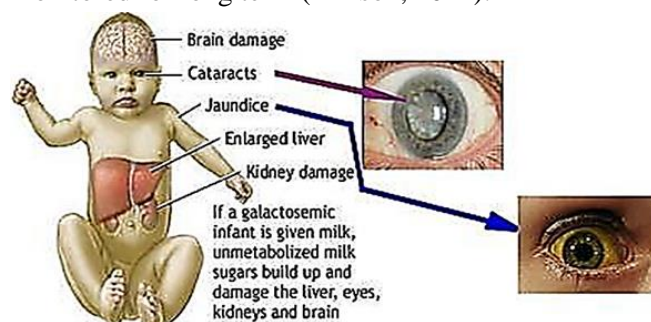


Figure 6: Symptoms of Galactosemia

The galactosemia symptoms appear in the early stage during the feeding of breast milk. The early symptoms are; vomiting, seizures, jaundice (yellow skin and the white eyes), hypoglycaemia and the liver enlargement. Some other symptoms are failure to gain weight, lethargy, irritability, poor feeding, diarrhoea, bacterial infection and convulsion. In the poor feeding children refuse the food that contains milk. If the disease is not diagnosed at new-born stage then kidney, liver, eye and brain damage may also occur. Moreover if the children are not treated at initial stage then delay in development

and delay in learning are most common in school going children. Neurological impairments in which involuntary muscles contract are known as tremors and dysmetria. In the female's ovarian failure, the premature ovarian insufficiency that is known as POI; a process in which release of the egg from the ovaries stops than the normal person. This is a major symptom of galactosemia in almost all the females (Elzouki, 2012).

IV. DIAGNOSIS OF GALACTOSEMIA

It can be diagnosed by different tests like genetic test, blood test and urine test. In the genetic test we detach pair of genes that cause galactosemia; in the child this test helps to identify the type of galactosemia moreover it is also very helpful to analyse gene changes in the child. This test is also called DNA testing for galactosemia. In the blood test for galactosemia we check the amount of enzyme in different blood cells like red blood cells and white blood cells and also in the liver. This test shows that there is no enzyme activity in the patients of galactosemia but in carrier parent has average, means half then the normal, enzyme activity. The patients who ingest the galactose, secretes it in urine where the amount of galactose can be detected. This is known as urine test for galactosemia. The blood and urine test is helpful to determine what type of treatment should be given to the patient. The galactosemia tolerance test is also useful to diagnose the disease but it is a harmful test so not preferable. During pregnancy galactosemia also diagnose by the DNA test in this we take the sample of amniocentesis or CVC. In case if this test fails then we can also do another test that is enzyme test in which we take the sample from the foetus and their sample also obtain from amniocentesis or CVC. New born screening is a test that is used for new born children for the detection of any harmful defect or disorder that have chances to appear at the birth. In this blood sample is required and by this sample we test that new-born have chances of this disease or not. This test can also be done for galactosemia, to check the amount of enzyme deficiency that causes galactosemia like GALT, GALT_K and UDP. According to the result of this we do the treatment and recommend that type of food which has no milk content to the new-born. NBS gives high risk for galactosemia when GALT enzyme activity is absent and galactose and galactose-1-phosphate levels are very high (Berry, 2012).

TREATMENT OF GALACTOSEMIA

Galactosemia could be controlled by controlling the diet. Foods that have high levels of galactose are not recommended for example dairy products and cow milk and others. Diet varies from patient to patient some are recommended to take minor amounts of plants, fruits and legumes, as these contain low sources of galactose, galactose oligomers and conjugative galactose like glycoproteins and glycolipids (Wolfrom, 1954) Plant polysaccharide secretes galactose which were produced during the ripening of fruits in the form of alpha and beta galactose (Dey & Campillo, 2006). In the food the amount of free galactose increases due to the storage and different techniques that breakdown the conjugated galactose (Hartnett, 2007). In the diet many fermented food products like miso, natto, soy sauce, sofu, tempeh etc. cannot be used for galactosemia. Some galactose rich food is mentioned in the table 3.

In the dairy food product like cheese, yogurt and others contain high concentration of galactose. But in the cow milk it is estimated that 2400 mg galactose is present in 100mL. Gruyere and the brick parmesan aged cheese contain galactose in very low amount. The patient of galactosemia must take food that is free of lactose or galactose. List of such food items are shown in Table 4. In this some other things are also included such as pre-packaged food, some candies, fruits and vegetable that have galactose, tomato sauces, some medication like capsule, tablets, sweetened liquid drops that have lactose, drugs that have lactulose, lactalbumin, casein, caseinate, curds, whey solids or whey. Moreover the new born patients of galactosemia are recommended soya protein isolate formulas. Calcium supplements and vitamin D supplements should be given to those galactosemic patients who have their deficiency (Calcar *et al.*, 2014). Following table shows the food products that could be used by galactosemic patients. It is observed now days that many disease are cause due to the inactivity of the specific enzyme reactions or steps in the new born errors of metabolism that is also called (IEM). Galactosemia is also caused by this. This is also treated by use of metabolic foods so that toxic compounds could not accumulate in body (Tang, 2012)

Table 3: List of food items rich in galactose

Sr. No.	Food
1.	Dairy-Based Products

2-	Cheddar Cheese
3-	Gruyere
4-	Emmentaler
5-	Jarlsberg
6-	Parmesan, Brick
7-	Parmesan
8-	Sodium or Calcium Caseinate
9-	Plant-Based Products
10-	Various Fruits (Raw or Processed)
11-	Various Vegetables (Raw or Processed)
12-	Fruit and Vegetable Juices
13-	Legumes
14-	Garbanzo beans
15-	(Cooked or Processed)

Table 4: List of food item allowed for galactosemic patients

Sr. No.	Food items with low galactose content
1.	Soy-based infant formulas containing soy protein isolate, amino acid-based elemental infant formulas
2.	All fruits, vegetables and their juices, pickled fruits and vegetables
3.	All legumes (e.g. navy beans, kidney beans, garbanzo beans, soybeans)
4.	Soy-based products that are not fermented (soy milk, tofu, textured soy protein, hydrolyzed vegetable protein, soy protein concentrate, meat analogues, unfermented soy sauce)
5.	Aged Cheeses: Jarlsberg, Emmentaler, Swiss, Gruyere, Tilsiter, Parmesan aged N10 months, grated 100% Parmesan cheese, sharp Cheddar cheese
6.	Sodium and calcium caseinate
7.	All cocoa products except milk chocolate
8.	Additional ingredients: natural and artificial flavorings, all gums including carrageenan Restricted Foods and Ingredients
9.	Breast milk, all milk-based infant formulas
10.	All milk-based foods and beverages except for caseinate and aged cheeses, listed above
11.	All milk-based ingredients including buttermilk solids, casein, dry milk protein, dry milk solids, hydrolyzed whey protein,

	hydrolyzed casein protein, lactose, lactalbumin, whey
12.	All cheese and cheese-based products except those listed above
13.	Organ meats, meat-by-products
14.	Soy products that are fermented (e.g. miso, natto, tempeh, sufu)
15.	Iso Fermented Soy sauce that has not been fermented is made from hydrolyzed soy protein.

FUTURE PERSPECTIVE: DEVELOPMENT OF DRUG TREATMENT OF CLASSIC GALACTOSEMIA

It is said that inhibition of galactokinase could be the treatment for galactosemia type-1 (Wierenga, 2008). Reason behind this was that it would reduce build-up of galactose-1-phosphate which is more toxic than free galactose or we can say that type II is more dangerous than type I. Recently lead compounds are discovered that could inhibit galactokinase for example IC50 (Tang, 2010). Inhibitor should not be toxic as patients have to take it for lifetime. GALK1 and GALK2 have high structural similarity so inhibitor should not inhibit GALK2 with GALK1 (Timson, 2011). Through experimentation it is also noted that other than Leloir pathway there are pathways which can metabolize galactose but pathways are unclear. Only known fact about them is that they somehow use UDP-galactose pyro-phosphorylase (Leslie *et al.*, 2005). Drug development is the challenge but further research is needed in this regard.

V. CONCLUSION

Galactosemia is a hereditary disease in which body fails to metabolize galactose. Inhibition of enzyme of Leloir pathway causes accumulation of galactose and its derivatives. Each enzyme characterizes a specific type of galactosemia which includes type I, type II, type III. Galactosemia patients show different physical and neurological impairments at early stages of their life. Uphill now there are methods to control the disorder via nutrition management i.e. using galactose free, diet mostly fermented food products. But little work is done

on drug treatment of galactosemia. There is a lot of scope in this area regarding galactosemia.

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