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# LACTOSE INTOLERANCE: GENETICS OF LACTASE POLYMORPHISMS, DIAGNOSIS AND NOVEL THERAPY

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*Lactose intolerance is a common disorder affecting an individual's ability to digest lactose present in milk or any food product. Lactose intolerance is caused by the deficiency of β-galactosidase (lactase) in the digestive tract. Diagnosis of lactose intolerance is not so simple and straightforward clinically. Many biochemical and genetic tests have been developed for the determination of lactose intolerance. Several case reports indicate wherein subjects have self-diagnosed being lactose intolerant. There is an emerging link of this disorder with human gene polymorphism, where genetic basis has been used as a diagnostic tool. The high prevalence of this condition among children and adults has compelled the production of lactose-free foods. Additionally, external enzyme supplementation has been looked at as an alternative protective mechanism in lactose intolerant subjects. This review highlights the genetic variants of lactase polymorphism and theranostic (therapeutic and diagnostic) strategies for lactose intolerance.* ***Biomed Rev*** **2014; 25: 35-44**

**Keywords:** Lactose intolerance, lactase (β-galactosidase) deficiency and persistence, LCT- gene polymorphism, biochemical and genetic tests, therapy

## INTRODUCTION

Lactose is a disaccharide present in mammalian milk and is essential for the nourishment of newborn infants. It is hydrolyzed by the intestinal brush-border enzyme, lactase, into absorbable sugars, namely glucose and galactose to provide energy. In most infants, intestinal lactase activity is maximal during the postnatal period. However, after 2–12 years of age, there occurs a segregation into two distinct groups, viz, "lactase non-persistence group" with low lactase activity (hypolactasia) and a "lactase-persistence group" of individuals who retain their neonatal level of lactase activity even in adulthood (1-3).

Lactose intolerance is caused by the deficiency of β-galactosidase (lactase) in the digestive tract. The typical

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clinical symptoms consist of abdominal pain and distension of gastrointestinal tract, borborygmi, and flatulence. Sometimes diarrhoea may occur from 30 minutes and 2 hours after the ingestion of lactose (4). Lactase deficiency results in lack of absorption of non-digested lactose, which causes luminal water retention and leads to iso-osmolarity of chyme. Consequently, excessive fluid retention causes abdominal pain, nausea, and diarrhoea. Bacterial fermentation of lactose in the distal small intestine and colon further aggravate these symptoms.

Because of the acute diarrhoeal condition that occurs after drinking milk, people who suffer from this disorder generally avoid a lactose-containing diet such as milk and milk products (5). Nevertheless, milk is calcium and nutrient-rich food and an important part of a healthy diet (6). Avoidance of milk during childhood is a significant risk factor for retarded growth and development as well as low bone density (7). Those who avoid milk, due to lactose intolerance, consume significantly less calcium and suffer from poorer health and bone formation, and higher risk of osteoporosis (7). Some studies have suggested that the prevalence of lactose intolerance is a global issue. It is estimated that the worldwide population incidence of this condition is around 30.5% in children of 11–13 years (8), and about 55.1% in Chinese adults (9). Also, high incidence of lactase deficiency has been reported among Hispanic people (50% to 80%), African-American and Ashkenazi Jewish population (60% to 80%), and is almost 100% in Asian and American Indian people (10).

As summarized in Table 1, lactose intolerance is observed in various distinct forms such as congenital, primary and secondary. Congenital lactase deficiency is associated with the least lactase activity. Congenital hypolactasia is a single autosomal recessive disorder and is a extremely rare condition (11). Primary adult-type hypolactasia is an autosomal recessive condition, resulting from the physiological decline of lactase enzyme activity in the intestinal cells, and occurs in a large proportion of individuals. A single nucleotide polymorphism, C/T-13910, 14 kb upstream the lactase gene, has recently been correlated with lactase persistence/non persistence in several populations (12, 13). Secondary causes of hypolactasia, such as celiac disease, gastroenteritis and Crohn's disease, may lead to transient lactase deficiency and

***Table 1.*** *Types of lactase deficiencies and their associated risk factors (References cited are shown in brackets)*

| **Type of lactase deficiency** | **Risk factors / causes/triggers** |
|---|---|
| Congenital lactase deficiency | This condition has been diagnosed in less than 50 people world-wide (15). It becomes apparent at birth with persistent diarrhoea soon after milk is introduced. These children otherwise have a normal intestinal mucosa (16). |
| Primary lactase deficiency | It occurs when there is a gradual reduction in lactase production. It may not become clinically evident until late adolescence (17). It's prevalent in those geographical groups where the ancestors did not drink milk as a nutrient (15, 18). |
| Secondary lactase deficiency | It occurs as a result of gastrointestinal illness that alters the nature of the gut mucosa (19). Cryptosporidiosis, giardiasis and other parasitic infections lead to lactose malabsorption; it is very common in the children with rotaviral diarrhoea (20). It may occur in association with celiac disease, Crohn's disease and HIV (21, 22). Secondary lactase deficiency may also occur due to certain drugs like tetracycline and methotrexate that causes villous atrophy. Alcohol may initiate or worsen the lactose intolerance (18). |
| Developmental lactase deficiency | Premature infants may experience this deficiency. However this condition is temporary and improves as the intestinal mucosa matures (23). |

appearance of adverse abdominal symptoms (14).

The present review highlights the genetic variants of lactase polymorphism and theranostic (therapeutic and diagnostic) strategies for lactose intolerance.

## BIOCHEMISTRY AND GENETICS OF LACTASE

Lactase is located on the apical surface (glycocalix) of brush border enterocytes where it is anchored into the membrane by its C-terminal, with the bulk of the molecule projecting into the lumen of the gut (24). It is a large glycoprotein, with two active sites, that can catalyze the hydrolysis of a variety of β-glucosides, such as phlorizin, flavonoid glucosides (25), and pyridoxine-5'-β, -D glucoside (26), and β-galactosides in addition to lactose. Lactase is encoded by a single gene (*LCT*) which is approximately 50 kbp located on chromosome 2 (27, 28). The gene has 17 exons (27) and encodes an mRNA transcript for a preproprotein of 1927 aminoacid residues (29). This is composed of a putative signal peptide of 19 aminoacid residues, a large pro-portion of 849 amino acids and a mature protein that contains two catalytic sites, and at the C-terminal, a membrane-spanning domain and short cytoplasmic domain. *LCT* shows a four-fold internal homology, which suggests that it arose by two duplication events (24). Pro-lactase is proteolytically processed to a smaller protein (30-32) and two of the four homologous regions occur in the cleaved pro-portion of the molecule, which does not have a catalytic function, but probably has a chaperone function, in that it seems to play a role in transporting the molecule to the cell surface (33-38). There is one active site in each of the domains of the mature protein. Although details have been disputed by some investigators, it is now considered that the active site at Glu1273 in domain III is responsible for hydrolysis of glucosides such as phlorizin, whereas the other in domain IV, at Glu1749, catalyzes the hydrolysis of galactosides such as lactose (39, 40).

It has been reported that lactase is O-glycosylated through serines and threonines as well as N-glycosylated (through asparagine), and this glycosylation probably affects enzymatic activity as well as folding and intracellular transport (41). Lactase expression is restricted to the enterocytes of small intestine, at highest level being in the mid-jejunum (42).

## LACTOSE INTOLERANCE SYMPTOMATOLOGY

### *Gut related symptoms*

Hypolactasia may not cause any discomfort unless lactose-containing food is consumed. Colonic microflora ferment undigested lactose in the intestinal lumen, which leads to production of short-chain fatty acids, hydrogen, carbon dioxide, and methane as by-products causing flatulence, bloating and abdominal pain. Undigested lactose acidifies the colon and increases the osmotic load, resulting in formation of foamy, voluminous and aqueous stools (43). However, some patients can experience constipation due to decreased intestinal motility, possibly caused by production of methane (43).

### *Other symptoms*

The clinical presentation of lactose intolerance is not just restricted to gut symptoms. Other complaints such as headache, vertigo, memory impairment, lethargy, muscle and joint pains, allergy, cardiac arrhythmia, mouth ulcers, and sore throat have been reported (4, 44). Colonic bacteria generate toxic metabolites by lactose fermentation such as acetaldehyde, ethanol, acetone, peptide and protein toxins, which can alter many cell signalling mechanisms and are possibly responsible for these symptoms (44).

There is a considerable individual variability in the severity of symptoms, depending upon the amount of lactose ingested and the patient's ability to digest it. Factors contributing to this variability include osmolarity and the fat content of lactose-containing food, ability of colonic microflora to ferment lactose, gastric emptying rate, colonic water absorption capacity, intestinal transit time and individual perception of abdominal pain and discomfort (43, 45).

## EMERGING LINKS WITH GENETICS: LACTASE PERSISTENT ALLELES AND LACTASE POLYMORPHISMS

As indicated above, the *LCT* gene is 49.3 kbp in length and is located on the long (q) arm of chromosome 2 at position 21. It contains 17 exons and is translated into a 6 kb transcript (27-29). Individuals with hypolactasia and lactase persistence have identical coding sequences, except for a few silent mutations; hence, both lactases are identical (46). Two variants were found to be associated with lactase persistence: A polymorph variant, *LCT*-13910C.T, in intron 13 of the *MCM6* gene that is 13 910 bp from the initiation codon of *LCT*, while the *LCT*-22018G, a variant in intron 9 of *MCM6* gene upstream of the *LCT* locus 22,018 bp was associated (1, 2, 47).This association was confirmed in a study of DNA collected from subjects of Finnish, South Korean, Italian, German, French, or Caucasian or African-North-American descent (1,47). Both genotypes of *LCT*-13910CT and *LCT*-13910TT were associated with the lactase-persistence pheno-

type, indicating that the presence of one single lactase-persistence allele in the heterozygous state has a dominant effect, rendering the person a lactose digester (48).

## PROMOTOR SEQUENCE STUDIES

Functional *in vitro* studies of these polymorphic alleles have shown that *LCT*-13910T (1, 49, 50), *LCT*-13907G, *LCT*-13915G, and *LCT*-14010C act as enhancers of the *LCT* promoter (51). These effects are most likely mediated by the Oct-1 transcriptional factor binding site in the variant enhancer and by HNF1α binding in the *LCT* promoter (49, 52). *LCT* gene regulation of lactase-persistence alleles occurs at the transcriptional level. *LCT* mRNA levels, which are distinguished by polymorphic markers in the coding region of *LCT*, were several times higher in individuals with *LCT*-13910T/-22018A alleles than in individuals with *LCT*-13910C/-22018G alleles (1). It has also been generally agreed that nonpersistent individuals have lower levels of lactase mRNA [53-55].

Numerous transcription factors (Cdx2, GATA-4, GATA-5, GATA-6, and HNF1α) activate the *LCT* promoter in intestinal cell culture at the -100 to -20 bp binding site regions of *LCT* which are repressed by PDX-1 (1). Mutation of the PDX-1 binding site does not prevent *LCT* promoter repression, which suggests that PDX-1 might function by binding to another DNA binding site or by inhibiting other transcriptional factors. PDX-1 over expression resulted in strong repression of Cdx2 and HNF1α activation of the *LCT* promoter (1). However, the exact mechanism for down regulation of *LCT* after weaning still remains unknown.

## CONGENITAL LACTASE DEFICIENCY

While secondary loss of lactase in children is a frequent problem, resulting from viral infection and allergy (56), true congenital deficiency of lactase is very rare indeed. Congenital lactase deficiency is one of the severe gastrointestinal disorders characterized by watery diarrhoea shortly after the first feed with breast milk or lactose containing formulas (57). It is a rare autosomal disorder which occurs due to mutations in the coding regions of lactase, LCT gene. The LCT gene consists of 17 exons encoding 1927 amino acids comprising four homologous domains I-IV. Domain IV harbours the lactase activity mutation in exon 9 and is responsible for the truncation of lactase. One such case was recently reported in a Japanese female infant who had two mutations in the LCT gene in a heterozygous form: c.4419C> G (p.Y1473X) in exon 10 and c.5387 delA (p.D1796fs) in exon 16, these mutations occurred in domain IV and was considered causative for congenital lactase deficiency (58).

## FALLACIES FOR SELF-DIAGNOSIS OF LACTOSE INTOLERANCE

Due to an increased public awareness about the prevalence of lactose intolerance, there have also been several misunderstandings about self-diagnosis (59). In addition, there is also a little understanding that lactose *maldigestion* (incomplete lactose digestion due to low levels of lactase) is not synonymous with lactose *intolerance* (symptoms such as bloating, cramps and diarrhoea that may or may not occur in association with undigested lactose in the intestinal tract). Many studies have identified that the majority of those with lactose maldigestion do not experience the symptoms of lactose intolerance after consuming moderate quantities of lactose (60-70), and consequently avoid the lactose containing foods. One such food is milk, which is the richest source of calcium, vitamins, fatty acids, and low intake of milk can lead to serious health complications, including chronic disease like osteoporosis (59). On the contrary, it has also been reported that consuming dairy foods may actually contribute to improve the tolerance to lactose. Thus, individuals with self-reported lactose intolerance are unlikely to meet the appropriate levels for calcium from food sources alone. Dietitians and other health professionals can play an important role in informing these individuals about how to include lactose-containing foods without experiencing adverse symptoms, as well as providing information on other food sources of calcium, and further if needed, advice on appropriate supplementations.

## IMPLICATIONS OF LACTOSE INTOLERANCE AND OTHER DISEASES

In addition to being a major cause of inconvenience in itself, lactose intolerance could be associated with other health disorders like cystic fibrosis, an autosomal recessive disorder characterized by the loss of function of the cystic fibrosis transmembrane conductance regulator (CFTR) (71, 72). This occurs due to more than 1700 different mutations in the *CFTR* gene, the most common of which is the loss of phenylalanine at the 508 position of the CFTR protein resulting in its misfolding, a faulty posttranslational processing and endoplasmic reticulum regulated degradation (73). Reduced bone mineral density is a common malady affecting cystic fibrosis patients. Since, meat cannot by itself serve as a nutritional source (due to absence of pancreatic elastase-1 in

pancreatic supplements, an enzyme required to digest elastin fibres in meat) for exocrine pancreatic insufficient cystic fibrosis patients, the only alternative is dairy products (71). Although not typical for cystic fibrosis, lactose intolerance can hamper dairy product consumption and fail to help cystic fibrosis patients to increase their bone mineral density in severe cases.

Lactose intolerance could also be associated with epilepsy, which affects about 45 million people across the globe (74-76). Neural cells contain glycosphingolipids whose biosynthesis requires galactose which is derived from lactose and other carbohydrates. Lactose intolerance impairs the production of galactose which could lead to malfunctioning of neurons resulting in epileptic episodes (77).

## DIAGNOSIS OF LACTOSE INTOLERANCE

Previously, the most reliable method available for detecting lactose intolerance was a direct biochemical assay of lactase activity from a jejunal sample which was performed with a glucose oxidase reagent, that detects glucose liberated from lactose, with a cut off value of 10 U/g protein (1,2). However due to the invasiveness of jejunal biopsy, this method was then replaced by endoscopic duodenal biopsy (78,79).

Lactose tolerance tests have been developed to confirm the ability of intestinal lactase to hydrolyze lactose so as to avoid intestinal biopsies. In this technique, blood glucose levels are measured before and after an oral load of lactose at prespecified time intervals, with a maximum rise of 20 mg/dL, indicating lactose tolerance (80). Oral ethanol administration before lactose load is used to inhibit galactose metabolism for the determination of the blood maximum rise of glucose (at least 20 mg/dL) and galactose (at least 10 mg/dL), thereby indicating lactose tolerance. Thus, galactose concentration in combination with glucose concentration improves the correlation with jejunal lactase activity than using only glucose maximum rise after lactose load (81).

Despite of all the indirect lactose tolerance tests currently available, breath hydrogen concentration after ingestion of 50 g of lactose was considered the most suitable test for population screening for lactase deficiency (82). However, the use of the 50 g lactose dose has been criticized, because it is equivalent to 4–5 cups of milk, an amount that is ideally far more than an individual can usually ingest at one time, so an oral load of 25 g may be considered a more appropriate amount, with high sensitivity and specificity (80,83).

Interestingly, the discovery of lactase-persistence alleles resulted in the advent of genetic tests for diagnosis of lactase non-persistence by polymerase chain reaction restriction fragment length polymorphism (84-86), real-time polymerase chain reaction [87-89], and pyrosequencing technology (90). Compared with the lactose hydrogen breath test, the genetic test has numerous advantages such as it is simple, non-invasive, and more comfortable examination that does not provoke symptoms of lactose intolerance and is less cumbersome (85). However, other polymorphic variants in Europeans (*LCT*-13914G.A)50 and in African and Arab populations (*LCT*-13907C.G, *LCT*-13913T.C, and *LCT*-13915T.G, close to *LCT*-13910C.T,) affect the diagnostic accuracy of *LCT*-13910C.T typing by altering the melting profiles of the real-time polymerase chain reaction kit [89]. The reverse-hybridization strip assay based on multiplex DNA amplification and ready-to-use membrane test strips that detect *LCT* polymorphic variants (-13907C.G, -13910C.T, -13913T.C, -13914G.A, -13915T.G, and -22018G.A) and inevitably represents a reliable tool for genetic diagnosis of lactase non-persistence helps in overcoming the interference of different melting profiles of the real-time polymerase chain reaction kit by the other polymorphic variants (91). The genetic test provides a more direct result, i.e., a hypolactasia or lactase persistence genotype, whereas interpretation of the lactose breath test depends on several variants such as the cut off level, dose of lactose given, and duration of the test and age of the individual, and is also expensive.

## NOVEL TREATMENT INTERVENTIONS FOR MANAGEMENT OF LACTOSE INTOLERANCE

The initial recommendation for management of lactose intolerance is to aim for remission of symptoms through temporary avoidance of milk and dairy products. Most individuals with lactose malabsorption can tolerate up to 12 g of lactose without significant symptoms. After the initially restricted diet, lactose should be gradually reintroduced until the patient's threshold for symptoms is reached (92). The main pharmacological measures include lactase supplements, lactose-hydrolyzed or lactose-reduced milk, probiotics (93), and colonic adaptation.

## NON-LACTOSE INFANT FORMULAS

Infants who cannot tolerate any lactose may only be treated by excluding lactose from diet. This could be achieved by using lactose-free infant formulas or incubating feeds with the enzyme lactase which can break the sugar into its component

parts. In case of primary lactose intolerance where the degree of lactase deficiency varies, the use of lactose-free formula may help to relieve symptoms. In lactose-free formula, the carbohydrate source is glucose rather than lactose, therefore these milk products have a greater potential to cause dental caries. This is because lactose is a non-cariogenic sugar whereas glucose is cariogenic (94).

Soy-based infant formulas have been often preferred to lactose-free formula. However, they may not be safe because contain phytoestrogens; note, soy formulas are no more in usage for infants. A recent report suggests that phytoestrogens in soy infant formulas are capable of inhibiting the action of an enzyme involved in iodination of thyroxine (thyroid peroxidase, TPO) through competitive inhibition (95). It has not yet clearly established that the levels of free phytoestrogen in infants' plasma are sufficient to significantly inhibit TPO. Hence, the clinical significance of phytoestrogen consumption in the presence of adequate iodine intake still remains unclear.

## ENZYME THERAPY: ENDOGENOUS β-GALACTOSIDASE TO ALLEVIATE LACTOSE INTOLERANCE

In addition to decreasing the lactose concentration in milk products, there have been several techniques that have been used to produce low-lactose milk, such as β-galactosidase enzymes hydrolysis techniques or combinative techniques with ultrafiltration and enzymes hydrolysis (96, 97). However, they have also been known to alter the quality of the milk products and their commercial value (98). Enhancing the intestinal β-galactosidase activity of lactose intolerant subjects has gained more importance. Exogenous β-galactosidase was usually prescribed for lactose intolerant subjects. However, most of the supplemental β- galactosidase displayed poor stability in human gut. Interestingly, the endogenous β-galactosidase expressed in intestinal microbes has been reported to help humans in lactose usage (99), and is one of the promising treatment strategies, since it is associated with the promotion of beneficial microorganism in the gut (100). Therefore, the enhancement of β-galactosidase in the intestinal microflora of humans together with selected probiotics may be a promising approach in lactose intolerance management. An evidence to this fact was provided by a recent *in vivo* study conducted to evaluate the alleviation of lactose intolerance symptoms in post-weaning Balb/c mice, which were orally administered with 1×106 CFU or 1×108 CFU of *L. lactis* MG1363/FGZW daily for 4 weeks before lactose challenge. It was observed that in comparison with naïve mice, the mice administered *L. lactis* MG1363/FGZW showed significant reduction of diarrhea symptoms accompanied by lesser weight of total feces within 6 h post-challenge and suppressed intestinal motility after lactose challenge.

## CONCLUSION

We have highlighted the genetic variants of lactase polymorphism and theranostic strategies for lactose intolerance. A significant progress has been made in our understanding of lactose intolerance. Management of lactose intolerance has improved over the years with the introduction of newer and better options which help to bypass the inevitable pitfalls of simple lactose avoidance. There is no single gold standard test available for the diagnosis of lactose intolerance. The lactose breath test, although considered the best method may be influenced by several factors. Genetic testing has been a new tool for the diagnosis of hypolactasia/lactase persistence, but may not detect all the single nucleotide polymorphisms associated with this disorder. It appears that up to 12 g of lactose is well tolerated by lactase non-persistence individuals, which may negate the need for restrictions on lactose-hydrolyzed milk, fermented and matured milk products, hence preventing any subsequent deleterious effects on bone mass density.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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