Review

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New insights into the molecular basis of lactase non-persistence/ persistence: a brief review

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SUMMARY Lactose, a disaccharide and main carbohydrate in milk, requires hydrolysis in the intestinal tract to release its monosaccharides galactose and glucose for use as energy source by enterocytes. This hydrolysis is catalyzed by the enzyme lactase, a β-galactosidase located in the brush border membrane of small intestinal enterocytes. In most mammals, lactase activity declines after the weaning, a condition known as lactase non-persistence (LNP). Lactase persistence (LP) is an autosomal dominant trait enabling the continued production of the enzyme lactase throughout adult life. Non-persistence or persistence of lactase expression into adult life being a polymorphic trait has been attributed to various single nucleotide polymorphisms in the enhancer region surrounding lactase gene (*LCT*). However, latest research has pointed to 'genetic-epigenetic interactions' as key to regulation of lactase expression. LNP and LP DNA haplotypes have demonstrated markedly different epigenetic aging as genetic factors contribute to gradual accumulation of epigenetic changes with age to affect lactase expression. This review will attempt to present an overview of latest insights into molecular basis of LNP/LP including the crucial role of 'genetic-epigenetic interactions' in regulating lactase expression.

Keywords Lactase non-persistence, lactase persistence, genetic-epigenetic interactions

1. Introduction

Lactase (EC 3.2.1.23.62), a bifunctional enzyme having lactase and phlorizin hydrolase activities is an integral glycoprotein of the microvillus membrane of small intestinal enterocytes (*1,2*). Lactase activity is responsible for hydrolysing the milk sugar lactose to glucose and galactose, phlorizin hydrolase activity is responsible for hydrolysing aryl and alkyl β-glycosides to phlorizin and β-glycosylceramides (*3-5*). The gene encoding lactase (*LCT*) is about 50 kb, composed of 17 exons, has a one kb promoter region preceding it and maps physically to chromosome 2q21 (*6-8*). Lactase is synthesized as a pre-pro enzyme having cleavable signal peptide, large pro-part, domains containing phlorizin hydrolase/lactase active sites and a short intracellular domain at the C-terminus (*9,10*). Processing of lactase to pro and mature form involves cleavage of signal peptide, formation of homodimers and glycosylation in ER/Golgi complex. During intracellular transport, the pro-lactase protein is both *N*- and *O*-glycosylated in the ER and the Golgi complex. This glycosylation is necessary for both intracellular transport and enzymatic activity (*11,12*). Subsequently mature lactase is sorted to the apical membrane of the enterocyte (*13,14*) (Figure 1).

Lactase is a critical enzyme for neonates that depend on their mother's milk for nourishment. In mammals, the normal course of events for the newborn is to subsist on milk over the first few months of life and then be weaned. Thus, mammals have evolved a developmental pattern of small intestinal gene expression that promotes high level production of lactase early in life, followed by a turn-off of lactase expression around the time of weaning. This is indeed what happens in almost all mammals, including most humans. In the majority of human populations, lactase activity declines after weaning, a condition known as lactase non-persistence (LNP or adult-type hypolactasia) (*15*). The age of onset of down-regulation is different in different populations (*16,17*). Lactase persistence (LP) is an autosomal dominant trait enabling the continued production of the enzyme lactase throughout adult life. In general, the frequency of lactase persistence is high in European populations, whereas it is low in the native populations of Australia, America, Africa, and Asia (*14*).

2. Lactase non-persistence (LNP)

Hypolactasia is a general term for very low activity of lactase in the jejunal mucosa. It may occur due to down-

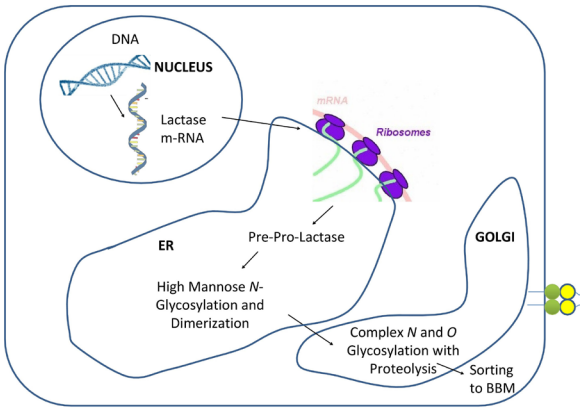

Figure 1. Transport and processing of lactase through ER and Golgi.

regulation of lactase activity after weaning (lactase non-persistence; LNP) or damage to intestinal mucosa leading to secondary lactase deficiency. Total lack of lactase activity from infancy is known as congenital lactase deficiency (CLD). In subjects with hypolactasia, most of lactose remains un-hydrolyzed causing an osmotic load in the small intestine that leads to influx of water into the lumen contributing to rapid intestinal transit (*18*). LNP is a genetically determined condition. It is the ancient phenotype and is characterized by decline in lactase activity during childhood (*19*). LNP causes primary lactose malabsorption (*19*). Secondary lactose malabsorption is caused by other reasons than genetically determined LNP, such as microbial infections, celiac disease or malnutrition that damage the intestinal villi (*20*). CLD is an autosomal recessive inherited severe gastrointestinal disorder in newborns. CLD manifests as a watery diarrhea during the first days of life of an infant fed with lactose-containing milk making them underweight with symptoms of dehydration and acidosis (*21,22*).

LNP is an autosomal recessive gastrointestinal condition that is the result of a decline in the activity of lactase in the intestinal lumen after weaning. Down-regulation of lactase is considered as a normal phenomenon among mammals, and symptoms are remarkably milder than experienced in CLD (*23*). Wang *et al.* (1995) studied the onset of LNP in children aged from 2 months to 11 years (*24*). Genetically programmed down-regulation of the lactase gene was observed starting from the second year of life, although the extent and onset was not constant (*24*). They concluded that a developmentally regulated *trans*-acting DNA-binding protein could bind to only one kind of lactase allele and influence transcription and/or mRNA stability (*24*). Various studies have pointed to a wide ethnic and regional variation in the age of onset of LNP. In majority of Thai children lactase activity decreases by the age of two years, in black populations LNP has been shown to manifest between one to eight years, whereas in white populations low lactase levels are rarely seen in children less than five years of age (*25-27*). Among Indian children, the onset of down-regulation of lactase expression and activity is between 3-5 years of age and it is complete between 7-8 years (*28*). Studies of the Finnish population have shown that LNP can manifest up to 20 years (*26*). However, data has also confirmed that the majority of Finns developed LNP by age of 10 years (*29*). In South America, Africa and Asia, over 50% of the population has LNP and in some Asian countries this rate is almost 100% (*4*).

3. Lactase persistence (LP)

LP is an autosomal dominant trait enabling the continued production of the enzyme lactase throughout adult life (*3*). LP is common among people of European ancestry, but with the exception of some African, Middle Eastern and Southern Asian groups, is rare or absent elsewhere in the world (*30*). To explain the highly geographic variations in the prevalence of LP, various researchers have produced some hypotheses to explain these variations focused on some selective factors related to milk supply because it is the only source of lactose, substrate of the lactase enzyme. These hypotheses are: (1) The culture historical hypothesis based on genetic selection and correlates the occurrence of LP with dairy culture (*31,32*); (2) The calcium absorption hypothesis put forward to explain the prevalence of LP in Northern Europe and based on fact that lactose could enhance absorption of calcium and thus individuals with LP will have less rickets and pelvic deformities resulting in a selection in favour of LP (*33*); (3) The arid climate hypothesis speculated that in desert climates (Middle and near East) where water and food were scarce, nomadic groups could survive by utilizing milk as a food source, and in particular, as a source of clean, uncontaminated water (*34*). In a phylogenetic approach, correlation of high lactose digestion frequency was tested with percentage dependence on pastoralism, levels of solar radiation and dry months/year or average rainfall and adjusted for relatedness in the analysis (*32*). These data revealed that percentage reliance on pastoralism best explained the variation observed between populations and concluded that lactose digestion capacity had most likely evolved as an adaptation to dairying, and that high frequency lactose digestion capacity had not evolved in the absence of milking (*32*). The co-evolution of genes for LP and milk consumption also becomes one of the most well-known gene-culture models for human evolutionary change (*35*). Table 1 presents the frequencies of various LP alleles around selected countries/populations of the world [as reviewed by Mattar *et al.* (2012) (*36*)].

4. Genetic-epigenetic interactions as molecular basis of LNP/LP

Table 1. Frequencies of various LP alleles around selected countries/populations

Country/Population	Allele Type and Frequency (%)				
	LCT –13910C>T	*LCT* –13915T>G	*LCT* –14010G>C	*LCT* –13907C>G	*LCT* –14009T>G
Finland	58.1	-	-	-	-
Tanzania	-	-	31.9	-	-
Sudan (Afro-Asiatic Beja)	-	-	-	20.6	-
Italy (North-East)	23.7	-	-	-	-
Italy (North-Central)	13.3	-	-	-	-
Italy (Southern)	5.5-8	-	-	-	-
Ethiopia (Amharic)	-	13.2	-	-	-
Sudan (Jaali)	-	14.2	-	-	6.6
Saudi Arabia	-	59.4	-	-	-
Ethiopia (Afar)	-	15	-	20	-
Ethiopia (Somali camel herders)	1.9	5.1	0.5	5.6	1.4
Xhosa (South Africa)	-	-	12.8	-	-
Sardinia	7.2	-	-	-	-
Jordan	-	39.1	-	-	-
Chile (Amerindians)	5.8	-	-	-	-
Xhosa (mixed ancestry)	-	-	8.1	-	-
Ethiopia (Somali camel herders)	-	-	0.5	-	-
Brazil (Caucasian)	24.7	-	-	-	-
Brazil (African origin)	18.3	-	-	-	-
Kenya	-	-	27.6	-	-
Estonia	51.4	-	-	-	-
Canary Islands	36.5	-	-	-	-
Chile (Hispanics)	22	-	-	-	-
Hungary	35.9	-	-	-	-
Russia (Northern)	38.9	-	-	-	-
US (African origin)	9	-	-	-	-
Mali (Fulbe)	37	-	-	-	-

Research on molecular basis of developmental down-regulation of lactase has been going on for a long time but the causes remained elusive. Initially, decreased production of lactase, synthesis of an inactive high molecular weight lactase and defective post-translational modifications were the factors attributed to down regulation of lactase activity after weaning (*37-40*). However, subsequent research indicated that a genetic polymorphism controlled by an element which acts in *cis* to the lactase gene might be responsible for LNP/LP trait (*41,42*). Evidence that LNP/LP is controlled by a *cis*-acting regulatory variant upstream of *LCT* came from linkage studies in the Finnish families (*43*). Total of 52 non-coding variants were identified using sequence analysis of the 47 kb region upstream of *LCT*. Two of the single nucleotide polymorphisms (SNPs), C/T –13910 (rs4988235), in intron 13 and G/A –22018 (rs182549), in intron 9 of *MCM6* gene (minichromosome maintenance 6) upstream of the *LCT* locus showed complete co-segregation with LNP/LP trait (*43*). Further studies revealed several new sequence variants in very close proximity to –13910*T (*44-47*), two of which are clearly associated with LP in different parts of East Africa (–13915*G and –14010*C). One of these, -13915*G, was also shown to be associated with high lactase expression in Saudi Arabia (*48*). A third SNP, –13907*G, revealed much weaker evidence, but was found in several studies and there were several other candidates found in lactase persistent or milk drinking people (*44-47*). Positive selection for LP which allows the dietary consumption of animal milk by adult humans without risk of symptoms of lactose intolerance has been attributed to present day frequencies of these alleles (*49-52*). Population expansion, migration, allele surfing and cultural/environmental processes may also have influenced the distributions of these alleles (*49,52*).

Transfection studies of promoter-reporter gene constructs, DNA-protein and protein-protein interaction have explored the functional significance of these SNPs in regulating the lactase expression (*53,54*). It has been proposed that multiple transcription factors and their interactions with *LCT* immediate promoter influence the decline of the lactase enzyme after childhood (*54,55*). A transcription factor, Oct-1, was identified which bound more strongly to the –13910*T containing motif than to the alternative *C* allele, providing a possible mechanism for up-regulation of *LCT* expression (*55*). In addition, binding sites to intestinal transcription factors GATA-6, HNF-4α, Fox and Cdx-2 were also identified in the –13910 region, providing further support that this region underlies the developmental regulation of lactase expression in human (*55*). In a recent study on *in-vitro* functional analyses of infrequent nucleotide variants in the lactase enhance, four allelic variants were chosen for *in-vitro* functional tests (*56*). These include (1) –14009*G (rs869051967), selected because it was strongly associated with digester status (*57*); (2) –14011*T (rs4988233), although too rare to test for

association, was analyzed as it is located immediately adjacent to the known functional variant –14010*C (*58*); (3) –13779*G (rs527991977) was of interest because it was found to be relatively common in some groups in India including milk drinking Toda and some hunter-gatherers (*58*); (4) –14028*C (rs759157971) had previously been found as the only *LCT* enhancer allele identified in the second highest expressing transcript of a homozygous lactase persistent person (*45,59*). Results indicate a clear effect on promoter activity upregulation as assessed by transfection assays in case of –14009*G and –14011*T, but the molecular interactions leading to such effects may be different (*56*). For –14028*C variant, the results suggest a clear change in transcription factor binding, but no obvious effect in transfections and –13779*G variant displays greater effect in transfections but less on transcription factor binding (*56*). Independent haplotypic backgrounds with different geographic distribution gave rise to each of the four variants (*56*).

Epigenetics is described as inherited alterations in gene expression or silencing that take place without changes in DNA sequence (*60,61*). Epigenetic mechanisms are often attributed to transcriptional variation within the same cell type, determining cell identity and affecting genomic functions in response to aging and environmental cues (*62-65*). DNA methylation is critical for the regulation of gene expression during differentiation in many self-renewing tissues, including the germline and embryonic, hematopoietic, and epidermal stem cells (*66,67*). Being one of the most rapidly renewing tissues in the human body, cooperation between transcription factors, signaling pathways and epigenetic mechanisms is essential for the tight control of the constant renewal of intestinal tissue (*68,69*).

Recently, the role DNA methylation in LNP/LP has also been reported (*70*). It was found that LNP occurs due to DNA variation dependent accumulation of methylation with the age. Research suggests that LNP haplotypes containing the –13910*C allele accumulates modified cytosine's that silence the regulatory elements in *LCT* rather than the haplotype containing the –13910*T allele (Figure 2). This genetic dependent epigenetic aging may account for age specific down-regulation and inter-individual variation of lactase activity in different human populations. Therefore, individual genetic landscape sets the epigenetic clock for regulation of lactase expression (*70,71*). Labrie *et al.* (2016), employing high density tiling microarrays, performed chromosome-wide profiling of DNA modification consisting of methylation and other epigenetic cytosine modifications followed by targeted bisulfite sequencing-based interrogation of the human and mouse lactase genes in intestinal cells and other tissues (*70*). Results indicated that gradual decline in lactase gene expression following infancy in mammals may be directed by changes in DNA modification densities at several distinct regulatory elements (*70*). This was followed by exploration of how genetic factors including C/T –13910 SNP containing haplotypes contributed to the epigenetic aging and could impact age-specific changes in epigenetic marks of *LCT-MCM6* (*70*). Epigenetically controlled regulatory elements for the lactase gene were validates using RNA interference (RNAi) in human tissue culture and CRISPR-Cas9-induced genetic deletions in the mouse models (*70*). It was revealed that accumulation of transcriptionally suppressive epigenetic changes on haplotypes carrying the –13910*C allele led to LNP, while haplotypes containing –13910*T allele escape from inactivation to facilitate LP (*70*). 35 CpG sites which clustered into seven distinct regions: *LCT* intron 5, intron 3, intron 2, exon 1, *MCM6* exon 17, exon 16 and intron 13 displayed significant DNA modification differences. Each of the seven regulatory regions had age-related DNA modification changes in the enterocyte samples stratified for C/T –13910 genotypes (*70*). Individuals with –13910*C allele of LNP exhibited a 4-fold higher density of modified cytosine's at *MCM6* intron 13-exon 13 compared to the –13910*T individuals with LP (*70*). Another study examined transcriptional and epigenetic variation of the *LCT* in enterocytes along the proximal-to-distal axis of the mouse small intestine (*72*). Aging and environmentally induced changes enabled by divergent epigenetic programming in gene transcription occurring in cells of the same type were reported (*72*). *MCM6* exon 13-intron 13 site emerged as a key modulator of the age-dependent establishment and maintenance of the *LCT* transcriptional gradient (*72*). Thus, aging and environmentally induced gradients of *LCT* mRNA have been supported by DNA modification patterns which could potentially affect phenotypic outcome by modifying transcriptional programs within same cell types (*72*). DNA modifications and chromatin

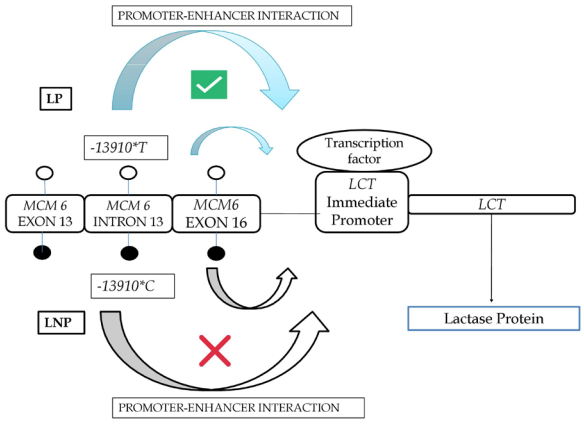

Figure 2. Relationship between lactase expression, promoter enhancer interactions and methylation status of selected regulatory sites where DNA variation dependent accumulation of methylation occurs with the age [As first reported by Labrie *et al.* (2016) and reviewed by Swallow and Troelsen (2016)]. Filled circles represent methylated DNA.

architectural protein CTCF (CCCTC-Binding Factor) may work in tandem with transcription factors to create and maintain age-dependent transcriptional gradients of *LCT* in cells of the same type (*72*). Furthermore, a recent study identified putative lactase meQTLs (methylation quantitative trait loci), which are differentially methylated between lactase persistent and lactase non-persistent individuals. –13910*T allele in genetically homogenous populations appears to be dominant, leading complete correlation of LP with the presence of the variant allele and tri-modal distribution in lactase enzymatic activities. However, methylation at the *LCT* enhancer and the *LCT* promoter are both affected by the genotype at rs4988235, and appear to be continuously associated with lactase phenotypes in heterogeneous populations (*73*). DNA methylation, rather than differential regulation of intestinal transcription factors like CDX2, POU2F1, GATA4/6 or HNF1α, in the enhancer and promoter site of the *LCT* gene was predictive of LNP/LP as indicated by regression (*73*).

5. Conclusion

Being a multifactorial phenomenon, regulation of lactase expression may involve complex spatial and developmental patterns in the small intestine. New avenues for basic and applied research in genetics of LNP/LP were unraveled with identification of the C/T –13910 and G/A –22018 polymorphisms. However, with recent advances in genomics and increasing role of DNA modifications (like DNA methylation) in tissue development and progression of many common diseases, genetic-epigenetic interactions may be pivotal to uncovering the molecular origin of complex disease (*74*). In this context, investigation of age-dependent epigenetic changes for disease-associated genetic variants has provided new insights into molecular basis of LNP/LP. Latest research into molecular basis of LNP/LP has made it clear that genetic variation seems to be setting up the stage, either allowing or preventing DNA modification accumulation with age at key regulatory sites (*70,72*). It would be interesting to further investigate and elaborate how an escape from inactivation is brought about with age-related epigenetic changes. In future, examining DNA methylation profiles of fetuses (with low lactase expression for all haplotypes) and of children (with high lactase expression for all haplotypes) could provide fascinating details into genetics induced epigenetic regulation of lactase expression.

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