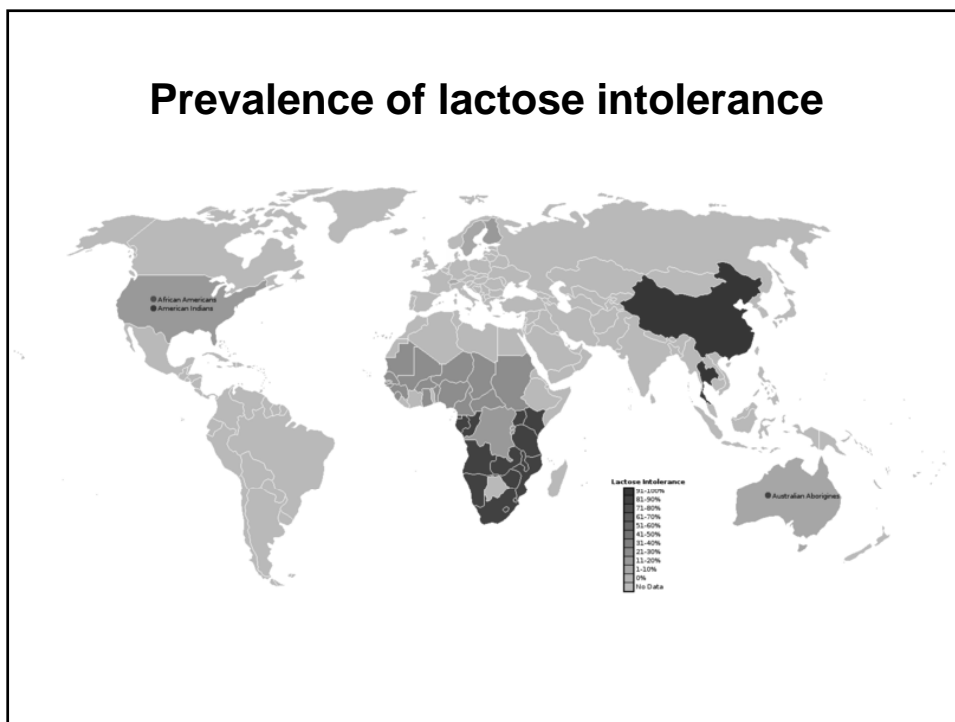


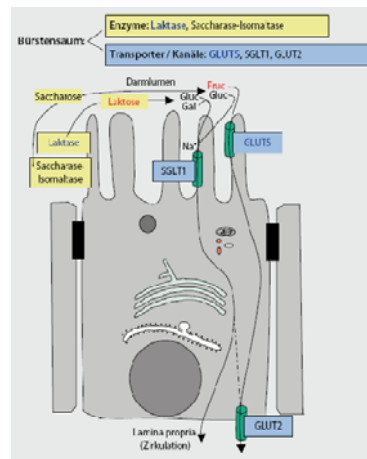
Health consequences of adult-type hypolactasia



Prevalence of lactose intolerance



Lactose intolerance



... is an inability to digest and absorb lactose.

Fig. X Molecular pathophysiology of lactose intolerance.

Zimmer, K.P.(2007) Monatsschr Kinderheilkd 155:565-567

Terminology and classification of human lactase deficiencies

Different types:

- a) Primary lactase deficiency (in which the lactase enzyme is selectively deficient):
 - Congenital lactase deficiency (lactase enzyme is almost nonexistent in the intestine of the newborn)
 - Adult-type hypolactasia (lactose non persistence – lactase enzyme is physiologically decreased in adulthood to a level about 1/10 of that in newborn)

Terminology and classification of human lactase deficiencies - II

b) Secondary lactase deficiency: (the lactase enzyme activity is affected with other disacchridase enzymes of the epithelial cells)

-> due to an injury to intestinal mucosa

- Inflammatory bowel disease
- Coeliac disease
- Acute enteritis
- Tropical sprue
- Parasitic infections
- Oral medicines (neomycin..)
- Gamma irradiation

Lactase deficiency

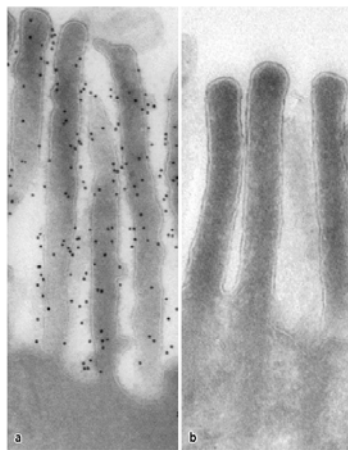


Fig. X Immunoelectronmicroscopy – location of lactase in the brush border membrane; a) lactase expression; b) lactase deficiency

Zimmer, K.P.(2007) Monatsschr Kinderheilkd 155:565-567

Congenital lactase deficiency (CLD)

- is an autosomal recessively inherited severe gastrointestinal disorder (pathological) in newborns
- so far 50 patients in 42 families have been diagnosed in Finland and several cases have been reported elsewhere in the world
- CLD has an incidence of 1:60000 in the Finnish population
- CLD is one of the 36 rare monogenic disorders enriched in Finland

Congenital lactase deficiency (CLD)

The birthplaces of great-grandparents of 31 Finnish CLD families demonstrate that CLD mutation is emphasized in central Finland



Figure x. The birthplaces of the great-grandparents of 31 CLD families in Finland. The figure is adapted from Järvelä et al. (1998).

Clinical features of CLD

- Pure watery diarrhea after first doses of breast fed milk
 - Feces are acidic (pH 4.5-5.5) and contain large quantities of lactose as a result of undeveloped bacterial flora
 - At the age of 1.7 years or older lactose is absent in feces (probably fermented by colonic bacterial flora – creating flatulence and abdominal pains)
- > severe diarrhea followed by dehydration, metabolic acidosis and weight loss are usually diagnosed during the first weeks or month of life!

Adult-type hypolactasia

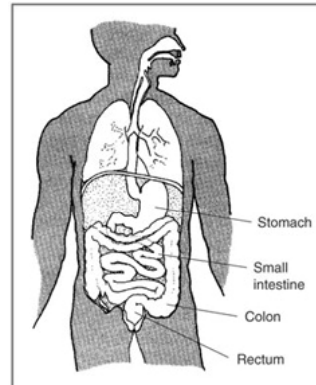
- unlike CLD not a disease but part of the normal development of mammals
- autosomal recessive gastrointestinal condition that is the result of a decline in the activity of lactase in the intestinal lumen after weaning (physiological)
- down-regulation of lactase is considered as a normal phenomenon among mammals, and symptoms are remarkably milder than experienced in CLD
- however, some humans have the ability to maintain lactase activity and digest lactose throughout their lives (lactase persistence)

Adult-type hypolactasia

...leads to symptoms of lactose intolerance when a person with low lactase activity consumes lactose-containing food.

Classical symptoms:

- Abdominal bloating and pain
- Fullness
- Cramps
- Borborygmi
- Flatulence
- Loose stools
- diarrhea



Adult-type hypolactasia - Symptoms

... the severity of the symptoms has a correlation with the amount of lactose consumed, and:

- the diet with which lactose is consumed
- the rate of abdominal emptying
- the small-intestinal transit time
- individual sensitivity to the stretching of the intestinal wall
- degree of adaptation to lactose
- composition of the colonic microbiota?

Adult-type hypolactasia - Management

- ... the basis for the treatment of lactose intolerance is to reduce the amount of lactose in the diet
- the degree of the restriction depends on the individual's tolerance (adults with lactose malabsorption tolerate approx. 100ml milk)
 - lactose-free milk has been shown to induce symptoms in as many lactose malabsorbers as a milk containing 7g of lactose
 - lactose is better tolerated when it is consumed with some other food or when it is divided between several meals

Diagnosis of adult-type hypolactasia

1) Direct method

- Intestinal biopsy

2) Indirect methods

- Lactose tolerance test (LTT)
- Lactose tolerance test with ethanol (LTTE)
- Breath hydrogen test (BHT)

Genetic analysis?????

Intestinal biopsy

- ... invasive method in which intestinal biopsy specimen are used for an assay of mucosal disaccharides (golden standard for diagnostics of adult - type hypolactasia)
- ... is not suitable for routine diagnostics
- ... the standardization of the biopsy site to obtain reproducible duodenal biopsies is essential
 - Lactase activity (hypolactasia is defined as $< 10\text{U/g}$)
 - Lactase/sucrase ratio (L/S-ratio) (hypolactasia is defined as a ratio < 3)

Lactose tolerance test (LTT) - LTTE

- ... is based on the measurements of the increase in blood glucose by serial determinations (intervals of 15 to 30 min up to two hours) after oral load of 50g of lactose (adults)
 - ... to increase the reliability of the test a LTT with ethanol to inhibit the conversion of galactose to glucose by liver has been used
- > a rise in blood glucose $> 1,7\text{mmol/l}$ is indicative of normolactasia and that of $< 1,1\text{ mmol/l}$ for hypolactasia



Breath hydrogen test (BHT)

- ... is based on the determination of exhaled hydrogen produced by the bacterial flora in the colon after an oral lactose load
- ... samples of hydrogen are taken at intervals of 15 to 60 min for two to six hours
- ... determination of the change in hydrogen concentration in the expired air
- ... lactose malabsorption is diagnosed when the difference between breath hydrogen concentration at baseline and maximum exceeded 20 ppm



Fig. X Ledochowski M et al. Journal für Ernährungsmedizin 2003;5(1)

Products produced by the bacterial flora in the colon after an oral lactose load

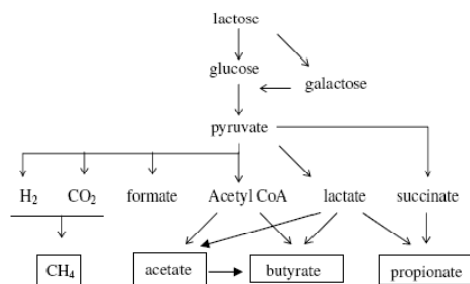


Figure 1. Fermentation of lactose by the colonic microbiota. (Modified based on Reilly & Rombeau (75) and Morrison *et al.* (161))

Breath hydrogen test - example

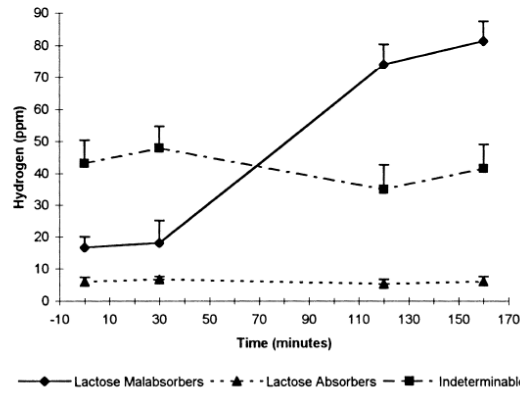


Fig. 1. Composite means (including standard errors) of breath hydrogen concentration before and 30, 120, and 160 minutes after ingestion of 50 g of lactose in 300 ml of water for individuals with lactose malabsorption, absorption, and indeterminable results.

CHEER, S.M and ALLEN, J.S (1997) Lactose Digestion Capacity and Perceived Symptomatic Response After Dairy Product Consumption in Tokelau Island Migrants AMERICAN JOURNAL OF HUMAN BIOLOGY 9:233-246

Specificity and sensitivity of the diagnostic methods of adult-type hypolactasia

Test	Specificity	Sensitivity
LTT	77-96%	76-94%
LTTE	96-100%	81-100%
BHT	89-100%	69-100%

Prevalence of adult-type hypolactasia

- Europe
 - Denmark and in Sweden 1-5%;
 - Finland 8-23%
 - Austria 20%
 - Spain 30%
 - Italy 40-50%
- America
 - American whites: 15%
 - African Americans: 80%
 - Mexican Americans: 70%
- Asia
 - Northern India 30% - Southern India 60-70%
 - Thailand 97-100%
 - China 90%

Onset of adult-type hypolactasia

... in a number of populations lactase activity starts to decline a few years after weaning

... but also the age at downregulation varies between populations:

- Thais: 1-2 years
- African children: 3 years
- Finnish children: 5-12 years

Reasons for these timing variations are unknown

Evolution of lactase persistence

... Lactase persistence varies greatly between different and within populations from almost 0 % in South East Asia to 99 % in Northern Europe

To explain these highly geographic variations in the prevalence of lactase persistence, various researchers have produced some hypotheses to explain these variations

- dairying is estimated to have originated approximately 7.000 - 10.000 years ago
- at this time man began to utilize milk after weaning
- lactase persistence became advantageous

Evolution of lactase persistence - 2

1) *The culture historical hypothesis*

The selection pressure for cultures that relied on milk as a main nutritional source for lactose tolerance was very high. Individuals with lactase persistence were able to use all the nutrients of milk, therefore, they were stronger, better equipped to survive and possibly had more children

Evolution of lactase persistence - 3

2) Calcium absorption hypothesis:

... to explain the prevalence of lactase persistence in Northern Europe:

In this region of the world the nutritional supply of vitamin D was low and it was proposed that lactose could enhance absorption of calcium and thus individuals with lactase persistence will have less rickets and pelvic deformities resulting in a selection in favour of lactase persistence.

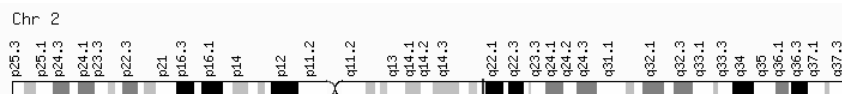
Evolution of lactase persistence - 4

3) Selective advantage of lactase persistence to survive cholera and other epidemics.

This hypothesis was put to explain the high frequency of lactase persistence in hot climates such as desert regions.

Polymorphisms and adult-type hypolactasia

- ... lactase persistence/non-persistence most likely due cis-acting differences – e.g. some polymorphism(s) within or near the lactase gene.



Identification of cis-acting variant associated with adult-type hypolactasia

Nature genetics (2002):

Identification of a variant associated with adult-type hypolactasia

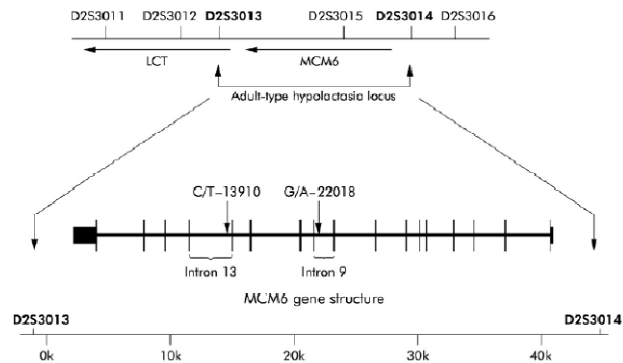
Nabil Sabri Enattah¹, Timo Sahi², Erkki Savilahti³, Joseph D. Terwilliger⁴, Leena Peltonen^{1,5*} & Irma Jarvelä^{1,6}

Published online: 14 January 2002, DOI: 10.1038/ng826

Adult-type hypolactasia, also known as lactase non-persistence (lactose intolerance), is a common autosomal recessive condition resulting from the physiological decline in activity of the lactase-phlorizin hydrolase (LPH) in intestinal cells after weaning. LPH hydrolyzes lactose into glucose and galactose. Sequence analyses of the coding and promoter regions of *LCT*, the gene encoding LPH, has revealed no DNA variations correlating with lactase non-persistence^{1,2}. An associated haplotype spanning *LCT*, as well as a distinct difference in the transcript levels of 'non-persistence' and 'persistence' alleles in heterozygotes, suggest that a cis-acting element contributes to the lactase non-persistence phenotype^{3,4}. Using linkage disequilibrium (LD) and haplotype analysis of nine

extended Finnish families, we restricted the locus to a 47-kb interval on 2q21. Sequence analysis of the complete region and subsequent association analyses revealed that a DNA variant, C/T₋₁₃₈₁₀, roughly 14 kb upstream from the *LCT* locus, completely associates with biochemically verified lactase non-persistence in Finnish families and a sample set of 236 individuals from four different populations. A second variant, G/A₋₂₂₀₁₉, 8 kb telomeric to C/T₋₁₃₈₁₀, is also associated with the trait in 229 of 236 cases. Prevalence of the C/T₋₁₃₈₁₀ variant in 1,047 DNA samples is consistent with the reported prevalence of adult-type hypolactasia in four different populations. That the variant (C/T₋₁₃₈₁₀) occurs in distantly related populations indicates that it is very old.

Discovery of the responsible variant



Enattah NS, Sahi T, Savilahti E, Terwilliger JD, Peltonen L, Jarvela I.
 Identification of a variant associated with adult-type hypolactasia. *Nat Genet.*
 2002;30:233-7.

C to T⁻¹³⁹¹⁰ and G to A⁻²²⁰¹⁸ SNP

- C/T variant is located 13.910 base pairs from the initiation codon of the LCT gene, in intron 13 of the MCM6 gene
- G/A variant 22.018 base pairs upstream of LCT, in intron 9 of MCM6
- complete cosegregation with lactase persistence (Enattah et al. 2002)
- The function of the MCM6 (minichromosome maintenance 6) gene where the variants are located is relatively poorly known.

Studies – C/T-13910

...several studies have explored its functional significance

- Quantitation of relative expression of the LPH mRNA transcripts from the C-13910 and T-13910 alleles by allele-specific reverse transcription polymerase reaction (RT-PCR) in Finnish adults, showed several times higher expression of LPH mRNA from the T-13910 allele
- Other studies showed that the –13910 region contains a strong enhancer, the T-13910 variant enhancing the LPH promoter more strongly

Biochemically determined lactase activity and C/T – 13910 variant

A statistically significant difference between each genotype and their corresponding L/S ratio was found

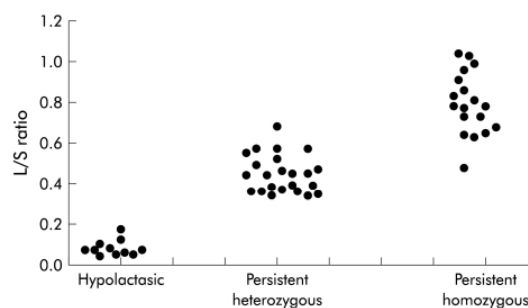


Fig. X Kuokkanen, M. et al. *Gut*. 2003 May; 52(5): 647–652

Correlation of the C/T-13910 genotypes with the phenotype

... few studies have assessed the correlation of the C/T-13910 genotypes with the lactase persistence phenotype in populations outside Finland

... weakness in these studies:

the lack of the biochemically determined lactase activity as a definition of the lactase persistence/non-persistence status of the subjects studied.

Studies – non-Finnish

Examples:

- Austrian study 1: the 24% frequency of the C/C-13910 was concordant with the frequency of lactose intolerance diagnosed by BHT
- Austrian study 2: a 97% correlation was observed between the C/C-13910 genotype and a positive test result in BHT. Of those with C/T-13910 and T/T-13910 genotypes 14%, however, had a positive BHT
- German cohort: frequency of the C/C-13910 genotype was 21.4%; somewhat higher than that diagnosed by BHT (15%)

New aspects

- In the sub-Saharan groups the T-13910 allele was found too seldom in order to underlie the lactase persistence phenotype
- it was suggested that C/T-13910 might not be the causing variant in these groups.
- possibility that another variant may underlie the lactase persistence in those populations; however, more studies on the issue are warranted

Identification of 3 „new“ SNPs

ARTICLES

nature
genetics

Convergent adaptation of human lactase persistence in Africa and Europe

Sarah A Tishkoff^{1,2}, Floyd A Reed^{1,2}, Alessia Ranciaro^{1,2}, Benjamin F Voight³, Courtney C Babbitt⁴, Jesse S Silverman⁴, Kvedi Powell¹, Holly M Mortensen¹, Jibril B Hirbo¹, Maha Osman², Muntaser Ibrahim², Sabah A Omar², Godfrey Lema², Thomas B Nyambo², Jilur Ghori², Suzannah Bumpstead⁶, Jonathan K Pritchard¹, Gregory A Wray¹ & Panos Deloukas⁸

Publishing Group <http://www.nature.com/naturegenetics>

A SNP in the gene encoding lactase (*lactase* (*CT*)-13910) is associated with the ability to digest milk as adults (lactase persistence) in Europeans, but the genetic basis of lactase persistence in Africans was previously unknown. We conducted a genotype-phenotype association study in 470 Tanzanians, Kenyans and Sudanese and identified three SNPs (G/C-14010, T/G-13915 and C/G-13907) that are associated with lactase persistence and that have derived alleles that significantly enhance transcription from the *lactase* promoter *in vitro*. These SNPs originated on different haplotype backgrounds from the European *CT*-13910 SNP and from each other. Genotyping across a 3-Mb region demonstrated haplotype homozygosity extending >2.0 Mb on chromosomes carrying C-14010, consistent with a selective sweep over the past ~7,000 years. These data provide a marked example of convergent evolution due to strong selective pressure resulting from shared cultural traits—animal domestication and adult milk consumption.

Functional role of SNPs

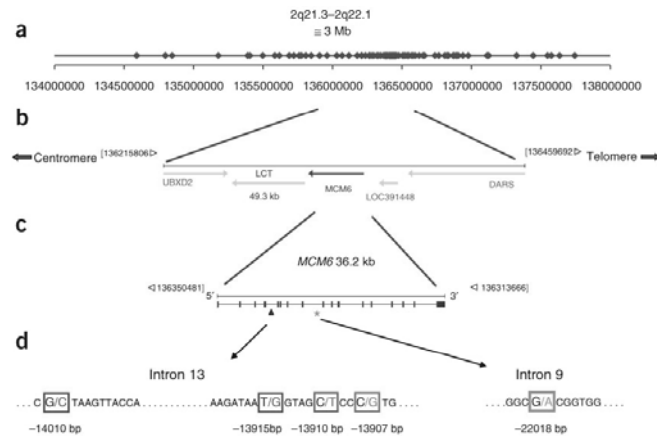


Figure 26. Map of the LCT and MCM6 gene region and location of genotyped SNPs
Tishkoff, S A et al. (2007) Nature genetics Volume 39; Number1

Adult-type hypolactasia – Nutritional consequences

Lactase non persistence can be manifested clinically as lactose intolerance by abdominal bloating, cramping, distention, flatulence, and diarrhea, causing many people to avoid drinking milk, the main source of calcium



Bone development and Osteoporosis

- role of calcium from milk and other dairy products has been shown to be essential for bone mass development
- lactose intolerance might lead to diminished calcium intake and reduced calcium absorption
- Thus lactase non persistence has been considered to be a risk factor for osteoporosis and fractures

Studies show different results....

Diabetes Mellitus and other diseases

Since people with lactase persistence supposedly drink more milk than people with lactase non persistence, high lactose consumption leads to a greater exposure to glucose and galactose.

This might have implications for many other disease risks.

- In humans, intestinal lactase activity is increased in diabetes and been shown to be normalized with insulin treatment
- milk lipids, contain high amount of energy, might contribute to obesity and increased risk of diabetes
- Possible role of lactose ingestion and galactose cytotoxicity in the pathogenesis of ovarian cancer?

Should a total restriction of milk and milk-products be suggested because of a positive genetic analysis??