THE ORIGINS OF LACTASE PERSISTENCE AND ONGOING CONVERGENT EVOLUTION

by

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ABSTRACT

As a primary factor in human evolution, natural selection is an important component of genetic research. Studies of lactase persistence suggest that positive selection has played a powerful role in the adaptation to a lifelong consumption of fresh milk. Using multiple research studies of lactase persistence and suspected corresponding single nucleotide genetic polymorphisms, this study combines data sources to determine whether evidence exists for natural selection of a specific cytosine-to-thymine genetic mutation located 13,910 base pairs (T-13910) upstream from the lactase gene. This polymorphism has potential to be a causal element for lactase persistence, and data suggest that natural selection has played a role in the rising frequency and distribution of this allele, if only in some regions. European and neighboring regions appear to have the highest frequencies with little or no frequency in Asia, Africa and Indonesia; however the presence of lactase persistence in those areas suggests convergent evolution may be occurring on a phenotypic level. To examine this possibility several other identified polymorphisms in the same region as the T-13910 will be included in this study.

Dedicated to my wonderful family whose
encouragement made all the difference.

ACKNOWLEDGMENTS

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CHAPTER 1: INTRODUCTION

The evolutionary process of human adaptation is an important premise of the study of physical anthropology. Understanding the role of natural selection as one of the key forces in adaptation is essential to determining the causes of diversity in the human species. The idea that we may have a recently evolved phenotypic adaptation, lactase persistence, which developed under tremendous selective pressure warrants a much closer examination. The forces behind this pressure most likely lie in a combination of natural and cultural adaptive processes that collectively fall under the umbrella of natural selection.

All mammals are capable of lactation, allowing a greater period of growth and development for offspring after birth. Lactose sugar is an excellent source of energy and is used to spur this postnatal development in nearly all mammals. The lactase enzyme required to digest the lactose sugar is produced in the intestinal tract of the human child until approximately 5 years of age, at which time it begins to taper off (Montgomery et al., 2007). When this downregulation of lactase does not occur, the individual is said to be “lactase persistent” (LP) (Swallow, 2003). People who are lactase persistent are able to digest milk products throughout adulthood.

Studies in the last two decades have increasingly addressed the existence of lactase persistence, and questions pertaining to the origin and cause of persistence have attracted a great deal of attention. Some of the research has revealed single nucleotide polymorphisms (SNP’s) that appear to be causal, particularly a polymorphism referred to as T-13910 due to its location 13,910 base pairs upstream from the gene that codes for lactase (LAC or LCT gene) (Swallow, 2003). This study will focus mainly on the T-13910 allele as a potential causal element in lactase persistence, while also determining its origin and spread, addressing the role of natural selection in this process. The possibility of convergent evolution in regions that do not exhibit the T-13910 SNP will also be examined.

Research questions and hypothesis

Three general research areas will be addressed. The first considers whether current studies indicate that lactase persistence is caused by different genetic mutations in different populations, generally located in separate geographical regions. The second examines the relationship between lactase persistence and the development of dairy consumption. The third examines the role of natural selection in the expansion of lactase persistence. If the research indicates the T-13910 mutation evolved under selective pressure, we can use it to predict lactase persistence in European populations. However, in African and Asian populations this allele does not appear at a high enough frequency to predict the persistence found in some populations of nomadic pastoralists, therefore the results should also indicate whether possible convergent evolution exists for this phenotypic trait.

Material sources

Most of the background source material used for this thesis will consist of peer-reviewed articles published by experts in this field. Research studies contained in these publications will be analyzed collectively to expose any consistencies or inconsistencies that may indicate convergent evolution or, alternatively, genetic drift. Statistical analyses will be conducted to reveal correlations between lactase persistence and suspected causal polymorphisms, particularly the T-13910 mutation. Should this mutation not appear to associate with lactase persistence in African and Asian populations, we will need to consider the possibility of phenotypic convergent evolution and examine other potential polymorphisms.

Analysis strategy

Using data from previous studies a comparison and analysis will be conducted across populations. The main focus will be the correlation of lactase persistence with the T-13910 SNP. Other researched polymorphisms will be briefly analyzed for additional correlations. These additional polymorphisms include A-22018, C-14010, G-13915, G-13917 and C-13913. This type of meta-analysis should reveal convergent evolutionary trends between Europe, Africa and Asia.

Analyses of data contained in published studies will be used to determine the most likely area of the origination of persistence. This can be compared to archaeological evidence of dairy consumption across the European region with the T-13910 mutation to indicate whether selective pressure was probably occurring in relationship to milk-consumption.

Frequency and gradient maps can be created between the T-13910 SNP, persistence and dairy consumption using ArcGIS and Mapviewer. This will allow for visualization of the evolution of persistence in Europe. Additional correlation maps may be included to examine the geographical relationship between the other polymorphisms included in this study and the evidence of lactase persistence outside of Europe.

CHAPTER 2: THE BASICS OF LACTOSE AND LACTASE

Most of us are already familiar with the term “lactose” and have been for much of our lives. Although today lactose is found in many types of commonly ingested substances, we are generally led to equate it with milk and other dairy products since lactose is the basic carbohydrate found in milk (Hollox et al., 1999). Human milk contains approximately 72 g of lactose per liter, and cow’s milk (which is consumed by adults in many societies) about 47 g per liter (Lomer et al., 2008). The overall taste, while far less sweet than sucrose and about half as sweet as glucose, has a hint of sweetness, adding an overall palatability in addition to its value as a carbohydrate energy source.

Lactose is a disaccharide consisting of the bonded sugar molecules glucose and galactose. Though found minutely in a variety of natural sources, lactose is present in large quantities in most mammalian milk, reaching as high as 75 g per liter in some species, with humans in the highest production range (Campbell et al., 2009; Lomer et al., 2008). As a common component of dairy products, the lactose sugar offers an excellent nutritional advantage to humans, particularly cultures that have domesticated high volume milk-producing animals.

Lactase is found in the mammalian small intestine and is the enzyme responsible for processing the lactose sugar found in the milk. The enzyme projects from the brush border of the intestinal lining into the lumen, where it can attach to and hydrolyze ingested lactose (Swallow, 2003). The lactase, also known as a β-galactosidase for its ability to hydrolyze a disaccharide, breaks down the lactose through hydrolysis into its separate sugar molecules, providing a usable source of nutrition for the mammalian organism (Swallow, 2003; Hollox, 1999).

The roles of lactose and lactase in mammalian growth and development

Lactose and lactase molecules are key components of mammalian growth and survival. Nearly all newborn mammals rely completely on mother's milk containing the lactose disaccharide for the first part of life, necessitating the lactase enzyme to separate the sugars for absorption into the bloodstream from the small intestine (Swallow, 2003). This lactose-lactase molecular codependency exists only in mammalian species. Once the sugars are divided into the monosaccharides glucose and galactose, they can be easily absorbed into the blood to provide a carbohydrate form of energy during the newborn and infancy stages of life. During late infancy the young are gradually weaned from mother's milk as they become reliant on the natural food sources of the species. This reduction in milk consumption, and therefore dietary lactose, generally coincides with a decline in lactase production as well. While the terms "nonpersistence" or "impersistence" denotes this reduction of lactase in humans, "lactose intolerance" refers to the gastrointestinal symptoms caused by the inability to digest lactose (Wiley, 2004).

Throughout fetal development the levels of lactase increase, peaking in humans at about 34 weeks of gestation (Lomer et al., 2008). Levels remain high until weaning for most mammals, at which time they decline rapidly to a minimal presence (Swallow, 2003). Humans, however, currently exhibit delayed or nonexistent reduction of lactase in some populations, leading to investigations of the development of lactase persistence. This persistence allows many people to continue producing high levels of lactase throughout their lifetimes.

The actual correlation of milk products with the symptoms of intolerance were first noted by Hippocrates over 2,000 years ago (Campbell et al., 2009). Up until the late 1960's, however, the common belief regarding lactase persistence was that it was the normal biological process (Wiley, 2004). Studies conducted during this time period indicated this was incorrect, concluding that nonpersistence was normal. These studies revealed the chemical process that led to intolerance and the ethnic relationships among populations (Campbell et al., 2009). Eventually the realization that the difference was genetically determined became the accepted theory.

Sources of lactase nonpersistence

Lactase nonpersistence in humans is due to one of three main sources: congenital, primary or secondary. Congenital hypolactasia is evident at birth and exhibits the lowest level of lactase activity (Lomer et al., 2008). Primary hypolactasia is a result of the normal downregulation upon weaning. Secondary hypolactasia occurs following intestinal damage caused by an illness, virus, trauma or infection. Congenital and secondary hypolactasia are less common than primary and are not relevant to the downregulation function of the lactase gene. Primary hypolactasia is the main focus from an anthropological point of view of examining adaptive mutational frequencies guided by selective pressures.

Congenital Lactase Deficiency. Human babies born with little or no functional lactase, Congenital Lactase Deficiency (CLD), are generally subject to dehydration and malnutrition due to the inability to digest the mammalian milk (Torniainen et al., 2009). It has been discovered that many mutations that lie within the lactase gene itself lead to the inability to produce more than a trace of lactase, suggesting that any coding change to the lactase gene will most likely result in a severe reduction or complete lack of the lactase enzyme. The effects of normal downregulation after weaning actually leave much more lactase action in the intestine than CLD.

Primary lactase deficiency. The natural reduction of lactase in the human intestine can lead to digestion difficulties when consuming lactose, especially in the high quantities found in fresh dairy products (Table 1). As the bacteria existing within the colon ferments the undigested lactose, many people who are lactase nonpersistent experience extremely uncomfortable symptoms (Ingram et al., 2009a). Water travels from the body into the intestinal lumen and various gasses and fatty acids are released leading to bloating, diarrhea, flatulence and general abdominal discomfort. People who experience these symptoms due to lactose consumption are said to be “lactose intolerant.” Though these symptoms are extremely uncomfortable in contemporary *Homo sapiens sapiens*, they may be more serious or fatal for less well-nourished or healthy individuals due to dehydration and weakness. Furthermore, the calories consumed from the milk remain predominantly unavailable, providing little or no nutritional benefit.

Table 1: Lactose presence in various dairy products

Food*	**Lactose % by Weight**
Human milk	7.2
Horse milk	6.5
Ice cream – vanilla	5.2
Sheep milk	5.1
Processed Cheese Slices	5.0
Yak milk	4.9
Cow milk – Skimmed	4.8
Yogurt – plain	4.7
Cow milk – Whole	4.6
Goat milk	4.4
Cottage cheese	3.1
Sour cream	2.7
Kurut	2.2
Feta cheese	1.4
Cheddar cheese	0.1
Mozzarella cheese	Trace
Edam/gouda cheese	Trace
Cream cheese	Trace

*Fermented products have less lactose due to microbial breakdown of the molecules.
(Lomer et al., 2008; McKinnon and Voss, 1993; Swallow, 2003; Wu et al., 2009)

Secondary lactase deficiency. Secondary hypolactasia is most commonly associated with intestinal issues such as giardiasis, coeliac disease, cow milk allergies or viral infections (Lomer et al., 2008; Crittendon & Bennett, 2005; Campbell et al., 2009). Though the symptoms are the same as primary hypolactasia, this type is usually reversible once the root cause is diagnosed and corrected (Lomer et al., 2008). As with primary hypolactasia, secondary usually still allows for a greater lactase production than CLD.

Health effects of milk

Attempts have also been made to associate numerous other pathologies with lactose intolerance, lactase persistence or lactase nonpersistence. Lactase activity was found to increase in diabetics, indicating a possible risk factor, although an association with lactase persistence genotypes could not be established (Enattah et al., 2004). Correlations between increased consumption of dairy foods and colorectal cancer mortality, supported by an inverse relationship between the cancer and lactase nonpersistence, suggests a potential connection as well (Szilagyi et al., 2006). Loss of bone density from insufficient calcium intake due to lactase nonpersistence has been tentatively put forth (Laaksonen et al., 2008). Bladder cancer in a Japanese study showed a reduced risk by intake of fermented milk (Salminen et al., 2004). Breast, ovarian, prostate, lung and stomach cancers, Crohn’s disease, colitis and more have all been closely studied in an effort to discover lactose and lactase correlations (Shrier et al., 2008).

Alternatively, when included as a nutritional component of a human diet, dairy products, especially fresh milk, can provide calcium, vitamins (A, B group, C), phosphorus, magnesium, zinc and even a small amount of essential fatty acids (Shortt et al., 2004). The nutrients found in dairy, especially calcium, can help increase bone density and avoid weakening and fracturing of bone material later in life. For example, the calcium content per 100 g of fresh milk is 119 mg, yogurt 121 mg, and cheddar 729 mg (Kitts & Kwong, 2004). In addition, symptoms of lactose intolerance can generally be avoided by consuming only small quantities of dairy products, especially fresh milk.

Fermented dairy also tends to have less actual lactose due to bacterial action (Salminen et al., 2004). This makes items, such as yogurt and cheese, more tolerable than milk to individuals with little lactase activity, enabling them to take advantage of the nutritional value of dairy products.

Lactose structure, function and formation

Carl Scheele began research on lactose in the late 1700's (Fox, 2009) and we now have an extensive knowledge of its molecular structure and function. Lactose is a disaccharide, or double sugar, consisting of a glucose and a galactose molecule (Swallow, 2003). The combined monosaccharides form a compound molecule designed in most mammals to provide a sustaining food source through mother's milk until the young are old enough to survive on the normal food substance of the species. The glucose provides energy, while the galactose is used in forming glycolipids and glycoproteins used in other biological functions (Lomer et al., 2008). The lactose carbohydrate found in mammalian milk "provides an excellent source of energy at a time of rapid growth and development" (Lomer et al., 2008).

Lactose is often used as filler in pharmaceuticals and processed foods (Lomer et al., 2008). It may also be used as a browning agent, an emulsifier or a bulking agent. Bread, processed meats, soft drinks and lager beers may all contain lactose. In the United States alone lactose production increased from 50 million kg per year in 1979 to 300 million kg per year in 2004.

Lactose is found in combination with polysaccharides, glycoproteins and glycolipids throughout the natural world (Campbell et al., 2009). Free lactose, however, is mainly found in mammalian milk in a combination of alpha and beta forms. α-lactose is different from β-lactose in the placement of the –OH on Carbon 1 in relation to Carbon 6 of the glucose molecule leading to a change in the rotation of the molecule (Fox, 2009). The alpha version exhibits the –OH on the opposite side of the molecular ring, while beta is on the same side as Carbon 6 (Fig. 1).

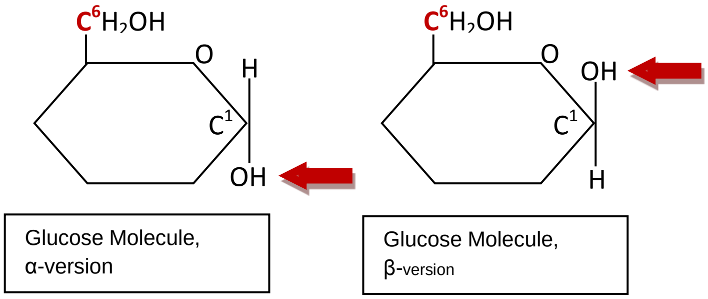


Fig. 1: Alpha and beta glucose molecules.

Lactose is a reducing sugar with an aldehyde group that allows a ring structure to form and open, interchanging between alpha and beta versions (Fox, 2009). This mutarotation leads to a fluctuating ratio of concentrations between the two versions based on the effects of temperature and acidity (Choi, 1958). The two isomers of lactose have different rotation and solubility properties, with β-lactose being more soluble, as well as slightly sweeter (Choi et al., 1949; Fox, 2009).

The glucose and galactose molecules are both sugars and therefore have similar atomic structures (Sinnott, 2007). The basic structure of the glucose ring consists of 5 Carbon atoms and 1 Oxygen atom bonded in a closed circle. Several Hydrogen and –OH groups as well as a sixth Carbon atom extend from this basic ring (Fig. 2a). The galactose molecule is also based on a carbon ring with a slightly different atomic structure (Fig. 2b). The two molecules form a bond at the Oxygen atom that extends from Carbon 1 with a β1,4 glycosidic bond to create the lactose disaccharide (Fig. 2c).

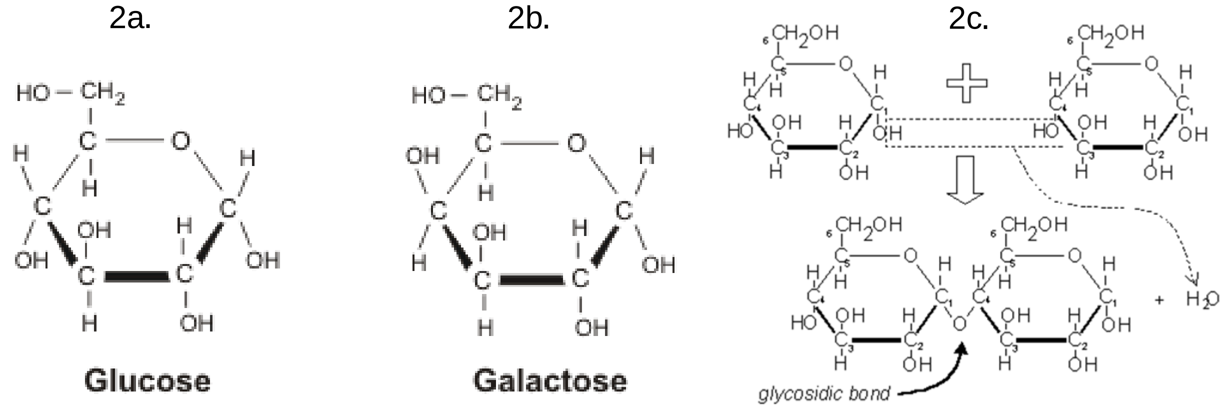


Fig. 2: (2a) glucose molecule, (2b) galactose molecule and (2c) lactose formation.
(2a, 2b) http://www.azaquar.com/en/iaa/index.php?cible=ca_glucides
(2c) http://image.wistatutor.com/content/feed/tvcs/sugar20bond.gif

In an interesting twist, the galactose molecule used in lactose is actually a converted molecule of glucose (Fox, 2009). Other than the minor assistance in glycolipid and glycoprotein production, it is not yet known why this conversion occurs since it costs energy to conduct this process, however the supposition is that there is a yet-to-be-found benefit to supplying lactose in milk, as opposed to maltose which consists of two glucose molecules. It is possible that there may be some relation to the maintenance of osmotic pressure. Lactose maintains enough pressure to keep mammalian milk at a usable viscosity. However, mammals in northern climates have little or no lactose, so they require adequate concentrations of inorganic salts to perform the same function in place of the missing lactose. Therefore an inverse relationship is also seen between lactose and inorganic salts in mammalian milk.

Lactation begins in the mammary gland in late pregnancy. Lactose is synthesized in the epithelial cells of the mammary gland and appears in mammalian milk directly and inversely proportionate to lipid concentration of the milk (Fox, 2009). Lipids are another source of energy contained in milk, and they provide twice the energy of lactose sugar. For this reason, mammals in colder climates generally have little or no lactose but do have high lipid concentrations in their milk. This includes sea lions, polar bears, seals and walruses.

Prior to the branching off of the first mammals, a non-mammalian housekeeping gene that produced the enzyme designated β1,4-galactosyltransferase (β4GalT-I) in vertebrates was recruited for the synthesis of lactose (Shaper et al., 1998). Research shows that β1,4-galactosyltransferase is a glycoprotein that is the precursor to lactose production in mammals (Shaper et al., 1997). Additional changes to the gene producing this enzyme eventually led to the specific cellular function of lactose production. Thus lactation became possible, leading to the genesis of the mammalian branch.

The β1,4-galactosyltransferase enzyme is also found in certain plant species, indicating the presence of the gene can be dated to before the divergence of animals about 1 billion years ago (Shaper et al., 1998). β1,4-galactosyltransferase is a common glycoprotein in the vertebrate system designed to catalyze galactose into biologically usable glycolipids and glycoproteins (Rajput et al., 1995). The β4GalT-I gene is generally considered a “housekeeping” gene, particularly in light of the high level of saturation of this enzyme throughout the vertebrate cellular structure. In mammals and mammals alone, the same gene that codes for β4GalT-I was enlisted to perform the vital function of lactose production in the mammary gland.

Lactose production begins as the β4GalT-I enzyme joins with α-lactalbumin to form lactose synthetase (a protein heterodimer) (Rajput et al., 1995). Exon 1 of the β4GalT-I gene (as shown in both murine and bovine species) contains two separate start sites for RNA transcription. The protein formed by using the first start site (4.1 kb) is found in all somatic cells, and is the main form used in all cells except those of the lactating mammary gland. In this gland the second start site at 3.9 kb becomes dominant, indicating its functional role in lactose biosynthesis. Each version of mRNA is transcribed based on specific and complex sequences of promoters for tissue-specific or housekeeping functions. It is also theorized that in nonmammary gland cells an active down-regulation occurs to the tissue-specific 3.9 kb protein, possibly by the GCBF protein which exists in large quantities in somatic tissues with low levels of mRNA production.

Control of the production of lactose in milk appears to be mainly based on the amount of available α-lactalbumin (Fox, 2009). α-lactalbumin is only created in the epithelial cells of the mammary gland (Rajput et al. 1995). An increase in β4GalT-I enzymes in these cells occurs in mid-pregnancy, stimulating lactose production. Hormone levels of insulin, prolactin and hydrocortisone also play key roles in the regulation of lactose production.

The lactase gene

The Lactase gene, often designated as either LAC or LCT, occupies approximately 50 kb on the long arm (q) of Chromosome 2, on the long arm (q), reverse strand (Campbell et al., 2009; Swallow, 2003). The location is formally referred to as 2q21 (Fig. 3). The gene contains 17 exons and transcribes to an mRNA section of 6,274 bases, which is reduced to a pre-proprotein of 1,927 amino acids. Following two separate cleavages and glycosylation the resulting protein contains 1,059 amino acids.

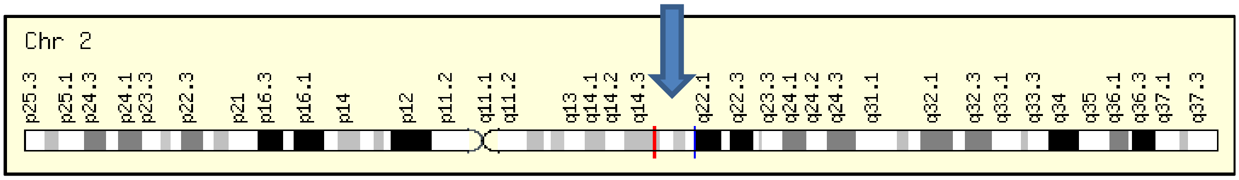
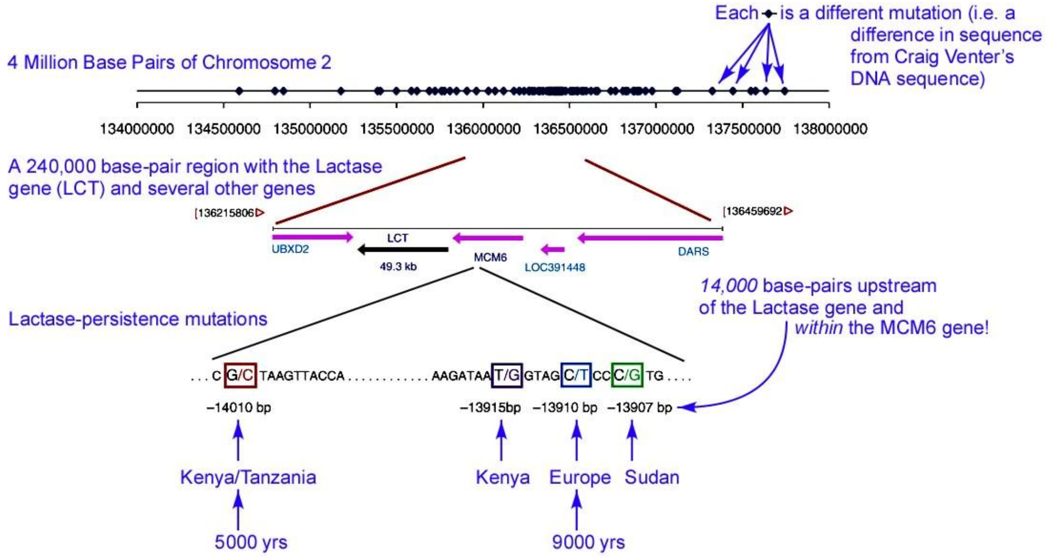


Fig. 3: Chromosome 2 with marked location of the Lactase Gene. Genecards version 3, http://www.genecards.org/pics/loc/LCT-gene.png

The production of lactase from this gene is naturally downregulated following weaning, however it remains active on a small scale for a specific biological purpose. The lactase enzyme contains a second site that is a competitive inhibitor of the lactase, designed to hydrolyze an aryl glycoside known a phlorizin (Campbell et al., 2009). Phlorizin was originally discovered in apple bark, and is made up of molecules of glucose and phloretin, a glucose transport inhibitor.

Individuals who retain normal or nearly normal function of the lactase gene without downregulation of production are considered persistent. The cause of lactase persistence does not appear to come from within the LCT gene itself, but rather within a maintenance gene located directly before the LCT gene. Some of the key genetic polymorphisms associated with lactase persistence are in the neighboring MCM6 (Minichromosome Maintenance 6) gene. This gene is part of a complex associated with

DNA helicase activity and (in conjunction with MCM genes 2, 3, 4, 5, and 7) is responsible for the initiation of DNA replication by unwinding the DNA strands (You et al., 1999). MCM6 occupies 36 kb containing at least 43 SNP’s and 9 insertion/deletions (Enattah et al., 2002). Combined information for linkage disequilibrium, haplotype/geographic association and correlations with persistence led to the conclusion that the T-13910 and A-22018 were strongly associated with, if not causal for, lactase persistence in Europe (Harris and Meyer, 2006). Further studies have identified additional SNP’s that appear to associate with persistence in other areas of the Old World as well, all residing within the MCM6 gene (Fig. 4). Subsequent in-vitro studies support these elements as causal through transcription enhancement by promoting the binding of the Oct-1 transcription factor, a protein responsible for initiating lactase production (Ingram et al., 2007; Ingram et al., 2009a; Olds et al., 2011).


Each ◆ is a different mutation (i.e. a difference in sequence from Craig Venter's DNA sequence)
4 Million Base Pairs of Chromosome 2
134000000
134500000
135000000
135500000
136000000
136500000
137000000
137500000
138000000
A 240,000 base-pair region with the Lactase gene (LCT) and several other genes
[136215806
[136459692
UBXD2
LCT
49.3 kb
MCM6
LOC391448
DARS
14,000 base-pairs upstream of the Lactase gene and within the MCM6 gene!
Lactase-persistence mutations
. . . C G/C TAAGTTACCA . . . . AAGATAA T/G GTAG C/T CC C/G TG . . . .
–14010 bp
–13915bp
–13910 bp
–13907 bp
Kenya/Tanzania
Kenya
Europe
Sudan
5000 yrs
9000 yrs

Sarah A Tishkoff, Floyd A Reed, Alessia Ranciaro, Benjamin F Voight, Courtney C Babbitt, Jesse S Silverman, Kweli Powell, Holly M Mortensen, Jibril B Hirbo, Maha Osman, Muntaser Ibrahim, Sabah A Omar, Godfrey Lema, Thomas B Nyambo, Jilur Ghori, Suzannah Bumpstead, Jonathan K Pritchard, Gregory A Wray & Panos Deloukas

Convergent adaptation of human lactase persistence in Africa and Europe
Nature Genetics 39, 31 - 40 (2006)

Fig. 4: MCM6 gene and associated polymorphisms: C-14010, G-13915, T13910 and G13907.

CHAPTER 3: NATURAL SELECTION

Determining the effects of natural selection on the human genome is a complicated process. It is estimated that 4 non-synonymous mutations arise in the human genome in each generation (Bamshad & Wooding, 2003). Many are negative mutations, usually removed immediately from the population due to low survival rates. Others are neutral and cause no effects that change the "fitness" of an individual. Some are beneficial and may quickly spread toward fixation. This may appear as a correlation between the environment and a specific trait, or a gene with an additional adopted function.

Distinguishing natural selection from demographic influences

Unfortunately factors of demography often exhibit the same outcomes as natural selection. Two-thirds of the genes that appear subject to selection may only be the result of demographic influences (Akey et al., 2004). Population expansion, bottlenecks, gene flow and genetic drift all contribute to the variation of the human genome and can leave footprints very similar to that of selection (Harris & Meyer, 2006). Some of the methods we can use to determine which factors are in effect include close examination of amino acid substitutions, diversity, linkage disequilibrium (LD), and substitution rates between species.

Amino acid substitution rates will appear irregular in the case of natural selection (Bamshad & Wooding, 2003). As the mutation enters different environments the amino acid substitution may be selected against due to adverse environmental conditions, or favored due to an advantage. In the negative environments, the mutation will move toward deletion, while moving toward fixation in the advantageous environments. Diversity will be reduced as linked sites to the mutation are removed (background selection) or retained (genetic hitchhiking) in accordance with the mutation itself.

Linkage disequilibrium decay can be compared to normal recombination rates to look for positive selection (Harris & Meyer, 2006). When DNA recombination occurs, the unlinked background regions surrounding the mutation will change composition as a mutation is selected for or against. Positive natural selection will allow the surrounding regions to travel with the mutation, decreasing diversity in those regions. Negative natural selection will lead to a reduction of the surrounding regions, also decreasing diversity. This also allows us to create haplotypes for a specific mutation, since far less recombination will have occurred, and we can often trace the mutation back to the root haplotype. A long block of LD around the mutation combined with a high frequency in the population is highly suggestive of positive selection (Bamshad & Wooding, 2003).

Between species substitution rates can be used to recognize selective action by comparing the ratio of nonsynonymous (k_a) to synonymous (k_s) mutations (Hu & Banzhaf, 2008). Mutations that are nonsynonymous result in a change of an amino acid, leading to a protein change. Most are unfavorable and are quickly removed through negative selection. Those few that are advantageous begin moving toward fixation. By examining the ratio of k_a/k_s, assuming a neutral value of 1, we can interpret a high ratio as leading toward fixation while a low ratio is being removed from the gene pool. According to the Neutral Theory, the latter is most often the case (Doyle & Gaut, 2000). The Neutral Theory of molecular evolution predicts that synonymous mutations will have no functional effect while the nonsynonymous would usually be disadvantageous, leading to a more common ratio of less than one.

If we have some prior information about the demography of a population we can use tests of neutrality that take them into consideration. Tajima's D is often used to measure the polymorphic diversity within a species (Harris & Meyer, 2006). Tajima's D finds the mean difference between DNA samples and returns a difference between them based on a neutral value of zero. However, in 2000 Justin Fay and Chung-I Wu recognized the inability of this test to identify the action of natural selection as opposed to demographic effects, so they developed a more reliable version, Fay and Wu's H, which compared data from related species to search for high frequency mutations.

In 1951 Sewall Wright developed the fixation index, F_{st}, to measure differentiation within populations (Harris & Meyer, 2006). F_{st} tests, such as ANOVA can show evidence of selection by comparing population data (Holsinger & Weir, 2009). Excessively high rates of alleles or traits reveal themselves among the populations tested. Geographical clines may also become evident. Both these factors can help to identify potential selective action, however errors may be introduced due to gene flow or genetic drift, necessitating additional testing to develop more support for results being attributable or not attributable to natural selection.

Identifying natural selection for lactase persistence

The next step is to use the methods of identifying natural selection on lactase persistence. We already know the obvious advantages to persistence, such as better nutrition, hydration, and calcium intake, so we have valid reasons to believe a causal mutation could survive and move toward fixation. Additional studies also reveal a milk-protein diversity in cattle that likely involved deliberate selection for greater milk yield and protein composition (Beja-Pereira et al., 2003).

Haplotype studies of a 60 kb region surrounding the lactase gene reveal four global versions: **A**, **B**, **C** and **U** (Hollox et al., 2001). All four differ from each other at three or more sites but are found in nearly all populations. The diversity within each haplotype is due to recent events affecting only a single population or subset. One probable cause is the selection for lactase persistence. The older events leading to these four major haplotypes, however, occurred prior to the spread across Eurasia and were more likely related to a combination of bottleneck and genetic drift.

Haplotype **A** is the most common, particularly in the European populations, although not as prevalent in the Sub-Saharan African populations. Haplotype **B** is found in all but the Bantu-speaking South African populations, but is most common in Papua New Guinea. Although also found in all populations, Haplotype **C** is not prevalent in European areas. Haplotype **U** is absent in European and Indian populations, but appears in Sub-Saharan Africa and East Asia with high frequencies.

Of these Haplotype **A** strongly correlates with the areas associated with lactase persistence in Europe, suggesting a mutation in this haplotype that is causal of persistence (Hollox et al., 2001). Linkage disequilibrium studies found a 1 Mb block at a frequency of 77% in northern Europeans, revealing that this haplotype is recent and was probably under intense positive selection to reach such a high frequency in less than 10,000 years (Hollox et al., 2005).

F_{st} testing was used to examine this area of linkage disequilibrium by first testing the values at 28,440 markers genome wide, then comparing with F_{st} values at markers located near the Lactase gene (Bersaglieri et al., 2004). The resulting p-value of .002 suggests a high significance in the distribution, clearly underlining a difference between genome wide frequencies and the local frequencies within and surrounding the Lactase gene. This also supports the likelihood of natural selection for lactase persistence in European populations.

In African populations the results are not as clear. It may be that results in European populations are highly significant due to a different set of selective pressures that forced a rapid adaptation, resulting in strong signatures (Akey et al., 2004). Various statistical tests offered similar results for multiple genes: Tajima's D, Fu and Li's D, Fu and Li's F and Fay and Wu's H. The identification of multiple genes that contain signatures of selection, yet are not shared between the non-African and African populations, also indicates different pressures upon the groups after humans migrated out of Africa.

Theories of lactase persistence expansion

We do not yet know what the main cause for the spread of lactase persistence was; however two leading theories have been suggested to explain the expansion of this phenotypic trait. The Culture-historical hypothesis was independently put forward by Simoons and McCracken in the early 1970's (Bloom and Sherman, 2005). The indication is that persistence leads to a selective advantage, therefore the lactase persistence mutation existed in rare proportions prior to fresh dairy consumption, then increased substantially in populations that adopted dairy farming. Alternatively, the Reverse-cause hypothesis states that the persistence mutation(s) arose first, then spread to higher frequencies before the advent of dairy consumption (Burger et al., 2007).

The Culture-historical hypothesis. This hypothesis is based on the premise that persistence mutational polymorphisms were rare but rose quickly to higher frequencies through selective pressures (Burger et al., 2007; Swallow, 2003). This led to an increase in persistence for dairy consuming pastoralists as milk-dependence became an integral part of the culture. Gerbault et al. (2009) studied 25 African populations and found evidence supporting this hypothesis by showing a high correlation between lactase persistence and pastoralism with clear genetic boundaries between pastoralists and non-pastoralists. Additional evidence of gene-culture coevolution between the milk protein genes in European cattle stock and human lactase persistence also supports the concept of intentional selection for milk protein genes by dairying cultures (Beja-Pereira et al., 2003). Finally, genetic studies of Neolithic human bones in Europe found no evidence of the T-13910 allele (Burger et al., 2007), believed to be the putative cause of persistence in this region of the world. For these reasons, the Culture-historical hypothesis is supported to some extent.

The Reverse-cause hypothesis. According to the Reverse-cause hypothesis, the mutations were already established; therefore the practice of dairying was adopted by those populations for which the frequency was high enough to sustain milk-dependence (Burger et al., 2007). This much differentiation prior to dairy consumption would probably not allow for the genetic boundaries that exist between neighboring populations that do or do not rely on dairy. So far, though it cannot be disproved, evidence undermines this hypothesis yet continues to accrue in support of the Culture-historical. The genetic boundaries, cattle coevolution, Neolithic DNA evidence and extended linkage disequilibrium of the lactase gene area (indicating recent expansion) all suggest the Reverse-cause hypothesis is relatively unlikely when compared to Culture-historical.

Regional variation and additional hypotheses. The Culture-historical and Reverse-cause hypotheses are not designed to explain the regional variation that occurs in the practice of dairying across all continents. Certain factors may contribute heavily to the sustainability of herding milk-producing animals, such as climate, forage and pathogens (Bloom and Sherman, 2005). For example, it may be too difficult to keep domestic animals alive in climates that become extreme for certain periods of the year. Many cultures are nomadic pastoralists for this reason, however if an area does not have a safe region to move to during inclement months, the herds would not survive. Food and water availability also are essential to maintaining livestock, and endemic diseases, such as sleeping sickness, can lead to decimation of the herd. Overall the expansion of lactase persistence appears to follow the Culture-historical process, however many specific regional landscapes may have resulted in the widely diverse range of frequencies.

Several ideas have been presented to explain the selective pressures that led to the expansion of the lactase persistence phenotype. Flatz and Ratthauwe (1973) presented the concept of milk consumption to boost calcium absorption in higher latitudes where sunlight was less available. In 1975, Cook and al-Torki developed the Arid Climate hypothesis, postulating that milk - specifically that of camels - is an excellent substitute for food and water in desert areas. Anderson and Vullo (1994) alternatively attempted to explain nonpersistence as a function of malarial conditions, since flavins, found in high quantities in milk, increase the risk of malarial infection.

Identification of lactase persistence mutations

Montgomery et al. (2007) hypothesize the production of repressors begin at about five years of age in a human child. These repressors act to prevent the lactase gene from manufacturing any significant amounts of lactase by effectively "switching off" production. Persistence is partly rooted in blocking these repressors, allowing the lactase gene to continue functioning. Additional studies suggest the T-13910 allele is also directly responsible for enhancing the production of lactase when it binds to the Oct-1 transcription factor (Enattah et al., 2007).

Many studies have been conducted in search of the causal genetic variant of lactase persistence. The T-13910 polymorphism is the one of the most promising associations found to date, lying 13,910 base pairs upstream from the Lactase (LCT) gene (Swallow, 2003). A strong correlation can be drawn between this allele and prevalence of persistence in Europe. Lactase persistence studies indicate the largest frequency of this SNP occurs in North Europe, decreasing geographically to the south and east, with minimal or no appearance in African and Asian regions.

The original genetic structure, or “wild-type,” of nonpersistence is determined by a pair of cytosines (CC) at this location. When Scandinavian studies revealed a thymine replacement of one or both cytosines in this position that almost completely correlated with persistence, investigations quickly began to determine if it could be the cause of persistence in these individuals (Swallow 2003). In addition, persistence existed in both heterozygous and T-homozygous individuals, suggesting that if this was a causal polymorphism, the thymine expressed dominance.

An additional mutation found in the same area is A-22018. This SNP has a relatively strong, though incomplete, association with persistence (Enattah et al., 2007). A small variation seems to occur in nonpersistent groups with CC-13910, with 2.5 to 11% of nonpersistent individuals exhibiting the G/A-22018 genotype.

In Africa there are a number of pastoralist cultures that exhibit lactase persistence, yet do not have the T-13910 (or A-22018) polymorphism, indicating another variant or variants must be associated. Subsequently additional variants were located that may be associated with persistence (Ingram et al., 2008). Two polymorphisms, C-14010 and C-13913, reveal a correlation with persistence in areas of Africa (Imtiaz et al., 2007). Two other variants, G-13915 and G-13907, do not appear to show the same level of association as the European SNP T-13910 (Ingram et al., 2008; Tishkoff et al., 2007). Geographically it appears that G-13915 originated in the Middle East while the other three SNP’s arose in East Africa.

Comparisons of linkage disequilibrium tracts suggest the C-14010 was subject to strong positive selection, even more so than the T-13910 SNP (Tishkoff et al., 2007).

With a high frequency, as high as 27%, and an average homozygous tract length of 1.8 Mb, compared to T/T-13910 average of 1.4 Mb, there is good evidence for selection. Additional research should be conducted to develop broad-based statistical support for natural selection.

Little progress has been made in determining the causes of lactase persistence in Asian populations, though there are relatively few persistent cultures. The A-22018 allele, already known for some association in European populations, appears to correlate with persistence in several of the northern Chinese populations (Xu et al., 2009). None of the other polymorphisms, however, were found in high enough frequencies to indicate cause of persistence.

CHAPTER 4: ARCHAEOLOGICAL DATA

Finding evidence of dairy production in the archaeological record is both easy and difficult at the same time. Evidence of dairy use certainly exists, however it is nearly impossible to determine how early the practice started. Sherrat's 1981 model of the "Secondary Products Revolution" surmised that dairy production originated in the 4th millennium in the Middle East, and the 3rd millennium in Europe, as "technical innovations" secondary to meat production (Vigne & Helmer, 2007). However, the earliest evidence now pushes the extensive use of milk from domesticated animals in the Middle East as far back as the 7th millennium (Evershed et al., 2008) narrowing the gap between origination of animal domestication and secondary dairy production. Actual domestication of goats and sheep, followed by cattle, is estimated to have occurred in the 8th millennium in the Middle East area, spreading outward into Central and North Europe.

Methods of detecting domestic animal strategies

One of the commonly used methods to determine the usage of domesticated animals is that of kill-off profiles. Domestic animal strategies can be deduced by examining the culling practices used (Greenfield et al., 1988). Age and sex distributions of slaughtered animals reveal patterns that work better for specific production strategies. Milk production may be indicated by the culling of young male stock, particularly first year, to ensure more grazing ground for lactating females. After the infant/juvenile age is passed, butchering dramatically decreases.

Other physical evidence of domestication can help us by identifying the use of secondary products, particularly dairy. For example, cheese strainers were found in Neolithic sites of Britain dating to 4,500 BC (Dudd and Evershed, 1998), while images and written records existed from upper Africa and Mesopotamia as early as 4,000 BC that confirmed dairy production.

Another line of evidence that uses the analysis of pottery sherd residue has become a popular method of detecting milk consumption. Recent understandings of lipid residue decay have opened up a new avenue for detecting dairy usage in prehistoric cultures. Stable carbon isotope analysis and lipid analysis can be used to test ancient unglazed pottery sherds for lipid compositions that were absorbed into the pottery material (Evershed et al., 2008).

Fresh milk contains short chain fatty acids and triacylglycerols that are key identifiers of dairy products (Craig et al., 2005). Burial, however, leads to decay and degradation of these identifiers resulting in alterations of the fatty acids and disappearance of the triacylglycerols, leaving a residue that is chemically very similar to adipose fats. The resulting saturated mid-chain fatty acids, however, will reveal differing carbon ratios of C_{16} to C_{18} (Carbon chain lengths of 16 or 18 atoms) between dairy and adipose fats, allowing us to use compound-specific measurements that can identify individual mid-chain fatty acids based on the number of carbon atoms. In dairy residue, the ratio of C_{16} to C_{18} is different from adipose residue with as much as 7% fewer C_{18} isotopes. The differences in carbon composition reveal ruminant versus nonruminant fats, as well as adipose fats versus dairy fats. Testing sherds for dairy fats allows us to trace dairy consumption to an earlier time period than originally surmised, possibly to the very beginning era of animal domestication during the Neolithic period.

Dairy residue studies

Craig et al. (2005) selected two Neolithic sample sites in the Danube basin specifically to test for dairy residues. Schela Cladovei lies on the Romanian side of the Danube and the pottery samples were taken from a period between 5,950 and 5,500 BC. Ecsegfalva 23 is found on the Hungarian side of the Danube in the Great Hungarian Plain and was occupied from 5,800 to 5,700 BC. Both sites are in areas that are believed to have influenced agricultural and pastoral development in Central and Northwest Europe.

The sherd samples tested from both sites revealed the processing of ruminant fats, both adipose and dairy. A small amount of intact triacylglycerols were also found, confirming the containment of milk in those vessels. This strongly suggests that dairy production was quite early in the domestication timeline, which is estimated at about 10,000 years ago for sheep and goats in the Middle East (Haenlein, 2007). Kill-off profiles also suggest that dairying and meat production were mixed in these Neolithic sites (Craig et al., 2005). It is likely that household pastoralism was more common than large community herds, as indicated by microwear on sheep teeth that is caused from enclosed overgrazing.

Additional findings include mid-chain ketones, which are produced by heating milk. Applying heat may help reduce the lactose content, allowing intolerant individuals to consume more dairy, as well as improve storage potential of dairy products. This offers an explanation of dairy consumption prior to lactase persistent mutations by allowing non-persistent people to consume large quantities of dairy without the debilitating side effects of intolerance. In addition, the use of bacterial fermentation would greatly reduce the lactose content and allow higher dairy consumption in nonpersistent individuals (Wiley, 2004). Based on these concepts, dairy production was not necessarily a function of lactase persistence.

The evidence from these studies suggests milk was in use prior to 6,000 BC in this area. Another stable carbon isotope study (Evershed et al., 2008) examined sherds from 23 sites in central and southeastern Europe, Greece, Anatolia and the Levant, and results push this estimate further back to sometime well before 6,500 BC. All of the sites had sherds that tested positive for dairy residue, with Northwestern Anatolia revealing intensive processing by the 7th millennia BC (70% of the sherds were positive). A positive correlation between sherds with dairy residue and cattle bones also appears, linking the importance of cattle to milk production. Kill-off profiles suggest a mixture of meat and dairy production was used, with higher values placed on dairy in sites with higher proportions of cattle.

Though evidence of ketones was not reported, it is highly likely that the milk was processed for consumption and storage as cheese, ghee or other less perishable products, particularly considering the intense consumption in the Anatolia region. This usage of dairy also precedes the pottery production in the area, making it difficult to find earlier evidence of milk consumption. It certainly must have occurred relatively close to the beginnings of domestication in the 8th millennium.

Development of animal domestication

Currently most archaeological dairy usage studies concentrate on the Middle East and European regions. Animal domestication for meat is believed to have originated in the Middle East about 10,000 years ago, spreading into Europe and other areas of the Eastern Hemisphere over the next few thousand years (Evershed et al., 2008, Haenlein, 2006). Dairy domestication followed this sometime later, with current archaeological evidence indicating goat and/or sheep milk was heavily used in the Middle East by 8,500 years ago. Over time, additional evidence attained in Africa, Asia and the Middle East will greatly contribute to the knowledge base of Neolithic and Mesolithic dairy consumption.

CHAPTER 5: MATERIALS AND METHODS

Evaluating the origin and spread of lactase persistence requires an extensive database of information. Datasets were collected from numerous sources to create a more comprehensive set of samples for analysis. A total of 73 population samples have been assembled from all regions excluding the New World and Australia. This provides T-13910 frequency data for more than 12,000 individuals, and lactase persistence frequency data for almost 10,000. For populations that did not provide the numbers of heterozygous and homozygous individuals, these numbers were obtained using Hardy Weinberg calculations of $p^2+2pq+q^2=1$, with C-frequency as p and T-frequency as q.

New World samples were removed from the original database since the recent influx of Old World populations scattered throughout the American continents would be nearly impossible to follow, and would probably not reveal any evolutionary processes in such a short span of time. Data were not included for Australia either due to a lack of substantial information.

Haplotype distribution

Examination of the long stretch of linkage disequilibrium surrounding the lactase gene revealed four common global haplotypes that exist at 5% or greater frequency: **A**, **B**, **C** and **U** (Hollox et al., 2001; Swallow, 2003). Haplotypes **A**, **B** and **C** are all found in European populations; however, haplotype **A** comprises the greatest part of Europe. The T-13910 SNP is found only on this haplotype (Harvey et al., 1998).

Additional regional polymorphisms

Data are also included for other polymorphisms suspected of associating with lactase persistence. A-22018 is found on the same haplotype (**A**) as the T-13910 polymorphism and shows a correlation with persistence, although far less robust (Swallow, 2003; Enattah et al., 2007), and appears to correlate with persistence in northern Chinese populations. In East-Central and Southern Africa the C-14010 polymorphism (on haplotype **B**) seems to be a strong indicator of lactase persistence (Coelho et al., 2009; Enattah et al., 2008; Ingram et al., 2009a). The G-13915 SNP is found on the **C** haplotype and is common in the North African and Middle Eastern regions (Ingram et al., 2007; Gerbault et al., 2009). G-13907 is found on the **A** Haplotype and exists mainly in the Ethiopia/Sudan region (Ingram et al., 2009a).

Data adjustments

In many cases samples were combined from multiple sources to create better regional representation or a more complete record for a given population. For example, since two population studies for the Hezhen of China were included in the study, they were combined into one record as a more efficient way to examine the data. Details of these actions are noted in Table 2.

Table 2: Combined data records.

Morocco	The Saharawi culture occupies a large area of Morocco and was combined with the sample for Morocco from source Enattah et al. 2007
Hezhen	Data pooled from sources Bersaglieri et al. 2004 and Sun et al. 2007
Oroqen	Data pooled from sources Bersaglieri et al. 2004 and Sun et al. 2007
Han	Data pooled from sources Enattah et al. 2007 and Bersaglieri et al. 2004
French Basque	Data pooled from sources Bersaglieri et al. 2004, Itan et al. 2009 and Enattah et al. 2007
England	Data pooled from sources Smith et al. 2009, Gerbault et al. 2009 and Itan et al. 2009
France (main)	Data pooled from sources Bersaglieri et al. 2004, Enattah et al. 2007 and Itan et al. 2009
Greece	Data pooled from sources Anagnostou et al. 2009 and Itan et al. 2009
Iran	Data pooled form sources Enattah et al. 2007 and Gerbault et al. 2009
Ireland	Data pooled from sources Itan et al. 2009 and Gerbault et al. 2009
Israel	Data combined for multiple populations in Israel listed in Bersaglieri et al. 2004.
Italy	Data pooled from sources Bersaglieri et al. 2004, Anagnostou et al. 2009, Itan et al. 2009, Bersaglieri et al. 2004, Coelho et al. 2005 and Gerbault 2009
Orkney	Data pooled from sources Itan et al. 2009 and Bersaglieri et al. 2004
Pakistan	Data pooled from sources Bersaglieri et al. 2004 and Enattah et al. 2007
Scandinavia	Data pooled from sources Itan et al. 2009, Coelho et al. 2007, Swallow 2003, Enattah et al. 2007 and Bersaglieri et al. 2004
Spain	Data pooled from sources Gerbault et al. 2009 and Agueda et al. 2010
Melanesia	Data combined for Papuan and Melanesian listed in Bersaglieri et al. 2004.

In cases of more distinct populations it makes less sense to combine them within a region. The Saami culture of the Northern Scandinavian Peninsula seems to be distinct from the rest of the Scandinavian region so was kept as a population sample. The Basque area between France and Spain also remains distinct from either neighbor so was included for analysis. A wide variety of Chinese and Russian populations were also retained to represent the large regional differences in populations and cultures for those countries. The other population records used were generally defined by country of origin.

In many cases the literature sources did not include complete information for both the T-13910 polymorphism and lactase persistence frequencies; therefore the information was obtained from different sources. The same situation applies to additional polymorphisms suspected to play a role in lactase persistence that will be included in this study.

Each population was designated a latitude and longitude location for mapping purposes. Populations which did not include the original locations were assigned based on central location or large city centers using Google Earth. Though this does leave some room for error, the adjustment to larger regions by combining data and using country of origin representation allows for a wider possible area of collection, reducing error margins. The estimated latitude and longitude locations are indicated in Table 3.

Table 3: Geographical location estimates and sources for latitude and longitude.

Population Record	Estimated Point of Lat/Long	Population Record	Estimated Point of Lat/Long
Adygei	Maykop	Algeria	Teniet Beni Mzab
Arabia	Ar Riyad	Cambodia	Phnom Penh
Cameroon	Douala	Canary Islands	Santa Cruz
Daghestan	Makhachkala	Dai	Sipsongpanna
Daur	Heilongjiang	England	Gerbault et. al. 2009
Erzas	Central Mordovia	Ethiopia	Addis Ababa
France (Main)	Itan et al. 2009	French Basque	Itan et al. 2009
Fulbe (Nigeria)	Aguie	Germany	Itan et al. 2009
Greece	Itan et al. 2009	Han	Shanghai
Hezhen	Sun et al. 2007	Hungary	Budapest
India	New Delhi	Iran	Gerbault et. al. 2009
Ireland	Itan et al. 2009	Israel	Jerusalem
Italy	Itan et al. 2009	Japanese	Tokyo
Kazak	Zhaosu, Ili, Xinjiang	Kenya	Nairobi
Komi	gorod Sosnogorskiy, Komi Republic	Lahu	Kunming
Malawi	Lilongwe	Man	Si Zhong Xian
Melanesia	Ctr Solomon Sea	Miaozu	Kunming
Mokshas	Central Mordovia	Mongol	Haifar
Mongola	Heilongjiang	Morocco	Marrakech
Mozambique	Maputo	Namibia	Windhoek
Naxi	Liangshang	Nigeria	Yoruba Island
North France	Paris	NW Russia	Center of Western Russia
Ob-Ugric	Reka Ob'	Orkney	Itan et al. 2009
Oroqen	Sun et al. 2007	Pakistan	Islamabad
Portugal	Lisbon	Russian	Center of Russia
Saami	Murmansk	Sao Tome	Sao Tome City
Scandinavian	Itan et al. 2009	Scotland	Glasgow
Senegal	Dakar	She	Fuzhou
Somalia	Mogadishu	South Africa	Johannesburg
South Korea	Daegu	Spain	Madrid
Sudan	Khartoum	Tanzania	Dar Es Salaam
Tu	Haibei	Tujia	Changde
Turkey	Itan et al. 2009	Udmurts	Udmurtskaya
Uganda	Kampala	Uygur	Urumqi
Xibo	Shenyang	Yakut	Sakha Republic
Yizu	Kunming		

Regional designations

Each sample record was assigned a regional designation. These designations will allow the creation of a base map that can be used to show isoclines for the analyzed alleles and persistence frequencies. The regional designations include North Africa, Middle East, East Africa, China, Western Russia, Asia (excludes China), the Caucasus, Western Europe, Eastern Europe, Indonesia and Sub-Saharan Africa.

Hardy Weinberg equilibrium

Hardy Weinberg equations were formulated for all population records for the 13910 polymorphic frequencies to help identify discrepancies in equilibrium. Using the Hardy Weinberg Equilibrium (HWE) formula $p^2 + 2pq + q^2 = 1$, expected homozygous values were calculated based on the number (N) multiplied by the square of the CC or TT frequency, while heterozygous values were calculated as 2N*CCfreq*TTfreq. By subtracting the observed number of each allelic type from the expected number calculated the trend toward allele fixation can be identified and graded for strength.

Hardy Weinberg equilibrium relies on a strict set of assumptions and most likely one or more will be violated. Mutations, gene flow, genetic drift, small populations and nonrandom mating will lead to deviation from equilibrium (Dvorak, 2009). This allows the results to be examined for changes that reveal the direction of evolutionary trends for an individual SNP. Increases in heterozygosity suggests a rare allele that is either moving toward fixation or toward extinction.

Methods of testing lactose metabolism in included studies

Multiple testing methods are available to determine lactase persistence. Tests range from non-invasive to extremely invasive depending on how the results are gathered. However, most tests only measure lactase activity and cannot distinguish between natural and pathological reductions of lactase, making them less reliable for subjects that may have other intestinal issues, such as IBS or giardiasis. To substantiate test results, studies are generally conducted using a minimum of two methods.

Hydrogen Breath Test. The Hydrogen Breath Test (HBT) is a non-invasive test that is one of the most commonly used methods of detecting lactase nonpersistence. Several hours after administering a lactose load, hydrogen levels in the exhaled breaths reveal carbohydrate digestion or maldigestion based on changes in elevation (Lomer et al., 2008). This test is cost-effective and relatively reliable, although reliability is questionable if certain protocols are not observed. As much as 20% of intolerant individuals have been known to provide false-negatives if conditions are not carefully followed. A study by Avallone et al. (2010) provides substantial evidence that, prior to the lactose load, a 24 hour period of carbohydrate restriction followed by 12 hours of fasting should be observed. This dramatically reduces false-negative errors. In addition, antibiotics should be avoided for several weeks prior to the test since they affect intestinal bacterial levels.

Most HBT tests begin with a basal H^2 level recorded for each individual, followed by an oral lactose load of 50g, as found in about a liter of milk (Lomer et al., 2008).

Breath excretions are taken at defined increments, usually 30 minutes, for 3-6 hours and submitted to gas chromatography analysis to measure H^2 in ppm. An increase exceeding 20 ppm is considered a positive response and indicates much of the lactose consumed is undigested and is being fermented by intestinal bacteria, releasing large quantities of H^2 that is partially absorbed into the bloodstream and subsequently excreted in the breath.

Lactose Tolerance Test. The Lactose Tolerance Test (LTT) uses a blood sample following a lactose load to measure glucose levels in the blood. As the lactose disaccharide is digested in the intestinal tract, the resulting glucose and galactose sugars are absorbed into the bloodstream. A rise in blood glucose greater than 1.1 mmol/l and galactose greater than 0.3 mmol/l is indicative of lactase persistence (Seppo et al., 2008). The reliability of the LTT can also be slightly increased by adding a small amount of ethanol (300 mg/kg of body weight) to the load. Unfortunately for the test subjects, both this and the HBT tests involve a lactose load, which can lead to extreme discomfort for intolerant individuals.

Jejunal biopsies. Although considered the "golden standard," jejunal biopsies to test for lactase activity are highly invasive and alternate methods are usually preferred (Vonk et al., 2001). Small samples are sectioned from the jejunum portion of the small intestine and analyzed for lactase concentrations. Although biopsies provide a reliable indicator of lactase activity, they cannot provide conclusive evidence that a lack of lactase is due to natural downregulation as opposed to a pathological illness or infection.

Methods of DNA sampling in included studies

Genotyping uses a simple blood test or buccal swab to genotype the regions suspected of affecting lactase production. The results can clearly identify a causal polymorphism, however the polymorphism must be known first. Swallow (2003) was able to demonstrate a complete association with T-13910 in Finnish studies, yet the absence of this SNP in other regions, such as Africa and Asia, indicates the need for determining putative polymorphisms with substantially supporting evidence in different regions. The genotyping assays also reveal the large section of linkage disequilibrium containing lactase gene, suggesting not only strong selection, but also that the cause of persistence does lie within this region in all four main haplotypes (Swallow, 2003). Pinpointing the actual causal polymorphism(s), however, is much more difficult.

Statistical evaluations

All statistical analyses were conducted with PASW Statistics 18, Release 18.0.0. The data set analyzed contains 73 population records, with a total count of 12,371 individuals. The population samples were drawn from areas throughout Africa, Europe and Asia, with one addition from Indonesia. No populations were included from the New World, Australia, Greenland or Iceland. Of the 73 populations, 44 contained information regarding lactase persistence frequencies (9,682 individuals).

The ideal statistical evaluations are the parametric versions which model the magnitude of associations between variables, as opposed to the nonparametric which are based on ranking mechanisms that compare the variables. Parametric tests, however, often have a specific set of requirements that must be met to obtain valid results, particularly a normal distribution of the data. Therefore, the first step in completing statistical analyses is to determine if the data for the variables to be tested are normally distributed. This can be done with two methods, one graphical and the other calculated.

The graphical method of examining variables for normal distribution is the histogram. When we look at the T-13910 (variable T-13910 freq) and the lactase persistence (variable LP freq) frequencies from the populations within the data set, the distribution does not appear normal for the T-allele frequency based on the normal curve shown (Fig. 5). The lactase persistence frequency, however, appears much closer to the normal curve, though there are some discrepancies.

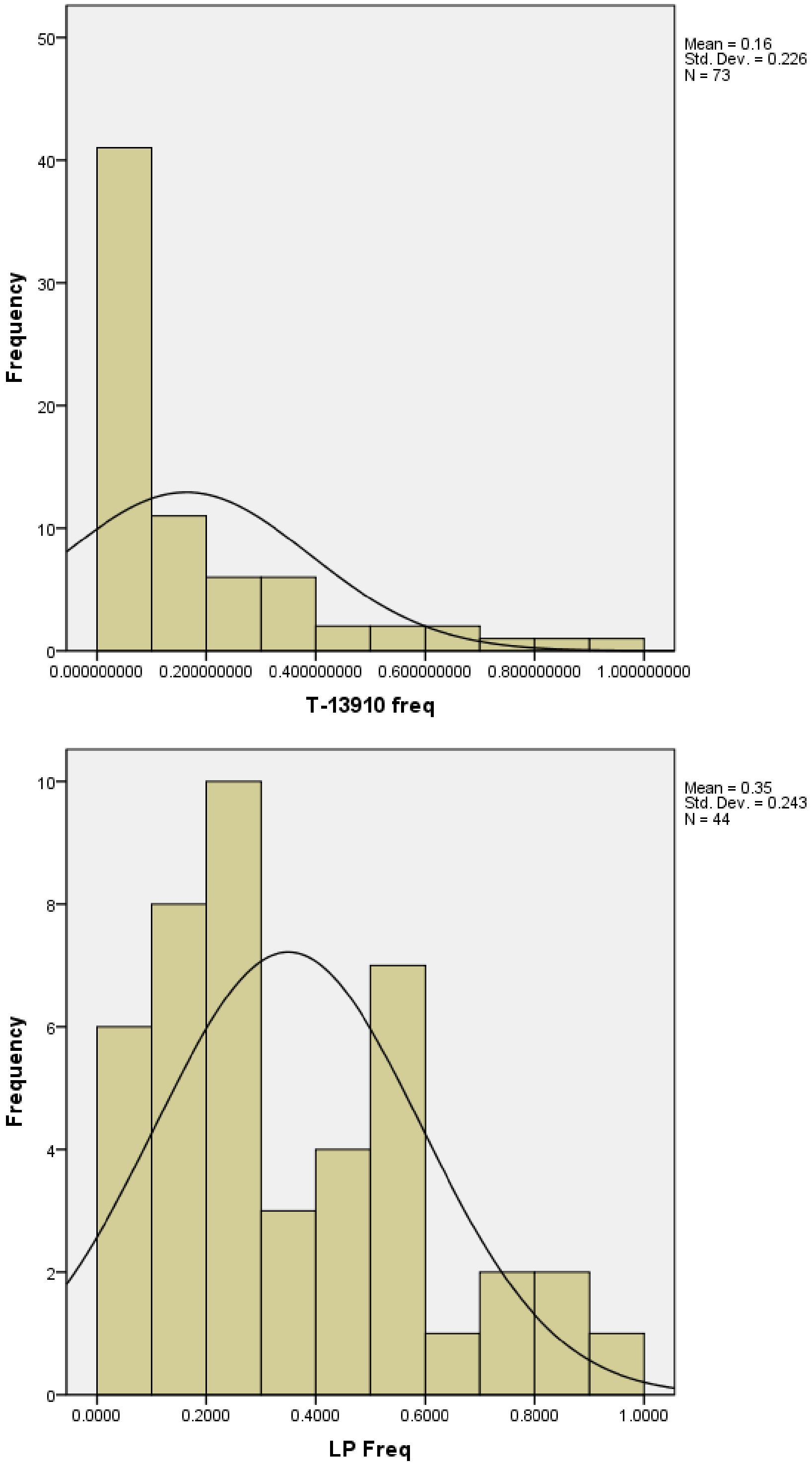


Fig. 5: Histograms exhibiting the frequency distributions for T-13910 and lactase persistence. The normal curves are shown.

To verify these results, particularly for the T-13910 distribution, we can use the Kolomogorov-Smirnoff nonparametric test for normality. The K-S test confirms the lack of normality for the T-13910 distribution with a significant p-value of less than .001 (Table 4). The Lactase Persistence frequency is not significant and confirms to a normal distribution. The non-normal distribution for T-13910 is an expected result from the inclusion of many groups in the data set that have little or none of the T-13910 mutation. Since this mutation appears generally relegated to the European area, the inclusion of populations throughout Asia and Africa causes a severe skew toward zero.

Table 4: One-Sample Kolmogorov-Smirnov test for the full data set.

		T-13910 freq	LP Freq
N		73	44
Normal Parameters[a,b]	Mean	.16354332926	.349809
	Std. Deviation	.225636351763	.2432213
Most Extreme Differences	Absolute	.234	.141
	Positive	.202	.141
	Negative	-.234	-.075
Kolmogorov-Smirnov Z		2.002	.937
Asymp. Sig. (2-tailed)		.001	.344

a. Test distribution is Normal. b. Calculated from data.

Due to the lack of a normal T-allele distribution it is necessary to use nonparametric testing to analyze the entire data set. Regional analyses may be re-conducted to determine if some areas, such as the European region, can be examined separately using parametric testing for more detailed information. For the full data set we will use the Mann-Whitney U test and Spearman's Rho to look for dependency and correlation between the T-13910 and lactase persistence variables. For a regional analysis, should the distributions be normal, we can use the parametric versions which are the T-test and Pearson's Correlation.

Full data set analysis

The Mann-Whitney U Test is designed to determine if the samples used are drawn from populations with the same mean, in this case of lactase persistence frequency. Only cases that have a value for the lactase persistence variable were used. The first test was conducted using groups based on the absence (≤ 0%) versus the presence of the T-13910 allele (Table 5). The second test was also grouped by the T-13910 variable, with all cases below a frequency 5% (≤ 5%) tested against the cases 5% or greater (Table 6). In both tests the results are significant with a p-value of less than .001, revealing the observations are not equally mixed and are from different populations. Although the samples are pulled from the same data set, the frequencies of lactase persistence do not overlap between the groups with little or no presence of the T-allele and the appearance of it at a significant level.

Table 5: Mann-Whitney test at 0% or > 0%

Freq var			N	Mean Rank	Sum of Ranks
T-13910 freq	dimension1	≤ 0%	23	12.00	276.00
		> 0%	50	48.50	2425.00
		Total	73		

	T-13910 freq
Mann-Whitney U	.000
Wilcoxon W	276.000
Z	-6.937
Asymp. Sig. (2-tailed)	.000

Table 6: Mann-Whitney test at ≤ 5% or > 5%

Freq var			N	Mean Rank	Sum of Ranks
T-13910 freq	dimension1	≤ 5%	35	18.00	630.00
		> 5%	38	54.50	2071.00
		Total	73		

	T-13910 freq
Mann-Whitney U	.000
Wilcoxon W	630.000
Z	-7.460
Asymp. Sig. (2-tailed)	.000

To examine the correlation between the two variables, T-13910 and LP, we use the Spearman's Rho analysis (Table 7). The result of .706 correlation coefficient (R^2 = .4984) suggests about half of the cases of persistence are associated with the presence of the T-allele. Some of the leftover variance is likely due to statistical and measurement errors. Statistical errors may occur due to the difference between the actual population frequencies and those recorded in the studies. Measurement errors may occur when testing for lactase persistence. Though this indicates that there are many other relationships outside of the T-allele that predict lactase persistence, at nearly 50% we still see a strong correlation between these two variables.

Table 7: Spearman's rho correlation between T-13910 and lactase persistence.

			T-13910 freq	LP Freq
Spearman's rho	T-13910 freq	Correlation Coefficient	1.000	.706**
		Sig. (2-tailed)	.	.000
		N	73	44
	LP Freq	Correlation Coefficient	.706**	1.000
		Sig. (2-tailed)	.000	.
		N	44	44

Revised data set analysis

In a separate study adjustments were made to the data set to compensate for regional differences. All cases were removed other than populations in the regions of Europe, North Africa, Western Russia, the Russian Caucasus and the Middle East. Removing the cases in Asia and Sub-Saharan/East Africa trims 38 populations (2,935 individuals). The included 35 populations (9,436 individuals) extend outward from the northwestern areas of Europe into North Africa, the Middle East and the western and southern portions of Russia. We can now use parametric testing to establish the correlation between the T-13910 allele and lactase persistence and establish the strength of the correlation between them as applicable to these combined regions. The histograms now produce a more normal distribution for this allele, while maintaining a relatively normal distribution for lactase persistence (Fig. 6).

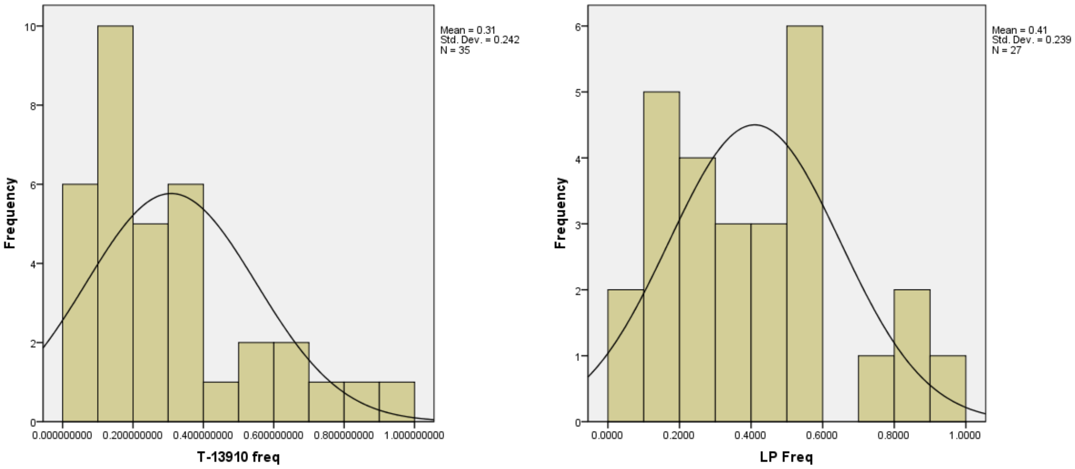


Fig. 6: Revised histograms exhibiting the frequency distributions for T-13910 and lactase persistence based on regional sections. The normal curves are shown.

The Kolmogorov-Smirnov test was run to confirm the normal distribution for both variables using the revised data set (Table 8). A finding of .579 indicates the T-13910 frequency is not significant, and therefore can be treated as normal for the purpose of using the parametric testing methods. With a p-value of .624 the lactase persistence is still not significant, and is therefore considered a normal distribution.

Table 8: One-Sample Kolmogorov-Smirnov test for the revised data set.

		T-13910 freq	LP Freq
N		35	26
Normal Parameters[a,b]	Mean	.30685664536	.409967
	Std. Deviation	.249199466473	.2392883
Most Extreme Differences	Absolute	.130	.145
	Positive	.130	.145
	Negative	-.112	-.085
Kolmogorov-Smirnov Z		.779	.752
Asymp. Sig. (2-tailed)		.579	.624

a. Test distribution is Normal.

b. Calculated from data.

A one-sample T-test reveals both variables are significant (Table 9). The 95% mean confidence interval of the T-13910 freq variable is 0.225 to 0.391, and the LP freq variable 0.329 to 0.517, indicating the sample frequencies are not identical. The paired T-test reveals that the means are also quite different between the two variables (Table 10). The T-13910 variable is .171 points smaller than the lactase persistence variable, and the significance of the paired T-Test reveals the strong dependency between the two variables.

Table 9: One-Sample T-test for revised data set

	Test Value = 0					
					95% Confidence Interval of the Difference	
	t	df	Sig. (2-tailed)	Mean Difference	Lower	Upper
T-13910 freq	7.531	34	.000	.308229886143	.22505367861	.39140609367
LP Freq	9.242	25	.000	.4233115	.328976	.517647

Table 10: Paired Samples T-test for the revised data set

		Paired Differences							
					95% Confidence Interval of the Difference				
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	Sig. (2-tailed)
Pair 1	T-13910, LP Freq	-.14917	.118288	.023198	-.19695	-.10139	-6.43	25	.000

Pearson's correlation analysis is then conducted to determine the correlation coefficient for this restricted data set (Table 11). The correlation between the T-13910 allele and lactase persistence is at .877, which is squared to provide a coefficient of .769. We now know that about 80% of the lactase persistence in this regional data set can be explained by the presence of the T-allele. Though this does not prove causation, it does indicate a strong association in a large region encompassing all of Europe and parts of Africa and the Middle East. However we also note the T-allele is not completely in association with persistence, suggesting other causes may be found.

Table 11: Pearsons Correlation between T-13910 and LP in the revised data set.

		T-13910 freq	LP Freq
T-13910 freq	Pearson Correlation	1	.877**
	Sig. (2-tailed)		.000
	N	35	26
LP Freq	Pearson Correlation	.877**	1
	Sig. (2-tailed)	.000	
	N	26	26

**. Correlation is significant at the 0.01 level (2-tailed).

Using the correlation tests for both the full data set and the regional data set demonstrates a powerful geographical association with lactase persistence for both the T-13910 allele and other unexplained factors, possibly additional polymorphisms. The demonstration of a reduction in the correlation coefficient between Europe and neighboring regions, as compared to lower African and Asian territories, reveals a higher concentration of the T-allele proceeding toward Europe. The association between the variables exhibited in the Mann-Whitney and T-Tests also suggests the T-allele may be used as a predictor of lactase persistence in European populations.

Additional polymorphisms analyzed

Outside of the European and neighboring regions the T-13910 allele appears at very low frequencies or does not exist. Since lactase persistence does exist in some populations in these areas, convergent evolution of the phenotype is indicated. Several other single nucleotide polymorphisms (mutations) have been discovered that may be linked to persistence in these regions. Research in these areas is limited; therefore only five potential candidates have been included in this study: C-14010, G-13915, G-13907, C-13913 and A-22018. All except the A-22018 are located in close proximity to the T-13910 allele.

Multiple populations are included in the tables indicating available information for each of the additional African polymorphisms, however the statistical analyses of these polymorphisms, as well as A-22018, only includes the studies that reveal a frequency greater than zero. The areas outside of the region where the polymorphism is found are therefore eliminated, removing data that would cause excessive skews. This provides a more accurate picture of the polymorphism correlations with lactase persistence.

C-14010. The C-14010 mutation so far has only been found in the eastern area of Africa including Ethiopia, Uganda, and Kenya (Table 12). Five additional areas of study involving 2,214 individuals revealed no frequency in Europe, the Middle East or Sub-Saharan Africa. A linear regression reveals nearly complete correlation in those regions between the C-14010 allele and lactase persistence (Table 13). The data strongly indicate that this mutation is a predictor of lactase persistence, however, with only three positive samples totaling 1,544 individuals, more studies should be conducted to strengthen the correlation.

Table 12: Samples of the C-14010 polymorphism.

Population	C-14010 Frequency	Sample Number	Study Source(s)
Arabia	0.000	1206	Ingram et al 2009b, Imtiaz et al 2001 and Enattah et al 2008
Italy	0.000	66	Ingram et al. 2009b
Israel	0.000	262	Bersaglieri et al. 2004 and Ingram et al. 2009b
Sudan	0.000	562	Tishkoff et al. 2007, Enattah et al. 2008 and Ingram et al. 2009b
Senegal	0.000	118	Ingram et al. 2009b
Ethiopia*	0.006	1080	Jones et al. 2009 in Itan et al. 2010 in Itan et al. 2010, Ingram et al. 2009b
Uganda*	0.030	76	Jones et al. 2009 in Itan et al. 2010
Kenya*	0.273	388	Tishkoff et al. 2007

*Populations included in regression / correlation analysis.

Table 13: C-14010 regression / correlation analysis.

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
Dimension0 1	.999[a]	.999	.998	.0072386

a. Predictors: (Constant), C-14010

G-13915. The G-13915 polymorphism is recorded in the Middle East, North Africa and East Africa, overlapping to some extent with the C-14010 mutation (Table 14). Areas located in Europe or the western region of Africa as well as areas south or west of Kenya reported no occurrence of the G-13915 allele. With six positive population studies totaling 3,522 individuals, this allele has more strength than the C-14010 allele, however it would still be better understood with additional population studies in Africa and the Middle East. Unlike the C-14010 allele, the G-13915 mutation does not appear to associate with lactase persistence (Table 15). The correlation coefficient, R^2, is 0.157, explaining persistence in less than 16% of the population. Based on these studies, this polymorphism does not predict lactase persistence.

Table 14: Samples of the G-13915 polymorphism.

Population	G-13915 Frequency	Sample Number	Study Source(s)
Italy	0.000	66	Ingram et al. 2009b
Senegal	0.000	118	Ingram et al. 2009b
Uganda	0.000	76	Jones et al. 2009 in Itan et al. 2010
Scandinavia	0.000	1876	Enattah et al. 2008
Tanzania	0.000	512	Tishkoff et al. 2007
Kenya*	0.039	388	Tishkoff et al. 2007
Israel*	0.061	262	Bersaglieri et al. 2004 and Ingram et al. 2009b
Ethiopia*	0.072	1080	Jones et al. 2009 in Itan et al. 2010, Ingram et al. 2009b
Morocco*	0.083	24	Enattah et al. 2008
Sudan*	0.137	562	Tishkoff et al. 2007, Enattah et al. 2008 and Ingram et al. 2009b
Arabia*	0.582	1206	Ingram et al 2009b, Imtiaz et al 2001 and Enattah et al 2008

*Populations included in regression / correlation analysis.

Table 15: G-13915 regression / correlation analysis.

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
dimension0 1	.396[a]	.157	-.012	.1861636

a. Predictors: (Constant), G-13915

G-13907. A third African polymorphism is G-13907, found almost exclusively in the East African coastal countries (Table 16). The linear regression for this polymorphism establishes less than 1% correlation in a total positive sample group of

3,748 individuals (Table 17). Based on these results it is fairly conclusive that the G-13907 is neither causal nor predictive of persistence.

Table 16: Samples of the G-13907 polymorphism.

Population	G-13907 Frequency	Sample Number	Study Source(s)
Italy	0.000	66	Ingram et al. 2009b
Senegal	0.000	118	Ingram et al. 2009b
Uganda	0.000	76	Jones et al. 2009 in Itan et al. 2010
Scandinavia	0.000	1876	Enattah et al. 2008
Israel	0.000	262	Bersaglieri et al. 2004 and Ingram et al. 2009b
Morocco	0.000	24	Enattah et al. 2008
Arabia*	0.002	1206	Ingram et al 2009b, Imtiaz et al 2001 and Enattah et al 2008
Sudan*	0.023	562	Tishkoff et al. 2007, Enattah et al. 2008 and Ingram et al. 2009b
Kenya*	0.023	388	Tishkoff et al. 2007
Ethiopia*	0.094	1080	Jones et al. 2009 in Itan et al. 2010, Ingram et al. 2009b
Tanzania*	0.293	512	Tishkoff et al. 2007

*Populations included in regression / correlation analysis.

Table 17: G-13907 regression / correlation analysis.

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
Dimension0 1	.030[a]	.001	-.332	.2348980

a. Predictors: (Constant), G-13907

C-13913. The C-13913 mutation has only been found so far at minimal frequencies in Sudan and Ethiopia (Table 18). Though the correlation is much higher for this polymorphism, 40% (Table 19), the lack of data and extremely low frequencies make this unlikely to have a strong enough association with persistence to use for prediction. More data would be necessary to make a more conclusive determination, particularly through the North African and Middle Eastern areas.

Table 18: Samples of the C-13913 polymorphism.

Population	C-13913 Frequency	Sample Number	Study Source(s)
Scandinavia	0.000	1876	Enattah et al. 2008
Israel	0.000	132	Ingram et al. 2007
Morocco	0.000	24	Enattah et al. 2008
Arabia	0.000	248	Enattah et al. 2008
Sudan	0.005*	185	Ingram et al. 2007
Cameroon	0.017*	117	Ingram et al. 2007
Ethiopia	0.026*	38	Ingram et al. 2007

*Populations included in regression / correlation analysis.

Table 19: C-13913 regression / correlation analysis.

Model		R	R Square	Adjusted R Square	Std. Error of the Estimate
Dimension0	1	.632[a]	.400	-.201	.2931602

a. Predictors: (Constant), C-13913

A-22018. Another allele that also appears to highly correlate with persistence is found in high association with the T-13910 allele. The A-22018 has been widely studied in European populations. Enattah et al. (2007) notes that the A-22018 allele, though not entirely associated, is certainly strongly associated with persistence. It is also noted that the allele may simply have hitchhiked due to the long region of linkage disequilibrium. Xu et al. (2010), however, has found a correlation with populations in North China in which the T-13910 allele is not significantly present. In these populations the A-22018 allele appears to mirror the frequency of persistence. When analyzed by linear regression the correlation coefficient is 89.6%, a high association with persistence (Table 20). Further studies of this allele may warrant consideration as predictive in some Chinese populations.

Table 20: A-22018 regression / correlation analysis.

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
dimension0 1	.946[a]	.896	.892	.085994

a. Predictors: (Constant), 0

Additional polymorphism results. The C-14010 polymorphism is a powerful indicator that lactase persistence is attributable to single nucleotide polymorphisms located in the MCM6 gene upstream from the lactase gene. The in-vitro studies have confirmed the likelihood of causality for both this and the T-13910 alleles that lie in what appears to be an enhancer sequence for increased mRNA production (Enattah et. al., 2007; Ingram et al., 2009b). This enhancer region binds to several transcription factors believed to affect lactase production. Studies currently indicate these two alleles are extremely important to adult digestion of lactose. The A-22018 polymorphism also appears to correlate with persistence in conjunction with T-13910, as well as alone in Northern Chinese populations.

Mapping analyses

Frequency maps were created with ArcMap, from the ArcGIS 9.3 software package. Gradient maps were created with ArcGIS shape files using Mapviewer 7 from Golden Software. In all allele frequency maps the frequencies of zero or missing entries were removed to clarify the geographical cline. The strength of the frequency is visually represented by the size of the box, with the largest boxes indicating the highest frequencies. The gradient maps interpolate the same data to indicate high to low frequencies, with missing areas filled in as a gradual decrease from the neighboring frequencies to no information (gray). For this reason these maps are also predictive for areas between and surrounding sample locations.

The first map (Fig. 7) indicates a decline in frequency of the T-13910 allele geographically from Europe toward Africa and Asia. However, traces of the polymorphism are found in the farthest reaches of these continents, suggesting either convergent evolution or, more likely, a rapid surge of the mutation across great distances, probably through migration. This map offers a strong visual reference for the origin of this allele as somewhere on the Western European coastline, most likely on the British Isles or Scandinavian Peninsula. The gradient map (Fig. 8) shows the high frequency areas and the spread outward and is consistent with the area of origin.

A single outlier location in Sudan has a much higher than expected frequency and may need to be further examined. One possible cause for this could be an excessively high rate of inbreeding within the tested population. However, the Hardy Weinberg expected homozygosity for CC is 1.10, and TT is 0.86, whereas inbreeding would have resulted in a higher rate of TT homozygotes. Another possibility is an influx of European genotypes that are not historically recorded, allowing the concentration of T-13910 to rise as it mixed with indigenous populations. The only other feasible answer would be errors of testing incorporated into the study itself. More studies need to be conducted in this region and surrounding areas to determine why the frequency is inconsistent.

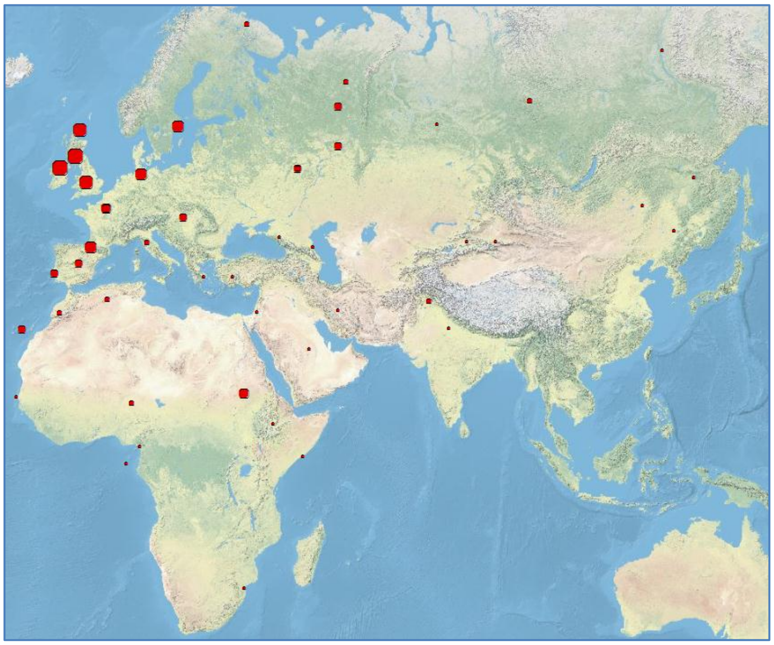


Fig. 7: T-13910 frequency distribution.

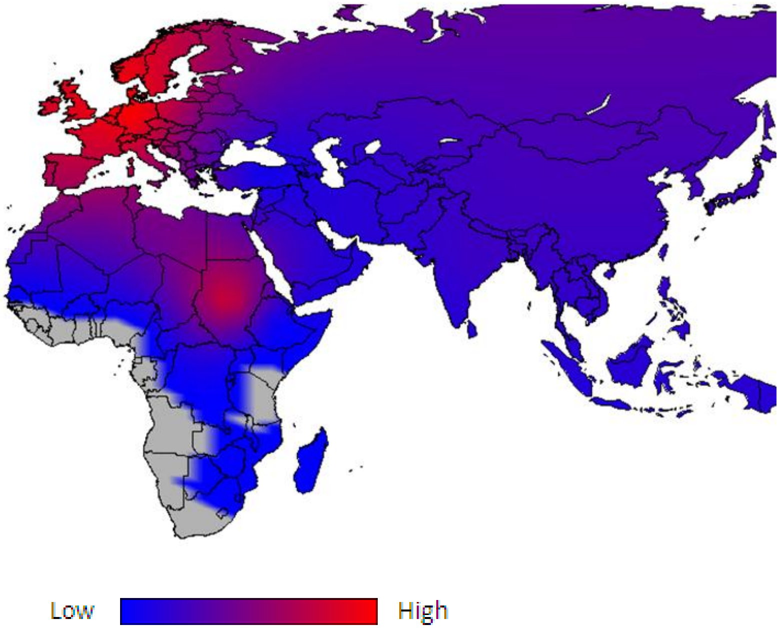


Fig. 8: T-13910 frequency gradient.

Covering the same general regions as the T-13910 allele, the A-22018 polymorphism appears to have originated in Europe and spread into Africa and Asia (Fig. 9). The strongest frequencies are found in Scotland and the Basque region between France and Spain, with substantial concentrations in Scandinavia and Sudan. New evidence has been presented (Xu et al. 2010) indicating that the A-22018 allele correlates with lactase persistence in northern Chinese populations. In addition, the frequency found in Sudan also suggests the high frequency of the T-13910 allele in the same location was due to a strong European influence rather than convergent evolution or research error.

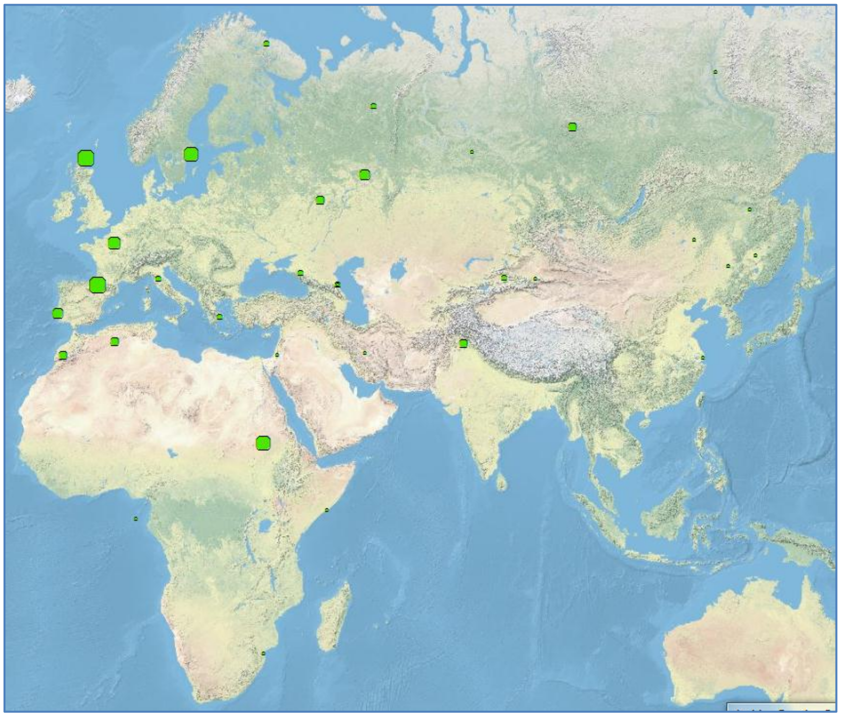


Fig. 9: A-22018 frequency distribution.

Maps for the other alleles included in this study reveal much more limited clines. In most cases only a few populations have been studied in a relatively small region.

This makes it much easier to examine the areas where the frequencies are found, however, it is possible that other regions not yet studied may contain these mutations.

The C-14010 is limited to the eastern coastline of Africa (Fig. 10). The mutation was found in high frequencies in Kenya and Uganda, with a trace existing to the north in Ethiopia. Studies conducted in North Africa, the Middle East (Israel) and Western Africa revealed no traces of the allele.

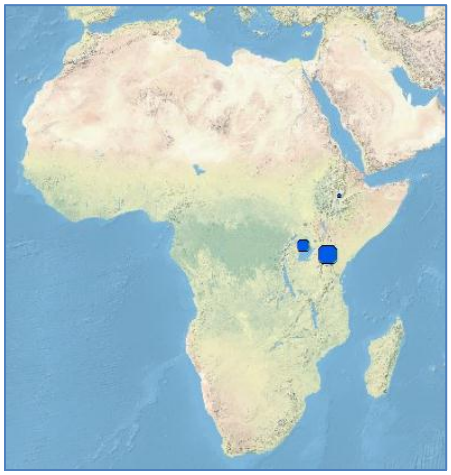


Fig. 10: C-14010 frequency distribution.

Figure 11 shows the G-13915 allele is only found at a high frequency in the Middle East (Saudi Arabia). Traces were found extending into North, Eastern and Western Africa as far south as Tanzania, indicating this allele may have a much wider range than expected. However, the lack of correlation with lactase persistence suggests it may be more worthwhile to pursue studies of other alleles that appear to be much stronger indicators of persistence.

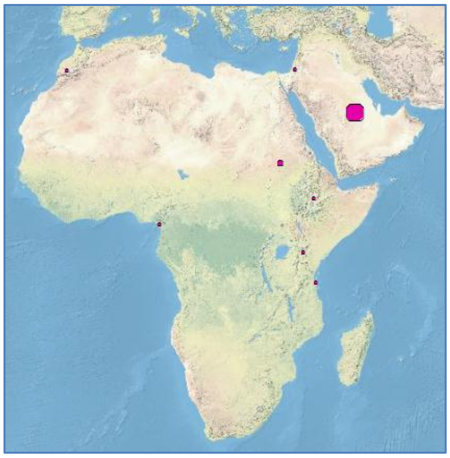


Fig. 11: G-13915 frequency distribution.

The G-13907 mutation in Figure 12 follows a relatively clear frequency decline from Tanzania northward through Ethiopia and Sudan, finally ending in the Middle East (Saudi Arabia) at 0.2%. This overlaps heavily with the C-14010 allele. It makes little sense that two causative alleles would overlap under selective pressures, however, the G-13907 polymorphism does not appear to correlate with lactase persistence.

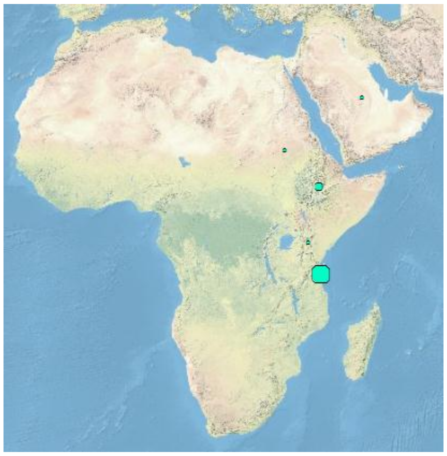


Fig. 12: G-13907 frequency distribution.

The C-13913 is only weakly present in any location, with the highest frequency discovered in Ethiopia at 2% (Fig. 13). The locations it is found in are on opposite coasts, with 1.7% found in Cameroon. The allele was not found in Middle East samples, however, very little information is available yet on this allele and many more studies are needed to determine if there is a presence in other locations.

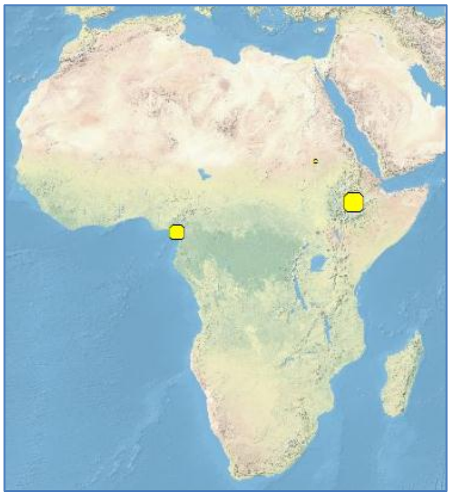


Fig. 13: C-13913 frequency distribution.

When viewing lactase persistence on a separate frequency map we see a similar cline to the T-13910 and A-22018 alleles with higher frequencies generally found in Europe, spreading east and south into Asia and Africa (Fig. 14). The gradient map indicates strong European frequencies with some high frequency locations scattered throughout Africa and moderate frequencies ranging throughout Asia (Fig. 15). Much of the frequency range through Asia is predictive based on the available data. The East African coast where the C-14010 is found also demonstrates persistence frequencies that uphold the correlation between this mutation and lactase persistence.

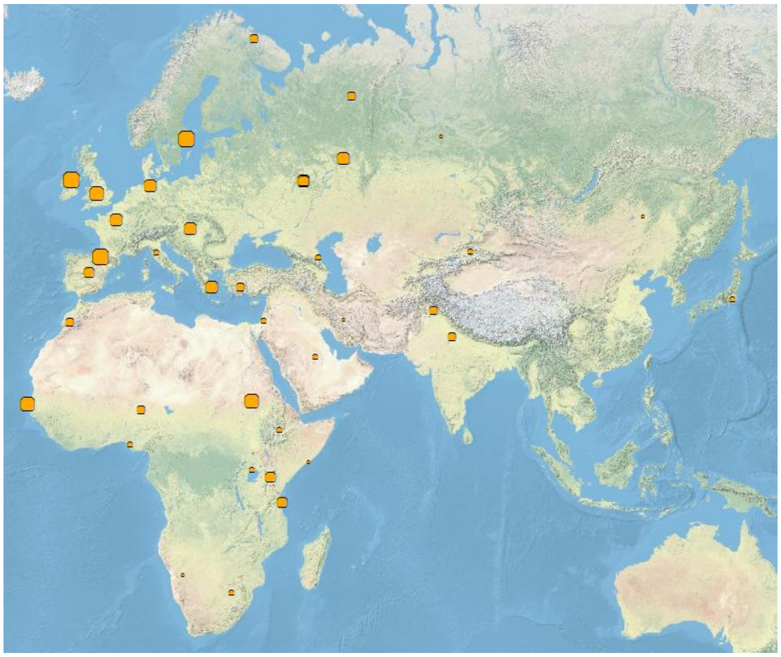


Fig. 14: Lactase persistence frequency distribution.

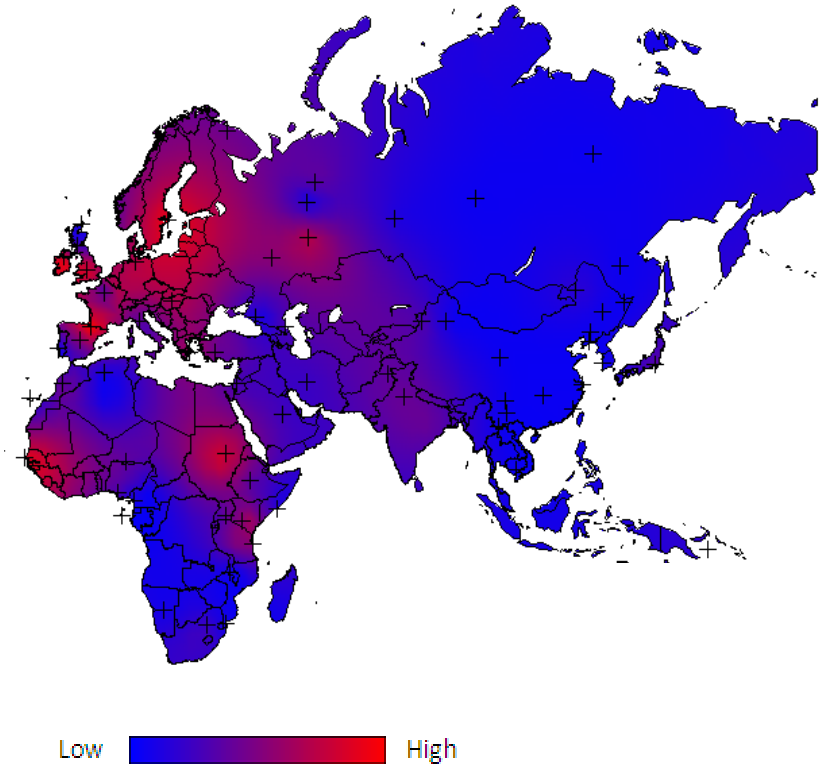


Fig. 15: Lactase persistence frequency gradient.

CHAPTER 6: CONCLUSIONS

Collecting individual data would better reveal clusters within populations, however, the impossibility of using individual data for studying large populations led to the use of population samples from published studies. The large geographical area covered further increases the difficulty of individual assessments, since the overall regions examined include billions of individuals. Using population samples may introduce some error due to a variation in sample sizes; however, this offers a more manageable representation of the frequency distributions throughout the Old World.

The origin of the T-13910 polymorphism, based on the map analysis appears to have begun in the coastal area of Western Europe, probably in the British Isles or nearby mainland coast. The mutation spread rapidly south and east, reaching notable frequencies in Western Russia and Northwestern Africa, with trace frequencies found extending into Northeastern China and Southeastern Africa. Population movements through the Eurasian steppes following horse domestication over the last six thousand years or more (Anthony, 2007) likely contributed to the traces seen in the far reaches of Asia, nearly reaching the Pacific. Slave trade and European colonization undoubtedly distributed some frequencies in the Sub-Saharan African region as well.

Examination of the lactase persistence frequencies indicates a similar geographical spread, confirming the high correlation of the mutation with persistence. The few cases in the European region with T-13910 frequencies that are high yet persistence frequencies are low may be a reflection of a variation in the subsets of the populations used in different studies that were then combined. Alternatively they may be a reflection of secondary nonpersistence factors.

The large area of linkage disequilibrium surrounding the lactase gene suggests a recent, rapid increase in the lactase persistence adaptation. Based on the Culture-historical hypothesis, this rise in frequency occurred in conjunction with, or immediately following, the spread of dairy consumption, naturally selecting for the change as populations quickly adopted dairy pastoralism. However, archaeological evidence indicates the use of dairy during the Neolithic period, prior to the appearance of the T-13910 mutation, beginning at least 8,500 years ago and spreading from the Middle East region into Europe, reaching Britain by the 5th millennium BC. All evidence collected indicates the T-13910 allele originated in Western Europe, then spread into Russia, the Middle East and North Africa. Based on the Culture-historical hypothesis, since the two clines flow in opposing directions the T-13910 mutation was probably much more recent than dairy pastoralism, and selection was even stronger than expected. Although the Culture-historical hypothesis applies to the concept of a rise in frequency after dairy practices are adopted, it should be noted that lactase persistence occurred and spread well after dairying, from Western Europe to the Middle East.

Haplotype **A** in the European area is the carrier of the T-13910 mutation and the widespread focus of lactase persistence correlation studies. The large block of linkage disequilibrium surrounding the lactase gene in 77% of the Europeans exhibiting this haplotype is another piece of evidence supporting intense selection of this region. F_{st} tests reveal a highly significant distribution between genome wide markers and the markers surrounding the lactase gene proving this block consists of about one million bases.

Between species studies that compare the lactase gene region of humans with other primates were used to determine the ancestral human haplotypes (Enattah et al., 2007). This allows an age estimate to be calculated for the T-13910 allele. Using a 25 year generation coefficient, Enattah et al. (2007) revealed the probable age as approximately 5,000 to 9,000 years in Finnish populations. In evolutionary terms this is an extremely rapid acceleration of an adaptive trait. This rate of adaptation is highly unlikely to have occurred without potent selective pressure.

Examining Hardy Weinberg Equilibrium equations in the populations that contain the T-13910 polymorphism reveals an average heterozygosity excess of 13%. An excess of heterozygotes is another indicator of natural selection as the new allele begins to move toward fixation. When comparing the homozygosity of the C-13910 allele, recessive for nonpersistence, to the expected Hardy Weinberg Equilibrium, a 67% deficiency is revealed. This not only indicates selective pressures, but the direction they are moving.

The analyses of several additional alleles suspected of association with persistence in the Middle East and Africa reveal what is most likely convergent evolution on a phenotypic level only. A robust correlation can be drawn between C-14010 and persistence, as well as a weaker correlation between C-13913 and persistence. The evidence of the T-13910 polymorphism in a Fulani Sudanese population was potentially a case of genotypic convergent evolution, however, the A-22018 polymorphism, predominantly located in the same European regions as T-13910, exhibits a presence consistent with the European genotype. It is highly unlikely two alleles would evolve collectively in two geographically separated populations.

Statistical analyses support the correlation between the T-13910, A-22018, C-14010 and C-13913 alleles and lactase persistence. In-vitro tests conducted on the T-13910, C-14010 and C-13913 polymorphisms suggest these alleles not only correlate with persistence, but are causal. This lends even greater importance to the correlation of these mutations, as opposed to only markers, with persistence, and allows them to be used for predictive purposes. Based on these in-vitro studies, the region containing these alleles appears to act as an enhancer region to the lactase gene. Transcriptional enhancers usually span 100 – 2,000 bases, and lay within 100,000 bases of the gene they affect (Arnosti and Kulkarni, 2005). Regardless of the distance between them, the nonlinear folding of DNA allows the enhancer to exist in close proximity to the gene it augments. Studying enhancers helps with understanding the interaction between genes, and the regulation of those genes.

Combining the data from different analyses and examinations including maps, linkage disequilibrium studies, haplotype studies, archaeological data, statistical analyses and Hardy Weinberg equations offers a detailed insight into the evolution of the T-13910 allele and lactase persistence in general. Assuming causality for the T-13910, C-14010 and C-13913 polymorphisms, the geographical correlation with persistence allows both prediction and identification of the presence of additional causal alleles through unexplained persistence frequencies. The effects of natural selection can never be proven absolutely, however a clear footprint exists in the data collected and analyzed for this study.

If the T-13910 allele were not subject to natural selection, we would see several differences in the data obtained. Although haplotype **A** would still be dominant in Europe, since the spread into Eurasia occurred after the four global haplotypes came into existence, we would see a greater diversity in the lactase gene region. The large region of linkage disequilibrium would be far smaller or nonexistent. The frequency of the mutation would take far longer to reach appreciable levels within the population of origin, and would be much more likely to reach equilibrium without becoming excessive for homozygosity. Migration across wide geographical regions may still take place but on a much slower and more sporadic scale, with more isolated populations less likely to be affected. To reach the high frequencies we see, as much as 77% in Europeans, would most likely require longer than the 35,000 (minimum) years humans have been in Europe.

The actual selective pressures have yet to be identified, although theories have been put forth regarding calcium intake in northern latitudes, food and water sources in desert regions or malarial prevention (as nonpersistence). However, it is more likely that different pressures would apply in different geographical locations. Further studies and examinations of specific geographical locations may help identify more potential causal factors and clarify the reasons for the strong selection for lactase persistence.

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