The Onset of Lactase Persistence in Europe

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Abstract

The genomic region containing the lactase (*LCT*) gene shows one of the strongest signals of positive selection in Europeans, detectable using a range of approaches including haplotype length, linked microsatellite variation and population-differentiation-based tests. Lactase is the enzyme that carries out the digestion of the milk sugar lactose. Its expression decreases at some point after the weaning period is over in most mammals and in around 68% of all living adult humans. However, in some humans, particularly those from populations with a history of dairying, lactase is expressed throughout adulthood. This trait is called lactase persistence (LP), and in people of European ancestry, it is associated with a single mutation (-13910^*T). Evidence from the detection of dairy fat residues in potsherds, and allele frequencies in ancient DNA samples suggest that LP arose after dairying practices had developed. However, the reasons why LP may have been advantageous are still debated, and the respective contribution of demography and natural selection remains to be disentangled. This paper discusses various studies, from archaeology to population genetics, that have shed some light on the subject by investigating the evolution of LP in Europe.

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Lactase Persistence and Natural Selection

The lactase gene (*LCT*) encodes the small intestine enzyme lactase or lactase phlorizin hydrolase, responsible for the cleavage of the milk sugar lactose into its two monosaccharide components glucose and galactose [1, 2]. Without lactase, lactose reaches the colon, where it is fermented by colonic bacteria. Products of this fermentation and osmotic effects generate symptoms of lactose intolerance, including abdominal cramps, bloating, flatulence, or diarrhea [3]. Lactase activity is high in young mammals, including most humans, but decreases after weaning [4]. This is the case for most adults worldwide [5], except for about 32% who show a persistence of lactase activity throughout their lifetimes. This phenotype is termed lactase persistence (LP) and has only been observed in humans.

The genomic region surrounding *LCT* has been widely identified as a striking example of a region that shows strong signals of positive directional selection. In European genomes, this effect is detected using tests which show extended haplotype lengths [6–8], a reduced diversity of linked microsatellites [9] and a high population differentiation [10, 11]. These signals of strong selection are associated with a single nucleotide polymorphism (rs4988235, C>T) of which allele -13910^*T , located at 13910 nucleotides upstream of the transcription start of *LCT*, is causal of LP [12–14]. Estimates of the selection coefficients of this allele range from 0.3 to 19% using a co-

alescent approach [15], from 0.8 to 1.8% using a demic approach [16], from 5.2 to 15.9% using a spatially explicit simulation modeling approach [17] and from 9 to 19% using genetic variation [6]. These high selection coefficients are attributable to the fact that the allele is so widespread, yet the date estimates for the expansion of the allele are relatively recent: 2,188–20,650 years before present (BP) using extended haplotype variation [6], 7,475–10,250 years BP using closely linked microsatellite variation [18], 6,256–8,683 years BP using simulation modeling [17] and 1,500–64,900 years BP using a coalescent approach [15].

The -13910^*T allele enhances lactase activity both in vitro [13, 14] and in vivo [12]. It is noteworthy that four additional variants, located in the same genomic region, are also associated with LP in other geographic regions [5] and have been shown to enhance lactase expression in vitro [19–23]. This suggests that the LP is a case of parallel selection, or convergent evolution [21–25]. The coexistence of several alleles in a single geographic location alters the patterns of diversity and provides a different signature of selection [20–22].

While the reasons why LP may have been positively selected remain to be addressed, they are likely to differ between distinct geographic regions [26, 27]. Interestingly, LP is not evenly distributed worldwide [3, 5, 28], but appears to be more frequent in populations with a history of dairying [29, 30].

The Archaeological Context of Dairying

The earliest archaeological evidence for dairying dates back to the beginning of an archaeological period called the Neolithic. The Neolithic refers to the transition from a subsistence strategy mainly based on hunted and gathered species to one where domesticated crop and animal species tended to predominate. The archaeological record shows that the most ancient evidence for farming economies lies in the Near East (approx. 10,500 years ago), while farming only appears between 2,000 and 3,000 years later in continental Europe and up to 4,000 years later in Northern and Northwestern Europe [31, 32]. How farming economies spread is still a matter of debate, but it seems that expanding farming populations from the Near East introduced their economies over much of Europe [33–35], with the exception of some areas, where it may have been adopted by local hunter-gatherer populations [31, 36]. This population expansion can be defined by a small population growing in size and by expanding its geographical range [37, 38]. This population expansion, associated with farming,

has been suggested from diverse sources of archaeological evidence including an increase in fertility [39] inferred from a higher proportion of young individuals in Neolithic cemeteries and a higher population density inferred from a higher density of radiocarbon dates in Neolithic times in comparison to earlier periods [31, 40].

With respect to the onset of dairying, the age and sex distribution of animals (at death) in archaeological skeletal assemblages have suggested that more female animals were kept alive to be milked in Near Eastern archaeological sites dated to around 10,500 years BP onwards than in earlier (late Mesolithic) sites [41–44]. Further archaeological evidence for the intensification of dairying comes from the detection of dairy fat residues in potsherds by a combination of individual fatty acid discrimination and measurements of stable isotope ratios. The use of dairy products has by these means been observed (fig. 1) as early as 8,500 years BP [45] in the Northwest of present-day Turkey, around 7,900–7,450 years BP [46] at Neolithic sites in Romania and Hungary and around 6,100 years BP in Britain [47]. These dates lie near or at the time when farming developed in the respective regions, indicating that dairying was an early feature of the Neolithic lifestyle.

The archaeological record provides additional information on the evolution of LP in Europe with recent developments in ancient DNA technologies (fig. 1). Indeed, the earliest evidence for the presence of -13910^*T in ancient DNA European samples is in late Scandinavian hunter-gatherers (with a frequency of 5%) dated from 5,400–3,400 years BP [48] and at about the same time (between 5,000 and 4,500 BP, with a frequency of 26 and 11%) in early Neolithic farmers from Northwestern Spain [49]. The allele is, however, absent from early Neolithic farmer samples from other regions [50–52]. Less surprisingly, it has been observed in Medieval samples from Northeastern Europe (a single sample dated to 400–600 AD, which was heterozygous [50]) and from Central-Eastern Europe (with a frequency of 11% in samples dated to 1012–1112 AD [53]). Considering both the low frequency/rarity of the -13910^*T allele in Neolithic samples [50–52] together with the archaeological evidence of dairying [45] suggests that dairying was practiced before LP arose or became common (fig. 1).

Natural Selection and Demography

Assuming dairying reflects the cultural trait of milk drinking and consequently a potential selection pressure, the selection pressure predates the occurrence of the ad-

Fig. 1. Ancient DNA evidence of the -13910^*T allele and occurrence of dairy fat residues in potsherds from the archaeological record. The chart size reflects the sample size and is color-coded, going from red (for older samples) to cream (for the most recent samples), representing the frequency of the ancestral -13910^*C allele (the frequency of the -13910^*T allele, associated with LP in Europe, is given in black). Blue symbols indicate the locations of Neolithic sites from which lipid residue analyses were performed on potsherds (with the detection of dairy residues based on isotopic analyses or molecular criteria for well-preserved residues): circles = milk fats were undetectable; diamonds = the detected proportion of milk fats was $<30\%$; triangles = the detected proportion of milk fats was $>30\%$. med. time = The median time of sampling of ancient DNA, given in years BP. The data were taken from: ** Burger et al. [50]; " Lacan et al. [52]; + Plantinga et al. [49]; ' Lacan et al. [51]; * Malmström et al. [48]; ^ Nagy et al. [101]. The data on lipid residue analyses are from [45–47, 77, 94–98]. The figure is adapted from Gerbault et al. [99].

vantageous allele (i.e. -13910^*T) in these populations. This model of selection is sometimes referred to as 'selection on a de novo mutation' in opposition to 'selection from standing variation', where alleles are already present at a reasonable frequency when the selective pressure occurs [54]. A recent study using a coalescent approach coupled with an approximate Bayesian framework [15] showed that the selection on *LCT* in European genomes was more likely to be a case of selection from a de novo mutation than from standing variation. The occurrence of the allele at a low frequency at the time when the selective pressure arose is perhaps the most reasonable scenario.

It is worth mentioning that whether selection from a de novo mutation or from standing variation is more likely may not only depend on the timing of when the mutation arises in relation to when the selective pressure occurs, but it may also depend on the effective size of the population considered. In other words, the larger the population, the larger the number of variants that can remain in that population and the more likely a selection from standing variation. This corroborates what we know

about the evolution of LP in Africa and Europe. Indeed, in the former, LP is associated with multiple beneficial alleles – and could be an example of 'soft sweep' – [22, 23, 25, 55], and the estimated population sizes are generally larger than those estimated for Eurasia [56, 57]. Accordingly, LP in Europeans has so far been associated with a single beneficial allele – and could be a case of 'hard sweep' [24, 58].

Furthermore, it is noteworthy that this recent study [15] used the observed allele frequency of the modern Finnish population sample for fitting the modeled -13910^*T allele trajectory. Amongst other features required for testing the case of selection from a de novo mutation or standing variation [15], this population sample was chosen because it has a high frequency of the positively selected allele, without it being fixed. Nonetheless, the -13910^*T allele frequency varies quite substantially within Europe (fig. 2). For example, while it can reach frequencies as high as 81.5% in Sweden or 68.8% in Great Britain [6], it can be as low as 3.1% in Turkey [18] or 8.5% in Sicily [59]. While it is often assumed that the location where an allele is most frequent is its location of origin, it

Fig. 2. The current geographic distribution of the -13910^*T allele in European populations. The chart size does not reflect the sample size. The data on the -13910^*T allele frequencies (portion in black) are from [6, 18, 59, 100–103].

has recently been shown that this is not always the case [60, 61]. Indeed, similar patterns can be produced by a phenomenon called ‘allele surfing’ occurring during a spatial population expansion [60–65], as may have been the case in the context of the Neolithic expansion.

Allele surfing can happen in any allele, derived or ancestral, neutral, advantageous or deleterious, but the spatial distribution of allele frequencies may be different depending on its selection status – neutral, advantageous or deleterious [62]. This phenomenon is nonetheless more likely to be identified when a rare allele has surfed successfully, generating a gradient of allele frequency between its location of origin and the wave front of advance. Allele surfing is more frequent when the population size and the migration rate are small, because then the founder effect in newly colonized habitat strengthens the effect of genetic drift and leaves more opportunities for an allele that has been rare to reach higher frequencies [61].

In the case of LP and the -13910^*T allele, Itan et al. [17] used a spatially explicit model, simulating the range expansion of farmers from the Near East into Europe, to infer the location where selection on this European allele

may have started: in Central Europe, at a place where (and a time when) an archaeological culture called the ‘Linear-bandkeramik’ (LBK) based on cattle economy was spreading Northwest. In this model, the allele remained at low frequencies where it first appeared and was selected and rose in frequency as farming populations expanded Northwest. During such a range expansion, allele surfing can also produce similar patterns for neutral alleles, and the extent to which the -13910^*T frequency trajectory can be obtained without selection during a spatial expansion remains to be formally assessed. Along these lines, a demographic simulation study [16] suggested that selection of the -13910^*T allele may not have been as high in Southern Europe as it may have been in Northern Europe.

Interestingly, Bersaglieri et al. [6] inferred that for a Scandinavian population sample of parent-offspring trios the selection pressure was stronger and the allele more recent than if other European-derived US pedigree samples were considered (i.e. a selection of 9–19 vs. 1.4–15% and a time range of between 1,625 and 3,188 vs. 2,188 and 20,650 years ago in Scandinavian vs. other European samples, respectively). However, the significance of these differences

on the continental scale may reflect other demographic or ancestry effects [33, 34, 66]. Furthermore, disentangling the effect of natural selection over that of demographic range expansion is made difficult because range expansions and allele surfing can substantially affect the genetic diversity of an expanding population [63, 65, 67, 68].

Why Would LP Have Been Advantageous in Europe?

Additional environmental features at the onset of the Neolithic need to be considered to have an appreciation of the context in which LP may have evolved. On the one hand, the development of farming and agriculture occurred during the Holocene, a period when the climate was becoming warmer and may have promoted human migrations in the Near East and in Europe [40, 69–71]. Climate may thus have affected the -13910^*T allele distribution to the extent that it affected human migrations. On another hand, with the intensification of agriculture and domestication, the food range of agricultural and farming populations is known to have become narrower in comparison to that of hunter-gatherers [72–75]. Even though the consumption of milk and dairy products may thus have provided a good nutritional component to the diet of farmers [76], the identification of cheese-strainers from 7,150 to 6,750 years BP [77] suggests that lactase-nonpersistent adults may have consumed dairy products without experiencing symptoms of lactose intolerance early in the Neolithic. However, boom-and-bust patterns of farming populations due to early farming practices [32] or the not yet locally adapted domesticated species [78–81] may have provided a temporally and spatially limited selective pressure sufficient to drive the -13910^*T allele to high frequencies in modern-day European populations. This, however, remains very speculative.

The hypothesis of an environmental variable shaping the co-evolutionary process between dairying and LP in Europe has been suggested previously in terms of the calcium assimilation hypothesis [82]. In this hypothesis, the positive correlation between LP frequency and latitude led Flatz and Rotthauwe [82] to suggest that because of the calcium content and small amount of vitamin D in milk, LP may have provided a good supplement of both against the lack of UVB sunlight at higher latitude. Latitude – or more specifically the lack of sunlight – would be the selective pressure advantaging LP in adults. Before Northern Europeans started to rely on farming, a lack of sunlight (at high latitudes) may not have been an issue, since the diet of European pre-Neolithic hunter-gatherers would have

been rich in marine sources [83]. However, while the archaeological record provides evidence for less healthy Neolithic populations in comparison to Upper Paleolithic and Mesolithic ones [74, 84–86], there is no evidence for a higher incidence of rickets (i.e. severe vitamin D deficiency) in Neolithic archaeological assemblages [86]. It is possible, however, that mild vitamin D deficiency, which may be more complex to assess from archaeological remains, would be selectively disadvantageous [87, 88].

Conclusion

There are molecular signals of strong directional selection in the genomic region surrounding a single nucleotide variant, present in Europeans, that has been shown to affect the expression of the *LCT* gene both in vitro and in vivo. The existence of other alleles in this sequence region, in adults from different populations, is evidence of parallel or convergent evolution. These alleles also affect the persistence of lactase activity and may show a different signature of selection. The correlation between LP – and LP-associated mutations identified so far – and pastoralist/milk drinking populations [5, 29, 30] provides strong support for a co-evolutionary process between a cultural (dairying) and a genetic trait. This underlines the evolution of LP as a good example of gene-culture co-evolution [26, 89–92]. This correlation between the cultural skill of dairying and the occurrence of LP in human populations also provides a good example of the type of dietary adaptation the Neolithic period entailed [73, 93]. The reasons why LP may have been advantageous remain to be clarified and the respective contribution of natural selection and spatial range expansion in shaping the -13910^*T allele trajectory to be disentangled. This research has nonetheless benefitted from diverse sources, including ancient and modern DNA data, lipid residue and archaeozoological analyses, ancient population demographic studies and population genetics. The integration of such a diverse source of information has shed a better understanding of the processes underlying the evolution of LP.

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