## Background

In the popular media and scientific literature, attempts have been made to question the scientific foundation of the Paleolithic diet. Critics of the Paleolithic diet have argued that the duplication of AMY1 provides evidence that humans are well adapted to consume large quantities of carbohydrates, including grains[1]. Similarly, it has been argued that high prevalence of lactase persistence (LP) in many populations illustrates adaptation to dairy consumption, as well as illustrating the potential for genetic adaptation to occur in the Neolithic era[2]. In reviewing the most up to date literature on diet-related genetic change in recent human history, this article addresses those critiques, thereby reaffirming the scientific basis of the Paleolithic diet.

## Introduction

Since the agricultural transition, humans have continued to undergo genetic change [1,2]. Changes in the salivary amylase (AMY1), alcohol dehydrogenase (ADH) and lactase (LCT) genes in very recent human history illustrate the potential for diet to drive genetic change in this time period [3-5]. However, genome scans searching for signatures of positive selection in recent human evolutionary history have found very little evidence of other genetic changes in response to diet. New insights into the selection of AMY1, ADH, and LCT variants suggest that unusually strong selection pressures were applied on these genes during, and even before, the Neolithic era. These genetic changes are therefore exceptional examples, and do not represent a broader level of genetic adaptation to the Neolithic diet.

[1] See for example: https://books.google.com/books?id=AB1hDgAAQBAJ&q=amylase#v=snippet&q=amylase&f=false ;https://books.google.com/books?id=4Ggf32BvMMC&q=amylase#v=snippet&q=amylase&f=false

[2] See, for example: https://www.theguardian.com/books/2013/apr/24/paleofantasy-evolution-sex-diet-review; https://books.google.com/books?id=AB1hDgAAQBAJ&q=lactase#v=snippet&q=lactase&f=false

## Detecting signatures of recent genetic change

Selection of a beneficial mutation causes a reduction in the diversity of SNPs either side of the selected allele. This pattern of reduced diversity is described as a selective sweep [6]. Recent research attempting to find evidence of recent genetic change has largely focused on the identification of selective sweeps in the genome. Since mutations that have occurred in recent human history are unlikely to have reached fixation in the current population, the genomic signature is likely to be a *partial* selective sweep (in which the beneficial mutation is carried by a proportion of the population, rather than by every member of the population) [6,7]. Genomic signatures of selective sweeps can also occur as a result of genetic caused by population bottlenecks; it is therefore important to rule out population bottlenecks when searching for adaptive change within the genome [8].

Standard statistical tests used to provide evidence of selection include Tajima's D test and Fay and Wu's H test, which detect deviations the ratio of intermediate-frequency mutations relative to low and high frequency mutations [8]. A low Tajima's D can result from positive selection, while a high Tajima's D can result from balancing selection, for example [8]. A negative Tajima's D result has been used as evidence for recent selective sweeps in LCT and AMY1 [8,21]. These standard tests are useful in candidate-gene studies in which there is a prior hypothesis of selection [14]. Recently, advanced statistical tests have been designed to detect signals of selection from whole-genome scans, in order to identify novel genes that may have been targets of recent selection. These include haplotype-based tests (a haplotype is a chromosomal segment defined by the specific array of single nucleotide polymorphisms, (SNPs) that it carries [9]) able to detect haplotypes of high homozygosity that extend over large chromosomal regions [8,10], and the linkage disequilibrium decay (LDD) test which detects a decrease in recombination rates either side of an SNP that has undergone recent positive selection [11,12]. Additionally, attempts have been made to search for signatures of *polygenic* adaptation, in which adaptation has occurred through subtle changes in allele frequencies at multiple loci [13]. Following the discovery of a SNP, further evidence (such as correlation with environmental factors, and comparison with the genomes of other species) can be used to determine whether a candidate SNP has likely been subject to positive selection [8].


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## Recent genome-wide scans

Several recent genome-wide scans have been carried out in order to search for genetic changes that have occurred in recent human history. These tests are sensitive so that they are able to detect weak signals of positive selection, involving weak partial sweeps within a population. Wang and colleagues carried out an LDD test and found evidence of selection in around the last 40,000 years of a small number genes related to diet. These include genes involved in protein metabolism (such as *ADAMTS19–20, APEH,* and *PLAU*) and organic compound metabolism (this has been suggested to be a result of increasing meat consumption) [11]. Voight and colleagues used an integrated haplotype score (iHS) test to identify genes that have been subject to recent selection. Diet-related genes that revealed signs of recent selection include genes involved in carbohydrate metabolism (MAN2A1 involved in metabolizing mannose, S1 involved in sucrose metabolism, and LCT involved in lactose metabolism), genes involved in lipid metabolism (including SLC27A4 and PPARD involved in uptake of lipids and SLC25A20 involved in oxidation of lipids), and genes involved in vitamin transport [14]. A composite likelihood ratio (CLR) test by Williamson and colleagues identified sterol carrier protein 2 (*SCP2*), involved in the intracellular movement of cholesterol as a gene that has been potentially subjected to recent change [15].

All of the above examples were detected in highly sensitive scans; the genetic changes therefore apply to only a small percentage of individuals within the population and do not illustrate population-wide genetic adaptation. In contrast, the examples discussed below, AMY1, LCT, and ADH, represent genetic changes that have either reached fixation, or are much closer to reaching fixation.

## AMY1

AMY1 encodes salivary amylase, a protein responsible for starch digestion in the oral cavity [16,17]. Chimpanzees have only two copies of AMY1, while humans have an average copy number of six (greater copy number has been shown to correspond to greater expression of salivary amylase and improved efficiency in digestion of starch) [3,18]. Sequence analysis of genomes from other great apes suggests that the AMY1 gene was duplicated in the human lineage after divergence from the great apes, as opposed to being lost in the great ape lineage [3]. It is assumed that at some point in human evolutionary history, positive

selection occurred for individuals with a greater copy number of AMY1 [3,19]. Perry and colleagues argue that positive selection for increased starch digestion occurred in the post-Neolithic era when starch consumption massively increased, a hypothesis that was supported by the correlation between starch consumption and average copy number of AMY1 in populations worldwide [3]. However, recent analyses suggest that positive selection occurred much earlier in human history. Genome analysis of an 8000 year old Mesolithic hunter-gatherer revealed that this individual already had 13 copies of AMY1, which is towards the high end of the number of copies of Europeans, suggesting that duplication occurred much earlier in human evolutionary history [20]. A recent analysis by Inchley and colleagues conducted various analyses on sequence data from 480 individuals worldwide, extinct hominids including Altai Neanderthals and Denisovans, and Mesolithic and Neolithic humans [21]. Phylogenetic analysis using BEAST (Bayesian Evolutionary Analysis Sampling Trees) estimated the divergence of the modern and archaic human haplotypes as 450KYA, following the divergence of the modern human lineage from a common ancestor of the Neanderthals and Denisovans. This is supported sequence data showing that Neanderthals and Denisovans have only two copies of AMY1 [21,22]. Hence, according to this most recent analysis, AMY1 duplications are likely to have occurred around 450KYA, and to have subsequently been subject to positive selection. Inchley and colleagues suggest that positive selection may have initially occurred around the time that food processing techniques increased the availability of starch from tubers during the Middle Pleistocene [21]. The presence of regional variation in AMY1 copy number, which has been shown by Perry and colleagues to correlate with starch consumption, suggests that more recent positive selection may have *maintained* higher copy numbers in populations consuming more starch, while allowing copies to be lost through genetic drift in populations consuming less starch [3,21]. Therefore, we can conclude that if positive selection for higher copy number of AMY1 occurred in the Neolithic era, the initial duplication and selection for higher copy number likely occurred before the Neolithic era.

**Lactase persistence**

Lactase (required for the digestion of lactose in milk) shows changes in expression in many populations, with lactase expression persisting into adulthood (known as lactase persistence, LP). Lactose tolerance evolved separately in Northern European, African, Arabian and South Asian populations [1]. It has


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been argued that this tolerance evolved in Neolithic herding societies. For example, in Europe the trait has been suggested to have evolved in cultures along the Danube and Northern German plain around 5500 BCE [1,23]. LP is associated with different mutations in different populations, and has thus evolved independently in several regions [6, 24-26]. Genomic analyses reveal signatures of positive selection in the genomes of the various populations that correspond with the time period in which milk consumption is thought to have originated in these populations [6, 24, 26, 27]. The speed at which lactose tolerance spread through several dairy farming populations has led to the 'cultural historical hypothesis'. This hypothesis suggests that cultural pressures in dairy farming societies drove the genetic changes in LCT, rather than dairy consumption alone [28]. Gene-culture co-evolutionary models consistently show that evolution can occur at a much faster rate than in the absence of cultural forces [28,29]. Theoretical models by Feldman and Cavalli-Sforza support the cultural historical hypothesis in explaining the rapid spread of lactose tolerance: these models showed that lactose tolerance could spread quickly through a population *only* if the children of dairy consumers also became dairy consumers, indicating a strong cultural pressure [28, 30]. There are currently no other known examples of genetic adaptation to dietary factors being driven by cultural pressures. This may be, in part, because 'cultural selection pressures may frequently arise and cease to exist faster than the time required for the fixation of the associated beneficial allele(s)' [28].

**Alcohol dehydrogenase**

ADH catalyses the oxidation of ethanol to acetoaldehyde. Recent research (including long-range haplotype scans) suggests that positive selection for a mutation in ADH, *ADH1B**47His, occurred in the last 7,000-10,000 years in Asian populations [5, 6, 31, 32]. This mutation dramatically speeds up the activity of this enzyme, increasing the efficiency of alcohol metabolism [6, 31]. This results in a rapid accumulation of acetaldehyde which causes the distinctive flushing reaction [6,31]. The time period at which *ADH1B**47His was selected coincides with the period of rice domestication in East Asia. Since ADH1B*47His has been shown to protect against alcoholism [33], it been proposed that the selection pressure was provided by the consumption of fermented food and drinks accompanying rice domestication, which may have had a large detrimental effect on those without the mutation [6,31]. If this hypothesis is correct, the selection of this mutation in the last 10,000 years is a

result of a particularly strong selection pressure, as in the case of selection for mutations in LCT.

**Grain consumption and autoimmune conditions**

Some genetic changes may have occurred in response to grain consumption [34]. The Human Leukocyte Antigen (HLA) system, which encodes proteins involved in the immune response, is likely to have been subject to recent selection [35]. HLA haplotypes HLA-DQ2 and HLA-B8, implicated in susceptibility to celiac disease (CD), follow a gradient of higher levels in northern Europe to lower levels in the Mideast, corresponding to a similar gradient of prevalence of CD [36]. This gradient reflects the spread of agriculture over this region: agriculture spread from the Mideast towards Europe. It has therefore been suggested that Natural Selection occurred for HLA haplotypes that confer less susceptibility to celiac disease and other autoimmune disorders during the spread of agriculture through Europe [34;36-37]. If this hypothesis is correct, whilst some negative selection has occurred as a result of the detrimental effects of CD, there has been insufficient time for whole populations to adapt to these detrimental effects.

Other HLA haplotypes associated with celiac disease, HLA-DQA1 and HLA-DQB1, show genetic signatures of balancing selection [38]. Balancing selection describes the maintenance of multiple alleles in a population due to opposing selection pressures, and commonly affects genes involved in the immune response (pathogens adapt to the more frequent genotypes, such that natural selection favors the less frequent genotypes) [39]. HLA has "long been observed to be under balancing selection in human populations" [40]. HLA-DQA1 and HLA-DQB1 show particularly strong signatures of balancing selection in regions in which CD is common [40]. Considering that balancing selection is most often associated with exposure to pathogens, Sams and Hawks hypothesize that regions relying heavily on grain agriculture suffered an increased pathogen load, which favored the selection of these CD-associated variants [40]. Thus, the selection pressure for a strong immune response may have offset negative selective pressures resulting from CD, preventing the evolution of genetic adaptations to protect against CD.

Selection pressure for a strong immune response may have also driven the selection of other genes associated with CD, supporting the hypothesis that

selection for a strong immune response during the Neolithic Era offset potential negative selection pressure resulting from CD. Genomic scans have found evidence of positive selection of CD-associated variants IL12A, IL18RAP, and SH2B3 [41]. Since IL12A and IL18RAP are involved in the proinflammatory activation of cytokine pathways, the variants conferring susceptibility to celiac disease has been suggested to provide a more vigorous immune response [41]. Similarly, SH2B3 is likely to have a role in the immune response, and carriers of the variant of SH2B3 that confers susceptibility to celiac disease have found to have increased production of proinflammatory cytokines, representing a more vigorous immune response [41]. Given the increased exposure of populations to pathogens in the Neolithic era [42], Zhernakova and colleagues suggest that the positive selection provided by pathogens may have overridden the selective pressures resulting from grain consumption. Therefore, the increased pathogen load during the Neolithic Era may have inhibited genetic adaptation to some of the negative health effects associated with grain consumption.

Given the number of genetic changes that have likely occurred in the immune system in recent human history [11,15, 42], it is possible that changes at other loci have similarly contributed to negative immune responses to substances in food, and prevented populations from adapting to the immune-activating properties of Neolithic foods.

## Conclusion

The three examples of mutations that have undergone positive selection in recent human history each involve a particularly strong selective pressure that allowed these mutations to increase in the population with unusual rapidity. Additionally, in two of the three cases, AMY1 and LCT, the mutations are in the regulatory regions of the genes, not the protein-encoding region. Haygood and colleagues suggest that throughout human evolution a particularly large number of diet-related genes have been affected by mutations in the regulatory regions of genes [43]. It can be speculated that mutations that change the function of a protein in an advantageous manner likely occur with much lower frequency. Considering the short length of time since the agricultural revolution, there has therefore been limited opportunity for such mutations to arise in response to modern foods. Conversely, the length of the Paleolithic era (around 2.5 million years) provided

far more opportunity for mutations of all kinds to arise and to undergo natural selection. Given the small number of genetic changes that have occurred in the last 10,000 years in response to diet, it can be concluded that a Paleolithic dietary template is most closely aligned with our genetic make-up.

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