Review

Genomic signatures of diet-related shifts during human origins

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There are numerous anthropological analyses concerning the importance of diet during human evolution. Diet is thought to have had a profound influence on the human phenotype, and dietary differences have been hypothesized to contribute to the dramatic morphological changes seen in modern humans as compared with non-human primates. Here, we attempt to integrate the results of new genomic studies within this well-developed anthropological context. We then review the current evidence for adaptation related to diet, both at the level of sequence changes and gene expression. Finally, we propose some ways in which new technologies can help identify specific genomic adaptations that have resulted in metabolic and morphological differences between humans and non-human primates.

Keywords: human evolution; natural selection; adaptation owing to diet

1. INTRODUCTION

Several recent studies have focused on signatures of natural selection in humans and non-human primates, both at the level of genome sequence as well as in gene expression data. Additionally, a number of studies have been very successful in identifying specific signatures of adaptation to dietary changes in the genome of modern humans (e.g. [1–4]). These case studies have shown that it is possible to identify specific coding, regulatory and copy number adaptive changes in the human genome related to changes in diet. However, the next challenge is understanding the larger sets of genes or pathways that have been under selection owing to shifts in the human diet, as compared with other primates, and in particular great apes. Gene set enrichment analyses, which focus on broader categories of genes showing signatures of positive selection, reveal a striking pattern across studies where many selective differences appear to be related to diet and metabolism. This consistent result suggests that selection acted to optimize differential metabolic requirements in humans and non-human primates. The challenge now is how to locate and make biological sense of the particular genes or pathways that underlie these metabolic adaptations. We propose that two sources of information need to be integrated to address this challenge: anthropological and comparative genomic analyses.

There are a number of prominent hypotheses in the anthropological literature concerning the importance of diet in human evolution. Comparisons with extant great apes as well as the fossil and archaeological record suggest that among the most important changes in diet was an increase in animal products (meat and fat) and starchy plant products during human evolution (reviewed in

[5]). Diet and foraging patterns are thought to have had a profound influence on the human phenotype, and dietary differences have been hypothesized to contribute to the dramatic morphological changes seen in modern humans [6–8]. These dietary changes can inform our understanding of sequence and gene expression differences found in metabolic genes between primates.

Here, we attempt to integrate the results of genomic studies within this well-developed anthropological context. We then review the current evidence for adaptation related to diet, both at the level of sequence and gene expression. Finally, we propose some ways in which genomic technologies can help identify specific adaptations owing to metabolic differences between humans and non-human primates.

2. THE IMPORTANCE OF METABOLIC CHANGES DURING HUMAN EVOLUTION

(a) Shifts in the diets of modern human populations as compared with ape diets

First we will compare the changes in diet between apes and modern human populations, and then review the changes that occurred in the time since the human lineage diverged from our most recent common ancestor with chimpanzees and bonobos. There is an enormous amount of geographical and temporal variation in the modern human diet, but the underlying strategy remains omnivory. The most dramatic change in the recent diet of humans is the domestication of animals and plants [9]. Nutrient intake varies widely among modern human populations, where some have a heavy reliance on carbohydrates in the form of cereals, roots and tubers from agriculture and gathering, while other populations have an emphasis on fat and protein extracted from animal husbandry, hunting and fishing [10]. These diets

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distinguish human populations from members of the larger family of living Hominidae, which includes the gorilla, chimpanzee and bonobo. All of the African apes have a diet that includes large quantities of fruit and/or structural plant parts. This is not to say these animals are exclusively vegetarian, as we know chimpanzees, bonobos and gorillas sometimes eat invertebrates [11], and chimpanzees [12,13] as well as bonobos hunt vertebrates [14,15].

(b) Dietary shifts in hominin evolution

The connection between diet and the appearance of possibly adaptive traits in hominins is of great importance for understanding human evolutionary history as well as the health and disease consequences of these adaptations for modern humans. Hominins, as a group, include humans and all of our ancestors arising after the human–chimpanzee divergence approximately 4.6–6.2 Ma [16]. In this review, we consider dietary traits acting at several scales, from molecular to organismal, associated with the intake and processing of food. In order to interpret the signatures of diet-related molecular changes, it is important to revisit the evolutionary history of humans at the organismal level. The phylogenetic affinities and the accompanying diets of many early hominin species remain unclear; however, ecological studies of extant primates and functional analyses of fossil remains suggest that hominins in general occupied an omnivore trophic niche [17] and provide us with several clear indicators of their dietary components. The fossil remains of several australopithecine and paranthropine species show that diet varied between 4.5 and 1.2 Ma, but that overall these hominins had large molars lacking well-developed shearing crests, thick enamel and powerful jaws [18–22]. These dental traits indicate crushing of hard food items during mastication and a diet that included seeds, rich in protein and fat, but do not preclude a diet including underground storage organs (USOs), such as roots and tubers, covered with abrasive soil and rich in carbohydrates [23–25]. The relative contribution of seeds versus USOs to the early hominin diet is currently an area of active discussion and research (reviewed in [26]). The genus *Homo* is first recognized in Africa approximately 2.5 Ma. The evolution of the genus *Homo* between 2.5 and 1.9 Ma is poorly understood because of the difficulties in assigning fossil specimens to distinct taxa. However, there appears to be a trend of gradually increasing brain size during this period [27–29]. Dental and skeletal traits of early *Homo* are difficult to interpret, though increased occlusal relief suggests an emphasis on shearing of food items during mastication [30]. It is notable that both stable isotope and dental microwear studies suggest that it is difficult to demonstrate a highly specialized diet for early hominins [31–35].

It is with the origin of *Homo erectus* approximately 1.9 Ma and the appearance of the Acheulean tool industry approximately 1.6 Ma that we see several traits that signify a clear shift towards the modern human form and a change in diet as compared with their more robust predecessors. With *H. erectus*, there is an increase in body size, skeletal indicators of a striding bipedal gait, a reduction in the size of the teeth and jaws and a substantial jump in relative brain size, which together with the evidence from the archaeological record suggest a dietary strategy that included bulk processing of a significant proportion of high-quality, calorie-rich food items [6,28,36–38] (figure 1). There is evidence at this time for extraction of marrow and flesh from large mammals using stone tools [39–41], although recent evidence argues that this may have occurred much earlier in *Australopithecus afarensis* [42]. Early representatives of the genus *Homo* probably used tools for the processing of both animal and plant materials and for wooden tool production [36,43]; however, hunting weapons do not show up unequivocally in the fossil record until about 400 thousand years ago [44]. While the archaeological record clearly shows that scavenging occurred at carnivore kill sites, there is little consensus as to what constitutes evidence for distinguishing passive scavenging, 'power scavenging', and hunting [45], where power scavenging is defined as actively driving another animal away from a carcass, as opposed to passive scavenging which involves harvesting food from an abandoned carcass [39]. The evidence from the feeding apparatus, particularly the reduction in post-canine tooth size, the increased occlusal relief and the gracilization of the jaws, indicates continued emphasis on shearing food items during mastication with a reduction in the hardness of foods consumed and/or the use of technology to process foods prior to ingestion [19,25,30,46]. The fossil and archaeological evidence suggests there was not only an increase in access to animal products during this period, but also the continued importance of plant material. Taken together, the hallmark of the early *Homo* diet is its great versatility and consistent access to high-quality foods [31,47]. Regardless of the predominate meat procurement mode, the increased availability of protein and fat in the diet of *H. erectus* via oil-rich seeds, USOs and meat [17] would provide consistently available, high-quality, calorie-rich fuels for such energetically expensive adaptations as a large brain.

(c) Trade-off hypotheses regarding diet

As discussed above, a hallmark of the evolution of human diet is the inclusion of a high percentage of nutrient-rich foods (including animal products). Several hypotheses have been put forth to connect changes in nutrition with evidence of adaptation, specifically the increase in brain size over the past 2 Myr, and many of them focus on tissue mass changes. The first, and most prominent, of these hypotheses is the expensive tissue hypothesis proposed by Aiello & Wheeler [6]. They noticed that the total mass-specific basal metabolic rate of humans is well within the range of other primates, but that we have a larger brain, which results in greater energy requirements. Taken together, they predicted that some other structure or structures had to decrease in mass in order to reduce energy consumption and allow for the expansion of our metabolically demanding human brain. Aiello & Wheeler [6] hypothesized that the increasing quality and digestibility of the hominin diet during evolution allowed for the reduction of the energetically expensive gut tissue. Then, the net gain in energy could be allocated to the human brain. The differences in gut size among mammal species are consistent with empirical observations relating digestive organs to diet quality [48]; herbivores usually have large guts to better extract
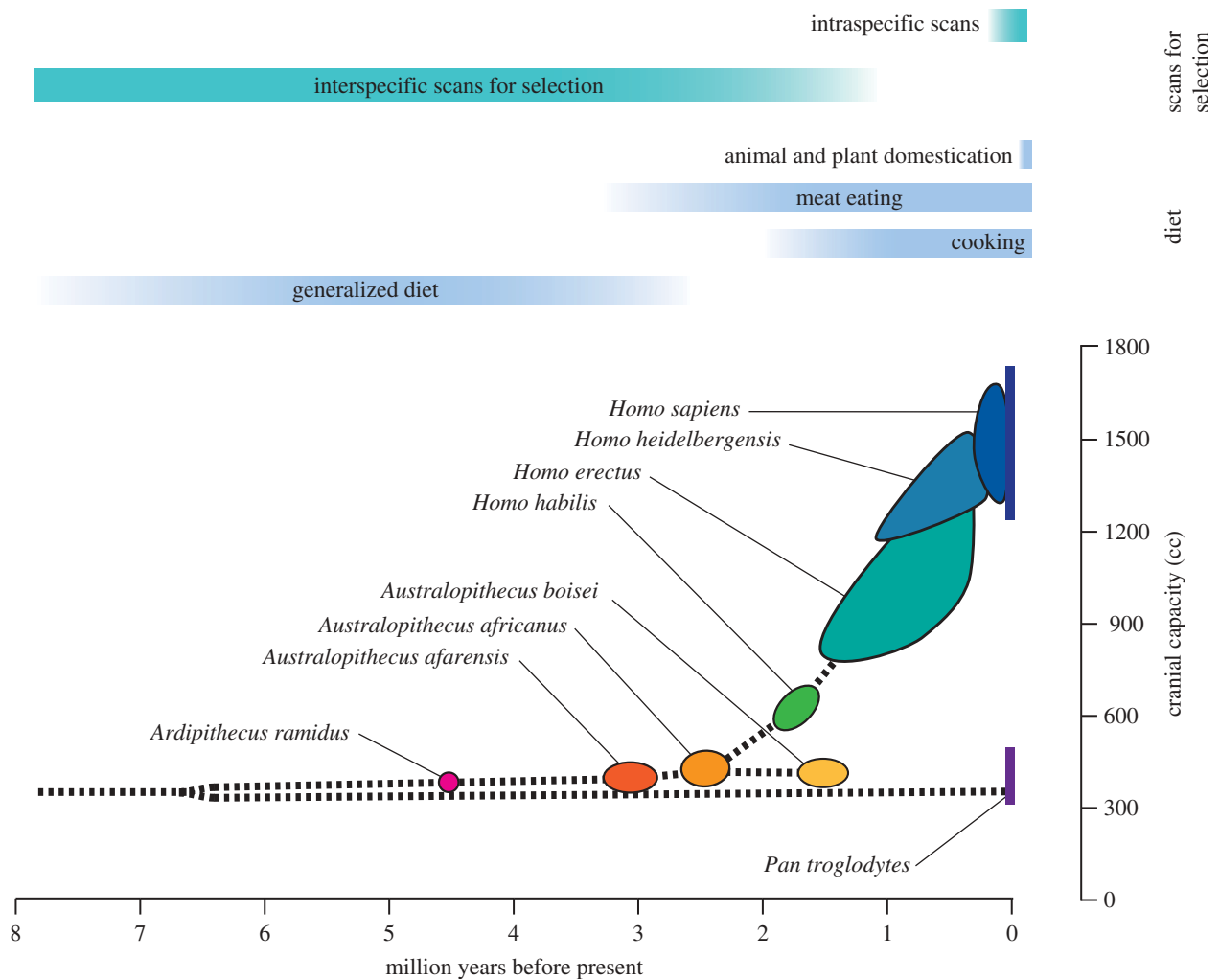
Figure 1. A timeline showing some of the temporal intersections of diet, natural selection and one of the many changes in human morphology. Green bars indicate the temporal range on which different methods for scanning for selection are optimized to identify relevant changes in the genome (reviewed in [111]). Blue bars indicate the times in which there is evidence for shifts in human dietary intake [6,17,28,36,112,113]. The coloured bubbles are a general schematic of the time and range in size of cranial capacity found in various hominin species adapted from Schoenemann [27] with additional data from White *et al.* [114].

nutrients from plant tissue, whereas the simpler gut of humans is more typically found in carnivores. Another trade-off prediction comes from Leonard *et al.* [7], who suggest that a decrease in muscle mass and an increase in adiposity provided a potential source of energy to fuel the evolution of the human brain in two main ways. First, an energetically expensive tissue, skeletal muscle, was reduced. Second, a tissue known for its ability to store energy, fat, was increased.

Together these hypotheses illustrate that diet may have acted as a 'releaser' and a 'challenger' in human evolution. Examples of a release of energetic constraint would be the re-allocation of energy-expensive tissues, allowing for the development and maintenance of the human brain, or an increase in diet quality in terms of energy value [6,49–51]. Diet can also act as a challenger as foraging for foods high in quality often provides both an energetic challenge as well as a cognitive challenge [52–56]. When considering diet as a 'releaser' and a 'challenger' of metabolism, we are in the position to identify and characterize interesting genetic and molecular candidates that could be responsible for adaptive traits.

3. GENOMIC ANALYSES ALLOW US TO IDENTIFY TRENDS ACROSS SETS OF GENES

There has been an exponential increase in genomic information available for humans and non-human primates in the last decade (reviewed in [57–59]). This includes genomic variation between human populations as well as our closest primate relatives. Using these data, a number of studies looked for signatures of natural selection in gene sequence and/or differential gene expression within many primate lineages, most with the goal of determining human-specific adaptations [60–71]. Many of these studies have performed categorical enrichment analysis, which tests whether larger, pre-defined, classes of genes, such as 'immunity and defence' or 'lipid metabolism' are statistically over-represented among the genes showing signatures of selection or differential expression. Even if there is only a moderate signal for the individual genes with the category, there might be an important biological signal if many genes involved in a process are changing function in concert along a lineage. The predefined categories for human genes come from databases such as the Gene

Ontology (GO) [72], or the Protein ANalysis THrough Evolutionary Relationships (PANTHER) [73] databases, which group genes in categories based on experimental evidence or predicted function. It is important to note that these annotations are partially hand-curated and so their interpretation presents some caveats: genes can be placed in multiple categories, some of the categories are not independent and some category labels can be difficult to interpret biologically. Nevertheless, by grouping genes into larger categories, we can begin to discern patterns of change at a genomic, rather than at a single gene, level. However, a change in expression or signature for positive selection in DNA sequence does not always imply a change in diet; even with a conserved diet, a change in gene expression may simply modify how the nutrients are processed and used. Therefore, it is critical to understand the biological context for all of these new datasets, and so it is essential to integrate them within the extensive anthropological framework concerning changes in diet during human evolutionary history.

4. SIGNATURES OF METABOLIC CHANGES BASED ON GENOME-WIDE SCANS FOR NATURAL SELECTION

In the search to uncover the genotypic changes underlying shifts in human phenotypes, there have been many in-depth accounts of polymorphisms segregating within human populations that confer some adaptive advantage in diet and digestion. The most prominent example is the ability to digest milk solids (lactose) into adulthood (lactase persistence) in some human populations [2,3,74,75]. Multiple regulatory polymorphisms in these different populations help drive *lactose-phlorizin hydrolase* (*LPH*) gene expression in adults. This regulatory locus shows one of the strongest signals of positive selection in the human genome [3,76–79]. There are now many examples of recent adaptations in genes as well as in gene families that appear to be shaped by more recent diet-related pressures (e.g. taste [80,81] and olfactory [82] receptor genes). However, as reviewed above, as hominoids diverged from our last common ancestors with the *Pan* lineage, there were significant changes in their diet—indicating that older adaptive changes might be found throughout the genome.

(a) *Comparisons of metabolic categories changing between protein-coding and regulatory genomic regions between species*

Comparative genomic studies looking for signatures of positive selection have taken two general approaches: the first is to scan the genome looking for regions where there is an overabundance of nucleotide substitutions as compared with nucleotides that are thought to be evolving neutrally [60–62,65,66]; the second is to look for regions that are extremely evolutionarily conserved, but show an accelerated number of changes in the human genome [63,64]. These studies have also differed in their focus on selection working on either protein-coding [60,62,65,66] or putative regulatory regions near genes [61,63,64]. Indeed, there appear to be different signatures coming from coding and regulatory regions [83] (i.e. coding and non-coding). Overall, however, many of these

studies have found signatures of selection in genes that may have played a role in adaptations to dietary changes.

In coding regions, genes involved in sensory perception (i.e. processes such as taste and smell) have been under positive selection [60,65,66], as well as genes related to immune responses, which are expected to be under lineage-specific selection. Looking between mammalian clades, many genes involved in conveying sensory perception to the brain also appear to be under positive selection in the primate lineage, but not in the rodent lineage, correlating with the increased brain size and complexity in the primate lineage [62]. Other studies of more recent signals of positive selection within and between human populations also show some enrichments for metabolism-related genes, such as protein, carbohydrate and phosphate metabolism (for an extensive review, see [84]).

Regulatory regions in humans also show signatures of positive selection in genes related to metabolism, especially glucose metabolism [61]. Glucose metabolism categories include carbohydrate metabolism, glycolysis, other polysaccharide metabolism and anion transport. Glucose metabolism-related genes scoring high in humans include *HK1*, *GCK* and *GPI*, which are all involved in steps of glycolysis. One test to see whether these diet-related signals are biologically meaningful is to perform branch-specific enrichments, where only regions under positive selection in each species are used in the enrichments. These analyses show that the metabolic categories for each species can be quite different, and that even for the same category the specific genes showing evidence of selection are usually distinct subsets on different species' branches [61,62,71]. A weaker signal for glucose metabolism-related categories is seen on the chimpanzee branch, but the specific genes involved differ on the human and chimpanzee branches. On the chimpanzee branch, metabolic categories that are not enriched on the human branch include glycogen metabolism, sulphur redox metabolism and acyl-CoA metabolism [61].

A formal comparison of the different categories showing evidence for positive selection in coding and putative regulatory regions on the human branch revealed that very different categories of genes are evolving through changes in these regions [83]. Specifically, selection in coding regions is more prevalent on genes involved in olfaction, immunity and male reproduction; whereas selection in regulatory regions was associated with genes involved in neural development.

(b) *Comparisons of metabolic categories changing between protein-coding and regulatory genomic regions within humans*

In a scan for more recent adaptation in the DNA sequence owing to diet between human populations, Hancock *et al.* [85] show that there have been shifts in allele frequencies between populations in different eco-regions and with different diets. The strongest correlations between SNPs and environmental variables were seen in genic and non-synonymous SNPs. The strongest signal related to diet is seen in populations where the main dietary components are roots and tubers. Roots and tubers are mainly composed of starch and have low levels of other essential nutrients. Populations with diets rich in roots and tubers show significant shifts in allele frequency in the

'starch and sucrose metabolism' and 'folate biosynthesis' categories [85]. This study illustrates that there are numerous genetic changes of small effect scattered through the genome related to more recent dietary shifts. In contrast, the functional impact of regulatory SNPs throughout the genome is currently not well understood. Specific examples such as the regulatory variants that confer lactose tolerance (the ability to drink milk after weaning) in multiple populations [2,3,74,75] are known, suggesting that more are present and may be found in future functional genomic studies.

5. EVIDENCE FROM THE EVOLUTION OF GENE EXPRESSION BETWEEN HUMANS AND NON-HUMAN PRIMATES

(a) *Evidence for changes in metabolic pathways*
One way to identify gene expression patterns that are consistent with positive selection is to look for lineage-specific changes in expression levels as compared with changes in multiple lineages. Cases where a lineage-specific shift in gene expression levels is seen in conjunction with low variance within the species are suggestive of directional selection driving that change [68,86]. There are clear changes in gene expression in humans, compared with non-human primates, that are centred in metabolic pathways in a number of different tissues [67–71], and some of these may be changes owing to directional selection. The metabolic pathways, again, differ on the human and chimpanzee branches [68]. For example, Blekhman *et al.* [68] found that fatty acid metabolism showed evidence of directional selection in gene expression in human heart tissue, whereas vitamin B6 metabolism and folate biosynthesis show evidence of directional selection on the chimpanzee branch. Additionally, Khaitovich *et al.* [67] found an enrichment for diet-related genes that both are differentially expressed between species and are in a large region of linkage disequilibrium (evidence of a selective sweep on that genomic region) in multiple human populations. Thus, it is likely that positive selection acted on gene expression changes before these populations diverged approximately 100 000 years ago [87–90], although many of these changes could have been selected for in morphologically modern human populations before moving out of Africa. Other changes in transcriptional regulation could also be important contributors to diet-related adaptations. For example, a recent study on alternative splicing within expressed genes in humans and non-human primates [70] found an enrichment for genes involved in metabolic processes in differentially spliced exons in humans as compared with chimpanzees and macaques.

(b) *Evidence for changes in energy transport*
In brain tissues, there is a consistent pattern of changes in expression of genes critical to aerobic energy metabolism [67,69,71,91]. This includes categories such as oxidative phosphorylation, electron transport and other nuclear-encoded genes that function in the mitochondria. This differential expression of aerobic energy metabolism-related genes could be due to the increased neural activity and metabolic requirements of the human brain. This trend is seen in adult human brain tissue, but not in foetal tissue, where selected enrichment categories are

related to neuronal signalling and connectivity [71]. Some aerobic energy metabolism genes also show evidence of positive selection in their amino acid sequence during anthropoid primate evolution [92–94]; these changes correlate with changes in brain size and lifespan in primate life histories.

Unsurprisingly, different categorical enrichments are seen from different tissues. For example, the differential expression of genes related to aerobic energy metabolism has only been found in studies examining expression in brain regions between species [67,69,91]. To get a more complete picture of the important functional shifts in gene expression between tissues and species, future studies will need to examine more tissues throughout the body.

6. OTHER SHIFTS IN THE GENOMIC LANDSCAPE OWING TO DIETARY SHIFTS
Shifts in diet will also have effects on other functional levels, such as chromatin structure or protein processing. For instance, epigenetic changes may also play a large role in differential gene expression between species, and recent work in mouse models has shown that diet can play a role in changes in chromatin modification [95–97]. These epigenetic changes can also have a lasting impact since changes in maternal diet can affect methylation status for multiple generations (reviewed in [98]). Two preliminary studies of methylation change between humans and chimpanzees noted significant differences in methylation patterns, with a higher amount of methylation seen in humans [99,100]. Dietary changes incorporating more sources of methyl groups (e.g. methionine or choline) and folate would change the methylation states for many genes, and these changes could be important in development and later disease susceptibility (reviewed in [101,102]).

A recent study has also measured shifts in the concentration of 21 metabolites between humans and non-human primates [103], and found that a statistically significant number of them differ in relative concentration. Specifically, the relative concentration of metabolites related to energy metabolism, such as lactate and creatine, appear to have changed rapidly during human evolution. Genes involved in these metabolic processes also show greater sequence and gene expression divergence than expected. The authors suggest that the human brain may be working at the edge of its metabolic capabilities, as suggested through additional comparisons of metabolites between schizophrenic and control individuals [103].

7. NEXT STEPS IN UNDERSTANDING THE IMPACT OF DIET ON PHENOTYPE
In order to empirically test whether these patterns of genome-wide metabolism and energy metabolism are biologically relevant, the next steps will be to take different experimental approaches for a better understanding of the intersection of genotype, phenotype and diet in primates.

What does the anthropological evidence suggest to test questions surrounding the genetic impact of dietary shifts? One possibility would be to look for genetic and genomic signatures of adaptations related to digestion of

meats, fats, and marrows, possibly even scavenged meats with attached immune challenges (rotting or parasite infested, for instance). This will be critical for distinguishing how during human evolution adaptations in gut, brain, muscle and fat and reproductive adaptations arose even though all are related to diet. A parallel investigation could be undertaken to see whether there is genetic evidence for more ancient adaptations to the adoption of cooking habits [104]. For instance, Blekhman et al. [68] hypothesized that signatures of selection in gene expression in the liver reflected the beginning of cooking meat during human evolution. Since it is challenging to detect specific dietary influences on musculoskeletal anatomy during human evolution, we contend that combining morphological approaches with genomic approaches is a next step in addressing these questions by looking at the evidence for natural selection at the molecular and tissue levels.

Experimentally, this could include exploring changes in pathways between species using other molecular approaches, such as in vitro cell culture assays. For example, one could treat a relevant cell type with varying levels of metabolites or oxygen, and then measure subsequent changes in gene expression or other metabolite concentrations between species. This would allow for a dissection of the genome-wide influence of a single factor between species, possibly helping to elucidate networks of genes that have changed.

Alternatively, detailed investigations of changes over large networks or pathways of genes would be informative. Like many complex traits, if changes in diet have been important in human evolution, we might expect many small changes at multiple loci. Likewise, genomic sequence and expression from other populations will increase our power to understand these adaptations. The recently published Neanderthal genome [105] will also be valuable in understanding the timing of certain specific mutations.

By comparing gene expression across tissues as well as between species, we may start to understand the genetic underpinnings of phenotypic changes related to dietary changes. For instance, paralogues within a gene family could be differentially 'tuned' to function in specific tissues. Phylogenetic histories of gene duplication, and gene family expansion, would help to illuminate this type of pattern, as seen in the olfactory receptors in humans [106]. Alternatively, if there are tissue changes (e.g. a reduced gut or enlarged brain) between species, analyses looking for shifting patterns of gene expression, protein function or methylation state in these tissues would be valuable. A similar effect might be expected at the level of natural selection on DNA sequence, showing an enrichment of selection on tissue-specific genes in tissues that have changed dramatically in size or energy consumption between species, whereas ubiquitously expressed genes may not show those enrichments.

The pattern we describe would also predict that other studies should see similar shifts in phenotypes (and the underlying genotypic shifts) in other organisms where diet has changed dramatically within a clade. With genome sequencing technologies rapidly advancing as costs decrease, it is now possible to create resources for new 'model' organisms to address specific questions. For example, measurements of brain and gut volume in

addition to gene expression studies in Onychomys, a small (approx. 30 g), highly carnivorous cricetid rodent [107], would be an interesting natural experiment in the morphological and genetic patterns that occur when a carnivore evolves from a seed-eating ancestor. Another system to investigate is the elephant-nose fish Gnathonemus petersii. Gnathonemus petersii has an extremely large brain (particularly the cerebellum) that is exceptionally expensive for an ectotherm, with 60 per cent of total oxygen consumption being used by the brain compared with 2–8% in most vertebrates and 20 per cent in humans [108]. The enlarged cerebellum of G. petersii may be due to an energetic trade-off with the digestive tract [109], and the size of the brain varies widely within closely related species [110]. Experimental taxa such as these would be a powerful source of detailed information on the interplay between the genetic, physiological and morphological changes involved in energetic re-allocations.

A difficult gap between genotype and phenotype remains, and so next steps need to look at physiological, developmental and morphological differences—challenging in human and non-human primate species and populations. A combination of the experimental data reviewed here may assist in gaining a comprehensive understanding of how dietary changes have moulded the modern human phenotype.

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