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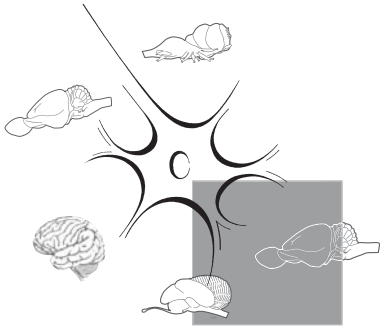

Epigenetic Information from Ancient DNA Provides New Insights into Human Evolution

Commentary on Gokhman D et al. (2014): Reconstructing the DNA Methylation Maps of the Neanderthal and the Denisovan. Science 344:523–527

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Phenotypic differences can be attributed to variation in both the DNA sequence and gene regulation. The latter is influenced by stochastic, environmental, and genetic factors. Epigenetic processes that are biochemical modifications of DNA and chromatin play a major role in gene regulation during development, differentiation, and disease processes [Feinberg, 2007; Jirtle and Skinner, 2007]. A mammalian body is composed of >200 different cell types following unique developmental trajectories during ontogenesis from fertilization to death of the organism. Because all these trajectories are blueprinted by the same DNA sequence in each somatic cell, one can imagine the impact of transcriptional control on phenotypic development and diversity [Smith and Meissner, 2013]. This is true for variation not only within but also between species, notably between human and nonhuman primates [Hernando-Herraez et al., 2013]. The striking phenotypic differences between chimpanzees and present-day humans cannot be fully explained by an approximately 5% DNA sequence divergence (counting indels) [Chimpanzee Sequencing and Analysis Consortium, 2005], in particular considering that mice and rats exhibit relatively small phenotypic differences and yet are much more diverged in their sequence.

The most thoroughly studied type of epigenetic modification is DNA methylation, which mainly occurs at position 5 of the pyrimidine ring in the context of CG dinucleotides. The human genome contains more than 28 million CpG sites; 7% of them are clustered within CpG islands and approximately 45% exist within repetitive elements [Rollins et al., 2006]. DNA methylation is not only sequence dependent; it is highly dynamic during development and susceptible to environmental factors. At least for a small number of well-characterized loci, such as imprinting control regions or certain gene promoters [Horsthemke, 2006; Galetzka et al., 2012], it is possible to infer phenotypic information from DNA methylation patterns, which cannot be inferred from the DNA sequence alone [Vidaki et al., 2013]. In this light, the study of methylation profiles in ancient DNA can provide an additional layer of information for paleoanthropologists to draw conclusions about human evolution. However, DNA methylation decays over years, which has rendered epigenetic analysis of prehistoric specimens an unrealistic ambition until very recently.

In a milestone paper, Gokhman et al. [2014] reconstructed DNA methylation profiles of the Neanderthal and the Denisovan, both extinct human antecessors, using sophisticated bioinformatic analyses of next-generation sequencing data. During tens of thousands of years, methylated and unmethylated cytosines in ancient DNA were degraded into thymines and uracils, respectively. Enrichment of the analyzed DNA sequences with thymines allowed the authors to estimate DNA methylation proxies

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in archaic human female limb bones. Comparison of the ancient methylation patterns with those of present-day humans revealed more than 1,900 differentially methylated regions: 307 in Neanderthals, 295 in Denisovans, 891 in present-day humans, and 412 unclassified. Since these results were produced with a method unprecedented in extinct human species, it may be helpful to appraise their applicability. Gokhman et al. [2014] considered many potential pitfalls in their study, including differences in deamination rates, adjustment to housekeeping genes and CpG islands, methylation variation between tissues, genders, and individuals, and methylation differences between active and inactive X chromosomes.

Various techniques currently used for genome-wide DNA methylation analyses in humans can reliably detect methylation differences (between samples) in the order of 10–20% [Bock et al., 2010; Marabita et al., 2013]. Detection of smaller (between-group) differences requires analysis of a sufficient number of samples (usually several dozens to several hundreds). Moreover, intraspecific variation in DNA methylation is much larger than DNA sequence variation [Horsthemke, 2006]. Functionally important locus-specific methylation values can vary by up to 30% among humans, and even monozygotic twins show age-dependent divergence of their methylation patterns [Schneider et al., 2010; Talens et al., 2012]. Small sample sizes are a major problem inherent to the field of paleoanthropology [Hawks, 2004], in particular when small effect sizes are considered. At the individual gene level, any methylation difference between the Neanderthal, the Denisovan, and present-day humans below the 20–30% threshold has to be interpreted with caution. Conclusions based on the annotation of multiple genes, i.e. the enrichment of differentially methylated region-containing loci in present-day humans with disease-relevant genes, particularly for neurological and psychiatric diseases, may be more robust. Interestingly, differentially methylated regions between human and chimpanzee cortices are also enriched with genes for neurological and psychiatric disorders. Compared to the chimpanzee brain, many disease-relevant gene promoters have shown a 20% lower methylation in present-day humans [Zeng et al., 2012]. Thus, DNA methylation-driven divergence between present-day and archaic humans, as well as between humans and chimpanzees, may have contributed to the evolution of specific disease susceptibilities in modern humans. On the other hand, it is important to emphasize that DNA methylation patterns are cell type and developmental stage specific, which makes it difficult to extrapolate epigenetic signatures from adult bone tissue to brain development and function.

One surprising result is hypermethylation of the *H19* imprinting control region in Neanderthal bone tissue compared to the Denisovan and present-day humans. *H19* is a paternally methylated and maternally expressed imprinted gene [Horsthemke, 2006]. Abnormal *H19* hypermethylation causes Beckwith-Wiedemann syndrome in present-day humans, which is characterized by fetal overgrowth, a large tongue, and a protruding lower jaw. At first glance, this may coincide with the distinctive facial features and early growth spurts in Neanderthals. However, Beckwith-Wiedemann syndrome is also associated with an increased risk of childhood cancer. Since *H19* imprinting has been highly conserved for 170 million years from therians to humans [Smits et al., 2008], loss of imprinting in Neanderthals seems unlikely. One possible explanation for the observed *H19* hypermethylation is allelic dropout of the unmethylated maternal allele due to DNA degradation or an amplification bias [Kim et al., 2013]. Allelic dropout may also have occurred for the imprinted genes *HYMAI* and *MEST,* where the computed methylation values suggest loss of imprinting in both the Neanderthal and the Denisovan, and *SNRPN,* suggestive of loss of imprinting in the Denisovan. All three genes are maternally methylated in human and nonhuman primates, and there is no evidence for changes in their imprinting status between humans and great apes [Schneider et al., 2012; Hernando-Herraez et al., 2013].

Arguably, the most interesting finding is a methylation difference between modern and archaic humans in the *HOXD* gene cluster, which for some genes exceeds 60%. The *HOXD9* promoter and the *HOXD10* gene body are hypermethylated in both the Denisovan and the Neanderthal. Since the *HOXD* cluster is important for limb development [Goodman, 2002], differential regulation of an *HOXD* gene(s) is a plausible molecular mechanism driving evolution of the human skeleton. Fossil records allow one to compare the skeletons of Neanderthals and anatomically modern humans, whereas, apart from teeth and finger bones, skeletal remains of Denisovans are missing. Considering that none of the *HOX* genes showed more than a 20% blood methylation difference between humans and great apes [Hernando-Herraez et al., 2013], the magnitude of the change between present-day and archaic humans is impressive. However, this may be due to the tissue specificity of methylation patterns. In contrast to primate blood, bone, which has been analyzed in the Neanderthal and the Denisovan, is a target tissue for *HOX* gene regulation. In this context, it is important to emphasize that functionally important methylation changes in one tissue, i.e. of genes for cognitive abilities

in the brain, are not necessarily represented in an accessible tissue, which in paleoanthropology are teeth and bones. Moreover, DNA methylation patterns are highly plastic during development and differentiation. If epigenetic changes in *HOX* gene regulation contribute to skeletal differences between present-day and archaic humans, the most critical time window should be during bone and limb development. In this light, the pronounced *HOX* methylation differences in adult bone tissue are all the more intriguing.

In summary, Gokhman et al. [2014] have inaugurated a new field: epigenetic paleoanthropology. Using the developed algorithms to reconstruct methylation patterns in ancient DNA samples, it has become possible for the first time to combine morphologic and epigenetic information to infer physiological traits of extinct human species. At this point we have a proof of principle, but this new technology will need further improvements and adjustments. The extrapolation of epigenetic signatures from adult bone and teeth to other organs (in particular the brain) and developmental stages remains an enormous task. This is true not only for paleoanthropology but also for the rapidly growing field of clinical epigenetics, which assumes that, despite enormous between-tissue differences, the easily accessible blood epigenome can reflect, at least to some extent, the functionally relevant methylation variation in the brain [Davies et al., 2012]. Just as sequencing of ancient DNA has provided many new insights into human evolution, epigenetic information from ancient DNA will likely have an impact along the same order of magnitude on paleoanthropology.

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