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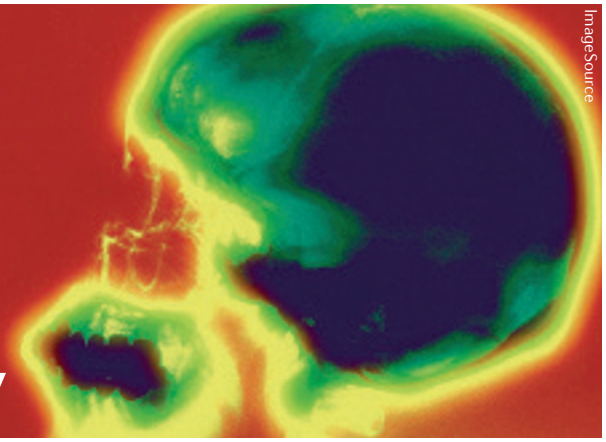
EVOLUTION

Regulatory evolution of human craniofacial morphology



Changes in enhancer sequences contributed to the emergence of species-specific craniofacial features in humans and chimpanzees, according to a new study. The findings indicate that alterations in transcription factor binding motifs at enhancers that regulate gene expression in cranial neural crest cells (CNCCs) underlie, at least in part, evolutionary divergence between these closely related primates.

Computational analyses of differences in non-coding DNA between humans and other primates have previously been performed, but functional data have been comparatively scarce. The researchers used induced pluripotent stem cells from chimpanzees, which have only been available in the past couple of years, to generate CNCCs *in vitro*. These cells were then analysed side-by-side with human CNCCs similarly derived from induced pluripotent or embryonic stem cells. Three humans and two chimpanzees provided cells for the study.

The researchers performed chromatin immunoprecipitation followed by sequencing (ChIP–seq), using antibodies against two transcription factors that drive gene expression in CNCCs, the general co-activator p300, and histone modifications. ChIP–seq and chromatin accessibility assays were used to identify candidate regions that could be active enhancers in CNCCs. The researchers identified ~1,000 putative enhancer regions with biased activity either towards human or towards chimpanzee.

To validate the *in vitro* identification of enhancers with a role in craniofacial development, the researchers cross-referenced these data with the VISTA Enhancer Browser database, which yielded a list of 247 regulatory regions overlapping CNCC enhancers, and performed functional analysis by transgenic expression in mouse embryos. This analysis showed that 208 of these regions were active in developing mice at embryonic day 11.5, particularly in head tissues derived from the neural crest.

Quantitative analysis of enrichment for histone H3 lysine 27 acetylation (H3K27ac) in human and chimpanzee CNCCs was used to investigate quantitative differences in enhancer activity, which the researchers hypothesized to be an important mechanism of enhancer divergence in closely related species. The analysis revealed species-biased patterns of enhancer activity, which were indeed associated with differences in transcription factor and p300 binding and in chromatin accessibility. Functional experiments *in vitro* and in transgenic mice showed that species-biased enhancers drive different patterns of gene expression in CNCCs and CNCC-derived tissues. The researchers conclude that divergent gene expression in CNCCs occurs owing to quantitative modulation of enhancer activity.

The sequences of species-biased enhancers were less conserved than those of enhancers that were active in both humans and chimpanzees. Species-biased enhancers were enriched in specific classes of retrotransposons, which might explain their evolutionary origin. Analysis of sequence substitutions in transcription factor binding motifs suggests that these differences could explain the differential activation or repression of gene expression in the two species.

High-throughput RNA sequencing (RNA-seq) analysis in CNCCs revealed that genes with differential expression between species are significantly enriched in species-biased enhancers. The researchers also identified 32 human and 65 chimpanzee clusters of species-biased enhancers, which are located in regions close to or within genes controlling craniofacial morphogenesis. Some of these genes have been associated with normal-range human facial variation.

According to the researchers, the identification of differentially expressed genes and regulatory elements in humans and chimpanzees provides a resource for further study of inter-specific and intra-specific evolution and genetic variation.

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ORIGINAL RESEARCH PAPER Prescott, S. L. *et al.* Enhancer divergence and *cis*-regulatory evolution in the human and chimp neural crest. *Cell* **163**, 68–83 (2015)