differentiation trajectory. Although chromatin accessibility was dynamic during cortex development across species, differences were noted between human and chimpanzee cortical NPCs and neurons. Importantly, many differentially accessible regions were associated with human-specific gene expression or genetic changes. Indeed, differentially accessible peaks and putative organoid-specific regulatory regions were identified near differentially expressed genes with human-fixed single-nucleotide changes or in proximity to human accelerated regions (HARs) or human-specific highly conserved deletions. For example, 1 of the 62 HARs that overlapped with differentially accessible peaks was located near the *CDH7* gene, which has human-specific expression in neurons.

Finally, to assess the potential and limitations of cerebral organoids, the persistence of the human-specific expression patterns observed during cortical development into adulthood was investigated. scRNA-seq-based mapping of gene expression in post-mortem prefrontal cortex tissues from three humans, two chimpanzees, one bonobo and three macaques uncovered shared and distinct developmental genetic patterns relative to cerebral organoids. Importantly, many genes were expressed in both adult cortical excitatory or inhibitory neurons and in organoid-derived NPCs representative of the forebrain telencephalon, illustrating that developmental differences do indeed persist into adulthood. Furthermore, in humans and chimpanzees, few species-specific genes (identified in organoids) were differentially detected in adult neurons and organoid trajectories, suggesting that cell-state-specific changes occur exclusively in the adult brain.

Overall, the findings illuminate the genetic features of primate forebrain development across the evolutionary continuum and could serve as a valuable resource for further investigation of the gene-regulatory mechanisms that characterize the developing human brain.

Conor A. Bradley

ORIGINAL ARTICLE Kanton, S. et al. Organoid single-cell genomic atlas uncovers human-specific features of brain development. *Nature* **574**, 418–422 (2019)

Credit: Pobytov/iStock/Getty

Using the V^G reference estimates from the GTEx skeletal muscle samples, ANEVA-DOT was applied to AE data from 70 patients with rare Mendelian muscular dystrophies and myopathies; of the 65 patients with high-quality data for analysis, 32 had a previous pathogenic genetic diagnosis (21 of which were expected to lead to allelic imbalance). ANEVA-DOT accurately detected genes with pathogenic variants in previously resolved cases. Indeed, of a median of 2,190 tested genes, ANEVA-DOT identified a median of 11 outlier genes per individual, which included the previously diagnosed gene in 76% of diagnosed patients. Furthermore, among the 33 patients who had not received a genetic diagnosis from previous DNA and RNA sequencing analysis, a median of 9 outlier genes were noted per sample, including at least 1 neuromuscular disease-related gene in 12 patients. For one of these potential new diagnoses, RNA sequencing and RT-PCR analysis led to a confirmed diagnosis in the Mendelian muscle disease gene *DES*, which had previously been missed as it was an intronic variant. These findings demonstrate how V^G reference estimates from GTEx data can inform rare disease diagnosis and identify non-coding pathogenic variants that might have been missed using current approaches.

Overall, the ANEVA–ANEVA-DOT statistical framework can provide insight into rare and pathogenic variants, including those in non-coding regions, to complement transcriptomics-based diagnostic pipelines for patients with rare Mendelian diseases.

Conor A. Bradley

ORIGINAL ARTICLE Mohammadi, P. et al. Genetic regulatory variation in populations informs transcriptome analysis in rare disease. *Science* **366**, 351–356 (2019)

GENOME EDITING

A prime alternative

A new genome editing strategy called prime editing uses a catalytically impaired Cas9 fused to an engineered reverse transcriptase to write desired genetic sequence information directly into a target locus. This 'search-and-replace' approach was able to perform targeted insertions, deletions and all possible base-to-base conversions in human cells.

CRISPR–Cas9 and other programmable nucleases can be used for targeted mutagenesis as they generate site-specific DNA double-strand breaks (DSBs), which induces homology-directed repair (HDR). However, HDR is dependent on a DNA donor template and is inefficient in most cell types, and DSBs can induce undesirable insertions and deletions (indels).

> "targeted insertions, deletions and all possible base-to-base conversions in human cells"

Prime editors comprise a reverse transcriptase fused to an RNA-guided DNA-nicking domain, such as Cas9 nickase, which introduces DNA single-strand cuts rather than DSBs. This fusion protein forms a complex with a prime editing extended guide RNA (pegRNA), which both specifies the target site and encodes the desired edit. Once bound to target DNA, the complex nicks the strand containing the protospacer adjacent motif (PAM). This process generates a free 3′ end that hybridizes to a primer-binding site on the pegRNA. Reverse transcription of new DNA including a desired edit then occurs from the template within the pegRNA extension. Preferential excision of the unedited 5′ single-stranded 'flap' and ligation of the edited 3′ flap creates a heteroduplex with one edited and one unedited strand that is subsequently resolved by DNA mismatch repair to stably 'install' the edit.

Anzalone et al. describe multiple prime editor variants optimized to increase editing efficiencies and minimize indel by-products. Applying prime editing to human HEK293T cells, the team corrected the most common mutation that causes Tay–Sachs disease by removing 4 bp in the gene *HEXA*. The team also used prime editing to generate and subsequently correct the major cause of sickle cell disease, which is a transversion point mutation in *HBB*. Moreover, prime editing was used to install a transversion in *PRNP* that confers resistance to prion disease.

Comparing prime editing to Cas9-initiated HDR, prime editors exhibited higher or similar efficiency with much lower indel formation and off-target activity. Similar to base editors, prime editors circumvent the need for DSBs and donor DNA templates; however, prime editors offer substantial advantages over current base editors when target bases are not well-positioned for editing or when multiple cytosines or adenines are present.

Linda Koch

ORIGINAL ARTICLE Anzalone, A. V. et al. Search-and-replace genome editing without double-strand breaks or donor DNA. *Nature* https://doi.org/10.1038/s41586-019-1711-4 (2019)
RELATED ARTICLE Rees, H. A. & Liu, D. R. Base editing: precision chemistry on the genome and transcriptome of living cells. *Nat. Rev. Genet.* **19**, 770–788 (2018)