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Genomic approaches for understanding the evolution of the human brain

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Human cognitive and social behaviors differ from those of other mammals, but the molecular, cellular and circuit-level changes that underlie these behavioral differences are poorly understood. The recent availability of thousands of mammalian, non-human primate, ancient human and modern human genomes now makes it possible to use quantitative approaches to identify genomic regions with signatures of selection in humans, which, when combined with comparative experimental approaches, can provide precise insights into the phenotypes that were the targets of adaptation across different evolutionary timescales. This Review presents a progress report on a 'genome-up' approach to understanding human brain evolution and lays out a framework for further advancement. Additional progress will require cohort expansion to improve the identification of genetic loci under selection, the application of comparative experimental approaches to additional milieus and the functional dissection of specific human-evolved loci.

Compared to other primates, humans have evolved dramatic changes to cognitive and social behaviors. However, little is known about the molecular, cellular and circuit-level modifications in the human brain that result in our distinctive behaviors. This is partly because we lack a complete understanding of how the brain develops, forms circuitry and executes behaviors, and this gap in knowledge hinders comparative studies. However, even when we can identify neural phenotypes that differ between humans and other species (examples illustrated in Fig. 1), it is difficult to know whether these changes are actually causal for the behavioral phenotypes, such as learning, communication and critical thinking, that make us distinctive from other species. We propose that an approach that starts by directly examining the genomes of present-day humans, ancient and archaic humans and extant, closely related species such as chimpanzees (what we term a 'genome-up' strategy) has major potential for identifying the genotypes, and their emergent phenotypes, that are under selection in humans. This approach allows researchers to examine human brain evolution along all timescales, including those for which comparative phenotyping is limited by the available fossil record between humans and other primates, between modern and archaic humans (for example, Neanderthals and Denisovans) and between present-day and ancient humans (for example, within the past 50,000 years).

Most human-specific sequence variants and molecular changes (for example, gene expression) are likely to have evolved neutrally and have little to no functional effect. Thus, a 'genome-up' strategy must first apply quantitative approaches to identify genomic regions that are under selection across evolutionary time. The identification of genetic loci with signatures of selection began around two decades ago with the release of human, mouse and select primate genomes. Recent releases of hundreds of mammalian, primate and ancient human genomes[1–4] and thousands of present-day human genomes[5–7] have made it increasingly tractable to identify signatures of selection across multiple timescales (Fig. 1). In parallel, transcriptomic, epigenomic and proteomic comparisons have identified human-specific molecular changes in particular cell types and developmental contexts. To enrich for likely functional variants, a 'genome-up' strategy intersects

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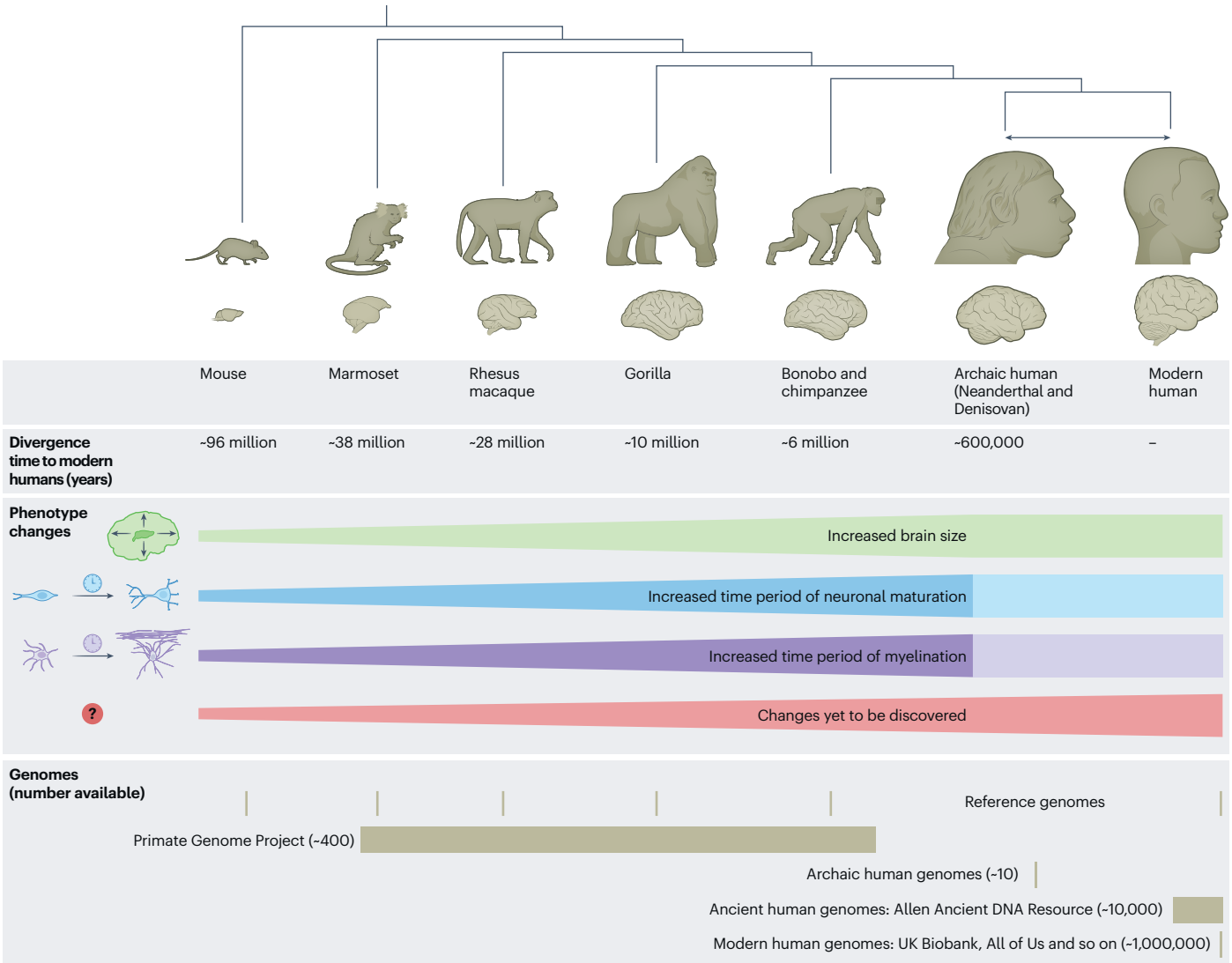

Fig. 1 | Phenotypic changes along the human evolutionary lineage and available genome resources. Top, phylogenetic tree with divergence times to modern humans. Middle, examples of phenotypes that have changed in humans, including an expansion in brain size[132], an increased period of neuronal maturation that extends into the third decade of life[133] and a correspondingly increased period of myelination[134]. There is no increase in brain size between archaic and modern humans. Differences in the timing of neuronal maturation and myelination are unknown between archaic and modern humans, as indicated by the pale shaded regions. The red bar represents phenotypic changes that have yet to be discovered. Bottom, bars indicate available genomes throughout the phylogenetic tree.

identified genetic loci with human-specific molecular changes to link genetic variants to when and where in the body they might act. These linkages can then be experimentally studied with both high-throughput and single-locus methods to determine their phenotypic effects. This Review examines each aspect of the 'genome-up' approach, revealing emerging insights and methodologies, current limitations and future research directions.

Selection and innovation of protein-coding genes in humans

To identify genomic regions with signatures of selection, one strategy has been to identify genes whose amino acid sequences are likely to be under positive selection along the human lineage (Fig. 2a). Two such genes are *FOXP2* and *NOVA1*, which have been of particular interest owing to their role in speech production[8–11]. Recreation of human-specific amino acid changes in these genes in mice resulted in vocalization differences[11–13], suggesting that these changes may have contributed to human speech (Fig. 2a). Other genes related to brain size and olfaction, such as *ASPM*, have also been found to show signatures of positive selection in humans[14,15], although their human-specific amino acid changes have not yet been investigated experimentally.

In addition to amino acid changes in existing genes, researchers have examined genes that arose from sequence duplication (Fig. 2a). Duplicated genes are common evolutionary substrates because they are functionally redundant with the ancestral gene copy, and, therefore, variation in the new gene copy may be tolerated. Of the ~100 gene duplications in humans, ~10 duplicated genes have been shown to be expressed, present in all examined human genomes and free of common variants that would truncate the protein-coding sequence[16]. Several of these have been found to perform neural functions: *ARHGAP11B* and *NOTCH2NL* affect neurogenesis, potentially contributing to the increased size of the human brain[17–23], whereas *SRGAP2B* and *SRGAP2C* affect dendrite formation and maturation, potentially contributing to the increased period of neuronal and glial maturation observed in humans[24–28]. Ongoing efforts to generate telomere-to-telomere genomes, which fill in the gaps that exist in current reference genomes, will further resolve the number and variation of human-specific gene duplications, as well as gene-loss events specific to humans[1,29].

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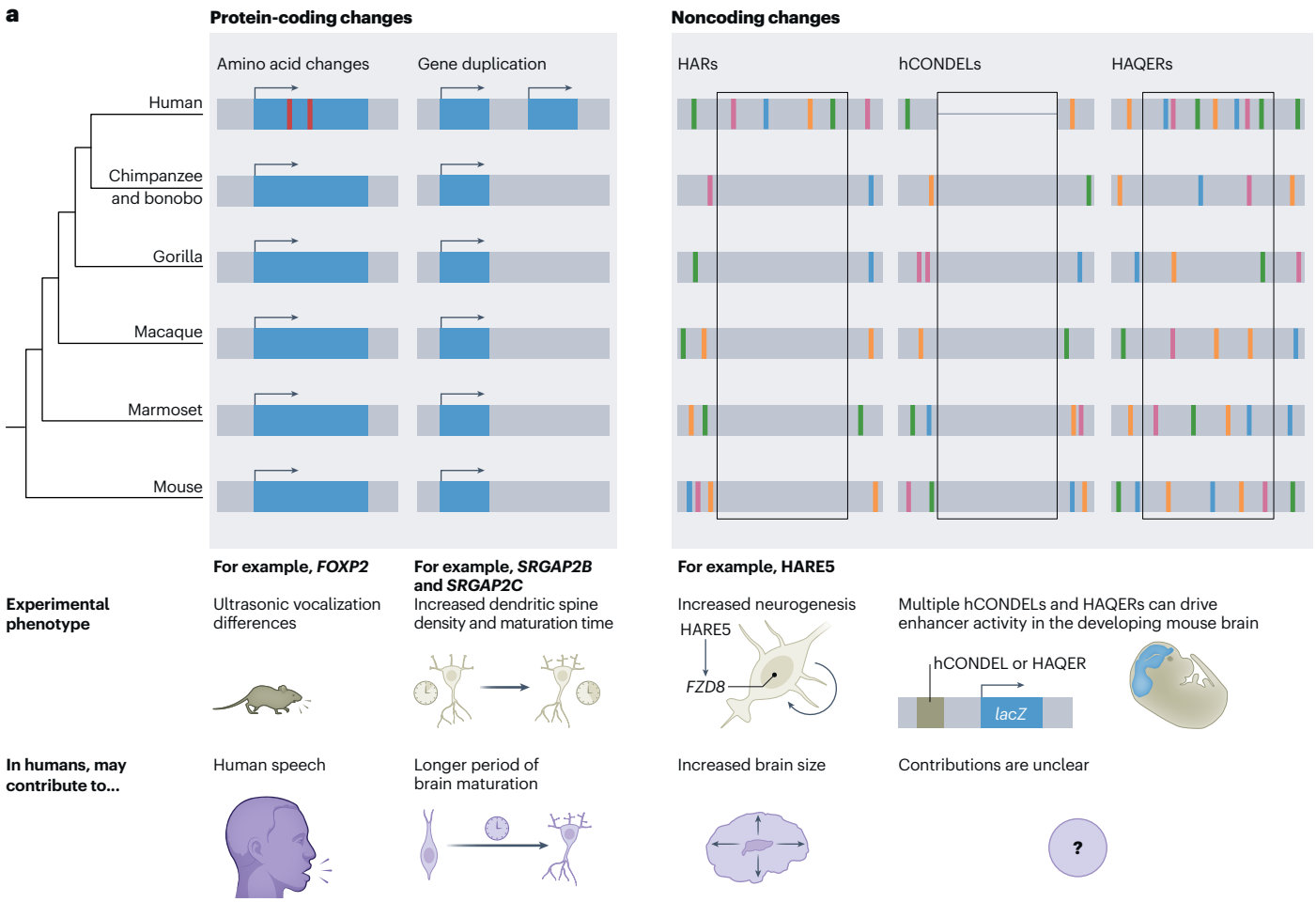

Fig. 2 | Examples of genomic changes between humans and other species and in recent human history. Vertical bars in genome diagrams indicate sequence variants. **a**, Types of genomic change between humans and other species, including an illustrative example[13,24,32,45,50] below each genome diagram. **b**, Genomic changes in recent human history. The graph at the bottom-right shows examples of changes in allele frequency between ancient and present-day humans that might be indicative of positive selection at specific time intervals.

Identifying noncoding regions under selection between humans and other mammals

Despite striking examples of amino acid changes and gene duplications that are likely to have affected human evolution, protein-coding sequences are 99% identical between humans and chimpanzees[30]. Evolutionary studies in multiple vertebrate systems suggest that >80% of adaptive sequence changes occur in noncoding, regulatory regions and that these noncoding changes can have comparable effect sizes to protein-coding changes[31–33]. The predominance of noncoding changes is plausibly because noncoding variants can restrict changes to specific spatiotemporal milieus and avoid the pleiotropic effects of protein-coding changes[31]. However, without the ability to use protein sequence as a predictor of function, it has been difficult to assess the consequences of the tens of millions of noncoding sequence changes between humans and other species[34].

In the early 2000s, the release of the first mammalian genomes allowed researchers to directly compare the human genome to that of other mammals. To prioritize functional noncoding elements, researchers focused on genomic regions that had high sequence conservation across non-human species, implying that these sequences are under purifying selection and are likely to perform critical functions. Researchers then searched for conserved regions whose

sequences changed specifically in humans, suggesting that they may have a human-specific function (Fig. 2a). This approach was used to identify genomic regions that have accelerated substitution rates in humans suggestive of positive selection (human accelerated regions (HARs))[35–40] and those that are deleted in humans (human conserved deletions (hCONDELs))[32]. These initial efforts identified 3,171 HARs and 583 hCONDELs and found that both sets were enriched near genes involved in neural development, consistent with selection along the human lineage for neurological traits[32,41]. Ensuing experimental characterization of these regions found that many have regulatory activity in neural cells[32,41–44] and identified specific elements such as HARE5 that are likely to contribute to human-specific traits, such as increased brain size[45] (Fig. 2a).

The recent release of new and improved mammalian genomes (including reference genomes for 241 mammalian species[2] and 800 genomes from 233 primate species[3]) has provided unprecedented resolution to identify sequence changes that are unique to humans and to pinpoint when these sequences were under selection relative to other mammals. For instance, a recent study identified 312 HARs when comparing humans to 240 other mammals[46], and another study identified 1,674 HARs when comparing the human genome to newly published primate genomes and other mammalian outgroups[47]. Differences between HAR sets arise from differences in defining conservation and acceleration (for example, which non-human species are used to identify conserved regions). Recently released genomes have also improved the identification of hCONDELs. The initial identification focused on deletions that were >25 base pairs (bp) in size, whereas new and improved genomes have enabled the recent identification of 10,032 hCONDELs that are <31 bp in size[48] and improved accuracy in identifying 930 hCONDELs that are >50 bp in size[49]. Future comparisons of recently published telomere-to-telomere primate genomes[29] will enable the identification of human-specific changes in previously gapped regions, such as centromeres and telomeres, for which prior investigation was not possible.

What about human-specific genetic changes in nonconserved, noncoding regions? Conservation indicates functionality across other species, but it is likely that novel human-specific functions arose in the 95% of the human genome that is not conserved[50]. An initial effort identified 1,596 regions with the highest accelerated base pair substitution rates in humans, suggesting that they are under positive selection (human ancestor quickly evolved regions (HAQERs)[50,51]; Fig. 2a). HAQERs were found to be enriched near genes involved in neural development and disease, and a number of HAQERs can act as species-specific enhancers in the developing brain[50]. We note that this study normalized accelerated substitution rates to gorilla. Because 15% of the human genome is more closely related to the gorilla genome than the chimpanzee genome[52], future reanalysis with normalization to more distantly related primates may improve the specificity of HAQER identification. Genome-wide scans for other classes of nonconserved regions with signatures of selection in humans, as well as de novo human insertions[53] and noncoding RNAs[54], have the potential to identify additional classes of noncoding regions associated with human evolution.

Identifying signatures of selection between archaic, ancient and present-day humans

Advances in sequencing ancient DNA over the last few decades has also made it possible to examine genetic material from archaic and ancient humans[55,56]. Neanderthals and Denisovans were archaic hominins harboring lineages that split from modern humans ~300,000 to 1 million years ago and lived primarily in Eurasia[57]. These archaic hominins interbred with modern humans moving out of Africa ~50,000 years ago before going extinct ~40,000 years ago[58]. Because these groups interbred with humans in Eurasia, comparisons between archaic and modern human genomes can identify archaic DNA that was transferred to humans, a process called introgression. These introgressed archaic alleles in non-African populations can reveal traits, such as skin pigmentation, hair morphology and the immune system, that were under positive selection in specific human populations within the past 50,000 years by comparing the frequency of introgressed alleles at specific sites to the background rate of introgression[59–61] (Fig. 2b). Conversely, researchers can identify regions where there is no evidence of introgression from archaic genomes[59,60], suggesting that the human alleles provided a selective advantage (Fig. 2b). Such a signal is observed in a large genomic region containing *FOXP2* on chromosome 7, which is consistent with the hypothesis that variants at the *FOXP2* locus may be specifically important to modern human biology[62]. Recent studies have also begun to examine protein-coding variants unique to modern humans in the context of brain development[63–67], but more work is needed to definitively determine which genetic changes between archaic and modern humans were under selection and contributed to neural traits.

What selective pressures acted on humans in the more recent past? During the past ~10,000 years, cultural and technological developments, such as farming and the formation of large, structured social hierarchies, may have changed human lifestyles and selective pressures[68]. Examining how allele frequencies change over time in humans can identify these signatures of selection (Fig. 2b). A particular advance in this area combined the Allen Ancient DNA Resource database, which compiles whole-genome information for >10,000 ancient humans[4,69] (Fig. 1), with a new statistical approach that tests for evidence of consistent selection over time to identify 347 independent loci with evidence for natural selection over the past 10,000 years of European history[70]. Consistent with prior studies, individual loci associated with cognitive traits were not under strong selection in this period compared to immunological traits, which were intensely selected. However, in aggregate, alleles associated with neuropsychiatric diseases such as bipolar disease and schizophrenia were under negative selection, whereas alleles that increase measures of cognitive performance were under positive selection. This suggests that there was coordinated selection in the past 10,000 years on neurally associated loci of small effect size[70]. We note that this work is limited to Europeans over the past 10,000 years. Extending these kinds of analysis to non-European populations and projecting back to a 50,000-year timescale, which is accessible with ancient DNA, would allow for more generalizable insights.

Whole-genome sequencing of ancient genomes can also provide greater resolution by characterizing variants that arose in the past but are no longer observed in present-day humans. Analyzing these variants can provide genetic clues about the selective forces that acted at different time points in human history and that are masked from analyses that rely solely on present-day human genomes (for example, HARs). More than 200 high-quality ancient genomes, analyzed through shotgun sequencing, which produces a complete genomic read out, are currently available through the Allen Ancient Genome Diversity Project[71]. Increasing the size of such databases will substantially extend the types of insight that are possible from analyzing these datasets.

Examining genomic constraint and phenotypic associations in healthy humans

Comparing the genomes of extant humans can also help us to understand how humans evolved. The falling cost of genome sequencing has facilitated the rapid generation of genetic data for more than 1 million healthy individuals and individuals with a disease[5–7]. These repositories provide a rich resource for functional inference. There are ~50 de novo germline mutations per individual[72], 3 billion base pairs in a haploid human genome and almost 8 billion people in the world. This suggests that, on average, every base in our genome is mutated in >100 people worldwide just in the current generation, with many more considering past generations. Thus, just as conservation across species can be used to assign a measure of functionality to base pairs across the genome,

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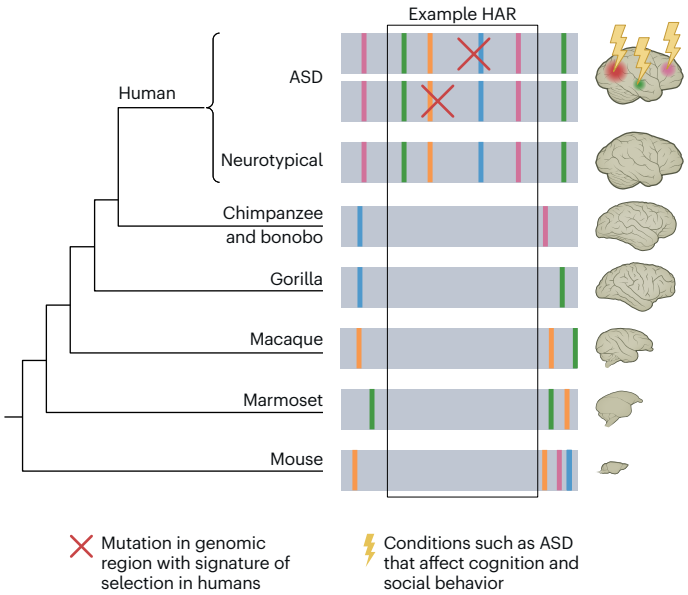

Fig. 3 | Genomic regions with signatures of selection in humans may be preferentially implicated in conditions that affect cognitive and social behaviors. We show an illustrative example of a HAR that is conserved through non-human mammals and has many sequence variants (vertical bars) specifically in humans, suggesting that it may be under positive selection. Mutations in HARs at human-specific base pairs or at surrounding base pairs (red crosses) have been shown to contribute to the development of conditions that affect cognitive and social behaviors, such as ASD[83].

genomic regions where there are fewer variants among healthy humans than expected (called constraint) indicate that variants in those regions are likely to be embryonic lethal or lead to deleterious disease consequences. With the recent availability of >100,000 whole genomes from healthy individuals, it has now become possible to assess constraint across the human genome, including in noncoding regions[73]. We posit that evolutionary sequence changes in regions constrained in modern humans are of particular interest because they are likely to contribute to essential, human-specific phenotypes. In addition, these datasets can be used to assess whether a variant identified between humans and other mammals or between modern and archaic humans is fixed or polymorphic in modern humans, which can also indicate its likely functional importance.

Concomitant with the increased availability of genetic data, databases such as the UK Biobank[6] and All of Us[7] (Fig. 1) provide matched genotype and phenotype information for hundreds of thousands of humans. These resources have been leveraged to link genetic loci with a variety of phenotypes, including skeleton proportions[74,75] and brain imaging measurements[76]. Strikingly, genetic loci associated with skeletal proportions related to bipedalism are enriched near HARs, suggesting that adaptive sequence changes in specific HARs may have contributed to the evolution of bipedalism[74]. The future intersection of loci linked to neural phenotypes with HARs and other human-evolved regions might similarly nominate specific sequence changes that affected the evolution of the human brain.

Human disease cohorts provide insights into human evolution

Genomes from cohorts of individuals with diseases can also provide an entryway into understanding the potential biological consequences of human-specific variants. Disease-associated mutations have been found in genomic regions with signatures of selection in humans[53,77–79], and these disease associations can indicate which cellular and developmental processes might be regulated by specific genomic regions. For instance, a rare variant in HAR426 that was found in individuals with autism spectrum disorder (ASD) is likely to regulate the expression of the transcription factor *CUX1*[78]. Similarly, a human-specific insertion containing a common variant associated with schizophrenia and bipolar disorder is likely to regulate the isoform expression of the calcium channel subunit *CACNA1C*[53,80]. For both *CUX1* and *CACNA1C*, de novo protein-coding mutations can lead to severe neurodevelopmental disorders[81,82], highlighting the importance of these genes to cognitive traits and raising the possibility that sequence variation in their regulatory elements may result in both evolutionary and disease consequences. Importantly, the known roles and disease outcomes of these genes provide focused directions for future investigations into the evolutionary functions of human-specific variants near *CUX1* and *CACNA1C*.

Because an obvious difference between humans and non-human primates is in our cognitive and social behaviors, the association of conditions that affect cognitive and social behaviors (for example, ASD and schizophrenia) with genomic regions under selection in humans has led to the hypothesis that variants in human-evolved genomic regions may preferentially contribute to these conditions (Fig. 3). In support of this hypothesis, a recent study found that the contribution of rare variants in HARs to ASD is more robust than the contribution from conserved neural enhancers, the largest previously observed noncoding association[83]. This suggests that the study of human-evolved genomic regions has the potential to enhance our understanding of diseases that affect human-evolved traits.

Connecting adaptive, human-specific genetic changes to their phenotypic effects

Challenges

Despite substantial progress in identifying genomic regions with signatures of selection along the human lineage and even linking them to potential phenotypes, experimentally studying their molecular, cellular, morphological and behavioral effects has been much more challenging, particularly for noncoding changes. Functional investigation has been hindered by multiple challenges (Fig. 4), including:

(1) What is the target gene? Studies have shown that noncoding changes can regulate genes over long distances[84], making it difficult to pinpoint which gene a noncoding change may regulate.
(2) At what developmental time point and in which cell type does the noncoding change affect the target gene? Genes are often expressed at multiple developmental time points and in different cell types, and these spatiotemporal gene expression patterns are controlled by distinct regulatory elements[85]. However, which regulatory elements control gene expression in which contexts is not known for most genes. As a result, even when the target gene of a particular noncoding change is identified, it can be difficult to know the developmental time point and cell type in which the gene is regulated by the noncoding change.
(3) What is the effect size? Many adaptive sequence changes are likely to have small effect sizes that may be hard to detect experimentally[86]. Strategies are needed (a) to determine whether a specific trait evolved via a small number of genomic changes with large effects or through many genomic changes with small effects and (b) to identify the genomic changes with effect sizes that are large enough to be studied experimentally.
(4) Is the effect dependent on the genomic background? Gene function and essentiality can differ between species[87]. Thus, if a human-specific variant affects a gene that performs different functions in humans compared with other species, the effect of the variant may not be apparent in non-human models.

Comparative omics

To begin to identify the developmental time point and cell type at which a human-specific genomic change might function (challenges 1 and 2), one common approach has been to directly compare human

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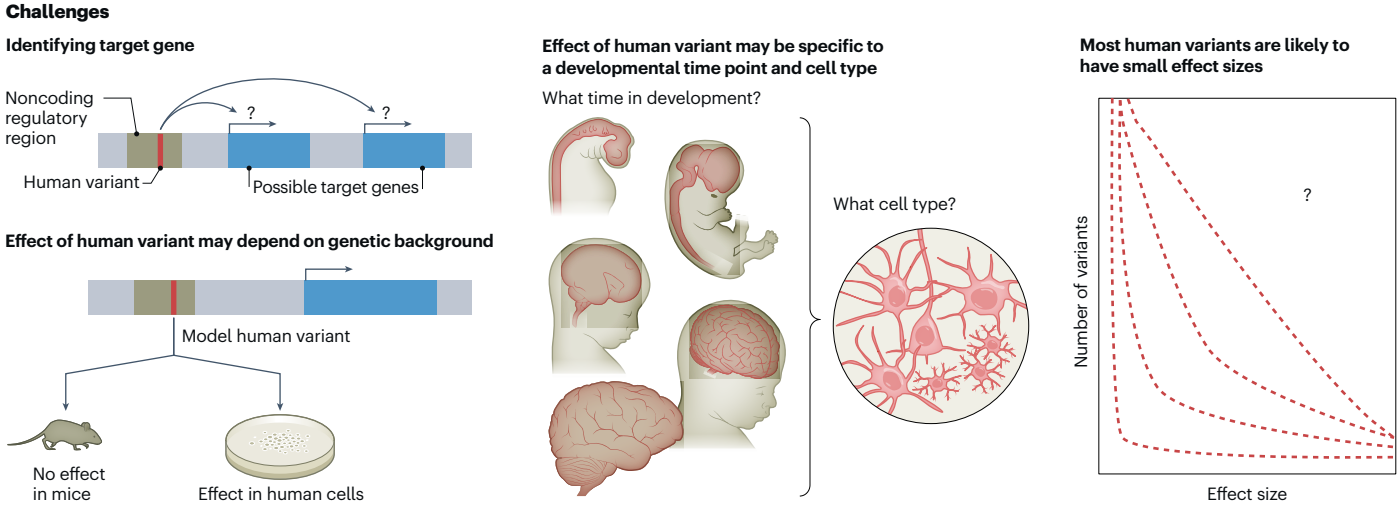

Fig. 4 | Challenges for identifying the phenotypic consequences of human-specific variants. The graph on the right illustrates uncertainty about which distribution of effect size and variant number is accurate.

and non-human primate cells and tissues to identify genes, proteins and noncoding regions that differ between species (Fig. 5). Because these comparative studies focus on particular developmental time points and brain regions, they can help to tie genic and noncoding changes to potential times and places of action. We note, however, that this approach is limited to extant organisms and cannot be easily applied to examine how present-day humans diverged from archaic or ancient humans.

Comparative studies using single-cell sequencing technologies have resolved human-specific molecular changes within specific cell types in the brain. Although a small number of cell types appear to be primate-specific[88,89], most cell types are conserved from mice to humans, and the proportion of each cell type within the brain is also largely conserved[90]. This suggests that human-specific traits primarily arose from molecular changes within specific cell types, rather than from changes to cell-type identity and specification. Within specific cell types, these studies have now identified thousands of genes with human-specific expression patterns[91–93]. Some studies have also profiled chromatin accessibility across the genome (greater accessibility is associated with genomic activity) and have identified thousands of noncoding regions that are differentially accessible in humans[90,92]. Recent work has even begun to identify proteomic differences[94]. In addition to cataloging molecular changes between species, these efforts have also identified cell types such as oligodendrocytes[92] and cellular pathways such as synapse assembly[93] that are different in humans compared to other primates.

For paradigms and time points that cannot be easily assessed with primary tissue, such as early neural development, in vitro comparisons between human and other mammalian cells have also proved powerful. Comparison of human and non-human primate brain organoid development has identified thousands of human-specific molecular changes[95,96], including a change in *ZEB2* expression that affects cell shape and progenitor number[97]. Similarly, evolutionary comparisons of the neuronal response to activity, which affects brain circuitry and behavioral outputs[98], have been largely restricted to in vitro approaches where neurons can be stimulated in a controlled manner[99–102]. These studies have identified *OSTN* (encoding osteocrin) as an activity-dependent gene in primates but not in mice that affects activity-dependent dendritic growth[99] and found that the regulatory landscape of the key activity-dependent transcription factor FOS differs substantially between human and chimpanzee neurons[102].

The recent explosion in comparative transcriptomic, epigenomic and proteomic data has motivated the study of specific molecular changes experimentally. Although these studies have generated intriguing findings[97,103], we caution that, just as most sequence changes between humans and other species are likely to be neutrally evolving, most gene expression and epigenomic changes between humans and other species are also likely to be neutral[104]. Thus, intersecting genes or candidate regulatory regions that change in a specific spatiotemporal context with genetic loci with signatures of selection in humans is a critical step to enrich for meaningful differences.

Linking human-specific noncoding variants to genes

Multiple strategies have been used to link genetic loci with signatures of selection in humans with the genes that they might regulate in specific contexts (challenges 1 and 2). Human-specific gene expression changes in specific cell types have been linked with nearby noncoding regions that have signatures of selection in humans and that have human-specific chromatin accessibility in the same cell type[92,93]. Other studies have profiled physical contacts between promoters and noncoding regions across human neurodevelopment to link candidate enhancers containing human-specific sequence changes, such as HARs, with the genes that they might regulate[105,106] (Fig. 5). Disease associations (as discussed in 'Human disease cohorts provide insights into human evolution') can also provide links to potential target genes and disease phenotypes (Fig. 5).

Although these and other putative linkages provide numerous, well-motivated starting points for experimental studies, the sheer number of molecular differences that have been identified in specific cell types, at specific developmental time points and with different environmental perturbations (each has hundreds to thousands of identified differences) makes connecting these differences to their causative genetic change an overwhelming challenge. One recent strategy to winnow the list of molecular differences is to identify which of these molecular differences are due to a nearby human-specific sequence change on the same DNA molecule. This is called *cis* regulation and contrasts with *trans* regulation, in which a molecular change occurs because of differences in diffusible factors, such as protein sequences or levels of transcription factors, in the cellular environment. Ultimately, adaptive noncoding changes regulate nearby genes in *cis* that lead to downstream changes in the cellular environment that then regulate the expression of other genes in *trans*. To distinguish *cis* and *trans* differences, multiple groups have recently generated human–chimpanzee tetraploid stem cell lines as a genetic model in which the human and chimpanzee genomes are in the same cellular environment and only *cis*-regulated changes are observed[103,107,108]. This model has

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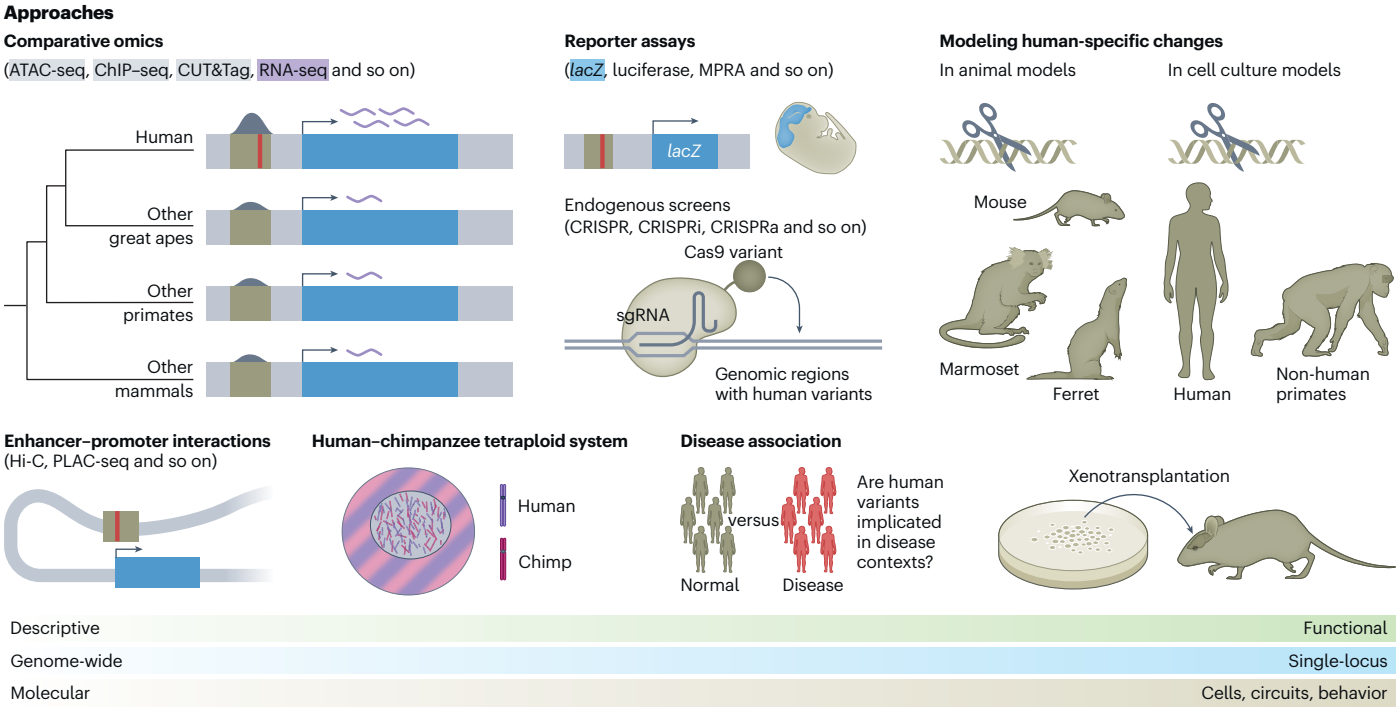

Fig. 5 | Current approaches for identifying the phenotypic consequences of human-specific variants. 'Comparative omics' shows an example of a human variant (red bar) that increases the activity of a regulatory region (tan rectangle), as evidenced by a larger pileup of sequencing reads (increased height of gray distributions) from assay for transposase-accessible chromatin with high-throughput sequencing (ATAC-seq), chromatin immunoprecipitation with sequencing (ChIP–seq) or CUT&Tag[135]. This change in regulatory activity leads to a nearby change in gene expression as assessed by RNA sequencing, where the wavy purple lines represent transcripts. 'Enhancer–promoter interactions' shows an example of how a regulatory region with a human variant can complex with transcription factors and other proteins (not shown) to contact the promoter of a nearby gene to affect gene expression, as assessed by Hi-C or proximity ligation-assisted chromatin immunoprecipitation with sequencing (PLAC-seq)[135]. 'Human–chimpanzee tetraploid system' shows a cartoon human–chimpanzee tetraploid cell. This system distinguishes *cis* effects due to a nearby variant from *trans* effects due to changes in the cellular environment. 'Reporter assays' shows an example regulatory region with a human variant (red bar) that can act as an enhancer in the developing mouse brain (light blue shading indicating *lacZ* reporter gene expression). 'Endogenous screens' represents a Cas9 variant (light and dark tan shape) bound to a single guide RNA (sgRNA) to target a genomic region with human variants; CRISPRi, CRISPR interference; CRISPRa, CRISPR activation.

begun to be used to distinguish *cis*- from *trans*-regulated changes and to link *cis*-regulated noncoding regions to nearby *cis*-regulated genes in multiple cell types (challenges 1 and 2), including neural progenitors, cortical excitatory neurons and neural crest cells[102,103,107,109–111]. Additionally, promoting recombination between the human and chimpanzee genomes in this system could directly identify the causative variants and their effect sizes for specific traits (challenge 3a)[107], although substantial work remains to fully realize this potential.

Functional testing

The approaches described above have linked thousands of human-specific genetic changes to gene expression changes in particular spatiotemporal contexts. How then can we move from these hypotheses to ultimately uncovering the phenotypic consequences of adaptive, human-specific genetic changes? There are multiple ways to prioritize genetic changes for testing, including the interests and expertise of different researchers, proximity to genes of interest and rank ordering based on the strength of selection at a given genomic region. The exemplar of these prioritization approaches has been HARE5 (Fig. 2a), which was nominated by selection strength, overlapped a region bound by the transcriptional coactivator p300 and is near the Wnt pathway gene *FZD8* (ref. 112). Generation of mice containing the HARE5 sequence and of human and chimpanzee in vitro models containing the reciprocal HARE5 sequence changes demonstrated that HARE5 regulates *FZD8* expression in neural progenitors to expand neurogenic potential and probably to contribute to the increased size of the human brain[45]. However, more than 20 years after the first human genome was released, this remains one of only a handful of examples[80,113,114] where any phenotype has been definitively linked to a noncoding, human-specific genetic change.

To approach this problem more agnostically, researchers have begun to apply high-throughput functional genomics approaches to screen hundreds to thousands of human-specific variants in a single experiment (challenges 1, 2 and 3b; Fig. 5). Massively parallel reporter assays have been used to compare the enhancer activity of thousands of human and chimpanzee HAR sequences in neural cells[41–44] and to compare over 10,000 variants between modern and archaic humans in diverse cell types[115,116]. Although these studies have identified hundreds of candidate variants (~3–30% of tested variants) that can affect enhancer activity, pervasive endogenous enhancer redundancy[117] complicates interpretation. More recently, CRISPR screens have been used to directly study human-evolved regions at their endogenous loci[111,118–120]. For instance, a CRISPR deletion screen showed that 15 of 409 tested HARs affected neural stem cell proliferation[118], and a CRISPR inhibition screen showed that 20 of 6,358 human-specific deletions acted as enhancers in pluripotent stem cells[120]. Thus far, these studies have been largely limited to enhancer and proliferation screens and have examined individual loci. Noncoding elements may also include silencers that inhibit gene expression, insulators that prevent interactions between genomic elements and range extender elements that allow enhancers to act over long genomic distances[85,121]. In addition, many cellular phenotypes outside proliferation are also implicated in the evolution of the human brain, and disrupting multiple loci simultaneously may be needed to overcome enhancer redundancy.

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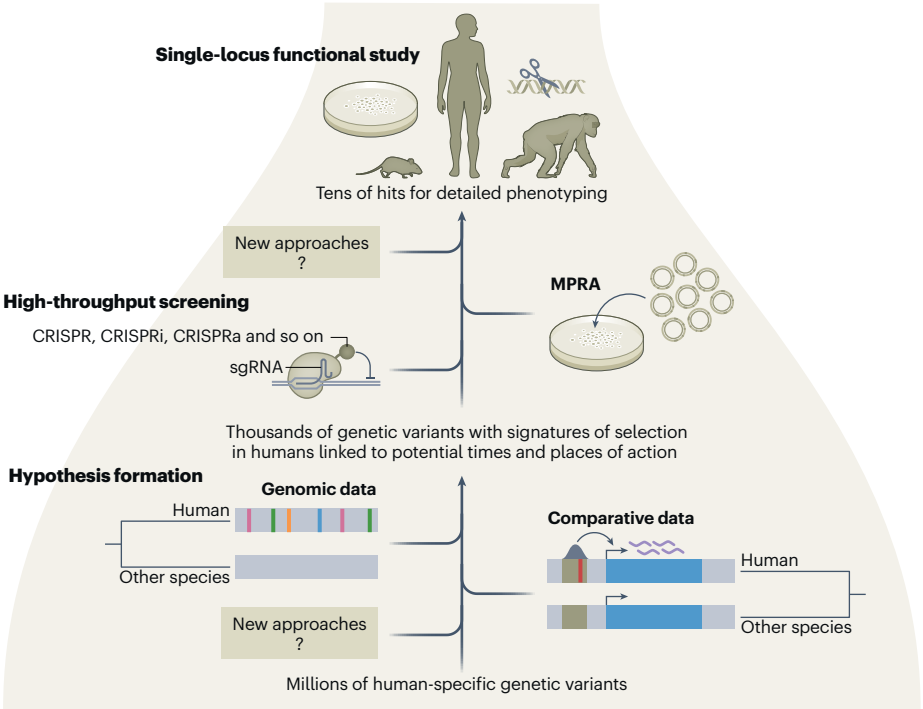

Fig. 6 | Proposed 'genome-up' approach. From the millions of genetic variants found when comparing present-day humans to other species, archaic humans or ancient humans, genomic approaches can identify variants with signatures of selection in humans that are linked to potential times and places of action using comparative transcriptomic and epigenomic data between humans and other species. These variants can then be further filtered using high-throughput screening approaches such as CRISPR screens or massively parallel reporter assays (MPRA). Finally, the top hits from these screens can be recreated in animal and cell culture models to identify their resulting phenotypes.

The continued application and development of new technologies will be needed to screen human-specific variants more agnostically across additional gene regulatory and cellular modalities.

Discoveries from these high-throughput approaches are top candidates for further study. To assess molecular, cellular, circuit-level, morphological and behavioral phenotypes, the gold standard experiment is to recreate human-specific sequence changes at their endogenous loci in both in vivo and in vitro models (Fig. 5). The mouse has long been the workhorse mammalian model owing to its advantages in gestation time, litter size and tool development. However, in contrast to the gyrencephalic (folded) human brain, the mouse brain is lissencephalic (smooth) and lacks features that are found in humans, such as outer radial glia cells. This has motivated the use of additional models. One gyrencephalic animal model is the ferret, which has relatively large litter sizes and short gestation times and can recapitulate neurological disease phenotypes not observed in mice[122]. Non-human primate models, such as marmosets and macaques, have also recently been used to study neurological diseases, although their long gestation times, small litter sizes and cost of care limit widespread adoption[123]. In addition to animal models, the rapid development of in vitro technologies over the last decade has enabled the direct study of genetic changes in human and non-human primate cells. Human and non-human primate pluripotent stem cells are now widely available[124] and can be differentiated into neural cell types[125–127] and region-specific brain organoids[128]. To examine complex phenotypes, human neurons or brain organoids have also been xenotransplanted into rodent models, where they can mature and form functional circuits in a species-specific manner[27,129–131]. We note that model choice will be constrained by whether a sequence orthologous to the human sequence of interest exists and that resultant phenotypes may be dependent on other changes in the human genetic background, rendering certain phenotypes undetectable or muted in non-human models (challenge 4; Fig. 5). Thus, careful analyses of the phenotypic effects of human variants will require both animal models, in which complex phenotypes including long-range circuitry and behavioral changes can be assessed, and human in vitro models, where variants can be studied in the human genetic background.

Summary and future directions

The confluence of decreasing sequencing costs and rapid genomic technology development charts a path forward for uncovering the human-specific genetic changes that result in neural adaptations. The recent and continued release of genomes from throughout the mammalian phylogenetic tree with dense sampling of primates, ancient humans and present-day humans allows for the identification and refinement of sets of genomic regions with signatures of selection in humans across different evolutionary time scales. At the same time, the use and development of comparative and functional genomics methods provides new approaches to link genomic regions to a particular spatiotemporal context and to screen these candidates in a high-throughput manner.

We propose that by intersecting the study of genomic regions with signatures of selection across different timescales in human evolution with data from comparative approaches and from extant humans, we can prioritize which of the millions of human-specific variants might be functionally important and form hypotheses about where in the body and when during development they might act. These thousands of resulting hypotheses can then be screened using high-throughput approaches for molecular or cellular phenotypes. Finally, the handful of resulting hits can be the target of focused, low-throughput and labor-intensive single-locus studies in which human-specific changes are recreated at the endogenous loci in both in vivo and in vitro models and their phenotypes are assessed across molecular to behavioral scales (Fig. 6). Together, this 'genome-up' approach has the potential to connect genetic variants with signatures of selection to meaningful phenotypes across different timescales of human evolution.

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Author contributions

Conceptualization: J.H.T.S., M.E.G., D.R. and C.A.W. Writing, original draft: J.H.T.S. Writing, review and editing: J.H.T.S., M.E.G., D.R. and C.A.W.

Competing interests

C.A.W. is on the Scientific Advisory Boards of Bioskyrb Genomics (cash, equity) and Mosaica Therapeutics (cash, equity) and is an advisor to Maze Therapeutics (equity) and CAMP4 (cash), but these have no relevance to this work. The remaining authors declare no competing interests.

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