Human brain evolution: transcripts, metabolites and their regulators

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Abstract | What evolutionary events led to the emergence of human cognition? Although the genetic differences separating modern humans from both non-human primates (for example, chimpanzees) and archaic hominins (Neanderthals and Denisovans) are known, linking human-specific mutations to the cognitive phenotype remains a challenge. One strategy is to focus on human-specific changes at the level of intermediate phenotypes, such as gene expression and metabolism, in conjunction with evolutionary changes in gene regulation involving transcription factors, microRNA and proximal regulatory elements. In this Review we show how this strategy has yielded some of the first hints about the mechanisms of human cognition.

Neuropile
A synaptically dense region between the neuronal and glial cell bodies, composed of dendrites, axons, synapses, glial cell processes and microvasculature.

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Humans differ from other primates in their ability to create, accumulate and transmit cultural knowledge between generations[1–4]. These abilities are the result of cognitive and behavioural features that are characteristic of humans, such as strong prosocial and cooperative behaviour, the extensive use of abstract symbols, spoken language and grammar[5,6]. But what molecular events during the course of human evolution gave rise to these cognitive changes? Answering this question has been anything but simple.

First, we still lack comprehensive knowledge of phenotypic differences between humans and other primates — such as cognitive differences between humans and apes — as well as differences in brain anatomy, histology and organization (see REFS 7–9 for reviews). Originally, chimpanzees were thought to lack many cognitive functions found in humans, including altruism, understanding another person's cognitive state, social cooperation, tool use and cultural transmission. All of these abilities, however, have subsequently been described in various forms in chimpanzees, bonobos and other great apes[7,10–17], which has stimulated continuing debate regarding the uniqueness of human cognitive traits[5,18].

With regard to brain anatomy, the most noticeable human feature is the large brain volume relative to body size[8]. In fact, the human brain/body ratio is less than that of mice[19] but is still unusually high among large mammals. Given how brain/body ratios change with body size among primates, the human brain would be expected to be ~50% larger than the chimpanzee brain, but it is ~200% larger[20]. The increase in volume reflects the increase in neuron number[21,22], which is hypothesized to be a result of increased symmetric neural progenitor cell divisions in the ventricular zone[19]. Brain volume growth in human development is accordingly more accelerated than in chimpanzees, both at the fetal[23] and postnatal stages[24] (reviewed in REF. 25). Notably, the proportion of high-level associative areas of the brain, such as the prefrontal cortex (PFC), is also higher in the human brain. However, whether this extent of cortical growth is expected under allometric brain growth patterns common to primates or whether it is an extraordinary feature that appeared only in human evolution remains unresolved[22,26,27]. Although an increased neuron number in association areas is likely to boost human information processing capacity, it is clear that brain size alone is unlikely to explain the entire range of cognitive functions characteristic of humans (BOX 1).

At the histological level, only a handful of studies have so far explored differences in brain cellular organization and neural connectivity between humans and apes. These studies have identified increased glia to neuron ratios[28], increased spacing between neurons[29], increased astrocyte complexity[30], and increased neuropile density[31] in the human brain. Some of these changes, however, may be a result of the overall brain size increase on the human evolutionary lineage[27]. Finally, the only comparative diffusion tensor imaging study known to us identified human-specific changes in the structure of the arcuate fasciculus, a white matter tract connecting language-associated regions in the human brain[32]. Overall, few studies so far have focused on the identification of human-specific cognitive, anatomical and histological features.

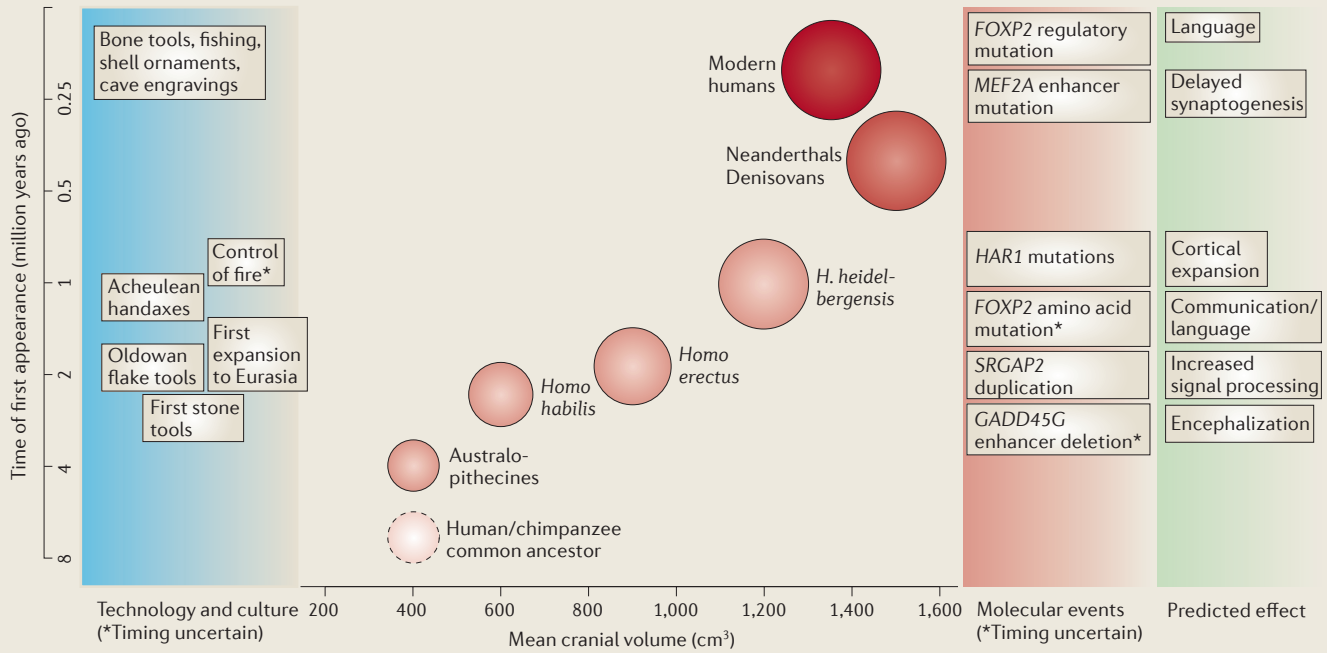
Box 1 | **Brain size evolution in the archaeological and genetic records**

Anatomical differences between human and chimpanzee brains, changes in cranial size in the human fossil record, and archaeological records of tools and cultural artefacts together present a general picture of the evolutionary steps that have led to the emergence of the human brain and cognitive abilities. From the most recent common ancestor of humans and chimpanzees 6–8 million years ago (mya)[33,35] until the dawn of the genus *Homo* approximately 2.5 mya, all early human ancestors had ape-like cranial volumes[145,146]. By contrast, subsequent *Homo* species had both higher absolute cranial capacities and higher cranium-to-body ratios (also known as encephalization quotients)[145,147,148] (see the figure). For example, the early *Homo habilis* (2.3–1.6 mya) had a cranium that was 1.5 times larger than that of modern chimpanzees. *Homo erectus*, a hominid species that populated our planet between 1.9 mya and 200 thousand years ago (kya), had a cranium that was initially twice as large[147,148]. *Homo heidelbergensis*, another hominin ancestor that appeared approximately 1 mya, had a cranial capacity approximately three times that of modern chimpanzees, overlapping with that of modern humans (*Homo sapiens sapiens*, 200 kya[149]), whereas Neanderthals (*Homo sapiens neanderthalis*, from 400–800 kya to 30 kya[35,39]) evolved cranial sizes that surpassed those of modern humans[147].

Compared to ancestral hominins, increased cranial capacity probably augmented the general and social intelligence of *Homo* species. Encephalization might have been the basis for the Oldowan (2.6–1.4 mya) and Acheulean (1.8–0.25 mya) stone tool technologies, and could have set the ground for the first out-of-Africa hominin colonizations undertaken by *Homo erectus*[145,150,151]. Tool use for scavenging, hunting and digging tubers, and the subsequent control of fire and invention of cooking[152] are thought to have promoted human encephalization by enriching nutritional content and increasing energy input[153,154]. Technological advance, access to high-quality food and encephalization thus appear to have evolved hand-in-hand. However, exceptions to this concept of parallel evolution might exist, such as the not yet fully understood case of the small-brained *Homo floresiensis* species, who used stone tools but had an ape-like cranial volume[155].

Recent comparative genomic studies have identified a number of mutations in the modern human genome that could underlie the evolution of larger brain size and behavioural traits. In agreement with the fossil record, these mutations are shared with extinct hominin species, indicating that encephalization and certain behavioural changes evolved early in the evolution of *Homo*. For example, a deletion of one enhancer of the growth arrest and DNA-damage-inducible gamma (*GADD45G*) gene, which is postulated to have led to brain size expansion, is shared with Neanderthals (data from REF. 45). Likewise, a human-specific duplication of a truncated version of the gene SLIT-ROBO Rho GTPase activating protein 2 (*SRGAP2*) was dated to approximately 2.4 mya[156,157]. This duplication is predicted to increase dendritic spine density in the cortex and could have enhanced signal processing in the hominin brains (see the figure). It is also worth noting that both Neanderthals and Denisovans — the two extinct hominin species that diverged from the human evolutionary lineage 400–800 kya[35,40] — differ from other primates by the same two amino acid changes as modern humans in the gene encoding forkhead box P2 (*FOXP2*)[158,159]. As these amino acid substitutions have been linked to the evolution of human language[160] and changes in brain connectivity[110], it is conceivable that Neanderthals and Denisovans also possessed certain types of human-like linguistic abilities.

Remarkably, although the late *Homo* species showed an increase in brain size to and beyond that of modern humans, their Acheulean technology (1.8–0.25 mya), mainly consisting of stone flakes and hand-axes, made only limited progress for nearly 1.5 million years[150]. This cultural and technological standstill contrasts with the rapid cultural explosion observed in the archaeological record that started approximately 250 kya and is associated with the appearance of the first fossil remains of modern humans, *Homo sapiens sapiens*[161]. The first modern human forms appear about 190 kya in East Africa[162], which is in agreement with the estimation for the origin of modern humans based on population genetic studies[149]. With the rise of modern humans in Africa, between 250 and 50 kya, bone tools began to be exploited, and spear heads and fishing appeared[163–165]. Besides technological innovation, this period also witnessed the first appearance of unequivocal symbolic artefacts, such as pigment use by 160 kya[166] and shell ornaments and cave engravings by 77 kya[167]. Producing these artefacts involved a large number of steps, and maintaining such a culture demanded efficient systems of information transfer across generations[165]. This cultural explosion was soon followed by successful colonization of other continents and could have accelerated the extinction of other hominin species[168]. Strikingly, the now-extinct hominins that coexisted with *Homo sapiens* — including Neanderthals — do not appear to have produced symbolic artefacts (but see REF. 169), despite Neanderthals having a larger cranial capacity than *Homo sapiens*[161].



Furthermore, these studies typically examined very limited numbers of individuals. Thus, it is likely that additional histological differences between human brains and the brains of other primates remain to be identified.

Second, although the completion of the human and the non-human primate genomes has provided us with a nearly complete list of genetic features specific to humans, the functional consequences of these features remain elusive. Genetically, humans are close to other apes: the genetic distance between humans and chimpanzees or bonobos is on the order of 1–5% (depending on the types of genetic divergence considered) and reflects the recent divergence of the species[33–35]. By comparison, the difference between the common mouse and rat species, *Mus musculus* and *Rattus norvegicus*, respectively, ranges from 20% to 50%[36]. Nonetheless, the relatively small genetic divergence between the human and chimpanzee genomes includes ~50,000 amino acid changes along with ~30,000,000 point mutations in non-coding sequences, as well as millions of insertions, deletions, inversions, genomic rearrangements, transposable element movements, and others[33].

Importantly, most of these genetic changes in all likelihood made no contribution to human phenotype and, therefore, are evolutionarily neutral[37,38]. How then can we recognize the few genetic changes that were responsible for the emergence of human cognition among the millions of evolutionarily neutral changes separating humans and non-human primates? One strategy is to limit the scope of investigation to known functional sites and to focus on human-specific genetic changes, that is, changes found in the human genome but not in the genomes of any other closely related primate species, including the extinct hominin species Neanderthals and Denisovans[39,40]. Another strategy is to screen the genome for regions that contain unusually large numbers of human-specific DNA sequence changes — an occurrence that implies adaptive evolution at such loci.

These approaches have led to the identification of changes in genes associated with language and speech (forkhead box P2 (*FOXP2*), protocadherin 11 X-linked (*PCDH11X*) and *PCDH11Y*), genes associated with brain size (microcephalin 1 (*MCPH1*), asp homologue, microcephaly associated (*Drosophila*) (*ASPM*), CDK5 regulatory subunit associated protein 2 (*CDK5RAP2*), solute carrier family 2 (facilitated glucose transporter), member 1 (*SLC2A1*), *SLC2A4*, neuroblastoma breakpoint family (*NBPF*) genes, growth arrest and DNA-damage-inducible gamma (*GADD45G*), ret finger protein-like 1 (*RFPL1*), *RFPL2* and *RFPL3*) and genes associated with neuronal functionality (dopamine receptor D5 (*DRD5*), glutamate receptor, ionotropic, NMDA 3A (*GRIN3A*), *GRIN3B* and SLIT-ROBO Rho GTPase activating protein 2 (*SRGAP2*)). Genome-wide scans have similarly identified both coding and non-coding transcripts and regulatory loci predicted or known to affect neural development[41–45]. These findings have shown that studies of human-specific genetic changes can be fruitful, especially when the phenotypic roles of the affected loci are partially known through studies of genetic linkage or association with cognitive disease or from experiments in model organisms (reviewed in REFS 38,46–48). An alternative avenue of the search for evolutionary changes underlying human brain evolution is the investigation of human-specific alterations at levels intermediate between the genome and phenotype (FIG. 1). There are several advantages to this approach. First, such data can be scrutinized to determine transcripts, proteins and metabolites that show shared patterns of species divergence across brain regions, cell types or developmental time points. Such coordinated differences that involve multiple genes may hint at the spatial and temporal rearrangements of existing functional processes, as well as the emergence of novel histological structures, metabolic fluxes or developmental patterns in the human brain. Second, coordinated changes in transcript, protein or metabolite abundance can be linked to changes in upstream regulation shared by these elements, thus facilitating identification of the causative evolutionary changes.

The difficulty of this approach, compared to genome-based studies, stems from the need for precise large-scale measurements conducted in specific brain regions and specific cell types, at various ontogenetic time points in multiple human and non-human primate individuals, and under known environmental conditions. The technologies allowing such measurements are available today (FIG. 1). In particular, high-throughput sequencing technologies, also known as next-generation sequencing (NGS), provide comprehensive quantification of human and non-human primate transcriptomes that are free of biases and limitations characteristic of microarray-based measurements[49,50]. For example, unlike most microarrays, NGS allows the identification of novel coding and non-coding transcripts expressed in the human brain[51–55]. Besides delivering comprehensive transcriptome measurements, NGS provides a means for determining the epigenetic states of chromatin, identifying regulatory binding sites and even estimating translational rate by ribosome profiling (reviewed in REF. 56). In addition to NGS, a less recognized area of recent technological advances is metabolomics, an approach that allows us to obtain high-throughput measurements of hundreds of hydrophilic and hydrophobic compounds present in the brain using mass spectrometry-based approaches[57]. This has opened new perspectives for the identification of functional features specific to the human brain. The current challenge to evolutionary studies of the human brain is, therefore, not technological. The main limiting factor is the availability of good quality human and non-human primate brain samples with precise histological, ontogenetic and demographic characteristics.

Nonetheless, recent studies based on the investigation of molecular phenotypes have provided important insights into the molecular mechanisms and the genetic changes that underlie human cognitive evolution. In this Review we specifically focus on a large body of studies examining human-specific features of the brain transcriptome, as well as the regulatory mechanisms driving

Hominid
A member of the family *Hominidae* (the great apes), including the subfamily *Homininae* (humans, gorillas and chimpanzees) and the subfamily *Ponginae* (orang-utans).

Hominin
Member of the tribe *Hominini*, including all species that evolved since the last common ancestor between humans and chimpanzees, and which were more closely related to humans than to chimpanzees, including the genera *Ardipithecus*, *Australopithecus*, *Paranthropus* and *Homo*.

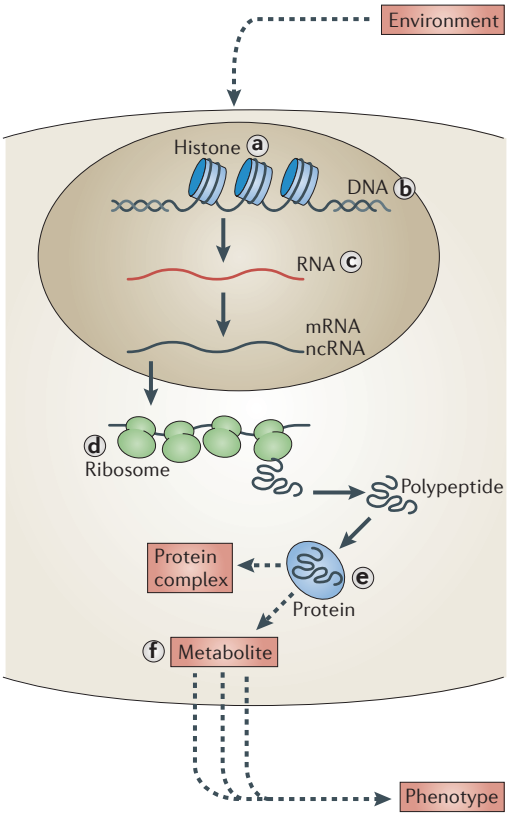
Neanderthals
An extinct species of the genus *Homo*, which inhabited Europe and parts of western Asia between 400,000 and 30,000 years ago. Notably, they had very similar morphology to modern humans, slightly bigger brains, and interbred with Eurasian modern humans for some period.

Denisovans
A recently discovered extinct *Homo* species identified by genome sequencing of a finger bone from Siberia. Denisovans were more closely related to Neanderthals than to modern humans.

mRNAs
Transcripts encoding sequences of protein-coding genes. mRNAs serve as messengers between genomic DNA and the protein translation machinery of the cell.

Non-coding RNAs
(ncRNAs). Transcripts that do not encode proteins but have regulatory, structural and catalytic roles in cells.

Broca's area
A region of the brain within the inferior frontal gyrus that is associated with speech in humans and manual gestures in other primates. The region tends to be larger in the left hemisphere.



	Molecular level	Methodology
a	Histone modification	ChIP–seq
b	DNA modification	Bisulphite sequencing
c	Transcriptome	Microarrays/high-throughput RNA-seq
d	Ribosome positioning	Ribosome position profiling
e	Proteome	Mass spectrometry
f	Metabolome	Mass spectrometry, NMR

Figure 1 | **A schematic representation of the complete molecular roadmap for deciphering human brain evolution.** Adult human and chimpanzee brains show thousands of mRNA abundance differences, which could be relevant to how the human CNS develops and functions differently from that of other primates. How these expression differences are regulated is, however, largely unknown. To address this, systematic identification of human-specific features at all levels of molecular phenotype is needed. Measurements of epigenetic regulatory marks, such as histone modifications (**a**) and DNA modifications (**b**), transcriptome abundance levels (**c**), ribosome positioning (**d**) and proteome abundance levels (**e**), in combination with metabolite concentration measurements (**f**), will allow us to identify the full spectrum of human-specific molecular features. With this knowledge we should then be able to propose and test mechanisms underlying human cognitive evolution. ChIP–seq, chromatin immunoprecipitation followed by sequencing; ncRNA, non-coding RNA; RNA-seq, RNA sequencing.

this transcriptomic divergence. Additionally, we introduce recent findings on human-specific aspects of brain functionality stemming from the emerging field of evolutionary metabolome studies.

Transcriptome studies

Transcription of genomic material in the form of mRNAs and non-coding RNAs (ncRNAs) represents the first step in the transfer of genetic information from the DNA sequence to the organismal phenotype (FIG. 1). Below, we summarize the results of studies that have investigated transcript expression features that are specific to the human brain.

Species comparisons of the adult brain. The first studies comparing RNA transcript abundance between human brains and the brains of closely related non-human primates such as chimpanzees, orang-utans and macaques measured mRNA abundance levels in few adult individuals and sampled a limited number of cortical areas, such as the PFC[58], anterior cingulate cortex (ACC)[59] or the cerebral cortex in general[60]. Despite such limitations, these studies uncovered several features of primate transcriptome evolution. First, they showed that for many genes, mRNA abundance variation within a species is lower than the divergence between species: that is, in the adult cerebral cortex, thousands of transcript abundance signatures are uniquely present in humans but not in other primates[58]. Second, they found that changes in mRNA abundance in the brain proceeded at a faster rate in the human than on the chimpanzee evolutionary lineage[58,60]. This observation is consistent with the notion that changes in brain functionality on the human evolutionary lineage could have been determined by changes in gene expression[61,62]. However, these and subsequent studies suggested that, in a similar way to DNA sequence changes, a substantial proportion of gene expression differences observed between human and other primates' brains may be unrelated to the cognitive differences among species or may have no effect on the organism's phenotype[63,64].

One approach to study the functionality of gene expression changes is to survey human-specific mRNA abundance profiles across brain regions with diverse functions. The first such study examined mRNA abundance in six human and chimpanzee brain regions — the dorsolateral PFC (DLPFC), Broca's area, primary visual cortex (V1), ACC, caudate nucleus and cerebellum — and found surprisingly few differences that were specific to a particular brain region[65]. Transcript abundance differences between humans and chimpanzees were distinct among the cerebellum, caudate nucleus and the cerebral cortex, but within the cerebral cortex only the V1 showed substantial divergence specific to this region. This paucity of human-specific mRNA abundance in particular cortical regions, including the DLPFC and Broca's area, was further confirmed by modular analysis of co-expression networks based on the original expression data[66]. Functionally, the DLPFC, ACC and, particularly, Broca's area show activation when people perform tasks that involve

Heterochrony
An evolutionary phenomenon involving changes in the rate and timing of development.

human-specific cognitive functions, including social behaviour, decision-making, attention, creativity and language[67–71]. Furthermore, damage to these brain areas results in a loss or decline of these cognitive functions, as in Broca's aphasia[72,73]. A lack of pronounced human-specific mRNA abundance changes that are particular to these cortical regions indicates that their novel functions evolved in humans without large-scale changes in functional organization and/or histological composition of these regions in the adult brain. This observation complements histological studies that show general homology between the human and macaque parietal and frontal cortex areas[74,75]. Moreover, there is a histological homologue of Broca's area in chimpanzees and macaques, but it functions in non-vocal communication, including gestures and orofacial responses[76,77]. Thus, brain regions involved in human-specific cognitive functions are likely to represent previously existing areas that have been recruited for novel functionality[8].

Nonetheless, it is important to note that current studies of brain-region-specific differences between humans and non-human primates were based on small numbers of individuals and examined bulk cortical samples that contained a mixture of functionally distinct cell types. As recent studies have demonstrated profound differences in transcript abundance between specific histological formations, such as cortical layers (BOX 2), and between specific regions of the human brain[78], many human-specific gene expression features particular to specific sites or cell types within the brain may still be undiscovered.

A developmental perspective. Human cognitive abilities, such as language, emerge and develop during childhood and adolescence[79]. Their development requires external input, such as interactions with conspecifics, during a specific developmental period. For example, human infants deprived of contact with other humans are not able to achieve normal levels of cognitive skills, such as verbal communication, when introduced into human society later in life[80]. Further, the potential to develop these abilities is specific to humans: babies of non-human primates raised in a human environment do not develop comparable cognitive skills[81]. Additional insight into the evolution of human cognition might, therefore, be gained through studies of human brain development.

The first comparative molecular study of primate brain development found that more than 70% of genes expressed in the human, chimpanzee or macaque brains undergo changes in mRNA abundance during postnatal development, with the most rapid changes taking place in early infancy[82]. Although the trajectories of these developmental changes were conserved across species for more than 75% of genes expressed in the brain, their timing differed substantially among species — a phenomenon termed heterochrony[83]. On average, such timing differences scaled with species' differences in maturation rates. However, this temporal scaling was not uniform across genes: for example, when comparing humans to chimpanzees, certain genes showed greater delay than expected from the difference in maturation rates, whereas others showed accelerated changes.

Subsequent studies provided further insights by separating gene expression differences among species into differences in the mean mRNA abundance level, thus preserving the shape of developmental trajectories, and into differences that altered the shape of mRNA abundance trajectories during postnatal brain development (FIG. 2a). Firstly, this showed that changes affecting mRNA abundance levels accumulated at similar levels on both the human and chimpanzee lineages (FIG. 2b). By contrast, many more changes in the shape of developmental trajectories took place on the

Box 2 | **Transcriptome studies at finer layers of cortical organization**

Within the cerebral cortex, neurons and their projections form a distinct laminar architecture that is commonly classified into six layers (for example, see REF. 170). Cortical layers differ from one another with respect to the neuronal types they contain, as well as the types of neuronal projections originating from and feeding into these layers (see REFS 171–173 for reviews). Recent studies have demonstrated that these differences in histology and connectivity are underlined by differential expression of genes across the layers. An *in situ* hybridization-based study investigated the expression localization in human cortex for approximately 1,000 genes associated with neural functions[174]. This study revealed profound differences in gene expression across the six cortical layers in all three brain regions examined: primary and secondary visual cortices, and part of the midtemporal cortex. This finding agrees with findings from studies using high-throughput transcriptome analysis methodologies, microarrays and RNA sequencing (RNA-seq) in macaques and mice: ~4,900 and 5,800 of examined transcripts showed distinct localization across the cortical layers in macaques[175] and mice[176], respectively. Among cortical regions, only 4% of examined genes showed differential expression across layers between human secondary visual and midtemporal cortices, whereas 18% of genes differed between primary and secondary visual cortices[174]. Similarly, in the rhesus macaque cortex, the primary visual cortex showed the most distinct gene expression signature across the layers among all ten cortical regions examined[175].

Importantly, despite the relatively high similarity of layer-specific gene expression profiles across human cortical areas, a comparison with the mouse *in situ* hybridization results reveals the rapid evolution of layer-specific expression pattern across species: as many as 21% of the neural-function-related genes examined showed clear differences in layer localization between the human and mouse cortices, with most of these genes being characteristic of neurons rather than glial cells and being expressed in a layer-specific manner in one of the two species[174]. These differences included a shift in the expression of genes involved in signal transduction and cell–cell communication from the mouse layer V to the large pyramidal neurons of the human layer III. This is interesting because layer V neurons send output projections to subcortical and contralateral regions, whereas layer III neurons send projections within the same hemisphere (ipsilateral projections). Owing to the enormous increase in brain size, layers III and II (which are similarly involved in the formation of ipsilateral projections) are greatly expanded in the human cortex and project over far greater distances compared to smaller brains, such as in mice[177,178]. This result highlights the fact that despite the apparent basic uniformity of cortical layer organization across mammals at the levels of basic histological architecture, neuronal connectivity and developmental origins[75,179–181], drastic differences in cortical organization exist between species at the level of gene expression localization across the cortical layers.

Divergence of layer-specific expression profiles might have important implications for the evolution of human cognition. Changes in synaptic density during human postnatal development as examined by electron microscopy showed substantial differences in the pace of synaptogenesis across cortical layers[88]. More recently, pyramidal neurons in layer IIIc and layer V of the human prefrontal cortex were shown to undergo extensive synaptic remodelling until the fourth decade of life[182]. Interestingly, the pace and the scale of this remodelling differ between layer IIIc and layer V pyramidal neurons and between different types of dendrites formed by these neurons. Given the recent discovery of a human-specific extension of cortical synaptogenesis observed in a prefrontal cortex sample homogenate[85], it is possible that timing of this extension varies among cortical layers, as well as among neuronal and projection types within the layers.

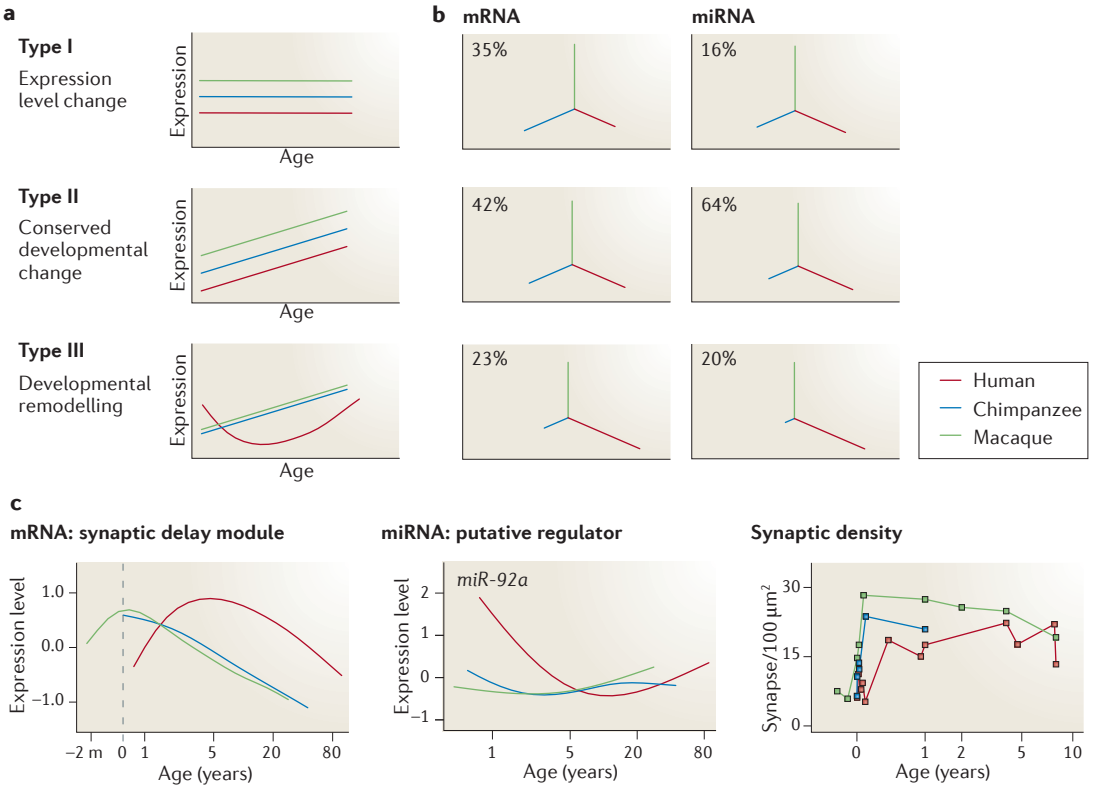


Figure 2 | **Expression divergence patterns in the prefrontal cortex. a** | There are three types of expression differences that distinguish human, chimpanzee and rhesus macaque brain development. Type I: no age-related changes, with constant mRNA abundance differences among species. Type II: conserved developmental patterns, with constant mRNA abundance differences among species. Type III: differences in the shape of developmental patterns of mRNA abundance among species (developmental remodelling). **b** | Evolutionary trees based on the three types of expression differences show that mRNA and microRNA (miRNA) expression during prefrontal cortex (PFC) development changed to a similar extent on the human (red) and chimpanzee (blue) evolutionary lineages with respect to Type I and Type II differences. By contrast, there are many more Type III differences on the human evolutionary lineage as compared to the chimpanzee lineage. The percentages within the panels show the percentage of genes showing this type of expression difference. **c** | An example of developmental remodelling in the human PFC: the average expression profile of 184 genes showing synchronized human-specific change in the shape of mRNA expression trajectories (left panel). Functions characteristic of these genes are shown in TABLE 1. Expression of this set of genes is correlated with putative regulators. The expression profile of one such putative regulator miRNA (miR-92a) is shown in the middle panel. The human-specific changes in expression of this set of genes are associated with synaptic functions and suggest extended synaptogenesis in the human PFC, a notion supported by direct measurements of synaptic density using electron microscopy (right panel). Part **b** is modified from REF. 84. Part **c** is modified, with permission, from REF. 85 © (2012) CSHL Press.

human lineage compared to the chimpanzee lineage[84]. Secondly, human-specific changes in developmental trajectories were approximately 3–5 times more pronounced in the PFC than in the cerebellum[84]. Thirdly, in the PFC the most prominent human-specific developmental trajectory change affected genes associated with synaptic functions[85] (FIG. 2c; TABLE 1). In summary, these results demonstrate that within the last 6–8 million years (see BOX 3 for more information on evolutionary timing), mRNA developmental trajectories changed more rapidly in the human PFC than in the chimpanzee PFC or human non-cortical regions such as the cerebellum. One of the major aspects of this remodelling has involved heterochronic expression of synaptic genes, resulting in their peak expression at approximately 5 years after birth in the human PFC compared to several months after birth in chimpanzees and macaques.

Implications of extended human synaptogenesis. After birth, the cerebral cortex of humans and other primates undergoes massive synaptic proliferation (synaptogenesis), followed by an extended period of synaptic pruning (synaptic elimination). Studies that directly monitored synaptic density changes during human brain development using electron microscopy showed that synaptic density peaks at 3.5–10 years of age in the PFC and at 0.4–3.5 years of age in the auditory cortex. In the V1, synaptic elimination begins substantially earlier, with the maximal synaptic density occurring at approximately 3 months of age[86–88]. By contrast, in the rhesus macaque brain peak synaptic density is reached shortly after birth in all cortical regions examined: that is, the prefrontal, visual, motor, somatosensory and limbic cortices[89]. A comparative examination of synaptic density in the PFC of humans, chimpanzees and rhesus macaques, although based on a limited number

Synaptogenesis
The formation of synapses between neurons. In the human cerebrum, synaptogenesis is especially intense starting from 30 weeks of pregnancy until 15 months of age.

Synaptic elimination
Also called synaptic pruning, this is the developmental process whereby immature synapses — that is, those not subject to activity-dependent strengthening — are removed. At least half of the synapses generated in the infant cortex are eventually eliminated.

Table 1 | **Examples of pathways that underwent developmental remodelling in the human PFC**

Database	Pathways	Genes	Adjusted *P*-value
KEGG	Calcium signalling pathway	*ATP2A2*, *CALM3*, *CAMK2B*, *CAMK4*, *CHRM1*, *CHRM3*, *PPP3CB*, *PRKCB*, *RYR3*, *SLC8A2*	8.00E-03
	Long-term potentiation	*CALM3*, *CAMK2B*, *CAMK4*, *PPP1CA*, *PPP3CB*, *PRKCB*	3.50E-02
GO	Synaptic transmission	*AKAP5*, *BDNF* precursor, *CAMK4*, *CHRM1*, *CPNE6*, *CRHR1* precursor, *DLG4*, *GABBR2* precursor, *GABRA2* precursor, *GABRA5* precursor, *GABRG1* precursor, *GRIA4* precursor, *GRM8* precursor, *NRXN3*, *PARK2*, *RASGRF1*, *SLC6A1*, *STX1A*, *TAC1*, *UNC13C*	2.44E-04

AKAP5, A-kinase anchor protein 5; *ATP2A2*, sarcoplasmic/endoplasmic reticulum calcium ATPase 2: *BDNF*, brain-derived neurotrophic factor; *CALM3*, calmodulin 3; *CAMK*, calcium/calmodulin-dependent protein kinase; *CHRM*, muscarinic acetylcholine receptor M; *CPNE6*, copine 6; *CRHR1*, corticotropin-releasing factor receptor 1; *DLG4*, discs, large homologue 4; *GABBR2*, GABA type B receptor subunit 2; *GABRA2*, GABA type A receptor, alpha 2; *GABRA5*, GABA type A receptor, alpha 5; *GABRG1*, GABA type A receptor, gamma 1; GO, Gene Ontology database; *GRIA4*, glutamate receptor 4; *GRM8*, glutamate receptor, metabotropic 8; KEGG, Kyoto Encyclopedia of Genes and Genomes; *NRXN3*, neurexin 3; *PARK2*, parkinson protein 2, E3 ubiquitin protein ligase; *PPP1CA*, protein phosphatase 1, catalytic subunit, alpha isozyme; *PPP3CB*, protein phosphatase 3, catalytic subunit, beta isozyme; *PRKCB*, protein kinase C beta type; *RASGRF1*, Ras-specific guanine nucleotide-releasing factor 1; *RYR3*, ryanodine receptor 3; *SLC6A1*, solute carrier family 6 (neurotransmitter transporter, GABA), member 1; *SLC8A2*, solute carrier family 8 (sodium/calcium exchanger), member 2; *STX1A*, syntaxin 1A; *TAC1*, tachykinin, precursor 1; *UNC13C*, unc-13 homologue C. Data from REF. 85.

of individuals, demonstrated that the pace of synaptic density changes in the chimpanzee PFC was faster compared to that in the human PFC, and resembled the pace in macaques[85] (FIG. 2c). Human-specific changes in the expression of the synaptic genes described above could therefore be correlated with synaptic density changes in the PFC. Thus, in human infants the PFC remains in an immature state with respect to synaptogenesis in comparison to chimpanzees and macaques. Furthermore, the period of synaptogenesis in the human PFC is stretched out over the first 3.5–10 years of childhood — a developmental window when most fundamental human cognitive abilities are formed[88] — whereas in chimpanzees and macaques it is limited to a few postnatal months. Remarkably, the amplitude of this heterochronic shift is far greater than the overall developmental delay observed in humans compared to other primates[85].

In all three species the initial increase in the expression of synaptic genes is followed by a decline in their mRNA abundance, reflecting synaptic elimination. This decline not only starts later but is also slower in humans, resulting in a higher expression of synaptic genes throughout the human lifespan (FIG. 2c). This finding corroborates previous observations in the adult human cortex[60,90] and may be linked to the higher neuropile density reported in the human frontopolar cortex (as well as other cortical areas) compared to adult chimpanzees[29,31]. It is appealing to speculate that the greater number of cortical neurons[22] combined with increased neuropile density (an indicator of better neuronal connectivity) might provide humans with improved cognitive abilities. However, the functional consequences of this and other observations would need to be tested experimentally in animal models.

How well do human-specific developmental changes in one region of the PFC correspond to changes in other cortical areas? A recent investigation of spatiotemporal expression profiles in 11 human cortical regions throughout fetal and postnatal development showed substantial similarities in the developmental mRNA abundance profiles among all cortical regions, except the V1 (REF. 91). Within the cerebral cortex, however, different cortical layers might have different developmental trajectories (BOX 2). Furthermore, imaging studies have reported substantial differences in the timing of maturation among cortical regions with respect to white and grey matter volume changes, with the PFC being among the later-maturing areas[92,93]. Thus, whether or not the human-specific shift in the timing of synaptogenesis occurs in the majority of cortical areas remains to be investigated.

Human-specific changes in the timing of cortical synaptic development and maturation are further supported by findings that axonal myelination rates differ between the human and the chimpanzee cortex during postnatal development and maturation. Axonal myelination is a sign of mature neuronal connectivity. It allows more efficient signal transduction (reviewed in REF. 92), potentially at the cost of reduced network plasticity. A recent survey of myelination changes spanning the full period of postnatal development from birth to adulthood, in histological preparations of four cortical regions from humans and chimpanzees, demonstrated that in the chimpanzee cortex myelination peaks at approximately the age of sexual maturity[94]. Thus, chimpanzee cortical myelination trajectories match those previously observed in macaques[95]. By contrast, all four regions of the human cortex myelination followed a different trajectory, characterized by a delayed period of axonal maturation extending well into the third decade of life. Similarly, an MRI study comparing white matter volume in humans, chimpanzees and macaques during the first decade of life showed a more immature white matter state in human infants compared to chimpanzee and macaque infants[96].

Box 3 | **Neoteny hypothesis of human evolution, revisited**

The record of fossil remains and cultural artefacts described in BOX 1 suggests that the increase in brain size on the human evolutionary lineage substantially pre-dated the recent cultural and technological explosion that is commonly associated with modern human cognitive abilities. If so, what was the basis of this novel behaviour? One plausible explanation could be the extension of the childhood period and/or remodelling of synaptic development in modern humans. Compared to chimpanzees, modern human infants mature 1.5–2 times more slowly and accordingly reach sexual maturity at a later chronological age[25,183]. However, the delay in synaptic maturation observed in the prefrontal cortex — from a few months in chimpanzees and macaques to more than 5 years in humans — is much more dramatic compared to this overall delay in maturation[85]. This developmental change suggests that humans retain higher numbers of cortical synapses[88] and thus may have greater neural plasticity for a longer time than other primates[184]. Consistently, adult humans show higher mRNA abundance levels of synaptic genes compared to chimpanzees and macaques. The shifted timing of peak synaptic density in the human frontal cortex can therefore be considered a form of neoteny, providing support for the long-standing hypothesis of human neotenic evolution[83] at the level of the molecular phenotype.

When did the extension of cortical synaptogenesis and, more generally, the extension of the childhood period occur? In this regard, the fossil record provides equivocal evidence. On the one hand, cranial growth patterns in Neanderthals might have been more similar to that of modern humans: both species display a rapid postnatal growth not seen in *Homo erectus* or in chimpanzees[24,25]. On the other hand, the rate of dental maturation in Neanderthals seems to have been rapid, occurring at a similar rate to that of *Homo erectus* and of chimpanzees, and unlike the delayed maturation observed in fossils of modern humans[185–187] (but see REF. 188). Thus, certain aspects of body and cranial growth rates were shared between modern human ancestors and Neanderthals, whereas finer changes in developmental patterns may have occurred after the two species split[189].

There is accumulating evidence that human brain development was fundamentally reshaped through several genetic events within the short time space between the human–Neanderthal split and the emergence of modern humans. First, a reanalysis of the positive selection signature associated with genetic changes in the transcription factor forkhead box P2 (*FOXP2*) has been suggested to involve changes in regulatory elements around this gene, rather than amino acid substitutions[190]. Notably, unlike the amino acid substitutions that pre-date the human–Neanderthal split, these putative regulatory changes are thought to have occurred within the last 250,000 years of human evolution. Second, a recent genome analysis noted that among 23 genes harbouring amino acid changes at conserved positions that are human-specific and not shared with Denisovans, eight have roles in neural and synaptic development, including contactin-associated protein-like 2 (*CNTNAP2*), which has been associated with autism[159]. Finally, a comparison between the modern human genome and the genome of Neanderthals revealed that the 50–100 kb upstream region of myocyte enhancer factor 2A (*MEF2A*) — one of the potential transcriptional regulators of extended synaptic development in the human cerebral cortex — is located in a region that shows an indication of recent positive selection in humans[85]. Specifically, this region contains three times as many modern human-derived single nucleotide polymorphisms compared to the genome average. Although this is not unequivocal evidence of positive selection, such a polymorphism pattern implies that a genetic variant located within this region — for example, a single nucleotide substitution leading to a regulatory change — rapidly increased in frequency on the human evolutionary lineage after the human–Neanderthal separation[39,85]. This suggests that changes in *MEF2A* expression, which potentially resulted in a delayed peak expression and an increase in the overall mRNA abundance of synaptic genes that is characteristic of the prefrontal cortex of modern humans, took place within the past half a million years of human evolution and had adaptive significance.

Metabolome studies

The investigation of human-specific changes in brain metabolism is a recent offshoot of research that focuses on intermediate molecular phenotypes. Brain metabolites encompass all small molecules present in the brain with a molecular weight below 1,500 Da and therefore represent all compounds that are involved in brain functionality: that is, the building blocks of proteins and polysaccharides, membrane lipids, neurotransmitters, energy substrates, as well as intermediate products of catabolic and anabolic reactions.

Metabolic pathways that are important for brain functionality — such as energy metabolism, neurotransmitter synthesis and degradation — as well as general protein and lipid synthesis pathways are highly conserved across diverse taxa[97]. Nevertheless, several observations have suggested that brain metabolism may have undergone substantial changes in primates and, specifically, on the human evolutionary lineage. First, as a result of the increase in brain size, humans allocate more of their total body energy to the brain: up to 20%, compared to 11–13% for non-human primates and 0.5–8% for other vertebrates[98]. Notably, energy consumption per unit volume is similar between humans and macaques[99], which is consistent with the constant brain metabolic rates across mammals[100]. It has been suggested, however, that the metabolic rate in human brains is higher than expected given its size[9], and such a rise in human brain energy consumption may be linked with the higher expression of genes involved in energy metabolism[59,60] and with possible recent positive selection of metabolic genes[101]. Finally, an additional version of glutamate dehydrogenase, *GLUD2*, which encodes an enzyme important for the recycling of glutamate (the major brain energy metabolite and excitatory neurotransmitter), emerged on the hominoid lineage, supporting the idea that brain metabolism has been evolving rapidly in humans and apes[102–104].

The first study to address this possibility examined concentrations of 16 metabolites in the PFC of ten adult patients with schizophrenia, 12 healthy adult humans, five adult chimpanzees and six adult rhesus macaques using proton-NMR (^{1}H-NMR) analysis[105]. In this study, 7 out of 16 metabolites showed statistically significant concentration differences among species. Somewhat contrary to the notion of higher metabolic rate of the human brain, concentrations of lactate (one of the non-glucose energy metabolites used by neurons[106]) were lower in the human brain compared to chimpanzees and macaques, and glutamate did not show concentration differences between humans and either chimpanzees or rhesus macaques.

The only other study of metabolic evolution of the human brain examined concentrations of 61 annotated metabolites by means of gas chromatography coupled with mass spectrometry in the PFC and cerebellum of 49 humans, 11 chimpanzees and 45 rhesus macaques[107]. This study included subjects with ages spanning postnatal brain development, maturation and ageing, and included a controlled assessment of post-mortem changes in metabolite concentrations. The results confirmed the clear separation of human, chimpanzee and macaque metabolic profiles in both brain regions and, additionally, demonstrated profound changes in metabolite concentrations over the lifespan in all three species. Most notably, the PFC showed substantially greater numbers of human-specific metabolic changes than the cerebellum: 11 (18%) versus three (5%) annotated

metabolites, respectively. Also, in agreement with the previous study[105], glutamate concentrations were similar among adults of the three species, but were significantly lower in the PFC of human newborns compared to chimpanzees and macaques.

Despite limitations in the scope of the metabolites and brain areas examined, these studies demonstrate that a large fraction of the brain metabolome has diverged among closely related primates. To what extent this metabolome divergence was affected by environmental factors such as diet remain to be investigated. Several potential associations between metabolic and functional features of the human and non-human brains could nonetheless be made on the basis of the current data. For example, similar concentrations of glutamate in the brains of adult humans, chimpanzees and macaques may reflect the similarities in metabolic rates per unit of volume reported across mammals[100]. Likewise, lower concentrations of glutamate in the human newborns may reflect the slower pace of excitatory synapse maturation in the human PFC[85], as well as the lag in glucose metabolism (which is linked with glutamate metabolism[108]) in the human PFC compared to the occipital, temporal and parietal cortices during the first 8 months after birth[109].

Mechanisms of human-specific changes

The studies summarized above have shown that the expression of thousands of genes, as well as the abundance of many metabolites, differs between the human brain and the brains of closely related primate species. The functional effects of these human-specific differences could potentially be tested in various experimental systems, including cell lines or laboratory animals[55,110,111]. Furthermore, recent advances in the generation of induced pluripotent stem (iPS) cells, which can be differentiated into various neural cell types, may represent a particularly promising experimental platform for the identification of key regulators and key genetic changes underlying the evolution of human-specific phenotypes[112]. First, however, these key regulatory and genetic changes driving human-specific transcriptome and metabolome divergence need to be identified among the thousands of genetic and gene expression differences that separate humans from other primate species.

Regulatory mutations can be identified using both bottom-up approaches (from the genome to the phenotype) and top-down approaches (from the phenotype to the genome). Examples of bottom-up approaches are genome-wide scans searching for regulatory regions, such as promoters and enhancers, which contain unusually high numbers of human-specific mutations. Studies have shown that such human-accelerated enhancer or promoter regions tend to occur around genes involved in neural development that are expressed in the brain[41,44]. Thus, the regulation of gene expression might have been altered more in the brain than in other tissues during human evolution[113]. However, other observations show that non-coding loci affecting neural development genes are generally evolving rapidly among mammals[114].

Top-down approaches search for common regulators of expression patterns specific to the human brain. These studies are based on the postulate that genes showing correlated expression change profiles across various biological states or conditions are co-regulated by shared trans-acting regulators (such as transcription factors and/or microRNAs (miRNAs)) and may be involved in the same biological process (for example, see REF. 115). The first study applying this approach to analyse human-specific expression in the brain looked at correlations of mRNA expression levels across human and chimpanzee brain regions with the aim of assessing the functional significance of expression differences between species[66]. The authors reported general evolutionary conservation of co-expression gene modules, and further suggested that changes in the co-expression network topology between species could be an indication of functional transcriptomic differences.

A recent analysis of mRNA and miRNA expression divergence during postnatal brain development has indicated that expression changes that affect mean transcript abundance levels may have different regulatory origins from expression changes that alter the shape of transcript abundance trajectories during development[84] (FIG. 2a). Expression changes that affect the mean mRNA abundance but preserve the shape of developmental trajectories have been associated with mutations in *cis*-elements, as estimated on the basis of correlations between *cis*-regulatory sequence divergence and expression divergence. Notably, despite their multiplicity, protein-coding genes displaying this type of expression divergence do not group into any functional categories, but represent a random selection of genes expressed across many human tissues. Furthermore, this type of expression divergence is equally widespread on both the human and the chimpanzee evolutionary lineages (FIG. 2b).

By contrast, expression changes that alter the shape of mRNA and miRNA abundance trajectories during postnatal brain development show distinct evolutionary and functional features. First, for both mRNA and miRNA, such expression differences are more common on the human than on the chimpanzee evolutionary lineage[84] (FIG. 2b). Second, protein-coding genes displaying these differences have highly conserved amino acid and regulatory sequences. Third, these expression differences are tissue- and even brain-region-specific and show clear associations with specific biological pathways that are directly relevant to neural functions (TABLE 1). Last, these changes could be associated with expression changes of the corresponding *trans*-regulators, such as transcription factors and miRNAs (FIG. 2c), rather than to changes in the sequence of their own *cis*-regulatory elements[84].

Thus, in contrast to the common hypothesis postulating a major role for *trans*-regulation in shaping the evolution of the human brain transcriptome[61], most gene expression differences that separate humans from non-human primates probably reflect evolutionarily neutral mutations in *cis*-regulatory elements that have spread through the population by genetic drift (FIG. 3).

Neoteny
A type of evolutionary change in timing — that is, heterochrony — brought about by a retardation of somatic development, resulting in adult features characteristic of the juvenile state of ancestors.

Hominoid
Member of the superfamily *Hominoidea*, which contains two families of extant species — the family *Hominidae* (the great apes, including humans, orang-utans, gorillas, chimpanzees and bonobos) and the family *Hylobatidae* (lesser apes, including gibbons).

Transcription factors
Proteins activating or repressing the expression of genes by binding to particular DNA sequence motifs proximal to a gene's transcription start site.

MicroRNAs
(miRNAs). miRNAs are commonly single-stranded RNA molecules of 20–23 nucleotides in length, generated endogenously from a single-stranded hairpin precursor. They act as post-transcriptional inhibitors in association with the RNA-induced silencing complex (RISC).

Cis-elements
DNA sequences, such as a transcription binding site, directly affecting the expression of a gene within the same chromosomal region.

Trans-regulator
An RNA or protein that regulates the expression of another, so-called target gene. Unlike a *cis*-element, it does not necessarily reside in the same chromosomal region as the target gene.

Neutral mutation
A mutation that has no effect on evolutionary fitness: that is, it is neither positively nor negatively selected, so its frequency changes only as a result of genetic drift.

Genetic drift
Random sampling effects, such as random variance in the number of offspring among individuals, that can increase or decrease a mutation's frequency in a population across generations. Such events can cause the loss or, more rarely, fixation of a neutral or nearly neutral mutation.

Zinc finger proteins
A family of proteins containing a zinc finger motif, where zinc ions take part in stabilizing the structure, and which usually function in DNA or RNA binding. The largest family of mammalian transcription factors consists of zinc finger proteins.

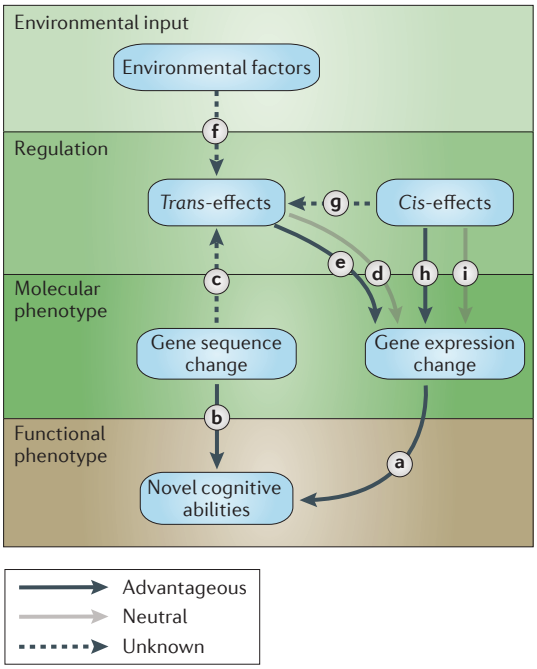


Figure 3 | **Schematic representation of potential contributors to the emergence of novel cognitive phenotypes.** It has been suggested that evolutionary changes in the human cognitive phenotype are more often based on changes in gene expression (**a**) than on changes in protein sequence (**b**)[61]. Generally, such transcriptome changes may arise by two major mechanisms. The first mechanism is based on changes in the sequence (**c**) or expression (**d**,**e**) of *trans*-regulators. Examples of such regulators include transcription factors, regulatory non-coding RNA and histone-modifying enzymes. This mechanism commonly leads to coordinated changes in the expression of multiple genes that are targeted by the same *trans*-regulator. Although some of these regulatory changes may not lead to changes in the organism's phenotype (evolutionarily neutral changes, grey arrows), coordinated changes in the expression of many genes commonly involved in similar functions are often either deleterious (and are therefore quickly removed by natural selection) or result in phenotypic changes that increase the organism's fitness (advantageous changes, black arrows). Notably, environmental differences between species, such as differences in diet or social learning, might also manifest themselves through this mechanism (**f**). The second mechanism is based on changes in the sequence or epigenetic state at the binding sites for *trans*-regulators. These changes usually occur in genomic regions that lie proximal to the affected gene and therefore are classified as *cis*-regulatory changes. Most genetic changes that affect the expression *trans*-regulators are likely to occur in their own *cis*-regulatory elements (**g**). Therefore, the distinction we are making here is not between *cis*- and *trans*-regulatory modes in general, but between *cis*- and *trans*-effects that are directly responsible for differences in mRNA abundance between species. Although some of the expression differences directly caused by *cis*-regulatory changes may result in advantageous changes to the organism's phenotype (**h**), most of them are probably evolutionarily neutral (**i**)[84].

Nevertheless, a small number of mutations that affect the expression of *trans*-regulators might have led to coordinated changes in the expression of hundreds of genes, thus reshaping certain aspects of human brain development. Although these developmental changes constitute a minority of expression differences between humans and non-human primates found in the brain, they are likely to play major parts in the evolution of human brain functions. This point is important because the total number of genetic changes that have led to cognitive adaptations in recent human evolution might be limited to a small number of events (BOX 4). Below, we explore the potential roles of several types of *trans*-regulators in human brain evolution.

Transcription factors. Among *trans*-regulatory changes with a possible contribution to human cognitive evolution, the bulk of evidence involves transcription factors. This evidence is partly based on genomic sequence comparisons between humans and other species and on searches for unusual patterns of sequence change, further combined with functional information from human disease studies. One important example is the discovery of two human-specific amino acid substitutions that change the regulatory specificity of the transcription factor *FOXP2* (REFS 110,111). In humans, loss of one copy of *FOXP2* leads to language impairment and abnormal cognitive activity[116]. In mice, expression of the human version of the *FOXP2* gene resulted in greater dendrite arborization and synaptic plasticity in the striatum[110]. The amino acid differences between the human and the chimpanzee versions of *FOXP2* were also associated with a different regulatory potential of this transcription factor in a human cell line, which could partly explain the differences in mRNA abundance between adult human and chimpanzee brains[111].

Functional analyses of genome scans have revealed accelerated sequence evolution of transcription factors, especially zinc finger proteins, along the primate and human lineages[42,117]. Transcription factors containing the zinc finger domain were also found to be over-represented among genes that have newly arisen on the human lineage through duplications[118]. Interestingly, an analysis of transcription factor expression differences between humans and chimpanzees in the PFC, liver, kidney, heart and testis revealed strong expression divergence of the Krüppel-associated box (KRAB) zinc finger proteins in the PFC, but not in other tissues[119].

Another line of evidence for the role of transcription factors in human cognitive evolution comes from studies showing that brain transcriptome divergence among species was associated with differences in the expression of specific transcription factors. For example, a recent study analysed frontal cortex, caudate nucleus and hippocampal expression profiles in humans, chimpanzees and macaques, and reported regulatory roles for both *FOXP2* and the circadian regulator (*CLOCK*) in driving human-specific gene expression in the PFC[49]. This is interesting, as deregulation of *CLOCK* is implicated in neuropsychiatric disorders such as mania-like behaviours (reviewed in REF. 120).

Box 4 | **Estimating the number of genetic events leading to modern human cognition**

One of the crucial questions in human evolution is how many genetic events were responsible for the evolution of human cognition[8]. Although millions of genetic differences separate humans from other species, most of these differences probably did not contribute to phenotypic evolution[37,191,192]. By contrast, mutations underpinning the appearance of novel cognitive skills on the human evolutionary lineage could have given the individuals carrying such mutations a selective advantage over conspecifics. Such advantageous mutations, after reaching a critical frequency, can sweep through a population and fix[193].

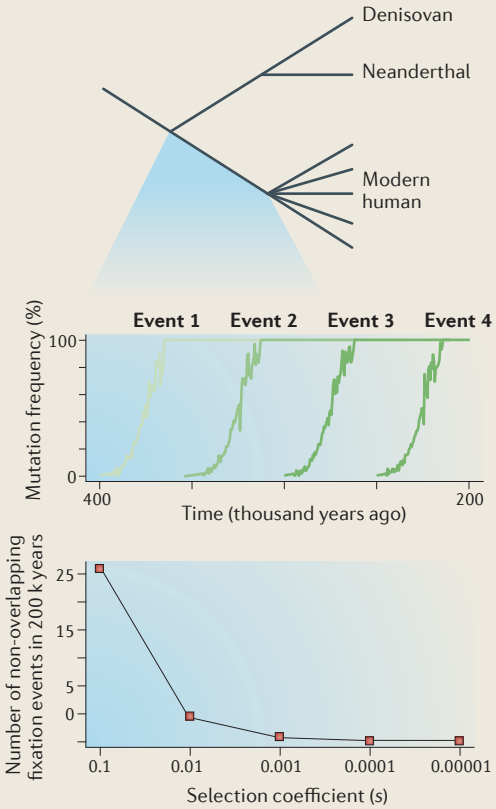
How many of these positive selection events related to brain functionality could have taken place in the time during which modern human cognition is assumed to have emerged, that is, from the split between the Neanderthal and the human evolutionary lineages (~400 thousand years ago (kya)[39]) to the start of cultural and technological explosion that accompanied the emergence of modern humans in Africa, ~200 kya[161] (BOX 1)? There are several possibilities. First, new advantageous mutations require time to occur. Second, such mutations may be lost by chance shortly after their appearance owing to the small numbers of individuals that carried them. Third, the adaptive effect of mutations should be large enough to allow them to reach fixation — that is, be present in all individuals of the species — within the time frame following the human–Neanderthal lineage divergence.

These three possibilities themselves depend on a number of parameters: the number of possible beneficial mutations in the genome affecting brain development and functionality; the mutation rate, μ, which is around ~10^{-8} per base pair per generation in humans[194]; the generation time, which is usually assumed to be 20 years for humans[195]; the selection coefficient of a new beneficial mutation, *s*, which describes its increase in frequency in each generation; and the effective population size, N_e, which refers to the number of reproducing individuals that are necessary to produce the observed levels of genetic diversity[196].

The two most important factors are *s* and N_e. In large populations, the probability of fixation of a new beneficial allele is ~2*s* (see REF. 197). Thus, a higher *s* increases the chance that a mutation eventually fixes. A higher *s* also results in a shorter time required for a mutation to sweep though the population[198]. How high would *s* for cognition-related mutations be? There are several examples in which selection coefficients of beneficial alleles have been calculated in humans. For example, the genetic mutations that affect transcriptional regulation of the lactase gene, which allows adults to digest milk, have an estimated *s* of ~0.10 (REFS 199,200). Notably, this selection event has left one of the strongest signatures of positive selection in the modern human genome[201]. Dozens of similar positive selection signatures have been detected, mainly associated with local adaptation to new diets and immune pressures within the past 50,000 years[202,203]. However, these are rare cases of extremely strong selection. Most positive selection events that are detectable in the human genome have lower selection coefficients, such as $s = 0.00025$ (REF. 192).

The second important factor is N_e. Human N_e is estimated to be around 10,000, which reflects the limited genetic diversity within humans compared to most other mammals[37,204]. With a small N_e, the chance of a new mutation arising is smaller. Moreover, slightly beneficial mutations may be more easily lost owing to genetic drift[205].

The average waiting time for a new mutation to arise at a single locus is $1/(2N_e\mu)$ generations. Assuming that the total number of genomic sites that, when mutated, could improve the cognitive abilities of our ancestors towards the modern human level is ~1,000, and that one out of the three possible substitutions at these sites is beneficial, the waiting time for any such mutation to appear would be ~300 years. Thus, within 200,000 years, ~700 beneficial mutations could arise. However, depending on the selection coefficient *s*, only a fraction of these would reach fixation in the population. For example, even if we assume a relatively strong selection coefficient for these mutations, $s = 0.01$, only 14 of the 700 beneficial mutations would not be lost (based on REF. 197). It would further mean that each event, from a new mutation arising until fixation, would take 50,000 years (based on REF. 198). Thus, if selection events related to brain functionality did not overlap (see the figure), a maximum of four mutations could fix in human ancestors after the human–Neanderthal split. If the selection coefficients were closer to the average predicted $s = 0.00025$ (REF. 192) in humans, the number of mutations that could fix would be even lower. Although these numbers seem to be extremely small, they match analyses of Neanderthal and Denisovan genomes, which found only 88 and 260 amino acid substitutions, respectively, that fixed on the human lineage after the species split, and the majority of these substitutions are presumed to be evolutionarily neutral[159,206]. Although this analysis involves a large number of assumptions, it suggests that the number of genetic changes underlying changes in cognitive abilities that took place after separation of the human and Neanderthal lineages should be on the order of ten, or less.



This approach has also been used to analyse the regulation of expression divergence during brain development. Liu *et al.* analysed the regulation of a group of 184 co-expressed genes involved in synaptogenesis and signal transduction that showed a human-specific delay in peak expression in the PFC[85]. In agreement with the notion of shared regulation of co-expressed genes involved in the same functional process, the promoter regions of genes with this human-specific expression profile shared binding sites for the transcription factors myocyte enhancer factor 2A (MEF2A), early growth response 1 (EGR1), EGR2 and EGR3. Notably, all four have been shown to be involved in the regulation of neuronal functions, including neuronal survival, synaptogenesis and long-term potentiation[121–124]. As shown using sensory stimulation in rats, *MEF2A* itself is activated by neuronal activity through dephosphorylation, and in turn is predicted to activate transcription of the three *EGR* genes, as well as genes encoding other major synaptic proteins[125]. These studies suggest that *MEF2A* may have a master role in the regulation of the human-specific delay in the expression of synaptic genes in the PFC. Intriguingly, there are indications that human-specific changes in *MEF2A* expression might have taken place after the human–Neanderthal split (BOX 3).

MicroRNAs. Along with transcription factors, small regulatory RNAs, such as miRNAs, have roles in neuronal development (reviewed in REFS 126,127). Although an initial study of miRNAs expressed in human and chimpanzee brains reported substantial differences in the miRNA repertoire between the two species[51], subsequent studies indicated that most miRNA genes are highly conserved among primates in terms of both sequence and expression[128]. miRNA expression changes during postnatal cortical development were also generally conserved among humans, macaques and mice[129]. Likewise, among more than 300 miRNAs expressed in the human, chimpanzee and macaque PFC, only ~30 showed expression differences between humans and chimpanzees, with the differences being approximately equally distributed between the human and chimpanzee evolutionary lineages[130]. Using the binding site enrichment and regulator-target expression correlation approach described above, these miRNA expression differences were estimated to explain 2–4% of mRNA expression divergence and 4–6% of protein expression divergence between the human and chimpanzee PFC. One of these miRNAs, miR-184 — which is abundantly present in the PFC and cerebellum of humans but not chimpanzees and macaques — had previously been shown to be an important driver of neural stem cell proliferation[131]. Another study used the same approach to search for miRNA regulators of genes showing species-specific changes in developmental trajectories of mRNA abundance in the PFC[84]. According to this analysis, about 10% of such species-specific developmental changes (140 genes) in gene expression may be explained by inversely correlated changes in the expression of their regulatory miRNA (17 miRNAs from 11 miRNA families). Notably, some of these miRNAs (for example, miR-92a, miR-320b and miR-454) were not previously known to have a role in brain development regulation. Similarly, an evolutionarily novel miRNA (miR-941) that is not present in the chimpanzee or other non-human primate genomes but is present in multiple copies (2 to 11 copies) in the modern human and Denisovan genomes was recently implicated in the regulation of genes linked to neuronal functions[55].

Chromatin structure. Only a modest proportion of mRNA expression divergence between species can be explained by miRNA or transcription factor expression divergence. This could be due, in part, to a poor knowledge of the actual transcription factor and miRNA targets in the brain, and to a lack of understanding of *trans*-regulatory roles in the context of an entire regulatory network. Another reason could be the potential prevalence of expression changes caused by mutations in *cis*-regulatory elements, rather than by the action of *trans*-regulators. *Cis*-regulatory changes can affect transcription directly (by altering transcription factor binding) or indirectly (by altering DNA methylation or histone modification patterns). Both types of modifications can strongly affect transcription by opening or condensing chromatin, are necessary for healthy brain development and are known to be dysregulated in cognitive disorders[132,133].

The first genome-wide scan of DNA methylation was recently conducted using bisulphite sequencing in three human and three chimpanzee PFC samples[134]. Despite high intra-species variation, CpG methylation levels were found to be higher in chimpanzees relative to humans. Higher methylation at promoter CpG sites was inversely correlated with gene expression levels, and the affected genes were associated with neural functions and neural disorders. Together with the reported crucial role of promoter DNA methylation in brain development[133], these results imply that promoter methylation differences may contribute to human-specific expression divergence.

Another recent genome-wide survey measured levels of a histone modification associated with active promoters — histone H3 lysine 4 trimethylation (H3K4me3) — in neuron nuclei isolated from PFC samples from infant and adult humans, as well as from adult chimpanzees and macaques[135]. The authors identified 410 loci with human-specific gain and 61 loci with human-specific depletion of the H3K4me3 mark. One of the most intriguing findings was human-specific gain of two H3K4me3 marks around the dipeptidyl-peptidase 10 (*DPP10*) gene, which has been associated with autism and schizophrenia[136]. The study further described a complex regulatory mechanism of *DPP10* expression regulation in the human PFC that is potentially mediated by the expression of a short non-coding RNA from the reverse strand of the gene.

Additional levels of regulation. Other regulatory mechanisms that may have contributed to the emergence of human-specific expression changes include RNA splicing, RNA editing, post-transcriptional regulation mediated by RNA-binding proteins and regulation mediated

RNA editing
A molecular process in which the information content in an RNA molecule is altered through a chemical change in the base make-up at the post-transcriptional level.

RNA-binding proteins
Proteins that bind to RNAs through an RNA-binding motif. The binding may regulate the translation of RNA or induce post-transcriptional changes, such as RNA splicing and editing.

PIWI-interacting RNA
(piRNA). A class of small non-coding RNA molecules of 26–31 nucleotides in length with a bias for a 5′ uridine, which is abundant in the germ line and has been implicated in the maintenance of genomic integrity by both epigenetic and post-transcriptional silencing of transposable elements and other genetic elements.

Large intergenic non-coding RNA
(lincRNA). Non-protein coding transcripts of more than 200 nucleotides in length, which are characterized by the complexity and diversity of their sequences. lincRNAs have emerged as key molecules involved in the control of transcriptional and post-transcriptional gene regulatory pathways.

by various types of ncRNA, such as PIWI-interacting RNA (piRNA) and large intergenic non-coding RNA (lincRNA). To date, a limited number of studies have investigated divergence between human and non-human primate brains at these levels of molecular phenotype. Two comparative studies of mRNA splicing in the brain have identified, using microarray technology, dozens to hundreds of differently spliced genes, including genes that encode transcriptional and post-transcriptional regulators[137,138]. Many human-specific splicing differences may, however, still remain unidentified by these studies owing to the limitations of microarray technology. A survey of RNA editing of *Alu* sequences located within six genes in the human, chimpanzee and macaque cerebellum found higher levels of A-to-I *Alu* editing in the human brain[139]. Nonetheless, a genome-wide survey of RNA editing features characteristic to the human brain is still lacking, despite the fact that, among several tissues, RNA editing is most widespread in the frontal cortex[140] and levels of editing are regulated during brain development[141].

With respect to ncRNA, it is notable that the first genome-wide scan for genetic loci that are conserved in primates and mammals but show many human-specific mutations identified a brain-expressed ncRNA, named human accelerated region 1F (*HAR1F*), as a primary candidate. Expression of this ncRNA was localized to Cajal–Retzius neurons in the fetal human brain, and its human-specific mutations were predicted to substantially change its secondary structure. The authors thus suggested that human *HAR1F* ncRNA might contribute to changes in the cortical layer organization[142].

In addition, genome-wide transcriptome profiling conducted in the brains of humans and non-human primates using tiling arrays or RNA sequencing have identified large numbers of novel ncRNA transcripts, many of which show human-specific expression[53,54,143]. Although these studies imply important roles for regulatory ncRNA in shaping human-specific gene expression and functional differences between species, additional studies are needed to form a more complete picture of the regulatory mechanisms that underlie gene expression patterns that are specific to the human brain.

The road ahead

A decade of molecular studies of human brain evolution has yielded a fair selection of discoveries, and answers to the genetic basis of human cognition are gradually emerging. RNA and metabolite profiling studies conducted in post-mortem brains of humans and non-human primates have illustrated how this approach can illuminate specific processes that may underlie human-specific cognitive abilities, such as extended cortical synaptogenesis that could boost learning abilities in human infants. Follow-up studies have further predicted the regulatory effects directing these processes. These results now await additional investigations aimed at confirming the identified *trans*-regulators and determining the genetic mutations driving these regulatory changes. The functional effects of these human-specific regulatory changes must also be tested in animal models.

With respect to the trajectory of human brain evolution, the existing data suggest two distinct phases: a long and gradual increase in brain size that was accompanied by cortical reorganization, followed by a more recent phase of region-specific developmental remodelling. This second phase, which led to the emergence of the cognitive traits that produced the human cultural explosion ~200,000 years ago, may have been driven by only a few mutations that affected the expression and/or primary structure of developmental regulators.

However, the emergence of cognitive skills that allowed humans to create, accumulate and transmit cultural knowledge between generations might have had a cost. A rapid phase of cognitive evolution may not have provided sufficient time to fine-tune these processes and to render them robust against environmental and natural genetic perturbations, and this may have resulted in the high frequency of human psychological disorders associated with abnormal brain development[144]. Accordingly, a number of recent studies have reported that many more neuropsychiatric disorder-associated genes than would be expected by chance show differential expression between humans and non-human primates[49,136]. The study of the molecular mechanisms of human cognitive evolution may therefore contribute to novel treatment and prevention strategies for common cognitive dysfunctions.

Although the findings obtained to date are encouraging, it is certain that at present we are only at the very beginning of a long journey towards understanding the evolution of human cognition. More studies conducting comparative analyses of human and non-human primate brains in different regions, cortical layers and cell types, surveying various stages of brain ontogenesis, and measuring all possible levels of the molecular phenotype are needed to build a complete roadmap of the molecular events leading to the emergence of the human brain.

1. Tomasello, M. *The Cultural Origins of Human Cognition* (Harvard Univ. Press, 1999).
2. Richerson, P. J. & Boyd, R. *Not By Genes Alone: How Culture Transformed Human Evolution* (Univ. of Chicago Press, 2004).
3. Boyd, R. & Richerson, P. J. *The Origin and Evolution of Cultures* (Oxford Univ. Press, 2005).
4. Hill, K., Barton, M. & Hurtado, A. M. The emergence of human uniqueness: characters underlying behavioral modernity. *Evol. Anthropol.* **18**, 187–200 (2009).
5. Whiten, A. & Erdal, D. The human socio-cognitive niche and its evolutionary origins. *Phil. Trans. R. Soc. B* **367**, 2119–2129 (2012).
6. Tennie, C., Call, J. & Tomasello, M. Ratcheting up the ratchet: on the evolution of cumulative culture. *Phil. Trans. R. Soc. B.* **364**, 2405–2415 (2009).
7. Roth, G. & Dicke, U. Evolution of the brain and intelligence in primates. *Prog. Brain Res.* **195**, 413–430 (2012).
8. Preuss, T. M. The human brain: rewired and running hot. *Ann. NY Acad. Sci.* **1225** (Suppl. 1), E182–E191 (2011).
A comprehensive review of the current issues of debate in human brain evolution, including questions on the uniqueness of frontal cortex expansion in humans, human brain lateralization and connectivity.
9. Preuss, T. M. Human brain evolution: from gene discovery to phenotype discovery. *Proc. Natl Acad. Sci. USA* **109** (Suppl. 1), 10709–10716 (2012).
10. Whiten, A. The second inheritance system of chimpanzees and humans. *Nature* **437**, 52–55 (2005).
11. Pollick, A. & de Waal, F. Ape gestures and language evolution. *Proc. Natl Acad. Sci. USA* **104**, 8184–8189 (2007).
12. Pruetz, J. & Bertolani, P. Savanna chimpanzees, *Pan troglodytes verus*, hunt with tools. *Curr. Biol.* **17**, 412–417 (2007).
13. Call, J. & Tomasello, M. Does the chimpanzee have a theory of mind? 30 years later. *Trends Cogn. Sci.* **12**, 187–192 (2008).

14. Hare, B. & Kwetuenda, S. Bonobos voluntarily share their own food with others. *Curr. Biol.* **20**, R230–R231 (2010).
15. Horner, V., Carter, J. D., Suchak, M. & de Waal, F. B. M. Spontaneous prosocial choice by chimpanzees. *Proc. Natl Acad. Sci. USA* **108**, 13847–13851 (2011).
16. Whiten, A., McGuigan, N., Marshall-Pescini, S. & Hopper, L. M. Emulation, imitation, over-imitation and the scope of culture for child and chimpanzee. *Phil. Trans. R. Soc. B* **364**, 2417–2428 (2009).
17. Luncz, L. V., Mundry, R. & Boesch, C. Evidence for cultural differences between neighboring chimpanzee communities. *Curr. Biol.* **22**, 922–926 (2012).
18. Boesch, C. What makes us human (*Homo sapiens*)? The challenge of cognitive cross-species comparison. *J. Comp. Psychol.* **121**, 227–240 (2007).
19. Roth, G. & Dicke, U. Evolution of the brain and intelligence. *Trends Cogn. Sci.* **9**, 250–257 (2005).
20. Marino, L. A comparison of encephalization between odontocete cetaceans and anthropoid primates. *Brain Behav. Evol.* **51**, 230–238 (1998).
21. Lent, R., Azevedo, F. A., Andrade-Moraes, C. H. & Pinto, A. V. How many neurons do you have? Some dogmas of quantitative neuroscience under revision. *Eur. J. Neurosci.* **35**, 1–9 (2011).
22. Herculano-Houzel, S. The remarkable, yet not extraordinary, human brain as a scaled-up primate brain and its associated cost. *Proc. Natl Acad. Sci. USA* **109** (Suppl. 1), 10661–10668 (2012).
23. Sakai, T. *et al.* Fetal brain development in chimpanzees versus humans. *Curr. Biol.* **22**, R791–R792 (2012).
24. Leigh, S. R. Brain growth, life history, and cognition in primate and human evolution. *Am. J. Primatol.* **62**, 139–164 (2004).
25. Zollikofer, C. P. E. & Ponce de León, M. S. The evolution of hominin ontogenies. *Semin. Cell Dev. Biol.* **21**, 441–452 (2010).
A review of the studies and debate on comparative developmental trajectories between humans and other primates.
26. Semendeferi, K., Lu, A., Schenker, N. & Damasio, H. Humans and great apes share a large frontal cortex. *Nature Neurosci.* **5**, 272–276 (2002).
27. Marino, L. Absolute brain size: did we throw the baby out with the bathwater? *Proc. Natl Acad. Sci. USA* **103**, 13563–13564 (2006).
28. Sherwood, C. *et al.* Evolution of increased glia–neuron ratios in the human frontal cortex. *Proc. Natl Acad. Sci. USA* **103**, 13606–13611 (2006).
29. Semendeferi, K. *et al.* Spatial organization of neurons in the frontal pole sets humans apart from great apes. *Cereb. Cortex* **21**, 1485–1497 (2011).
30. Oberheim, N. A. *et al.* Uniquely hominid features of adult human astrocytes. *J. Neurosci.* **29**, 3276–3287 (2009).
31. Spocter, M. A. *et al.* Neuropil distribution in the cerebral cortex differs between humans and chimpanzees. *J. Comp. Neurol.* **520**, 2917–2929 (2012).
32. Rilling, J. K. *et al.* The evolution of the arcuate fasciculus revealed with comparative DTI. *Nature Neurosci.* **11**, 426–428 (2008).
This study compares human and chimpanzee cortices using diffusion tensor imaging and reports a prominent human-specific change in temporal connectivity.
33. Chimpanzee Sequencing and Analysis Consortium. Initial sequence of the chimpanzee genome and comparison with the human genome. *Nature* **437**, 69–87 (2005).
34. Prüfer, K. *et al.* The bonobo genome compared with the chimpanzee and human genomes. *Nature* **486**, 527–531 (2012).
35. Langergraber, K. E. *et al.* Generation times in wild chimpanzees and gorillas suggest earlier divergence times in great ape and human evolution *Proc. Natl Acad. Sci. USA* **109**, 15716–15721 (2012).
36. Gibbs, R. A. *et al.* Genome sequence of the Brown Norway rat yields insights into mammalian evolution. *Nature* **428**, 493–521 (2004).
37. Harris, E. E. Nonadaptive processes in primate and human evolution. *Am. J. Phys. Anthropol.* **143** (Suppl. 51), 13–45 (2010).
38. Vallender, E. J., Mekel-Bobrov, N. & Lahn, B. T. Genetic basis of human brain evolution. *Trends Neurosci.* **31**, 637–644 (2008).
A comprehensive summary of brain-related genetic changes in human evolution, including protein-coding changes, duplications and regulatory changes.
39. Green, R. E. *et al.* A draft sequence of the Neandertal genome. *Science* **328**, 710–722 (2010).
40. Reich, D. *et al.* Genetic history of an archaic hominin group from Denisova Cave in Siberia. *Nature* **468**, 1053–1060 (2010).
41. Prabhakar, S., Noonan, J., Pääbo, S. & Rubin, E. Accelerated evolution of conserved noncoding sequences in humans. *Science* **314**, 786 (2006).
42. Bustamante, C. D. *et al.* Natural selection on protein-coding genes in the human genome. *Nature* **437**, 1153–1157 (2005).
43. Pollard, K. *et al.* Forces shaping the fastest evolving regions in the human genome. *PLoS Genet.* **2**, e168 (2006).
44. Haygood, R., Fedrigo, O., Hanson, B., Yokoyama, K.-D. & Wray, G. A. Promoter regions of many neural- and nutrition-related genes have experienced positive selection during human evolution. *Nature Genet.* **39**, 1140–1144 (2007).
45. McLean, C. Y. *et al.* Human-specific loss of regulatory DNA and the evolution of human-specific traits. *Nature* **471**, 216–219 (2011).
46. Sabeti, P. *et al.* Positive natural selection in the human lineage. *Science* **312**, 1614–1620 (2006).
47. Laland, K. N., Odling-Smee, J. & Myles, S. How culture shaped the human genome: bringing genetics and the human sciences together. *Nature Rev. Genet.* **11**, 137–148 (2010).
48. Sholtis, S. J. & Noonan, J. P. Gene regulation and the origins of human biological uniqueness. *Trends Genet.* **26**, 110–118 (2010).
49. Konopka, G. *et al.* Human-specific transcriptional networks in the brain. *Neuron* **75**, 601–617 (2012).
50. Marioni, J. C., Mason, C. E., Mane, S. M., Stephens, M. & Gilad, Y. RNA-seq: an assessment of technical reproducibility and comparison with gene expression arrays. *Genome Res.* **18**, 1509–1517 (2008).
51. Berezikov, E. *et al.* Diversity of microRNAs in human and chimpanzee brain. *Nature Genet.* **38**, 1375–1377 (2006).
52. Wetterbom, A., Ameur, A., Feuk, L., Gyllensten, U. & Cavelier, L. Identification of novel exons and transcribed regions by chimpanzee transcriptome sequencing. *Genome Biol.* **11**, R78 (2010).
53. Babbitt, C. *et al.* Both noncoding and protein-coding RNAs contribute to gene expression evolution in the primate brain. *Genome Biol. Evol.* **2010**, 67 (2010).
54. Xu, A. G. *et al.* Intergenic and repeat transcription in human, chimpanzee and macaque brains measured by RNA-Seq. *PLoS Comput. Biol.* **6**, e1000843 (2010).
55. Hu, H. Y. *et al.* Evolution of the human-specific microRNA miR-941. *Nature Commun.* **3**, 1145 (2012).
56. Dunham, I. *et al.* An integrated encyclopedia of DNA elements in the human genome. *Nature* **489**, 57–74 (2012).
57. Kaddurah-Daouk, R. & Krishnan, K. R. Metabolomics: a global biochemical approach to the study of central nervous system diseases. *Neuropsychopharmacology* **34**, 173–186 (2009).
58. Enard, W. *et al.* Intra- and interspecific variation in primate gene expression patterns. *Science* **296**, 340–343 (2002).
59. Uddin, M. *et al.* Sister grouping of chimpanzees and humans as revealed by genome-wide phylogenetic analysis of brain gene expression profiles. *Proc. Natl Acad. Sci. USA* **101**, 2957–2962 (2004).
60. Cáceres, M. *et al.* Elevated gene expression levels distinguish human from non-human primate brains. *Proc. Natl Acad. Sci. USA* **100**, 13030–13035 (2003).
61. King, M. & Wilson, A. Evolution at two levels in humans and chimpanzees. *Science* **188**, 107–116 (1975).
A classic paper that put forward the hypothesis that human brain evolution involved gene expression differences, probably during development, rather than changes in protein sequence.
62. Carroll, S. Genetics and the making of *Homo sapiens*. *Nature* **422**, 849–857 (2003).
63. Khaitovich, P. *et al.* A neutral model of transcriptome evolution. *PLoS Biol.* **2**, e132 (2004).
64. Khaitovich, P., Enard, W., Lachmann, M. & Pääbo, S. Evolution of primate gene expression. *Nature Rev. Genet.* **7**, 693–702 (2006).
A review of the first half decade of comparative transcriptome studies, describing how patterns of gene expression differences among primates are shaped by positive selection, negative selection and neutral drift.
65. Khaitovich, P. *et al.* Regional patterns of gene expression in human and chimpanzee brains. *Genome Res.* **14**, 1462–1473 (2004).
66. Oldham, M., Horvath, S. & Geschwind, D. Conservation and evolution of gene coexpression networks in human and chimpanzee brains. *Proc. Natl Acad. Sci. USA* **103**, 617973–617978 (2006).
This paper introduces the use of co-expressed gene groups for analysing species transcriptome data.
67. van den Bos, W., van Dijk, E., Westenberg, M., Rombouts, S. A.R. B. & Crone, E. A. Changing brains, changing perspectives: the neurocognitive development of reciprocity. *Psychol. Sci.* **22**, 60–70 (2011).
68. Knoch, D. & Fehr, E. Resisting the power of temptations: the right prefrontal cortex and self-control. *Ann. NY Acad. Sci.* **1104**, 123–134 (2007).
69. Dietrich, A. & Kanso, R. A review of EEG, ERP, and neuroimaging studies of creativity and insight. *Psychol. Bull.* **136**, 822–848 (2010).
70. Gernsbacher, M. A. & Kaschak, M. P. Neuroimaging studies of language production and comprehension. *Annu. Rev. Psychol.* **54**, 91–114 (2003).
71. Halsband, U. Bilingual and multilingual language processing. *J. Physiol. Paris* **99**, 355–369 (2006).
72. Burgess, P. W., Gilbert, S. J. & Dumontheil, I. Function and localization within rostral prefrontal cortex (area 10). *Phil. Trans. R. Soc. B* **362**, 887–899 (2007).
73. Burns, M. S. & Fahy, J. Broca's area: rethinking classical concepts from a neuroscience perspective. *Top. Stroke Rehabil.* **17**, 401–410 (2010).
74. Petrides, M. & Pandya, D. N. Comparative cytoarchitectonic analysis of the human and the macaque ventrolateral prefrontal cortex and corticocortical connection patterns in the monkey. *Eur. J. Neurosci.* **16**, 291–310 (2002).
75. Petrides, M., Tomaiuolo, F., Yeterian, E. H. & Pandya, D. N. The prefrontal cortex: comparative architectonic organization in the human and the macaque monkey brains. *Cortex* **48**, 46–57 (2012).
76. Petrides, M., Cadoret, G. & Mackey, S. Orofacial somatomotor responses in the macaque monkey homologue of Broca's area. *Nature* **435**, 1235–1238 (2005).
77. Taglialatela, J. P., Russell, J. L., Schaeffer, J. A. & Hopkins, W. D. Communicative signaling activates 'Broca's' homolog in chimpanzees. *Curr. Biol.* **18**, 343–348 (2008).
This study demonstrates that an area in the chimpanzee brain that is homologous to Broca's area (which is involved in language in humans) is activated during gesture-based signalling.
78. Hawrylycz, M. J. *et al.* An anatomically comprehensive atlas of the adult human brain transcriptome. *Nature* **489**, 391–399 (2012).
79. Johnson, M. Functional brain development in humans. *Nature Rev. Neurosci.* **2**, 475–483 (2001).
80. Reynolds, C. R. & Fletcher-Janzen, E. *Concise Encyclopedia of Special Education* (John Wiley and Sons, 2004).
81. Tomasello, M. *Origins of Human Communication* (MIT Press, 2008).
82. Somel, M. *et al.* Transcriptional neoteny in the human brain. *Proc. Natl Acad. Sci. USA* **106**, 5743–5748 (2009).
83. Gould, S. J. *Ontogeny and Phylogeny* (Harvard Univ. Press, 1977).
84. Somel, M. *et al.* MicroRNA-driven developmental remodeling in the brain distinguishes humans from other primates. *PLoS Biol.* **9**, e1001214 (2011).
85. Liu, X. *et al.* Extension of cortical synaptic development distinguishes humans from chimpanzees and macaques. *Genome Res.* **22**, 611–622 (2012).
This paper reports an extreme delay in synaptic maturation in the human PFC compared to other primates. The study further predicts the regulatory network involved in this change.
86. Huttenlocher, P. R., de Courten, C., Garey, L. J. & Van der Loos, H. Synaptogenesis in human visual cortex — evidence for synapse elimination during normal development. *Neurosci. Lett.* **33**, 247–252 (1982).
87. Glantz, L. A., Gilmore, J. H., Hamer, R. M., Lieberman, J. A. & Jarskog, L. F. Synaptophysin and postsynaptic density protein 95 in the human prefrontal cortex from mid-gestation into early adulthood. *Neuroscience* **149**, 582–591 (2007).
88. Huttenlocher, P. R. & Dabholkar, A. S. Regional differences in synaptogenesis in human cerebral cortex. *J. Comp. Neurol.* **387**, 167–178 (1997).
89. Rakic, P., Bourgeois, J. P., Eckenhoff, M. F., Zecevic, N. & Goldman-Rakic, P. S. Concurrent overproduction of

synapses in diverse regions of the primate cerebral cortex. *Science* **232**, 232–235 (1986).
90. Caceres, M., Suwyn, C., Maddox, M., Thomas, J. & Preuss, T. Increased cortical expression of two synaptogenic thrombospondins in human brain evolution. *Cereb. Cortex* **17**, 2312–2321 (2007).
91. Kang, H. J. *et al.* Spatio-temporal transcriptome of the human brain. *Nature* **478**, 483–489 (2011).
92. Toga, A., Thompson, P. & Sowell, E. Mapping brain maturation. *Trends Neurosci.* **29**, 148–159 (2006).
93. Shaw, P. *et al.* Neurodevelopmental trajectories of the human cerebral cortex. *J. Neurosci.* **28**, 3586 (2008).
94. Miller, D. J. *et al.* Prolonged myelination in human neocortical evolution. *Proc. Natl Acad. Sci. USA* **109**, 16480–16485 (2012).
This recent study reports that the prolonged myelinization process, which is known to extend into the third and fourth decades in humans, subsides by sexual maturity in chimpanzees and thus could represent another extreme case of delayed human neurodevelopment.
95. Gibson, K. *Sequence of Myelinization in the Brain of Macaca mulatta*. Thesis, Univ. of California (1970).
96. Sakai, T. *et al.* Differential prefrontal white matter development in chimpanzees and humans. *Curr. Biol.* **21**, 1397–1402 (2011).
97. Peregrín-Alvarez, J. M., Sanford, C. & Parkinson, J. The conservation and evolutionary modularity of metabolism. *Genome Biol.* **10**, R63 (2009).
98. Mink, J. W., Blumenschine, R. J. & Adams, D. B. Ratio of central nervous system to body metabolism in vertebrates: its constancy and functional basis. *Am. J. Physiol.* **241**, R203–R212 (1981).
99. Noda, A. *et al.* Age-related changes in cerebral blood flow and glucose metabolism in conscious rhesus monkeys. *Brain Res.* **936**, 76–81 (2002).
100. Karbowski, J. Global and regional brain metabolic scaling and its functional consequences. *BMC Biol.* **5**, 18 (2007).
101. Khaitovich, P. *et al.* Positive selection on gene expression in the human brain. *Curr. Biol.* **16**, R356–R358 (2006).
102. Burki, F. & Kaessmann, H. Birth and adaptive evolution of a hominoid gene that supports high neurotransmitter flux. *Nature Genet.* **36**, 1061–1063 (2004).
103. Kanavouras, K., Mastorodemos, V., Borompokas, N., Spanaki, C. & Plaitakis, A. Properties and molecular evolution of human *GLUD2* (neural and testicular tissue-specific) glutamate dehydrogenase. *J. Neurosci. Res.* **85**, 3398–3406 (2007).
104. Rosso, L., Marques, A. C., Reichert, A. S. & Kaessmann, H. Mitochondrial targeting adaptation of the hominoid-specific glutamate dehydrogenase driven by positive Darwinian selection. *PLoS Genet.* **4**, e1000150 (2008).
105. Khaitovich, P. *et al.* Metabolic changes in schizophrenia and human brain evolution. *Genome Biol.* **9**, R124 (2008).
106. Tanaka, M. *et al.* Role of lactate in the brain energy metabolism: revealed by bioradiography. *Neurosci. Res.* **48**, 13–20 (2004).
107. Fu, X. *et al.* Rapid metabolic evolution in human prefrontal cortex. *Proc. Natl Acad. Sci. USA* **108**, 6181–6186 (2011).
108. Sibson, N. R. *et al.* Stoichiometric coupling of brain glucose metabolism and glutamatergic neuronal activity. *Proc. Natl Acad. Sci. USA* **95**, 316–321 (1998).
109. Chugani, H. T. & Phelps, M. E. Maturational changes in cerebral function in infants determined by 18FDG positron emission tomography. *Science* **231**, 840–843 (1986).
110. Enard, W. *et al.* A humanized version of Foxp2 affects cortico-basal ganglia circuits in mice. *Cell* **137**, 961–971 (2009).
111. Konopka, G. *et al.* Human-specific transcriptional regulation of CNS development genes by FOXP2. *Nature* **462**, 213–217 (2009).
The authors show that the two human-specific amino acid changes in FOXP2 can cause differential expression in cell lines, which overlaps with human and chimpanzee brain expression differences.
112. Romero, I. G., Ruvinsky, I. & Gilad, Y. Comparative studies of gene expression and the evolution of gene regulation. *Nature Rev. Genet.* **13**, 505–516 (2012).
113. Haygood, R., Babbitt, C. C., Fedrigo, O. & Wray, G. A. Contrasts between adaptive coding and noncoding changes during human evolution. *Proc. Natl Acad. Sci. USA* **107**, 7853–7857 (2010).
A meta-analysis of genome-scans for human-specific changes in protein-coding sequence and *cis*-regulatory changes. They find that human-specific genetic changes in neural genes mainly involve *cis*-regulatory but not protein-coding changes.
114. Lambert, N. *et al.* Genes expressed in specific areas of the human fetal cerebral cortex display distinct patterns of evolution. *PLoS ONE* **6**, e17753 (2011).
115. Eisen, M., Spellman, P., Brown, P. & Botstein, D. Cluster analysis and display of genome-wide expression patterns. *Proc. Natl Acad. Sci. USA* **95**, 14863–14868 (1998).
116. Lai, C. S., Fisher, S. E., Hurst, J. A., Vargha-Khadem, F. & Monaco, A. P. A forkhead-domain gene is mutated in a severe speech and language disorder. *Nature* **413**, 519–523 (2001).
117. Nielsen, R. *et al.* A scan for positively selected genes in the genomes of humans and chimpanzees. *PLoS Biol.* **3**, e170 (2005).
118. Zhang, Y. E., Landback, P., Vibranovski, M. D. & Long, M. Accelerated recruitment of new brain development genes into the human genome. *PLoS Biol.* **9**, e1001179 (2011).
119. Nowick, K., Gernat, T., Almaas, E. & Stubbs, L. Differences in human and chimpanzee gene expression patterns define an evolving network of transcription factors in brain. *Proc. Natl Acad. Sci. USA* **106**, 22358–22363 (2009).
120. Menet, J. S. & Rosbash, M. When brain clocks lose track of time: cause or consequence of neuropsychiatric disorders. *Curr. Opin. Neurobiol.* **21**, 849–857 (2011).
121. Davis, S., Bozon, B. & Laroche, S. How necessary is the activation of the immediate early gene *zif268* in synaptic plasticity and learning? *Behav. Brain Res.* **142**, 17–30 (2003).
122. Flavell, S. W. *et al.* Activity-dependent regulation of MEF2 transcription factors suppresses excitatory synapse number. *Science* **311**, 1008–1012 (2006).
123. Shalizi, A. *et al.* A calcium-regulated MEF2 sumoylation switch controls postsynaptic differentiation. *Science* **311**, 1012–1017 (2006).
124. Li, L. *et al.* Egr3, a synaptic activity regulated transcription factor that is essential for learning and memory. *Mol. Cell. Neurosci.* **35**, 76–88 (2007).
125. Flavell, S. W. *et al.* Genome-wide analysis of MEF2 transcriptional program reveals synaptic target genes and neuronal activity-dependent polyadenylation site selection. *Neuron* **60**, 1022–1038 (2008).
126. Kosik, K. S. The neuronal microRNA system. *Nature Rev. Neurosci.* **7**, 911–920 (2006).
127. Schratt, G. MicroRNAs at the synapse. *Nature Rev. Neurosci.* **10**, 842–849 (2009).
128. Liang, H. & Li, W.-H. Lowly expressed human microRNA genes evolve rapidly. *Mol. Biol. Evol.* **26**, 1195–1198 (2009).
129. Somel, M. *et al.* MicroRNA, mRNA, and protein expression link development and aging in human and macaque brain. *Genome Res.* **20**, 1207–1218 (2010).
130. Hu, H. Y. *et al.* MicroRNA expression and regulation in human, chimpanzee, and macaque brains. *PLoS Genet.* **7**, e1002327 (2011).
131. Liu, C. *et al.* Epigenetic regulation of miR-184 by MBD1 governs neural stem cell proliferation and differentiation. *Cell Stem Cell* **6**, 433–444 (2010).
132. Borrelli, E., Nestler, E. J., Allis, C. D. & Sassone-Corsi, P. Decoding the epigenetic language of neuronal plasticity. *Neuron* **60**, 961–974 (2008).
133. Houston, I. *et al.* Epigenetics in the human brain. *Neuropsychopharmacology* **38**, 183–197 (2012).
134. Zeng, J. *et al.* Divergent whole-genome methylation maps of human and chimpanzee brains reveal epigenetic basis of human regulatory evolution. *Am. J. Hum. Genet.* **91**, 455–465 (2012).
135. Shulha, H. P. *et al.* Human-specific histone methylation signatures at transcription start sites in prefrontal neurons. *PLoS Biol.* **10**, e1001427 (2012).
The first genome-wide analysis of histone modification differences among human, chimpanzee and macaque brains. It identified hundreds of H3K4me3 differences, some of which included genes associated with neurological disorders.
136. Marshall, C. R. *et al.* Structural variation of chromosomes in autism spectrum disorder. *Am. J. Hum. Genet.* **82**, 477–488 (2008).
137. Calarco, J. *et al.* Global analysis of alternative splicing differences between humans and chimpanzees. *Genes Dev.* **21**, 2963–2975 (2007).
138. Lin, L. *et al.* Evolution of alternative splicing in primate brain transcriptomes. *Hum. Mol. Genet.* **19**, 2958–2973 (2010).
139. Paz-Yaacov, N. *et al.* Adenosine-to-inosine RNA editing shapes transcriptome diversity in primates. *Proc. Natl Acad. Sci. USA* **107**, 12174–12179 (2010).
140. Li, J. B. *et al.* Genome-wide identification of human RNA editing sites by parallel DNA capturing and sequencing. *Science* **324**, 1210–1213 (2009).
141. Wahlstedt, H., Daniel, C., Enstërö, M. & Ohman, M. Large-scale mRNA sequencing determines global regulation of RNA editing during brain development. *Genome Res.* **19**, 978–986 (2009).
142. Pollard, K. *et al.* An RNA gene expressed during cortical development evolved rapidly in humans. *Nature* **443**, 167–172 (2006).
143. Khaitovich, P. *et al.* Functionality of intergenic transcription: an evolutionary comparison. *PLoS Genet.* **2**, e171 (2006).
144. Varki, A., Geschwind, D. H. & Eichler, E. E. Explaining human uniqueness: genome interactions with environment, behaviour and culture. *Nature Rev. Genet.* **9**, 749–763 (2008).
145. Plummer, T. Flaked stones and old bones: biological and cultural evolution at the dawn of technology. *Am. J. Phys. Anthropol. Suppl.* **39**, 118–164 (2004).
146. Schoenemann, P. T. Evolution of the size and functional areas of the human brain. *Annu. Rev. Anthropol.* **35**, 379–406 (2006).
147. Rightmire, G. P. Brain size and encephalization in early to Mid-Pleistocene *Homo*. *Am. J. Phys. Anthropol.* **124**, 109–123 (2004).
148. Antón, S. C. Natural history of *Homo erectus*. *Am. J. Phys. Anthropol.* **122** (Suppl. 37), 126–170 (2003).
149. Cann, R. L., Stoneking, M. & Wilson, A. C. Mitochondrial DNA and human evolution. *Nature* **325**, 31–36 (1987).
150. Stout, D. Stone toolmaking and the evolution of human culture and cognition. *Phil. Trans. R. Soc. B.* **366**, 1050–1059 (2011).
151. Lepre, C. J. *et al.* An earlier origin for the Acheulian. *Nature* **477**, 82–85 (2011).
152. Organ, C., Nunn, C. L., Machanda, Z. & Wrangham, R. W. Phylogenetic rate shifts in feeding time during the evolution of *Homo*. *Proc. Natl Acad. Sci. USA* **108**, 14555–14559 (2011).
153. Navarrete, A., van Schaik, C. P. & Isler, K. Energetics and the evolution of human brain size. *Nature* **480**, 91–93 (2011).
154. Babbitt, C. C., Warner, L. R., Fedrigo, O., Wall, C. E. & Wray, G. A. Genomic signatures of diet-related shifts during human origins. *Proc. Biol. Sci.* **278**, 961–969 (2011).
155. Brown, P. *et al.* A new small-bodied hominin from the Late Pleistocene of Flores, Indonesia. *Nature* **431**, 1055–1061 (2004).
156. Charrier, C. *et al.* Inhibition of SRGAP2 function by its human-specific paralogs induces neoteny during spine maturation. *Cell* **149**, 923–935 (2012).
Together with reference 157, this paper shows how a human-specific duplication in recent human history may have lead to prolonged neurodevelopment and increased spine density.
157. Dennis, M. Y. *et al.* Evolution of human-specific neural *SRGAP2* genes by incomplete segmental duplication. *Cell* **149**, 912–922 (2012).
158. Krause, J. *et al.* The derived *FOXP2* variant of modern humans was shared with Neandertals. *Curr. Biol.* **17**, 1908–1912 (2007).
159. Meyer, M. *et al.* A high-coverage genome sequence from an archaic Denisovan individual. *Science* **338**, 222–226 (2012).
160. Enard, W. *et al.* Molecular evolution of *FOXP2*, a gene involved in speech and language. *Nature* **418**, 869–872 (2002).
161. Tattersall, I. Human evolution and cognition. *Theory Biosci.* **129**, 193–201 (2010).
162. McDougall, I., Brown, F. H. & Fleagle, J. G. Stratigraphic placement and age of modern humans from Kibish, Ethiopia. *Nature* **433**, 733–736 (2005).
163. McBrearty, S. & Brooks, A. S. The revolution that wasn't: a new interpretation of the origin of modern human behavior. *J. Hum. Evol.* **39**, 453–563 (2000).
164. Backwell, L., d'Errico, F. & Wadley, L. Middle Stone Age bone tools from the Howiesons Poort Layers, Sibudu Cave, South Africa. *J. Archaeol. Sci.* **35**, 1566–1580 (2008).
165. Brown, K. S. *et al.* An early and enduring advanced technology originating 71,000 years ago in South Africa. *Nature* **491**, 590–593 (2012).
166. Marean, C. W. *et al.* Early human use of marine resources and pigment in South Africa during the Middle Pleistocene. *Nature* **449**, 905–908 (2007).

167. Henshilwood, C. S. *et al.* Emergence of modern human behavior: Middle Stone Age engravings from South Africa. *Science* **295**, 1278–1280 (2002).
168. Mellars, P. Neanderthals and the modern human colonization of Europe. *Nature* **432**, 461–465 (2004).
169. Zilhão, J. *et al.* Symbolic use of marine shells and mineral pigments by Iberian Neandertals. *Proc. Natl Acad. Sci. USA* **107**, 1023–1028 (2010).
170. Brodmann, K. *Vergleichende Lokalisationslehre der Grosshirnrinde in ihren Prinzipien dargestellt auf Grund des Zellenbaues* (J. A. Barth, 1909).
171. Molyneaux, B. J., Arlotta, P., Menezes, J. R. L. & Macklis, J. D. Neuronal subtype specification in the cerebral cortex. *Nature Rev. Neurosci.* **8**, 427–437 (2007).
172. Leone, D. P. Srinivasan, K., Chen, B., Alcamo, E. & McConnell, S. K. The determination of projection neuron identity in the developing cerebral cortex. *Curr. Opin. Neurobiol.* **18**, 28–35 (2008).
173. Vitalis, T. & Rossier, J. New insights into cortical interneurons development and classification: contribution of developmental studies. *Dev. Neurobiol.* **71**, 34–44 (2011).
174. Zeng, H. *et al.* Large-scale cellular-resolution gene profiling in human neocortex reveals species-specific molecular signatures. *Cell* **149**, 483–496 (2012).
175. Bernard, A. *et al.* Transcriptional architecture of the primate neocortex. *Neuron* **73**, 1083–1099 (2012).
176. Belgard, T. G. *et al.* A transcriptomic atlas of mouse neocortical layers. *Neuron* **71**, 605–616 (2011).
177. Rilling, J. K. & Insel, T. R. Differential expansion of neural projection systems in primate brain evolution. *Neuroreport* **10**, 1453–1459 (1999).
178. Herculano-Houzel, S., Mota, B., Wong, P. & Kaas, J. H. Connectivity-driven white matter scaling and folding in primate cerebral cortex. *Proc. Natl Acad. Sci. USA* **107**, 19008–19013 (2010).
179. Walker, A. A cytoarchitectural study of the prefrontal area of the macaque monkey. *J. Comp. Neurol.* **73**, 59–86 (1940).
180. Deacon, T. Rethinking mammalian brain evolution. *Am. Zool.* **30**, 629–705 (1990).
181. Finlay, B. & Darlington, R. Linked regularities in the development and evolution of mammalian brains. *Science* **268**, 1578–1584 (1995).
This work demonstrates how evolutionary changes in brain size follow common rules across mammals.
182. Petanjek, Z. *et al.* Extraordinary neoteny of synaptic spines in the human prefrontal cortex. *Proc. Natl Acad. Sci. USA* **108**, 13281–13286 (2011).
183. Bogin, B. Childhood, adolescence, and longevity: a multilevel model of the evolution of reserve capacity in human life history. *Am. J. Hum. Biol.* **21**, 567–577 (2009).
184. Thompson-Schill, S. L., Ramscar, M. & Chrysikou, E. G. Cognition without control: when a little frontal lobe goes a long way. *Curr. Dir. Psychol. Sci.* **18**, 259–263 (2009).
185. Smith, T. *et al.* Earliest evidence of modern human life history in North African early *Homo sapiens*. *Proc. Natl Acad. Sci. USA* **104**, 6128–6133 (2007).
186. Smith, T. M. *et al.* Dental evidence for ontogenetic differences between modern humans and Neanderthals. *Proc. Natl Acad. Sci. USA* **107**, 20923–20928 (2010).
187. Smith, T. M., Toussaint, M., Reid, D. J., Olejniczak, A. J. & Hublin, J.-J. Rapid dental development in a Middle Paleolithic Belgian Neanderthal. *Proc. Natl Acad. Sci. USA* **104**, 20220–20225 (2007).
188. Macchiarelli, R. *et al.* How Neanderthal molar teeth grew. *Nature* **444**, 748–751 (2006).
189. Dean, M. C. Tooth microstructure tracks the pace of human life-history evolution. *Proc. Biol. Sci.* **273**, 2799–2808 (2006).
190. Ptak, S. *et al.* Linkage disequilibrium extends across putative selected sites in FOXP2. *Mol. Biol. Evol.* **26**, 2181– 2184 (2009).
191. Kimura, M. Evolutionary rate at the molecular level. *Nature* **217**, 624–626 (1968).
192. Boyko, A. R. *et al.* Assessing the evolutionary impact of amino acid mutations in the human genome. *PLoS Genet.* **4**, e1000083 (2008).
193. Maynard Smith, J. & Haigh, J. The hitch-hiking effect of a favourable gene. *Genet. Res.* **23**, 23–35 (1974).
194. Kong, A. *et al.* Rate of *de novo* mutations and the importance of father's age to disease risk. *Nature* **488**, 471–475 (2012).
195. Walker, R. *et al.* Growth rates and life histories in twenty-two small-scale societies. *Am. J. Hum. Biol.* **18**, 295–311 (2006).
196. Wright, S. Evolution in Mendelian populations. *Genetics* **16**, 97–159 (1931).
197. Haldane, J. B. S. A mathematical theory of natural and artificial selection, part V: selection and mutation. *Proc. Cambridge Phil. Soc.* **23**, 838–844 (1927).
198. Kimura, M. & Ohta, T. The average number of generations until fixation of a mutant gene in a finite population. *Genetics* **61**, 763–771 (1969).
199. Bersaglieri, T. *et al.* Genetic signatures of strong recent positive selection at the lactase gene. *Am. J. Hum. Genet.* **74**, 1111–1120 (2004).
200. Tishkoff, S. *et al.* Convergent adaptation of human lactase persistence in Africa and Europe. *Nature Genet.* **39**, 31–40 (2007).
201. Voight, B., Kudaravalli, S., Wen, X. & Pritchard, J. A. Map of recent positive selection in the human genome. *PLoS Biol.* **4**, e72 (2006).
202. Nielsen, R., Hellmann, I., Hubisz, M., Bustamante, C. & Clark, A. Recent and ongoing selection in the human genome. *Nature Rev. Genet.* **8**, 857–868 (2007).
203. Pritchard, J., Pickrell, J. & Coop, G. The genetics of human adaptation: hard sweeps, soft sweeps, and polygenic adaptation. *Curr. Biol.* **20**, R208–R215 (2010).
204. Takahata, N. Allelic genealogy and human evolution. *Mol. Biol. Evol.* **10**, 2–22 (1993).
205. Kimura, M. On the probability of fixation of mutant genes in a population. *Genetics* **47**, 713–719 (1962).
206. Burbano, H. A. *et al.* Targeted investigation of the Neandertal genome by array-based sequence capture. *Science* **328**, 723–725 (2010).

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Competing interests statement
The authors declare no competing financial interests.

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