Genetic basis of human brain evolution

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Human evolution is characterized by a rapid increase in brain size and complexity. Decades of research have made important strides in identifying anatomical and physiological substrates underlying the unique features of the human brain. By contrast, it has become possible only very recently to examine the genetic basis of human brain evolution. Through comparative genomics, tantalizing insights regarding human brain evolution have emerged. The genetic changes that potentially underlie human brain evolution span a wide range from singlenucleotide substitutions to large-scale structural alterations of the genome. Similarly, the functional consequences of these genetic changes vary greatly, including protein-sequence alterations, cis-regulatory changes and even the emergence of new genes and the extinction of existing ones. Here, we provide a general review of recent findings into the genetic basis of human brain evolution, highlight the most notable trends that have emerged and caution against overinterpretation of current data.

Introduction

A hallmark of human biology is advanced cognitive capacity. It lies at the heart of the unparalleled explosion in behavioral repertoire from tool use and language to science and art. It is because of complex cognition that we as a species are uniquely capable of cultural evolution – the creation and dissemination of knowledge that transcends behaviors hardwired in our genes.

Millions of years of hominid evolution have transformed our brain in both size and structural complexity. The volume of the human brain has more than tripled since the divergence from chimpanzees and is approximately eight times that of the New World monkeys [1]. This expansion, occurring heterogeneously across brain regions, has also changed the relative proportions of the areas of the brain. The cerebral cortex has become particularly pronounced, with the most notable expansion occurring in the prefrontal cortex, an area thought to have a crucial role in regulating social behavior [2]. Less obvious but no less important are changes in the wiring and physiology of the brain that affect how brain cells communicate with each other and with the rest of the body.

The convergence of two important advances in recent years has greatly facilitated the study of human brain evolution at the genetic level. The first is the ability to readily read and analyze the sequences of genes (and in many cases whole genomes) across species. Comparative genomics, which entails sequence comparisons of genes

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across many genomes both within and between species, is enabling researchers to probe the very unit (i.e. mutations in DNA) of evolutionary adaptation. These studies are aided not only by the availability of large amounts of sequence data but also by the development of sophisticated analytical methods and computational tools for inferring salient evolutionary events (such as the action of position selection on specific genes) from the otherwise static sequences. The second advance is the growing understanding of the genetic basis of neurobiology. An increasing number of genes have been implicated in normal and disease processes of the brain. When functional knowledge of brain-related genes is coupled with the analysis of gene evolution, many links can be hypothesized between genetic evolution and phenotypic evolution with respect to human brain biology. In addition to comparisons of gene and genome sequences, insights into human brain evolution have also been gained from studies of gene expression differences and genome structural variations between species.

Here, we provide a general review of recent studies into the genetic basis of human brain evolution. The insights gleaned from these studies can be broadly divided into three categories: (i) positive selection on protein-coding regions of the genome that lead to changes in the sequences of existing proteins; (ii) duplication and deletion of genes; and (iii) evolutionary changes in non-coding regions of the genome, especially those in *cis*-regulatory sequences that lead to altered gene expression (Figure 1).

Changes in protein sequence

There are numerous differences in the sequences of existing proteins between humans and other mammals. Indeed, even when comparing humans and chimpanzees most proteins show at least one amino acid difference. Many of these differences are likely to have little functional consequence and are not relevant to the phenotypic evolution of humans. However, some changes undoubtedly carry important functional effects and a subset might contribute to human-specific brain characters. When positive selection acts upon non-synonymous mutations (i.e. coding-region mutations that alter the encoded proteins), it often leaves behind telltale signatures in the affected genes. The chief approach in studying the genetics of human brain evolution has been to search for these signatures of positive selection in brain-related genes in primates (Figure 2).

One fruitful area of research relates to genes associated with microcephaly, a congenital condition characterized by a severe reduction in brain size. Among the many forms of microcephaly, primary microcephaly manifests as a

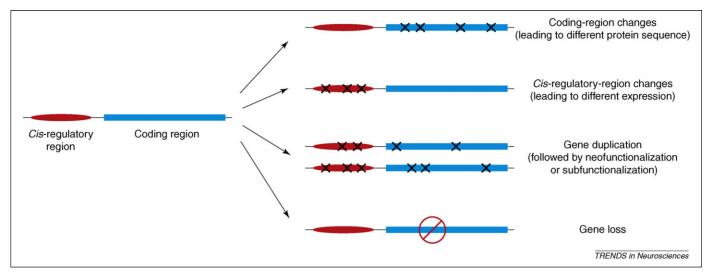


Figure 1. Schema of several types of evolutionary changes in a gene that could potentially contribute to human brain evolution.

reduction in brain size without gross abnormalities in brain architecture or gyral formation [3–5]. Typically, the reduction in the size of the cerebral cortex is much more severe than that of other regions of the brain. Thus, primary microcephaly can be considered as an 'atavistic' condition in that it recapitulates some aspects of the earlier evolutionary stages of the hominid brain (smaller size overall but especially of the cerebral cortex). Primary microcephaly is genetically heterogeneous and has been mapped to six regions of the human genome, named *MCPH1* to *MCPH6* (microcephaly, primary autosomal recessive 1 to 6), with null mutations identified in four loci: *microcephalin* (*MCPH1*), *CDK5RAP2* (CDK5 regulatory-subunit-associated protein 2; *MCPH3*), *ASPM* (abnormal spindle-like, microcephaly-associated; *MCPH5*) and *CENPJ* (centromeric protein J; *MCPH6*) [6–9].

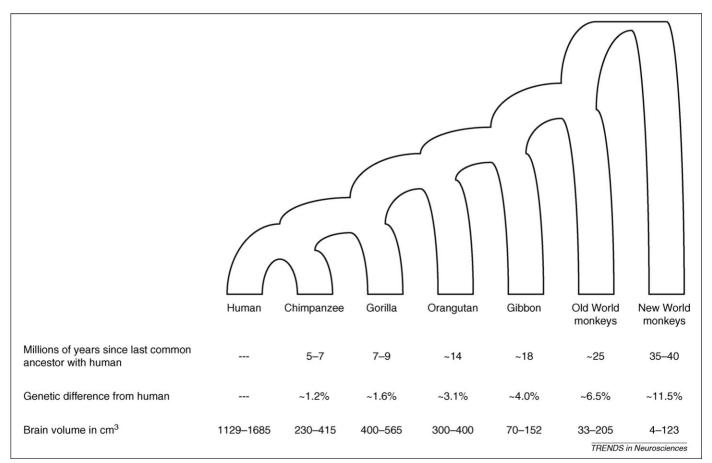


Figure 2. Phylogenetic relationship of humans and other primates. The separation between humans and other species in evolutionary time and genetic difference is indicated. Also indicated are the brain volumes in these species. Values in genetic difference are based on nucleotide substitutions without considering other types of differences such as insertions or deletions and structural changes. Values for Old World monkeys and New World monkeys are each based on groups of species within each clade.

Review

ASPM has been shown to have undergone positive selection throughout the primate lineage leading to humans, including both the lineage separating the great apes from the Old World monkeys and the lineage within the great apes leading to humans [10-12]. On the other hand, *microcephalin* shows a strong signature of positive selection primarily in the lineage leading from the ancestral primates to the great apes [13,14]. Both CDK5RAP2 and CENPJ show higher rates of non-synonymous substitutions in primates than rodents, and CDK5RAP2 shows especially high rates in the human and chimpanzee terminal lineages [15]. ASPM. CDK5RAP2 and CENPJ have been implicated in mitotic spindle formation [9,16,17], whereas *microcephalin* has been implicated in DNAdamage response and DNA condensation during mitosis [18-20]. Thus, all four primary microcephaly genes seem to function in cell-cycle control, and their ability to impact brain size is likely to stem from their role in regulating the proliferative potential of neural precursor cells during embryogenesis. Although more functional studies are needed, this might prove to be the first genetic evidence of the long-standing hypothesis that the evolutionary expansion in the human and primate brain is the result of an increased number of neural precursor divisions during neurogenesis [21]. It is important to note, however, that these genes are also expressed outside of the brain, although their non-brain functions, if any, are likely to be minor because null mutations in these genes cause notable defects only in the brain. It is thus possible (for these genes and also genes discussed later) that the positive selection identified therein does not exclusively target the biology of the brain.

Another gene involved in neural precursor proliferation, *ADCYAP1* (adenylate-cyclase-activating polypeptide 1), has also been found to bear signatures of accelerated protein-sequence evolution in humans. *ADCYAP1* has been shown to play a part in regulating the transition from proliferative to differentiated states during neurogenesis [22,23]. It exhibits an exceptionally high rate of nonsynonymous substitutions in the human lineage since the divergence from chimpanzees and is one of the most divergent genes identified to date [24].

A second aspect of neural development that might have been a substrate for positive selection in humans is axon guidance. *AHI1* (Abelson helper integration site 1), one of the genes associated with a rare brain malformation called Joubert syndrome, is involved in directing axons from the brain to the spinal cord. Like *ASPM* and *ADCYAP1*, *AHI1* shows an accelerated rate of non-synonymous sequence change along the human lineage since the divergence from chimpanzees [25].

SHH (Sonic hedgehog) is a highly conserved developmental gene that has been intensely studied for decades. It encodes a signaling molecule that has a central role in developmental patterning of many tissues, especially the nervous and skeletal systems. SHH protein has two functional domains: the signaling peptide and an auto-catalytic region responsible for cleaving off the signaling peptide. The auto-catalytic portion of SHH shows a markedly accelerated rate of protein sequence evolution in primates relative to other mammals, most prominently along the lineage leading to humans [26]. Intriguingly, the evolution of *SHH* in the lineage leading to humans is characterized by a rampant and statistically highly non-random gain of serines and threonines, residues that are potential substrates of post-translational modifications. This indicates that *SHH* might have evolved more complex post-translational regulation in the lineage leading to humans. Collectively, these findings implicate *SHH* as a potential contributor to the evolution of primate- or human-specific morphological traits in the nervous and/or skeletal systems.

Signatures of positive selection have also been found in genes associated with language in humans. The most notably example is FOXP2 (forkhead box P2). Loss-offunction mutations in this gene lead to developmental verbal dyspraxia, a disorder characterized by difficulties in the production of speech believed to result from defects in the part of brain that translates intended speech into specific muscle movements [27]. Interestingly, FOXP2 is implicated in verbal communication in other species including mice and songbirds [28–31]. Despite near-perfect amino-acid-sequence conservation in mammals, FOXP2 shows two non-synonymous changes in the human lineage since its divergence from chimpanzees, indicating a possible role for positive selection [32]. The X-linked gene SRPX2 (sushi-repeat-containing protein, X-linked 2) is also associated with speech processing [33] and exhibits an accelerated rate of non-synonymous substitutions in the human lineage, although there was insufficient statistical power to demonstrate the action of positive selection [34]. Finally, it has recently been suggested that the primary microcephaly genes ASPM and microcephalin might also be involved in the capacity for language, indicating that positive selection on these genes might, in part, target their putative role in language [35].

The X-lined *MAOA* (monoamine oxidase A) gene encodes a mitochondrial enzyme that catabolizes several neurotransmitters including dopamine, serotonin and norepinephrine. As such, functional alterations in this gene can potentially have numerous physiological and behavioral consequences [36–39]. It has been suggested that a non-synonymous change in this gene that occurred in the human lineage after human-chimpanzee divergence might have created a functional shift in the encoded enzyme [40].

All the studies discussed here have focused on identifying signatures of adaptive evolution in genes selected for their known functions in the brain. Another category of studies looks instead at large cohorts of genes to see if they, on average, show patterns of sequence evolution consistent with positive selection [41–45]. Results of such large-scale surveys, however, have been difficult to interpret owing to the use of different gene sets and/or different analytical methods. An early study based on 214 genes selected by known functions in the brain showed an accelerated rate of non-synonymous substitutions in the primate lineage leading to humans [41]. Importantly, this trend is most prominent for \mathbf{the} subset of genes involved in neurodevelopment but is barely significant for the subset of genes involved in neurophysiology, indicating that protein-sequence evolution in neurodevelopment genes might have played an important part in the emergence of the human brain. This observation is corroborated by another study [46]. However, two subsequent studies focusing on genes expressed in the adult brain failed to show accelerated evolution in the human lineage [47,48]. This discrepancy calls into question interpretations of the role that evolutionary changes in protein sequences have had in human brain evolution. There are two possible explanations for the discrepancy: either the initial study selected a non-representative set of genes or the latter studies focused on the 'wrong' sets of genes. Of note is the fact that the latter studies used adult-brain expression as the criteria for selecting genes. However, genes prominently expressed in the adult brain tend to be involved in neurophysiology whereas genes functioning in neurodevelopment are likely to be expressed in the embryonic brain. Thus, it is possible that the focus of the latter studies on genes expressed in the adult brain has created a bias toward the inclusion of neurophysiology genes, and it is this bias that resulted in the lack of signal. It is important to recognize that genome-scale evolutionary studies often suffer from biases in the selection of genes and the choice of analytical tools. As such, interpretations of results in these studies need to be done with a good measure of caution.

Novel genes

There is mounting evidence that the creation of novel genes might have contributed to the evolution of the human phenotype. The introduction of new genes into the genome is triggered by duplication events, often within large gene families that might be predisposed to such event. The relaxation of evolutionary constraint on duplicated genes provides a unique opportunity for either neofunctionalization or subfunctionalization [49]. Neofunctionalization involves the acquisition of novel function by one or both duplicated genes, whereas subfunctionalization involves the partitioning of the ancestral function among duplicates [50,51].

The recent sequencing of complete genomes from multiple primate species, coupled with new technologies such as comparative genome hybridization, has greatly facilitated the identification of gene-duplication events during human evolution [52–54]. Among these are genes with known brain-related functions, which are good candidates for studying how the emergence of novel genes might have contributed to human brain evolution.

The first gene family for which there is compelling evidence of gene duplication followed by neofunctionalization is the *morpheus* family [52]. This gene family expanded dramatically in human and great ape lineages and was accompanied by extremely intense positive selection on the encoded protein sequences. Indeed, positive selection on some duplicated copies is so strong that their coding regions bear little resemblance to their ancestral precursors. The function of *morpheus* is unknown, however, and it not clear whether the dramatic adaptive evolution of this gene family has anything to do with the brain.

Another study identified a family of genes characterized by the presence, within each gene, of multiple copies of the DUF1220 domain (protein domain of unknown function 1220) [54]. This family has undergone rapid expansion in primates and, interestingly, the number of genes in the family increases as one moves across the primate phylogeny toward the human lineage, with the greatest copy number found in humans. The function of this gene family (or its DUF1220 domain) is not known, but it is prominently expressed in the brain and specifically in neurons. The expansion of this gene family in primates is coupled with an accelerated rate of non-synonymous substitutions, indicating the action of positive selection. Taken together, these data raise the possibility that this gene family might underlie some aspect of human brain evolution.

The MRG (MAS-related gene) family encodes a group of G-protein-coupled receptors expressed specifically in nociceptive neurons of the spinal cord and are implicated in the modulation of nociception [55,56]. The multiple copies of MRG in humans is likely to be the result of gene amplification after human-mouse divergence, and sequence comparisons among the various human copies reveal strong signatures of positive selection in regions of the genes encoding the extracellular ligand-binding domains [57]. Thus, it can be hypothesized that the evolutionary changes in MRG might have altered the sensitivity and/or selectivity of nociceptive neurons to aversive stimuli. Of note, the MRG family in mice also shows evidence of gene amplification coupled with positive selection on the extracellular domains [57].

One case in which the functional consequence of a duplication event is clearer is the birth of the GLUD2 (glutamate dehydrogenase 2) gene [58]. Rather than originating from a large gene family, GLUD2 arose from the retrotransposition, or reintegration, of a processed mRNA from a single ancestral precursor, GLUD1. In most mammalian species, GLUD1 is the only gene encoding glutamate dehydrogenase, which, in the brain, catalyzes the recycling of the chief excitatory neurotransmitter, glutamate. The retrotransposition event occurred in the ape lineage after its divergence from the Old World monkeys, giving rise to *GLUD2*, which encodes a second glutamate dehydrogenase gene specific to apes and humans [58]. Although GLUD1 is broadly expressed in many tissues, GLUD2 has circumscribed expression in nerve tissues and testis [59]. After its birth, GLUD2 underwent a period of positive selection at the amino-acid-sequence level [60]. The resulting ape- and human-specific GLUD2 encodes an enzyme that seems optimized for function in the brain, including a high enzymatic activity despite the high GTP levels in the brain [60,61].

The best-established instance of adaptation through the creation of a novel gene is the emergence of trichromatic color vision in primates after the duplication and subsequent selection of the X-linked *opsin* genes [62]. In this case, a duplication of a 'green' *opsin*, which detects medium-wavelength light, was followed by neofunctionalization creating a 'red' *opsin* that detects longer wavelengths. This duplication occurred in the common ancestor of catarrhines, a primate clade that includes humans, leading to a shift from dichromatic to trichromatic vision in catarrhines. It was argued that this shift coincides with the increased reliance on vision for sensory perception.

Gene loss

Although the creation of novel genes can produce pronounced phenotypic effects, the loss of existing genes in an organism can have even more drastic consequences. Gene loss during evolution is much rarer than gene duplication because, presumably, the removal of a functional gene is far more likely to be deleterious. In some instances, however, gene loss can occur due to changes in selective constraints over time.

The best-known example of gene loss during primate and human evolution is the olfactory receptor (OR) gene family [63–66]. It is estimated that mice have ~ 1200 functional OR genes whereas the corresponding number for humans is only ~ 350 [64,65]. This is mainly owing to the fact that many OR genes in the human genome are mere pseudogenes (nonfunctional relics of the ancestral genes). The degeneration of OR genes is not unique to humans but seems to affect many primate species, although there are cases of recent decay of specific OR genes in the human lineage after divergence from chimpanzees [66]. The rampant degeneration of OR genes in primates is thought to result from a diminished reliance on smell for sensing and communication. Nevertheless, there is indicative evidence that positive selection might have operated on some intact OR genes in the human lineage, implying that differences in environment might have led to distinct olfaction needs during human evolution [67].

There is also an intriguing example of gene loss that might have played a part in the emergence of the larger human brain [68]. MYH16 (myosin, heavy chain 16) encodes a myosin heavy-chain protein present in skeletal muscles. In nonhuman primates, MYH16 is expressed exclusively in muscles of the head including those involved in mastication. In humans, however, a frameshift mutation resulted in the loss of function of this gene. Because, compared with other primates, humans show an underdeveloped masticatory system, it was suggested that the loss of MYH16 was partly responsible and that the changes released the cranium from geometric constraints, enabling it to expand to accommodate increased brain size [69]. Thus, the loss of *MYH16* might have come about through the relaxation of functional constraints on masticatory muscles coupled with positive selection for increased brain size. However, this interpretation was challenged on the ground that the loss of *MYH16* is much older than the reduction in the masticatory system during hominid evolution [70].

Changes in gene expression

It has long been postulated that changes in gene expression might have played an important part in the emergence of the human phenotype. In particular, it has been argued that small changes in non-coding regulatory elements could strongly impact the spatial and temporal expression patterns of key developmental genes, which could have profound phenotypic effects [71–73].

One approach in probing how changes in gene expression might have contributed to human brain evolution is to compare *cis*-regulatory regions of brain-related genes across many species to identify those bearing signatures of positive selection. One example is *PDYN* (prodynorphin), which encodes a precursor for a suite of opioid neuropeptides involved in numerous neural processes. An upstream *cis*-regulatory element of this gene exhibits an exceptionally rapid rate of sequence change in the human lineage after divergence from chimpanzees consistent with the action of positive selection [74]. Interestingly, the human element is capable of driving much higher levels of expression than the corresponding chimpanzee element in a cell-culture expression system [74].

In addition to single-gene studies, several genome-wide analyses have been performed to systematically identify *cis*-regulatory elements that show accelerated rates of change in the human lineage [75–78]. Collectively, these studies indicate that the *cis*-regulatory regions of many brain-development genes might have experienced positive selection during human evolution; however, in nearly all cases there is, as yet, no corroborating evidence that the *cis*-regulatory changes indeed altered the expression patterns of the corresponding genes.

Besides *cis*-regulatory sequences, changes in the protein sequences of transcription factors can have profound effects on the expression of the genes that they regulate. Several genome-wide studies have found significant overrepresentation of transcription factors among genes that are likely to have experienced positive selection in their protein-coding regions, although it is not clear whether the expression patterns of their downstream targets are affected [42,79].

Another approach is to compare directly patterns of gene expression in the brain across various species including humans [72,73]. These studies have considered the brain as a whole [46], in addition to specific regions of the brain [80] such as cerebral cortex [81], frontal cortex [82], prefrontal cortex [83] and anterior cingulate cortex [84]. As has been the case in sequence-based analyses of positive selection, these studies have also focused largely on comparisons between humans and either chimpanzees or macagues. Some studies have also attempted to validate different expression patterns between species observed for some genes by cell-culture expression experiments [85]. Two broad trends have emerged. First, total gene expression in the brain has been considerably upregulated during human evolution. Second, gene expression profiles in the brain are more similar between humans and chimpanzees than is the case for other tissues.

Even though comparative expression studies on the brain have produced some important insights, efforts to interpret these studies are limited by several confounding factors. One is the effect of sequence divergence on hybridization efficiency. In particular, differential hybridization can occur from sequence divergence rather than differential gene expression, a problem that can affect not only oligonucleotide-based arrays such as Affymetrix arrays but also cDNA-based arrays [86]. The second confounding factor is the difficulty in ascertaining the precise anatomical homologies between specific brain regions in different species. Whole-brain studies are not very informative because they provide extremely limited resolution, but when specific regions of the brain are examined imprecise identification of anatomical homologies between species can have a profound effect on the resulting data. This is not a trivial problem because the regions that these studies focus on tend to be those that have diverged the most at the anatomical level between humans and other species. Finally, the environment can have a profound influence on expression profiles of the brain [87]. Nonhuman primate tissues are almost always collected from animals in captivity, where environmental variables might be considerably different from the wild. Furthermore, human brain tissue is often collected from hospital patients of advanced age and is always considered to be post hoc. By contrast, nonhuman primate brain tissue might not be subject to these effects. Owing to these confounding factors, comparative expression studies of the brain need to be interpreted with great care.

Non-coding RNA genes

An emerging area of research is the identification of rapidly evolving non-coding RNAs. Methodologically, these studies are similar to studies seeking to identify rapidly evolving *cis*-regulatory elements. Consequently, many of the techniques developed for identifying rapidly evolving promoter or enhancer regions can be used to study the evolution of non-coding RNAs [75–78]. Indeed, it was during a large-scale scan for rapidly evolving *cis*regulatory elements that the first positively selected human non-coding RNA was found [88]. The gene for this RNA, dubbed HAR1 (human accelerated region 1), is expressed in neurons of the developing human neocortex. Evolutionary analysis revealed that, although this gene is only 118 bp in length, it contained 18 changes in the human lineage since the divergence from chimpanzees more than ten times the neutral rate. This large number of changes over such a short evolutionary period is truly striking when compared with the chimpanzee-chicken divergence; although far greater in their evolutionary distance, comparisons between these two species showed only two changes in the entire region. Structural analysis of *HAR1* revealed that these changes alter the secondary structure of the human RNA compared with other amniotes. Thus, it is suggested that the human-specific changes in HAR1 might have played a part in the evolution of the human cerebral cortex.

Is the human brain so unique?

There is a pervasive notion that the human brain is a qualitative break from all other species. By this notion, only the human brain can be placed in the 'superior' category whereas the brains of the other species can all be relegated to one 'less-evolved' group. This anthropocentric notion is incomplete at best. First, the superior human brain is the result of progressive changes over a prolonged period of 60-70 million years in the lineage leading from ancestral primates to modern humans, although the rate of change has been particularly dramatic in the last few million years [89–91]. As such, species that branched off more recently from this lineage, such as apes, tend to possess larger and more complex brains than species that branched off at earlier stages, such as New World monkeys. Second, mammals in general, and birds to some degree, exhibit a trend of brain expansion over evolutionary time that is absent in other vertebrates

[89]. Thus, humans are not the only species that have experienced brain expansion.

The view that the human brain is the result of a trend also affecting other primates is consistent with many studies. Both large-scale surveys of evolutionary changes in brain-related genes, in addition to studies of many single genes such as ASPM, microcephalin, SHH and GLUD2, have shown that these genes experienced adaptive evolution in various time periods along the lineage leading to humans, often affecting humans and other related primates rather than being specific to humans only. Thus, available data point away from the anthropocentric notion of human brain evolution to a more nuanced view, which sees the human brain as resulting from a trend of increasing size and complexity that also affected other living primates, although the impact on humans is undoubtedly most profound. More plainly stated, the salient features of the human brain did not all come about in the terminal human branch after divergence from chimpanzees. Rather, many changes have occurred in much earlier stages of the human lineage. Given this new view, genetic studies of human brain evolution should focus on comparisons across many primates and even non-primate species instead of being limited to only comparing humans and chimpanzees.

Future directions

It is only in the last few years that the genetic basis of brain evolution has come under serious investigation. Much insight has been gained since, mostly relating to the identification of brain-related genes (or their *cis*-regulatory elements) that bear signatures of positive selection during primate or human evolution. However, there are two important drawbacks in almost all of these studies.

The first is the lack of certainty in the conclusions. The link between a gene (or a group of genes) and a role in brain evolution is typically based on inference from two observations: (i) the pattern of sequence changes in the gene acquired during primate or human evolution can be best explained by the action of positive selection; and (ii) the gene has some function in the brain. In almost no case are there experimental data linking sequence changes of a gene to alterations of brain phenotype. As such, the proposition that a gene has contributed to brain evolution is almost never definitive and has to be viewed as a hypothesis. The second limitation is the lack of resolution. When studies indicate an involvement of a gene in brain evolution, they typically do so in general terms rather than in specific terms. This, again, is due to the lack of experimental data linking changes in DNA sequence to specific alterations of phenotype.

The best experiments to demonstrate a definitive causal relationship between the sequence evolution of a gene and the phenotypic evolution of the human brain would involve introducing human genes into nonhuman primates (for example chimpanzees) and vice versa. Given that such experiments are not feasible, the lack of certainty and resolution will probably haunt genetic studies of human brain evolution for many years to come. There are, however, ways to at least partially circumvent the problem. One promising approach is to place genes from humans and other primates into mice and examine the effect on phenotype. Although interpretations of such experiments are hampered by many confounding factors, especially the question of whether mice are evolutionary too divergent to provide the right context for testing the function of primate genes, the approach might, nevertheless, yield useful insights.

Particularly fruitful might be the use of transgenic mice to study the evolution of *cis*-regulatory sequences. For example, when a *cis*-regulatory element is found to bear evidence of positive selection in the human lineage, different versions of the element from humans and other primates can be placed into a reporter construct and introduced into mice as transgenes. Expression of the transgenes can be readily studied at the appropriate developmental stages and functional differences between versions of the element might result in different expression patterns. Another approach is to examine the ability of *cis*regulatory elements to drive expression in cell culture [85], although insight from such *in vitro* experiments is likely to be limited.

In summary, an important future challenge of the field is to go beyond the vague hypotheses that dominate current studies and bring greater certainty and finer resolution to the understanding of how the human brain has evolved at the genetic level. To this end, comparative genomics, which has been the main workhorse of current studies, needs to be complemented by cleverly designed *in vivo* and *in vitro* functional experiments aimed at probing the exact phenotypic consequence of evolutionary changes in DNA sequence.

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