Hemby & Bahn (Eds.)
Progress in Brain Research, Vol. 158
ISSN 0079-6123

CHAPTER 14

Human brain evolution

Hilliary Creely and Philipp Khaitovich*

Max-Planck Institute for Evolutionary Anthropology, Deutscher Platz, D-04103 Leipzig, Germany

Anatomical evolution

The most prominent feature distinguishing humans from all other organisms is our mind. From a biological perspective, study of the mind begins with a study of the brain. For our body size, the human brain is the largest among primate species, roughly three times the size that of our nearest living relative, the chimpanzee (Fig. 1). However, it is not likely that human-specific cognitive abilities can be explained by brain size alone. Several mammalian species, such as dolphins, whales and elephants, surpass humans in brain size but not in cognitive function. Furthermore, certain human congenital disorders lead to a drastic reduction in brain size without an equivalent decrease in cognitive abilities. Mutations in the *MCPH1* (*microcephalin*) and the *ASPM* (*abnormal spindle-like, microcephaly associated*) genes cause a disorder known as primary microcephaly, which is characterized by a severe decrease in human brain size, typically down to 430 g, with retention of overall brain structure (Kumar et al., 2002). Thus, brain size in primary microcephaly patients is comparable to the average adult chimpanzee brain size (410 g) (Herndon et al., 1999). Although patients with this disorder suffer from mental retardation that ranges from mild to severe, their mental abilities far exceed those of the chimpanzee. Nonetheless, the normal human brain is indisputably large. Its size is approximately five times than expected for a mammal of the same size and three times larger than expected for an ape of the same size (Woods et al., 2005). Having such a large brain is quite costly. At the metabolic level, the brain is an expensive organ that consumes 20–25% of total body energy at rest. At the organismal level, the enlarged cranium of a human fetus causes birthing difficulties resulting in high rate (0.5–1%) of labor-related maternal mortality, a rate much higher than observed in other mammals. As a consequence of this problem, the human female has a broader pelvis that decreases the efficiency of bipedal locomotion (reviewed in Gilbert et al., 2005). In light of such high fitness costs for the disproportionately large human brain, the increase in brain size must have conferred a great enough adaptive benefit to our ancestors to justify this cost. Thus, while brain size alone cannot account for the whole of human cognitive abilities, it appears to be one essential human brain feature.

Although decades of studies have brought us incrementally closer to deciphering the functional mechanisms underlying human-specific cognitive abilities, our knowledge remains very limited. Anatomical studies have provided some insight on functional differences between human and chimpanzee brain; however, these differences are not always obvious. On the anatomical level, all differences between humans and other primates known to date are quantitative, rather than qualitative. Compared to chimpanzees and other non-human primates, humans possess an enlarged cerebral cortex (Rilling and Insel, 1999). This enlargement is believed to reflect a longer period of neuronal formation during pre-natal development in humans

*Corresponding author. E-mail: khaitovich@eva.mpg.de

DOI: 10.1016/S0079-6123(06)58014-8

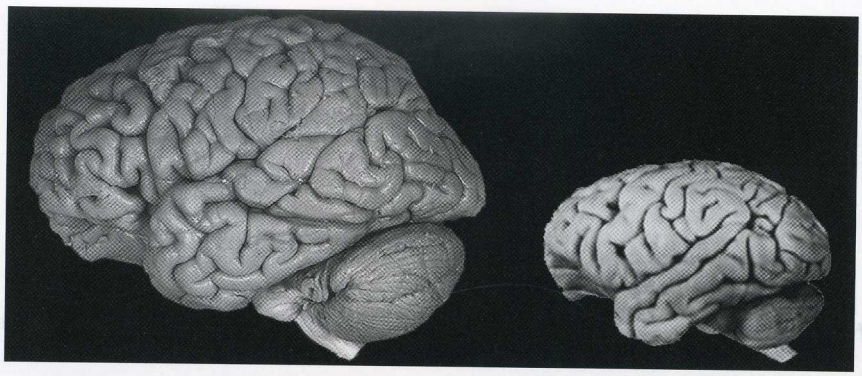

Fig. 1. Relative size comparison between adult human (left) and adult chimpanzee (right) brains.

(Kornack and Rakic, 1998). However, the precise molecular mechanisms determining the neuronal formation process in humans and other primates remain unknown. While brain size is one clearly definable feature, most functional differences distinguishing human brains from the brains of other primate species are much less obvious. For example, upper layers of the cerebral cortex are overrepresented in humans compared to other primates (Marin-Padilla, 1992). Similarly, special types of neurons called spindle cells are overrepresented in anterior cingulate cortex in the human brain compared to those of great apes (Nimchinsky et al., 1999). One of the most well-defined quantitative human-specific brain features is functional asymmetry of the cerebral cortex (Kandel et al., 2000). This asymmetry is most clearly reflected in fact that approximately 90% of humans are right handed. Other species, including chimpanzees, do have "handedness" on the individual level and some degree of right hand preference for certain tasks (Hopkins et al., 2005). However, they do not show the strong left–right asymmetry on the population level observed in humans. Distribution of other cognitive functions in humans is also asymmetric, most famously illustrated by language, which is preferentially localized in the left hemisphere (Broca, 1861; Wernicke, 1874). Indeed, functional specialization between human brain hemispheres may be a direct result of new cognitive abilities acquired during human evolution, with new functions, such as language, replacing an existing specialization in a given brain area (Corballis et al., 2000). This functional lateralization of the human brain is accompanied by anatomical asymmetry in the frontal and temporal lobes. Namely, the size of the cortical areas involved in language and speech production in humans, located in the frontal and temporal lobes, are typically larger in the left than in the right hemisphere (Geschwind and Levitsky, 1968; Amunts et al., 1999). These anatomical features were believed to be directly associated with language abilities and thus to be human-specific. However, recent studies indicate that chimpanzee brains show some asymmetries in the frontal and temporal lobes on the gross-anatomical level as well, although these asymmetries are less pronounced than those in human brains (Gannon et al., 1998; Hopkins et al., 1998; Cantalupo and Hopkins, 2001). Thus, the anatomical asymmetry observed in humans in the frontal and temporal lobes is another quantitative, rather than a qualitative, human-specific feature. With the exception of such quantitative differences, no anatomical features uniquely specific to the human brain have yet been identified.

In conclusion, although anatomical studies provided some important clues about human brain features that may be related to human-specific cognitive abilities, the molecular mechanisms underlying these cognitive abilities remain obscure. Another approach to understand these mechanisms is to identify molecular changes associated with the appearance of human-specific cognitive traits. Recent advances in large-scale analysis of gene structure and gene regulation in humans and non-human primates (predominantly chimpanzees) provide insight on how such approaches

can help uncover changes important for human-specific cognitive abilities. These efforts, the new perspectives they create, and the difficulties that arise from them, will be the focus of the remainder of this chapter.

Protein sequence evolution

The molecular basis for the functional features that distinguish the human brain from that of our ancestors and other primate species lies in DNA sequence changes that happened on the human lineage after separation from the common human–chimpanzee ancestor 5–7 million years ago (Glazko and Nei, 2003). DNA sequence changes may influence phenotype by altering either amino acid sequence in proteins or changing gene expression regulation (Fig. 2). With the nearly completed human and chimpanzee genome sequences, all DNA sequence changes that determine phenotypic differences between species, including those related to cognitive functions, can, in principle, be identified. While changes in protein amino acid composition are relatively easy to identify based on genome sequence information, the vast majority of these changes are expected to be neutral with respect to phenotype. The notion that most amino acid substitutions that accumulate during evolution do not affect phenotype was first suggested by Motoo Kimura in 1968 and formally described in 1983 as the "Neutral Theory of Molecular Evolution" (Kimura, 1983). In practical terms, this means that only a small fraction of all DNA sequence changes observed between humans and chimpanzees are relevant to phenotypic differences between these two species. This makes identification of non-neutral changes a daunting task. As a consequence, only a very limited number of studies to date have been successful in bridging DNA sequence and phenotypic differences between species in general, and between humans and chimpanzees in particular. Nevertheless, there are several examples where protein changes have been linked to the evolution of human-specific cognitive features.

Arguably, the most prominent example of such changes discovered to date is given by the gene *FOXP2* (*forkhead box P2*). In humans, disruption of this gene causes a speech impairment, usually characterized by poor oralfacial movement control and accompanied by a disregulation of brain activity associated with speech (Lai et al., 2001; Vargha-Khadem et al., 2005). Although *FOXP2* is one of the most conserved genes in mammals, with only three amino acid substitutions occurring since the divergence of human and mouse species more

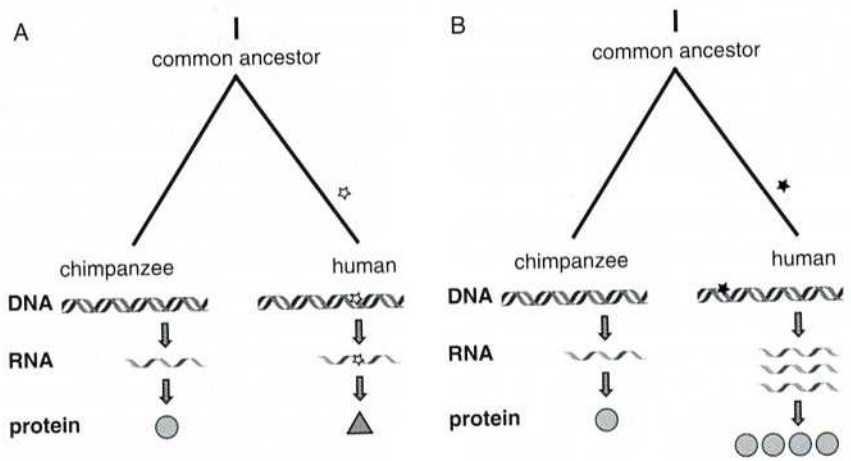

Fig. 2. Schematic representation of DNA sequence mutations on the human lineage (represented by a star) inducing structural (A) or regulatory (B) changes. Structural changes happen due to mutations within the protein-coding region and lead to an alteration in protein functional properties. Regulatory changes occur due to mutations that can take place almost anywhere in a genome and lead to an alteration in protein quantity.

than 80–120 million years ago, two of these substitutions have occurred on the human lineage within last 5–6 million years ago (Enard et al., 2002b). Additionally, an unusual pattern of nucleotide polymorphism around this gene is observed in extant humans and indicates that these mutations likely occurred within the last 200,000 years of human evolution, and then spread among the entire human population due to positive selection. These data strongly implicate *FOXP2* in the appearance of speech and language during recent human evolution, traits that likely provided a selective advantage to our ancestors. Interestingly, there are indications that *FOXP2* may be involved in evolution of vocal communication in other species as well. In mice, disruption of both copies of the *FOXP2* gene completely abolishes ultrasonic vocalization normally observed when pups are temporally removed from their mothers, while disruption of one copy of the gene significantly reduces these vocalizations (Shu et al., 2005). In song-learning birds such as zebra finches, the striatal nucleus Area X, necessary for vocal learning, expresses more *FOXP2* than the surrounding tissue during the period when vocal learning occurs (Haesler et al., 2004). Similarly, in adult canaries, transient increase in *FOXP2* expression in Area X is associated with instances of increased song variation. These examples strengthen the link between changes in FOXP2 and evolution of vocal communication, while highlighting the fact that even this trend cannot be characterized as uniquely human. Interestingly, while evolutionary adaptations in human *FOXP2* took place on the gene sequence level, in birds, these adaptations seem to have occurred on the level of gene expression regulation.

Other prominent examples of positive selection on the human lineage at the level of protein sequence are two genes involved in the regulation of brain size, *MCPH1* and *ASPM1*. Disruption of either of these genes in humans leads to primary microcephaly, a disorder described earlier in this chapter that is characterized by a dramatic reduction in brain size (Kumar et al., 2002). For both genes, an unusual increase in the number of amino acid substitutions is observed during ape evolution and particularly within the human lineage (Evans et al., 2004a, b). Moreover, adaptive differences between isoforms of these two proteins exist among extant humans (Evans et al., 2005; Mekel-Bobrov et al., 2005). Thus, although the major brain size increase on the human evolutionary lineage likely began as much as 2 million years ago (McHenry, 1994), more recent positive selection on *MCPH1* and *ASPM1* indicates that brain size regulation has probably played an important role in establishing modern brain functionality. It is particularly intriguing to think that the human brain may have been undergoing adaptive evolution in our very recent past. However, differences between distinct forms of *MCPH1* and *ASPM1* found in contemporary humans have not yet been directly linked to differences in cognitive abilities. Moreover, it is not exactly clear whether slight differences in cognition may confer any benefit to individuals in terms of reproductive success in contemporary society.

Other known examples of genes that show adaptive evolutionary changes at the protein sequence level include *MAOA* (*monoamine oxidase A*), *AHI1* (*Abelson helper integration site 1*), *GLUT2* (*glutamate dehydrogenase 2*) and *MYH16* (*myosin, heavy polypeptide 16*), genes potentially involved in the formation of human brain. Monoamine oxidase A (*MAOA*) has been implicated in different aspects of human behavior and cognition in a multitude of studies, though not without controversy (Andres et al., 2004; and references therein). Interestingly, patterns of polymorphism observed in this gene among contemporary humans indicate that it has undergone positive selection recently in human evolution (Gilad et al., 2002). This selection is human-specific and is not observed in apes (Andres et al., 2004). Structurally, the human form of *MAOA* contains only one non-synonymous amino acid substitution that may potentially influence protein dimerization properties. Although it is not clear whether selection acted on this structural change or on an as yet unknown regulatory change, the fact that *MAOA* was positively selected during human evolution certainly warrants further investigation of its potential role in the rise of human cognitive abilities.

Mutations disrupting the *AHI1* gene cause Joubert syndrome, a disorder characterized by abnormal axonal crossing in the brain that results in

motor disturbances, such as severe clumsiness and abnormal breathing and eye movements. In addition, patients with Joubert syndrome suffer from cognitive impairments and autistic behaviors (Saraiva and Baraitser, 1992; Ozonoff et al., 1999). While *AHI1* evolved unusually rapidly in all apes at the amino acid sequence level, this trend is accelerated on the human lineage (Ferland et al., 2004). Although this acceleration cannot be taken as proof that changes in the *AHI1* gene were directly connected to the evolution of human brain functions, it provides an indication for the adaptive value of these changes during human evolution.

GLUT2 and its closely related homologue *GLUT1* encode two forms of an enzyme (glutamate dehydrogenase) essential for the recycling of glutamate, a major excitatory neurotransmitter (Plaitakis et al., 2003). While *GLUT1* encodes an evolutionarily old, housekeeping-form of an enzyme, *GLUT2* is an ape-specific gene that that is believed to have originated by retrotransposition of a processed *GLUT1* copy less than 23 million years ago (Burki and Kaessmann, 2004). *GLUT1* remained unchanged after this event, whereas *GLUT2* rapidly evolved resulting in an enzyme form that is adapted to functioning in brain. Thus, *GLUT2* may have contributed to the evolution of enhanced brain function by accelerating neurotransmitter turnover rates in humans and apes. This adaptation took place both on the structural level, through multiple substitutions in the amino acid sequence of the protein, and on the regulatory level, resulting in preferential expression of the modified enzyme version in neural tissue.

MYH16 is an isoform of a sarcomeric myosin, a main structural component of muscle tissue. *MYH16* has been inactivated by a fame shift mutation in humans and this inactivation is associated with marked reduction of chewing musculature. The emergence of this inactivating mutation was dated to 2.4 million years ago, a time point that coincides with the commencement of brain size expansion on the hominid lineage (Stedman et al., 2004). Thus, it has been speculated that loss of the massive chewing musculature attached to the scull may have facilitated brain expansion by allowing an unconstrained increase in the scull volume. However, this link is contested based on the observation that Neanderthals possessed massive chewing musculature and, at the same time, brains that were on average slightly greater in volume than the brains of modern humans (Pennisi, 2004). In addition, another study analyzed a larger data set and estimated the age of inactivating mutation at 5.3 million years ago (Perry et al., 2005). The new date puts a large time gap between loss of *MYH16* function and brain size expansion on the hominid limeade, making association between these two events less plausible.

The preceding examples highlight the fact that genes involved in the evolution of human cognition can potentially be identified. However, these individual gene studies do not provide any estimate of the overall number of proteins that had to undergo changes during human evolution in order for the human brain to reach its contemporary state. A recent study compared protein evolution rates in primates, including humans, and rodents in two manually selected gene sets: 214 proteins implicated in various aspects of human nervous system function and 95 housekeeping genes (Dorus et al., 2004). The set of nervous system proteins evolved 30% faster in primates than in rodents, while no significant difference was found for the housekeeping set. This finding would imply that evolution of the human nervous system required a large number of amino acid changes in many proteins. However, amino acid sequence comparisons between humans and chimpanzees for more than 13,000 proteins did not confirm this observation (Consortium, 2005). In contrast, this study found that genes involved in neuronal function or genes expressed in human brain tend to show a slower rate of change in amino acid composition compared to other genes (Consortium, 2005; Khaitovich et al., 2005). Despite this rate difference, genes expressed in brain tend to have more amino acid substitutions on the human lineage than on the chimpanzee lineage (Khaitovich et al., 2005). Thus, it appears that human brain evolution required substantially more changes in protein sequence than evolution of the chimpanzee brain. This finding may not be too surprising given that, since the separation from the most recent common ancestor, much more profound changes in brain size and capacity happened on the human than on

the chimpanzee lineage. Further, it indicates that many more genes involved in the evolution of human cognition may be identified based on the patterns of protein structure changes between humans and other primates.

Gene expression evolution

As mentioned earlier in this chapter, the phenotypic differences we observe between species can be caused by changes in protein sequence as well as by changes in regulation of gene expression. Indeed, differences in gene expression can cause dramatic phenotypic changes. At all stages of development, an organism's genome remains unchanged. Thus, all of the organism's phenotypic changes throughout development, from egg to adult, are caused by changes in gene regulation. Similarly, within an organism, the great phenotypic and functional variation seen between different tissues is caused by differences in gene regulation. So, it is conceivable that in the most extreme case, all observed phenotypic differences between humans and chimpanzees could have been caused by differences in gene expression alone. The notion that the majority of phenotypic differences between humans and chimpanzees are caused by such gene regulation differences, and not by differences in protein sequence, was first introduced by Allen Wilson and Mary Claire King in 1975 (King and Wilson, 1975). Their pioneering work comparing both genome annealing kinetics and protein electrophoresis migration patterns in the two species revealed that the human and chimpanzee genomes differ, on average, in about 1% of their sequences and that a large proportion of proteins do not show any structural differences between species. Thirty years later, comparison between the completed human and chimpanzee genome sequences provided undisputable verification of these measurements. Indeed, human and chimpanzee genome sequences differ from one another by an average of 1.23% on the point mutation level and approximately 1/3 of the 13,454 proteins compared between the two species have no amino acid substitutions (Consortium, 2005). Nevertheless, an average sequence difference of 1.23% adds up to more than 30 million point mutations between humans and chimpanzees. In addition to these point mutations, genomic differences between the two species include a multitude of small and large sequence insertions, deletions, duplications and inversions. These rearrangements vary in scale from genome regions spanning only a few nucleotides to whole chromosome changes. Such large-scale rearrangement is most dramatically illustrated by the fusion of two ancestral chromosomes during human evolution producing modern human chromosome 2, thereby creating the chromosome number difference between humans and chimpanzees we observe today (Wienberg et al., 1994). All of these genomic differences can potentially contribute to differences in gene regulation between the two species. However, while differences in protein amino acid composition can be deduced from comparisons between the human and chimpanzee genomes, differences in gene regulation cannot, at present, be recognized based on genomic information alone. Several factors contribute to this limitation: first, regulatory sequence elements for a given gene can be located anywhere in a genome; second, multiple sequence elements and protein factors regulate expression of each gene; third, expression regulation differs between tissues; and fourth, expression regulation can change with time depending on input from within and from outside the organism.

Fortunately, the gene expression levels of all known genes can be measured directly using microarray technology and this information can be used to study evolution (Fig. 3). In recent years, several studies used microarrays to investigate expression differences between humans, chimpanzees and other primates in brain and other tissues. As a result of this work, the following conclusions emerge. First, few genes can be described as "brain-specific" in terms of their expression in humans (Su et al., 2002, 2004). The vast majority of genes are expressed in many tissues and in many different brain regions. Thus, differences in gene expression between tissues are mainly quantitative rather than qualitative. Second, approximately 5–10% of genes expressed in adult brain are expressed at significantly different levels in humans and chimpanzees (Enard et al., 2002a, b; Caceres

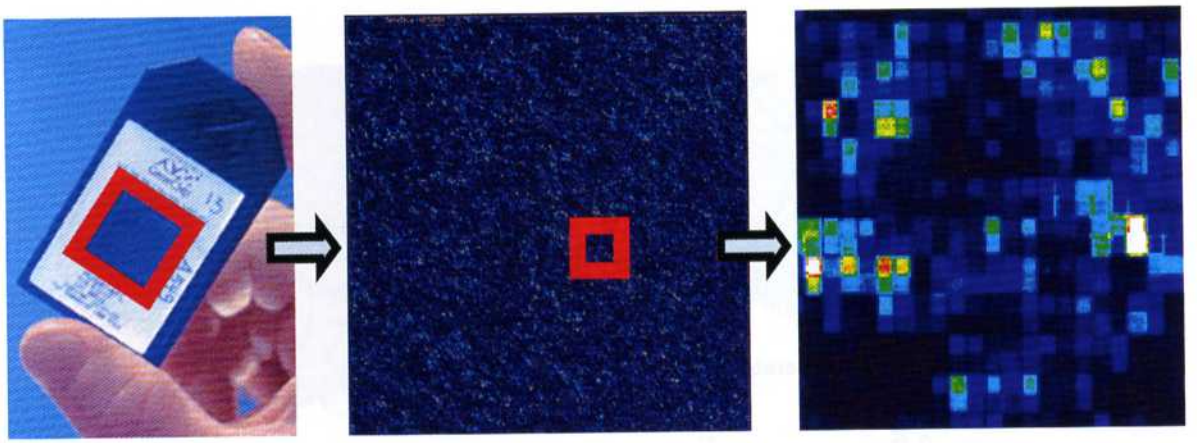

Fig. 3. Schematic representation of an Affymetrix® microarray, one of the most common types of microarrays currently used in large-scale gene expression analysis. While the size of the microarray field highlighted in red on the first panel is approximately 1 × 1 cm, the size of features corresponding to different microarray probes is approximately 10 × 10 μm.

et al., 2003; Enard et al., 2002a; Khaitovich et al., 2004a; Uddin et al., 2004). This may seem like a small percentage of genes but, given that more than half of all known human genes are expressed in brain, this adds up to approximately 1000 differently expressed genes. The proportion of genes differently expressed between humans and chimpanzees in brain is similar to that observed in other tissues like heart, kidney or liver (Khaitovich et al., 2005). However, the amplitude of expression differences observed in brain in significantly smaller than that observed in the other tissues studied to date, indicating that brain is one of the most conserved tissues in terms of gene regulation. Interestingly, genes expressed in brain show significantly fewer amino acid differences between humans and chimpanzees compared to genes expressed in the other tissues (Khaitovich et al., 2005). Thus, brain-expressed genes are under more constraint on both regulatory and structural levels than genes expressed in other tissues. Third, when expression differences observed between humans and chimpanzees are divided into two categories, one that occurred on the human lineage and the other one that occurred on the chimpanzee lineage, the differences appear to be distributed unequally. Namely, in brain, noticeably more regulatory changes have occurred on the human than on the chimpanzee lineage (Caceres et al., 2003; Enard et al., 2002a; Gu and Gu, 2003; Hsieh et al., 2003; Khaitovich et al., 2005). This asymmetry is observed in other tissues as well, but never to the extent seen in brain. Fourth, distinct brain regions involved in very different cognitive functions, such Broca's area, prefrontal, cingulate and primary visual regions of the cerebral cortex, and even non-cortical areas such as caudate nucleus and cerebellum, show almost the same expression differences between humans and chimpanzees (Fig. 4) (Khaitovich et al., 2004a). Thus, functionally distinct areas of the human brain appear to diverge equally far on the gene regulation level between humans and chimpanzees. Fifth, within brain, differences between various brain regions appear to be highly conserved in primate species. For example, all expression differences observed between various cortical regions in humans are also found in chimpanzees and even in rhesus macaque brains (Khaitovich et al., 2004a). Additionally, more ancient regions of the brain show more expression differences compared to younger brain regions (Fig. 5) (Khaitovich et al., 2004b). Hence, regulatory divergence of different brain areas does occur with time, but at a much slower pace than regulatory divergence between different species. This is not surprising given that regulatory changes within the brain must occur on the same genotypic background while between species genetic

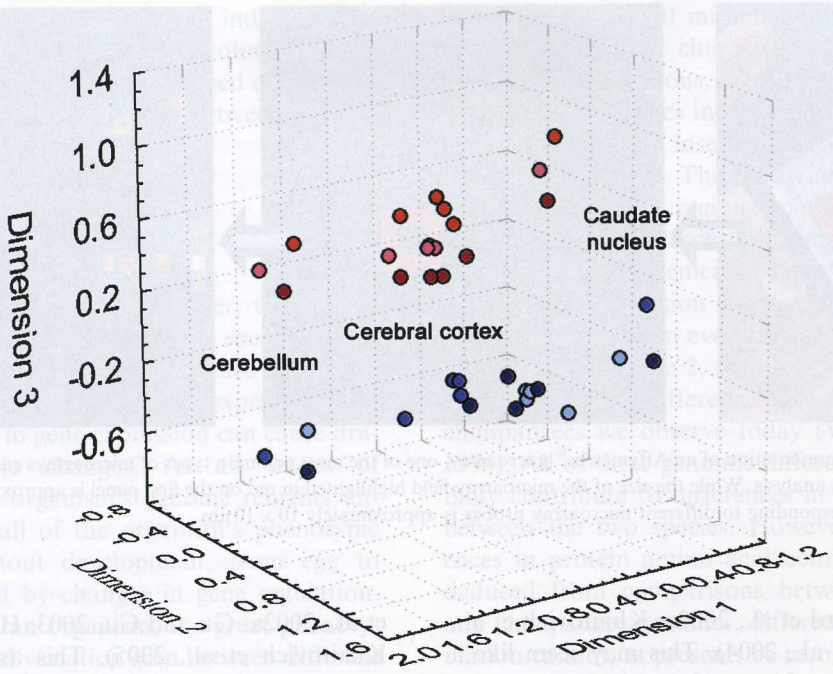

Fig. 4. Multidimensional scaling plot representing expression differences observed in brain within and between species. Each circle represents one sample. Chimpanzee samples are shown in red and human in blue. Different shades of color represent different individuals. Different brain regions form the same pattern in both species with most expression differences found between cerebellum, cortex and caudate nucleus. Despite large differences in expression between these regions within species, they all differ between species to the same extent.

divergence can cause more rapid accumulation of regulatory differences. However, genotypic changes between species are the result of random mutations that should be largely neutral or deleterious for the organism and only very rarely lead to adaptive changes. Thus, the question once asked for structural changes during the early days of genome studies now arises for studies of regulatory evolution: How many of the regulatory differences observed between species are relevant to differences in phenotype and how many differences are neutral?

Theory of gene expression evolution

In the 1950–1960s, at the dawn of DNA sequence studies, every mutation resulting in an amino acid substitution was thought to yield a functional change that would be either deleterious or advantageous for the organism. Within a population, it was believed that advantageous mutations would be quickly swept to fixation by Darwinian (positive) selection, while deleterious mutations would be weeded out by purifying (negative) selection (Darwin, 1859). This view persisted until the end of 1960s, when Motoo Kimura proposed the neutral theory of molecular evolution. As mentioned earlier in this chapter, this groundbreaking theory postulated that the vast majority of DNA sequence substitutions observed both within and between species have no effect on an organism's phenotype and thus are evolutionary neutral (Kimura, 1983). This theory stemmed from the observation that the number of amino acid substitutions between species far exceeds the number expected, assuming total functionality for each substitution (Kimura, 1968). Kimura's theory that most mutations are evolutionary neutral solves this contradiction and, at the same time, predicts that DNA mutations leading to amino acid substitutions should accumulate linearly with time and show little

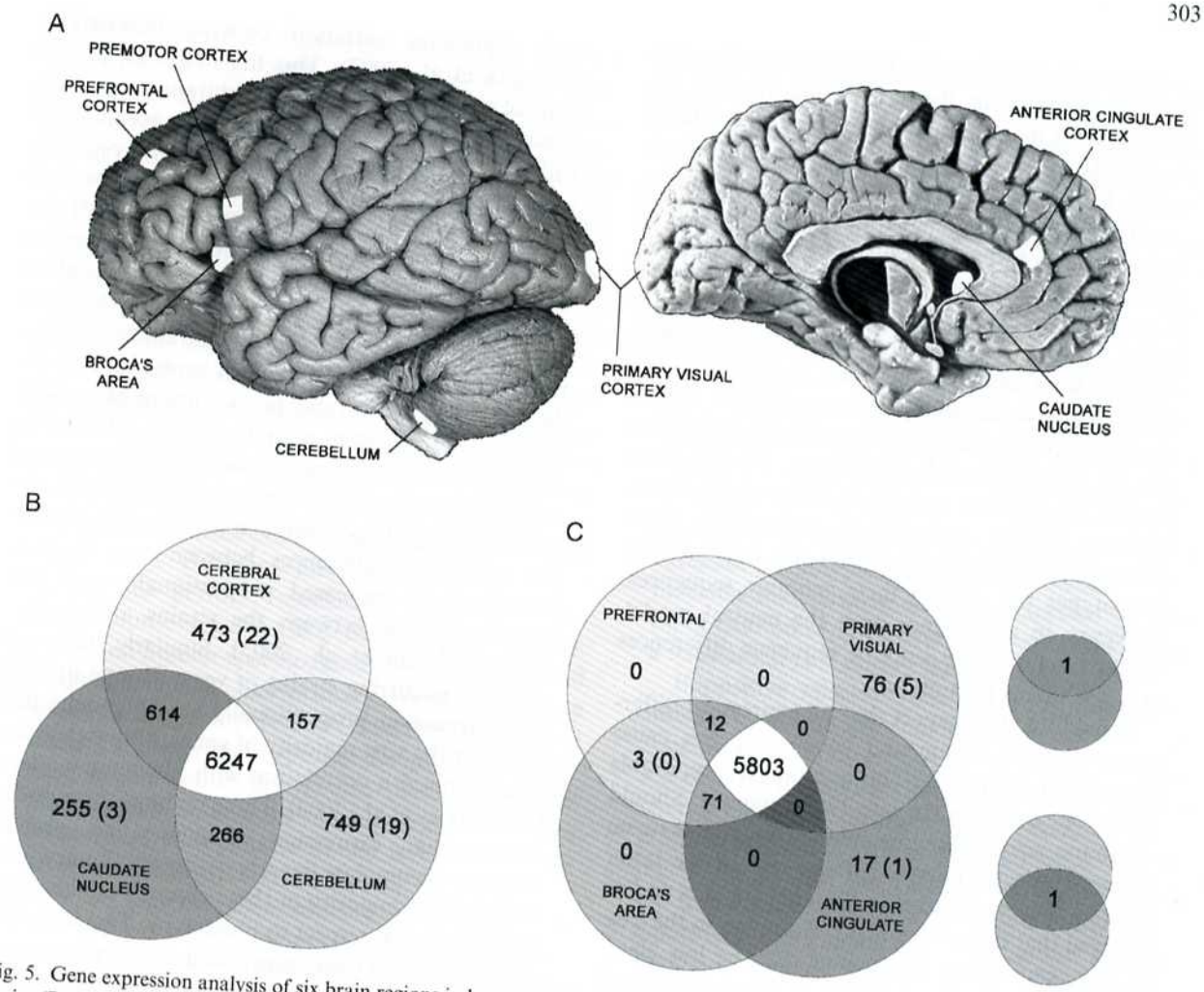

Fig. 5. Gene expression analysis of six brain regions in humans and chimpanzees. (A) Location of studied brain regions in the human brain. (B and C) Diagram showing numbers of significant expression differences observed between different brain regions in humans. Numbers in parenthesis indicate numbers of genes where differences between brain regions found in humans were not seen in chimpanzees. While different brain regions like cortex, cerebellum and caudate nucleus are substantially different from each other in their expression, almost no differences are seen between different cortical regions. Moreover, the vast majority of expression differences between brain regions found in humans are also observed in chimpanzees.

correlation with phenotypic changes. Decades of experimental studies unambiguously confirmed these predictions, making the neutral theory of molecular evolution a widely accepted null-hypothesis for every observed structural change. Importantly, this theoretical model of neutral evolution (also called the neutral model) allows for the development of tests designed to find significant deviation from the substitution patterns predicted by the model. Such unusual substitution patterns likely indicate sequence differences influenced by positive selection and thus suggest functional significance for the change. In the decades following Kimura's initial observation, several statistical tests to identify such patterns were introduced and have proven instrumental in finding genomic patterns bearing signatures of functional adaptations (Hudson et al., 1987; Tajima, 1989; McDonald and Kreitman, 1991; Fu and Li, 1993; Fay and Wu, 2003). In fact, all examples of genes involved in human brain evolution given thus far in this chapter were identified using these tests.

As described earlier, the introduction of microarray technology in the late 1990s allowed the simultaneous measurement of expression levels for thousands of genes. This important technical advance led to a multitude of studies measuring changes in gene expression due to physiological differences in test organisms, for example, comparisons between control and diseased individuals in cancer studies or between different cell cycle stages in yeast. In most of these cases, expression changes were observed between groups with the same genomic sequence, thus expression differences reflect regulatory variation due to phenotypic or environmental differences. Evolutionary studies, in contrast, usually involve comparisons between groups representing individuals from different species and thus differ not only in their phenotypes but also in their genomic sequences. However, in accordance with the neutral model, the vast majority of genomic sequence differences may not affect phenotype.

Should we then expect that all expression differences observed within and between species cause phenotypic differences? This question is comparable to the one asked in the late 1960s, namely: Should all amino acid differences seen within and between species cause phenotypic differences? In the latter case, as discussed earlier in this chapter, it is widely believed that the answer is no. However, in the case of gene expression, the answer may be less clear. Even for closely related species, such as humans and chimpanzees or different species of mice, at least 5% of all expressed genes show significantly different expression levels between species in every tissue studied thus far (Khaitovich et al., 2005). Adding further complexity to the question, unlike amino acid substitutions, there is no theory to date that allows estimation of the number of expected, functional expression differences. However, it is possible to test whether the predictions of the neutral theory formulated for amino acid changes can also be applied to gene expression evolution. More specifically, do expression changes accumulate linearly with time and show little correlation with phenotypic changes? Experiments in several naturally occurring populations of teleost fish of the genus *Fundulus* showed that, for most genes, expression variation within populations positively correlates with expression variation between populations (Oleksiak et al., 2002). This fits well with predictions of the neutral theory of evolution, which postulates that for most genes variation accumulates with time at the same rate within and between populations. However, a large proportion of expression variation between fish populations in this study depended on the environment in which these populations lived. Interestingly, this expression variation persisted even when all fish were raised in the same environment. This observation contradicts expectations from the neutral theory of evolution and indicates that a measurable proportion of expression changes can correlate with functional alterations. Despite this contradiction, most evolutionary gene expression studies show strong agreement with the neutral theory. Experiments in fruit fly showed that the expression differences between two developmental stages increased proportionally with the genetic distance between the strains and species studied (Rifkin et al., 2003). Similarly, different naturally occurring strains of yeast diverge in their gene expression proportionally to the genetic distance for the vast majority of genes, although a few genes showed a correlation with phenotypic adaptations (Fay et al., 2004). These findings support the notion that expression changes, like genetic changes, accumulate linearly with evolutionary time and that the majority of these changes are likely to be neutral with respect to phenotype. This idea was explicitly tested using expression data from several primate and mouse species. As predicted by the neutral theory, expression divergence between these species accumulates linearly with time in the tissues studied and expression variation within species correlates with expression divergence between species (Khaitovich et al., 2004). These findings provide strong support for the idea that most expression changes, like structural/sequence changes, comply with the neutral theory of evolution.

Here it must be noted that on both the gene expression and gene structure levels, many changes that occur in the population are not neutral but rather deleterious to the organism. Such changes will not become fixed in a population, but will be eliminated by negative selection. Indeed, studies in nematodes, fruit flies and primate tissues clearly demonstrate that negative selection has a strong

effect on evolutionary gene expression changes (Denver et al., 2005; Khaitovich et al., 2005; Rifkin et al., 2005). Research in model organisms provides additional support for the observation that most expression changes that are not removed by negative selection do not lead to phenotypic changes, and are therefore evolutionary neutral. These studies indicate that up to 32% of yeast genes and up to 85% of nematode genes can be individually inactivated without any detectable phenotypic alterations (see, for instance, Brookfield, 1997; Kim, 2001). Most expression changes seen between populations or between species are much less drastic than complete gene inactivation and are thus even less likely to induce phenotypic changes. Additionally, expression divergence between species does not appear to reflect functional divergence. Different human brain regions are known to perform specialized cognitive tasks. While some regions, like Broca's area, are involved in human-specific functions like speech and language, others, like primary visual cortex, are thought to be involved in functions conserved among most primate species. Despite this difference in functional divergence, these distinct regions appear to have the same divergence between human and chimpanzees at the level of gene expression (Khaitovich et al., 2004a).

Taken together, these observations suggest that a large proportion of expression changes are neutral with respect to phenotype. Thus, use of the neutral model as a null hypothesis for gene expression evolution is warranted. As described for gene structure evolution, significant deviation in gene expression from patterns expected under the neutral model would indicate changes influenced by positive selection. However, the current absence of a solid theoretical basis for modeling expression evolution and the lack of reliable markers allowing estimation of the neutral change rate prevents assessment of the actual proportion of expression changes influenced by positive selection.

Adaptive human brain evolution

While the genetic distance between humans and our closest living relatives, chimpanzees, just slightly exceeds 1%, the phenotypic differences between the two species are far greater than between any other pair of primate species with similar genetic divergence. Based on current knowledge about the two species' common ancestor, most of these phenotypic changes, including increase in brain size, happened on the human lineage. This is especially interesting because genetic mutation, the molecular substrate of phenotypic change, occurs at approximately the same frequency in humans and chimpanzees. While the majority of genetic mutations have either no effect on phenotypic traits or are deleterious, a small proportion of mutations can confer an individual with a selective advantage. Such mutations spread rapidly within populations due to Darwinian (positive) selection. The apparent excess of phenotypic changes on the human lineage indicates that positive selection, expected to cause rapid fixation of advantageous phenotypes, played a substantial role during human evolution.

If positive selection was pervasive throughout human evolution, we might expect to see more functional changes occurring on the human lineage than on that of the chimpanzee. On the level of overall genomic divergence, however, no such effect is apparent. This is expected since, according to the neutral theory of molecular evolution, the overwhelming majority of genomic mutations separating the human and chimpanzee genomes have no effect on phenotype and accumulate in proportion to divergence time. However, when differences in protein sequence and expression regulation for genes expressed in human brain are considered, there are indeed more changes on the human lineage than on the chimpanzee lineage for genes expressed in this tissue, but not in any other tissues studied (Enard et al., 2002a; Caceres et al., 2003; Khaitovich et al., 2005). Thus, during human evolution, a detectable amount of genetic change affecting human brain function gave a selective advantage to the ancestors of modern humans. Further, the genetic features that conferred this advantage can potentially be identified.

Why is it important to identify positively selected changes? One of the central aims of modern biology is identification of gene structure and gene regulation changes responsible for phenotypic alteration. This statement is just as applicable to studies of the

genetic basis for human diseases as it is to evolutionary studies. In disease research, detection of genes responsible for dysfunction may shed light on the molecular mechanisms of human disorders. Similarly, in evolutionary studies of human brain, finding genes related to human-specific brain functions would illuminate the molecular mechanisms involved with cognition. In the case of human brain, such information is particularly valuable since the molecular pathways underlying human cognition are largely unknown. How can one identify genes involved with such pathways? Studies of human psychiatric and neurological disorders affecting human-specific cognitive functions can lead to identification of genetic changes. However, genes identified in this way are not necessarily causal to the affected function and may also be involved in more basic neural functions not limited to humans. Here, evolutionary data may provide critical, additional information. If a gene found through analysis of a psychiatric or neurological disorder also bears a signature of recent-positive selection, this would strongly indicate its direct involvement in human-specific cognitive mechanisms. Several such cases of recent positive selection in genes associated with human cognition, including *FOXP2*, *MCPH1*, *ASPM1*, *MAOA*, *AHI1*, *GLUT2* and *MYH16* were discussed earlier in this chapter.

The only known example of positive selection at the level of gene regulation was shown in the altered regulation of *prodynorphin* (*PDYN*), a precursor molecule for a number of endogenous opioids and neuropeptides with roles in perception, behavior and memory (Rockman et al., 2005). This study showed that the promoter variant causing elevated expression of *PDYN* was positively selected during recent human evolution prior to the human spread from Africa. Furthermore, positive selection on different promoter variants continued in different human populations, potentially yielding regulatory differences adaptive to certain environmental or cultural conditions. The fact that only one example of positive selection on the gene regulation level has been discovered does not necessarily mean that regulatory changes were subjected to positive selection with less frequency during human evolution than structural changes. Rather, it may reflect the fact that positive selection on regulatory changes is more difficult to identify because regulatory sequences can be located almost anywhere in the genome and regulatory changes themselves are much less well defined than the structural ones. Given that in brain, but not in other tissues, there are more regulatory changes overall on the human lineage than on chimpanzee lineage, a substantial influence of positive selection on gene regulation likely occurred in our evolutionary history. On the other hand, the excess of regulatory changes observed on the human lineage in brain may indicate relaxation of negative selection, allowing genes expressed in human brain to vary more without any detrimental effect on phenotype. Given the increased complexity and capacity of the human brain, the latter explanation may not seem the most plausible but our limited knowledge of molecular mechanisms determining human brain functions prevents it from being ruled out. It is, however, possible to distinguish between these two alternative explanations using data on genome-wide patterns of human polymorphism. These patterns can illuminate positive selection events that took place in the last 200,000–500,000 years. If positive selection played substantial role in shaping regulatory changes that took place during recent human evolution, we would expect to see a positive correlation between such patterns and expression differences that can be currently observed between humans and other primates. Indeed, such a correlation can be seen in brain, but not in the other tissues studied (Khaitovich et al., 2006). This finding indicates that regulatory changes played a substantial role in shaping human-specific cognitive abilities. Identification of these changes remains a challenge, but this challenge is no longer insurmountable.

Conclusion

Studies of human brain function and dysfunction have provided a wealth of data, including the identification of many genes essential for the normal human condition. Though less extensive by comparison in terms of scope and amplitude, molecular evolution studies have provided evidence that changes in some of these genes gave a selective

advantage to our ancestors, and are thus primary candidates in the search for genes responsible for the evolution of human-specific cognitive functions. Of course, many more genes involved in the evolution of human cognition, with changes affecting both gene structure and gene regulation, are yet to be identified. Since the rise of new cognitive abilities during human evolution should have given the individuals possessing these abilities a selective advantage, recently selected changes can be recognized in the nucleotide polymorphism patterns among contemporary humans. Identification of genes affected by positive selection, either on regulatory or structural levels, may help us discover the molecular mechanisms underlying human-specific brain functions. The advantage of this evolutionary approach is that it is not limited to genes already implicated in neurological function through disease studies, but rather allows the identification of completely new and unexpected functional changes. There is no doubt that dramatic expansion and improvement of genetic and regulatory data in humans and non-human primates, combined with advances in medical and neurobiological studies, will provide new insights into human cognition — perhaps even before this book is published.

Acknowledgments

We thank Svante Pääbo for guidance, and all members of our research group for their practical assistance and inspiring discussions. Special thanks to Wolfgang Enard, Ines Hellmann and Michael Lachmann for their insight and helpful suggestions. The original work described in this review was financially supported by the Max Planck Society and the Bundesministerium für Bildung und Forschung.

References

Amunts, K., Schleicher, A., Burgel, U., Mohlberg, H., Uylings, H.B., et al. (1999) Broca's region revisited: cytoarchitecture and intersubject variability. J. Comp. Neurol., 412: 319–341.

Andres, A.M., Soldevila, M., Navarro, A., Kidd, K.K., Oliva, B., et al. (2004) Positive selection in MAOA gene is human exclusive: determination of the putative amino acid change selected in the human lineage. Hum. Genet., 115: 377–386.

Broca, P.P. (1861) Perte de la parole, ramollissement chronique et destruction partielle du lobe antérieur gauche. [Sur le siège de la faculté du langage.]. Bull. Soc. Anthropol., 2: 235–238.

Brookfield, J.F. (1997) Genetic redundancy: screening for selection in yeast. Curr. Biol., 7: R366–R368.

Burki, F. and Kaessmann, H. (2004) Birth and adaptive evolution of a hominoid gene that supports high neurotransmitter flux. Nat. Genet., 36: 1061–1063.

Caceres, M., Lachuer, J., Zapala, M.A., Redmond, J.C., Kudo, L., et al. (2003) Elevated gene expression levels distinguish human from non-human primate brains. Proc. Natl. Acad. Sci. USA, 100: 13030–13035.

Cantalupo, C. and Hopkins, W.D. (2001) Asymmetric Broca's area in great apes. Nature, 414: 505.

Consortium, C.S.a.A. (2005) Initial sequence of the chimpanzee genome and comparison with the human genome. Nature, **437**: 69–87.

Corballis, P.M., Funnell, M.G. and Gazzaniga, M.S. (2000) An evolutionary perspective on hemispheric asymmetries. Brain Cogn., 43: 112–117.

Darwin, C. (1859). The Origin of Species by Means of Natural Selection; or, the Preservation of Favored Races in the Struggle for Life. Murray, London.

Denver, D.R., Morris, K., Streelman, J.T., Kim, S.K., Lynch, M., et al. (2005) The transcriptional consequences of mutation and natural selection in Caenorhabditis elegans. Nat. Genet., 37: 544–548.

Dorus, S., Vallender, E.J., Evans, P.D., Anderson, J.R., Gilbert, S.L., et al. (2004) Accelerated evolution of nervous system genes in the origin of Homo sapiens. Cell, 119: 1027–1040.

Enard, W., Khaitovich, P., Klose, J., Zollner, S., Heissig, F., et al. (2002a) Intra- and interspecific variation in primate gene expression patterns. Science, 296: 340–343.

Enard, W., Przeworski, M., Fisher, S.E., Lai, C.S., Wiebe, V., et al. (2002b) Molecular evolution of FOXP2, a gene involved in speech and language. Nature, 418: 869–872.

Evans, P.D., Anderson, J.R., Vallender, E.J., Choi, S.S. and Lahn, B.T. (2004a) Reconstructing the evolutionary history of microcephalin, a gene controlling human brain size. Hum. Mol. Genet., 13: 1139–1145.

Evans, P.D., Anderson, J.R., Vallender, E.J., Gilbert, S.L., Malcom, C.M., et al. (2004b) Adaptive evolution of ASPM, a major determinant of cerebral cortical size in humans. Hum. Mol. Genet., 13: 489–494.

Evans, P.D., Gilbert, S.L., Mekel-Bobrov, N., Vallender, E.J., Anderson, J.R., et al. (2005) Microcephalin, a gene regulating brain size, continues to evolve adaptively in humans. Science, 309: 1717–1720.

Fay, J.C. and Wu, C.I. (2003) Sequence divergence, functional constraint, and selection in protein evolution. Annu. Rev. Genomics Hum. Genet., 4: 213–235.

Fay, J.C., McCullough, H.L., Sniegowski, P.D., Eisen, M.B., et al. (2004) Population genetic variation in gene expression is

associated with phenotypic variation in *Saccharomyces cerevisiae*. Genome Biol., 5: R26.

Ferland, R.J., Eyaid, W., Collura, R.V., Tully, L.D., Hill, R.S., et al. (2004) Abnormal cerebellar development and axonal decussation due to mutations in AHI1 in Joubert syndrome. Nat. Genet., 36: 1008–1013.

Fu, Y.X. and Li, W.H. (1993) Statistical tests of neutrality of mutations. Genetics, 133: 693–709.

Gannon, P.J., Holloway, R.L., Broadfield, D.C. and Braun, A.R. (1998) Asymmetry of chimpanzee planum temporale: humanlike pattern of Wernicke's brain language area homolog. Science, 279: 220–222.

Geschwind, N. and Levitsky, W. (1968) Human brain: left–right asymmetries in temporal speech region. Science, 161: 186–187.

Gilad, Y., Rosenberg, S., Przeworkski, M., Lancet, D. and Skorecki, K. (2002) Evidence for positive selection and population structure at the human MAO-A gene. Proc. Natl. Acad. Sci. USA, 99: 862–867.

Gilbert, S.L., Dobyns, W.B. and Lahn, B.T. (2005) Genetic links between brain development and brain evolution. Nat. Rev. Genet., 6: 581–590.

Glazko, G.V. and Nei, M. (2003) Estimation of divergence times for major lineages of primate species. Mol. Biol. Evol., 20: 424–434.

Gu, J. and Gu, X. (2003) Induced gene expression in human brain after the split from chimpanzee. Trends Genet., 19: 63–65.

Haesler, S., Wada, K., Nshdejan, A., Morrisey, E.E., Lints, T., et al. (2004) FoxP2 expression in avian vocal learners and non-learners. J. Neurosci., 24: 3164–3175.

Herndon, J.G., Tigges, J., Anderson, D.C., Klumpp, S.A. and McClure, H.M. (1999) Brain weight throughout the life span of the chimpanzee. J. Comp. Neurol., 409: 567–572.

Hopkins, W.D., Marino, L., Rilling, J.K. and MacGregor, L.A. (1998) Planum temporale asymmetries in great apes as revealed by magnetic resonance imaging (MRI). Neuroreport, 9: 2913–2918.

Hopkins, W.D., Russell, J.L., Cantalupo, C., Freeman, H. and Schapiro, S.J. (2005) Factors influencing the prevalence and handedness for throwing in captive chimpanzees (Pan troglodytes). J. Comp. Psychol., 119: 363–370.

Hsieh, W.P., Chu, T.M., Wolfinger, R.D. and Gibson, G. (2003) Mixed-model reanalysis of primate data suggests tissue and species biases in oligonucleotide-based gene expression profiles. Genetics, 165: 747–757.

Hudson, R.R., Kreitman, M. and Aguade, M. (1987) A test of neutral molecular evolution based on nucleotide data. Genetics, 116: 153–159.

Kandel, E.R., Schwartz, J.H. and Jessell, T.M. (2000) Principles of Neural Science. McGraw-Hill Health Professions Division, New York.

Khaitovich, P., Hellmann, I., Enard, W., Nowick, K., Leinweber, M., et al. (2005) Parallel patterns of evolution in the genomes and transcriptomes of humans and chimpanzees. Science, 309: 1850–1854.

Khaitovich, P., Muetzel, B., She, X., Lachmann, M., Hellmann, I., et al. (2004a) Regional patterns of gene expression in human and chimpanzee brains. Genome Res., 14: 1462–1473.

Khaitovich, P., Tang, K., Franz, H., Kelso, J., Hellmann, I., et al. (2006) Positive selection on gene expression in the human brain. Curr. Biol., 16: R356–R358.

Khaitovich, P., Weiss, G., Lachmann, M., Hellmann, I., Enard, W., et al. (2004b) A neutral model of transcriptome evolution. PLoS Biol., 2: E132.

Kim, S.K. (2001) *C. elegans*: mining the functional genomic landscape. Nat. Rev. Genet., 2: 681–689.

Kimura, M. (1968) Evolutionary rate at the molecular level. Nature, 217: 624–626.

Kimura, M. (1983) The neutral theory of molecular evolution. Cambridge University Press, Cambridge.

King, M.C. and Wilson, A.C. (1975) Evolution at two levels in humans and chimpanzees. Science, 188: 107–116.

Kornack, D.R. and Rakic, P. (1998) Changes in cell-cycle kinetics during the development and evolution of primate neocortex. Proc. Natl. Acad. Sci. USA, 95: 1242–1246.

Kumar, A., Markandaya, M. and Girimaji, S.C. (2002) Primary microcephaly: microcephalin and ASPM determine the size of the human brain. J. Biosci., 27: 629–632.

Lai, C.S., Fisher, S.E., Hurst, J.A., Vargha-Khadem, F. and Monaco, A.P. (2001) A forkhead-domain gene is mutated in a severe speech and language disorder. Nature, 413: 519–523.

Marin-Padilla, M. (1992) Ontogenesis of the pyramidal cell of the mammalian neocortex and developmental cytoarchitectonics: a unifying theory. J. Comp. Neurol., 321: 223–240.

McDonald, J.H. and Kreitman, M. (1991) Adaptive protein evolution at the Adh locus in Drosophila. Nature, 351: 652–654.

McHenry, H.M. (1994) Tempo and mode in human evolution. Proc. Natl. Acad. Sci. USA, 91: 6780–6786.

Mekel-Bobrov, N., Gilbert, S.L., Evans, P.D., Vallender, E.J., Anderson, J.R., et al. (2005) Ongoing adaptive evolution of ASPM, a brain size determinant in Homo sapiens. Science, 309: 1720–1722.

Nimchinsky, E.A., Gilissen, E., Allman, J.M., Perl, D.P., Erwin, J.M., et al. (1999) A neuronal morphologic type unique to humans and great apes. Proc. Natl. Acad. Sci. USA, 96: 5268–5273.

Oleksiak, M.F., Churchill, G.A. and Crawford, D.L. (2002) Variation in gene expression within and among natural populations. Nat. Genet., 32: 261–266.

Ozonoff, S., Williams, B.J., Gale, S. and Miller, J.N. (1999) Autism and autistic behavior in Joubert syndrome. J. Child Neurol., 14: 636–641.

Pennisi, E. (2004) Human evolution. The primate bite: brawn versus brain? Science, 303: 1957.

Perry, G.H., Verrelli, B.C. and Stone, A.C. (2005) Comparative analyses reveal a complex history of molecular evolution for human MYH16. Mol. Biol. Evol., 22: 379–382.

Plaitakis, A., Spanaki, C., Mastorodemos, V. and Zaganas, I. (2003) Study of structure-function relationships in human glutamate dehydrogenases reveals novel molecular mechanisms for the regulation of the nerve tissue-specific (GLUD2) isoenzyme. Neurochem. Int., 43: 401–410.

Rifkin, S.A., Houle, D., Kim, J. and White, K.P. (2005) A mutation accumulation assay reveals a broad capacity for rapid evolution of gene expression. Nature, 438: 220–223.

Rifkin, S.A., Kim, J. and White, K.P. (2003) Evolution of gene expression in the *Drosophila melanogaster* subgroup. Nat. Genet., 33: 138–144.

Rilling, J.K. and Insel, T.R. (1999) The primate neocortex in comparative perspective using magnetic resonance imaging. J. Hum. Evol., 37: 191–223.

Rockman, M.V., Hahn, M.W., Soranzo, N., Zimprich, F., Goldstein, D.B., et al. (2005) Ancient and recent positive selection transformed opioid cis-regulation in humans. PLoS Biol., 3: e387.

Saraiva, J.M. and Baraitser, M. (1992) Joubert syndrome: a review. Am. J. Med. Genet., 43: 726–731.

Shu, W., Cho, J.Y., Jiang, Y., Zhang, M., Weisz, D., et al. (2005) Altered ultrasonic vocalization in mice with a disruption in the Foxp2 gene. Proc. Natl. Acad. Sci. USA, 102: 9643–9648.

Stedman, H.H., Kozyak, B.W., Nelson, A., Thesier, D.M., Su, L.T., et al. (2004) Myosin gene mutation correlates with anatomical changes in the human lineage. Nature, 428: 415–418.

Su, A.I., Cooke, M.P., Ching, K.A., Hakak, Y., Walker, J.R., et al. (2002) Large-scale analysis of the human and mouse transcriptomes. Proc. Natl. Acad. Sci. USA, 99: 4465–4470.

Su, A.I., Wiltshire, T., Batalov, S., Lapp, H., Ching, K.A., et al. (2004) A gene atlas of the mouse and human protein-encoding transcriptomes. Proc. Natl. Acad. Sci. USA, 101: 6062–6067.

Tajima, F. (1989) Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics, 123: 585–595.

Uddin, M., Wildman, D.E., Liu, G., Xu, W., Johnson, R.M., et al. (2004) 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.

Vargha-Khadem, F., Gadian, D.G., Copp, A. and Mishkin, M. (2005) FOXP2 and the neuroanatomy of speech and language. Nat. Rev. Neurosci, 6: 131–138.

Wernicke, K. (1874) Der Aphasische Symptomencomplex. Eine Psychologische Studie auf anatomischer Basis. M. Crohn und Weigert, Breslau.

Wienberg, J., Jauch, A., Ludecke, H.J., Senger, G., Horsthemke, B., et al. (1994) The origin of human chromosome 2 analyzed by comparative chromosome mapping with a DNA microlibrary. Chromosome Res., 2: 405–410.

Woods, C.G., Bond, J. and Enard, W. (2005) Autosomal recessive primary microcephaly (MCPH): a review of clinical, molecular, and evolutionary findings. Am. J. Hum. Genet., 76: 717–728.