# OPINION

# Genetic links between brain development and brain evolution

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Abstract | The most defining biological attribute of *Homo sapiens* is its enormous brain size and accompanying cognitive prowess. How this was achieved by means of genetic changes over the course of human evolution has fascinated biologists and the general public alike. Recent studies have shown that genes controlling brain development - notably those implicated in microcephaly (a congenital defect that is characterized by severely reduced brain size) — are favoured targets of natural selection during human evolution. We propose that genes that regulate brain size during development, such as microcephaly genes, are chief contributors in driving the evolutionary enlargement of the human brain. Based on the synthesis of recent studies, we propose a general methodological template for the genetic analysis of human evolution.

As a species, *Homo sapiens* is markedly different from other mammals in several biological domains, particularly the nervous and reproductive systems, skeleton, skin and vocal organs. Perhaps none is as notable as the brain, which is significantly larger and more complex in humans than in other mammals<sup>1</sup>, and which allows our species to exhibit its uniquely rich and highly sophisticated behavioural repertoires, such as language, tool use, self-awareness, symbolic thought and cultural learning<sup>2</sup>.

From a genetic perspective, the larger and more complex human brains must arise from human-specific functional properties of the genes that underlie brain biology<sup>3,4</sup>. Despite a long-standing interest in the genetic basis of human brain evolution, and despite the success of modern genetics in dissecting the relationship between genes and phenotypes, little is known about which genetic changes in the human lineage are responsible for the markedly altered brain phenotype. For many decades, investigators have focused on the neuroanatomical, neurophysiological and behavioural differences between humans and other species<sup>1,5-7</sup>, with scant effort devoted to finding the molecular changes that are responsible for these differences. Several basic questions about the genetics of human brain evolution have not been addressed. For example, does the increase in human brain size and organizational complexity involve functional modifications of many genes or just a limited number of genes? Do these functional modifications stem from changes in gene expression or protein sequence? How important is the gain (or loss) of genes as compared with functional modifications of existing genes? And, are post-transcriptional regulations such as alternative splicing more complex (and functionally more important) in the human brain than in the brains of other species.

Recent progress in comparative genomics, population genetics and molecular phylogenetics is beginning to offer a glimmer of hope for addressing these and other important questions about the genetics of human brain evolution. In particular, several recent studies have identified genes in the nervous system that show atypical patterns of molecular evolution in primates (and especially in the lineage leading to humans), indicating that these genes might have contributed to the evolution of the human brain<sup>8–21</sup>. Intriguingly, although perhaps not surprisingly, many of these genes are involved in regulating the size and organization of the brain during development<sup>13–17,20,21</sup>.

Mutations in several of these genes in humans lead to congenital microcephaly - a disorder that is characterized by marked reduction in brain size, with or without other abnormalities<sup>22,23</sup>. From a developmental perspective, microcephaly can be thought of as ATAVISTIC — a 'throwback' disorder whereby the brain size of affected individuals reverts to that of early HOMINID ancestors<sup>22,24</sup>. However, from a genetic perspective, this view is superficial because mutations that cause microcephaly typically disrupt gene function rather than revert a gene to its ancestral sequence. Nevertheless, given that disruption of certain genes results in a brain phenotype that resembles an ancestral state, we can speculate that genetic changes in these genes that were acquired during human evolution might contribute to the derived brain phenotype of modern humans.

Although the pronounced brain expansion in H. sapiens undoubtedly resulted from genetic changes that accumulated during human evolution, we do not know whether variations in brain phenotype in modern humans are primarily due to genes or the environment. Nonetheless, growing evidence indicates that within-human differences in brain structure and function also have a strong genetic basis. Recent studies have shown that genes exert a strong influence on the overall volume of the brain in humans<sup>25</sup> and on the size and morphology of specific functional domains in the brain, such as the FRONTAL LOBES, the SENSORIMOTOR CORTEX and BROCA'S AND WERNICKE'S SPEECH AREAS<sup>26</sup>. Cognitive abilities and personality traits in humans also have a strong genetic component<sup>27-30</sup>, and cognitive abilities and brain size are moderately correlated<sup>31-34</sup> (but see also REF. 35 for contradicting data). In light of these observations, the search for the genetic basis of human brain evolution is not



Figure 1 | Values of the encephalization quotient (EQ) for representative primates and hominids. EQ is calculated by one of two allometric scaling equations:  $EQ = E/P^{0.28}$  and  $EQ = E/P^{0.59}$ , where E is brain weight and P is body weight. Although exponents of 0.67 (REF. 1) and 0.75 (REFS. 102,103) have been postulated for mammals, these high values are only suitable for comparisons at broad taxonomic levels and are not appropriate for closely related species<sup>36,104–108</sup>. For related species, much lower exponents have been proposed, ranging from 0.28 (REFS. 36,104) to 0.59 (REF. 105). Given the uncertainty in the exponent and the debate over the relevance of EQ in gauging an animal's brain capacity (see REF. 109 and accompanying commentaries), two sets of EQ values are presented, one calculated from the lower-bound exponent of 0.28, the other from the upper-bound value of 0.59. a | EQ values for species residing along the primate lineage leading to Homo sapiens. The red circles follow the vertical axis on the left, and indicate EQ values that are calculated from an exponent of 0.28. The blue squares follow the vertical axis on the right, and indicate EQ values that are calculated from an exponent of 0.59. EQ at time point A is estimated from Eocene and Oligocene prosimians<sup>1,110</sup>, B is from Aegyptopithecus zeuxis<sup>110,111</sup>, C is from Proconsul africanus<sup>112,113</sup>, D is from Australopithecus afarensis<sup>37</sup>, E is from Homo habilis<sup>37</sup>, F is from Homo erectus<sup>37</sup>, G is from archaic H. sapiens<sup>37</sup> and H is from modern H. sapiens<sup>37</sup>. **b** | EQ values for extant and extinct primates. Two values are given for each species — the top one is calculated from an exponent of 0.28 and the bottom one (in parentheses) is calculated from an exponent of 0.59. For ancestral nodes, A is estimated from Eocene and Oligocene prosimians<sup>1,110</sup>, B is from A. zeuxis<sup>110,111</sup> and C is from P. africanus<sup>112,113</sup>. EQ values of A. zeuxis and P. africanus should be treated provisionally because they are based on limited fossil data.

only relevant to understanding the emergence of *H. sapiens* as a species, but might also shed light on the differences in brain phenotype such as brain size and organization, cognitive abilities, personality traits and perhaps even psychiatric conditions — among individual humans. In this article, we describe the latest findings linking genes that are involved in brain development — many of which have been identified through studies of brain malformations — to brain evolution. We place particular emphasis on genes that are implicated in microcephaly, and propose that a subset of microcephaly genes have had important roles in the evolutionary enlargement of the human brain. We discuss the implications of this hypothesis in the search for further genes involved in human brain evolution. We also suggest the converse strategy — that evolutionary analysis might aid in the identification of further microcephaly genes. Finally, we lay out a methodological template that combines large-scale surveys with individual-gene analyses, and argue that this template might be broadly applicable to the genetic analysis of human-specific biological traits (or more generally, species-specific traits in diverse taxa).

# Brain expansion in human evolution

There is a prevailing sense among the general public — and even among some biologists — that the human brain is so highly evolved relative to the brains of other primates that it must belong to a class of its own. By extension, the brains of all other primates, including our closest kin, the chimpanzees, are perceived as being so inferior to ours that they are all relegated to a single 'less-evolved' cohort.

Such an anthropocentric view might have some justification. After all, H. sapiens is the only extant species that can achieve advanced culture through its cognitive endowment. However, this view is incomplete at best. First, there has been a progressive increase in brain size and complexity over the 60-70 million year interval leading from ancestral primates to anatomically modern humans<sup>1,36</sup> (FIG. 1a). Although this increase might seem progressive over extended evolutionary time, it contains many bursts and periods of stasis on a finer timescale. For example, the past 2-3 million years of hominid evolution witnessed the most pronounced burst of brain expansion<sup>37</sup>. Given the progressive (although fitful) history of brain expansion along the lineage to humans, species that branched off more recently from this lineage, such as apes, tend to have larger and more complex brains compared with species that branched off at an earlier stage, such as prosimians (FIG. 1b). Second, studies have shown that mammals in general — and to some degree birds show a trend for increasing encephalization over evolutionary time that is not found in most other vertebrates<sup>1</sup>. This phenomenon probably reflects an elevated importance of cognitive abilities in the fitness of mammals and birds<sup>1,36</sup>. So, the trend of brain expansion is not the exclusive domain of humans (or even primates), although this trend is clearly the most striking in the primate

# Box 1 | The K<sub>a</sub>/K<sub>s</sub> ratio and the McDonald-Kreitman test

Non-synonymous substitutions are nucleotide changes in the coding region of a gene that alter the encoded amino acids. Because non-synonymous changes alter the biochemical properties of the protein product, they are typically subject to selection. Synonymous substitutions occur in the degenerate positions of codons and therefore do not alter the encoded amino acids. Because synonymous substitutions do not affect protein sequence, they are generally considered to be functionally neutral. The rate of synonymous substitutions is therefore taken as an indicator of the neutral mutation rate. A useful parameter in molecular evolutionary studies is the ratio of non-synonymous substitution rate (commonly denoted as  $K_a$ ) to synonymous substitution rate ( $K_s$ ) that is observed in a gene over a certain evolutionary period. It is, in essence, a measurement for the pace by which the protein product of a gene has evolved compared with that expected under selective neutrality. A high (or low)  $K_a/K_s$  ratio is interpreted to mean that the protein encoded by the gene has evolved rapidly (or slowly). A ratio greater than 1 means that the rate of non-synonymous substitutions is faster than that expected under selective neutrality, presumably owing to the presence of positive selection that favours amino-acid replacements in the protein product.

The McDonald–Kreitman test for positive selection is used to test whether the  $K_a/K_s$  ratio for a gene in an evolutionary lineage exceeds that expected under selective constraint alone<sup>87</sup>. The test first examines the intraspecies polymorphism pattern in the coding region of a gene, and obtains the  $K_a/K_s$  ratio for that polymorphism. It then examines the interspecies divergence pattern within the same gene, and obtains the  $K_a/K_s$  ratio for that divergence. The test considers the intraspecies ratio to be what is expected under selective constraint alone. If the interspecies ratio is significantly higher than the intraspecies ratio, the data are taken to mean that positive selection has elevated the interspecies ratio above that expected under selective constraint alone.

lineage leading to humans<sup>1,37</sup>. Third, the capacity for advanced culture might, on the surface, indicate a qualitative difference between the human brain and that of other primates. However, it is possible that sophisticated cultural capabilities might have resulted from incremental changes in brain function. By this argument, once brain size and structural complexity surpassed a certain threshold, as in the case of humans, cognitive abilities might increase disproportionately with physical improvements of the brain.

These arguments notwithstanding, the human brain is by far the most complex — and powerful — among extant species. Therefore, we speculate that the evolution of the human brain has been a 'privileged' process — one that involves selective regimes that are distinct from those operating in other taxa. A recent study suggested that the evolution of the genes of the nervous system experienced a pronounced acceleration in the lineage leading to humans as compared with other taxa<sup>20</sup>, lending some support to the view that the human brain is the product of distinct evolutionary forces (see below for further discussion).

Having big brains exacts high fitness costs. First, the brain is one of the most metabolically expensive organs in humans, accounting for approximately 20% of total energy consumption at rest, even though it constitutes only about 2% of body

weight<sup>38-40</sup>. Second, enlarged cranial capacity creates maladaptive birthing difficulties that contribute directly to the high rate (0.5-1%) of labour-related maternal mortality that existed before modern medicine — by far the highest among mammals<sup>41–43</sup>. To compensate for the birthing difficulties, pelvic apertures increased, thereby reducing the efficiency of bipedal locomotion<sup>44</sup>. Third, larger brains take much longer to mature, which significantly extends gestation and child-rearing times, therefore exerting high demands on the mother and reducing the total number of offspring that she can have<sup>45-47</sup>. Given these significant fitness costs associated with increased encephalization, large brains must confer substantial adaptive benefits that more than offset the high costs.

In light of the above points, and considering the fact that many mutations are probably needed to create the larger and more complex human brain<sup>20</sup>, we posit that the selective forces driving the pronounced encephalization in primates (especially in the human lineage) must have been exceptionally strong — far stronger than the selection that operates on species with smaller, less complex brains, such as rodents. We further argue that such intense selection probably left a rich collection of tell-tale signatures in primate genomes. Identification of such signatures will be a crucial first step towards understanding the genetic basis of human brain evolution.

## **Evolution of nervous system genes**

So far, most efforts to address the genetic basis of human brain evolution have focused on one gene or one gene family at a time. The ad hoc nature of these studies (and their scarcity) made it difficult to discern broad evolutionary trends. A recent study took a more systematic approach by examining the evolution of 214 genes that are implicated in diverse aspects of the human nervous system<sup>20</sup>. Coding sequences of these genes were compared across four mammalian taxa - human, macaque (an Old World monkey), rat and mouse. For each gene, the ratio of non-synonymous to synonymous substitution rates — that is, the  $K_1/K_1$  ratio (BOX 1) - was calculated separately for primates (on the basis of a human-macaque comparison) and for rodents (on the basis of a rat-mouse comparison). On average, the  $K_{a}/K_{a}$  ratio was higher in genes of the nervous system in primates than in rodents (by over 30%), indicating that the proteins encoded by these genes have evolved 30% faster in primates. The study showed that this trend is due to many genes rather than a few exceptional outliers. Moreover, when examining only the subset of genes that function predominantly during nervous-system development, the primate-rodent disparity in the  $K_c/K_c$ ratio became even more pronounced (over 50% higher in primates than rodents). By contrast, genes that function primarily in the routine physiology and maintenance of the nervous system showed much less disparity between primates and rodents. Importantly, within primates, the increase in the  $K_{a}/K_{c}$  ratio is most prominent in the lineage leading from ancestral primates to humans. Collectively, these observations indicate that the remarkable phenotypic evolution of the human nervous system might be correlated with accelerated evolution of the underlying genes, particularly those involved in the development of the nervous system.

One 'trivial' explanation for these findings is that the EFFECTIVE POPULATION SIZE  $(N_{a})$ in primates, and particularly in the lineage leading to humans, is far smaller than that of the other lineages included in the study. In theory at least, a small  $N_{o}$  is predicted to relax selective constraint, thereby allowing the fixation of slightly deleterious mutations<sup>48</sup>. By this argument, the elevated  $K_{L}/K_{L}$  ratio in certain classes of genes during human evolution is not due to positive selection, but is rather the result of relaxed selective constraint under a small  $N_{i}$  that allowed the fixation of many slightly deleterious mutations. This argument is unlikely for several reasons. First, if a small  $N_{a}$  is indeed at work,

Table 1   Known human microcephaly genes and syndromes							
Gene	Syndrome	Microcephaly*	Mental retardation <sup>‡</sup>	Motor defect§	Seizure	Early death	References
High-functioning group							
ASPM	Primary microcephaly	++	+	-	-	-	58,59
MCPH1	Primary microcephaly	++	+	-	-	-	60–62
SHH	Microcephaly with facial anomalies <sup>¶</sup>	+	+	-	+/#	-	63–66
Low-functioning group							
ARFGEF2	Microcephaly with heterotopia	++	++	+	+	+	70,71
ATR	Seckel syndrome**	++	++	+	+	+	72–76
SLC25A19	Amish lethal microcephaly	++	++	+	+	+	77–79

\*Microcephaly is defined as mild when the occipitofrontal circumference at birth is at -2 to -3 standard deviations (SD) (+), or severe when it is at or below -3 SD (++). \*Mental retardation is defined as moderate to severe (+), or profound (++). \*Hypotonia or mixed axial hypotonia and limb spasticity. "Early death is defined as death that typically occurs within the first several years of life. \*Heterozygous sonic hedgehog (*SHH*) mutations cause microcephaly and mild facial anomalies without holoprosencephaly in one-third of cases. \*Variable manifestation. \*\*Seckel syndrome is characterized by severe and proportionate microcephaly and growth deficiency. *ARFGEF2*, ADP-ribosylation factor guanine nucleotide-exchange factor 2; *ASPM, asp* (abnormal spindle)-like, microcephaly-associated; *ATR*, ataxia telangiectasia and Rad3-related; *MCPH1*, microcephaly, primary autosomal recessive 1; *SLC25A19*, solute carrier family 25 (mitochondrial deoxynucleotide carrier), member 19; –, indicates the absence of a defect.

it should cause other classes of genes (not just those of the nervous system) to show an elevated  $K_a/K_s$  ratio as well. Second, among the genes of the nervous system surveyed, those implicated in development — but not those involved in the routine physiology and maintenance of the nervous system — showed elevated  $K_a/K_s$  ratios in primates<sup>20</sup>. This specificity is inconsistent with a small  $N_c$  in itself. Finally, further studies on several of the genes of the nervous system that were surveyed found compelling evidence that their evolution was driven by positive selection rather than relaxed constraint (see below for further discussion).

The large-scale study shed light on several long-standing questions about the genetic basis of human brain evolution<sup>4</sup>. The first is whether functionally important mutations have occurred predominantly in the regulatory sequences or protein-coding regions of genes. Data from the study argue that changes that alter amino-acid sequences are likely to be important in driving brain evolution, although this conclusion does not in any way imply that regulatory changes are necessarily less important. Indeed, some studies have suggested that changes in gene regulation might also have a role in the evolution of the human brain<sup>49-51</sup>, although this idea is not without contention<sup>52</sup>. The study also asked whether many genes or just a few key genes confer the increase in brain size and structural complexity<sup>4</sup>. The results indicate that a large number of mutations in coding sequences of many genes were probably needed to produce the pronounced structural changes observed in the primate brain. Indeed, it can be roughly estimated

that there is an excess of 1-2 non-synonymous substitutions per gene in the nervous system of primates over rodents. The excess rises to 3-4 non-synonymous substitutions per gene in primates over rodents when considering only the developmental gene subgroup. So, thousands of mutations in many hundreds (or possibly even thousands) of genes might have contributed to the evolution of the human brain. The third question addressed by the study is how easily detectable these functionally important mutations are. The results indicate that the human genome, together with the genomes of other species, might contain many 'smoking guns' that are informative about the important genetic changes involved in the emergence of the human form.

It is worth noting that the genes of the nervous system that were surveyed in the study were selected on the basis of a limited set of criteria, including prior knowledge of their function in the nervous system, prominent expression in the brain and/or involvement in diseases of the nervous system. Admittedly, these criteria are rather arbitrary. It would be of interest for future studies to examine whether other means of choosing genes, such as using GENE ONTOLOGY (GO) categories<sup>53</sup> (see also Onlne links box), would produce similar findings.

Among the 214 genes of the nervous system that were surveyed, the ones that showed statistically higher  $K_a/K_s$  values in primates than in rodents were referred to as the 'primate-fast outliers'<sup>4</sup>. Further analyses of these outlier genes found that many bore evolutionary signatures that were consistent with their having had a role in human brain evolution (REFS. 13–17,20; S.L.G., W.B.D.

and B.T.L., unpublished observations). Remarkably, most of the primate-fast outliers are involved in the regulation of either brain size or behaviour<sup>20</sup>. Of the genes that regulate brain size, three are of particular interest because their loss-of-function mutations in humans cause microcephaly. These are *asp* (abnormal spindle)-like, microcephaly-associated (*ASPM*), microcephaly, primary autosomal recessive 1 (also known as microcephalin; *MCPH1*) and sonic hedgehog (*SHH*), and are the focus of subsequent discussions.

# Microcephaly: a developmental defect

Congenital microcephaly or small head size is usually defined as an OCCIPITOFRONTAL CIRCUMFERENCE that is at or below -2 standard deviations (SD) at birth. By this definition, microcephaly is found in ~2% of newborns and constitutes a feature found in more than 400 genetic syndromes. So, a more restrictive definition of microcephaly has been proposed — birth occipitofrontal circumference that is at or below -3 SD<sup>23,54</sup>. The head size of long-term survivors in these severe cases typically ranges between -5 and -10 SD later in life, resulting in adult head circumferences of 40-45 cm (the normal range is 53-59 cm). These head sizes are in the same range as those of extant great apes, or early hominid ancestors such as those from the genus Australopithecus.

Clinical observations have shown that most patients with severe congenital microcephaly fall into one of two groups: a 'high-functioning' group characterized by relatively mild phenotypes, and a 'low-functioning' group with much more severe phenotypes<sup>23,55,56</sup>



Figure 2 | **Brain magnetic resonance images. a** | Brain of a normal 8-month-old child. **b** | Brain of an 8-month-old child with primary microcephaly.

(TABLE 1). The high-functioning group consists of children with marked congenital microcephaly (FIG. 2), who nevertheless reach many developmental milestones. Most learn to walk by 2 years of age and develop limited language skills<sup>54,55,57</sup>. Brain imaging shows a simplified brain-folding pattern that is presumably due to the marked reduction in cerebral cortical area, but there are no other apparent neuroanatomical abnormalities (FIG. 2). Patients with homozygous mutations of ASPM and MCHP1 belong to this group<sup>58-62</sup>. Even less severe forms of microcephaly are observed in some patients with heterozygous mutations of SHH (REFS 63-65) (TABLE 1). However, many patients with SHH mutations have a more severe brain malformation known as holoprosencephaly that is caused by defects in the development of the ventral aspect of the neural tube<sup>63-67</sup>.

In the low-functioning group, affected children suffer from, in addition to marked microcephaly, many defects including profound mental retardation, failure to achieve developmental milestones, severe HYPOTONIA or SPASTICITY from birth, earlyonset intractable epilepsy, and frequent early death54-56,68,69. Brain-imaging studies show a simplified folding pattern and often other abnormalities. These phenotypes are typically associated with mutations of the other three known microcephaly genes (TABLE 1). For example, patients with homozygous mutations of ADP-ribosylation factor guanine nucleotide-exchange factor 2 (ARFGEF2) have extensive **PERIVENTRICULAR NODULAR HETER**-OTOPIA<sup>70,71</sup>. Patients with Seckel syndrome and homozygous mutations in ataxia telangiectasia and Rad3-related (ATR) or related pathways

have severe intrauterine and postnatal growth deficiency, skeletal and brain anomalies, and sometimes lymphoma or PANCYTOPENIA<sup>72–76</sup>. Finally, patients that have Amish lethal microcephaly, which is due to homozygous mutations of solute carrier family 25 (mitochondrial deoxynucleotide carrier), member 19 (*SLC25A19*), have 2-KETOGLUTARIC ACIDURIA, severe hypoplasia of the brainstem and cerebellum, and pathological evidence of malformation and degeneration of various brain regions<sup>77–79</sup>.

An obvious interpretation of these observations is that patients in the high-functioning microcephaly group have defects that are restricted to the proliferation of neural progenitor cells (neuroblasts), with little disruption of other developmental processes. By contrast, patients in the low-functioning group have major disruptions of several developmental processes, as well as in neuroblast proliferation.

Recent molecular evolutionary studies of these six microcephaly genes have also revealed two distinct groups: those with signatures of adaptive evolution in the lineage leading to humans (ASPM, MCPH1 and SHH) and those without such signatures (ARFGEF2, ATR and SLC25A19) (REFS. 13-17,20; S.L.G., W.B.D. and B.T.L., unpublished observations). Remarkably, the grouping that is based on evolutionary criteria is the same as that based on phenotypic severity. Genes associated with adaptive evolution in the human lineage are linked to high-functioning microcephaly (that is, relatively mild phenotypes and prolonged survival despite marked microcephaly), whereas genes that are not associated with

evidence of adaptive evolution are linked to low-functioning microcephaly (that is, very severe phenotypes and typically early death, as well as microcephaly).

We speculate that the concordance between adaptive evolution and phenotypic severity reflects something about the 'evolutionary plasticity' of a given gene. There are two mutually compatible hypotheses to account for this evolutionary plasticity. First, non-synonymous mutations in genes involved in high-functioning microcephaly are perhaps more likely to be advantageous (given the highly specific function of these genes in regulating brain size). These advantageous mutations might lead to brain enlargement with few deleterious 'side effects'. Second, non-synonymous mutations in genes implicated in low-functioning microcephaly are more likely to be deleterious (given the pleiotropic effect of these genes in several tissues), and therefore poorly tolerated by selection. In either case, genes implicated in high-functioning microcephaly are more likely to show signatures of adaptive evolution (such as elevated  $K_{L}/K_{r}$  ratios) in the lineage leading to humans, where brain expansion has been the most pronounced.

# **Evolution of microcephaly genes**

Of the three genes associated with highfunctioning microcephaly, ASPM and MCPH1 have been studied extensively with respect to their molecular evolution. These two genes are implicated in a subclass of congenital microcephaly known as primary (or true) microcephaly, also referred to as microcephalia vera. In primary microcephaly, affected individuals have a significantly smaller brain size at birth (and are also intellectually impaired), but are free of other gross neurological deficits or dysmorphic features. On reaching adulthood, brains of primary microcephaly patients typically have a volume of around 400 cm<sup>3</sup>, far smaller than the 1200–1600 cm<sup>3</sup> of a normal adult human brain<sup>23</sup>. So far, six autosomal recessive loci, MCPH1-MCPH6, have been linked to clinically indistinguishable forms of primary microcephaly<sup>60,80-85</sup>. For two of these MCPH loci, the underlying genes have been identified by genetic linkage studies. One is microcephalin, which corresponds to the MCPH1 locus<sup>60-62</sup>; the other is ASPM, which corresponds to the MCPH5 locus58,59.

For both genes, comparisons of coding sequences across many species revealed features that are consistent with strong adaptive evolution in the lineage leading to humans<sup>13–17</sup>. First, the rates of non-synonymous substitutions are markedly accelerated in primates



Figure 3 | **Molecular evolution of ASPM and MCPH1 in primates.** The  $K_a/K_s$  (non-synonomous substitution rate to synonomous substitution rate) ratio is given for each branch of the phylogenetic trees. High ratios indicate rapid non-synonymous changes after scaling to the neutral mutation rate. The primate lineage leading to humans is shown in red. *ASPM, asp* (abnormal spindle)-like, microcephaly-associated; *MCPH1*, microcephaly, primary autosomal recessive 1. Adapted, with permission, from REFS. 14,17 © (2004) Oxford University Press.

compared with non-primate mammals. Second, within primates, this acceleration is most prominent in the lineage leading to humans (FIG. 3). Indeed, for both genes, several phylogenetic segments in the lineage leading to humans show  $K_{c}/K_{c}$  ratios that are greater than 1; a signature of positive selection<sup>86</sup> (FIG. 3). Third, using the McDonald-Kreitman test, which is a relatively stringent test of positive selection<sup>87</sup> (BOX 1), it can be shown for both genes that the rate of non-synonymous substitutions in the lineage to humans far exceeds that expected under functional constraint alone — providing a statistically robust signature of positive selection<sup>87,88</sup>. Finally, accelerated evolution seems to be highly localized within specific regions of these genes, indicating that positive selection has targeted certain domains more intensely than others. Taken together, the above observations provide compelling evidence that ASPM and MCPH1 have been the target of strong positive selection during primate evolution, particularly in the primate lineage that leads to humans.

Although both ASPM and MCPH1 show accelerated evolution in the primate lineage leading to humans, their evolutionary rates are not consistently high throughout this lineage. For ASPM, the most pronounced acceleration occurred in the lineage from ape ancestors to humans. By contrast, evolutionary acceleration of MCPH1 is most pronounced in early portions of the lineage to humans (from the last common ancestors of SIMIANS to the last common ancestors of the great apes) (FIG. 3). These observations indicate that if ASPM and MCPH1 did contribute to the continuous brain expansion that is evident in the trajectory from ancestral primates to modern humans, then mutations in ASPM might have been particularly important during the late portion of this lineage, whereas alterations in MCPH1 might have contributed more to an earlier episode of primate encephalization. Indeed, there is no reason to expect that a gene that is important for brain evolution should only exert its influence in the terminal human branch

after the human-chimpanzee divergence. As discussed earlier, there is ample evidence that brain size and complexity have increased progressively throughout the lineage from ancestral primates to anatomically modern humans, although the increase is the most pronounced in the past 2–3 million years of hominid evolution<sup>37</sup>.

How many advantageous non-synonymous substitutions have occurred in ASPM and MCPH1 in the lineage leading to humans? A rough estimate that is based on the McDonald-Kreitman test indicates that about 43 advantageous substitutions have taken place in ASPM over the 18-20 million years of evolution from the last common ape ancestors to humans (or ~2 favourable changes per million years)14. For MCPH1, roughly 45 advantageous changes occurred over 25-30 million years in the lineage from simian ancestors to humans (also ~2 favourable changes per million years)<sup>17</sup>. These estimates are probably conservative<sup>87</sup>. Such high rates of adaptive evolution are remarkable for any gene, but especially so for brain-related genes where non-synonymous mutations are more likely to be deleterious than mutations in genes of other, less complex tissue systems<sup>89</sup>.

Given that the biochemical functions of the proteins encoded by ASPM and MCPH1 are poorly understood, it is premature to speculate on the exact mechanisms by which sequence changes in these two genes resulted in enlarged brains. However, preliminary evidence does indicate that both genes are involved in regulating neuroblast proliferation during embryonic development. The mammalian ASPM protein is a putative orthologue of the Drosophila melanogaster protein, abnormal spindle (ASP), which organizes the spindle structures during cell division, including the division of neuroblasts<sup>90-92</sup>. The MCPH1 protein contains several copies of the so-called breast cancer carboxy-terminal (BRCT) domain, which is found in cell-cycle regulators such as the tumour suppressor breast cancer 1, early onset protein (BRCA1) (REF. 93), indicating that this protein might be involved in cellcycle regulation during neuroblast proliferation. This is supported by a recent study, which shows that chromosomes decondense prematurely during mitosis in cells with disrupted MCPH162. If ASPM and MCPH1 are involved in regulating neuroblast proliferation, then evolutionary changes in these genes might alter the rate of division and/or differentiation of neuroblasts during the neurogenic phase of embryogenesis, which in turn could alter the size and structure of the resulting brain.

## A scheme for studying human evolution

Large-scale evolutionary studies that involve many genes can reveal genome-wide trends that are not visible to individual gene studies, but the logistical limitations of these studies typically prohibit tailored analysis of specific genes. By contrast, individual gene studies can uncover nuanced evolutionary features of a particular gene, but they are illequipped to address genome-wide trends. Using several recent studies<sup>13-17,20</sup>, we propose a methodological template that begins with large-scale studies and moves progressively towards tailored analysis of specific genes (FIG. 4). Although here we show how it can be applied to the genetic dissection of human brain evolution, we propose that it can be used broadly to study the genetics of species-specific traits.

The first stage requires large-scale comparisons of genes across several strategically selected species. Genes are chosen to include both a set of 'test genes' (that is, genes that are related to some or all aspects of brain function) and a set of 'control genes' (that is, genes that are involved in general housekeeping functions or in tissue systems with limited phenotypic differences among taxa). Similarly, two pairs of species are chosen that include both a test pair and a control pair (FIG. 4a). The test pair contains, in addition to the human, a species that has a much simpler brain and an appropriate level of evolutionary divergence from humans. If the species is too distant from humans, such as a non-primate mammal, the biological framework for interpreting the evolutionary data might become compromised. If the species is too close to humans, such as the chimpanzee, informative evolutionary patterns might be overwhelmed by the stochastic noise that is endemic to comparisons of closely related species. A reasonable choice is therefore a primate of intermediate divergence from humans, such as the Old World monkey (OWM). For the control pair, the rat and mouse are a good choice for several reasons. First, the evolutionary divergence between them is about 19 million years, which is comparable to the roughly 23 million years separating the human and OWM. Second, unlike humans and OWMs, which show considerable structural differences in the brain, the rat and mouse are roughly comparable in brain organization. Third, both rat and mouse are convenient model organisms that have been studied extensively and for which the complete genome sequences are available. Using these two pairs of species, evolutionary rates of genes can be derived for primates



Figure 4 | A methodological template for investigating the genetic basis of human brain evolution. a | Large-scale comparisons of brain-related genes across four strategically selected species that include the human, Old World monkey, rat and mouse. These comparisons can reveal broad genome-wide trends and uncover specific genes of interest (for example, genes with significantly higher rates of evolution in primates than rodents). b | Analysis of interesting genes identified through (a) in a wider range of species. This analysis allows a more detailed evolutionary investigation of individual genes to address questions such as whether the evolution of these genes is specifically accelerated in the lineage leading to humans compared with that in other primate and non-primate taxa. c | Polymorphism studies of interesting genes in humans. Each line represents a copy of a locus under investigation and each cross represents a mutational polymorphism. d | Correlating polymorphisms in humans with variations in brain phenotype (such as brain size). The phylogenetic relationships and evolutionary timescales depicted in (a) and (b) are based on data from REFS 114–118.

on the basis of human–OWM sequence comparison, and for rodents on the basis of rat–mouse comparison. Broad trends can be discerned by comparing primate rates with rodent rates of evolution. In the second stage, outlier genes identified in the initial large-scale survey, such as those with highly elevated rates of evolution in primates, are compared over a much wider range of

taxa that includes both non-primate and primate species (FIG. 4b). For nonprimates, extra pairs of related species are used to extend the pair-wise analysis (for example, dog-cat comparison for evolutionary rates in carnivores, rhinoceros-horse in PERISSODACTYLS and cow-sheep in ARTIODACTYLS). For primates, species that occupy key positions of the primate phylogeny are included to show whether elevated rates of evolution are concentrated in the lineage leading to humans.

In the third stage, genes showing the most interesting evolutionary patterns are subject to polymorphism studies in humans. Such studies serve two purposes. First, polymorphism data could be combined with divergence data to strengthen the case of positive selection (for example, by using the McDonald–Kreitman test as described in BOX 1). Second, polymorphism studies might reveal genetic variants that are evolving adaptively within human populations (FIG. 4c). Indeed, if a gene has experienced strong positive selection during the evolutionary lineage leading to *Homo sapiens*, there is no reason to think that such selection should have stopped after the emergence of anatomically modern humans.

In the final stage of our methodological template, polymorphisms identified in humans, especially those associated with signatures of positive selection, are subject to association studies to examine whether they correlate with phenotypic differences among individual humans (FIG. 4d). In the case of brain-related genes, phenotypes such as brain size and morphology, cognitive abilities, personality traits and even psychiatric conditions can be investigated.

# From function to evolution and back

The above discussions show that functional knowledge of the genes involved in a biologi-

## Glossary

# ARTIODACTYLS

These are even-toed ungulates (hoofed mamals).

#### ATAVISM

The reappearance in an organism of characteristics that are present in the organism's remote ancestors.

## BROCA'S AND WERNICKE'S SPEECH AREAS

The cortical regions of the brain that are primarily involved in speech. Broca's area is located in the left frontal lobe, and is required for the production of language. Wernicke's area is located in the left temporal lobe, and mediates the understanding of language.

#### EFFECTIVE POPULATION SIZE

The size of an idealized population that is stable over time and practises random mating. This size, denoted as  $N_e$ , is typically much smaller than the real population size, denoted as N, because individuals in a population do not choose mates at random.

#### FRONTAL LOBES

The front portion of the brain to which functions such as movement, speech, reasoning, planning, emotions and problem solving map.

#### GENE ONTOLOGY

A database that classifies genes into functional categories.

#### HOMINID

A family of bipedal primates that includes humans and related fossil species that post-date the human-chimpanzee divergence.

#### HYPOTONIA

An abnormal decrease in passive resistance to movement (muscle tone) in the extremities. Hypotonia is typically associated with poor head control and loosely extended arms and legs. It is indicative of diseases of the central nervous system or muscle.

#### OCCIPITOFRONTAL CIRCUMFERENCE

A measurement of the circumference of the head around the most posterior aspect of the skull (the occiput) to the most anterior portion of the frontal bone (the forehead). It is used to monitor brain growth by comparing the measurement with standard graphs of the expected head size for a given set of ages.

#### PANCYTOPENIA

A shortage of all types of blood cell, including red and white blood cells, as well as platelets.

## PERISSODACTYLS These are odd-toed ungulates.

PERIVENTRICULAR NODULAR HETEROTOPIA In the brain, nodules comprising nerve cells and supporting cells (glia) that are abnormally located along the walls of the lateral ventricles. The cells should have migrated from the ventricular wall up to the cortex during embryonic development, but failed to do so.

#### SENSORIMOTOR CORTEX

The part of cerebral cortex that is directly concerned with movement of the body and perception of stimuli (especially related to touch, pressure, temperature and pain).

#### SIMIANS

A suborder of primates that contains apes (including humans), Old World monkeys and New World monkeys. Simians are also known as anthropoids or higher primates. The other suborder of primates, prosimians (also known as lower primates), is characterized by more primitive features. Prosimians are considered to be basal to simians.

#### SPASTICITY

An abnormal increase in muscle tone, which might be associated with loss of strength and coordination in voluntary movement. It is a common complication of cerebral palsy, spinal-cord injuries, stroke and some developmental defects of the central nervous system.

## 2-KETOGLUTARIC ACIDURIA

A disorder of the metabolism whereby elevated concentrations of 2-ketoglutaric acid, a naturally occurring chemical formed as a part of the tricarboxylic acid cycle, are excreted in urine. cal system can be a useful guide when probing the genetic basis of how that system evolved. This methodological model might be especially powerful for identifying genes that are involved in traits that show salient differences between humans and other species, such as those that control brain organization<sup>13–17,20</sup>, craniofacial musculature<sup>21</sup>, olfaction<sup>18</sup>, speech<sup>9</sup> and behaviour<sup>20</sup>. More specifically, we argue that genes involved in regulating brain size, particularly microcephaly genes, are likely to be important contributors to the pronounced brain enlargement that is seen in the evolutionary lineage leading to *H. sapiens*.

Just as genetic analysis of gene function can inform evolutionary studies, we argue that the converse might also be true. Evolutionary analysis provides a tool that can potentially complement genetic studies. For example, evolutionary studies might accelerate the identification of several microcephaly genes that have been mapped but not yet cloned. Genes that occur at crucial intervals can be subjected to phylogenetic analysis, and it might be worth screening for mutations in those genes that show accelerated evolution in the human lineage. This approach has the advantage of not requiring any functional information, which is often limiting.

Evolutionary analysis might also contribute to understanding biological differences among humans. As noted earlier, brain-related genes that show signatures of positive selection might be subject to continuing selection within present-day human populations. Polymorphism studies of these genes might uncover variants that are under positive selection, and association studies might correlate these variants with brain phenotype variation in humans. Of particular interest is the possibility that genetic polymorphisms in brain-related genes that have experienced positive selection might contribute to diseases of the brain such as psychiatric disorders. Indeed, genetic variants that have quickly reached a high frequency under positive selection might carry secondary, deleterious effects that are offset by the strong fitness advantages they confer. Deleterious effects can occur when a mutation that confers a strong advantage in one trait reduces the fitness of another. Alternatively, the deleterious effect might be due to a closely linked unfavourable mutation that hitchhikes with the favourable mutation. Examples of this 'beneficialdeleterious duality' have been described for the evolution of several genes, although mostly outside the nervous system<sup>94-101</sup>. It is conceivable that similar phenomena might

also apply to brain-related genes that have experienced strong selection.

We predict that future studies of brain function will continue to provide fodder for the understanding of brain evolution. Conversely, evolutionary studies will have an increasingly important role in the understanding of human biology in general, and neurobiology in particular. So, the study of the brain might be uniquely suited to fostering cross-fertilization of two traditionally distinct disciplines.

## Note added in proof

Two further primary microcephaly genes have just been identified. One is cyclindependent kinase 5 regulatory protein 2 (*CDK5RAP2*), which corresponds to the *MCPH3* locus; the other is centromereassociated protein J (*CENPJ*), which corresponds to the *MCPH6* locus. Preliminary analysis of these two genes shows that, consistent with our prediction, their rates of evolution seem to be accelerated in primates relative to other mammals, and this acceleration seems to be particularly prominent in the primate lineage leading to humans.

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- 1. Jerison, J. H. *Evolution of the Brain and Intelligence* (Academic Press, New York, 1973).
- Spuhler, J. N. The Evolution of Man's Capacity for Culture (Wayne State Univ. Press. Detroit, 1959).
- Olson, M. V. & Varki, A. Sequencing the chimpanzee genome: insights into human evolution and disease. *Nature Rev. Genet.* 4, 20–28 (2003).
- Carroll, S. B. Genetics and the making of Homo sapiens. Nature 422, 849–857 (2003).
- 5. Noback, C. R. & Montagna, W. *The Primate Brain* (Meredith Corporation, New York, 1970).
- Armstrong, E. & Falk, D. Primate Brain Evolution: Methods and Concepts (Plenum, New York, 1982).
- Matsuzawa, T. Primate Origins of Human Cognition and Behavior (Springer, Tokyo, 2001).
- Jacobs, G. H., Neitz, M., Deegan, J. F. & Neitz, J. Trichromatic colour vision in New World monkeys. *Nature* 382, 156–158 (1996).
- Enard, W. *et al.* Molecular evolution of *FOXP2*, a gene involved in speech and language. *Nature* **418**, 869–872 (2002).
- Choi, S. S. & Lahn, B. T. Adaptive evolution of MRG, a neuron-specific gene family implicated in nociception. *Genome Res.* 13, 2252–2259 (2003).
- Shi, P., Zhang, J., Yang, H. & Zhang, Y. P. Adaptive diversification of bitter taste receptor genes in mammalian evolution. *Mol. Biol. Evol.* **20**, 805–814 (2003).
- Mundy, N. I. & Cook, S. Positive selection during the diversification of class I vomeronasal receptor-like (V1RL) genes, putative pheromone receptor genes, in human and primate evolution. *Mol. Biol. Evol.* **20**, 1805–1810 (2003).
- <sup>2</sup>Dhang, J. Evolution of the human ASPM gene, a major determinant of brain size. *Genetics* **165**, 2063–2070 (2003).

- Evans, P. D. et al. Adaptive evolution of ASPM, a major determinant of cerebral cortical size in humans. Hum. Mol. Genet. 13, 489–494 (2004).
- Kouprina, N. *et al.* Accelerated evolution of the ASPM gene controlling brain size begins prior to human brain expansion. *PLoS Biol.* 2, e126 (2004).
- Wang, Y. Q. & Su, B. Molecular evolution of microcephalin, a gene determining human brain size. *Hum. Mol. Genet.* **13**, 1131–1137 (2004).
- Evans, P. D., Anderson, J. R., Vallender, E. J., Choi, S. S. & Lahn, B. T. Reconstructing the evolutionary history of microcephalin, a gene controlling human brain size. *Hum. Mol. Genet.* 13, 1139–1145 (2004).
- Gilad, Y., Wiebe, V., Przeworski, M., Lancet, D. & Paabo, S. Loss of olfactory receptor genes coincides with the acquisition of full trichromatic vision in primates. *PLoS Biol.* 2, e5 (2004).
- Ferland, R. J. et al. Abnormal cerebellar development and axonal decussation due to mutations in *AHI1* in Joubert syndrome. *Nature Genet.* 36, 1008–1013 (2004).
- Dorus, S. et al. Accelerated evolution of nervous system genes in the origin of Homo sapiens. Cell 119, 1027–1040 (2004).
- Stedman, H. H. et al. Myosin gene mutation correlates with anatomical changes in the human lineage. *Nature* 428, 415–418 (2004).
- Mochida, G. H. & Walsh, C. A. Molecular genetics of human microcephaly. *Curr. Opin. Neurol.* 14, 151–156 (2001).
- Dobyns, W. B. Primary microcephaly: new approaches for an old disorder. Am. J. Hum. Genet. 112, 315–317 (2002)
- Komai, T., Kishimoto, K. & Ozaki, Y. Genetic study of microcephaly based on Japanese material. *Am. J. Hum. Genet.* 47, 51–65 (1955).
- Tramo, M. J. et al. Brain size, head size, and intelligence quotient in monozygotic twins. *Neurology* 50, 1246–1252 (1998).
- Thompson, P. M. et al. Genetic influences on brain structure. Nature Neurosci. 4, 1253–1258 (2001).
- Bouchard, T. J. Jr. Genes, environment, and personality. Science 264, 1700–1701 (1994).
- Plomin, R., Owen, M. J. & McGuffin, P. The genetic basis of complex human behaviors. *Science* 264, 1733–1739 (1994).
- Plomin, R., DeFries, J. C., McClearn, G. E. & Rutter, M. Behavioral Genetics (W. H. Freeman, New York, 1997).
   Bouchard, T. J. Jr & McGue, M. Genetic and
- environmental influences on human psychological differences. J. Neurobiol, **54**, 4–45 (2003).
- Rushton, J. P. & Ankney, C. D. Brain size and cognitive ability: correlations with age, sex, social class, and race. *Psychon. Bull. Rev.* 3, 21–36 (1996).
- Vernon, P. A., Wickett, J. C., Bazana, P. G. & Stelmack, R. M. in *Handbook of Intelligence* (ed. Sternberg, R. J.) 245–264 (Cambridge Univ. Press, Cambridge, UK, 2000).
- Posthuma, D. *et al.* The association between brain volume and intelligence is of genetic origin. *Nature Neurosci.* 5, 83–84 (2002).
- Gray, J. R. & Thompson, P. M. Neurobiology of intelligence: science and ethics. *Nature Rev. Neurosci.* 5, 471–482 (2004).
- Schoenemann, P. T., Budinger, T. F., Sarich, V. M. & Wang, W. S. Brain size does not predict general cognitive ability within families. *Proc. Natl Acad. Sci. USA* 97, 4932–4937 (2000).
- Williams, M. F. Primate encephalization and intelligence. Med. Hypotheses 58, 284–290 (2002).
- McHenry, H. M. Tempo and mode in human evolution. *Proc. Natl Acad. Sci. USA* 91, 6780–6786 (1994).
- Aschoff, J., Gunther, B. & Kramer, K. Energiehaushalt und Temperatureregulation (Urban and Schwarzenberg, Munich, 1971).
- Armstrong, E. Brains, bodies and metabolism. *Brain Behav. Evol.* 36, 166–176 (1990).
- Aiello, L. C. & Wheeler, P. The expensive tissue hypothesis: the brain and the digestive system in human and primate evolution. *Curr. Anthropol.* **36**, 199–221 (1995).
- Smith, B. H. The cost of a large brain. *Behav. Brain Res.* 13, 365–366 (1990).
   Loudon I. Maternal mortality in the past and its relevance
- Loudon, I. Maternal mortality in the past and its relevance to developing countries today. *Am. J. Clin. Nutr.* 72, 241S–246S (2000).
- Rosenberg, K. R. & Trevathan, W. R. The evolution of human birth. Sci. Am. 285, 72–77 (2001).
- Lovejoy, C. O. in *Primate Functional Morphology and Evolution* (ed. Tuttle, R. H.) 291–326 (Mouton, The Hague, 1975).
- Sacher, G. A. in *Primate Brain Evolution: Methods and Concepts* (eds Armstrong, E. & Falk, D.) 97–112 (Plenum, New York, 1982).

- Harvey, P. H. & Clutton-Brock, T. H. Life history variation in primates. *Evolution* **39**, 559–581 (1985).
- Martin, R. D. Scaling of the mammalian brain: the maternal energy hypothesis. *News Physiol. Sci.* 11, 149–156 (1996).
- Ohta, T. Slightly deleterious mutant substitutions in evolution. *Nature* 246, 96–98 (1973).
- Enard, W. et al. Intra- and interspecific variation in primate gene expression patterns. Science 296, 340–343 (2002).
- Caceres, M. et al. Elevated gene expression levels distinguish human from non-human primate brains. Proc. Natl Acad. Sci. USA 100, 13030–13035 (2003).
- Preuss, T. M., Caceres, M., Oldham, M. C. & Geschwind, D. H. Human brain evolution: insights from microarrays. *Nature Rev. Genet.* 5, 850–860 (2004).
- Uddin, M. et al. Sister grouping of chimpanzees and humans as revealed by genome-wide phylogenetic analysis of brain gene expression profiles. *Proc. Natl Acad. Sci. USA* 101, 2957–2962 (2004).
- Harris, M. A. et al. The Gene Ontology (GO) database and informatics resource. Nucleic Acids Res. 32 (Database issue). D258–D261 (2004).
- Barkovich, A. J. *et al.* Microlissencephaly: a heterogeneous malformation of cortical development. *Neuropediatrics* 29, 113–119 (1998).
- Tolmie, J. L., McNay, M., Stephenson, J. B. P., Doyle, D. & Connor, J. M. Microcephaly: genetic counseling and antenatal diagnosis after the birth of an affected child. *Am. J. Med. Genet.* 27, 583–594 (1987).
- Baraitser, M. The Genetics of Neurological Disorders 456 (Oxford Univ. Press, Oxford, 1997).
- Peiffer, A., Singh, N., Leppert, M., Dobyns, W. B. & Carey, J. C. Microcephaly with simplified gyral pattern in six related children. *Am. J. Med. Genet.* 84, 137–144 (1999).
- Bond, J. et al. ASPM is a major determinant of cerebral cortical size. Nature Genet. 32, 316–320 (2002).
- Bond, J. et al. Protein-truncating mutations in ASPM cause variable reduction in brain size. Am. J. Hum. Genet. 73, 1170–1177 (2003).
- Jackson, A. P. et al. Primary autosomal recessive microcephaly (MCPH1) maps to chromosome 8p22-pter. Am. J. Hum. Genet. 63, 541–546 (1998).
- Jackson, A. P. et al. Identification of microcephalin, a protein implicated in determining the size of the human brain. Am. J. Hum. Genet. **71**, 136–142 (2002).
- brain. Am. J. Hum. Genet. **71**, 136–142 (2002).
   Trimborn, M. et al. Mutations in microcephalin cause aberrant regulation of chromosome condensation. Am. J. Hum. Genet. **75**, 261–266 (2004).
- Cohen, M. M. Jr. Perspectives on holoprosencephaly: Part I. Epidemiology, genetics, and syndromology. *Teratology* 40, 211–235 (1989).
- Muenke, M. et al. Linkage of a human brain malformation, familial holoprosencephaly, to chromosome 7 and evidence for genetic heterogeneity. Proc. Natl Acad. Sci. USA 91, 8102–8106 (1994).
- Roessler, E. *et al.* Mutations in the human sonic hedgehog gene cause holoprosencephaly. *Nature Genet.* 14, 357–360 (1996).
- Roessler, E. *et al.* Mutations in the C-terminal domain of sonic hedgehog cause holoprosencephaly. *Hum. Mol. Genet.* 6, 1847–1853 (1997).
- Dubourg, C. et al. Molecular screening of SHH, ZIC2, SIX3, and TGIF genes in patients with features of holoprosencephaly spectrum: mutation review and genotype-phenotype correlations. Hum. Mutat. 24, 43–51 (2004).
- Sztriha, L., Al-Gazali, L. I., Varady, E., Goebel, H. H. & Nork, M. Autosomal recessive micrencephaly with simplified gyral pattern, abnormal myelination and arthrogroposis. *Neuropediatrics* 30, 141–145 (1999)
- ten Donkelaar, H. J. Major events in the development of the forebrain. *Eur. J. Morphol.* **38**, 301–308 (2000).
- Sheen, V. L. *et al.* Mutations in *ARFGEF2* implicate vesicle trafficking in neural progenitor proliferation and migration in the human cerebral cortex. *Nature Genet.* 36, 69–76 (2004).
- Sheen, V. L. et al. Autosomal recessive form of periventricular heterotopia. *Neurology* 60, 1108–1112 (2003).
- Shanske, A., Caride, D. G., Menasse-Palmer, L., Bogdanow, A. & Marion, R. Central nervous system anomalies in Seckel syndrome: report of a new family and review of the literature. *Am. J. Med. Genet.* **70**, 155–158 (1998).
- Goodship, J. et al. Autozygosity mapping of a Seckel syndrome locus to chromosome 3q22.1–q24. Am. J. Hum. Genet. 67, 498–503 (2000).
- Capovilla, G. et al. Seckel's syndrome and malformations of cortical development: report of three new cases and review of the literature. J. Child. Neurol. 16, 382–386 (2001).

- O'Driscoll, M., Ruiz-Perez, V. L., Woods, C. G., Jeggo, P. A. & Goodship, J. A. A splicing mutation affecting expression of ataxia-telangiectasia and Rad3-related protein (ATR) results in Seckel syndrome. *Nature Genet.* 39, 497–501 (2003).
- Alderton, G. K. *et al.* Seckel syndrome exhibits cellular features demonstrating defects in the ATR-signalling pathway. *Hum. Mol. Genet.* **13**, 3127–3138 (2004).
- pathway. *Hum. Mol. Genet.* **13**, 3127–3138 (2004).
  77. Kelley, R. I., Robinson, D., Puffenberger, E. G., Strauss, K. A. & Morton, D. H. Amish lethal microcephaly: a new metabolic disorder with severe congenital microcephaly and 2-ketoglutaric aciduria. *Am. J. Med. Genet.* **112**, 318–326 (2002).
- Rosenberg, M. J. *et al.* Mutant deoxynucleotide carrier is associated with congenital microcephaly. *Nature Genet.* 32, 175–179 (2002).
- Strauss, K. A., Pfanni, R. & Morton, D. H. The neuropathology of Amish lethal microcephaly. *Am. J. Hum. Genet.* **71**, A517 (2002).
- Roberts, E. et al. The second locus for autosomal recessive primary microcephaly (MCPH2) maps to chromosome 19q13.1–13.2. Eur. J. Hum. Genet. 7, 815–820 (1999).
- Moynihan, L. *et al.* A third novel locus for primary autosomal recessive microcephaly maps to chromosome 9q34. *Am. J. Hum. Genet.* 66, 724–727 (2000).
- Jamieson, C. R., Govaerts, C. & Abramowicz, M. J. Primary autosomal recessive microcephaly: homozygosity mapping of MCPH4 to chromosome 15. Am. J. Hum. Genet. 65, 1465–1469 (1999).
- Jamieson, C. R., Fryns, J. P., Jacobs, J., Matthijs, G. & Abramowicz, M. J. Primary autosomal recessive microcephaly: *MCPH5* maps to 1q25–q32. *Am. J. Hum. Genet.* 67, 1575–1577 (2000).
- Pattison, L. *et al.* A fifth locus for primary autosomal recessive microcephaly maps to chromosome 1q31. *Am. J. Hum. Genet.* **67**, 1578–1580 (2000).
   Leal, G. F. *et al.* A novel locus for autosomal recessive
- Leal, G. F. *et al.* A novel locus for autosomal recessive primary microcephaly (*MCPH6*) maps to 13q12.2. *J. Med. Genet.* 40, 540–542 (2003).
- Li, W. H. *Molecular Evolution* (Sinauer, Sunderland, Massachusetts, 1997).
   McDonald, J. H. & Kreitman, M. Adaptive protein
- McDonald, J. H. & Kreitman, M. Adaptive protein evolution at the *Adh* locus in *Drosophila*. *Nature* 351, 652–654 (1991).
- Kreitman, M. Methods to detect selection in populations with applications to the human. *Annu. Rev. Genomics Hum. Genet.* 1, 539–559 (2000).
- Duret, L. & Mouchiroud, D. Determinants of substitution rates in mammalian genes: expression pattern affects selection intensity but not mutation rate. *Mol. Biol. Evol.* 17, 68–74 (2000).
- Ripoll, P., Pimpinelli, S., Valdivia, M. M. & Avila, J. A cell division mutant of *Drosophila* with a functionally abnormal spindle. *Cell* **41**, 907–912 (1985).
- Saunders, R. D., Avides, M. C., Howard, T., Gonzalez, C. & Glover, D. M. The *Drosophila* gene abnormal spindle encodes a novel microtubule-associated protein that associates with the polar regions of the mitotic spindle. *J. Cell. Biol.* **137**, 881–890 (1997).
- do Carmo Avides, M., Tavares, A. & Glover, D. M. Polo kinase and Asp are needed to promote the mitotic organizing activity of centrosomes. *Nature Cell Biol.* 3, 421–424 (2001).
- Huyton, T., Bates, P. A., Zhang, X., Sternberg, M. J. & Freemont, P. S. The BRCA1 C-terminal domain: structure and function. *Mutat Res* 460, 319–332 (2000).
- Haldane, J. B. S. The rate of mutation of human genes. Hereditas 35 (Suppl. 1), 267–272 (1949).
- 95. Mears, J. G. *et al.* Sickle gene. Its origin and diffusion from West Africa. J. Clin. Invest. **68**, 606–610 (1981).
- Flint, J. *et al.* High frequencies of α-thalassaemia are the result of natural selection by malaria. *Nature* **321**, 744–750 (1986).
- Lell, B. *et al.* The role of red blood cell polymorphisms in resistance and susceptibility to malaria. *Clin. Infect. Dis.* 28, 794–799 (1999).
- Tishkoff, S. A. *et al.* Haplotype diversity and linkage disequilibrium at human G6PD: recent origin of alleles that confer malarial resistance. *Science* 293, 455–462 (2001).
- Sabeti, P. C. *et al.* Detecting recent positive selection in the human genome from haplotype structure. *Nature* 419, 832–837 (2002).
- Aidoo, M. *et al.* Protective effects of the sickle cell gene against malaria morbidity and mortality. *Lancet* **359**, 1311–1312 (2002).
   Ayi, K., Turrini, F., Piga, A. & Arese, P. Enhanced
- 101. Ayi, K., Turrini, F., Piga, A. & Arese, P. Enhanced phagocytosis of ring-parasitized mutant erythrocytes: a common mechanism that may explain protection against falciparum malaria in sickle trait and β-thalassemia trait. *Blood* **104**, 3364–3371 (2004).

- Bauchot, R. Encephalization in vertebrates. A new mode of calculation for allometry coefficients and isoponderal indices. *Brain Behav. Evol.* **15**, 1–18 (1978).
- Martin, R. D. Relative brain size and basal metabolic rate in terrestrial vertebrates. *Nature* 293, 57–60 (1981).
- 104. Lapicque, L. Sur la relation du poids de l'encéphale au poids du corps. C. R. Seances Soc. Biol. Fil. 50, 62–63 (1898).
- Martin, R. D. & Harvey, P. H. in Size and Scaling in Primate Biology (ed. Jungers, W. L.) (Plenum, New York, 1985).
- Pilbeam, D. & Gould, S. J. Size and scaling in human evolution. *Science* 186, 892–901 (1974).
- Martin, R. D. Primate Origins and Evolution: a Phylogenetic Reconstruction (Princeton Univ. Press, Princeton, 1990).
- Kruska, D. C. On the evolutionary significance of encephalization in some eutherian mammals: effects of adaptive radiation, domestication, and feralization. *Brain Behav. Evol.* **65**, 73–108 (2005).
- 109. Finlay, B. L., Darlington, R. B. & Nicastro, N. Developmental structure in brain evolution. *Behav. Brain Sci.* 24, 263–278; discussion 278–308 (2001).
- Radinsky, L. Early primate brains: facts and fiction. J. Hum. Evol. 6, 79–86 (1977).
- Radinsky, L. Aegyptopithecus endocasts: oldest record of a pongid brain. Am. J. Phys. Anthropol. 39, 239–247 (1973).
- 112. Gingerich, P. D. Correlation of tooth size and body size in living hominoid primates, with a note on relative brain size in Aegyptopithecus and Proconsul. Am. J. Phys. Anthropol. 47, 395–398 (1977).
- Walker, A., Falk, D., Smith, R. & Pickford, M. The skull of *Proconsul africanus*: reconstruction and cranial capacity. *Nature* 305, 525–527 (1983).
- Kumar, S. & Hedges, S. B. A molecular timescale for vertebrate evolution. *Nature* **392**, 917–920 (1998).
- Murphy, W. J. *et al.* Resolution of the early placental mammal radiation using Bayesian phylogenetics. *Science* 294, 2348–2351 (2001).

- Page, S. L. & Goodman, M. Catarrhine phylogeny: noncoding DNA evidence for a diphyletic origin of the mangabeys and for a human-chimpanzee clade. *Mol. Phylogenet. Evol.* **18**, 14–25 (2001).
- Poux, C. & Douzery, E. J. Primate phylogeny, evolutionary rate variations, and divergence times: a contribution from the nuclear gene *IRBP*. *Am. J. Phys. Anthropol.* **124**, 1–16 (2004).
- 118. Springer, M. S., Murphy, W. J., Eizirik, E. & O'Brien, S. J. Placental mammal diversification and the Cretaceous–Tertiary boundary. *Proc. Natl Acad. Sci. USA* 100, 1056–1061 (2003).

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#### Competing interests statement

The authors declare no competing financial interests.

# **Online links**

#### DATABASES

## The following terms in this article are linked online to:

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Seckel syndrome

#### FURTHER INFORMATION

Gene Ontology: http://www.geneontology.org The Lahn Laboratory web site: http://hominid.uchicago.edu William Dobyns' web site: http://www.uchospitals.edu/ physicians/william-dobyns.php

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## ERRATUM

# GARDENING THE GENOME: DNA METHYLATION IN ARABIDOPSIS THALIANA

Simon W.-L. Chan, Ian R. Henderson and Steven E. Jacobsen

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In Figure 3, the bottom box in the maintenance section was printed incorrectly. The correct version of the figure is shown below. This correction has been made to the online enhanced text and PDF version of this review.

